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Review and revision of empirical critical loads of nitrogen for Europe

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Abstract

This report describes the scientific background and results from the review and revision of empirical critical loads of nitrogen that had been established for Europe in 2011 under the auspices of the UNECE Convention on Long-range Transboundary Air Pollution (LRTAP Convention). In 2020, the Coordination Centre for Effects started a project under the LRTAP Convention to bring empirical critical loads up to date. New relevant information from studies (2010 - summer 2021) on the impacts of nitrogen on natural and semi-natural ecosystems was incorporated in the existing European database on empirical critical loads of N ($CL_{emp}N$). The current review and revision used for the first time gradient studies to evaluate and determine the $CL_{emp}N$. The $CL_{emp}N$ were structured according to the updated classification used within the European Nature Information System (EUNIS).

Consensus on the results was reached in a UNECE expert workshop on empirical critical loads of nitrogen (26-28 October 2021, Berne, Switzerland), organised by the Swiss Federal Office for the Environment (BAFU), the Coordination Centre for Effects, and the B-WARE Research Centre. The results, as presented in Table 1 of the Executive Summary, show that in many cases the outer ranges of the empirical critical loads have decreased. The resulting 2021 European database includes both revised and newly established value ranges of $CL_{emp}N$ for each EUNIS class. The outcomes of this report are of major importance for the protection of N-sensitive natural and semi-natural ecosystems across Europe. This knowledge is used to support European policies to reduce air pollution.

Kurzzusammenfassung

Dieser Bericht beschreibt den wissenschaftlichen Hintergrund und die Ergebnisse der Überprüfung und Überarbeitung der empirischen Critical Loads für Stickstoff ($CL_{emp}N$), die 2011 im Rahmen des UNECE-Übereinkommens über weiträumige grenzüberschreitende Luftverunreinigung (Convention on Long-range Transboundary Air Pollution, LRTAP) für Europa festgelegt wurden. Im Jahr 2020 startete das Coordination Centre for Effects ein Projekt im Rahmen des LRTAP-Übereinkommens, um die empirischen Critical Loads auf den neuesten Stand zu bringen. Neue relevante Informationen aus Studien (2010 - Sommer 2021) zu den Auswirkungen von Stickstoff auf natürliche und naturnahe Ökosysteme wurden in die bestehende europäische Datenbank zu empirischen Critical Loads für N ($CL_{emp}N$) aufgenommen. Bei der aktuellen Überprüfung und Überarbeitung wurden zum ersten Mal Gradientenstudien zur Bewertung und Bestimmung der $CL_{emp}N$ herangezogen. Die $CL_{emp}N$ wurden gemäß der aktualisierten Klassifizierung des Europäischen Naturinformationssystems (EUNIS) strukturiert.

Ein Konsens über die Ergebnisse wurde in einem UNECE-Expertenworkshop zu empirischen Critical Loads für Stickstoff (26.-28. Oktober 2021, Bern, Schweiz) erzielt, der vom Schweizer Bundesamt für Umwelt (BAFU), dem Coordination Centre for Effects und dem Forschungszentrum B-WARE organisiert wurde. Die Ergebnisse, die in Tabelle 1 der Zusammenfassung dargestellt sind, zeigen, dass sich in vielen Fällen die äußeren Bereiche der empirischen Critical Loads verringert haben. Die sich daraus ergebende europäische Datenbank für 2021 enthält sowohl überarbeitete als auch neu festgelegte Wertebereiche für $CL_{emp}N$ für jede EUNIS-Klasse. Die Ergebnisse dieses Berichts sind von großer Bedeutung für den Schutz von N-empfindlichen natürlichen und naturnahen Ökosystemen in ganz Europa. Dieses Wissen wird zur Unterstützung der europäischen Politik zur Verringerung der Luftverschmutzung genutzt.

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Summary

I. Introduction

- ▶ This report describes the scientific background and results from the review and revision of empirical critical loads of nitrogen ($CL_{emp}N$) as established for Europe in 2011 under the auspices of the UNECE Convention on Long-range Transboundary Air Pollution (LRTAP Convention). In June 2020 the Coordination Centre for Effects (CCE) started a project under the LRTAP Convention to bring the empirical critical loads of nitrogen up to date. New relevant information on the impacts of nitrogen on natural and semi-natural ecosystems was incorporated.
- ▶ This report describes the scientific background and results from the review and revision of empirical critical loads of nitrogen ($CL_{emp}N$) as established for Europe in 2011 under the auspices of the UNECE Convention on Long-range Transboundary Air Pollution (LRTAP Convention). In June 2020 the Coordination Centre for Effects (CCE) started a project under the LRTAP Convention to bring the empirical critical loads of nitrogen up to date. New relevant information on the impacts of nitrogen on natural and semi-natural ecosystems was incorporated.
- ▶ The workshop on the review and revision of empirical critical loads for Europe was held under the LRTAP Convention, in Berne, from 26 to 28 October 2021. The workshop was organised by the Coordination Centre for Effects (CCE) and hosted by the Swiss Federal Office for the Environment (BAFU).
- ▶ The workshop was attended by 37 participants from Austria, Canada, Finland, France, Germany, the Netherlands, Norway, Spain, Sweden, Switzerland, the United Kingdom, and the United States, and by representatives from the International Cooperative Programme (ICP) on ICP Waters, ICP Vegetation and ICP Modelling and Mapping. The secretariat to the Convention was not represented.
- ▶ The decision to organise the workshop was adopted at the 39th session of the Working Group on Effects (ECE/EB.AIR/144/Add.2), following recommendations from 35th session of the Task Force on Modelling and Mapping (2-4 April 2019) held as web-conference.
- ▶ The meeting was opened by Reto Meier on behalf of the BAFU and Alice James Casas as chair of the ICP Modelling and Mapping.

II. Objectives and structure of the workshop

- ▶ The objective of the workshop was the revision of the $CL_{emp}N$ for natural and semi-natural ecosystems, which were set at the last expert workshop held in Noordwijkerhout from 23 to 25 June 2010 (see Bobbink and Hettelingh, 2011), based on additional scientific information available for the period from 2010 to 2021. The workshop discussions were based on the new and updated background document that was sent to all participants prior to this workshop.
- ▶ Most of the European Nature Information System (EUNIS) classes, which were addressed in the present revision, have changed since the last update by Bobbink and Hettelingh (2011): marine habitats (EUNIS class MA, formerly A), coastal habitats (EUNIS class N, formerly B),

inland surface waters (EUNIS class C), mires, bogs and fens (EUNIS class Q, formerly D), grasslands and lands dominated by forbs, mosses or lichens (EUNIS class R, formerly E), heathland, scrubland and tundra (EUNIS class S, formerly F), woodland, forest and other wooded land (EUNIS class T, formerly G).

- ▶ An international team of 43 scientists prepared the background document for the workshop in the period from June 2020 to September 2021, after a web-based kick-off meeting in June 2020:
 - EUNIS class MA – Roland Bobbink
 - EUNIS class N – Laurence Jones, Emiel Brouwer and Eva Remke
 - EUNIS class C – Heleen De Wit, Roland Bobbink, Christin Loran, Linda May and Jan-Erik Thrane
 - EUNIS class Q - Chris Field, Julian Aherne, Roland Bobbink and Hilde Tomassen
 - EUNIS class R - Carly Stevens, Rocio Alonso, Vegar Bakkestuen, Erika Hiltbrunner and Lukas Kohli
 - EUNIS class S - Leon Van den Berg, Julian Aherne, Andrea Britton, Simon Caporn, Hector García Gómez and Liv Guri Velle
 - EUNIS class T – Sabine Braun, Rocio Alonso, Frank Ashwood, Tomas Chuman, Lucienne de Witte, Thomas Dirnböck, Per Erik Karlsson, Sirkku Manninen, Michael Perring, Hans Tömmervik, Simon Tresch, Liisa Ukomaanah and Elena Vanguelova
 - Chapter 10 “Use of empirical critical loads of nitrogen in risk assessment and nature protection” – Markus Geupel, Khalid Aazem, Sabine Augustin, Jesper Bak, Alice James Casas, Laurence Jones, Christin Loran, Reto Meier, Anne-Katrin Prescher, Thomas Scheuschner, Axel Ssymank and Susan Zappala
- ▶ The background document was, after the internal review round, reviewed by Sabine Augustin, Ariel Bergamini, Leonor Calvo Galván, Tara Greaver, Kevin Hicks, Raúl Ochoa-Huesa, Tonje I. Økland and Jan Roelofs.
- ▶ The working groups exchanged their progress in short plenary sessions. Results, conclusions, and recommendations were discussed and summarised in a final plenary session chaired by Christin Loran.

III. Conclusions

- ▶ Statistically and biologically significant outcomes of field addition experiments were the basis for the assessment of the CL_{empN} . Only studies which have independent N treatments and realistic N loads, and durations were used for updating and refining CL_{empN} values.
- ▶ In the present review and revision period an increasing number of gradient studies on atmospheric N deposition have been published in several EUNIS habitat types and proved to be useful for evaluation and setting of the CL_{empN} .
- ▶ Studies with higher N additions or shorter experimental periods were only interpreted with respect to the understanding of effects mechanisms, possible N limitation or sensitivity of the system. The methods used in these studies were carefully scrutinised to identify factors

related to the experimental design or data analysis, which may constrain their use in assessing CL_{emp}N ranges.

- ▶ CL_{emp}N were agreed on for a range of N deposition values for all treated EUNIS classes (level 2 or 3). New results regarding nitrogen effects in surface waters could be included based on activities presented by the ICP Waters. Novel findings for some Mediterranean habitats could be adopted as well.
- ▶ The new Chapter 10 “Use of CL_{emp}N in risk assessment and nature protection” was presented and discussed. It contains a selection of examples of the use of CL_{emp}N on different scales and in different European countries to provide guidance to practitioners and policy makers on how CL_{emp}N can be used in practice.
- ▶ The assessment of the reliability of the CL_{emp}N ranges was continued from the last update in 2011; distinguishing between ‘reliable’, ‘quite reliable’ and ‘expert judgement’ symbolised by ##, # and (#), respectively.
- ▶ CL_{emp}N ranges resulting from the reviewing and revising procedure were agreed by consensus at the workshop, as summarised in Table 1. In more than 40% of the EUNIS types presented, the lower value of the range had become lower than in 2011. The same applies for the upper value. In only one case did the upper value increase.

IV. Recommendations

- ▶ More research and data are required to establish a CL_{emp}N for the following ecosystems: several grasslands and steppe meadows; all Mediterranean vegetation types; wet (swamp) forests; many mires and fens and several coastal habitats; in addition, more research is needed for all distinguished EUNIS habitat types that have an ‘expert’ judgement rating.
- ▶ Impacts of N enrichment in (sensitive) freshwater and shallow marine ecosystems (including coastal waters) need further research.
- ▶ More well-designed gradient studies with both (very) low and high N loads are needed, especially in EUNIS habitat types that are hardly investigated. Furthermore, combining the results of both experimental and gradients studies increases the reliability of the CL_{emp}N.
- ▶ More research is needed on the differential effects of the deposited N forms (NO_x or NH_y) to be able to determine the critical loads for oxidised and reduced nitrogen separately in the future.
- ▶ To refine the current CL_{emp}N, long-term experiments (10-20 years) with a high N addition frequency of between 5 and 50 kg N ha⁻¹ yr⁻¹ in regions with low background deposition are crucial. This would increase the reliability of the derived CL_{emp}N if the lowest treatment level does not exceed the current critical load.
- ▶ Climate change and nitrogen deposition are likely to have strong interactive effects on ecosystem functioning, with climate change altering ecosystem responses to nitrogen deposition and vice versa. More experimental studies are needed to investigate these interactions, as well as more gradient studies that explicitly examine the impacts of nitrogen deposition in combination with climatic gradients.
- ▶ In conclusion, it is crucial to understand the long-term effects of increased N deposition on ecosystem processes in a representative range of ecosystems. It is therefore important to

quantify the effects of N deposition by manipulating N inputs in long-term ecosystem studies in both pristine and affected areas. These data in combination with gradient studies are essential to validate critical loads and develop robust dynamic ecosystem models and/or multiple correlative species models that are reliable enough to use in calculating $CL_{emp}N$ for natural and semi-natural ecosystems, and to predict natural recovery rates for N-affected systems.

Table 1. Overview of empirical N critical loads ($kg\ N\ ha^{-1}\ yr^{-1}$) to natural and semi-natural ecosystems (column 1), classified according to EUNIS (column 2), as established in 2011 (column 3), and as revised in 2022 (column 4). The reliability is indicated by ## reliable; # quite reliable and (#) expert judgement (column 5). Column 6 provides a selection of effects that may occur when critical loads are exceeded. Finally, changes with respect to 2011 are indicated as values in bold.

Ecosystem type	EUNIS code	2011 $kg\ N\ ha^{-1}\ yr^{-1}$	2022 $kg\ N\ ha^{-1}\ yr^{-1}$	2022 reliability	Indication of exceedance
Marine habitats (MA)					
Atlantic upper-mid salt marshes	MA223	20-30	10-20	(#)	Increase in dominance of graminoids; decline positive indicator species
Atlantic mid-low salt marshes	MA224	20-30	10-20	(#)	Increase in late successional species; decline positive indicator species
Atlantic pioneer salt marshes	MA225	20-30	20-30	(#)	Increase in late successional species; increase in productivity species
Coastal habitat (N)					
Shifting coastal dunes	N13, N14	10-20	10-20	#	Biomass increase; increased N leaching; reduced root biomass
Coastal dune grasslands (grey dunes)	N15	8-15	5-15	##	Increased biomass and cover of graminoids and mesophilic forbs; decrease in oligotrophic species including lichens; increased tissue N; increased N leaching; soil acidification
Coastal dune heaths	N18, N19	10-20	10-15	#	Increased plant production; increased N leaching; accelerated succession; typical lichen C:N decrease; increased yearly increment <i>Calluna</i>
Moist and wet dune slacks	N1H	10-20	5-15	#	Increased cover of graminoids and mesophilic forbs; decrease in oligotrophic species; increased Ellenberg N
Dune-slack pools (freshwater aquatic communities of)	N1H1, N1J1	10-20	10-20	(#)	Increased biomass and rate of succession

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
permanent Atlantic and Baltic or Mediterranean and Black Sea dune-slack water bodies)					

Inland surface water habitats (C) ^a

Permanent oligotrophic lakes, ponds and pools (including soft-water lakes)	C1.1	3-10	2-10 ^b	##	Increased algal productivity and a shift in nutrient limitation of phytoplankton from N to P; shifts in macrophyte community
Alpine and sub-Arctic clear water lakes	C1.1		2-4	##	Increased algal productivity and a shift in nutrient limitation of phytoplankton from N to P
Boreal clear water lakes	C1.1		3-6	##	Increased algal productivity and a shift in nutrient limitation of phytoplankton from N to P
Atlantic soft water bodies	C1.1, elements C1.2	3-10	5-10	##	Change in species composition of macrophyte communities
Permanent dystrophic lakes, ponds and pools	C1.4	3-10	5-10 ^c	(#)	Increased algal productivity and a shift in nutrient limitation of phytoplankton from N to P

Mire, bog and fen habitats (Q)

Raised and blanket bogs	Q1	5-10	5-10	##	Increase in vascular plants; decrease in bryophytes; altered growth and species composition of bryophytes; increased N in peat and peat water
Valley mires, poor fens and transition mires	Q2	10-15	5-15	##	Increase in sedges and vascular plants; negative effects on bryophytes
Palsa and polygon mires	Q3		3-10	(#)	Increase in graminoids, tissue N concentrations and decomposition rate
Rich fens	Q41-Q44	15-30	15-25	#	Increase in tall vascular plants (especially graminoids); decrease in bryophytes
Arctic-alpine rich fens	Q45	15-25	15-25	(#)	Increase in vascular plants; decrease in bryophytes

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
Grasslands and tall forb habitats (R)					
Semi-dry Perennial calcareous grassland (basic meadow steppe)	R1A	15-25	10-20	##	Increase in tall grasses; decline in diversity; change in species composition; increased mineralisation; N leaching; surface acidification
Mediterranean closely grazed dry grasslands or Mediterranean tall perennial dry grassland or Mediterranean annual-rich dry grassland	R1D or R1E or R1F	15-25	5-15	(#)	Increased production; dominance by graminoids; changes to soil crusts; changes to soil nutrient cycling
Lowland to montane, dry to mesic grassland usually dominated by <i>Nardus stricta</i>	R1M	10-15	6-10	##	Increase in graminoids; decline of typical species; decrease in total species richness
Oceanic to subcontinental inland sand grassland on dry acid and neutral soils or Inland sanddrift and dune with siliceous grassland	R1P or R1Q	8-15	5-15	(#)	Decrease in lichens; increase in biomass
Low and medium altitude hay meadows	R22	20-30	10-20	(#)	Increase in tall grasses; decrease in diversity; decline of typical species
Mountain hay meadows	R23	10-20	10-15	#	Increase in nitrophilous graminoids; changes in diversity; decline of typical species
Moist or wet mesotrophic to eutrophic hay meadow	R35	15-25	15-25	(#)	Increase in tall graminoids; decreased diversity; decrease in bryophytes
Temperate and boreal moist and wet oligotrophic grasslands	R37	10-20	10-20	#	Increase in tall graminoids; decreased diversity; decrease in bryophytes

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
► Moss and lichen dominated mountain summits	(Earlier E4.2)	5-10	5-10	#	Change in species composition; effects on bryophytes or lichens
► Temperate acidophilous alpine grasslands	R43	5-10	5-10	#	Changes in species composition; increase in plant production
Arctic-alpine calcareous grassland	R44	5-10	5-10	#	Changes in species composition; increase in plant production

Heathland, scrub and tundra habitats (S)

Tundra	S1	3-5	3-5 ^d	#	Changes in biomass; physiological effects; changes in bryophyte species composition; decrease in lichen species richness
Arctic, alpine and subalpine scrub habitats	S2	5-15	5-10 ^d	#	Decline in lichens; bryophytes and evergreen shrubs
Lowland to montane temperate and submediterranean <i>Juniperus</i> scrub	S31		5-15	(#)	Shift in vegetation community composition; reduced seed viability
Northern wet heath	S411				
► U' Calluna-dominated wet heath (upland)	S411	10-20	5-15 ^e	##	Decreased heather dominance; decline in lichens and mosses; increased N leaching
► 'L' Erica tetralix-dominated wet heath (lowland)	S411	10-20	5-15 ^e	##	Transition from heather to grass dominance; decrease in heather cover; shift in vegetation community composition
Dry heaths	S42	10-20	5-15 ^e	##	Transition from heather to grass dominance; decline in lichens; changes in plant biochemistry; increased sensitivity to abiotic stress

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
Maquis, arborescent matorral and thermo-Mediterranean scrub	S5	20-30	5-15	(#)	Change in plant species richness and community composition; nitrate leaching; acidification of soil.
Garrigue	S6		5-15	#	Changes in species composition; decline in shrub cover; increased invasion of annual herbs

Forest habitats (T)

Broadleaved deciduous forest	T1	10-20	10-15	##	Changes in soil processes; nutrient imbalance; altered composition mycorrhiza and ground vegetation
<i>Fagus</i> forest on non-acid and acid soils	T17, T18	10-20	10-15	(#)	Changes in ground vegetation and mycorrhiza; nutrient imbalance; changes in soil fauna
Mediterranean <i>Fagus</i> forest on acid soils	T18		10-15	(#)	Annual height and volume tree growth; analogy to temperate <i>Fagus</i> forest
Acidophilous <i>Quercus</i> forest	T1B	10-15	10-15	(#)	Decrease in mycorrhiza; loss of epiphytic lichens and bryophytes; changes in ground vegetation
<i>Carpinus</i> and <i>Quercus</i> mesic deciduous forest	T1E	15-20	15-20	(#)	Changes in ground vegetation
Mediterranean evergreen <i>Quercus</i> forest	T21	10-20	10-15	(#)	NO ₃ in soil water and streams
Coniferous forests	T3	5-15	3-15	##	Changes in soil processes; nutrient imbalance; altered composition mycorrhiza and ground vegetation; increase in mortality with drought
Temperate mountain <i>Picea</i> forest, Temperate mountain <i>Abies</i> forest	T31, T32	10-15	10-15	(#)	Decreased biomass of fine roots; nutrient imbalance; decrease in mycorrhiza; changed soil fauna

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
Mediterranean mountain <i>Abies</i> forest	T33		10-15	(#)	Tree foliar stoichiometry; tree physiology; soil N losses
Temperate continental <i>Pinus sylvestris</i> forest	T35	5-15	5-15	#	Changes in ground vegetation and mycorrhiza; nutrient imbalances; increased N ₂ O and NO emissions
Mediterranean montane <i>Pinus sylvestris</i> - <i>Pinus nigra</i> forest	T37		5-17	(#)	Lichen chemistry and community changes in Mediterranean mixed-conifer forests in USA
Mediterranean lowland to submontane <i>Pinus</i> forest	T3A	3-15	5-10	(#)	Reduction in fine-root biomass; shift in lichen community
Dark taiga	T3F	5-10	3-5^f	##	Changes in epiphytic lichen and ground-layer bryophyte communities; increase in free-living algae; decline in N-fixation
<i>Pinus sylvestris</i> light taiga	T3G	5-10	2-5^f	#	Changes in epiphytic lichen and ground-layer bryophyte communities; increase in free-living algae; decline in N-fixation

- a) The lower part of the CL_{emp}N range should be applied for lakes in small catchments (with high lake to catchment ratios), because these are most exposed to atmospheric deposition, given that a relatively high fraction of their N inputs is deposited directly on the lakes and is not retained in the catchments. Similarly, the lower part of the range should be applied for lakes in catchments with thin soils, sparse vegetation and/or with a high proportion of bare rock.
- b) This CL_{emp}N should only be applied to oligotrophic waters with low alkalinity and with no significant agricultural or other human inputs. Apply the lower end of the range to clear-water sub-Arctic and alpine lakes, the middle range to boreal lakes and the higher end of the range to Atlantic soft waters.
- c) This CL_{emp}N should only be applied to waters with low alkalinity and with no significant agricultural or other direct human inputs. Apply the lower end of the range to boreal dystrophic lakes.
- d) Use towards high end of range if phosphorus limited, and towards lower end if phosphorus is not limiting.
- e) Use towards high end of range with high intensity management, and use towards lower end of range with low intensity management.
- f) Mainly based on N deposition impacts on lichens and bryophytes.

Zusammenfassung

I. Einleitung

- ▶ Dieser Bericht beschreibt den wissenschaftlichen Hintergrund und die Ergebnisse der Überprüfung und Überarbeitung der empirischen Critical Loads für Stickstoff (CL_{empN}), die 2011 im Rahmen des UNECE-Übereinkommens über weiträumige grenzüberschreitende Luftverunreinigung (Convention on Long-range Transboundary Air Pollution, LRTAP) für Europa festgelegt wurden. Im Juni 2020 startete das Coordination Centre for Effects (CCE) ein Projekt im Rahmen des LRTAP-Übereinkommens, um die empirischen Critical Loads für Stickstoff auf den neuesten Stand zu bringen. Es wurden neue relevante Informationen über die Auswirkungen von Stickstoff auf natürliche und naturnahe Ökosysteme aufgenommen.
- ▶ Im Rahmen des LRTAP-Übereinkommens fand vom 26. bis 28. Oktober 2021 in Bern ein Workshop zur Überprüfung und Überarbeitung der empirischen Critical Loads für Europa statt. Der Workshop wurde vom Coordination Centre for Effects (CCE) organisiert und vom Schweizer Bundesamt für Umwelt (BAFU) ausgerichtet
- ▶ Der Workshop wurde von 37 Teilnehmenden aus Österreich, Kanada, Finnland, Frankreich, Deutschland, den Niederlanden, Norwegen, Spanien, Schweden, der Schweiz, dem Vereinigten Königreich und den Vereinigten Staaten sowie von Vertretern des International Cooperative Programme (ICP) Waters, ICP Vegetation und ICP Modelling and Mapping besucht. Das Sekretariat der Konvention war nicht vertreten.
- ▶ Die Entscheidung, den Workshop zu organisieren, wurde auf der 39. Sitzung der Working Group on Effects (ECE/EB.AIR/144/Add.2) nach Empfehlungen der 35. Sitzung der Task Force Modelling and Mapping (2. - 4. April 2019) getroffen, die als Webkonferenz stattfand.
- ▶ Die Sitzung wurde von Reto Meier im Namen des BAFU und Alice James Casas als Vorsitzende des ICP Modelling and Mapping eröffnet.

II. Ziele und Struktur des Workshops

- ▶ Ziel des Workshops war die Überarbeitung der CL_{empN} für natürliche und naturnahe Ökosysteme, die auf dem letzten Expertenworkshop vom 23. bis 25. Juni 2010 in Noordwijkerhout festgelegt wurden (siehe Bobbink und Hettelingh, 2011), auf Grundlage zusätzlicher wissenschaftlicher Informationen für den Zeitraum von 2010 bis 2021. Die Diskussionen auf dem Workshop basierten auf dem neuen und aktualisierten Hintergrunddokument, das allen Teilnehmern vor dem Workshop zugesandt worden war.
- ▶ Die meisten Habitatklassen des Europäischen Naturinformationssystems (European Nature Information System, EUNIS), die in der vorliegenden Überarbeitung behandelt wurden, haben sich seit der letzten Aktualisierung durch Bobbink und Hettelingh (2011) geändert: Marine Lebensräume (EUNIS-Klasse MA, früher A), Küstenlebensräume (EUNIS-Klasse N, früher B), Binnengewässer (EUNIS-Klasse C), Moore und Sümpfe (EUNIS-Klasse Q, früher D), Grasland und von krautigen Pflanzen, Moosen oder Flechten dominierte Flächen (EUNIS-Klasse R, ehemals E), Heideland, Buschland und Tundra (EUNIS-Klasse S, ehemals F), Wälder, Forste und andere bewaldete Flächen (EUNIS-Klasse T, ehemals G).

- ▶ Ein internationales Team von 43 Expertinnen und Experten erstellte das Hintergrunddokument für den Workshop im Zeitraum von Juni 2020 bis September 2021, nach einem digitalen Kick-off-Meeting im Juni 2020:
 - EUNIS Klasse MA – Roland Bobbink
 - EUNIS-Klasse N – Laurence Jones, Emiel Brouwer und Eva Remke
 - EUNIS-Klasse C – Heleen De Wit, Roland Bobbink, Christin Loran, Linda May und Jan-Erik Thrane
 - EUNIS-Klasse Q - Chris Field, Julian Aherne, Roland Bobbink und Hilde Tomassen
 - EUNIS-Klasse R - Carly Stevens, Rocio Alonso, Vegar Bakkestuen, Erika Hiltbrunner und Lukas Kohli
 - EUNIS-Klasse S - Leon Van den Berg, Julian Aherne, Andrea Britton, Simon Caporn, Hector García Gómez und Liv Guri Velle
 - EUNIS-Klasse T – Sabine Braun, Rocio Alonso, Frank Ashwood, Tomas Chuman, Lucienne de Witte, Thomas Dirnböck, Per Erik Karlsson, Sirkku Manninen, Michael Perring, Hans Tömmervik, Simon Tresch, Liisa Ukomaanah und Elena Vangelova
 - Kapitel 10 “Verwendung von empirischen Critical Loads für Stickstoff für Risikobewertung und Naturschutz” - Markus Geupel, Khalid Aazem, Sabine Augustin, Jesper Bak, Alice James Casas, Laurence Jones, Christin Loran, Reto Meier, Anne-Katrin Prescher, Thomas Scheuschner, Axel Ssymank und Susan Zappala
- ▶ Nach der internen Überprüfung, wurde das Hintergrundpapier überprüft durch Sabine Augustin, Ariel Bergamini, Leonor Calvo Galván, Tara Greaver, Kevin Hicks, Raúl Ochoa-Huesa, Tonje I. Økland und Jan Roelofs.
- ▶ Die Arbeitsgruppen tauschten sich in kurzen Sitzungen über ihre Fortschritte aus. Ergebnisse, Schlussfolgerungen und Empfehlungen wurden in einer abschließenden Vollversammlung unter dem Vorsitz von Christin Loran diskutiert und zusammengefasst.

III. **Schlussfolgerungen**

- ▶ Statistisch und biologisch signifikante Ergebnisse von Feldversuchen waren die Grundlage für die Bewertung der $CL_{emp}N$. Für die Aktualisierung und Verfeinerung der $CL_{emp}N$ -Werte wurden nur Studien mit unabhängigen N-Behandlungen und realistischen N-Frachten und -Zeiträumen herangezogen.
- ▶ Im aktuell überprüften Zeitraum wurde eine zunehmende Anzahl von Gradientenstudien zur atmosphärischen N-Deposition in mehreren EUNIS-Lebensraumtypen veröffentlicht, die sich als nützlich für die Bewertung und Festlegung der $CL_{emp}N$ erwiesen haben.
- ▶ Studien mit höheren N-Zugaben oder kürzeren Versuchszeiträumen wurden nur im Hinblick auf das Verständnis der Wirkungsmechanismen, einer möglichen N-Limitierung oder der Empfindlichkeit des Systems interpretiert. Die in diesen Studien angewandten Methoden wurden sorgfältig geprüft, um Faktoren des Versuchsaufbaus oder der Datenanalyse zu identifizieren, die ihre Verwendung bei der Bewertung der $CL_{emp}N$ -Bereiche einschränken könnten.

- ▶ Für alle untersuchten EUNIS-Klassen (Stufe 2 oder 3) wurde eine Einigung über CL_{emp}N für eine Reihe von N-Depositionswerten erzielt. Neue Ergebnisse zu den Auswirkungen von Stickstoff in Oberflächengewässern konnten auf der Grundlage der von ICP Waters vorgestellten Aktivitäten einbezogen werden. Neue Erkenntnisse für einige mediterrane Lebensräume konnten ebenfalls aufgenommen werden.
- ▶ Das neue Kapitel 10 "Verwendung von CL_{emp}N in der Risikobewertung und im Naturschutz" wurde vorgestellt und diskutiert. Es enthält eine Auswahl von Beispielen für die Verwendung von CL_{emp}N auf unterschiedlichen Skalen und in unterschiedlichen europäischen Ländern, um Fachleuten und politischen Entscheidungsträgern eine Anleitung zu geben, wie CL_{emp}N in der Praxis eingesetzt werden kann.
- ▶ Die Bewertung der Zuverlässigkeit der CL_{emp}N-Bereiche wurde seit der letzten Aktualisierung im Jahr 2011 fortgesetzt, wobei zwischen "zuverlässig", "ziemlich zuverlässig" und "Expertenurteil" unterschieden wurde, symbolisiert jeweils durch ##, # bzw. (#).
- ▶ Die aus dem Überprüfungs- und Revisionsverfahren resultierenden CL_{emp}N-Bereiche wurden auf dem Workshop einvernehmlich festgelegt und sind in Tabelle 1 zusammengefasst. Bei mehr als 40 % der vorgestellten EUNIS-Typen war der untere Wert der Spanne niedriger als 2011. Das Gleiche gilt für den oberen Wert. Nur in einem Fall hat sich der obere Wert erhöht.

IV. Empfehlungen

- ▶ Für die folgenden Ökosysteme sind weitere Untersuchungen und Daten erforderlich, um ein CL_{emp}N zu erstellen: mehrere Gras- und Steppenlandschaften, alle mediterranen Vegetationstypen, feuchte (Sumpf-)Wälder, viele Moore und Flachmoore sowie mehrere Küstenlebensräume; darüber hinaus ist weitere Forschung für alle EUNIS-Lebensraumtypen erforderlich, die ein Experten-Rating haben.
- ▶ Die Auswirkungen der N-Anreicherung in (sensitiven) Süßwasser- und flachen Meeresökosystemen (einschließlich Küstengewässern) müssen weiter erforscht werden.
- ▶ Es werden mehr gut konzipierte Gradientenstudien mit sowohl (sehr) niedrigen als auch hohen N-Belastungen benötigt, insbesondere in EUNIS-Lebensraumtypen, die kaum untersucht werden. Darüber hinaus erhöht das Zusammenführen von Ergebnissen aus experimentellen und Gradientenstudien die Zuverlässigkeit der CL_{emp}N.
- ▶ Die unterschiedlichen Auswirkungen der abgelagerten N-Formen (NO_x oder NH_y) müssen weiter erforscht werden, um die Critical Loads für oxidierten und reduzierten Stickstoff in Zukunft getrennt bestimmen zu können.
- ▶ Zur Weiterentwicklung der aktuellen CL_{emp}N sind Langzeitexperimente (10-20 Jahre) mit einer hohen N-Zugabehäufigkeit zwischen 5 und 50 kg N ha⁻¹ yr⁻¹ in Regionen mit geringer Hintergrunddeposition von entscheidender Bedeutung. Dies würde die Zuverlässigkeit der abgeleiteten CL_{emp}N erhöhen, wenn die niedrigste Behandlungsstufe den derzeitigen Critical Load nicht überschreitet.
- ▶ Der Klimawandel und die Stickstoffdeposition haben wahrscheinlich starke interaktive Auswirkungen auf die Funktionsweise von Ökosystemen, wobei der Klimawandel die Reaktionen der Ökosysteme auf die Stickstoffdeposition verändert und umgekehrt. Es werden mehr experimentelle Studien benötigt, um diese Wechselwirkungen zu untersuchen,

sowie mehr Gradientenstudien, die ausdrücklich die Auswirkungen der Stickstoffdeposition in Kombination mit klimatischen Gradienten untersuchen.

- Zusammenfassend lässt sich sagen, dass es von entscheidender Bedeutung ist, die langfristigen Auswirkungen einer erhöhten N-Deposition auf Ökosystemprozesse in einer repräsentativen Auswahl von Ökosystemen zu verstehen. Daher ist es wichtig, die Auswirkungen der N-Deposition durch manipulierte N-Einträge in langfristigen Ökosystemstudien sowohl in unberührten als auch in beeinträchtigten Gebieten zu quantifizieren. Diese Daten sind in Verbindung mit Gradientenstudien unerlässlich, um Critical Loads zu validieren und robuste dynamische Ökosystemmodelle und/oder Modelle für mehrere korrelierende Arten zu entwickeln, die zuverlässig genug sind, um CL_{empN} für natürliche und naturnahe Ökosysteme zu berechnen und natürliche Erholungsraten für N-belastete Systeme vorherzusagen.

Table 1. Überblick über die empirischen Critical Loads von Stickstoff ($\text{kg N ha}^{-1} \text{a}^{-1}$) für natürliche und naturnahe Ökosysteme (Spalte 1), klassifiziert nach EUNIS (Spalte 2), wie 2011 ermittelt (Spalte 3) und wie 2022 überarbeitet (Spalte 4). Die Verlässlichkeit wird durch ## zuverlässig; # ziemlich zuverlässig und (#) Expertenurteil angegeben (Spalte 5). Spalte 6 zeigt eine Auswahl von Effekten, die bei Überschreitung der Critical Loads auftreten können. Änderungen im Vergleich zu 2011 sind als fettgedruckte Werte angegeben

Ökosystemtyp	EUNIS Code	2011 $\text{kg N ha}^{-1} \text{a}^{-1}$	2022 $\text{kg N ha}^{-1} \text{a}^{-1}$	2022 Verlässlichkeit	Überschreitungsindikator
Marine Habitate (MA)					
Atlantische mäßig-stark salzbeeinflusste Wiesen	MA223	20-30	10-20	(#)	Zunahme der Dominanz von Graminoiden; Rückgang positiver Indikatorarten
Atlantische niedrige-mäßig salzbeeinflusste Wiesen	MA224	20-30	10-20	(#)	Zunahme von Arten später Sukzessionsstadien; Abnahme von positiven Indikatorarten
Atlantische Pionier salzbeeinflusste Wiese	MA225	20-30	20-30	(#)	Zunahme von Arten später Sukzessionsstadien; Zunahme von produktiven Arten
Küstenhabitate (N)					
Wanderdünen der Küsten	N13, N14	10-20	10-20	#	Zunahme der Biomasse; verstärkte N-Auswaschung; reduzierte Wurzelbiomasse
Stabile Küstendünen (graue Dünen)	N15	8-15	5-15	##	Erhöhte Biomasse und Bodenbedeckung durch Graminoiden und mesophilen Gräsern; Rückgang der oligotrophen Arten, einschließlich Flechten; erhöhter N-Gehalt im Gewebe; verstärkte N-Auswaschung; Bodenversauerung
Dünenheiden an der Küste	N18, N19	10-20	10-15	#	Erhöhte Pflanzenproduktion; verstärkte N-Auswaschung; beschleunigte Sukzession; typische C:N Abnahme in Flechten; erhöhter jährlicher <i>Calluna</i> Zuwachs
Feuchte und nasse Dünentümpel	N1H	10-20	5-15	#	Erhöhte Bodenbedeckung durch Graminoiden und mesophilen Gräsern; Abnahme von oligotrophen Arten; erhöhter Ellenberg N-Wert
Dünetümpel Wasserbecken (Aquatische Süßwassergemein-	N1H1, N1J1	10-20	10-20	(#)	Zunehmende Biomasse und Sukzessionsrate

schaften von permanenten atlantischen und baltischen oder mediterranen und Schwarzmeer Dünentümpelgewässern)					
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Süßwasserhabitate (C) ^a

Dauerhaft oligotrophe Stillgewässer (einschließlich Weichwasserseen)	C1.1	3-10	2-10 ^b	##	Erhöhte Algenproduktivität und Wechsel der Nährstofflimitierung für Phytoplankton von N auf P; Veränderungen der Makrophytengemeinschaft
Alpine und subarktische Klarwasserseen	C1.1		2-4	##	Erhöhte Algenproduktivität und Wechsel der Nährstofflimitierung für Phytoplankton von N auf P
Boreale Klarwasserseen	C1.1		3-6	##	Erhöhte Algenproduktivität und Wechsel der Nährstofflimitierung für Phytoplankton von N auf P
Atlantische weiche Gewässer	C1.1, Elemente C1.2	3-10	5-10	##	Veränderung der Artenzusammensetzung von Makrophytengemeinschaften
Dauerhaft dystrophe Stillgewässer	C1.4	3-10	5-10 ^c	(#)	Erhöhte Algenproduktivität und Wechsel der Nährstofflimitierung für Phytoplankton von N auf P

Sumpf- und Moorhabitate (Q)

Hoch- und Deckenmoore	Q1	5-10	5-10	##	Zunahme der Gefäßpflanzen; Rückgang der Moose; verändertes Wachstum und veränderte Artenzusammensetzung von Moosen; erhöhter N-Gehalt in Torf und Torfwasser
Nährstoffärmere und nährstoffarme Niedermoore	Q2	10-15	5-15	##	Zunahme von Riedgräsern und anderen Gefäßpflanzen; negative Auswirkungen auf Bryophyten
Palsa- und Polygonmoore	Q3		3-10	(#)	Zunahme der Graminoiden, der N-Konzentration im Gewebe und der Zersetzungsrate
Nährstoffreiche Niedermoore	Q41-Q44	15-30	15-25	#	Zunahme hoher Gefäßpflanzen (insbesondere Graminoide); Rückgang von Moosen

Nährstoffreiche arktische und Berg-Flachmoore	Q45	15-25	15-25	(#)	Zunahme von Gefäßpflanzen; Rückgang von Moosen
Graslandhabitate (R)					
Kalkreicher Halbtrockenrasen (basische Wiesensteppe)	R1A	15-25	10-20	##	Zunahme der Hochgräser; Rückgang der Artenvielfalt; Veränderung der Artenzusammensetzung; verstärkte Mineralisation; N-Auswaschung; Oberflächenversauerung
Mediterraner, dicht beweideter Trockenrasen oder Mediterraner hoher mehrjähriger Trockenrasen oder Mediterraner jährlicher Trockenrasen	R1D oder R1E oder R1F	15-25	5-15	(#)	Erhöhte Produktion; Dominanz von Graminoiden; Veränderung der Bodenkruste; Veränderung des Nährstoffkreislaufs im Boden
Tief- bis Bergland, trockener bis mesischer Rasen, gewöhnlich dominiert von <i>Nardus stricta</i>	R1M	10-15	6-10	##	Zunahme der Graminoiden; Rückgang der typischen Arten; Rückgang des gesamten Artenreichtums
Ozeanischer bis subkontinentaler Binnen-Sandrasen auf trockenen sauren und neutralen Böden oder Sandverwehungen und Dünen mit silikatischem Grasland	R1P oder R1Q	8-15	5-15	(#)	Rückgang der Flechten; Zunahme der Biomasse
Mähwiesen tiefer und mittlerer Lagen	R22	20-30	10-20	(#)	Zunahme an Hochgräsern; Rückgang der Artenvielfalt; Rückgang typischer Arten
Bergmähwiesen	R23	10-20	10-15	#	Zunahme der nitrophilen Graminoiden; Veränderung der Artenvielfalt; Rückgang typischer Arten
Feuchte oder nasse mesotrophe bis eutrophe Mähwiesen	R35	15-25	15-25	(#)	Zunahme der hohen Graminoiden; geringere Artenvielfalt; Rückgang der Moose
Gemäßigte und boreale feuchte und nasse oligotrophe Rasen	R37	10-20	10-20	#	Zunahme der hohen Graminoiden; geringere Artenvielfalt; Rückgang der Moose

Moos- und flechtendominierte Berggipfel	(Früher E4.2)	5-10	5-10	#	Veränderungen in der Artenzusammensetzung; Auswirkungen auf Bryophyten oder Flechten
Gemäßigte acidophile alpine Rasen	R43	5-10	5-10	#	Veränderungen in der Artenzusammensetzung; Zunahme der Pflanzenproduktion
Arktisch-alpine kalkhaltige Rasen	R44	5-10	5-10	#	Veränderungen in der Artenzusammensetzung; Zunahme der Pflanzenproduktion

Heiden- und Strauchhabitats (S)

Tundra	S1	3-5	3-5 ^d	#	Veränderungen in der Biomasse; physiologische Effekte; Veränderungen in der Artenzusammensetzung der Moospflanzen; Rückgang des Flechtenartenreichtums
Arktische, alpine und subalpine Zwergstrauchheiden	S2	5-15	5-10 ^d	#	Rückgang der Flechten, Bryophyten und immergrünen Sträucher
Tief- bis bergländisches gemäßigtes und submediterranes <i>Juniperus</i> Buschland	S31		5-15	(#)	Verschiebung der Zusammensetzung der Vegetationsgemeinschaft; geringere Lebensfähigkeit der Samen
Nördliche feuchte Heide	S411				
► 'U' <i>Calluna</i> -dominierte feuchte Heide (Bergland)	S411	10-20	5-15 ^e	##	Abnehmende Heidedominanz; Rückgang von Flechten und Moosen; erhöhte N-Auswaschung
► 'L' <i>Erica tetralix</i> -dominierte feuchte Heide (Tiefland)	S411	10-20	5-15 ^e	##	Übergang von der Dominanz von Heidekraut zu Gräsern; Rückgang der Heidekrautbedeckung; Veränderung der Zusammensetzung der Vegetationsgemeinschaft
Trockene Heiden	S42	10-20	5-15 ^e	##	Übergang von der Heidekraut- zur Grasdominanz; Rückgang der Flechten; Veränderungen in der Biochemie der Pflanzen; erhöhte Empfindlichkeit gegenüber abiotischem Stress
Macchia, baumförmige Hartlaubgebüsche und	S5	20-30	5-15	(#)	Veränderung des Artenreichtums und der Zusammensetzung der

thermo-mediterranes Buschland					Pflanzengemeinschaften; Nitratauswaschung; Versauerung des Bodens.
Garrigue	S6		5-15	#	Veränderungen in der Artenzusammensetzung; Rückgang der Strauchbedeckung; zunehmende Invasion von einjährigen Kräutern

Wälder und Forsten (T)

Sommergrüne Laubwälder	T1	10-20	10-15	##	Veränderungen in Bodenprozessen; Nährstoffungleichgewicht; Veränderungen der Mykorrhiza und Bodenvegetation
Buchenwald auf nicht- sauren und sauren Böden	T17, T18	10-20	10-15	(#)	Veränderungen der Bodenvegetation und Mykorrhiza; Nährstoffungleichgewicht; Veränderungen der Bodenfauna
Mediterraner bodensaurer Buchenwald	T18		10-15	(#)	Jährliches Höhen- und Volumenwachstum der Bäume; Analogie zu Buchenwäldern der gemäßigten Zonen
Bodensaurer eichendominierter Wald	T1B	10-15	10-15	(#)	Rückgang der Mykorrhiza; Verlust von epiphytischen Flechten und Bryophyten; Veränderungen der Bodenvegetation
Eichen-Hainbuchen mesischer Laubwald	T1E	15-20	15-20	(#)	Veränderungen der Bodenvegetation
Mediterraner immergrüner Eichenwald	T21	10-20	10-15	(#)	NO ₃ im Bodenwasser und Wasserläufen
Nadelwälder	T3	5-15	3-15	##	Veränderungen der Bodenprozesse; Nährstoffungleichgewicht; veränderte Zusammensetzung von Mykorrhiza und Bodenvegetation; Zunahme der Sterblichkeit bei Trockenheit
Gemäßigter Fichtenbergwald Gemäßigter Tannenberwald	T31, T32	10-15	10-15	(#)	Verminderte Biomasse der Feinwurzeln; Nährstoffungleichgewicht; Rückgang der Mykorrhiza; veränderte Bodenfauna

Mediterraner Tannenbergwald	T33		10-15	(#)	Blattstöchiometrie der Bäume; Baumphysiologie; N-Verluste im Boden
Gemäßigter kontinentaler <i>Pinus sylvestris</i> Wald	T35	5-15	5-15	#	Veränderungen der Bodenvegetation und der Mykorrhiza; Nährstoffungleichgewicht; erhöhte N ₂ O- und NO-Emissionen
Mediterraner <i>Pinus sylvestris</i> - <i>Pinus nigra</i> Bergwald	T37		5-17	(#)	Flechtenchemie und Veränderungen der Lebensgemeinschaften in mediterranen Mischwäldern in den USA
Mediterraner Tiefland- bis submontaner Kiefernwald	T3A	3-15	5-10	(#)	Verringerung der Feinwurzelbiomasse; Veränderung der Flechtengemeinschaft
Dunkle Taiga	T3F	5-10	3-5 ^f	##	Veränderungen der epiphytischen Flechten- und Bodenschicht-Bryophytengemeinschaften; Zunahme der freilebenden Algen; Rückgang der N-Fixierung
<i>Pinus sylvestris</i> helle Taiga	T3G	5-10	2-5 ^f	#	Veränderungen der epiphytischen Flechten- und Bodenschicht-Bryophytengemeinschaften; Zunahme der freilebenden Algen; Rückgang der N-Fixierung

- a) Die Untergrenze des CL_{emp}N-Bereichs sollte für Seen in kleinen Einzugsgebieten (mit einem hohen Verhältnis zwischen See und Einzugsgebiet) angewandt werden, da diese der atmosphärischen Deposition am stärksten ausgesetzt sind, da ein relativ hoher Anteil ihrer N-Einträge direkt auf den Seen abgelagert und nicht in den Einzugsgebieten zurückgehalten wird. In ähnlicher Weise sollte die Untergrenze des CL_{emp}N-Bereichs für Seen in Einzugsgebieten mit dünnen Böden, spärlicher Vegetation und/oder einem hohen Anteil an nacktem Felsen angewendet werden.
- b) Dieser CL_{emp}N-Wert sollte nur auf oligotrophe Gewässer mit geringer Alkalinität und ohne nennenswerte landwirtschaftliche oder sonstige menschliche Einträge angewendet werden. Die Untergrenze des Bereichs gilt für subarktische und alpine Klarwasserseen, der mittlere Bereich für boreale Seen und die Obergrenze des Bereichs für atlantische Weichgewässer.
- c) Dieser CL_{emp}N-Wert sollte nur auf Gewässer mit geringer Alkalinität und ohne nennenswerte landwirtschaftliche oder sonstige direkte menschliche Einträge angewendet werden. Die Untergrenze des Bereichs sollte auf boreale dystrophe Seen angewendet werden.
- d) Verwendung der Obergrenze, wenn der Phosphorgehalt begrenzt ist, und der Untergrenze, wenn der Phosphorgehalt nicht begrenzt ist.
- e) Verwendung der Obergrenze bei hoher Intensität und Verwendung der Untergrenze bei niedriger Intensität.
- f) Hauptsächlich basierend auf die Auswirkungen der N-Deposition auf Flechten und Moose

1 Introduction

Adapted by Roland Bobbink, Christin Loran and Hilde Tomassen

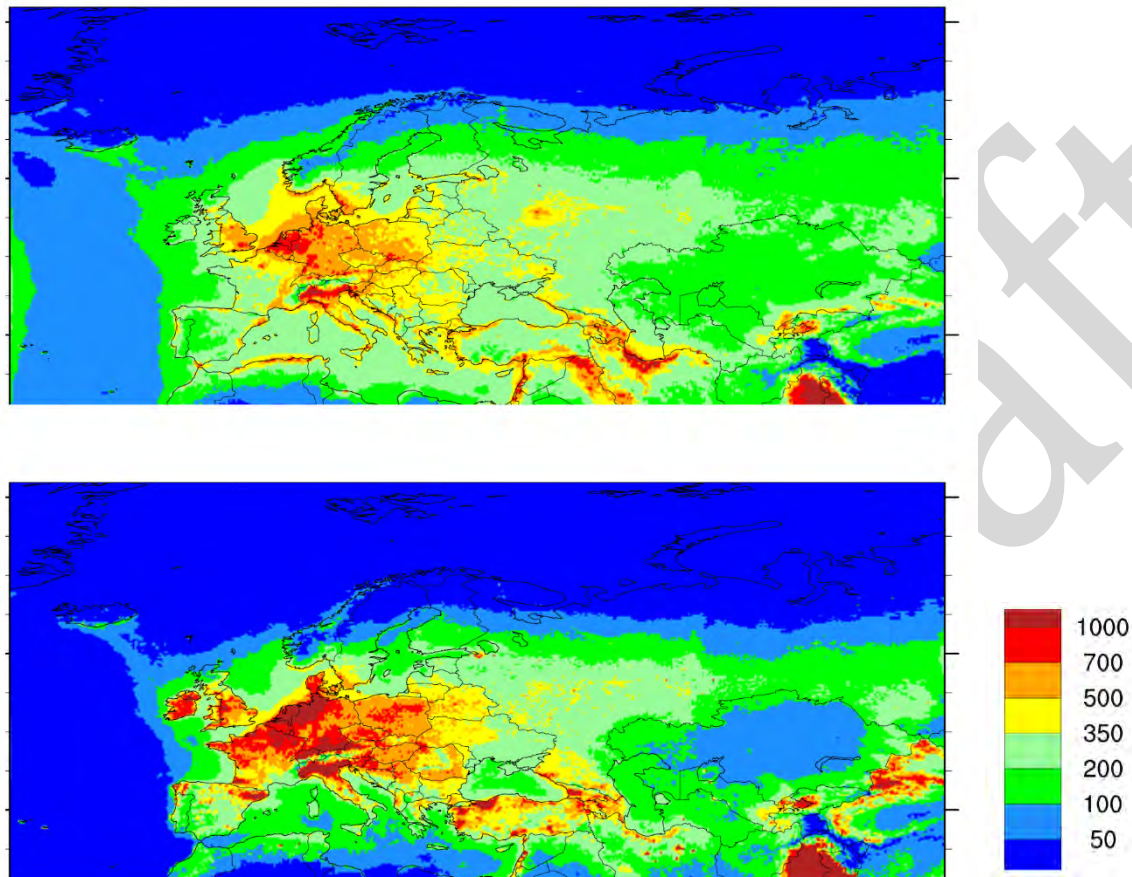


Nitrogen addition experiments are of utmost importance for the setting of empirical critical loads: overview of the experimental N addition facility at Whim Bog, Scotland, UK. Photo: Roland Bobbink.

1.1 Impacts of N deposition

Emissions of ammonia (NH_3) and nitrogen oxides (NO_x) strongly increased in the second half of the 20th century. Ammonia is volatilised from intensive agricultural systems, such as dairy farming and intensive animal husbandry, whereas nitrogen oxides originate mainly from burning of fossil fuel by traffic, industry and households. Because of short- and long-range transport of these nitrogenous compounds, atmospheric nitrogen (N) deposition has clearly increased in many natural and semi-natural ecosystems across the world. Areas with high atmospheric N deposition ($20\text{--}80 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) nowadays are central and western Europe, eastern United States and, since the 1990s, eastern Asia (e.g. Galloway and Cowling, 2002; Dentener et al., 2006; Fowler et al., 2020). The modelled deposition for Europe is published annually by EMEP/MSC-West (Figure 1.1).

Figure 1.1. Modelled total depositions of oxidised (upper map) and reduced (lower map) nitrogen ($\text{mg N m}^{-2} \text{yr}^{-1}$) across Europe in 2019 (EMEP Status Report, 2021). $1000 \text{ mg N m}^{-2} = 10 \text{ kg N ha}^{-1}$.



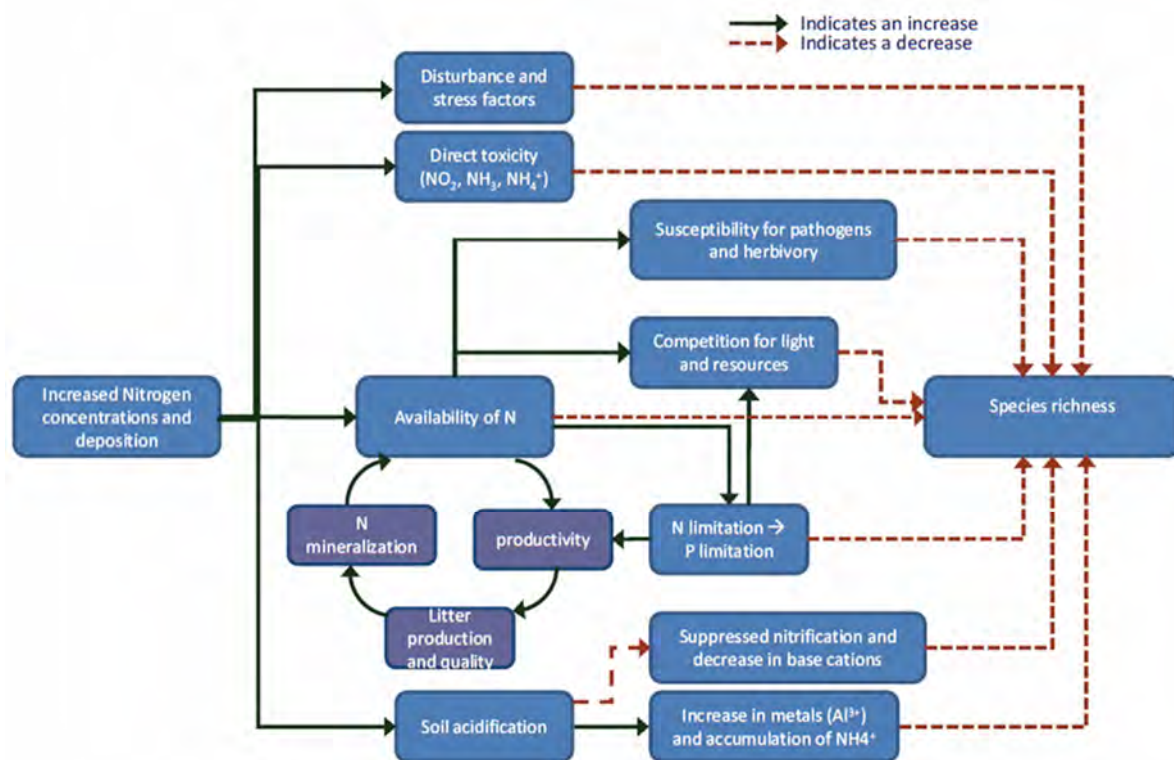
Source: EMEP Status Report, 2021

The availability of nutrients is one of the most important abiotic factors which determine plant species composition in ecosystems. N is the primary limiting nutrient for plant growth in many natural and semi-natural ecosystems, especially for oligotrophic and mesotrophic habitats. Most of the plant species in such ecosystems are adapted to nutrient-poor conditions and can only survive or compete successfully on soils with low N availability (e.g. Tamm, 1991; Aerts and Chapin, 2000). The series of events which occurs when N inputs increase in an area with originally low background deposition rates is highly complex. Many ecological processes interact and operate at different temporal and spatial scales. As a consequence, high variations in sensitivity to atmospheric N deposition have been observed between different natural and semi-natural ecosystems. Despite this diverse sequence of events, the most obvious effects of increased N deposition are significant changes in the N cycle, vegetation composition and biodiversity. For more details, see Bobbink et al. (1998, 2010), Dise et al. (2011) and Stevens et al. (2020).

Many ecological processes interact and operate at different temporal and spatial scales. Furthermore, N is the limiting nutrient for plant growth in many natural and semi-natural ecosystems, especially oligotrophic and mesotrophic habitats. The severity of the impacts of atmospheric N deposition depends on a number of factors, of which the most important are (numbers not being a ranking): 1) the duration and total amount of inputs, 2) the chemical and physical form of the airborne N input, 3) the intrinsic sensitivity of the plant and animal species present, 4) the abiotic conditions, including climate, and 5) the past and present land use or

management. Acid neutralising capacity (ANC), soil nutrient availability, and soil factors that influence the nitrification potential, N immobilisation and denitrification rates, are especially important. As a consequence, different ecosystems show high variability in sensitivity to atmospheric N deposition (Bobbink et al., 2010; Pardo et al., 2011). Despite this highly diverse sequence of events, it is possible to generalise some types of impacts. A schematic overview of the potential sequence of events is given in Figure 1.2.

Figure 1.2. Scheme of the main impacts of increased N deposition on ecosystems. Stress is considered to occur when external constraints limit the rate of dry matter production of the vegetation, whereas disturbance consists of mechanisms which affect soils and plant biomass by causing its partial or total destruction.



Source: Bobbink and Hettelingh, 2011

a) **Direct toxicity of N gases and aerosols to individual species**

An important effect of nitrogenous gases, aerosols and dissolved compounds (NH_3 , NO_2 , NO , HNO_3 and NH_4^+) can be direct toxicity to the above-ground parts of individual plants. The impacts have been mostly studied in crops and saplings, but studies with native plant species or mixtures of species in open-top chambers (OTCs) and free-air fumigation have also demonstrated leaf injury, changes in physiology, and growth reductions at increased concentrations of just-mentioned N pollutants (e.g. Pearson and Stewart, 1993; Grupa, 2003; Sheppard et al., 2009). Direct toxicity impacts of NO_2 were observed in parts of Europe and North America in the 1980s, but are currently rare in these regions, except in cities or in the direct neighbourhood of roads with heavy traffic. However, concentrations of these nitrogen oxides in air are now increasing in large areas of Asia (primarily China and India), possibly leading again to direct foliar impacts. In addition, lichens are clearly the most sensitive group in the vegetation with respect to direct toxicity of NH_3 (e.g.

Hallingbäck, 1992; Van Herk et al., 2003). This, based on data from the United Kingdom, Italy and Portugal, has led to a significant lowering of the long-term critical level of NH_3 for ecosystems in which lichens and bryophytes are important (Cape et al., 2009; Sutton et al., 2009). Furthermore, it became obvious that the exceedances of this critical level occur in many areas of North-western Europe (Sutton et al., 2009).

b) Eutrophication

N is the limiting nutrient for plant growth in many natural and semi-natural terrestrial ecosystems, especially under oligotrophic and mesotrophic conditions. Increased N deposition results in an increase in the availability of inorganic N in the topsoil, in the short term, except in bogs and fens. This gradually leads to an increase in plant productivity in N-limited vegetation, and thus to higher annual litter production and litter with high concentrations of N. Because of this, N mineralisation will also gradually increase, which, in turn, may increase plant productivity. This is positive feedback, because higher N mineralisation leads to higher N uptake and its subsequent effects. Local plant species diversity increases with increasing resource availability at originally very low levels of resource availability. Above a certain level of primary productivity, however, local plant species diversity declines as production increases. Observational studies across N deposition gradients, and many N addition experiments, demonstrate this effect in the long term. Competitive exclusion ('overshading') of characteristic species of oligotrophic or mesotrophic habitats occurs in the presence of relatively fast-growing nitrophilic species, with rare species at low abundances being especially at risk (Figure 1.3) (e.g. Bobbink et al., 1998; Suding et al., 2005).

Figure 1.3. A calcareous grassland (*Mesobromion erecti*) (R1A) in the Netherlands without N addition (left) and after three years of N addition ($100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) (right). Photo: R. Bobbink.



Source: R. Bobbink

The rate of N cycling in the ecosystem is clearly increased in such situations, although the response time to increased N inputs can be long in highly organic soils (with high C:N ratios), or, indeed in any soil with large potential N sinks. When N is no longer limiting in the ecosystem, plant growth becomes limited by other resources, such as phosphorus (P), potassium (K), magnesium (Mg), or water. In this situation, the productivity of the vegetation will not increase any further with continuing increases in N. However, N

concentrations within the plants do tend to increase when N availability continues to increase. This may affect the palatability of the vegetation for herbivores or the sensitivity to pathogens (see below), and will influence microbial communities, too. Recently, it has been suggested that after a shift from N to P limitation or in highly P-limited situations, changes in plant species composition can gradually still occur under long-term N inputs (see Chapter 6 for examples).

c) **Acidification**

Soil acidification is characterised by a wide variety of long-term effects. It is defined as the loss of acid neutralising capacity (ANC) and may lead to a decrease in soil pH. Changes in pH are dependent on the buffering capacity of the soil (e.g. Ulrich 1983, 1991). Acidifying compounds (N and S) deposited on calcareous soils (including substrates of young moraine regions) at first will not change soil acidity. In these soils HCO_3^- and Ca^{2+} ions leach from the system, but the pH remains the same until almost all of the calcium carbonate has been depleted. In soils dominated by silicate minerals (pH 6.5-4.5), buffering is taken over by cation exchange processes of the soil adsorption complexes. In this situation, protons are exchanged with Ca^{2+} and Mg^{2+} , and these cations are leached from the soil together with anions (mostly nitrate or sulphate). Because of the restricted capacity of this buffering system, soil pH will soon start to decrease. However, in mineral soils with a large cation exchange capacity and high base saturation, this buffering may continue for several decades, even at relatively high inputs.

At low pH (< 5.0), hydrous oxides of several metals dissolve. This causes a strong increase in the levels of toxic Al^{3+} and other metals in the soil solution. As a result of the decrease in pH, nitrification is strongly hampered or even completely absent in most of these highly acidic soils. This may lead to accumulation of ammonium, with nitrate levels decreasing to almost zero (e.g. Roelofs et al., 1985). In addition, the decomposition rate of organic material in the soil is lower in these acidified soils, which leads to increased accumulation of litter (e.g. Van Breemen et al., 1982; Ulrich, 1983, 1991). As a result of this cascade of changes, plant growth and species composition of the vegetation can be seriously affected: acid-resistant plant species will gradually become dominant, and several species typical to intermediate and higher soil pH will disappear.

d) **Differences in effects of oxidised versus reduced N**

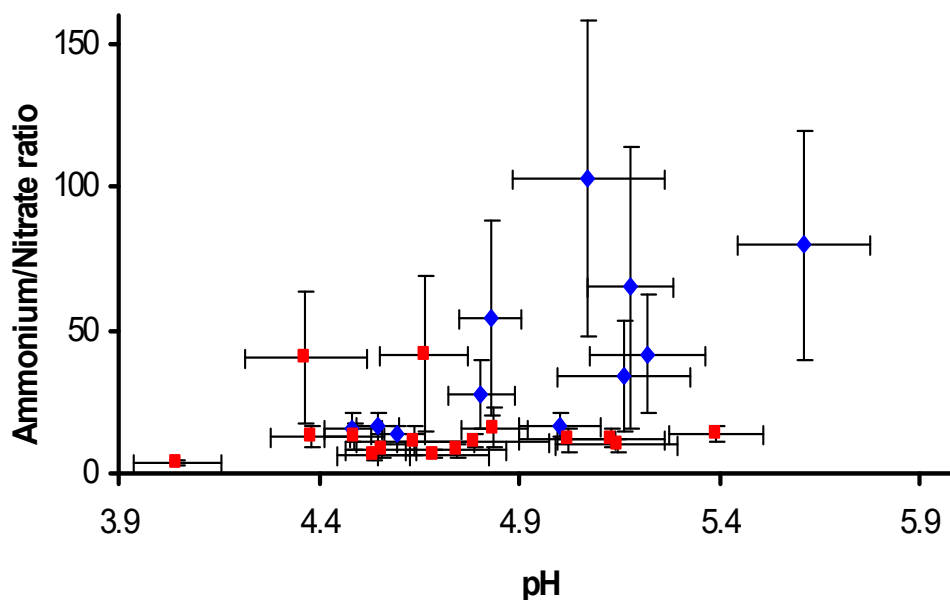
Emissions of ammonia (NH_3) and nitrogen oxides (NO_x) both contribute to atmospheric N deposition. Ammonia is volatilised from agricultural systems, such as dairy farming and intensive animal husbandry, whereas nitrogen oxides originate mainly from burning of fossil fuels in economic sectors including traffic (also by vehicle catalysts which may be a locally important source of N deposition), households and industry (Truscott et al., 2005). Because of this difference in sources (i.e. agriculture vs industry, households or traffic) and different rates of deposition from the atmosphere, the spatial and temporal patterns of deposition differ between reduced and oxidised compounds. Oxidised N deposition prevails in urban or industrial areas, whereas reduced N deposition clearly dominates in agricultural or rural regions. Furthermore, in most regions with a relatively high rate of N deposition, a high proportion of the deposited N originates from NH_y (e.g. Asman et al., 1998; Fowler, 2002; Sutton et al., 2008). This may cause a change in the dominant N form in the soil from nitrate to ammonium, especially in habitats with low rates of nitrification (pH < 4.5).

The response of sensitive plant species can be significantly affected by this change in N form. Species of calcareous or slightly acidic soils are able to use nitrate, or a combination

of nitrate and ammonium, as their N source, whereas early studies showed that species of acidic habitats generally use ammonium (e.g. Gigon and Rorison, 1972; Kinzel, 1982), because at least some of these plants do not have nitrate reductase (Ellenberg, 1996). For several plant species reduced N appeared to be only toxic at low pH (Lucassen et al., 2003). Laboratory and field studies demonstrate that the performance of most forest understory species of deciduous forests in southern Sweden improves when not only ammonium but also nitrate can be taken up (Falkengren-Grerup, 1998; Olsson and Falkengren-Grerup, 2000).

One of the impacts of increased ammonium uptake is a reduced uptake of base cations and exchange of these cations (K^+ , Ca^{2+} and Mg^{2+}) to the rhizosphere. Ultimately this can lead to severe nutritional imbalances, which are important in the decline in tree growth in areas with high ammonia/ammonium deposition (e.g. Nihlgård, 1985; Van Dijk et al., 1990; references in Bobbink et al., 2003). High concentrations of ammonium in the soil or water layer are also toxic to many sensitive plant species, disrupting cell physiology, cell acidification, accumulation of N-rich amino acids, poor root development, and finally, inhibition of shoot growth. Strong evidence exists that many endangered vascular plant species of grasslands, heathlands and soft-water lakes, and fen bryophytes, are very intolerant to increased concentrations of reduced N and to high $NH_4^+ : NO_3^-$ ratios (De Graaf et al., 1998; Paulissen et al., 2004; Kleijn et al., 2008; Van den Berg et al., 2008) (Figure 1.4).

Figure 1.4. Characterisation of growth sites of common (blue diamonds) and rare (red squares) species typical to Dutch heaths, matgrass swards and fen meadows in terms of pH and $NH_4^+ : NO_3^-$ ratio in the soil. Symbols indicate mean \pm SE. In contrast to common species, almost all rare species occur only at a low $NH_4^+ : NO_3^-$ ratio (from Kleijn et al., 2008).



Source: Kleijn et al., 2008

e) Increased susceptibility to secondary stress and disturbance factors

The sensitivity of plants to stress (defined here as external constraints, such as drought, frost, pathogens or herbivores, which limit dry-matter production rate), or disturbance factors, (mechanisms which affect plant biomass by causing its partial or complete destruction), may be significantly affected by N deposition. With increasing N deposition,

susceptibility to fungal pathogens and attacks by insects also increases. This is probably due to altered concentrations of phenolic compounds (leading to lower resistance) and soluble N compounds, such as free amino acids, together with a lower vitality of individual plants as a result of polluted deposition. Increased levels of pathogenic fungi have been found for several tree species in N addition experiments and field surveys, but for most ecosystems data are lacking and the influence of such pathogens on diversity is still unclear (e.g. Flückiger et al., 2002; Bobbink et al., 2003).

In general, herbivory is affected by the palatability of plant material, which is strongly determined by its N content. Increased organic N content in plants, caused by N deposition, can thus result in increased insect herbivory (e.g. Throop and Lerdau, 2004). Data on herbivory and N deposition are very scarce, but a link has been demonstrated in dry *Calluna* heathlands. The frequency and intensity of infestations of heather beetle (*Lochmaea suturalis*) are clearly related to atmospheric N inputs and N concentrations in the heather (e.g. Brunsting and Heil, 1985; Berdowski, 1993; Bobbink and Lamers, 2002; for details see Chapter 8). N-related changes in plant physiology, phenology, biomass allocation (root:shoot ratios) and mycorrhizal infection can also differentially influence the sensitivity of plant species to drought or frost stress, leading to reduced growth in some species and possible changes in plant interactions.

1.2 Background to, and aims of the report

Within the UNECE Convention on Long-range Transboundary Air Pollution (LRTAP Convention), procedures have been developed to model and map critical loads for airborne N deposition in support of effect-based European policies for the abatement of air pollution (Bull et al., 2001; Hettelingh et al., 2001, 2007). Both the steady-state mass balance method and the empirical approach are used to scientifically support European policies aiming at effective emission reductions of air pollutants (ICP M&M, 2017). For the support of these policies it is important that scientific knowledge be regularly updated with new findings. This report focuses on recent knowledge for the review and revision of empirical critical loads of N ($CL_{emp}N$).

$CL_{emp}N$ are in almost all cases based on observed changes in the structure and functioning of ecosystems, primarily in a) species abundance, composition and/or diversity ('structure'), or b) N leaching, decomposition or mineralisation rate ('functioning'). For a more complete overview of indicators, see Løkke et al. (2000). Effects have been evaluated for specific ecosystems. Statistically and biologically significant results from field addition experiments and mesocosm experiments conducted under close-to-field conditions have been used for quantifying $CL_{emp}N$. Only studies on independent N treatments with a duration of two years or more have been used. In particular data from long-term experiments in low-background areas are most useful for observing effects of N enrichment. However, since experimental studies have been conducted for a variety of reasons, their designs differ, and the methods used are carefully scrutinised to identify factors related to the experimental design or data analysis that may constrain their use. This includes evaluation of the accuracy of estimated values of background N deposition at experimental sites. In addition, the results from correlative or retrospective field studies have been used, but only as additional evidence to support conclusions from experiments, or as a basis for an 'expert judgement' rating.

$CL_{emp}N$ for natural and semi-natural ecosystems were first presented in a background document for the 1992 workshop on critical loads held under the UNECE Convention at Lökeberg (Sweden) (Bobbink et al., 1992). After detailed discussions, before and during the meeting, the proposed values were set at that meeting (Grennfelt and Thörnelöf, 1992). Additional

information from the 1992-1995 period was evaluated and summarised in an updated background paper and published as Annex III (Bobbink et al., 1996) of the UNECE manual on methodologies and criteria for mapping critical levels and loads. The updated $CL_{emp}N$ were discussed and set with full consensus at the December 1995 expert meeting held under the UNECE Convention in Geneva (Switzerland). They were also used for the development of the second edition of the Air Quality Guidelines for Europe by the World Health Organization's Regional Office for Europe (WHO, 2000). Furthermore, the $CL_{emp}N$ deposition was extensively reviewed and updated in 2001-2002 (Berne workshop; Achermann and Bobbink, 2003). In that update, classification of the receptor ecosystems was brought in line with that of the European Nature Information System (EUNIS) (mostly level 3) (Davies and Moss, 2002; Hall et al., 2003; Davies et al., 2004), in addition to the incorporation of results from new N-impact studies from the 1996-2002 period (Bobbink et al., 2003). The last review and revision were in 2009/2011 (Noordwijkerhout workshop, Bobbink and Hettelingh, 2011). That revision incorporated N-impact studies from November 2002 to spring 2010. The CCE workshop in Noordwijkerhout included separate sessions of three Working Groups each addressing one or more EUNIS classes. The summary reports of Working Group 1, 2 and 3 can be found in the Appendices in Bobbink and Hettelingh (2011).

It was recognised at the CCE workshop and Task Force meetings of the International Cooperative programme on Modelling and Mapping Critical Loads & Levels and Air Pollution Effects, Risks and Trends in Madrid (ICP M&M, 2019) that considerable new insights into, and data on, the impacts of N deposition on natural and semi-natural vegetation have become available since the compilation of the last background document in 2011. An update of the background material based on the availability of new scientific evidence for many N-sensitive ecosystems is thus pertinent and was adopted by the Working Group on Effects at its 39th session (WGE, 2020), under the LRTAP Convention, and was included in the work plan 2020-2021. This new report will be the basis for the revision of Chapter 5.2 of the modelling and mapping manual (ICP M&M, 2017).

The aims and structure of this report are as follows:

- ▶ New relevant information from studies (2010 - summer 2021) on the impacts of N on semi-natural and natural ecosystems, with emphasis on Europe, to be added to the existing database on $CL_{emp}N$
- ▶ To update the recently revised EUNIS classes (Chytrý et al., 2020) and link, where possible, the $CL_{emp}N$ based on the EUNIS classification with Natura 2000 Annex 1 habitats
- ▶ To review and revise Bobbink and Hettelingh (2011) and provide a revised table on $CL_{emp}N$ for Europe, using the new scientific data including gradient studies (Chapters 2 to 9)
- ▶ To synthesise examples of how $CL_{emp}N$ can be used on different scales and in different European countries (Chapter 10)

Furthermore, the report ends with three appendices. The correspondence between ecosystems classified according to EUNIS and the EU Habitats according to Directive Annex 1 is provided in appendix 1. Appendices 2 and 3 present the agenda and the list of participants from the expert workshop in Berne (Switzerland, October 2021).

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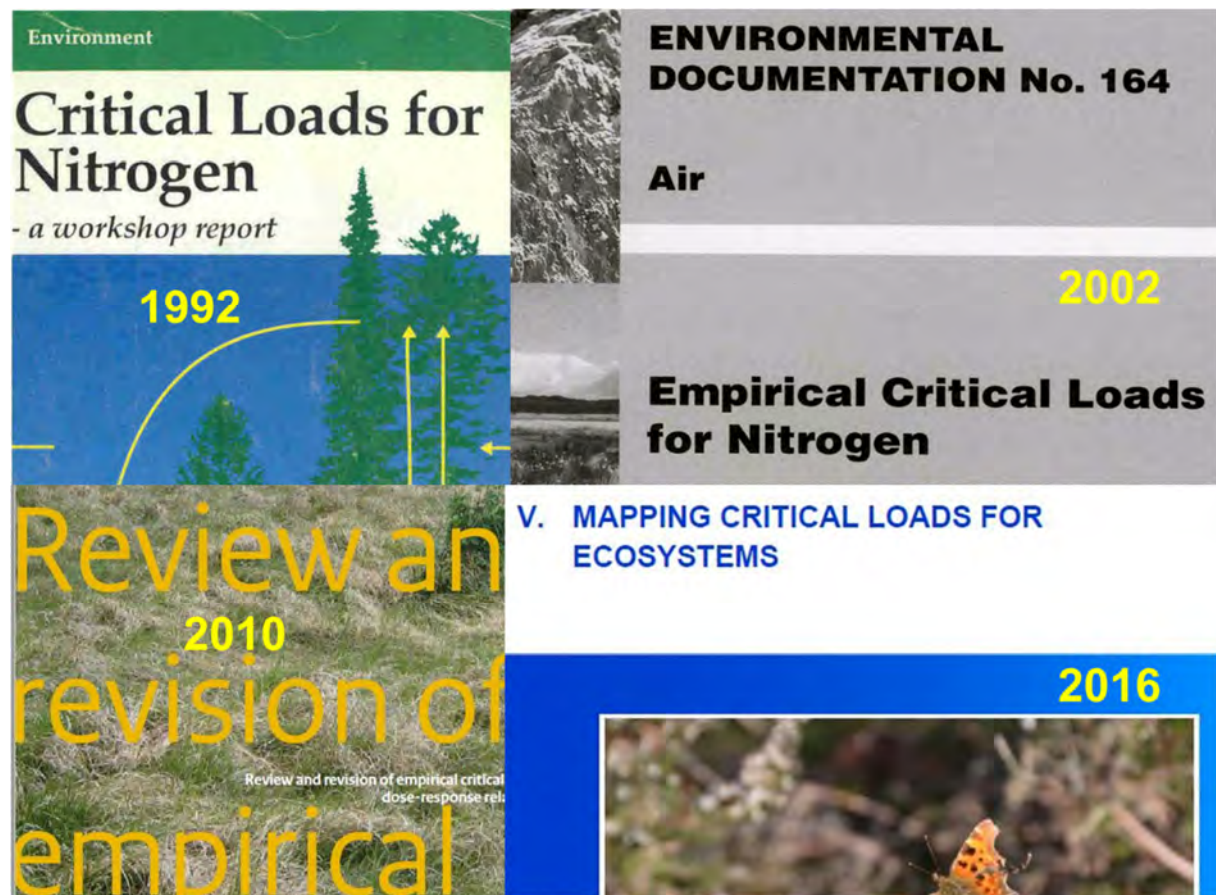
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2 Updating and reviewing procedures for empirical critical loads of nitrogen (CL_{emp}N)

Adapted by Roland Bobbink



Compilation of cover pages of previous reports on empirical critical loads of nitrogen (CL_{emp}N).

2.1 Updating procedure

In this updating procedure, an '**empirical approach**' has been used, similar to that of the previous background documents (Bobbink et al., 2003; Bobbink and Hettelingh, 2011), with the following phases: 1) Kick-Off meeting (June 2020), 2) data collection, 3) first drafting of the different chapters (per class according to the European Nature Information System (EUNIS)), 4) optimisation of the drafts after exchange between the contributing authors (internal review round), 5) review of the second draft by an external expert team, and 6) finalisation of the background document for the UNECE CCE expert workshop, held in October 2021 (Figure 2.1). Following the expert workshop, the background document was finalised after addition and incorporation of the comments of the workshop participants.

Figure 2.1. Schematic representation of the working procedure



a) Data collection

A comprehensive collection of European publications on the effects of nitrogen (N) in natural and semi-natural ecosystems has been made for the period from early 2010 to September 2021. Peer-reviewed publications, PhD theses, book chapters, nationally published papers, and 'grey' reports by institutes or organisations (if digitally available) were used. Relevant information from these studies has been collected, including location, background deposition (if available), and EUNIS classification. The correspondence between the EUNIS class and the Natura-2000 habitat type has been added in an appendix (Appendix 1), as in Bobbink and Hettelingh (2011).

In principle, only European studies have been used as the basis for the assessment of empirical critical loads of N ($CL_{emp}N$). Exceptionally, when no or very few studies were available for a particular important ecosystem, such as steppe grasslands or Mediterranean vegetation, non-European (mostly Northern American and Chinese) literature was used for an 'expert judgement' rating of ecosystem sensitivity to N deposition.

b) Drafting of the chapters

Following data collection, drafts of the several chapters (per EUNIS class) of the background document were produced by groups of authors, using the 2011 document (Chapters 3 to 9 in Bobbink and Hettelingh (2011)) as a starting point. When no new data were available for a specific habitat, the 2011 text was used. When new data were found to be available, the 2011 text was updated accordingly, which in places resulted in completely rewritten chapters. At the end of each chapter, a concluding table presents the $CL_{emp}N$.

c) **Optimisation of the chapter drafts**

All drafts of the different chapters were circulated in the author group of that chapter for discussion and review. This was coordinated by the lead author of the chapter. Comments of co-authors were discussed and incorporated into the main version of the chapter by the lead author, which resulted in the generation of several drafts. All drafts of the different chapters were then reviewed and checked for consistency by the team of lead authors. This internal review round led to a draft version of each chapter, which was then checked and integrated into the main document.

d) **External review**

The consolidated drafts of the chapters were presented to a team of international experts on the impacts of N enrichment in natural and semi-natural ecosystems. This reviewing team consisted of experts from different parts of Europe and the USA. Each chapter on a specific EUNIS class was evaluated by at least two to three experts.

e) **Finalisation of the background document**

Review comments on the second draft were incorporated into the text by the leading authors of each chapter, in close collaboration with the coordinator(s) of the revision. After a final check, the background document was sent to the participants of the UNECE CCE expert workshop on CL_{emp}N (October 2021, in Berne, Switzerland). The comments and additions by participants were used to finalise the final CL_{emp}N table and background document for the formal revision of the Manual on Methodologies and Criteria for Modelling and Mapping Critical Loads & Levels and Air Pollution Effects, Risks and Trends (ICP Modelling & Mapping, in prep.), within the framework of the UNECE Convention on Long-range Transboundary Air Pollution.

2.2 Reviewing and setting values for CL_{emp}N

2.2.1 Types of empirical evidence

The type of “empirical” evidence used to set values for CL_{emp}N varies between terrestrial and aquatic ecosystems. Long-term field manipulations in lakes are highly ecologically relevant but are prohibitively expensive, and are therefore also limited in terms of duration and replication. Experiments of more limited time-span in more controlled conditions in aquatic systems (mesocosms) can supply valuable additional evidence on ecosystem effects related to N addition, especially when they are done along environmental gradients. Therefore, the following text on the type of evidence largely relates to terrestrial ecosystems, including wetlands, while for aquatic ecosystems evidence has been gathered to a larger extent from gradient studies in combination with controlled experiments.

There are two major types of “empirical” evidence available to relate atmospheric N deposition to changes in the structure and/or functioning of ecosystems. The first is from long-term field addition (or manipulation) experiments, in which N deposition is artificially increased, normally by application of increased concentrations of mineral N (NH₄⁺ and/or NO₃⁻). If significant impacts were detected compared to the untreated controls, it was inferred with confidence that simulated N deposition would have been the cause. Experiments can provide information on how long it takes for different components of the system to respond to N addition, and can be designed to assess interactions, for example with other stresses (e.g. drought, warming), land management type or ecosystem type. Experiments can also identify thresholds for the effects of N on biodiversity. However, since most experiments examine effects of increases in deposition, it is difficult to identify thresholds from experiments in areas with a relatively long history of elevated levels of N deposition, where there may already have been significant impacts of N

deposition and where those thresholds may have already been crossed in the past. Other limitations of experimental studies are that they typically assess relatively short-term responses (even the longest-running experiments seldom exceed 20-25 years) and that peculiarities of the experiment (e.g. very high concentrations of the applied pollutant compared with environmentally realistic loads) or site-specific factors might also explain part of the observed response.

A second approach – besides of the evaluation of N addition experiments - is through studies exploring changes in ecosystem structure and/or functioning over an observational N deposition gradient. Such targeted N-gradient studies may provide information on longer-term responses of increased atmospheric N deposition and serve to demonstrate that the responses observed in experiments can also be found in the real-world. They usually cover, if present, a more differentiated range of N deposition than added in the N addition experiments, particularly at the lower end of N deposition, as experiments typically deal with high N doses that are often based on future projections. Most of the studies used species richness of the vegetation – or of components of the vegetation – as a bioindicator, but sometimes (a)biotic factors including plant tissue chemistry and soil biogeochemistry were also considered. In addition, these studies avoid experimental artefacts and allow for analysis of interactions with other environmental stressors (e.g. drought). However, since gradients of N deposition may also be correlated with gradients of other potential important drivers of ecosystem structure and functioning (e.g. S deposition, rainfall, temperature or management), gradient studies need a careful and appropriate design and statistical evaluation. Thus, gradient studies have some advantages and disadvantages compared with experimental evidence. Advantages include adequate sampling of the range of climate space and variability in other factors such as soil type, elevation and management, as well as the range of N deposition present across a habitat type. The disadvantage is greater variability in ecological responses, which requires careful consideration of confounding variables, as discussed above, and larger sample sizes, needed to give sufficient statistical power to detect effects of N deposition. Confounding abiotic and biotic variables may also be considered as modulating variables when they alter the response of the ecosystem to N deposition. One special class of gradient study is the structured survey (defined here as survey not explicitly set up to detect effects of N deposition). This can be analysed post-hoc to detect N impacts (e.g. Maskell et al. 2010). As decided at the Kick-Off meeting of the 2020-2022 review and revision of the CL_{emp}N and based upon the outcome of discussions within an expert group of the authors, the outcome of published N gradient studies have been incorporated in the CL_{emp}N approach as important insights.

2.2.2 Setting CL_{emp}N

In this background document, the authors focus particularly on statistically and biologically significant outcomes of field N addition experiments and mesocosm studies for the assessment of CL_{emp}N. Only studies which have independent N treatments and realistic N loads and durations (below 100 kg N ha⁻¹ yr⁻¹; 2 years or more, optimally > 5-10 years in low background areas) were used for the update and refinement of CL_{emp}N values. Studies with higher N additions or shorter experimental periods have only been interpreted with respect to the understanding of effect mechanisms, possible N limitation or sensitivity of the studied ecosystem. The methods used in these studies have been carefully scrutinised to identify factors related to the experimental design or data analysis, which may constrain their use in assessing critical loads. This includes evaluation of the precision of the estimated values of background deposition at the experimental site, which are often based on models instead of on-site measurements. This is necessary to get insight into the total N load in both the N-treated and the control vegetation.

In general, pot or microcosm studies were not used for setting $CL_{emp}N$ values, except for bryophyte layer studies. However, the outcome of these studies, in some selected cases, was used as an indication of the N sensitivity of the most important or sensitive plant species of an ecosystem (e.g. in coastal habitats).

When available, the outcomes from dynamic ecosystem models provided additional insight into underlying mechanisms of ecosystem decline, which are difficult to incorporate or analyse in experimental studies, such as increased frequencies of pests and diseases and a greater sensitivity to environmental stressors such as frost, drought and heatwaves.

2.2.3 N gradient studies

The outcomes of N gradient studies have been used to assess the impacts of atmospheric N deposition in this 2020-2022 review and revision of the $CL_{emp}N$, combined with the results of N addition experiments. Principally, peer-reviewed publications have been analysed, together with some “grey” publications. N gradient studies show the relationships between (modelled) atmospheric annual total inorganic N deposition and ecological parameters, e.g. species richness of the vegetation (or of taxonomical or functional groups of the vegetation), species composition, plant tissue stoichiometry, soil chemistry, drought resistance and sensitivity against pathogens and pests. In almost all cases, significant relationships between the selected indicators and N deposition have been found. Several functions have been fitted through the data, such as linear, negative exponential or S-shaped curves and in several instances more rapid responses are observed at lower N loads. The relationships as presented in many N gradient studies have been originally quantified to demonstrate the potentially negative influence of N deposition on ecosystem structure or functioning, mostly not to identify N thresholds per se. Several recent studies have, however, been purposely set up to reveal $CL_{emp}N$ values/ranges and used statistical techniques/models to identify these thresholds. These techniques may include change point models (Roth et al., 2017; Tipping et al., 2013), community change point analysis with Threshold Indicator Taxa ANalysis (TITAN) (Payne et al., 2013; Wilkins and Aherne, 2016; Wilkins et al., 2016) or simple comparison between classes of N deposition (e.g. per 5 kg N ha⁻¹ yr⁻¹) (Roth et al., 2013). A comparison of the just mentioned techniques to reveal $CL_{emp}N$ values or ranges is given in the next chapter (1.3), demonstrating a good agreement between the different techniques if applied to the same data set.

To evaluate the published N gradient studies in the empirical approach, two types of studies have been identified: a) studies that did not use special techniques to quantify critical loads (“only general” regressions) and b) studies using the previously mentioned special statistical techniques. With respect to the first group of evidence (a), visual inspection of the basic figures was used to reveal a “change range” in the curves (data points). For the second group of studies (b) the presented technique has been shortly described and the outcome given as evidence with respect to the setting of the $CL_{emp}N$ of the studied ecosystem type.

Most of the N gradient studies used have been published in peer-reviewed, international scientific journals. In this case it is likely that the (modelling of) atmospheric N deposition is accurate and at a relevant scale (e.g. Braun et al., 2017) for the specific situation and thus useable for $CL_{emp}N$ evaluation, as done for N addition experiments too. If relevant, possible weaknesses in the presented N deposition values are evaluated, including suggestions with respect to the setting of $CL_{emp}N$. In addition, it has been checked if confounding/modulating predictors (e.g. temperature, rainfall, S deposition) were included sufficiently and if appropriate statistical procedures were used. Some of the N gradient studies have so far only been available in “grey” literature reports. In that case, special attention has been paid to the outcome of the study with respect to both the method of disentangling the contributions of different predictor

variables and the N deposition values used (which model etc). After this evaluation, (parts of) the results have been used to find CL_{empN} values.

2.3 A comparison of methods to estimate empirical critical loads from gradient studies

Authors: Tobias Roth, Julian Aherne, Kayla Wilkins and Roland Bobbink

2.3.1 Introduction

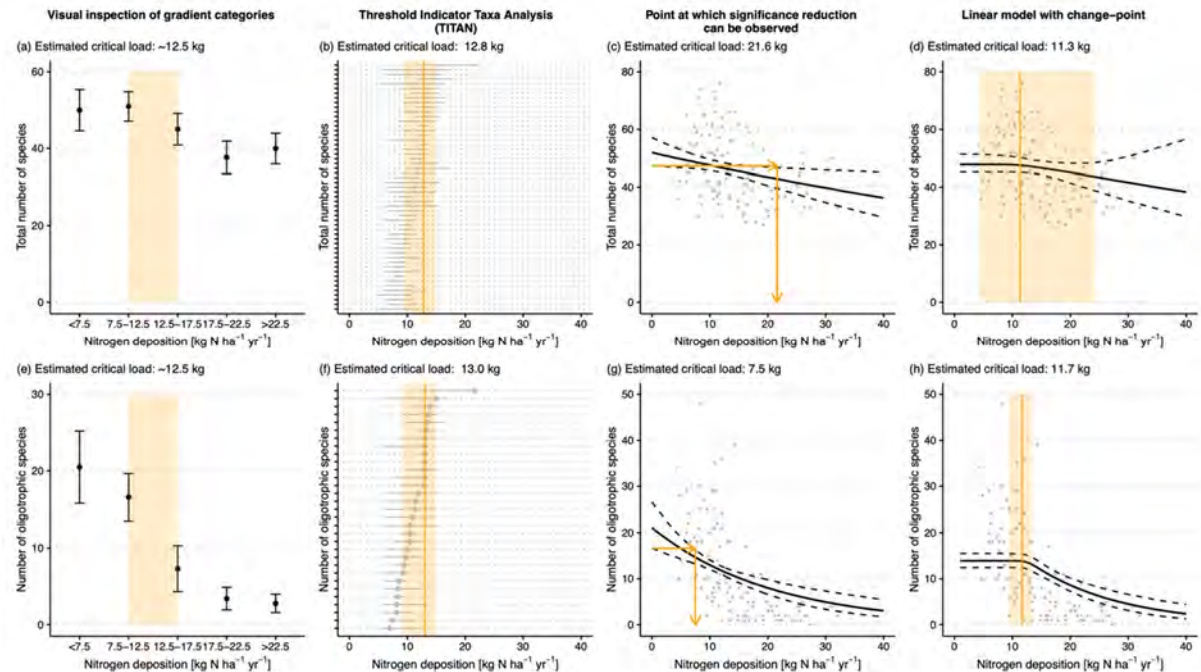
There are numerous methods to estimate an abrupt change or ‘change-point’ along a gradient. Several studies have used an abrupt change in plant species along a nitrogen deposition gradient as a ‘quantitative estimate’ of the empirical critical loads for nitrogen (e.g. Wilkins et al., 2016; Roth et al., 2017). To our knowledge, these methods have never been applied to the same data. It is thus unknown whether the different methods result in systematic differences between the estimated critical loads. Consequently, it is difficult to compare critical load estimates between studies. Here we apply four methods that were previously used in publications to estimate empirical critical loads from gradient studies to the same data sets as indicated in Chapter 2. The four methods are listed in Table 2.1. Our goals were 1) to give an overview of the differences between the methods and list their potential advantages and shortcomings, and 2) to show whether and how the results differ if they are applied to identical data sets.

2.3.2 Case studies: Mountain hay meadows in Switzerland and Atlantic oak woodlands in Ireland

We applied the four methods (Table 2.1) to mountain hay meadow data from the biodiversity monitoring in Switzerland (Weber et al., 2004) and Atlantic oak woodland data (species abundances) from the Irish National Parks and Wildlife Service (Perrin et al., 2008). For mountain hay meadows, we selected the same sites as in Roth et al. (2013) but used for each site the most recent survey conducted between 2016–2020. For the recent surveys, species cover (abundance) was recorded, whereas for the data in Roth et al. (2013) only presence / absence data were available. For these surveys, we analysed all recorded species together and the subset of species that are typically found on nutrient poor sites (i.e. oligotrophic species with N-values of one and two; Landolt et al., 2010). For Atlantic oak woodlands, we used the same sites, plant species data (surveyed between 2003–2007), and nitrogen deposition data as Wilkins and Aherne (2016). We analysed all recorded species together and the subset of species identified as positive indicators by the Irish National Parks and Wildlife Service. All analyses were conducted in R. Data sets and scripts to reproduce the analyses are available on GitHub¹.

¹ <https://github.com/TobiasRoth/eCL-methods>

Figure 2.2. Comparison of the results applying the four methods (a and e: visual inspection of gradient categories; b and f: Threshold Indicator Taxa Analysis for plant species with negative change-points; c and g: point at which significant reduction can be observed; d and h: linear model with change-point) to the plant data of mountain hay meadows in Switzerland. The upper panels (a–d) give the results based on all plant species and the lower panels (e–g) give the results based on oligotrophic species only. Critical load estimates (vertical orange line) with estimate range (orange areas) are given if provided by the method.

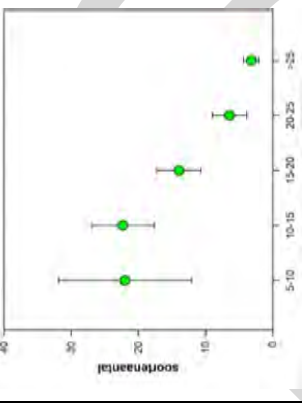
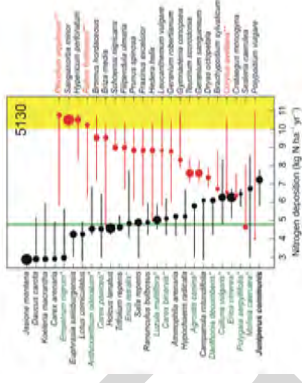
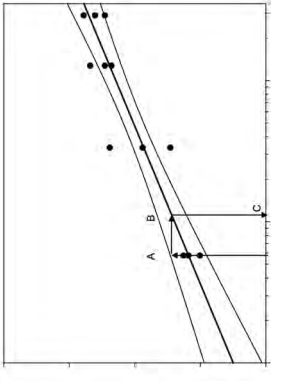
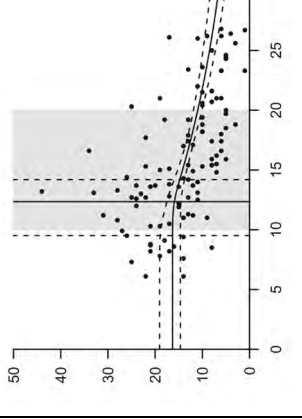


Source: <https://github.com/TobiasRoth/eCL-methods>

For the mountain hay meadows, the critical load estimates based on the visual inspection of gradient categories, TITAN and linear model with change-point were very similar and were stable, independent of whether the analyses were based on all recorded species or on only the oligotrophic species (Figure 2.2). The estimates were all in the range of the critical load that was established in this revision. In contrast, the method of Cape et al. (2009), based on the point at which significant reduction of species richness could be observed, showed a difference between the total and subset species data and the critical load found was outside of the established range for both (Figure 2.2). This method suggested the lowest critical load based on oligotrophic species, but the highest critical load when based on all recorded species (Figure 2.2).

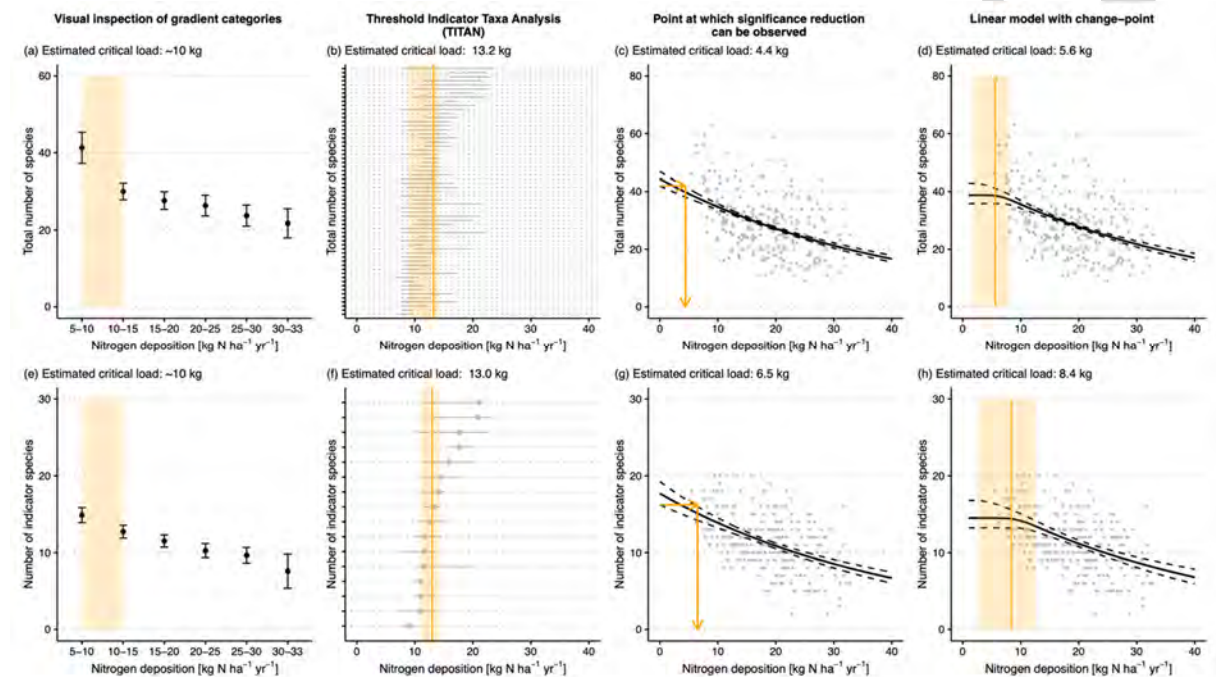
With respect to the Atlantic oak woodland, the critical load estimates of the visual inspection of gradient categories and TITAN were again very similar and within the range of the established critical load (Figure 2.3). They both showed very little difference in estimated critical loads based on the total number of species or the subset. In contrast, the method of Cape et al. (2009) and the linear model with change-points both suggested critical loads below the established critical load with lower estimates for total species richness than for the number of indicator species.

Table 2.1. Comparison of four methods to estimate empirical critical loads from nitrogen deposition gradient studies.

V.	VI. Visual inspection of gradient categories	VII. Threshold Indicator Taxa Analysis (TITAN)	VIII. Point at which significance reduction can be observed	IX. Linear model with change-point
References	Wamelink et al. (2021)	Baker and King (2010); Wilkins et al. (2016)	Cape et al. (2009)	Tipping et al. (2013); Roth et al. (2017)
Short description	The gradient (nitrogen deposition) is aggregated to equal sized classes (e.g. 5 kg N ha ⁻¹ yr ⁻¹ deposition ranges) and the average and confidence interval of the biodiversity measure is calculated for each class. The critical load is estimated 'by eye' between the classes at which the first significant decrease in the biodiversity measure occurs.	A two-step approach; 1) Individual taxa with significant change-points in species occurrence or abundance along a nitrogen deposition gradient are identified. 2) The point along the gradient at which these single species change-points accumulate (the community change-point) is identified, and interpreted as the critical load.	(Generalized) linear (mixed) model that describes the relationship between the gradient and the biodiversity measure and other variables (covariates). The nitrogen deposition at which a significant change in the biodiversity measure can be observed is considered as the critical load.	(Generalized) linear (mixed) model that describes the relationship between the gradient and the biodiversity measure using a change-point (or segmented, piecewise, broken-stick). The nitrogen deposition at which the change-point occurs is considered as the critical load.
Example figure from cited references				
Advantages	<ul style="list-style-type: none"> Easy to apply and understand. 	<ul style="list-style-type: none"> Explicitly considers individual species' behaviour. 	<ul style="list-style-type: none"> Can account for covariables. 	<ul style="list-style-type: none"> Can account for covariables. Estimates uncertainty in critical load setting.

V.	VI. Visual inspection of gradient categories	VII. Threshold Indicator Taxa Analysis (TITAN)	VIII. Point at which significance reduction can be observed	IX. Linear model with change-point
Potential shortcomings		<ul style="list-style-type: none"> Estimates uncertainty in change-point. 		
	<ul style="list-style-type: none"> Arbitrary classification of the continuous gradient into discrete ranges. 	<ul style="list-style-type: none"> For some individual species the estimated change-points seem unrealistic (e.g. eutrophic species with low change-points and vice versa). 	<ul style="list-style-type: none"> Since the confidence interval decreases with the sample size, the estimated critical load will also change with sample size. 	<ul style="list-style-type: none"> Brute force application of the change-point model to the data; a critical load is also estimated if nitrogen deposition does not negatively affect biodiversity.
	<ul style="list-style-type: none"> An exact change-point is not identified (generally set to middle of range). 	<ul style="list-style-type: none"> Not possible to account for covariates. 	<ul style="list-style-type: none"> Assumes that statistical significance corresponds to biological relevance. 	<ul style="list-style-type: none"> Limited to a summary statistic across all species (e.g. species richness, total number of individuals).
	<ul style="list-style-type: none"> Sufficient sample sites are needed for all categories. 			
	<ul style="list-style-type: none"> Limited to a summary statistic across all species (e.g. species richness, total number of individuals). 		<ul style="list-style-type: none"> Limited to a summary statistic across all species (e.g. species richness, total number of individuals). 	<ul style="list-style-type: none"> Limited to a summary statistic across all species (e.g. species richness, total number of individuals).

Figure 2.3. Comparison of the results applying the four methods (a and e: visual inspection of gradient categories; b and f: Threshold Indicator Taxa Analysis for plant species with negative change-points; c and g: point at which significant reduction can be observed; d and h: linear model with change-point) to the plant data of Atlantic Oak Woodland in Ireland. The upper panels (a–d) give the results based on all plant species and the lower panels (e–g) give the results based on positive indicator species only. Critical load estimates (vertical orange line) with estimate range (orange areas) are given if provided by the method.



Source: <https://github.com/TobiasRoth/eCL-methods>

2.3.2 Discussion

- ▶ In general, three of the four methods (i.e. visual inspection of gradient categories, TITAN and linear model with change-point) provided critical load estimates that were similar in most cases suggesting that there is no systematic difference in the results they provide.
- ▶ Two of the methods (visual inspection of gradient categories and TITAN) appear to be less influenced by the number of species (or sample size), as they showed very little difference in estimated critical loads based on the total number of species or the species subset (oligotrophic or indicator species).
- ▶ In the two example habitats presented here, the highest and most stable critical load estimates were generally obtained from TITAN. We suggest that this may be related to the greater resolution provided by individual species abundance and frequency data compared with the summation to species richness from presence / absence data only used in the other methods.
- ▶ In some cases, the TITAN approach estimates single-species change-points that seem unrealistic (e.g. eutrophic species with low change-points and vice versa) and it was argued that this may affect the estimated community change-points. If that is the case, one would expect biased results particularly if all species (including all eutrophic species) are used in the analyses. For the mountain hay meadows and Atlantic oak woodlands, however, the

TITAN estimates were almost identical if applied to all species and if applied to the oligotrophic or indicator species subsets only. Furthermore, for mountain hay meadows the TITAN results were very similar ($< 1.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) to the results obtained from the linear model with a change-point.

- ▶ The unrealistic single-species change-points estimated by TITAN may reflect a species with a unimodal response to nitrogen that was not captured by the observed deposition range in the case studies. Further, the unrealistic single-species response was present in the other methods, which used the species and nitrogen deposition data. However, the response of the single-species' was masked in the summation to species richness.
- ▶ Based on our results we are confident that estimates of critical loads are comparable between studies regardless of whether they were obtained using the visual inspection of gradient categories, TITAN or linear models with change-points.

2.4 Ecosystem classification

In this background document, the groups of natural and semi-natural ecosystems have been classified and ordered according to the EUNIS (European Nature Information System) habitat classification for Europe. For a general description of the updated EUNIS classification and an introduction to its use, see Davies et al. (2004) and the supporting website (<https://www.eea.europa.eu/data-and-maps/data/eunis-habitat-classification>). With respect to the classification in 2011, the codes for two classes changed: heathland, scrub and tundra became Class S (instead of F), whereas forest and other wooded land changed from G to T. In addition, very recently, also marine habitats changed into MA (instead of A), coastal habitats to N (formerly B), grasslands and lands dominated by forbs, mosses or lichens into R (instead of E) and wetlands to Q (instead of D) (Chytrý et al., 2020). Level 2 and 3 numerical codes changes in many cases as well, but not always. In general, the ecosystems described in this document have been classified down to level 3 of the EUNIS hierarchy, and the EUNIS code is given in the text and tables in brackets, for example, perennial calcareous grasslands and basic steppes (R1A). The Natura-2000 habitat type (e.g. H6210) have been presented in Appendix 1. Finally, as in 2011, additional attention has been paid to the classification of forest and other wooded land (T), in order to better differentiate the CL_{empN} between the wide range of European forest types. As before, studies based on pure plantation stands were not included in the chapter on forest habitats. The critical loads of N for these intensively managed systems are based upon steady-state mass balance methods, see the UNECE Mapping Manual (2015-17). Ground-living lichens and bryophytes, as before, have been incorporated in the chapters of the appropriate ecosystems, because many experimental and gradient studies incorporated these species groups. Epiphytic lichens and epiphytic bryophytes are mostly not part of the empirical approach for N critical loads, as they often were not treated or exposed in experiments, but they have been included in the critical levels for ammonia.

2.5 Revision, reliability and interpretation of CL_{empN} ranges

The indication of reliability of the CL_{empN} (Bobbink and Hettelingh, 2011) have been adapted because of the implementation of N gradient studies in this review and revision. The following guidelines have now been used:

- ▶ expert judgement (#): we only use this indication if no empirical data (experimental and/or gradient studies) were available for this type of ecosystem. For this, the CL_{empN} was based upon expert judgement and knowledge of ecosystems which were likely to be similar;

- ▶ if, for a given ecosystem, few experimental data exist (and no N gradient study) showing a significant effect at a certain range, then the CL_{emp}N is considered as quite reliable #;
- ▶ if, for a given ecosystem, no experimental data exist, but an appropriate gradient study showed a significant effect at a certain range, then the CL_{emp}N is considered as quite reliable #;
- ▶ if several good quality studies (both experimental and/or N gradient) revealed N deposition effects at a certain level/range, then the CL_{emp}N is considered as reliable ##.

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Roth, T., Kohli, L., Rihm, B., Meier, R. and Achermann, B. (2017). Using change-point models to estimate empirical critical loads for nitrogen in mountain ecosystems. *Environmental Pollution* **220**, 1480-1487.

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Final Draft

3 Effects of nitrogen deposition on marine habitats (EUNIS class MA, formerly A)

Adapted by Roland Bobbink



Upper-mid salt marsh (MA223) on a Wadden Island in the Netherlands. Photo: Bas van de Riet.

Summary

In this chapter empirical N critical loads ($CL_{emp}N$) for Atlantic coastal salt marshes (MA223, MA224 and MA225) have been updated and revised, if necessary. Unfortunately, no experimental field studies with application of nitrogen compounds with low enough N loads have become available in the present revision period. However, the outcome of two N gradient studies of Atlantic salt marshes (MA223 and MA224) indicated that the $CL_{emp}N$ of these two salt marsh types should be lowered to 10-20 kg N ha⁻¹ yr⁻¹ (expert judgement). Finally, it has been concluded that long-term N addition studies with low doses of N application are highly needed in these typical intertidal communities of high conservation value. In addition, no data are presently available for ecologically important Mediterranean salt marshes (MA25).

3.1 Introduction

Marine habitats, categorised in the European Nature Information System (EUNIS) under class MA, are distinguished from other ecosystems by their direct connection to the sea. Most of these systems are either not covered with vascular plants or fully aquatic, and therefore out of the scope of this background document on the effects of atmospheric N deposition and empirical critical loads. However, coastal salt marshes around and above the high (spring) tide in tidal regions are included in marine habitats (Class MA) and therefore treated in this chapter. Since the 2011 update, hardly any new evidence has become available for this EUNIS class, thus, the content of this chapter is more or less identical to that of 2011 (Bobbink and Hettelingh, 2011), except for a few corrections and the outcome of two additional “gradient” studies for salt marshes. No N addition studies with low enough N loads have been published in the present update and revision period.

3.2 Atlantic coastal salt marshes and saline reed beds (MA22)

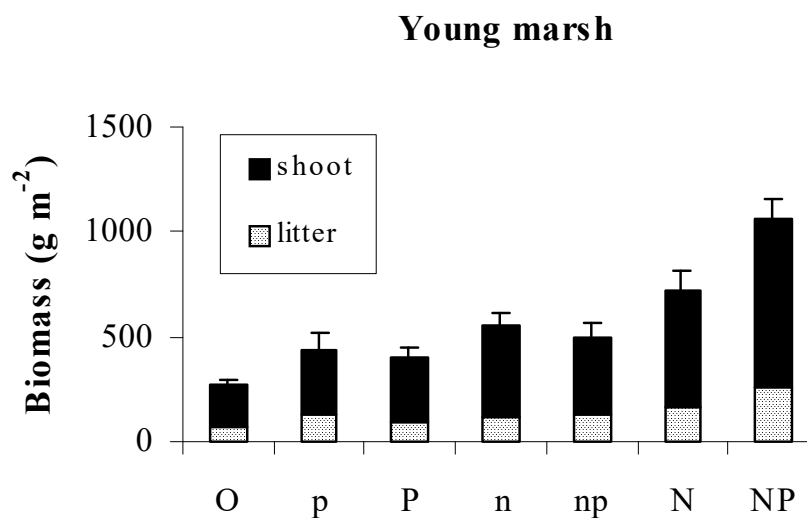
Salt marshes develop where fine sediments accumulate along sheltered coastlines. They are often associated with estuaries, but also frequently occur in areas where the coastline is protected by islands and sandbars. They are typically intertidal, i.e. located in areas that lie between lowest and highest tide and are periodically covered with salty water. The dominant plants are rooted macrophytes and shrubs which are adapted to the environmental stresses associated with sea water inundation (Archibold, 1995). They are characterised by an open nutrient cycle, receiving large amounts of nutrients from surface water, and exporting similarly large amounts of nutrients through surface water and denitrification (for N). This has led to the conclusion, in accordance with Morris (1991), that these systems are not vulnerable to the effects of increased atmospheric N deposition, at least not at most current deposition rates.

However, it is generally accepted that salt-marsh vegetation is primarily N limited (Mitsch and Gosselink, 2000) and N limitation has been demonstrated, for example, in European salt marshes at the Dutch island of Schiermonnikoog (Kiehl et al., 1997) and in Norfolk, in the United Kingdom (Jefferies and Perkins, 1977). During salt-marsh succession, N accumulates in organic material, and N mineralisation increases as marshes age, as shown by Olff et al. (1993) and Van Wijnen et al. (1999). This accumulation of N is considered as a major driving force behind succession, as competition for nutrients is replaced by competition for light.

Van Wijnen and Bakker (1999) added 50 kg N ha⁻¹ yr⁻¹ for three years to a 15-year-old salt marsh (EUNIS category MA224 - Atlantic mid-low salt marshes) and a 100-year-old salt marsh (EUNIS category MA223 - upper-mid salt marshes) in the Netherlands (background deposition 15-20 kg N ha⁻¹ yr⁻¹). Biomass increased significantly after N application from the first growing season in the young salt marsh, and it continued to be higher during all three years of this treatment than in the control treatment (Figure 3.1). In the older salt marsh, however, the addition of 50 kg N ha⁻¹ yr⁻¹ had no significant effect on biomass, although the response to a much higher N application (250 kg N ha⁻¹ yr⁻¹) showed that the vegetation was at least partly N limited (Van Wijnen and Bakker, 1999). Fertilisation increased biomass of late-successional species and decreased the floristic differences between the young and old marshes. However, these species-composition responses were measured only in the combined high N (250 kg N ha⁻¹ yr⁻¹) and high P treatments, compared with the control situation. Thus, the effect of N on species composition could not be separated from the effect of adding P. However, as the effects of added P on biomass were either non-significant or quite small compared to the effects of N, there is a clear indication that increased N availability does increase the rate of succession. The successional age of these salt marshes is an important determinant of their quality as staging

areas for Brent and Barnacle geese (*Branta bernicla* and *Branta leucopsis*, respectively) (Bakker, 1985). The increases in N deposition might decrease the surface area of early successional vegetation on the marsh and thereby the foraging area that is suitable for these migratory birds. Salt marsh areas that are also particularly important for migratory birds are those located in the southernmost part of Europe (e.g. Doñana National Park and Bay of Cádiz in Spain). However, there are to date no available studies that have evaluated the effects of increased N deposition on Mediterranean-type salt marshes (MA25).

Figure 3.1. Above-ground biomass of young salt marsh vegetation (MA224) in the Netherlands, after a 1-year addition of differential nutrients; lower case n = 50 kg N ha⁻¹ yr⁻¹, capital N = 250 kg N ha⁻¹ yr⁻¹, lower case p = 20 kg P ha⁻¹ yr⁻¹, capital P = 100 kg P ha⁻¹ yr⁻¹ (adapted from Van Wijnen and Bakker, 1999).



Source: Van Wijnen and Bakker, 1999

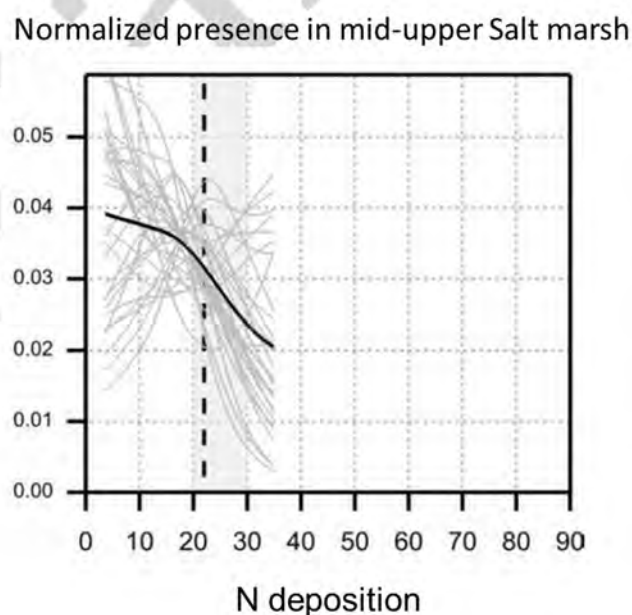
During primary succession N accumulates in organic material in the soil and is one of the main driving forces of succession. Increased N deposition will accelerate this natural process, but, because it does not affect the sediment accretion rate of salt marshes, this will result in a net loss of salt marshes of a low successional age (EUNIS categories MA225 and MA224). Information from long-term monitoring (25 years) of vegetation on one Dutch island with an estimated total N deposition of 15 to 20 kg ha⁻¹ yr⁻¹ showed a trend towards more eutrophic vegetation, both in grazed and ungrazed mid-successional salt marshes (Dijkema et al., 2005). This study was based primarily on mid-successional salt marshes (MA223); in early successional salt marshes the trend was less pronounced, partly because of the very low number of species. This has been considered as an indication for a CL_{emp}N of 15-20 ha⁻¹ yr⁻¹ in the previous update and revision.

Recently, changes in species composition – with positive indicator species – along a nitrogen deposition gradient has been studied in Ireland (Aherne et al., 2020). Vegetation data (relevés) of Irish salt marshes (EUNIS code MA22x) were obtained from the Irish National Parks & Wildlife Service (Salt Marsh Monitoring Program [SMMP]). Unfortunately, no differentiation could be made in the analysis between the different salt marsh types in this data set, but the proposed habitat maps suggest that the relevés predominantly (68%) represented MA223 and MA224. The TITAN (Threshold Indicator Taxa Analysis) model has been used to detect changes in plant species distributions (e.g. plant species abundance) along an environmental gradient, such as N deposition; the location along the gradient where the greatest change occurs is called the ‘change point’ (Baker and King, 2010). Evidence for community thresholds is suggested by a

convergence (or synchrony) of individual species change points along the environmental gradient. TITAN produces two change points: one represents the species that significantly decrease in abundance along the environmental gradient (z^-), and the other represents the species that significantly increase along the environmental gradient (z^+). This study focused on changes in positive indicator species of the salt marshes (Aherne et al., 2020). 65 plant species were present in the Irish salt marshes dataset (EUNIS code MA22x), with 11 of the 15 species with a z^- change point were positive indicator species, and the z^- community change point was $7.8 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. The authors suggested a new range for Atlantic salt marshes of $5\text{-}10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, well below the previously set up CL_{empN} range ($20\text{-}30 \text{ kg N ha}^{-1} \text{ yr}^{-1}$).

Furthermore, dose-response relations for 60 Annex 1 habitat types and N deposition based on the responses of individual plant species were estimated (Wamelink et al., 2021). The deposition was linked to the plant species present in over 400,000 vegetation relevés of the European Vegetation Archive (EVA) database (<http://euroveg.org/eva-database>). In this study relevés from 14 countries, from Portugal to Finland, and from Ireland to Austria were used. The total N deposition (EMEP) at the site was calculated as the average deposition of the previous five years. A response curve for N deposition was estimated for each species. From this spline function, the percentiles were used to estimate the response of a habitat type to N deposition. The percentiles and the occurrence of positive indicator species of a habitat type were added together – after normalisation – and subsequently a mean response curve per habitat was estimated (Wamelink et al., 2021). This approach did not reveal a “change range” for pioneer salt marshes dominated by *Salicornia* spp. (MA225), but for mid-low salt marshes (MA224) and upper-mid salt marshes (MA223) a “change range” of $15\text{-}20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ was observed (Figure 3.2). Thus, the results of this study also indicate a lower CL_{empN} , especially for the two just-mentioned salt marshes.

Figure 3.2. Normalised presence (Y-axis) of all indicator species (grey lines) and the mean of the positive indicator species (black line) for the upper-mid salt marsh (MA225) against total N deposition ($\text{kg N ha}^{-1} \text{ yr}^{-1}$). Grey area is the CL_{empN} range in 2011, striped vertical line the CL_{empN} according to the Dutch modelling approach (Wamelink et al., 2021).



Source: Wamelink et al., 2021

Based on the previous expert judgement and the new evidence from the two “gradient studies”, a new critical load range of 10 to 20 kg N ha⁻¹ yr⁻¹ is recommended for mid-low and upper-mid salt marsh systems (MA224 and MA223; Table 3.1). The lower limit of 10 is the mean of 5 (Aherne et al., 2020) and 15 (Wamelink et al., 2021). The upper limit represents the maximum value proposed by Wamelink et al. (2021). For pioneer salt marshes (MA225) the range of the CL_{emp}N has not been revised. However, field experiments with lower N additions over a longer period of time and/or additional gradients studies to improve the reliability for these adaptations are urgently needed, particularly in southern European latitudes that harbour areas of extremely high importance for migratory birds.

Table 3.1. CL_{emp}N and effects of exceedances on marine habitats (MA). ## reliable, # quite reliable and (#) expert judgement. Changes with respect to 2011 are indicated as values in bold.

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2011 reliability	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
Atlantic upper-mid salt marshes	MA223	20-30	(#)	10-20	(#)	Increase in dominance of graminoids; decline positive indicator species
Atlantic mid-low salt marshes	MA224	20-30	(#)	10-20	(#)	Increase in late successional species; decline positive indicator species
Atlantic pioneer salt marshes	MA225	20-30	(#)	20-30	(#)	Increase in late successional species; increase in productivity species

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4 Effects of nitrogen deposition on coastal habitats (EUNIS class N, formerly B)

Adapted by Laurence Jones, Emiel Brouwer and Eva Remke



Coastal landscape, Terschelling (The Netherlands). Photo: Eva Remke.

Summary

In this chapter empirical N critical loads ($CL_{emp}N$) for coastal habitats have been updated and revised where necessary. Since the last $CL_{emp}N$ update in 2011, a number of experimental and gradient studies have been published for coastal dunes and sandy shores (N1). Based on new evidence, the $CL_{emp}N$ ranges were confirmed or narrowed down to a lower level. For shifting coastal dunes (N13, N14), the $CL_{emp}N$ range of 10 to 20 kg N ha⁻¹ yr⁻¹ was confirmed. However, it should be kept in mind that the mechanisms of N impact may differ in temperate Europe compared with the Mediterranean. Compared to the last review in 2011, it is proposed to decrease the $CL_{emp}N$ range for coastal dune grasslands (N15), coastal dune heaths (N18, N19) and moist to wet dune slacks (N1H). For these subcategories, the evidence status should be increased to reliable (dune grasslands) or quite reliable (dune heaths, dune slacks).

New in this chapter, compared to the last update by Bobbink and Hettelingh (2011), is the inclusion of the EUNIS class dune slack-pools which belong to the freshwater aquatic communities of permanent Atlantic and Baltic (N1H1) or Mediterranean and Black Sea (N1J1) dune-slack water bodies (Chytrý et al., 2020). Due to relatively little new evidence for this habitat the $CL_{emp}N$ range did not change.

Beside this good evidence for coastal dunes and sandy shores (N1), there remain major knowledge gaps for coastal shingle (N2) and rock cliffs, ledges and shores, including the supralittoral (N3), for which $CL_{emp}N$ could not be established due to a lack of evidence.

4.1 Introduction

This chapter presents an evaluation of the impacts of atmospheric nitrogen (N) deposition on coastal habitats (class N, formerly B) of the European Nature Information System (EUNIS), with respect to the setting of empirical critical loads of N ($CL_{emp}N$). Coastal habitats are defined as situated above the high spring tide limit (or above mean water level in non-tidal waters) with coastal features and characterised by their proximity to the sea. They include coastal dunes (dry grasslands, wet to moist dune slacks, dune-slack pools, scrub and wooded dunes), beaches and cliffs (Davies et al., 2004). Dune-slack pools in the EUNIS system were classified under permanent oligotrophic waters in Bobbink and Hettelingh (2011). However, due to their coastal location, this habitat is now incorporated within the coastal sand dunes chapter for the purposes of $CL_{emp}N$ evaluation.

The first subdivision within class N in EUNIS is based on underlying substrates, that is, sand, shingle or rock, but data to support proposals for $CL_{emp}N$ are only available for sand substrates (N1; coastal dune and sandy shores). The following chapter integrates information from the last review of $CL_{emp}N$ (Bobbink and Hettelingh, 2011) with information published since the last review to evaluate the total evidence base and determine whether to retain or revise the $CL_{emp}N$ values. In this background document, separate critical load values for N deposition are evaluated and reviewed for major habitats within the EUNIS category N1. There remain major knowledge gaps on the effects of atmospheric N on the other coastal habitats, including coastal shingle (N2) and rock cliffs, ledges and shores, including the supralittoral (N3).

4.2 Coastal dunes and sandy shores (N1)

Dune ecosystems in the coastal areas of Europe retain a large part of their original plant and animal life, and are thus a major reservoir of European biodiversity, especially for lowland species adapted to calcareous substrates which have been lost elsewhere due to agricultural expansion. They are found on sandy, nutrient-poor soils, and considered to be sensitive to the impacts of both eutrophication and acidification (e.g. Ellenberg, 1988a; Wellburn, 1988; De Vries et al., 1994). With respect to the setting of $CL_{emp}N$ in coastal dune and sand habitats, evidence exists only for some EUNIS categories, namely those of shifting coastal dune (N13 and N14), coastal dune grassland (grey dune) (N15), coastal dune heaths (N18 and N19), Atlantic and Baltic moist and wet dune slack (N1H), and dune slack pools (N1H1, N1J1).

4.2.1 Shifting coastal dunes (N13, N14)

Shifting coastal dunes are coastal, mobile sand habitats of the boreal, nemoral, steppe, Mediterranean and warm-temperate humid zones of Europe. They include embryonic shifting dunes at the beach and shifting dunes along the shoreline with *Ammophila arenaria* ('white dunes'). There is only little evidence available on this EUNIS category. This includes two experimental studies: one from Iceland with rather high N addition rates but low ambient deposition (Greipsson and Davy, 1997) and evidence from a new study in Italy with low additions and also low ambient deposition (Menicagli et al., 2020). The other evidence comes from a gradient study in the UK (Jones et al., 2004). There are other, non-European studies on shifting dunes, but the studies are carried out at relatively high ambient N deposition ($15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) and with high experimental additions of three to nine times ambient N deposition (Bird and Choi, 2017). For these reasons, their results are not included in this review.

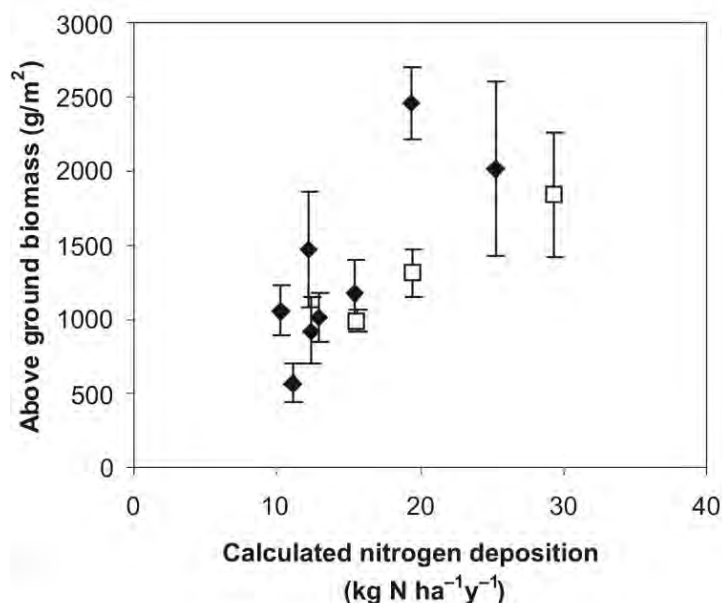
In the Iceland study (Greipsson and Davy, 1997), the effects of N addition over a two-year period were reported for coastal dunes (probably Atlantic and Baltic shifting coastal dune EUNIS N13) in a region with very low atmospheric N deposition. The number of flowering spikes and total

seed weight of the tall dune grass *Leymus arenarius* strongly increased within the first year of application of either 50 or 100 kg N ha⁻¹. This study is not ideal as nitrogen fertiliser has been applied only once (in June) and in high doses, but as there are only limited studies available for this habitat type it has been included in this review.

The Italian study consisted of a one-year field experiment in Mediterranean, Macaronesian and Black Sea shifting coastal dunes (N14) on the Tuscan sandy coast (Menicagli et al., 2020). The study examined the individual and combined effects of N deposition (current and predicted in the Mediterranean for 2050), macro-plastic and biotic condition on the performance of vegetative propagules of dune plants. Two clonal grass species typical for the Mediterranean embryonic dunes were chosen: *Thinopyrum junceum* (L.) A. Love (previously known as a *Elymus farctus* or sand couch), a typical dune-building and endemic species, and *Sporobolus pumilus* (Roth) P.M. Peterson and Saarela (previously *Spartina patens*), a generalist species not involved in the dune formation process and probably introduced from North America. Background wet N deposition rate along the Tuscan coast ranged from 4.7 to 5 kg N ha⁻¹ yr⁻¹ (Marchetto et al., 2014). As a necessary simplification to estimate total N deposition, dry deposition has been assumed to be broadly equivalent to wet deposition (this is typical of seasonally dry Mediterranean environments), giving a background load of approximately 10 kg N ha⁻¹ yr⁻¹. In experimental plots an amount of 5.2 kg N ha⁻¹ yr⁻¹ was added, which would give a total N input of ~15 kg N ha⁻¹ yr⁻¹, which is in the range of the atmospheric N inputs predicted in the Mediterranean for 2050 (10-15 kg ha⁻¹ yr⁻¹, Phoenix et al., 2006). Nitrogen additions were applied in four doses per year (liquid solution of NH₄NO₃). After one year, *T. junceum* plants had a significantly lower root biomass with added N; shoot biomass and establishment probability was slightly lower, though not significantly due to low replicate numbers. *S. pumilus* showed no changes to N addition alone, but it did so in interaction with added bio-degradable plastic where N and plastic together were found to increase root growth. This study indicates that elevated N could hinder growth of the dune-building function of the endemic species *T. junceum*.

Supporting evidence for the effects of N deposition is also available in the form of a targeted gradient study in the United Kingdom (Jones et al., 2002, 2004). Eleven sand dune sites were surveyed with a range of atmospheric N inputs from 10 to 30 kg N ha⁻¹ yr⁻¹. The relationship between site parameters and N deposition was examined using linear regression. Each parameter was also checked for significant soil pH effects. Where significant relationships with pH occurred, pH was included as the first term of the regression to separate these effects from those of atmospheric N, with the assumption that these represented existing underlying gradients in sand parent material rather than effects of acidification due to atmospheric deposition in generally well-buffered systems. Above-ground biomass ($p < 0.05$) and sward height ($p < 0.10$) related positively to N inputs (Figure 4.1). Consequently, there was also a positive correlation between N deposition and the pool of N in the vegetation. The increase in biomass was largely caused by the increased height and cover of the typical grass species *Ammophila arenaria*. In general, the observed effects start to become apparent above 10 kg N ha⁻¹ yr⁻¹, if there are no other modifying factors. The authors suggested that, in the long term, this increase in biomass may also lead to enhanced organic matter accumulation and thus accelerated soil development and increased succession rates. This agrees with the evidence provided by Jones et al. (2008), where accelerated dune soil development was positively correlated with both N deposition and temperature. The longer-term consequences of increased grass height and vigour in shifting dunes are likely to be a decrease in the area of bare sand which are important for many rare invertebrate and other species (Howe et al., 2010), and reduced natural dynamics which is important for a healthy dune system (Jones et al., 2021). As with all gradient studies, this survey indicates an association and not causality, but on the basis of these results it appears likely that the sites with the higher N inputs have been impacted.

Figure 4.1. Above ground biomass (g m^{-2}) in relation to N deposition in a UK survey of mobile and semi-fixed dunes (Jones *et al.*, 2004). Filled diamonds represent calcareous sites, open squares represent acidic sites. Bars show ± 1 SE.



Source: Jones *et al.*, 2004

Summary for shifting coastal dunes (N13, N14)

The 2011 $CL_{\text{emp}}N$ range was $10\text{--}20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (expert judgement). Taken together, the British gradient study and the new experimental evidence from the Mediterranean region support a negative impact on shifting coastal dunes from $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ upwards. However, the mechanisms of N impact may differ in temperate Europe compared with the Mediterranean. In the drier Mediterranean there may be interactions with drought stress since N reduces root biomass of dune building species common in embryonic dunes, while in temperate dunes N generally led to increased total cover or biomass of the dominant grasses. Despite differences in potential mechanisms acting in the Mediterranean compared with temperate Europe, the evidence for embryonic dunes suggests retaining the $CL_{\text{emp}}N$ range for shifting coastal dunes of $10\text{--}20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ but increasing the evidence status to quite reliable. Further experimental work is required to improve the evidence base for this habitat, and also to better understand the mechanisms of impact across different European climatic regions.

4.2.2 Coastal dune grassland (grey dune) (N15)

A large number of stable dune grasslands are located along the coasts of Europe, from the boreal to the Mediterranean and warm-temperate zones. They are found in fixed dunes, usually with herbs and graminoids as the dominant life form, although in certain areas in the northern and western systems, bryophytes make up a substantial component of the biomass – up to 70 %, particularly where grazed (e.g. Plassmann *et al.*, 2009). In early *Corynephorus* stages in drier dunes, mosses and lichens may dominate, both in abundance and in species richness. They occur in dune habitats which are mostly out of reach of the water table (typically Mean Spring water Level values deeper than 85 cm) and occur on calcareous to acidic sandy soils, thus from high to low base status (e.g. Davies *et al.*, 2004). In general, these stable dune grasslands have a high species diversity and many characteristic plant and animal species.

Synthesis of observed effects in dune grasslands

In many Dutch dry dune grasslands, tall grasses have increased since the 1970s, a period with relatively high N loading (20-30 kg N ha⁻¹ yr⁻¹) and sulphur (S) deposition. The dominant tall grass species are mainly *Calamagrostis epigejos*, *Elymus repens* and *Elymus athericus* (Kooijman and De Haan, 1995; Remke, 2010). In more acidic or decalcified (i.e. partially acidified) dunes, *Ammophila arenaria* and *Carex arenaria* are usually the dominant species. Because of reduced light penetration through the tall grass canopies formed by these species, the development of several prostrate species has been reduced and management is now necessary to maintain the diversity of these systems. In the past, tall graminoids were usually not dominant on these low nutrient sandy dune soils in the Netherlands. A survey in the 1990s of dry dune grasslands along the Dutch coast revealed that non-calcareous, iron-poor dry dune ecosystems were N limited, but that in calcareous, iron-rich dunes there was co-limitation of N and phosphorus (P) (Kooijman et al., 1998; Kooijman and Besse, 2002). Kooijman et al. (1998) concluded that atmospheric N deposition may cause tall grass dominance encroachment in non-calcareous dunes, but probably only accelerates the process in calcareous dune grasslands. Yet, a strong, negative correlation between the percentage of open dunes and total N deposition, especially above 15 kg N ha⁻¹ yr⁻¹, has been seen in both Dutch dune regions (Van Hinsberg and Van der Hoek, 2003). The hypothesis that the present dominance of tall grasses and increased rate of succession in the Netherlands might be a result of increased atmospheric N deposition is also supported by the fact that in many coastal areas of Britain, receiving relatively lower N deposition loads (approximately 10 kg N ha⁻¹ yr⁻¹), stable dune grasslands are still rich in species (Jones et al., 2002; Field et al., 2014; Jones et al., 2018). N deposition is recognised as a major, although not the only, factor altering dune vegetation since approximately the 1960s. Other factors include reduction in traditional grazing, climate change and reductions in rabbit populations (Provoost et al., 2011). All of these factors, in combination with increased N deposition, tend to drive vegetation change in the same direction.

The principal new sources of evidence to add to the previous assessment come from Rowe et al. (2011), Hall et al. (2011), Jones et al. (2013), Field et al. (2014), Ford et al. (2016), Pakeman et al. (2016), Aherne et al. (2021) and Payne et al. (2020).

Evidence from short-term, high dose, N-manipulation experiments

The effects of nutrients in dry dune grasslands on sandy soils (calcium carbonate 1%) were experimentally studied at Braunton Burrows (Devon, UK) by Willis (1963). Nutrients were applied during a two-year period and complete NPK fertilisation strongly stimulated the growth of grasses, such as *Festuca rubra*, *Poa pratensis* and *Agrostis stolonifera*, which significantly reduced the abundance of many small plants, such as prostrate phanerogamic species, mosses and lichens. The impacts of different combinations of N, P and potassium (K) were also investigated and N (> 100 kg N ha⁻¹ yr⁻¹) proved to be more limiting for plant growth than P. Although the changes in vegetation were clearly less profound than after complete fertilisation, reduction in species numbers (especially annual species, lichens and mosses) was observed under N addition (Willis, 1963). Boorman and Fuller (1982) examined the effects of nutrient additions on species composition of rabbit grazed dune grassland in Norfolk (UK) over a five-year period. They added 80 kg N ha⁻¹ yr⁻¹ as (NH₄)₂SO₄ and NaNO₃ in five replicates in April, June and September of each year. The grazing prevented *F. rubra* from becoming dominant, but several species (especially annuals, mosses and lichens) declined, while two species (*Carex arenaria* and *Calystegia soldanella*) increased under all treatments containing N (80 kg N ha⁻¹ yr⁻¹). In this study, no evidence was found for reduced diversity in plots that had received P and K additions. In a one-year experiment with additions of N (20, 40, 80 and 160 kg N ha⁻¹ yr⁻¹; atmospheric load 15 kg N ha⁻¹ yr⁻¹) or P, the above-ground biomass of a stable dune grassland at

the Dutch Wadden island of Schiermonnikoog proved to be strongly N limited; plant biomass significantly increased above additions of 40 kg N ha⁻¹ yr⁻¹ in this 30-year-old stable dune grassland (Olf et al., 1993). In summary, these short-term experiments clearly indicate the importance of N limitation in several stable dune grasslands, but the high N doses applied prevent a reliable determination of CL_{emp}N. Moreover, in some cases, co-limitation with P has been observed, or rabbit grazing may have prevented the dominance of tall grasses, suggesting the importance of these two drivers as modulators of the response of these ecosystems to increased N deposition.

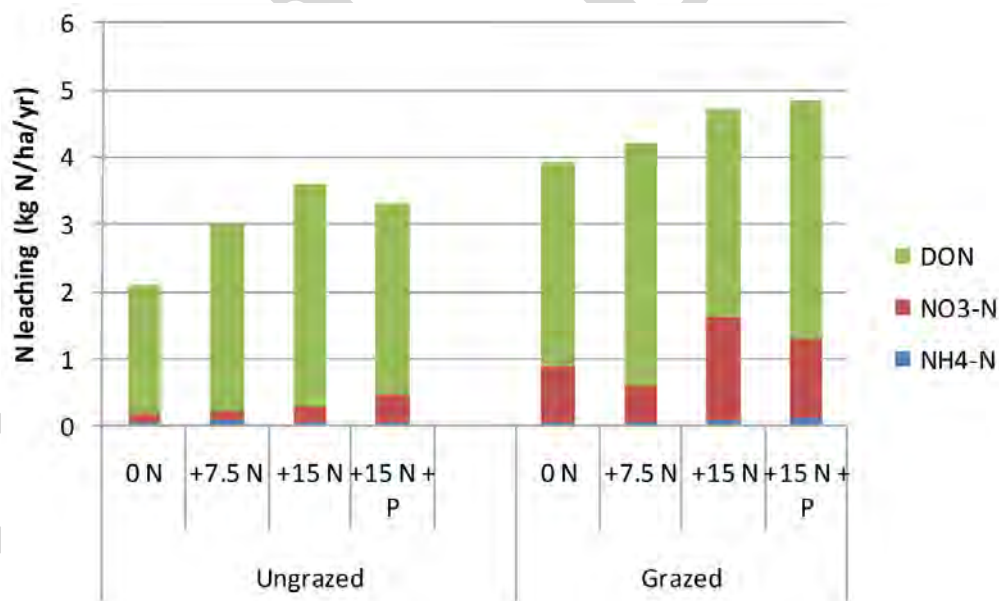
Evidence from longer-term, low dose, N-manipulation experiments

Two low-dose N addition experiments have been carried out in stable dune grasslands, one in the Netherlands, and one in the UK, with the specific objective to examine the effects of N deposition. In the Dutch N addition experiment, the effects of N additions and the interaction with rabbit grazing were investigated in a factorial design in two coastal stable dune grasslands, one calcareous and one partly decalcified, by Ten Harkel and Van der Meulen (1995) and Ten Harkel et al. (1998). After four years of N additions twice a year (25 kg N ha⁻¹ yr⁻¹ in the form of ammonium nitrate pellets; background deposition approximately 23 kg N ha⁻¹ yr⁻¹) no significant changes were found in species composition, neither in the grazed nor the ungrazed situation. Exclusion of grazing by rabbits and horses, through the use of enclosures, resulted in graminoid dominance (*Festuca rubra*, *Festuca ovina* and *Poa pratensis*), especially where N additions were made, which suggested that grazing may prevent grass dominance in stable dune grasslands (Ten Harkel and Van der Meulen, 1995). Because of the high, direct leaching losses resulting from the added fertiliser pellets used in the first phase of the experiment, for the last year and a half of the experiment the treatment was changed to a fortnightly solute addition by watering of 50 kg N ha⁻¹ yr⁻¹ as ammonium sulphate (Ten Harkel et al., 1998). In the last year and a half, even in the no added-nitrogen controls in the ungrazed enclosures, the data suggest that there was leaching of 36% of N inputs from background deposition in the foredune plots and 13% of N inputs in the older decalcified plots, suggesting a degree of N saturation already at background deposition of 23 kg N ha⁻¹ yr⁻¹ (Ten Harkel et al., 1998). From this experiment in stable dune grasslands, experimental N enrichment had no effect on species composition after four years of increased N addition. This may have been related to a shift towards P limitation after the long period of high atmospheric N inputs in the Netherlands, during which time some botanical changes may already have occurred, and the relatively high rabbit grazing pressure in that period which may enhance nutrient co-limitation. However, the high N leaching from the control unfertilised vegetation is also a strong indication of N saturation of these dune grasslands, probably because of the high N deposition rates (> 20 kg N ha⁻¹ yr⁻¹) over several decades, but also because the young dune soils have a thin top layer with relatively low organic matter content, lack clay particles, and therefore have fewer exchange sites with which to bind ammonium ions.

In the N addition experiment in the UK, the effects of low levels of N additions (and P) were studied on the Isle of Anglesey under relatively low background deposition (10-12 kg N ha⁻¹ yr⁻¹) in a calcareous, heavily grazed fixed dune grassland (Plassmann et al., 2009). The experiment also looked at the impacts of grazing management in combination with N. Four N treatments (unwatered control, watered control, 7.5 kg N ha⁻¹ yr⁻¹, 15 kg N ha⁻¹ yr⁻¹, on top of the background N deposition) were nested within three grazing treatments. In a separate treatment, effects of fertilisation with both N (15 kg ha⁻¹ yr⁻¹) and P (15 kg ha⁻¹ yr⁻¹) on top of background N were investigated. After two years, N addition resulted in significantly greater amounts of total above-ground biomass and bryophyte biomass, under both low and high N treatment, compared to the control situation. In bryophytes, the tissue N concentration was significantly greater

under high N treatment ($15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), whereas the total N pool in the bryophytes was significantly greater under both N addition treatments. No effects on vegetation composition, sward height or soil parameters occurred within the two-year research period. Furthermore, combined addition of N and P together had a greater impact on above-ground biomass, sward heights and sward structure than N addition alone. Demonstrating similarities with the Dutch experiment, further work at the site after six years of nutrient additions, on N and P mineralisation suggests the site is N and P co-limited, and that there were no longer differences in moss biomass due to elevated N, unless P was also added (Ford et al., 2016). Leaching calculations at this site show that the majority of deposited N is retained within the soil-plant system (Hall et al., 2011). A maximum of 6 % of inputs was leached at the highest N load in the fully grazed treatments (combined experimental and background load of $27 \text{ kg ha}^{-1} \text{ yr}^{-1}$) in the form of inorganic N (or up to 18%) if dissolved organic N compounds were included in the calculations (Figure 4.2). Dynamic modelling of various scenarios of increased N deposition from a nearby poultry unit suggests that the accumulated soil N will eventually trigger changes in botanical composition over longer time scales at additions above current background levels of $\sim 11 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Rowe et al., 2011). These experimental and modelling results suggest that N and P co-limitation may prevent species composition changes in the short-term in dune grasslands, but that N still accumulates in the soil and plant system, and is likely to eventually cause species change at additions above $\sim 11 \text{ kg N ha}^{-1} \text{ yr}^{-1}$.

Figure 4.2. Leaching fluxes of inorganic and organic N with N additions ($\text{kg N ha}^{-1} \text{ yr}^{-1}$) above a background of $10\text{--}12 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ under two grazing regimes on a partially decalcified calcareous fixed dune grassland at Newborough Warren, North Wales, UK. (DON = dissolved organic nitrogen) (Hall et al., 2011).



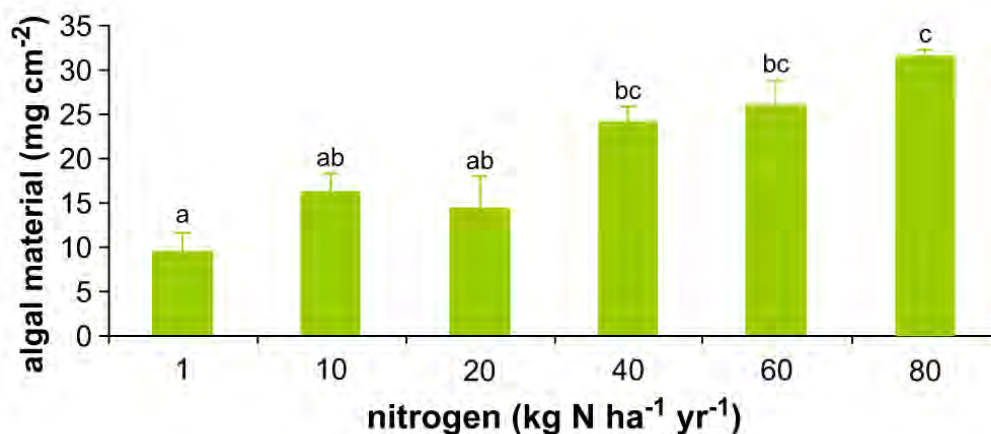
Source: Hall et al., 2011

Evidence from mesocosm studies

The effects of N loads have also been studied in a series of mesocosm studies in the Netherlands and the UK. The effects of elevated N loads in a situation of low background deposition ($< 5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) have been studied during two to three years, in recreated dry dune calcareous grassland mesocosms in a greenhouse ($1 \times 1 \text{ m}$ in size) (Tomassen et al., 1999; Van den Berg et

al., 2005). After a pre-treatment period of two months with clean rainwater that removed the excess of nitrate from the soil, N was added twice a week in the form of ammonium nitrate (1, 5, 10, 15, 20, 40, 60 and 80 kg N ha⁻¹ yr⁻¹). The effects on soil-pore water chemistry and on two characteristic graminoid species (*Calamagrostis epigejos* and *Carex arenaria*) and two endangered herb species were monitored. Within one year of N additions, a clear difference was found in the amount of green algae (Chlorophyta) growing on the surface of the sand. The amounts of green algae increased under the treatments of between 10 and 20 kg N ha⁻¹ yr⁻¹, but the difference with the two lowest treatments was especially distinct above 20 kg N ha⁻¹ yr⁻¹ (Figure 4.3). The strong increase in algae on the soil layer due to N deposition may have important implications as the algae prevent sand drifts that are caused by wind action. Such 'blowouts' are important for renewed vegetation succession, and biodiversity will decrease when young successional stages decline. Concentrations of nitrate in soil-pore water showed a strong seasonal fluctuation. During the first winter period an increase in nitrate was measured for the treatments ≥ 40 kg N ha⁻¹ yr⁻¹. During spring, nitrate concentrations rapidly decreased. During the second and third winter, an increase in nitrate could only be observed at the highest N addition level. Ammonium concentrations remained consistently at a very low level (< 5 μmol l⁻¹) most likely due to fast nitrification.

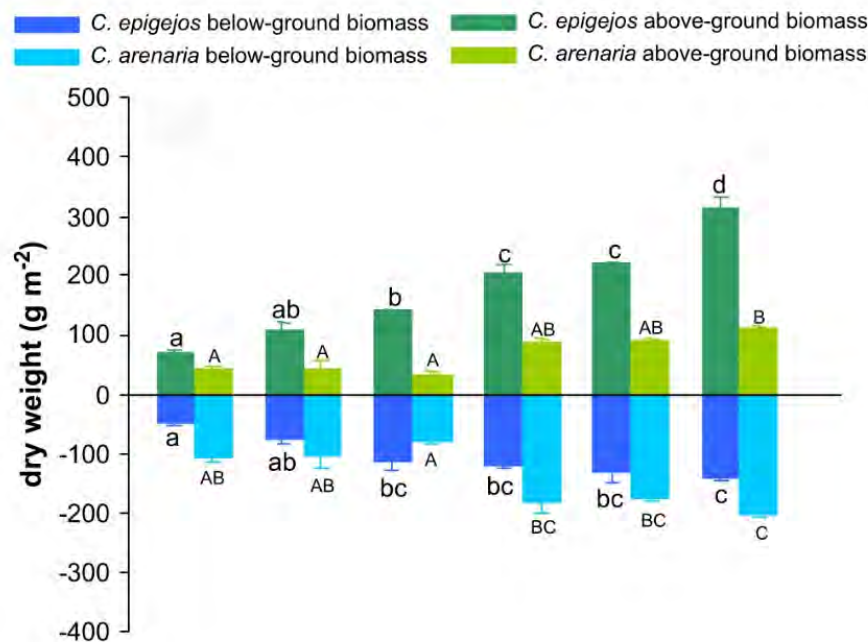
Figure 4.3. Algal material, measured as active chlorophyll concentration (mg cm⁻²; means ± standard error; n = 4), in the top layer of the soil at different N addition rates (Van den Berg et al., 2005).



Source: Van den Berg et al., 2005

Differences in plant growth were first observed after one year, and they became more obvious after two to three years. The total cover of the vegetation increased with elevated N inputs. This increase could almost completely be attributed to the growth of *Calamagrostis epigejos*. Biomass of this tall grass species increased significantly, above 20 kg N ha⁻¹ yr⁻¹ for shoots and above 15 kg N ha⁻¹ yr⁻¹ for roots (Figure 4.4). No clear effects of elevated N deposition rates on the two herbaceous species *Galium verum* and *Carlina vulgaris* were measured over the initial two years, however, in the third year, the number of individuals and dry weight of *G. verum* decreased significantly above 20 kg N ha⁻¹ yr⁻¹ (Van den Berg et al., 2005). After two years of treatment, the total amount of N stored in the vegetation was strongly elevated due to increased N deposition (Tomassen et al., 1999).

Figure 4.4. Above- and below-ground biomass (g m^{-2}) of *Calamagrostis epigejos* and *Carex arenaria* after two years of N application in coastal dune grassland (N15) mesocosms (Tomassen et al., 1999; Van den Berg et al., 2005). From left to right: 1, 10, 20, 40, 60 and 80 $\text{kg N ha}^{-1} \text{yr}^{-1}$.



Source: Tomassen et al., 1999; Van den Berg et al., 2005

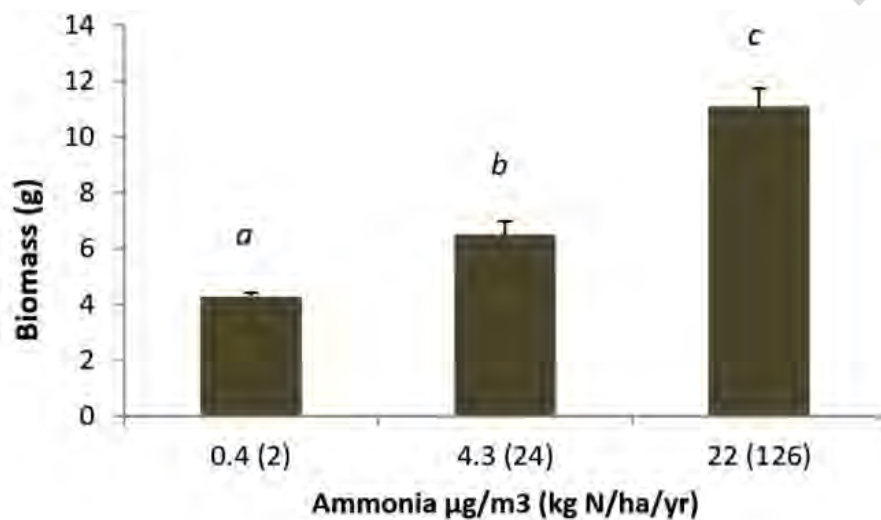
A series of mesocosm experiments were conducted in the UK (Mohd-Said, 1999; Jones et al., 2013), using a mix of seven dry dune grassland species including the following grasses and forbs: *Dactylis glomerata*, *Festuca rubra*, *Plantago lanceolata*, *Centaurea nigra*, *Achillea millefolium*, *Leontodon hispidus* and *Galium verum*. The species were planted in 4 litre, 20 cm diameter pots filled with sand, containing two individuals of each species in random positions, and exposed to N in three experiments: wet deposition, ammonia fumigation, and an *in-situ* exposure along a field-gradient of ammonia concentration. The simulated wet N deposition rates were an additional 2, 10, 20 and 55 $\text{kg N ha}^{-1} \text{yr}^{-1}$ on top of an estimated background of $\sim 4 \text{ kg N ha}^{-1} \text{yr}^{-1}$. At additions of 10 $\text{kg N ha}^{-1} \text{yr}^{-1}$ and over, the cover of the grass *Festuca rubra* increased, while there was no significant change in the cover of other grass or herb species (Figure 4.5). A separate experiment used the same species, exposed to different ammonia concentrations in open-top chambers, with treatments ranging from $0.4 \mu\text{g m}^{-3} \text{NH}_3$ (equivalent to 2 $\text{kg N ha}^{-1} \text{yr}^{-1}$) up to $35 \mu\text{g m}^{-3}$ (200 $\text{kg N ha}^{-1} \text{yr}^{-1}$). The background deposition in this experiment was not measured, but it was estimated around 10-15 $\text{kg N ha}^{-1} \text{yr}^{-1}$. The total above-ground biomass (Figure 4.5a) showed a significant increase above an ammonia concentration of $0.4 \mu\text{g m}^{-3}$ (equivalent to N addition of 2 $\text{kg N ha}^{-1} \text{yr}^{-1}$ on top of background of at least 10 $\text{kg N ha}^{-1} \text{yr}^{-1}$), and again above ammonia concentration of $4.3 \mu\text{g m}^{-3}$ (equivalent to 24 $\text{kg N ha}^{-1} \text{yr}^{-1}$ on top of background).

When biomass was analysed by species (Figure 4.5b), both *P. lanceolata* and *F. rubra* showed significant biomass increases above an ammonia concentration of $0.4 \mu\text{g m}^{-3}$, and *D. glomerata* showed significant biomass increase above an ammonia concentration of $4.3 \mu\text{g m}^{-3}$. The other species showed no significant effects of ammonia fumigation. However, all species showed significant increases in tissue N concentration with each successive treatment, i.e. showing luxury N accumulation, except *D. glomerata* which showed significant increases in tissue N at all but the lowest ammonia treatment. The third experiment involved *in situ* exposure to elevated

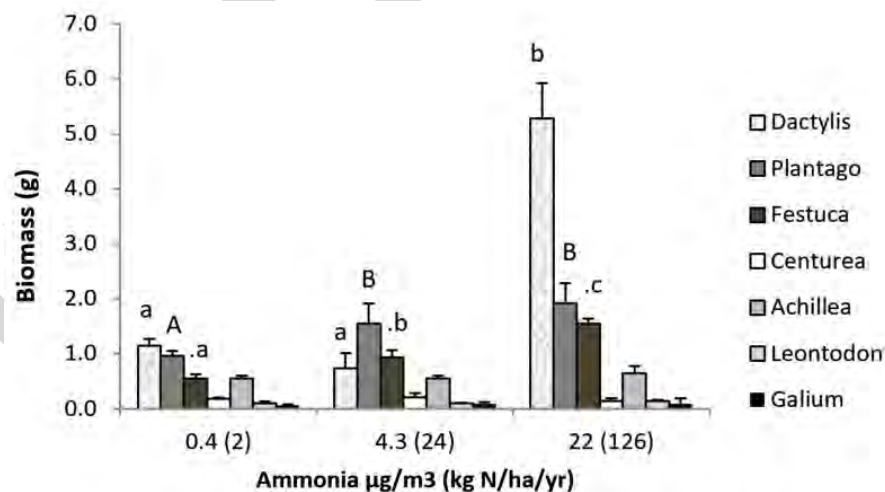
ammonia concentrations in a field gradient study away from a poultry unit point source. Here, total biomass (Figure 4.6a), and for individual species *D. glomerata* and *P. lanceolata* (Figure 4.6b), increased between exposures of 8.3 and 11.1 kg N ha⁻¹ yr⁻¹. Tissue N increased in all seven species between N exposure of 11.1 and 40 kg N ha⁻¹ yr⁻¹.

Figure 4.5. Above-ground biomass showing a) total biomass of all species and b) individual dry dune grassland species after exposure to ammonia fumigation (Jones et al., 2013). Data from highest ammonia treatment (35 µg m⁻³) not shown. Differing letters denote significant differences between treatments for each species ($p < 0.05$). Bars represent ± 1 s.e.

a)



b)

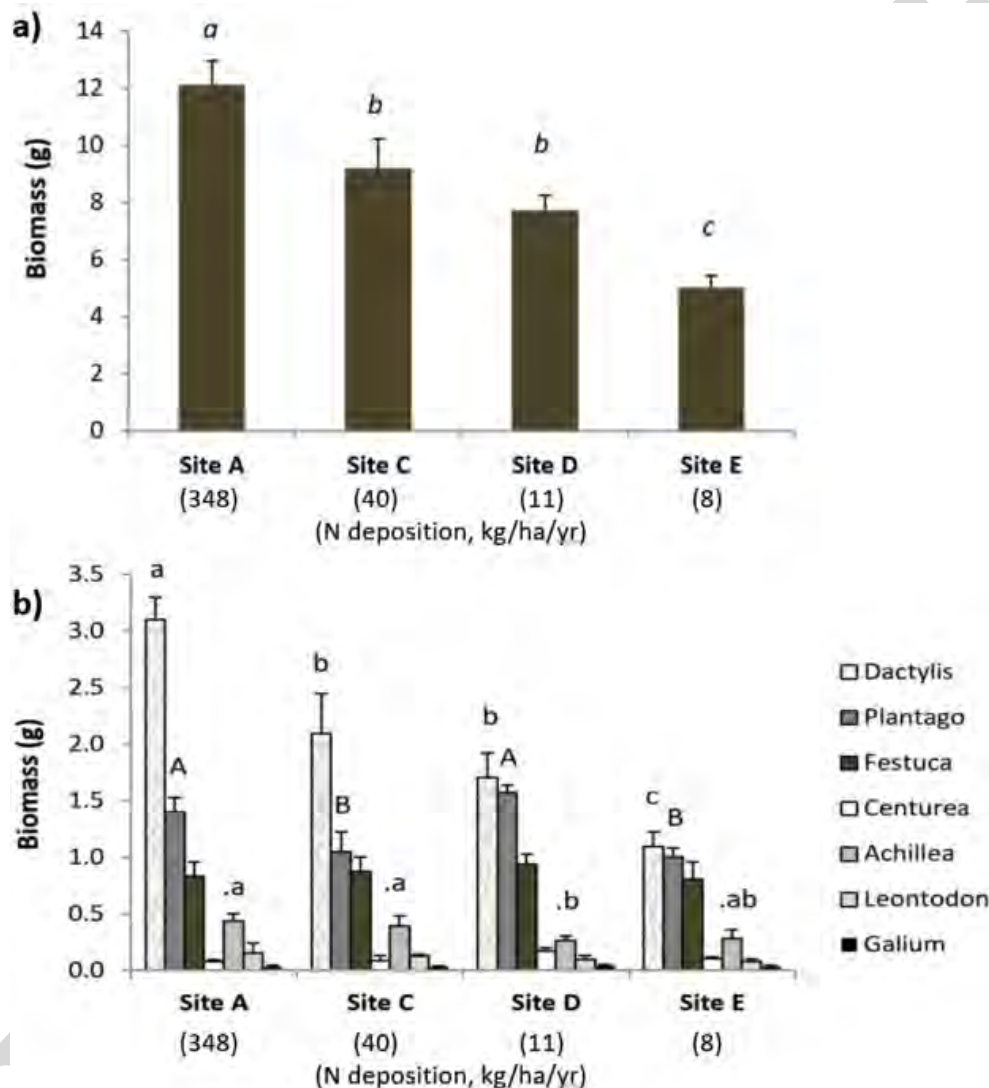


Source: Jones et al., 2013

As a general conclusion, all the mesocosm experiments showed a significant increase in species cover that was often attributed to the response of a few dominant graminoid species, with the three separate UK mesocosm experiments consistently showing effects on the mainly calcareous species between total N loads of around 8 to 14 kg N ha⁻¹ yr⁻¹. In all experiments, higher loads led either to dominance of graminoid species within a few years (Dutch experiment), or

considerably greater biomass, primarily of graminoid species, and increased tissue N concentrations in most species with increasing N (UK experiments).

Figure 4.6. Above-ground biomass of a) all species combined, and b) seven different dry dune grassland species after exposure to a field-gradient of ammonia concentrations away from a point source (Jones et al., 2013). Differing letters denote significant differences between treatments for each species ($p < 0.05$). Bars represent ± 1 s.e.



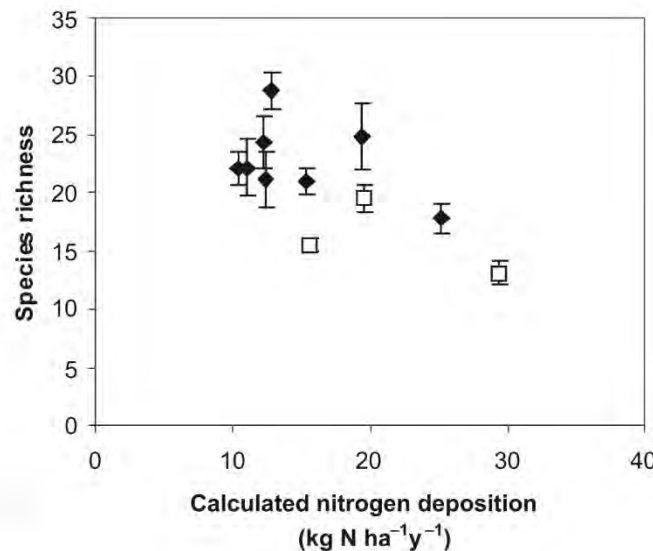
Source: Jones et al., 2013

Evidence from gradient studies

Additional field evidence on the effects of N deposition of dune grasslands is currently available from a series of gradient studies. A targeted survey in the coastal dune areas across England and Wales, in regions with much lower N deposition than in the Netherlands (Jones et al., 2004), surveyed eleven coastal dune sites with atmospheric N deposition ranging from 10 to 30 kg N ha⁻¹ yr⁻¹. In these stable dune grasslands, above-ground biomass was related positively to N deposition, while species richness showed a weak negative relationship (Figure 4.7). In addition, in these grasslands, a strong positive relationship was found between dissolved organic N in groundwater and N deposition, which may be an indicator of high inorganic N inputs, or perhaps increased mineralisation in response to N deposition. Furthermore, the cover of *Carex arenaria*

also related positively to N inputs. In general, the observed effects in this study started to become apparent in the range 10 to 20 kg N ha⁻¹ yr⁻¹.

Figure 4.7. Species richness (2 x 2 m) of vascular plants, bryophytes and lichens in relation to N deposition in a UK survey of stable dune grasslands (Jones et al., 2004). Filled diamonds represent calcareous sites, open squares represent acidic sites. Bars show ± 1 s.e.



Source: Jones et al., 2004

The impacts of atmospheric N deposition on dry lichen-rich dune grasslands (N151 – acidic to slightly calcareous) around the Baltic Sea was also studied in a targeted survey by Remke et al. (2009a, 2009b) and Remke (2010). Coastal dunes around the Baltic Sea are rather pristine ecosystems, to date receiving small amounts of atmospheric N. Across the 19 investigated dune sites, atmospheric wet N deposition ranged between 3 to 8 kg N ha⁻¹ yr⁻¹ (wet N deposition of nearby EMEP certified weather stations). The dry deposition at these cleaner sites was subsequently estimated to be at least 3 kg N ha⁻¹ yr⁻¹. The N content of the dominant lichen, *Cladonia portentosa*, was demonstrated to be a suitable bio-indicator of N deposition at these low to medium N deposition levels, with tissue N concentrations increasing above 5 kg N ha⁻¹ yr⁻¹ wet N deposition. Comparison with EMEP deposition data showed that tissue N concentrations in *Cladonia portentosa* also reflected the deposition history of the last three to six years. Moreover, a shift from lichen-rich short grass vegetation towards species-poor vegetation dominated by *Carex arenaria* also correlated with increasing wet N deposition loads. Plant species richness, however, was not shown to correlate with these low to medium N deposition loads (Remke et al., 2009a). Accelerated soil acidification, as well as increased growth of *Carex* and accumulation of organic matter, was observed only in acidic grasslands with pH_{NaCl} of the parent material between 5.0 and 6.0. At sites with more calcareous parent material (pH_{NaCl} 6-7), these relationships with N deposition were not apparent. A trigger for grass encroachment seems to be high acidification in early successional stages to below pH_{NaCl} 4.0. Metals such as aluminium (Al) were more freely available and may inhibit more sensitive species. From the acidic sites, N mineralisation was higher at those sites with higher N deposition, which may further stimulate *Carex*. *Carex*-dominated dune grasslands are species-poor (Figure 4.8). The number of foliose lichen species, forbs and grasses was lower in regions with wet deposition

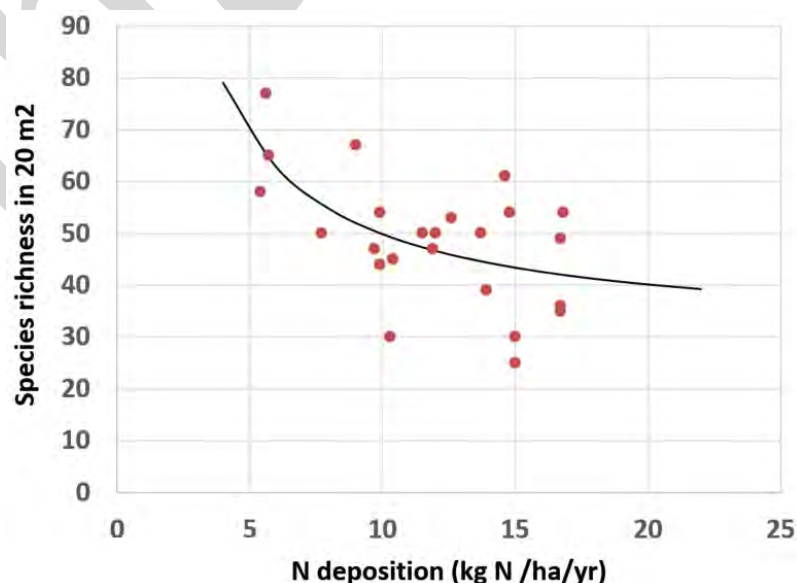
above 5 kg N ha⁻¹ yr⁻¹, compared with ‘clean’ areas (2-5 kg N ha⁻¹ yr⁻¹) at the investigated acidic sites (Remke et al., 2009b). Including the estimated 3 kg N ha⁻¹ yr⁻¹ in dry deposition, it is likely that 5 kg N ha⁻¹ yr⁻¹ wet deposition will correspond with approximately 8 kg N ha⁻¹ yr⁻¹ total deposition.

Figure 4.8. Picture of a *Carex arenaria*-dominated stable dune grassland in the Baltic (Korshage DK; photo E. Remke).



A further targeted gradient survey in the UK was carried out in 2009 in acidic/decalcified stable dune grasslands (Field et al., 2014; data also re-analysed in Jones et al., 2018). The gradient covered a range from 5.4 to 16.8 kg N ha⁻¹ yr⁻¹ total N deposition. This showed a decrease in vegetation species richness almost from the lowest N deposition points along the gradient (> 5 kg N ha⁻¹ yr⁻¹), with the most rapid change in species richness of the fitted curve occurring at the cleanest sites (Figure 4.9). The number of bryophyte species was also lower above 5-10 kg N ha⁻¹ yr⁻¹.

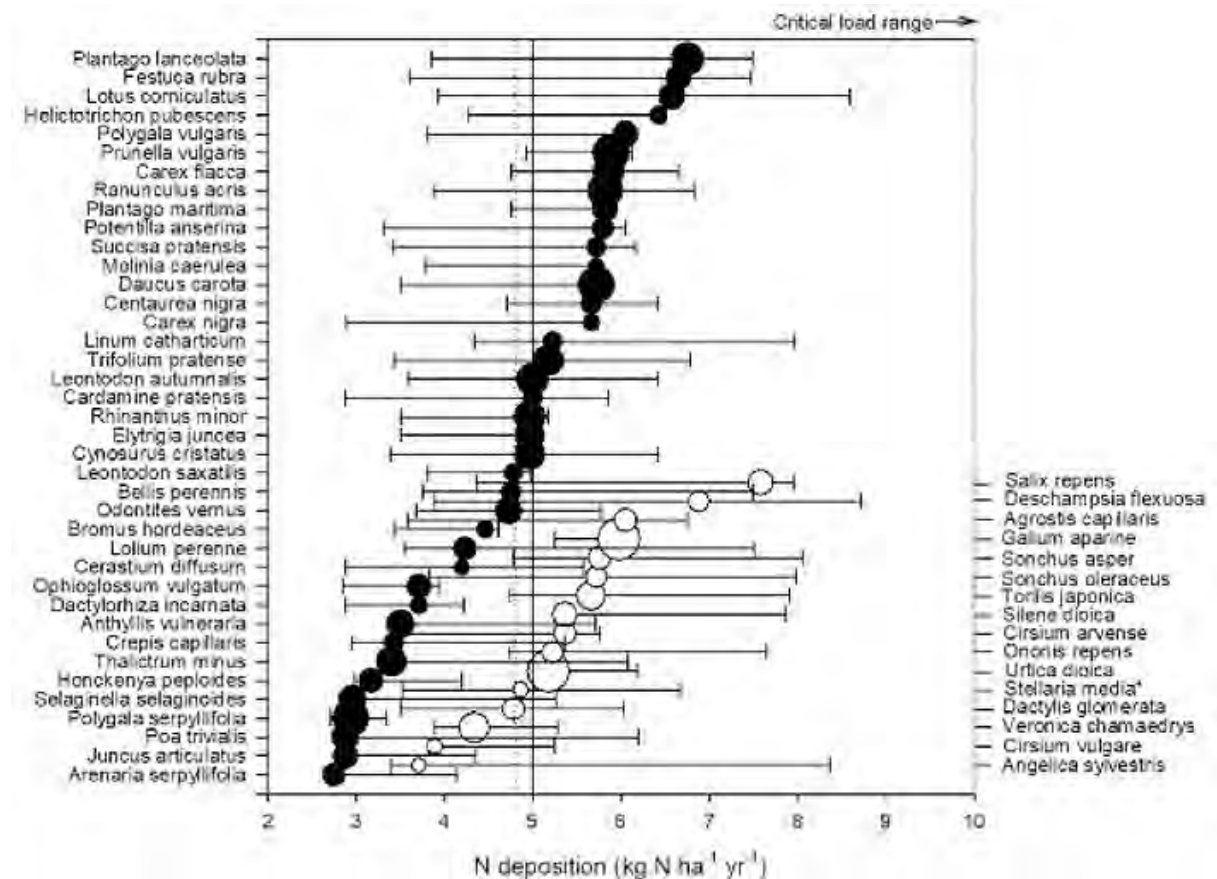
Figure 4.9 Species richness in acidic or decalcified dry dune grassland communities of UK. Line shows fitted relationship, $y = 98.351 - 20.4662 (\ln(x))$; $R^2 = 33\%$, $p = 0.003$. Data from Field et al. (2014) and Jones et al. (2018).



Source: Field et al., 2014; Jones et al., 2018

A series of datasets including gradient surveys designed to detect impacts of N deposition, and untargeted surveys were collated by Payne et al. (2020) for analysis of N impacts. They collected 36 vegetation datasets across multiple habitats, including dunes, to conduct a TITAN (Threshold Indicator Taxa Analysis). In the final analysis, only five datasets were used as most did not pass initial screening stages, including the criterion that studies were only included when N deposition (current annual or 30-year cumulative) explained significant variation in the redundancy analysis with co-variables partialled out. Therefore, this represents a highly robust analysis of N impacts from large datasets with co-variables explicitly accounted for. The analysis used both annual deposition and 30-year cumulative deposition. Annual N deposition was calculated with the Concentration Based Estimated Deposition (CBED) model for the UK, cumulative N deposition for the past 30 years via the Fine Resolution Atmospheric Multi-Pollutant Exchange (FRAME) model. Two dune habitats, fixed dune grasslands and dune slacks (see Chapter 4.2.2 and 4.2.5), were analysed in this study. The analysis of fixed dune grasslands used data from Scottish coastal dunes (Pakeman et al., 2016), covering mostly the very clean end of the N deposition gradient, 2.7-11.8 kg N ha⁻¹ yr⁻¹ total N deposition, and included mostly calcareous dune habitats, but some acidic ones. In total, 39 species showed significant negative change points (i.e. a defined point above which there was a significant decrease in the abundance of that species) and 16 species showed a positive change point, analysed against annual total N deposition. The overall community sum(-z) change point was determined as 5 kg N ha⁻¹ yr⁻¹ (Figure 4.10).

Figure 4.10. Negative (black circles) and positive (white circles) change points for dry dune grassland species. Vertical lines show overall community sum(z-) [solid line] and sum(z+) [dotted line] change-points. Circles represent species showing high purity and reliability negative (black circles) and positive (white circles) change points in response to nitrogen deposition and bootstrap 5% and 95% quantiles. From Payne et al. (2020).



Source: Payne et al., 2020

An entirely different dataset, this time from Ireland, also used TITAN analysis of dune habitats (Aherne et al., 2021). Their analysis was conducted across a mixture of predominantly calcareous dry dune and dune slack habitats, although the species showing significant change points were more commonly found in dry dune habitats (*Trifolium repens*, *Plantago lanceolata*, *Cerastium fontanum*, *Rhytidadelphus squarrosus*, *Linum catharticum*, *Bellis perennis*, *Achillea millefolium*). Their estimated community change point was 6.2 kg N ha⁻¹ yr⁻¹.

Lastly, additional evidence comes from a re-survey of Scottish coastal vegetation over a 35-year interval (1975-2010) (Pakeman et al., 2016). The study looked at mobile dune grasslands, fixed dune grasslands, and dune slacks, as well as other coastal vegetation types. This showed increased fertility of fixed dune grasslands, from mean Ellenberg N score of 4.003 to 4.195 over time, consistent with an increase in N deposition over that period (CBED model for N deposition). In fixed dune habitats, the greatest increases in the Ellenberg N index occurred at the more polluted end of the gradient at 4.1-5.9 kg N ha⁻¹ yr⁻¹ total deposition. At this higher end, the species richness declined with an average of 3.12 species per quadrat, although former S deposition cannot be excluded as a confounding factor for the change in species richness. Based on changes in overall vegetation composition and Ellenberg N indices, Pakeman et al. (2016) suggested a CL_{emp}N of 4-6 kg N ha⁻¹ yr⁻¹ for fixed dune grasslands.

Summary for coastal dune grasslands (grey dunes) (N15)

The 2011 CL_{emp}N range for coastal dune grassland (grey dune) (N15) was 8 to 15 kg N ha⁻¹ yr⁻¹ (quite reliable) (Bobbink and Hettelingh 2011). For this habitat, there is now strong evidence from a wide range of studies including *in situ* N additions, experimental mesocosms under a range of exposure methods, gradient studies and analysis of untargeted surveys. There is new evidence, from multiple sources, of ecological changes in both calcareous and acidic dunes at the lower end of this range. Based on this evidence we propose a reduction of the lower limit of the CL_{emp}N range for coastal dune grasslands to 5 kg N ha⁻¹ yr⁻¹. However, phosphorus limitation may lead to fewer botanical responses in calcareous dunes compared with acidic or decalcified dune sites. The higher end of the range is kept at 15 kg N ha⁻¹ yr⁻¹ as there are calcareous rich sites with P limitation where there are few botanical changes, but other receptors are still affected, such as nitrate leaching. In summary, the revised CL_{emp}N range for coastal dune grasslands is 5 to 15 kg N ha⁻¹ yr⁻¹, and the authors increase the evidence status to reliable.

4.2.3 Coastal dune heaths (N18 and N19)

Besides dry dune grasslands, heathland vegetation is also present in the coastal dunes in Europe (Gimingham et al., 1979; Ellenberg, 1988b). These natural coastal dune heaths are mostly dominated by the typical dwarf shrub *Empetrum nigrum*, while *Calluna vulgaris* is less common. Within EUNIS, there are now two categories of coastal heaths: Atlantic and Baltic coastal *Empetrum* heath (N18) and Atlantic coastal *Calluna* and *Ulex* heath (N19), which are both classified as a subcategory of coastal dune and sand habitats. There is one new study to add to the previous assessment for dune heaths, Bähring et al. (2017).

Only two N manipulation experiments have been performed in coastal heaths, one in Denmark within the Danish HEATH experiment (Riis-Nielsen, 1997; Nielsen et al., 2000), located in a coastal heath at Lodbjerg, and a new study at the island of Fehmarn, Germany (Bähring et al., 2017).

The Danish coastal heathland, dominated by *Empetrum nigrum* and *Ammophila arenaria*, is located in an area of approximately 250 to 300-year-old dunes on the coast of Jutland, with a relatively low naturally occurring N deposition (13 kg N ha⁻¹ yr⁻¹) (Nielsen et al., 2000). Ammonium nitrate (0, 15, 35 and 70 kg N ha⁻¹ yr⁻¹, dissolved in demineralised water) has been applied six times per year and was carried out over a period of two years. As a response to N additions, vascular plant species increased in cover, whereas lichens and bryophytes showed a very slight but non-significant decrease over those two years (Riis-Nielsen, 1997). *Hypnum cupressiforme*, a typical heathland moss, declined linearly with applications of N, whereas the cover of *Empetrum* and *Carex arenaria* increased linearly. Thus, plant productivity in this coastal heath system was obviously controlled by N. However, observed drought effects on *Empetrum* showed no interaction with N inputs (Tybirk et al., 2000). The leaching of both nitrate and ammonium was also quantified in this coastal Danish heath. In the control plots and those receiving 15 kg N ha⁻¹ yr⁻¹, virtually no N leached to the subsoil. However, with higher N additions, especially 70 kg N ha⁻¹ yr⁻¹, a considerable part of the N leached as nitrate from the B horizon, accompanied by aluminium, leading to soil acidification (Nielsen et al., 2000). Johansson (2000) found no effects of N additions on ericoid mycorrhizal infection of *Calluna vulgaris* in this coastal heath experiment.

At the island of Fehmarn, in the Baltic Sea, an experiment has been conducted within dry heaths on sand (EUNIS type S42; Bähring et al., 2017). Background deposition was 9 kg N ha⁻¹ yr⁻¹ based on modelled data of Schaap et al. (2015). Six levels of N fertilisation (0, 2.5, 5, 10, 20, and 50 kg N ha⁻¹ yr⁻¹) were applied for three years in 12 doses during the growing season (May to October) as dissolved NH₄NO₃ solution. The growth responses of different plant species of different life

forms (dwarf shrubs, graminoids, bryophytes, and lichens) as well as shifts in the C:N ratios of plant tissue and humus horizons were quantified. The current year's shoot increment of the dominant dwarf shrub *Calluna vulgaris* was the most sensitive indicator of N fertilisation. Shoot increment was significantly greater after additions of $\geq 5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (on top of background, i.e. total load $> 14 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) already in the first year. *Cladonia* spp. tissue C:N ratios decreased with N additions $\geq 5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in the second year of study. After three years, an increase in the cover of graminoids and a corresponding decrease of cryptogams at N fertilisation rates of $\geq 10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ were observed.

Summary for coastal dune heaths (N18 and N19)

The CL_{empN} of coastal dune heathlands established in 2011 was $10\text{-}20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (expert judgement). Since then, only one additional relevant study has been performed (Bähring et al., 2017). The responses observed after only a few years at total N loads of $\geq 14 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in this well-designed study suggest it would be appropriate to lower the upper end of the range of the CL_{empN} to 15, giving a CL_{empN} of $10 \text{ to } 15 \text{ N ha}^{-1} \text{ yr}^{-1}$, and to increase the evidence status to quite reliable. However, more long-term information from even cleaner environments and additional evidence would be desirable to inform this CL_{empN} .

4.2.4 Moist to wet dune slacks (N1H)

Atlantic and Baltic moist and wet dune slack (EUNIS N1H) of primary or secondary origin are hot spots of plant diversity in the sandy dune regions of Europe. They are characterised by typical graminoids (sedges, rushes and grasses), together with a high diversity of forb species and bryophytes, including many rare basiphilous species. Groundwater level is usually at or above soil level in winter, whereas in the growing season the groundwater level is considerably lower in these dune slacks. A defining feature of these dune slack vegetation communities is that they dry out in most years in summer, otherwise the vegetation tends towards mire or swamp communities. Typical Mean Spring Level (as average depth from soil surface to the groundwater in March, April and May) is less than 85 cm from the ground level, but varies by community (Jones et al., 2021), and can be near the ground surface for the wetter communities. Dune slacks in nature reserves are often maintained through management, such as grazing, hay production and harvest or sod cutting (e.g. Ellenberg, 1988b; Lammerts and Grootjans, 1997; Davies et al., 2004). Because of their isolation in the landscape and their successional position, they mostly receive nutrients via atmospheric inputs, but some sites are also affected by groundwater nutrients (Rhymes et al., 2014). In addition, they are very sensitive to hydrological changes from groundwater extraction.

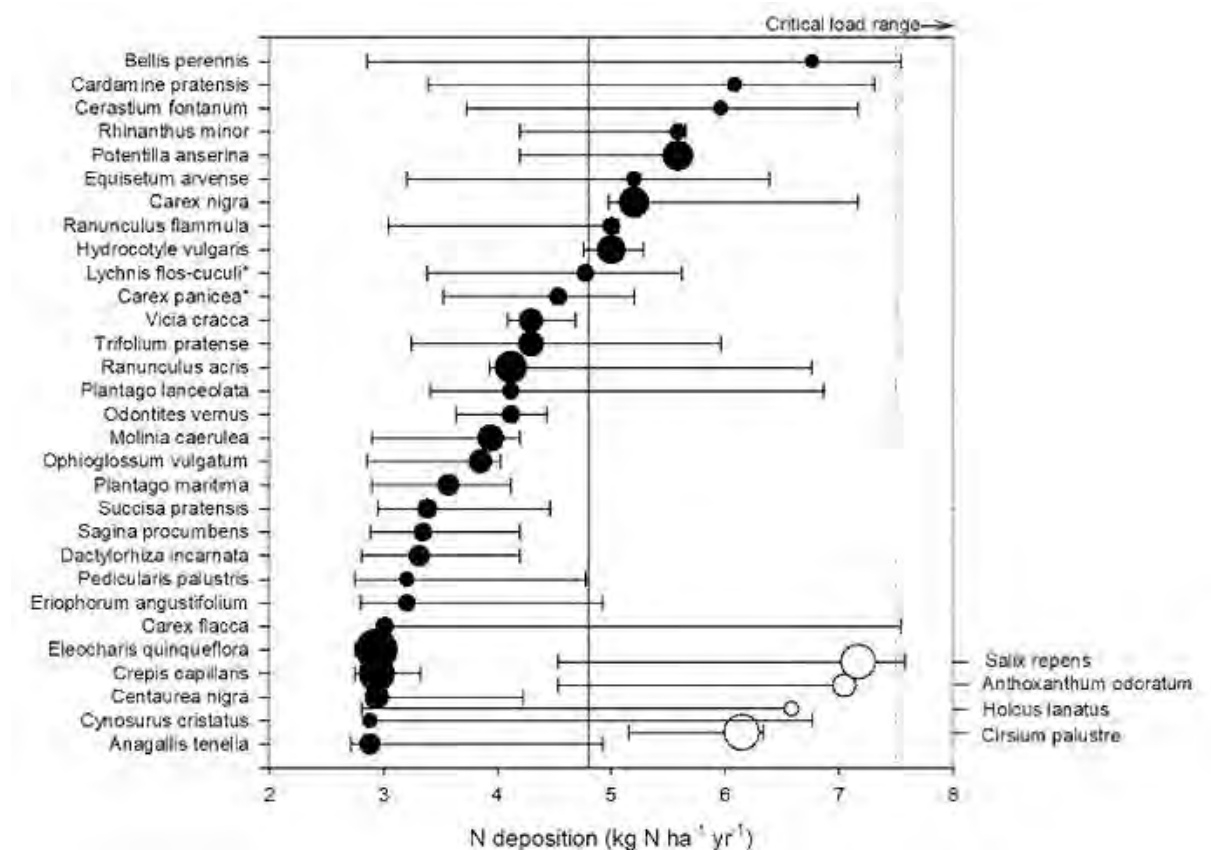
The limitation of dune slack vegetation by nutrients has been the topic of several studies (e.g. Willis, 1963; Olff et al., 1993) and reviewed by Lammerts and Grootjans (1997). Factorial fertilisation experiments have shown that in almost all studied moist to wet, primary or secondary dune slacks in the United Kingdom, the Netherlands and the United States, the above-ground biomass production is limited by N availability. Primary P limitation was found only once in a dune slack where sod cutting had been applied shortly before. Single N additions (above $100 \text{ N kg ha}^{-1} \text{ yr}^{-1}$) have led to increased dominance of *Carex* and *Juncus* species, and of tall grasses such as *Agrostis stolonifera* and *Calamagrostis epigejos*. In some studies, typical forb species had declined in such situations (for an overview and references, see Lammerts and Grootjans, 1997). Unfortunately, none of the studies have been carried out with low N additions ($< 100 \text{ N kg ha}^{-1} \text{ yr}^{-1}$; $> 1 \text{ year}$), and thus they are not adequate for setting a CL_{empN} . The following new studies add to the previous assessment of CL_{empN} for dune slacks: Rhymes et al. (2014, 2015), Pakeman et al. (2016), and Payne et al. (2020).

A targeted field survey of eleven dune systems in the United Kingdom, with a calculated total N deposition ranging from 6.9 to 29.4 kg N ha⁻¹ yr⁻¹ (Jones et al., 2004), showed no significant relationship between atmospheric N deposition and either soil or bulk vegetation parameters in dune slacks. This may, in large part, be due to the relatively small sample size and the absence of wet dune slacks in two of the eleven sites. However, the cover of *Carex arenaria* and *Hypochaeris radicata* related positively to total N deposition, suggesting a response in some vegetation species of dune slacks to N enrichment at rather low loads. A second gradient study focusing on rare dune slack species at 12 sites in the United Kingdom, in a total N deposition range of 4 to 20 kg N ha⁻¹ yr⁻¹, also showed no significant effects of N on species richness or soil parameters such as total N content, or available N (Jones, 2007).

However, there is experimental evidence that N may cause major shifts in the germination community of dune slacks. In a UK seedbank germination experiment, 15 kg N ha⁻¹ yr⁻¹ (in the form of NH₄NO₃) was applied to soils from dune slacks with a site background deposition of approximately 11 kg N ha⁻¹ yr⁻¹. The emerging seedling community on N treated soil differed strongly from communities on untreated dune slack soil. Germination in response to N was generally greater in species with low Ellenberg N indicator values (Plassmann et al., 2008). This enhanced germination may deplete the seedbank of early successional species which depend on seed longevity to survive until the next sand dune mobility phase or disturbance event which exposes bare sand allowing germination.

There is new evidence since the 2011 review from gradient studies on dune slack data from Scotland, generally covering the lower part of the N deposition range, that suggest there are impacts of N on dune slack vegetation. Payne et al. (2020) analysed non-targeted survey data using TITAN change-point analysis for dune slacks in Scotland, along a gradient of 2.7-11.8 kg N ha⁻¹ yr⁻¹ and showed that 30 species had negative change points within this deposition range. Four species also showed positive change points (i.e. started to increase) within this range (Figure 4.11). The overall community change point for species declines was estimated at 5 kg N ha⁻¹ yr⁻¹. Further analysis of the dune slack data used by Payne et al. (2020) was undertaken, since the species exhibiting change points and the order in which they responded to the N deposition gradient did not always match expectations based on their ecology. This analysis showed that there was an increase in the unweighted Ellenberg N at around 5-6 kg N ha⁻¹ yr⁻¹, and a decrease in species richness at around the same point on the N deposition gradient (Figure 4.12). This is consistent with the observed community change point reported in Payne et al. (2020).

Figure 4.11. Negative (black circles) and positive (white circles) change points for dune slack species. Vertical lines show overall community sum(z-) [solid line] and sum(z+) [dotted line] change-points. Circles represent species showing high purity and reliability negative (black circles) and positive (white circles) change points in response to nitrogen deposition and bootstrap 5% and 95% quantiles. From Payne et al. (2020).



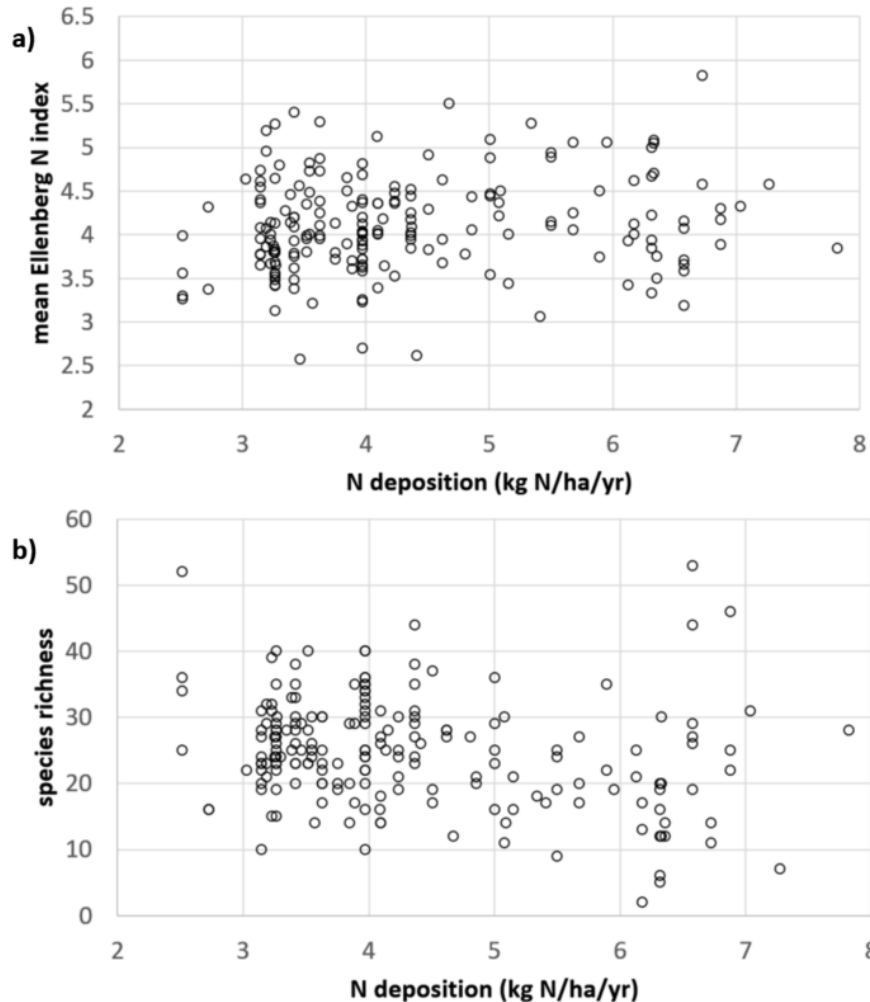
Source: Payne et al., 2020

Data looking at change in dune slack vegetation over time also provides evidence of impacts within this deposition range. The Scottish re-survey of coastal vegetation by Pakeman et al. (2016) over a 35-year interval (1975-2010) also showed a shift towards more nitrophilous vegetation in dune slacks, from a mean Ellenberg N score of 3.997 to 4.147 over time, consistent with the increase in N deposition over that period.

Separate evidence comes from a UK study (Rhymes et al., 2014, 2015) which looked at N impacts on dune slack vegetation and soils through observation of within-site gradients in groundwater contamination. While this represents a different nutrient pathway, this study provides strong additional evidence for the sensitivity of dune slacks to N. The Aberffraw dunes in Wales, UK, contain an N gradient in groundwater coming from adjacent farmland. Analysis of soils and vegetation along this gradient suggests higher available nitrate in soil where dissolved inorganic N (DIN) concentrations in groundwater were greater than $0.084 \pm 0.034 \text{ mg L}^{-1}$, and altered vegetation composition where DIN concentrations in groundwater were greater than $0.0224 \pm 0.011 \text{ mg L}^{-1}$ (Rhymes et al., 2014), by comparison with locations towards the middle of the site where the vegetation was least impacted, and assumed only to receive N from background atmospheric deposition with no additional groundwater inputs of N (Rhymes et al., 2015). The background deposition to the site is approximately $11 \text{ kg N ha}^{-1} \text{yr}^{-1}$, therefore, regardless of the source and pathway of N, this study provides evidence for further impacts of N

above this level. This also shows the importance of understanding all significant N inputs at any given site.

Figure 4.12. Re-analysis of dune slack data used in Payne et al. (2020), data originally from Lewis et al. (2016); Pakeman et al. (2015, 2016, 2017), showing a) mean unweighted Ellenberg N index and b) species richness, against N deposition.



Source: Payne et al., 2020; Lewis et al., 2016; Pakeman et al. (2015, 2016, 2017)

Summary for moist to wet dune slacks (N1H)

The 2011 $CL_{emp}N$ range was 10-20 kg ha⁻¹ yr⁻¹ (expert judgement). There is new evidence suggesting that dune slacks are sensitive to N deposition. This is based on surveys, and from monitoring of change over time, particularly from studies focused in areas of relatively low N deposition. Together, these studies suggest that the $CL_{emp}N$ range should be lowered to 5 to 15 kg N ha⁻¹ yr⁻¹ with the evidence status improved to quite reliable. This is reinforced by evidence of impacts from additional N inputs via groundwater in a site receiving atmospheric deposition of 11 kg N ha⁻¹ yr⁻¹. Experimental studies to reinforce this new value would certainly be desirable. Due to new evidence of impacts at low N deposition loads in calcareous systems, we no longer recommend to apply a different part of the $CL_{emp}N$ range to slacks of low base status. However, acid to acidic slacks may still show greater impacts than well-buffered slacks for a given level of N deposition.

4.2.5 Dune-slack pools (freshwater aquatic communities of permanent Atlantic and Baltic (N1H1) or Mediterranean and Black Sea (N1J1) dune-slack water bodies)

These relatively small pools are found in the European coastal dune areas, both in the temperate as well as the Mediterranean zone. Dune pools can dry out totally in some years and the very shallow and small ones also every year, but they have a waterbody for more than $\frac{3}{4}$ of the year. Wet dune slacks have typically less than half year with an open water body. Dune slack pools are a typical part of young, expanding coastal dune areas. These pools with young sediments are oligotrophic to mesotrophic nutrient poor, but not extremely so. Whereas large, permanent water bodies in such dunes are often rather eutrophic, dune slack pools are characterised by clear water and a diverse submerged macrophyte vegetation (e.g. *Potamogeton* and *Chara* species, and littoral isoetids). Relative to oligotrophic inland surface waters, they show a high succession rate towards large helophytes and shrubs. A distinct influence of local groundwater from the surrounding dunes is present, transporting dissolved iron that ensures phosphorus limitation in the water layer. Contaminated groundwater may also transport nutrients to such pools. Desiccation with aeration of a large part of the sediment may frequently occur and aeration of the sediments also occurs by radial oxygen loss of the root system of isoetids like *Littorella uniflora* (Adema et al., 2005). Oxygenation of the rhizosphere stimulates denitrification of N_2 to the air via nitrate production, reducing the amount of N in the pool. In addition, the fluctuation of the water table improves phosphorus binding to iron, reducing its availability. The interactions between carbonate content, desiccation, N deposition and succession rates are yet not well studied.

Despite their well-defined geographic location, their functioning can differ in several ecologically important aspects. Water bodies can be permanent, or annual desiccation may occur. This may also vary from year to year depending on precipitation and the local hydrology. Dune sands can also vary from highly calcareous to almost without calcium carbonate and slightly acidic. Accumulation of organic matter is a dominant factor in determining the succession rates. Both desiccation as well as calcareous soils stimulate decomposition of organic material, thereby slowing down succession (Sival and Grootjans, 1996). Water eutrophication and N deposition accelerate succession rates and shorten the lifespan of dune slack pools. Eutrophication in these dune slack pools is more likely to be caused by atmospheric inputs or by high densities of waterfowl than by the inflow of enriched surface water, because of the hydrological isolation of these habitats and their location in (large) natural coastal areas but could in principle occur from contaminated groundwater.

Very few experimental data exist on the sensitivity of dune slack pools with respect to CL_{empN} setting, despite the generally well-known N limitation of dune slack wetlands (Lammerts and Grootjans, 1997; Romo et al., 2016). In Australian dune lakes, algal growth was stimulated by N additions as low as $50 \mu g L^{-1}$ ammonium nitrate, but these lakes are ancient and rather acidic (Hadwen, 2002). The impact of atmospheric N deposition was quantified in dune-pool mesocosms (approximately 2-m diameters) during a two-year experiment with different liquid N loads (1, 20, 40 and $120 kg N ha^{-1} yr^{-1}$ as $(NH_4)_2SO_4$ in an unheated glasshouse (Brouwer et al., 1996). No acidification of the water was found in those two years, but total biomass of water plants and helophytes increased strongly at over $20 kg N ha^{-1} yr^{-1}$. Nitrogen additions clearly accelerated the rate of succession in these dune slack mesocosms, leading to more helophytes and less open water. This phenomenon has also been observed in many dune slack pools in the Netherlands receiving relatively high atmospheric N loads ($15-20 kg N ha^{-1} yr^{-1}$). A recent problem in temperate dune pools is invasion by an exotic plant species, *Crassula helmsii* (Dean, 2015). Experiments have shown that the colonisation of phosphorus poor soils is strongly enhanced after three months of adding N at a rate of $15 kg N ha^{-1} y^{-1}$ (Brouwer et al., 2017).

Summary for dune-slack pools (N1H1, N1J1)

The 2011 $CL_{emp}N$ range was 10-20 kg ha⁻¹ yr⁻¹ (expert judgement) rated as 'expert judgement'. There is relatively little new evidence for this habitat and it does not change the previous assessment. Therefore, we retain the existing $CL_{emp}N$ range.

In this case we also recommend that long-term field experiments are held, with realistic doses of N added to these waters, especially in regions with low naturally occurring N deposition. Mediterranean dune pools differ from pools in the Atlantic/Baltic zone by higher temperatures, longer desiccation periods, on average more alkaline conditions and less accumulation of organic matter (Romo et al., 2016). This highly dynamic situation stimulates N losses through cycles of nitrification and denitrification and prevents accumulation of nitrogen in organic matter. Therefore, Mediterranean pools might be less sensitive to increased N deposition but could be more affected by N pollutants from surrounding agricultural areas.

4.2.6 Coastal forests

Coastal forests on sand occur as natural Atlantic dunes woodland or other natural assemblages, in addition to plantation woodland. For N impacts on these woodlands, please refer to the forest chapter (EUNIS class T).

4.3 Overall summary of $CL_{emp}N$ for coastal dunes and sandy shores (N1)

The $CL_{emp}N$ for coastal habitats (N) are summarised in Table 4.1.

Table 4.1. $CL_{emp}N$ and effects of exceedances on coastal habitats (N). ## reliable, # quite reliable and (#) expert judgement. Changes with respect to 2011 are indicated as values in bold.

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2011 reliability	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
Shifting coastal dunes	N13, N14	10-20	(#)	10-20	#	Biomass increase; increased N leaching; reduced root biomass
Coastal dune grasslands (grey dunes)	N15	8-15	#	5-15	##	Increased biomass and cover of graminoids and mesophilic forbs; decrease in oligotrophic species including lichens; increased tissue N; increased N leaching; soil acidification
Coastal dune heaths	N18, N19	10-20	(#)	10-15	#	Increased plant production; increased N leaching; accelerated succession; typical lichen C:N decrease; increased yearly increment <i>Calluna</i>
Moist and wet dune slacks	N1H	10-20	(#)	5-15	#	Increased cover of graminoids and mesophilic forbs; decrease in oligotrophic species, increased Ellenberg N

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2011 reliability	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
Dune-slack pools (freshwater aquatic communities of permanent Atlantic and Baltic or Mediterranean and Black Sea dune-slack water bodies)	N1H1, N1J1	10-20	(#)	10-20	(#)	Increased biomass and increased rate of succession

4.4 References

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5 Effects of nitrogen deposition on inland surface water habitats (EUNIS class C)

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Helgetjerne, Hemsedal municipality, Norway. Photo: Jacqueline Knutson.

Summary

In this chapter empirical N critical loads ($CL_{emp}N$) for standing inland surface water habitats (C1) have been reviewed and revised. The assessment is given for the permanent oligotrophic lakes, ponds and pools (C1.1) and for the permanent dystrophic lakes, ponds and pools (C1.4). New, compared to the last report (Bobbink and Hettelingh, 2011), is the differentiation of the permanent oligotrophic class (C1.1) into the following three subcategories: alpine and sub-Arctic clear-water lakes (C1.1), boreal clear-water lakes (C1.1) and Atlantic soft-water bodies (C1.1/C1.2). New $CL_{emp}N$ ranges are proposed for the first two subcategories, respectively 2-4 and 3-6 $N\ ha^{-1}\ yr^{-1}$. For the permanent dystrophic class (C1.4) the lower end of the $CL_{emp}N$ range has been increased from three to five (5 to 10 $kg\ N\ ha^{-1}\ yr^{-1}$). New in this chapter, compared to the last update by Bobbink and Hettelingh (2011), is the exclusion of the EUNIS class dune slack-pools, which has been moved to Chapter 4 in this report (EUNIS class N). It belongs to the freshwater aquatic communities of permanent Atlantic and Baltic (N1H1) or Mediterranean and Black Sea (N1J1) dune-slack water bodies (Chytrý et al., 2020).

5.1 Introduction

In this chapter, the effects of atmospheric N deposition on freshwater ecosystems (inland surface water habitats; class C of the European Nature Information System (EUNIS)) are evaluated. Inland surface water habitats are non-coastal, open, fresh or brackish water bodies (e.g. lakes and pools, rivers, streams, ditches and springs), including their littoral zones. This class includes constructed inland freshwater, brackish or saline water bodies (e.g. canals and ponds) which support a semi-natural community of both plants and animals, and seasonal water bodies which may dry out for part of the year (temporary or intermittent rivers and lakes and their littoral zones). Freshwater littoral zones include those parts of banks or shores that are sufficiently frequently inundated to prevent the formation of closed terrestrial vegetation. Permanent snow and ice are excluded from this EUNIS class.

The main subcategories of EUNIS class C are: surface standing waters (C1), surface running waters (C2), and the littoral zone of inland surface water bodies (C3) (Davies et al., 2004). This chapter summarises field and experimental evidence to establish critical loads for atmospheric N deposition with respect to eutrophication or effects of adverse ammonium. This chapter only discusses surface standing waters (C1) because of limited data availability for class C2 and C3. As usual in earlier empirical critical load reports, rivers (C2) were not assessed because most rivers with potentially available data received high loads of nitrogen from agricultural sources. Only surface waters with no significant agricultural or other human impacts besides atmospheric deposition are considered in this chapter. Atmospheric nitrogen inputs also has acidifying effects on surface waters (Stoddard et al., 1994; Marchetto et al., 1994; Wright et al., 2001). Critical loads for acidification in relation to atmospheric N deposition have been established using mass- and charge balance models (Forsius et al., 2021).

Surface standing waters vary according to nutrient status, depth, sediment type, alkalinity and colour (Moe et al., 2008; Arts, 2002) and these characteristics are also used in the classification of ecological status of surface waters for the EU Water Framework Directive (European Communities, 2003). These characteristics determine their suitability as habitat for aquatic organisms, most importantly fish, phytoplankton (free-floating algae), water plants (macrophytes), organisms growing on solid substrates (epilithon), zooplankton and macro-invertebrates. Nitrogen leaching from semi-natural catchments, which remains challenging to predict (Dise et al., 2009), is related to both N deposition and catchment characteristics, including habitat management and land use within the catchment (Dise and Wright, 1995; Aber et al., 1998; Oulehle et al., 2013). Lake nitrate is often found to be positively related to the catchment ratio (Helliwell et al., 2001; Helliwell et al., 2007).

Nutrient status, in particular phosphorus (P) concentrations, is likely to be an important control of aquatic biological responses to changes in N inputs (Bergström, 2010). Terrestrial and aquatic food webs have similarities and differences related to nutrient limitations (Elser et al., 2000). There are structural contrasts between terrestrial and aquatic food webs that regulate trophic pathways, apparently related to nutritional quality of autotrophs (Shurin et al., 2006). The effects of N deposition on biology in freshwater habitats will depend on catchment N retention capacity, and on the sensitivity of habitats and organisms to changed N availability. The assessment is given for three subcategories of C1, i.e. Atlantic soft-waters, clear-water boreal and alpine lakes, and dystrophic boreal waters. No changes were made in empirical critical loads for Atlantic soft-waters whereas new ranges are now supplied for clear-waters and dystrophic surface waters.

5.2 Surface standing waters (C1)

The main division of permanent standing waters containing fresh water (i.e. non-saline) is based on the nutrient status of the waters, from oligotrophic (C1.1) to mesotrophic (C1.2) and eutrophic lakes, ponds and pools (C1.3). In addition, dystrophic water bodies (C1.4) which are rich in humic substances and often with a brown colour, are also included in C1. Unfortunately, the important gradient in alkalinity (from very soft to hard water), which is at least partly independent of the nutrient status of the water (e.g. Moss, 1988; Brouwer et al., 2002), is not separately treated in the EUNIS habitat hierarchy, making it difficult to classify these systems under EUNIS. As in the previous update (Bobbink and Hettelingh, 2011), the impacts of N deposition are first discussed for the elements of category C1.1 Atlantic soft-water lakes. The effects of N deposition in pristine boreal and alpine oligotrophic water bodies across Europe and North America have received considerable attention in the last 15 years, leading to new evidence, experimental and from gradient studies, to set $CL_{emp}N$ for this important group of category C1.1.

5.2.1 Atlantic soft-water lakes (part of permanent oligotrophic lakes, pools and ponds (C1.1) and some elements of permanent mesotrophic water bodies (C1.2))

In the lowlands of western Europe, many shallow soft waters are found on sandy sediments that are poor in, or almost devoid of, calcium carbonate. These waters are poorly buffered and their concentrations of calcium are very low. Also, they are shallow, fully mixed water bodies with fluctuating water levels that are mainly fed by rain water or water from acidic catchment soils. Thus, they are oligotrophic. In the EUNIS classification scheme, these waters are classified as either permanent oligotrophic (C1.1) or permanent mesotrophic (C1.2) lakes, pools and ponds. These ecosystems are characterised by plant communities from the phytosociological alliance Littorelletea (Schoof-Van Pelt, 1973; Wittig, 1982; Arts, 1990; 2002). Stands of these communities are characterised by the presence of rare and endangered isoetids (with the growth form of *Isoetes*), such as *Littorella uniflora*, *Lobelia dortmanna*, *Isoetes lacustris*, *Isoetes echinospora*, *Echinodorus* species, *Luronium natans* and other soft-water macrophytes. Nowadays, these soft-waters have become very rare in western Europe (Arts, 2002) and are almost all located within nature reserves. This decline is illustrated by the fact that *Littorella uniflora*, which was recorded at more than 230 sites in the Netherlands in the early 1950s, was found at about only 40 locations by the end of the 1980s. In addition, these soft waters have also seen a marked decline in their amphibian species (Leuven et al., 1986). It has been suggested that systems in western Europe (e.g. The Netherlands, northern Germany, Belgium) have been affected more than in other European countries (e.g. Poland) because they are smaller and shallower than elsewhere (Arts, 2002).

The effects of atmospheric N pollutants on these hydrologically isolated soft-water systems – with (hardly) any catchment – have been studied intensively in the Netherlands, in both field surveys and experimental studies. Field observations in approximately 70 soft-water systems that supported well-developed isoetid vegetation in the 1950s showed that the waters in which characteristic macrophytes were still abundant in the early 1980s were those that were poorly buffered (alkalinity 50-500 meq l⁻¹), circumneutral (pH 5-6) and very low in N (Roelofs, 1983; Arts et al., 1990). The 53 soft-water sites from which these plant species had disappeared fell into two groups. In 12, eutrophication resulting from the inflow of enriched water seemed to be the main cause of the decline. In this group of non-acidified waters, plants such as *Lemna minor* had become dominant and high concentrations of phosphate and ammonium were measured in the sediment of these waters. In some of the larger water bodies in this group, no macrophytes were found due to dense plankton blooms. In the second group of lakes and pools (41 out of 53),

the isoetid species were found to have been replaced by dense stands of *Juncus bulbosus* or aquatic mosses, such as *Sphagnum cuspidatum* and *Drepanocladus fluitans*. This indicates, clearly, that acidification has occurred in these soft-waters over recent decades, probably as a result of increases in atmospheric N deposition. Also, this field study found that N levels in the water were higher in ecosystems from which the natural vegetation had disappeared, compared to those in which isoetid stands were still present (Roelofs, 1983). This strongly suggests a detrimental effect of atmospheric N deposition on these lakes.

A number of ecophysiological studies have revealed that 1) low inorganic carbon availability due to intermediate levels of alkalinity, and 2) low N concentrations in the water are important to the success of endangered isoetid macrophytes communities. Furthermore, most of the typical soft-water plants had a relatively low potential growth rate. Increased acidity and higher concentrations of ammonium in the water clearly stimulated the development of *Juncus bulbosus* and submerged mosses, such as *Sphagnum* and *Drepanocladus* species (Roelofs et al., 1984; Den Hartog, 1986) (Figure 5.1). Laboratory experiments have also shown that the form of N involved (ammonium or nitrate) differentially influences the growth of aquatic plant species. For example, almost all of the characteristic soft-water isoetids developed more effectively when nitrate was added compared to ammonium, whereas the growth of *Juncus bulbosus* and aquatic mosses (*Sphagnum* and *Drepanocladus*) were stimulated by higher levels of ammonium (Schuurkes et al., 1986). The importance of ammonium for the growth of these aquatic mosses is also reported by Glime (1992).

Figure 5.1. Mass growth of *Juncus bulbosus* in a soft-water in an area with high deposition of reduced N (photo E. Lucassen).



Source: E. Lucassen

The effects of atmospheric deposition on macrophyte communities were studied over a two-year period in soft-water mesocosms contained in an unheated greenhouse; the mesocosms were treated with different types of artificial rain (Schuurkes et al., 1987). Acidification due to sulphuric acid, without N inputs, did not result in increased growth of *Juncus bulbosus*, and a diverse isoetid vegetation remained. However, when N concentrations of 19 kg N ha⁻¹ yr⁻¹ or higher were applied in the form of ammonium sulphate, changes in floristic composition were observed compared to the control treatments (< 2 kg N ha⁻¹ yr⁻¹). These changes were similar to those seen under field conditions, i.e. a dramatic increase in dominance of *Juncus bulbosus*, submerged aquatic mosses and *Agrostis canina* (Schuurkes et al., 1987). This strongly suggests that the observed changes in the field may have occurred due to ammonium sulphate deposition, which led to both eutrophication and acidification. The increased levels of ammonium in the system also stimulated the growth of plants such as *Juncus bulbosus*, and any surplus ammonium

was nitrified in these soft-water systems ($\text{pH} > 4.0$). During this nitrification process, H^+ ions are produced that increase the acidity of the system. The results from this study demonstrate that large changes occurred within two years of treatment with $\geq 19 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. In addition, the strongest decline in macrophyte species composition in Dutch soft-water communities was found to occur in areas with atmospheric N loads of approximately 10 to $13 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Arts, 1990). In addition, Brouwer et al. (1997) showed that, after ten years of treatment with clean rainwater, there was only a partial recovery within the soft-water mesocosms that had been treated with ammonium sulphate, and that *Juncus* and *Molinia* were still the dominant species, suggesting a strong legacy effects of the N deposition impacts.

The previously established CL_{empN} for shallow soft-water bodies (most in C1.1, but some elements in C1.2) was based on experimental evidence and was set at 3 to $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ and was considered as reliable (Bobbink et al., 1996, 2003; Bobbink and Hettelingh, 2011). As no new evidence from experimental studies or targeted gradient studies has been published since then on the impacts of N deposition in (shallow) soft-water lakes, the CL_{empN} range for these systems remains unchanged. This has been incorporated into the more general CL_{empN} range for oligotrophic lakes, ponds and pools (C1.1) of 2 to $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (reliable), with a recommendation that the upper part of the range (5 to $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) is applied in practice (see also Chapter 5.2.2).

5.2.2 Oligotrophic boreal and alpine lakes (part of C1.1 and C1.4)

There is ample evidence that an increase in acidic and acidifying compounds in atmospheric deposition has resulted in the acidification of lakes and streams in geologically sensitive regions of Scandinavia, western Europe, Canada and the United States (e.g. Hultberg, 1988; Muniz, 1991). This acidification is characterised by a decrease in pH, alkalinity and acid neutralising capacity (ANC), and by increases in concentrations of sulphate, aluminium, and sometimes nitrate and ammonium. Since the 1970s, various research approaches (field surveys, laboratory studies, whole-lake experiments) have shown that surface water acidification can have dramatic consequences for plant and animal species (macrofauna, fish), and for the functioning of these aquatic ecosystems (Havas and Rosseland, 1995). However, due to the strong reduction in sulphur deposition over the last decades, a (partial) chemical recovery from acidification in these very sensitive waters has been observed in both North America and Europe (e.g. Stoddard et al., 1999; Skjelkvale et al., 2005; Van Kleef et al., 2010; Monteith et al., 2014; Garmo et al., 2014). Currently, critical loads for acidification of surface waters take into account acidifying effects of S and N deposition (Forsius et al., 2021).

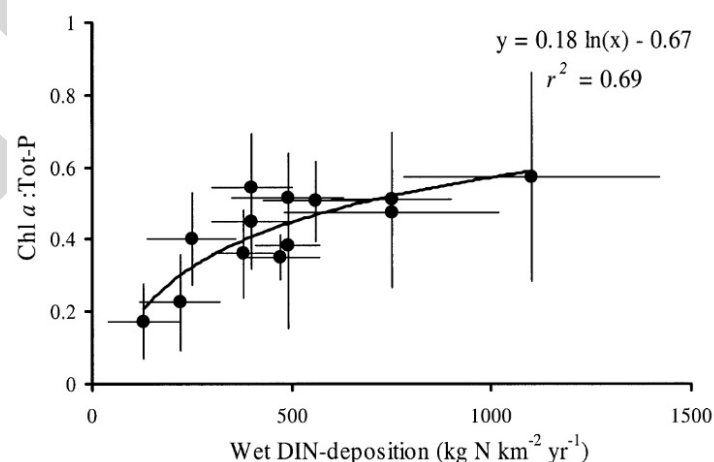
Before the 1990s, effects of eutrophication by atmospheric N deposition were hardly viewed as relevant with regards to surface waters, except for some permanent oligotrophic or mesotrophic water bodies (see Chapter 5.2.1). This is because primary production in almost all surface waters was thought to be limited by phosphorus (e.g. Moss, 1988). However, in the past decades it has become obvious that this paradigm does not hold for all freshwater systems, especially in pristine areas of alpine, sub-Arctic, Arctic or boreal regions and that N limitation or co-limitation is widespread (e.g. Saros et al., 2005; Bergström and Jansson, 2006; Wolfe et al., 2006; Sterner, 2008; Elser et al., 2007; De Wit and Lindholm, 2010). This view appears to be widely accepted now, as evidenced in the review from Howarth et al. (2021), on N and P limitation in aquatic ecosystems. The authors conclude that for freshwaters located in catchments with no local N and P sources, and in low N deposition areas, N limitation or co-limitation is common and that lakes can be driven towards P-limitation when N deposition is high and/or catchment N retention is low (Howarth et al., 2021). An update of the literature review in De Wit and Lindholm (2010)

also concludes that evidence for the limiting role of N in alpine and boreal lakes has increased (Thrane et al., 2021).

Bergström et al. (2005, 2008) conducted lake sampling and in situ nutrient enrichment enclosure experiments with N and P in oligotrophic boreal lakes (C1.1) along a gradient of increasing atmospheric N deposition (0.9 to 8.0 kg N ha⁻¹ yr⁻¹ wet deposition) in Sweden. Regional and seasonal patterns of nutrient limitation of phytoplankton were clearly related to the levels of N deposition and catchment N inputs that these lakes received. In areas of low N deposition in northern Sweden (< 3 kg ha⁻¹ yr⁻¹ wet deposition), N limitation of phytoplankton growth was evident in the summer season due to high N retention in the catchment areas and very low dissolved inorganic N (DIN) inputs during the early summer. Higher N deposition in the south (> 3 kg ha⁻¹ yr⁻¹ wet deposition) was accompanied by high DIN concentrations in the lakes during the early summer and subsequent P limitation of phytoplankton. However, in these lakes P limitation did not persist over the summer and, as a result of a declining DIN pool, co-limitation by N and P subsequently occurred, followed by N limitation only. Generally, in the summer, the studied oligotrophic Swedish lakes were N limited rather than P limited. The authors concluded that N limitation is probably a natural state of boreal and sub-Arctic oligotrophic lakes, but that P limitation of varying intensities and duration had been induced by elevated atmospheric N deposition (> 3-4 kg ha⁻¹ yr⁻¹ wet deposition).

In a lake survey that included over 4000 oligotrophic lakes in Europe and North America, Bergström and Jansson (2006) showed that phytoplankton biomass per unit P increased with increasing wet N deposition. The range in wet N deposition was < 1 to 14 kg N ha⁻¹ yr⁻¹, and the largest increase in algal biomass per unit P occurred at wet N deposition < 5 kg N ha⁻¹ yr⁻¹. Lake sampling, bioassays and physiological assays in four acid-sensitive lakes in the United States (5 to 9 kg N ha⁻¹ yr⁻¹) demonstrated that N enrichment gave growth responses in phytoplankton that were similar to or larger than those of P enrichment, and that N deposition was large enough to satisfy daily algal demand for N (Axler et al., 1994). These results support findings from Sweden (Bergström et al., 2005; Figure 5.2) and indicate that N deposition has contributed to higher algal productivity in oligotrophic lakes, including dystrophic ones.

Figure 5.2. The relationship between mean ratios of chlorophyll a and total phosphorus (Chl a: tot P, g to g) and mean wet inorganic nitrogen deposition (wet DIN deposition) in unproductive lakes in different Swedish regions for the period 1995-2001 (Bergström et al., 2005).



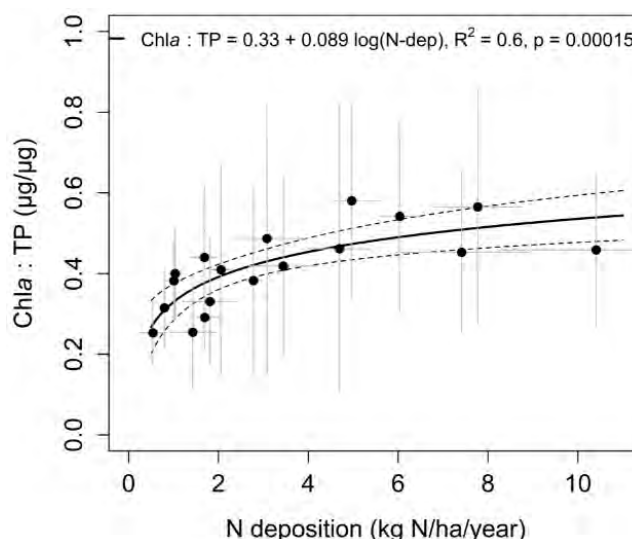
Source: Bergström et al., 2005

The impact of atmospheric N deposition on element stoichiometry was investigated in a survey of oligotrophic lakes in Norway, Sweden and the United States (Colorado), in low and high N-deposition areas, including dystrophic waters, but the dystrophic waters were not addressed separately (Elser et al., 2009). The survey showed that atmospheric wet N deposition increased the stoichiometric N:P ratio in these lakes, indicative of a shift in ecological nutrient limitation. In a subset of lakes, bioassays indicated that phytoplankton growth was generally found to be N limited under low N deposition (approximately 4.5 kg N ha⁻¹ yr⁻¹ in Norway and approximately 2 kg N ha⁻¹ yr⁻¹ in Sweden). However, in lakes with high N deposition (approximately 8.5 kg N ha⁻¹ yr⁻¹ in Norway and approximately 6 kg N ha⁻¹ yr⁻¹ in Sweden), the growth of phytoplankton was consistently limited by P. Elser et al. (2009) concluded that even relatively low levels of N deposition affected nutrient limitation of phytoplankton growth in oligotrophic lakes in Scandinavia (and the United States). The authors hypothesised that in the long term, in regions with still increasing atmospheric N loads, future functioning of the food web, even in lakes far away from direct human disturbance may be seriously disrupted. Whole-lake manipulations designed to examine effects of experimentally added N on lake food webs (Deininger et al., 2017a, 2017c) have not found strong cascading impacts of N at higher trophic levels.

The referred studies on nutrient limitation in lakes from surveys in Europe and North America are based on data collection from the 1990s and early 2000s. Deposition of N in Europe has declined since 1990 (Engardt et al., 2017; Torseth et al., 2012) while concentrations of DIN have either remained constant or have declined, probably driven by trends in deposition and climate (Kaste et al., 2020; Lucas et al., 2016; Rogora et al., 2012). Surface waters in catchments that receive high N deposition have a higher risk for elevated inorganic nitrogen concentrations (Wright et al., 2001), but this risk is mediated by catchment properties such as vegetation cover or lack thereof, which is found in a dataset of water chemistry, catchment characteristics, climate and deposition from North America and Europe (Austnes et al., 2022).

A data analysis from 2006-2018 from > 300 Nordic lakes in natural catchments (range in wet N deposition from < 2 to > 12 kg N ha⁻¹ yr⁻¹) shows that chlorophyll *a* to total phosphorus (Chl*a*:TP) ratios increase with N deposition where total N deposition is below 2 to 4 kg N ha⁻¹ yr⁻¹ (Thrane et al., 2021; Figure 5.3), suggesting N limitation, while no further responses are found above deposition of 3 to 5 kg N ha⁻¹ yr⁻¹. Thus, the threshold range where the lakes turn from N limitation to P or co-limitation is in the same range as indicated by Bergström and Jansson (2006) and Bergström et al. (2005).

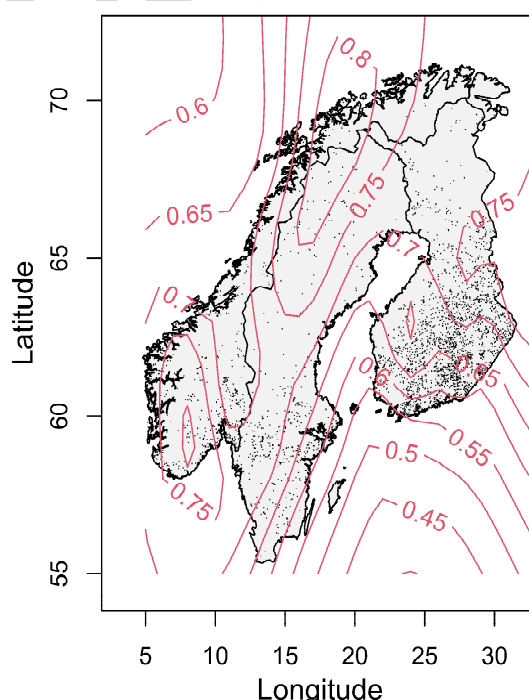
Figure 5.3. The relationship between median values of chlorophyll *a* per unit total phosphorus (chl*a* : TP) and median values of total N deposition for lakes in 18 regions in Norway, Sweden and Finland. Grey lines show median \pm one standard deviation for chl*a* : TP (vertical lines) and N deposition (horizontal lines) within each region (Thrane et al., 2021)



Source: Thrane et al., 2021

It should be noted that Thrane et al. (2021) use the sum of dry and wet deposition, while many other studies, including Bergström and Jansson (2006), use wet N deposition. Thrane et al. (2021) calculate that median wet N deposition in their dataset is 68% (25% and 75% percentiles: 63% and 73%, respectively) of total N deposition (EMEP data, for 2006-2018) (Figure 5.4).

Figure 5.4. Mean fraction of wet N deposition to total N deposition in the Nordic countries for 2006-2018 (Thrane et al., 2021).



Source: Thrane et al., 2021

Although this chapter on surface waters does not include an assessment of running waters, it is worthwhile to note that Thrane et al. (2021) also present seasonal element stoichiometry (DIN to TP) from natural rivers in Norway. They find that DIN:TP in river from catchments receiving less than $2.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (total deposition) are most frequently in the range indicative for N limitation established by Bergström (2010) for lakes. River catchments receiving more than $4 \text{ ha}^{-1} \text{ yr}^{-1}$ had DIN:TP ratios that usually indicated P-limitation. Thus, the critical threshold for the shift from N limitation to P limitation for these natural rivers is similar to what the analysis of ratios of chlorophyll-a to total phosphorus from lakes indicates, i.e. 2 to $4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Myrstener et al. (2018) found that biofilm communities in oligotrophic Arctic streams display N limitation, based on nutrient bioassays. Others have found N and co-limitation of biofilm in polluted rivers (Reisinger et al., 2016), suggesting that rivers may display somewhat similar responses to increases in N availability as standing waters.

In recent years, additional focus has been drawn to the role of dissolved organic matter (DOM) as a possible factor that can affect productivity in contrasting ways, i.e. by limiting light availability and by transporting organic forms of N and P (Karlsson et al., 2009; Seekell et al., 2015). Northern lakes have become more DOM-rich in the past decades, related to reduced acid deposition (Monteith et al., 2007) and to increased precipitation (De Wit et al., 2016). DOM in boreal and alpine lakes varies with land cover (forests and peatlands) and climate (Larsen et al., 2011). Results from a whole lake experiment (Deininger et al., 2017b) suggested that effects of enhanced N are weakened along a positive DOM gradient, indicating that algal productivity in clear-water lakes is more sensitive to increased N availability than darker lakes. This implies that clear-water lakes are especially sensitive to N deposition because low DOM and low N retention capacity are causally linked through low pools of soil organic matter in their catchments, a consequence of little vegetation and shallow soils (Evans et al., 2006).

Combined effects of DOM and nutrient availability on food web dynamics suggested that DOM affected food quality for secondary producers (Deininger et al., 2017b). A meta-analysis of field studies and experimental data from a global lake dataset focused on effects of nutrients and DOM on primary and secondary lake productivity supported that N was a limiting nutrient but could not draw definite conclusions on interactions between DOM and N availability for lake productivity (Faithfull et al., 2011). However, the nutritional value of pelagic lake food webs appears to be negatively related with nutrient availability (DIN to total P ratios) and lake temperature (Lau et al., 2021).

Studies of nutrient limitation in epilithic communities of oligotrophic Swedish lakes along a north-south gradient concluded that increased atmospheric N deposition ($10\text{--}12 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) intensified P limitation of epilithic algae and invertebrate grazers, compared to those in low deposition areas ($2\text{--}6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), although more studies would be needed to generalise these findings (Liess et al., 2009). In-situ nutrient-diffusion strata placed in alpine lakes in French Alps receiving N deposition of $7 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, with and without grazing, showed that phyto-benthic biomass was higher in N-enriched substrates, and green filamentous algae were favoured over diatoms and cyanobacteria in N-enriched substrates (Lepori and Robin, 2014). Myrstener et al. (2018) presented nutrient-diffusion strata in Arctic streams in northern Sweden, receiving less than $1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ to test for various limitations on microbial communities in biofilms and found strong responses to N availability clearly signaling N limitation, but also found that such responses were mitigated by temperature and light availability.

The CL_{empN} for Atlantic soft-water lakes (see Chapter 5.2.1) has been based on experiments. For boreal, sub-Arctic and alpine lakes, the CL_{empN} was based on survey and gradient studies supported by experimental evidence from bioassays and whole-lake experiments. Emerging evidence points at factors that mitigate responses to nitrogen – such as DOM (regulating light)

and temperature. Still, many studies published after 2010 have further substantiated that N limitation is common in boreal, sub-Arctic and alpine lakes. Studies from North America and Europe come to similar conclusions. This is in particular illustrated by the empirical critical loads developed in the US shortly after the $CL_{emp}N$ developed for the LTRAP Convention (Bobbink and Hettelingh, 2011) presented in Baron et al. (2011). Baron et al. (2011) differentiate between the high-elevation western lakes with sparse vegetation and north-eastern lakes in forest-covered regions, considering catchment differences in capacity to retain atmospherically deposited N. Their results are summarised as follows: The nutrient enrichment $CL_{emp}N$ for western lakes ranged from 1.0 to 3.0 kg N ha⁻¹ yr⁻¹, reflecting the nearly non-existent watershed vegetation in complex, snowmelt-dominated terrain. The nutrient enrichment $CL_{emp}N$ for north-eastern lakes ranged from 3.5 to 6.0 kg N ha⁻¹ yr⁻¹.

In the 2011 report (Bobbink and Hettelingh 2011), a single $CL_{emp}N$ range for C1.1 (permanent oligotrophic water bodies) of 3 to 10 kg ha⁻¹ yr⁻¹ (reliable) was proposed. The range should only be applied to oligotrophic waters with low alkalinity with no significant agricultural or other direct human inputs. Furthermore, the lower end of the range would apply to boreal, sub-Arctic and alpine lakes, whereas the upper end of the range applies to Atlantic soft-water lakes. It was also concluded in 2011 that dystrophic lakes (C1.4) may be sensitive to N deposition. This is now further substantiated and it can be stated with more confidence that dystrophic lakes are indeed sensitive to N deposition, but less so than clear-water lakes, which is due to the fact of a) the effect of DOM on light availability, that limits the ecological response to increases in nitrogen, b) the catchment properties that lead to high DOM also lead to higher retention of atmospheric nitrogen deposition.

The evidence that has accumulated since 2010 allows for a better quantification of the $CL_{emp}N$ for C1.1 and C1.4. Clear-water sub-Arctic and alpine lakes (C1.1) in catchments with little vegetation and limited soil cover have a slightly lower threshold for responses to N deposition than boreal lakes (C1.1) from forested regions. Based on the evidence referred to above, we propose a $CL_{emp}N$ range of 2-6 kg ha⁻¹ yr⁻¹, where Alpine and sub-Arctic clear-water lakes have a $CL_{emp}N$ range of 2-4 kg N ha⁻¹ yr⁻¹ (reliable) while boreal (non-dystrophic) lakes have a $CL_{emp}N$ range of 3-6 kg N ha⁻¹ yr⁻¹ (reliable). Dystrophic, humic lakes (C1.4) in catchments with forests, wetlands and well-developed forest soils are less sensitive to N deposition because more N is retained in the catchment, while high DOM limits the eutrophying effects because of light limitation. Hence, their $CL_{emp}N$ range is proposed to be 5-10 kg N ha⁻¹ yr⁻¹ (expert judgement).

5.3 Overall summary of $CL_{emp}N$ for inland surface waters (C)

Table 5.1 provides an overview of the $CL_{emp}N$ for inland surface waters (C). The $CL_{emp}N$ should only be applied to waters with low alkalinity and no significant agricultural or other direct human inputs.

Table 5.1. $CL_{emp}N$ and effects of exceedances on surface standing water habitats (C1)^a. ## reliable, # quite reliable, and (#) expert judgement. Changes with respect to 2011 are indicated as values in bold.

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2011 reliability	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
Permanent oligotrophic lakes, ponds and pools	C1.1	3-10	##	2-10 ^b	##	Increased algal productivity and a shift in nutrient limitation of phytoplankton

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2011 reliability	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
(including soft-water lakes)						from N to P; shifts in macrophyte community
► Alpine and sub-Arctic clear-water lakes	C1.1			2-4	##	Increased algal productivity and a shift in nutrient limitation of phytoplankton from N to P
► Boreal clear-water lakes	C1.1			3-6	##	Increased algal productivity and a shift in nutrient limitation of phytoplankton from N to P
► Atlantic soft-water bodies	C1.1, elements C1.2	3-10	##	5-10	##	Change in species composition of macrophyte communities
Permanent dystrophic lakes, ponds and pools	C1.4	3-10	(#)	5-10 ^c	(#)	Increased algal productivity and a shift in nutrient limitation of phytoplankton from N to P

- a) The lower part of the CL_{emp}N range should be applied for lakes in small catchments (with high lake to catchment ratios), because these are most exposed to atmospheric deposition, given that a relatively high fraction of their N inputs is deposited directly on the lakes and is not retained in the catchments. Similarly, the lower part of the range should be applied for lakes in catchments with thin soils, sparse vegetation and/or with a high proportion of bare rock.
- b) This CL_{emp}N should only be applied to oligotrophic waters with low alkalinity and with no significant agricultural or other human inputs. Apply the lower end of the range to clear-water sub-Arctic and alpine lakes, the middle range to boreal lakes and the higher end of the range to Atlantic soft waters.
- c) This CL_{emp}N should only be applied to waters with low alkalinity and with no significant agricultural or other direct human inputs. Apply the lower end of the range to boreal dystrophic lakes.

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Final Draft

6 Effects of nitrogen deposition on mire, bog and fen habitats (EUNIS class Q, formerly D)

Adapted by Chris Field, Julian Aherne, Roland Bobbink and Hilde Tomassen



Sphagnum moss species in the Forest of Alyth Mires, Scotland, UK. Photo: Chris Field.

Summary

Mire, bog and fens (EUNIS class Q) are among the most sensitive habitats to nitrogen deposition, and this is reflected in their low critical load ranges. A limited number of new experimental addition studies have been published since the last update; in contrast, a large number of gradient studies have been published. For raised and blanket bogs (Q1), recent experimental research and gradient studies confirm the previous recommended empirical N critical load ($CL_{emp}N$) of 5-10 kg $ha^{-1} yr^{-1}$. However, several studies indicate that effects already occur at the lower end of this range and additional work is needed to understand these responses further. In addition, recent experimental studies show that reduced nitrogen is probably more harmful than oxidised nitrogen. For poor fens (Q22) based on limited new evidence we recommend a reduction to the low end of the critical load and a revised $CL_{emp}N$ range of 5 to 15 kg $N ha^{-1} yr^{-1}$. In rich fens (Q41-Q44) a recent gradient study provides strong indications that the upper limit of 30 kg $N ha^{-1} yr^{-1}$ is too high, so we propose a new range of 15 to 25 kg $N ha^{-1} yr^{-1}$. This range corresponds with the $CL_{emp}N$ for arctic-alpine rich fens (Q45). Based on expert judgement, we were able to set a new $CL_{emp}N$ range for palsa and polygon mires (Q3) of 3 to 10 kg $N ha^{-1} yr^{-1}$.

6.1 Introduction

Class Q of the European Nature Information System (EUNIS) includes a wide range of wetland systems that have their water table at or above soil or sediment level for at least half of the year, dominated by either herbaceous or ericoid vegetation (Davies and Moss, 2002; Davies et al., 2004). EUNIS Q encompasses the broad habitat classifications: Raised and blanket bogs (Q1), Valley mires, poor fens and transition mires (Q2), Palsa and polygon mires (Q3), Base-rich fens and calcareous spring mires (Q4) and Helophyte beds (Q5). Nutrient budgets in these wetland ecosystems are characterised by inputs and outputs of nutrients via groundwater and surface water and are tightly linked with local hydrology. The extent to which these systems receive and lose nutrients with in- and out-flowing water largely determines their sensitivity to excess nitrogen (N) from atmospheric deposition. Little to very little effects from N deposition are to be expected in several open wetland systems, such as reed marshes and sedge beds (EUNIS category Q5, e.g. Morris, 1991). A larger impact of atmospheric N deposition is expected in systems with a closed N cycle. This, of course, is especially clear in the case of ombrotrophic bogs (EUNIS Q1), which receive the majority of their nutrients from the atmosphere. In addition, attention should be paid to the existing hydrological state of the studied wetlands, as decreasing water tables may enhance decomposition and thus nutrient availability and allow encroachment by shrubs, whereas in areas of higher water table, hydrology may constrain responses to N.

The following chapter builds on the last review of $CL_{emp}N$ in 2011 (Bobbink and Hettelingh, 2011) and adds a synthesis of information published since this review to determine retention or revision of the $CL_{emp}N$ values. Most research undertaken to date has been on raised and blanket bogs (Q1), which is the focus of much of this chapter. However, we also cover valley mires, poor-fens and transition mires (Q2), base-rich fens and calcareous spring mires (Q4), and for the first time, we are able to provide a $CL_{emp}N$ for palsa and polygon mires (Q3).

6.2 Raised and blanket bogs (Q1)

Introduction

Ombrotrophic (raised) and blanket bogs are nutrient-poor habitats that receive all their nutrients from the atmosphere, and therefore are particularly sensitive to airborne N deposition. These bogs are systems of acidic, wet areas and are very common in the boreal and temperate regions of Europe. Due to a high-water table and often anaerobic conditions, decomposition rates are low, favouring the development of peat. Typical plant species of bogs include peat mosses (*Sphagnum*), sedges (*Carex*, *Eriophorum*) and ericaceous plants (*Andromeda*, *Calluna* and *Erica*). Within the EUNIS system, these communities have been classified under Q1 (raised and blanket bogs) for which the criterion is that precipitation is their continuous or primary water supply. EUNIS category Q1 is subdivided into raised bogs (Q11) and blanket bogs (Q12). Raised bogs are highly oligotrophic, strongly acidic peatlands with a raised centre from which water drains towards the edges. Blanket bogs are formed on flat or gently sloping grounds with poor surface drainage, in oceanic climates with high levels of precipitation (north-western Europe) (Davies et al., 2004). For the purpose of critical load definition, there is no basis on which to differentiate between raised and blanket bogs. Both bog types support the same Ericoid vegetation, and both are acidic and oligotrophic which suits the formation of carpets of *Sphagnum* spp. and hummocks of *Eriophorum vaginatum*.

The current $CL_{emp}N$ for ombrotrophic and blanket bogs is between 5-10 kg ha⁻¹ yr⁻¹ based on evidence gathered up to 2011. Since the last update of the $CL_{emp}N$ for ombrotrophic and blanket bogs (Bobbink and Hettelingh, 2011), a significant number of publications on the effects of N on bogs have appeared. The results from these studies confirm the importance of *Sphagnum* mosses

for the immobilisation of N; when *Sphagnum* becomes saturated, N availability increases for vascular plants, leading to increased vascular plant biomass (Berendse et al., 2001; Bobbink and Hettelingh, 2011) and shifts in species composition. Experiments with realistic additions of N that are within the range of the $CL_{emp}N$ (5-10 kg N ha⁻¹ yr⁻¹) and that may be used to validate the existing critical load, are still limited. However, evidence is emerging from experimental N addition studies from Scotland (addition levels from 8-56 kg N ha⁻¹ yr⁻¹) and Canada (addition levels from 5-25 kg N ha⁻¹ yr⁻¹ in a low-N area (bulk [wet] deposition < 2 kg N ha⁻¹ yr⁻¹)), as well as gradient studies from Canada, Ireland, Norway and the UK that support experimental responses.

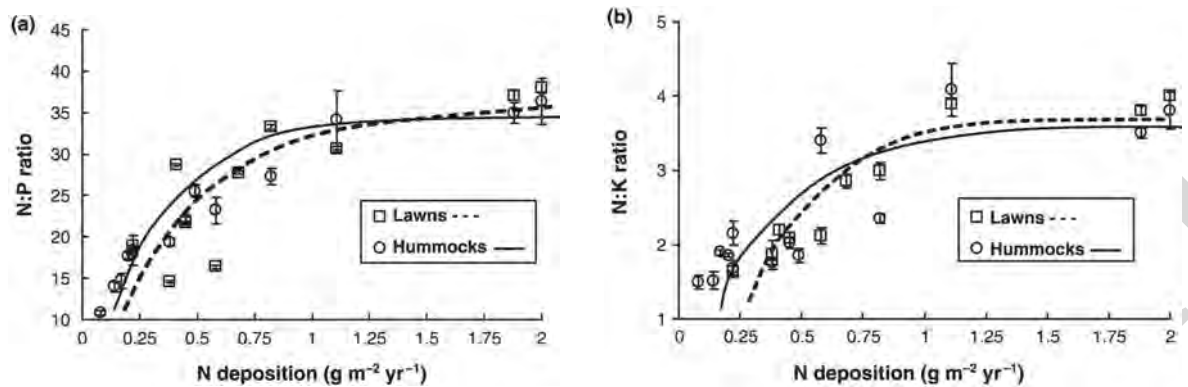
Physiological responses in bryophytes and lichens

This section on physiological responses draws heavily on work captured during the previous review of $CL_{emp}N$ in 2011, however, recent work from Canada and northern Europe is incorporated alongside the earlier body of evidence. Several studies on British bogs have shown that increased supplies of N are rapidly absorbed and utilised by bog mosses (*Sphagnum* species), reflecting the significance of N deposition as a nutrient and its scarcity in unpolluted regions (e.g. Press et al., 1988). High N loadings, however, are supra-optimal for the growth of many characteristic *Sphagnum* species, as demonstrated by restricted development in growth experiments and transplantation studies between clean and polluted locations. In areas with high N loads such as the Pennines, the growth of *Sphagnum* in general was lower than in unpolluted areas (Lee and Studholme, 1992). After transplantation of *Sphagnum* from an 'unpolluted' site to a bog in the southern Pennines, a rapid increase in N content from ca 12 to 20 mg g⁻¹ dry weight was observed (Press et al., 1988). Furthermore, a large increase in N-containing amino acids (arginine) in the shoots of these bog mosses was found after application of N, indicating nutritional imbalance in this species.

At Whim Moss, Scotland, *Sphagnum papillosum* tissue N content increased in response to N additions of 8 kg N ha⁻¹ yr⁻¹ on top of ambient N deposition of around 8-11 kg N ha⁻¹ yr⁻¹ (Millet et al., 2012). The same experiment also revealed tissue stoichiometry responses in the lichen *Cladonia portentosa*, albeit to higher levels of N addition (+56 kg N ha⁻¹ yr⁻¹; Hogan et al., 2010) and changes in proteins responsible for physiological processes such as respiration and photosynthesis (Munzi et al., 2017; 2020), highlighting a potential underlying response mechanism. The work highlighted that elevated N levels can also shift nutrient limitation to phosphorus (P) availability (Hogan et al., 2010) as found in earlier studies (e.g. Aerts et al., 1992; Gunnarsson and Rydin, 2000).

Bragazza et al. (2004) determined nutrient limitation of ombrotrophic *Sphagnum* plants across a natural gradient of bulk atmospheric N deposition ranging from 1 to 20 kg N ha⁻¹ yr⁻¹ in Europe. Nutrient ratios increased steeply at low atmospheric input, but above a threshold of ~10 kg N ha⁻¹ yr⁻¹ the N:P and N:K ratios tended to saturate (Figure 6.1) and *Sphagnum* growth changed from being N limited to K and P co-limited.

Figure 6.1. Mean values (± 1 SE) of (a) N: P and (b) N: K ratios in hummock and lawn *Sphagnum* plants across a natural gradient of bulk atmospheric N deposition ranging from 1 to 20 kg ha⁻¹ yr⁻¹ in Europe (1 g m⁻² yr⁻¹ is equal to 10 kg ha⁻¹ yr⁻¹). Dashed and continuous lines represent the theoretical patterns based on regression model calculations (Bragazza et al., 2004).



Source: Bragazza et al., 2004

Phuyal et al. (2008) studied the effects of increased atmospheric N deposition on phosphatase activity in *Sphagnum capillifolium* tissue in the lowland ombrotrophic bog at Whim Moss (Scotland) over a four-year period. Phosphatase activity (an enzyme that catalyses the cleavage of inorganic P from organic P compounds) of *Sphagnum capillifolium* was found to be significantly increased by addition of 56 kg N ha⁻¹ yr⁻¹ in both ammonium (NH₄Cl) and nitrate (NaNO₃) treatments, ambient N deposition was around 8 kg ha⁻¹ yr⁻¹. Phosphatase activity was found to be positively related with tissue N and negatively related to tissue P concentrations. This implies that, when N starts to accumulate in *Sphagnum* tissue, the relative availability of P may be (temporarily) increased by increasing phosphatase activity, leading to increased growth and thereby dilution of tissue N concentration.

Although these and other studies strongly indicate the detrimental effects of high N deposition rates on the development of lichens and bog-forming *Sphagnum* species, there is also evidence of growth stimulation in response to small increments in N deposition. Field experiments by Aerts et al. (1992) at a site with low atmospheric deposition (0.4 kg N ha⁻¹ yr⁻¹) in northern Sweden showed that *Sphagnum balticum* increased its growth four-fold within three years of N addition (20 and 40 kg N ha⁻¹ yr⁻¹), whilst no effect was found on *Sphagnum magellanicum*² at southern Sweden sites with higher atmospheric deposition (7-9 kg N ha⁻¹ yr⁻¹). Responses at very low atmospheric deposition of N have also been observed in lichens including the epiphyte *Evernia mesomorpha* which, in a Canadian study, showed increases in chlorophyll fluorescence as N deposition increased above a background of 1.3 kg N ha⁻¹ yr⁻¹ (Vitt et al., 2020).

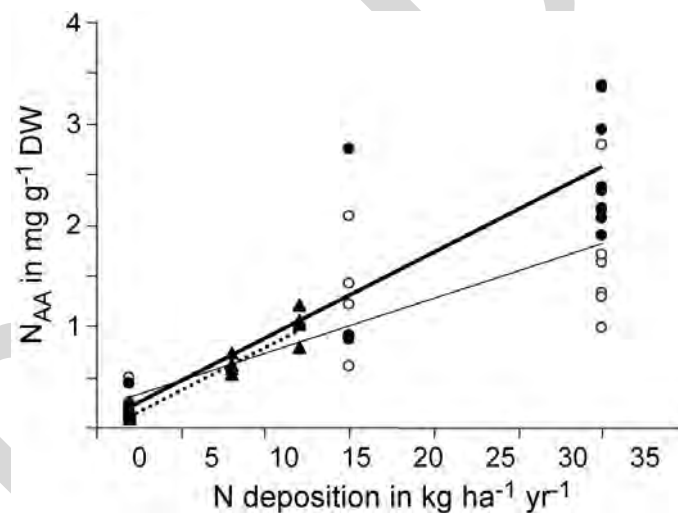
A three-year field manipulation experiment used a range of N addition rates (0, 10, 30, 50, 100 kg N ha⁻¹ yr⁻¹) at two Swedish mires with differing existing atmospheric loads (Gunnarsson and Rydin, 2000; Nordin and Gunnarsson, 2000). N:P ratios and experimental results confirmed that *Sphagnum* growth at the site in central Sweden, with very low ambient deposition (~3-4 kg N background), was N limited, while the southern site (~7-11 kg N background) was P limited (Gunnarsson and Rydin, 2000). After two years, the experimental addition of N increased free amino acid concentrations in *Sphagnum* capitula, whereas it decreased *Sphagnum* extension growth at deposition rates of 30 kg N ha⁻¹ yr⁻¹ and over. However, at low N deposition rates

² *Sphagnum magellanicum* has recently been split into three species: *S. magellanicum*, *S. divinum* and *S. medium* (Hassel et al., 2018), however, it is not possible to determine which of these specific species in this review of critical loads are referred to hence we used the collective name '*S. magellanicum*'.

(lower than 7-11 kg N ha⁻¹ yr⁻¹), no correlation was observed between *Sphagnum* total amino acid N concentrations and growth rates (Nordin and Gunnarsson, 2000). After three years of treatment, biomass production and length increment decreased, while capitulum dry mass increased, with increasing N addition. This experiment included a treatment of 10 kg N ha⁻¹ yr⁻¹, but the biomass and length responses showed a steady decline from the control treatment, especially in areas dominated by *Sphagnum rubellum*. Thus, even if the control and 10 kg N ha⁻¹ yr⁻¹ treatments are not statistically distinguished, these data suggest a threshold for effects on such a bog community exists below 10 kg N ha⁻¹ yr⁻¹.

Accumulation of free amino acids in *Sphagnum* capitula was found in several other studies. Tomassen et al., (2003) treated transplanted turfs from an ombrotrophic floating bog with a range of N deposition rates in the laboratory over three years. Tissue N concentrations in *Sphagnum fallax* showed a linear response to the experimental N addition. Excess N was accumulated as N-rich free amino acids, starting already at very low N addition rates (> 2.5 kg ha⁻¹ yr⁻¹), indicating N saturation. Wiedermann et al., (2009b) also concluded that N accumulation in the form of free amino acids (N_{AA}) is a sensitive indicator signalling N saturation and future shifts in vegetation composition, although no clear relationship between this indicator and a shift in plant diversity could be drawn (Figure 6.2).

Figure 6.2. The relationship between soluble amino acid N tissue content (mg g⁻¹ DW) and N deposition (kg N ha⁻¹ yr⁻¹) for *S. balticum* samples from a gradient study (filled triangles, dashed line), and from a field experiment in 1997 (open circles, thin line) and 2004 (filled circles, solid line) (Wiedermann et al., 2009b).



Source: Wiedermann et al., 2009b

The linear increase in total N and amino acid N concentrations in lichen tissue paralleling increased N deposition (Figure 6.2) raises the question whether or not *Sphagnum* species are capable of adjusting to high N supply through N uptake regulation. Wiedermann et al. (2009a) exposed *S. balticum* and *S. fuscum* from three peatland sites differing in N deposition (2, 8 and 12 kg N ha⁻¹ yr⁻¹) to different N forms labelled with 15N (15NH₄⁺, 15NO₃⁻ and the amino acids [15N] alanine and [15N] glutamic acid). All the forms of applied N were taken up by both *Sphagnum* species. Uptake rates were highest for NH₄⁺, followed by alanine and glutamic acid, with only very small amounts of NO₃⁻ taken up. At the site with the highest background deposition (12 kg N ha⁻¹ yr⁻¹) N uptake was lower compared to the two other sites (2 and 8 kg N ha⁻¹ yr⁻¹). The potential of *Sphagnum* to adjust to high N exposure through N uptake regulation

will not prevent tissue N accumulation but is likely to delay the toxic effects of high tissue NH_4^+ concentrations.

Increased pathogen infections in *Sphagnum* have also been linked to N. Following three consecutive years of N fertilisation in field experiments in Ireland and the Netherlands, Limpens et al., (2003b) observed increased fungal infections, caused by the parasite *Lyophyllum palustre* (a basidiomycete fungus). Total background N deposition in these experiments ranged from 15 (Irish site) to 37 (Dutch sites) $\text{kg N ha}^{-1} \text{yr}^{-1}$, with additions of 40 $\text{kg N ha}^{-1} \text{yr}^{-1}$ in N treatments. In a subsequent greenhouse experiment, they inoculated *Sphagnum* to verify that the necrosis found had indeed been caused by *Lyophyllum palustre* and was related to tissue N concentration. This experiment confirmed that *Lyophyllum palustre* was responsible for the necrosis or defoliation of *Sphagnum* and disease severity was related to the N concentration in the capitula (Limpens et al., 2003b).

In a north–south transplant experiment, covering a latitudinal N deposition gradient across northern Europe ranging from 2.8 $\text{kg ha}^{-1} \text{yr}^{-1}$ in the north to 14.9 $\text{kg ha}^{-1} \text{yr}^{-1}$ in the south, Granath et al. (2009a) measured photosynthetic responses to increasing N deposition in *Sphagnum balticum* and *Sphagnum fuscum*. The maximum photosynthetic rate increased southwards and was mainly explained by tissue N concentrations. For *Sphagnum fuscum* photosynthetic rate continued to increase up to a deposition level of 14.9 $\text{kg N ha}^{-1} \text{yr}^{-1}$, whereas for *Sphagnum balticum* this seemed to level out at 11.4 $\text{kg N ha}^{-1} \text{yr}^{-1}$. The results suggest that *Sphagnum* species may be able to adapt or physiologically adjust to higher N depositions, and that *Sphagnum balticum* might be more sensitive to N deposition than *Sphagnum fuscum*. The maximum photosynthetic rate was not (*Sphagnum balticum*) or only weakly (*Sphagnum fuscum*) correlated with biomass production, indicating that production is largely governed by factors other than photosynthetic capacity (Granath et al., 2009a).

However, in a 12-year field fertilisation experiment in Sweden. The maximum photosynthetic rate in *Sphagnum balticum* did not differ between the control (2 $\text{kg ha}^{-1} \text{yr}^{-1}$) and the high N treatment (30 $\text{kg ha}^{-1} \text{yr}^{-1}$) but was higher for the middle N treatment (15 $\text{kg ha}^{-1} \text{yr}^{-1}$) (Granath et al., 2009b). The optimum tissue N concentration for photosynthetic rate in *S. balticum* was found to be $\approx 13 \text{ mg N g}^{-1}$. Granath et al., (2009b) suggest that negative effects on *Sphagnum* productivity under high N deposition are not related to negative effects on the photosynthetic apparatus. However, differences in optimum N concentration levels between the various *Sphagnum* species may affect their competitive ability under different N deposition regimes. In a subsequent greenhouse experiment, increases in tissue N concentrations were observed when a range of N between 0 and 5.6 $\text{kg N ha}^{-1} \text{yr}^{-1}$ were added to plants collected from a background N deposition area of around 2 $\text{kg N ha}^{-1} \text{yr}^{-1}$ (Granath et al., 2012). *Sphagnum balticum* showed the same unimodal response as the field experiment, whilst productivity of *S. fallax* and *S. fuscum* declined with the authors suggesting P limitation.

For the assessment of CL_{empN} , these pot or microcosm studies, generally, are not accepted, except for bryophyte layer studies (which are relevant for bogs). We have focused particularly on statistically and biologically significant outcomes of field addition experiments and mesocosm studies. Wiedermann et al. (2009b) examined whether small-scale field experiments could predict the direction and magnitude of ecosystem responses to increased N supply. In order to do so, they compared data from a ten-year field experiment [involving deposition of 2 (ambient), 15 and 30 $\text{kg N ha}^{-1} \text{yr}^{-1}$] with field data from sites representing a gradient of increasing N deposition (2, 8 and 12 $\text{kg N ha}^{-1} \text{yr}^{-1}$). They found a highly significant correlation between the two data sets which was attributed to the key function of *Sphagnum* species that monopolise N availability and control the water balance, creating an environment hostile to

vascular plants (Wiedermann et al., 2009b). These results support the use of data from small-scale experiments on the effects of N deposition on vegetation dominated by bryophytes.

Effects on bryophytes and lichen species composition

This section on species composition summarises earlier work included during the previous review of $CL_{emp}N$ in 2011 and brings in more recent experiment work from Canada and Scotland. Nitrogen has differential effects on the growth of different *Sphagnum* species that causes changes in species composition across the N deposition gradient; this has been demonstrated in several experimental studies. For example, in an early controlled-environment experiment, Risager (1998) examined the growth responses of *Sphagnum fallax* at low N addition rates (0, 5, 10 and 20 kg N ha⁻¹ yr⁻¹). Growth was significantly stimulated by the application of N (especially with 5 and 10 kg NH₄-N ha⁻¹ yr⁻¹), but because of the growth dilution effect, tissue N concentration did not change. Risager (1998) also investigated the responses of *Sphagnum magellanicum* after addition of N in a comparable study involving similar levels of N addition, *Sphagnum magellanicum* showed no increase in height, but addition of N decreased the production density of capitula. In contrast to *Sphagnum fallax*, the tissue N concentration of *Sphagnum magellanicum* increased with increasing additions of N. In both cases, uptake of NO₃⁻ was considerably lower than of NH₄⁺. Risager (1998) concluded that the form of N is also important (see later in this chapter) in species response, and that increased N availability may cause shifts in species composition in favour of *Sphagnum fallax* at higher N deposition levels. This expansion of *Sphagnum fallax* under high N deposition rates was studied in a field experiment at sites with low and high background deposition of N (Limpens et al., 2003c). At the low N deposition site *Sphagnum fallax* area expanded when extra N (40 kg ha⁻¹ yr⁻¹) was applied. At the high N deposition sites such expansion was limited by P. The authors concluded that *Sphagnum fallax* will gradually colonise an increasing number of new habitats in areas with low, albeit increasing, N deposition, but may only grow to dominate when P supply is adequate (Limpens et al., 2003c).

Clear effects of N eutrophication have also been observed in Dutch ombrotrophic bogs. The composition of the moss layer in small remnants of formerly large bog areas has changed markedly as N loads increased to between 20 and 40 kg N ha⁻¹ yr⁻¹, especially in the form of ammonium/ammonia; the most characteristic *Sphagnum* species were replaced by more nitrophilous moss species (Greven, 1992). These dramatic changes in species composition were also observed in British bogs (e.g. Lee and Studholme, 1992); many characteristic *Sphagnum* species were eradicated from affected ombrotrophic bog areas, such as those in the South Pennines in England, where atmospheric N deposition increased to over 30 kg N ha⁻¹ yr⁻¹ following many decades of elevated sulphur deposition.

More recent experimental work at the low end of the N range (bulk [wet] deposition < 2 kg N ha⁻¹ yr⁻¹) in Canada observed responses in different species to N additions of 5, 10, 15 and 20 kg N ha⁻¹ yr⁻¹ over five years. Initially, *Sphagnum fuscum* growth responded positively to N, then declined. It also decreased in abundance whereas *S. magellanicum* increased. No effects were observed on *S. angustifolium*. The authors recommended a $CL_{emp}N$ of 3 kg N ha⁻¹ yr⁻¹ (Wieder et al., 2019).

Bog micro-habitat may also be important in determining species responses. A two-year German N addition experiment showed such effects on species composition in an established *Sphagnum* community (Lütke Twenhöven, 1992) in the field. In bog hollows, *Sphagnum fallax* was significantly promoted by the addition of both nitrate and ammonium (10 kg N ha⁻¹ yr⁻¹ with an estimated ambient atmospheric deposition of 5 kg N ha⁻¹ yr⁻¹), but less so on the bog lawns. This resulted in *Sphagnum fallax* outcompeting *Sphagnum magellanicum* in the hollows and, when

water supply was sufficient, also on the lawns. However, on the hummocks in the bog, nitrate and, to a lesser extent, ammonium reduced the growth of both species. Results from Gunnarsson and Rydin (2000) also suggest that lawn communities are less vulnerable to increased N deposition than hummock communities. Because of the differences in vegetation structure, the rate of supply of N to a hummock community dominated by dwarf shrubs is about 40% greater than to a lawn community (Bobbink et al., 1992; Malmer and Wallén, 1999).

Sphagnum capillifolium is often associated with good quality bogs and its vitality has been observed to suffer at 16 kg N ha⁻¹ yr⁻¹ (background was 8 kg N ha⁻¹ yr⁻¹) at Whim Moss, Scotland (differential responses of N form were observed - see section Effect of nitrogen form) (Sheppard et al., 2011). Declines in lichens were also observed at 16 kg N ha⁻¹ yr⁻¹ (Phoenix et al., 2012), and the pleurocarpous bryophyte *Pleurozium schreberi* declined from 16 kg N ha⁻¹ yr⁻¹ and was eradicated by 64 kg N ha⁻¹ yr⁻¹ (Sheppard et al., 2014) whilst in contrast, *Hypnum jutlandicum* increased at 16, 32 and 64 N ha⁻¹ yr⁻¹ to oxidised N addition.

The importance of competition between moss species is also indicated by the earlier study of Mitchell et al. (2002) using higher rates of N deposition. This experiment examined the effect of an addition of 30 kg N ha⁻¹ yr⁻¹ in a cutover bog in the Jura Mountains of Switzerland, where ambient deposition was estimated to be around 15 kg N ha⁻¹ yr⁻¹. The normal pattern of succession in the restoration of these sites is that keystone species such as the moss *Polytrichum strictum* create favourable micro- environments for the establishment of *Sphagnum fallax* and hence of typical bog vegetation. However, three years of study showed that cover and density of *Polytrichum strictum* was strongly increased while cover and density of *Sphagnum fallax* decreased; *Polytrichum* was observed to overgrow the *Sphagnum* which might prevent the typical regeneration process in central European bogs. Thus, in the study period, biomass production of *P. strictum* increased by almost 75% in response to N addition, whereas production of *Sphagnum fallax* decreased by close to 50% (Mitchell et al., 2002).

Effects on vascular plants and community composition

This section synthesises information on N effects on vascular plant growth and species composition, it builds on work included in the previous reviews of critical loads by incorporating evidence from N addition experiments in Canada and Scotland. A Danish survey of national ombrotrophic bogs showed a decline in the original bog vegetation together with an increase in more N-demanding grass species (such as *Molinia caerulea* and *Deschampsia flexuosa*) and trees (such as *Betula pubescens*) in areas with wet ammonium (NH₄⁺) deposition loads of more than 10 to 15 kg N ha⁻¹ yr⁻¹ (Aaby, 1994), together with increased NH₃ concentrations, rapidly deposited to ombrotrophic mires. For a bog in southern Sweden, in a region with an ambient N deposition of 7 to 9 kg ha⁻¹ yr⁻¹, Gunnarsson et al. (2002) showed that the total number of species (vascular plants + mosses) per plot did not change much between 1954 and 1997. However, there were large changes in species composition that were indicative particularly of a drier mire surface and an increased availability of N. The increased growth of trees may also have triggered further changes in plant cover.

Responses from other gradient surveys are discussed later in this chapter, however, such observations highlight that increased N deposition may influence the competitive relationships between mosses and vascular plants in nutrient-deficient vegetation such as bogs. For mineral nutrients, interactions between vascular plants and mosses are partly unbalanced as *Sphagnum* mosses rely only on atmospheric supply while vascular plants also rely on mineralisation (Malmer et al., 1994). Thus, in a field experiment in southern Sweden (ambient N deposition rate 7-9 kg ha⁻¹ yr⁻¹) a supply of both N and P (20 and 4 kg ha⁻¹ yr⁻¹, respectively) only affected the

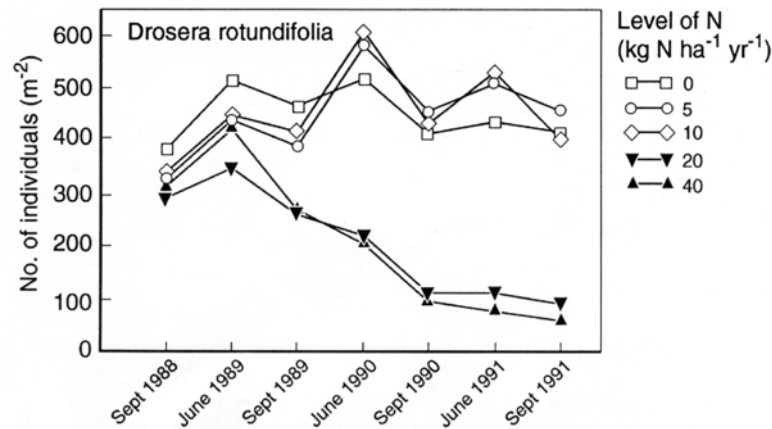
growth of the mosses if N and P were applied on the surface, and only affected the vascular plants if applied below the moss surface.

To determine the N available to quantitatively important boreal bog plants, Nordbakken et al. (2003) measured the $\delta^{15}\text{N}$ signature after a three-year N addition experiment in a Norwegian bog with an estimated total N background deposition of $7.9 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. An addition of only $5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ was sufficient to significantly increase the N concentration in *Sphagnum* mosses, liverworts and shallow rooted vascular plants. An addition of $40 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, however, was not sufficient to increase the N concentration in deep rooted plants even after three years of N addition. Over the course of three years, the *Sphagnum* layer was able to completely immobilise the relatively high N load of $40 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ and is therefore, likely to have significant effects on the N availability to vascular plants. However, an earlier synthesis of *Sphagnum* tissue N concentrations across a number of studies covering a gradient of N deposition, suggested saturation and failure of the 'natural nitrogen filter' at above $20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Lamers et al., 2000). This highlights the importance of long-term experiments in reproducing real-world effects and that experiments of short durations at higher N loads may not produce effects observed in the real world over a longer period at lower N. N accumulation and saturation are therefore important.

These responses were also demonstrated experimentally by Heijmans et al. (2001), who studied the effect of added N deposition ($50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) at an ambient atmospheric deposition of the same magnitude, on peat monoliths taken from a mire in the northern Netherlands. The N:P ratio in the mosses indicated P limitation (24:1), which corresponded with the observed lack in growth response to N addition. The mosses were still able to capture a large part of the deposited N (Heijmans et al., 2002a) and three years after the start of the experiment, all species showed increased N concentrations. The mosses showed decreased height increment, but no changes in dry matter production, indicating an effect on moss morphology (Heijmans et al., 2001). The fertilised mesocosms showed a significantly higher N uptake by deep-rooting vascular plants (based on ^{15}N enrichment; Heijmans et al., 2002a), but only the growth, in terms of cover area, of *Vaccinium oxycoccus* had increased significantly. There was a negative relationship between litter (also increased by N) and vascular plant cover, on the one hand, and *Sphagnum* species, on the other hand (Heijmans et al., 2001), suggesting that *Sphagnum* growth might also be limited by increased shading.

Sphagnum mosses are not the only plants that encounter negative effects from N deposition due to changes in competitive interactions with vascular plants. The effects of the supply of extra N on the population ecology of *Drosera rotundifolia* were studied in a four-year fertilisation experiment in Swedish ombrotrophic bogs, using a range of deposition rates (Redbo-Torstensson, 1994). It was demonstrated that experimental applications of $20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (as NH_4NO_3 above an ambient deposition of $5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) significantly reduced the survivorship of the plants after one year, and also negatively affected flowering after two years (Figure 6.3). The N driven decrease in the population density of the characteristic carnivorous bog species *Drosera* was associated with increased density of tall species such as *Eriophorum* and *Andromeda*, which resulted in increased competition for light and further reduction in *Drosera*.

Figure 6.3. Numbers of individuals of *Drosera rotundifolia* in an ombrotrophic raised bog (D1) near Stockholm (Sweden) during three years of N additions (Redbo-Torstensson, 1994).



Source: Redbo-Torstensson, 1994

Limpens et al. (2004) conducted a three-year N fertilisation experiment (+40 kg ha⁻¹ yr⁻¹) at six sites; one with moderate N deposition and five with high N deposition. Adding N increased the concentration of inorganic N in the rhizosphere at the site with moderate deposition and at two of the sites with high deposition. The addition of N depressed *Sphagnum* height increment but shading by vascular plants was of minor importance in explaining the negative effects of N on *Sphagnum*. P alleviates the negative impact that N has on *Sphagnum* by enhancing its capability to assimilate the deposited N. P availability is therefore a major factor determining the impact of deposition on *Sphagnum* production and, thus, on carbon sequestration in bogs (Limpens et al., 2004).

Gerdol et al. (2007) studied the effect of N addition over a period of four years in an Italian bog. *Sphagnum* production was depressed by high levels (30 kg ha⁻¹ yr⁻¹) of N addition, but not at an intermediate level of 10 kg ha⁻¹ yr⁻¹. Vascular plant cover increased at the expense of *Sphagnum* mosses, but this was probably triggered by an exceptional heat wave in one of the summer periods (year 2003; Gerdol et al., 2008). A proportionally greater accumulation of vascular plant litter, together with an increased potential decay of *Sphagnum* litter, may result in decreased carbon fixation. Gunnarsson et al. (2008) also suggested that one of the main causes of the low carbon input rates to the peat layer was the high level of N deposition, which increased decomposition and changed the vegetation from peat-forming *Sphagnum*-dominance to dominance by dwarf shrubs and graminoids.

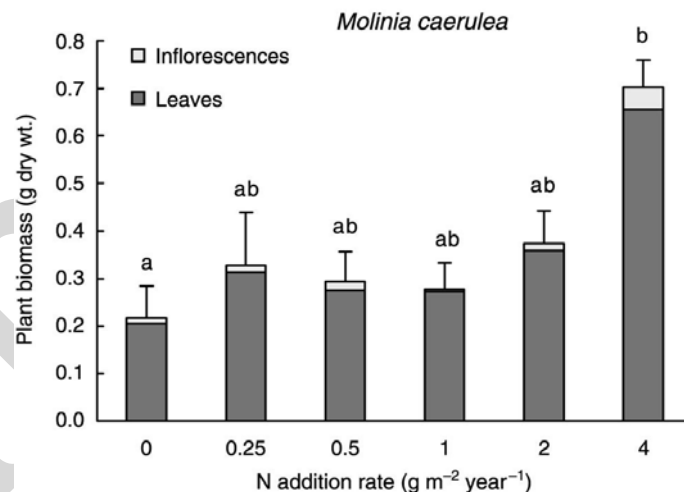
There is information about the effects of N addition on species composition at high levels of deposition. In a greenhouse competition experiment between two graminoid species, *Eriophorum vaginatum* and *Rhynchospora alba*, and two ericoid species, *Calluna vulgaris* and *Vaccinium oxycoccus*, Kool and Heijmans (2009) found that at a high N supply (50 kg ha⁻¹ yr⁻¹), ericoid species responded more strongly than graminoids. This suggests that under increased N availability, bogs could potentially turn into dwarf-shrub-dominated ecosystems than into grasslands. However, the balance between the functional groups is likely to be also mediated by hydrology, with *Calluna* favouring drier sites and sedges, wetter areas - in another greenhouse study, Heijmans et al. (2002b) found that *Rhynchospora alba* became the dominant vascular plant species at a high N deposition of 50 kg ha⁻¹ yr⁻¹.

Experimental work at Whim Moss, Scotland, showed increases of *Eriophorum vaginatum* at high levels of N (64 kg N ha⁻¹ yr⁻¹) at the expense of *Calluna vulgaris*. In the early stages of the

experiment, other shrubs including *Erica tetralix*, *Vaccinium myrtillus* and *Empetrum nigrum* all responded positively to N as a result of the *Calluna* canopy opening (Phoenix et al., 2012), although after seven years the positive increase in *E. tetralix* had subsided and other ericoids had declined (Sheppard et al., 2014). The nitrophilous fern *Dryopteris dilatata* became invasive at a high rate of 64 kg N ha⁻¹ yr⁻¹ (Sheppard et al., 2011). N form effects were observed and are discussed in section Effect of nitrogen form. The potential negative effects of N to *Erica tetralix* were also observed in Danish wet heath where in these drier conditions, *Calluna* did well (Strandberg et al., 2012). Acidification due to N was determined as the cause.

In Dutch and Danish bogs, the first signs of the negative effect of high atmospheric N deposition of around 40 kg were invasions of the graminoid species *Molinia caerulea* and tree species such as *Betula pubescens* and *Pinus* species (e.g. Aaby, 1994; Tomassen et al., 2002). In order to confirm the hypothesis that high atmospheric N loads had triggered the observed vegetation changes, Tomassen et al. (2003) studied the effects of N on *Molinia caerulea* and *Betula pubescens* in a three-year N addition laboratory experiment. *Betula pubescens* and *Molinia caerulea* plants were introduced into transplanted turfs from an ombrotrophic floating bog in the Netherlands, and were treated with various N deposition rates. After three years, above-ground biomass of *Molinia caerulea* plants was significantly higher in the turfs that received 40 kg N ha⁻¹ yr⁻¹ (Figure 6.4). *Betula pubescens* was unable to increase its above-ground biomass, probably due to P limitation.

Figure 6.4. Individual above-ground biomass of *Molinia caerulea* after three years at different rates of experimental N addition (means \pm 1 SE; n = 4). Different letters indicate significant differences ($P < 0.05$) between N treatments (one-way ANOVA) (Tomassen et al., 2003). Discussion in text has converted to kg ha⁻¹ by multiplying g m⁻² by 10.



Source: Tomassen et al., 2003

In addition, Tomassen et al. (2004) studied the effects of N on *Molinia caerulea* and *Betula pubescens* in a three-year N addition experiment in an Irish raised bog. The water table at the experimental site had been drastically reduced by peat cutting activities in the past. Tomassen et al. (2004) concluded that the invasion of *Molinia* and *Betula* in bogs is likely to be less affected by desiccation than by increased N availability. *Molinia* is known to be well adapted to P-limiting conditions (Ellenberg, 1988; Kirkham, 2001), which may explain its success in regions with increased N deposition.

Limpens et al. (2003a) examined the effects of N deposition on the competition between shrubs and mosses and the establishment and growth of the invasive *Betula pubescens* and *Molinia caerulea* in intact bog vegetation removed from a site subject to $40 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. *Molinia* biomass was positively related to the inorganic N concentration in the interstitial water which was positively affected by N additions. N deposition increased the N availability to vascular plants in the rhizosphere, thus encouraging vascular plant growth. Water-table level and availability of P were found to be important in explaining species-specific responses to N deposition (Limpens et al., 2003a).

Shifts in species composition have also been observed much lower down the N range (bulk deposition $< 2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) at a bog N addition experiment in Alberta, Canada, where increased N above background produced an increase in shrub cover and vascular plants in general (Wieder et al. 2019). The authors recommended a CL_{empN} of $3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ and commented that responses above background N had no clear threshold but were linear across the N deposition range, however, it is important to note that, depending on the relative contribution of dry N deposition, bulk deposition may underestimate total N deposition, and this would mean their critical load should be slightly higher.

Whilst peatlands in Alberta receive relatively low N deposition compared to most of Europe, bogs in northern and western Europe in Finland, Norway, Sweden, Ireland, and areas of the UK are at these low levels of N.

Effect of nitrogen form

Recently, several studies have been focussed on the effects of different forms of N on bog vegetation. In most fertilisation experiments N is added as NH_4NO_3 , reflecting the current ratio of reduced and oxidised N in precipitation (e.g. Boxman et al., 2008), but a number of experiments have studied the effects of ammonium (NH_4Cl) and nitrate (NaNO_3), separately. As discussed earlier, the uptake of nitrate by *Sphagnum* mosses is much lower than the uptake of ammonium (Risager, 1998; Phuyal et al., 2008; Wiedermann et al., 2009a), something that could lead to different effects on vegetation.

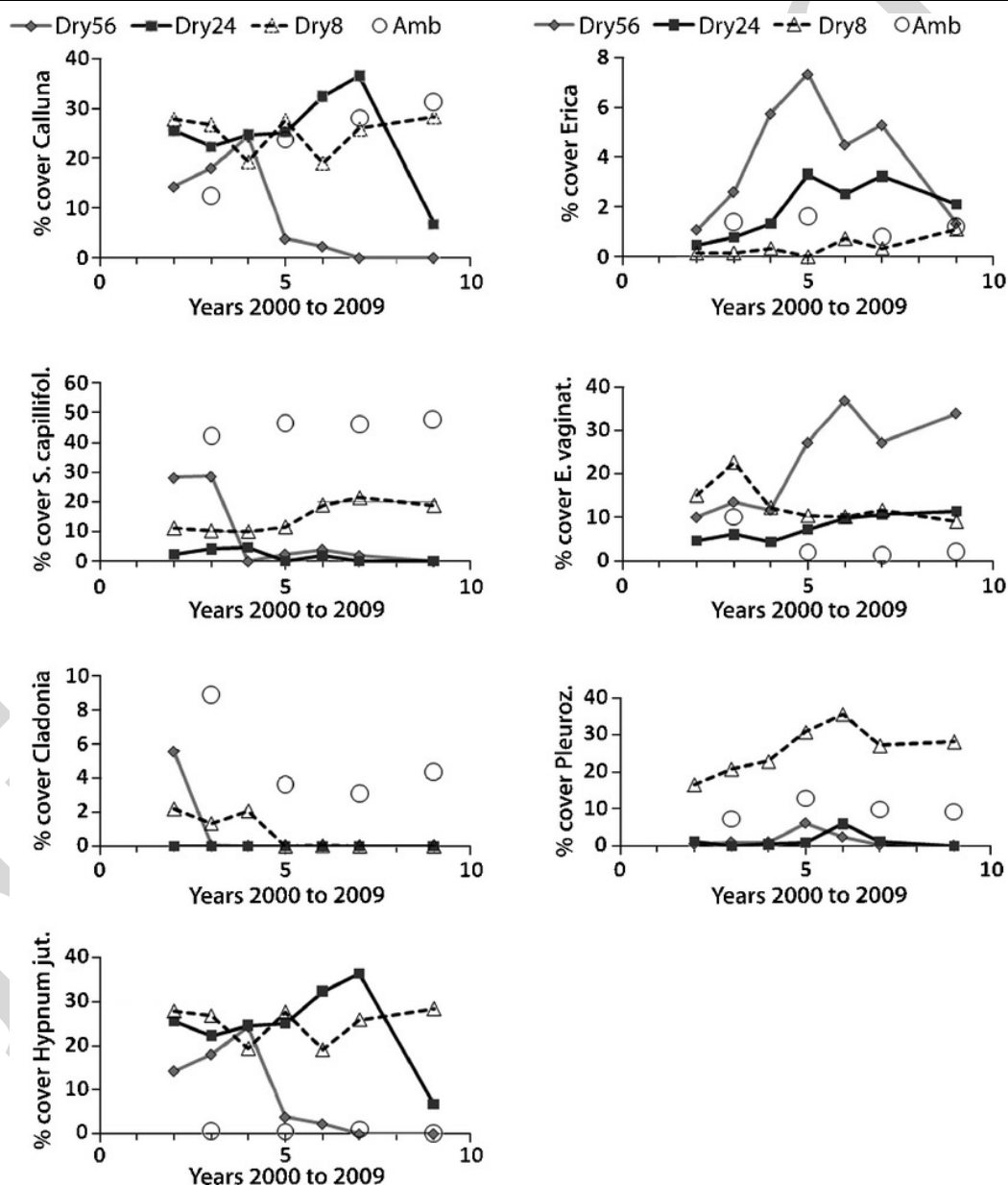
In an early controlled-environment experiment, Risager (1998) examined the growth responses of *Sphagnum fallax* to different forms of N (NO_3^- , NH_4^+ or NH_4NO_3) at low addition rates (0, 5, 10 and $20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). This growth was significantly stimulated by the application of reduced N only, especially with 5 and $10 \text{ kg NH}_4\text{-N ha}^{-1} \text{ yr}^{-1}$.

Differential evidence of N uptake is also evident outside of manipulation experiments. Bragazza et al. (2005) determined the $\delta^{15}\text{N}$ isotopic signatures of *Sphagnum* plants collected from sites with an ambient N deposition ranging from 2 to $20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. The $\delta^{15}\text{N}$ isotopic signatures were found to be more related to the ratio of reduced to oxidised N forms in atmospheric deposition than to the total amount of atmospheric N deposition, indicating that $\delta^{15}\text{N}$ signatures can be used as an integrated measure of $\delta^{15}\text{N}$ signature of atmospheric precipitation. Bogs located in areas dominated by NH_3 emissions had *Sphagnum* plants with a more negative $\delta^{15}\text{N}$ signature, compared to areas dominated by NO_x emissions (Bragazza et al., 2005).

At Whim Moss, Scotland, a large-scale, automated N addition field experiment with different forms of N (ammonium and nitrate) at 8 (background), 16 and $64 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ was started in 2002 (Sheppard et al., 2004). Within one or two years of increased N input, branching and the height of photosynthetically active material of *Sphagnum capillifolium* were reduced. The effect was more pronounced when the water table was low, but responses did not differ between oxidised and reduced forms of N addition (Carfrae et al., 2007). The experiment also contains a gaseous ammonia (NH_3) release to mimic an intensive farm where rapid responses in vegetation were observed following the start of the experiment including the total loss of *Calluna vulgaris*,

Sphagnum capillifolium and *Cladonia portentosa* at a high 56 kg N ha⁻¹ yr⁻¹ dry NH₃ deposition (calculated from NH₃ concentration data) on top of ambient deposition of 8-11 kg N ha⁻¹ yr⁻¹ (Figure 6.5, Sheppard et al., 2011). Visible injury was observed in *Calluna vulgaris* at 17 kg N ha⁻¹ yr⁻¹ and *Sphagnum capillifolium* at 22 kg N ha⁻¹ yr⁻¹ in the dry NH₃ treatment (not shown), with *Sphagnum capillifolium* vitality reducing in the wet plots (N applied in solution as either NaNO₃ or NH₄Cl) at 16 kg N ha⁻¹ yr⁻¹ (Figure 6.6, Sheppard et al., 2011). The N load at which the responses were observed declined over time. Wet N did not have a detrimental effect on *Calluna* cover (Sheppard et al., 2013).

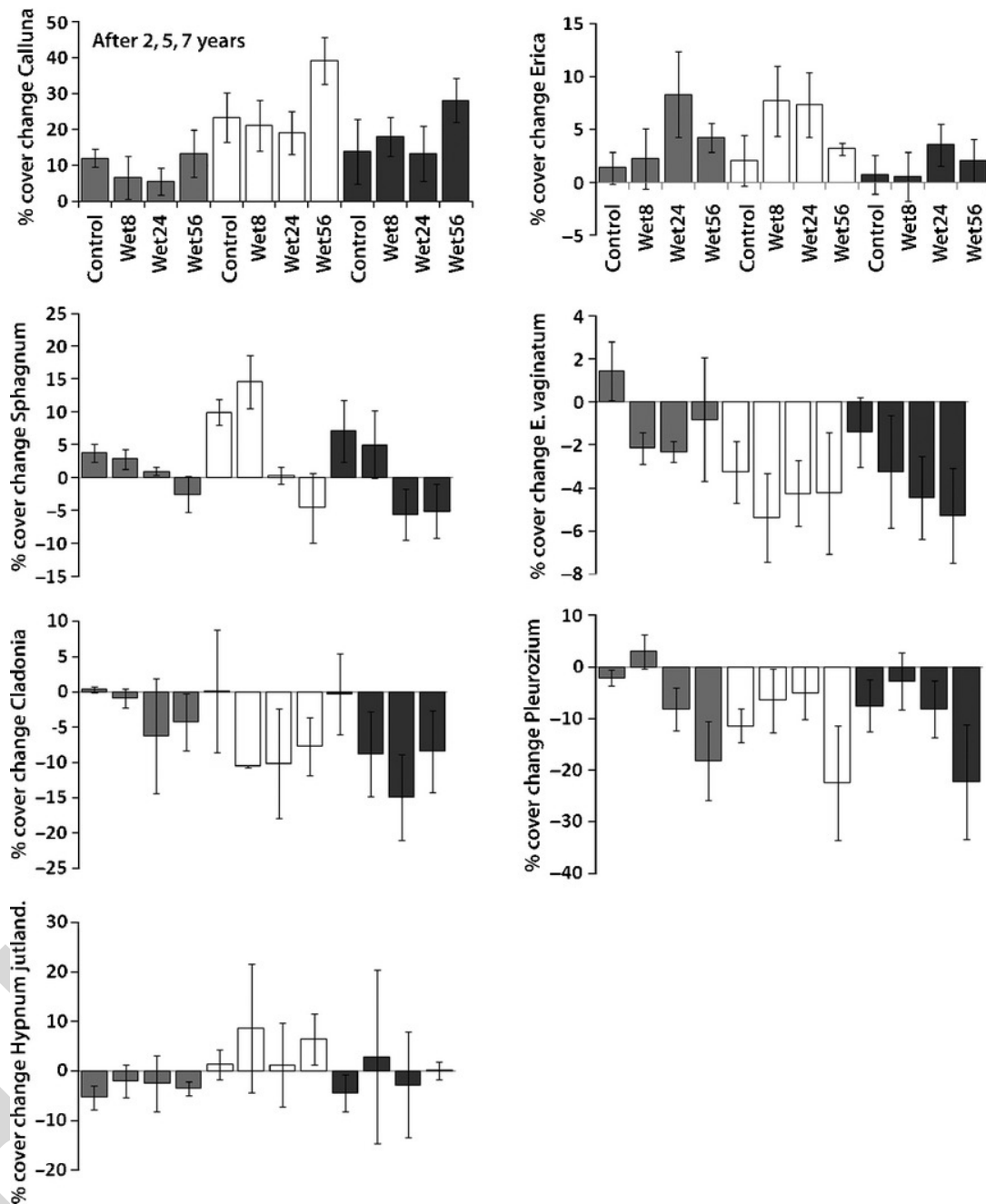
Figure 6.5. Chronological percent cover of *Calluna*, *Erica tetralix*, *Sphagnum capillifolium*, *Eriophorum vaginatum*, *Cladonia portentosa*, *Pleurozium schreberi* and *Hypnum jutlandicum* in permanent quadrats receiving 8, 24 and 56 kg N ha⁻¹ yr⁻¹ dry N deposition and ambient (assessed from Year 1, 2003) from the start (2002) through to 2009. (Sheppard et al., 2011).



Source: Sheppard et al., 2011

In pleurocarpous bryophytes, *Pleurozium schreberi* was eradicated at high doses of 56 kg N ha⁻¹ yr⁻¹ in the wet-reduced N plots, whereas *Hypnum jutlandicum* increased with oxidised N at +8 and +56 kg N ha⁻¹ yr⁻¹ (Figure 6.6, Sheppard et al., 2011). The lichen *Cladonia portentosa* was also strongly responsive to dry NH₃, declining rapidly close to the ammonia source (~56 kg N ha⁻¹ yr⁻¹) in the first year of the experiment and disappearing entirely up to 48 m away from the source (~16 kg N ha⁻¹ yr⁻¹) after four years, with damage at lower N levels emerging (Sheppard et al., 2011). *Cladonia* also decreased in the wet-N plots but much more slowly (Figure 6.6). Responses in lichen proteomics as discussed earlier also exhibit differential responses to N form, although the direction of change is inconsistent (Munzi et al., 2017; 2020).

Figure 6.6. Changes (difference from start) in *Calluna*, *Erica tetralix*, *Sphagnum capillifolium*, *Eriophorum vaginatum*, *Cladonia portentosa*, *Pleurozium schreberi* and *Hypnum jutlandicum* in permanent quadrats in control and treatments receiving 8, 24 and 56 kg N ha⁻¹ yr⁻¹ wet N deposition, pre-treatment and repeated after 2 (grey), 5 (open) and 7 (black) years, \pm SEM (Sheppard et al., 2011).



Source: Sheppard et al., 2011

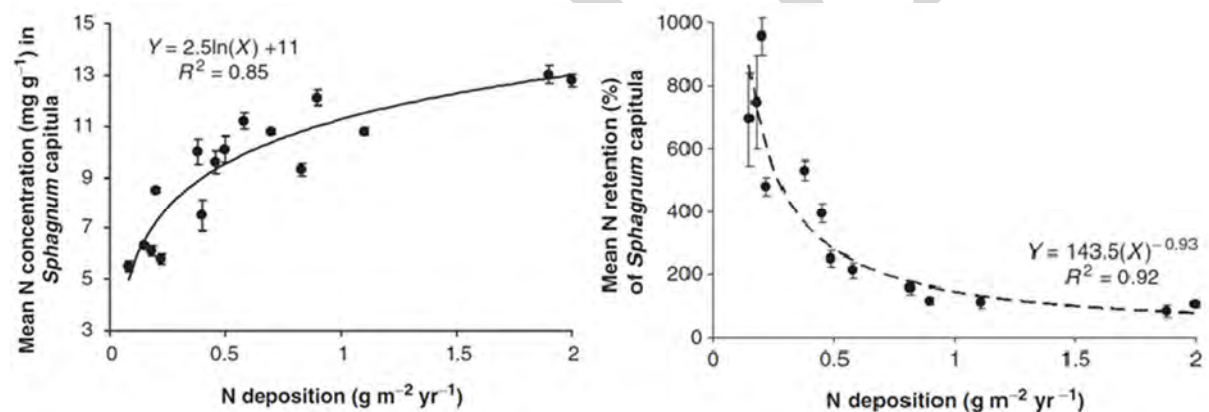
Consistently, the responses to reduced N, especially gaseous, appear more acute than responses to oxidised N, particularly in non-vascular plants (Sheppard et al., 2014). The authors recommend the lowest value of the $CL_{emp}N$ range where vegetation communities include sensitive lower plants and where deposition is dominated by reduced N (Sheppard et al., 2014). However, the authors highlight that cumulative effects of N are important too, with oxidised N appearing to drive a greater response at low N and reduced N at higher N loads. The accumulation of the ammonium cation in soil in contrast to the mobility of the anion nitrate was

suggested as the driving mechanism behind this response (Sheppard et al., 2014). Response of soil processes and leaching to N forms are incorporated into the following section.

Effects on *Sphagnum*, peat and peat water chemistry as evidence of nitrogen saturation

Along a natural gradient of bulk atmospheric N deposition varying from 2 to 20 kg N ha⁻¹ yr⁻¹, Bragazza and Limpens (2004) found that concentrations of dissolved inorganic N (DIN) and dissolved organic N (DON) in pore water increased with N deposition. The increase in concentrations of DIN was related to the reduced capacity of the moss layer to trap atmospheric N, which in turn was a result of the moss layer's N saturation. The increased concentrations of DON appeared to be the result of increased leaching of organic compounds by *Sphagnum* (Bragazza and Limpens, 2004). Survey work demonstrated increasing tissue N concentrations as N deposition increased which saturated at low N loads (Bragazza et al., 2005), with the percentage of N retained falling rapidly from around 2 kg N ha⁻¹ yr⁻¹ (Figure 6.7). This in turn led to elevated inorganic N in pore waters which increased exponentially at N deposition loads lower than the top of the current CL_{emp}N range of 5-10 kg N ha⁻¹ (Bragazza et al., 2005).

Figure 6.7 a) Trend of mean (\pm SE) nitrogen (N) concentration in *Sphagnum* capitula along the gradient of atmospheric N and b) Mean (\pm SE) N retention by *Sphagnum* capitula along the gradient in atmospheric N deposition (Bragazza et al., 2005). To convert g m⁻² to kg ha⁻¹ multiply by 10. N retention was calculated as the ratio between capitulum N concentration per square metre and atmospheric N deposition.

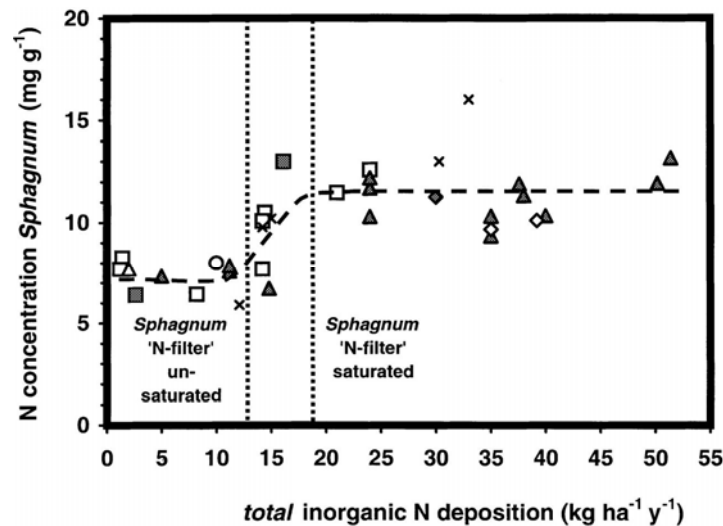


Source: Bragazza et al., 2005

Lamers et al. (2000) had previously used field data from Ireland and the Netherlands to produce an assessment of the capacity of moss layers to filter the amount of N deposition reaching the rhizosphere, thereby decreasing the growth of competitive graminoids and other species (Figure 6.8). The estimates were based on field data on *Sphagnum* species from sites covering a range of N- deposition values. At a deposition level of below 10 kg N ha⁻¹ yr⁻¹, Lamers et al. (2000) suggest that growth stimulation may absorb additional N inputs, while between 10 and 20 kg ha⁻¹ yr⁻¹ an increase in N content of *Sphagnum* species, primarily from accumulation of amino acids, might assimilate further increase in N deposition.

Above 20 kg ha⁻¹ yr⁻¹, the 'natural nitrogen filter' of *Sphagnum* is suggested to fail completely, as was observed in regions with high deposition levels (Lamers et al., 2000). It is important to note that Lamers et al. (2000) estimated the total deposition to be double the measured bulk deposition, however, at the remote sites with low deposition levels, where dry deposition is likely to be very low, their method would significantly overestimate the actual deposition inputs; according to this figure, this would thus reduce the threshold N deposition rate for response as illustrated by Braggaza et al. (2005).

Figure 6.8. The N concentration ($\text{mg g dry weight}^{-1}$) in raised bog (D1) *Sphagnum* species (apical parts) in Europe and the United States, in relation to total atmospheric N inputs (estimated at twice the wet deposition) (Lamers et al., 2000). Data taken from literature (◆ Ferguson et al., 1984; □ Malmer, 1988; ■ Aerts et al., 1992; ♦ Lütke Twenhöven, 1992; ○ Van der Molen, 1992; × Pitcairn et al., 1995; Δ Johnson and Maly, 1998); ▲ collected by the authors in 1998 in Europe and the United States).



Source: Ferguson et al., 1984; Malmer, 1988; Aerts et al., 1992; Lütke Twenhöven, 1992; Van der Molen, 1992; Pitcairn et al., 1995; Johnson and Maly, 1998

The significance of the N saturation of the *Sphagnum* layer, and increased availability of N in peat and in peat waters, has also been investigated in several experimental studies. Tomassen et al. (2003) found that peat water ammonium concentrations had significantly increased after three years of N addition ($40 \text{ kg N ha}^{-1} \text{yr}^{-1}$). Ammonium concentrations increased to $25 \mu\text{mol l}^{-1}$ (a common level in Dutch ombrotrophic bogs); in all other N-treatment experiments, levels were between 5 and $10 \mu\text{mol l}^{-1}$ due to the very high N retention in the peat mosses. Limpens et al. (2003a) found that lowering of the N input from 40 to $0 \text{ kg ha}^{-1} \text{yr}^{-1}$ decreased both interstitial water and *Sphagnum* N concentrations, while doubling the N input to $80 \text{ kg ha}^{-1} \text{yr}^{-1}$, increased N concentrations.

A study at Whim Moss, Scotland, analysed responses in the upper profile of peat following five years of experimental N additions, revealed rapid increases in soil solution N in response to N addition above a background N of $8 \text{ kg N ha}^{-1} \text{yr}^{-1}$ (Field et al., 2013). The authors suggested that bog vegetation was not efficient at buffering N deposition and commented that N retention in the peat appeared to be very poor in comparison to organic heath soils with the site leaching N at high C:N ratios (Field et al., 2013). However, an experiment on boreal peatland at very low background N ($\sim 1.6 \text{ kg N ha}^{-1} \text{yr}^{-1}$) in Canada did not find increases in pore water N in response to added N over the period of the study (Wieder et al., 2019). The authors suggested that additional N was being taken up by shrubs which increased in growth at the expense of *Sphagnum*. At Whim Moss, a positive growth response in shrubs (*Erica tetralix*) was observed in the early years of the experiment but subsequently declined (Sheppard et al., 2014). Hydrology can also play a role in the balance between shrubs and peat mosses, limiting the response of the former in wet situations.

Effects on carbon and nutrient cycling and biological processes

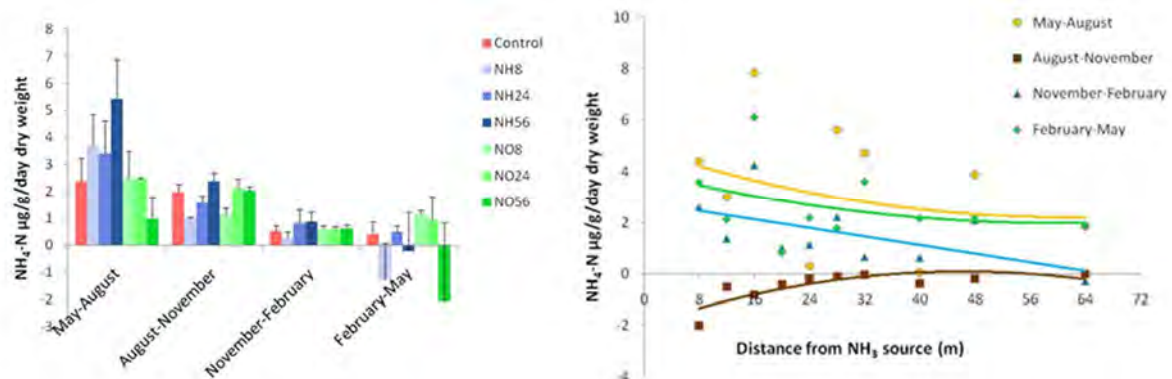
Concern over rising levels of atmospheric CO₂ has directed increasing attention towards bogs and peatlands due to their large storage capacity of carbon (Joosten, 2009; Nichols and Peteet, 2019), with the sequestration of carbon in peat determined by the ratio between primary production and decomposition of plant material, mainly bryophytes (Risager, 1998; Mitsch and Gosselink, 2000).

Increased carbon as well as N availability may increase primary production under pristine (nutrient- poor) conditions, whilst increased N will reduce the C:N ratio of litter and thus hypothetically increase peat decomposition rates. However, peat mosses (*Sphagnum* species), similar to bryophytes in other systems (e.g. grasslands, forests), have been proved to be sensitive to increasing N availability, and to react with decreased growth to high doses of N. Furthermore, the C stores within peat are themselves threatened by climate change (e.g. by increased drying of the peat layer and thus increased mineralisation).

This section of the chapter considers the effect of increasing N addition on carbon and nutrient cycling and biological processes. It builds on previous work through the addition of recent experimental work from Canada, Scotland and Sweden, and a European gradient study. Early work, based on studies on the northern and southern Swedish sites discussed previously in this chapter, concluded that a high atmospheric N supply may affect the carbon balance of ombrotrophic bogs (Aerts et al., 1992), because productivity under these circumstances is P limited, rather than N limited, but decomposition is probably increased by the high N loads. This hypothesis was supported by results from short-term experiments by Williams et al. (1999) and Williams and Silcock (2000) where N was added for three years on Moidach More, Scotland. The addition of 30 kg N ha⁻¹ yr⁻¹ resulted in a decrease in peat C:N ratios (Williams et al., 1999), which could increase rates of first-stage organic matter decomposition and N mineralisation (Aerts et al., 1992). Furthermore, additions of N to cores of *Sphagnum capillifolium* and *Sphagnum recurvum*, although taken up mainly by the moss, had significant effects on C and N values of the microbial biomass in the underlying peat (Williams and Silcock, 2000).

Nitrogen stimulates mineralisation across many habitats (e.g. Curtis et al., 2004, Pilkington et al., 2005; Emmet et al., 2007) including peatlands where N driven increases in mineralisation were observed at the Whim Moss experiment, Scotland (Field et al., 2010, 2013). In this study, N increased mineralisation but only in warmer periods when the water table was lower. Due to high site heterogeneity, the data were only significant in the reduced-N plots (Figure 6.9a, Field et al., 2010, 2013). The authors highlighted strong responses along the gaseous NH₃ transect over a concentration range equivalent to 8 to 56 kg N ha⁻¹ yr⁻¹, with elevated mineralisation closer to the NH₃ source in all but one season (Figure 6.9b; Field, 2010) and observed that the site appeared to leach at high C:N ratios, with N being poorly retained in the soil (not shown). The authors suggested that other factors such as hydrology or P or potassium (K) availability may constrain responses.

Figure 6.9. Summer 2006 to Spring 2007 seasonal net daily mineralisation rates from the top 10 cm surface peat from Whim a) Wet N plots (reduced N addition plots in blue, oxidised N addition in green, control plots in pink) and b) dry NH₃ release transect. Ambient deposition was circa 8-11 kg N ha⁻¹ yr⁻¹. Source: Field et al. (2010).



Source: Field et al., 2010

An early study of *Sphagnum* peat in Swedish ombrotrophic bogs along a gradient of N deposition (Hogg et al., 1994) also indicated that the decomposition rate of *Sphagnum* peat was more influenced by the P content of the material, than by N. This finding that P content is significant is consistent with results from a further study on the same two Swedish sites by Aerts et al. (2001), involving four years of fertilisation with 5 or 10 kg N ha⁻¹ yr⁻¹. These treatments were found to have no significant effects on potential decay rates at either site, measured by taking litter formed in the first three years of the experiment and monitoring time courses of CO₂ evolution in the laboratory. There was a significant relationship between the potential rate of peat decay and nutrient concentrations in litter, but the effects of the N and P content in the litter were comparable, and these relationships were primarily governed by differences between sites rather than treatments. Moreover, Tomassen et al. (2002) determined the decay rate of peat, which had been treated with six levels of N deposition for three years (see previous section), by measuring both CH₄ and CO₂ evolution. Despite significant differences in C:N ratios, carbon mineralisation rates were not at all affected by N treatments. Tomassen et al. (2004) also found no effect of N addition on the C mineralisation, despite significant differences in C:N ratios.

However, decomposition rates of recently formed peat litter collected in nine European countries under a natural gradient of atmospheric N from ca 2 to 20 kg N ha⁻¹ yr⁻¹, increased with increasing N deposition rates, resulting in higher carbon dioxide emissions and dissolved organic carbon (DON) release (Bragazza et al., 2006). Increasing N concentrations in *Sphagnum* litter, as a result of increased exogenous N availability, was found to be accompanied by a decreasing concentration of polyphenols (Bragazza and Freeman, 2007). The lower content of decay-inhibiting polyphenols could accelerate litter peat decomposition. Other studies have observed that an increase in vascular plant cover in response to N can lead to increased peat decomposition (Breeuwer et al., 2008).

In contrast, Saarnio et al. (2003) found that, over a two-year period, additions of 30 kg N ha⁻¹ yr⁻¹ had very little effect on the C gas exchange in lawns of boreal oligotrophic mires. To verify long-term changes in C balance, however, experiments over longer periods of time would be needed. Kivimäki et al. (2013) observed increased ecosystem respiration at Whim Moss, Scotland, but only between the control and a high +56 kg N ha⁻¹ yr⁻¹ treatment. Currey et al. (2010) observed enhanced mineralisation of labile carbon at Whim Moss, but either a decrease or no change in the mineralisation of complex carbon with an increasing effect of N. Effects were strongest at the highest N of +56 kg ha⁻¹ yr⁻¹ but no thresholds were identified. Eriksson et al. (2009) studied the

effects of long-term N addition (12 years) on the production of methane. Long-term deposition of N increased methane production, which may be attributed to a shift in plant community composition. The percentage of cover of the sedge *Eriophorum vaginatum* and the dwarf shrubs *Andromeda polifolia* and *Vaccinium oxycoccos* increased in response to experimental N deposition, with a concomitant reduction in the cover of *Sphagnum* species (Wiedermann et al., 2007); *Eriophorum* species in particular have been linked to elevated methane emissions due to the presence of aerenchyma. These findings differ from other studies, which found no effects of long-term deposition of N in the field on methane production in peat samples, despite similar changes in vegetation cover (Nykänen et al., 2002; Keller et al., 2005). These studies, however, lasted for five to six years, which may not be enough to induce changes in community structure of the methanogenic population or its substrate supply (Eriksson et al., 2009).

Lund et al. (2009) conducted a short-term N fertilisation experiment ($+40 \text{ kg ha}^{-1} \text{ yr}^{-1}$) in two Swedish bogs subjected to low ($2 \text{ kg ha}^{-1} \text{ yr}^{-1}$) and high ($15 \text{ kg ha}^{-1} \text{ yr}^{-1}$) background N deposition. At the low background deposition site, after two years, both gross primary production and ecosystem respiration were already significantly increased by N addition. At the site with high N background deposition, primary production was limited by P. N addition had no effect on CH_4 exchange, but elevated N_2O emissions were detected in N-fertilised plots. This corresponds with the results from Glatzel et al. (2008), who examined the effects of atmospheric N deposition on greenhouse gas release in a restoring peat bog in north-western Germany. They found that N fertilisation did not increase decomposition of surface peat, but under high N deposition it would be important to avoid frequent water table fluctuations which may increase N_2O release. Lund et al. (2009) concluded that, in the long term, increased nutrient availability will cause changes in plant composition, which will further act to regulate peatland greenhouse gas exchange. Higher N_2O emission were associated with elevated N at Whim Moss but only in the $+56 \text{ kg ha}^{-1} \text{ yr}^{-1}$ wet-oxidised N and dry-reduced N plots (Sheppard et al., 2013); in the wet-reduced N plots, a lower pH was suggested to inhibit denitrification. The authors here also highlighted the poor N-retention of the system, linking it to P or K limitation; growth responses in *Sphagnum capillifolium* when P/K was added were previously observed at the site (Carfrae et al., 2007). The potential of increase methane release was suggested following an 18-year fertilisation and warming experiment in a Swedish oligotrophic peatland. Marti et al. (2019) studied the response of the methanogen community to an N addition of $30 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (above ambient deposition of around $2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) and simultaneous warming, they found that N amplified the effects of warming with an increase in the abundance of methanogens.

Whilst much of this work has occurred at N deposition loads at or above the upper end of the existing CL_{empN} range, experimental work in Canada at a low background $\text{N} < 2 \text{ kg ha}^{-1} \text{ yr}^{-1}$, found that biological nitrogen fixation (BNF) was progressively inhibited at N deposition $> 3 \text{ kg}$ (Wieder et al., 2019). The study also observed stimulation of cellulose decomposition by N but no response in mineralisation rates. Similar N mediated declines in BNF were observed in a comparative study of 4 bogs across Europe spanning a N range of $2\text{--}27 \text{ kg ha}^{-1} \text{ yr}^{-1}$; BNF at the second lowest N site at $6 \text{ kg ha}^{-1} \text{ yr}^{-1}$ was lower than BNF at $2 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Saiz et al. 2021). Whilst the number of sites in this study were low, replication was high (39 at each over two years) and a simultaneous experiment which added $+15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ found similar responses in BNF, demonstrating the link with N (Saiz et al. 2021). Hinting at an underlying mechanism and demonstrating a link with ecosystem functioning, a survey of 25 European bogs over a 7 to 30 kg N range found linear declines in the diversity and community structure of ericoid mycorrhizal fungi (EMF) in response to increasing N deposition (Van Geel et al., 2020). The authors also linked soil P with a reduction in EMF richness.

In summary, these results do indicate that effects of elevated N deposition on *Sphagnum* and shrub growth, litter chemistry, and on the microbial community microbial biomass, are likely to affect the decomposition processes and ecosystem functioning, but that this process is regulated by more than just the C:N ratio of the peat with hydrology, climate and the availability of other nutrients being key. However, further evidence is still necessary to properly evaluate the long-term effects of increased N supply on the decomposition of *Sphagnum* peat, rates of nutrient cycling and the stability of carbon stored within peat.

Observed responses in vegetation composition from real-world gradient surveys

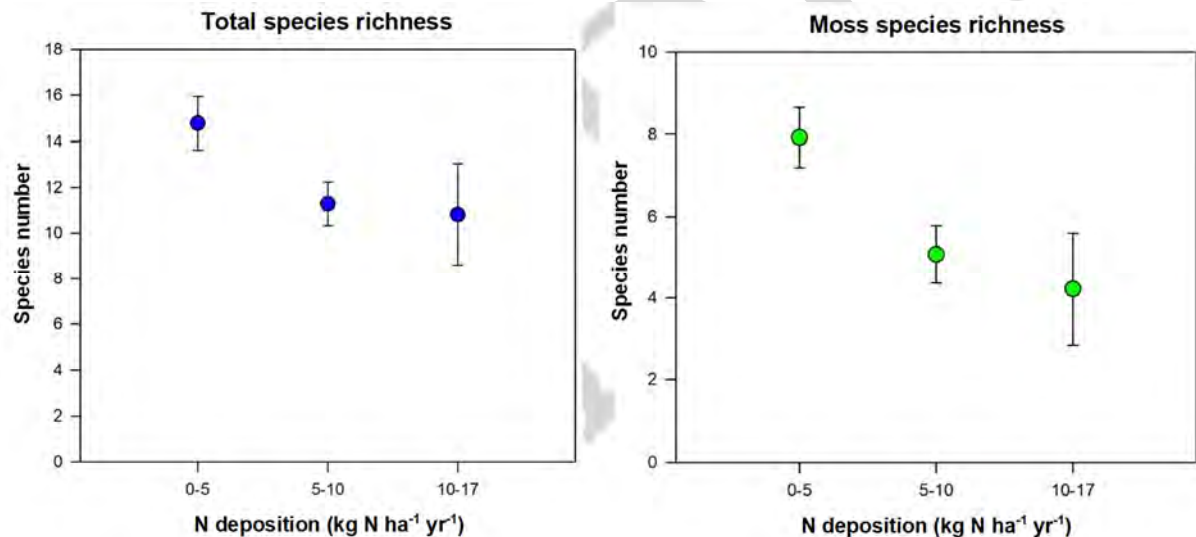
Since Bobbink and Hettelingh (2011), published gradient studies have provided strong, field-scale evidence of links between N deposition (in both oxidised and reduced forms) and widespread changes in the composition and diversity of plant species in bogs (Q1). Many of these studies have used vegetation data from national-scale surveys to assess changes in species richness and plant community composition along a N deposition gradient (Stevens et al., 2011; Tipping et al., 2013; Wilkins et al., 2016; Aherne et al., 2021). A number of these studies have attempted to identify an N deposition threshold where change occurs (Tipping et al., 2013; Wilkins et al., 2016; Aherne et al., 2021).

Further, these gradient studies typically include regions with low N deposition (e.g. 5 kg N ha⁻¹ yr⁻¹) allowing for the assessment of impacts at lower N inputs compared with experimental additions.

A number of broad-scale gradient studies have assessed the impacts of N deposition on bogs in the UK (Stevens et al., 2011; Tipping et al., 2013; Field et al., 2014; Van den Berg et al., 2016; using total N deposition, CBED model at 5 × 5 km resolution). Field et al. (2014) assessed species richness and plant community composition along gradients of climate and pollution for five habitats including bogs (n = 29) with N deposition ranging from 5.9 to 30.9 kg N ha⁻¹ yr⁻¹. They found linear reductions species richness and changed species composition associated with higher N deposition along the N gradient studied. Furthermore, N deposition was the dominant correlate to species richness of lichens (as a negative relationship). Species richness declined by about 20% of maximum species richness from the lowest to the highest N deposition bog sites; 80% of maximum species richness occurred at 7.1 kg N ha⁻¹ yr⁻¹. Similarly, van den Berg et al. (2016) assessed species richness of vascular plants as a measure of biodiversity in bogs and mires (n = 1136 quadrats) from the 2007 UK Countryside Survey; total N deposition ranged from 5.1 to 54.2 kg N ha⁻¹ yr⁻¹. Their results provide clear evidence that N deposition affects species richness, after factoring out correlated explanatory variables (climate and sulphur deposition). The observed decline in species richness was accompanied by an increased grass:forb ratio. Approximately 80% of species richness occurred at 11 kg N ha⁻¹ yr⁻¹. Stevens et al. (2012) analysed the probability of presence of individual lichen taxa (n = 6) in bogs at a given level of N deposition together with driver data for climate, change in sulphur deposition, and land-use using generalised additive models. Two taxa showed a significant relationship with N deposition, with *Cladonia portentosa* showing a steep reduction in the chance of occurrence from 10 to 25 kg N ha⁻¹ yr⁻¹. Tipping et al. (2013) used a broken stick median regression to estimate the thresholds above which N deposition definitely had an effect on plant species richness in bogs (n = 203) from the 1998 UK Countryside Survey under an N deposition gradient of 5.3 to 40 kg N ha⁻¹ yr⁻¹. The average relative loss of species in bogs was 1.7% per kg N ha⁻¹ yr⁻¹. The threshold N deposition for declines in species richness was estimated to be 14.3 kg N ha⁻¹ yr⁻¹ with a 95% confidence interval range of 13.2 to 15.9 kg N ha⁻¹ yr⁻¹, suggesting a conservative threshold of 13.2 kg N ha⁻¹ yr⁻¹.

Outside of the UK, Jokerud (2012) evaluated the possible impact of N deposition on ombrotrophic mire vegetation (species richness and composition) in Western Norway. Linear multiple regression with backward selection was used to assess which environmental gradient best explained species richness along a gradient between the northern and southern study areas (20 sites with N deposition ranging from 2.7 to 17.5 kg N ha⁻¹ yr⁻¹; observation-based total N deposition at 50 × 50 km resolution). Latitude showed a strong correlation with total species richness, vascular plant species richness and bryophyte species richness. Nitrogen deposition was the only variable that explained the significant decrease in total species richness and bryophyte species richness southwards. However, vascular plant species richness showed only a weak relationship with nitrogen. There was a significant decrease in average total species richness between the two deposition ranges 0-5 kg N ha⁻¹ yr⁻¹ and 5-10 kg N ha⁻¹ yr⁻¹ (Figure 6.10). However, the greatest change occurred after a threshold deposition of 5.8 kg N ha⁻¹ yr⁻¹.

Figure 6.10. Total species richness of the vegetation (left) and moss species richness (right) in bogs in Norway over a gradient in nitrogen deposition from 2.6 to 17.5 kg. Mean values (0-5 kg: n = 5; 5-10 kg: n = 7 and 10-17 kg: n = 8) ± 95% confidence intervals are shown. Wamelink et al. (2021) after Jokerud (2012).



Source: Wamelink et al., 2021; Jokerud, 2012

Wilkins et al. (2016), with updates in Aherne et al. (2021), identified vegetation community change points (thresholds) for raised and upland blanket bogs in Ireland using species abundance data from relevé plots (389 and 247, respectively) spanning a deposition gradient of 4 to 17 kg N ha⁻¹ yr⁻¹ (observation-based total N deposition at 5 × 5 km resolution). For raised bogs, species data were limited to 15 indicator species, compared with all species data for blanket bogs (i.e. 115 plant species). The community change point for declining species in raised bogs was estimated at 6.7 kg N ha⁻¹ yr⁻¹ with 4 species decreasing in abundance (*Trichophorum germanicum*, *Sphagnum cuspidatum*, *Sphagnum denticulatum*, *Sphagnum capillifolium* s. *rubellum*). For blanket bogs the threshold for declining species was 6.2 kg N ha⁻¹ yr⁻¹ with 15 species decreasing in abundance (8 identified as positive indicator species; *Trichophorum germanicum*, *Sphagnum tenellum*, *Racomitrium lanuginosum*, *Pleurozia purpurea*, *Sphagnum denticulatum*, *Breutelia chrysocoma*, *Rhynchospora alba*, *Schoenus nigricans*).

The community change points or thresholds for bogs in Norway and Ireland align with the lower end of the existing $CL_{emp}N$ range (5-10 kg N ha⁻¹ yr⁻¹), whereas those for the UK centre on approximately 10 kg N ha⁻¹ yr⁻¹, the upper end of the existing range for bogs (Q1).

Summary of Q1

Even though there has been only a limited number of long-term N manipulation experiments in bog ecosystems, a clear picture has emerged of the potential impact of elevated N deposition on bog habitats. In Canadian and Swedish work, responses to N deposition as low as 2 kg N ha⁻¹ yr⁻¹ have been observed in a number of bog species (in terms of survivorship, flowering, and density).

Bryophyte species, in particular *Sphagnum* species, appear to be susceptible to the rise in anthropogenic N pollution, showing a decline in favour of grass and other competitive species, changes in competition between *Sphagnum* species, and changes in physiological and biochemical characteristics. Experimental work in Canada and a gradient survey of European bogs observed an inhibition of biological nitrogen fixation (BNF) and a consequent disruption of N cycling with onward impacts of vegetation communities has been observed at above 3 kg N ha⁻¹ yr⁻¹. There appears to be a limited capacity for retention of N in the moss layer, above which N availability in the rhizosphere increases, offering a tool for assessment of factors which may modify the critical loads for these systems. Studies of N cycling and retention are consistent with a long-term response threshold of around 10 kg N ha⁻¹ yr⁻¹ but with an indication that N retention is less effective from around 2 kg N ha⁻¹ yr⁻¹. It is likely that there is a time component linked to N saturation and the accumulation of N in a system when it is added at loads above which biological processes can accommodate. There are also likely to be more severe effects when N is added over a shorter time period or a site is subject to high concentrations of N.

The $CL_{emp}N$ recommended by Bobbink and Hettelingh (2011), 5-10 N kg ha⁻¹ yr⁻¹, was based on a considerable body of field and experimental evidence and, hence, was judged to be reliable. Additional studies since then have generally provided results that further support this rating, although experimental work at very low background N as highlighted above has now suggested that responses are observed at even lower N deposition. Gradient surveys in the UK (Field et al., 2014), Ireland (Wilkins et al., 2016; Aherne et al., 2021) and Norway (Jokerud, 2012) have all observed changes in species richness or composition at the low end of the $CL_{emp}N$ range. Whilst we propose that the $CL_{emp}N$ range for bog ecosystems (Q1) encompassing lowland raised bogs (Q11) and blanket bogs (Q12) remains at 5 to 10 kg ha⁻¹ yr⁻¹ (classification 'reliable'), we highlight the emerging body of evidence of responses at very low N loads and recommend further experimental study to understand the long-term effects on ecosystem functioning of these loads.

Much of Europe, North America, Central and Western Asia has received N deposition well above the critical load for many decades and, as a result, we have witnessed a shifted baseline across many of our peatlands compared to northern latitude systems with low deposition histories. However, many of the studies in this chapter highlight the lack of an N threshold and the progressive nature of N responses as deposition increases from a low background, this suggests that any reduction in N deposition is worthwhile and will, over time, lead to a recovery of ecosystem functioning.

6.3 Valley mires, poor fens and transition mires (Q2)

The last review of Q2 habitats established a $CL_{emp}N$ of 10-15 kg N ha⁻¹ yr⁻¹ based on the evidence available at that time. In this review, we summarise this earlier evidence and incorporate results from experimental work at very low background N levels in Canada and a gradient study in The

Netherlands. Valley mires, poor fens and transition mires are weakly to strongly acidic peatlands, flushes and vegetated rafts formed in situations where they receive water from the surrounding landscape or are intermediate between land and water (Davies et al., 2004). All systems have permanently waterlogged soils, with groundwater just below or at the soil surface. This water supply is rather poor in base cations, leading to an acidic system with a pH slightly above bog systems, where peat mosses, but also small sedges and some brown moss species, dominate the vegetation. The distinction between valley mires, poor fens and transition mires is made on the basis of water level and water origin and may have some implication for the level of their critical loads. However, the low number of studies does not allow a further distinction, and the limited information that is available to date comes mainly from poor-fen systems (Q22). When compared to poor fens, based on the generalisation from Morris (1991) on the link between N sensitivity and hydrology (see introduction Chapter 6.1), valley mires are expected to be slightly less sensitive, and quaking bogs and transition mires to be more sensitive to excess N.

The significance of competition for light to the N response in *Sphagnum* was demonstrated by the study of Hogg et al. (1995) in a small valley mire near York in the United Kingdom. The growth of the mosses *Sphagnum palustre* and *Sphagnum fimbriatum* was reduced by 50% after additions of 12 kg N ha⁻¹ yr⁻¹ over two years; a rate which was probably comparable to ambient deposition. Where *Sphagnum* was growing poorly and the dominant grass species *Molinia caerulea* was abundant, adding N had no effect, but cutting *Molinia caerulea* in the summer was beneficial to *Sphagnum*, re- invigorating its growth.

In an experiment in central France, Francez and Loiseau (1999) studied the fate of N in poor fens in Côte de Braveix by tracking 5 kg N ha⁻¹ yr⁻¹ labelled with 15N (background deposition 10 kg N ha⁻¹ yr⁻¹). All of this N, added between June and August, remained in the system until October. Most of this N (55-65%) had accumulated in the *Sphagnum* layer. The upper peat layer of up to 10 cm accumulated about 15 to 30% of the added N, and from all deeper peat layers less than 5% of the added N was retrieved (Francez and Loiseau, 1999). These results indicate that *Sphagnum* functions as a N filter in poor fens as it does in ombrotrophic bogs (Lamers et al., 2000; Malmer and Wallén, 2005). Microcosm studies with *Sphagnum magellanicum*, a species characteristic of poor fens in Scandinavia, have shown significant negative effects of N additions (30 kg N ha⁻¹ yr⁻¹) on concentrations of nutrients such as P, K and Ca, in mosses after a period of three months (Jauhiainen et al., 1998b).

Malmer et al. (2003) studied the competition between vascular plants and peat mosses in a fertilisation experiment, and with respect to competition for light in a removal experiment in poor fens during two growing seasons. N was added in the form of ammonium nitrate at a level of 20 kg N ha⁻¹ yr⁻¹ (4-5 times the ambient supply rate) and was added both on and below the moss surface. Adding N confirmed the hypothesis that mosses rely on atmospheric supply, while *Narthecium ossifragum* depends on mineralisation in the peat (Malmer et al., 2003). N addition significantly increased shoot length of *Narthecium* and length increment of *Sphagnum* (but not its biomass). The negative relationship between the growth of *Narthecium* and of *Sphagnum* demonstrated a symmetric competition for light, the intensity of which increased with an increasing availability of N (Malmer et al., 2003).

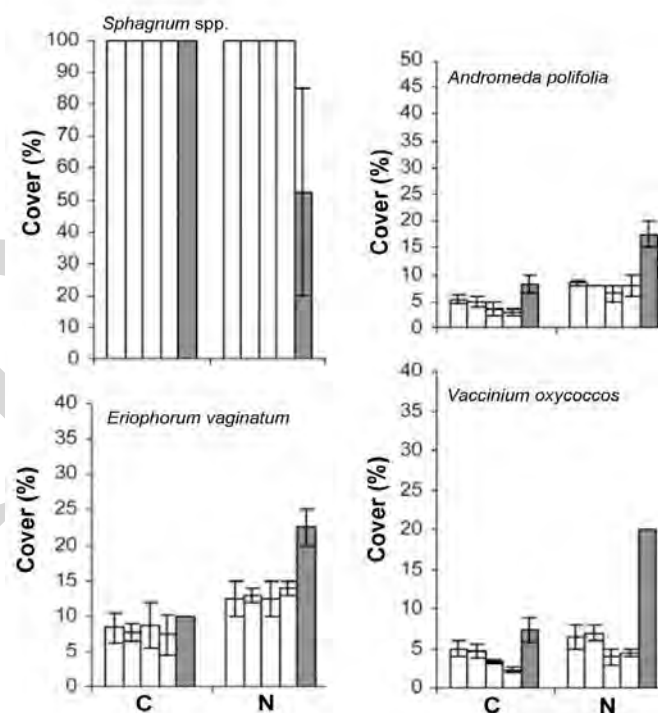
In 1995, a long-term field experiment (addition of 15 and 30 kg N ha⁻¹ yr⁻¹) was started in northern Sweden on a poor fen, at an ambient deposition of 2 kg N ha⁻¹ yr⁻¹ (Granberg et al., 2001). After three growing seasons, Granberg et al. (2001) reported results on the possible effects of climate change on CH₄ emission. They expected (and confirmed) sedges to be an important factor in CH₄ release into the atmosphere. Similar to effects found in ombrotrophic bogs (see Chapter 6.2), sedge cover significantly increased with additions of increasing amounts of N (Granberg et al., 2001). Unfortunately, the regression analysis used did not permit a

distinction between effects at 15 and 30 kg N ha⁻¹ yr⁻¹. Using sedge cover as a covariate, the effect of N addition on CH₄ emission changed over time from non-significant in the first year (1995) to a significant negative effect during the last year (1997). This cumulative effect was probably linked to the significant accumulation of total N in the upper 5 (15 kg N ha⁻¹ yr⁻¹ treatment) or 10 (30 kg N ha⁻¹ yr⁻¹ treatment) cm of the soil (Granberg et al., 2001).

In the same field fertilisation experiment, Gunnarsson et al. (2004) focused on the growth and production of *Sphagnum balticum* and interspecific competition between *S. balticum* and either *Sphagnum lindbergii* or transplanted *Sphagnum papillosum*. Production and length increment of *Sphagnum balticum* in nutrient-poor lawn communities was significantly reduced after four years of N addition. The area covered by *Sphagnum lindbergii* was increased on the N-treated plots, which may reflect its greater tolerance to high N influx in relation to *Sphagnum balticum* (Gunnarsson et al., 2004). The hummock-forming *Sphagnum papillosum* was found to increase at the expense of *Sphagnum balticum* under climatic regimes with a more negative water balance, probably because of the low tolerance of *Sphagnum balticum* to drier conditions. Reduced growth of peat mosses may have positive effects on vascular plants, as numerous studies on bogs have shown (see Chapter 6.2).

Gunnarsson et al. (2004) concluded that increased N deposition may transform mires that are dominated by *Sphagnum* into vascular-plant-dominated mires.

Figure 6.11. Time series for the years 1995, 1996, 1997 and 1998 (white bars) and 2003 (grey bars). Data represent cover (mean ± SE) of *Sphagnum* species, *Eriophorum vaginatum*, *Andromeda polifolia* and *Vaccinium oxycoccos* for two treatments (C = control and N = +30 kg N ha⁻¹ yr⁻¹). Adapted from Wiedermann et al. (2007).

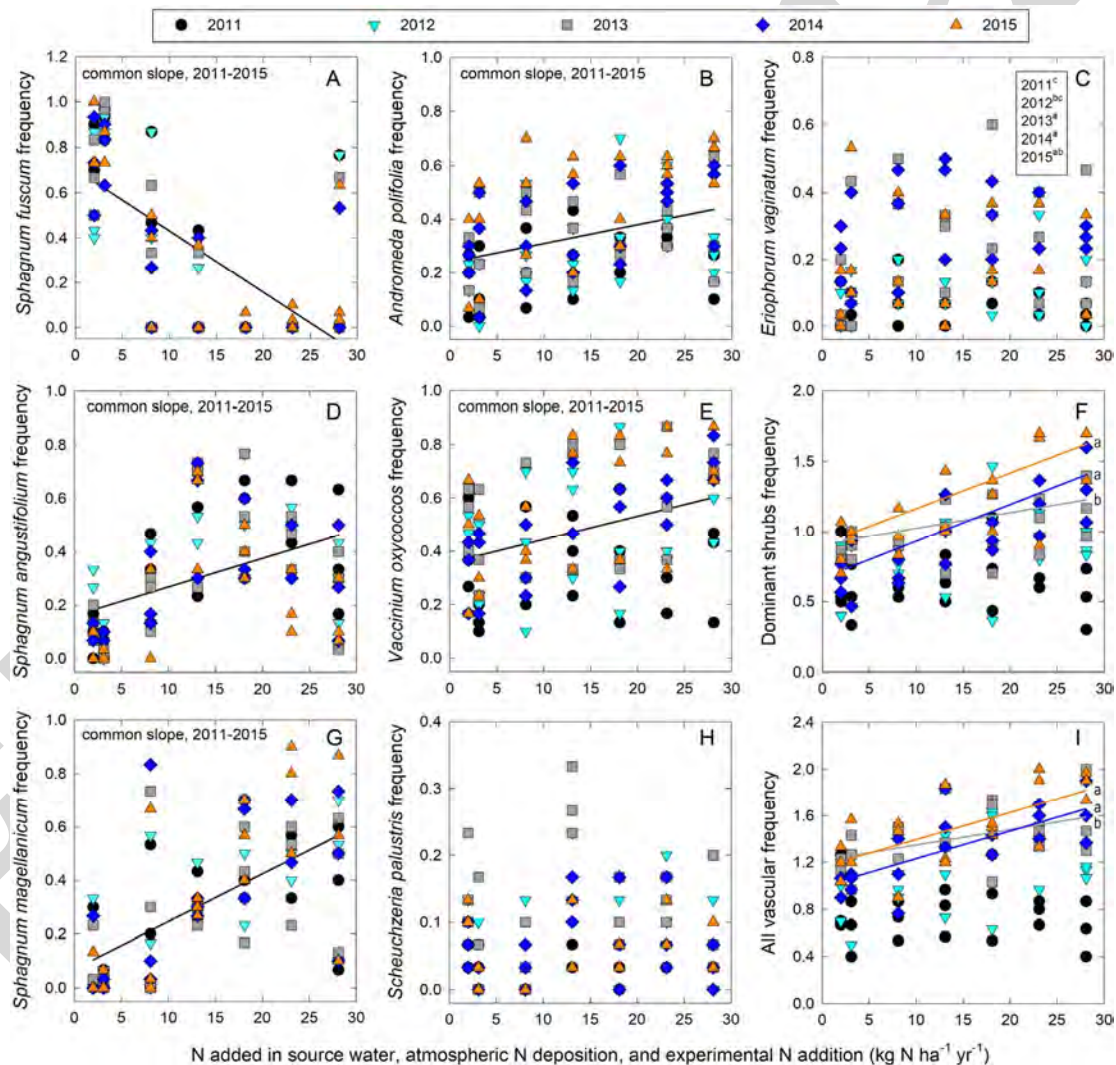


Source: Wiedermann et al., 2007

The experiment described above was continued for four more years, but unfortunately only the results from the highest treatment dose of 30 kg N ha⁻¹ yr⁻¹ were provided. Wiedermann et al. (2007) found that the vegetation responses were negligible for the first four years, but after

eight years of continuous N addition, the closed *Sphagnum* carpet had been drastically reduced from 100% down to 41% (Figure 6.11). The total vascular plant cover (*Eriophorum vaginatum*, *Andromeda polifolia* and *Vaccinium oxycoccus*) increased from 24% to an average of 70% (Figure 6.10). Wiedermann et al. (2007) stress that both bryophytes and vascular plants in boreal mires receiving background levels of N of only about 2 kg ha⁻¹ yr⁻¹ exhibit a time lag of more than five years before responding to N, emphasising the need for long-term experiments. However, it should be observed that climate was also observed to be higher in 2003 and could therefore play an interactive role in the response.

Figure 6.12. Frequency of occurrence from point frame measurements for the three most frequently occurring *Sphagnum* species (*Sphagnum fuscum*, *Sphagnum angustifolium*, *Sphagnum magellanicum*), the two most frequently occurring shrub species (*Andromeda polifolia*, *Vaccinium oxycoccos*), the most frequently occurring graminoid species (*Eriophorum vaginatum*), the most frequently occurring forb species (*Scheuchzeria palustris*), dominant shrubs combined (*Andromeda polifolia*, *Vaccinium oxycoccos*, *Chamaedaphne calyculata*, *Kalmia polifolia*), and all vascular plant species combined (*Scheuchzeria palustris*, *Eriophorum vaginatum*, *Andromeda polifolia*, *Vaccinium oxycoccos*, *Chamaedaphne calyculata*, *Kalmia polifolia*, *Drosera rotundifolia*, *Rubus chamaemorus*, *Maianthemum trifolium*, *Carex aquatilis*, *Carex limosa*, *Picea mariana* below 1 m tall) as a function of N addition. When there were differences between years, but no significant effect of N addition, years with the same letter superscript do not differ significantly (ANCOVA, a posteriori Tukey's Honestly Significant Difference test). Slopes with the same lower-case letter to the right of regression lines plotted for individual years do not differ significantly (ANCOVA). (Wieder et al., 2020).



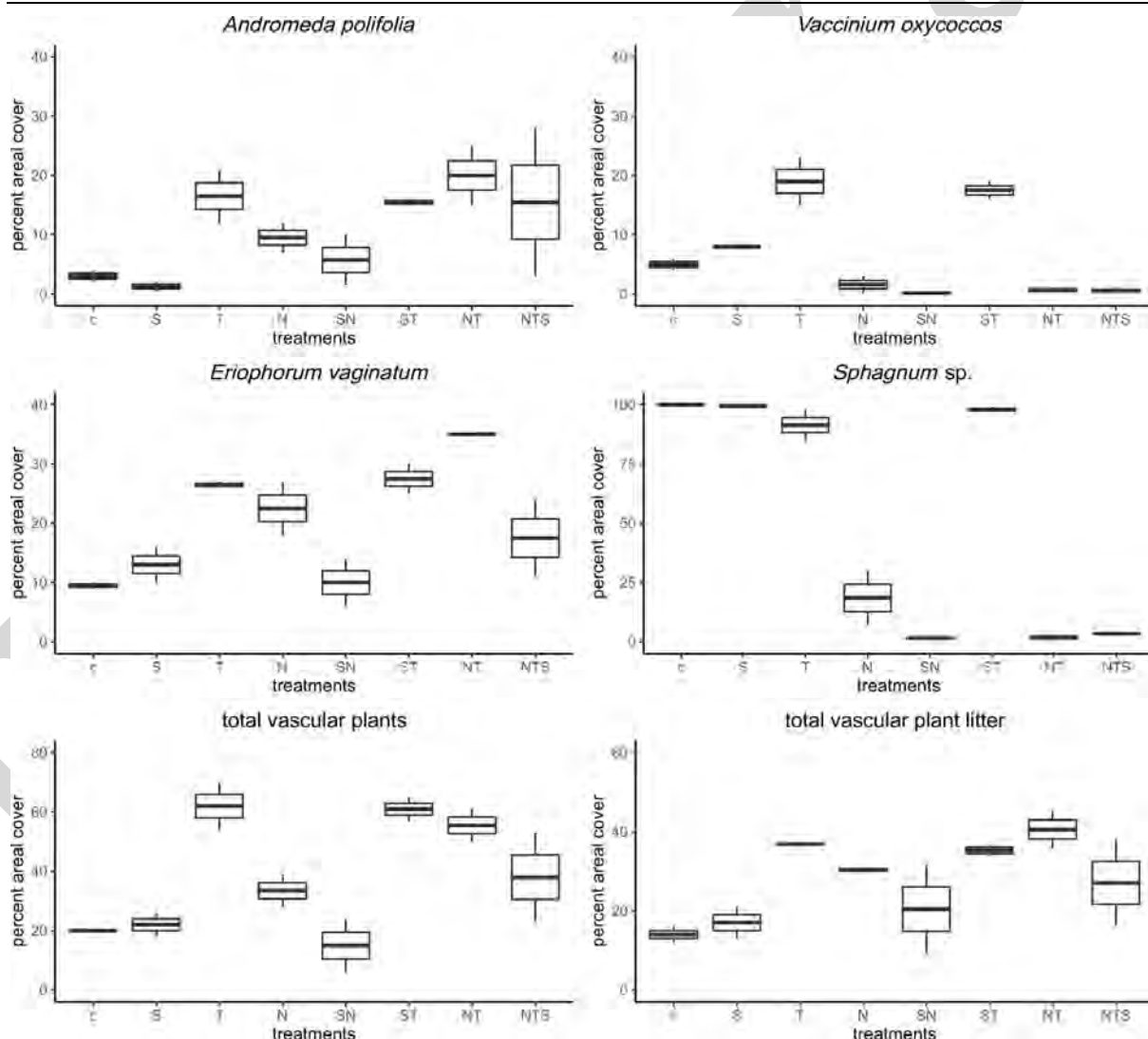
Source: Wieder et al., 2020

Experimental work completed since the last review of CL_{emp}N has taken place on a poor fen in Alberta, Canada, at very low background N (bulk deposition < 2 kg N ha⁻¹ yr⁻¹), where Wieder et al., (2020) found N-driven reductions in biological nitrogen fixation (BNF), decreased abundance

of *Sphagnum fuscum* and increases in *S. angustifolium*, and increases in vascular plants in general (Figure 6.12). A stimulation in decomposition of cellulose was also observed but not of more recalcitrant *Sphagnum* or vascular plant litter. No response thresholds were observed, with changes suggested as progressive from above background N. A $CL_{emp}N$ of $3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ was proposed by the authors (Wieder et al., 2020) based on the observation that, above this level, N cycling and species composition was disrupted.

A recent gradient study at higher N loads in the Netherlands, spanning a range of $17\text{--}24 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, observed decreased base saturation at higher N in poor fens and an observation that Dutch fens are more acidic than fens in regions with lower N deposition (Van Diggelen et al., 2018).

Figure 6.13. Cover estimates (% areal cover) of vascular plants and *Sphagnum* vegetation as well as vascular plant litter at the long-term fertilisation experiment at Degerö Stormyr in July 2016; y-axes: cover estimate; x- axes: experimental treatments: c = control, S = sulfur ($20 \text{ kg ha}^{-1} \text{ yr}^{-1}$), T = temperature ($+3.6 \text{ }^{\circ}\text{C}$), N = nitrogen ($30 \text{ kg ha}^{-1} \text{ yr}^{-1}$), SN = sulfur + nitrogen, ST = sulfur + temperature, NT = nitrogen + temperature, NTS = nitrogen + temperature + sulfur; total number of plots = 16 (Wiedermann and Nilsson, 2020).



Source: Wiedermann and Nilsson, 2020

Interactions with climate change and warming have also been observed in fens. A long-term (21 year) experiment in a *Sphagnum* dominated poor-fen peatland in Degerö Stormy, Sweden, applied 30 kg N ha⁻¹ yr⁻¹ on top of a low background N ~2 kg N ha⁻¹ yr⁻¹ in conjunction with, and separately from, a warming effect created by open topped chambers (Wiedermann and Nilsson, 2020). The authors found that *Sphagnum* cover fell sharply when N was added (from ~80% to ~20%) and further still in conjunction with elevated temperature (Figure 6.13). *Vaccinium oxycoccus* reduced a little with N alone (but not significantly so) but declined further with elevated temperature and N (note that with temperature alone, the shrub increased in cover). *Andromeda polifolia* decreased with N alone but elevated temperature reversed this. As with Q1, *Eriophorum vaginatum* increased with N but more so when temperature was also elevated. In general, the overall cover of vascular plants was increased by both temperature and N (Figure 6.13; Wiedermann and Nilsson, 2020).

Other work on a transition alpine mire consisting of hummocks and lawns added 0, 10, and 30 kg N for eight years (background N ~8 kg) also observed temperature and N effects, although in response to a short-term natural heatwave event (Brancaleoni and Gerdol, 2014). In hummocks *Sphagnum* mosses recovered better from heat wave under N addition, however, in lawns vascular plants expanded faster under high N, suggesting that persistent climate change could reduce the carbon sink. Species richness also fell in lawns (Brancaleoni and Gerdol, 2014). The differential response between hummocks and lawns highlights the susceptibility of some bog species to climate change.

Summary of Q2

The CL_{emp}N previously recommended for poor fens (Q22) by Bobbink and Hettelingh (2011) was 10-15 kg N ha⁻¹ yr⁻¹, with the low end of the range emphasised for oceanic valley mires (Q21) where limited studies at low N were available. These critical loads represented a reduction on the earlier critical load based on the evidence of the failure of the *Sphagnum* N filter function observed in bogs. Recent studies in Q2 are still limited, however, emerging work at the low end of N deposition has found responses at very low N loads (e.g. Wieder et al., 2020). Based on this new but limited evidence we would recommend a reduction of the low end of the CL_{emp}N and a new range of 5 to 15 kg N ha⁻¹ yr⁻¹ (classification 'reliable') and encourage further research into low N responses in these habitats to determine if the higher end of the range is appropriate.

6.4 Palsa and polygon mires (Q3)

Palsa and polygon mires are patterned mire complexes of the arctic, subarctic and northern boreal zones. The formation and maintenance of these types of mires are dependent on the action of frost or ice. Palsa mires (Q31) are formed by elevated frozen mounds or ridges (palsas) 0.5 to 8 m high and up to 50 m in diameter, interspersed with wet hollows of similar area (Davies et al., 2004). Polygon mires (Q32) are complex mires of the arctic and subarctic patterned by surface microrelief of large, 10-30 m in diameter, low-centre or high-centre polygons formed by the juxtaposition of dry, 0.3-0.5 m high ridges (Davies et al., 2004). The distinction between palsa and polygon mires is made on the perpetual presence of ice, which is only the case for palsa mires. Intact palsa mounds show a patterning of weakly minerotrophic vegetation with different assemblages of mosses, herbs and sub-shrubs on their tops and sides (Schaminee et al., 2019). In polygon mires, cover of non-sphagnaceous mosses and lichens outweigh *Sphagnum* species and together with dwarf shrubs occur on the ridges (Schaminee et al., 2019). Wet hollows are occupied by grasses, sedges and mosses including *Sphagnum* species. According to Schaminee et al. (2019) palsa and polygon mires cannot be distinguished on floristic criteria. Since both types of mires are limited to the arctic, subarctic and northern boreal zones, we assume that the N sensitivity for both types of mires will be comparable.

For palsa and polygon mires no $CL_{emp}N$ was set by Bobbink and Hettelingh (2011), due to the lack of empirical data. While there are no experimental studies available from Europe, there is a study from polygon mires in the Canadian arctic (Bylot Island; Marchand-Roy, 2009). The aim of this fertilisation experiment was to simulate the nutrient input by the annual presence of the Great Snow Goose. Nitrogen was added as NH_4NO_3 at a dose of 10, 30 and 50 kg N ha⁻¹ yr⁻¹ for five years (ambient yearly nitrogen deposition is not given). Nitrogen was added in one yearly dose in late June or early July, after the snow melted and the polygon was no longer flooded. At an N fertilisation rate above 10 kg N ha⁻¹ yr⁻¹ a significant effect on the growth of graminoid plants was observed (Marchand-Roy, 2009). Compared to the control, the aboveground biomass of graminoid plants increased by 24% at the lowest dose of 10 kg N ha⁻¹ yr⁻¹, 41% at the intermediate dose of 30 kg N ha⁻¹ yr⁻¹ and 103% at the highest dose of 50 kg N ha⁻¹ yr⁻¹. This significant effect was not observed after two years of fertilisation, due the N filtering effect of bryophytes (Pouliot et al., 2009). Nitrogen input had no effect on the primary productivity of bryophytes. Tissue N concentrations of both graminoid plants and bryophytes were increased significantly by N addition. In addition, the decomposition of organic matter was significantly promoted by N.

Summary of Q3

The experiment in the Canadian arctic showed significant effects of 10 kg N ha⁻¹ yr⁻¹ on the production of graminoids, tissue N concentrations and decomposition rate within a short experimental period of five years. However, ambient nitrogen deposition is very low in the region (< 1-2 kg N ha⁻¹ yr⁻¹), indicating the $CL_{emp}N$ is likely to be below 10 kg N ha⁻¹ yr⁻¹. Since (*Sphagnum*) mosses function as an N filter in polygon mires, like ombrotrophic bogs, we think that the $CL_{emp}N$ for polygon mires is similar to that of raised and blanket bogs (Q1). However, as palsa and polygon mires only occur in pristine areas, we believe that the low end of the range is should be reduced. We therefore propose, based on expert judgement, a $CL_{emp}N$ range for palsa and polygon mires (Q3) of 3 to 10 kg N ha⁻¹ yr⁻¹.

6.5 Base-rich fens and calcareous spring mires (Q4)

Similar to poor fens, base-rich fens and calcareous spring mires have developed on permanently waterlogged soils, but in these systems there is a base-rich, nutrient-poor and often calcareous water supply buffering the system from high levels of acidity. They are largely occupied by calcicolous small sedges and brown moss communities (Davies et al., 2004). Although rich fens are the habitat of a range of specialised and rare species, very few field experiments have been conducted with enrichments of ecologically relevant doses of N to determine the effects of increased N deposition. Early work set the $CL_{emp}N$ range for mesotrophic fen ecosystems at between 15 and 35 kg N ha⁻¹ yr⁻¹ (Bobbink et al., 2003). This was based mainly on nutrient budget studies on rich fens (Q41-Q44) in the Netherlands and on several field experiments, but these were all with (very) high N additions (> 100 kg N ha⁻¹ yr⁻¹) (e.g. Beltman et al., 1996; Boeye et al., 1997; Wassen et al., 1998). This was then revised down in the last review of $CL_{emp}N$ to 15-30 kg N ha⁻¹ yr⁻¹ for rich fens (Q41-Q44) (Bobbink and Hettelingh, 2011) and 15-25 kg N ha⁻¹ yr⁻¹ for arctic-alpine rich fens (Q45). These reductions were based on experiments in Ireland that observed severe negative effects on the bryophyte layer within five years after additions of 35 kg N ha⁻¹ yr⁻¹ under low ambient N depositions (6-8 kg N ha⁻¹ yr⁻¹). This previous work is briefly summarised below.

Koerselman and Verhoeven (1992) assumed that the input of N needed to be counterbalanced by the output of N through usual management (mowing). Increased N input, compared to N removal, results in a considerable increase in tall graminoids and a subsequent decrease in diversity of the subordinate plant species (Vermeer, 1986; Verhoeven and Schmitz, 1991).

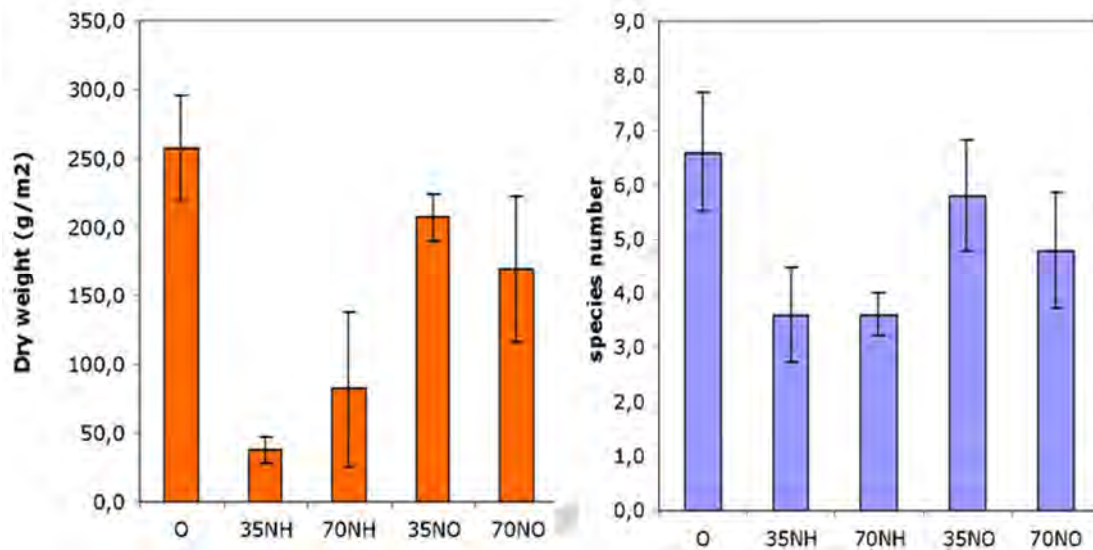
Although in some fens strong P limitation inhibits changes in diversity with increased N, it is expected that such a situation leads to increased losses of inorganic N to the surface or groundwater, thus leading to a similar critical load as those for P or N limited situations (Bobbink et al., 1996). Five locations in Belgium, Ireland and Poland were used in factorial fertilisation experiments with both N and P. Two sites (in the north-eastern part of Belgium and in Poland) gave clear evidence of N as the most important growth-limiting factor, while growth at the other three sites was strongly limited by P (Beltman et al., 1996; Boeye et al., 1997; Wassen et al., 1998).

Microcosm experiments with bryophytes (*Sphagnum wanstorffii*) and vascular plants (*Carex rostrata*) collected from rich fens at higher latitudes have indicated sensitivity to additions of ammonium nitrate of 30 kg N ha⁻¹ yr⁻¹ and higher. Within three months, a decrease in moss growth could be measured, as well as an increase in N and free amino acid concentrations in both mosses and vascular plants, both of which are clear indications of N enrichment effects (Jauhiainen et al., 1998a; Saarinen, 1998). Although, by themselves, the conditions in these experiments were too artificial to justify a decrease of the present critical load, they do indicate that moss species in particular, which are an important and prominent part of biodiversity in rich fens, may be very sensitive to increased N deposition.

Paulissen (2004) studied the effects of different forms of pollutant N in a rich fen in central Ireland (ambient load 6-10 kg N ha⁻¹ yr⁻¹). N was added in the form of ammonium or nitrate, at a level of 50 kg N ha⁻¹ yr⁻¹. After two years, no effects of N treatment could be found in the vascular plant cover, nor in the dominant bryophyte species (*Scorpidium revolvens* and *Sphagnum contortum*). Tissue N:P ratio indicated that the bryophyte layer was P limited (Paulissen, 2004). The surface phosphatase (an enzyme that allows plants to take up organically bound P under conditions of increased P limitation) activity of the typical brown moss *Scorpidium revolvens* was significantly stimulated by nitrate addition, whereas ammonium addition did the same in the invasive *Sphagnum contortum*. After four years, ammonium significantly reduced the biomass of *Scorpidium revolvens* although *Sphagnum contortum* biomass was not affected by N addition (Paulissen et al., 2016). This implies that, in the longer term, *Sphagnum* will profit from high ammonium deposition, while it will negatively affect the typical brown mosses. Paulissen (2004) concluded that, in the short term, the vegetation of rich fens would not be very sensitive to increased N deposition. In the longer term (> 2 years), however, growth of brown mosses, such as *Scorpidium revolvens*, in P-limited fens could become negatively affected by ammonium, although not (yet) by nitrate. In contrast, *Sphagnum contortum* was found not to be sensitive to increased ammonium availability. The negative effect of ammonium on brown mosses was confirmed in additional experiments (Paulissen et al., 2004; 2005).

The effects of different loads and forms of N on rich fen vegetation were studied in the same fen in central Ireland (ambient load 6-10 kg N ha⁻¹ yr⁻¹), for five years (Dorland et al., 2008; Verhoeven et al., 2011 – this later study as noted as ‘in-press’ in the last review). N was added either as ammonium or nitrate in the following doses: 35 and 70 kg N ha⁻¹ yr⁻¹. Peat water was characterised by a high pH, very low concentrations of nitrate, ammonium and phosphate, and high base-cation concentrations (especially Calcium; Verhoeven et al., 2011). Ammonium additions at rates of 35 and 70 kg N ha⁻¹ yr⁻¹ significantly increased tissue N concentrations in the bryophyte *Calliergonella cuspidata* and reduced the number of bryophyte species and bryophyte biomass production (Figure 6.14; Dorland et al., 2008; Verhoeven et al., 2011). In contrast, vascular plant species were not affected by N addition, and their biomass was even increased as a result of ammonium addition (Dorland et al., 2008). Vascular plants benefited from the opening up of the thick bryophyte layer, and from the reduced N uptake by bryophytes.

Figure 6.14. Dry biomass (left) and species number (right) of bryophytes after five years of N addition in a rich fen in central Ireland (Scragh Bog). Treatments: O = control treatment; NH = ammonium addition; NO = nitrate addition. Number before code = annual load of element in $\text{kg ha}^{-1} \text{yr}^{-1}$. Source: Verhoeven et al. (2011).



Source: Verhoeven et al., 2011

There are limited new publications to consider since the last review of CL_{empN} . A survey of alkaline fens (Q41) over a low N deposition range (4.2 to $10.0 \text{ kg N ha}^{-1} \text{yr}^{-1}$) in Ireland used TITAN change point analysis on vegetation data collected from 32 relevé plots (Wilkins et al., 2016; Aherne et al., 2021). The authors identified a change point threshold at $5.5 \text{ kg N ha}^{-1} \text{yr}^{-1}$ with decreases in the abundance of 5 species including 2 positive indicator species. The threshold in this study is substantially below the existing CL_{empN} range of 15 - $30 \text{ kg N ha}^{-1} \text{yr}^{-1}$, however, the study was limited by the small number of plots spanning a narrow deposition gradient. In contrast, Van den Berg et al. (2016) assessed the effects of total N deposition on species richness across eight habitats. They found clear evidence that N deposition affects species richness in all habitats except base-rich mires ($n = 274$ over a deposition gradient of 5.1 to $54.2 \text{ kg N ha}^{-1} \text{yr}^{-1}$), after factoring out correlated explanatory variables (climate and sulphur deposition).

Summary of Q4

It is difficult to make recommendations to drastically change the existing CL_{empN} based upon the limited new evidence. We have strong indications that the upper limit of $30 \text{ kg N ha}^{-1} \text{yr}^{-1}$ for rich fens is too high. Therefore, we propose a CL_{empN} range of 15 - $25 \text{ kg N ha}^{-1} \text{yr}^{-1}$ for rich fens (Q41-Q44), similar to arctic-alpine rich fens (Q45). In addition, we have increased the reliability for rich fens from 'expert judgement' to 'quite reliable'. Long-term fertilisation experiments with ecologically relevant additions of N and observational studies in temperate regions, northern countries and arctic-alpine fens would increase the reliability of these figures. For arctic-alpine rich fens (Q45; CL_{empN} based on expert judgement), it is likely that the critical load should be lower with further research particularly recommended in this habitat.

6.6 Overall summary for mire, bog and fen habitats (EUNIS class Q)

An overview of the CL_{emp}N ranges for mire, bog and fen habitats is presented in Table 6.1.

Table 6.1. CL_{emp}N and effects of exceedances on different mire, bog and fen habitats (Q). **##** reliable, **#** quite reliable and **(#)** expert judgement. Changes with respect to 2011 are indicated as values in bold.

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2011 reliability	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
Raised and blanket bogs	Q1	5-10	##	5-10	##	Increase in vascular plants, decrease in bryophytes; altered growth and species composition of bryophytes; increased N in peat and peat water
Valley mires, poor fens and transition mires	Q2	10-15 ^a	#	5-15	##	Increase in sedges and vascular plants; negative effects on bryophytes
Palsa and polygon mires	Q3			3-10	(#)	Increase in graminoids; tissue N concentrations and decomposition rate
Rich fens	Q41-Q44	15-30	(#)	15-25	#	Increase in tall vascular plants (especially graminoids); decrease in bryophytes
Arctic-alpine rich fens	Q45	15-25	(#)	15-25	(#)	Increase in vascular plants; decrease in bryophytes

^{a)} The CL_{emp}N previously recommended for poor fens (Q22) by Bobbink and Hettelingh (2011) was 10-15 kg N ha⁻¹ yr⁻¹, with the low end of the range emphasised for oceanic valley mires (Q21) where limited studies at low N were available.

6.7 Recommendations and knowledge gaps

Since the last update a limited number of experiments with realistic additions of N that are within or below the range of the critical load have been published. We recommend establishing field experiments in areas with low deposition histories to understand responses at low N. We also recommend experimental work in habitat types where the reliability of the critical load is based on expert judgement or is only quite reliable (e.g. Q3, Q41–Q44). Especially for arctic-alpine montane fens (Q45), fertilisation experiments are urgently needed to increase the reliability of its critical load (still based on expert judgement). For poor fens (Q2) we encourage further research into low N responses to determine if the higher end of the range (15 kg N ha⁻¹ yr⁻¹) is appropriate. In this ecosystem, ground water N is also important but the interactive effects of this and atmospheric deposition are poorly understood.

Long-term fertilisation experiments (> 10 years) are needed to determine the cumulative effects of nitrogen. In addition, reduced nitrogen appears to be more harmful than oxidised nitrogen, but the number of studies on the form of nitrogen are very limited (only for Q1). Nitrogen research is often limited to the effects on the vegetation, leaving the fauna (e.g. birds and butterflies) underexposed.

6.8 References

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Final Draft

7 Effects of nitrogen inputs in grasslands and lands dominated by forbs, mosses and lichens (EUNIS class R, formerly E)

Adapted by Carly Stevens, Rocio Alonso, Vegar Bakkestuen, Erika Hiltbrunner and Lukas Kohli



Annual xeric grassland constituting the understory of a Dehesa, an open evergreen oak woodland, in Tres Cantos (central Spain). Dehesas and montados are traditional agroforestry systems characteristics of the Mediterranean area. Photo: CIEMAT.

Summary

In this chapter empirical N critical loads ($CL_{emp}N$) for grasslands and lands dominated by forbs, mosses and lichens have been updated and revised, if necessary. A number of experimental N addition experiments and gradient studies have become available in the present revision period. Based on these studies the $CL_{emp}N$ of four grassland types should be lowered. Calcareous grassland communities (R1A and R1B) are given a $CL_{emp}N$ of 10-20 kg N ha⁻¹ yr⁻¹, Mediterranean grassland communities (R1D, R1E and R1F) are given a $CL_{emp}N$ of 5-15 kg N ha⁻¹ yr⁻¹, acidic grassland communities (R1M) are given a $CL_{emp}N$ of 6-10 kg N ha⁻¹ yr⁻¹, and inland dune grassland communities (R1P and R1Q) are given a $CL_{emp}N$ of 5-15 kg N ha⁻¹ yr⁻¹. Although there is good evidence to support the $CL_{emp}N$ in some habitats long-term N addition studies with low doses of N application and gradient studies are needed in Mediterranean grassland (R1D, R1E and R1F), inland dune grasslands (R1P and R1Q), moist or wet mesotrophic to eutrophic hay (R35) and low and medium altitude hay meadows (R22) where $CL_{emp}N$ are based on expert judgement.

7.1 Introduction

A large number of grassland ecosystems (class R of the European Nature Information System (EUNIS), formerly E) are found across Europe: from very dry to wet habitats, acidic to alkaline conditions, inland saline soils, those adapted to high concentrations of heavy metals and very different climatic regimes (e.g. Ellenberg, 1988; Davies et al., 2004; Dengler et al., 2020). Only a small proportion of these grasslands are of natural origin (e.g. dry steppe grasslands, alpine grasslands), while some of these habitats are strongly intensified, some are covered by semi-natural vegetation (Dengler et al., 2020). Traditional agricultural use or management is thus an important ecological factor influencing the structure and function of these grassland systems. These grasslands have long been an important part of the European landscape and contain many rare and endangered plant and animal species; a number of them have been set aside as nature reserves in several European countries (e.g. Ellenberg, 1988; Dengler et al., 2020). Semi-natural grasslands of conservation importance are generally nutrient-poor, because of the agricultural use involving low levels of manure addition and the removal of plant material by grazing or hay making. The vegetation is often characterised by many species of low stature due to the nutrient-poor nature of soils (Ellenberg, 1988). However, some semi-natural meadow communities of high nature conservation value, particularly those on deep alluvial soils in river flood plains subjected to periodic inundation or inputs of manure, can be moderately fertile with soil macro-nutrients levels at the higher end of the spectrum covering species-rich grasslands. These are likely to have a higher proportion of relatively fast-growing species than, for example, oligotrophic acidic or calcareous grasslands. To maintain high species diversity, it is recommended that artificial fertilisers and slurry should be avoided. Given the sensitivity to artificial fertilisers, it is thus likely that several of these species-rich grasslands, especially those of oligotrophic or mesotrophic soils, will also be sensitive to increased atmospheric nitrogen (N) inputs. Moreover, several of the most species-rich grasslands are found under weakly buffered or almost neutral soil conditions, which make them sensitive to soil acidification and very sensitive to the negative impacts of ammonium accumulation in the case of high deposition of reduced N (see Chapter 1).

Grasslands and lands dominated by forbs, mosses or lichens (EUNIS class R), which are dry or only seasonally wet (i.e. with the water table at or above ground level for less than half of the year) usually have a vegetation cover greater than 30%. The dominant part of the vegetation is grasses, sedges and other non-woody plants, including moss-, lichen- and fern- and sedge-dominated communities. An important level of division is based on soil water availability (dry (R1), mesic (R2) and wet (R3) grasslands). Most of the studies on the effects of N in grassland habitats have been carried out for ecosystems which are classified as dry grasslands (R1). Furthermore, the impacts of N inputs have only been studied in parts of the other major EUNIS categories (R2-R7). Some information exists on mesic grasslands (R2) and wet grasslands (R3), the classification of which particularly relates to present land use or management. Evidence also exists on the impacts of N deposition in alpine and subalpine grasslands (R4).

7.2 Dry grasslands (R1)

7.2.1 Semi-dry perennial calcareous grassland (meadow steppe, R1A)

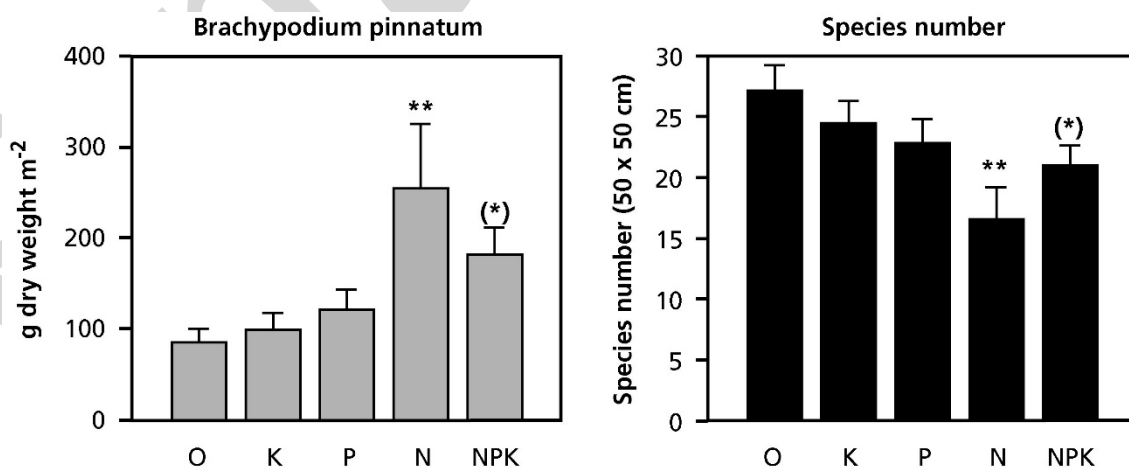
Calcareous grasslands (EUNIS category R1A) are communities developed on soils derived from limestone as parent materials, which are widespread in the hilly and mountainous regions of western and central Europe. Subsoils consist of different kinds of limestone with high contents of calcium carbonate (> 90%), covered by shallow rendzina soils, low in plant-available phosphorus (P) and N (pH of the top soil: 7-8 with a calcium carbonate content of approximately

10%). Plant productivity is low and calcareous grasslands are among the most species-rich plant communities in Europe, including a large number of rare and endangered species (Dengler et al., 2020). These grasslands decreased strongly in area during the second half of the last century due to urbanisation and conversion to agriculture (e.g. Wolking and Plank, 1981; Ratcliffe, 1984). To maintain the characteristic calcareous vegetation, a specific management is needed in most situations to prevent its natural succession towards woodland (Wells, 1974; Dierschke, 1985).

In the late 1970s and early 1980s, a gradual increase in one grass species (*Brachypodium pinnatum*) had been observed in Dutch calcareous grasslands, although management of these grasslands (hay making in autumn) had not been changed since the mid-1950s. Since then, it has been hypothesised that the increase in atmospheric deposition of N (from 10-15 kg N ha⁻¹ yr⁻¹ in the 1950s to 25-35 kg N ha⁻¹ yr⁻¹ in the 1980s) caused this drastic change in vegetation composition (Bobbink and Willems, 1987). These effects of N enrichment were investigated in two field experiments in the Netherlands (Bobbink et al., 1988; Bobbink, 1991). Application of ammonium nitrate (50-100 kg N ha⁻¹ yr⁻¹, in addition to an ambient load of 30-35 kg N ha⁻¹ yr⁻¹) over a three-year period resulted in a drastic increase in the grass *B. pinnatum*, and in a strong reduction in species diversity (including several Dutch Red List species) (Figure 7.1).

B. pinnatum is very efficient at both acquiring N from the soil and re-allocating it from senescing shoots to its well-developed rhizome system. It benefits from the extra N invested into the below-ground rhizomes by enhanced growth in the next spring. In this way *B. pinnatum* strongly monopolised the N storage (>75%) in both the above-ground and below-ground plant compartments in response to increasing N availability (Bobbink et al., 1988; 1989; De Kroon and Bobbink, 1997). Besides this decrease in phanerogamic plant species due to increased N deposition and the spread of *Brachypodium pinnatum*, many characteristic lichens and mosses have disappeared from Dutch calcareous grasslands since the 1960s (During and Willems, 1986). This has partly been caused by the (indirect) effects of extra N inputs, as shown in experiments by Van Tooren et al. (1990).

Figure 7.1. Above-ground biomass of *Brachypodium pinnatum* (g m⁻²) and vascular plant species number (per 50 x 50 cm) on Dutch perennial calcareous grassland (R1A) after three years of N application in the form of ammonium nitrate (100 kg N ha⁻¹ yr⁻¹) (after Bobbink, 1991). O = control.



Source: Bobbink, 1991

From the 1950s to the mid-1980s, almost all of the calcareous grasslands in the Netherlands were mowed and the hay was removed. By the removal of the hay, between 17 and 22 kg N ha⁻¹ yr⁻¹ was taken away from the system under the usual land management (Bobbink, 1991).

Legume species (*Fabaceae*) also occur in these grasslands, and provide an additional N input associated with the N-fixing bacteria in their root nodules (approximately 5 kg N ha⁻¹ yr⁻¹). The N mass balance of Dutch calcareous grasslands was summarised in Bobbink et al. (1992), and a critical load of N was estimated using a steady-state mass balance model (e.g. De Vries et al., 1994). Assuming a long-term immobilisation rate for N of between 0 and 6 kg N ha⁻¹ yr⁻¹, the critical load of N could be derived by adding the N fluxes due to net uptake, denitrification and leaching, corrected for the N input from fixation. In this way, the authors determined 15 to 25 kg N ha⁻¹ yr⁻¹ as the critical load for N (Bobbink et al., 1992). This range is in close accordance with the results found by Neitzke (1998, 2001) for calcareous grasslands in the eastern Eifel in south-west Germany. In a gradient of nutrient enrichment extending along a transect away from an agricultural field, N mineralisation was found to explain the variation in species composition and species degradation, from the nutrient enriched border zone to the intact central calcareous grassland. Comparing the soil N mineralisation rates of the undisturbed calcareous grasslands and the plots with significantly altered species composition, *B. pinnatum* increased in cover, and species diversity decreased when N mineralisation increased from 6 to 10 kg N ha⁻¹ yr⁻¹ in the unaffected parts to between 35 and 55 kg N ha⁻¹ yr⁻¹ in the areas adjacent to the agricultural fields (Neitzke, 1998, 2001).

Following a survey of data from a number of conservation sites in southern England, Pitcairn et al. (1991) concluded that *B. pinnatum* had expanded in the United Kingdom (UK) during the last century. They considered that much of the early spread could be attributed to a decreased grazing pressure, but that more recent increases in the grass, in some cases, had occurred despite grazing or mowing, and may have been related to increased N inputs. A retrospective study of a heavily grazed calcareous grassland at Parsonage Downs (UK), however, showed no substantial change in species composition between 1970 and 1990, a period for which N deposition is thought to have increased from probably below 10 kg N ha⁻¹ yr⁻¹ to 15 to 20 kg N ha⁻¹ yr⁻¹ (Wells et al., 1993). *B. pinnatum* was present in the sward but did not expand as in the Dutch grasslands.

In studies on calcareous grasslands in England, additions of N hardly stimulated a dominance of grasses (Smith et al., 1971; Jeffrey and Pigott, 1973). However, with applications of 50 to 100 kg N ha⁻¹ yr⁻¹ and further additions of P, a dominance of the grasses *Festuca rubra*, *Festuca ovina* or *Agrostis stolonifera* was observed. However, *B. pinnatum* or *Bromus erectus*, the most frequent species on continental calcareous grasslands, were absent from these British sites, so the data are not comparable in that respect. Van den Berg et al. (2011) conducted a survey of 46 plots on calcareous grassland in nature reserves in the UK. The plots were first surveyed between 1990 and 1993 and then re-surveyed between 2005 and 2009. The survey found a linear increase in the grass:herb ratio that was apparent from the lowest levels of deposition. The results also showed a decline in Shannon diversity and evenness as well as a decline in characteristic calcareous grassland species and rare species. The change in both Shannon diversity and evenness became a negative one around 20-25 kg N ha⁻¹ yr⁻¹. Structural equation modelling indicated that direct effects of N deposition were the dominant mechanism for change in Shannon diversity. Changes in the abundance of individual species were observed even in the lowest N deposition range of 0-15 kg N ha⁻¹ yr⁻¹. Despite these changes in species composition there was no decline in species richness associated with N deposition nor a change in average Ellenberg N or the number of eutrophic or oligotrophic species.

Diekmann et al. (2014) also found changes in species composition and richness by analysing published data from 1061 plots in *Bromion erecti* grasslands in north-west Germany dating from 1936 onwards plus 125 plots sampled in 2008. Whilst declines in species richness were not observed, there was a decline in specialist and rare species and an increase in generalist and

taller species. Detrended correspondence analysis (DCA) showed that Ellenberg N score was aligned with the primary axis of variation. *B. pinnatum* did not increase in relation to N deposition.

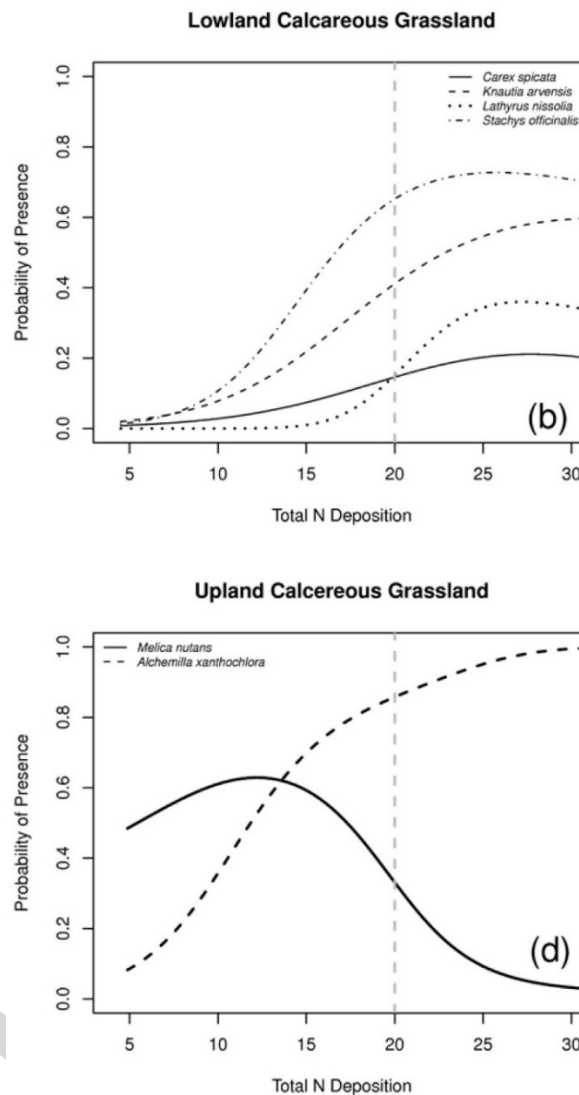
A series of studies from the UK have used data from the UK Countryside Survey to examine relationships between species richness and composition and N deposition. The UK Countryside Survey is a national survey of randomly located 1 x 1 km squares across the UK. Within these squares are various quadrat sizes including 2 x 2 m plots where vegetation is recorded. Surveys were conducted in 1998 and 2007. There were 94 calcareous grassland plots used in the analysis by Maskell et al. (2010), and 92 in the analysis by Tipping et al. (2013) from 1998, and 869 in Van den Berg et al. (2016) from 2007 where a different definition of calcareous grasslands was used. Maskell et al. (2010) found no relationship between species richness and N deposition for calcareous grassland. Using cover-weighted values from trait databases they noticed significant increases in canopy height, grass:forb ratio, Ellenberg N and leaf N concentration. Also using data from 2007, Tipping et al. (2013) applied non-parametric quantile regression with a breakpoint aiming at estimating the breakpoint where there was a clear effect of N deposition on species richness. They estimated 23.6 kg N ha⁻¹ yr⁻¹ as the breakpoint. Van den Berg et al. (2016) undertook further analysis once new data were collected in 2007. They found that total N deposition was positively related to species richness. The larger number of plots enabled them to examine relationships with NH_x and NO_y separately. NH_x:NO_y ratio was significantly negatively related to richness. Grass:forb ratio significantly increased as N deposition increased. There was a large variation between communities classified as calcareous grasslands in Van den Berg et al. (2016) and the authors found that breaking the category down to examine the most common grassland groups showed no significant relationships with N deposition. The authors suggested variation in local management may be responsible for the lack of observable impact.

A four-year N addition experiment was performed in a calcareous grassland (R1A) in Voeren, Belgium (Jacquemyn et al., 2003). In this study, the effects of N additions at three levels (30, 60 and 90 kg N ha⁻¹ yr⁻¹), in the form of ammonium nitrate (added once a year), were examined in a factorial experiment with two management treatments (high density cattle grazing and mowing with removal of the hay). The background deposition at the site was approximately 20 kg N ha⁻¹ yr⁻¹ (modelled EMEP data). Species richness decreased significantly, from around 25 to 29 vascular plant species per m² in the control area, to between 18 and 20 species after additions of 30 kg N ha⁻¹ yr⁻¹ and between 15 and 18 species after the two highest N additions. Much of the reduction in species richness could be attributed to decreased light availability, resulting from increased above-ground productivity of tall grasses and forb species. In contrast to the results in Dutch calcareous grasslands, *B. pinnatum* was not present on this study site, although other tall grasses and forbs became very abundant.

Henrys et al. (2011) undertook a spatial analysis using data collected from two surveys on species presence across the UK. The Vascular Plant Database records vascular plant species at 10 km resolution for the period 1987-1999. The second data set was the BSBI Local Change Database which records wild vascular plants in 811 2 x 2 km tetrads set within a regular grid of hectads (10 x 10 km) across the UK. For both datasets, species were assigned to a habitat, habitat generalists were excluded, and survey squares were designated as upland or lowland. Generalised additive models (GAMs) were employed to look at relationships between species occurrence or Ellenberg scores averaged per habitat, and N deposition, whilst accounting for the effect of other environmental variables (intensity of land-use in each grid square; minimum January temperature; maximum July temperature; total annual rainfall; and change in sulphur deposition). For the Vascular Plant Database 40 species in lowland calcareous grasslands were

analysed by the GAM models, 17 of these species showed a significant relationship in their presence with N deposition, 9 of these declined from 10 kg N ha⁻¹ yr⁻¹ or less. Four species showed positive relationships, all increasing their presence at around 25 kg N ha⁻¹ yr⁻¹. In upland calcareous grasslands, there were only sufficient data to analyse 7 species, 2 of these showed significant relationships with N, one increased steadily from lowest levels of N deposition the other declined rapidly after 15 kg N ha⁻¹ yr⁻¹ (Figure 7.2). In the BSBI Local Change dataset 17 species were analysed in lowland calcareous grasslands, 8 of these showed a significant relationship with N deposition, some of these were complex relationships but a number showed steep declines from the lowest levels of deposition. In upland grasslands, there were three species which showed significant relationships with N deposition and all showed a hump-shaped relationship. Only one species showed a decrease in both datasets (*Ononis repens*- a typical Mesobromion species), while other species tended to have a significant relationship in one dataset but non-significant in the other. In the Vascular Plant database, Ellenberg N increased with increasing N deposition for lowland and upland calcareous grasslands, whereas in the BSBI dataset these increases were not always linear, but tended to increase from lowest levels of N deposition in lowland calcareous grasslands.

Figure 7.2. Change in probability of presence for species showing statistically significant relationships to N deposition in calcareous grasslands in the Vascular Plant database against increasing total inorganic N deposition ($\text{kg N ha}^{-1} \text{ yr}^{-1}$) (from Henrys et al., 2011).



Source: Henrys et al., 2011

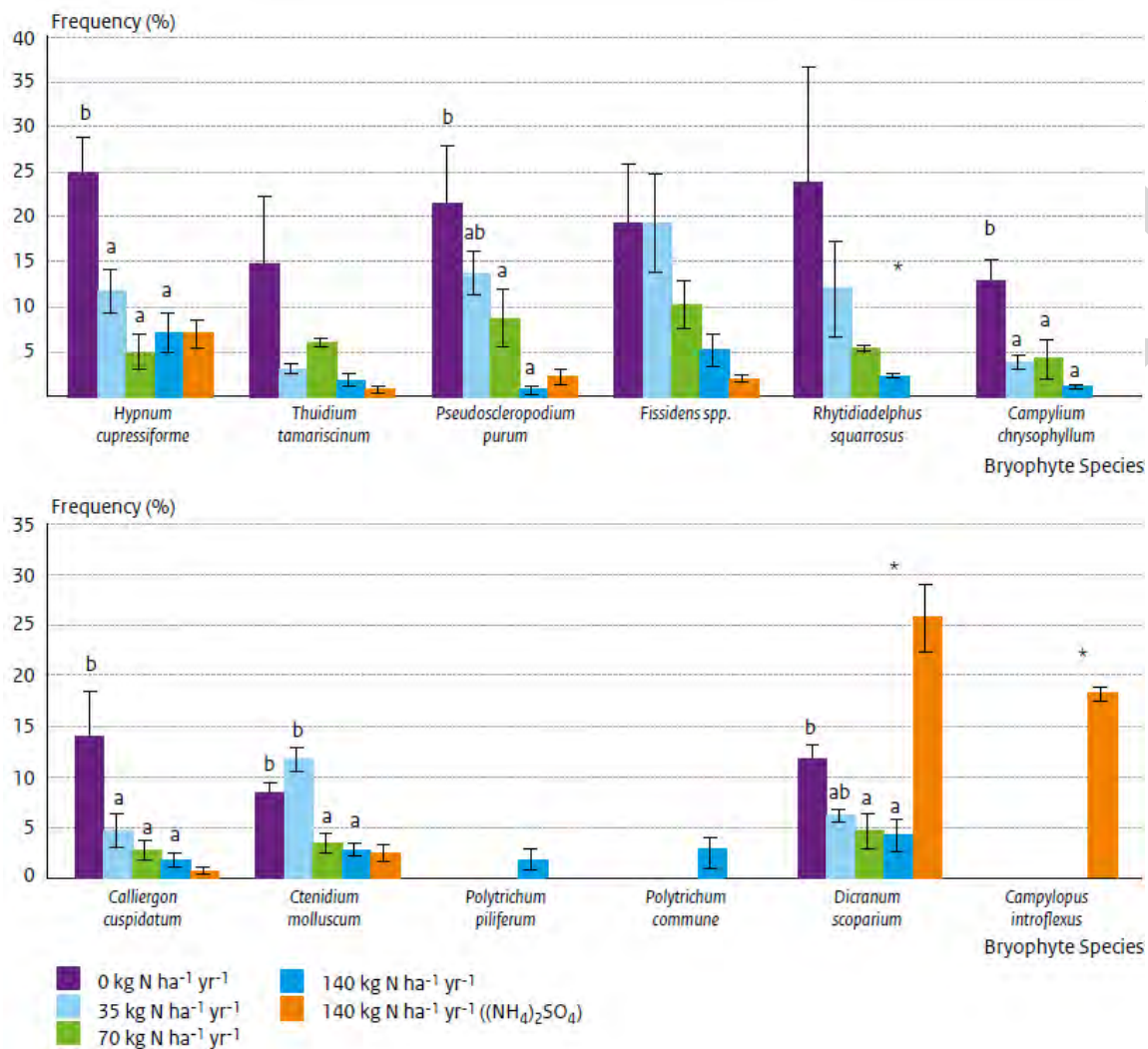
Stevens et al. (2012) took a very similar approach using spatial analysis with data from the British Lichen Society database over a 50-year period. Data were analysed at a 10 km resolution and as in Henrys et al. (2011), generalised additive models were applied to look at occurrence in relation to N deposition accounting for other environmental drivers. To be included in the analysis, species had to be terricolous (i.e. growing on the soil or ground), largely associated with one habitat, not too scarce or regionally distributed and accurately represented in the database. Sixteen lichen species were analysed of which three showed a significant relationship with N deposition. *Cladonia foliacea* declined significantly with increasing N deposition above $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$.

Long-term effects of ammonium nitrate additions ($35, 70$ and $140 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) were studied between 1990 and 2008, in a calcareous grassland area (Wardlow Hay Cop) with a shallow soil ($< 10 \text{ cm}$) on limestone bedrock in Derbyshire, in the UK (Morecroft et al., 1994; Carroll et al., 1997; Lee and Caporn, 1999; Carroll et al., 2003; Phoenix et al., 2003; 2004; Haworth et al., 2007; Horswill et al., 2008, O'Sullivan, 2011). The highest N addition was also applied in the form of

ammonium sulphate. Within the first four years of N addition, no significant change in species composition of vascular plants was observed. From the sixth year onwards, however, there was a marked and significant dose-related decline in vascular plant cover with increased N addition (Carroll et al., 1997; Lee and Caporn, 1999; Carroll et al., 2003). The species that were negatively affected by N included a range of subordinate species (e.g. *Thymus*) typical of calcareous grasslands. In contrast, *Hypochaeris radicata* increased in the N-enriched vegetation (Carroll et al., 1997; Lee and Caporn, 1999). Overall, vegetation responses of vascular plants were slow, and significant changes in individual species cover were found mainly at the higher levels of N addition, although the trend could be witnessed from the lowest treatment upwards. Where growth was concerned, no significant increases in response to N were seen at any stage in this experiment, suggesting the strong P limitation of plant production on this calcareous grassland area (Carroll et al., 2003).

Significant changes in bryophyte species composition were also observed in this UK experiment after 12 years of N additions (Haworth et al., 2007) (Figure 7.3). The lowest NH_4NO_3 additions ($35 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) produced significant declines in frequency of *Hypnum cupressiforme*, *Campyllum chrysophyllum*, and *Calliargon cuspidatum*. Significant reductions in frequency at higher NH_4NO_3 application rates were recorded for *Pseudoscleropodium purum*, *Ctenidium molluscum*, and *Dicranum scoparium*. The highest NH_4NO_3 and $(\text{NH}_4)_2\text{SO}_4$ additions provided acidic conditions for the establishment of two typical calcifuges – *Polytrichum* species and *Campylopus introflexus*, respectively. Substrate-surface (0–2.5 cm) pH measurements showed a dose-related reduction in pH with increasing NH_4NO_3 addition rates, with a difference of 1.6 pH units between the control and highest deposition rate, and a further significant fall in pH of more than 1 pH unit, between the NH_4NO_3 and $(\text{NH}_4)_2\text{SO}_4$ treatments. *Dicranum scoparium* was strongly promoted by the $(\text{NH}_4)_2\text{SO}_4$ treatment, maybe also due to the strongly decreased pH. There is a clear indication that in this experiment increased N inputs led to soil acidification, especially in the top centimetres of the soil, as was also seen for the full soil layer after ten years of N addition (Horswill et al., 2008). They also found significant losses of soil base cations and increases in aluminium (Al) and manganese (Mn) following the highest N treatments ($140 \text{ kg N ha}^{-1} \text{ yr}^{-1}$; especially with $(\text{NH}_4)_2\text{SO}_4$); clear signs of soil acidification, which might lead to declines in sensitive calcicole species.

Figure 7.3. Frequency of bryophyte species at Wardlow Hay Cop after 12 years of treatment with different N loads and the impact of sulphate rather than nitrate at 140 kg N a⁻¹ yr⁻¹. Significance between N forms is denoted by * (based on Haworth et al., 2007, adapted by Bobbink and Hettelingh, 2011)



Source: Haworth et al., 2007; Bobbink and Hettelingh, 2011

In contrast to the slow vegetation responses, N concentrations in shoots, nitrate reductase activities, and soil N mineralisation and nitrification rates significantly increased during the early years of increased inputs of N (≥ 70 kg N ha⁻¹ yr⁻¹) (Morecroft et al., 1994; Carroll et al., 1997). After six years of treatments, N mineralisation rates in summer were significantly higher following all N treatments, but in autumn or winter they were only significant after the two highest N additions. Nitrification was also clearly higher in summer on all N-treated plots, but in autumn and winter, nitrification rates following the 35 kg N ha⁻¹ yr⁻¹ addition were not significantly different from those of the controls. Soil microbial activity was not significantly affected by six years of ammonium nitrate additions, but root-surface phosphomonoesterase activity increased significantly following the addition of ammonium sulphate (Johnson et al., 1998). In the eighth year of N additions, significant increases in root-surface phosphomonoesterase activities were found for three plant species (a forb, a grass and a sedge) after additions of both 35 and 140 kg N ha⁻¹ yr⁻¹ in the form of ammonium nitrate (Phoenix et al., 2004). This showed an increased demand for P induced by N enrichment.

Nitrogen cycling and accumulation in calcareous grasslands can be significantly reduced by two major processes: 1) leaching from the soil, and 2) removal through management regimes. N losses by denitrification in dry calcareous grasslands are low ($< 1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$; e.g. Mosier et al., 1981). The N budget and fluxes of this calcareous grassland site have been reported by Phoenix et al. (2003) following six years of N additions. Their major finding was that even sustained, very high inputs of N did not lead to large losses of N. In general, 80 to 90% of the additional N was retained in the system; even 65% remained in the plots with the high $140 \text{ N ha}^{-1} \text{ yr}^{-1}$ additions, a treatment that represented a seven-fold increase of the ambient N deposition. The major fluxes of N loss from this grassland were from biomass removal (simulated grazing) and leaching of organic N, constituting 90% of leached N under ambient conditions. Leaching of nitrate contributed significantly to the output flux of N under the highest N treatment only. Even in this P-limited grassland, a (very) high fraction of the extra N was accumulated (immobilised) in the soil system. After 12 years of N addition O'Sullivan et al. (2011) reported no significant differences in soil ammonium N concentrations between treatments and controls but increases in concentrations of oxidised N.

Unkovich et al. (1998) also found N limitation in their field study in Wytham (UK). They noticed more than a doubling in plant production after weekly additions of $11.5 \text{ kg N ha}^{-1}$ for 6 weeks (total N addition of almost 70 kg N ha^{-1}), independently of N form (ammonium or nitrate) and no response to P or any other nutrient. Adding the N in the form of ammonium sulphate or ammonium nitrate increased foliar N concentrations significantly, compared with the controls and also with plots where N was added in the form of potassium nitrate. Plants took up more than 40% of the added N; the remaining additions (almost 60%) were immobilised in the soil (Unkovich et al., 1998).

In a gradient study of 120 plots across 21 calcareous and 19 acidic grasslands in eight countries in Europe with a N deposition range of $4\text{--}31 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, Ceulemans et al. (2014) performed a molecular analysis to identify the impacts of N deposition on mycorrhizal fungi. Richness of arbuscular mycorrhizal operational taxonomic units (OTUs) was negatively related to N deposition in calcareous grasslands, declining steadily from the lowest levels of N deposition. Fungal community composition was significantly affected by N deposition. There were also signs of soil acidification related to N deposition. TITAN (threshold indicator taxa analysis, Baker and King, 2010) was conducted on a combined acidic and calcareous grassland dataset to identify a breakpoint in the data. This analysis revealed a threshold of $7.7 \text{ kg N ha}^{-1} \text{ yr}^{-1}$; however, this is difficult to relate to $\text{CL}_{\text{emp}}\text{N}$ for a specific type of habitat because calcareous and acidic habitats were combined.

Less information is available for calcareous grasslands in the Mediterranean areas of Europe. A three-year N addition experiment performed in central Italy (Bonamoni et al., 2006) was considered in the 2010 update, but it was included in the 'Mediterranean xeric grassland' (Chapter 7.2.2). In this experiment, the effects of N enrichment, cutting and litter removal were investigated in a species-poor dry Mediterranean grassland. The site represented an abandoned area dominated by *Brachypodium rupestre*, 15 to 20 years after it had last been in agricultural use (rotating crops with legumes), and no management had been carried out since. In the experiment, N had been added once a year, in the form of commercial urea fertiliser, at a rate of $35 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Estimated background deposition was less than $15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. As for most grasslands without appropriate management, cutting of the vegetation highly reduced above-ground biomass, compared with the uncut controls. However, N additions significantly increased total above-ground biomass in each of three management treatments (uncut, litter removal and cutting), with the cutting treatment leading to the smallest increase in the vegetation. In this experiment, species diversity was quantified using the Shannon index (H), with diversity

increasing particularly after cutting of the vegetation, mostly of annual and biennial species, and to a lesser extent after litter removal. Species diversity was lower following all N addition treatments, but this effect was only significant in combination with litter removal. Furthermore, it was evident that the species diversity was highly negatively correlated ($R^2 = 0.85$) with the biomass percentage of *B. rupestre*, a finding similar to that for *B. pinnatum* in Dutch calcareous grasslands (Bobbink and Willems, 1987).

A similar experiment was conducted in an area nearby but in a species-rich mowed grassland (Bonamoni et al., 2009). In this case it was a calcareous grassland ascribed to the association *Briza mediae*–*Brometum erecti*, which is rich in rare and endemic species. The experiment was performed in both the mowed grassland and in an adjacent abandoned area. The mowed site was co-dominated by the grasses *Bromus erectus*, *Briza media*, *Anthoxanthum odoratum* and *Festuca circummediterranea* and the forbs *Rhinanthus personatus*, *Tragopogon pratensis*, *Onobrychis viciifolia* and *Trifolium pratense*, while the abandoned treatment was dominated by the perennial grasses *B. rupestre* and *Dactylis glomerata*. In this experiment a similar methodology to Bonamoni et al. (2006) was followed, investigating the interactive effects of three years of N enrichment, cutting and litter removal. Nitrogen was added once a year, in the form of commercial urea fertiliser, at a rate of 35 kg N ha⁻¹ yr⁻¹ and estimated background deposition was less than 15 kg N ha⁻¹ yr⁻¹. As found in the previous experiment, cutting significantly increased species diversity in the abandoned area expanding the cover of almost all annual and biennial species and several perennial forbs, and reducing the dominance of perennial grasses. On the other hand, in the mowed area, interrupting cutting did not reduce species diversity, although some rare species reduced their abundance while the perennial grasses started to increase their cover. Litter removal in this case had no significant effect on increasing species diversity, contrasting with the results found in the previous study. Nitrogen enrichment over three growing seasons did not affect species diversity in the abandoned and mowed areas and caused a limited increase of plant biomass, suggesting that these communities are only partially limited by nitrogen.

Summary for semi-dry perennial calcareous grassland (meadow steppe, R1A)

Considering the findings from gradient studies and spatial analyses, which revealed changes in species composition beginning from the lowest levels of deposition, it is necessary to reduce the CL_{emp}N from 15 to 25 kg N ha⁻¹ yr⁻¹ down to 10 to 20 kg N ha⁻¹ yr⁻¹ (reliable). Whilst there was some evidence of impacts below 10 kg N ha⁻¹ yr⁻¹, the number of sites below this level was relatively low since many of the gradient studies were from the UK with very few areas with low deposition. Increased N availability is probably of major importance in a number of European calcareous grasslands. In N-limited calcareous grasslands, increased availability of N is indicated by increased growth of some 'tall' grasses, especially of species which have a slightly higher potential growth rate and more efficient N utilisation. Nitrogen retention by the system is very high with hardly any leaching, and N mineralisation rates may also be increased because of N inputs. However, under P-limited conditions, vegetation responses are slow and loss of species is associated with changing soil conditions (acidification and decreased base saturation). Nitrogen mineralisation and nitrification are increased under P limitation, and in N-saturated systems with shallow soils this would result in somewhat higher leaching losses, although a large proportion of the N inputs would still be retained in the system. Most data from calcareous grasslands are from studies conducted in temperate, sub-Atlantic regions, and from sites with relatively high atmospheric N deposition. There is a need for experimental studies in continental regions, and for experiments with low N doses at sites with low ambient atmospheric deposition (< 10 kg N ha⁻¹ yr⁻¹).

7.2.2 Mediterranean closely grazed dry grassland (R1D) or Mediterranean tall perennial dry grassland (R1E) or Mediterranean annual-rich dry grassland (R1F)

EUNIS categories R1D, R1E and R1F include xeric, thermophilic and mostly open Mediterranean perennial grasslands rich in therophytes growing on usually eutrophic, but also oligotrophic soils. The diversity of plants, but also of invertebrate and vertebrate species, is usually high. The conservation of Mediterranean xeric grasslands has been favoured by traditional management and contributes to prominent cultural landscapes. Grazing is essential for the long-term preservation of these communities and a reduction in grazing will result in scrub encroachment. Mineral fertilisation with P has been traditionally used to improve pasture quality since it increases spontaneous legume species and therefore forage protein content. There was limited information available on the effects of N enrichment on these grasslands in previous CL_{emp}N revisions, but new studies have recently been published.

One of the main communities of Mediterranean xeric grasslands in the Iberian Peninsula are the annual pastures constituting the understory of Dehesas/Montados, a traditional agroforestry system, with a high biodiversity of species and high economic and ecological value. Nitrogen fertilisation experiments have been performed on the herbaceous stratum of a Dehesa in Central Spain (Migliavacca et al., 2017; Martini et al., 2019). The ecosystem is dominated by an annual grassland with low density of oak trees, mostly *Quercus ilex* (20% cover, 25 trees ha⁻¹). The herbaceous stratum is dominated by species of three main plant functional groups (grasses, forbs and legumes) whose proportion varied seasonally according to their phenological status. The plants are generally active from October to end of May. A first experiment assessed the effects of N or P alone or in combination at a small scale (9 x 9 m plots). In spring, N was applied as potassium nitrate (KNO₃) and ammonium nitrate (NH₄NO₃) at a rate of 100 kg N ha⁻¹ and P was added as monopotassium phosphate (KH₂PO₄) in one application of 50 kg P ha⁻¹. The total doses of N were approximately 10 times higher than the current N deposition rate in the area (Morris et al., 2019). One of the challenges found in this experiment is that response variables showed wide variations over time. Additions of N changed the abundance of different plants affecting the community architecture and biochemistry (Migliavacca et al., 2017; Martini et al., 2019). In particular, higher N increased forbs while graminoid cover decreased. Relative abundance of legumes was marginal and did not show significant changes with nutrient additions (Martini et al., 2019). Changes in the abundance of different plant functional groups modified ecosystem processes such as increasing gross primary productivity (GPP), transpiration and albedo (Martini et al., 2019). The results of P addition alone showed negligible response on vegetation structure and soil properties (Pérez-Priego et al., 2015).

An additional experiment was performed at large scale (24 ha) in the same area using eddy covariance and intense monitoring to analyse carbon and water fluxes and their response to climate variability at ecosystem level. In this case only three treatments were established: control, nitrogen and nitrogen + phosphorus (NP). Nitrogen was applied as calcium ammonium nitrate (Ca(NO₃)₂NH₄NO₃) in the N treatment and as ammonium nitrate (NH₄NO₃) in the NP treatment in one application of 100 kg N ha⁻¹ in the spring of first year and 20 kg N ha⁻¹ the following year. Phosphorus was added as triple superphosphate (Ca(H₂PO₄)₂) in one application of 50 kg P ha⁻¹ the first year and 10 kg P ha⁻¹ the second year. A P only treatment was not applied this time since the previous study indicated that the ecosystem is N-limited and did not respond to P fertilisation alone (Migliavacca et al., 2017; Nair et al., 2019). Results were collected throughout four years showing a large inter-annual variability with, for example, larger differences in Gross Primary Productivity (GPP) in those years with precipitation above average rainfall compared to dry years. Nitrogen fertilisation increased root biomass and root length density (Nair et al., 2019) favouring nutrient absorption and increases of N pools in leaves.

These changes caused a rise in the photosynthetic capacity and higher GPP and leaf area index in fertilized areas (Nair et al., 2019; Luo et al., 2020). As a result, there was a higher evapotranspiration and consumption of water that accelerated grass senescence (Luo et al., 2020). The structure of the grassland community also varied with N fertilisation decreasing the abundance of forbs in some years (Luo et al., 2020), similar to the results found in the experiment at small scale. The results of these experiments highlight that the interaction between the availability of nutrients and water determine the functioning, composition and structure of Mediterranean xeric grasslands linked to Montado/Dehesas. The projected climate warming and increase in regional drought could compensate the effects of N enrichment due to atmospheric deposition (Luo et al., 2020).

N effects on annual grasslands have also shown a significant interaction with ozone (O_3), the most important air pollutant in the Mediterranean region. Two experiments were performed in open top chambers in Spain with mesocosm simplified communities exposed to four treatments with increasing O_3 concentrations and three treatments of N enrichment representing background (around 5 kg N ha^{-1}), 20 and 40 kg N ha^{-1} . Nitrogen supplementation was applied biweekly as ammonium nitrate (NH_4NO_3) in four equal applications during the experiment covering the active growing season. Ozone exposure reduced the fertilisation effect of enhanced N availability, while N could counteract pernicious O_3 effects on plant biomass production, but only at moderate O_3 levels (Calvete-Sogo et al., 2014, 2017). Grass species were more responsive to N enrichment compared with legumes but O_3 was a more important factor than N in explaining plant responses (Calvete-Sogo et al., 2016).

A N deposition gradient has been studied in semiarid ecosystems in central, southern and eastern Spain covering a range from 4.3 to $7.3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Ochoa-Hueso et al., 2013). The gradient included 16 sites dominated by grasslands (10 sites), shrublands (4 sites) or woodlands (2 sites). Nitrogen deposition affected soil nutrient cycling and fertility, and altered the functioning of biological soil crusts (Ochoa-Hueso et al., 2013). Soil bacteria and cyanobacteria and fungi abundance decreased with N deposition while green algae and cyanobacteria richness increased, contributing to ecosystem eutrophication (Ochoa-Hueso et al., 2013, 2016). Biological soil crusts formed by terricolous lichen and bryophyte communities are important for ecosystem functioning and nutrient cycling in these semiarid ecosystems. Based on the linear responses found in most soil indicators, a threshold of $4.3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ was proposed (Ochoa-Hueso et al., 2013).

In the previous update of $CL_{emp}N$ (Bobbink and Hettelingh, 2011), a N fertilisation experiment in a species-poor grassland dominated by *B. rupestre* performed in central Italy was included as Mediterranean xeric grassland (Bonamoni et al., 2006). The site represented an abandoned area 15-20 years after it had last been in agricultural use (rotating crops with legumes). An additional experiment performed in an area nearby in a stable species-rich mowed grassland has been added since then (Bonamoni et al., 2009). In this case, the community was a calcareous grassland ascribed to the association *Brizo mediae-Brometum erecti*. When this area was abandoned, the perennial grasses *B. rupestre* and *Dactylis glomerata* become dominant indicating this experiment should be considered in the calcareous grasslands section.

Summary for Mediterranean closely grazed dry grassland (R1D) or Mediterranean tall perennial dry grassland (R1E) or Mediterranean annual-rich dry grassland (R1F)

In the U.S., a $CL_{emp}N$ for similar Mediterranean grasslands of $6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ has been proposed, based on annual grasses replacing native herbs in a nutrient-poor serpentine grassland (Pardo et al., 2011). In the previous $CL_{emp}N$ revision in Europe, a first estimate of the $CL_{emp}N$ for the EUNIS categories R1D, R1E and R1F was 15 to $25 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, based on expert judgement that

included information from an experiment in Italy that has now been transferred to the calcareous grasslands section R1A-R1B. Based on the gradient study outlined above and on the $CL_{emp}N$ available for scrublands (S5, S6) and forest (T21) communities, with which xeric grasslands are commonly associated, a lower range of 5-15 kg N ha⁻¹ yr⁻¹ is proposed as $CL_{emp}N$ for Mediterranean xeric grasslands based on expert judgement. However, further research is needed to disentangle the fate of N deposition effects in Mediterranean grasslands while interacting with a warmer and drier climate and high O₃ concentrations.

7.2.3 Lowland to montane, dry to mesic grassland usually dominated by *Nardus stricta* (R1M)

This EUNIS category groups all dry to mesic, base-deficient grasslands on acidic and neutral, weakly buffered, often sandy soils with closed vegetation in Atlantic or sub-Atlantic lowland and montane regions of northern and middle Europe and the western part of the Iberian Peninsula (R1M). Typical phytosociological units are *Violion caninae*, *Nardetalia strictae* and *Agrostion curtisii*. Species that are rare in parts of Europe, such as *Arnica montana*, *Antennaria dioica*, *Thymus vulgaris* and *Dactylorhiza maculata*, have been observed to disappear from these grasslands before tall and dense growing grasses started to dominate the vegetation in the Netherlands (e.g. Bobbink et al., 1996). In the Netherlands, these species are rare and endangered species are extremely sensitive to acidification and ammonium accumulation (e.g. Roelofs et al., 1996; De Graaf et al., 1998, 2009; Van den Berg et al., 2005; Kleijn et al., 2008). The input of acidifying nitrogenous deposition decreases the acid neutralising capacity (ANC) and subsequently the soil pH in these grasslands, which have weakly buffered soils. The deposited ammonium starts to accumulate once the pH significantly hampers nitrification (pH < 4.5). Thus, for these systems, species changes and loss of diversity are likely to be strongly associated with soil acidification inputs and/or changes in N form as well as the direct effects of N as a result of N.

Experimental N applications were carried out over three years on an *Agrostis capillaris* and a *Festuca ovina* grassland, respectively, both with a different initial fertility, in the province of Småland, in southern Sweden. This resulted in increased above-ground biomass as well as proportionately greater graminoid biomass following additions of 19 kg N ha⁻¹ yr⁻¹ for the low fertility *Festuca ovina* grassland (atmospheric load 13 kg N ha⁻¹ yr⁻¹). No significant response was found for additions of 37 kg N ha⁻¹ yr⁻¹ on the more fertile *Agrostis capillaris* grassland area (with atmospheric deposition of 15 kg N ha⁻¹ yr⁻¹), within three years of N additions (Berlin, 1998).

The effects of N additions (35, 70, 140 kg N ha⁻¹ yr⁻¹ in the form of ammonium nitrate) have been studied in a *Festuca-Agrostis-Galium* grassland in Derbyshire (UK) (atmospheric deposition approximately 25 kg N ha⁻¹ yr⁻¹) (Morecroft et al., 1994; Johnson et al., 1999; Lee et al., 2000; Carroll et al., 2003; Phoenix et al., 2003; Horswill et al., 2008; Arróniz-Crespo et al., 2008). During the second year of the experiment a significant decline in bryophytes (especially *Rhytidiadelphus squarrosus*) was found following all levels of N treatment. Although this may partly have been an artefact, resulting from the relatively high N concentration applied, it indicates a high sensitivity to N. Moreover, *R. squarrosus* collected from the plots showed an increasingly higher N concentration with each N addition (Carroll et al., 1997, 2000). Nitrate reductase activities and soil N mineralisation rates clearly increased with increasing inputs of N during the first three years (≥ 35 kg N ha⁻¹ yr⁻¹), this did not significantly alter the cover of vascular plant species, diversity or species composition, during the first four years (Morecroft et al., 1994; Carroll et al., 1997). From 1995 (sixth year of treatment) onwards, there has been a clear trend of decreased overall cover of the vegetation as well as decreased herb cover with increasing N additions. In 1995, an additional experiment with a factorial N x P design was

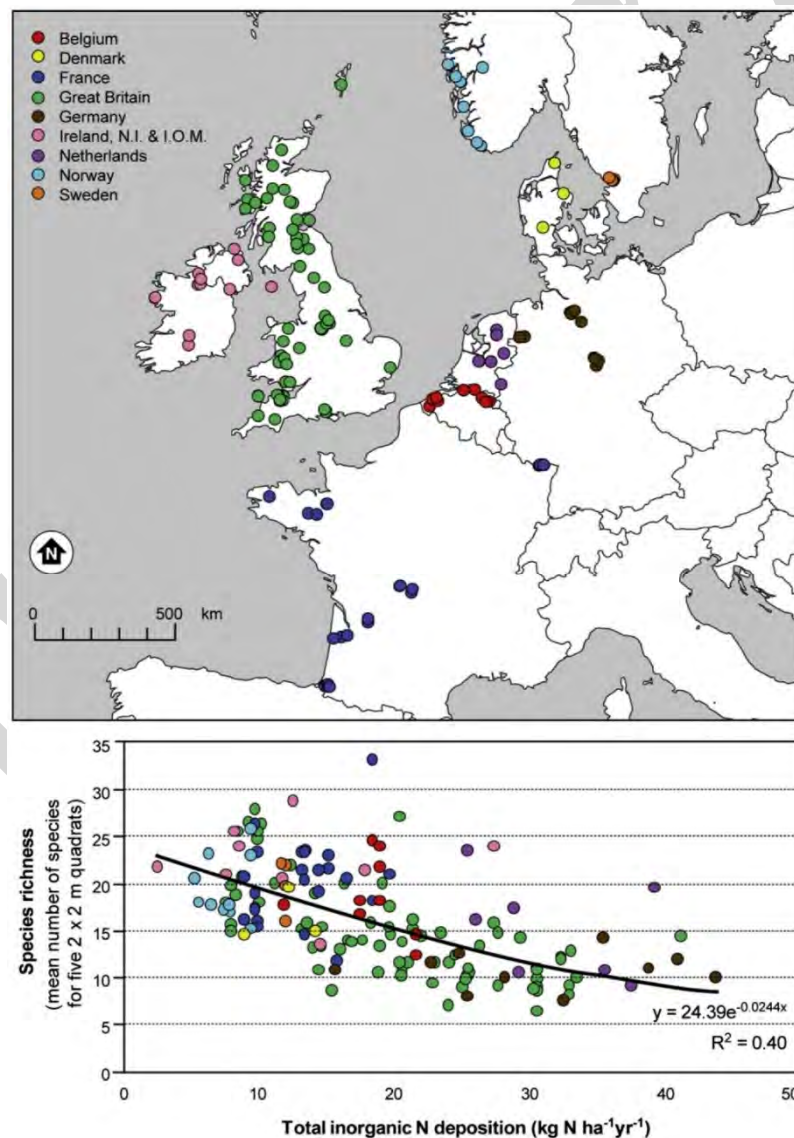
initiated with N additions of 35 and 140 kg N ha⁻¹ yr⁻¹. The results from the N only treatments corroborated the results found in the longer-term experiment, with a clear decrease in *Festuca ovina*, *Luzula campestris* and *Potentilla erecta*, and an increase in *Nardus stricta* (Lee and Caporn, 2001). This experiment also confirmed that this grassland was strongly limited by P rather than N (Lee and Caporn, 2001). N accumulation in the soil was not significant in any of the treatment areas, and N leaching was only significant at addition rates of ≥ 35 kg N ha⁻¹ yr⁻¹.

Phosphomonoesterase activity (plays a critical role in controlling P cycling), increased in the soil during the long-term experiment at the lowest N addition (35 kg N ha⁻¹ yr⁻¹) within three to four years (Carroll et al., 1997). Additionally, microcosm studies on soils from this field experiment (after seven years of N addition) showed increased root-surface phosphomonoesterase activity on the roots of *Agrostis stolonifera* seedlings, at N inputs of 35 kg N ha⁻¹ yr⁻¹ and higher. Thus, the increased N addition eventually also affected the P cycle in this severely P-limited system. A similar experiment with soils that had received N for only one and a half years showed no effect, indicating that the effect was the result of long-term changes in the soil (Johnson et al., 1999). After 12 years of N addition in the form of ammonium nitrate, ammonium-N concentrations in the soil showed strong dose-related treatment effects on the mineralisation-immobilisation N cycle in both organic and mineral horizons that were strongly dose related (O'Sullivan et al., 2011). Basto et al. (2015) studied the effect of N additions on the seed bank in the acidic grassland in the same experiment. They found that the seed bank size (i.e. number of seeds) was reduced by 61% in the 140 kg N ha⁻¹ yr⁻¹ N treatment and by 34% in the 35 kg N ha⁻¹ yr⁻¹ treatment. Seed bank richness was also reduced by 41% and 29%, respectively. Seed bank composition was also significantly changed with the largest reduction seen in forbs (73% at 140 kg N). Seed bank shifts were not the same as species composition changes observed in above-ground vegetation, resulting in an increased dissimilarity between above- and below ground species composition.

A field survey carried out by Stevens et al. (2004) revealed the relationship between atmospheric N deposition and plant species richness (including bryophytes) in acidic *Agrostis-Festuca* grasslands (*Violion caninae*) across the United Kingdom. The authors sampled 68 sites of high nature conservation interest in 2002 and 2003, with N deposition ranging from just above 5 kg N to 35 kg N ha⁻¹ yr⁻¹. Of the 20 variables measured, total N deposition was the most important predictor of the variability in species richness. A clear negative linear (or negative exponential, see Emmett (2007)) relationship between species richness of these grassland communities and N deposition was found (Figure 7.4). This dataset was further analysed by Field et al. (2014) who used a subset of 22 sites with deposition between 7.8 and 30.3 kg N ha⁻¹ yr⁻¹ showing a consistent rate of species loss with N deposition. Species composition changed and richness of bryophytes, lichens, forbs and graminoids declined due to N. Payne et al. (2011, 2013) investigated changes in individual species abundance. The moss *Hylocomium splendens*, hemi-parasitic forb *Euphrasia officinalis* and forb *Plantago lanceolata* were the species most strongly negatively associated with N deposition, whereas the moss *Hypnum cupressiforme* responded positively to N deposition. TITAN analysis was used to reveal change points in individual species abundances. The lowest possible change point could be identified at 7 kg N ha⁻¹ yr⁻¹. Further analysis provided by Payne et al. (2020) supported this with change points emerging below 10 kg N ha⁻¹ yr⁻¹. A community level threshold of 14.2 kg N ha⁻¹ yr⁻¹ was identified where the community shifts to a more N tolerant assemblage, however, one third of individual species, mostly forbs, had change points below 10 kg N ha⁻¹ yr⁻¹. Very few change points were noticed above 25 kg N ha⁻¹ yr⁻¹, suggesting that changes have already occurred by this level. It should be noted though that some of the grasslands would also be slightly wetter than those found in other parts of Europe.

To investigate if this relationship holds for the whole Atlantic range of these acidic grasslands, and to gain more insights on the lower and higher ends of the N deposition ranges, the European Science Foundation (ESF) funded a programme named BEGIN (Biodiversity of European Grasslands: The Impact of Atmospheric Nitrogen Deposition), that carried out a survey identical to the UK study of Stevens et al. (2004) in nine countries, from Norway to the south of France, in the 2007-2009 period (Stevens et al., 2010b). This survey has shown that on this scale, species richness is again negatively correlated with total N deposition (Figure 7.4). Across the region, 9.8% of variation in species composition was driven by N deposition (Stevens et al., 2011a) with the proportion of grasses increasing with increasing N deposition (Stevens et al., 2011b). Helsen et al. (2014) combined this data with an additional data set (Ceulemans et al., 2011), on 297 semi-natural *Nardus* grasslands with a N deposition from 2 to 43 kg N ha⁻¹ yr⁻¹. They found high soil N was associated with higher proportion of graminoids and long-lived, clonal species and a decrease in therophytes and forbs. Among other changes there was also a decrease in N₂-fixers, and orchids.

Figure 7.4. Species richness of acidic grasslands across a gradient of N deposition in the Atlantic region of Europe (Stevens et al., 2010b).



Source: Stevens et al., 2010b

In addition to this, Maskell et al. (2010) reported results from the UK countryside survey using data collected in 1998 which broadly support these findings. Analysis of this data using non-parametric quantile regression with a breakpoint identified a threshold at $7.8 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Tipping et al., 2013). Analysis of data from the same survey but using data collected in 2007 also showed a negative relationship between species richness and N deposition, reporting an increase in grass:forb ratio (Van den Berg et al., 2016). Results from both these reports confirm the findings in Stevens et al. (2004). Furthermore, an analysis of the changes in species composition across this gradient demonstrated that soil acidification is likely to be the most important driving factor of the observed decline in species alongside the other effects of N (Stevens et al., 2010a). Based on an evaluation with a Bayesian approach, Pescott and Jitlal (2020) suggested that the estimated negative effects of N deposition have been over-estimated. However, additional analyses showed their results were unreliable (Smart et al., 2021).

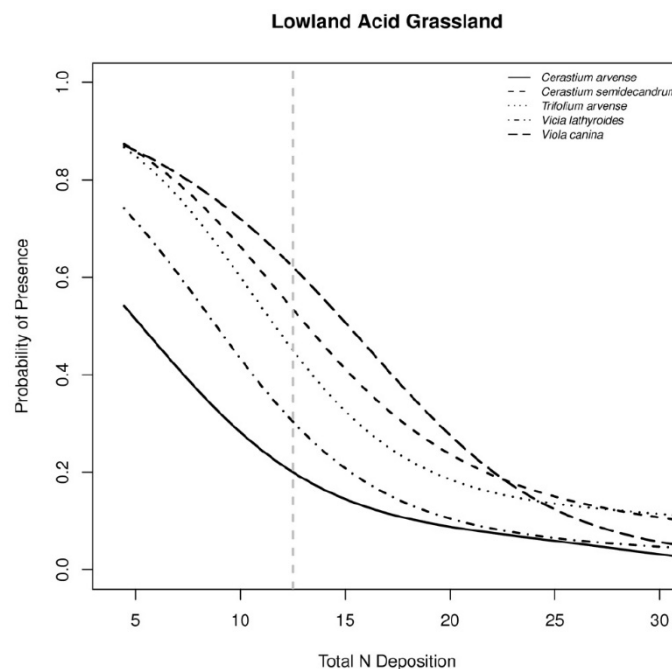
Other surveys in different countries have similarly confirmed the relationships identified by Stevens et al. (2004). Data from the Danish National habitat monitoring programme were adjusted to allow comparison with the Stevens et al. (2010) data. There was a significant correlation between the two data sets and the decrease in species richness along a N deposition gradient was evident. Whilst the range of N deposition in Denmark was too narrow to see this impact on its own, when combined with the larger European survey, it was apparent (Damgaard et al., 2011). Wilkins et al. (2016) examined data from the Irish National Parks and Wildlife Service. They examined 108 relevés for Natura 2000 community 6230 with a range of N deposition of 2.4 to $22.3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Results suggested loss of species richness with N deposition and indicated a community change point between 3.9 and $6.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$.

A number of surveys have also investigated changes in species composition over time. Duprè et al. (2010) conducted a temporal analysis utilising data sets published between 1939-2007 from Great Britain, the Netherlands, Germany, Denmark, and Sweden. The analyses of 1114 plots belonging to the *Violion caninae* showed that Ellenberg R (soil reaction) scores increased up to 1980 and then stabilised or even slightly declined. Cumulative N deposition showed a negative relationship with species richness in all countries. Pannek et al. (2015) used these data combined with Stevens et al. (2010b) to show that 16 species (55%) responded significantly to N deposition, 75% of those responded negatively. Change in species frequency over time was related to N deposition, suggesting declining species were predominantly sensitive to N deposition. Species with high relative growth rate tended to respond positively to N deposition. Gaudnik et al. (2011) collated 162 published records over a period of 25 years, 1980-90 and 1995-2005 and some additional data collected in 2007 from nutrient poor, acidic grassland in Atlantic region of France. Ordinations suggested that N deposition was an influential driver of species composition at local scales although there were difficulties in disentangling the effects of N deposition and climate change. In a repeated survey of *Nardus* grasslands in central Germany between 1971-87 and 2012-2015, significant increases in soil pH and Ellenberg R were observed, indicating recovery from acidification. Increases in Ellenberg N and high nutrient indicators together with a decrease in low nutrient indicator species, and *Nardus* grassland specialist species indicated eutrophication. In contrast to other studies, forbs increased and graminoids declined (Peppler-Lisbach et al., 2019), which could be related to species identity or interaction with other driver variables.

Spatial analysis using data collected from the UK Vascular Plant Database, which gives vascular plant species at 10 km resolution between 1987 and 1999 was analysed as described in Chapter 7.2.1. Eleven species were analysed for lowland acidic grassland with five showing significant negative relationships with N, including declines below $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Figure 7.5). Only one species could be analysed for upland acidic grassland, but this did not show a

significant relationship with N deposition. Lowland acidic grasslands showed a clear increase in Ellenberg N with increasing N deposition but there was no significant relationship for upland acidic grasslands in either database (Henrys et al., 2014). In a similar study using 10 km resolution data on presence /absence from the British Lichen Society over the last 50 years (methods as described in Chapter 7.2.1) 16 taxa were analysed for acidic grasslands, three showed significant relationships with N deposition. *Cladonia foliacea* occurred less frequently above 10 kg N ha⁻¹ yr⁻¹. The two other species showed variable responses (Stevens et al., 2012).

Figure 7.5. Relationships between probability of presence for species showing statistically significant relationships to N deposition in lowland acidic grasslands in the Vascular Plant database against increasing total inorganic N deposition (kg N ha⁻¹ yr⁻¹) (Henrys et al. 2011).



Source: Henrys et al., 2011

Summary for Lowland to montane, dry to mesic grassland usually dominated by *Nardus stricta* (R1M)

These studies indicate that many dry to mesic acidic grasslands are sensitive to N loads. The effects of N deposition became apparent through the use of spatial and temporal national and regional surveys (gradient studies). All of these identified impacts of N from low levels of deposition. Four of these studies (Payne et al., 2020, 2013; Tipping et al., 2013; Wilkins et al., 2016) specifically estimate change points for individual species and the community as a whole. These results provide change points ranging from 3.9 to 6.6 in Ireland (Wilkins et al., 2016) to the highest of 14.2 (Payne et al., 2013) but with large numbers of individual species showing change below this point. It is clear from these numbers that the CL_{emp}N range of 10 to 15 kg N ha⁻¹ yr⁻¹ should be reduced. Since there are few sites with deposition at and below 5 kg N ha⁻¹ yr⁻¹ uncertainty regarding the effects of N is high. The range is thus reduced to 6 to 10 kg N ha⁻¹ yr⁻¹, quantified as reliable. There remains a need for more field studies in areas with very low atmospheric deposition.

7.2.4 Oceanic to subcontinental inland sand grassland on dry acid and neutral soils (R1P) or Inland sanddrift and dune with siliceous grassland (R1Q)

These habitats contain a special flora (red list species and a high proportion of cryptogams, particularly lichens) and fauna (insects, birds). The impacts of N loads have been studied for a pioneer community on sandy grassland (*Koelerion glauca*) in the Upper Rhine valley in Germany (Storm and Süss, 2008), with a background N deposition of 17 kg N ha⁻¹ yr⁻¹. The vegetation was treated with two levels of N (25 and 100 kg N ha⁻¹ yr⁻¹; in the form of ammonium nitrate), between 2000 and the summer of 2004. The above-ground biomass of the vascular plants significantly increased following the high N addition, whereas the biomass of the cryptogams declined during the last two years of the experiment. Other nutrients did not cause additional increases in above-ground productivity, thus indicating that this vegetation was clearly N limited. The cover of 10 species increased after high N addition. No significant changes in diversity were found between treatments, although effects in this originally very open community could occur in the long term.

Other investigations in the Netherlands have demonstrated that areas receiving high levels of N deposition (41 kg N ha⁻¹ yr⁻¹) have reduced microbial biomass, increased net N mineralisation, higher soil N and higher microbial N:P ratios compared to lower deposition (24 kg N ha⁻¹ yr⁻¹) sites (Sparrius and Kooijman, 2013). A gradient study spanning deposition between 17 and 50 kg N ha⁻¹ yr⁻¹ showed evidence of invasion of *Campylopus introflexus*, a neophytic moss species, at higher deposition (Sparrius et al., 2011). A N addition experiment incorporating 21 dunes in the Netherlands with deposition between 21 and 47 kg N ha⁻¹ yr⁻¹ showed that high deposition induced higher algal cover, reduced vascular species richness, reduced lichen richness and reduced occurrence of some plant species as well as reductions in soil pH (Sparrius et al., 2012). Comparing dunes from high (34-44.8 kg N ha⁻¹ yr⁻¹) and lower (22.6-33.7 kg N ha⁻¹ yr⁻¹) deposition areas, Sparrius et al. (2013a) showed that there was an increased vegetation cover resulting in greater loss of bare sand in high deposition areas. Furthermore, 2.5 years of N addition at a rate of 42.9 kg N ha⁻¹ yr⁻¹ at two sites (background deposition 25 kg N ha⁻¹ yr⁻¹ and 34 kg N ha⁻¹ yr⁻¹) had a range of impacts including increased grass cover, reduced lichen cover and increased water extractable N and higher tissue N content of grasses and in lichen dominated vegetation (Sparrius et al., 2013b). However, whilst these investigations show that this habitat is sensitive to N deposition, all these studies have been carried out at high background levels of N deposition.

Summary for Oceanic to subcontinental inland sand grassland on dry acid and neutral soils (R1P) or Inland sanddrift and dune with siliceous grassland (R1Q)

Defining a CL_{emp}N based on these findings is difficult, since a high proportion of the investigations have taken place at levels of deposition far exceeding the CL_{emp}N. These inland dune grasslands have a species composition and ecological functioning comparable with that of coastal dune grasslands (see Chapter 4 for details). Because of this similarity, the CL_{emp}N for inland sand grassland on dry acid and neutral soils (R1P) or inland sanddrift and dune with siliceous grassland (R1Q) is set at the same level as that of coastal dune grasslands (see Chapter 4, 5-15 kg N ha⁻¹ yr⁻¹). Inland dunes are acidic or strongly decalcified and so would likely lie to the more sensitive lower end of this range. However, this estimation is based completely on expert judgement and there is thus a significant need for long-term research on these ecosystems to substantiate the effects.

7.3 Mesic grasslands (R2)

7.3.1 Low and medium altitude hay meadows (R22)

In semi-natural grasslands situated at low or medium elevation that are managed for making hay (R22) field experiments have been conducted with N-only treatments in realistic doses. The famous Park Grass experiment at Rothamsted (UK) has been running since 1856 (Williams, 1978; Dodd et al., 1994; Crawley et al., 2005; Silvertown et al., 2006). Nitrogen has been annually applied (single dose) as ammonium sulphate or sodium nitrate ($48 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) to plots of mesic, low altitude hay meadow (R22). On N-treated plots, within five to ten years the vegetation became dominated by a few grasses, such as *Alopecurus pratensis*, *Arrhenatherum elatius*, *Holcus lanatus* or *Agrostis* species. For species diversity a negative correlation was found with total biomass and soil acidity. Ammonium sulphate, through its acidifying effects, reduced the diversity of higher plant and bryophyte species significantly more than other forms of N within ten years, and this difference was still present after more than 150 years (Goulding et al., 1998; Virtanen et al., 2000; Silvertown et al., 2006). The experiment shows a positive response of biodiversity to reducing N addition from either atmospheric pollution or fertilisers. As atmospheric N deposition declined from $45 \text{ kg ha}^{-1} \text{ yr}^{-1}$ in 1996 to $21 \text{ kg ha}^{-1} \text{ yr}^{-1}$ in 2010, the proportion of legumes, plant species richness and species diversity increased. An important driver for the development was the increase in soil pH. Biodiversity has also partly recovered on plots formerly fertilised with $96 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. However, biodiversity is still below that of the control plots. As biodiversity has only partly recovered within the $\text{CL}_{\text{emp}}\text{N}$ range ($20\text{-}30 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) this may be an indication that the $\text{CL}_{\text{emp}}\text{N}$ is currently set too high (Storkey et al., 2015).

In addition to an increase in biomass, a 25% reduction in species diversity was observed after relatively long-term (>4 years) additions of N ($100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), applied to a hay meadow along the river Rhine in the Netherlands (Beltman and Barendregt, 2002; Beltman et al., 2007). The effects of N inputs became less pronounced because of a large flooding event after eight years, although differences between N treatments and the controls remained significant. P additions did not affect the vegetation within the timeframe of this experiment.

Wilkins et al. (2016) analysed data from the Irish National Parks and Wildlife Service. They examined 125 relevés belonging to Annex I habitat type 6510 of the EU habitat directive with a range of N deposition between 2 and $22 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Results pointed toward a loss of species richness with higher N deposition and indicated a community change point of between 7.5 and $8.7 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. In an update, the analyses were revised, additional habitats were added and deposition mapping was improved (Aherne et al., 2020). For the Atlantic region they suggested a $\text{CL}_{\text{emp}}\text{N}$ range for low and medium altitude hay meadows (R22) of $5\text{-}15 \text{ kg ha}^{-1} \text{ yr}^{-1}$.

Summary for low and medium altitude hay meadows (R22)

Previously, the $\text{CL}_{\text{emp}}\text{N}$ for low and medium altitude hay meadows was set at $20\text{-}30 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ based on expert judgement. Although new findings from a field experiment and gradient studies have been published since the last review, the data basis is still uncertain. Therefore, the $\text{CL}_{\text{emp}}\text{N}$ range for low and medium altitude hay meadows is as expert judgement specified as $10\text{-}20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. There is, however, still a need for field addition studies in different countries, especially in regions with low atmospheric deposition.

7.3.2 Mountain hay meadows (R23)

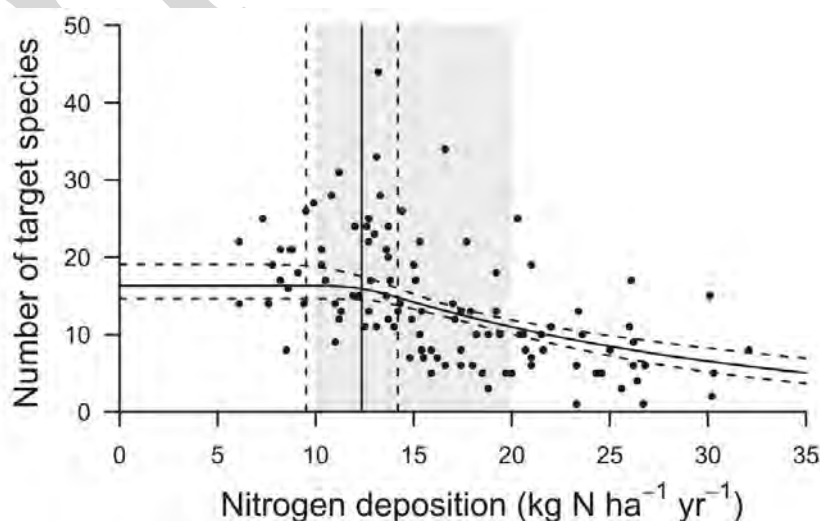
Many semi-natural grassland types occur in montane regions across Europe, containing many rare and endangered plant and animal species (e.g. Ellenberg, 1996). However, experimental studies with low doses of N are not available for this grassland type. The Rengen Grassland

Experiment in Germany has fertiliser treatments in a mountainous hay meadow but, however, N was applied in combination with Ca and Mg, thus preventing soil acidification after N inputs and also any significant impact of N deposition on these acidic meadows (Hejcman et al., 2007).

The previous $CL_{emp}N$ range ($10\text{--}20\text{ kg N ha}^{-1}\text{ yr}^{-1}$) for mountain hay meadows was based on expert judgement only. In a gradient study using data on vascular plants and bryophytes from the Biodiversity Monitoring of Switzerland (2006–2010), Roth et al. (2013) inferred whether N deposition is negatively related to species richness and Simpson diversity in mountain hay meadows. The relationship between N deposition and species richness and Simpson diversity was analysed for all species together, but also for high and low nutrient indicators and for species of conservation concern. Non-linear effects of confounding variables such as elevation and mean or annual precipitation were accounted for in the generalised additive models (GAM) used to analyse effects of N deposition. The average ($\pm SD$) background N deposition on the 122 plots was $18 (\pm 6)\text{ kg N ha}^{-1}\text{ yr}^{-1}$. Species richness and diversity of vascular plants and bryophytes were negatively related to N deposition. Furthermore, a negative relationship between the number of low nutrient indicator species and N deposition was found. As low nutrient indicator species are generally rare, the species composition became more similar with increasing N deposition because the rare species disappeared at higher level of N deposition.

Using data from the Biodiversity Monitoring of Switzerland, but with recordings from 2010–2014, the $CL_{emp}N$ for mountain hay meadows could be directly estimated by a change-point model. Seven co-variables (elevation, inclination, precipitation, calcium carbonate content, aspect and mean indicator values for humidity and light) were taken in account. The results of this study suggest that the 2011 $CL_{emp}N$ range is too broad (Roth et al., 2017). The number of target species of this meadow type starts declining at a N deposition rate of $13\text{ kg N ha}^{-1}\text{ yr}^{-1}$ (Fig. 7.6). Roth et al. (2017) thus proposed to reduce the $CL_{emp}N$ upper limit to $15\text{ kg N ha}^{-1}\text{ yr}^{-1}$.

Figure 7.6. Number of species of conservation concern in mountain hay meadows (EUNIS R23) across a gradient of nitrogen (N) deposition in Swiss mountains. The dots represent the raw counts of species numbers in the sampling sites of 10 m^2 . The black line represents the change-point regression of species richness on N deposition that simultaneously accounted for seven confounding variables (see text). The vertical line represents the position of the change-point, the dashed lines the corresponding 95% credible intervals. The grey shaded area represents the expert-based empirical critical load range from 2011 (Roth et al., 2017).



Source: Roth et al., 2017

Data from three Monitoring programs in Switzerland were recently pooled to check the CL_{empN} given above (Roth et al., unpublished data). The same change-point model was applied as by Roth et al. (2017) including elevation, inclination, precipitation, calcium carbonate content, aspect as well as mean indicator values for humidity and light as confounding variables. The gradient of N deposition ranged from 4 to 37 kg N ha⁻¹ yr⁻¹. When data from three Swiss monitoring programmes are combined, the change-point in mountain hay meadows for the target species was estimated at 7-9 kg N ha⁻¹ yr⁻¹ (Roth et al., unpublished data).

An experimental NPK fertiliser study on Swiss mountain hay meadows supports this finding. The number of vascular plant species decreased significantly at a nitrogen input of 16 kg N ha⁻¹ yr⁻¹ and the forb species richness at 15 kg N ha⁻¹ yr⁻¹ (Boch et al., 2021). However, the results may not be used directly for the revision of the CL_{empN} , as the background N load was not accounted for, and the number of cuts increased from one to two cuts during the experiment.

Summary for mountain hay meadows (R23)

Since 2010, the CL_{empN} range can be delineated more precisely with data from gradient studies. The CL_{empN} for mountain hay meadows (R23) is set at 10-15 ha⁻¹ yr⁻¹ (previously 10-20 ha⁻¹ yr⁻¹) and classified as reliable.

7.4 Seasonally wet and wet grasslands (R3)

7.4.1 Moist or wet mesotrophic to eutrophic hay meadow (R35) or Temperate and boreal moist and wet oligotrophic grasslands (R37)

Temperate and boreal moist and wet oligotrophic grasslands (R37) are characterised by oligotrophic and moist to wet peaty soil conditions. This EUNIS category consists mostly of hay meadows under original agricultural management that are especially rich in typical plant and animal species. R37 combines two subcategories that were distinguished previously, namely (i) *Molinia caerulea* meadows (previously E3.51; 'litter meadows' or 'fen meadows') and (ii) heath meadows and humid *Nardus stricta* swards (previously E3.52). R35 covers moist or wet mesotrophic to eutrophic hay meadows. Because of their long history of traditional land use with low additional inputs of nutrients, these grassland communities are likely to be sensitive to extra nutrient inputs. Several fertilisation experiments in these wet oligotrophic grasslands have demonstrated limitation by either N or P or even co-limitation by these elements (e.g. Vermeer, 1986; Egloff, 1987; Spink et al., 1998; Van Duren et al., 1998; Olde Venterink et al., 2001). In the case of N limitation, grass productivity, especially of the dominant *Molinia caerulea*, increased and species diversity declined (e.g. Vermeer, 1986). However, almost all of the studies that have been performed in moist or wet oligotrophic grasslands were either carried out with high to very high loads (> 100 kg N ha⁻¹ yr⁻¹) or had a time span that was too short to be used for setting a CL_{empN} range.

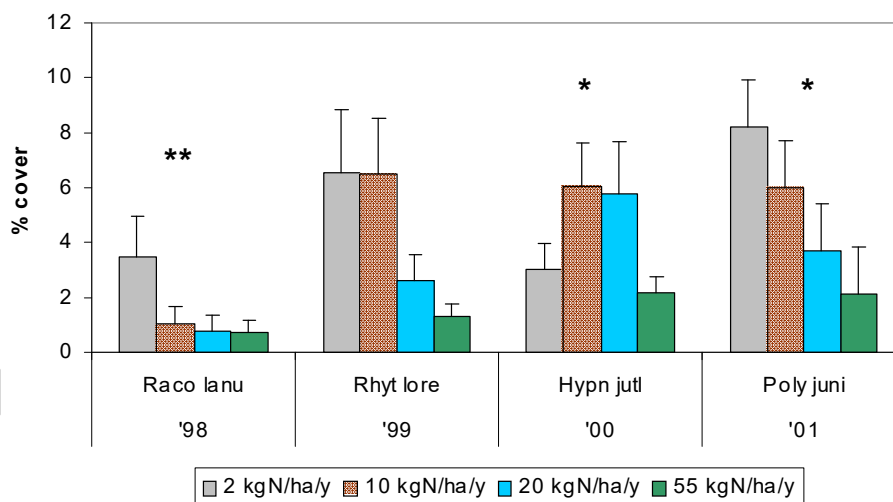
Fortunately, the impacts of N additions on species richness have been quantified in flower-rich, oligotrophic wet hay meadows (R35) in Somerset (UK) (Mountford et al., 1994; Tallwin et al., 1994; Kirkham et al., 1996). Nitrogen additions of 25 kg N ha⁻¹ yr⁻¹ and more (with an estimated background atmospheric load of 15-25 kg N ha⁻¹ yr⁻¹) for six years significantly reduced the number of species, while several grasses increased in dominance (*Lolium perenne*, *Holcus lanatus* and *Bromus hordeaceus*). The forbs, characteristic of these meadows, declined sharply in number, and some, for example, *Cirsium dissectum*, *Lychnis flos-cuculi* and *Lotus pedunculatus* disappeared from N treated plots altogether.

More recently, a N manipulation experiment examined the effects of additions of ammonium sulphate (10 and 20 kg N ha⁻¹ yr⁻¹) or sodium nitrate (20 kg N ha⁻¹ yr⁻¹ only) to an upland grass

heath in Wales (R37) (Emmett et al., 2001, 2007). This site, at an altitude of 600 m, had been overgrazed in the 1970s and 1980s, resulting in degradation of *Calluna*-dominated moorland to a sward dominated by *Nardus stricta*, *Vaccinium myrtillus* and *Festuca*. The treatments were applied to paddocks which had different rates of experimental sheep grazing from 1989 up to the start of the experiment in 1997. After four years of treatment, relatively small effects were observed on the vascular plants, although there was evidence of increased *Festuca* cover on the lightly grazed paddock, and greater frost injury to *Vaccinium* (only on the plot treated with nitrate), which may have been linked to earlier bud break in the spring. The observed lack of response in palatable grasses in the heavily grazed paddock may reflect selective grazing by sheep. In later years of this experiment, high N addition significantly reduced the cover and species richness of bryophytes, although grazing intensities modified this response; it only became obvious at a low grazing intensity, while no effect could be observed when the grazing intensity was higher (Emmett et al., 2007).

Measurements of soil water chemistry at this site (lightly grazed plots) showed significant leaching losses in the control plots, representing 25% of inorganic inputs, which increased from 5 to 7 kg N ha⁻¹ yr⁻¹, during the 20 kg N ha⁻¹ yr⁻¹ treatment. These high leaching rates suggested that N levels at this site were already above the CL_{emp}N. Only for the ammonium treatments, there was also increased base cation leaching and decreased pH. However, there were no significant treatment effects on mineralisation or nitrification rates.

Figure 7.7. Cover of moss species in *Nardus stricta* grassland mesocosms, exposed since 1997 to four N treatments (2, 10, 20, and 55 kg N ha⁻¹ yr⁻¹; from left to right). The mosses are *Racomitrium lanuginosum*, *Rhytidiadelphus loreus*, *Hypnum jutlandicum* and *Polytrichum juniperinum* (Jones et al., 2002).



Source: Jones et al., 2002

The atmospheric deposition at the grass heath site in Wales was estimated at 20 kg N ha⁻¹ yr⁻¹. To assess the impacts of lower deposition rates, Jones and Ashenden (2000) applied a range of N deposition treatments above and below that of the existing site estimate (2, 10, 20 and 55 kg N ha⁻¹ yr⁻¹ in the form of ammonium nitrate) to mesocosms that were taken from the site and grown in a greenhouse. To assess possible interactions with grazing pressure, three levels of simulated grazing (clipping) were also applied. Within one to two years, there were strong effects of N treatments of below 20 kg N ha⁻¹ yr⁻¹, increasing the cover of certain moss and lichen species, but only in combination with heavy clipping, presumably because of the lower

competition from vascular plants. Subsequent data (Jones et al., 2002) have shown the emergence of different optima for bryophyte species – that for *Racomitrium lanuginosum* and *Polytrichum juniperinum* lying below $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ – while for *Hypnum jutlandicum* this would be around $20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Figure 7.7). Although results have shown an increase in fine grass cover and a decrease in *Nardus* following increasing N additions, these effects occurred primarily at between 20 and $55 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. In contrast to results from the parallel experiment on the calcareous mesocosms and results from the field site, there was no evidence of effects of nitrate leaching in the first two years of the experiment (Jones et al., 2002). More recent work at these experimental sites has indicated that whilst plant species composition varied strongly between treatments, N did not increase vegetation height compared to control plots (Stiles et al., 2017). Nitrogen treatments also had lower CO_2 fluxes and lower CH_4 uptake than in the controls (Stiles et al., 2018).

Summary for Moist or wet mesotrophic to eutrophic hay meadow (R35) or Temperate and boreal moist and wet oligotrophic grasslands (R37)

In summary, several moist or wet oligotrophic grasslands of high conservation value have been shown to be sensitive to N eutrophication. Increases in dominant grasses and decreases in species richness have been observed following increasing levels of N inputs. The study of a degraded upland heath meadow (Emmett et al., 2001, 2007) has provided evidence of response in bryophyte cover to relatively low levels of N deposition, and suggested increases in leaching and acidification at levels above $20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. In view of these UK studies, the CL_{empN} for R37 moist to wet oligotrophic grasslands is set at $10\text{-}20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ specifically for *Nardus stricta* swards and considered to be 'quite reliable'. Studies for other parts of Europe are needed, especially as some studies included in the section on closed non-Mediterranean dry acid and neutral grassland span a gradient from dry to moist.

The CL_{empN} for moist or wet mesotrophic to eutrophic hay meadow (R35) however, is based on expert judgement only, and estimated to be somewhat higher ($15\text{-}25 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), because, to date, this habitat has barely been studied and the one experiment for R35 grasslands has high levels of N addition relative to the CL_{empN} range. The CL_{empN} for both of these categories have not changed from those in the 2010 document. Base status is likely to be a significant modifying factor as systems with low base status are likely to be more sensitive to N deposition, while fluctuations in the water table may cause habitats in the wet hay meadows to be less sensitive.

7.5 Alpine and subalpine grasslands (R4)

7.5.1 Temperate acidophilous alpine grasslands (R43) or Arctic-alpine calcareous grassland (R44)

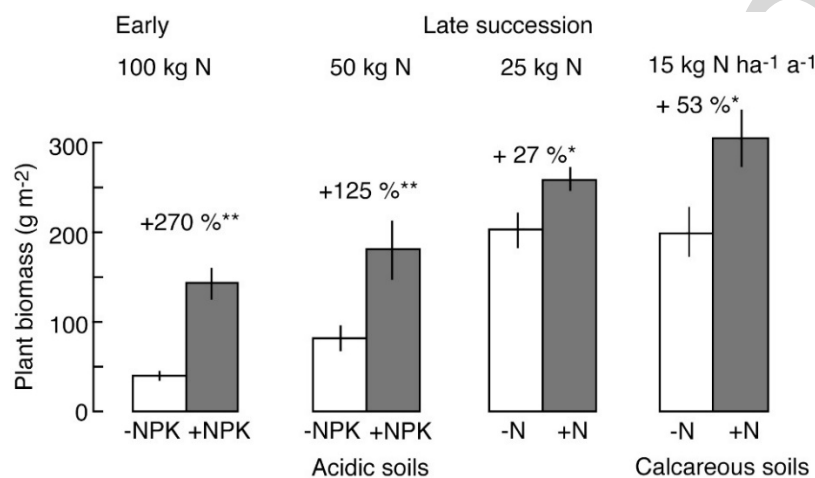
Many natural grassland types are found in the alpine and subalpine regions of Europe and other mountains systems on both acidic (R43) and calcareous (R44) soils. These grasslands often encompass an outstandingly high biodiversity with many rare and endemic plant species (Körner, 2021). Alpine is defined as the vegetation zone above the upper natural treeline. The alpine vegetation zone is the only vegetation zone which can be clearly defined globally (Körner, 2021). As subalpine is not a clearly defined term, the term montane or mountainous areas below the treeline should be used.

Land use often contributes as a source of N emissions to increased atmospheric N deposition, although sustainable land use by mowing and grazing contributes to preservation of montane grasslands by keeping them open. Complete abandonment of land use leads to encroachment of

woody taxa and biodiversity losses in the montane vegetation zone. Land use within the alpine vegetation zone is commonly less intensive and patchy (Körner, 2021).

There is clear evidence of impacts of high rates of N addition ($> 40 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) in alpine grasslands. Late successional acidic grassland at a 2,500 m elevation in the central Swiss Alps (Furkapass area) showed a rapid and large response to additions of 40 to 50 $\text{kg N ha}^{-1} \text{ yr}^{-1}$ (Körner et al., 1997; Figure 7.8). Part of this early experiment was conducted with a multi-nutrient fertiliser, but there is little other evidence from these habitats. Biomass was doubled already in the second year, with sedges (*Carex curvula*) profiting most.

Figure 7.8. Responses of vegetation of high elevation ($> 2450 \text{ m}$) grassland and glacier foreland (most left) to two to four years of nutrient addition in early and late successional habitats, control plots are shown as -NPK or -N (Heer and Körner, 2002; Körner et al., 1997; Hiltbrunner, unpublished data).



Source: Heer and Körner, 2002; Körner et al., 1997; Hiltbrunner, unpublished data

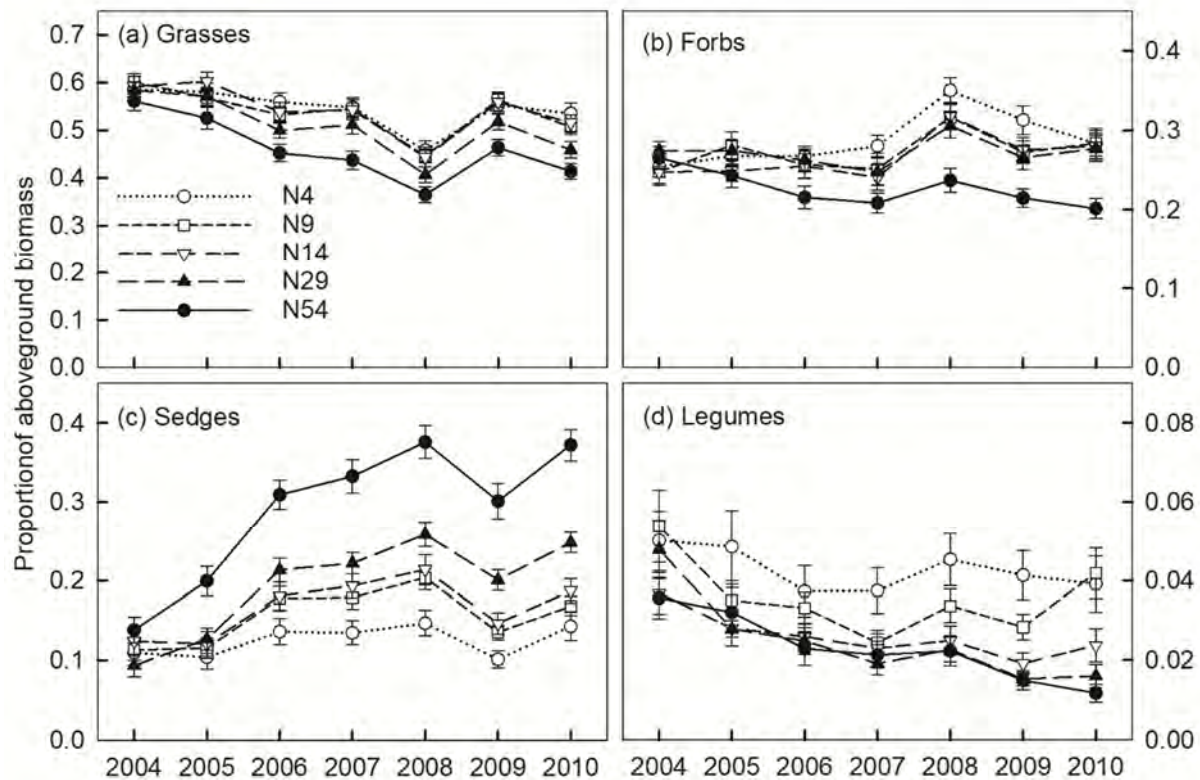
A $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ addition converted an open vegetation with cushion plants ($< 10\%$ cover) into a lush grassland (100% cover) within two years only (Heer and Körner, 2002). Earlier experiments with similarly high doses of N applied to alpine dwarf-shrub heath confirmed sensitivity of alpine vegetation to nutrient addition. These experiments ended with a rather unexpected response after four years of nutrient addition: a complete collapse of *Loiseleuria procumbens* and *Calluna vulgaris* stands due to snow mould infestation exclusive to the nutrient addition area (Körner, 1984). Hence, there is no doubt that cold-climate, alpine vegetation is responsive to an elevated N supply. Several longer-term studies on N additions have been set up in Switzerland in alpine acidic (R43) and alpine calcareous grasslands (R44), to quantify the impacts of lower N loads.

The effects of N addition on plant production in acidic alpine grasslands (R43; *Caricetum curvulae*) was studied from 2002 to 2005 in the central Alps (Furka) in Switzerland, at two elevations (2450 and 2650 m a.s.l.) (Hiltbrunner and Körner, 2004; Hiltbrunner et al., 2005). In the fourth year of this experiment, the addition of $25 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, in the form of ammonium nitrate, resulted in a significant increase (27-45%) in the above-ground biomass, compared to the control vegetation and irrespective of elevation. Background deposition at the research site was around 4 to $5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$.

In a seven-year N addition experiment, in a species-rich acidic grassland area (Geo-montani Nardetum), at 2000 m a.s.l. in the Swiss Alps, 5, 10, 25 and $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ were applied to grassland monoliths, with an ambient background of approximately $4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Bassin et al.,

2007, 2009). The above-ground biomass of the vegetation significantly increased with the added N levels. The sedges *Carex sempervirens* and *Carex ornithopoda* tripled their fractional biomass at the expense of legumes (*Trifolium alpinum*), grasses (*Agrostis capillaris*, *Briza media*, *Festuca* spp.), and forbs, the latter of which responded inconsistently. Compositional changes were significant with +5 kg N ha⁻¹ y⁻¹; at all levels of N, but changes ceased after five years (Bassin et al., 2013, Figure 7.9).

Figure 7.9. Relative contribution (means \pm SE) of the four functional groups (a-d): grasses, forbs, sedges, and legumes to the total aboveground biomass following N additions (incl. background N deposition) of 4 (N4 - control), 9, 14, 29, and 54 kg N ha⁻¹ y⁻¹ (background plus N additions) during the experimental years 2004-2010 (Bassin et al., 2013).



Source: Bassin et al., 2013

In this same experiment, on plots receiving 50 kg N ha⁻¹ yr⁻¹, the net ecosystem productivity (NEP) has been shown to yield losses of 54 g C m⁻² per season from the grassland compared to the control area (Volk et al., 2010). However, after seven years of N addition, cumulative NEP was not significantly altered due to N addition, and NEP was slightly higher at 10 kg N ha⁻¹ yr⁻¹ than at 50 kg N ha⁻¹ yr⁻¹ (Volk et al., 2016).

In a fully factorial three-way transplant experiment with three-levels of N addition (0, 3, 15 kg N ha⁻¹ year⁻¹), water treatment and transplants along an elevation gradient to expose grassland monoliths to temperature differences in the range of -1.4 to +3.0 °C revealed that the stimulating effect of increased N on sedge cover was dampened by increased temperatures. Unexpectedly, no changes in above-ground biomass formation were found due to N addition after four years (Wüst-Galley et al., 2021; Volk et al., 2021).

Based on the evidence provided, alpine acidic grasslands (R43) are likely to be sensitive to N loads. The $CL_{emp}N$ for these habitats remains unchanged at 5 to 10 kg N ha⁻¹ yr⁻¹, which is considered to be 'quite reliable'.

Arctic-alpine calcareous grasslands (R44) are especially species-rich communities. The impacts of N enrichment have been investigated in a calcareous alpine grassland area in Switzerland, at approximately 2500 m a.s.l. Background deposition at the experimental site ranged between 3 and 5 kg N ha⁻¹ yr⁻¹, and six N application levels, of 2.5, 5.0, 10, 15, 20 and 25 kg N ha⁻¹ yr⁻¹ were applied. In the fourth year of this N addition experiment, the dominant sedge species *Carex firma*, showed a positive response in cover following additions of 5 kg N ha⁻¹ yr⁻¹ and more. Total above-ground biomass increased by 53 % at N addition of 15 kg N ha⁻¹ yr⁻¹. However, after seven years of N addition, above-ground biomass was significantly stimulated at 25 kg N ha⁻¹ yr⁻¹ only, indicating a declining responsiveness to N addition over time (Hiltbrunner, unpublished data), similar to the observation on weaker compositional changes in the acidic grassland after five years of N addition (Bassin et al., 2013).

There is no additional evidence for alpine calcareous grasslands (R44) since the $CL_{emp}N$ was set in 2010. So, based on the results of this experiment, the $CL_{emp}N$ remains at 5 to 10 kg N ha⁻¹ yr⁻¹, which is considered to be 'quite reliable'. There is a clear need for further investigation in this habitat.

7.5.2 Moss- and lichen-dominated mountain summits, ridges and exposed slopes

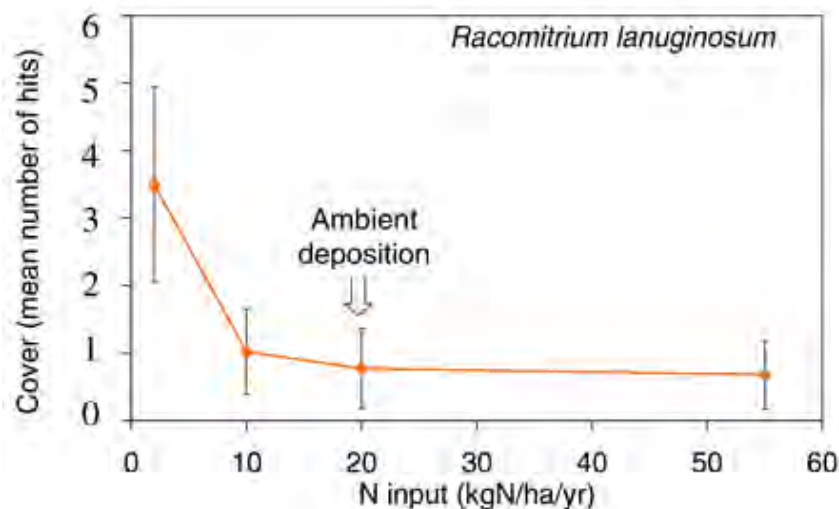
Within the old EUNIS system, an important sub-category of alpine and subalpine grasslands was communities without continuous snow cover which are dominated by moss and lichen species. They previously formed category E4.2, but no allocation exists for these habitats as a separate category for the new EUNIS classification (Chytrý et al., 2020). However, best represented is E4.2 in the new category R42 (Boreal and arctic acidophilous alpine grassland).

Since these communities are nutrient limited and many moss and lichen species are highly responsive to increased N deposition, it is likely that they are sensitive and should be assigned a low $CL_{emp}N$. The majority of N addition experiments and more recent gradient studies on this habitat type have been carried out in the *Racomitrium*-moss dominated heath, which is found on mountain summits in Britain and in montane areas of arctic and sub-arctic zones.

In the United Kingdom, there has been a serious decline in *Racomitrium* heath, over recent decades, with them being replaced by grass communities. Increasing rates of N deposition may be one of the main factors involved in the deterioration of *Racomitrium* heath (Thompson and Baddeley, 1991). However, evidence from experimental manipulation studies suggests that changes in grazing pressure also contribute to these changes. Pearce and Van der Wal (2002) set up an experiment in the north-east Scottish Highlands with montane *Racomitrium lanuginosum*-*Carex bigelowii* heath. In this experiment, plots on the summit were subject to low (10 kg ha⁻¹ yr⁻¹) and high (40 kg ha⁻¹ yr⁻¹) additions of N in two separate forms (NO₃⁻ and NH₄⁺), during two consecutive summer seasons. Background deposition was estimated at 15 to 18 kg N ha⁻¹ yr⁻¹. *Racomitrium* was shown to be extremely sensitive to even low N addition rates, responding with a raised tissue N concentration, and shoot growth rates which were less than 50% of those on the control plot. After only two growing seasons, Pearce and Van der Wal (2002) also demonstrated how quickly *Racomitrium* was replaced by graminoid species; *Racomitrium* cover was reduced by 31% at 10 kg ha⁻¹ yr⁻¹, while graminoid cover increased by 57%. These results could reflect both a toxic effect and increased competition for light by graminoids, which utilised excess N.

The high sensitivity of *Racomitrium lanuginosum* to N deposition is also supported by the results from a glasshouse experiment that used monoliths taken from montane grassland in central Wales. In this experiment, N deposition and simulated grazing were manipulated over a four-year period (Jones, 2005). The applied N treatments were both above and below 20 kg N ha⁻¹ yr⁻¹. *Racomitrium* only persisted under heavy simulated grazing, confirming its sensitivity to competition for light by grasses. In these monoliths, a significant effect of N application became apparent within one year, with the cover of *Racomitrium* reducing from 10% at 2 kg ha⁻¹ yr⁻¹ to 2% or less at 10 kg ha⁻¹ yr⁻¹ and more (Figure 7.10).

Figure 7.10. Change in cover, detected by the number of hits using the pinpoint method, of the moss *Racomitrium lanuginosum* following N additions (> 20 kg N ha⁻¹ yr⁻¹) as well as N reductions (< 20 kg N ha⁻¹ yr⁻¹) on acidic grassland mesocosms in an experimental misting facility that excluded ambient N deposition (Jones, 2005; Emmett, 2007).



Source: Jones, 2005; Emmett, 2007

However, a three-year manipulation study by Jónsdóttir et al. (1995), in which very low levels of N addition (4 kg N ha⁻¹ yr⁻¹) were applied to a *Racomitrium-Carex* heath in Iceland with a background deposition of about 2 kg ha⁻¹ yr⁻¹, found small non-significant increases in *Racomitrium* growth and shoot density. It was assumed that the small response to the low deposition rates used in this experiment was associated with growth limitations due to other factors.

The impacts of N deposition relative to those of climatic and grazing conditions on the composition of *Racomitrium* heath were examined along a N deposition gradient of 0.6-39.6 kg N ha⁻¹ yr⁻¹, and combined with climatic conditions across 36 European sites (Armitage et al., 2014). Besides climatic conditions, N deposition was the second most important driver, explaining 15% of variability, and it was more important than soil factors or current grazing. Along the N gradient, species richness declined by five species m⁻² and there was a 30% shift in cover from mosses to graminoids. Changes in community composition were noticed across the whole range of N deposition, thus, there was no evidence for a threshold below which no change in composition occurred. Further analysis of this data suggests a substantial change in species richness between 0-5 and 5-10 kg N ha⁻¹ yr⁻¹ deposition categories (Wamelink et al., 2021).

Across 15 sites in the UK with N deposition ranging from 6.4-35.4 kg N ha⁻¹ yr⁻¹, Britton et al. (2018) assessed the effects of N deposition on bryophyte litter quality, decomposition and C and N stocks in *Racomitrium* moss-sedge heath. Increasing N deposition reduced C:N in bryophyte

litter, which in turn enhanced its decomposition. Thus, N-induced decomposition accelerated the depletion of the moss mat and allowed the graminoids to take over rapidly, pointing to an additional mechanism in addition to the light competition by graminoids.

At higher elevation and higher latitude, mountain summits are often dominated by the occurrence and abundance of lichens. Specific studies on lichens should therefore be accounted for. In a ten-year experiment, Fremstad et al. (2005) studied cover changes of different lichen species in low alpine and mid-alpine vegetation communities in south-central Norway. Nitrogen was added at 7, 35, and 70 kg ha⁻¹ yr⁻¹; background N deposition was 2 to 4 kg ha⁻¹ yr⁻¹. The most sensitive species at the warmer, low alpine site were the lichens *Alectoria nigricans* and *Cetraria ericetorum*, decreasing in cover at the lowest N application rate of 7 kg ha⁻¹ yr⁻¹. Cover of another six lichens species declined at an N rate of 35 kg ha⁻¹ yr⁻¹. Beside declines in cover, lichens developed discoloured and smaller thalli. Interestingly, the only lichen unaffected by any level of N application was the N₂-fixing lichen *Stereocaulon paschale*. However, at the colder, mid-alpine site, the lichen *Cetrariella delisei* was the only species in which cover changes over ten years due to N application of 70 kg ha⁻¹ yr⁻¹ were found and authors assumed a slower response time in colder climates.

In a meta-analysis referring to 39 articles and 31 experimental sites, Gutierrez-Larruga et al. (2020) found that N addition (in most studies between 10 and 56 kg N kg ha⁻¹ yr⁻¹) accelerates lichen metabolism in both terricolous and epiphytic lichens in the short-term and decreases their abundance in the longer term. Early senescence of lichens is proposed as a possible mechanism linking the two observed responses. Currently, long-term effects of low N addition rates on lichens have not yet been adequately assessed in these habitats.

Summary for moss- and lichen-dominated mountain summits, ridges and exposed slopes

The experiments and more recent gradient studies described above and suggest that moss- and lichen-dominated mountain summits, ridges and exposed slopes habitats where background deposition is currently low have considerable potential for changes in the abundance of sensitive species and a decrease in lichen and bryophyte cover with even small increases in N. The 2011 CL_{emp}N for moss and lichen dominated mountain summits, ridges and exposed slopes was set at 5-10 kg N ha⁻¹ yr⁻¹. The more recent evidence supports that CL_{emp}N and it should remain 5-10 kg N ha⁻¹ yr⁻¹ and is be considered 'quite reliable'.

7.6 Overall summary for grasslands and lands dominated by forbs, mosses or lichens (EUNIS class R)

A summary of the CL_{emp}N and their reliability is included in Table 7.1. Six CL_{emp}N (presented in bold) have changed from the 2010 recommendations with all showing reductions either through a lowering of the whole CL_{emp}N range or a narrowing of the range.

Referring to the entire grassland class R, there are some biogeographical regions in Europe, where no information is yet available on the effects of increased N deposition. This is the case of Pannonian, Steppic and Black Sea regions. Also in the Mediterranean region, regarded as a global biodiversity hotspot for conservation priorities, there are many grassland communities whose sensitivity to N deposition has not been assessed. In particular, future attention should be paid to Mediterranean mountain areas as well as to alpine vegetation belts in other mountain systems (Pyrenees, Carpathians) where there are high levels of plant diversity and endemism.

Table 7.1. **CL_{emp}N and effects of exceedances on grasslands and lands dominated by forbs, mosses and lichens (R). ## reliable, # quite reliable and (#) expert judgement. Changes with respect to 2011 are indicated as values in bold.**

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2011 reliability	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
Semi-dry perennial calcareous grassland (meadow steppe)	R1A	15-25	##	10-20	##	Increase in tall grasses; decline in diversity; change in species composition; increased mineralisation; N leaching; surface acidification
Mediterranean closely grazed dry grasslands or Mediterranean tall perennial dry grassland or Mediterranean annual-rich dry grassland	R1D or R1E or R1F	15-25	(#)	5-15	(#)	Increased production; dominance by graminoids; changes to soil crusts; changes to soil nutrient cycling
Lowland to montane, dry to mesic grassland usually dominated by <i>Nardus stricta</i>	R1M	10-15	##	6-10	##	Increase in graminoids; decline of typical species; decrease in total species richness
Oceanic to subcontinental inland sand grassland on dry acid and neutral soils or Inland sanddrift and dune with siliceous grassland	R1P or R1Q	8-15	(#)	5-15	(#)	Decrease in lichens; increase in biomass
Low- and medium altitude hay meadows	R22	20-30	(#)	10-20	(#)	Increase in tall grasses; decrease in diversity; decline of typical species
Mountain hay meadows	R23	10-20	(#)	10-15	#	Increase in nitrophilous graminoids; changes in diversity; decline of typical species
Moist or wet mesotrophic to eutrophic hay meadow	R35	15-25	(#)	15-25	(#)	Increase in tall graminoids, decreased diversity; decrease in bryophytes

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2011 reliability	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
Temperate and boreal moist and wet oligotrophic grasslands	R37	10-20	#	10-20	#	Increase in tall graminoids, decreased diversity; decrease in bryophytes
Temperate acidophilous alpine grasslands	R43	5-10	#	5-10	#	Changes in species composition; increase in plant production
Arctic-alpine calcareous grassland	R44	5-10	#	5-10	#	Changes in species composition; increase in plant production
Moss and lichen dominated mountain summits	(Earlier E4.2)	5-10	#	5-10	#	Change in species composition; effects on bryophytes or lichens

7.7 References

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Final Draft

8 Effects of nitrogen deposition on heathland, scrub and tundra habitats (EUNIS class S, formerly F)

Adapted by Leon Van den Berg, Julian Aherne, Andrea Britton, Simon Caporn, Hector García Gómez and Liv Guri Velle



Alpine heath (S2) in Scotland. Photo: Andrea Britton.

Summary

The amount of new (long-term) experimental evidence for N impacts on heathland, scrub and tundra habitats published since the review of 2010 is limited. However, a large number of gradient studies have become available, covering many of these habitats.

From the experimental and gradient studies, a clear picture is emerging that deposition rates of as low as $5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ can affect tundra ecosystems (S1) and arctic, alpine and subalpine scrub habitats (S2). Therefore, empirical N critical loads (CL_{empN}) for these habitats were maintained or narrowed to $3 \text{ to } 5 \text{ kg ha}^{-1} \text{ yr}^{-1}$ and $5 \text{ to } 10 \text{ kg ha}^{-1} \text{ yr}^{-1}$ respectively.

*Gradient studies also allowed a first CL_{empN} of $5 \text{ to } 15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ to be set for lowland to montane temperate and submediterranean *Juniperus* scrub (S31). In addition, a number of gradient studies provided evidence for N deposition impacts on vascular plant and bryophyte species composition at or below the lower end of the previous CL_{empN} range for upland *Calluna*-dominated wet heath (S411), lowland *Erica tetralix*-dominated wet heath (S411) and dry heaths (S42). Therefore, the CL_{empN} ranges for these habitats were lowered to $5 \text{ to } 15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$.*

Maquis, arborescent matorral and thermo-Mediterranean scrub (S5) are less well studied but are expected to show similar N responses to related Garrique and woodland habitats. Therefore, the CL_{empN} range for Maquis, arborescent matorral and thermo-Mediterranean scrub (S5) was lowered to $5 \text{ to } 15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, classifying it as 'expert judgement'. New experimental evidence on the Garrique habitat (S6) allowed a first estimate of CL_{empN} of $5 \text{ to } 15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$.

8.1 Introduction

Historically, heathlands have played an important role in the western European landscape. The term heath generally describes various types of plant communities, but here the term is being applied to those plant communities for which the dominant life form is that of small-leaved dwarf shrubs that form a canopy at one metre or less above soil surface. Dwarf shrub canopies may be discontinuous and interspersed with grasses and forbs, and a ground cover of mosses or lichens is often present (Gimingham et al., 1979; De Smidt, 1979). Heathlands are classified together with scrub and tundra habitats in class S of the European Nature Information System (EUNIS). This class includes all dry and seasonally wet inland vegetation (cover > 30%) that is dominated by shrubs, dwarf shrubs or scrubs (Davies and Moss, 2002; Davies et al., 2004). In some subcategories of this class, the vegetation is determined by climate. Here, succession towards woodland is inhibited by drought, low temperature, or growing season length (e.g. categories S1, and S2). In contrast, the extensive inland, lowland dwarf-shrub heathlands in Atlantic and sub-Atlantic Europe are man-made and have existed for several centuries. In these heaths, the development towards woodland is prevented by regular mowing, burning, sheep grazing or sod removal. They are dominated by *Ericaceae*, especially *Calluna vulgaris* in the dry heathlands and *Erica tetralix* in the wet heathlands, or *Erica cinerea* in the western Atlantic heathlands (e.g. Gimingham et al., 1979). These communities are found on nutrient-poor mineral soils with a low pH (3.5-4.5), which makes them sensitive to the effects of both eutrophication and acidification caused by elevated nitrogen (N) deposition. Shrublands in the Mediterranean (e.g. categories S3, S5, S6) occur on a wide variety of soil types, including limestone and sandy soils. Succession towards woodland in these categories is often inhibited by severe drought, occasional fires and grazing by livestock. Because of their high nature conservation value, many scrub and heathlands have been designated as nature reserves.

In accordance with the EUNIS habitat classification, this chapter distinguishes the following categories and subcategories. Tundra (S1), arctic, alpine and subalpine scrub habitats (S2), temperate and Mediterranean montane scrub (S3), temperate shrub heathland habitats (S4), with subcategories of wet (S41) and dry (S42) heaths. In view of their functional differences, wet heaths are subdivided, according to climate, into northern (S411) and southern (S412) wet heaths. For southern wet heaths, no data are available to assign them a $CL_{emp}N$. For northern wet heaths a separate $CL_{emp}N$ was assigned when dominated by *Calluna vulgaris* (upland *Calluna* moorlands) or *Erica tetralix*. Coastal dune heaths have been categorised as coastal habitats (Chapter 4.2.3; N18, N19), and acidic grasslands with some heather species as grassland habitats (Chapter 7.2; R1M). Lowland Mediterranean scrub lands are covered in the maquis, arborescent matorral and thermo-Mediterranean scrubs (S5) and Garrigue (S6). Both habitats are characterised by temperate to Mediterranean climate and consist of evergreen and sclerophyllous shrublands and heathlands. For other EUNIS categories in class S (S7, 8 and 9), including other heathland types, no $CL_{emp}N$ have been determined, due to a lack of data availability.

8.2 Tundra (S1) and arctic, alpine and subalpine scrub habitats (S2)

Alpine and arctic habitats have many ecological characteristics in common, although climatic conditions are more severe in arctic regions than in most alpine regions. The growing season is short, temperatures are low, winds are frequent and strong, and the distribution of plant communities depends on the distribution of snow during winter and spring. Most alpine and all arctic zones are influenced by frost or solifluction. A continuous snowpack in winter insulates the soils from freezing temperatures. Although soil organic matter contents are typically high, decomposition and nutrient cycling are frequently slow, and the low nutrient supply limits

primary production (Robinson and Wookey, 1997). Despite these constraints, there are a number of plant species growing in the tundra and arctic and sub-arctic, including herbaceous species, small dwarf shrubs, sedges and tussock grasses, reindeer and other lichens (crustose and foliose) and bryophytes (mosses and liverworts).

In classifying these communities under the EUNIS system, it is necessary to distinguish between tundra (S1) and arctic, alpine and subalpine scrub habitats (S2). Tundra is defined as vegetated land with graminoids, shrubs, mosses and lichens, overlying permafrost (Davies et al., 2004). The presence of permafrost prevents root penetration and often keeps the ground waterlogged in summer. European tundras are limited to Spitsbergen, Norway and northern Russia. A similar vegetation type occurs on boreal mountains and in the low arctic region, far away from the main permafrost region, for instance in Fennoscandia and Iceland. These habitats are listed under alpine and subalpine grasslands (R4), or arctic, alpine and subalpine scrub habitats (S2). The latter comprise scrub habitats that occur north of or above the climatic tree line, but outside the permafrost zone, and scrub occurring close to but below the climatic tree line where trees are locally suppressed by late lying snow, wind or repeated grazing.

8.2.1 Tundra (S1)

Tundra habitats are divided into two subcategories: shrub tundra (S11) and moss and lichen tundra (S12). Shrub tundras have extensive cover of small dwarf-shrubs over herbs, mosses, and lichens. They are sporadically found on permafrost soils of the southern arctic and subarctic zones, often grazed into mosaics dominated by grasses. Dominant species are typically dwarf shrubs such as *Empetrum nigrum*, *Rubus chamaemorus*, and *Cassiope tetragona* with mosses such as *Pleurozium schreberi*. Moss and lichen tundras are found in the middle and northern high arctic zone where permafrost soils dominate. These have sparse cover of mosses, lichens, and low-stature herbs. Dominating species are dwarf shrubs such as *Empetrum nigrum* and *Betula nana* with lichens and mosses including *Cladonia stellaris*, *Racomitrium lanuginosum* and *Cetraria nivalis* (Schaminée et al., 2014). There are not enough data at the sub-category level available to assign a $CL_{emp}N$ to these tundra subcategories. However, studies are available that provide information at the category level.

Numerous nutrient addition field studies have been conducted in manipulating tundra ecosystems. Most of the early studies added NPK fertiliser (e.g. Robinson et al., 1998; Press et al., 1998; Schmidt et al., 2000). There are also studies that used single and large applications of N (50 and 250 kg ha⁻¹ yr⁻¹) (see Henry et al., 1986; Shaver and Chapin, 1995). Unfortunately, such high application rates in a single dose do not realistically simulate atmospheric N depositions and, since they do not allow conclusions regarding $CL_{emp}N$, they are excluded from the following review.

In an experimental study on dwarf shrub tundra, significant effects have been reported at much lower N application rates (Baddeley et al., 1994). In that mixed tundra heath near Svalbard, Spitsbergen (with an estimated background deposition of 1.5 kg N ha⁻¹ yr⁻¹) plots were located in three different tundra heath vegetation types, which received factorial combinations of N (10 and 50 kg N ha⁻¹ yr⁻¹) and phosphorus (5 kg P ha⁻¹ yr⁻¹) in four to five applications during the growing seasons. Plots were treated for specific periods of time; those dominated by *Dryas octopetala* were treated from 1991 to 1998, those dominated by *Salix polaris* from 1991 to 1997, and those dominated by *Cassiope tetragona* from 1991 to 1993. Baddeley et al. (1994) reported early responses to the N additions (after one year of treatment); *Salix polaris* had increased levels of foliar N, increased leaf biomass and an increased photosynthetic rate at both N levels. *Cassiope tetragona* showed no response to the N addition, whilst *Dryas octopetala* showed an intermediate response in these measured variables.

After cessation of the treatment applications (in 1993, 1997 and 1998, respectively), Gordon et al. (2001) re-examined the impacts of N on these plots with particular attention to the bryophyte communities. Overall bryophyte cover was unaffected by increased N addition, although this was the net result of different individual species responses. For example, *Polytrichum juniperinum* increased in cover, whilst *Dicranum scoparium* cover declined. Tissue N concentration increased with increasing addition. Importantly, a number of significant persistent effects were observed at additions of 10 kg N ha⁻¹ yr⁻¹. Nitrate reductase activity was clearly inhibited in *Polytrichum juniperinum*, suggesting N saturation at 10 kg N ha⁻¹ yr⁻¹. This N saturation of bryophytes is of ecosystem importance, since N inputs may pass through the bryophyte layer (e.g. Britton et al., 2008; Britton et al., 2019), becoming available for soil microbes and higher plants. This may lead to increased growth of vascular plant species and hence, changes in species composition over time. Nitrogen addition also increased the proportion of green bryophyte shoots to a small extent, thus, apparently increasing bryophyte biomass production. The increased 'greenness' of the bryophyte cover on the fertilised *Cassiope* heath plots was independent of the P additions and was observed five years after N additions had ceased. This suggests that the added N was retained within the bryophyte layer of the *Cassiope* heath.

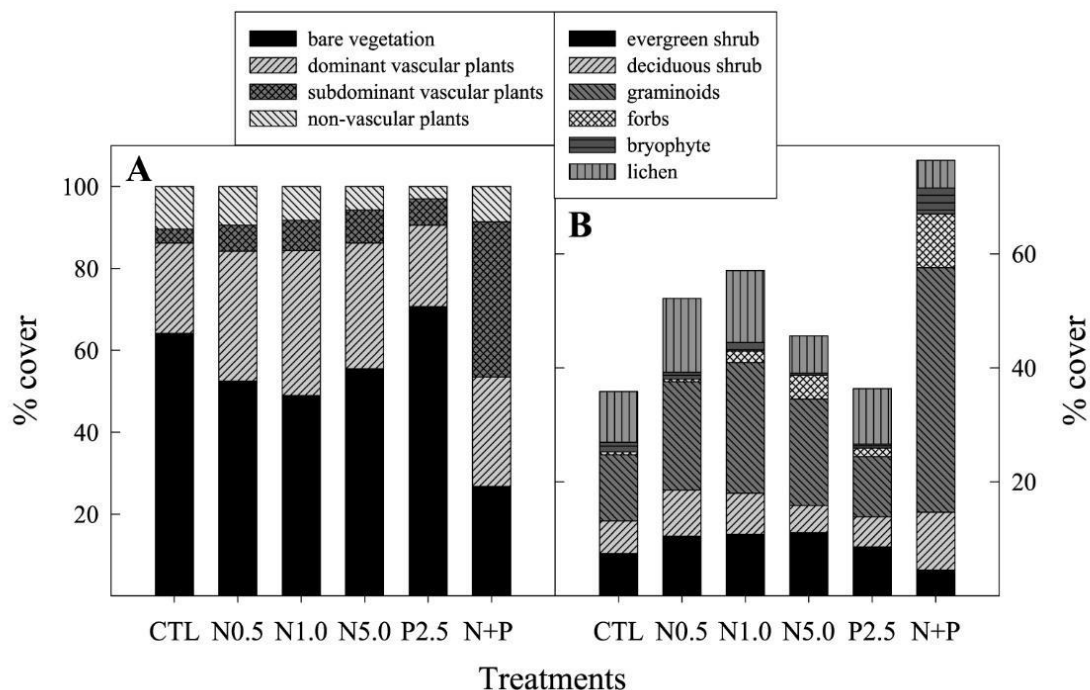
A subsequent revisit to this experiment 18 years after cessation of the additions showed that the effects of N treatments persisted and were P-dependent (Street et al., 2015). In plots where P was added, there were still effects of low N addition on community composition and nutrient dynamics. Further, N + P additions caused increased moss abundance, which influenced nutrient dynamics. These results show a lack of recovery in tundra and show that even small amounts of N deposition may potentially cause long-term ecological changes. Similar conclusions were reached by Liu et al. (2020) who examined the effects of continued nutrient additions or recovery (no further additions) in plots in northern Sweden that had previously been treated with high levels of N (50 kg N ha⁻¹ yr⁻¹) for eight to ten years. After the initial nutrient additions (eight to ten years), no further responses to additional N treatments (four years) were observed, suggesting that these subarctic habitats were resilient to further changes six years after cessation of additions. They concluded that recovery of a subarctic ecosystem to conditions prior to nutrient amendment may be very slow due to the slow responsiveness and possibly the high levels of N retention. Choudhary et al. (2016) assessed the fate of N deposition (0, 4, 40, and 120 kg N ha⁻¹ yr⁻¹) to high arctic bryophyte dominated tundra in a ¹⁵N labelling experiment. They found that more than 95% of the total ¹⁵N applied was recovered after one growing season, demonstrating the considerable capacity of this ecosystem to retain N from deposition events in arctic tundra. Regardless of the application rate or form, the following sinks were found (in order of magnitude): non-vascular plants > vascular plants > organic soil > litter > mineral soil.

In many experiments in tundra ecosystems, N additions were studied in relation to P additions or climatic variables. Co-limitation by N and P was clearly demonstrated on tundra heath in Svalbard (Street et al., 2015). Hence, CL_{emp}N for tundra ecosystems may be dependent on factors such as P availability. Shaver et al. (1998) suggested that wet tundra sites are more likely to be P limited than moist sites with a thinner peat layer, while dry tundra deserts are primarily N limited. Cornelissen et al. (2001) examined relationships between lichen and vascular plant abundances in arctic manipulation experiments (manipulated factors were temperature and nutrient availability). They concluded that negative correlations were greater at sites in milder climates with a greater above-ground biomass, where increased shading and litter production negatively affected the lichens.

Arens et al. (2008) studied the effects of additions of N (5, 10 and 50 kg N ha⁻¹ yr⁻¹) on vegetation characteristics and CO₂ exchange in a high arctic prostrate dwarf-shrub, herb tundra in north-

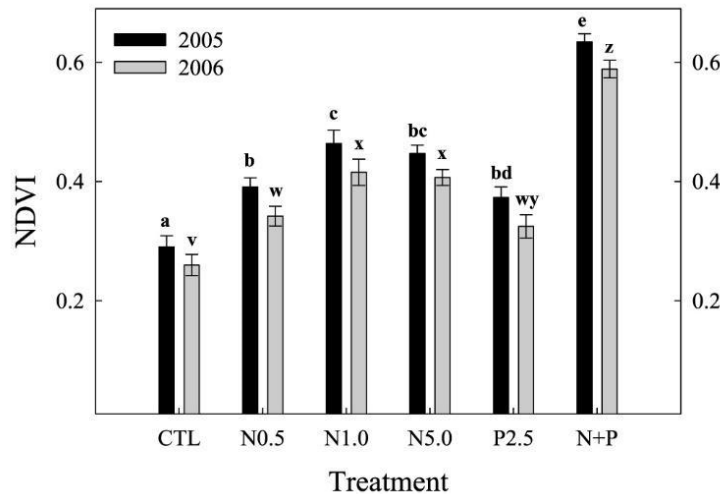
western Greenland (background deposition $< 1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). They used factorial additions of N and P ($25 \text{ kg P ha}^{-1} \text{ yr}^{-1}$) to test for potential co-limitations. Dry ammonium nitrate and/or commercial phosphate fertiliser were applied twice during the growing seasons of three consecutive years. At the study site, approximately 40% of the area was covered by vascular plants, of which *Salix arctica*, *Carex rupestris* and *Dryas integrifolia* were the dominant species, and 60% of the area was unvegetated. Vegetation cover and composition and ecosystem CO_2 exchange appeared to be very sensitive to low rates of N inputs ($5 \text{ kg ha}^{-1} \text{ yr}^{-1}$). Additions of $5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ led to a significant increase in *Salix arctica*, $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ almost doubled the cover of graminoids, and $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ resulted in a more than seven-fold increase in the cover of forbs (Figure 8.1). The mean NDVI (Normalised Difference Vegetation Index = cover of green biomass) was calculated for each plot ($20 \times 40 \text{ cm}$) and showed a saturation response to increasing levels of N addition, such that the largest NDVI response occurred at $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, while no further increase in NDVI was observed at $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Figure 8.2). Next to a decrease in ecosystem respiration and photosynthesis in the plots receiving $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, the results suggest N saturation in the ecosystem between 10 and $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Arens et al., 2008). Combined additions of both N ($50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) and P dramatically increased ecosystem photosynthesis and respiration, leading to a drastic increase in the cover of graminoids (especially *Festuca brachyphylla*; Figure 8.1).

Figure 8.1. Percentage of cover per (a) cover type and (b) functional group, in 2006, following three years of N additions in a prostrate dwarf-shrub, herb tundra (north-western Greenland). Each bar represents the mean of six plots for each treatment. N+P treatment = $2.5 \text{ g P m}^{-2} \text{ yr}^{-1} + 5.0 \text{ g N m}^{-2} \text{ yr}^{-1}$. Numbers behind the type of treatment indicate nutrient load in $\text{g m}^{-2} \text{ yr}^{-1}$ (times 10 for $\text{kg N ha}^{-1} \text{ yr}^{-1}$). Source: Arens et al. (2008).



Source: Arens et al., 2008

Figure 8.2. Mean NDVI (Normalised Difference Vegetation Index; a vegetation index that is correlated with the presence of photosynthetically active vegetation), in 2005 and 2006, representing the second and third growing season, respectively. Values represent the treatment mean ($n = 6$) \pm 1.0 SE. Bars with the same letter are not significantly different at $\alpha = 0.05$. N+P treatment = $2.5 \text{ g P m}^{-2} \text{ yr}^{-1} + 5.0 \text{ g N m}^{-2} \text{ yr}^{-1}$. Numbers behind the type of treatment indicate nutrient load in $\text{g m}^{-2} \text{ yr}^{-1}$ (times 10 for $\text{kg N ha}^{-1} \text{ yr}^{-1}$). Source: Arens et al. (2008).



Source: Arens et al., 2008

Summary Tundra (S1)

Despite the limited number of long-term experiments, a clear picture is emerging of the potential impact of long-term N deposition on tundra ecosystems. Ecosystem response to N has been observed at deposition rates of as low as $5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. To a large extent, however, the response to atmospheric N within tundra ecosystems may well depend on other factors, such as P status or climate.

In the CL_{empN} revision by Bobbink and Hettelingh (2011), the experiment by Arens et al. (2008) confirmed that tundra ecosystems are very sensitive to additional loads of N. Since significant effects were already seen at additions of as low as $5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, a new CL_{empN} was set at 3 to $5 \text{ kg ha}^{-1} \text{ yr}^{-1}$ and considered as 'quite reliable'. The increasing number of studies documenting effects of N deposition within this critical load, strengthen the arguments for setting the CL_{empN} at 3 to $5 \text{ kg ha}^{-1} \text{ yr}^{-1}$. The CL_{empN} for the North American tundra ecoregion has been suggested to be set at 1 to $3 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Nadelhoffer and Geiser, 2011; Pardo et al., 2011). However, to do so for Europe, studies documenting effects of N additions of $3 \text{ kg ha}^{-1} \text{ yr}^{-1}$ and below are needed.

The strong responses to N in situations where P was also applied are an indication of N and P co-limitation, identifying P as an important modifier of the CL_{empN} . Thus, higher critical loads should be applied to systems that are limited by P, and lower critical loads to systems that are not.

8.2.2 Arctic, alpine and subalpine scrub habitats (S2)

The EUNIS class of arctic, alpine and subalpine scrub habitats is subdivided into seven categories: subarctic and alpine dwarf willow scrubs (S21), alpine and subalpine ericoid heath (S22), alpine and subalpine juniper scrub (S23), subalpine *Genista* scrub (S24), subalpine and subarctic deciduous scrubs (S25), subalpine *Pinus mugo* scrub close to tree line (S26) and scrub close to the tree line with conifers other than *Pinus mugo* (S27) (Chytrý et al., 2020). Dwarf

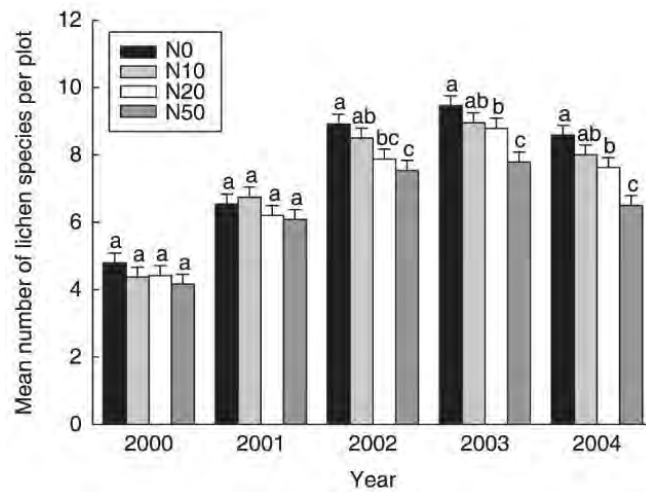
willow scrubs (S21) are well developed in boreal and arctic mountains and in subarctic lowlands. Alpine and subalpine ericoid heaths (S22) are dwarf or prostrate shrub formations in alpine and subalpine mountainous zones, dominated by ericaceous species or other woody species such as *Dryas octopetala*, *Kalmia procumbens* or *Rhododendron* species. Alpine and subalpine juniper scrub (S23) is dominated by dwarf junipers and may occur as either primary vegetation or as a result of deforestation and long-term grazing at high elevations. Subalpine *Genista* scrub (S24) occurs on high mountains around the Adriatic region. Subalpine deciduous scrubs (S25) include the subalpine scrubs of *Alnus*, *Betula*, *Salix* and *Rosaceae* (*Amelanchier*, *Potentilla*, *Rubus*, *Sorbus*), which are less than five metres tall, often accompanied by tall herbs. The last categories of conifer scrub close to the tree line (S26 and S27) relate to scrubland with dwarf conifers (krummholz) shaped by strong winds, often with incomplete canopy cover. Treelines dominated by *Pinus mugo* are classified as S26, and those dominated by other conifers as S27. The tree species at the arctic tree line can grow to large stature under favourable conditions. Despite the distinction of seven subcategories, most of the studies for this class have focussed on the alpine and subalpine ericoid heath (S22). Only a few studies have incorporated subalpine and subarctic deciduous scrubs (S25).

Experimental studies

A study in the Dovre mountains in Norway at 1000 to 1400 m above sea level, investigated the effects of three years of fertilisation of a *Betula nana* dominated community (S25) with 12 and 61 kg N ha⁻¹ yr⁻¹, at a site with an estimated background deposition of 2 to 4 kg N ha⁻¹ yr⁻¹ (Paal et al., 1997). There was no significant effect on plant growth, and no evidence of increased N content in vegetation or soils. In two other studies (Möls et al., 2001; Fremstad et al., 2005), two lichen-dominated communities were investigated, one in low-alpine and the other in middle-alpine regions. After ten years of applications of 7, 35 and 70 kg ha⁻¹ yr⁻¹, there was no significant effect on vascular plants. Lichens proved to be more sensitive; the cover of the lichens *Alectoria ochroleuca* and *Cetraria nivalis* had already decreased at the lowest dose of 7 kg N ha⁻¹ yr⁻¹. A possible reason for the limited effects of N on vascular plants and soils in this long-term experiment could be that other factors such as climate, soil properties and community structure may have been more important for determining species composition and cover (Fremstad et al., 2005).

Britton and Fisher (2007a) studied the effects of N deposition on low-alpine *Calluna* – *Cladonia* heath (S22) in Scotland (background deposition of 10 kg N ha⁻¹ yr⁻¹). Three levels of N addition (10, 20 and 50 kg N ha⁻¹ yr⁻¹) were applied over a five-year period. After five years, *Calluna vulgaris* shoot extension was stimulated by N additions of 10 kg ha⁻¹ yr⁻¹, indicating that low-alpine heathlands are very sensitive to low levels of N deposition (Britton and Fisher, 2008). Diversity of lichens was significantly reduced at additions of more than 10 kg N ha⁻¹ yr⁻¹ (Figure 8.3). Nitrogen addition caused rapid and significant increases in N content and N:P and N:K ratios of *Calluna vulgaris* following the two highest N treatments, suggesting increasing P and potassium limitation of growth. Soil C:N ratios declined significantly with N addition (only 50 kg N ha⁻¹ yr⁻¹), indicating N saturation and increasing likelihood of N leakage (Britton et al., 2008). A gradient study in low-alpine heathlands across Scotland suggested, based on N:P ratios, that growth of *Calluna vulgaris* on most sites is usually co-limited by N and P or P limited, due to the accumulated long-term N deposition in these mountain regions (Britton and Fisher, 2007b).

Figure 8.3. Effect on mean lichen species richness (+ 1 SE) of N addition treatments of 10, 20 and 50 kg ha⁻¹ yr⁻¹ (background deposition of 10 kg N ha⁻¹ yr⁻¹). Means for years not sharing the same letter are significantly different ($P < 0.05$); error bars show the standard error of the mean. Source: Britton and Fisher (2007a).



Source: Britton and Fisher, 2007a

In the N addition experiment on low-alpine *Calluna* heath described above (Britton and Fisher, 2007a), Nitrogen addition was also combined with burning and grazing (clipping) management treatments. Burning had a large effect on plant diversity and vegetation composition, but both recovered quickly. Nitrogen addition interacted with burning; burned plots showed no significant effect of N on species diversity, while the diversity on unburned plots was significantly reduced following the 10 kg N ha⁻¹ yr⁻¹ treatment (Britton and Fisher, 2007a; 2008). Clipping had no effect on plant diversity. This suggests that burning mitigates the impacts of low-dose N addition on species diversity.

The effects of 14 kg N ha⁻¹ yr⁻¹ addition on the emergence and survival of seedlings in subalpine heath dominated by *Empetrum hermaphroditum* and *Vaccinium uliginosum* was studied in Swedish Lapland (Milbau et al., 2017). Nitrogen addition significantly enhanced seedling emergence and survival for *Betula nana*, had no effect on *Solidago vigaurea* and reduced seedling establishment in *Vaccinium myrtillus*. Also utilising N additions at the lower limit of the critical load, Britton and Fisher (2010) tested the effects of both N load and concentration on thallus chemistry and growth of five terricolous alpine lichen species in a laboratory study. Responses to N addition varied between species; thallus N concentration was positively related to N load in *Cetraria islandica*, *Cladonia rangiferina* and *Flavocetraria nivalis*, with the greatest change occurring between 2.5 and 12.5 kg N ha⁻¹ yr⁻¹, but there was no relationship for *Platismatia glauca*. In this short (three month) study, impacts of the N additions on growth were only seen in two species (*Alectoria nigricans* and *Cetraria islandica*) both of which exhibited negative, linear relationships between growth rate and N concentration. In the Pacific Northwest of the USA, Simpson et al. (2019) tested the effects of low levels of N addition (3, 5 and 10 kg N ha⁻¹ yr⁻¹) on soil chemistry in alpine heaths dominated by *Phyllodoce*, *Cassiope* and *Vaccinium* species with a background deposition of 0.2-2 kg N ha⁻¹ yr⁻¹. This three-year study showed increases in soil NO₃⁻ and NH₄⁺ availability in response to additions of 5 kg N ha⁻¹ yr⁻¹ and declines in extractable soil organic carbon and increases in extractable soil NO₃⁻ and microbial N in response to additions of 10 kg N ha⁻¹ yr⁻¹.

New experimental studies also contribute further evidence of the impacts of N deposition above the current CL_{emp}N range. Papanikolaou et al. (2010) studied the effects of N additions of 10, 20

and 50 kg N ha⁻¹ yr⁻¹ on litter decomposition and associated enzyme activities and microbial communities in a Scottish *Calluna*-dominated alpine heath with a background deposition of 10 kg N ha⁻¹ yr⁻¹. They found no effect of N addition on litter mass loss over a two-year period, but activity of phosphomonoesterase in the litter was significantly increased by the 10 kg ha⁻¹ yr⁻¹ N addition. Bacterial community richness in litter harvested after two years increased with N addition and bacterial and fungal community composition (as determined by terminal restriction fragment length polymorphism) were also altered by N additions, although the magnitude of these effects was small. The effects of N additions on multiple ecosystem parameters over an eleven-year period at the same Scottish alpine heath are summarised by Phoenix et al. (2012). Additions of 10 kg N ha⁻¹ yr⁻¹ above background significantly increased *Calluna* productivity and flowering, increased availability of NO₃⁻ and NH₄⁺ in the soil and increased leaching of NH₄⁺. With larger additions of 20 and 50 kg N ha⁻¹ yr⁻¹ there was also evidence of increased frost damage of *Calluna*, enhanced N mineralisation in the soil and leaching of NO₃⁻ into soil water (Helliwell et al., 2010; Phoenix et al., 2012).

Gradient studies

In addition to the experimental studies, gradient studies including areas of low background N deposition provide some of the best evidence of N deposition impacts on vegetation composition at or below the current critical load. Three studies, which used vegetation composition data to identify community change points (thresholds) associated with N deposition, have been published since the previous critical loads update. In Swiss alpine heaths (S22) with data spanning a deposition range of approximately 5-19 kg N ha⁻¹ yr⁻¹ (modelled total N deposition at 0.1 × 0.1 km resolution), Roth et al. (2017) reported a community change point of 10.7-10.8 kg N ha⁻¹ yr⁻¹ above which total vascular plant richness, the richness of species associated with oligotrophic conditions and richness of conservation target species all declined. A study of alpine heaths in the UK (Payne et al., 2020) over a deposition range of 4.9-19.4 kg N ha⁻¹ yr⁻¹ (CBED model, total N deposition at 5 × 5 km resolution) that included data on lichens and bryophytes, found individual species change points occurring from the lowest deposition levels in the dataset, with negatively responding taxa clustered into two groups responding around 5-7 and 10-14 kg N ha⁻¹ yr⁻¹. A third study from Ireland (Wilkins et al., 2016) included slightly lower N deposition levels (range of 4.0-19.9 kg N ha⁻¹ yr⁻¹ for alpine heaths, observation-based total N deposition at 5 × 5 km resolution). In this study, change points for the complete vegetation community (including bryophytes and lichens) were identified and considered to be evidence of 'significant harmful effects' if the number of positive indicator species (plant species indicative of good habitat status) that declined in cover was greater than the number that increased in cover. In alpine heaths (S2) twenty species declined in response to N deposition, including nine positive indicator species, while only five species (including one positive indicator) increased. Eleven of the twenty declining species were bryophytes or lichens. The community change point for declining species was estimated at 5.5 kg N ha⁻¹ yr⁻¹. Additional support for the impacts of low levels of N deposition on terricolous lichen species in heathland is provided by Stevens et al. (2012). In this study, records of lichen presence on a 10 km grid across the UK were used to model probability of presence at a given level of N deposition (CBED model, total N deposition at 5 × 5 km resolution). Several heathland lichen taxa were found to decline in response to N deposition, with declines in prevalence occurring from the lowest levels of N deposition (approximately 5 kg N ha⁻¹ yr⁻¹) and many taxa reaching a very low probability of presence by 20 kg N ha⁻¹ yr⁻¹.

Summary Arctic, Alpine and subalpine scrub (S2)

Studies on the effects of N addition to the vegetation types within the S2 class are scarce and most use N additions which fall above or within the CL_{emp}N range when background deposition

is incorporated (Aerts, 2010, Phoenix et al., 2012, Manninen and Tolvanen, 2013). A few gradient studies that include areas of low background N deposition have provided evidence for impacts around the lower $CL_{emp}N$ limit (Wilkins et al., 2016; Payne et al., 2020) but almost all evidence for N impacts in the EUNIS S2 category relates to alpine and subalpine ericoid heaths (S22). Since there is very limited evidence for impacts in other alpine and subalpine heath and scrub habitats, critical loads are only provided for the S2 level. Previously, the $CL_{emp}N$ for arctic, alpine and subalpine scrubs was set at 5 to 15 kg ha⁻¹ yr⁻¹ (Bobbink and Hettelingh, 2011). Data from new experimental studies with low background deposition provide additional evidence for biological and biogeochemical impacts occurring within this range. In addition, survey studies encompassing low background deposition areas provide evidence for N deposition impacts at the lower end of the current $CL_{emp}N$ range, particularly affecting bryophytes and lichens. Therefore, we propose to narrow the $CL_{emp}N$ range, setting it at 5 to 10 kg ha⁻¹ yr⁻¹ for arctic, alpine and subalpine scrub habitats (S2), classifying it as 'quite reliable'. For this habitat, similarly to S1, responses to N deposition depend on N and P co-limitation. Higher critical loads should be applied to systems that are limited by P, and lower critical loads to systems that are not.

8.3 Temperate and Mediterranean-montane scrub (S3)

Temperate and Mediterranean-montane scrub (S3) habitats are subdivided into eight categories. However, few of these habitats have published studies relevant to $CL_{emp}N$, with the exception of two gradient studies in lowland to montane temperate and submediterranean *Juniperus* scrub (S31). Gruwez and colleagues (Gruwez et al., 2014) collected seeds from 42 populations of *Juniperus communis* throughout its distribution in Europe to assess the effects of climate and atmospheric depositions on seed viability. Seed viability was determined using seed dissection. Nitrogen deposition, which ranged from 1.8 to 36.0 kg ha⁻¹ yr⁻¹ across the study area (EMEP total N deposition at 50 × 50 km resolution), was negatively related to seed ripening time, with the proportion of seeds ripening in two vs. three years rapidly increasing across the deposition range of ~2 to 10 kg N ha⁻¹ yr⁻¹. Potentially acidifying deposition, including N deposition was also associated with a reduction in seed viability. The authors suggested that the failure of natural regeneration in many European juniper populations might be attributed to enhanced atmospheric deposition of N. Wilkins et al. (2016), with updates in Aherne et al. (2021), identified vegetation community change points for *Juniperus* scrub using species abundance data from 191 relevés in Ireland covering a deposition gradient of ~3 to 25 kg N ha⁻¹ yr⁻¹ (observation-based total N deposition at 5 × 5 km resolution). The community change point for declining species was estimated at 6.2 kg N ha⁻¹ yr⁻¹ with 19 species decreasing in abundance (16 of which were positive indicator species considered typical for this habitat).

Summary Temperate and Mediterranean-montane scrub (S3)

The gradient studies support the possible sensitivity of *Juniperus* scrub to N deposition. Here we propose a $CL_{emp}N$ range of 5 to 15 kg N ha⁻¹ yr⁻¹ for lowland to montane temperate and submediterranean *Juniperus* scrub (S31), classifying it as 'expert judgement' given the limited studies.

8.4 Temperate shrub heathlands (S4)

As discussed above, both wet and dry heathlands (S41 and S42) have been placed within EUNIS class S4 (temperate shrub heathlands), because they occur in the Atlantic climate region and are dominated by ericoid shrubs. This EUNIS class has been divided into subcategories of wet heaths (S41), which are characterised by wet, organic-rich to peat soils, and dry heaths (S42) characterised by free-draining sandy-podzol soils. Both upland *Calluna* moorlands and lowland

wet heaths dominated by *Erica tetralix* fall within the category of ‘northern’ wet heaths (S411). However, since these communities are clearly ecologically different, it is important that this distinction is retained. Therefore, each habitat has been assigned a different critical load. Given that there is no elevation-based cut-off that can be recommended to distinguish the two habitats, the primary criterion must be that of species dominance.

Since Bobbink and Hettelingh (2011), the number of new relevant experimental studies is limited, with many using high N additions, e.g. 50+ kg N ha⁻¹ yr⁻¹ or in regions with high background deposition, e.g. 20+ kg N ha⁻¹ yr⁻¹. However, continuation of some of the original studies provide valuable data on long term effects. Several publications on the effects of N on upland *Calluna vulgaris* heath have focused on management measures to counteract the negative effects of N on locations where critical loads are being exceeded. In contrast, a notable number of new gradient studies have been published since the Bobbink and Hettelingh (2011) update. These studies have generally considered plant species data from large-scale surveys and have primarily focused on changes in species richness and plant community composition along a N deposition gradient either in the UK (Maskell et al., 2010; Henrys et al., 2011; Tipping et al., 2013; Field et al., 2014; Van den Berg et al., 2016), Denmark (Strandberg et al., 2012; Damgaard et al., 2014), or Ireland (Wilkins et al., 2016; Aherne et al., 2021). A number of these studies have used change-point analysis, such as TITAN, or similar statistical analysis to identify a deposition threshold where change occurs (Strandberg et al., 2012; Tipping et al., 2013; Wilkins et al., 2016; Aherne et al., 2021). Further, these gradient studies typically include regions with low N deposition, e.g. less than 5 or 10 kg N ha⁻¹ yr⁻¹, allowing for the assessment of impacts at lower N inputs compared with experimental additions.

8.4.1 Upland *Calluna*-dominated wet heath (upland moorland) (S411)

Calluna vulgaris dominated, upland moorland heaths (S411) are generally developed on acidic, peaty organic soils, and are characterised by a dominance of dwarf shrubs (in particular *Calluna vulgaris*) and a high abundance of bryophyte species. The effects of N deposition on upland *Calluna vulgaris* heaths have been studied in the UK using both field surveys and experiments. The CL_{emp}N range of 10 to 20 kg N ha⁻¹ yr⁻¹ that was recommended by Bobbink et al. (1996; 2003) and Bobbink and Hettelingh (2011) for this community, was based on three types of evidence from these UK studies: effects on growth and species composition, effects on shoot nutrient concentration, and effects on soils and root characteristics. Experimental studies reported after 2010 support this range (described below), while gradient studies encompassing low background deposition regions provide evidence for N deposition impacts at the lower end of the current CL_{emp}N range.

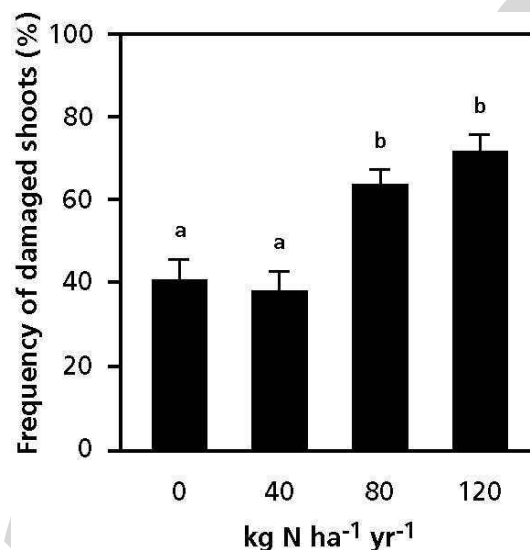
Effects on growth and species composition

The longest running N manipulation experiment in *Calluna vulgaris* moorland is at Ruabon, north Wales, where experiments were established in May 1989 on moorland at an altitude of 470 metres (Caporn et al., 1994). This site was estimated recently to receive an atmospheric N deposition of approximately 19 kg N ha⁻¹ yr⁻¹ (APIS 2021). In this experiment, additions of N as ammonium nitrate at 40, 80 and 120 kg N ha⁻¹ yr⁻¹, have been applied since 1989, at monthly intervals (Caporn et al., 1994; Lee and Caporn, 1998; Carroll et al., 1999; Pilkington et al., 2007a). The initial period from 1989 to 1993 was characterised by positive effects of N on *Calluna vulgaris* in terms of increased shoot growth, N concentration and flowering, with no indication that the dose applied exceeded the capacity of the plants for uptake and subsequent growth. The following three years of the study, however, showed a much-reduced effect of the treatment on shoot extension, and no clear dose response to increasing N inputs. The 1996 data, in particular, showed no effect at all of N on shoot extension after seven years (Carroll et al.,

1999). One interpretation of the *Calluna vulgaris* growth responses in this experiment is that additions of N accelerated the natural *Calluna vulgaris* cycle, with earlier ageing and opening of the canopy in the plots that received the highest doses.

One factor causing increased canopy opening, with potential for grass invasion, may be greater damage in winter to *Calluna vulgaris* shoots. Detailed experimental studies on frost tolerance in *Calluna vulgaris* shoots collected in the early years of the study (1989-1994) demonstrated that N addition had actually improved frost tolerance in autumn (Caporn et al., 1994). However, field surveys in 1996 and 1998 showed large increases in 'winter browning' of heather shoots in the late winter, most notably following additions of 80 and 120 kg N ha⁻¹ yr⁻¹ (Figure 8.4) (Carroll et al., 1999; Lee et al., 2000). The mechanism behind this damage may have been frost injury.

Figure 8.4. Effects of seven years of ammonium nitrate additions (kg N ha⁻¹ yr⁻¹) on the frequency (means ± SE) of damaged *Calluna vulgaris* shoots in northern wet heath (S411) in North Wales (Carroll et al., 1999). Columns sharing a letter are statistically not significantly different.



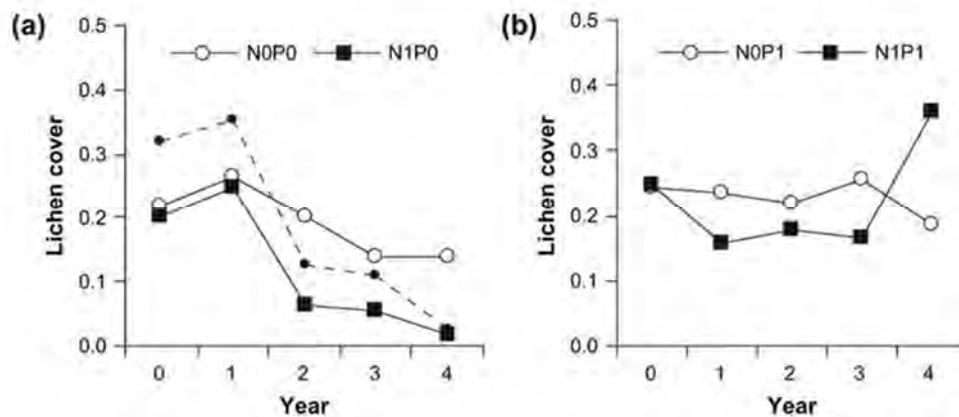
Source: Carroll et al., 1999

In the earlier years, both bryophytes and lichens had disappeared from below the *Calluna vulgaris* canopy following all N addition treatments, although *Vaccinium myrtillus* persisted (Carroll et al., 1999). It is not certain, however, whether this was a direct effect of N addition or a response to changes in *Calluna vulgaris* canopy cover and increased litter production resulting in reduced light penetration. A later survey, following ten years of treatment, showed not only that bryophytes, notably *Hypnum jutlandicum*, had returned to the N treated plots, but also their cover had actually increased with increasing N depositions, probably as a result of the accelerated ageing and opening up of the *Calluna vulgaris* canopy (Lee et al., 2000). No such response was found in lichen species.

Adjacent to the above original experiment, Pilkington et al., (2007a) treated experimental plots in a upland mature growth phase *Calluna* heath with factorial combinations of N (0 and 20 kg N ha⁻¹ yr⁻¹) and P (0 and 5 kg ha⁻¹ yr⁻¹). They found that lichen cover had virtually disappeared within four years from plots that had received 20 kg N ha⁻¹ yr⁻¹ and from separate plots that had received 10 kg N ha⁻¹ yr⁻¹ (background N deposition of 16.4 kg N ha⁻¹ yr⁻¹). However, this effect was reversed by the addition of P (Figure 8.5). Continued research on the same experiment by Edmondson (2007) demonstrated similar effects of N and P treatments on the mosses and liverworts as well as lichens. The adverse effects of N were evident for species such as the

liverwort *Lophozia ventricosa* but less so for the more nutrient tolerating *Hypnum jutlandicum* (Edmondson et al., 2013).

Figure 8.5. Effect of factorial combinations of N and P additions on the cover of lichens in an upland *Calluna vulgaris* heath (background N deposition of $16.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). $\text{N0} = 0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, $\text{N1} = 20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, $\text{P0} = 0 \text{ kg P ha}^{-1} \text{ yr}^{-1}$ and $\text{P1} = 5 \text{ kg P ha}^{-1} \text{ yr}^{-1}$. The dotted line in Figure (a) indicates a separate N addition of $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Source: Pilkington et al. (2007a). The decline in Lichen cover in the control (N0P0) reflects the increased growth of the *Calluna* canopy.



Source: Pilkington et al., 2007a

The experimental plots at Ruabon were subject to a controlled burn in 2000 in order to simulate traditional management of these systems. Subsequent post-burn regrowth, primarily from stem bases, was significantly lower in the plots with higher N treatments (Lee and Caporn, 2001). Pilkington et al. (2005) completed an N budget for the site based on harvesting data. Significant increases in green tissue, wood and litter biomass, and litter depth were found for all levels of N treatments. Although most of the added N in the $40 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ treatment was found in green tissue and litter, increasing amounts of added N were found in the peat horizons in the plots with higher N treatments (Pilkington et al., 2005).

Effects on shoot nutrient content

Several studies found that N content in shoots of *Calluna vulgaris* and bryophytes was significantly higher in areas with higher N inputs ($> 10\text{--}15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), substantially higher compared with measurements of historical plant material (Pitcairn et al., 1995). Indeed, the N concentration of *Calluna* shoots, common mosses (e.g. *Hypnum jutlandicum*) and leaf litter were suggested to be reliable indicators of atmospheric N deposition across UK heathlands (Caporn et al., 2014). Additionally, increases in N concentration in shoots have been linked to increased performance of winter moths (*Operophtera brumata*); infestations of which have led to extensive damage to heather moorlands in Scotland over the last decade (Kerslake et al., 1998).

In the long-term experiment in north Wales, analysis of foliar nutrients between 1989 and 1992 showed increased N concentrations with increasing doses of N, and measurements taken in 1996 still showed a significant increase in response to the earlier N additions (Carroll et al., 1999). By 1996, N:P ratios had clearly increased as a result of the earlier treatments, with values of 23:1 in the plots that had received the highest N treatment ($120 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), compared to values of 16:1 in control plots (Carroll et al., 1999). Carroll et al. (1999) compared these values with the critical threshold for the N:P ratio of 14:1 to 16:1, as proposed by Koerselman and Meuleman (1996), to indicate a switch from N to P limitation, and suggested that the onset of P

limitation might explain the declining response to N in shoot extensions, which occurred at this stage of the experiment. This interpretation is supported by evidence of increased phosphomonoesterase activity in peat and root surfaces (Johnson et al., 1998; Lee et al., 2000).

This observation is also consistent with Kirkham (2001), who sampled *Calluna vulgaris* shoots from a number of sites in England and Wales. The N:P ratios in shoots of *Calluna vulgaris* were above 16:1 at about half of the sampled sites, suggesting that N deposition had changed a substantial proportion of *Calluna*-dominated uplands in the UK from N-limited ecosystems into P-limited or N:P co-limited heaths, again (see also tundra) stressing the modulating effects of P.

Effects on soil and root characteristics

Calluna vulgaris roots characteristically exhibit a substantial degree of ericoid mycorrhizal infection (Yesmin et al., 1996), which is important for the degradation of complex organic substances in order to give plants access to N sources that would otherwise be unavailable to them. The N addition experiment in North Wales consistently showed little effect on mycorrhizal infection levels (Caporn et al., 1995; Lee et al., 2000), using either the ergosterol method or visual assessments. In contrast, Yesmin et al. (1996) reported a negative correlation between N deposition and mycorrhizal infection rate at five remote Scottish sites with a total deposition in the range of 2 to 10 kg N ha⁻¹ yr⁻¹. In addition, a separate greenhouse study showed a small but significant decrease in infection rate when deposition rates were increased from 12 to 24 kg N ha⁻¹ yr⁻¹ for one year.

Soil studies at Ruabon, north Wales, found a number of responses to N additions. Leaching rates, although showing a small response to the higher N addition treatments, account for only a very small percentage of the added N (Pilkington et al., 2005). There was also evidence of increased mineralisation and decreased C:N ratio in the litter and rhizosphere with increasing N treatments, although this effect was not found below two centimetres in the soil (Lee and Caporn, 2001). Recent work at this site showed that N addition of 20 kg N ha⁻¹ yr⁻¹ significantly increased storage of C and N in the litter layer and surface soil horizons (Field et al., 2017). Overall, results implied a high retention of the added nitrate and ammonium, probably through microbial immobilisation; Johnson et al. (1998) demonstrated that the long-term application of N at a site increased soil microbial biomass. Curtis et al. (2005) used a stable isotope tracer (¹⁵N) to determine the fate of N inputs in a gradient study (varying from 6.4 to 30.7 kg N ha⁻¹ yr⁻¹) applied in small doses over a one-year period. The purpose of the study was to determine the fate of ¹⁵N-labelled N additions at four sites selected from the UK Acid Waters Monitoring Network. The sites represented gradients of total N deposition and leaching losses of inorganic N (in north-east Scotland, mid-Wales and north-west England) measured as part of a larger N budget study. Mosses and lichens showed far greater ¹⁵N recovery per unit of biomass than grasses or ericaceous shrubs. High N deposition rates reduced the biomass of mosses and lichens and thereby the absorption capacity of the cryptogams and the proportion of N recovered; this may lead to increased nitrate leaching.

N deposition and management measures

These upland *Calluna vulgaris* heaths are actively managed by cutting or burning. In the UK, rotational burning of upland moorland (once every 7 to 20 years) is a commonly used management practice to maintain *Calluna vulgaris* stands. Pilkington et al. (2007b) studied the effects of moorland burning on N pools and leaching in a long-term N manipulation experiment in North Wales. Burning increased leaching of total dissolved inorganic N and dissolved organic N from organic and mineral soil horizons. Nitrogen additions magnified the effect of burning on leaching losses but reduced this effect on the N pools in the mineral layer. Pilkington et al. (2007b) concluded that burning approximately every ten years may be effective in removing N

retained in the system, at N deposition rates of up to 56 kg N ha⁻¹ yr⁻¹, although burning exacerbates the threat of N loading to groundwater in heavily N-polluted areas.

In addition, grazing is likely to be an important regulating factor. In general, active management of *Calluna vulgaris* moorlands is thought to reduce the impact of increased N deposition and allows for higher critical loads but the effect strongly depends on the grazing levels (Alonso et al., 2001; Hartley et al., 2003; Hartley and Mitchell, 2005).

Gradient studies

Since Bobbink and Hettelingh (2011), a large number of broad-scale gradient studies in the UK have assessed the impacts of N deposition on upland and lowland heaths (Edmondson et al., 2010; Field et al., 2014; Henrys et al., 2011; Stevens et al., 2012; Southon et al., 2013; Tipping et al., 2013; Van den Berg et al., 2016). These studies provide some of the best evidence of N impacts on vegetation composition at or below the current critical load. In general, upland and lowland heath habitats have primarily been categorised as dry heaths (S42) in these studies but they likely include wet heaths (S411) given their underlying organic soil; only one of the broad-scale studies directly identified S411 (Tipping et al., 2013). Field et al. (2014) and Southon et al. (2013) studied 25 upland heaths along a deposition gradient of 5.9 to 32.4 kg N ha⁻¹ yr⁻¹ (CBED model, total N deposition at 5 × 5 km resolution). Here, heathlands were ascribed to S411 given their underlying organic soil, dominance by *Calluna vulgaris* and high rainfall. Field et al. (2014) found reduced species richness and changed species composition associated with higher N deposition. Species richness declined by about 40% of maximum species richness from the lowest to the highest N deposition sites (32 kg N ha⁻¹ yr⁻¹), with a steeper decline in species richness in the lower deposition range < ~11 kg N ha⁻¹ yr⁻¹. Similarly, Southon et al. (2013) found that the abundance of nitrophilous species increased with increasing N deposition (CBED model, total N deposition at 5 × 5 km resolution). The number of forb and graminoid species also decreased with increasing total N deposition. The biggest losses were seen at the lower end of the N deposition gradient, with an average of 13 species lost per site between 5 to 10 kg N ha⁻¹ yr⁻¹, compared to an average of only three species lost as N increased from 10 to 20 kg N ha⁻¹ yr⁻¹.

Summary Upland *Calluna*-dominated wet heath (S411)

Since the last update (Bobbink and Hettelingh, 2011), the number of new addition experiments with N loads within the range of the current CL_{emp}N is limited. In general, the available experimental data are based on total N inputs (background plus treatments) that are significantly above the previously established CL_{emp}N range of 10 to 20 kg N ha⁻¹ yr⁻¹. In contrast, a growing number of gradient studies have incorporated regions with low N deposition (< 10 kg N ha⁻¹ yr⁻¹); they provide evidence for N deposition impacts on plant species composition at or below the lower end of the current CL_{emp}N range. Therefore, we propose to revise the CL_{emp}N range, setting it at 5 to 15 kg N ha⁻¹ yr⁻¹ for upland *Calluna*-dominated wet heath (S411), classifying it as 'reliable'. This CL_{emp}N range is dependent on management practices, with the high end of the range applying to wet *Calluna*-dominated heath with high intensity management, and the low end of the range to wet *Calluna*-dominated heath with low intensity management.

8.4.2 Lowland *Erica tetralix*-dominated wet heath (S411)

The wet habitats in western European lowland heathlands are dominated by the dwarf-shrub *Erica tetralix* (Ellenberg, 1988) and classified within EUNIS as northern wet heath (S411). A drastic change in species composition has been observed in Dutch wet heathlands, from *Erica tetralix* dominated dwarf shrub vegetation to monospecific stands of the grass *Molinia caerulea*. Alongside *Erica tetralix*, almost all of the rare plant species have disappeared from this habitat type. It has been hypothesised that this change was caused by eutrophication induced by

elevated atmospheric N deposition. This favours the highly competitive grass *Molinia caerulea*, which is able to outcompete *Erica tetralix* and other key species from this habitat (Berendse and Aerts, 1984; Aerts and Berendse, 1988).

Using the competitive relationship between *Molinia caerulea* and *Erica tetralix*, the biomass production of these species and taking into account management measures, Berendse (1988) suggested that 17 to 22 kg N ha⁻¹ yr⁻¹ is the CL_{emp}N range for the transition of lowland wet heath towards a grass-dominated sward. This CL_{emp}N range was also the value recommended by Bobbink et al. (1996). However, because of the lack of natural variation in the modelling approach, the range of 17 to 22 kg N ha⁻¹ yr⁻¹ was considered too narrow. The model by Berendse (1990) was based on the intensive management of sod (turf) with cutting every 50 years, in combination with grazing. Sod cutting is a common restoration measure in the Netherlands that effectively removes the N-rich and acidified top layer of the soil in order to decrease eutrophication and eliminate thick *Molinia* tussocks. It is a very intensive measure, setting back succession of the vegetation. In the UK, Allchin et al. (2001) applied a similar model to dry heaths with less intensive management regimes, such as mowing every 15 years, which gave a threshold for changes in species composition of about 10 kg N ha⁻¹ yr⁻¹. Similar effects of management regimes were expected for wet heaths and hence the lower end of the CL_{emp}N range was reduced to account for the effects of N under less intensive management.

There is no clear evidence of a differential response in *Calluna vulgaris* and *Erica tetralix* to simulated N depositions; Smart et al. (2004) found similar spatial relationships between changes in cover and N deposition in the UK for the two species. Therefore, Bobbink and Hettelingh (2011) recommended that the lower end of the CL_{emp}N range should be 10 kg N ha⁻¹ yr⁻¹, the same as that for *Calluna*-dominated wet and dry heaths. The upper end of the CL_{emp}N was also reduced, to make it equivalent with that for other heathland (S411 (upland) and S42) habitats. Bobbink and Hettelingh (2011) proposed a revised CL_{emp}N of 10-20 kg N ha⁻¹ yr⁻¹, based on expert judgment.

Gradient studies

Since Bobbink and Hettelingh (2011), gradient studies have provided new evidence with respect to the CL_{emp}N for *Erica tetralix*-dominated wet heaths. A number of gradient studies from the UK (e.g. Maskell et al., 2010; Henrys et al., 2011; Van den Berg et al., 2016) have repeatedly shown reduced species richness and changed species composition in heathlands associated with higher N deposition. Tipping et al. (2013) used a broken stick median regression to estimate the threshold above which N deposition had an effect on plant species richness data from the 1998 Countryside Survey in the UK. The analysis included 457 heathlands sites (classified as S411 and S42) spanning a deposition gradient of 4.9 to 40 kg N ha⁻¹ yr⁻¹ (CBED model, total N deposition at 5 × 5 km resolution). They estimated a threshold of 8.8 kg N ha⁻¹ yr⁻¹ (4.7-10.1 kg N ha⁻¹ yr⁻¹) with an average relative loss of species with increasing N deposition of 2.3% per kg N ha⁻¹ yr⁻¹.

In Denmark, Damgaard et al. (2014) applied a structural equation modelling (SEM) approach to 89 wet heathland sites (spanning a deposition range of 8 to 22 kg N; NOVANA model, total N deposition) to understand the underlying causes for the decrease in cover of *E. tetralix*. The most important causal effect revealed by the SEM was a significant negative effect of N deposition on the cover of *E. tetralix*. Strandberg et al. (2012) analysed *E. tetralix* cover data from 500 wet heathland plots (S411) in the Danish National Monitoring Programme. They found a significant negative relation between the cover of *E. tetralix* and N deposition, indicating a threshold at a deposition between 8.3 and 13.2 kg N ha⁻¹ yr⁻¹. Wilkins et al. (2016), with updates in Aherne et al. (2021), identified a vegetation community change point (threshold) for northern Atlantic wet heaths in Ireland using species abundance data from 231 relevés spanning a deposition gradient

of ~3 to 24 kg N ha⁻¹ yr⁻¹ (observation-based total N deposition at 5 × 5 km resolution). The community change point for declining species was estimated at 4.7 kg N ha⁻¹ yr⁻¹ with eleven species decreasing in abundance (four identified as positive indicator species).

Summary Lowland *Erica*-dominated wet heath (S411)

Gradient studies incorporating regions with low N deposition provide strong evidence of N deposition impacts on vegetation composition at or below the current critical load. Therefore, we propose to revise the CL_{emp}N range, setting it at 5 to 15 kg N ha⁻¹ yr⁻¹ for lowland *Erica tetralix*-dominated wet heath (S411), classifying it as 'reliable'. This CL_{emp}N range is dependent on management practices, with the high end of the range applying to wet *Calluna*-dominated heath with high intensity management, and the low end of the range to wet *Calluna*-dominated heath with low intensity management.

8.4.3 Dry heaths (S42) (mostly sub-Atlantic *Calluna-Genista* heaths (S422))

Despite the conservation and management efforts in nature reserves, many lowland heaths (S42) in western Europe have become dominated by grass species. By using aerial photographs, it was demonstrated that more than 35% of Dutch heaths developed into grasslands during the 1980s (Van Kootwijk and Van der Voet, 1989). It was suggested that a strong increase in atmospheric N deposition contributed to this transition towards grassland. Similar patterns were found in the UK over the past 20 to 50 years. Pitcairn et al. (1991) showed declines in abundance of *Calluna vulgaris* at three heathland areas in East Anglia and an increase in grasses; the authors concluded that increased N deposition (up to 30 to 40 kg N ha⁻¹ yr⁻¹) had at least been partly responsible for these changes, but also noted that the management had changed. A wider assessment of heathlands in eastern England showed that in some cases *Calluna vulgaris* had declined and subsequently been outcompeted by grasses, while other areas were still dominated by dwarf shrubs (Marrs, 1993). Although a move away from traditional management practices, such as grazing, burning or sod cutting, may be partly responsible, the decline in British heathlands has been linked with the historic increase in N deposition during the past 50 years. Furthermore, it has been hypothesised that, besides important changes in land use, increased N deposition is an additional cause of the decline in heaths in the southern parts of the Nordic countries (e.g. Fremstad, 1992; Tybirk et al., 1995).

Plant productivity and nutrient limitation

In N-limited systems, one of the first effects of increased N availability through atmospheric deposition is an increase in biomass production of the vegetation. Many studies found increased productivity of dwarf shrubs following experimental N enrichment in dry heathlands in several north-western European countries (e.g. Heil and Diemont, 1983; Van der Eerden et al., 1991; Aerts and Heil, 1993; Power et al., 1995; 1998a; Lee and Caporn, 2001). Gaining reliable estimates of critical loads is difficult in many European regions where background N deposition is already moderate to high (10 to 20 kg N ha⁻¹ yr⁻¹). However, Bahring et al. (2017) studied lowland coastal heaths on the Baltic island of Fehmarn where the background deposition was 9 kg N ha⁻¹ yr⁻¹. In this relatively clean environment, the addition of 5 kg N ha⁻¹ yr⁻¹ significantly increased *Calluna* shoot extension. This indicates that most of these lowland dry heath ecosystems are primarily limited by N, although some inland dry heaths are limited by P (Riis-Nielsen, 1997; Nielsen et al., 2000).

An illustrative example of the growth stimulation of *Calluna vulgaris* was found in a field experiment in the UK. The experiment was set up in 1989 to assess long-term impacts of realistic N loads on lowland dry heaths (S422) in southern Britain (Uren, 1992; Uren et al., 1997; Power et al., 1995; 1998a; 2001). After seven years of applications of ammonium sulphate (7.7

and 15.4 kg N ha⁻¹ yr⁻¹ with a background deposition of 8 kg N ha⁻¹ yr⁻¹, Power and Barker (2003) found no negative effects on *Calluna vulgaris*. Indeed, a significant stimulation of flower production, shoot density, and litter production occurred and, after six to seven years, the canopy was 50% taller at 15.4 kg N ha⁻¹ yr⁻¹ than in the control plots (Power et al., 1995; 1998a). The increased shoot growth for the N-treated vegetation was not reflected by root growth, and an increased shoot:root ratio was inferred. Nitrogen concentrations in shoots also increased, with significant effects found in the months of July and/or October of several of the years that were assessed (Power et al., 1995; 1998a). In 1998, a parallel experiment was set up at the same site involving the addition of N (30 kg ha⁻¹ yr⁻¹ in two-weekly additions of ammonium sulphate) over a 12-year period. Similar to the earlier study, this experiment also demonstrated large and sustained increases in above-ground productivity, increased foliar N concentrations and an acceleration in the rate of N cycling and storage within the ecosystem (Barker, 2001; Jones, 2009). Similar growth stimulation of *Calluna vulgaris* was observed in a dry lowland heath in northern England. Since 1996, N has been applied in the form of ammonium nitrate (20, 60 and 120 kg N ha⁻¹ yr⁻¹) in Cheshire; the atmospheric deposition at this site was estimated at 20 kg N ha⁻¹ yr⁻¹. Within two years, shoot growth and flowering in *Calluna vulgaris* increased following the two highest N addition rates; after five years of N addition these effects were maintained in terms of canopy density, while the canopy height of *Calluna vulgaris* increased by ~20 centimetres (Cawley, 2001; Lee and Caporn, 2001). Collectively, these experiments demonstrated continued N limitation of this lowland heathland under prolonged N inputs.

Nitrogen accumulation and mineralisation

During secondary dry heath succession, an increase in the amount of organic material and N in the soil has been observed (Chapman et al., 1975; Gimingham et al., 1979). The accumulation of organic matter and N after sod removal was quantified in dry heaths in the Netherlands by Berendse (1990). A large increase was reported in plant biomass, soil organic matter and total N storage in the first 20 to 30 years of succession suggesting an annual increase in N in the system of ~33 kg N ha⁻¹ yr⁻¹. These values are in good agreement with the measured N inputs in Dutch heathlands (Bobbink et al., 1992b).

Owing to their high organic matter and microbial biomass content, ammonium (the dominant form of mineral N in these systems) immobilisation in the soil is high, and hardly any leaching losses to deeper layers have been measured in Dutch, British or Danish dry non-coastal heaths even under high N inputs (De Boer, 1989; Van der Maas, 1990; Power et al., 1998a; Kristensen and McCarty, 1999; Kristensen, 2001; Nielsen et al., 2000), very similar to wet heaths S411. This means that almost no N is lost from these systems. Indeed, the N content of the soil (upper 10 cm) significantly increased from 35.0 g N m⁻² to 45.5 g N m⁻², following seven years of 15.4 kg N ha⁻¹ yr⁻¹ addition applied to Thursley dry heath, while N leaching remained very low (Power et al., 1998a, 1998b; Barker, 2001). Significant relationships between N deposition and concentrations of extractable N and total soil N (in addition to foliar N) have also been found in an English lowland heath survey (Jones, 2010; Jones and Power, 2012), providing field-based evidence of an accumulation of N in lowland heathlands in response to N inputs. At the Budworth lowland heath site, Caporn et al. (2002) reported leaching only at additions of 120 kg N ha⁻¹ yr⁻¹, and constituting less than 10% of the added N. At the same experimental plots but following several years more N additions, Field et al. (2013) found leaching even at the lowest 20 kg N addition and observed that dissolved N concentrations in the soil solution were much higher than in the upland moorland experiment at Ruabon, north Wales. They suggested that the much lower N concentrations in the latter were due to immobilisation in the *Calluna* litter layer and more organic surface soils in the moorland. Only after severe damage to the *Calluna vulgaris*

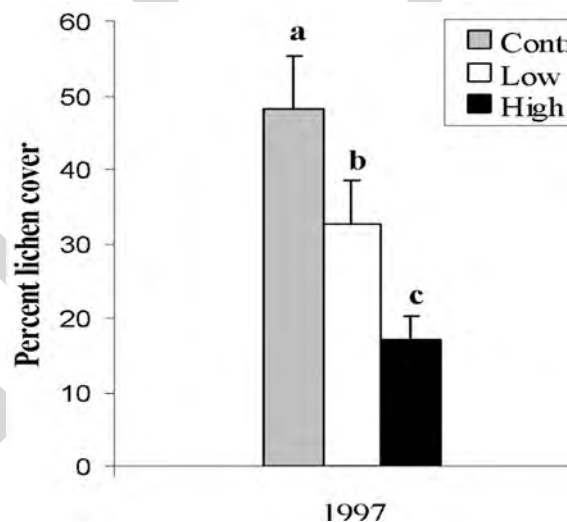
canopy, caused by heather beetles, was significant N leaching to the subsoil observed at lower rates of deposition (Van der Maas, 1990; Nielsen et al., 2000).

The accumulation of N in the soil and litter layers affects decomposition and soil N mineralisation. Power et al. (1998a) found that adding $15.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ resulted in faster rates of cotton strip degradation, a clear indication of stimulated decomposer activity. Furthermore, the estimated time for incorporation of litter into the soil humus pool, based on measurements of annual litter production and the size of the litter pool, decreased from 8.6 years in control areas to 6.3 years in plots with low additions of N ($7.7 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), and to 6.1 years in plots with high additions of N ($15.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). The increased mineralisation will lead to enhanced availability of N. This will alleviate the N limitation on plant production and may lead to a shift to more nitrophilous species.

Changes in species composition

Competition experiments in containers and in the field have clearly demonstrated an important effect of increased N availability on the competitive interactions between *Calluna vulgaris* and grasses in the early phase of secondary succession in dry lowland heath. In the Netherlands, following experimental N additions (7 and $28 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) over four years during the 1980s (background deposition of approximately $25\text{--}35 \text{ N ha}^{-1} \text{ yr}^{-1}$), grasses (*Festuca ovina*) strongly outcompeted *Calluna vulgaris*, under low initial vegetation cover (e.g. Heil and Diemont, 1983). However, under dense vegetation *Calluna vulgaris* clearly is a stronger competitor than grass species in mature heath vegetation, even at high N loads (Aerts et al., 1990; Aerts, 1993).

Figure 8.6. Lichen cover (%) following seven years of N additions applied to dry heath (S42) at plots in Thursley, Surrey (UK) with low ($7.7 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) and high ($15.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) additions of N (redrawn from Barker, 2001).



Source: Barker, 2001

Understorey species, especially the typical and frequently occurring lichen and moss species, can be negatively affected by increased growth of vascular species. On the Baltic island of Fehmarn, Bähring et al. (2017) found that the increased N accumulation in the system and falling C:N ratios were linked to an increase in graminoid cover at additions of $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ and subsequent decline in bryophytes and *Cladonia* lichens at $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. After seven years of N additions in Surrey (UK), the cover of lichens and lichen diversity (*Cladonia* species; *Parmelia*) significantly decreased, following additions of 7.7 and $15.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (background deposition $8 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) (Barker, 2001; Figure 8.6). Because of weekly applications of relatively low

concentrations of additional N, this decline was almost certainly not caused by the direct toxic effects of N, but probably by increased shading through the greater canopy density of *Calluna vulgaris*. The same was found for moss understorey in the N experiment in Cheshire (UK). The cover of *Hypnum* species, a nitrophilous moss, was also considerably lower following four or five years of N additions (60 and 120 kg N ha⁻¹ yr⁻¹) (Lee and Caporn, 2001).

Opening of the *Calluna vulgaris* canopy and heather beetle infestations

When the productivity of *Calluna vulgaris* is no longer primarily limited by N, the growth response is less or absent, and luxury consumption will lead to increased N concentrations in the plant. In an experiment on a P-limited heathland in Denmark (background deposition of 18 kg N ha⁻¹ yr⁻¹), N concentrations in shoots increased significantly following N additions of 15, 35 and 70 kg N ha⁻¹ yr⁻¹ in the form of ammonium nitrate (Johansson, 2000), while there was no significant growth response in this plant species (Riis-Nielsen, 1997). However, a relationship has been established between increased foliar N concentrations and pathogenic infestations as well as sensitivity to secondary stresses such as frost and drought.

Infestations of heather beetles (*Lochmaea suturalis*), a chrysomelid beetle, occur frequently in dry lowland heaths. These beetles forage exclusively on the green parts of *Calluna vulgaris*. Insect herbivory is generally affected by the nutritive value of the plant material, with N concentration being especially important (e.g. Crawley, 1983; Brunsting and Heil, 1985; Van der Eerden et al., 1990, 1991). Increased *Calluna* tissue N concentrations have been found to be associated with faster development of heather beetle larvae and increased adult weights (Van der Eerden et al., 1990, 1991; Power et al., 1998b). Infestations of these beetles may lead to opening of the closed *Calluna vulgaris* canopy over large areas, greatly reducing light interception (Berdowski, 1987; 1993), thus leading to enhanced growth of understorey grasses, such as *Deschampsia flexuosa* (*Avenella flexuosa*) or *Molinia caerulea*. It is thus likely that the frequency and intensity of insect infestations are stimulated by increased atmospheric N loads. This is supported by the observations by Blankwaardt (1977), who reported that, from 1915 onwards, heather beetle infestations occurred at ca 20-year intervals, in the Netherlands, whereas in the last 15 years of the observation period, this interval decreased to less than eight years. In addition, it has been observed that *Calluna vulgaris* plants are more severely damaged in N-fertilised vegetation during a heather beetle infestation, in the Netherlands (Heil and Diemont, 1983), in Denmark (Tybirk et al., 1995; Riis-Nielsen, 1997) and in the UK (Lee and Caporn, 2001).

Opening of the *Calluna vulgaris* canopy: secondary stresses

Similar to upland *Calluna*-dominated wet heath (S411), impacts of N deposition on the frost sensitivity of *Calluna vulgaris* have been suggested as the cause of observed die-back of *Calluna vulgaris* shoots in successive severe winters of the mid-1980s in dry heathlands in the Netherlands (Van der Eerden et al., 1990). Nitrogen addition experiments with ammonia (fumigation) or ammonium sulphate suggest that N addition may increase frost sensitivity in *Calluna vulgaris* during late winter (Van der Eerden et al., 1991; Uren, 1992). Van der Eerden et al. (1991) studied the frost sensitivity of *Calluna vulgaris* vegetation that was artificially sprayed with different levels of ammonium sulphate (3-91 kg N ha⁻¹ yr⁻¹). After five months the frost sensitivity of *Calluna vulgaris* had already increased significantly in vegetation treated with the highest level of ammonium sulphate (400 µmol l⁻¹; 91 kg N ha⁻¹ yr⁻¹), compared to the control vegetation. However, another study that measured the effects of low levels of N addition (7.7 and 15.4 kg N ha⁻¹ yr⁻¹) on frost sensitivity of *Calluna vulgaris* showed only limited effects after seven years of treatment (Power et al., 1998b). Hence, the significance of increased frost sensitivity at ambient N loads is very uncertain.

In addition to effects during winter, summer ‘browning’ of *Calluna vulgaris* canopies in the Netherlands was frequently seen in dry summers of the 1980s— the decade with the highest N loads. It was suggested that N enrichment increased the sensitivity of *Calluna vulgaris* to periods of drought due to reduced root growth and greater shoot:root ratio or as a result of a decrease in mycorrhizal infection. Indeed, the root weight ratio (RWR) of *Calluna* significantly decreased with increasing N additions (Aerts et al., 1991) and a small reduction in root to shoot ratio was found after seven years of N additions ($15.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) in a dry lowland heathland in the UK (Power et al., 1998a). However, experimentally imposed drought on roofed split plots in an N-enriched lowland heath in the UK showed that increased sensitivity in *Calluna* and grass encroachment may be the result of a number of interacting effects such as root:shoot ratio and heather beetle infestation (Cawley et al., 1998; Lee and Caporn, 2001; Green, 2005). In addition, ericoid mycorrhizal infection of heather roots could also be influenced by increased N load but studies on this subject report highly variable results (Caporn et al., 1995; Yesmin et al., 1996; Aerts and Bobbink, 1999; Johansson, 2000). It is obvious that the sensitivity of *Calluna vulgaris* to drought stress might be increased by a shift in root:shoot ratio, and that grasses might profit from this damage to the heather canopy, but the precise importance of this process has yet to be clarified.

Gradient studies

Edmondson et al. (2010) assessed potential bio-indicators of N deposition (e.g. litter phenol oxidase activity, bryophyte species richness, N:P ratios, litter extractable N) in dry heaths (S42); they investigated 18 managed upland heather moorland sites throughout the UK spanning an N deposition gradient from ~ 7 to $31 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (CBED model, total N deposition at $5 \times 5 \text{ km}$ resolution). They found that litter phenol oxidase activity had a significant negative association with N deposition, notable at the lower end of the deposition range, ~ 7 to $11 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Field et al. (2014) assessed species richness and plant community composition along gradients of climate and pollution in upland heaths ($n = 25$) with N deposition from 7.4 to $32.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ and lowland heaths ($n = 27$) with N deposition from 5.9 to $29.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (CBED model, total N deposition at $5 \times 5 \text{ km}$ resolution). They found reduced species richness and changed species composition (more grasses) associated with higher N deposition. Species richness declined by about 40% of maximum species richness from the lowest to the highest N deposition sites in both types of heathlands, with a steeper decline in species richness in the lower deposition range $< \sim 11 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Maskell et al. (2010) observed a significant reduction in species richness with N deposition (5 to $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) in 459 heathland sites from the 1998 UK Countryside Survey even after fitting covarying factors. Similarly, Van den Berg et al. (2016) assessed species richness of vascular plants as a measure of biodiversity in upland ($n = 267$) and lowland ($n = 182$) dry heaths from the 2007 UK Countryside Survey; total N deposition ranged from 5.1 to $54.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (CBED model, total N deposition at $5 \times 5 \text{ km}$ resolution). Their results provide clear evidence that N deposition affects species richness, after factoring out correlated explanatory variables such as climate and sulphur deposition. The strongest negative coefficient was found for dry lowland heath followed by dry upland. Henrys et al. (2011) examined the response of individual vascular plant species to N in heathlands using the Vascular Plant Database and Botanical Society of the British Isles Local Change survey data. In lowland heaths, *Viola canina* had a negative relationship with N deposition declining considerably in its probability of presence between 10 and $25 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. In upland heaths, *Arctostaphylos uva-ursi* had a negative relationship with N deposition declining in probability of presence between 5 and $15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, and *Vaccinium vitis-idaea* showed a clear decline in response to N deposition from 5 to $25 \text{ kg N ha}^{-1} \text{ yr}^{-1}$.

Stevens et al. (2012) analysed the probability of presence of individual lichen taxa ($n = 26$) in heathlands at a given level of N deposition together with driver data for climate, change in sulphur deposition, and land-use using generalised additive models. Nine species showed a significant relationship with N deposition, with the majority starting from the lowest levels of deposition ($< 5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). Southon et al. (2013) assessed plant species richness in 52 heathlands (25 upland and 27 lowland) across an N deposition gradient of 5.9 to $32.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Plant species richness declined with increasing temperature and N deposition, and the abundance of nitrophilous species increased with increasing N. The number of forb and graminoid species decreased with increasing total N deposition. The relationships were broadly similar between upland and lowland sites, with the biggest reductions in species number associated with increasing N inputs at the low end of the deposition range, with an average of 13 species lost per site between $5\text{--}10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Tipping et al. (2013) estimated the thresholds above which N deposition definitely had an effect on plant species richness in (dry and wet) heathlands ($n = 457$) from the 1998 UK Countryside Survey under an N deposition gradient of 4.9 to $40 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. The threshold N deposition was estimated to be $8.8 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (4.7 to $10.1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) with an average relative loss of species of 2.3% per $\text{kg N ha}^{-1} \text{ yr}^{-1}$.

Outside of the UK, Wilkins et al. (2016), with updates in Aherne et al. (2021), identified a vegetation community change point (threshold) for European dry heaths in Ireland using species abundance data from 161 relevés spanning a deposition gradient of ~ 3 to $24 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (observation-based total N deposition at $5 \times 5 \text{ km}$ resolution). The community change point for declining species was estimated at $5.6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ with 17 species decreasing in abundance.

8.4.4 Dry heaths (S42) (Submontane *Vaccinium* – *Calluna* heaths (S421))

The effects of fertilisation and experimental cutting were intensively studied in three heathlands in Spain where the dominant species were *Calluna vulgaris*, *Vaccinium myrtillus* and *Erica tetralix* (Marcos et al., 2003; Calvo et al., 2005, 2007; Cuesta et al., 2008). Additions of $56 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ did not significantly alter soil characteristics. At these sites, background N deposition ranged between 7.5 and $15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, according to the EMEP and CHIMERE models for Spain (García-Gómez et al., 2014). Nitrogen addition led to increased plant N concentration in *Calluna* and, to a lesser extent, in *Erica*. Nitrogen addition favoured perennial herbaceous graminoid species (e.g. *Nardus stricta*, *Festuca rubra* and *Deschampsia flexuosa*). Calvo et al. (2005) concluded that, in the short term, increased nutrients at twice the estimated current atmospheric deposition for the area, would not significantly alter the composition of the mountain heathlands. However, once stands matured, the capacity of the community to regenerate after a severe disturbance would diminish. A drastic impact such as cutting may not result in re-growth of the same shrub species, but in replacement by herbaceous species, which would also benefit from the increased nutrients. In the *Calluna* and *Erica* heathlands studied, cutting plus fertilisation led to an increase in plant diversity over time. Cutting patches of heathland was recommended as a mechanism for maintaining high vegetation diversity, when grazing is not possible (Calvo et al., 2007). Recent research at this site highlighted the sensitivity of these montane heathlands to N deposition. Calvo-Fernandez et al. (2018) reported on the ten-year impact of $56 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ and also a three-year experiment using lower N treatments of 0, 10, 20 and $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. While several biogeochemical measures were not significantly affected by the high ten-year treatment, a number of variables were significantly changed by addition of only $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ including soil available NH_4^+ and *Calluna* tissue N and P concentration. Taboada et al. (2018) reported results of plant and community changes from the same experiments at the Cantabrian mountain location. The most sensitive responses were increased shoot extension in *Calluna* and number of flowers, similar to findings from other European heathland experiments. Community species changes were only significant following

the highest treatment (56 kg N ha⁻¹ yr⁻¹) over nine years, and these were linked to reductions in bryophyte and lichen cover.

Summary Dry heaths (S42)

The impacts of increased N inputs to dry inland heaths (S42) are complex and occur at different time scales. Firstly, increased N availability stimulates biomass and litter production of *Calluna vulgaris* in most situations. This N is strongly retained in the system, gradually leading to higher N mineralisation rates in the soil. However, *Calluna* can be a strong competitor with respect to grasses, even at very high N availability if its canopy is closed. A shift from dwarf shrub towards grass dominance is clearly triggered by opening of the canopy caused by heather beetle attacks, winter injury or drought. If *Calluna* canopy cover is reduced, grasses quickly profit from the increased light availability, together with the high N availability. Within a few years, this may lead to a drastic increase in grass cover. Because of the random nature of several processes (e.g. heather beetle infestations, winter injury, drought) and the many long-term processes that interact with them, it is very difficult to model and clarify all these stochastic relationships without results from long-term (10-20 years) and large-scale experiments.

The CL_{emp}N range for dry inland heaths was previously set at 10 to 20 kg N ha⁻¹ yr⁻¹ (Bobbink and Hettelingh, 2011). This range, based primarily on a long-term field experiment in the UK, was also supported by the results from simulation modelling using low intensity management regimes. Since the last update, no experimental data have become available that would warrant an adjustment of this. However, gradient studies encompassing low N deposition areas provide evidence for impacts at the lower end of the current CL_{emp}N range. Given the strong evidence of N deposition impacts on vegetation composition at or below the current critical load, we propose to revise the CL_{emp}N range, setting it at 5 to 15 kg N ha⁻¹ yr⁻¹ for dry heaths (S42), classifying it as 'reliable'. It should be stated that most N addition studies and gradient studies have been conducted on a subcategory of dry heaths and sub-Atlantic *Calluna-Genista* heaths (S422), but it seems reasonable for this CL_{emp}N to be applied to all habitats in the S42 category. The intensity of management of *Calluna* heathlands may affect the impact of increased N deposition (Power et al., 2001). The high end of the CL_{emp}N range applies to dry *Calluna*-dominated heath with high intensity management, and the low end of the range to dry *Calluna*-dominated heath with low intensity management. The relative importance of P availability in some dry heath areas and habitat management as modifiers of dry heath response to increased N deposition needs further investigation.

8.5 Maquis, arborescent matorral and thermo-Mediterranean scrub (S5)

Maquis, arborescent matorral and thermo-Mediterranean scrub (S5; in short Mediterranean scrub) are important habitats in terms of diversity and cover in Mediterranean areas of Europe. Maquis is dominated by deep-rooting small evergreen shrubs and occasionally dense oak vegetation. This class generally consists of complex mosaics of herbaceous, shrub and even arborescent strata with a high diversity that hinders a detailed categorisation in habitat classification systems when using floristic composition.

The first N manipulation field study on these habitats was started in 2007, in southern Portugal, investigating the effects of N doses (40 and 80 kg N ha⁻¹ yr⁻¹) and forms (ammonium as a 1:1 NH₄Cl to (NH₄)₂SO₄ mixture or ammonium nitrate (NH₄NO₃)) on maquis vegetation at the Natura 2000 Arrábida/Espichel site. Nitrogen additions were applied in three equal doses throughout the year (spring, summer and between autumn and winter) on top of a low background deposition of <4 kg N ha⁻¹ yr⁻¹ (Dias et al., 2014b). This study was performed in an area that was

burnt four years prior to the start of the experiment and therefore showed the early stages of post-fire succession.

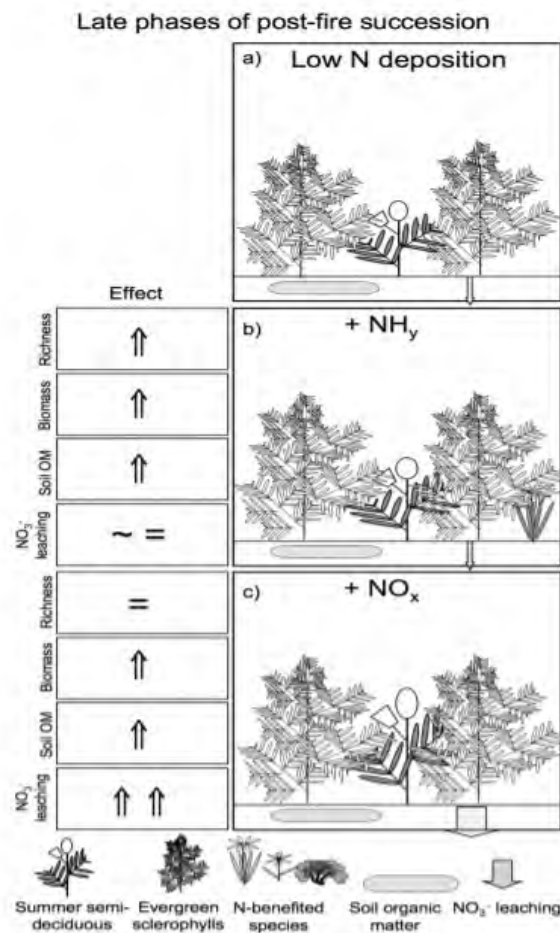
In contrast to most N addition studies (see Bobbink et al., 2010), the results suggested that one year of N enrichment had already caused shifts in the soil bacterial community structure, arbuscular mycorrhizal fungal community and plant composition (Dias et al., 2014a). Plant species richness increased within one year, with ruderal and herbaceous Maquis species particularly benefitting from the N addition.

Perennial shrubs such as evergreen *Cistus ladanifer* showed a negative effect of N addition; after seven years *C. ladanifer* had declined in cover with N applications of 40 kg N ha⁻¹yr⁻¹ (Dias et al., 2017). In addition, lower mineral weathering and lower N fixation were observed with applications of 40 kg N due to impacts on ectomycorrhizal fungi and N-fixing bacteria. The form in which N was applied to these ecosystems strongly affected the responses of the shrubs. *C. ladanifer* showed low tolerance to NH₄⁺ application, which led to a lower photosynthetic N use efficiency for this species and reduced cover (Dias et al., 2014b, 2017). As a result, N addition at 40 kg N ha⁻¹ yr⁻¹ resulted in decreased soil protection (increased patches of bare soil), which may lead to increased erosion. In these naturally N limited systems, added N was largely retained via recycling of N in the biomass (Dias et al., 2012). Based on these findings and considering the importance of ammonium as a driving force in Mediterranean ecosystems, Dias et al. (2017) suggested a critical threshold at 20-40 kg ammonium-N ha⁻¹ yr⁻¹.

In addition to the Arrábida/Espichel N-application experiment, a gradient study in central, southern and eastern semiarid Mediterranean Spanish shrublands, showed that increased N deposition above 4.4 kg N ha⁻¹ yr⁻¹ resulted in soil acidification with potential to affect the N-cycle (Ochoa-Hueso et al., 2014a). Additionally, studies of a Mediterranean habitat in the USA (Chaparral), very similar to the EUNIS category S5, provide additional information on the sensitivity of this habitat type to N deposition (Fenn et al., 2010). Chaparral showed elevated nitrate leaching to stream water with increased N deposition (above 10 kg N ha⁻¹yr⁻¹), which contrasts with the N retention described by Dias et al. (2012). However, the soils at the Portuguese experimental site were low in nutrients compared to the Californian soils, potentially muting the effects of N through P co-limitation. Moreover, the Portuguese experimental site was in an early successional state, while the longer N-deposition history in Chaparral systems in the USA may already have altered their structure and composition in the past. It has been suggested that in later phases of post-fire succession in European Mediterranean scrub, increased N deposition would be expected to cause increased nitrate leaching, particularly under elevated nitrate deposition (Figure 8.7; Dias et al., 2012).

Changes in the epiphytic lichen community towards dominance by eutrophic lichen species has also been linked to elevated N deposition (Fenn et al., 2010). Based on these responses, a CL_{emp}N range for Chaparral was set between 5.5 and 10 kg N ha⁻¹ yr⁻¹. Chaparral habitats and coastal sage scrubs (see Dias et al., 2012) were estimated to have lower critical loads than the Mediterranean scrubs (Pardo et al., 2011, 2015). This difference between American and European habitats could be attributed to several factors, including methodological reasons (e.g. lack of N deposition gradient studies and long-term addition experiments) but also differences between land use, N-deposition history, soil fertility and plant communities.

Figure 8.7. Effects of increased N deposition in the form of ammonium or nitrate (respectively b and c) on Mediterranean Maquis (source: Dias et al., 2012).



Source: Dias et al., 2012

Summary Maquis, arborescent matorral and thermo-Mediterranean scrub (S5)

The habitat Maquis, arborescent matorral and thermo-Mediterranean scrub is spatially and ecologically related to other Mediterranean habitat like Mediterranean evergreen (*Quercus*) woodland, Mediterranean xeric grasslands and Garrigue. The CL_{emp}N for Mediterranean evergreen (*Quercus*) woodland is 10-15 kg N ha⁻¹ yr⁻¹ (see Chapter 9), Mediterranean xeric grasslands 5-10 kg N ha⁻¹ yr⁻¹ (see Chapter 7) and Garrigue 5-15 kg N ha⁻¹ yr⁻¹ (this chapter). It is expected that the Maquis habitat responds in a similar way to nitrogen. In addition, the CL_{emp}N for the related Mediterranean scrubs in USA (Chaparral 3.1-14 kg N ha⁻¹ yr⁻¹; Coastal sage scrub 7.8-10 kg N ha⁻¹ yr⁻¹) correspond with these ranges. Therefore, we propose a CL_{emp}N range for Maquis, arborescent matorral and thermo-Mediterranean scrub (S5) of 5 to 15 kg N ha⁻¹ yr⁻¹, classifying it as 'expert judgement'. This value should be applied with caution in mature habitats and in habitats that are not on calcareous soils.

8.6 Garrigue (S6)

Garrigue is a Mediterranean shrub habitat that is generally considered to be a drier and more open habitat than Maquis. Garrigue consists of sclerophyllous shrubs on shallow ground with generally > 10% bare soil or biocrust.

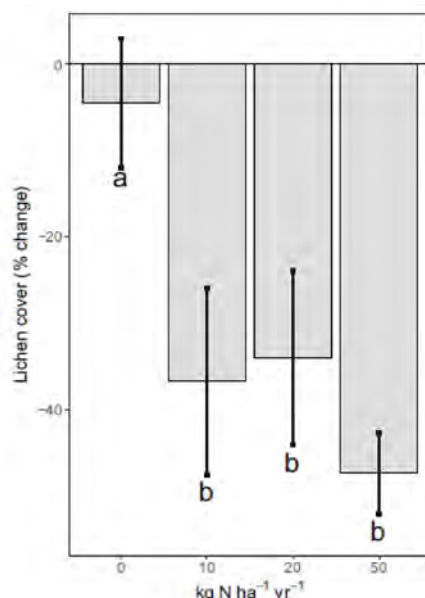
A long-term N addition study in Spain run by R. Ochoa-Hueso and colleagues has improved our knowledge and understanding of Mediterranean shrub vegetation responses to N deposition. In

the Nature Reserve “El Regajal-Mar de Ontígola”, N was applied as NH_4NO_3 at levels of 0, 10, 20, 50 over background ($\sim 6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) on a mosaic of open and dense shrubland habitat dominated by the sclerophyllous scrub Kermes oak (*Quercus coccifera*) and *Rosmarinus officinalis* with bare soil and a diverse and well-developed biological soil crust with short-lived therophytes. The denser parts of this study site could be designated as Mediterranean maquis (EUNIS classification S5) but because of its structure and location, the plant species description and the open, low canopy structure in the fertilisation experimental area, we suggest that this habitat is classified as S61: Basiphilous Garrigue. This semi-arid calcareous shrubland could be considered highly representative of other shrublands from the Mediterranean basin (Ochoa-Hueso et al., 2017), and here we consider that the $\text{CL}_{\text{emp}}\text{N}$ derived from this long-term experiment might be applied to other Mediterranean shrublands where canopy openness, dry conditions and basic soils are the dominant characteristics (i.e. Garrigue S6), regardless of their *a priori* habitat categorisation.

In the open areas of the study site, Ochoa-Hueso and Stevens (2015) showed that the characteristically dominant annual forbs responded negatively to N addition (0, 10, 20 and 50 $\text{kg N ha}^{-1} \text{ yr}^{-1}$) after 2.5 years. Annual species such as *Limonium echiodides* and small chamaephytes such as *Helianthemum violaceum* decreased in biomass and cover with increasing N application. In contrast, the nitrophilous forbs (mainly crucifers) increased with N after 2.5 years, but this response was conditioned by between-year variations in rainfall and the heterogeneous distribution of P availability, which limited growth of these species. These ecosystems proved to be highly sensitive, as responses to N were recorded at all N addition levels above background for biomass of *L. echiodides*. Additionally, soil macrofauna abundance (*Collembola* and *Paupopoda*) changed due to the eutrophying effects, acidification and increased NH_4^+ concentrations in soils (Ochoa-Hueso et al., 2014a). In addition to acidification, potassium became less available after four years as a result of increased nitrate leaching (Ochoa-Hueso et al., 2013). Although the experiment was executed on calcareous soils, these slight but significant effects were evident in the experimental plots that received 20 or 50 $\text{kg N ha}^{-1} \text{ yr}^{-1}$. It was found that N deposition resulted in changes in pigment ratios in the Mediterranean bryophyte *Pleurochaete squarrosa* and the lichen *Cladonia foliacea*, which may lead to changes in physiological priorities either for photosynthesis or protection against photooxidation (Ochoa-Hueso et al., 2014b). Responses in physiological parameters such as PME/NR ratios are widely used as bioindicators to identify effects of atmospheric N deposition (Arróniz-Crespo et al., 2008). In the Kermes oak vegetation, PME (phosphomonoesterase) enzyme activity increased and nitrate reductase (NR) activity respectively increased in both *P. squarrosa* and *C. foliacea* and decreased in *P. squarrosa* following N addition.

In a follow up study, Cabal et al. (2017) showed that N application reduced the cover and leaf lifespan (measured as increased defoliation) of shrubs (*Rosmarinus officinalis*) at additions of 10 $\text{kg N ha}^{-1} \text{ yr}^{-1}$ and higher. Shrubs in these semiarid ecosystems provide shelter, soil nutrient input (via litter accumulation) and shading, affecting soil moisture, colonisation potential of other plant species and protection against erosion. Nitrogen deposition also gradually reduced the cover of the lichen *Cladonia foliacea*, an important component of biocrusts (Ochoa-Hueso et al., 2017; Benvenuto-Vargas and Ochoa-Hueso, 2020), with significant effects observed at the lowest level of N addition (Figure 8.8). In contrast, moss cover did not change in response to N. The plots treated with 50 $\text{kg N ha}^{-1} \text{ yr}^{-1}$ showed a 50% reduction in lichen cover compared to the control plots after ten years (from 41% to 20%).

Figure 8.8. Effects of N addition on percentage of change of lichen cover (mean \pm SE) between the years 2008 and 2012. The different letters indicate the statistically significant differences ($P < 0.05$). After Ochoa-Hueso et al. (2017).



Source: Ochoa-Hueso et al., 2017

Summary Garrigue (S6)

The effects of additional N deposition to these semiarid N-limited ecosystems are complex, and clear, direct effects of N manipulations were not found on a single ecosystem response variable. However, when multiple (and interacting) ecosystem responses were evaluated in European studies, it was clear that at 10 kg N ha⁻¹ yr⁻¹, ecosystem disruption was visible after four years of application (Ochoa-Hueso, 2016). Based on these results, and the fact that they correspond well with similar habitats from the USA that show similar critical loads (Ochoa-Hueso et al., 2017), the CL_{emp}N for the Garrigue (S6) is set between 5 and 15 kg N ha⁻¹ yr⁻¹, classifying it as 'quite reliable'.

8.7 Overall summary of CL_{emp}N for heathland, scrub and tundra habitats (S)

An overview of the CL_{emp}N for heathland, scrub and tundra habitats (S) is presented in Table 8.1.

Table 8.1. CL_{emp}N and effects of exceedances on heathland, scrub and tundra habitats (S). ## reliable, # quite reliable and (#) expert judgement. Changes with respect to 2011 are indicated as values in bold.

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2011 reliability	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
Tundra	S1	3-5	#	3-5 ^a	#	Changes in biomass; physiological effects; changes in bryophyte species composition; decrease in lichen species richness
Arctic, alpine and subalpine scrub habitats	S2	5-15	#	5- 10 ^a	#	Decline in lichens; bryophytes and evergreen shrubs

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2011 reliability	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
Lowland to montane temperate and submediterranean <i>Juniperus</i> scrub	S31			5-15	(#)	Shift in vegetation community composition; reduced seed viability
Northern wet heath	S411					
► 'U' <i>Calluna</i> -dominated wet heath (upland)	S411	10-20	#	5-15 ^b	##	Decreased heather dominance; decline in lichens and mosses; increased N leaching
► 'L' <i>Erica tetralix</i> -dominated wet heath (lowland)	S411	10-20	#	5-15 ^b	##	Transition from heather to grass dominance; decrease in heather cover; shift in vegetation community composition
Dry heaths	S42	10-20	##	5-15 ^b	##	Transition from heather to grass dominance; decline in lichens; changes in plant biochemistry; increased sensitivity to abiotic stress
Maquis, arborescent matorral and thermo-Mediterranean scrub	S5	20-30	(#)	5-15	(#)	Change in plant species richness and community composition; nitrate leaching and acidification of soil.
Garrigue	S6			5-15	#	Changes in species composition; decline in shrub cover and increased invasion of annual herbs

a) use towards high end of range if phosphorus limited, and towards lower end if phosphorus is not limiting.

b) use towards high end of range with high intensity management, and use towards lower end of range with low intensity management.

8.8 Recommendations and knowledge gaps

Based on the number of studies that were published we can conclude that long-term experimental studies with low N dose additions at a low N deposition background are particularly needed for Mediterranean, alpine and arctic habitats. In addition, the inclusion of the differential effects of reduced and oxidised N is needed to further disentangle mechanisms of effect.

The arctic, alpine and subalpine scrub habitats category (S2) is particularly varied, encompassing a range of habitats variously dominated by evergreen and deciduous shrub species and with varying importance of bryophytes and lichens. The sensitivity of these habitats to N deposition may well vary and more low-dose experimental studies and well-designed gradient studies which include low deposition areas are needed to refine critical loads for these communities.

New experimental or gradient evidence is also needed for the major heath and scrub categories for which no $CL_{emp}N$ is currently defined. These include Spiny Mediterranean heaths (S7), Thermo-Atlantic xerophytic scrub (S8) and riverine and fen scrub habitats (S9).

As well as vascular plants, new studies should always include bryophytes and lichens where relevant to the habitat since these are often the most sensitive component of the vegetation and are also important to ecosystem functioning. New studies addressing biodiversity impacts of N deposition beyond vegetation, should also be a high priority. Soil biodiversity (fungi, bacteria, micro- and meso-fauna) and invertebrate biodiversity are particularly important for ecosystem functioning.

Climate change and nitrogen deposition are likely to have strong interactive effects on ecosystem functioning and climate change may alter ecosystem responses to nitrogen deposition and vice versa. More experimental studies are needed to examine these interactions and also more gradient studies which explicitly examine the impacts of nitrogen deposition in combination with climatic gradients.

At present there are only a few studies which have looked at both impact of and recovery from nitrogen deposition. More studies on this topic are needed to understand the reversibility of nitrogen deposition effects and the long-term prognosis for N impacted ecosystems.

8.9 References

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9 Effects of nitrogen deposition on forests and other wooded land (EUNIS class T, formerly G)

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Signs of strong eutrophication in a mixed forest stand in Switzerland (beech/Norway spruce). Photo: Sabine Braun.

Summary

*In this chapter, empirical N critical loads ($Cl_{emp}N$) for forests have been updated and revised based on current reviewed scientific evidence. Compared to the previous report, no new experimental results could be included, but a number of gradient studies using modelled total N or throughfall N deposition were reviewed. Recommendations for $Cl_{emp}N$ were derived using TITAN and change point analyses. A number of studies suggested that the upper level of the previous $Cl_{emp}N$ for deciduous broadleaved forests (T1) was too high. It has, therefore, now been set at $10\text{--}15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Furthermore, the lower level of the previous $Cl_{emp}N$ for coniferous forests (T3) has been reduced to $3\text{--}15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Information on N effects on lichens and on N leaching led to a lowering of the $Cl_{emp}N$ for northern forests to $3\text{--}5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (*Picea abies*, dark taiga or spruce taiga woodlands, T3F) and to $2\text{--}5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (*Pinus sylvestris*, light taiga or pine taiga woodlands T3G). The Mediterranean forests could now be included in more detail. This led to the inclusion of new forest types and the updating of the $Cl_{emp}N$ for broadleaved evergreen forests (T2) to $10\text{--}15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$.*

9.1 Introduction

Woodland, forests and other wooded lands (i.e. class T ecosystems of the EUNIS) include habitats where the vegetation is, or was until very recently, dominated by trees with canopy cover of at least 10%.

According to the EUNIS classification, woodland and forest habitats (EUNIS level 2; T1, T2 and T3) are separated from other wooded habitats (T4), such as lines of trees, small anthropogenic woodlands (< 0.5 ha), recently felled woodlands, early-stage woodlands and coppice. In EUNIS forests are characterised by the dominant tree types, which may be mixtures of species within the categories *deciduous broadleaved forest* (T1), *broadleaved evergreen forest* (T2) or *coniferous forest* (T3). The EUNIS classification emphasises the dominant tree species, soil hydrology and management practices, more so than soil chemistry. For more details, see Chytrý et al. (2020) and the EUNIS website.

As in the previous updating procedure, non-forest ecosystems (Chapters 3 to 8) have been classified and listed according to the EUNIS habitat classification for Europe. In the previous background document, the then available empirical data on forest ecosystems did not allow for a differentiation below EUNIS level 2 (Bobbink and Hettelingh, 2011). Empirical critical loads of nitrogen ($CL_{emp}N$) were set in 2011 for T1 (broadleaved deciduous forests) and T3 (coniferous forests), with the latter being divided into boreal and temperate types.

One of the main aims of this background document was to achieve a more detailed differentiation (down to level 3) of the $CL_{emp}N$ for forest ecosystems (class T) by updating the literature. However, this approach has been restricted by the following major constraints:

- ▶ Several studies cannot be classified below EUNIS level 2 because of a lack of original data or a combination of different forest types within data sets;
- ▶ Lack of data from nitrogen (N) addition studies and/or gradient studies on major habitat types, such as all riparian forests, wet forest types and broadleaved evergreen woodland in the Mediterranean region (T2);
- ▶ Often, EUNIS classes referring to the dominant tree species do not represent the natural communities because the tree species composition is of anthropogenic origin. This is particularly the case in central Europe, where the native deciduous tree cover has often been replaced by conifers.

To address these constraints, the following structure was adopted in this chapter. First, Chapter 9.2 contains some general remarks on the quantification of N deposition in forests. The overall $CL_{emp}N$ for European forest ecosystems are discussed in Chapter 9.3. It summarises the main effects of N deposition on habitats in classes T1 and T3, without specification down to level 3 categories, as was the case in the 2011 document. A separate evaluation of the impact of N deposition on the boreal forest zone (Taiga woodlands, T3F and T3G) is presented in Chapter 9.4, as sufficient experimental data were now available to distinguish effects on this important subtype of coniferous forests in Europe (T3). The Mediterranean ecosystems are dealt with in Chapter 9.5. Furthermore, an additional differentiation between EUNIS types is proposed in Chapter 9.6, based on overview tables that summarise the data from N addition studies and gradient studies across EUNIS types (if classification was possible). In this way, $CL_{emp}N$ values could be allocated for an additional six to eight level 3 categories. Finally, an overview of the $CL_{emp}N$ for class T are summarised in Chapter 9.7. As before, any studies based solely on plantation stands or short rotation forestry (e.g. EUNIS categories T1H, T29, T3M) were

excluded. This is because critical loads of N for these intensively used systems were obtained via the steady-state mass balance method (UNECE Mapping Manual, 2004).

9.2 Atmospheric deposition of N in forest ecosystems

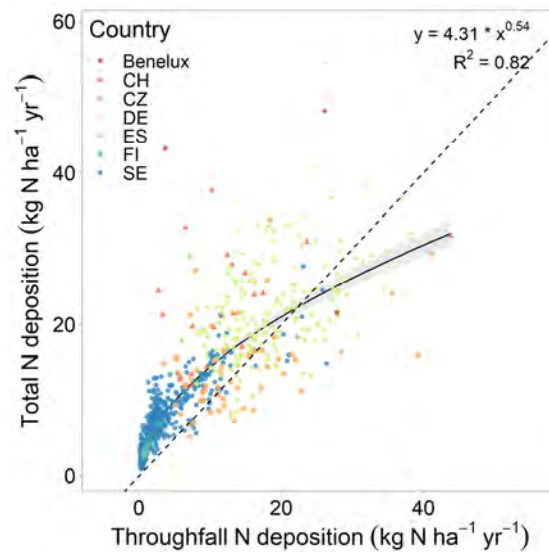
The atmospheric deposition of reactive N species in forest ecosystems occurs mainly as dry and wet deposition, with some “occult” deposition from fog and low clouds. Forests are particularly efficient at scavenging N via dry and occult deposition due to their aerodynamically rough canopies (Fowler et al., 1989). The type and form of N deposition (e.g. NH_4^+ , NO_3^- or dissolved organic nitrogen (DON)) may be relevant particularly in terms of the impact on lichen and bryophyte communities. N deposition in forests is usually measured using throughfall (TF), i.e. precipitation collected below canopy plus stemflow (for trees with a smooth bark, such as beech). The deposition collected in the open field is called bulk deposition (BD) (UNECE, 2015). The BD samplers mainly collect wet deposition, while the TF samplers also collect a fraction of dry deposition which varies depending on the atmospheric species (Fenn et al., 2015). The collection of NH_3 may be incomplete as NH_3 is also taken up by stomata (Cape et al., 2009). Particulate NO_3^- and NH_4^+ can also be taken up directly in the canopies (Karlsson et al., 2019). This leads to the underestimation of total N deposition in forests measured as TF, especially in boreal forests where the leaf area index is high, and the precipitation is low (Esseen et al., 2016).

The canopy uptake of part of the deposited N leads to an underestimation of total N deposition by TF. A fraction of the deposited N is taken up directly by forest canopies, without passing through the soil, through the process of canopy exchange (Adriaenssens et al., 2012; Draaijers et al., 1996; Harrison et al., 2000; Karlsson et al., 2019). In Mediterranean areas dry deposition typically exceeds wet deposition in evergreen broadleaf and conifer forests (Aguillaume et al., 2017; Avila et al., 2017; Garcia-Gomez et al., 2018; Sanz et al., 2002). Consequently, TF cannot be used as a direct measure of total N deposition but is used, together with the BD, to calculate the total deposition using a canopy budget model (UNECE, 2015) or in combination with measurements with surrogate surfaces (Karlsson et al., 2019). In polluted areas, TF is higher than the BD alone (outside or above the canopy). This reflects the accumulated dry deposition and the wash-off from the canopy (Vanguelova et al., 2011). However, measurements of TF and BD in Sweden and Finland clearly demonstrated that this was the case only in the southern parts with higher N deposition, while in the northern parts of both Sweden and Finland the amount of N in TF can be lower than in BD (Karlsson et al., 2019; Mustajärvi et al., 2008; Salemaa et al., 2020). Similar observations were made in high elevation spruce forests in Switzerland. Thimonier et al. (2019) published comparisons of TF measurements and total deposition obtained from the inferential measurement of single components. An updated analysis of these comparisons is given in Figure 9.1. At $2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ throughfall deposition, $6.0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ total deposition is estimated which is in line with the results discussed previously (Karlsson et al., 2019). Above $20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ canopy uptake can no longer be observed. The relation can be used to estimate total deposition from TF deposition. This equation should not, however, be applied in northern Fennoscandia where wet deposition is a better estimate of total N deposition than TF (Karlsson et al., 2019; Figure 9.2).

In Mediterranean broadleaf evergreen forests, canopy uptake and retention show characteristic seasonal variations linked to water availability for biological activity (Garcia-Gomez et al., 2016). Moreover, significant alterations of the N deposited when passing through tree canopy have been described in these forests due to microbial activity (Guerrieri et al., 2020). It is thus essential that gradient studies clearly state the fraction of N deposition used for the analysis: studies with only TF deposition and total deposition (either inferential, canopy budget models

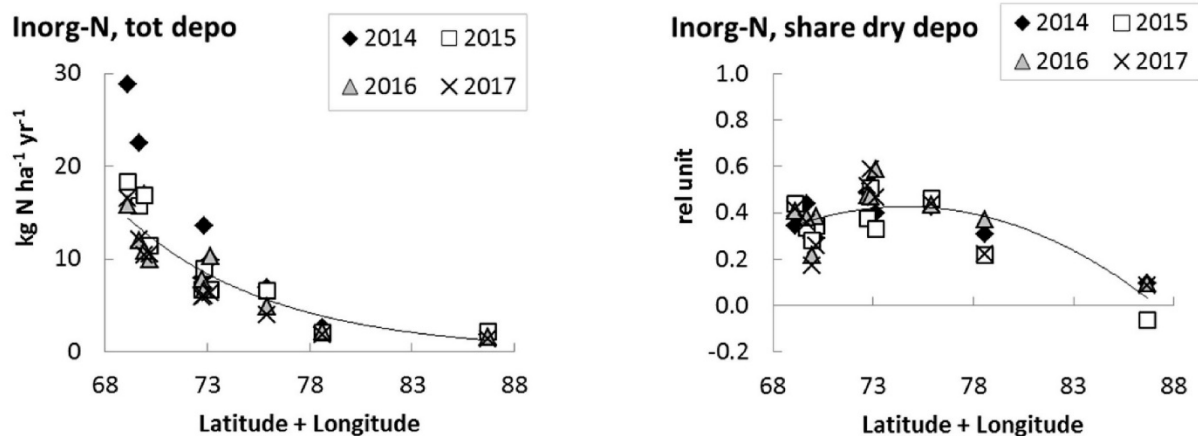
or modelled total deposition with a high spatial resolution) must be treated separately and, for the former, an estimate of canopy uptake should be added.

Figure 9.1. Relation between total N deposition (dry deposition and wet deposition) and throughfall deposition from coniferous (n = 103), deciduous (n = 33), broadleaved evergreen (n = 4) and mixed (n = 2) forests. Swiss data: coniferous stands from Thimonier et al. (2019) with one additional measurement site (Muri) from Braun et al. (2018). German data: Ahrends et al. (2020) and Schmitz (pers. Comm), Swedish data: Karlsson et al. (2019) and Pihl-Karlsson (pers. Comm), Spanish data: García-Gómez et al. (2018). Additional datasets included were from Zimmermann et al. (2006), Neirynck et al. (2007; 1 site each from Germany and Belgium), Flechard et al. (2011), Korhonen et al. (2013) as well as 36 plots from the Czech Republic (Hůnová et al., 2016). Figure from Braun et al. (2022a).



Source: Braun et al., 2022a

Figure 9.2. The importance of dry deposition decreases towards northern latitudes (Karlsson et al., 2019). The geographical index used on the x-axis, latitude + longitude, decreases from south-west towards north-east. It strongly reflects the gradient in N deposition over Sweden.



Source: Karlsson et al., 2019

The relative importance of the different pathways by which deposited N enters the forest ecosystems has been discussed (Wang et al., 2017), but no overall conclusions have been drawn. More particularly, it is unclear whether and how much N is retained in canopies, re-emitted and/or altered by chemical or biological reactions and what portion and chemical form of deposited N eventually reaches the soil as washed-out N compounds. Some of the N taken up through canopy exchange is, however, likely to end up in the epiphytes growing on tree branches, leaves, needles, trunks (Dahlman et al., 2004; Woods et al., 2012) and on bryophytes in the forest floor layer (Liu et al., 2013; Meyer et al., 2015; Salemaa et al., 2020). Studies using multiple isotope tracers provided evidence of important canopy N transformation processes which should not be ignored and merit further exploration (Guerrieri et al., 2015, 2020). It will not be possible to draw any conclusions on whether it is necessary to provide differentiated CLempN for dry and wet N deposition until further research has been conducted.

Continuous N deposition results in an accumulation in the ecosystem (Aber et al., 1998; Emmett, 2002). Based on vegetation surveys in various ecosystems (grassland, heath, coastal, peatland), Payne et al. (2019) propose 30-year cumulative deposition as an optimum metric for changes in vegetation composition. Rowe et al. (2016) further developed this by proposing a habitat-specific, integrated exceedance of the critical load, over the preceding 30 years for soil-based habitats or three years for epiphytic/epilithic sub-habitats (see also the explicit application of this metric with ICP Integrated Monitoring data in Forsius et al. (2021)). However, the critical load is based on actual deposition data. Currently there is no measure of accumulation.

Meta-analyses suggest that reduced N produces effects at lower loads than the oxidised forms (Yan et al., 2019). In trees and forbs, a greater response of plant growth to $\text{NH}_4^+\text{-N}$ (+6.3% per g N) than to $\text{NO}_3^-\text{-N}$ (+1.0% per g N) addition was detected. As noted above, any conclusions on whether it is necessary to provide critical loads for reduced and oxidised N species will require further research.

9.3 Effects of N deposition on temperate broadleaved and coniferous forests (T11, T31, T34, T35 and T37)

Forest ecosystems consist of different compartments which may be affected differently by increased N deposition. The soil may be acidified or eutrophied, both processes having consequences for microbiology, groundwater quality, soil fauna and vegetation. The species composition of the ground vegetation and of the mycorrhizal fungi may also be affected, with N-demanding or acid-tolerant species increasing and other species decreasing. Acidification and eutrophication also affect tree nutrition and growth. This may alter trees' resistance to abiotic and biotic stress factors. Additionally, forest ecosystems may release NO , N_2 and N_2O into the atmosphere, the last of which contributes to global warming and depletion of the stratospheric ozone layer. Furthermore, an ecosystem approach requires understanding of the interactions between different compartments that lead to an overall forest ecosystem response. The aim is to find threshold loads for N deposition that correspond to changes in system structure and functioning. This section describes the following indicators for the impacts of N deposition with respect to critical loads:

- **Soil processes:**
Eutrophication, acidification, mineralisation, nitrification, leaching, N trace-gas emission, litter decomposition and nutrient cycling.
- **Trees:**
Nutrition, physiology, phenology, recruitment and susceptibility to pest and pathogens.

► **Biodiversity:**

Macrofungi and mycorrhiza, lichens, free-living green algae, cyanobacteria (i.e. blue-green algae in lichens and bryophytes), ground- and field-layer vegetation and fauna.

9.3.1 Effects on soil processes

Soil plays an important role in mediating N effects on the whole forest ecosystem. The following processes are important:

- c) **Soil eutrophication.** A surplus of N, originating from deposition or enhanced nitrification due to accumulated N in the soil, will lead to eutrophication. Field-based ¹⁵N studies demonstrated that a large proportion of incoming N (11-56%) was retained in the forest floor through biotic and abiotic processes within the first two years of N enrichment (Emmett et al., 1998; Tietema et al., 1998). An accumulation of ammonium on the soil ion exchange complex may occur in areas with high deposition of reduced N (Boxman et al., 1991; Roelofs et al., 1985; Schulze et al., 1989; Van Dijk and Roelofs, 1988). Ammonium is usually not detectable in forest soil solution, as evidenced by data from ICP Forests plots (De Vries et al., 2003). The need for a multiple indicator approach to monitor and detect forest eutrophication and recovery from N saturation was demonstrated by Verstraeten et al. (2017). The ratio between DON and dissolved inorganic nitrogen (DIN) in soil solution, DON:DIN, has been used as an indicator of N saturation in forests (Park and Matzner, 2006; Williams et al., 2001, 2004). Similarly, low ratios of DON to total dissolved nitrogen (TDN) in soil solution, DON:TDN, and of dissolved organic carbon (DOC) to NO₃⁻, DOC: NO₃⁻, are also often used as indicators of soil N saturation (Currie et al., 1996; Sleutel et al., 2009). To determine the stage of N saturation, Williams (2004) proposed critical limits of the DON:TDN ratio (stage 0: > 67% DON, stage 1: 33-67% DON, stage 2: < 33% DON).
- d) **Nitrate leaching.** Nitrate that is not taken up by the plants or incorporated into microbial biomass or organic matter is leached. Nitrate leaching is an indicator of ecosystem N status. It increases with N deposition (Figure 9.6). Nitrate leaching depends on the C:N ratio in the organic matter (Augustin et al. 2005; Gundersen et al. 1998a; Dise et al., 2009), the tree species and the vegetation cover as well as disturbance of the forest stand (Braun et al., 2020b). Below a C:N ratio of approximately 25 and above an annual N deposition of 10 kg N ha⁻¹ yr⁻¹, the level of nitrate leaching drastically increases and endangers groundwater quality (Borken and Matzner, 2004; UNECE, 2005). However, nitrate leaching could be site specific and depends on the N saturation of the ecosystem. Moldan et al. (2018). Tahovská et al. (2020) reported only limited leaching from the Gårdsjön catchment after more than 20 years of 40 kg N ha⁻¹ yr⁻¹ addition. As with N deposition, N leaching is influenced by forest type, age and soil type amongst other factors and also depends on the amount and fate of N deposition. Trends of N leaching are region and scale specific.
- e) **Soil organic matter decomposition and carbon and nutrient cycling.** Generally, short-term and low-dose N inputs to N-poor forests tend to stimulate microbial activity, root autotrophic respiration and organic matter decomposition whereas long-term and high-dose N addition have obvious inhibition effects on soil decomposer activity and heterotrophic respiration in N-rich forests (DeForest et al., 2004; Janssens et al., 2010). Moreover, N addition accelerates the decomposition of soil organic matter (SOM) in high C:N soil but reduces decomposition of soil organic carbon (SOC) in low C:N soil (Lu et al., 2011). Thus, the varying effects of N inputs on soil carbon (C) dynamics observed in studies could be attributed to different responses of forests with differing in N status, suggesting an N input threshold for soil C storage increases or decreases. The C:N ratio is a good indicator of soil organic matter quality as it determines how much N can potentially

be mineralised per unit of C respired (Lehtonen et al., 2016). Root trait (e.g. adsorptive root biomass, specific roots length, root tissues density, ectomycorrhizal root biomass) dynamics are strongly related to the soil C:N ratio across a European north to south gradient (Ostonen et al., 2017). For example, fine roots biomass per tree basal area decreased with decreasing soil C:N ratio suggesting lower C input to the soil from fine roots with increasing N accumulation in soils.

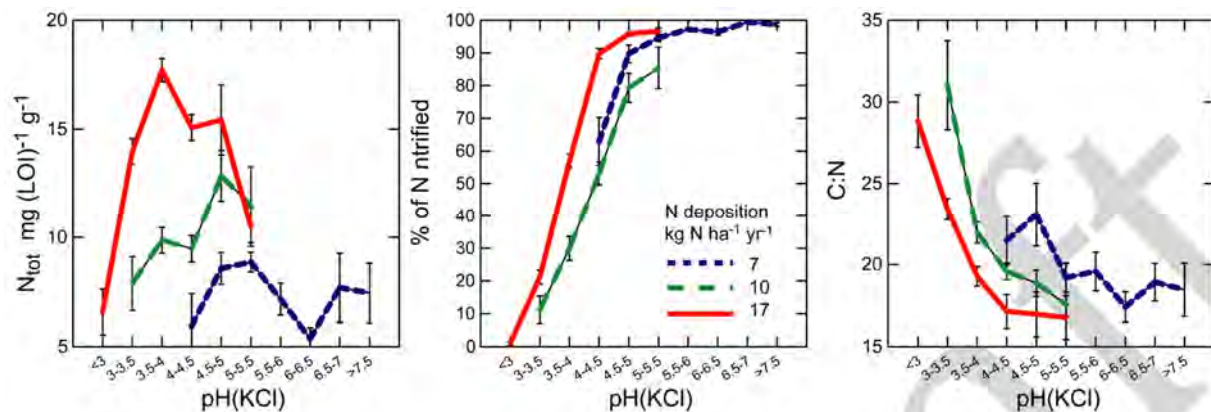
- f) **Exchange of trace gases between soils and the atmosphere.** The production of N-trace gases in forest soils is mainly due to microbiological processes such as nitrification and denitrification (Bahl-Butterbach et al., 1997; Davidson, 1991). The uptake of atmospheric CH₄ by forest soils is also catalysed by soil microorganisms (Dunfield et al., 1999; King and Schnell, 1998). Various authors have shown a positive correlation between the magnitude of NO and N₂O emissions and the amount of N deposition, as well as a negative correlation between CH₄ uptake and the amount of N deposition for different temperate forest ecosystems (Butterbach-Bahl et al., 2002; Davidson and Kinglerlee, 1997; Fenn et al., 1996; Gasche and Papen, 1999; Jenssen et al., 2002). Oulehle et al. (2021) found a strong correlation between the denitrification and precipitation to runoff ratio.
- g) **Soil acidification.** This occurs as a result of the nitrification of ammonium and leaching of nitrate. This process leads to mobilisation and leaching of base cations. Below a pH of 4.5, aluminium is increasingly dissolved. This can damage fine root development and mycorrhiza, thereby reducing nutrient and water uptake (Ritter, 1990; Sverdrup and Warfvinge, 1993). Soil acidification and critical loads for acidification are addressed in a separate UNECE document (CLRTAP, 2017).

9.3.2 N mineralisation, nitrification, NO₃⁻ leaching, N accumulation

N mineralisation and nitrification

N mineralisation and nitrification rates may both be stimulated by N deposition. In a field study on 600 deciduous forests in four geographically separate regions of southern Sweden, the N mineralisation and nitrification rates were by far the highest in the region, with the highest N deposition (17 kg N ha⁻¹ yr⁻¹), especially in the most acidic soils. Soil N mineralisation rates increased by 40 to 80%, nitrification rates increased by 20 to 90%, and the C:N ratio decreased by 10 to 25%, compared to the region with a deposition of 7 to 10 kg N ha⁻¹ yr⁻¹. (Falkengren-Grerup et al., 1998; Falkengren-Grerup and Diekmann, 2003). Differences in N mineralisation, nitrification and the relationship between C:N ratio and pH of the soil were also observed between areas with 7 and 10 kg N ha⁻¹ yr⁻¹ (Figure 9.3). The effect on mineralisation may depend on deposition history and background N deposition. Cheng et al. (2020) suggested that the responses of gross rates of N mineralisation, nitrification, and NO₃ immobilisation to experimental N addition changed from positive to negative as background N deposition increased. N deposition of > 15 kg N ha⁻¹ yr⁻¹ significantly increased the soil nitrification rate in comparison to N inputs of 8-12 kg N ha⁻¹ year⁻¹. NITREX experiments, for example, did not identify any effects on mineralisation (Emmet, 1999). Lovett et al. (2013) showed that elevated N deposition (after six years of 50 kg N ha⁻¹ yr⁻¹ addition) led to significant decline in potential N mineralisation and nitrification rates in the mineral horizon but not in the forest floor. The response varied from species to species. Heuck et al. (2018) reported that N addition did not affect N mineralisation in coniferous organic soil horizons, but N mineralisation rates significantly increased in deciduous organic soil horizons.

Figure 9.3. N mineralisation, nitrification and C:N ratio in 10 pH classes (topsoil) and three regions with varying N depositions. Means \pm SE. From Falkengren-Grerup and Diekmann (2003).



Source: Falkengren-Grerup and Diekmann, 2003

N accumulation

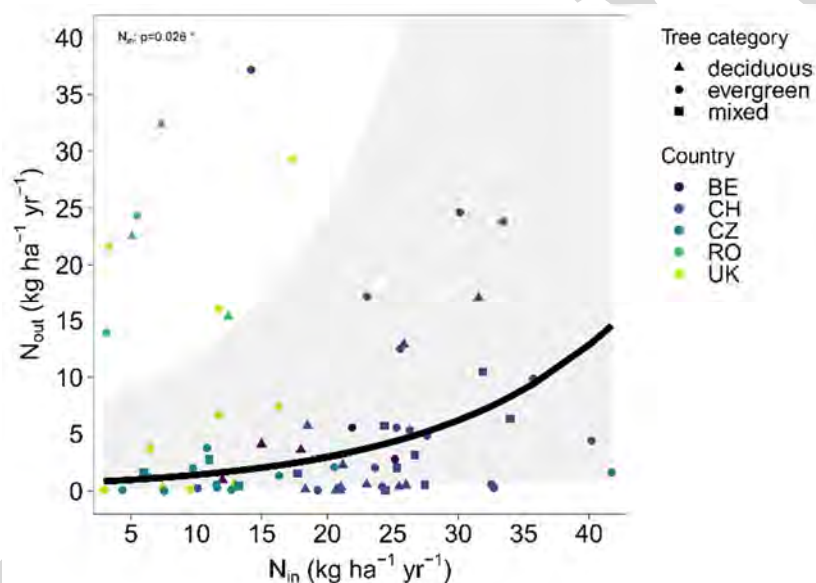
Nitrification is less important in acidic forest soils with raw humus cover and C:N ratios of more than approximately 25. In these soils, N losses are negligible and N is accumulated in all compartments of the ecosystem. The amount of soil C is very important in determining the fate of N in soils. Different soil types have a differing capacity for N retention. For example N stocks in the top 1 m of the soil could vary between 6 t N ha⁻¹ in sandy soils and more than 15 t N ha⁻¹ in organic soils (Vanguelova et al., 2018). Soil type and texture can also influence the risk of N leaching or N accumulation capacity of various N forms. For example, NH₄⁺ can be fixed by some clay types (2:1 clay minerals), be bound to negatively charged soil particles or be bound firmly and irreversibly between clay layers. It can account for up to 20 to 40% of the total mineral soil N in the subsoil and 5%-10% in the surface soil (Weil and Brady, 2017). Regarding accumulation of N, it is worth noting that a high level of retention of airborne N is widespread in European catchments (Vuorenmaa et al., 2017), even in catchments where N deposition was > 20 kg N ha⁻¹ yr⁻¹ for many decades (Dirnböck et al., 2020). The accumulation of N as NH₄⁺ in the soil may shift the ratio between NH₄⁺ and base cations (K⁺, Ca²⁺ or Mg²⁺) in the soil with severe consequences for tree nutrition (see Chapter 9.3.2; Roelofs et al., 1985b; Boxman et al., 1988; Van Dijk and Roelofs, 1988) although usually little NH₄⁺ can be detected in soil solution (De Vries et al., 2003). Forstner et al. (2019) reported a significant increase in extractable NH₄⁺ in the forest floor in N addition plots. The harmful effects of N accumulation on biodiversity are discussed in Chapter 9.3.3.

N leaching

Excess N is leached out from the soil (Figure 9.4), especially at low C:N ratios (Gundersen et al., 2006). The relationship between N input and N leaching may depend on stand structure, tree age, the proportion of dry deposition in total N load (with coniferous stands having a higher potential dry deposition), the forest floor (usually with higher C:N ratios in coniferous stands) and the actual growth rate (which may be higher in coniferous stands than in deciduous ones, if there are no other factors limiting conifer growth). Different leaching patterns between broadleaved and coniferous forests do not necessarily imply a differentiation between CL_{emp}N. In forest soils with moder-like and mull-like humus and C:N ratios of below approximately 25, the nitrification effect of tree species on N leaching is not always clear. De Vries et al. (2003) observed that the relationship between N input and output was significantly steeper in deciduous stands than in coniferous ones, whereas Borken and Matzner (2004) and Rothe and

Mellert (2004) found the opposite. After comparing directly adjacent stands of Norway spruce and European beech, Braun et al. (2020b) found significantly higher N leaching rates under spruce than under beech trees. The relations between N input and N output are confounded by disturbances and clearcutting which can increase soil mineralisation and NO_3^- leaching (Akselsson et al., 2004; Reynolds et al., 1995; Mannerkoski et al., 2005; Braun et al., 2020b).

Figure 9.4. Europe-wide forest soil data analysis of N deposition in throughfall (BE, CZ, RO, UK) or total deposition (CH) (N_{in} kg N ha⁻¹ yr⁻¹) and NO_3^- leaching (N_{out} kg N ha⁻¹ yr⁻¹) measured in the mineral soil solution (50-80 cm). Annual data from 70 forest sites averaged for the years 2015-2019 including six tree species (beech, oak, Norway spruce, Sitka spruce, Scots pine and Corsican pine) and tree species mixtures (beech/spruce and oak/beech) for UK, see Vanguelova et al. (2010) and for CH see Braun et al. (2020b). The linear model (LMEM) included besides N_{in} the C:N ratio and tree group (deciduous, evergreen or mixed) as confounding factors and the countries as random effect. The estimated explained variances are $R^2_{\text{marginal}} = 0.16$, $R^2_{\text{conditional}} = 0.65$. The line indicates the estimated effect based on the LMEM with the 95% confidence interval as the shaded area. Data analysis made for this background document by Simon Tresch.

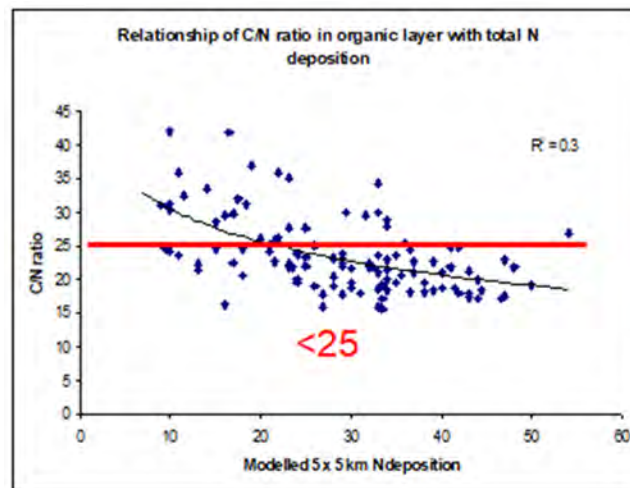


Source: Vanguelova et al., 2010; Braun et al., 2020b; Simon Tresch

Data from the UK's BioSoil network (Vanguelova et al., 2013) showed that the forest floor C:N ratio of conifer woodland is negatively related to modelled 5 x 5 km total N deposition and falls below the suggested critical C:N ratio of 25 when total N deposition is > 20 kg N ha⁻¹ yr⁻¹ (Figure 9.5). Tree species had an impact on the relationship between N deposition and forest floor C:N ratio (Villada et al., 2013). In areas with higher N loads this indicator is, however, no longer useful (Desie et al., 2020). Forest floor C:N decrease has been found at the UK's ICP forest Level I plots with C:N ratios of > 25 at 75 % of the conifer plots in 1995 (Kennedy, 2003) compared to 40% of conifer plots with C:N ratio > 25 in 2008 (RoTAP, 2012).

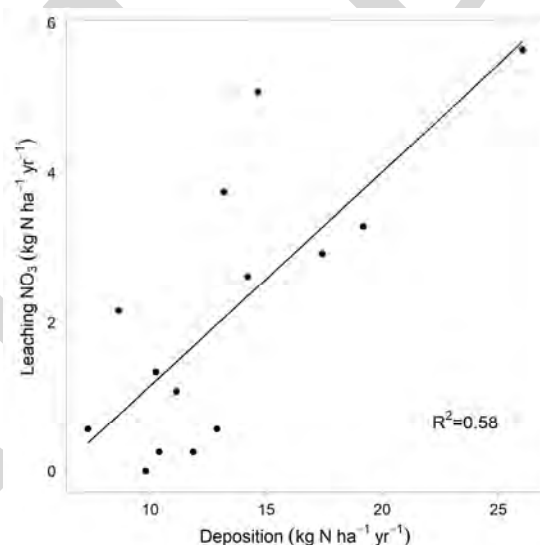
Data from the long-term monitoring GEOMON forested watersheds (Figure 9.6, Oulehle et al., 2021) also show that N leaching increases as the forest floor C:N ratio decreases and with increasing N:P ratio. This is an indication that N saturation might have shifted our forests toward phosphorus (P) limitation.

Figure 9.5. Relationship between forest soil organic layers C:N ratio and total N deposition. Data from 167 BioSoil sites across England, Scotland and Wales. Data from Vanguelova et al. (2013). The red line is the organic layer C:N threshold for NO_3 leaching as suggested by Gundersen (1998a).



Source: Gundersen, 1998a

Figure 9.6. Runoff leaching losses of NO_3 related to N deposition. Linear regression coefficients shown (R^2) at $\alpha = 0.05$ (Oulehle et al., 2021).



Source: Oulehle et al., 2021

Summary N mineralisation, nitrification, NO_3 leaching, N accumulation

In summary, the available data on soil processes suggest a $\text{CL}_{\text{emp}}\text{N}$ range of 10 to 15 $\text{kg N ha}^{-1} \text{yr}^{-1}$ for mineralisation, nitrification and nitrate leaching in both coniferous and broadleaved forests ('quite reliable').

Soils N and C trace-gas fluxes

In order to identify the effects of atmospheric N deposition on the magnitude of N and C trace-gas fluxes, a series of Scots pine forest sites with different loads of atmospheric N input was investigated in the north-east of the German Northern Lowland (Butterbach-Bahl et al., 2002;

Jenssen et al., 2002). These studies showed a strong increase in NO and N₂O emissions, both related to humus quality (transition from raw humus to moder, or C:N ratio of humus below 25). The N deposition varied from 10 to 25 kg N ha⁻¹ yr⁻¹ (throughfall deposition). Furthermore, a decrease in atmospheric CH₄ uptake by forest soils was measured with increasing N deposition.

Unmanaged forest sites were treated with simulated increased N deposition in the range of 10–30 kg N ha⁻¹ yr⁻¹ over two years. N₂O emissions increased three-fold in the coniferous sites in the first growing season in response to the low N treatment of 10 kg N ha⁻¹, although the response was barely significant ($p < 0.06$). In deciduous forests, increased levels of soil mineral N were observed during the second year of N fertilisation. However, the N₂O fluxes did not increase. Rates of methane oxidation were similar in all sites with no impact of field N application. No effects were observed in soil CO₂ efflux in response to N additions (Ambus and Robertson, 2006).

Litter decomposition, carbon sequestration, DOC leaching, CO₂ release

Litter decomposition is an important part of nutrient cycling in forests. Litter decomposition processes are affected by several drivers in time and space such as litter quality, climate, soil properties and soil biological activity. These drivers are, in turn, influenced by N deposition which means that decomposition rates can be affected as well. The effect of increased N deposition on litter decomposition seems to depend on the lignin concentration of the substrate (Carreiro et al., 2000; Frey et al., 2004; Knorr et al., 2005; Sinsabaugh et al., 2002) and on the decomposition stage (Magill and Aber, 1998). The activity of phenol oxidases, which decompose lignin, is sometimes decreased by N while cellulase activity is stimulated. Thus, the decomposition of lignin-rich litter and litter of the late decomposition stages are delayed. Moreover, in a meta-analysis of 106 long-term studies on litter decomposition, encompassing 21 litter types, the litter mass remaining after decomposition was significantly negatively related to N concentrations. The higher the N concentration in the litter (i.e. the lower the C:N ratio), the more organic matter was left when litter decomposition reached its limit value (Berg and Meentemeyer, 2002). Růžek et al. (2021) analysed the N addition effect (50 kg N ha⁻¹ yr⁻¹) on spruce needles, beech leaves, green tea and rooibos tea for 24 months in a spruce and a beech forest in the Czech Republic. They reported only spruce needle decomposition reduction by N addition. Kwon et al. (2021) analysed the response of incubated green and rooibos tea across nine biomes and observed decreased litter decomposition with increasing N deposition in temperate biomes, where atmospheric N deposition rates were high (up to 22 kg N ha⁻¹ yr⁻¹). Carreiro et al. (2000) observed significant effects of experimental NH₄NO₃ application on litter decomposition of dogwood (*Cornus florida*) and oak (*Quercus rubra*) at N applications of 20 kg N ha⁻¹ yr⁻¹, with an atmospheric deposition of 10 kg N ha⁻¹ yr⁻¹. The mean litter residence time of oak litter was increased from 3.4 years to 4.0 years (20 kg N ha⁻¹ yr⁻¹) and 4.5 years (80 kg N ha⁻¹ yr⁻¹), respectively.

Long-term N fertilisation in the range of 4–75 kg N ha⁻¹ yr⁻¹ in northern temperate zones (including roof enclosures) has been estimated to enhance C storage by 0.25 Pg C yr⁻¹ (Nadelhoffer et al., 1999). This estimate does not, however, include the effects on soil organic matter (SOM) processes such as the stability increase of soil with long-term elevated N deposition (Hyvönen et al., 2008; Swanston et al., 2004). Concomitant decreases in rates of microbial respiration (the release of CO₂) and decreases in C mineralisation in forest soil (Bowden et al., 2004; Sjöberg et al., 2003; Swanston et al., 2004) increase the potential for C sequestration. Forstner et al. (2019) concluded that the long term N addition of 22 and 35 kg N ha⁻¹ yr⁻¹, respectively, increased SOC in the organic horizon but decreased it in the mineral soil. Thus, it led to vertical redistribution of SOC pools but the overall SOC storage within the topmost 30 cm of soil was unaffected.

Up to now, no consistent DOC leaching response to N addition has been documented. Evans et al. (2008) reviewed 17 field N manipulation studies across northern Europe and north-eastern United States and found that DOC concentrations depended on the form of N used for manipulation: increases (9 experiments) were documented with NaNO_3 additions or gaseous NH_3 exposure, and decreases (8 experiments) with most NH_4 additions. Lovett et al. (2013) reported that DOC in soil solution was unaffected by the six years of $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ addition but did correlate with the C stock in the forest floor. However, although northern temperate forests might now function as significant CO_2 sinks, N deposition only accounted for < 20% of the sink. Predicting the future role of forests in the global carbon budget requires the identification of the mechanisms behind changes in C sequestration (Nadelhoffer et al., 1999).

Soil acidification

Soil acidification is only briefly reviewed in this document because critical loads for acidity, set on the basis of base cation to aluminium ratios and tree growth, are well established and addressed in separate guidelines (Nilsson et al., 1988; Sverdrup and Warfvinge, 1993, CLRTAP, 2017). However, the significance of N compounds in acidification is increasing as sulphur emissions are decreasing. In western Europe, their contribution increased from 53% (1990) to 72% (1999) (Vigdis, 2001). N addition significantly decreased soil exchangeable Ca^{2+} , Mg^{2+} and K^+ in forest ecosystems and significantly increased free Al^{3+} (Tian et al., 2015; Braun et al., 2020b).

Conclusions

The effect of N deposition on soil processes in forest ecosystems is regional- and scale-specific; it depends on the age of ecosystems or soil conditions. Some studies show only a limited effect of N deposition on soil processes, even at doses around $40\text{--}50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. However, we consider the leaching of nitrates that affects water quality, causes acidification and potentially shifts ecosystems from N limitation to limitation by other nutrients, for instance, base cations or P, to be an essential indicator for setting the critical load. Nitrate leaching increases significantly with N deposition over $10\text{--}15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ throughout European forests. The results from Falkengren-Grerup et al. (1998) and Falkengren-Grerup and Diekmann (2003a) from 600 Swedish hardwood forests on N mineralisation rates, nitrification rates and the C:N ratio also suggested a $\text{CL}_{\text{empN}} < 17 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. The results of Thorpe (2011) for nitrification rates suggested a CL_{empN} of < 15. Therefore, a range of $10\text{--}15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ is proposed as CL_{empN} for soil processes (N mineralisation and nitrification classified as 'quite reliable', and NO_3^- leaching classified as 'reliable').

9.3.3 Effects of N deposition on growth, nutrition, physiology and parasite attacks on trees

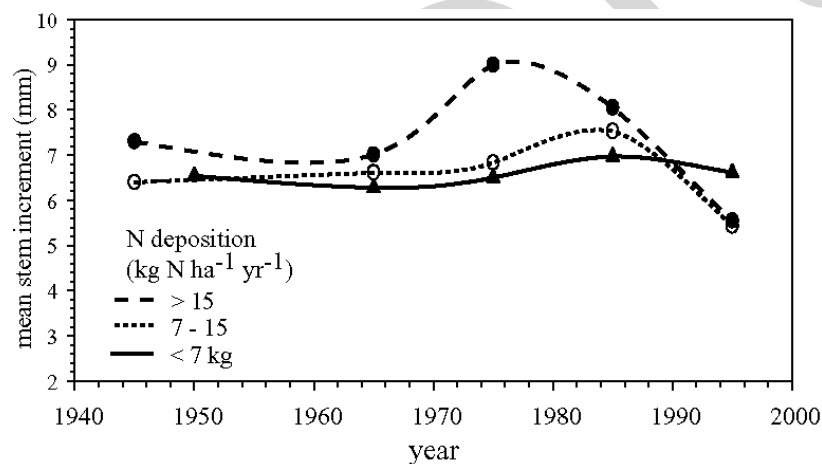
The growth of a vast majority of the forest tree species in the northern hemisphere was originally limited by N (Tamm, 1991). An increase in the supply of any essential nutrient, including N, will stimulate tree growth as long as growth is not limited by other factors. The initial impact of increased N deposition, therefore, most often has a fertilising effect. However, chronic N deposition may result in 'N saturation'. At this point, increased N inputs no longer stimulate tree growth but start to disrupt ecosystem structure and function (Aber et al., 1989; Agren, 1983; Tamm, 1991).

9.3.4 Growth of aboveground plant parts

Based on the definition of critical load (Grennfelt and Thörnelöf, 1992), all changes in growth due to anthropogenic N input have to be regarded as undesirable effects, and this includes

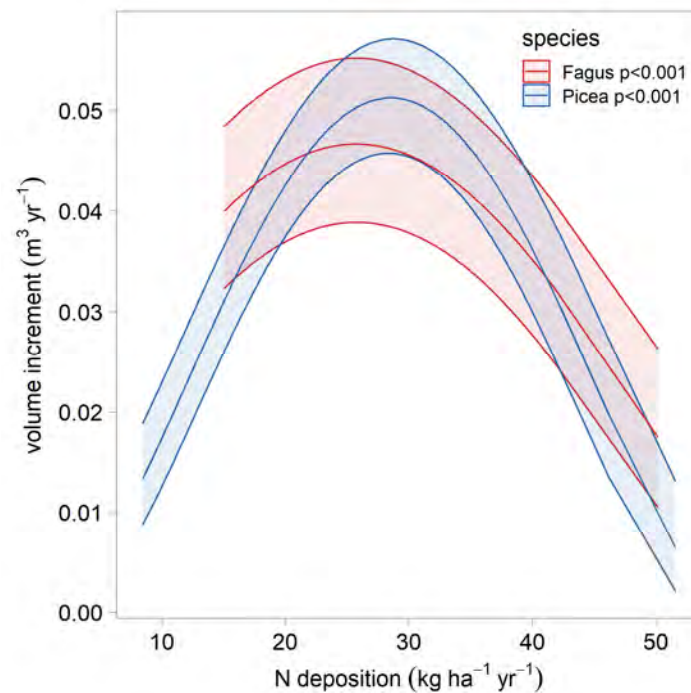
increased growth. The promoting effect of N on forest growth in temperate European regions has been demonstrated in the past 40 to 50 years where Spiecker et al. (1996) showed growth increases and EFI (2002) concluded that increased N deposition was the main cause. In the meantime, this increase has, however, changed to a growth decrease especially in regions with high N deposition (Kint et al., 2012; Nabuurs et al., 2013). Such a reverse of the response is in line with an increment core study from southern Norway where an initial stem growth increase shifted to a decrease in regions with a modelled wet N deposition of 7 to 15 kg N ha⁻¹ yr⁻¹ in the 1990s. No change was observed in plots with a modelled wet N deposition below 7 kg N ha⁻¹ yr⁻¹ (Figure 9.7; Nellemann and Thomsen, 2001). Relationships between growth and N deposition often show a growth maximum at 20-40 kg N ha⁻¹ yr⁻¹ (e.g. Braun et al. (2017): gradient with beech and Norway spruce from 10 to 42 kg N ha⁻¹ yr⁻¹ (Figure 9.8), and Etzold et al. (2020): gradient with spruce, pine, beech and oak from 2-48 kg N ha⁻¹ yr⁻¹).

Figure 9.7. Stem increment of spruce in Norway, grouped in relation to the amount of wet N deposition. Growth increase in the highest deposition class, as well as growth decrease in the two highest classes are significant at $p < 0.01$ (Nellemann and Thomsen, 2001). R. Wright (personal communication) estimated dry deposition in southern Norway to add another 10 to 20% to these N deposition rates.



Source: Nellemann and Thomsen, 2001

Figure 9.8. Volume increment of beech and Norway spruce in Switzerland in relation to total N deposition. Dataset described in Braun et al. (2022b).



Source: Braun et al., 2022b

The gradient studies with mature *Fagus sylvatica* presented by Braun et al. (2017) are similar in the type of change to the ones derived from experimental N addition to a young beech forest on calcareous and acidic soils, with modelled background deposition of 15 or 30 kg N ha⁻¹ yr⁻¹, respectively (Flückiger and Braun, 2011). But the responses in the gradient study are more sensitive, suggesting that gradient studies, with a number of observations at low N depositions and long-term exposures, may predict more sensitive reactions. Growth responses seem to be similar on acidic and calcareous soils (Flückiger and Braun, 2011).

A growth reduction due to N was also shown on the NITREX experimental plot in the Netherlands, where ambient N deposition was reduced from 56 to 4 kg N ha⁻¹ yr⁻¹. Trees in a roofed environment with low N, grew better than in the roofed control environment with high N (Boxman et al., 1998). In the Swedish Skogaby experimental plot, Norway spruce fertilised with 100 kg N ha⁻¹ yr⁻¹ (in the form of (NH₄)₂SO₄, background deposition 16 kg N ha⁻¹ yr⁻¹) grew better for the first three years than those on the control plots, but after ten years their growth fell below that of the control plot (Jönsson et al., 2004b).

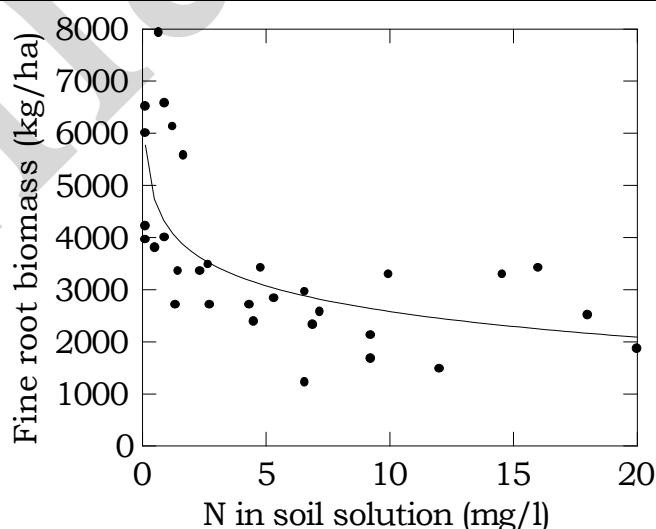
A study using a unique dataset of 80 forest FLUXNET sites (Fleischer et al., 2013) showed that forest canopy photosynthetic capacity relates positively to N deposition for evergreen needleleaf forests below an observed critical load of ~ 8 kg N ha⁻¹ yr⁻¹, with a slope of 2.0 ± 0.4 (S.E.) $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ per kg N ha⁻¹ yr⁻¹. Above this threshold canopy photosynthetic capacity levels off, exhibiting a saturating response in line with the N saturation hypothesis. Climate effects on canopy photosynthetic capacity cannot be separated from the effect of N deposition due to considerable covariation. For deciduous broadleaf forests and forests in the temperate (continental) climate zones, the analysis shows the N deposition effect to be either small or absent (Fleischer et al., 2013).

Root growth

N may also stimulate root growth (Heinsdorf and Schulzke, 1969; Persson, 1980) in ecosystems which are not N saturated. For example, fine root biomass increased significantly after 20 years of N addition ($35 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ at the Danish site Klosterhede; Forstner et al., 2019). However, some studies showed that root growth is inhibited by excessive N supply. There is strong evidence of increased N deposition causing reduced fine-root biomass and root length.

Increasing root biomass and root vitality in Scots pine, Douglas-fir and Norway spruce were reported when trees were protected from N deposition in the NITREX roofed experiments (Boxman et al., 1995; Murach and Parth, 1999; Persson and Ahlström, 2002). The treatments consisted of a reduction in N deposition from 56 to $4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in The Netherlands (Scots pine) (Gundersen et al., 1998b), from 36 to less than $5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in Germany (Douglas-fir, Norway spruce) and from 13 to less than $4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in Sweden (Norway spruce). In a gradient study, Matzner and Murach (1995) observed a relation between total fine-root biomass of Norway spruce saplings and N concentrations: it decreased significantly when NO_3^- and NH_4^+ in soil water were more than 2 mg N l^{-1} (Figure 9.9). From the relationship between N deposition and NO_3^- concentration as published by De Vries et al. (2001), this concentration may be attributed in coniferous stands to an average N throughfall load of $25 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (range 13 – 33). Magill et al. (2004) also provided evidence of declining fine-root biomass although they found only a trend of a 20 to 25% fine-root biomass reduction in the organic horizons after a 15 -year N addition experiment at 50 and $150 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, on pine and hardwood stands, respectively (Harvard Forest, Massachusetts (USA), background deposition $8 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). Fenn et al. (2008) reported a 26% reduction in fine-root biomass of *Pinus ponderosa* at a $17 \text{ kg ha}^{-1} \text{ yr}^{-1}$ N deposition from throughfall. Additionally, Braun et al. (2005) observed decreasing fine-root lengths ($< 0.25 \text{ mm } \varnothing$) of young beech in a gradient study in Switzerland, which was related to modelled N deposition, although in this study a confounding effect from soil acidification could not be excluded (range of modelled N deposition of 18 to $35 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). Altogether, increased N deposition will lead to a less developed fine-root system, possibly resulting in reduced tree stability. Forests experiencing high N depositions may become more vulnerable to storms.

Figure 9.9. Fine-root biomass of Norway spruce in relation to N in soil solution (Matzner and Murach, 1995).



Source: Matzner and Murach, 1995

Fine-root density and specific root length were found to be either reduced at increased N availability in several gradient studies and N experiments focussing on ectomycorrhizal fungal communities (Almeida et al., 2019; De Witte et al., 2017; Kj  ller et al., 2012) or unchanged (Morrison et al., 2016).

Stem increment data are more difficult to interpret as the relative change depends on current nutrition status. The dataset by Braun et al. (2017) showed a significant increase of volume increment of Norway spruce at N deposition of $< 15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Root growth data are more relevant for tree vitality, but they are less frequently collected. The root biomass results from the NITREX roof clean experiments (Gundersen et al., 1998b) suggested a CL_{empN} range of 10 to $15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. The CL_{empN} can be set for both coniferous and broadleaved temperate forests and is considered as 'quite reliable'. This is lower than the currently valid CL_{empN} for broadleaved temperate forests of $10\text{-}20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$.

Nutrition of trees

It is a well-known fact that P and N are known to be limiting nutrients in forests (Johnson and Taylor, 1989). However, increasing N deposition rates during the last decades of the 20th century could have exacerbated P deficiency in forest ecosystems that already had a low P supply (Tamm, 1991). Increased N deposition may change the nutrition for trees by increasing the N concentration in the foliage and/or decreasing the (relative) uptake of other nutrients. Usually, P concentrations and – depending on the soil – also K and/or Mg concentrations are lowered in parallel. Thus, the ratios between N on the one hand and P, K and Mg on the other, tend to increase. These changes were found in both field observations and experimental N additions. The mechanisms behind the changes may differ from element to element. In the case of Mg, soil acidification and leaching of nutrients seem to play an important role (Braun et al., 2020b; Cape et al., 1990; Elling et al., 2007). Competition from NH_4^+ in root uptake may be important in areas with very high NH_y deposition (Roelofs et al., 1985). Nonetheless, the main reason is probably the result of N impacts on mycorrhizal fungi (see Chapter 9.3.3). In ecosystems where N is still promoting growth, a decrease in nutrient contents may also be the result of a dilution effect (Thelin et al., 1998).

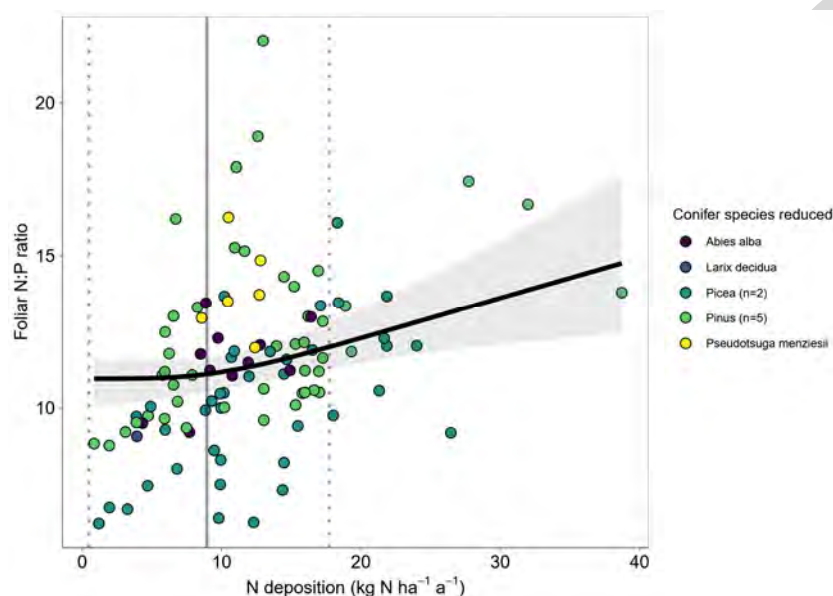
An increase in the N:P ratio seems to be a quite general response as shown in the meta-analysis by Peng et al. (2019), either by increasing the N or decreasing the P concentration. While earlier studies described rising foliar N concentrations with increasing N deposition (Duquesnay et al., 2000; De Vries et al., 2003), this relationship seems to have disappeared as N concentrations decreased between 1992 and 2009 (Jonard et al., 2015). While the studies of Duquesnay and Jonard compared time series with no direct relationship to N deposition, Braun et al. (2020a) observed a changing relationship in the foliar N concentrations of *Fagus sylvatica* in Switzerland between 1984 and 2015. Whereas the relationship with N current deposition was positive in the 1980s, it disappeared later, suggesting a saturation process. At the same time, the relationships between foliar P concentrations and N deposition became more negative. The N deposition gradient over the 1984-2015 period was between 12 and $45 \text{ kg N ha}^{-1} \text{ yr}^{-1}$.

Experimental N addition to saplings of beech and Norway spruce in young stands on both acidic and calcareous soils induced nutrient imbalances and deficiencies. They were significant at added N loads of more than 10 to $20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, after four and six years of N treatment (with modelled atmospheric depositions of 15 and $20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, respectively). On acidic soil, N treatment led to acute Mg deficiency, whereas on calcareous soil, K and P became limiting (Fl  ckiger and Braun, 1999). N concentrations remained unchanged. Foliar nutrient concentrations in mature forest trees showed significant relationships of P and K concentrations with N deposition, in a range of $10\text{-}50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Braun et al., 2020a). These relationships

observed in the field suggested a more sensitive response than in the experiment. Additions of 35 kg N ha⁻¹ yr⁻¹ to an ambient N input of 15 to 20 kg N ha⁻¹ yr⁻¹, in a spruce forest at Klosterhede (Denmark), led to increased concentrations of N in the needles, and to decreased P and Mg concentrations in the foliage, during the three years of treatment (Gundersen, 1998).

With high N inputs, concentrations of organic N in needles may increase to levels above the optimum range (De Kam et al., 1991; Van Dijk and Roelofs, 1988). N-rich free amino acids, especially arginine, were found to have increased significantly in needles with high tissue N concentrations (Hällgren and Näsholm, 1988; Näsholm and Ericsson, 1990; Pahlsson, 1992; Pietilä et al., 1991; Van Dijk and Roelofs, 1988). It has been suggested that arginine concentrations in foliage are a sensitive indicator of N input (Edfast et al., 1990; Huhn and Schulz, 1996). In Sweden, arginine concentrations in coniferous foliage of more than 5 µmol g dw⁻¹ have been linked to NO₃⁻ leaching, because the arginine accumulation in trees correlated with decreased uptake rates of NH₄⁺, leaving NH₄⁺ ions available for nitrification. This was subsequently followed by NO₃⁻ leaching (Näsholm et al., 1997). In permanent observation plots in Sweden, arginine concentrations in Norway spruce were higher in areas with high inputs of N (Ericsson et al., 1995). In Swiss plots, arginine concentrations in spruce foliage, although not in beech leaves, correlated with modelled N depositions in the range of 14 to 37 kg N ha⁻¹ yr⁻¹ (Braun et al., 2010; Quiring et al., 1997). The strongest correlation, however, was observed between arginine and P concentrations in the foliage of both tree species, suggesting P limitation. By decreasing N deposition in the NITREX roofed experiment, arginine concentrations in needles of Scots pine decreased significantly (Boxman et al., 1995; Boxman and Van Dijk, 1994). Similarly, cessation of N additions resulted in a rapid decrease in arginine in Scots pine growing in central Sweden (Edfast et al., 1996) and northern Sweden (Quist et al., 1999). In 109 ICP Forest plots, median N deposition for plots with a balanced nutrition (ratio between N and the other macronutrients) was 9.6 kg N ha⁻¹ yr⁻¹, and for the unbalanced plots it was 21 kg N ha⁻¹ yr⁻¹ (De Vries et al., 2003). N deposition was total deposition derived from throughfall and bulk deposition using a canopy exchange model (ICP Forests, 2001). The old critical load for nutrition imbalances was based on this analysis. An update has been published by Du et al. (2021). These authors showed a relation between foliar N:P and N deposition from EMEP (0.1° grid resolution). An analysis of these data using change-point regression according to Roth et al. (2017) by Tresch, Braun and Roth yielded a CL_{emp}N of 14.3 kg N ha⁻¹ yr⁻¹ (Figure 9.10).

Figure 9.10. Relationship between foliar N:P ratio of European conifer tree species (103 sites, 10 conifer species from 1995-2017) and N deposition (EMEP data spatial resolution 0.1° (~ 11.1 km)) provided by Du et al. (2021). The estimated change point from the non-linear Bayesian change-point regression is given with 95% CI as shaded grey areas ($R^2=0.42$). The dashed lines are the 95% CrI from the change-point model, with an estimated CL of N of 9.0 (SD = 4.3) $\text{kg N ha}^{-1} \text{yr}^{-1}$. Analysis made for this background report by Roth et al. (2022).



Source: Du et al., 2021; Roth et al., 2022

From the data analysis on N:P ratio by Du et al. (2021), a $\text{CL}_{\text{emp}}\text{N}$ of $15 \text{ g N ha}^{-1} \text{yr}^{-1}$ is proposed. The gradient study presented by Braun et al. (2020a) suggested a $\text{CL}_{\text{emp}}\text{N}$ for coniferous temperate forests of $10\text{-}15 \text{ kg N ha}^{-1} \text{yr}^{-1}$ and for deciduous temperate forests of $15\text{-}20 \text{ kg N ha}^{-1} \text{yr}^{-1}$. Both ranges are considered 'quite reliable'. Although calcareous and acidic soils may have different types of nutrient imbalances, there is no fundamental difference in the sensitivity of the response.

Tree physiology

Winter injury

Changes in nutrient status may influence frost hardiness by affecting carbon production, respiration and allocation, as well as via changes in membrane properties and osmotic potential (Bigras et al., 2001). Increased N concentrations in foliage may increase respiration rates and thereby reduce non-structural carbohydrate reserves, including the sugars that protect against frost during the winter. Winter injury may be caused by either low temperatures (frost sensitivity) or drought stress (winter desiccation). Most studies found a decreased sensitivity of needles to frost, after N addition (DeHayes et al., 1989; Klein et al., 1989; L'Hirondelle et al., 1992). Sensitivity to drought also seemed to increase. For example, long-term fertilisation with various loads of N (15.7 , 19.8 , 25.6 and $31.4 \text{ kg N ha}^{-1} \text{yr}^{-1}$, atmospheric bulk precipitation $5.4 \text{ kg N ha}^{-1} \text{yr}^{-1}$) between 1988 and 1996, in the eastern United States, significantly increased winter injury in montane Red Spruce (*Picea rubens*) foliage at N additions of more than $15.7 \text{ kg N ha}^{-1} \text{yr}^{-1}$, although cold tolerance was not affected and N treatment decreased dehardening (Perkins et al., 1999). Jönsson et al. (2004a) observed increased frost sensitivity of the inner bark of Norway spruce after eleven years of continuous application of ammonium sulphate (Skogaby experimental plot, $100 \text{ kg N ha}^{-1} \text{yr}^{-1}$). They attributed this observation mainly to nutrient

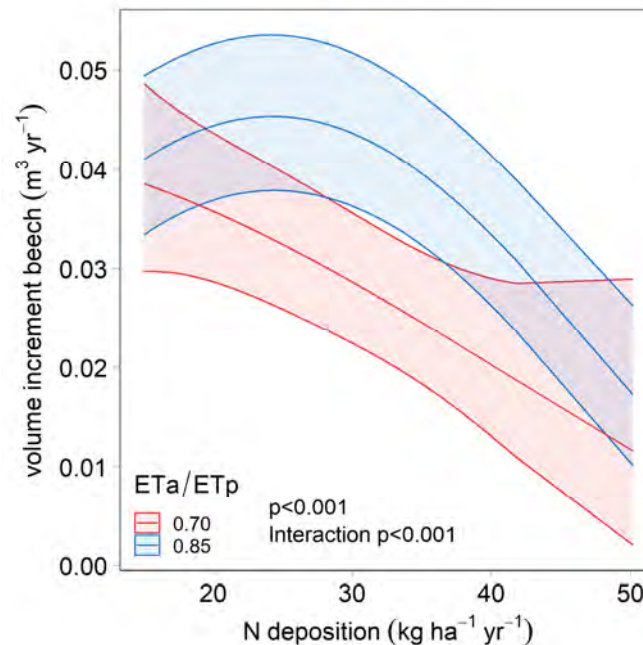
deficiency with Mg of the N treated trees in the deficient range. In another experiment, Jönsson et al. (2004a) found that spruce needles with a lower K and P status were more sensitive to frost. This may be of importance as an N surplus often lowers the supply of these elements (see section on tree nutrition). Moreover, in field fertilisation experiments it is often observed that tree growth starts earlier in the season, which may increase damage by late frosts (Jönsson et al., 2004a).

Drought tolerance

There are a number of reports indicating that N addition increases drought sensitivity. These interactions are especially important in the light of climate change. Increased water loss, a consequence of increased growth, has often been mentioned as a reason for increased drought sensitivity in response to N addition (Valliere et al., 2017). But there may be other reasons: increased competition from grass vegetation (Anders et al., 2002; Valliere et al., 2017), increased stomatal conductance (Liang et al., 2020; Nilsen, 1995), decreased root growth (see Chapter 9.3.2), decrease of the mycorrhizal symbionts (De Witte et al., 2017; van der Linde et al., 2018) and unbalanced nutrition as a consequence of N deposition (see Chapter 9.3.2). The effect of an interaction between drought and increased N on water relations at the leaf level, which is often analysed with carbon isotopes (intrinsic water use efficiency), seems to depend very much on species. Increased water use efficiency has been observed in temperate China for *Quercus variabilis* (Hu et al., 2019) while the contrary was found in California for Mediterranean-type shrubs including *Artemisia californica* (Valliere et al., 2017) or for European beech (*Fagus sylvatica*) (Braun et al., 2009). Several dendrochronological studies with temperate European tree species observed stronger drought effects when N deposition was high (Hess et al., 2018; Kint et al., 2012; Latte et al., 2016; Maes et al., 2019). In a forest monitoring study in Switzerland, N deposition enhanced the drought effect on the stem increment of *Fagus sylvatica* (Braun et al., 2017, Figure 9.11).

Spatial and temporal (over 30 years) changes in tree-ring $\delta^{13}\text{C}$ -derived intrinsic water-use efficiency (iWUE), $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ were studied for four species in twelve forests across climate and atmospheric deposition gradients in Britain (Guerrieri et al., 2020). Six of the sites underwent N deposition $> 10\text{--}12 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ and six sites deposition $< 10\text{--}12 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. There was an increase in iWUE but this was not uniform across sites and species-specific underlying physiological mechanisms reflected the interactions between climate and atmospheric drivers (oak and Scots pine), but also an age effect (Sitka spruce). Most species showed no significant trends for tree-ring $\delta^{15}\text{N}$, suggesting no changes in N availability. An increase in iWUE was mostly associated with an increase in temperature and a decrease in moisture conditions across the south-north gradient and over a period of 30 years. Overall, climate had the prevailing effect on changes in iWUE across the investigated sites, while detection of N deposition signals was partially confounded by structural changes during stand development (Guerrieri et al., 2020).

Figure 9.11. Volume increment of beech in Swiss monitoring plots in relation to N deposition. The interaction with drought is shown as regression estimates for two levels of drought (ratio between actual and potential evapotranspiration, E_t/E_{tp}). 0.85 is moist, 0.7 is dry. Dataset described in Braun et al. (2022b). Data: 7400 trees, 8 increment periods, 91 sites.



Source: Braun et al., 2022b

Herbivores and pathogens

With increasing N deposition, the susceptibility of trees to fungal pathogens and insects may change. Increased drought sensitivity in response to N, as outlined in the previous section, may play a role as many pathogens and herbivores require weakened hosts. Decreased rates of mycorrhizal infection (see Chapter 9.3.3) may increase susceptibility to root pathogens (Branzanti et al., 1999; Marx, 1969). Furthermore, altered concentrations of phenolic compounds and soluble N compounds, such as free amino acids, may also play a role (Bolsinger and Flückiger, 1989; Huber, 1980; McClure, 1980; Nordin et al., 2005; Nybakken et al., 2018). A fertilisation experiment involving additions of 10 kg N ha⁻¹ yr⁻¹ at two Swiss sites (with an atmospheric background deposition of 12 and 20 kg N ha⁻¹ yr⁻¹, respectively) showed that such additions were enough to alter the concentrations of fungistatic phenolic compounds in the fine roots of young beech and spruce after seven years of N treatment, with mostly decreasing levels (Tomova et al., 2005). In the same experiment, two fungistatic phenolic compounds in the leaves correlated negatively with the N:P ratio in leaves. The total amount of phenolic compounds in *Fagus* leaves in a 120-year stand in southern Sweden also decreased by more than 30% after four years of fertilisation with approximately 45 kg N ha⁻¹ yr⁻¹, compared with the control trees (Balsberg-Påhlsson, 1992).

Fungal pathogens

In the Netherlands, an epidemic of the pathogenic fungi *Brunchorstia pineae* and *Sphaeropsis sapinea* on *Pinus nigra* in coniferous forests was observed in the 1980s, especially in the south-eastern part of the country with high levels of atmospheric N deposition (Roelofs et al., 1985). Affected trees in the infested stand had significantly higher foliar N concentrations and higher soil ammonium levels than uninfected trees. Most of the additional N in the needles of the

affected stands was stored as free amino acids, especially arginine, but proline concentrations were also increased in the infected trees, indicating an enhanced degree of water stress (Van Dijk et al., 1992). Both high N supply and water stress increased the trees' susceptibility to attacks from *Sphaeropsis sapinea* (Blodgett et al., 1997).

In permanent beech monitoring plots in Switzerland (modelled N deposition of 15-35 kg N ha⁻¹ yr⁻¹) and N fertilisation experiments, a significant positive correlation was found between the N:K ratio in leaves and the necroses caused by the beech canker *Nectria ditissima* (Flückiger et al., 1986; Flückiger and Braun, 1998). In addition, beech bark lesions caused by the fungal pathogen *Nectria coccinea*, var. *faginata*, were found in 25 out of 48 studied sites in Scania (south Sweden). They were more frequent at sites with higher N deposition (20-25 kg N ha⁻¹ yr⁻¹) than those with lower N deposition (15-20 kg N ha⁻¹ yr⁻¹) (Westling et al., 1992). In a gradient study in two US regions, Latty et al. (2003) observed a positive correlation between the incidence of beech bark disease on *Fagus grandifolia* with bark N content and tree size. In two N fertilisation experiments, one on acidic and the other on calcareous soil, with additions of 10, 20, 40, 80 and 160 kg N ha⁻¹ yr⁻¹, damage to young beech by the pathogenic fungi *Apiognomonia errabunda* and *Phomopsis* species, was found to be significantly increased after treatments of > 10 kg N ha⁻¹ yr⁻¹ (atmospheric N depositions of 20 and 15 kg N ha⁻¹ yr⁻¹). In addition, a strong positive correlation was found between the extent of twig necroses and both N:P and N:K ratios in leaves (Flückiger and Braun, 1999). The effects in the experiments on acidic and calcareous soils were similar.

Effects of N on pathogen and insect infestations have also been observed in understory shrubs. They are addressed in Chapter 9.3.3.

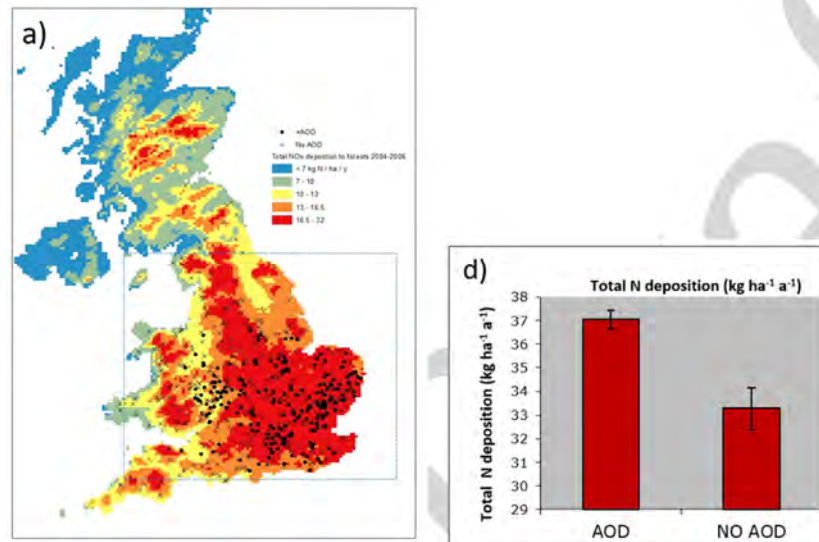
Insects

The Ellenberg N indicator value of the host plant and, by extension soil eutrophication, was positively related to the body size of butterflies and moths (Pöyry et al., 2017). Thus, soil N enrichment amplifies the diverging trends of herbivorous insects feeding on nitrophilous versus nitrophobous plants through differential plant-herbivore interactions. N deposition-induced changes in food quality, quantity and phenology may alter herbivore population dynamics. For insect herbivores, the N concentration of the host plants very much controls processes such as growth, survivorship, population levels and outbreak frequency. Changes to these processes result from both the direct effects of N on host plant quality and its influences on plant defensive chemistry. N deposition may also affect plant-herbivore interactions by altering relationships between herbivores and their natural enemies, leading to changes in herbivore survival and population dynamics (Throop and Lerdau, 2004). Many of the insects have been found to respond to N fertilisation feed by sucking. Infestation by the beech aphid *Phyllaphis fagi*, in an N fertilisation experiment, increased significantly with increasing foliar N concentrations and N:P ratios (Flückiger and Braun, 1998). On Swiss permanent observation plots, damage to beech nuts by the tortricid *Cydia amplana*, a non-sucking insect, was also found to have increased significantly with increasing foliar N:P ratios (N deposition 15-60 kg N ha⁻¹ yr⁻¹) (Flückiger and Braun, 2004).

The occurrence of insect damage to pine needles in permanent observation plots in the UK correlated positively with modelled N deposition (range 7-22 kg N ha⁻¹ yr⁻¹), but only within Scotland. For these plots, a negative relationship between needle retention and modelled N deposition was also reported (NEG-TAP, 2001). Another study revealed a significant influence of total and dry N deposition on the occurrence of Acute Oak Decline syndrome (AOD), across England and Wales (Denman et al., 2014). It suggested that low rainfall, high temperature and

high N deposition ($> 8 \text{ kg dry NO}_x \text{ ha}^{-1} \text{ yr}^{-1}$) and $> 33 \text{ kg total N ha}^{-1} \text{ yr}^{-1}$) could increase the oak's predisposition to bacterial and pest attacks (Figure 9.12; Brown et al., 2018).

Figure 9.12. Map of modelled total NO_x deposition (5 x 5 km grid; data from Centre of Ecology and Hydrology, UK) is shown with black dots representing oak forests with Acute Oak Decline syndrome (AOD, $n = 241$), grey crosses representing healthy oak forests (NO AOD, $n = 291$) (a) and annual mean modelled total N deposition in both AOD and healthy forests (d) (Brown et al., 2018).



Source: Brown et al., 2018

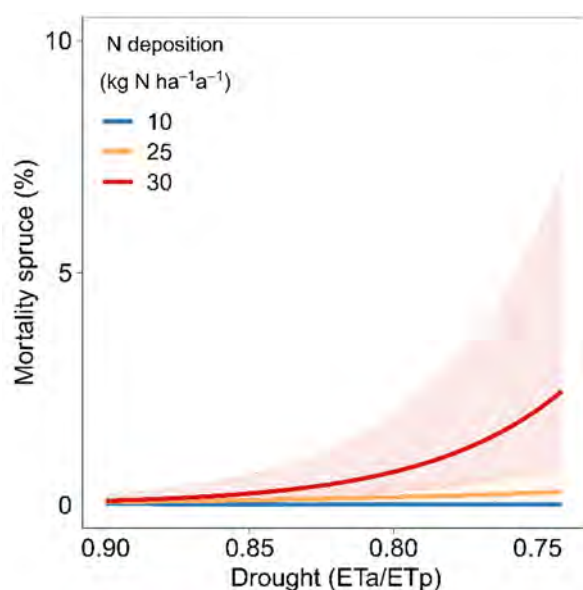
While the greater presence of the insects listed above may be explained by higher concentrations of soluble N compounds in the leaves or needles, the situation is more complicated in the case of *Haematoloma dorsatum*, a cicade. Originating from the Mediterranean region, this insect causes severe needle damage in pine stands in the Netherlands. Nymphs of this bug suck exclusively on the roots or basal stem parts of *Deschampsia flexuosa*. Only the adults cause damage to trees. *Deschampsia flexuosa* seems to be crucial for nymph development. As wintergreen grass, it is a food source in wintertime and early spring. The abundance of this grass in pine forests is thus an important ecological factor for the development of *Haematoloma dorsatum* (Moraal, 1996). Various studies have shown that the abundance of *Deschampsia flexuosa* increases significantly when N deposition is more than 10 to 15 $\text{kg N ha}^{-1} \text{ yr}^{-1}$ (see Chapter 9.3.3). Although this is a correlation study, it may explain the mechanism behind vegetation changes.

Tree mortality

Tree mortality is often the result of infestation by an insect or a pathogen. In a US-wide study with 71 tree species, Horn et al. (2018) observed a positive relationship between N deposition and mortality in 29 species, with effect peaks as low as $< 2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (average 6.9). Only in six species did mortality decrease as N deposition increased. In a gradient study in Switzerland (N deposition range $8\text{--}47 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), N deposition increased the mortality of Norway spruce (*Picea abies*) by the bark beetle *Ips typographus* (Tresch et al., 2022). In a drought situation, the effect of N deposition on mortality was much stronger (Figure 9.13). Mortality also increased when K nutrition was out of balance which may itself be the result of increased N deposition (Braun et al., 2020a). An increased bark beetle attack was also observed by Eatough Jones et al. (2004) in pine stands in California in response to elevated N deposition. The incidence of bark

beetle activity on pines at the site with low ambient N throughfall deposition ($18 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) was 20% higher in stands receiving N additions of 50 and $150 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ as slow release urea-formaldehyde. Bark beetle activity at the site with high atmospheric N input ($94 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) was generally high, with 30 to 57% of trees affected, regardless of the amount of additional N. Between 1999 and 2003, severe drought throughout the region was a major factor in decreased tree resistance. It was found that both ozone exposure and N deposition further increased pine susceptibility to beetle attacks.

Figure 9.13. Mortality of Norway spruce in Switzerland in relation to drought (quantified as ratio between actual and potential evapotranspiration averaged over the season, average lagged effect over three previous years), with the interaction effect of N deposition. Number of dead trees: 1132, 76 sites, 131,819 tree observations for 36 years, gradient of total N deposition $8.5\text{--}81 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Tresch et al., 2022). Change point regression yields critical load estimate of $10.1 \pm 3.5 \text{ (SD)} \text{ kg N ha}^{-1} \text{ yr}^{-1}$.



Source: Tresch et al., 2022

N addition of $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (background deposition of $15\text{--}20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) sufficed to increase an attack on *Fagus sylvatica* by two pathogenic fungi and change the phenolic concentration in the fine roots (background deposition of $14 \text{ kg N ha}^{-1} \text{ yr}^{-1}$).

Summary tree physiology

Given the importance of drought interactions on beech stem increments (Figure 9.11) and on Norway spruce mortality (Figure 9.13), a CL_{empN} range of 10 to $15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ is recommended, based on 'expert judgement'. However, to date, not enough data are available to permit differentiation between forest types.

9.3.5 Effects of N deposition on the biodiversity of temperate forests

Effects on soil microbiota

It is well known that N deposition and N fertilisation (Treseder, 2004; Wallenda and Kottke, 1998) can influence the growth of soil microbiota. Mycorrhizal fungi are still the most studied group of soil organisms in relation to N deposition because of the close symbiosis between many tree species and their fungal partners. All the same, research on rhizosphere bacteria, which are also essential for plant health and growth, has gained considerably in importance.

Early studies focused on fungal fruit body formation aboveground (Treseder, 2008; Van Strien et al., 2018) and still offer opportunities for long-term evaluation (Andrew et al., 2018). These fungi include species with both types of nutritional modes, saprophytes and mycorrhiza. However, belowground mycorrhizal communities are most relevant for the trees, as the external mycelium's surface area increase is essential to the trees' uptake of nutrients and water, and provides protection against root pathogens. As molecular approaches became affordable and methods to study bacterial and fungal communities in soil have improved, there have been reports of more and more belowground changes in biomass, community composition and species richness with increasing N availability (Lilleskov et al., 2018; Morrison et al., 2016; Ochoa-Hueso, 2016). Most recently, partial community recovery was also observed after reducing deposition or halting fertilisation experiments for conifer and birch forests in the Netherlands (Van Strien et al., 2018) and at a boreal conifer forest site in Sweden (Choma et al., 2017).

A very important finding throughout these studies is that responses are species-specific in both fungal and bacterial communities and that this can have an important impact on the magnitude of effects. For example, saprophytic and mycorrhizal fungi might show contrasting responses as their nutritional mode differs and mycorrhizal fungi are more involved in the nutrient cycling of trees (Gillet et al., 2010; Maaroufi et al., 2019; Morrison et al., 2016)

Effects on aboveground fungal fruit-body formation

Data from long-term N-deposition studies on the formation of fruit bodies indicate that there are prominent effects discernible aboveground. Fruit-body formation in 'generalist' species, forming a symbiosis with a wide range of tree species, seems to be less affected by increased N availability than in 'specialist' species. *Laccaria*, *Paxillus*, *Thelephora*, *Scleroderma* and *Lactarius* are examples of the less sensitive group, whereas *Tricholoma*, *Cortinarius* and *Suillus* have been found to be more sensitive (Arnolds, 1991). Fruit-body formation increased in *Paxillus involutus*, *Lactarius rufus* and *Laccaria bicolor* after N fertilisation with up to 240 kg N ha⁻¹ yr⁻¹ (Hora, 1959; Laiho, 1970; Ohenoja, 1988). The easily cultivated species that are used in culture experiments are adapted to higher N concentrations. This makes it difficult to derive a critical load from this type of experiment (Wallenda and Kottke, 1998).

There are numerous reports of decreases in species diversity and an abundance of mycorrhizal fungi in forests based on long-term monitoring and gradient studies of fruit-body formation. In the Netherlands, the average number of ectomycorrhizal species declined significantly, from 71 to 38, between 1912 and 1954 and between 1973 and 1983, while wood-colonising saprophytic and parasitic fungi increased from 38 to 50 (Arnolds, 1985, 1991). Similar observations were made by Rücker and Peer (1988) in forests in the Salzburg region (Austria). Data collected in 1937 showed 110 and in 1987 48 species of ectomycorrhizal fungi. The number of wood-colonising saprophytic and parasitic species increased from 17 to 19. Grosse-Branckmann and Grosse-Branckmann (1978) compared the occurrence of sporocarps in the Darmstadt area of Germany between 1970 and 1976 with data collected between 1918 and 1942. From the 236 species that were encountered during the first period, only 137 were still found to be there in the second period, corresponding to a loss of 99 species, including many ectomycorrhizal fungi. Termorshuizen and Schaffers (1987) found a negative correlation between the total N input in mature *Pinus sylvestris* stands in the Netherlands and the abundance of fruit bodies of ectomycorrhizal fungi (EMF). Schlechte (1986) compared two *Picea abies* sites in the Göttingen area in Germany. He found a negative relationship between N deposition and ectomycorrhizal species. At the site with N depositions of 23 kg N ha⁻¹ yr⁻¹, 85 basidiomycetes were found, including 21 ectomycorrhiza (25%), while at the site with 42 kg N ha⁻¹ yr⁻¹, 55 basidiomycetes were recorded, including 3 ectomycorrhiza (5%).

Effects on belowground fungal communities

Many studies on the belowground effects of N deposition focus on the formation of mycorrhiza on root tips (Suz et al., 2014). Others aim to investigate the production and distribution of mycorrhizal mycelium in the soil (e.g. Bahr et al., 2013). The former type of studies cannot easily draw conclusions about the actual fungal growth or biomass production because many mycorrhiza are expected to outlast adverse conditions as persistent tissue on root tips (fungal mantle). Both types of studies are usually based on measurements in the upper soil horizons (usually 10-15 cm). However, root tip colonisation and fungal mycelium growth can reach deep soil horizons (Dickie et al., 2002; Preusser et al., 2017; Rosling et al., 2004). Consequently, the effects of N deposition on fungal growth and microbial biomass production in deeper soils may be underestimated. Studies on rhizosphere fungi and bacteria became popular over the last ten years and first results indicate that these microbiotas may also be affected by N availability. Soil microbial communities have been studied in experiments and in gradient studies. The major differences observed in sensitivities between fungal species inhibited or promoted by N deposition may be caused by differences in their enzymatic capability to acquire N directly from complex soil organic compounds. Indeed, fungi that use organic N tended to be negative indicators of N deposition, and fungi that use inorganic N tended to be positive (Van der Linde et al., 2018).

Experimental N addition

N experiments also showed reduced fruit-body production of mycorrhizal fungi. Termorshuizen (1990) applied 30 and 60 kg N ha⁻¹ yr⁻¹ over a three-year period to young *Pinus sylvestris* stands, in the form of (NH₄)₂SO₄ and NH₄NO₃. In general, fruit-body production was more negatively influenced by the higher ammonium treatment than by the ammonium-nitrate mixture. In N addition experiments in both pine and hardwood stands of the Harvard Forest Long Term Ecological Research Program (US), fertilisation with 50 and 150 kg N ha⁻¹ yr⁻¹ on top of 8-15 kg N ha⁻¹ yr⁻¹, background deposition reduced species diversity and changed the species composition of mycorrhizal fungi, while ascomycetes and saprotrophs responded positively to N enrichment (Frey et al., 2004; Morrison et al., 2016). Avis et al. (2008) observed a decrease of approximately 20% in ectomycorrhizal fungal richness, with only a three-fold increase in experimental N deposition in two North American oak forests. The amount of N applied (in the form of KNO₃ and (NH₄)₂SO₄) was 21 kg N ha⁻¹ yr⁻¹, the ambient deposition approximately 7 kg N ha⁻¹ yr⁻¹.

Arbuscular mycorrhiza (AMF) play a less important role in forest ecosystems, but effects on this type of mycorrhiza have also been described. Phospholipid analysis of fine roots identified a significant decline in AMF fungi in 2 out of 4 *Acer species* stands after 12 years of additions of 12 kg N ha⁻¹ yr⁻¹ (background deposition of 4.8-8.3 kg N ha⁻¹ yr⁻¹) (Van Diepen et al., 2007, 2011).

Gradient studies

In a gradient study from less than 1 to up to 18 kg N ha⁻¹ yr⁻¹ (bulk deposition) near an industrial ammonia production facility in Alaska, which had operated for almost 30 years, sporocarps of 14 mycorrhizal fungi species were found at plots with the highest N loads, compared to 144 mycorrhizal species at the six plots with the lowest N loads (Lilleskov et al., 2001). The authors hypothesised that N-efficient species, which prevail under N-limiting conditions, were replaced by species that best functioned in nutrient-rich soils and, subsequently, by P-efficient species under high N conditions. However, no information was provided about the importance of dry (and therefore total) deposition at the investigated site. It is, therefore, difficult to use these data to derive a critical load.

Erland and Taylor (2001) used a gradient from low deposition in northern Europe (< 2 kg N ha⁻¹ yr⁻¹) to higher deposition in southern Europe (25 kg N ha⁻¹ yr⁻¹). They did not find any apparent

negative effects on ectomycorrhizal fungal diversity in beech forests. However, ectomycorrhizal root tips in spruce forests appeared to be more sensitive to high levels of N deposition. The diversity in root morphotypes decreased with increasing deposition from north to south. Interestingly, the proportion of species that could take up organic N declined as mineral N availability increased. In two similar 60-year old *Picea abies* forests in south Sweden, with different rates of N deposition (Vedby, 14-15 kg N ha⁻¹ yr⁻¹ and Skrylle, 24-29 kg N ha⁻¹ yr⁻¹), the level of mycorrhizal colonisation was almost 100%. However, the total number of mycorrhizal species was 30 to 42% higher at the site with low N deposition. The total number of mycorrhizal roots and the number of mycorrhizal morphotypes were also significantly lower at Skrylle than at Vedby.

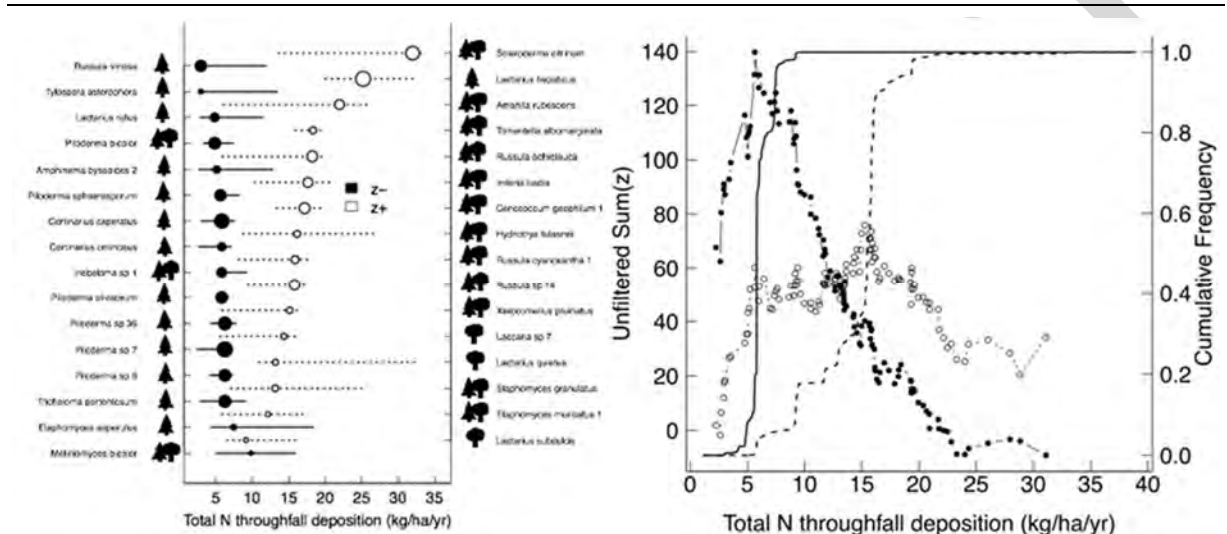
In temperate oak forests on a broad N deposition gradient in Central Europe (5 to 36 kg N ha⁻¹ yr⁻¹), variable sensitivities were detected for ectomycorrhizal fungi (EMF) species belowground but species with large mycelial networks tended to be more sensitive (Suz et al., 2014). This study also found a moderate (9.5-13.5 kg N ha⁻¹ yr⁻¹) and drastic (17 kg N ha⁻¹ yr⁻¹) effect of N throughfall deposition on EMF community composition. In a gradient study in oak-dominated deciduous forests in southern Sweden, a trend was observed towards reduced ectomycorrhizal mycelial growth at higher N depositions (20 kg N ha⁻¹ yr⁻¹), compared to depositions of 10 kg N ha⁻¹ yr⁻¹ (Nilsson et al., 2007). In a gradient study on forests dominated by European beech across Switzerland with N deposition levels between 16 and 33 kg N ha⁻¹ yr⁻¹, fungal mycelial growth and species diversity were found to be reduced along the gradient. Most indicator taxa that decreased in abundance as N deposition increased showed response thresholds between 22 and 23 kg N ha⁻¹ yr⁻¹ (De Witte et al., 2017).

In a European scale gradient study covering Mediterranean and northern ecosystems too, threshold indicator taxa analyses were carried out to identify distinct thresholds of ectomycorrhizal responses to key environmental variables (Van der Linde et al., 2018). A threshold was detected for fungi in coniferous forests negatively affected by N throughfall deposition at 5.8 kg N ha⁻¹ yr⁻¹ and in deciduous forests at 9.2 (Figure 9.14). Based on the relationship between throughfall and total deposition shown in Figure 9.1, a critical load derived from this number was estimated of 10.6 kg N ha⁻¹ yr⁻¹ for conifer forests and of 13.7 kg N ha⁻¹ yr⁻¹ for deciduous forests. Fungal species identified as indicator taxa were mainly conifer specialists which thrive in poor soils and at pre-industrial N levels (approximately < 2 kg N ha⁻¹ yr⁻¹). Potentially, however, they are unable to adapt to currently far higher N deposition levels. Positively affected fungi have a much broader response range. A less defined peak at 15.5 kg N ha⁻¹ yr⁻¹ for these fungi suggests that adaptation by positively affected fungi to increased N deposition varies greatly. Smaller-scale studies in Norway spruce forests in Sweden confirm a low N throughfall deposition threshold around 5-6 kg N ha⁻¹ yr⁻¹ (Bahr et al., 2013; Cox et al., 2010a). In the UK, Jarvis et al., (2013) concluded a CL_{emp}N of 5-10 kg N ha⁻¹ yr⁻¹ for Scots pine, using a gradient of total N deposition from 3.1-9.9 kg N ha⁻¹ yr⁻¹.

A recent review by Lilleskov et al. (2019) concluded that EMF communities respond much more strongly to N availability than AMF communities with the disappearance of a key taxa. However, estimates of critical loads for AMF in forests are still sparse. And within EMF communities, it is the conifer forest communities that are more sensitive to N deposition than broadleaf-associated EMF (Cox et al., 2010b; Van der Linde et al., 2018). Responses of saprophytic fungi to changing N availability seem to be less pronounced (e.g. Arnolds, 1991; Gillet et al., 2010; Morrison et al., 2016) but need further confirmation. Congruent species-specific changes for ectomycorrhizal fungi in response to N availability were found for *Cenococcum geophilum* (decrease; De Witte et al., 2017; Morrison et al., 2016); *Boletus*, *Tricholoma*, *Cortinarius* and *Piloderma* species (decrease : De Witte et al., 2017; Morrison et al., 2016; Suz et al., 2014; Van der Linde et al.,

2018); and *Russula ochroleuca*, *Tylospora asterophora*, *Elaphomyces* and *Laccaria* species (increase; De Witte et al., 2017; Morrison et al., 2016; Van der Linde et al., 2018).

Figure 9.14. ECM threshold indicator taxa analysis results reproduced from Van der Linde et al. (2018). Left) Individual OTU abundances in response to N throughfall deposition. Black symbols show taxa declining with increasing N deposition (z-), open symbols depict increasing taxa (z+). Symbol size is proportional to magnitude of response (z-score). Horizontal lines represent the 5th and 95th quantiles of values resulting in the largest change in taxon z-scores among 1,000 bootstrap replicates. Tree shapes indicate host generalist, conifer-specific or broadleaf-specific. Right) Community-level output of accumulated z-scores per plot is shown in response to N deposition.



Source: Van der Linde et al., 2018

After correction from throughfall to total N deposition, the European-wide results from Van der Linde et al. (2018) suggested a $CL_{emp}N$ of $11 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for coniferous and of $14 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for deciduous forests, while the study of Jarvis et al. (2013) from UK proposed a $CL_{emp}N$ of $5\text{--}10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for Scots pine.

Critical loads for soil microbiota

Mainly based on the gradient study by Van der Linde et al. (2018) in temperate ecosystems, it is concluded that conifer ectomycorrhizal fungal communities are more sensitive than broadleaf ectomycorrhizal fungal communities. Total deposition was estimated from throughfall deposition using the relation presented in Figure 9.1. For conifer forests, a $CL_{emp}N$ of $10\text{--}13 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ and for broadleaf forests a $CL_{emp}N$ of $10\text{--}15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ are proposed. In semiarid Mediterranean ecosystems, current $CL_{emp}N$ are estimated at $5\text{--}10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$.

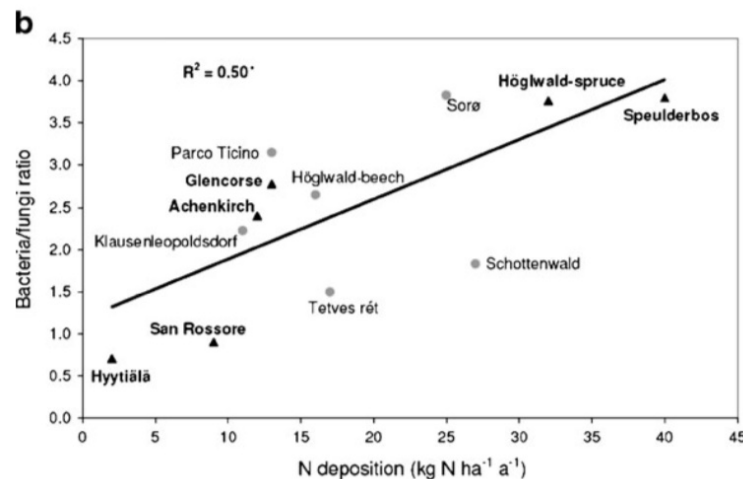
Effects on soil microbial communities

According to a review of N effects on soil bacteria by Lladó et al. (2017) and references therein, biomass and activity of soil microbial communities in many different soil environments, including temperate and boreal forests, changed as N deposition increased. Changes in abundance, however, seemed to depend on the level of background N deposition and N addition.

Gradient studies

In a N gradient study across Europe and different forest types from northern boreal, temperate and Mediterranean ecosystems, Zechmeister-Boltenstern et al. (2011) observed, in addition to a decrease in fungal species richness and changes in ratios of AM to EM fungi, an increase in the bacteria to fungi ratio especially in Central European forest stands with N deposition between 30 and 40 kg N ha⁻¹ yr⁻¹ (Figure 9.15).

Figure 9.15. Ratio of bacteria to fungi on a wide N gradient across Europe, reproduced from Zechmeister-Boltenstern et al. (2011).



Source: Zechmeister-Boltenstern et al., 2011

Experimental N addition

Frey et al. (2014), for example, observed a shift to greater dominance by bacteria in the N amended plots and a parallel reduction of fungal abundance after 25 years of N addition (50 kg N ha⁻¹ yr⁻¹) in mixed temperate forest stands in northern America with 8 kg N ha⁻¹ yr⁻¹ background deposition.

After 20 years of N addition (40 kg N ha⁻¹ yr⁻¹) in boreal conifer forests in central Sweden, soil microbial biomass increased, while the fungi to bacteria ratio did not change (Tahovská et al., 2020). N in open field precipitation at this site was 9 kg N ha⁻¹ yr⁻¹. Similarly, Choma et al. (2020) reported that the addition of 50 kg N ha⁻¹ yr⁻¹ had no significant effect on fungal and bacterial responses after four years. Background deposition at the experimental site was 3.2 N ha⁻¹ yr⁻¹. After 20 years of N addition (30 kg N ha⁻¹ yr⁻¹) in maple forests in Michigan, comparisons of soil bacterial and fungal communities did not reveal any changes in abundance or species richness in these communities. However, the enzyme activity profiles did indicate negative responses to N addition (Hesse et al., 2015). The background throughfall deposition at the two experimental sites was 7.1 and 6.4 kg N ha⁻¹ yr⁻¹, respectively. These studies in temperate and boreal forests suggested that, while fungal communities respond to N addition far below 30 kg N ha⁻¹ yr⁻¹, bacterial communities seemed to respond only to higher-level N inputs. However, in a 16-year N addition experiment (12.5 and 50 kg N ha⁻¹ yr⁻¹) in boreal conifer forests in northern Sweden (background deposition 2 kg N ha⁻¹ yr⁻¹), Maaroufi et al. (2015) found that total fungal and microbial biomass decreased already at the lower-level N input. This was in line with a study on an N deposition gradient in temperate forests in England (Thorpe, 2011). In this case, N deposition of > 15 kg N ha⁻¹ yr⁻¹ (versus 8-12 kg N ha⁻¹ yr⁻¹) increased the soil nitrification rate while the diversity of soil microbiota was reduced. In a broadleaf forest in Belgium (background deposition of 25.3 kg N ha⁻¹ yr⁻¹), that combined increased light, N (10 kg N ha⁻¹ yr⁻¹) and

warming treatments (Ma et al., 2018), N addition and warming did not significantly affect the soil microbial biomass and plant community composition. Warming did, however, significantly alter the composition of the soil bacterial community. Yet, the number of unique operational taxonomic units of plants was higher in plots with N addition, and there were significant interactive effects of light and N addition.

Critical loads for soil microbial communities

Soil bacteria show a potential response to N deposition, but it depends heavily on other factors. Based on the results presented, it is not possible to propose a critical load for this receptor.

Effect on ground-living and epiphytic lichens and algae

Since the end of the 18th century, epiphytic or tree-bark-inhabiting lichens have been used in air pollution mapping studies. In the Netherlands, the forest vegetation of one Scots pine stand in the central part of the country, with a deposition of around 20 kg N ha⁻¹ yr⁻¹, was investigated in 1958, and then re-investigated in 1981 when the deposition was around 40 kg N ha⁻¹ yr⁻¹. In the intervening period, all lichens had disappeared (Dirkse and Van Dobben, 1989). This could also have been a sulphur (S) or acidity effect, but results from fertilisation experiments in northern Sweden with low background depositions of both N and S showed that all ground-living *Cladina*-species had disappeared after 28 years of N additions (34 kg N ha⁻¹ yr⁻¹). They were, however present on the unfertilised control plots (background deposition 2 kg N ha⁻¹ yr⁻¹) (Strengbom et al., 2001). This is an indication that N deposition may be partly responsible for observed reductions in the abundance of ground-living lichens, although other factors such as changes in forestry practices must also be considered (Sandström et al., 2016). A reduction of air pollution does not guarantee the recovery of lichen communities. Many epiphytic lichen species have a limited ability to colonise new sites (Kiebacher et al., 2017). In previously polluted areas, the presence of sensitive species can depend as much on their dispersal abilities as on current air quality (Hawksworth and McManus, 1989). While much of the research into recovery has focused on acid-sensitive species, the same problem of recolonisation applies where N sensitive species have become locally extinct.

Around 10 percent of all lichen species in the world have blue-green algae (cyanobacteria) as the photobiont (Insarova et al., 1992). These blue-green algae lichens are negatively affected by both acidity and N deposition. In an international survey that stretched from the Netherlands via Denmark to Sweden, the decline in lichens with blue-green algae was found to correlate significantly with N deposition rates of over 5 to 10 kg N ha⁻¹ yr⁻¹ (Göransson, 1990). Instead of being caused by N deposition, the negative effects on ground-living lichens may have been an indirect effect of competition with N-favouring vascular plants (Cornelisen et al., 2001). Free-living green algae, especially of the genus *Pleurococcus* (syn. *Protococcus*, *Desmococcus*), are strongly stimulated by enhanced N deposition. They cover outdoor surfaces which are not subject to frequent desiccation in regions with high N deposition, above approximately 10 kg N ha⁻¹ yr⁻¹ (Bobbink et al., 1996).

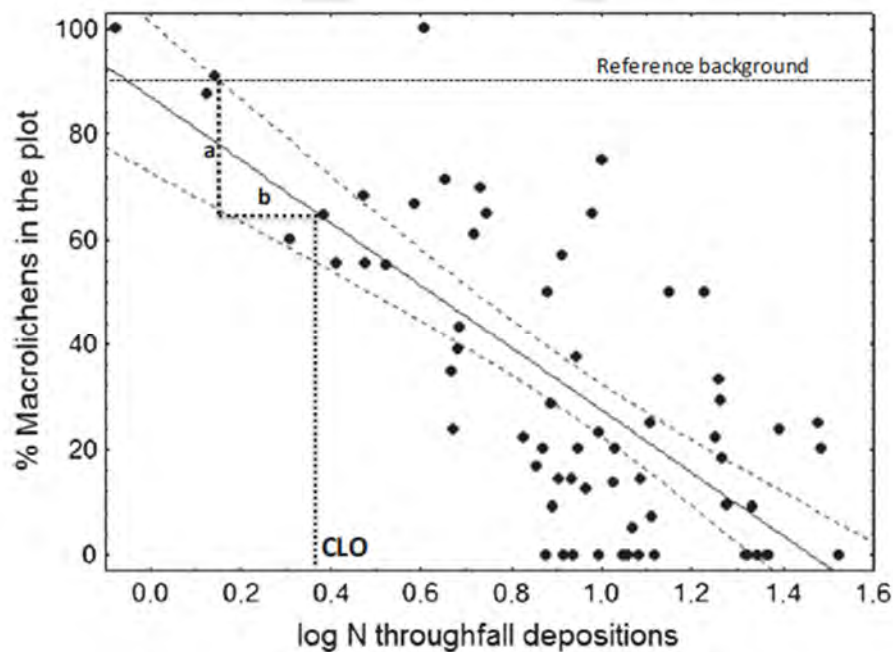
Epiphytic lichens, such as *Lobaria pulmonaria* and *Lobaria amplissima*, have been shown to be effective indicators of N pollution, for instance in Atlantic oak woods in Scotland and north-western England. In a comparison of sites with low (9.6-17.6 kg N ha⁻¹ yr⁻¹) and high N depositions (11.2-53 kg N ha⁻¹ yr⁻¹), these epiphytes were only found at sites with low N (Mitchell et al., 2003). Earlier data on epiphytic lichens also suggested a CL_{emp}N range of 5 to 10 kg N ha⁻¹ yr⁻¹, which was considered 'reliable'. Although most of these data referred to boreal forests, results from Bobbink et al. (1996) confirmed the range.

In a recent study, Geiser et al. (2019) assessed ecological risks from atmospheric N and S deposition in TF to the community composition of epiphytic macrolichens in US forests. They

used 20%, 50%, and 80% declines in selected biodiversity responses as cut-offs for low, moderate and high ecological risk from total deposition of N or S. The low ecological risk (20% decline) critical loads for total species richness, sensitive species (oligotroph) richness, forage lichen abundance and cyanolichen abundance were 3.5, 3.1, 1.9 and 1.3 kg N ha⁻¹ yr⁻¹, respectively. High ecological risk (80% decline), excluding total species richness, occurred at 14.8, 10.4 and 6.6 kg N ha⁻¹ yr⁻¹. The 'low-risk' critical loads proposed by Geiser et al. (2019) are supported by the fact that N deposition ranges from 1-4 kg N ha⁻¹ yr⁻¹ in many ecoregions in the USA (Pardo et al., 2011).

The critical load of 2.4 kg N ha⁻¹ yr⁻¹ (based on N deposition in throughfall) by Giordani et al. (2014) was linked to a significant decrease in the percentage of oligotrophic macrolichens, (Figure 9.16). An N dose of 4 kg N ha⁻¹ yr⁻¹ in TF was related to an approximate decrease > 90-50% in microlichens. The results were consistent when considering all forest types and for coniferous forests only. According to Figure 9.1, total N deposition for these two throughfall deposition estimates is 8.0 and 9.4 kg N ha⁻¹ yr⁻¹, respectively. The analysed dataset of 286 epiphytic lichen species was collected from 83 plots of the ForestBIOTA project (Forest Biodiversity Test Phase Assessments) covering the whole of Europe from the Mediterranean to Finnish Lapland.

Figure 9.16. Critical loads of N deposition on European ICP Forest plots considering the % macrolichens. Plots with the lowest depositions for which the response of % macrolichens was significantly different in the model was set as the background reference (horizontal dotted line). Their lower 95% confidence limit is calculated (line a) and the point corresponding to where this is met on the regression line is identified (line b). The x coordinate (logN deposition) of this latter represents the critical load (Giordani et al., 2014).



Source: Giordani et al., 2014

Critical load for lichens and algae

Given the effect of canopy retention of inorganic N in TF, the results of Giordani et al. (2014) from Europe and those of Geiser et al. (2019) from the USA support a CL_{emp}N range of 3-5 kg N ha⁻¹ yr⁻¹.

Effect on forest field-layer vegetation

Introduction

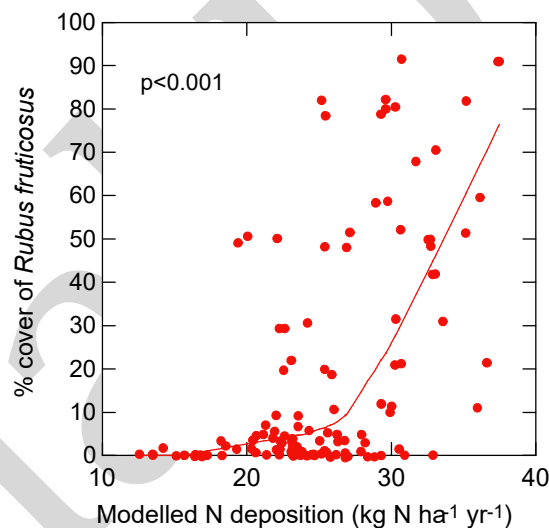
Since the last report in 2011, more case studies have examined the impacts of N deposition on forest understorey species (or the forest field-layer vegetation; defined here as: herbaceous and woody species, typically less than 1 to 2 m in height, including tree and shrub seedlings). They looked at time series, gradient studies and, less frequently, fertilisation experiments. Unfortunately, the experiments tended to be relatively short-term and often used higher levels of N addition than are found in the field, with designs hampering extrapolation of results to a regional (or global) level (Hettelingh et al., 2015). Case studies have typically shown increases in the abundance of nitrophilous species. Many of the local to regional studies were recently supplemented by synthetic analyses that consolidated datasets across temperate deciduous European forests and allowed spatio-temporal analyses across scales (e.g. Bernhardt-Römermann et al., 2015). Alternatively, they used innovative experimental platforms (e.g. Blondeel et al., 2020). Synthetic analyses also used ICP Forests and ICP Integrated Monitoring data, as well as independent initiatives, for instance, forestREplot (www.forestreplot.ugent.be) or LTER Europe (www.lter-europe.net/lter-europe). They covered a broader set of temperate forest types. Using TITAN analysis in a gradient study with total N deposition in a 5x5 km grid, Wilkins et al. (2016) found community change points in Atlantic oak woodland of 15.3 kg N ha⁻¹ yr⁻¹, and Wilkins and Aherne (2016) in sessile oak woodland of 8.8 kg N ha⁻¹ yr⁻¹. We used these findings to suggest that the CL_{emp}N for temperate forest field-layer vegetation in Europe remained in the range 10-15 kg N ha⁻¹ yr⁻¹. We emphasise that synthetic analyses have brought new insight into the context dependency of critical loads. It has always been important to stress this context dependency in critical load assessments. It means that forest understorey vegetation can be more or less sensitive to instantaneous rates of N deposition, depending on the underlying abiotic environmental conditions, the overstorey composition, current and prior management actions and previous atmospheric deposition (and therefore the cumulative amounts) (Perring et al., 2018b). Deposition history is also important: presurvey levels of N deposition determined subsequent diversity changes of vegetation. Environmental conditions such as light availability and density of large herbivores were also important. These different elements of context-dependency at specific locations are the reason for the range of the suggested CL_{emp}N for ground vegetation in temperate European forests. There also needs to be enhanced awareness of how field-layer vegetation changes in response to N relate to alterations in other aspects of the forest ecosystem, also mediated by N deposition. This includes competition, herbivory, mycorrhizal infection, disease and species invasions (Gilliam, 2006).

From individual case studies to synthetic analyses

Previously, a large number of observations showed an increase in the abundance of nitrophilous species in forests, with N deposition typically ranging (historically) from 15 to more than 40 kg N ha⁻¹ yr⁻¹. These species include *Galeopsis tetrahit*, *Rubus idaeus*, *Rubus fruticosus*, *Deschampsia flexuosa*, *Calamagrostis epigejos*, *Prunus serotina*, *Poa trivialis*, *Milium effusum*, *Molinia caerulea*, *Urtica dioica*, *Epilobium angustifolium*, *Frangula alnus*, *Arrhenaterum elatius*, *Impatiens parviflora*, *Galium aparine*, *Aegopodium podagraria*, *Sambucus* species, *Stellaria media*, *Stellaria holostea*, *Stellaria nemorum*, *Dryopteris filix mas*, *Dryopteris dilatata* and *Dryopteris cathusiana*. Such changes, with subsets of the species mentioned above, have been recorded in Dutch forests (Dirkse, 1993; Dirkse and van Dobben, 1989), in German mixed fir and spruce forests (Kraft et al., 2000; Rodenkirchen, 1992), in young moraine forests in Germany (also with a comparison to N deposition experiments) (Hofmann, 1987; Hofmann et al., 1990; Anders et al., 2002;; Jenssen and Hofmann, 2005; Jenssen, 2009), in beech, oak and hornbeam forests in north east France (Bost, 1991), in 17 out of 18 forest sites in two regions in Switzerland (Kuhn et al., 1987), in the

central Swiss plateau (Walther and Grundmann, 2001), in mixed deciduous Belgian forests (Lameire et al., 2000), in Atlantic old sessile oak woodlands (Wilkins and Aherne, 2016), and in woodland shelter belts of differing overstorey composition, along N deposition gradients from livestock farms in the UK (Pitcairn et al., 1998). As an example of an individual species, in Switzerland the cover of *Rubus fruticosus* agg. increased markedly in forest plots with a modelled N deposition rate of $> 25 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Flückiger and Braun, 2004, Figure 9.17). According to its Ellenberg N value, *Rubus fruticosus* would not be classified as a nitrophilous plant, but its shoot development seems to be highly stimulated by N. Gilliam et al. (2016) observed a significant N-mediated increase in *Rubus* spp. after the long-term application of $35 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in the temperate forests of Fernow Experimental Forest (FEF), West Virginia. Interestingly, the degree of canopy openness determines whether light will be a limiting factor once N availability increases through deposition. Species such as *Oxalis acetosella* and *Mercurialis perennis* profit from high nutrient levels under closed canopies while open forest species such as *Melampyrum pratense* and *Tanacetum corymbosum* disappear (Heinrichs and Schmidt, 2017). Under open-forest conditions, the growth of light-demanding species such as *Anemone nemorosa* increases markedly at the expense of species such as *Primula elatior* and *Viola reichenbachiana* (Bernhardt-Römermann et al., 2010; Jantsch et al., 2013).

Figure 9.17. Percentage of cover by *Rubus fruticosus* agg. in Swiss forest observation plots, in relation to modelled N deposition (Flückiger and Braun, 2004).



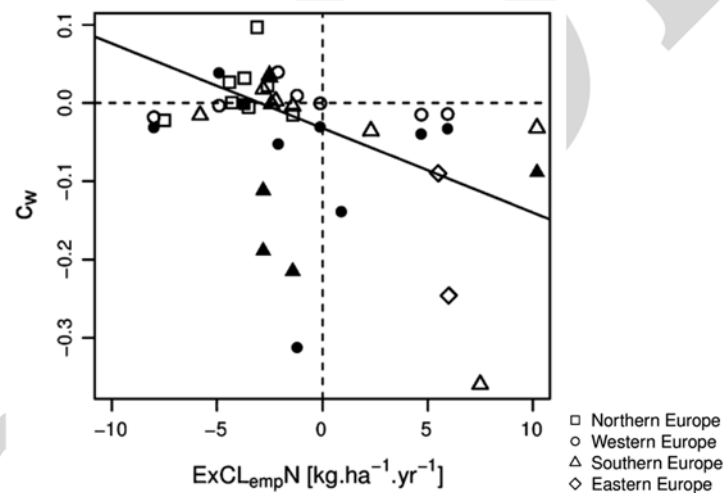
Source: Flückiger and Braun, 2004

By way of further examples, large-scale changes in vegetation have been observed in Scots pine forests in north-eastern Germany since the 1970s. N deposition of between 10 and $15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (bulk deposition) over several decades led to N accumulation in oligotrophic Scots pine forests. This induced a shift in vegetation types over large areas. Mainly Scots pine forest types that were dominated by lichens, heather and bryophytes (C:N ratio 30-35) disappeared almost completely and were replaced by common forest types dominated by grasses (*Deschampsia flexuosa*, *Festuca ovina*). The N deposition gradient study by Wilkins and Aherne (2016), which involved applying TITAN to infer plant species changes in Atlantic oak woodland, resulted in a $\text{CL}_{\text{emp}}\text{N}$ of $13.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$.

In parallel to these findings of taxonomic shifts, the average N indicator number (according to Ellenberg, 1988) had been shown to rise using ICP Forests data (Van Dobben and De Vries, 2017). However, the authors also noted that the N deposition effect could not be clearly delimited from the overall signal (see also Verheyen et al., 2012). In contrast, the abundance of

species with low N indicator values – comprising many endangered species (Ellenberg, 1985) – had been declining significantly in European forests with N deposition above the currently defined CL_{empN} (Dirnböck et al., 2014) (Figure 9.18). Combined taxonomic and indicator analyses from resurveys in north-east France confirmed a community increase in N indicator values, an increase in N demanding species, and an increased nutrient status as indicated by trophic level analyses (Thimonier et al., 1992). However, the clear attribution of vegetation changes to N deposition was not always straightforward, as seen for similar forests in north-east France (Thimonier et al., 1994). In Austria, N deposition effects on the forest floor vegetation of temperate spruce-fir-beech and pure Norway spruce forests were studied at the ICP Integrated Monitoring site Zöbelboden (northern Limestone Alps). Annual throughfall deposition of between 11 and 19 kg N ha⁻¹ yr⁻¹ (1994-2013) did cause homogenisation in the plant composition across the entire 90 ha study area (Hülber et al., 2008), as predicted by the N homogeneity hypothesis (Gilliam, 2006). However, wind and bark beetle disturbances hid any further eutrophication signal after 2005 (Helm et al., 2017).

Figure 9.18. Relationships between weighted averaged changes (cw) in the cover of nitrotrophic (Ellenberg N value 7-9: filled symbols) and oligotrophic plant species (Ellenberg N value 1-3, open symbols) and critical load exceedance ($ExCL_{empN}$). Significant linear regression ($P < 0.05$, t-test) was found for oligotrophic species. Adapted from Dirnböck et al. (2014).



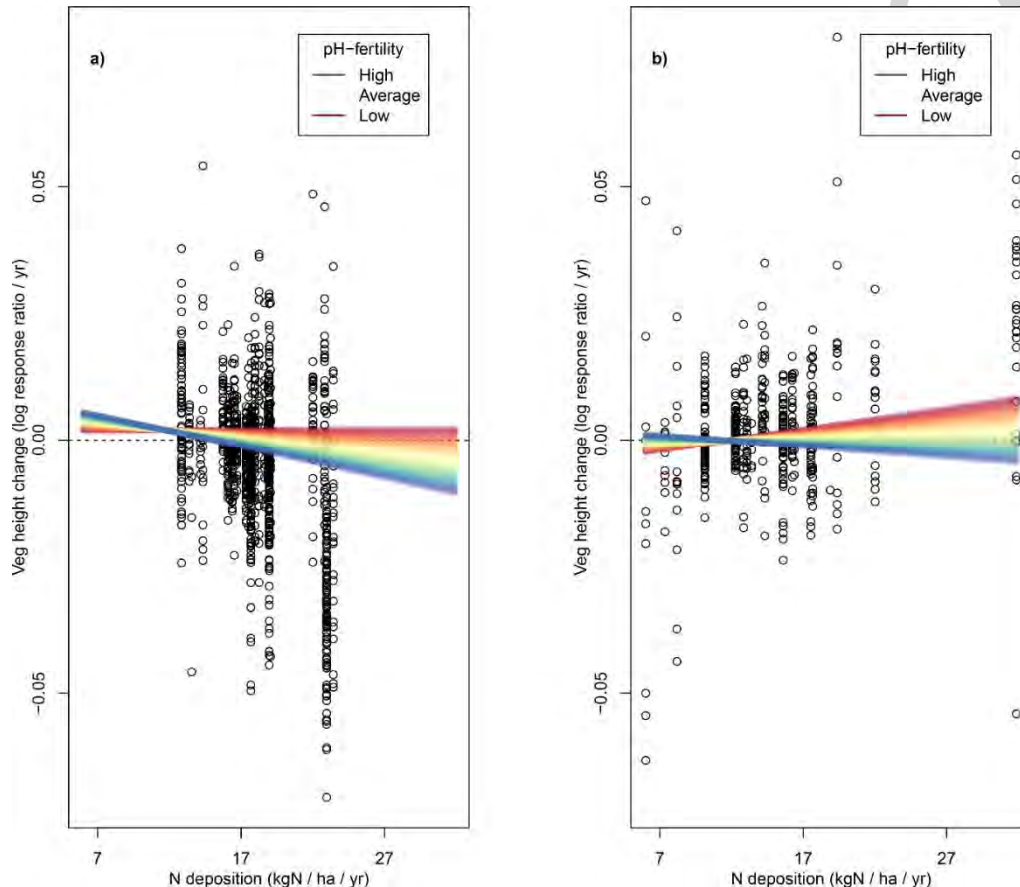
Source: Dirnböck et al., 2014

It is difficult to ascribe understorey vegetation changes to specific driving factors, specifically N (in the case of deriving critical loads), in single case or gradient studies with co-occurring environmental changes. This led to the awareness that combining studies from across sites and regions, coupled with careful analyses that account for study characteristics, can advance understanding within global change research (Verheyen et al., 2017). Such combination studies revealed the critical importance of overstorey dynamics in explaining an apparent eutrophication shift in European deciduous forests (Verheyen et al., 2012). Indeed, cumulative N deposition by the time of an initial survey had been shown to explain subsequent diversity changes that were related more to temporal changes in light availability and large herbivore density than to instantaneous N deposition rates (Bernhardt-Römermann et al., 2015). However, N deposition was shown to lead to declining herb cover in an observation resurvey dataset of forest understorey vegetation across Europe, but only in forests that have a land use history of continuous forest cover (Depauw et al., 2020). The importance of previous land use in dictating community responses to N deposition was also demonstrated in a functional trait framework for

a greater number of plots, albeit with a less orthogonal design (Perring et al., 2018b). In Perring et al. (2018b) higher rates of N deposition actually led to greater species richness (and plant height) in forests managed less intensively in 1800 (i.e. as high forests), compared to decreases that were seen in forests managed more intensively through coppicing. Such results, across Europe, reinforce earlier findings from site-based studies. For instance, at forest sites with earlier litter raking in Germany, the average Ellenberg N indicator values had increased by 0.6 units while at forest sites without litter raking the increase was 0.3 units (Rodenkirchen, 1992). N deposition is the major factor driving the decline of specialised species with low distribution ranges in temperate forest understoreys (Staudé et al., 2020). Hence, species compositional rather than richness changes are the predominant effects of N deposition in temperate forests.

Awareness that the impact of N deposition may be masked or may depend on other site factors, led to a review of forest understorey responses to this driving factor, and the development of a conceptual framework with the focus on critical load derivation (Perring et al., 2018b). They suggested that land and forest management, overstorey composition, climate change, forest continuity, landscape context, browsers and grazers can all affect forest understorey communities, with potential interactions with cumulative N deposition, and the forms and amounts of contemporary N deposition. They then focused on how light and soil pH value gradients influenced the community response to N deposition (while N deposition itself can influence these gradients). This was because soil pH can precondition subsequent community responses to the effects of increased N (and reduced sulphur deposition) (Smart et al., 2014; Stevens et al., 2011). In the case of light, as explained above, individual species (and plant communities) may respond to additional N in high-light environments but not when there are other resource limitations (De Frenne et al., 2015; Heinrichs and Schmidt, 2017).

Figure 9.19. Plant community responses to N deposition between surveys across European deciduous temperate forests. Shown are predicted (a) graminoid (including sedges, grasses and rushes) and (b) forb responses to N deposition based on the best-fit model using 1814 plots across 40 forest regions. Changes in cover of these structural groups between surveys depend on the magnitude of N deposition and whether or not initial survey plots are shown to be covered by light demanding species (blue line) or shade tolerant species (red line). Black circles show the actual data. Reproduced from Perring et al. (2018b).



Source: Perring et al., 2018b

Using resurveys of 1814 plots from 40 forest regions across Europe, Perring et al. (2018b) confirmed the importance of context dependency in determining understorey community changes to N deposition. Plant community change, as measured by species richness, structural composition and functional traits, rarely depended on N deposition as the main effect (Perring et al., 2018b). Instead, and as shown by Figure 9.19a, cover change for graminoids is negative in response to N deposition in open environments, while it is positive in closed environments. What this means is that in sites initially characterised by high-light species assemblages (blue line), large amounts of N deposition can lead to decreased graminoid cover, while low amounts of N can lead to an increase. On the other hand, in communities initially characterised by plants with a low demand for light (red line), higher N deposition led to increased graminoid cover, and low N deposition decreased cover. In contrast, forbs (Figure 9.19b) show a quadratic response to N deposition, with different curvature depending on the light environment at the time of the initial survey. Community change, as measured by the change in community-weighted mean height, depended on N deposition, but only in a complex manner related to historical forest management (Perring et al., 2018a) and the position of sites on a pH-fertility gradient (Perring et al., 2018b).

These synthetic results, and others that show conditional vulnerability of plant communities to N deposition (Simkin et al., 2016), have implications for our understanding of $CL_{emp}N$. As well as awareness of how $CL_{emp}N$ can vary among species within communities and within species depending on environmental context (Henrys et al., 2011; Payne et al., 2013), the results described above mean that critical loads need to be set reflecting other environmental conditions, especially forest-management history, fine-resolution light availability and nutritional condition of the site. For instance, in forests with infrequent biomass removal in the past (i.e. high forests) critical loads could be less risk averse (i.e. higher) than for those forests with frequent biomass removal in the past (i.e. former coppices). $CL_{emp}N$ can be adjusted according to the species pool and fertility context. As explained in Perring et al. (2018b), fertile sites predisposed light-demanding species to decline in high forests exposed to elevated rates of N deposition. This decline was even more pronounced in former coppices. In a different context of lower fertility, shade-tolerant species increased in high forests while they held steady in coppices. Low $CL_{emp}N$ in one context are not apparent in another. Dynamic plant-soil modelling approaches to calculate $CL_{emp}N$ can explain context dependency, for instance by taking into account how historical management effects, such as litter raking, alter soil nutrient depletion and acidity. Expected future climate change can likewise be factored into these models to explore likely joint impacts together with N deposition trends (Dirnbock et al., 2017, 2018; Dirnböck et al., 2020). The main conclusions from these studies were that climate-induced tree growth enhancement may increase the competition for soil N, thereby relaxing eutrophication in the future. However, it is important to note that a significant degree of uncertainty is still associated with these kinds of models (De Vries et al., 2010).

Empirical critical loads can also be set by experiments where N is directly added, allowing the attribution of N effects, although the exact mechanism (i.e. responses due to eutrophication and/or acidification) would require careful interpretation. Until recently, there have been few experimental investigations of forest herb layer responses to N deposition, especially in temperate deciduous forests in Europe (Gilliam, 2006). However, as with observational datasets, the increase in research over the last years does allow synthetic investigation, such as meta-analyses, often incorporating responses in forests as well as other biome types. In such an analysis focusing on biomass and species richness, De Schrijver et al. (2011) showed no significant response of forest understorey vegetation to in situ instantaneous N addition. It also showed that, in general, species loss occurs faster at low levels of cumulative N input with slower species loss as cumulative N increases. These findings complement observational studies highlighting the importance of cumulative effects of N addition (e.g. Bernhardt-Römermann et al., 2015). In a more recent meta-analysis using experimental results, Midolo et al. (2019) discovered that no response metrics showed an interaction between ecosystem type and N application. Forests, therefore, exhibited similar declines as did other ecosystems regarding species richness, individual abundance, mean species abundance and geometric mean abundance with increasing N addition.

Critical load for forest field-layer vegetation

Taking into account all the new findings from predominantly gradient studies, we suggest that the $CL_{emp}N$ for temperate forest field-layer vegetation in Europe remains in the range of 10 to 15 kg N ha⁻¹yr⁻¹ and is 'reliable'. Since the last review of $CL_{emp}N$, substantial work has been carried out on the detailed responses of forest field-layer vegetation when considering the multitude of other drivers (land use change, management, climate change, etc.). This opens up the possibility that $CL_{emp}N$ can be adjusted according to the species pool and fertility context.

Soil fauna

The effects of increased anthropogenic N deposition on soil fauna are still an understudied area, with few long-term studies from which to identify conclusive impacts or critical loads for the vast range of forest soil fauna. Most research on the impacts of N on soil biota to date focussed on plant, fungi and microbial communities. However, the responses of organisms at higher trophic levels, such as mesofauna and earthworms, are less well understood and may be species-specific (Gan et al., 2013). Since these organisms are important for mediating key ecosystem functions such as litter decomposition and N mineralisation (Bardgett and Wardle, 2010), this is an area that merits more attention in future research. Most invertebrate and microarthropod groups generally show a negative short-term effect of N deposition on species richness and abundance, followed by varying long-term responses dependent upon the type, duration and dosage of N input (Gan et al., 2013; Lohm et al., 1977; Vilkamaa and Huhta, 1986; Xu et al., 2019).

A short-term (five years) application of N at a dose of 57 kg N ha⁻¹ yr⁻¹ showed little effect on the biomass of soil microarthropods from litterbags in a *Pinus sylvestris* stand at the low-background-deposition NITREX site Ysselsteyn in Sweden (Boxman et al., 1998). The dominance of Proturans increased in the fertiliser treatment. However, the abundance and species richness of *Oribatida* mites and soil mesofauna generally decreased in the N addition treatment compared with the control plot.

Conversely, repeated N deposition to forest systems appears to result in N saturation and chronic declines in both the abundance and diversity of soil invertebrates. Negative responses in *Collembola* were shown at a site in the Swiss Alps (NITREX site; background deposition of 12 kg N ha⁻¹ yr⁻¹) by Xu et al. (2009) after 13 years of N addition (25 kg N ha⁻¹ yr⁻¹). Total *Collembola* density and the density of *Isotomiella minor*, the most abundant species, decreased significantly in the upper soil layer (0-5 cm). In addition, the genera *Tomocerus*, *Arrhopalites*, *Sminthurus* and *Neanura* were completely absent from the N-treated plots, and the density group index of the community was negatively affected. Gan et al. (2013) observed a decline in abundance of both detritivores (*Oribatida* and *Collembola*) and predaceous mites (*Mesostigmata*) after 17 years of experimental N deposition (30 kg N ha⁻¹ yr⁻¹) on four sugar maple (*Acer saccharum*) forest stands in North America. There was a shift in the community composition of *Oribatida* mites between N treatments, indicating species-specific responses to N deposition (Gan et al., 2013). In a state forest in Brittany, France (estimated background deposition 10-20 kg N ha⁻¹ yr⁻¹), a single application of 100 kg N ha⁻¹ yr⁻¹ (in the form of NH₄NO₃) produced an effect on soil microorganisms that was still significant 23 years later (Deleporte and Tillier, 1999). There were also decreases in populations of the microarthropods *Acarina* (genera *Oribatida* and *Gamarida*), *Collembola*, *Symphyla* and *Pseudoscorpionida*. Seven years of N fertilisation (20 kg N ha⁻¹ yr⁻¹) of a young beech stand (atmospheric deposition of 12 kg N ha⁻¹ yr⁻¹) resulted in a significant decrease (66%) in the abundance of earthworms (Flückiger and Braun, 1999). This decline may also have been the result of soil acidification, as the pH of the upper soil layer (30 cm) decreased from 3.7 to 3.5, and earthworms are sensitive to changes in soil pH (Muys and Granval, 1997). In Sweden, a significant decrease in snails was observed over a period of 14 to 46 years in areas with N depositions of 15 to 25 kg N ha⁻¹ yr⁻¹, while in areas with N depositions of 3 to 6 kg N ha⁻¹ yr⁻¹, no significant changes were found (Gårdenfors et al., 1995). However, in this Swedish study, there was a sulphur deposition gradient (soil acidification) as well. Hence, no CL_{emp}N could be defined on the basis of these data.

The observed short-term decreases in forest soil invertebrate populations after N fertiliser application were mostly attributed to acute ammonium toxicity and/or the 'salt effect' of increased osmotic potential in soil solutions (Lohm et al., 1977). Ammonium is very toxic to microarthropods, and hydrophilous soil organisms such as nematodes and earthworms are most

likely to be negatively impacted by such osmotic stress and changes in forest soil pH (Lohm et al., 1977; Muys and Granval, 1997). Subsequent recoveries and increases in soil fauna populations after singular fertiliser applications were attributed to increases in the populations of the microorganisms upon which they feed, and increased litter input by the fertilised vegetation (Gan et al., 2013; Lohm et al., 1977). In contrast, the chronic declines in soil fauna populations observed after long-term N deposition are linked to decreasing pH and changes in the soil microbial community, particularly a reduction in soil fungi (Berg and Verhoef, 1998; Xu et al., 2009). Many species of *Collembola* and *Acarina* are fungivores and will thus experience population decline due to loss of food, which then leads to a population reduction in their predators, such as *Mesotigmata* mites (Gan et al., 2013; Xu et al., 2009). N deposition-induced changes in microarthropod community composition and microbial activity can significantly reduce litter decomposition and soil respiration. This leads to an increase in both C storage in organic matter and soil nutrient leaching (Gan et al., 2013; Xu et al., 2009; Zak et al., 2008). Owing to the lack of long-term research into soil fauna responses to N deposition, $CL_{emp}N$ have not yet been defined conclusively. Based on microarthropod community responses, Ochoa-Hueso et al. (2014) suggest a $CL_{emp}N$ of between 20 and 50 kg N ha⁻¹ yr⁻¹ for a semi-arid Mediterranean shrub system, similar to the critical threshold of 37 kg N ha⁻¹ yr⁻¹ previously reported for a Swiss subalpine forest (Xu et al., 2009). Research thus far indicates that the abundance, diversity and species richness of N-sensitive soil mesofauna such as *Collembola* (Figure 9.20) may be promising biological indicators of $CL_{emp}N$ and N saturation in forest systems (Jandl et al., 2003; Xu et al., 2009).

Figure 9.20. Springtails (*Collembola*) such as this *Tomocerus* sp. are sensitive to N deposition and may be useful bioindicators of forest soil N saturation (Image copyright: Frank Ashwood).



Source: Frank Ashwood

9.3.6 Summary of $CL_{emp}N$ for coniferous and deciduous temperate forests

This section provides an overview of the impacts of N deposition on different components of general forest classes. The $CL_{emp}N$ ranges for the different classes are summarised in Table 9.1.

Table 9.1. $CL_{emp}N$ and effects of exceedances on different components of temperate forest classes. ## reliable; # quite reliable and (#) expert judgement. Changes with respect to 2011 are indicated as values in bold.

Component	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
Soil processes			
Deciduous and Coniferous	10-15	#	increased N mineralisation, increased nitrification
	10-15	##	increased NO ₃ ⁻ leaching

Component	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
Trees			
Temperate forests	10-15 ^a	(#)	Nutrient imbalances, increased N and decreased concentrations of P, K and Mg in foliage, increased susceptibility to pathogens and pests, change in fungistatic phenolics
Mycorrhiza			
Deciduous	13-16	##	Reduced sporocarp production, reduced belowground growth, reduced species richness and changed species composition
Coniferous	10-13	##	
Ground vegetation			
Temperate forests	10-15	##	Changed species composition, increase in nitrophilous species, decrease in oligophilic species,
Lichens and algae			
Temperate forests	3-5	#	Decline of epiphytic lichens and N ₂ fixation by cyanolichens, increase in free-living algae

a) Use the lower end under drought.

9.4 Effects of N deposition on spruce and pine taiga woodlands (T3F and T3G)

Together, spruce (T3F - dark taiga) and pine (T3G - light taiga) taiga woodlands constitute the westernmost part of the continuous Eurasian northern taiga belt (Chytrý et al., 2020). Spruce taiga woodlands include the boreal spruce and spruce-pine forests of Fennoscandia, north-eastern Poland, the Baltic States, Belarus and European Russia. Pine taiga woodlands include the boreal pine forests in the regions mentioned above. Broadleaved deciduous taiga woodlands include forests with broadleaved deciduous species, with extended cold winters and short mild summers. Tree height rarely exceeds 15 meters and may be as low as two meters in subarctic conditions. Lichens and mosses frequently dominate the ground layer.

Both spruce and pine taiga woodlands are mostly very poor in N. Any increase in N input may influence major soil processes as well as tree growth rates, changes in the species composition and diversity of forest understorey vegetation including bryophytes (mosses), epiphytic lichens on tree trunks and branches and the abundance of free-living algae and/or cyanobacteria living in association with bryophytes. Because soil responses to increased N input are likely to be similar in both spruce and pine taiga woodlands, the effects of N deposition on soil processes are presented together, whereas the impacts on biodiversity are treated separately.

9.4.1 Soil processes in spruce and pine taiga woodlands

Soil acidification

A long-term N fertilisation experiment in northern Sweden showed that 34 years of N additions (30 kg N ha⁻¹ yr⁻¹, background deposition 2-3 kg N ha⁻¹ yr⁻¹) led to increased N mineralisation rates in Scots pine forests (Chen and Högberg, 2006). Furthermore, N addition has been shown to result in soil acidification (Högberg et al., 2006; Solberg et al., 2004) which is reflected in a decrease in base cation saturation. This decrease in base cations was recorded only in the

mineral soil, whereas for mor-layer base cation concentrations, no differences were detected between N treated plots and control plots (Högberg et al., 2006). In a long-term experiment (45 to 52 years with N additions every 5-10 years) carried out in Finland on 28 sites (spruce or pine), the added N varied from 10.5 to 37 kg N ha⁻¹ yr⁻¹. During the experimental period, background N deposition decreased from 9 kg N ha⁻¹ yr⁻¹ to 4 kg N ha⁻¹ yr⁻¹. Stand production, amounts of organic matter, carbon and most nutrients increased, but C:N ratio and pH decreased in surface soil layers. This was an indication of a slight soil acidification effect (Saarsalmi et al., 2014). A study of 204 sites with Norway spruce and Scots pine across mid and south-eastern Norway showed that N deposition (6-8.5 kg N ha⁻¹ yr⁻¹) correlated negatively with base saturation in the humus layer, with soil pH and with Ca:Al ratio. It correlated positively with Al³⁺ concentration in the mineral soil layer (Solberg et al., 2004). However, Solberg et al. (2004) suggested that climate, geology and natural processes may also explain these relationships. Acidification of soil water at sites with low N depositions in northern Norway suggested other sources of acidification, such as plant and microbial uptake of base cations and NH₄⁺ and increased production of organic acids (Kvaalen et al., 2002).

N leaching

N deposition may lead to increased N leaching from coniferous forests. Boreal forest ecosystems have large capacities to retain N (Petrone et al., 2007; Moldan et al., 2018). There are empirical studies that show a positive relationship between N leaching and N deposition, and others that show no such relationship (Akselsson et al., 2010; Futter et al., 2009; Gundersen et al., 1998b; Kaste et al., 2004; Nadelhoffer et al., 1999; Sjøeng et al., 2009). Under undisturbed conditions, boreal forests have a high N-retention capacity. However, forest disturbances such as clearcut harvests (Zanchi et al., 2014), storm fellings (Hellsten et al., 2015) and bark beetle attacks (Karlsson et al., 2018) have all been shown to initiate the leakage of NO₃⁻ to the soil water. Furthermore, it has been shown that the N leached to the soil water after clearcut harvests can cause increased NO₃⁻ concentrations in groundwater (Kubin, 1995; Norrström, 2002) and surface waters, too (Grip, 1982; Karlsson et al., 2021; Löfgren et al., 2009).

In south Sweden it has been demonstrated that increased N depositions elevated the risk of N leaching (Akselsson et al., 2004). Furthermore, studies in Finland indicated that timber harvesting has effects on N leaching, because the uneven distribution of logging residues (tree tops, foliage and branches) can lead to hot spots for N cycling and losses on clear cut sites. For example, studies in spruce, pine and birch forests demonstrated that logging residues increase the rates of net N mineralisation and net nitrification in the humus layer under the piles of logging residue (Adamczyk et al., 2016; Smolander et al., 2019; Törmänen et al., 2018). These effects can last for decades (Smolander et al., 2010).

Bahr et al. (2013) studied the growth of ectomycorrhizal fungi and their effect on N leakage on a broad N gradient in Norway spruce forests across Sweden. They concluded that mycorrhizal fungi are probably important for N retention capacity since high N leaching coincided with low fungal growth. But they could not differentiate between the effects of mycorrhizal fungal growth and the direct effect of N deposition on N leaching. Decreasing N deposition may have rapid positive effects on soil processes. In Finland, the long-term monitoring of deposition and run-off chemistry over a period of 12 to 25 years, showed decreased SO₄²⁻ and NO₃⁻ concentrations in bulk deposition since the 1980s. This had led to a rapid decrease in concentrations of these elements in run-off (Forsius et al., 2021; Moldan et al., 2001).

Effects on litter decomposition and soil C stocks

Boreal forest soils store a major proportion of global terrestrial carbon. Belowground inputs contribute as much as aboveground plant litter to the total C stored in the soil (Pan et al., 2011).

Clemmensen et al. (2013, 2014) demonstrated that mycorrhizal fungi and root-associated fungi are important contributors to soil carbon stocks and that variation in belowground C and N sequestration is affected by mycorrhizal fungal type and growth. Recent studies have shed some light on the effects of N deposition on litter decomposition and soil C stocks. In boreal pine (*Pinus sylvestris* L.) and spruce (*Picea abies* Karst.) forests in northern Sweden with a low background deposition ($2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), Maaroufi et al. (2019) found that long-term N enrichment (ten years of 3, 6, 12 and $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) impeded mass loss of litter but not of humus, only slightly at low levels but significantly at the highest deposition level. They also found that saprophytic fungi and especially actinomycetes were more important for driving litter decomposition, whereas mycorrhizal fungi appeared more important for reducing N and P concentrations in humus and litter. In the same N experiment, Forsmark et al. (2020) were able to show that only at the highest N addition rate did litter accumulation increase by 46% and soil respiration per mass unit of soil C decrease by 31.2%, mainly by lowering autotrophic respiration. Thus, under the actual ambient low N deposition levels in boreal forest ecosystems, litter decomposition, soil respiration and soil carbon cycle seemed to be unaffected. From this study it was concluded that N deposition in temperate forest soils, where N is not limiting, impeded organic matter decomposition by reducing microbial growth, thereby stimulating carbon sequestration. The authors stated that the concomitant reduction in soil carbon emissions was substantial and equivalent in magnitude to the amount of carbon taken up by trees owing to N fertilisation. These findings seem to be in line with previous findings from a meta-analysis of Janssens et al. (2010).

Although the decomposition of high-quality litter, which is low in lignin, may be stimulated after increased N input, decomposition of litter with a high lignin concentration was found to be reduced by N (Knorr et al., 2005). Accordingly, the increase in soil C after N additions to coniferous forests was attributed not only to decreased decomposition rates of lignin-rich litter (Knorr et al., 2005) but also to N-induced increases in litter production (Franklin et al., 2003).

Soil microbial biomass

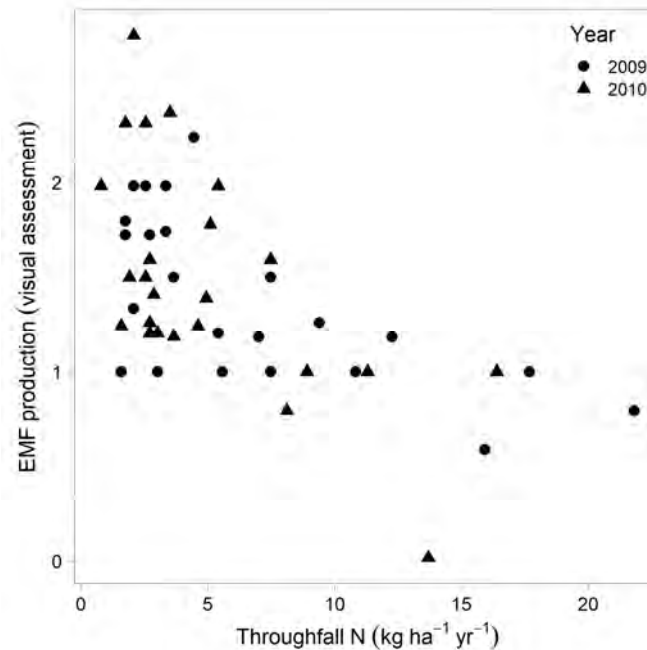
A review of N effects on soil microbial biomass by Treseder (2008) showed that N deposition reduced microbial biomass in boreal forests by up to 18%. Bacterial response ratios increased in boreal forests and decreased in tundra. After excluding very young forests and elevated CO_2 treatments from a meta-analysis by Janssens et al. (2010), the average reduction in microbial biomass due to N deposition was 16%. But the authors state that in N limited boreal forests or in open forests and very young plantations, bacterial biomass may even rise with increasing N deposition due to increasing rates of photosynthesis.

In a long-term N enrichment experiment (ten years of 0, 3, 6, 12, $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) in boreal conifer forests with very low background deposition ($2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), Maaroufi et al. (2019) found that microbial biomass (fungi, gram-positive and gram-negative bacteria) decreased significantly in the high N treatment compared to the control and low N treatments. The biomass of actinomycetes (bacteria) grew with increasing N addition. The results of this study also showed that the biomass of fungi and some bacteria was reduced in the $12 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ treatment, suggesting a negative effect already at low N deposition levels commonly found in some boreal ecosystems.

In a N gradient study across conifer forests in Sweden, Bahr et al. (2013) reported a reduction in mycelial biomass of ectomycorrhizal fungi even at moderate levels of N deposition (throughfall N load $< 10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$; Figure 9.21). This study included 29 thoroughly monitored Norway spruce stands from a large geographical region in Sweden and evaluated the importance of N

deposition on growth of extramatrical mycelia, N leaching and C sequestration in a broader context.

Figure 9.21. Moderate levels of N deposition reduce the biomass of ectomycorrhizal fungi (EMF) in conifer forests across Sweden (Bahr et al., 2013).



Source: Bahr et al., 2013

A different result was reported by Tahovská et al. (2020). They found that the soil microbial biomass was much larger in a spruce forest catchment in south-west Sweden that had received an N addition of 40 kg N ha⁻¹ yr⁻¹ over 24 years (cumulative N input of > 1200 kg N ha⁻¹), compared to a control catchment. The N addition did not change the fungi:bacteria ratio, but a larger share of the bacterial community was made up of copiotrophs. Furthermore, fungal community composition shifted to more nitrophilic ectomycorrhiza fungi. The restructured microbial community showed faster net N mineralisation and nitrification.

Effects on greenhouse gases

N addition also affects emissions of the important greenhouse gases, CH₄ and N₂O. It has been shown that boreal forest soils may be an important sink for atmospheric CH₄ as they contain CH₄-oxidising microbes. Long-term additions (27 years) of around 31 kg N ha⁻¹ yr⁻¹ to a Norway spruce stand in south-eastern Finland showed that the CH₄ uptake by soil was not negatively affected by N fertilisation (Saari et al., 2006) in contrast to results for a temperate system described in Chapter 9.3.1.

On organogenic forest soils (forests on former peatlands) in Sweden and Finland, a strong correlation between N₂O emissions and soil C:N ratio was demonstrated: the lower the C:N ratio, the higher the N₂O emissions (Klemedtsson et al., 2005). For other forests soils, not more than 0.5 to 1% of the N input evaporated as N₂O (Maljanen et al., (2006)): boreal soil, Papen and Butterbach-Bahl (1999); temperate soil (Germany)).

The information on the effect of N deposition on greenhouse gases is important for climate change models. There are not enough data to set a specific critical load.

9.4.2 Effects on mycorrhiza in spruce and pine taiga woodlands

Productivity in spruce- and pine-dominated taiga forests is primarily limited by a cold climate and low N availability (Tamm, 1991). Increased N input in boreal ecosystems alters growth and species composition of mycorrhizal fungi, as measured both in the production of fruit bodies (aboveground) and mycelia (belowground) (Lilleskov et al., 2001, 2002; Treseder, 2004); Lilleskov et al., 2011, 2019). In Norway spruce forests in southern Sweden aboveground fruit-body production in species of the genera *Cortinarius* and *Russula* decreased, while species such as *Paxillus involutus* and *Lactaria rufus* were less sensitive, or even responded positively to N additions of 35 kg N ha⁻¹ yr⁻¹, for 4.5 years (background deposition 13 kg N ha⁻¹ yr⁻¹) (Brandrud and Timmermann, 1998). Strengbom et al. (2003) found that the production of fruit-bodies in *Cortinarius* species in two conifer forests in northern Sweden was around 300 times higher on control plots than on plots receiving 34 kg N ha⁻¹ yr⁻¹ (background deposition 2-3 kg N ha⁻¹ yr⁻¹); on the latter, production was very small. In Scots pine forests in northern Sweden ectomycorrhizal sporocarp production and richness were investigated in several N addition treatments (20, 35, 70 and 110 kg N ha⁻¹ yr⁻¹, 2 kg N ha⁻¹ yr⁻¹ background deposition). The authors reported an elimination of sporocarps at the highest N treatment and a shift in community composition and dominant sporocarp taxa at 35 kg N ha⁻¹ yr⁻¹ toward a higher abundance of nitrophilic taxa, especially *Lactarius* (Hasselquist and Högberg, 2014). On a natural N gradient in boreal forests of northern America (1 to 20 kg N ha⁻¹ yr⁻¹), sporocarp production and richness were found to be strongly reduced and community composition was found to change dramatically with increasing N availability (Lilleskov et al., 2001, 2002). Nitrophobic and nitrophilic taxa could be identified. Species of the genera *Cortinarius*, *Piloderma*, *Amphinema*, *Tomentella*, *Russula*, *Tricholoma*, *Lactarius* and *Hebeloma* declined in species richness or abundance with increasing organic horizon mineral N, while *Lactarius theiogalus*, *Laccaria*, *Paxillus involutus*, *Hygrophorus olivaceoalbus*, *Tylospora fibrillosa*, *Tomentella subulilacina* and *Thelephora terrestris* increased with rising N enrichment.

Mycorrhizal fungi on ericaceous dwarf-shrubs in boreal ecosystems appear to be less sensitive than ectomycorrhizal fungi associated with trees. Ishida and Nordin (2010) found that, although species composition of fungi on roots of *Vaccinium* species differed between spruce and pine forests, N addition had no negative effect on fungal communities (12.5 and 50 kg N ha⁻¹ yr⁻¹ for four years in pine and 12 years in spruce forests, with background depositions of 2 kg N ha⁻¹ yr⁻¹). Maaroufi et al. (2019) found that N treatments (ten years of 0, 3, 6, 12, 50 kg N ha⁻¹ yr⁻¹) in boreal conifer forests with very low background deposition (2 kg N ha⁻¹ yr⁻¹) caused substantial changes in microbial community abundance and composition. Many of these effects occurred independently of trenching, and despite a minor decline in soil pH at the highest N application (0.22 pH units). Abundance was reduced for fungi and several bacterial groups, except for the bacterial group actinomycetes which increased in response to N enrichment. This also caused significant changes in both the mycorrhizal and saprotrophic fungal community composition, indicating that different species that are likely more N demanding became dominant as plots were enriched with N. While many species contributed to the compositional differences that emerged in response to N, the mycorrhizal fungi *Xerocomus ferrugineus* and *Tylospora asterophora* increased the most in response to N, whereas a variety of other operational taxonomic units decreased (e.g. *Tylospora*, *Echinosporea* and *Sebacinales*). Other studies also reported an increase in *Xerocomus* sp. in response to N addition (Almeida et al., 2019). The genus *Xerocomus* belongs to the long-distance exploration type (i.e. ectomycorrhizal fungi foraging strategy) that produces larger hyphal biomass contributing to fungal necromass with slow turnover compared to the short-distance exploration type.

Termination of N fertilisation experiments in conifer forests in Sweden led to residual fungal community effects even after 23 years (Choma et al., 2017) or 47 years (Strengbom et al., 2001). The former study revealed that the relative abundance of mycorrhizal fungal species shifted closer to, but did not reach, control levels. The latter study showed that sporocarp production did not fully recover and that species composition differed largely from the control plots. However, Högberg et al. (2014) reported a recovery of many ectomycorrhizal fungal species (mainly *Atheliaceae* and *Russulaceae*, but not *Cortinariaceae* and *Thelephoraceae*) 14 years after termination of high-level N treatment (108 kg N ha⁻¹ yr⁻¹ for 19 years) in a Scots pine forest in northern Sweden. Meanwhile, bacterial species in this forest did not show any sign of recovery.

Bahr et al. (2013) showed that ectomycorrhizal fungi contributed substantially to carbon sequestration, but their growth was limited as nitrate leaching increased. This correlated positively with N deposition. They used a gradient of 29 plots with throughfall deposition between 0.95 and 24.6 kg N ha⁻¹ yr⁻¹.

Along a soil fertility gradient in boreal forests in Sweden with NH₄⁺ values between 1 and 53 µg N per g organic matter, Sterkenburg et al. (2015) observed changes in the community composition of ectomycorrhizal fungi. They also reported a reduction in litter and root associated ascomycetes as well as yeast and mould fungi while basidiomycetes increased in abundance in more fertile forests sites.

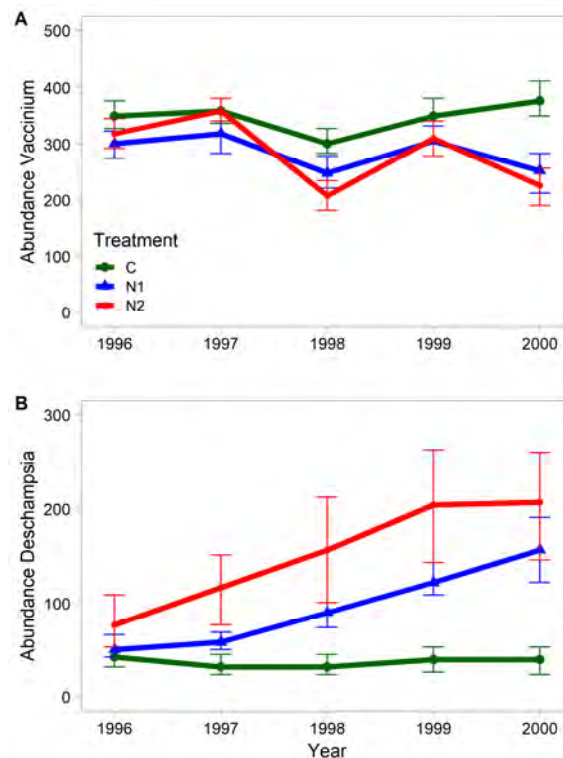
The N addition experiments did not permit any conclusions on a critical load as either the treatments were too high or an effect threshold was not indicated. However, the gradient study of Bahr et al. (2013) supports the results presented in the chapter on temperate forests.

9.4.3 Spruce taiga woodlands (T3F - dark taiga)

Field- and ground-layer vegetation

The responses in understorey vegetation in boreal spruce forests have been comprehensively studied. A common initial response after N addition in areas with low background depositions (2-6 kg N ha⁻¹ yr⁻¹) is elevated tissue N concentration in plants, such as bryophytes, grasses and ericaceous dwarf-shrubs (Forsum et al., 2006; Mäkipää, 1998; Nordin et al., 1998, 2006; Strengbom et al., 2002). Several studies also reported that the composition of field-layer vegetation was sensitive to increased N input. Over a period of 20 years, Rosén et al. (1992) observed a significant positive correlation between *Deschampsia flexuosa*-dominated coniferous forests in Sweden and the pattern of N deposition, based on comparisons between field-layer vegetation surveys in the Swedish Forest Inventories of 1973 to 1977 and 1983 to 1987. *Deschampsia flexuosa* increased significantly during this period. These changes occurred above an N deposition of 7 to 11 kg N ha⁻¹ yr⁻¹. Other studies reported altered abundance of commonly occurring dwarf shrubs such as *Vaccinium myrtillus* and *Vaccinium vitis-idaea* and the grass *Deschampsia flexuosa*. There have been reports of such changes already at low (5 kg N ha⁻¹ yr⁻¹: Kellner and Redbo-Torstensson (1995), 12.5 kg N ha⁻¹ yr⁻¹: Nordin et al. (2005)) or moderate levels (40 kg N ha⁻¹ yr⁻¹: Manninen et al. (2009)). In northern Sweden, four years of additions of 6 kg N ha⁻¹ yr⁻¹ (background deposition of around 2 kg N ha⁻¹ yr⁻¹) increased the abundance of *Deschampsia flexuosa* by around 50% (UNECE, 2007). Additions of 12.5 kg N ha⁻¹ yr⁻¹ over five years (background deposition of around 2 kg N ha⁻¹ yr⁻¹), resulted in a 300% higher abundance of *Deschampsia flexuosa*, and a 34% lower abundance of *Vaccinium myrtillus* (Figure 9.22).

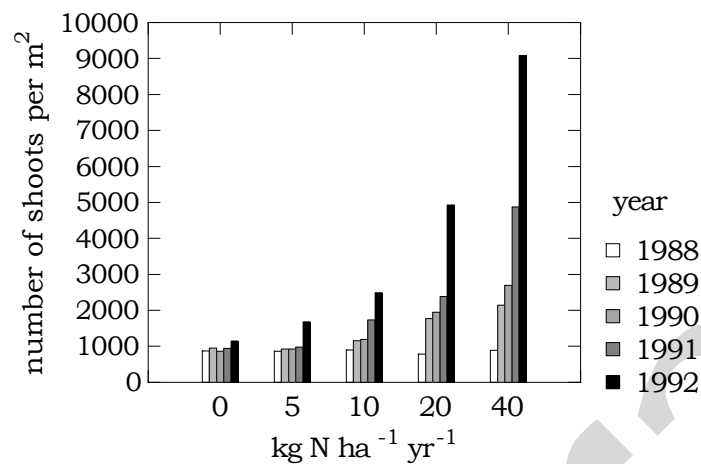
Figure 9.22. Response in the two dominant plant species of the ground vegetation (the dwarf shrub *Vaccinium myrtillus* (A) and the grass *Deschampsia flexuosa* (B)) in a Swedish boreal forest (T3F) that had been exposed to N additions corresponding to 12.5 (N1) and 50 kg N ha⁻¹ yr⁻¹ (N2) for five years. The fertilised plots were sized 1000 m² and each treatment was replicated six times (n = 6). Vertical bars show S.E. From Nordin et al. (2005).



Source: Nordin et al., 2005

In central Sweden (background deposition of 2-6 kg N ha⁻¹ yr⁻¹), the shoot density of *Deschampsia flexuosa* increased by 70, 250, 430 and 780%, after four years of additions of 5, 10, 20, and 40 kg N ha⁻¹ yr⁻¹ (Figure 9.23). At the same site, the shoot density of *Trientalis europaea* showed significant increases when N additions exceeded 10 kg N ha⁻¹ yr⁻¹.

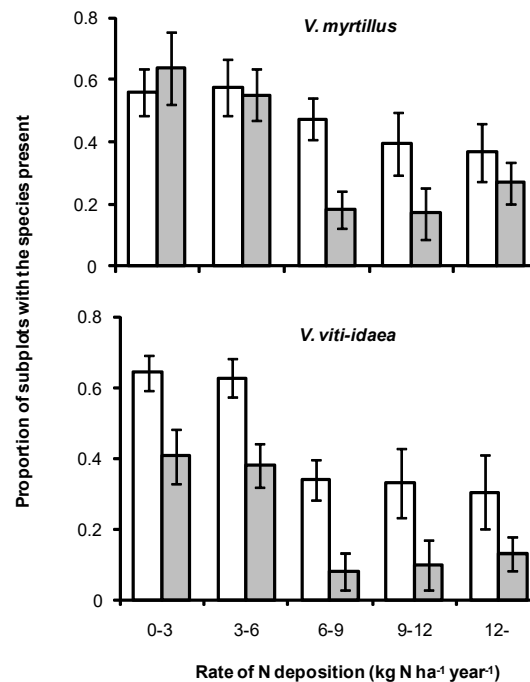
Figure 9.23. Number of shoots of *Deschampsia flexuosa* at different levels of N supply ($\text{kg N ha}^{-1} \text{yr}^{-1}$), between 1988 and 1992, in a boreal forest (T3F) located outside Söderhamn, in central Sweden (Kellner and Redbo-Torstensson, 1995).



Source: Kellner and Redbo-Torstensson, 1995

The above-listed effects after increased N inputs are supported by surveys along N deposition gradients and changes in understorey composition over time. A field survey in Sweden, examining the occurrence of understorey species at 557 sites (a few located outside T3F and T3G classes), showed that occurrences of *Vaccinium myrtillus* and *Vaccinium vitis-idea* (Figure 9.24) were less frequent in areas where N depositions had been greater than or equal to $6 \text{ kg N ha}^{-1} \text{yr}^{-1}$ than in areas with lower N depositions (Strengbom et al., 2003). In Norway, the occurrence of *Deschampsia flexuosa* increased in spruce forests between 1988 and 1993 (background deposition (wet) of $7.9 \text{ kg N ha}^{-1} \text{yr}^{-1}$) (Økland, 1995). In addition, *Vaccinium myrtillus* proved to be more susceptible to the leaf pathogen *Valdensia heterodoxa* in areas with high levels of N deposition (Strengbom et al., 2003).

Figure 9.24. Proportion of subplots with *Vaccinium myrtillus* and *Vaccinium vitis-idaea* in forest stands with various N deposition rates. White bars represent Scots pine and dark bars represent stands dominated by Norway spruce. Vertical bars: mean and confidence intervals (95%). Redrawn from Strengbom et al. (2003).



Source: Strengbom et al., 2003

Results from experiments showed that understorey responses to high levels of N may depend on forest type. At experimental sites with high tree canopy cover, for example sites with high spruce domination, the effects of the input of high levels of N could be limited by reduced light availability as tree canopy cover increased. In comparison, light limitation for more open experimental sites, for instance with a higher proportion of pine trees, could be less severe and understorey responses to N additions more linear. In a study by Kellner and Redbo-Torstensson (1995), the density of *Deschampsia flexuosa* rose as N additions increased (Figure 9.23). Nordin et al. (2005) reported relatively small differences in effects on *Deschampsia flexuosa* and *Vaccinium myrtillus* resulting from five years of N additions of 12.5 and 50 kg N ha⁻¹ yr⁻¹ (Figure 9.24). Kellner and Redbo-Torstensson (1995) studied a mixed pine-spruce forest, while Nordin et al. (2005) studied a spruce-dominated forest, in which light limitation for understorey species may have limited N responses at high input rates. This illustrates that the responses to increased N deposition may differ depending on site conditions, and that complex biotic interactions, including altered shading from the tree canopy at high levels of N, may complicate the interpretation of the effects of N addition (Gilliam, 2006; Nordin et al., 2009).

Bryophytes depend directly on the wet and dry deposition of N and are, therefore, considered to be highly sensitive, even to small changes in supply. Additional N doses of between 12.5 and 50 kg N ha⁻¹ yr⁻¹ to coniferous forests in northern Sweden, led to an increase in arginine concentrations of *Pleurozium schreberi* and *Dicranum majus* in an irrigation experiment (Nordin et al., 1998). This was an indication that the mosses were unable to respond to N additions by increased growth and that, instead, N was accumulated in the form of arginine. High amino acid concentrations may be harmful to bryophytes, and correlated with reductions in the growth length of *Sphagnum* (Nordin and Gunnarsson, 2000). Additionally, it was found that N-induced

decreases in the abundance of specific bryophytes may persist long after N input has been terminated (Nordin et al., 2005).

The concentration of total N in moss sampled in Sweden, Norway and Finland correlated linearly with the wet deposition of N in the range of 0 to 15 kg N ha⁻¹ yr⁻¹ (Harmens et al., 2011). A recent field study (Salemaa et al., 2020) showed accumulation of free NH₄⁺ and total N concentrations of up to 2% in *Pleurozium schreberi* and *Hylocomium splendens* at throughfall N deposition < 3.5 kg N ha⁻¹ yr⁻¹ in background areas in Finnish boreal forests. Overall, the bryophyte total N concentrations were higher in Norway spruce than in Scots pine forests, and higher inside than outside forests, as the throughfall especially in southern Finnish spruce forests was enriched with DON leached from the canopy. The high total N concentration in bryophytes at such a low total N deposition level highlights the role of dry-deposited N and DON in areas with low precipitation (400-700 mm yr⁻¹) and low temperatures. In support of this, Pitcairn et al. (2006) concluded that bryophytes reached high N concentrations in areas with high precipitation only when rainfall constantly contained large concentrations of N. Pearce and Van der Wal (2008) demonstrated in experiments the importance of NO₃⁻ and NH₄⁺ concentrations in wet deposition for the growth of a sensitive bryophyte species, for instance *Racomitrium lanuginosum*, irrespective of the N dose.

Biological N-fixation is a major driver of terrestrial ecosystem processes in natural ecosystems (Chapin et al., 1986). For instance, it was suggested that the symbiosis of feather mosses such as *Pleurozium schreberi* with cyanobacteria (*Nostoc*) was the main source of ecosystem N in natural boreal forest and tundra ecosystems (DeLuca et al., 2002). N deposition may affect biological N-fixation already at low levels. A CL_{emp}N < 5 kg N ha⁻¹ yr⁻¹ is proposed based on biological N₂ fixation by cyanobacteria living in association with bryophytes (Gundale et al., 2011; Salemaa et al., 2019; Zackrisson et al., 2004). Zackrisson et al., (2004) found that a surface application of 4.5 kg N ha⁻¹ yr⁻¹ as NH₄NO₃ eliminated N₂ fixation in *Pleurozium schreberi* from northern Sweden (65-66°N). In their field experiment with NH₄NO₃ granules applied within one week of snow melt, the lowest N addition (3 kg N ha⁻¹ yr⁻¹) resulted in a nearly 50% reduction in N₂ fixation per unit mass of *Pleurozium schreberi* relative to control (background deposition of 2 kg N ha⁻¹ yr⁻¹) in a northern Swedish (64°N) Scots pine forest (Gundale et al., 2011). Salemaa et al. (2019) reported, in turn, that biological N₂ fixation was switched off in *Pleurozium schreberi* and *Dicranum* sp. in northern Finnish Scots pine and Norway spruce forests at a total N deposition in BD of 1-2 kg N ha⁻¹ yr⁻¹, while *Hylocomium splendens* still showed very weak N₂ fixation in southern Finland at 3-4 kg N ha⁻¹ yr⁻¹ and a N concentration of 1.48% in bryophyte tissue. The amounts of N in TF were about 0.5-1.5 kg N ha⁻¹ yr⁻¹ in northern and 2-3 kg N ha⁻¹ yr⁻¹ in southern Finland. Inorganic N was a significant predictor of N₂ fixation in both BD and TF deposition models (Salemaa et al., 2019).

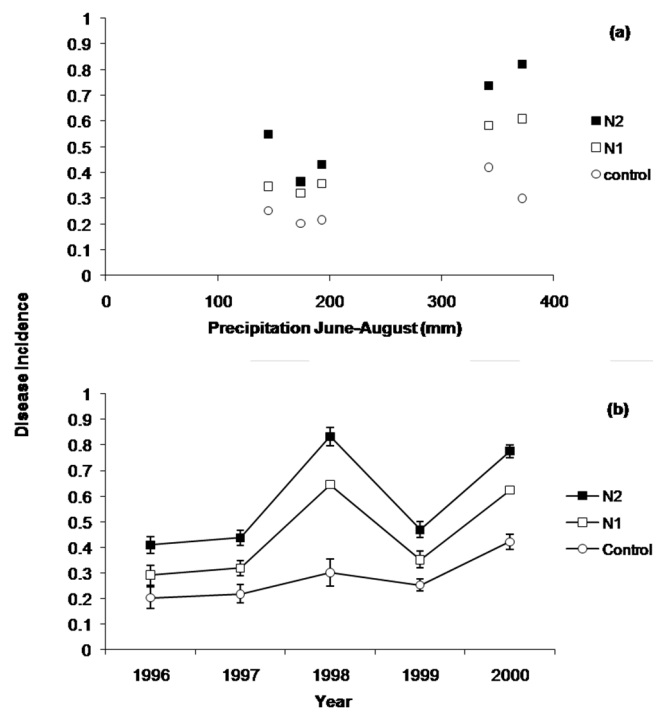
Increased sensitivity to pathogens and herbivores

Higher concentrations of N in plant tissue after increased N input may result in greater damage from pathogens and herbivores. Attacks by foliar pathogens on *Vaccinium myrtillus* and *Deschampsia flexuosa* increased after N additions of 12.5 kg N ha⁻¹ yr⁻¹ for 5-10 years (background deposition of around 2 kg N ha⁻¹ yr⁻¹; Nordin et al. (1998, 2005, 2006)). In areas with N depositions of over 6 kg N ha⁻¹ yr⁻¹, *Vaccinium myrtillus* was more susceptible to leaf pathogens than in areas with lower levels of N deposition (Strengbom et al., 2003).

For the host plants, the effects of increased N availability on pathogens often depend on interactions with weather conditions. For example, the disease incidence of *Valdensia heterodoxa* infecting *Vaccinium myrtillus* correlates positively with summer precipitation. Higher precipitation levels increase the effect of added N (Figure 9.25). This means that the effect of

increased N input may be small during dry years and large during wet years. In *Deschampsia flexuosa*, the two pathogens *Uromyces airae-flexuosae* and *Telimenella gangraena* showed opposite responses to drought stress. *Uromyces airae-flexuosae* decreased, while *Telimenella gangraena* increased in response to drought treatment (Nordin et al., 2006).

Figure 9.25. Disease incidence (proportion of diseased leaves) from the parasitic fungus *Valdensia heterodoxa* on *Vaccinium myrtillus* leaves, in relation to summer precipitation (a), and N addition (b). N corresponds to 0 (control), and to treatments of 12.5 (N1), and 50 kg N ha⁻¹ yr⁻¹ (N2). Each treatment was replicated six times (n = 6). Vertical bars show one SE. From Strengbom et al. (2006).



Source: Strengbom et al., 2006

Plant damage caused by insect herbivores may increase after increased N input as insect population densities may be limited by low concentrations of N in plants. Nordin et al. (1998) demonstrated that damage caused by insect herbivores in *Vaccinium myrtillus* increased after N additions corresponding to 12.5 kg N ha⁻¹ yr⁻¹ (background deposition of around 2 kg N ha⁻¹ yr⁻¹). Strengbom et al. (2005) demonstrated that *Operophtera brumata* larvae feeding on N-fertilised *Vaccinium myrtillus*, showed larger adult mass (indicating higher fecundity). Although N addition may have a positive effect on the population densities of *Operophtera* larvae (Nordin et al., 2009), increased predation or higher parasitoid load may limit such positive effects (Kytö et al., 1996; Strengbom et al., 2005), resulting in no or only minor effects on insect population densities.

Effects of different N forms

When assessing the effects of N deposition, it is important to bear in mind that plant species exhibit differences in their capacities to utilise N. Although the effect of N deposition on an ecosystem is mainly related to the quantity of N deposited, the qualitative aspect also needs to be acknowledged. Few studies have addressed the quantitative differences of N deposition on taiga habitats. Nordin et al. (2006) compared the effects of additions of 12.5 and 50 kg N ha⁻¹ yr⁻¹ in the form of NH₄NO₃, (NH₄)₂SO₄, or KNO₃, to a spruce forest in northern Sweden (background

deposition of around 2 kg N ha⁻¹ yr⁻¹). There was also a K₂SO₄ treatment to test the effect of the counter ions. *Deschampsia flexuosa* took up more NO₃⁻ than NH₄⁺. Consequently, NO₃⁻ addition induced more grass growth than additions of NH₄⁺. It was concluded that increased grass growth is less likely to occur when N is deposited as NH₄⁺ than when it is deposited as NO₃.

Epiphytic lichens

Whole mature Norway spruces were fertilised during four vegetation seasons with an automated irrigation-fertilisation system administered daily as a diffuse spray across the entire canopy, with N in the form of NH₄NO₃ at five levels, equivalent to 0.6, 6, 12.5, 25 and 50 kg N ha⁻¹ yr⁻¹ at Vindeln Experimental Forests in northern Sweden (Johansson et al., 2012). The background deposition in TF was 2 kg N ha⁻¹ in the area between late May and early October with 78% NH₄⁺, 17% amino acid N and 5% NO₃⁻ (Forsum et al., 2006). The composition of the epiphytic lichen community was monitored on a yearly basis. N deposition reduced the species richness of the epiphytic lichen communities even at the lowest level of fertilisation application (6 kg N ha⁻¹ yr⁻¹). The authors concluded that the applied wet deposition of NH₄NO₃ of 6 kg N ha⁻¹ yr⁻¹ (over the background deposition) was above the critical load of N deposition for boreal epiphytic lichen communities.

Esseen et al. (2016) studied the distribution of epiphytic hair lichens in the lower canopy of Norway spruce throughout Sweden from temperate to boreal and subalpine forests. They calculated the mean annual N deposition of N (dry + wet depositions) for 20 km x 20 km grid cells from data provided by the Swedish Meteorological and Hydrological Institute and then extracted the data for each study plot. The results suggest a CL_{emp}N of 3.9 kg N ha⁻¹ yr⁻¹ for *Alectoria* and of 5.7 kg N ha⁻¹ yr⁻¹ for *Usnea*.

Most hair lichens have been classified as oligotrops in nutrient-poor sites (McCune and Geiser, 2009). The lower end of the total deposition range of 3.5-5 kg N ha⁻¹ yr⁻¹ by Esseen et al. (2016) corresponds to the moderate-risk critical load of 3.5 kg N ha⁻¹ yr⁻¹ resulting in a 50% decline in the diversity and abundance of forage lichens (including hair lichens) estimated by Geiser et al. (2019). A CL_{emp}N of 3-5 kg N ha⁻¹ yr⁻¹ is proposed for taiga spruce forests (T3F), especially those with low precipitation but high concentrations of dissolved N, based on the European studies by Esseen et al. (2016) and Giordani et al. (2014).

Reversibility of N-induced effects

Although more than 47 years had passed since N addition (~100 kg N ha⁻¹ yr⁻¹) to a spruce forest in northern Sweden had ceased, the production of fruit-bodies by N-sensitive mycorrhizal fungi such as *Russula* and *Cortinarius* species were still 39 to 85% lower than in unfertilised control plots (background deposition of around 2-3 kg N ha⁻¹ yr⁻¹ total N). In addition, the abundance of the bryophyte *Hylocomium splendens* was still 70% lower in plots formerly treated with N than in control plots, while N-favouring bryophytes such as *Brachytecium* and *Plagiothecium* species only occurred in plots previously treated with N (Strengbom et al., 2001). This is an indication that the reversibility of N-induced effects on this forest habitat is low, and that spruce taiga woodlands are very susceptible to high N depositions.

Summary

In Sweden, additions of 6 kg N ha⁻¹ yr⁻¹ over a four-year period (background deposition of around 2 kg N ha⁻¹ yr⁻¹) were found to increase the abundance of *Deschampsia flexuosa* by around 50% (UNECE, 2007), whereas the abundance and occurrence of *Vaccinium myrtillus* was lower when N deposition exceeded this level (Strengbom et al., 2003). Additions of 5 kg N ha⁻¹ yr⁻¹ over four years resulted in 70% higher shoot densities of *Deschampsia flexuosa* in N-treated plots, compared to control plots (Kellner and Redbo-Torstensson, 1995). In addition, the

increased occurrence of *Deschampsia flexuosa* in Norway, between 1988 and 1993, was also attributed to N deposition (Økland, 1995). Large effects on species composition and increased sensitivity to leaf pathogens have been reported from N additions of 12.5 kg N ha⁻¹ yr⁻¹ within a decade (Nordin et al., 2005, 2006; Strengbom et al., 2002). The low reversibility of N-induced effects (Strengbom et al., 2001) support the suggestion that spruce taiga woodlands is a habitat class that is sensitive to N deposition. The most sensitive organisms in terms of biodiversity are the epiphytic lichens and the critical loads intended to protect these organisms very much depend on the N concentration of the water on their surfaces. Based on the current European data on epiphytic lichens and bryophytes, a CL_{emp}N of 3-5 kg N ha⁻¹ yr⁻¹ – the total deposition of inorganic N – is considered as a ‘reliable’ critical load for spruce taiga woodlands (T3F).

9.4.4 Pine taiga woodlands (T3G – light taiga)

Ground vegetation

Response to N deposition may partly depend on initial site productivity. In general, forests dominated by Scots pine, *Pinus sylvestris* (T3G), tend to be less productive than forests dominated by Norway spruce, *Picea abies* (T3F). There are indications that ground vascular plant species in forests dominated by Scots pine may be more resistant to increased N input than forests dominated by Norway spruce. Correlative data along the Swedish N deposition gradient show that in spruce forests, there is a significant drop in the occurrence of *Vaccinium myrtillus* when N deposition exceeds 6 kg N ha⁻¹ yr⁻¹. However, in pine-dominated forests, this effect is seen not until N deposition exceeds 12 kg N ha⁻¹ yr⁻¹ (Figure 9.24; Strengbom et al., 2003). For *Vaccinium vitis-idaea* there appears to be less difference in response to N deposition between pine- and spruce-dominated forests. Effects on plant community compositions from increased N inputs often depend on how light availability is influenced by increased N availability (Hautier et al., 2009; Strengbom et al., 2004). This is especially important in forested systems where the response in understory vegetation is often determined by the overstorey response to increased N availability (Gilliam, 2006; Oberle et al., 2009). Compared to Norway spruce stands, Scots pine stands tend to have less canopy cover with more light reaching the forest floor, due to lower productivity and different plant architecture. The implication here is that such stands will be less sensitive to the reduced light availability resulting from N-induced increased tree growth. This may explain why *Vaccinium myrtillus* shows lower responsiveness to elevated N input in pine forests than in spruce forests (Strengbom et al., 2003). The lower productivity of pine forests may also explain why, in these habitats, *Vaccinium myrtillus* may initially be unresponsive or may even increase (at the expense of other dwarf shrubs such as *Vaccinium vitis-idaea*) after N addition (Kellner and Redbo-Torstensson, 1995). However, if N input is sufficiently high, or lasts long enough, the abundance of *Vaccinium myrtillus* will also be reduced in these pine-dominated stands (at the expense of grasses and herbs) (Strengbom et al., 2001). The pattern of gradual replacement of species according to their N strategy (e.g. nutrient-use efficiency) may also explain why the species richness of ground- and field-layer vegetation in low-productivity habitats such as boreal forests, may remain unchanged or may initially even increase in response to increased N deposition (Bobbink, 2004). Despite these indications of differences in N sensitivity between spruce- and pine-dominated forests, the available data on such differences are sparse, and more data are needed to elucidate these differences.

Ground-living lichens often make up most of the species’ richness of the ground vegetation in pine forests. Several experiments identified this group of plants as sensitive and one of the first plant groups to disappear as a consequence of increased inputs of N (Dirkse and Martakis, 1992; Mäkipää, 1994, 1998; Skrindo and Økland, 2002; Strengbom et al., 2001). In Sweden, all ground-living lichens disappeared from plots receiving 30 to 60 kg N ha⁻¹ yr⁻¹ (background deposition of

around 2-3 kg N ha⁻¹ yr⁻¹) for 20 to 30 years (Strengbom et al., 2001; Van Dobben et al., 1999). In Norway the reduced occurrence of lichens was observed after N additions of 30 to 90 kg N ha⁻¹ yr⁻¹ (background deposition of 5-6 kg N ha⁻¹ yr⁻¹) (Skrindo and Økland, 2002). The mechanism by which N deposition negatively influences the abundance of ground-living lichens is not clearly understood. Mäkipää and Heikkinen (2003) reported that the relative abundance of *Peltigera aptosa*, which has cyanobacteria as its photobiont (and is expected to be N sensitive), decreased in Finland between 1951 and 1986, and again in 1995. It should be noted that the observed changes may not have been due solely to N deposition. Although several studies reported that increased N availability may disrupt physiological processes in lichens (Dahlmann et al., 2002; Kytöviita and Crittenden, 2007), the decreased abundance of lichens may also be an effect of increased competition from vascular plants that respond positively to N (Cornelisen et al., 2001).

Together with ground-living lichens, bryophytes make up the plant group that appears to be most sensitive to elevated N deposition. There are, however, major differences in responses between species, ranging from strong negative effects to positive effects. In pine forests, the decreased abundance of *Hylocomium splendens* and *Pleurozium schreberi*, and increased abundance of litter-dwelling species, such as *Brachythecium* and *Plagiothecium* species, are frequently reported after increased N input (Dirkse and Martakis, 1992; Mäkipää, 1994; Nordin et al., 2005; Skrindo and Økland, 2002; Strengbom et al., 2001; Van Dobben et al., 1999). Strengbom et al. (2001) reported a more than 70% reduction in the abundance of *Pleurozium schreberi* and the increased abundance of *Brachythecium* species, after 29 years of N additions of 34 kg N ha⁻¹ yr⁻¹ (background deposition of around 2-3 kg N ha⁻¹ yr⁻¹). Skrindo and Økland (2002) reported that the occurrence of *Ceratodon purpureus*, *Dicranum fuscescens*, *Dicranum polysetum* and *Dicranum spurium* decreased with increasing N additions (30-90 kg N ha⁻¹ yr⁻¹).

Optimal growth in relation to N inputs varies between species, and may partly explain why some species are more sensitive to increased N input than others (Salemaa et al., 2008). Moreover, the abundance of *Pleurozium schreberi* correlated negatively with tissue concentrations of amino acids (Nordin et al., 2005), indicating that excess N may be detrimental to this moss species. In addition, as for lichens, the reduced abundances of species such as *Hylocomium splendens* and *Pleurozium schreberi*, may also be partly explained by more intense competition from vascular plants under increased levels of N. The CL_{emp}N of 3-5 kg N ha⁻¹ yr⁻¹ based on bryophyte responses in spruce taiga woodlands also applies to pine taiga woodlands (Salemaa et al., 2019, 2020).

Effects of different N forms

As far as we know, no data are available on the specific effects on pine forests of the different forms of N, except that of Salemaa et al. (2020). These authors found that the relationship of N concentrations for mosses in clearings was best with bulk deposition outside the forest and for mosses in the forest it was DON in throughfall. The responses reported for spruce forests are mostly thought to be valid for pine forests, too (see Chapter 9.4.3). However, the dry deposition of N is most likely higher in Norway spruce than in Scots pine forests (Lövblad et al., 1992), although dry deposition in northern coniferous forests is difficult to monitor (Karlsson et al., 2019).

Green algae and epiphytic lichens

Most of the recent studies on epiphytic lichens and N pollution, especially those done in Europe, focussed on assessing the critical levels of reactive gaseous N compounds for lichen diversity etc. instead of the critical loads. A Canadian study on epiphytic lichens in Jack pine (*Pinus banksiana* forests) in northern Alberta and Saskatchewan proposed a total deposited N threshold (TDN) of 1.5-3 kg N ha⁻¹ yr⁻¹ (Vadinther, 2019). The modelled N deposition data used by Vadinther (2019)

accounted for 12 different forms of N. The TDN correlated highly with dry nitrogen oxide deposition (DNO) and dry nitrogen dioxide deposition (DNO₂), both of which were highly relevant drivers in the gradient Forest model which was used. Lichen community thresholds (based on epiphytic macrolichens such as *Vulpicida pinastri* and *Evernia mesomorpha* and a ground-living lichen *Cladina mitis*) across both DNO and DNO₂ gradients corresponded to a TDN threshold of 1.4-2.4 kg N ha⁻¹ yr⁻¹. The results of Vadinther (2019) from a smaller scale study suggest, however, a biodiversity-based community CL_{emp}N of 5.6 kg N ha⁻¹ yr⁻¹. The latter CL_{emp}N is based on several vascular plant species and bryophytes in addition to epiphytic lichens in Canadian pine taiga woodlands.

N concentrations in lichens can be used to estimate the throughfall deposition of inorganic N in areas for which no measured throughfall data are available (Root et al., 2013). Average N concentrations of 0.3-0.6% have been reported in *Platismatia glauca* and *Hypogymnia physodes* in background areas receiving 0.5-2.0 kg N ha⁻¹ yr⁻¹ as total or TF deposition (Dahlman et al., 2003; Johansson et al., 2010, 2011)), about 2.5 kg NO₃⁻-N ha⁻¹ yr⁻¹ as WD (Bruteig, 1993) or 0.02-0.1 mg NH₄⁺-N l⁻¹ in precipitation (Bruteig, 1993; Geiser and Neitlich, 2007). Based on the lichen N concentrations, atmospheric NO₂ and NH₃ concentrations, the frequencies of green algae + *Scoliocosporum chlorococcum* and selected acidophytic macrolichen indicator species on Scots pine trunks, Manninen (2018) proposed a CL_{emp}N of 2-3 kg N ha⁻¹ yr⁻¹ as a total NO₂+NH₃ deposition for Scots pine forest in Finland. There, the precipitation is low and the concentration of dissolved N may thus be higher than in areas with similar atmospheric concentrations of gaseous N pollutants but high precipitation. In support of the importance of dry deposition and N concentration, Frahm (2013) related the N sensitivity of *Hypogymnia physodes* to its low conductivity (osmotic value) and low water uptake from salt solutions compared to the nitrophyte species such as *Phaeophyscia orbicularis* and *Physcia adscendens* with high osmotic tolerance of the salt effects on N compounds. Frahm (2013) also concluded that the nitrophilous lichen species were drought resistant thanks to their high osmotic values and were, therefore, more competitive in areas with low humidity than other lichen species.

The responses of epiphytic lichens highlight the need for measurements and more detailed data on dry deposition of N compounds to assess the role of dry deposition on the most sensitive organisms in taiga forests.

Reversibility of N-induced effects

Quist et al. (1999) reported swift recovery in soil N concentrations after the cessation of 20 years of N additions of 108 kg N ha⁻¹ yr⁻¹ (Norrliden site, background deposition of around 2 kg N ha⁻¹ yr⁻¹). However, data on the reversibility of the N-induced effects on biodiversity at this site, suggest that reversibility is a slow process since the composition of understorey differed markedly from control. Strengbom et al. (2001) did not observe any recovery of plant species composition or fruit-body production nine years after N additions had ceased. And 14 years later, Chen and Högberg (2006) noted that the N mineralisation rates in Norrliden were still elevated for the plots that had been treated with N. In a gradient study, Weldon and Grandin (2021) also observed only slow recolonisation of N- and/or S-sensitive epiphytic lichen species in pine or spruce dominated forests over 20 years (1997-2016) in Sweden, despite the current suitable environmental conditions.

Summary

Compared to spruce taiga woodlands, there are indications that, for pine forests, negative effects on the biodiversity of vascular plant species from increased N input start to occur at higher N input rates. However, the data that support that pine forests are less sensitive to increased N input compared to spruce forests are not conclusive. Strengbom et al. (2003) found that, in pine

forests, *Vaccinium vitis-idaea* had a significantly lower occurrence when N depositions were higher than 6 kg N ha⁻¹ yr⁻¹. However, they also found that N depositions had to be over 12 kg N ha⁻¹ yr⁻¹ before *Vaccinium myrtillus* would occur at significantly lower rates. Experiments using higher N loads (over 20-30 kg N ha⁻¹ yr⁻¹) often show major effects on the composition of understorey vegetation (Dirkse and Martakis, 1992; Strengbom et al., 2001; Van Dobben et al., 1999). The effects of N on plant community composition (Strengbom et al., 2001) and N mineralisation (Chen and Högberg, 2006) also appear to reverse only slowly when external N inputs are halted. In summary, these results suggest that pine taiga woodlands are sensitive to increased N deposition. With the support of gradient studies that revealed the effects at N loads of less than 10 kg N ha⁻¹ yr⁻¹, it was recommended in the previous review version to set the CL_{emp}N range for Pine taiga woodlands (T3G, formerly G3B) at 5 to 10 kg N ha⁻¹ yr⁻¹. It was considered as 'quite reliable'. The study of Giordani et al. (2014) included both northern and southern European pine forests. A 'quite reliable' CL_{emp}N of 2-5 kg N ha⁻¹ yr⁻¹ is proposed for pine taiga woodland (T3G) based on epiphytic lichen responses, especially species' composition, to inorganic N deposition in TF reported in Europe, Canada and the USA, where there are still more pristine areas in terms of N deposition than in Europe. In comparison, the study by Geiser et al. (2021) indicated initial shifts from pollution-sensitive toward pollution-tolerant species already at 1.5 kg N ha⁻¹ yr⁻¹ and 2.7 kg S ha⁻¹ yr⁻¹. Moreover, these CL_{emp}N values are considered constant under any climate regime nationwide in the USA.

9.4.5 Summary T3F and T3G

Table 9.2 summarises the CL_{emp}N ranges on spruce and pine taiga woodlands (T3F and T3G)

Table 9.2. CL_{emp}N and effects of exceedances on spruce and taiga woodlands. ## reliable; # quite reliable and (#) expert judgement. Changes with respect to 2011 are indicated as values in bold.

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2011 reliability	2021 kg N ha ⁻¹ yr ⁻¹	2021 reliability	Indication of exceedance
Dark taiga	T3F	5-10	##	3-5^a	##	Changes in epiphytic lichen and ground-layer bryophyte communities; increase in free-living algae; decline in N-fixation
<i>Pinus sylvestris</i> light taiga	T3G	5-10	#	2-5^a	#	Changes in epiphytic lichen and ground-layer bryophyte communities, increase in free-living algae; decline in N-fixation

a) Mainly based on N deposition impacts on lichens and bryophytes.

9.5 Effects of N deposition on Mediterranean ecosystems

The Mediterranean biogeographical region is characterised by a climate with hot dry summers and rainy mild-cool winters. Spring and autumn are usually the growing seasons since the typical summer drought imposes limitations on biological activity. Besides the strong seasonal changes, the climate also presents high interannual variability. In Europe, this biogeographical region is located around the Mediterranean Basin. The climate variability acts together with a contrasting topography and is the driver behind a very rich biodiversity, particularly of plants.

25,000 flowering plants were identified that represent 10% of all known plants on earth. Half of the plants are also endemic. Mediterranean forests are also very diverse, with up to 100 different tree species. Almost half of the plants and animals listed in the Habitats Directive occur in the Mediterranean region. This high biodiversity makes the Mediterranean area one of the biodiversity hotspots for conservation priorities. The main threats to biodiversity in this region include land occupation for construction, forest fires, chronic water shortage linked to climate change, invasive species and an abandonment of traditional agriculture and pastoral regimes (Commission, 2009). Atmospheric N deposition has only recently been recognised as an additional pressure for Mediterranean biodiversity (Ochoa-Hueso et al., 2011; García-Gómez et al., 2020). High ozone concentrations are another particularity of the area. They are caused by the typical high air temperatures and solar radiation, together with the stability of air masses that constitute an additional threat to plant functioning and development (Ochoa-Hueso et al., 2017).

9.5.1 Deciduous broadleaved forest (T1)

Fagus forest on acid soils (T18)

The long-term variability of growth rates was analysed in four beech-dominated forests selected along a latitudinal transect across the Italian peninsula in the montane belt. The long-term median values of atmospheric N deposition, estimated with the WCRP-CMIP6 model, ranged from 4 to 9 kg N ha⁻¹ yr⁻¹ with maximum annual values up to 15 kg N ha⁻¹ yr⁻¹ (Gentilesca et al., 2018). The positive N deposition effect on annual height and volume growth N was more important than climate variation and this effect reached a threshold value of 10 kg N ha⁻¹ yr⁻¹. On the basis of the results of this study and the joint consideration of the results of similar forests growing in temperate areas, a CL_{emp}N of 10-15 kg N ha⁻¹ yr⁻¹ is proposed based on 'expert judgement'.

9.5.2 Broadleaved evergreen forest (T2)

Mediterranean evergreen Quercus forest (T21)

A CL_{emp}N lower than 26 kg N ha⁻¹ yr⁻¹ was proposed for evergreen cork-oak (*Quercus suber*) woodlands in Portugal along a gradient from a point-source barn using epiphytic lichen functional groups diversity (Pinho et al., 2012). Increasing N deposition promoted a change from oligotrophic to nitrophytic communities. A long-term monitoring study in a dense forest dominated by holm-oak (*Quercus ilex*) and beech (*Fagus sylvatica*) in north-eastern Spain found that an average total atmospheric inorganic N deposition of 14.3 kg N ha⁻¹ yr⁻¹ was mostly retained in the catchments and only 2% was exported as DIN (Avila et al., 2020; Avila and Rodà, 2012). However, NO₃⁻ concentrations in the stream increased in recent years probably linked to forest maturation and climate warming. Another study comparing four holm-oak forests in Spain with a range of measured total inorganic N deposition of 9.4-28.9 kg N ha⁻¹ yr⁻¹ (Garcia-Gomez et al., 2018) found that the forest with the lowest deposition registered events of high concentrations of NO₃⁻ in the soil water (up to 28.15 mg NO₃-N l⁻¹ at 20 cm depth) linked to ephemeral pulses of N deposition with the first rainfall events after the summer drought (García-Gomez et al., 2016). This phenomenon of temporal losses of N caused by a temporal asynchrony between N availability and plant N demand has been described as typical in Mediterranean ecosystems (Meixner and Fenn, 2004).

A fertilisation experiment of a holm-oak forest dominated by the evergreen tree *Quercus ilex* and the tall shrub *Phillyrea latifolia*, accompanied by other Mediterranean shrub species, with 60 kg N ha⁻¹ yr⁻¹ over background deposition, showed that after one year of N addition, the biomass of all microbial groups increased except for fungi. This increased the relative dominance of bacteria

(Peguero et al., 2021). The higher bacterial biomass strongly favoured oribatid mites over springtails, which caused changes in the structure of the mesofauna arthropod community. This propagated the effects of N inputs throughout the soil food web. The effect of increasing N availability may be compensated by the strong constraint of drought on soil microbe activity and biomass.

With regard to forest ground vegetation, a $CL_{emp}N$ of 5-15 kg N ha⁻¹ yr⁻¹ is proposed for Mediterranean xeric grasslands (see Chapter 7.2.2), communities that typically constitute the understory vegetation of Dehesas (Spain) and Montados (Portugal), a traditional agroforestry system with open evergreen oak woodland and an herbaceous layer.

Based on the new information available, a $CL_{emp}N$ of 10-15 kg N ha⁻¹ yr⁻¹ is proposed for Mediterranean evergreen *Quercus* forests (T21), based on 'expert judgement'.

9.5.3 Coniferous forests (T3)

Mediterranean mountain *Abies* forest (T33)

N effects have been described in Mediterranean mountain *Abies* forests (T33, formerly G3.1) of the endemic fir *Abies pinsapo* along a gradient of atmospheric N deposition in the south of Spain. Three comparable fir forests were analysed in a bulk N deposition gradient ranging from 3.5 to 10.4 kg N ha⁻¹ yr⁻¹. Chronic N deposition reduced fine root biomass, decreased photosynthetic nutrient use efficiency and shifted forests from N l to P limitation (Blanes et al., 2013a, b). The site with the highest N deposition showed changes in foliar N:P stoichiometry, smaller photosynthetic nitrogen use efficiency (PNUE), higher photosynthetic phosphorus use efficiency (PPUE) and indications of initial soil N saturation and N losses, compared to sites with up to 4.9 kg N ha⁻¹ yr⁻¹ of N deposition (Blanes et al., 2013a, b). The results indicated that the atmospheric N bulk deposition of 10.4 kg N ha⁻¹ yr⁻¹ had already exceeded the threshold where increasing N availability was beneficial for tree growth and physiology. Since dry deposition needs to be considered, a $CL_{emp}N$ of 10-15 kg N ha⁻¹ yr⁻¹ can be proposed for the protection of Mediterranean fir forests (T33), based on 'expert judgement'.

Mediterranean montane *Pinus sylvestris*- *Pinus nigra* forest (T37)

No specific fertilisation experiments or gradient studies are available for montane pine forests in the Mediterranean region. Sardans et al. (2016) described how N deposition was causing nutrient imbalances with increasingly limiting roles of P and other nutrients such as K in European *P. sylvestris* forests, including forests in the Mediterranean region. However, these effects were more evident in the central area of distribution of this species where higher levels of N deposition were observed. In the most recent revision of empirical critical loads, a value of 3 to 15 kg N ha⁻¹ yr⁻¹ was established for Mediterranean *Pinus* woodland based on 'expert judgement' of the results of Californian studies on N deposition effects in *Pinus ponderosa* (Fenn et al., 2008). A subsequent review established a $CL_{emp}N$ of 3 to 39 kg N ha⁻¹ yr⁻¹ for Mediterranean mixed-conifer forests (Pardo et al., 2011). The lowest $CL_{emp}N$ was based on lichen chemistry and community changes. For NO₃⁻ leaching and fine root biomass the critical load was 17 kg N ha⁻¹ yr⁻¹. For soil acidification and understory biodiversity the critical load was 26 kg N ha⁻¹ yr⁻¹. These values took into account throughfall N deposition. Since some N deposited on leaf surfaces could have been absorbed by plants or the phyllosphere, particularly in smaller deposition areas, a $CL_{emp}N$ of 5 to 17 kg N ha⁻¹ yr⁻¹ is proposed, based on 'expert judgement', for the protection of Mediterranean montane *Pinus* forests (T37).

Mediterranean lowland to submontane *Pinus* forest (*P. pinaster*, *P. halepensis*, *P. pinea*) (T3A)

The structure and functioning of microbial communities in biocrusts were studied in a N gradient study across semiarid Mediterranean ecosystems throughout Spain including shrubland, grassland and woodland sites (Ochoa-Hueso et al., 2013, 2016). This gradient included two *Pinus halepensis* woodlands. The authors found that the species abundance of both soil bacteria and fungi was reduced with N availabilities while green algae and cyanobacteria richness increased, thereby contributing to ecosystem eutrophication (Ochoa-Hueso et al., 2013, 2016). Since linear responses were found in most soil indicators along the 4.3-7.3 kg N ha⁻¹ yr⁻¹ gradient, a CL_{emp}N of 4.3 kg N ha⁻¹ yr⁻¹ was proposed as the lowest end of the gradient (Ochoa-Hueso et al., 2013). In another N gradient in Spain including 28 sites of *Quercus coccifera* shrublands (n=18) and *P. halepensis* forests (n=10) covering a range of modelled inorganic N deposition between 4.4 and 8.1 kg N ha⁻¹ yr⁻¹, soil acidification was detected in *Q. coccifera* but not in *P. halepensis* sites (Ochoa-Hueso et al., 2014). On the other hand, N deposition increased the C and N stored in the soils of Aleppo pine forests. A CL_{emp}N of 5-10 kg N ha⁻¹ yr⁻¹ may be sufficient to protect Mediterranean *Pinus* forests (T3A), based on 'expert judgement'. This critical load is a good fit with the CL_{emp}N of 5-15 kg N ha⁻¹ yr⁻¹ established as 'quite reliable', for Garrigue shrublands (S6) that frequently accompany these forests.

Effects on soil microbiota in Mediterranean ecosystems

The abundance of soil bacteria and fungi was studied in an N gradient study across semiarid Mediterranean ecosystems throughout Spain including sites in shrubland, grassland and woodland (Ochoa-Hueso, 2016). The author observed a reduction in the species abundance of both bacteria and fungi with N availabilities above 4-7 kg N ha⁻¹ yr⁻¹. In an earlier study on AMF spore abundance in a coastal sage scrub vegetation in California, also representative for Mediterranean ecosystems, a shift in AM community composition along a gradient in N deposition from 10 to 35 kg N ha⁻¹ yr⁻¹ was observed (Egerton-Warburton and Allen, 2000).

9.5.4 Summary

Table 9.3 summarises the CL_{emp}N ranges of the Mediterranean ecosystems.

Table 9.3. CL_{emp}N and effects of exceedances on Mediterranean forest ecosystems (EUNIS class T). ## reliable, # quite reliable and (#) expert judgement. Changes with respect to 2011 are indicated as values in bold.

Ecosystem type	EUNIS code	2021 kg N ha ⁻¹ yr ⁻¹	2021 reliability	Indication of exceedance
Mediterranean <i>Fagus</i> forest on acid soils	T18	10-15	(#)	Annual height and volume tree growth; analogy to temperate <i>Fagus</i> forest
Mediterranean evergreen <i>Quercus</i> forest	T21	10-15	(#)	NO ₃ ⁻ in soil water and streams
Mediterranean mountain <i>Abies</i> forest	T33	10-15	(#)	Tree foliar stoichiometry, tree physiology; soil N losses
Mediterranean montane <i>Pinus sylvestris</i> - <i>Pinus nigra</i> forest	T37	5-17	(#)	Lichen chemistry and community changes in Mediterranean mixed-conifer forests in USA
Mediterranean lowland to submontane <i>Pinus</i> forest	T3A	5-10	(#)	C and N stored in soils; changes in soil microorganism communities

Ecosystem type	EUNIS code	2021 kg N ha ⁻¹ yr ⁻¹	2021 reliability	Indication of exceedance
(<i>P. pinaster</i> , <i>P. halepensis</i> , <i>P. pinea</i>)				

9.6 Overall summary for forests and other wooded land (EUNIS class T)

Table 9.4 gives an overview of the CL_{emp}N ranges for forests and other wooded land.

Table 9.4. CL_{emp}N and effects of exceedances on forests and other wooded land (T). ## reliable, # quite reliable and (#) expert judgement. Changes with respect to 2011 are indicated as values in bold.

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2011 reliability	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
Broadleaved deciduous forest	T1	10-20	##	10-15	##	Changes in soil processes; nutrient imbalance; altered composition mycorrhiza and ground vegetation
<i>Fagus</i> forest on non-acid and acid soils	T17, T18	10-20	(#)	10-15	(#)	Changes in ground vegetation and mycorrhiza; nutrient imbalance, changes in soil fauna
Mediterranean <i>Fagus</i> forest on acid soils	T18			10-15	(#)	Annual height and volume tree growth; analogy to temperate <i>Fagus</i> forest
Acidophilous <i>Quercus</i> forest	T1B	10-15	(#)	10-15	(#)	Decrease in mycorrhiza; loss of epiphytic lichens and bryophytes; changes in ground vegetation
<i>Carpinus</i> and <i>Quercus</i> mesic deciduous forest	T1E	15-20	(#)	15-20	(#)	Changes in ground vegetation
Mediterranean evergreen <i>Quercus</i> forest	T21	10-20	(#)	10-15	(#)	NO ₃ in soil water and streams
Coniferous forests	T3	5-15	##	3-15	##	Changes in soil processes, nutrient imbalance; altered composition mycorrhiza and ground vegetation; increase in mortality with drought
Temperate mountain <i>Picea</i> forest, Temperate mountain <i>Abies</i> forest	T31, T32	10-15	(#)	10-15	(#)	Decreased biomass of fine roots; nutrient imbalance; decrease in mycorrhiza; changed soil fauna

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2011 reliability	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
Mediterranean mountain <i>Abies</i> forest	T33			10-15	(#)	Tree foliar stoichiometry; tree physiology; soil N losses
Temperate continental <i>Pinus sylvestris</i> forest	T35	5-15	#	5-15	#	Changes in ground vegetation and mycorrhiza; nutrient imbalances; increased N ₂ O and NO emissions
Mediterranean montane <i>Pinus sylvestris</i> - <i>Pinus nigra</i> forest	T37			5-17	(#)	Lichen chemistry and community changes in Mediterranean mixed-conifer forests in USA
Mediterranean lowland to submontane <i>Pinus</i> forest	T3A	3-15	(#)	5-10	(#)	Reduction in fine-root biomass; shift in lichen community
<i>Picea abies</i> , dark taiga	T3F	5-10	##	3-5 ^a	##	Changes in epiphytic lichen and ground-layer bryophyte communities; increase in free-living algae; decline in N-fixation
<i>Pinus sylvestris</i> light taiga	T3G	5-10	#	2-5 ^a	#	Changes in epiphytic lichen and ground-layer bryophyte communities; increase in free-living algae; decline in N-fixation

^{a)} Mainly based on N deposition impacts on lichens and bryophytes

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9.8 References

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Final Draft

10 Use of empirical critical loads of nitrogen ($CL_{emp}N$) in risk assessment and nature protection

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10.1 Introduction

In Europe, empirical critical loads of nitrogen ($CL_{emp}N$) are mainly used by the Parties to the Geneva Air Convention to quantify risks to ecosystems from air pollution, and to set emission reduction targets. The levels of implementation range from more common national broad-scale use in national risk assessments to specific regional and local applications in several countries to inform decisions on new and existing emission sources near protected habitats.

$CL_{emp}N$ for natural and semi-natural ecosystems were first presented in a background document at the 1992 workshop on critical loads staged during the UNECE Convention in Lökeborg (Sweden). Since then, they have been continuously developed on the basis of the latest scientific findings over the past 30 years. They pursue a harmonised, scientific approach developed with international experts. Based on field evidence, $CL_{emp}N$ are derived from observed N addition studies and N gradient studies which identify dose-effect relationships. The original rationale behind developing the $CL_{emp}N$ was to include the growing empirical evidence of negative nitrogen effects with a view to increasing the credibility of nitrogen CLs in policy support and regulation. This has proved very successful. $CL_{emp}N$ have come to play an important role used alone or in combination with computed critical loads. $CL_{emp}N$ constitute a powerful tool for setting (interim) targets (e.g. reducing the negative impacts of nitrogen deposition in the UK (Rowe et al., 2020)) for the protection of ecosystems or habitats.

The aim of this chapter is to provide examples of how $CL_{emp}N$ can be used on different scales and in different European countries. This is not a complete list of currently available applications. It is, rather, a selection in order to provide guidance for practitioners and policy makers on how $CL_{emp}N$ can be used in practice. The values are valid for all habitats regardless of the legal protection status of the ecosystem. Furthermore, this chapter considers how $CL_{emp}N$ are used in nature conservation practice. It also illustrates the extent to which they are a suitable metric to assess the risks for biodiversity and the diversity of ecosystems from atmospheric nitrogen inputs.

10.2 Examples of applications of $CL_{emp}N$ in risk assessments in Europe

In the Call for Data 2019-2021, the Parties to the Convention were asked if and how $CL_{emp}N$ are used at national or local level (CCE, 2019). In summary, countries use $CL_{emp}N$ at national level to determine eutrophication status, and to estimate nitrogen exceedances in all relevant natural and semi-natural ecosystems (e.g. Switzerland (FOEN, 2016) and Germany (UBA-DzU, 2021)), or only for terrestrial non-forest habitats (e.g. Austria, Ireland). In the UK, $CL_{emp}N$ are used to assess both the eutrophication of terrestrial habitats (except for managed woodland) and the acidification of soils in impacts assessments. Some specific examples of use are presented in the following section.

10.2.1 Critical load for the eutrophication ($CL_{eut}N$) approach

Since 2005, $CL_{emp}N$ have been part of the call for data published by the CCE. In 2015, the CCE published a combined dataset entitled the critical load for eutrophication ($CL_{eut}N$). The $CL_{eut}N$ of

an ecosystem is defined as either the empirical ($CL_{emp}N$) or the modelled ($CL_{nut}N$) critical load of nitrogen or – if a site has been assigned both values – the lower of the two. Since 2017, $CL_{eut}N$ have been reported by several NFCs to the CCE.

10.2.2 Parameter to verify modelled critical loads

$CL_{emp}N$ corroborate modelled critical loads from an effect-oriented perspective. Some countries use $CL_{emp}N$ to adjust the results for modelled simple mass balance (SMB) critical loads thereby increasing their reliability (e.g. Austria, Switzerland, UK). For example, based on $CL_{emp}N$, the minimum modelled CL for productive forests in Switzerland is set at $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$.

10.2.3 NEC Directive reporting (2016/2284/EU) and other National Emissions Ceilings Regulations

Critical loads and exceedances are not listed in the required parameters specified under the reporting on effects in Article 9 of the NEC Directive. The reporting is, however, required to include sites under pressure from N deposition. Furthermore, critical load exceedances have been reported by several countries as a detail for the sites (EEA, 2020).

The UK measures and reports on the impacts of air pollution on ecosystems in line with Part 5 of the National Emissions Ceilings (NEC) Regulations 2018. The obligation set out in Article 9 of the NEC Directive 2016 was transposed to the national regulations prior to the exit of the UK from the EU. $CL_{emp}N$ values feature in supplemental monitoring data and statistics that compare the ecological risk from air pollution to habitats at country level. Data are collated from the UK APIENs monitoring network. The reporting template mentioned in Article 9 is used as a starting point but both it and the monitoring network are being reviewed following the first round of Article 9 reporting (WER, 2019).

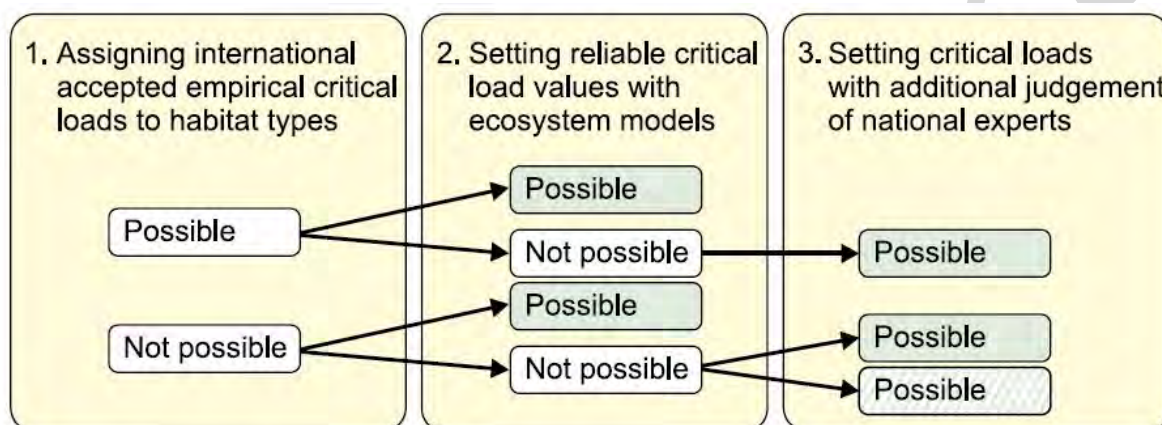
10.2.4 Use of empirical critical loads in the integrated approach in the Netherlands

Critical loads for Natura 2000 habitats have been identified using an integrated method of the empirical and the modelling approach (Van Dobben et al., 2014). The method for setting a unique critical load value per habitat is as follows: for each Dutch habitat type it was determined whether a $CL_{emp}N$ was available (Bobbink and Hettelingh, 2011). If so, the critical load was further specified with results from the simulation models for the combined effect of eutrophication and acidification (Van Dobben et al., 2006). If no $CL_{emp}N$ was available, the critical load value was based on the mean value of the results from the national simulation model. Furthermore, if no result was available from the simulation models either, the critical load value was based on expert judgement. The EUNIS type for which the critical load range was set, had to be clearly related to the habitat type. For an overview of the procedure, see Figure 10.1.

The procedure yielded N critical loads for most Annex I habitat types in the Netherlands. In most cases, empirical ranges and/or reliable model estimates were available, and critical loads could be set based on published information. In about 70 per cent of all the habitats, the models yielded critical load values within the given empirical range. In those cases where simulated critical loads were outside the empirical range, the critical load was set to the nearest extreme of the $CL_{emp}N$ values. The difference between the middle of the $CL_{emp}N$ range and the modelled values per habitat type was on average less than $1 \text{ kg ha}^{-1} \text{ yr}^{-1}$. 60 out of the 75 habitat types found in The Netherlands were sensitive to N deposition ($CLN < 34 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). These critical loads for N deposition (Van Dobben et al., 2014) are the given standard in The Netherlands, and are also accepted by the government and by the judges of the highest administrative court. And because these critical loads have unique values instead of ranges, it is clear whether or not the critical load of a specific habitat is exceeded. The habitat-specific critical loads are used together

with detailed maps of habitat occurrences and detailed deposition maps to reveal exceedances across Natura 2000 nature reserves.

Figure 10.1. Procedure used in the Netherlands for setting critical loads for Annex I habitat types. In most cases, reliable critical loads could be set after steps 1 and 2 (green boxes). In some cases, additional judgement from national experts was used for setting reliable or relatively reliable critical loads (green boxes), or even to give a best possible estimate (striped box).



10.2.5 Air Pollution Information System in the UK

At local level, the UK publishes habitat and site-specific $CL_{emp}N$ values on the Air Pollution Information System (APIS) along with other information about current and historic concentrations and deposition (CEH, 2016). APIS is a publicly available resource for risk assessment used in decision-making by regulators, local authorities, and land managers to understand air pollution effects on ecosystems. The use of site-specific evidence to ascertain which part of the $CL_{emp}N$ range (i.e. lower or higher value) should be utilised has been harmonised for UK practitioners (see APIS risk assessment guidance) in most cases (APIS Steering Group, 2013). Where $CL_{emp}N$ are less certain or require additional information prior to their application, users are advised to consult the relevant national nature conservation body. The way in which that critical load then feeds into risk assessment and decision-making processes currently varies across UK countries and decision-making bodies. For example, current approaches compare the predicted change in concentration or deposition that is expected to result from a licensing proposal with a percentage of the critical level or load in two stages: screening for likely significant effects and determination of whether adverse effects can be ruled out.

$CL_{emp}N$ also form an important part of the UK characterisation of risks to ecosystems from air pollution and promote understanding of where emission reduction measures can be targeted to maximise the benefits to those habitats. Although the form of these interventions and the locations where they have the most impact may vary between UK countries, $CL_{emp}N$ account for a large part of this activity. The national and international reporting approaches are harmonised for UK level reporting by habitat type, and are similar for the UK countries and protected sites in Scotland, Wales, Northern Ireland and England (Rowe et al., 2021). This is demonstrated both in the annual reporting for CLRTAP and in the biodiversity indicator for air pollution pressure reported for international biodiversity goals and targets (Defra, 2020). As increasing attention is paid to air pollution effects on ecosystems, the UK is investing in new evidence to support the use of critical loads and their underpinning data in risk assessment. The aims here are to

streamline their use and make the meaning of critical loads clearer for policy makers, proposers, and decision-makers.

10.2.6 Guidelines for immission control approval

In several countries, $CL_{emp}N$ are used as part of national standardised guidelines for the determination and assessment of the impact of nitrogen deposition on nearby ecosystems caused by installations requiring immission control approval. In Germany for example, the assessments are carried out for facilities that are to be newly licensed and that reach a certain size according to the Federal Immission Control Act, i.e. exceed a certain number of animals in the case of livestock facilities or a certain firing rate in the case of incineration plants. For all biotopes and ecosystems located in the vicinity of such a new facility, nitrogen sensitivity must be defined with the help of empirical critical loads (LAI N-Dep, 2012). The guideline is intended to contribute to a greater degree of legal certainty in the authorisation of installations and, by extension, to simplify and accelerate enforcement. In Denmark, $CL_{emp}N$ have served as a basis for defining classes of nitrogen sensitivity for different ecosystem types used in ammonia regulation and agricultural approval (Bak, 2014).

However, the interpretation of the ecosystem damage caused by nitrogen and the evaluation of the impact in any licensing process should also consider other non-atmospheric sources of nitrogen. This is particularly relevant for wetland habitats and aquatic systems where there are additional pathways of N input into sites, for instance, from surface water or groundwater (Rhymes et al., 2015) or from seawater for coastal habitats. There are no easy solutions to this problem, but some studies are starting to address this issue, such as trial-based approaches which calculate combined fluxes from atmospheric and other sources (e.g. Farr et al., 2019).

10.3 Empirical critical loads in nature conservation practice

Nitrogen deposition poses a serious threat to sensitive ecosystems throughout Europe. This has been confirmed by the latest international or national assessments that compare critical loads to modelled nitrogen deposition estimates (EMEP, 2020; Schaap et al., 2018; Rowe et al., 2021), and also by national survey data (Jones et al., 2016; Britton and Ross, 2018; Heinze et al., 2019). In large parts of sensitive ecosystems and habitats, the exceedance of critical loads may lead to significant changes in site conditions and biodiversity. This threatens many ecosystems, regardless of their protection status under both national and EU nature conservation law. Exceedances also have highly adverse effects on restoration and management efforts in oligotrophic and mesotrophic ecosystems and EU protected habitats, and result in far higher costs of the measures to compensate for the high nitrogen input.

Despite the evidence that nitrogen deposition is a major threat to European biodiversity, $CL_{emp}N$ have not been widely used up to now in European nature conservation practice and monitoring: For example, in the reporting guidelines for the obligatory, periodical reporting on the conservation status of the protected habitat types (reporting under Article 17 of the Habitats Directive 92/43/EEC), issued by the European Environment Agency (EEA) (DG Environment, 2017), the reporting of anthropogenic “eutrophication” is spread over the different sources of pollution in various subcategories of the main pressures and threats such as agriculture, forestry or air pollution, etc. Furthermore, only a limited number of main pressures can be reported using a ranking system and not all threats are reported in full. Anthropogenic nitrogen deposition is not a predefined threat category and the assessment with critical loads is not foreseen. Consequently, it is difficult to obtain a complete picture of eutrophication as a choice in the threat characterisation tables.

10.3.1 Critical loads for quantitative assessment of conservation status

To date, there is no guidance or recommendation on the application of critical loads to quantitatively assess conservation status and the threat of nitrogen deposition. This topic was also discussed at a workshop organised in 2013 by the Joint Nature Conservation Committee (JNCC) (Whitfield and McIntosh, 2014). The participants in the workshop already at that time recommended *“to establish a mechanism to bring together experts on the impacts of nitrogen deposition and conservation status to define common desired outcomes and develop integrated actions for consideration, how air pollution impacts relate to conservation status, and how critical load exceedance relates to achieving favourable conservation status.”* The suggestion was also made to further develop *“appropriate indicators or metrics of air pollution impacts ... for specific habitats, to support evaluation of air pollution policy”*. The recommendations of the workshop were partly taken over into national activities.

Both JNCC and NatureScot (formerly known as Scottish Natural Heritage (SNH)) have explored decision frameworks for assessing whether nitrogen deposition is a threat leading to adverse habitat condition on protected sites (JNCC 579, 2016; SNH 958, 2018). Both sets of guidelines give practical advice on how to make use of and apply $CL_{emp}N$. However, in both cases, limitations of the methodology were reported, for instance, the required $CL_{emp}N$ are not available for all of the covered biotopes or the robustness of critical load data was too low for some of the habitats. While NatureScot found that the approach for determining conservation status, for the aforementioned reasons, required further development, the JNCC approach is being considered in the UK by the other national nature conservation agencies. For example, Natural England has been developing the Nitrogen Decision Framework (NDF) to refine the assessment of exceedances of nitrogen deposition on designated sites. The NDF will help to support the assessment of the degree of risk or threat arising from a proposal as a consequence of emissions to air, based on national exceedance data and habitat quality information where it is available. The intention is to produce an automated method by which Natural England can apply the NDF to each “Site of Special Scientific Interest” unit (SSSI) in England. The application of this method will be discussed internally and more widely with the nature conservation bodies.

10.3.2 Recommendations for nature conservation practice

In Germany, $CL_{emp}N$ were used to assess the relevance of various threat factors for endangered habitat types on the Red List of Threatened Habitat Types (Heinze, 2019). Given the availability of $CL_{emp}N$ data, a nitrogen sensitivity value could be attributed to 47% of the habitats. However, in contrast, this meant that no sensitivity value could be attributed to 53% of the open-land biotopes to date. Nevertheless, the assessment suggests that nitrogen deposition is one of the predominant threats to protected habitats in Germany.

Furthermore, the latest German Interpretation Manual of Natura 2000 habitats (Ssymank et al., 2021) paves the way for the use of $CL_{emp}N$ in a) appropriate assessments according to Article 6 (3-4) of the Habitats Directive, b) site management and c) the assessment of the conservation status with respect to atmospheric nitrogen deposition as a threat. In this manual an indicative value of nitrogen sensitivity is attributed as an expert judgement to all habitat types in order to fill the gaps where $CL_{emp}N$ are still missing and need to be developed in future.

In summary, it is possible and beneficial to use $CL_{emp}N$ for both air pollution and biodiversity assessments at national and international levels. Technically, crosswise usage in both areas of application is possible. Transfer matrices of the $CL_{emp}N$ systematics and the Natura 2000 systematics have been available since the publication of the report of Bobbink and Hettelingh

(2011), and have been updated in this report (see Appendix 1). Furthermore, it is possible to narrow down the range values to concrete values with the help of limiting factors. This is already being done successfully for local approval procedures, for instance in Germany or The Netherlands.

Therefore, the recommendation is to include supplementary information on $CL_{emp}N$ also in the EU guidelines and manuals used for reporting on the conservation status of European habitat types. It is expected that a concurrent application of $CL_{emp}N$ in the area-wide assessments of risks from nitrogen deposition (EMEP, 2020; Rowe et al., 2021; Defra, 2020), as well as in national licensing practice and nature conservation, will further increase the visibility of the values, and help to harmonise risk assessment approaches at different application sites and for different pollutants.

10.4 A metric to assess risks to ecosystem biodiversity from atmospheric N inputs

Threats to ecosystems from nutrient imbalances, high nitrogen saturation, and biodiversity loss have become an increasingly important issue under the Geneva Air Convention. The Long-term Strategy 2020-2030, which was adopted in 2018, specifically identifies the protection of biodiversity and the prevention of biodiversity loss as a goal. Indeed, this strategy states that the “disruption of global and regional nitrogen cycles is one of the most important environmental challenges (...). Current and future exceedances of critical loads of nitrogen as an indicator of biodiversity loss over large areas are dominated by ammonia emissions from agriculture (...) (Long-Term Strategy Decision 2018/5: p. 7)”.

In general, biological diversity is the term used to depict the variety of life on Earth. It encompasses all organisms, species, and populations, the genetic variation among them and their complex assemblages of communities and ecosystems (UNEP, 2017). Each species within an ecosystem has its own niches and requirements. Changes in the ability to access these requirements – in this report through changes in nitrogen availability – affect the population size of a species resulting in changes to the composition of biological communities and ecosystems (CSS, 2020).

The results of the present review demonstrate and confirm that excessive atmospheric nitrogen deposition negatively affects species assemblages, and thus poses a serious risk to biodiversity. In many cases, it has already caused a decline. The scientific evidence available for the various existing dose-response relationships between nitrogen deposition and the risks for ecosystems and habitat quality of European ecosystems is summarised in Chapters 3 to 9 of this report. These chapters present many different scientific studies from around the world which provide evidence that exceedances of $CL_{emp}N$ can clearly be linked to reduced plant species richness in some major European ecosystem types.

Therefore, the revised $CL_{emp}N$ (Chapter 11, Table 11.1) are an appropriate and relevant metric to quantitatively assess risks to biodiversity (risks to ecosystem diversity) from nitrogen deposition.

It should, however, be noted, that the $CL_{emp}N$ approach also has some limitations. At first, it is sometimes difficult to establish the lower end of $CL_{emp}N$ ranges because current background depositions are above $CL_{emp}N$ in many places, and data has sometimes been collected in a situation where the system was not in a state of equilibrium. Secondly, the published studies draw on quite different impact indicators, which may not have included all relevant effects on the most sensitive species, and often do not include effects occurring over longer timescales. Thirdly, the type of habitat is not the only factor influencing $CL_{emp}N$ because climate conditions

or soil type impact sensitivity as well. Therefore, $CL_{emp}N$ ranges tend to be quite broad (Bobbink and Hettelingh, 2011). Last but not least, for large-scale application in risk assessment, the accurate mapping of ecosystems and, by extension, the spatial attribution of thresholds still reveals uncertainties. As a consequence, it will often be necessary or preferential to base risk assessment on a mixture of methods, for instance $CL_{emp}N$ combined, where appropriate, with static or dynamic soil geochemistry models, plant competition, and plant occurrence models.

10.5 Conclusions

The various uses of $CL_{emp}N$ range from broad-scale, national risk assessments of negative impacts of area-wide atmospheric nitrogen deposition to very local appraisals of a single protected ecosystem affected by nitrogen emissions from a single source. $CL_{emp}N$ provide a way to include consideration of effects on ecosystem function in relation to the pressure from nitrogen deposition, and to quantify the tolerance of ecosystems. A major strength of the $CL_{emp}N$ is that they are based on observations in the field. Values for different habitats were first established more than 30 years ago and are continuously being updated with due consideration of the latest scientific findings. Existing values apply to the scientific and natural entity of an ecosystem or habitat regardless of the legal protection status of that system. It is important to emphasise that $CL_{emp}N$ are not only applicable to the risk assessment of Natura 2000 habitats but also to the same ecosystems with other classifications such as EUNIS and without protection under Natura 2000 legislation.

Nevertheless, continuous experimental and survey work is essential to further improve the robustness of $CL_{emp}N$, and to establish $CL_{emp}N$ for habitats that have not yet been studied, for instance coastal shingle, temperate and Mediterranean-montane scrub. The different methods for modelling critical loads should be further developed concurrently and their integration into risk assessment is an important task for the future. Also, the Europe-wide, closer linking of environmental monitoring and air pollution measurements could help to effectively close knowledge gaps. Joint efforts and a more in-depth exchange between experts in air pollution control and biodiversity conservation are necessary to further increase knowledge. Last but not least, for large-scale assessments it is important to have an accurate mapping procedure that offers robust information on the location of ecosystems and the attribution of critical loads.

$CL_{emp}N$ are a suitable indicator to identify risks and damage to biodiversity at the ecosystem level, which can be linked to policy-relevant biodiversity targets (e.g. the EU Biodiversity Strategy for 2030 which aims to ensure no net loss of biodiversity and ecosystem services), and to the Convention on Biodiversity (CBD).

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Final Draft

11 Conclusions and gaps in knowledge

11.1 Conclusions

Within the UNECE Convention on Long-range Transboundary Air Pollution (LRTAP Convention), procedures have been developed to model and map critical loads for airborne N deposition in support of effect-based European policies for the abatement of air pollution (Bull et al., 2001; Hettelingh et al., 2001; 2007). Both the steady-state mass balance method and the empirical approach are used to scientifically support European policies aiming at effective emission reductions of air pollutants (ICP M&M, 2017).

Based on observed changes in the structure and function of ecosystems, empirical critical loads of N ($CL_{emp}N$) have been evaluated for specific receptor groups of natural and semi-natural ecosystems, reported in a range of publications (Bobbink et al., 1992; 1996; 2003). A synthesis of the knowledge for use of $CL_{emp}N$ under the LRTAP Convention was published by Achermann and Bobbink (2003) and by Bobbink and Hettelingh (2011). $CL_{emp}N$ were also included in the second edition of the Air Quality Guidelines for Europe of the World Health Organization Regional Office for Europe (WHO, 2000). Available new insights and data on the impacts of N deposition on natural and semi-natural ecosystems vegetation since the last publication of Bobbink and Hettelingh (2011) have justified a review and revision of $CL_{emp}N$ of which the results have materialised in this report.

The review and revision was conducted using a similar '*empirical approach*' (see Chapter 2 for more details) as described in Bobbink and Hettelingh (2011). For this purpose, the authors have reviewed all relevant European publications – as comprehensively as possible – on the effects of N on natural and semi-natural ecosystems for the period from 2010 to mid-2021. The authors used peer-reviewed publications, book chapters, nationally published papers and 'grey' reports by institutes or organisations, when available on request. For the classification of ecosystems the EUNIS habitat classification for Europe was used (Chytrý et al., 2020). To improve incorporation of the $CL_{emp}N$ in the EU habitat type classification, an appendix has been added, coupling the EUNIS system with the EU habitat classification (Appendix 1). Chapters 3 to 9 present evaluations of the effects of N enrichment per EUNIS class and $CL_{emp}N$ updated with newly published evidence, when available. $CL_{emp}N$ have been revised, if necessary, and summarised in separate tables, per chapter. In Chapter 10 the authors provide examples of how $CL_{emp}N$ can be used on different scales and in different European countries. In preparation of the CCE workshop (Berne, Switzerland, 26-28 October 2021), the draft of the background document was reviewed by a number of European experts and subsequently sent to all participants, after the suggested amendments had been processed.

11.2 Overview of the revised empirical N critical loads 2022

The text of this background document was intensively discussed and evaluated during the CCE workshop in Berne, held under the auspices of the Convention on Long-range Transboundary Air Pollution (CLRTAP Convention). At the end of this meeting, consensus was reached on the updated and revised list of empirical critical loads of N for natural and semi-natural ecosystems. Table 11.1 presents the new, revised empirical N critical loads.

Table 11.1. Overview of empirical N critical loads ($\text{kg N ha}^{-1} \text{yr}^{-1}$) to natural and semi-natural ecosystems (column 1), classified according to EUNIS (column 2), as established in 2011 (column 3), and as revised in 2022 (column 4). The reliability is indicated by ## reliable; # quite reliable and (#) expert judgement (column 5). Column 6 provides a selection of effects that may occur when critical loads are exceeded. Finally, changes with respect to 2011 are indicated as values in bold.

Ecosystem type	EUNIS code	2011 $\text{kg N ha}^{-1} \text{yr}^{-1}$	2022 $\text{kg N ha}^{-1} \text{yr}^{-1}$	2022 reliability	Indication of exceedance
Marine habitats (MA)					
Atlantic upper-mid salt marshes	MA223	20-30	10-20	(#)	Increase in dominance of graminoids; decline positive indicator species
Atlantic mid-low salt marshes	MA224	20-30	10-20	(#)	Increase in late successional species; decline positive indicator species
Atlantic pioneer salt marshes	MA225	20-30	20-30	(#)	Increase in late successional species; increase in productivity species
Coastal habitat (N)					
Shifting coastal dunes	N13, N14	10-20	10-20	#	Biomass increase; increased N leaching; reduced root biomass
Coastal dune grasslands (grey dunes)	N15	8-15	5-15	##	Increased biomass and cover of graminoids and mesophilic forbs; decrease in oligotrophic species including lichens; increased tissue N; increased N leaching; soil acidification
Coastal dune heaths	N18, N19	10-20	10-15	#	Increased plant production; increased N leaching; accelerated succession; typical lichen C:N decrease; increased yearly increment <i>Calluna</i>
Moist and wet dune slacks	N1H	10-20	5-15	#	Increased cover of graminoids and mesophilic forbs; decrease in oligotrophic species; increased Ellenberg N
Dune-slack pools (freshwater aquatic communities of permanent Atlantic and Baltic or Mediterranean and Black Sea dune-slack water bodies)	N1H1, N1J1	10-20	10-20	(#)	Increased biomass and rate of succession

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
Inland surface water habitats (C) ^a					
Permanent oligotrophic lakes, ponds and pools (including soft-water lakes)	C1.1	3-10	2-10 ^b	##	Increased algal productivity and a shift in nutrient limitation of phytoplankton from N to P; shifts in macrophyte community
Alpine and sub-Arctic clear water lakes	C1.1		2-4	##	Increased algal productivity and a shift in nutrient limitation of phytoplankton from N to P
Boreal clear water lakes	C1.1		3-6	##	Increased algal productivity and a shift in nutrient limitation of phytoplankton from N to P
Atlantic soft water bodies	C1.1, elements C1.2	3-10	5-10	##	Change in species composition of macrophyte communities
Permanent dystrophic lakes, ponds and pools	C1.4	3-10	5-10 ^c	(#)	Increased algal productivity and a shift in nutrient limitation of phytoplankton from N to P
Mire, bog and fen habitats (Q)					
Raised and blanket bogs	Q1	5-10	5-10	##	Increase in vascular plants; decrease in bryophytes; altered growth and species composition of bryophytes; increased N in peat and peat water
Valley mires, poor fens and transition mires	Q2	10-15	5-15	##	Increase in sedges and vascular plants; negative effects on bryophytes
Palsa and polygon mires	Q3		3-10	(#)	Increase in graminoids, tissue N concentrations and decomposition rate
Rich fens	Q41-Q44	15-30	15-25	#	Increase in tall vascular plants (especially graminoids); decrease in bryophytes
Arctic-alpine rich fens	Q45	15-25	15-25	(#)	Increase in vascular plants; decrease in bryophytes
Grasslands and tall forb habitats (R)					
Semi-dry Perennial calcareous grassland (basic meadow steppe)	R1A	15-25	10-20	##	Increase in tall grasses; decline in diversity; change in species composition; increased

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
					mineralisation; N leaching; surface acidification
Mediterranean closely grazed dry grasslands or Mediterranean tall perennial dry grassland or Mediterranean annual-rich dry grassland	R1D or R1E or R1F	15-25	5-15	(#)	Increased production; dominance by graminoids; changes to soil crusts; changes to soil nutrient cycling
Lowland to montane, dry to mesic grassland usually dominated by <i>Nardus stricta</i>	R1M	10-15	6-10	##	Increase in graminoids; decline of typical species; decrease in total species richness
Oceanic to subcontinental inland sand grassland on dry acid and neutral soils or Inland sanddrift and dune with siliceous grassland	R1P or R1Q	8-15	5-15	(#)	Decrease in lichens; increase in biomass
Low and medium altitude hay meadows	R22	20-30	10-20	(#)	Increase in tall grasses; decrease in diversity; decline of typical species
Mountain hay meadows	R23	10-20	10-15	#	Increase in nitrophilous graminoids; changes in diversity; decline of typical species
Moist or wet mesotrophic to eutrophic hay meadow	R35	15-25	15-25	(#)	Increase in tall graminoids; decreased diversity; decrease in bryophytes
Temperate and boreal moist and wet oligotrophic grasslands	R37	10-20	10-20	#	Increase in tall graminoids; decreased diversity; decrease in bryophytes
► Moss and lichen dominated	(Earlier E4.2)	5-10	5-10	#	Change in species composition; effects on bryophytes or lichens

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
mountain summits					
► Temperate acidophilous alpine grasslands	R43	5-10	5-10	#	Changes in species composition; increase in plant production
Arctic-alpine calcareous grassland	R44	5-10	5-10	#	Changes in species composition; increase in plant production

Heathland, scrub and tundra habitats (S)

Tundra	S1	3-5	3-5 ^d	#	Changes in biomass; physiological effects; changes in bryophyte species composition; decrease in lichen species richness
Arctic, alpine and subalpine scrub habitats	S2	5-15	5-10 ^d	#	Decline in lichens; bryophytes and evergreen shrubs
Lowland to montane temperate and submediterranean <i>Juniperus</i> scrub	S31		5-15	(#)	Shift in vegetation community composition; reduced seed viability
Northern wet heath	S411				
► 'U' <i>Calluna</i> -dominated wet heath (upland)	S411	10-20	5-15 ^e	##	Decreased heather dominance; decline in lichens and mosses; increased N leaching
► 'L' <i>Erica tetralix</i> -dominated wet heath (lowland)	S411	10-20	5-15 ^e	##	Transition from heather to grass dominance; decrease in heather cover; shift in vegetation community composition
Dry heaths	S42	10-20	5-15 ^e	##	Transition from heather to grass dominance; decline in lichens; changes in plant biochemistry; increased sensitivity to abiotic stress
Maquis, arborescent matorral and thermo-	S5	20-30	5-15	(#)	Change in plant species richness and community composition; nitrate leaching; acidification of soil.

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
Mediterranean scrub					
Garrigue	S6		5-15	#	Changes in species composition; decline in shrub cover; increased invasion of annual herbs
Forest habitats (T)					
Broadleaved deciduous forest	T1	10-20	10-15	##	Changes in soil processes; nutrient imbalance; altered composition mycorrhiza and ground vegetation
<i>Fagus</i> forest on non-acid and acid soils	T17, T18	10-20	10-15	(#)	Changes in ground vegetation and mycorrhiza; nutrient imbalance; changes in soil fauna
Mediterranean <i>Fagus</i> forest on acid soils	T18		10-15	(#)	Annual height and volume tree growth; analogy to temperate <i>Fagus</i> forest
Acidophilous <i>Quercus</i> forest	T1B	10-15	10-15	(#)	Decrease in mycorrhiza; loss of epiphytic lichens and bryophytes; changes in ground vegetation
<i>Carpinus</i> and <i>Quercus</i> mesic deciduous forest	T1E	15-20	15-20	(#)	Changes in ground vegetation
Mediterranean evergreen <i>Quercus</i> forest	T21	10-20	10-15	(#)	NO ₃ in soil water and streams
Coniferous forests	T3	5-15	3-15	##	Changes in soil processes; nutrient imbalance; altered composition mycorrhiza and ground vegetation; increase in mortality with drought
Temperate mountain <i>Picea</i> forest, Temperate mountain <i>Abies</i> forest	T31, T32	10-15	10-15	(#)	Decreased biomass of fine roots; nutrient imbalance; decrease in mycorrhiza; changed soil fauna
Mediterranean mountain <i>Abies</i> forest	T33		10-15	(#)	Tree foliar stoichiometry; tree physiology; soil N losses
Temperate continental <i>Pinus sylvestris</i> forest	T35	5-15	5-15	#	Changes in ground vegetation and mycorrhiza; nutrient imbalances; increased N ₂ O and NO emissions

Ecosystem type	EUNIS code	2011 kg N ha ⁻¹ yr ⁻¹	2022 kg N ha ⁻¹ yr ⁻¹	2022 reliability	Indication of exceedance
Mediterranean montane <i>Pinus sylvestris</i> - <i>Pinus nigra</i> forest	T37		5-17	(#)	Lichen chemistry and community changes in Mediterranean mixed-conifer forests in USA
Mediterranean lowland to submontane <i>Pinus</i> forest	T3A	3-15	5-10	(#)	Reduction in fine-root biomass; shift in lichen community
Dark taiga	T3F	5-10	3-5 ^f	##	Changes in epiphytic lichen and ground-layer bryophyte communities; increase in free-living algae; decline in N-fixation
<i>Pinus sylvestris</i> light taiga	T3G	5-10	2-5 ^f	#	Changes in epiphytic lichen and ground-layer bryophyte communities; increase in free-living algae; decline in N-fixation

- a) The lower part of the CL_{emp}N range should be applied for lakes in small catchments (with high lake to catchment ratios), because these are most exposed to atmospheric deposition, given that a relatively high fraction of their N inputs is deposited directly on the lakes and is not retained in the catchments. Similarly, the lower part of the range should be applied for lakes in catchments with thin soils, sparse vegetation and/or with a high proportion of bare rock.
- b) This CL_{emp}N should only be applied to oligotrophic waters with low alkalinity and with no significant agricultural or other human inputs. Apply the lower end of the range to clear-water sub-Arctic and alpine lakes, the middle range to boreal lakes and the higher end of the range to Atlantic soft waters.
- c) This CL_{emp}N should only be applied to waters with low alkalinity and with no significant agricultural or other direct human inputs. Apply the lower end of the range to boreal dystrophic lakes.
- d) Use towards high end of range if phosphorus limited, and towards lower end if phosphorus is not limiting.
- e) Use towards high end of range with high intensity management, and use towards lower end of range with low intensity management.
- f) Mainly based on N deposition impacts on lichens and bryophytes.

Modifying factors

The modifying factors, i.e. general relationships between abiotic factors and empirical N critical loads, presented in Table 11.2, were defined as part of the earlier revision by Bobbink and Hettelingh (2011). This table was discussed at the CL_{emp}N workshop in Noordwijkerhout in 2010. Each modifying factor was addressed by the workshop participants for each individual EUNIS category, with the aim of providing as specific instructions as possible for application in the different EUNIS classes (for more detailed information, see Bobbink and Hettelingh (2011), Appendices 5 to 7).

Table 11.2. Suggestions to apply lower, middle or upper parts of the set critical load ranges for terrestrial ecosystems (excluding wetlands), if national data are insufficient.

	Temperature/ frost period	Soil wetness	Base cation availability	Management intensity
Action				
Move to lower part	COLD/LONG	DRY	LOW	LOW
Use middle part	INTERMED	NORMAL	INTERMED	USUAL
Move to higher part	HOT/NONE	WET	HIGH	HIGH

As there is no consensus on how to quantify the modifying factors for broad regional scale assessments, it is proposed to use the minimum value of the ranges of $CL_{emp}N$ in each EUNIS class. This will enable comparison of their exceedances between different air pollution abatement scenarios. Furthermore, countries are advised to identify the highly sensitive receptor ecosystems within the EUNIS classification relating to their individual interest.

Several new national approaches have been developed in the UK and in the Netherlands to estimate or calculate critical loads for specific ecosystems that are part of a larger EUNIS class with an agreed range of $CL_{emp}N$ (Hall and Wadsworth, 2010; Van Dobben et al., 2014). Although this is an important development to provide a more complete list of critical loads for the large number of ecosystem types, it should be extended to a more European-based approach.

11.3 Gaps in knowledge and research needs

Most of the Earth's biodiversity is present in semi-natural and natural ecosystems. It is therefore crucial to control atmospheric N pollution to prevent negative impacts on these ecosystems. Effort should be directed to produce fine-resolution maps of sensitive ecosystems of high conservation value for each country in order to map critical loads of N for these systems. It is advisable to use both the mass balance and empirically derived N critical loads to improve the robustness of impact-based assessments of natural areas at risk from nitrogen deposition scenarios on a broad regional scale (see Hettelingh et al., 2007).

More information is needed on the relative effects of oxidised and reduced N deposition. At recent UNECE expert meetings, it was emphasised that there is increasing evidence that NH_x has a greater impact than NO_y . In particular, lichens and sometimes bryophytes in different ecosystems, as well as weakly buffered ecosystems, are likely to be more sensitive to deposition of reduced N. However, at present it is not possible to set critical loads for both forms of N, separately. In addition, the critical levels of NH_3 are in a process of revision in 2022 to 2023.

Serious gaps in knowledge exist on the effects of increased N deposition on semi-natural and natural ecosystems, although considerable progress has been made for several habitat groups, between 2010 and 2021. The following gaps in knowledge have been recognised as most important:

- more research and data are required to establish a critical load for the following ecosystems: several grasslands and hay meadows; all Mediterranean vegetation types; wet swamp forests; many mires and fens and several coastal habitats;
- more research is needed for all distinguished EUNIS habitat types that have an 'expert' judgement rating or for which only few studies are available;

- ▶ impacts of N enrichment in (sensitive) freshwater and shallow marine ecosystems (including coastal waters) need further research;
- ▶ additional efforts are needed to assign the observed N effects to the corresponding EUNIS forest habitat subtypes (level 3);
- ▶ in the last decade an increasing number of gradient studies on atmospheric N deposition in several EUNIS habitat types have been published and proved to be useful for evaluation of the ClempN. More gradient studies with both low and high N regions are needed, especially in EUNIS habitat types that are hardly investigated;
- ▶ more research is needed on the differential effects of the deposited N forms (NO_x or NH_y) in order to be able to determine the critical loads for oxidised and reduced nitrogen separately in the future;
- ▶ in order to refine the current critical loads, long-term (10-20 years) N addition experiments with a high frequency of N treatments between 5 and 50 kg N ha⁻¹ yr⁻¹ in regions with low background depositions are useful. This would increase the reliability of the derived critical loads if the lowest treatment level does not exceed the critical load;
- ▶ climate change and nitrogen deposition are likely to have strong interactive effects on ecosystem functioning and climate change may alter ecosystem responses to nitrogen deposition and vice versa. More experimental studies are needed to investigate these interactions, and also more gradient studies that explicitly examine the impacts of nitrogen deposition in combination with climatic gradients;
- ▶ at present, there are only a few studies that have looked at both the impact of and recovery from nitrogen deposition. More studies on this topic are needed to better understand the reversibility of nitrogen deposition impacts and the long-term projections for N-polluted ecosystems.

In conclusion, it is crucial to understand the long-term effects of increased N deposition on ecosystem processes in a representative range of ecosystems. Therefore, it is important to quantify the effects of N deposition through the manipulation of N inputs in long-term ecosystem studies in both pristine and affected areas. These data in combination with gradient studies are essential to validate critical loads and develop robust dynamic ecosystem models and/or multiple correlative species models that are reliable enough to use in the calculation of CL_{empN} for natural and semi-natural ecosystems, and to predict natural recovery rates for N-affected systems.

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Glossary

Glossary Item	Description
Acidification (referring to soil)	Acidification is defined by the loss of acid neutralizing capacity (ANC) (soil buffering capacity). It is a gradual process leading to leaching of base cations (calcium, magnesium, potassium) from the topsoil, through leaching, and replacement by acidic elements (especially aluminium).
Aerosols	Solid or liquid particles suspended in the air. This includes dust, soot and sea-salt crystals, size 1 nm to 100 µm.
Al	Aluminium
Ammonia	NH ₃ is a colourless gas with a pungent odour in high concentration. Ammonia is product of 'biological nitrogen fixation' (see entry), it can also be synthesised using the 'Haber–Bosch' process (see entry). Livestock farming largely contributes to NH ₃ emissions.
Ammonium	Is a monovalent cation (NH ₄ ⁺), closely linked to production and solution of ammonia in water. Is a form of reactive nitrogen. Constituent of many synthetic fertilisers, such as ammonium nitrate.
ANC	Acid Neutralizing Capacity
Anthropogenic	Effects which relate specifically to human activities, i.e. anthropogenic reactive nitrogen production, through the 'Haber–Bosch' process.
Anthroposphere	All parts of the planetary system which are affected by human activities.
Arborescent strata	Layer of trees in a plant community.
Arginine	N-rich amino acid, the arginine concentration in plant material often increased with higher N deposition.
Atmospheric deposition	Removal of suspended material from the atmosphere, this can be classified as either 'wet' or 'dry'. Wet deposition occurs when material is removed from the atmosphere by precipitation. In dry deposition, the material is removed from the atmosphere by interference with surfaces.
Autotroph	Organism (primary producer) that converts energy (sun light) into organic compounds which can be used by other organisms (see Heterotroph).
Biodiversity	Biodiversity is the variability of life on earth. It is a measure of variation at the genetic, species, and ecosystem level. The value of biodiversity is multifold, from preserving the integrity of the biosphere as a whole, to providing fodder, food and medicine, to spiritual and aesthetic well-being. Biodiversity and plant diversity are often used as synonyms.
Biological Nitrogen Fixation (BNF)	"Fixing" of unreactive di-nitrogen (N ₂) to reactive nitrogen species by microorganisms (Rhizobium, Frankia, free-living etc.).

Glossary Item	Description
Bulk deposition	Deposition collected in the open field with a funnel, measuring wet deposition with some dry deposition (15-30%).
Bryophytes	Earliest land plants on earth, consisting of mosses, liverworts and hornworts. They are non-vascular plants.
Carbon sequestration	The capture and removal of carbon dioxide from the atmosphere and storing it in a reservoir, storage in soil organic matter is one of the most efficient ways.
CLRTAP	Convention on Long-range Transboundary Air Pollution, under the United Nations Economic Commission for Europe (UNECE).
C:N ratio	Ratio of carbon (C) to nitrogen (N) in soil and of litter. C:N < 25 mineralisation dominates, C:N > 25 immobilisation dominates.
Chlorophyll a	Photosynthetic pigment. In aquatic ecosystem, the chlorophyll a is used as a proxy for algal biomass production.
CH ₄	Methane, is a colourless, odourless, flammable gas that is the simplest hydrocarbon and is the major constituent of natural gas.
CO ₂	Carbon dioxide, is a chemical compound composed of two oxygen atoms and one carbon atom.
Critical level	Concentration of an atmospheric pollutants above which direct harmful effects on sensitive vegetation elements may occur according to present knowledge.
Critical loads	A quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge.
Cryptogam	A cryptogam (scientific name Cryptogamae) is a plant (in the wide sense of the word) or a plant-like organism that reproduces by spores, without flowers or seeds.
Denitrification	A microbially facilitated process (mainly by heterotrophic bacteria, but also by fungi) where nitrate is reduced and di-nitrogen (N ₂) is ultimately produced through series of intermediate or nitrous oxide products, including the greenhouse gas N ₂ O.
DCA	Detrended Correspondence Analysis
DIN	Dissolved Inorganic Nitrogen
DOM	Dissolved Organic Matter
DON	Dissolved Organic Nitrogen
Di-nitrogen	N ₂ , a colourless, odourless, and inert gas which makes up approximately 78% of the atmosphere. Di-nitrogen is the thermodynamically stable state ('unreactive nitrogen') in contrast to many different reactive nitrogen forms. Also called nitrogen gas.

Glossary Item	Description
Dystrophic	Description for water bodies (lakes), rich in dissolved organic carbon (humic acids), low in productivity (see oligo- and eutrophic).
Ellenberg N indicator values	Ellenberg indicator values (EIVs) are ordinal estimates of species ecological optima along seven main ecological gradients - light, temperature, continentality, moisture, nutrients, soil reaction and salinity. Here for nutrients.
EMEP	European Monitoring and Evaluation Programme. Co-operative programme for monitoring and evaluation of the long-range transmission of air pollutants in Europe.
EUNIS	Stands for European Nature Information System. The EUNIS habitat classification is a comprehensive pan-European system for habitat identification. The classification is hierarchical and covers all types of habitats from natural to artificial, from terrestrial to aquatic. The habitat types are identified by specific codes (first revision with code changes started in 2015).
Eutrophication	The enrichment of the nutrient load in ecosystems (terrestrial and aquatic), especially by nitrogen and/or phosphorus compounds. This disturbs the balance of organisms in the ecosystem, affecting terrestrial and aquatic biodiversity and water quality.
Exceedance	The concentration or amount of pollution above a 'critical level' or 'critical load'.
Food webs	Terrestrial, aquatic food webs, interconnected food chains on "what/who" eats "what/who".
Forb	An herbaceous flowering plant that is not a graminoid (grass, sedge, or rush). The term is used in biology and in vegetation ecology, especially in relation to grasslands and understory.
Garrigue	Mediterranean shrub habitat, consisting of sclerophyllous shrubs, more open than maquis.
Gradient	Variable atmospheric N deposition in space or time. In the present revision, spatial gradients are included to assess critical N loads.
Greenhouse Gas	GHG includes carbon dioxide (CO ₂), nitrous oxide (N ₂ O), methane (CH ₄), ozone (O ₃), water vapour and various other gases.
Haber–Bosch process	The high-pressure, chemical process which synthesises reactive nitrogen as ammonia (NH ₃) from the reaction of N ₂ and H ₂ . Fritz Haber discovered the process (1908) and Carl Bosch developed the technique at an industrial scale.
Heathland	Various types of plant communities, commonly with a dominance of dwarf shrubs.
Herbaceous	A plant whose stem does not become woody and persistent (as in a tree or shrub) but remains soft and succulent, and dies (completely or down to the root) after flowering.
Herbivores	Organisms (insects, mammals) that feed on plants.

Glossary Item	Description
Heterotroph	Organism that cannot produce its own food.
Isotopes	Same chemical element (non-radioactive) with same number of electrons in the nuclei, but different number of neutrons in the nuclei (stable isotopes).
Immobilisation (referring to soil)	The incorporation of reactive nitrogen compounds into soil microbial biomass.
Leaching	The washing out of soluble ions (mainly of mobile nitrate ions) and compounds by water draining through soil.
Legumes	Herbs that fix di-nitrogen from the atmosphere, by means of symbiotic microorganisms in the plant roots.
Lichen	Lichen is a symbiosis between fungi and algae. The algae photosynthesise (in some cases they also fix N ₂) and the fungi provide nutrients from the environment. Terricolous lichens grow on soil, epiphytic lichens on branches or bark of trees. Most lichens can dehydrate completely without damage.
Litter	Dead, detached plant material. Litter decomposition (mineralisation) is an important part of the nutrient cycle.
Littoral	Part of a sea, lake, or river that is close to the shore, includes the intertidal zone.
Natura 2000	Protected areas in European Union, implanted by the EU Habitats Directive (1992).
Mineralisation	Decomposition process where organic compounds are transformed to inorganic compounds (N and C mineralisation).
Maquis	Mediterranean shrub habitat, consisting of densely growing evergreen shrubs (see Garrigue).
Macrophytes	Aquatic plants, term to distinguish them from algae and other microphytes.
N	Nitrogen
NDVI	Normalised Difference Vegetation Index = Cover of green biomass
NEP	Net Ecosystem Productivity
Nitrification	The biological oxidation of ammonia via nitrite to nitrate (aerobic process).
Nitrophilous	Translates to 'nitrogen-loving'. A species which may be nitrogen limited and then benefits from increased nitrogen availability in the environment.
N ₂ O	Laughing gas, produced through denitrification, strong greenhouse gas. Not accounted for the total atmospheric N deposition.
NPK fertiliser	Artificial fertiliser containing the three major plant nutrients (nitrogen, phosphorus and potassium).

Glossary Item	Description
Oligotrophic	‘Poor in nutrient’. The opposite of eutrophic (see Eutrophication).
Retention	Retention of N in the soil, mainly in the soil organic matter (SOM). Expected N losses due to high N deposition are lower than expected.
Phosphorus limitation	Plant phosphorus (P) limitation is common, primarily because soil P forms often have a low plant availability. Chronically elevated N deposition often affects plant P availability (so called N-induced P limitation, increased foliar N:P ratios).
Photooxidation	The light-dependent generation of active oxygen species is termed photooxidative stress. This can occur in two ways: 1) the donation of energy or electrons directly to oxygen as a result of photosynthetic activity; 2) exposure of tissues to ultraviolet irradiation. Photooxidation is responsible for the accumulation of harmful reactive oxygen species in plant tissues.
PME:NR ratio	Phosphomonoesterase:nitrate reductase ratio
Pristine	State or area which is not affected by atmospheric N deposition or other pollutants.
Relevés	A vegetation relevé is a recording of a sample of a plant community. For each plant species, the cover, abundance, frequency, vitality, fertility and/or the phenological condition in a specific test area and of the spatial vegetation structure are recorded.
Sclerophyllous shrub	A type of shrub that is adapted to long periods of dryness and heat. The plants feature hard leaves, short internodes (the distance between leaves along the stem) and leaf orientation which is parallel or oblique to direct sunlight. The word comes from the Greek sklēros (hard) and phyllon (leaf).
Synthetic fertiliser	Industrially produced fertiliser, using the ‘Haber–Bosch’ process.
Simple mass balance	Calculation method based on inputs and outputs of the variable of concern (nitrogen, acidity, base cations).
Throughfall	Rainwater (precipitation) falling through the canopy (foliage) of a forest (or crop). Throughfall N is commonly lower than total atmospheric N.
TITAN	Stands for Threshold IndicatorTaxa ANalysis (TITAN). Analytical method to detect changes in taxa distributions along an environmental gradient (N gradient) over space or time.
TP	Total Phosphorus
Vascular plant	A vascular plant is any one of a number of plants with specialised vascular tissue. The two types of vascular tissue, xylem and phloem, are responsible for moving water, minerals, and the products of photosynthesis throughout the plant.

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Appendix 1: Classification of habitats according to EUNIS and EU Habitats Directive (Natura 2000, Annex I)

This appendix gives the correspondence between ecosystems, classified according to EUNIS (European Nature Information System) (Chytrý et al., 2020) and the EU Habitats according to Directive Annex I. Only the EUNIS classes for which $Cl_{emp}N$ has been set are included in the table below. Empirical critical loads of N, as set in this background document, can be assigned to EU habitats with the same reliability as for the corresponding EUNIS classifications (see Table 11.1).

Ecosystem type	EUNIS code	EU habitats ^a
Marine habitats (MA)		
Atlantic upper-mid salt marshes	MA223	1330
Atlantic mid-low salt marshes	MA224	1330
Atlantic pioneer salt marshes	MA225	1310, 1320, 1330
Coastal habitat (N)		
Shifting coastal dunes	N13, N14	2120, 2110
Coastal dune grasslands (grey dunes)	N15	2130, 21A0
Coastal dune heaths	N18, N19	2140, 2150
Moist and wet dune slacks	N1H	2190, 21A0
Dune-slack pools (freshwater aquatic communities of permanent Atlantic and Baltic or Mediterranean and Black Sea dune-slack water bodies)	N1H1, N1J1	x
Inland surface water habitats (C)		
Permanent oligotrophic lakes, ponds and pools (including soft-water lakes)	C1.1	3110, 3130
Alpine and sub-Arctic clear water lakes	C1.1	3110, 3130
Boreal clear water lakes	C1.1	3110, 3130
Atlantic soft water bodies	C1.1, elements C1.2	3110, 3130
Permanent dystrophic lakes, ponds and pools	C1.4	3160
Mire, bog and fen habitats (Q)		
Raised and blanket bogs	Q1	7110, 7130
Valley mires, poor fens and transition mires	Q2	7140
Palsa and polygon mires	Q3	7320
Rich fens	Q41-Q44	7230
Arctic-alpine rich fens	Q45	7240
Grasslands and tall forb habitats (R)		

Ecosystem type	EUNIS code	EU habitats ^a
Semi-dry perennial calcareous grassland (and basic meadow steppe) or Continental dry grassland (true steppe)	R1A or R1B	6210, 6280, 62A0, 6240, 6250, 6270, 62C0 or 6240, 6250, 62C0
Mediterranean closely grazed dry grasslands or Mediterranean tall perennial dry grassland or Mediterranean annual-rich dry grassland	R1D or R1E or R1F	6220
Lowland to montane, dry to mesic grassland usually dominated by <i>Nardus stricta</i> or Boreal and arctic acidophilous alpine grassland	R1M or R42	6230 or 6150
Oceanic to subcontinental inland sand grassland on dry acid and neutral soils or Inland sanddrift and dune with siliceous grassland	R1P or R1Q	6120, 2330, 6270 or 2330, 2340
Low- and medium altitude hay meadows	R22	6270, 6510
Mountain hay meadows	R23	6520
Moist or wet mesotrophic to eutrophic hay meadow	R355	x
Temperate and boreal moist and wet oligotrophic grasslands	R372	x
► Moss and lichen dominated mountain summits	(Earlier E4.2)	x
► Temperate acidophilous alpine grasslands	R43	6140, 6150, 62D0, 6230
Arctic-alpine calcareous grassland	R44	6170
Heathland, scrub and tundra habitats (S)		
Tundra	S1	x
Arctic, alpine and subalpine scrub habitats	S2	4060, 4080
Lowland to montane temperate and submediterranean <i>Juniperus</i> scrub	S31	5130
Northern wet heath	S411	4010
► 'U' <i>Calluna</i> -dominated wet heath (upland)	S411	4010
► 'L' <i>Erica tetralix</i> -dominated wet heath (lowland)	S411	4010
Dry heaths	S42	2310, 2320, 4030, 4040, 5140
Maquis, arborescent matorral and thermo-Mediterranean scrub	S5	x
Garrigue	S6	x

Ecosystem type	EUNIS code	EU habitats ^a
Forest habitats (T)		
Broadleaved deciduous forest	T1	x
<i>Fagus</i> forest on non-acid and acid soils	T17, T18	9110, 9120, 9130, 9140, 9150, 91W0, 9210, 9220, 9270, 9280, 91K0, 91S0, 91V0, 91W0, 91X0, 9260
Mediterranean <i>Fagus</i> forest on acid soils	T18	9110, 9120, 91W0, 9260
Acidophilous <i>Quercus</i> forest	T1B	9190, 9260, 91A0
<i>Carpinus</i> and <i>Quercus</i> mesic deciduous forest	T1E	9020, 9160, 9170, 91G0, 91L0, 91Y0, 9260
Mediterranean evergreen <i>Quercus</i> forest	T21	9330, 9340, 9390, 93A0
Coniferous forests	T3	x
Temperate mountain <i>Picea</i> forest, Temperate mountain <i>Abies</i> forest	T31, T32	9410, 91BA, 91P0, 9110, 9120, 9140, 9270, 9410
Mediterranean mountain <i>Abies</i> forest	T33	9510, 9520, 9270
Temperate continental <i>Pinus sylvestris</i> forest	T35	91C0, 91T0, 91U0, 9060
Mediterranean montane <i>Pinus sylvestris</i> - <i>Pinus nigra</i> forest	T37	9530
Mediterranean lowland to submontane <i>Pinus</i> forest	T3A	9540
Dark taiga	T3F	9050, 9010
<i>Pinus sylvestris</i> light taiga	T3G	9010, 9060

- a) Relationships concern revised EUNIS level 3 only. When the revised EUNIS habitat has no relationship to Annex I habitat types this is indicated with x in field 'EU habitats'. *Italics* indicate relationships of low importance, in the sense that only a very small part of the Annex I habitat is crosslinked with the EUNIS habitat. In many cases low importance indicates that the relationship exist only under a specific interpretation of the Annex I habitat.

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Appendix 2: Agenda of the expert workshop on empirical critical loads of nitrogen

Tuesday 26 th October 2021		
8:30	Welcome coffee / Registration	
Welcome and introduction <i>Chair: Reto Meier</i>		
9:00	Welcome Address and Organizational Information	Reto Meier
9:15	Objectives of the workshop	Alice James Casas, Christin Loran
Presentation of scientific background information <i>Chair: Roland Bobbink</i>		
9:30	Review and revision procedure	Roland Bobbink
10:00	Marine habitats (EUNIS class MA)	Roland Bobbink
10:20	Coastal habitats (EUNIS class N)	Laurence Jones
10:40	Coffee Break	
11:00	Inland surface waters (EUNIS class C)	Christin Loran, Heleen de Wit
11:20	Discussion (EUNIS class MA, N, C)	
11:40	Mires, bogs and fens (EUNIS class Q)	Chris Field
12:00	Coffee Break	
13:30	Grasslands and lands dominated by forbs, mosses or lichens (EUNIS class R)	Vegar Bakkestuen
13:50	Heathland, scrub and tundra (EUNIS class S)	Leon van den Berg
14:10	Discussion (EUNIS class Q, R, S)	
14:30	Coffee Break	
15:00	Woodland, forest and other wooded land (EUNIS class T)	Sabine Braun
15:20	Discussion (EUNIS class T)	
15:40	General Discussion	
Wednesday 27 th October 2021		
Application of empirical Critical Loads <i>Chair: Reto Meier</i>		
9:00	Aspects of application	Markus Geupel
10:30	Coffee Break	Alice James Casas, Christin Loran

Wednesday 27th October 2021

Working group specific discussions

Discussions chaired by lead authors

11:00	Working group 1 - EUNIS class MA, N, C	
	Working group 2 - EUNIS Q, R	
	Working group 3 - EUNIS S, T	
12:30	<i>Lunch Break</i>	
14:00	Working group 1 - EUNIS class MA, N, C	
	Working group 2 - EUNIS Q, R	
	Working group 3 - EUNIS S, T	
19:00	<i>Light show "Rendez-vous Bundesplatz"</i>	
20:00	<i>Dinner at Restaurant Altes Tramdepot</i>	

Thursday 28th October 2021

Concluding session

Chair: Christin Loran

09:00	Reporting of working groups to plenary	
10:40	<i>Coffee Break</i>	
11:00	Discussion	
12:00	Conclusion	

Appendix 3: List of participants of the expert workshop on empirical critical loads of nitrogen

Surname	Name	Institute
Aazem	Khalid	Joint Nature Conservation Committee
Aherne	Julian	Trent University
Alonso	Rocío	CIEMAT
Augustin	Sabine	BAFU
Bakkestuen	Vegar	NINA
Bobbink	Roland	B-WARE
Braun	Sabine	IAP
Britton	Andrea	James Hutton Institute
Caporn	Simon	Manchester Metropolitan University
de Wit	Heleen	NIVA
Field	Chris	Manchester Metropolitan University
Garcia	Hector	CIEMAT
Geupel	Markus	UBA
Greaver	Tara	US EPA
Hayes	Felicity	CEH
Hicks	Kevin	University of York
Hiltbrunner	Erika	University of Basel
James Casas	Alice	INERIS
Jones	Laurence	CEH
Karlsson	Per Erik	IVL
Kohli	Lukas	Hintermann & Weber
Loran	Christin	UBA
Manninen	Sirkku	University of Helsinki
Meier	Reto	BAFU
Perring	Mike	CEH
Posch	Max	IIASA/CIAM
Prescher	Anne-Katrin	Thuenen Institute
Roth	Tobias	Hintermann & Weber
Rowe	Ed	CEH
Scheuschner	Thomas	UBA

Surname	Name	Institute
Tomassen	Hilde	B-WARE
Tresch	Simon	IAP
Ukonmaanaho	Liisa	Luke
van den Berg	Leon	Bosgroep Zuid Nederland
Vanguelova	Elena	Forest Research UK
Velle	Liv Guri	Moreforskning
Zappala	Susan	Joint Nature Conservation Committee