

Review and revision of empirical critical loads and dose-response relationships

Proceedings of an expert workshop, Noordwijkerhout, 23-25 June 2010

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Roland Bobbink and Jean-Paul Hettelingh (eds.)

Cover photo: Grass encroachment in Danish Dunes, Korshage (source: Eva Remke, Bargerveen Foundation).

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This research is performed by order and for the account of the Directorate for Climate and Air Quality of the Dutch Ministry of Infrastructure and the Environment within the framework of PBL project M/500090/01 and RIVM project E/680359/11, 'UNECE Coordination Centre for Effects (CCE)', in collaboration with the Swiss Federal Office for the Environment (FOEN) and the German Federal Ministry of the Environment, Nature Conservation and Nuclear Safety/ Federal Environment Agency (UBA), and for the account of (the Working Group on Effects within) the trust fund for the partial funding of effect-oriented activities under the Convention on Long-range Transboundary Air Pollution.

ISBN: 978-90-6960-251-6 RIVM report: 680359002

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Acknowledgements

This report is a product of the collaboration within the Effects Programme of the UNECE Convention on Longrange Transboundary Air Pollution, involving many institutions and individuals throughout Europe. We are grateful to all participants in the UNECE Workshop on the 'Review and revision of empirical critical loads and dose-response relationships' (23-25 June 2010, Noordwijkerhout, the Netherlands) for the fruitful discussions and their useful suggestions to improve the background documentation that finally resulted in this report.

In addition, the Coordination Centre for Effects (CCE) and B-WARE Research Centre acknowledge:

- Mariette van Empel, MSc and Dr Wil Prins of the Climate and Air Quality Directorate of the Dutch Ministry of Infrastructure and the Environment (I&M; www. rijksoverheid.nl/ministeries/ienm), Dr Martin Schiess and Mr Beat Achermann of the Swiss Federal Office for the Environment (FOEN; www.bafu.admin.ch) and Ms Gudrun Schütze of the German Federal Environment Agency (UBA; www.umweltbundesamt.de) for helping to facilitate the UNECE workshop and this report;
- Dr Anne Christine Le Gall of INERIS (France), chair of the Task Force of the International Cooperative Programme on the Modelling and Mapping of Critical Levels & Loads and Air Pollution Effects, Risks and Trends, and the members of the National Focal Centres, for their support;

- The UNECE secretariat of the Convention on Long-range Transboundary Air Pollution, for routing the executive summary of this report of the UNECE workshop in the official document ECE/EB.AIR/WG.1/2010/14 for the 29th session of the Working Group on Effects (Geneva, 22-24 September 2010);
- The review team consisting of Professor Mike Ashmore, Dr Martin Jenssen, Dr Mark Fenn, Professor Jan Roelofs and Dr Sarah Woodin, for their thorough scientific support and expert comments during the updating and drafting phase of the background document to the UNECE workshop. Furthermore, we gratefully acknowledge the detailed scientific comments from Professor Christian Körner, Dr Laurence Jones, Dr Eva Remke and Dr Seraina Bassin;
- Ms Karin van Doremalen, former management assistant of the CCE, for organising and managing the logistics of the UNECE workshop;
- Ms Annemieke Righart of Dunfield Editing, for her commitment and efficiency with respect to the editing of the language of the report, and for improving the consistency in terms between the chapters;
- Mr Martin Middelburg and Mr Gert Boer of the RIVM graphics department, for their collaborative and pro-active involvement to ensure the correct lay-out and printing of this report.

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Rapport in het kort

Het RIVM heeft in opdracht van het ministerie van Infrastructuur en Milieu (I&M) de wetenschappelijke basis beschreven voor nieuwe drempelwaarden voor stikstofdepositie (empirische kritische depositiewaarden). De hoeveelheid stikstof die vanuit de lucht neerslaat op de bodem is van invloed op de biodiversiteit. De empirische kritische depositiewaarden worden gebruikt om effecten van overmatige stikstofdepositie te schatten. De waarden zijn op basis van empirische veldwaarnemingen bepaald op diverse natuurtypen in Europa en zijn een update van resultaten uit 2003. In vergelijking met deze cijfers zijn nieuwe empirische kritische depositiewaarden tot stand gekomen, zoals voor oppervlaktewateren en mediterrane bossen. Daarnaast zijn enkele waarden verlaagd, zoals voor naaldbossen. Andere rapporten van het RIVM over effecten van luchtverontreiniging bevestigen dat stikstof in Europa een belangrijke risicofactor is voor onder meer veranderingen in de biodiversiteit.

Het Coordination Centre for Effects (CCE) aan het RIVM heeft het onderzoek uitgevoerd in nauwe samenwerking met Onderzoekcentrum B-WARE (Nederland), enkele Europese RIVM-zusterinstellingen en andere Europese onderzoeksinstituten. De update is in 2009 gestart door het CCE onder de auspiciën van de UNECE Conventie voor grootschalige grensoverschrijdende luchtverontreiniging. Het CCE is in 1990 op verzoek van Nederland bij de UNECE opgericht. Het werd bij het RIVM gevestigd om met diens Europese netwerk van circa dertig instituten het Europese luchtbeleid te ondersteunen. Nieuwe wetenschappelijke informatie over de effecten van stikstof op (half-) natuurlijke ecosystemen is nu opgenomen in de Europese databank van empirische kritische depositiewaarden van stikstof. Deze zijn geclassificeerd volgens het European Nature Information System (EUNIS).

Tijdens een door het CCE georganiseerde UNECEworkshop in juni 2010 hebben wetenschappers uit Europese lidstaten consensus bereikt over de resultaten. Consensus is belangrijk, omdat de effecten van stikstof binnen Europa kunnen verschillen als gevolg van variaties in meteorologische en bodemcondities.

Trefwoorden: Biodiversiteit, Kritische waarde, LRTAP Conventie, N-additie experimenten, Stikstofdepositie

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 2003 under the auspices of the UNECE Convention on Longrange Transboundary Air Pollution (LRTAP Convention). In 2009 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 (autumn 2002 – spring 2010) on the impacts of nitrogen on natural and semi-natural ecosystems was incorporated in the existing European database on empirical critical loads of N. Empirical critical loads were structured following the classification used in the European Nature Information System (EUNIS).

Consensus on the results was obtained in a UNECE workshop on the 'Review and revision of empirical critical loads and dose-response relationships' (23-25 June 2010, Noordwijkerhout, the Netherlands), organised by the Coordination Centre for Effects and the B-WARE Research Centre. The results, as provided in Table 1 of the Executive Summary, show that in many cases the outer ranges of the empirical critical loads have decreased. The resulting European database of 2011 includes both revised and newly established value ranges of empirical critical loads of nitrogen for each EUNIS class. The outcome of this report is of major importance for the protection of N-sensitive natural and semi-natural ecosystems across Europe. This knowledge is used in support of European policies to abate air pollution.

Keywords: Biodiversity, Diversity, Empirical critical load, EUNIS, LRTAP Convention, N-addition experiments, Nitrogen deposition, Soil, Vegetation

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Executive Summary

Jean-Paul Hettelingh, Hilde Tomassen, Roland Bobbink

Workshop on the Review and Revision of Empirical Critical Loads and Dose-response Relationships

Under the UNECE Convention on Long-range Transboundary Air Pollution at the NH Conference Centre Leeuwenhorst, Langelaan 3, 2211 XT Noordwijkerhout, The Netherlands, 23-25 June 2010.

I Introduction

- The workshop on the review and revision of empirical critical loads and dose-response relationships was held under the Convention on Long-range Transboundary Air Pollution, in Noordwijkerhout, from 23 to 25 June 2010. The workshop was organised by the Coordination Centre for Effects (CCE) and supported by the Dutch Ministry of Housing Spatial Planning and the Environment, the Swiss Federal Office for the Environment and the German Federal Environment Agency.
- 2. The workshop was attended by 51 participants from the Czech Republic, France, Germany, Ireland, the Netherlands, Norway, Portugal, Romania, 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.

- 3. The decision to organise the workshop was adopted at the 27th session of the Working Group on Effects, following recommendations from the 18th CCE workshop (21-23 April 2008) as supported by the 24th session of the Task Force on Modelling and Mapping (24-25 April 2008) held in Berne.
- 4. The meeting was opened by M.G. van Empel, Director of the Climate and Air Quality Directorate of the Dutch Ministry of Housing, Spatial Planning and the Environment (VROM).

5. The Status of the Convention was presented by A.C. Le Gall, chair of the Task Force on Modelling and Mapping, on behalf of the Secretariat to the Convention

II Objectives and structure of the workshop

- 6. The workshop had the following objectives:
 - a. Review and revise the empirical critical loads of nitrogen for natural and semi-natural ecosystems, set at an expert workshop held in Berne from 11 to 13 November 2002 (see Achermann and Bobbink, 2003)¹, on the basis of additional scientific information available for the period from late 2002 to 2010, as presented in a new and updated background document.
 - b. Provide guidance on how to use site-specific, modifying factors to improve the national application of the empirical approach.
 - c. Review relationships between exceedances of the empirical critical loads and species diversity on a European scale, together with possible regional applications.
- 7. The following classes according to the EUropean Nature Information System (EUNIS) were addressed: marine habitats (EUNIS class A), coastal habitats (EUNIS class B), inland surface waters (EUNIS class C), mires, bogs and fens (EUNIS class D), grasslands and lands dominated by forbs, mosses or lichens (EUNIS class E), heathland, scrubland and tundra (EUNIS class F), woodland, forest and other wooded land (EUNIS class G)
- An international team of scientists (R. Bobbink, S. Braun, A. Nordin, K. Schütz, J. Strengbom, M. Weijters, H. Tomassen) prepared the background documentation for each EUNIS class. This documentation was reviewed by B. Achermann, M. Ashmore, M. Fenn, J-P. Hettelingh, M. Jenssen, S. Power, J.G.M. Roelofs, G. Schütze, and S. Woodin.
- 9. Deliberations on the background documentation, empirical critical loads, modifying factors and further work were structured in three Working Groups, the tasks of which were outlined in guidelines designed by R. Bobbink and J-P. Hettelingh:

- a. Working Group on marine habitats, coastal habitats, inland surface waters and grassland habitats (chair: J. Roelofs; Rapporteur: M. Ashmore)
- b. Working Group on mire, bog and fen habitats and heathland, scrub and tundra habitats (chair: S. Woodin; Rapporteur : S. Power)
- c. Working Group on forest and woodland habitats (chair: J. Strengbom and M. Jenssen; Rapporteur: M. Fenn)
- 10. 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 J-P. Hettelingh.

III. Conclusions

- 11. Statistically and biologically significant outcomes of field addition experiments and mesocosm studies were the basis for the assessment of empirical N critical loads. Only studies which have independent N treatments and realistic N loads and durations (below 100 kg N ha⁻¹ yr⁻¹; more than 1 yr) were used for the updating and refinement of critical load values. In cases where no appropriate N-addition studies were available, gradient and retrospective studies were given a higher weight.
- 12. 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 critical loads. This includes evaluation of the precision of the estimated values of background deposition at the experimental site.
- 13. Empirical critical loads for levels 2 and 3 of the EUNIS classification were agreed on for a range of deposition values for all EUNIS classes, including forest and woodland habitats (EUNIS class G). New results regarding nitrogen effects in surface waters could be included on the basis of activities presented by the ICP Waters. Novel findings for some Mediterranean species could be adopted as well. The reliability of empirical critical loads was qualitatively established, distinguishing between 'reliable', 'quite reliable' and 'expert judgement' symbolised by ##, # and (#), respectively.

¹ Achermann, B. and Bobbink, R. (eds.) (2003). Empirical critical loads for nitrogen. Environmental Documentation No.164 Air, pp. 43-170. Swiss Agency for Environment, Forest and Landscape SAEFL, Berne.

- 14. Empirical critical loads for nitrogen resulting from the reviewing and revising procedure were agreed by consensus at the workshop, as summarised in Table 1. Table 1 also includes the range and reliability of the empirical critical loads established in Achermann and Bobbink (2003)¹, for comparison.
- 15. Additional qualitative information, in comparison to recommendations provided in Achermann and Bobbink (2003)¹, on how to interpret the agreed ranges of critical loads in specific situations for an ecosystem was assigned to a number of modifying factors. However, short of agreement on how to quantify modifying factors for assessments on broad regional scales, consensus was reached to use the minimum value of the ranges of empirical critical loads in every EUNIS class to enable the comparison of their exceedances between different air pollution abatement scenarios.
- 16. To assess effects of exceedances on broad regional scales, it was agreed that specific relationships between the nitrogen load and relevant indicators (see Chapter 10) could be considered, provided that results would only be presented to compare the environmental risk of scenarios in relative terms.

IV. Recommendations

- 17. More well-designed experiments with a wide range of N additions at sites with low background deposition are still urgently needed for several (possible) sensitive EUNIS classes or in regions with many unstudied ecosystems, if any more significant progress is to be made in defining and improving empirical critical loads in the coming years.
- 18. An increasing number of gradient (survey) studies with respect to atmospheric N deposition have been reported or recently initiated. More rigorous guidelines should be identified for evaluation of these studies, covering the estimation of deposition rates, the quantification of confounding factors and the application of methods for statistical analysis. It is recommended to organise a separate expert workshop on this topic.

Table 1 Overview of empirical critical loads of nitrogen deposition (kg N ha⁻¹ yr⁻¹) to natural and semi-natural ecosystems (column 1), classified according to EUNIS (column 2), as originally established in 2003 (column 3), and as revised in 2010 (column 4).The reliability is qualitatively indicated by ## reliable; # quite reliable and (#) expert judgement (column 5). Column 6 provides a selection of effects that can occur when critical loads are exceeded. Finally, changes with respect to 2003 values are indicated in bold.

selection of effects that car	TOCCUT WHEN	i ci ilical loads ale	exceeded. I III	any, changes with	respect to 2005 values are indicated in bold.
Ecosystem type	EUNIS code	2003 kg N ha ⁻¹ yr ⁻¹ and reliability	2010 kg N ha ⁻¹ vr ⁻¹	2010 reliability	Indication of exceedance
Marine habitats (A)					
Mid-upper salt marshes	A2.53		20-30	(#)	Increase in dominance of graminoids
Pioneer and low-mid salt	A2.54 and	30-40 (#)	20-30	(#)	Increase in late-successional species,
marshes	A2.55				increase in productivity
Coastal habitats (B)					
Shifting coastal dunes	B1.3	10-20 (#)	10-20	(#)	Biomass increase, increased N leaching
Coastal stable dune	B1.4ª	10-20 #	8-15	#	Increase in tall graminoids, decrease in
grasslands (grey dunes)					prostrate plants, increased N leaching, soil
					acidification, loss of typical lichen species
Coastal dune heaths	B1.5	10-20 (#)	10-20	(#)	Increase in plant production, increased N
	D1 Ob	10.25 (#)	10.00	(#)	leaching, accelerated succession
Inland curface water babit	$D 1.0^{-}$	10-25 (#)	10-20	(#)	increased biomass of tail grammolds
Dermanent eligetrephic		5 10 ##	7 10	##	Change in the species composition of
	C1.1*	5-10 ##	5-10	##	macrophyte communities increased algol
iakes, ponds and pools					productivity and a chiff in putriant limitation
					of phytoplankton from N to P
Dune slack pools	C1 16	10-20 (#)	10-20	(#)	Increased biomass and rate of succession
(permanent oligotrophic	C1.10	10 20 (#)	10 20	(#)	
waters)					
Permanent dystrophic	C1.4 ^d		3-10	(#)	Increased algal productivity and a shift in
lakes, ponds and pools					nutrient limitation of phytoplankton from N
Mire, bog and fen habitat	s (D)				
Raised and blanket bogs	D1 ^e	5-10 ##	5-10	##	Increase in vascular plants, altered growth
C C					and species composition of bryophytes,
					increased N in peat and peat water
Valley mires, poor fens	D2 ^f	10-20 #	10-15	#	Increase in sedges and vascular plants,
and transition mires					negative effects on bryophytes
Rich fens	D4.1 ^g	15-35 (#)	15-30	(#)	Increase in tall graminoids, decrease in
					bryophytes
Montane rich fens	D4.2 ^g	15-25 (#)	15-25	(#)	Increase in vascular plants, decrease in
					bryophytes
Grasslands and lands dom	ninated by fo	orbs, mosses and	lichens (E)		
Sub-Atlantic semi-dry	E1.26	15-25 ##	15-25	##	Increase in tall grasses, decline in diversity,
calcareous grasslands					increased mineralisation, N leaching; surface
					acidification
Mediterranean xeric	E1.3		15-25	(#)	Increased production, dominance by
grasslands					graminoids
Non-Mediterranean dry	E1.7⁵	10-20 #	10-15	##	Increase in graminoids, decline in typical
acidic and neutral closed					species, decrease in total species richness
grasslands					
Inland dune pioneer	E1.94 ^₅	10-20 (#)	8-15	(#)	Decrease in lichens, increase in biomass
grasslands					
Inland dune siliceous	E1.95 ^b	10-20 (#)	8-15	(#)	Decrease in lichens, increase in biomass,
grasslands					increased succession
Low and medium altitude	E2.2	20-30 (#)	20-30	(#)	Increase in tall grasses, decrease in diversity
nav meadows					

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Table 1Overview of empirical critical loads of nitrogen deposition (kg N ha⁻¹ yr⁻¹) to natural and semi-natural ecosystems (column 1),classified according to EUNIS (column 2), as originally established in 2003 (column 3), and as revised in 2010 (column 4). Thereliability is qualitatively indicated by ## reliable; # quite reliable and (#) expert judgement (column 5). Column 6 provides aselection of effects that can occur when critical loads are exceeded. Finally, changes with respect to 2003 values are indicated in bold.

Ecosystem type	EUNIS code	2003 kg N ha ⁻¹ yr ⁻¹ and reliability	2010 kg N ha ⁻¹ yr ⁻¹	2010 reliability	Indication of exceedance
Mountain hay meadows	E2.3	10-20 (#)	10-20	(#)	Increase in nitrophilous graminoids, changes
					in diversity
Moist and wet					
oligotrophic grasslands		15 25 (1)	15 25		
Molinia caerulea	E3.51	15-25 (#)	15-25	(#)	Increase in tall graminoids, decreased
meadows	57.52	10.00#	10.20		diversity, decrease in bryophytes
Heath (Juncus)	E3.52	10-20#	10-20	#	increase in tail graminoids, decreased
Meadows and numid					diversity, decrease in bryophytes
(Naraus strictu) swards	E4 2	5 10 #	5 10	#	Effects on bryonbytes and/or lisbons
dominated mountain	E4.2	5-10#	5-10	#	Effects on bryophytes and/or lichens
cummite					
Alpine and subalpine	F/1 3		5-10	#	Changes in species composition: increase in
acidic grasslands	L4.J		5-10		plant production
Alpine and subalpine	F4 4		5-10	#	Changes in species composition: increase in
calcareous grasslands	24.4		5 10		plant production
Heathland, scrub and tun	dra habitats	(F)			
Tundra	F1	5-10 #	3-5	#	Changes in biomass, physiological effects,
					changes in species composition in bryophyte
					layer, decrease in lichens
Arctic, alpine and	F2	5-15 (#)	5-15	#	Decline in lichens, bryophytes and evergreen
subalpine scrub habitats					shrubs
Northern wet heath	F4.11				
• 'U' Calluna-dominated	F4.11 ^{e,h}	10-20 (#)	10-20	#	Decreased heather dominance, decline in
wet heath (upland					lichens and mosses, increased N leaching
moorland)					
• 'L' Erica tetralix-	F4.11 ^{e,h}	10-25 (#)	10-20	(#)	Transition from heather to grass dominance
dominated wet heath					
(lowland)					
Dry heaths	F4.2 ^{e,h}	10-20 ##	10-20	##	Transition from heather to grass dominance,
					decline in lichens, changes in plant
					biochemistry, increased sensitivity to abiotic
					stress
Maquis, arborescent	F5		20-30	(#)	Change in plant species richness and
matorral and thermo-					community composition
Mediterranean brushes					
Woodland, forest and oth	er wooded l	and (G)			
Fagus woodland	G1.6		10-20	(#)	Changes in ground vegetation and
					mycorrhiza, nutrient imbalance, changes in
					soil fauna
Acidophilous Quercus-	G1.8		10-15	(#)	Decrease in mycorrhiza, loss of epiphytic
dominated woodland					lichens and bryophytes, changes in ground
Mana and a state				(#)	vegetation
Meso- and eutrophic	G1.A		15-20	(#)	Changes in ground vegetation
Quercus woodland	62.15		10.20	(#)	Changes in an industrial list and
Wediterranean evergreen	G2.1"		10-20	(#)	changes in epipnytic lichens
(Quercus) woodland					

Table 1 Overview of empirical critical loads of nitrogen deposition (kg N ha⁻¹ yr⁻¹) to natural and semi-natural ecosystems (column 1), classified according to EUNIS (column 2), as originally established in 2003 (column 3), and as revised in 2010 (column 4). The reliability is qualitatively indicated by ## reliable; # quite reliable and (#) expert judgement (column 5). Column 6 provides a calculation of affect that can accurate an article loads are eveneded. Finally, changes with respect to 2007 values are indicated in hold.

selection of effects that can occur when critical loads are exceeded. Finally, changes with respect to 2005 values are indicated in bold.							
Ecosystem type	EUNIS	2003	2010	2010	Indication of exceedance		
	code	kg N ha ⁻¹ yr ⁻¹	kg N ha ⁻¹	reliability			
		and reliability	yr '				
Abies and Picea woodland	G3.1		10-15	(#)	Decreased biomass of fine roots, nutrient		
					imbalance, decrease in mycorrhiza, changed		
					soil fauna		
Pinus sylvestris woodland	G3.4		5-15	#	Changes in ground vegetation and		
south of the taiga					mycorrhiza, nutrient imbalances, increased		
					N ₂ O and NO emissions		
Pinus nigra woodland	G3.5		15	(#)	Ammonium accumulation		
Mediterranean Pinus	G3.7		3-15	(#)	Reduction in fine-root biomass, shift in		
woodland					lichen community		
Spruce taiga woodland	G3.A ⁱ	10-20 #	5-10	##	Changes in ground vegetation, decrease in		
					mycorrhiza, increase in free-living algae		
Pine taiga woodland	G3.B ⁱ	10-20 #	5-10	#	Changes in ground vegetation and in		
					mycorrhiza, increase in occurrence of free-		
					living algae		
Mixed taiga woodland	G4.2		5-8	(#)	Increased algal cover		
with Betula							
Mixed Abies-Picea Fagus	G4.6 ¹		10-20	(#)			
woodland							
Overall							
Broadleaved deciduous	G1 ^{k,I}	10-20 #	10-20	##	Changes in soil processes, nutrient		
woodland					imbalance, altered composition mycorrhiza		
					and ground vegetation		
Coniferous woodland	G3 ^{k,I}	10-20 #	5-15	##	Changes in soil processes, nutrient		
					imbalance, altered composition mycorrhiza		
					and ground vegetation		

^{a)} For acidic dunes, the 8 to 10 kg N ha⁻¹ yr¹ range should be applied, for calcareous dunes this range is 10 to 15 kg ha⁻¹ yr¹.

^{b)} Apply the lower end of the range to habitats with a low base availability; and the higher end of the range to those with high base availability.

^o This critical load should only be applied to oligotrophic waters with low alkalinity with no significant agricultural or other human inputs. Apply the lower end of the range to boreal, sub-Arctic and alpine lakes, and the higher end of the range to Atlantic soft waters.

^{d)} This critical load should only be applied to waters with low alkalinity with no significant agricultural or other direct human inputs. Apply the lower end of the range to boreal, sub-Arctic and alpine dystrophic lakes.

^{e)} Apply the high end of the range to areas with high levels of precipitation and the low end of the range to those with low precipitation levels; apply the low end of the range to systems with a low water table, and the high end of the range to those with a high water table. Note that water tables can be modified by management.

^{f)} For EUNIS category D2.1 (valley mires): use the lower end of the range (#).

^{g)} For high-latitude systems, apply the lower end of the range.

^{h)} Apply the high end of the range to areas where sod cutting has been practiced; apply the lower end of the range to areas with low-intensity management.

ⁱ⁾ In 2003 presented as overall value for boreal forests.

¹⁾ Included in studies that were classified under G1.6 and G3.1.

^{k)} In 2003 presented as overall value for temperate forests.

¹⁾ For application at broad geographical scales.

^{m)} This critical load has been based on one European study in Portugal and evidence from studies in Mediterranean woodlands in California. During the final editing procedure of this report it became clear that the ambient background deposition of N in the Portuguese study had not been taken into account; therefore, the critical load was subsequently adapted to this value.

ⁿ⁾ See Appendix 4 for recent findings by the International Cooperative Programme on Waters under the LRTAP Convention.

Opening address at the Workshop on Review and Revision of Empirical Critical Loads and Dose-Response Relationships,Noordwijkerhout, 23-25 June 2010

Scientific work provides the foundation under successful air quality policy

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The Workshop on the Review and Revision of Empirical Critical Loads and Dose Response Relationships in Noordwijkerhout will evaluate and possibly revise the empirical critical loads of nitrogen for natural and seminatural ecosystems, set in 2002. Since then additional scientific information has become available, which has provided new insights into the relationships between nitrogen deposition and its effects on ecosystems and health.

This expert meeting, which is co-organised together with the Swiss Federal Office for the Environment and the German Federal Environment Agency, serves to review and evaluate this new knowledge and its significance for policy makers. With the help of this new knowledge we will be able to determine with more accuracy the maximum levels of nitrogen deposition which do not affect biodiversity and health, the so-called no-effects level. These revised critical loads are crucial to setting ambitious targets for the reduction of nitrogen emissions. Therefore, this workshop is of importance to the revision of the Gothenburg Protocol which is currently underway.

For over three decennia the Netherlands has implemented a two-track policy towards the mitigation of transboundary air pollution. The first track, being based on studying the effects of nitrogen deposition on ecosystems and human health, provides the scientific basis for the second, policy track, which aims at setting targets and establishing measures for the reduction of nitrogen emissions. However because air pollution is not halted by national borders, our aim is also to reach consensus on reduction policy between the countries within the UNECE region and beyond. In this respect special attention is given to the countries of Eastern Europe, Caucasus and Central Asia. Supported by like-minded countries, many of which are represented here, this two-track policy approach has been successfully introduced to the Convention of Long-range Transboundary Air Pollution (CLRTAP). This workshop is a clear example of the effects-based approach of the Convention.

The Netherlands strongly supports the scientific work which provides the foundation under the Convention. The Coordination Centre for Effects (CCE), which has prepared the workshop in Noordwijkerhout, is the Programme Centre for the International Cooperative Programme (ICP) on Modelling and Mapping. It develops methodologies for modelling and mapping the effects of transboundary air pollution. The Netherlands is also the lead country of the Task Force on Integrated Assessment Modelling (TFIAM), which calculates possible national emission ceilings with the help of computer models.

Nitrogen increasingly challenges environmental quality, biodiversity, human wellbeing and health. Because of its reactive properties nitrogen compounds occur in different forms in the air, water and on the soil. Moreover, one single nitrogen molecule can, in time, affect biodiversity and public health and contribute to climate change as well. What is needed, therefore, is an integrated policy approach which means that measures for the reduction of all types of nitrogen, such as ammonia, nitrate and nitrous oxide, are taken simultaneously.

This in order to prevent that the problem is just passed on and, for example, a reduction of ammonia emissions from agriculture to the air leads to the increase of nitrate in the ground water.

We are also increasingly becoming aware of the important role of nitrogen towards climate change. Although these relationships are complicated and yet not fully understood, it is nevertheless clear that any successful climate policy needs to take into account measures related to nitrogen. It is necessary to look for synergies between policies towards air pollution and climate change in order to reinforce the mutual effects and to prevent counteracting measures. Therefore, in addition to the effects of nitrogen with respect to eutrophication, the relationship with climate change is the second reason why the Netherlands calls for high ambition in the revision of the Gothenburg Protocol.

The Netherlands has much to gain from the Convention on Long-Range Transboundary Air Pollution. The reason is that the Netherlands is small and has to deal with many foreign countries. This has implications for many policy issues, including the economy and the environment. Half of the concentrations and depositions of all well-known air pollutants in the Netherlands stem from foreign countries. In addition, the major share of Dutch emissions is exported out of the Netherlands. Therefore, an international coordinated abatement policy will contribute to a level playing field which is both beneficial to our economic competitiveness as well as to environmental quality. Furthermore, I would like to stress the importance of the outcome of this workshop. First of all, critical loads are at the basis of all international agreements on air quality. The revised critical loads resulting from this workshop will be incorporated in the GAINS-model that is used to calculate desirable emission ceilings for nitrogen. That makes them an important building stone for the revision of the Gothenburg Protocol, as well as for the NEC directive.

However, the scientific work to improve empirical critical loads will not only be used to support air pollution policies. It is also of importance in support of policies in the field of biodiversity, for example, to maintain ecosystem-services that are important to human wellbeing. Globally, nitrogen deposition is a *headline indicator* under the Convention on Biological Diversity. In Europe, data on the exceedance of critical loads of nitrogen are used in support of the targets to halt biodiversity loss in Natura 2000 areas.

Although this workshop is first of all a scientific meeting, I would also like to stress the urgency of the outcomes of your work in support of air pollution policies in the near future.

As a consequence, I am looking forward to the report and the application of the results of this workshop in the mapping manual. In addition, I hope that the outcome of this workshop will contribute to maintain the ecological richness and the beautiful landscapes of natural areas in the whole UNECE region.

Chapter 1

Introduction

20 | Review and revision of empirical critical loads and dose-response relationships

1 Introduction

1.1 Impacts of N deposition

Emissions of ammonia (NH₃) 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 (20-80 kg N ha⁻¹ 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).

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).

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). Despite this highly diverse sequence of events, it is possible to generalise some types of impacts. A schematic overview of **Figure 1.1** 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.



the potential sequence of events is given in Figure 1.1.

(a) Direct toxicity of N gases and aerosols to individual species An important effect of nitrogenous gases, aerosols and dissolved compounds (NH₂, NO₂, NO, HNO₂ and NH₂⁺) 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 NO₂ 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, (e.g., Hallingbäck, 1992; Van Herk et al., 2003). This, based on data from the United Kingdom, Italy and Portugal, has recently led to a significant lowering of the long-term critical level of NH_ 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 new 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 a 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

Figure 1.2 A chalk grassland vegetation (*Mesobromion erecti*) (E1.2) in the Netherlands without N addition (left) and after three years of N addition (100 kg N ha⁻¹ yr⁻¹) (right). Photo: R. Bobbink.



rare species at low abundances being especially at risk (Figure 1.2) (e.g., Bobbink *et al.*, 1998; Suding *et al.*, 2005).

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 HCO₂⁻ and Ca²⁺ 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 Ca2+ and Mg2+, 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 Al³⁺ 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 (NH₂) and nitrogen oxides (NO₂) 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⁺, Ca²⁺ and Mg²⁺) 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 1.3).

(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

Figure 1.3. 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 $NH_{a}^{+}:NO_{3}^{-}$ ratio in the soil. Symbols indicate mean ± SE. In contrast to common species, almost all rare species occur only at a low $NH_{a}^{+}:NO_{3}^{-}$ ratio (from Kleijn *et al.*, 2008).



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 steadystate 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, 2010). 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.

Empirical critical N loads 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 empirical critical loads. Only studies on independent N treatments with a duration of 2 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.

Empirical critical loads of 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 critical loads of 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 empirical critical loads for N deposition were 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).

It was recognised at CCE workshops and Task Force meetings of the International Cooperative programme on Modelling and Mapping Critical Loads & Levels and Air Pollution Effects, Risks and Trends in Berne (ICP M&M, 2008) and Stockholm (ICP M&M, 2009) 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. 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 28 session (WGE, 2009) under the LRTAP Convention. This report will be the basis for the revision of Chapter 5.2 of the modelling and mapping manual (ICP M&M, 2010).

The aims and structure of this report are as follows:

- To add new relevant information from studies (November 2002 – spring 2010) on the impacts of N on semi-natural and natural ecosystems, with emphasis on Europe, to the existing database on empirical critical loads of N
- To review and revise Achermann and Bobbink (2003) and provide a revised table on empirical loads of nitrogen for Europe, using the new scientific data, including on background N deposition (Chapters 2 to 8)
- To differentiate and quantify empirical critical loads of N for more specific EUNIS forest ecosystem types (Chapter 9)
- To synthesise the relationships between N exceedances and diversity at a European scale (Chapter 10);
- To refine and provide further guidance on the use of critical load ranges, including consideration of site-specific 'modifying factors' (Appendices 5,6 and 7 and Chapter 11)
- To link, where possible, the empirical critical loads of N based on the EUNIS classification with Natura2000 Annex 1 habitats (Appendix 1)

Finally, the report is completed with a number of appendices. Appendix 1 addresses the link between the EUNIS classification and Natura 2000 habitats. Appendix 2 and 3 provide background information to chapter 9. Appendix 4 provides additional information on empirical critical loads of surface waters that became available after the CCE workshop in Noordwijkerhout, following the 26th session of the Task Force of the ICP Waters (October 4-6, 2010, Helsinki, Finland). The CCE workshop included separate sessions of three Working Groups each addressing one or more EUNIS classes. The summary reports of Working Group 1 (EUNIS classes A, B, C and E), Working Group 2 (EUNIS classes D and F) and of Working group 3 (EUNIS class G) can be found in Appendices 5, 6 and 7 respectively. Finally, Appendix 8 and 9 consist of the list of participants and workshop agenda respectively.

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Chapter 2

Updating and reviewing procedures for empirical critical loads of nitrogen



Photo p. 29 Compilation of cover pages of previous reports on empirical critical loads of nitrogen.

Updating and reviewing procedures for empirical critical loads of nitrogen

Updating procedure 2.1

In this updating procedure, a 'empirical approach' has been used, similar to that of the previous background document (Bobbink et al., 2003), with the following phases: 1) data collection, 2) drafting of the different sections (per class according to the European Nature Information System (EUNIS)), 3) optimisation of the drafts after exchange between the contributing authors, 4) review of the second draft by external expert team, and 5) finalisation of the background document for the UNECE CCE expert workshop, held in June 2010 (Figure 2.1). Following the expert workshop, the background document was finalised after addition and incorporation of the comments of the workshop participants. In addition, a draft workshop summary report has been produced for formal use in the UNECE.

a) Data collection

A comprehensive collection of European publications on the effects of N in natural and semi-natural ecosystems has been made for the period from mid 2002 to March 2010. Peer-reviewed publications, PhD theses, book chapters, nationally published papers, and 'grey' reports by institutes or organisations (if available on request) were used. Relevant information from these studies has been put in an electronic database, including location, background deposition (if available), and EUNIS classification. When feasible, the Natura2000 habitat type was also added in a special appendix (Appendix 1).

In principle, only European studies have been used as the basis for the assessment of critical loads of N. However, when no or very few studies were available for a particular habitat, non-European (mostly Northern American) literature was used for an 'expert judgement' rating of ecosystem sensitivity to N.

b) Drafting of the chapter sections

Following data collection, drafts of the several sections (per EUNIS class) of the background document were written, using the 2003 document as a starting point. When no new data were available, the 2003 text was used. When new data were found to be available, the 2003 text



Figure 2.1. Schematic representation of the working procedure

was modified, which in places resulted in completely rewritten sections. At the end of each section, a concluding table presents the critical loads. The authors have paid particular attention to include any available Natura2000 habitat codes in the different sections and final table.

c) Optimisation of the section drafts

All drafts of the different chapter sections were sent to the author team for discussion and review. Comments of co-authors were discussed and results incorporated into the main versions per chapter section, after which corrected drafts were checked for consistency and integrated into the main document.

d) External review

Second draft versions of the chapter sections were presented to a team of international experts on the impacts of N in natural and semi-natural ecosystems. This reviewing team consisted of experts from all the different parts of Europe'. Each chapter on a specific EUNIS class was evaluated by at least 2 to 3 experts.

e) Finalisation of the background document

Review comments on the second draft were incorporated into the text by the leading author, in close collaboration with the section authors. After a final check, the background document was sent to the participants of the UNECE CCE expert workshop on empirical critical loads of N (23-25 June, 2010, in Noordwijkerhout, the Netherlands). The comments and additions by participants (for reports of the working groups see Appendices 5 to 7) were used to finalise the final 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, 2004), within the framework of the UNECE Convention on Long-range Transboundary Air Pollution.

2.2 Reviewing and setting values for empirical critical loads of nitrogen

There are three major types of evidence available to relate N deposition to changes in 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 NH_4^+ and/or NO_3^- in water. If significant impacts were detected, it was inferred with confidence that 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 or management. Experiments can also identify thresholds for effects

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. Other limitations of experimental studies are that they typically assess relatively short-term responses (even the longest-running experiments seldom exceed 20 years) and that peculiarities of the experiment (e.g., very high concentrations of the applied pollutant compared with environmentally realistic burdens) or site-specific factors might also explain part of the observed response.

A second approach is through targeted field surveys of sites covering a gradient of N deposition. These may use short but intense gradients of N deposition (e.g., close to intensive animal husbandry units) or have a national or regional focus. Such field surveys may provide information on longer-term responses, cover a wider range of N deposition than experiments, and avoid experimental artefacts. Since gradients of N deposition may be highly correlated with those of other potential drivers (e.g. S deposition, climate or management), these need to be measured and considered in both statistical analyses and interpretation. For this reason, targeted surveys cannot prove causality, but can infer the role of N deposition as a possible driver of changes in, for instance, biodiversity. Targeted field surveys performed in this way may help to clearly identify the effects of N deposition, and are used as additional evidence to support conclusions from experimental N additions.

Targeted surveys and experiments allow effects on relatively common species and functional groups to be evaluated, but they have little statistical power to detect effects on rare or very scarce species. An alternative to targeted surveys is the use of data sets on broader ecological surveillance which cover a wider range of communities representative of the region of interest. These typically record the presence or absence of species in larger areas (e.g., 10×10 km squares). Such data reflect the impact of land use and a range of climatic, edaphic and management factors, and hence attribution of any change to N deposition is difficult. However, they do provide important signals of change in biodiversity at regional and national scales. These data sets, potentially, are also able to detect effects on (very) rare and scarce species, but they are not directly useful to set critical loads.

Setting critical loads

In this background document, we focus particularly on statistically and biologically significant outcomes of field addition experiments and mesocosm studies for the assessment of empirical critical loads of N. Only studies which have independent N treatments and realistic N loads and durations (below 100 kg N ha⁻¹ yr⁻¹; more than 1 year) were used for the updating and refinement of critical load 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 system. 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. This is necessary to get insight into the total N load in both the N-treated and the control vegetation.

Furthermore, the results from targeted field studies were only used as additional evidence in respect to the outcome of N-application studies, or to estimate a critical load value based on expert judgement, if experimental studies were lacking. When available, the outcomes from dynamic ecosystem models provided additional insight into underlying mechanisms, which are difficult to incorporate in experimental studies, such as increased frequencies of pests and diseases. In general, pot or microcosm studies were not used for setting critical loads, 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.

2.3 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 (Davies and Moss, 2002; Davies et al., 2004). For a general description of the updated EUNIS classification and an introduction to its use, see Davies et al. (2004) and the supporting website (http://eunis.eea.europa.eu/). In general, the ecosystems described in this document have been classified down to, at least, 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 (E1.2). In this background document special attention has been paid to the classification of woodland and forest habitats (G), in order to better differentiate the critical loads of N between the different forest types. As before, studies based on pure plantation stands were not included in the chapter on forest habitats. For the critical loads of N for these intensively managed systems, based upon simple steady-state mass balance methods, see the UNECE Mapping Manual (2004). Furthermore, an appendix has been compiled to relate the EUNIS classification to

Natura2000 habitat types (Appendix 1). Lichens and bryophytes, as before, have been incorporated in the sections of the appropriate ecosystems.

2.4 References

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Chapter 3

Effects of nitrogen deposition on marine habitats (EUNIS class A)



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

3.1 Introduction

Marine habitats, categorised in the European Nature Information System (EUNIS) under class A, are distinguished from other ecosystems by their direct connection to the sea. Most of these systems are either not covered with 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 above the high spring tide in tidal regions are included in marine habitats (Class A) and therefore included in this chapter. Since the 2003 update, hardly any evidence has been found for this EUNIS class, thus, the content of this chapter is more or less identical to that of 2003 (Bobbink *et al.*, 2003), except for a few small corrections and one additional study.

3.2 Coastal salt marshes and saline reed beds (A2.5)

Salt marshes develop where fine sediments accumulate along sheltered coastlines in the temperate and highlatitude regions of the world. 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, that is, they are located in areas that lie between lowest and highest tide, and are periodically covered with salt water. The dominant plants are rooted macrophytes which are adapted to the environmental stresses associated with sea water inundation (Archibold, 1995). They are characterised by an open nutrient cycle, receive large amounts of nutrients from surface water, and export 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 effects of increased atmospheric N deposition, at least not at 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 A2.54 – Low-mid salt marshes) and a 100-year-old salt marsh (EUNIS category A2.53 – Mid-upper salt marshes) in the Netherlands (background deposition 15-20 kg N ha-1 yr¹). Biomass increased significantly after N application from the first growing season in the young salt marsh, and 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 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. As 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), 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.

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 accretion rate of salt marshes, this will result in a net loss

Figure 3.1 Above-ground biomass of young salt marsh vegetation (A2.64) 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).



of salt marshes of a low successional age (EUNIS categories A2.55 and A2.54). New information from long-term monitoring (25 years) of vegetation on one Dutch island showed a trend towards more eutrophic vegetation in both grazed and ungrazed salt marshes (Dijkema et al., 2005). This study was based primarily on mid-successional salt marshes; in early successional salt marshes the trend was less pronounced, partly because of the low number of species. The total N deposition range in that study is estimated to be 15 to 20 kg ha⁻¹ yr⁻¹. On the basis of expert judgement and the evidence from this one study, a new critical load range of 20 to 30 kg N ha⁻¹ yr⁻¹ is recommended as a conservative estimate for salt marsh systems (A2.5). This new critical load should be extended to mid-upper successional salt marshes (A2.53). However, field experiments with lower N additions over a longer period of time would be needed to improve the reliability for this estimate (Table 3.1).

Table 3.1 Empirical critical loads of N and effects of exceedances on marine habitats (A). ## reliable, # quite reliable and (#) expert judgement. Changes with respect to 2003 are indicated in bold.

EUNIS code	kg N ha ⁻¹ yr ⁻¹	Reliability	Indication of exceedance
A2.53	20-30	(#)	Increase in dominance of
			graminoids
A2.54	20-30	(#)	Increase in
and			late-
A2.55			successional
			species,
			increase in
			productivity
	EUNIS code A2.53 A2.54 and A2.55	EUNIS codekg N ha'' yr'A2.5320-30A2.5420-30and A2.5520-30	EUNIS codekg N ha ⁻¹ yr ⁻¹ ReliabilityA2.5320-30(#)A2.5420-30(#)and A2.55(#)(#)

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Chapter 4

Effects of nitrogen deposition on coastal habitats (EUNIS class B)



Photo p. 41 Coastal dune landscape in the Dutch Wadden area with in the back of the picture grey dunes with strong dominance of Ammophila arenaria due to enhanced atmospheric N inputs (photo R. Bobbink).

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

4.1 Introduction

This chapter presents an evaluation of the impacts of atmospheric N deposition on coastal habitats (class B of the European Nature Information System (EUNIS), with respect to the setting of critical loads of N. Coastal habitats are those 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, scrub and wooded dunes), beaches and cliffs (Davies et al., 2004). Dune slack pools are considered in Chapter 5, because of their position in EUNIS class C (inland surface waters), although a consistency between critical loads for dune slacks and dune slack pools is clearly important in practical applications of empirical critical loads. The first subdivision within class B in EUNIS is based on underlying substrates, that is, sand, shingle or rock, but data to support proposals for empirical critical loads are only available for sand substrates (B1; coastal dune and sandy shores). In this background document, separate critical load values for N deposition are evaluated and reviewed for major habitats within B1, using the studies from Bobbink et al. (2003) as a base and updating values with newer information, where available.

4.2 Coastal dunes and sandy shores(B1)

Many dune ecosystems in the coastal areas of Europe are almost completely natural in origin as well as rich in typical plant and animal life, and are thus a major part of European biodiversity. They are found on sandy, nutrientpoor 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 critical loads of N in coastal dune and sand habitats, evidence exists only for some EUNIS categories, namely those of shifting coastal dunes (B1.3), coastal stable dune grasslands (B1.4), coastal dune heaths (B1.5), and moist and wet dune slacks (B1.8).

4.2.1 Shifting coastal dunes (B1.3)

Shifting coastal dunes are coastal, mobile sand habitats of the boreal, nemoral, steppe, Mediterranean and warmtemperate humid zones of Europe. They include embryonic shifting dunes and shifting dunes along the shoreline with *Ammophila arenaria* ('white dunes'). Hardly any evidence from N addition experiments is available on this habitat type, except for one study in Iceland with rather high N addition rates (Greipsson and Davy, 1997). These authors studied the effects of N addition during a two-year period in the coastal dunes of Iceland (probably EUNIS B1.3, shifting coastal dunes) 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. Supporting evidence for the effects of N deposition is also available in the form of a targeted field survey 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. 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 mentioned effects start to become apparent in the range of 10 to 20 kg N ha⁻¹ yr⁻¹ and over. In the long term, this increase in biomass may lead to enhanced organic matter accumulation and thus accelerated soil development and increased succession rates. See Jones et al. (2008), where accelerated dune soil development was positively correlated with both N deposition and temperature.

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.



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. Based on the observed relationships and generally low nutrient status of these soils, it is realistic to set the critical load range of N for shifting coastal dunes (B1.3) at 10 to 20 kg N ha⁻¹ yr⁻¹ (expert judgement).

4.2.2 Coastal stable dune grasslands (grey dunes) (B1.4)

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, mosses and lichens may dominate, both in abundance and in species richness. They are characterised by dry soil conditions 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.

Tall grasses, however, have increased in many Dutch dry dune grasslands, over recent decades (> 1970); a period with relatively high N loading (20-30 kg N ha⁻¹ yr⁻¹). In the primarily non-calcareous dunes, Ammophila arenaria is usually the dominant species, whereas in more calcareous areas Calamagrostis epigejos, Elymus repens and Elymus athericus dominate (Kooijman and De Haan, 1995). 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 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 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, with relatively low N deposition (ca 10 kg N ha⁻¹ yr⁻¹), stable dune grasslands are still rich in species (Jones *et al.*, 2002)

The effects of nutrients in dry dune grasslands (calcium carbonate 1%) on sandy soils 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 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 treatments with N (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 (NH)_SO and NaNO, 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 (Olff et al., 1993). In summary, these short-term experiments with relatively high N applications clearly indicate the importance of N limitation in several stable dune grasslands. However, in some cases, co-limitation with P has been observed, or rabbit grazing may have prevented the dominance of tall grasses.

Two N addition experiments have been carried out in stable dune grasslands, one in the Netherlands, and one in the United Kingdom, with the specific objective to examine the effects of N deposition. In addition, the effects of N loads have also been studied in a 3-year mesocosm study in the Netherlands. 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, in the Netherlands, by Ten Harkel and Van der Meulen

(1995) and Ten Harkel et al. (1998). After 4 years of N additions twice a year (25 kg N ha⁻¹ yr⁻¹ in the form of ammonium nitrate pellets; background deposition ca 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 from the added pellets, the plots were watered fortnightly with ammonium sulphate (50 kg N ha⁻¹ yr⁻¹) during the last year and a half of the experiment. In the course of the experiment, it had become obvious that ≥80% of the total N inputs in the N-treated vegetation had leached from the soil as nitrate, due to nitrification at the calcareous sites, while 70% of N input leached even from the control grazed plots at these sites. At the partly decalcified site, nitrate leaching was substantially smaller, but still between 7 and 40% (4-11 kg N ha⁻¹ yr⁻¹) of the experimental N inputs (Ten Harkel et al., 1998). It, thus, became clear that in these stable dune grasslands experimental N enrichment had no effect on species composition. This may have been be related to a shift towards P limitation after the long period of high atmospheric N inputs in the Netherlands and the relatively high rabbit grazing pressure in that period. The high N leaching from the control 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 thin dune soils have a relatively low organic matter content, lack clay particles, and therefore have fewer exchange sites with which to bind ammonium ions. It is also possible that biological responses may have already occurred as a result of previous inputs and thus had little further response potential.

The effects of elevated N loads in a situation of low background deposition (<5 kg N ha⁻¹ yr⁻¹) have been studied during 2 to 3 years, in dry dune calcareous grassland mesocosms in a greenhouse (1x1 m) (Tomassen et al., 1999; Van den Berg et al., 2005). After a pre-treatment period of 2 months with clean rain water 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 algae 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 4.2). The strong increase in algae on the soil top 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).

Figure 4.2 Algal material, measured as active chlorophyll concentration (mg cm⁻²; means \pm standard error; n=4), in the top layer of the soil at different N addition rates (Van den Berg *et al.*, 2005). Different letters indicate significant differences between the treatments.



Figure 4.3 Above- and below-ground biomass (g m⁻²) of *Calamagrostis epigejos* and *Carex arenaria* after 2 years of N application in coastal stable dune grassland (B1.4) mesocosms (Tomassen *et al.*, 1999; Van den Berg *et al.*, 2005). From left to right: 1, 10, 20, 40, 60 and 80 kg N ha⁻¹ yr⁻¹.



Differences in plant growth were observed after one year and became 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 *Calamagrostis epigejos*. The shoot and root biomass of this tall grass species increased with higher N additions, significantly above 20 and 15 kg N ha⁻¹ yr⁻¹, respectively (Figure 4.3).

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). These results are consistent with those of Mohd-Said (1999), who exposed mesocosms with dry dune grassland vegetation to a range of N deposition rates in the range of 2 to 55 kg ha⁻¹ yr⁻¹. At 10 kg N ha⁻¹ yr⁻¹ and over, the cover of the grasses Festuca rubra and Dactylis glomerata increased, while there was no change in cover of herb species, including Galium verum. These mesocosm experiments with hardly any background N load (1 kg N ha⁻¹ yr⁻¹) have demonstrated that, even in calcareous dune grasslands, N loads of ≥15 kg ha⁻¹ yr⁻¹ changed the algae and grass cover, while higher loads (when not grazed) led to dominance of graminoid species within a few years.

Recently, the effects of low levels of N additions (and P) were studied in the United Kingdom (Isle of Anglesey) under low background deposition (10-12 kg N ha⁻¹ yr⁻¹) in a calcareous, heavily grazed fixed dune grassland (Plassmann et al., 2009). In addition, the impacts of grazing management were examined. Four N treatments (unwatered control, watered control, 7.5 kg N ha⁻¹ yr⁻¹, 15 kg N ha⁻¹ yr⁻¹) were combined with three grazing treatments. In a separate experiment, effects of fertilisation with both N (15 kg ha⁻¹ yr⁻¹) and P (15 kg ha⁻¹ yr⁻¹) 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 N concentration was significantly higher under high N treatment (15 kg N ha⁻¹ yr⁻¹), whereas the total N pool in the bryophytes were significantly higher under both N-addition treatments. No effects on vegetation composition, sward height or soil parameters occurred within the two-year research period. Furthermore, additions of both N and P had a greater impact on above-ground biomass, sward heights and sward structure than N addition alone. The changes observed after only two years of experimental N addition may lead to community changes over longer time scales. Effects were observed even under heavy grazing

with co-limitation of P. The outcome of this experiment clearly suggests that significant changes can be seen in a clean area with a total N load of ca 20 kg N ha⁻¹ yr⁻¹.

Additional field evidence is now available from 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). Eleven coastal dune sites were surveyed, representing a range of atmospheric N deposition from 10 to 30 kg N ha⁻¹ yr⁻¹. In these stable dune grasslands, above-ground biomass related positively to N deposition, while for species density this relationship was negative (Figure 4.4). In addition, in these grasslands, a strong positive relationship was found between dissolved organic N in groundwater and N deposition. Furthermore, the cover of Carex arenaria also related positively to N inputs. In general, the observed effects started to become apparent in the range 10 to 20 kg N ha⁻¹ yr⁻¹ and over, although the number of measurements above 20 kg N ha⁻¹ yr¹ was very limited and statistically uncertain.

Figure 4.4 Species richness (2x2 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 SE.



The impacts of atmospheric N deposition on dry lichenrich dune grasslands (B1.4 – acidic to slightly calcareous) around the Baltic Sea was recently 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. In 19 investigated dune sites the atmospheric wet N deposition was 3 to 8 kg N ha⁻¹ yr⁻¹ (wet N deposition of nearby EMEP certified weather stations). The N content of the typical lichen, *Cladonia portentosa*, was demonstrated to be a suitable biomonitor of these low to medium deposition levels: the N concentrations were higher above 5 kg N ha⁻¹ yr⁻¹ wet N deposition. Comparison with EMEP deposition data showed that tissue N concentrations in *Cladonia portentosa* reflected the deposition history of the last 3 to 6 years. A shift from lichen-rich short grass vegetation towards species-poor vegetation dominated by *Carex arenaria* also correlated with the higher wet depositions. Plant species richness per field site, however, was not shown to decrease directly 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 4.5). The number of foliose lichen species, forbs and grasses was lower in regions with wet deposition over 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). Because of the low roughness of intact lichen-rich stable dune grasslands, it is likely that 5 kg N ha⁻¹ yr⁻¹ wet deposition will correspond with ca 8 kg N ha⁻¹ yr⁻¹ total deposition, suggesting a lower critical load than previously set. Preliminary data from a targeted survey in the United Kingdom, in acidic stable dune grasslands, in 2009, also showed a decrease in vegetation species number in the same N deposition range as that for the coastal dunes of the Baltic (personal communication M.L.M. Jones).

Previously, the critical load range for stable dune grasslands (grey dunes) was set at 10 to 20 kg N ha⁻¹ yr⁻¹ (quite reliable; Bobbink *et al.*, 2003). Based on new evidence (Remke *et al.*, 2009a; 2009b; Plassmann *et al.*, 2009) it is realistic to lower both the lower and higher limits of the critical load range for coastal stable dune grasslands (B1.4) to 8 to 15 kg N ha⁻¹ yr⁻¹ (quite reliable). In addition, it is suggested to use a range of 8 to 10 N ha⁻¹ yr⁻¹ for acidic or decalcified dune grasslands, and 10 to 15 kg N ha⁻¹ yr⁻¹ for calcareous stable dune grasslands. The impacts of N additions have been studied in coastal dune grasslands in several regions of Europe, but 'long-term' experimental studies (5 - 10 yrs) with realistic N loads in areas with low naturally occurring deposition levels are still needed to validate this critical load value.



Figure 4.5 Picture of a Carex arenaria-dominated stable dune grassland in the Baltic (Korshage DK; photo E. Remke)

4.2.3 Coastal dune heaths (B1.5)

Besides dry dune grasslands, heathland vegetation is also present in the coastal dunes in north-western 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, coastal heaths (B1.5) are classified as a subcategory of coastal dune and sand habitats.

Only one N manipulation experiment has been performed in coastal heaths, namely within the Danish HEATH experiment (Riis-Nielsen, 1997; Nielsen et al., 2000), in coastal heath at Lodbjerg, Denmark. This coastal heathland, dominated by Empetrum nigrum and Ammophila arenaria, is located in an area of ca 250 to 300-year-old dunes on the coast of Jutland, with a relatively low naturally occurring deposition (13 kg N ha⁻¹ yr⁻¹) (Nielsen et al., 2000). A high-frequency – low-dose application of ammonium nitrate (0, 15, 35 and 70 kg ha⁻¹ yr⁻¹) was carried out over a period of 2 years. As a response to N additions, vascular plant species increased in cover, whereas lichens and bryophytes showed a very slight and insignificant decrease over those 2 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 limitation. However, drought effects on *Empetrum* were clearly not related to 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 with 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.

It was concluded that coastal heaths are affected by moderate N additions (\geq 35-70 kg N ha⁻¹ yr ⁻¹), but because of the short experimental period at only one site, it is difficult to set a critical load for this ecosystem type. However, leaching data may indicate a higher sensitivity to extra N inputs in coastal heaths than in inland dry heaths. This is in accordance with the shallow organic layer in most of these coastal heaths. Moreover, these dune heaths are generally unmanaged, and thus have low N removal from the system. Because of this, the empirical critical load of N for coastal heath (B1.5) has been established at 10 to 20 kg ha⁻¹ yr⁻¹ (expert judgement). It is evident that more long-term information would be needed to verify this value.

4.2.4 Coastal moist to wet dune slacks (B1.8)

Moist to wet dune slacks (EUNIS B1.8) 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 many rare, basiphilous forb species. Groundwater level is at or above soil level in winter, whereas in the growing season groundwater level is considerably lower in these dune slacks. Nowadays, many dune slacks have been incorporated in nature reserves, and these are sometimes maintained though management, such as 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. In addition, they are very sensitive to desiccation 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 N kg ha⁻¹ 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 N kg ha⁻¹ yr⁻¹; >1 year), and thus they are not adequate for setting a N critical load.

A targeted field survey of dune slacks in the United Kingdom, with a calculated total N deposition ranging from 6.9 kg N ha⁻¹ yr⁻¹ 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. This may, in part, be due to the absence of wet dune slacks in 2 of the 11 high deposition sites. However, the cover of *Carex arenaria* and *Hypochaeris radicata* related positively to total N deposition, suggesting a response in the vegetation 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, 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 NH_4NO_3) was applied to soils from dune slacks with a site background deposition of ca 11 kg N ha⁻¹ yr⁻¹. The emerging seedling community on N treated soil differed strongly from communities on other soils. Germination was generally greater in species with low Ellenberg N indicator values (Plassmann *et al.*, 2008). This may also deplete the seedbank of early successional species which depend on seed longevity to survive until the next mobility phase.

It is likely that most dune slacks are sensitive to N inputs. Lowering the critical load range to 10 to 20 kg N ha⁻¹ yr⁻¹ is recommended for moist to wet dune slacks (B1.8) (expert judgement) rather than that of 10 to 25 kg N ha⁻¹ yr⁻¹ as recommended in 2003. This is based on relevant information from a UK study which showed positive effects on germination in greenhouse experiments, adding 15 kg N ha⁻¹ yr⁻¹ to columns taken from field, and from the fact that in areas of the Netherlands with a deposition of 20 to 25 kg ha⁻¹ yr⁻¹, there is a need to remove sludge or sods every 20 years. In addition, the resemblance of these slacks to wet, oligotrophic grasslands and base-rich fens has been taken in account, too. Based on expert judgement, the lower end of the range should be used with low base availability and the higher end should be used with high base availability. Of course, field validation of this new value would certainly be necessary.

The empirical critical loads of N for coastal habitats (B) are summarised in Table 4.1.

Table 4.1. Empirical critical loads of N and effects of exceedances on coastal habitats (EUNIS class B). ## reliable, # quite reliable and (#) expert judgement. Changes compared to the 2003 document are indicated in bold.

Ecosystem type	EUNIS code	kg N ha ⁻¹ yr ⁻¹	Reliability	Indication of exceedance
Shifting coastal dunes	B1.3	10-20	(#)	Biomass increase, increase N leaching
Coastal stable dune grasslands (grey dunes) ^a	B1.4	8-15	#	Increase tall graminoids, decrease prostrate plants, increased N leaching, soil acidification, loss of typical lichen species
Coastal dune heaths	B1.5	10-20	(#)	Increase plant production, increase N leaching, accelerated succession
Moist to wet dune slacks ^b	B1.8	10-20	(#)	Increased biomass and tall graminoids
^{a)} For acidic dunes, use the 8 to 10 kg N ha ⁻¹ yr ⁻¹ range, for				

calcareous dunes use the 10 to 15 kg N ha⁻¹ yr⁻¹range. ^{b)} Use the lower end of the range with low base-cation availability. Use the higher end of the range with high base-cation availability.

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52 | Review and revision of empirical critical loads and dose-response relationships

Chapter 5

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



Photo p. 53 Oligotrophic soft-water lakes are still common across Scandinavia (photo E. Lucassen, SW Norway).

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

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. Class C also includes dune slack pools (Davies et al., 2004).

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 N deposition with respect to eutrophication or effects of adverse ammonium. This chapter only discusses surface standing waters (C1) because of data availability. It briefly summarises the acidifying effects of airborne N compounds in surface waters, as critical loads for acidity, including N, based on Steady-State Water Chemistry models (SSWC). These are well established for most aquatic ecosystems (Henriksen, 1988; Kämäri *et al.*, 1992; Henriksen and Posch, 2001; UNECE, 2004).

Surface standing waters vary according to trophic status, depth, sediment type, alkalinity and colour (Moe *et al.*, 2008; Arts, 2002). These characteristics determine their suitability as habitat for all kinds of organisms and probably also their sensitivity to changed N availability. N leaching from semi-natural catchments, however poorly understood, is related to both N deposition and catchment characteristics (Dise and Wright, 1995; Aber *et al.*, 1998). Thus, effects of N deposition on biology in freshwater habitats will depend both on catchment N-retention capacity and on the sensitivity of habitats and organisms to changed N availability.

5.2 Surface standing waters (C1)

The main division of permanent standing waters containing fresh water (i.e. non-saline) is based on the trophic status of the waters, from oligotrophic (C1.1) via mesotrophic (C1.2) to 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 (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 et al., 2003), the impacts of N deposition are first discussed for two specific elements of category C1.1: a) Atlantic soft-water lakes (Subsection 5.2.1) and b) dune slack pools (Subsection 5.2.2), as for both elements experimental evidence existed. However, the effects of N deposition in pristine boreal and alpine oligotrophic water bodies across Europe and North America have become a hot topic in the last 5 years, leading to new experimental evidence to set empirical critical loads for this important group of category C1.1 (Subsection 5.2.3). Unfortunately, it is not possible to distinguish in EUNIS this important subcategory from the Atlantic soft-water lakes, both are part of 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, which are poor in or almost devoid of calcium carbonate. The waters are poorly buffered and the concentrations of calcium in the water are very low; they are shallow and fully mixed water bodies, with fluctuating water levels, and are mainly fed by rain water or water from acidic catchment soils, and thus oligotrophic. In the EUNIS approach, these waters are classified as either permanent oligotrophic (C1.1) or permanent mesotrophic (C1.2) lakes, pools and ponds. These soft-water ecosystems are characterised by plant communities from the phytosociological alliance Littorelletea (Schoof-Van Pelt, 1973; Wittig, 1982; Arts, 1990; 2002). The 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 many other soft-water macrophytes. These soft waters are nowadays almost all located within nature reserves and have become very rare in western Europe. This decline may be illustrated by the fact that Littorella uniflora was known to occur at more than 230 sites in the Netherlands in the early 1950s, while at the end of the 1980s this number had dwindled to ca 40. Furthermore, amphibians have also strongly declined in these soft waters (Leuven et al., 1986).

The effects of N pollutants on these soft-water systems have been intensively studied in the Netherlands, both in field surveys and experimental studies. Field observations in ca 70 soft waters (with well-developed isoetid vegetation in the 1950s) showed that the waters in which characteristic macrophytes were still abundant in the early 1980s, 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 soft-water sites from which these plant species had disappeared could be divided into two groups. In 12 of the 53 soft-water sites eutrophication, resulting from inflow of enriched water, seemed to be the cause of the decline. In this group of non-acidified waters, plant species such as *Lemna minor* had become dominant. High concentrations of phosphate and ammonium were measured in the sediment in these waters. In some of the larger water bodies no macrophytes were found at all, as a result of dense plankton bloom. In the second group of lakes and pools (41 out of 53) a different situation had developed: the isoetid species were replaced by dense stands of Juncus bulbosus or aquatic mosses, such as Sphagnum cuspidatum and Drepanocladus fluitans. This clearly indicates acidification of these soft waters during recent decades, probably caused by increased atmospheric deposition. The same field study showed that N levels in the water were higher in ecosystems from which natural vegetation had disappeared, compared with ecosystems in which isoetid stands were still present (Roelofs, 1983). This strongly suggests the detrimental effects of atmospheric N deposition on these soft-water lakes.

A number of ecophysiological studies have revealed that (i) inorganic carbon status of the water (as a result of intermediate levels of alkalinity), and (ii) low N concentrations, are important for the growth of endangered isoetid macrophytes. Furthermore, almost all 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 influenced growth of aquatic plant species. Almost all of the characteristic soft-water isoetids developed more effectively under nitrate addition than that of ammonium, whereas Juncus bulbosus and aquatic mosses (Sphagnum and Drepanocladus) were clearly stimulated by high levels of ammonium (Schuurkes et al., 1986). The importance of ammonium for the growth of these aquatic mosses is also reported by Glime (1992).

The effects of atmospheric deposition on soft-water mesocosms have been studied during a two-year treatment with different types of artificial rain (Schuurkes *et al.*, 1987). Acidification due to sulphuric acid, without N inputs, did not result in an increased mass growth of *Juncus bulbosus*, and a diverse isoetid vegetation remained



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

present. However, after increasing the N concentration in the precipitation (19 kg N ha⁻¹ yr⁻¹ or higher, in the form of ammonium sulphate; control < 2 kg N ha⁻¹ yr⁻¹), changes in floristic composition were observed, similar to those seen under field conditions: a dramatic increase in dominance of Juncus bulbosus, of submerged aquatic mosses and of Agrostis canina (Schuurkes et al., 1987). This demonstrates that the observed changes occurred because of the effects of ammonium sulphate deposition, leading to both eutrophication and acidification. The increased levels of ammonium in the system directly stimulated growth of plants such as Juncus bulbosus, and any surplus ammonium was nitrified in these soft-water systems (pH>4.0). During this nitrification process H⁺ ions are produced, which increase the acidity of the system. The results from this study clearly demonstrated that large changes already occurred after two years of treatment with 19 kg N ha⁻¹ yr⁻¹ or more. In addition, the strongest decline in the species composition of the macrophytes in Dutch soft-water communities was observed to occur in areas with atmospheric N loads of ca 10 to 13 kg N ha⁻¹ yr⁻¹ (Arts, 1990). Furthermore, Brouwer et al. (1997) showed that, after 10 years of clean-rainwater treatment, the recovery of these soft-water mesocosms, which before had been treated with ammonium sulphate, was only partial and Juncus and Molinia species were still the dominant species.

The critical load range of N for shallow soft-water bodies (most in C1.1, but some elements in C1.2) was set, based on experimental evidence, at 5 to 10 kg N ha⁻¹ yr⁻¹ (reliable) in 1996, and remained unchanged in the 2002-2003 update (Bobbink *et al.*, 1996; 2003). Since then, no new evidence from experimental studies has been published on the impacts of N deposition in (shallow) soft-water lakes. Therefore, the critical load range of N for these systems remains unchanged, and has been incorporated in the general critical load range for oligotrophic lakes, ponds and pools (C.1.1) of 3 to 10 kg N ha⁻¹ yr⁻¹ (reliable), with the recommendation to apply the upper part of the range (see also Subsection 5.2.3).

5.2.2 Dune slack pools (permanent oligotrophic lakes, pools and ponds C1.16)

Another studied subcategory in the EUNIS class of permanent oligotrophic waters is that of dune slack pools (C1.16). These relatively small but permanent clear-water pools are found in the European coastal dune areas. Despite their coastal location, they have been classified as part of the inland surface water habitats in EUNIS. The surface water is nutrient-poor and these dune slack pools are mostly insensitive to acidification, because of their higher alkalinity (>1000 µeq l⁻¹). They are also characterised by clear water and a diverse submerged macrophyte vegetation (e.g. Potamogeton and Chara species, and littoral isoetids). 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. Very few experimental data exist on the sensitivity of these dune slack pools with respect to critical load setting, despite the generally well-known N limitation of dune slack wetlands (Lammerts and Grootjans, 1997). However, the impacts of atmospheric N deposition were quantified in dune-pool mesocosms (ca 2-m diameters) during a two-year experiment with different N loads (1, 20, 40 and 120 kg N ha⁻¹ yr⁻¹) (Brouwer et al., 1996). No acidification of the water was found in those 2 years, but total biomass of water plants and helophytes increased strongly at over 20 kg ha⁻¹ yr⁻¹. N 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, with relatively high N loads (15-20 kg N ha⁻¹ yr⁻¹). However, experimental field studies or targeted surveys are lacking. Therefore, we propose to retain the 2002-2003 critical load range of 10 to 20 kg N ha⁻¹ yr ⁻¹ for dune slack pools (C1.16) (Bobbink et al., 2003), rated as 'expert judgement'. 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.

5.2.3 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 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 has had 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) recovery from acidification in 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).

The critical loads of acidity for aquatic ecosystems were elaborated in publications by UNECE in 1988, 1992, 1996 and 2004, using Steady-State Water Chemistry (SSWC) models. The latest SSWC models incorporate acidity from both sulphur and N deposition, and critical loads are calculated depending on (i) base-cation deposition, (ii) internal alkalinity production or base-cation concentrations, and (iii) nitrate leaching from the water system. The calculated critical loads are site specific (sensitive or non-sensitive geological regions) and also depend on local hydrology and precipitation. For more details see Henriksen (1988), Kämäri *et al.* (1992) and Henriksen and Posch (2001). Table 5.1 provides the critical loads of N compounds for acidifying effects, assuming N deposition as the only cause of acidification, for the most sensitive oligotrophic lakes and streams.

Table 5.1 Critical loads of acidity, caused by N compounds, for
sensitive oligotrophic lakes and streams, according to SSWC
model calculations.

Ecosystem type	Critical load of acidity kg N ha ⁻¹ yr ⁻¹	Reference
Scandinavian waters	1.4-4.2	(Henriksen, 1988;
(C1.1)		Kämäri et al., 1992)
Alpine lakes	3.5-6.1	(Marchetto et al.,
(C1.1)		1994)
Humic moorland pools	3.5-4.5	(Schuurkes et al.,
(C1.4)		1987; Van Dam and
		Buskens, 1993)

To date, the effects of eutrophication by atmospheric N deposition have hardly been incorporated in the setting of empirical critical loads for waters, except for some permanent oligotrophic or mesotrophic water bodies (see Section 5.2). This is because primary production in almost all surface waters has been thought to be limited by phosphorus, and thus N enrichment is considered to be unimportant in this respect (e.g., Moss, 1988). However, over recent years it has become obvious that this paradigm does not holds for all freshwater systems, especially in pristine areas of alpine, (sub)Arctic, Arctic or boreal regions (e.g., Saros *et al.*, 2005; Bergström and Jansson, 2006; Wolfe *et al.*, 2006; Sterner, 2008; Elser *et al.*, 2007; De Wit and Lindholm, in press).

Bergström *et al.* (2005; 2008) conducted lake sampling and in situ nutrient enrichment enclosure experiments with N and phosphorus (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) from the north to the south of Sweden. Regional and seasonal patterns of nutrient limitation of phytoplankton were clearly related to the amounts of N deposition and 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) 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 N deposition. The range in N deposition was <1 to 14 kg N ha⁻¹ yr⁻¹, and the largest increase in algal biomass per unit P occurred at 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 and indicate that N deposition has contributed to higher algal productivity in oligotrophic lakes, including dystrophic ones.

Epilithic communities have been studied in a targeted survey of oligotrophic Swedish lakes along a north-south gradient (Liess et al., 2009). Atmospheric N deposition in northern lakes ranged between 2 and 6 kg N ha⁻¹ yr⁻¹ and in southern lakes this was between 10 and 12 kg ha⁻¹ yr⁻¹. Epilithic communities appeared to be generally more N limited in the northern lakes and more P limited in the southern lakes. Lake water total N and epilithic N:P ratios were lower in northern lakes than in those in the south, and the proportion of N₂-fixing cyanobacteria was higher in northern lakes than in the southern ones. In northern lakes, gastropod grazers had less nutrient imbalances and cycled less N relative to P, than in the southern lakes. Atmospheric N deposition showed a strong positive correlation with lake water total N and a much weaker positive correlation with epilithic N:P ratios. Atmospheric N deposition was also negatively correlated with the proportion of N₂-fixing cyanobacteria. These data clearly suggest that increased atmospheric N deposition (10-12 kg N ha⁻¹ yr⁻¹) intensifies P limitation of epilithic algae and invertebrate grazers, compared to those in low deposition areas (2-6 kg N ha⁻¹ yr⁻¹), although more studies would be needed to generalise these findings.

The impacts of atmospheric N deposition have also been 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 these were not addressed separately in this survey (Elser et al., 2009). The survey showed that atmospheric N deposition increased the stoichiometric N:P ratio in these lakes, and, as a result, caused patterns of ecological nutrient limitation to shift. Under low N deposition (ca 4.5 kg N ha⁻¹ yr⁻¹ in Norway and ca 2 kg N ha⁻¹ yr⁻¹ in Sweden), phytoplankton growth was generally found to be N limited. However, in lakes with high N deposition (ca 8.5 kg N ha⁻¹ yr⁻¹ in Norway and ca 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). In the long term, in regions with still increasing atmospheric N loads, this may seriously disrupt the future functioning of the food web, even in lakes far away from direct human disturbance.

The critical load for Atlantic soft-water lakes (see Subsection 5.2.1) has been based on experiments. For boreal, sub-Arctic and alpine lakes, the critical load was based on survey and gradient studies supported by experimental evidence from bioassays. Although some of the individual gradient studies may not have accounted for confounding factors in formal statistical analysis, the interpretation that N deposition is the causal factor is supported by results from bioassays for N limitation, the fact that several independent gradient studies were involved, and by the fact that similar effects have been reported from several regions in Europe and North America (for more details, see De Wit and Lindholm, in press). In conclusion, it would be clearer to provide a single critical load range for C1.1 (permanent oligotrophic water bodies). On the above-mentioned basis, the new critical load range of 3 to 10 kg ha⁻¹ yr⁻¹ (reliable) is proposed. This critical load 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 applies to boreal, sub-Arctic and alpine lakes, whereas the upper end of the range applies to Atlantic soft-water lakes.

In addition, evidence has been found that dystrophic lakes (C1.4) may be sensitive to N deposition. There is insufficient basis for assessing the sensitivity of C1.4 relative to C1.1, but many of the studies given in Subsection 5.2.3 also incorporate dystrophic lakes. Based on expert judgement, the critical load range of 3 to 10 kg ha⁻¹ yr⁻¹ is proposed for dystrophic lakes (C1.4), with the advice of applying the lower end of the range to boreal, sub-Arctic and alpine dystrophic lakes. The critical load should only be applied to waters with low alkalinity and no significant agricultural or other direct human inputs.

For the future setting of reliable critical loads for freshwater ecosystems, not only greater insights into responses of aquatic organisms to N enrichment and into changes in the functioning of the system are required, but a better understanding of N leaching from surrounding catchments to surface waters is also necessary. To date, relations between N deposition and N leaching are not completely understood (Dise and Wright, 1995; Goodale *et al.*, 2005; Wright *et al.*, 2001; Aber *et al.*, 1998; 1989). Consequently, future N leaching, especially given prolonged N enrichment and climate change, is difficult to predict (Wright *et al.*, 2006; Stoddard, 1994).

Table 5.2 provides an overview of the empirical critical loads of N for inland surface waters (C).

Table 5.2. Empirical critical loads of nitrogen and effects ofexceedances on surface standing water habitats (C1)*. ##reliable, # quite reliable, and (#) expert judgement. Bold:changes compared to the 2003 document.

Ecosystem type	EUNIS code	kg N ha ⁻¹ yr ⁻¹	Reliability	Indication of exceedance
Permanent oligotrophic lakes, ponds and pools (including soft-water lakes)	C1.1ª	3-10	##	Change in species composition of macrophyte communities, increased algal productivity and a shift in nutrient limitation of phytoplankton from N to P
Dune slack pools (permanent oligotrophic waters)	C1.16	10-20	(#)	Increased biomass and rate of succession
Permanent dystrophic lakes, ponds and pools	C1.4 ^ь	3-10	(#)	Increased algal productivity and a shift in nutrient limitation of phytoplankton from N to P

* For all waters sensitive to acidification, also apply the critical loads of acidity calculated with Steady-State Water Chemistry (SSWC) models; Table 5.1).

^{a)} This critical load 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 boreal, Sub-Arctic and alpine lakes, and the higher end of the range to Atlantic soft waters.

^{b)} This critical load 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, Sub-Arctic and alpine dystrophic lakes.

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Chapter 6

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



Photo p. 63 Dominance of peat mosses, a typical phenomenon in ombrotrophic bogs with low background deposition of nitrogen (photo H. Tomassen, S. Norway).

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

6.1 Introduction

Class D 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). Nutrient budgets in 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 N. Little to very little effects from N enrichment are to be expected in several open wetland systems, such as reed marshes and sedge beds (EUNIS category D5 and D6) (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 raised bogs, which receive their major 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.

Several wetland types (e.g., those in categories D1, D2 and D4) are characterised by a partly or incomplete decomposition of the plant litter, resulting in peat formation (Mitsch and Gosselink, 2000; Malmer *et al.*, 2003). The capacity for peat formation is a major component in the nutrient balance of these wetland systems, and in many situations is strongly linked to the presence of peat mosses (*Sphagnum* species) (Van Breemen, 1995). These peat mosses are capable of efficiently accumulating N supplied to the moss surface (e.g., Clymo, 1970; Woodin, 1986). Their litter decays much slower than that of other plants. During the slow decomposition of litter (residence time of carbon up to 50-100 years), N is conserved in the top layer of the peat. During peat accumulation, mineral storage actively removes N and other nutrients from the moss layer and rhizosphere, making them thus unavailable for plant life (e.g., Mitsch and Gosselink, 2000; Malmer and Wallén, 2004).

In recent years, concern over high levels of atmospheric CO₂ has directed increasing attention towards bogs. Bogs and peatlands are large reservoirs of carbon, and the sequestration of carbon in peat depends on the ratio between primary production and decomposition of plant material, mainly bryophyte (Risager, 1998; Mitsch and Gosselink, 2000). Increased carbon as well as N availability may increase primary production under pristine (nutrientpoor) conditions, while it should also have opposing effects on the C:N ratio of litter and thus hypothetically also on peat decomposition rates. However, peat mosses (*Sphagnum* species), similar to mosses 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. Thus, the interactions between carbon and N under expected global change are still not clear. This sensitivity of peat mosses may also have implications for other systems that are characterised by a large bryophyte component, but for which, to date, reliable evidence for a critical load is lacking.

6.2 Raised and blanket bogs (D1)

a) Introduction

Ombrotrophic (raised) bogs, which receive all their nutrients from the atmosphere, are particularly sensitive to airborne N loads. These bogs are systems of acidic, wet areas and are very common in the boreal and temperate regions of Europe. Because abiotic conditions prevail, decomposition rates are low, favouring the development of peat. In western Europe and far northern latitudes, typical plant species include bog mosses (Sphagnum species), sedges (Carex, Eriophorum) and heathers (Andromeda, Calluna and Erica). Within the EUNIS system, these communities have been classified under D1 (raised and blanket bogs) for which the criterion is that precipitation is their continuous or primary water supply. EUNIS category D1 is subdivided into raised bogs (D1.1) and blanket bogs (D1.2). 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.

Since the last update of the empirical critical loads of N for ombrotrophic bogs (Bobbink *et al.*, 2003), a significant number of publications on the effects of N on European bogs have appeared. The results from these studies confirm the importance of *Sphagnum* mosses for the immobilisation of N, and, when *Sphagnum* becomes saturated, N availability increases for vascular plants, leading to increased vascular plant biomass (Bobbink *et al.*, 2003). The numbers of experiments with realistic additions of N that are within the range of the critical load of N (5-10 kg ha⁻¹ yr⁻¹) and that may be used to validate the existing critical load, are still limited.

b) Effects on peat moss growth

Clear effects of N eutrophication have been observed in Dutch ombrotrophic bogs. The composition of the moss layer in small remnants of formerly large bog areas has markedly changed in recent decades, as N loads have increased to 20 to 40 kg N ha⁻¹ yr⁻¹, especially in the form of ammonium/ammonia; the most characteristic *Sphagnum* species have been replaced by more nitrophilous moss species (Greven, 1992).

The effects of atmospheric N deposition on ombrotrophic bogs have also been intensively studied in Britain (e.g., Lee and Studholme, 1992). Many characteristic Sphagnum species have largely disappeared from affected ombrotrophic bog areas in Britain, such as those in the southern Pennines in England, where atmospheric N deposition has increased to ca 30 kg N ha-1 yr-1. Several studies on British bogs have shown that increased supplies of N are rapidly absorbed and utilised by bog mosses (Sphagnum species), reflecting the importance of N as a nutrient and its scarcity in unpolluted regions. 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 is 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.

Although these and other studies, strongly indicate the detrimental effects of high N deposition rates on the development of the 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* at sites with higher atmospheric deposition (7-9 kg N ha⁻¹ yr⁻¹). Because of the increase in the N deposition during the decades before the experiment, the growth of *Sphagnum* at the site with high atmospheric N deposition rates was thought to become P limited (Malmer, 1990).

More recently, 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 ha⁻¹ yr⁻¹ in Europe. Nutrient ratios increased steeply at low atmospheric input, but above a threshold of ca 10 kg ha⁻¹ yr⁻¹ the N:P and N:K ratios tended to saturation (Figure 6.1). Bragazza *et al.* (2004) suggest a critical load of N of 10 kg ha⁻¹ yr⁻¹, above which *Sphagnum* growth changes from being N limited to K and P co-limited, at an N:P ratio of more than 30 and an N:K ratio of more than 3.

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 yr¹ in Europe. Dashed and continuous lines represent the theoretical patterns based on regression model calculations (Bragazza *et al.*, 2004).



At high atmospheric N deposition rates, Sphagnum growth often becomes P limited (Aerts et al., 1992). Phuyal et al. (2008) studied the effects of increased atmospheric N deposition on the phosphatase activity of Sphagnum capillifolium in a lowland ombrotrophic bog (UK) over a four-year period. Ambient N deposition was 8 kg ha⁻¹ yr⁻¹ and was increased to 64 kg N ha⁻¹ yr⁻¹. N was added in the form of ammonium (NH₂Cl) or nitrate (NaNO₂). 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 both the ammonium and nitrate treatments. 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.

The importance of taking ambient atmospheric deposition into account when interpreting the results from field manipulation experiments is demonstrated by Berendse *et al.* (2001), who analysed the results from the Bog Ecosystem Research Initiative (BERI), with sites in Sweden, Finland, the Netherlands and Switzerland. They found that adding 30 kg N ha⁻¹ yr⁻¹ for 3 consecutive growing seasons (50 kg N ha⁻¹ yr⁻¹ in the Netherlands) increased *Sphagnum* N concentrations at all sites. However, this treatment decreased *Sphagnum* production only at the two sites with the highest atmospheric deposition (in Switzerland and the Netherlands, with an atmospheric deposition of 18 and 39 kg N ha⁻¹ yr⁻¹, respectively), and these sites also had the highest N concentrations at the start of the experiment.

A three-year field manipulation experiment used a range of N addition rates at two Swedish mires with differing existing atmospheric loads (Gunnarsson and Rydin, 2000; Nordin and Gunnarsson, 2000). N:P ratios and experimental results confirmed the idea that Sphagnum growth at the site in northern Sweden, with very low ambient deposition, was N limited, while at the southern site it 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. At low N deposition rates (lower than 7-11 kg N ha⁻¹ yr⁻¹), however, 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 below 10 kg N ha⁻¹ yr⁻¹.

Accumulation of free amino acids in *Sphagnum* capitula has been 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 3 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 to date 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).



The linear increase in total N and amino acid N concentrations paralleling increased N supply (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 N ha-1 yr-1) to different N forms labelled with 15N $(15NH^{+}, 15NO^{-})$ and the amino acids [15N] alanine and [15N]glutamic acid). All the applied N sources were taken up by both Sphagnum species. Uptake rates were highest for NH⁺, followed by alanine and glutamic acid, with only very small amounts of NO₂ being taken up. The negligible Sphagnum NO₂⁻ uptake may make any NO₂⁻ deposited readily available to co-occurring vascular plants (Wiedermann et al., 2009a). At the site with the highest background deposition (12 kg N ha⁻¹ yr⁻¹) N uptake was reduced, 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 N concentrations.

In a recent study, Sphagnum cores from three different sites in Sweden, with background depositions ranging from 3 to 11 kg N ha⁻¹ yr⁻¹, were exposed in a greenhouse experiment to 40 kg N ha⁻¹ yr⁻¹ for two consecutive growing seasons (Breeuwer *et al.*, 2009). N addition negatively affected height increment and production of all *Sphagnum* mosses, with the southern species (high background deposition) being affected the most (Breeuwer *et al.*, 2009). These negative responses were probably the result of the direct toxic effect of high tissue N concentrations (Gunnarsson and Rydin, 2000; Limpens and Berendse, 2003a; Nordin and Gunnarsson, 2000). High N addition also led to severe fungal infection in this greenhouse experiment.

Limpens *et al.* (2003b) also found increased infections in *Sphagnum*, caused by the fungal parasite *Lyophyllum palustre*, after three consecutive years of N fertilisation (field experiments in Ireland and the Netherlands). Total background deposition of N ranged from 15 kg (Irish site) to 37 kg (Dutch sites) N ha⁻¹ yr⁻¹, with additions of 40 kg N ha⁻¹ 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).

Granath et al. (2009b) tried to sort out the physiological mechanisms behind the strong negative effect of N on *Sphagnum* mosses. The maximum photosynthetic capacity in Sphagnum balticum was determined in a 12-year field fertilisation experiment. The maximum photosynthetic rate in Sphagnum balticum did not differ between the control (2 kg ha⁻¹ yr⁻¹) and the high N treatment (30 kg ha⁻¹ yr⁻¹), but was higher for the middle N treatment (15 kg ha⁻¹ yr⁻¹). The optimum tissue N concentration for photosynthetic rate in S. balticum was found to be \approx 13 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.

For the assessment of critical loads of N, pot or microcosm studies, generally, are not accepted, except for bryophyte layer studies (which are relevant for bogs). We have focused particularly one 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 10-year field experiment (involving deposition of 2 (ambient), 15 and 30 kg N ha⁻¹ yr⁻¹) with field data from sites representing a gradient of increasing N deposition (2, 8 and 12 N ha⁻¹ 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.

c) Effects on moss species composition

Because of differential effects of N on the growth of different Sphagnum species, the moss composition is known to change at high atmospheric N loads. This has also been demonstrated in several experimental studies. For example, in a controlled-environment experiment, Risager (1998) examined the growth responses of Sphagnum fallax to different forms of N (NO⁻, NH⁺ or NH NO,) at low addition rates (0, 5, 10 and 20 kg N ha⁻¹ yr¹). This growth was significantly stimulated by the application of NH_{a}^{+} (especially with 5 and 10 kg $NH_{a}^{-}N$ ha⁻¹ yr¹), but, logically, was not reflected in the tissue N concentration. NO₂⁻ only additions did not influence the growth of this species. Risager (1998) also investigated the responses of Sphagnum magellanicum after addition of NH_.NO₋-N in a comparable study involving similar levels of N addition, but without differentiation between reduced and oxidised N. 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 NO⁻ was considerably lower than of NH⁺. Risager (1998) concluded that the form of N is also important in species response, and that increased N availability may cause shifts in species composition in favour of Sphagnum fallax.

A German N addition experiment showed such effects on species composition in an established Sphagnum community (Lütke Twenhöven, 1992) in the field. In this two year study, the Sphagnum species responded in different ways to the N type applied, depending on micro-habitat (soil moisture content). 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 these species. Results from Gunnarsson and Rydin (2000) also suggest that lawn communities are less vulnerable to increased N deposition than are 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)

More recently, 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 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).

The importance of competition between moss species is also indicated by the study by Mitchell et al. (2002) on rather higher rates of N deposition. This experiment examined the effect of an addition of 30 kg ha⁻¹ yr⁻¹ in a cutover bog in the Jura Mountains of Switzerland, where ambient deposition was estimated to be 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 a cumulative differential height growth in response to added N deposition between overgrowing Polytrichum strictum and Sphagnum capitula, which might prevent this typical regeneration process in central European bogs. Thus, in the study period, biomass production of P. strictum almost doubled in response to N addition, whereas production of Sphagnum fallax decreased by close to 50%.

Granath et al. (2009a) tried to sort out the physiological mechanisms behind the competition between Sphagnum mosses at high N loads. In a north-south transplant experiment, covering a latitudinal N deposition gradient ranging from 2.8 kg ha⁻¹ yr⁻¹ in the north, to 14.9 kg ha⁻¹ 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 kg N ha⁻¹ yr⁻¹, whereas for Sphagnum balticum this seemed to level out at 11.4 kg N ha⁻¹ yr⁻¹. The results suggest that Sphagnum species may be able to adapt or physiologically adjust to high 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).

d) Effects on competition between mosses and vascular species 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 (NH⁺) deposition loads of more than 10 to 15 kg N ha⁻¹ yr⁻¹ (Aaby, 1994), together with increased NH, concentrations, rapidly deposited to ombrotrophic mires. Increased growth of trees, particularly Pinus sylvestris, was also demonstrated for many ombrotrophic sites in southern Sweden (Åberg, 1992; Gunnarsson et al., 2002), as well as reduced Sphagnum cover (Malmer and Wallén, 1999). 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. On a mire in central Sweden, in a region with lower N deposition, species numbers decreased over a similar period (Gunnarsson et al., 2000). However, this decrease was mainly found at sites with a pH of more than 5.0. At sites with lower pH, as that in southern Sweden, species numbers hardly decreased at all.

Such observations suggest that increased N deposition may influence the competitive relationships between mosses and vascular plants in nutrient-deficient vegetation such as bogs. For light, the competitive interactions between Sphagnum species and vascular plants are symmetric, while for mineral nutrients these interactions are partly asymmetric, 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 yr', 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. It is likely that the mosses were mostly N saturated and the target vascular plants, Narthecium ossifragum, had a N:P ratio as high as about 40 (Malmer et al., 2003).

Malmer and Wallén (2005) investigated the effects of increasing N deposition on *Sphagnum* mire vegetation in southern Sweden by comparing above-ground tissue concentrations and biomass variables in five vascular plants and two *Sphagnum* species, over a period of 50 years. During this period wet deposition rates increased from approximately 5 to 12 kg N ha⁻¹ yr⁻¹. Increased N deposition has had great effects on vegetation with the highest *Sphagnum* biomass and peat accumulation rate. Increased N deposition influenced the growth of *Sphagnum* mosses directly, and that of vascular plants indirectly, via increased mineralisation.

To determine the N source of quantitatively important boreal bog plants, Nordbakken et al. (2003) measured the δ^{15} N signature after a 3-year N addition experiment in a Norwegian bog with an estimated total N background deposition of 7.9 kg N ha⁻¹ yr⁻¹. An addition of only 5 kg N ha-1 yr-1 was sufficient to significantly increase the N concentration in Sphagnum mosses, liverworts and shallow rooted vascular plants. An addition of 40 kg N ha⁻¹ yr⁻¹, however, was not sufficient to increase the N concentration in deep rooted plants. The N added remained in the top layer of the peat, indicating the capacity of the living Sphagnum mosses and the surface peat to take up deposited N, and thereby function as a filter (Nordbakken et al., 2003). This experiment demonstrates the importance not only of ambient atmospheric N deposition, but also of the duration of fertilisation experiments. Over the course of three years, the Sphagnum layer was able to completely immobilise the relatively high N load of 40 kg N ha⁻¹ yr⁻¹. Long-term N deposition levels of 40 kg N ha⁻¹ yr⁻¹, therefore, likely to have significant effects on the N availability to vascular plants.

These responses were also demonstrated experimentally by Heijmans et al. (2001), who studied the effect of added N deposition (50 kg N ha⁻¹ 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, 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 ¹⁵N enrichment; Heijmans et al., 2002a), but only the cover 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.
Figure 6.3 Numbers of individuals of *Drosera rotundifolia* in an ombrotrophic raised bog (D1) near Stockholm (Sweden) during 4 years of N additions (Redbo-Torstensson, 1994).



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 4-year fertilisation experiment in Swedish ombrotrophic bogs, using a range of deposition rates (Redbo-Torstensson, 1994). It was demonstrated that experimental applications of 10 kg N ha⁻¹ yr⁻¹ (as NH₄NO₂ at an ambient deposition of 5 kg N ha⁻¹ yr¹) significantly reduced the survivorship of the plants after 4 years, while additions of 20 and 40 kg ha⁻¹ yr⁻¹ already had this effect after one year, and also negatively affected flowering after two years (Figure 6.3). The decrease in the population density of the characteristic bog species Drosera was associated with increased density of tall species such as Eriophorum and Andromeda, which resulted in increased competition for light.

Limpens et al. (2004) conducted a 4-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*. Because P alleviates the negative impact that N has on *Sphagnum* by enhancing its capability to assimilate the deposited N, P availability is 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 4 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 suggest 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.

In a competition experiment between two graminoid species, *Eriophorum vaginatum* and *Rhynchospora alba*, and two ericoid species, *calluna vulgaris* and *Vaccinium oxycoccos*, 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 are more likely to turn into dwarf-shrubdominated ecosystems than into grasslands. In another greenhouse study, however, 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.

In Dutch and Danish bogs, the first signs of the negative effect of high atmospheric N deposition 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 3-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 3 years, above-ground biomass of Molinia caerulea plants was significantly higher in the turfs which 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 3 years at different rates of experimental N addition (means + 1 SE; n = 4). Different letters indicate significant differences (P < 0.05) between N treatments (one-way ANOVA) (Tomassen *et al.*, 2003). Different letters indicate significant differences between the treatments.



In addition, Tomassen *et al.* (2004) studied the effects of N on *Molinia caerulea* and *Betula pubescens* in a 3-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* seems to be well adapted to P-limiting conditions, which may explain its success in regions with increased N deposition.

Limpens *et al.* (2003a) studied 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 kg N ha⁻¹ yr⁻¹. *Molinia* biomass was positively related to the inorganic N concentration in the interstitial water. 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).

e) 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 NH₄NO₃, reflecting the current ratio of reduced and oxidised N in precipitation (e.g., Boxman *et al.*, 2008), but there are several experiments that have studied the effects of ammonium (NH₄Cl) and nitrate (NaNO₃), separately. As mentioned 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.

Bragazza *et al.* (2005) determined the δ^{15} N isotopic signatures of *Sphagnum* plants collected from sites with an ambient N deposition ranging from 2 to 20 kg N ha⁻¹ yr⁻¹. The δ^{15} 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 δ^{15} N signatures can be used as an integrated measure of δ^{15} N signature of atmospheric precipitation. Bogs located in areas dominated by NH₃ emissions had *Sphagnum* plants with a more negative δ^{15} N signature, compared to areas dominated by NO_x emissions (Bragazza *et al.*, 2005).

In a Scottish bog, a large-scale, automated N-addition field experiment with different forms of N (ammonium and nitrate) at 8 kg (background), 16 kg and 64 kg N ha⁻¹ 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).

f) Effects on peat and peat water chemistry as evidence of nitrogen saturation

The influence of N deposition entering the moss layer on N dynamics in the underlying peat was investigated in experiments on a raised bog, Moidach More, in the north-east of Scotland (Williams et al., 1999b; Williams and Silcock, 2000). The bryophytes Sphagnum capillifolium and Sphagnum recurvum were chosen because they colonised contrasting sites; Sphagnum capillifolium is a hummockforming species, whereas Sphagnum recurvum occupies hollows and pools. The addition of 30 kg N ha⁻¹ vr⁻¹ increased the total N concentration in the mosses. Dissolved organic N in the moss water associated with Sphagnum capillifolium was proportional to the quantity of added N, suggesting an accumulation of amino acids in the mosses that had received N. This relationship was undetectable for Sphagnum recurvum, but this may have been due to its pool habitat, where surface waters in winter could dilute the water associated with the moss. Three seasons of N fertilisation (30 kg N ha⁻¹ yr⁻¹) had few effects on P cycling in the peat layer. The main effect was an increase in the C:P ratio of dissolved organic P (DOP) (Williams and Silcock, 2001).

However, the difficulties of generalising these experimental findings is indicated by Williams *et al.* (1999a), who reported the effects of adding a range of N loads to Sphagnum magellanicum carpets at four contrasting sites, in Finland, Estonia and France, in addition to the Moidach More site in the United Kingdom. These experiments were only conducted over a four-month period, and hence of little value in terms of assessing critical loads, but showed clear contrasts in response between sites, in terms of growth, increases in N content of the mosses, and in ammonium concentrations in the peat at 30-cm depths. The results suggest that both P limitation and a deep water table may decrease the retention of deposited inorganic N, and Williams *et al.* (1999a) suggest that peat drainage may reduce retention of deposited N by Sphagnum carpets.

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) 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).

The significance of the N saturation of the Sphagnum layer, and increased availability of N in peat and in peat waters, has been investigated in several recent studies. Tomassen et al. (2003) found that peat water ammonium concentrations had significantly increased after 3 years of N addition (40 kg N ha⁻¹ yr⁻¹). Ammonium concentrations increased to 25 µmol l⁻¹ (a common level in Dutch ombrotrophic bogs); in all other N-treatment experiments, levels were between 5 and 10 µmol 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 kg ha⁻¹ yr⁻¹ decreased both interstitial water and Sphagnum N concentrations, while doubling the N input to 80 ha⁻¹ yr⁻¹ increased N concentrations.

Lamers *et al.* (2000) used field data from Ireland and the Netherlands to produce an assessment of the capacity of moss layers to limit the amount of N deposition reaching the rhizosphere, thereby decreasing the growth of competitive graminoids and other species (Figure 6.5). 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. Other nutrients, however, especially P, may become a secondary limiting factor to plant growth when N inputs reach a certain threshold Figure 6.5. The N concentration (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; △ Johnson and Maly, 1998); ▲ collected by the authors in 1998 in Europe and the United States).



(Aerts *et al.*, 1992; Bragazza *et al.*, 2004). Above 20 kg ha⁻¹ yr⁻¹, this 'natural filter' is suggested to fail, and N availability in the rhizosphere to increase, as has also been observed in regions with high deposition levels (Figure 6.5). It is important to note that Lamers *et al.* (2000) estimated the total deposition to be double the measured bulk deposition, in constructing Figure 6.5. However, at the remote sites with low deposition levels, dry deposition is likely to be very low, and their method would significantly overestimate the actual deposition inputs; according to this figure, this would thus reduce the threshold N deposition rate for response

g) Effects on decomposition and nutrient cycling

Aerts *et al.* (1992) concluded, based on the studies on the northern and southern Swedish sites discussed above, that a high atmospheric N supply may affect the carbon balance of ombrotrophic bogs, because productivity under these circumstances is P limited, rather than N limited, but decomposition is probably increased by the high N loads. This hypothesis is supported by results from short-term experiments by Williams *et al.* (1999a) and Williams and Silcock (2000) (for details, see above). The addition of 30 kg ha⁻¹ yr⁻¹ N resulted in a decrease in peat C:N ratios (Williams *et al.*, 1999a), 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).

However, a study of Sphagnum peat in Swedish ombrotrophic bogs along a gradient of N deposition (Hogg et al., 1994) 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 4 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 3 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 during 3 years (see previous section), by measuring both CH₂ 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.

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 would accelerate litter peat decomposition.

In contrast, Saarnio *et al.* (2003) found that, over a 2-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. 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). These findings differ from results 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 5 to 6 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 kg ha⁻¹ yr⁻¹) in two Swedish bogs subjected to low (2 kg ha⁻¹ yr⁻¹) and high (15 kg ha⁻¹ 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 CH₂ exchange, but elevated N₂O 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 northwestern 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 N₂O 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.

Breeuwer et al. (2008) found that increased N deposition increased Sphagnum litter N loss (confirmed by Aerts et al., 2001; Limpens and Berendse, 2003b; Bragazza et al., 2005). N deposition had no effect on mass and N loss in Eriophorum vaginatum (Breeuwer et al., 2008), but vascular plant litter still decomposed faster than Sphagnum that was enriched with N. As a result of increased N deposition, vascular plants will increase at the expense of Sphagnum, and will form a larger fraction of the litter, leading to increased peat decomposition (Breeuwer et al., 2008).

A shift in litter N concentration is not the only thing that may lead to increased peat decomposition. Limpens and Berendse (2003b) found that mass loss of *Sphagnum* litter was positively related to the N:C ratio, but depended strongly on the range in observed N:C ratios. Above a N:C ratio of about 0.015 (C:N ratio lower than 66), net N loss was observed for all four *Sphagnum* species studied.

In summary, these results do indicate that effects of elevated N deposition on *Sphagnum* growth and litter chemistry, and on microbial biomass, are likely to affect

the decomposition process, but that this process would certainly be regulated by more than just the C:N ratio of the peat. However, further evidence would still be necessary to properly evaluate the long-term effects of increased N supply on the decomposition of *Sphagnum* peat and rates of nutrient cycling.

h) Reversibility of N effects

Numerous studies have addressed the effects of increased N deposition on bogs, but only a few studies have been conducted on the extent to which reductions in atmospheric N lead to recovery of a system and on what time scale. A considerable part of the negative effects of elevated N deposition may be related to an imbalance in tissue nutrient concentrations in Sphagnum. Limpens and Heijmans (2008) used changes in Sphagnum nutrient concentrations as an early indicator of ecosystem response. After ceasing N additions (40 kg N ha⁻¹ yr⁻¹ over a 3-year period), elevated Sphagnum N concentrations returned to normal levels within a fifteen-month period. Sphagnum nutrient concentrations quickly respond to reductions in N supply, indicating that a management policy that is aimed at reducing atmospheric nutrient input in bogs may yield results within a few years.

i) Summary

Even though there has been only a limited number of long-term (above 3-4 years) N manipulation experiments in bog ecosystems, a clear picture is emerging of the potential impact of elevated N deposition on bog habitats. Responses to N additions of as low as 10 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. The concept of a limited capacity for retention of N in the moss layer, above which N availability in the rhizosphere increases, offers a tool for assessment of factors which may modify the critical loads for these systems. Initial estimates using this method are consistent with a long-term response threshold of 10 kg ha⁻¹ yr⁻¹.

The critical load previously recommended by Bobbink *et al.* (2003) (5-10 N kg ha⁻¹ yr⁻¹) had been based on a considerable body of field evidence and experiments and, hence, was judged to be 'reliable' at the time. Additional studies since then have generally provided results that further support this rating and it is therefore proposed that the critical load range for bog ecosystems (D1) is continued at 5 to 10 kg ha⁻¹ yr⁻¹. Expert judgement, based on observations that responses to N are smaller in wetter bog areas, has found that bogs receiving high effective precipitation (e.g., in northern and western United Kingdom, Norway) are less sensitive to N than those in drier regions (e.g., in the Netherlands, Sweden). Therefore, precipitation is recommended as a modifying factor to be taken into account when assigning critical loads to individual sites.

6.3 Valley mires, poor fens and transition mires (D2)

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, 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 (D2.2). When compared to poor fens, based on the generalisation from Morris (1991) on the link between N sensitivity and hydrology (see introduction Section 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 2 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 Molina 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 *Sphagna* 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 3 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 depend 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, 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 CH. emission. They expected (and confirmed) sedges to be an important factor in CH₂ release into the atmosphere. Similar to effects found in ombrotrophic bogs (see Section 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 $\mathsf{CH}_{\scriptscriptstyle\!\mathcal{A}}$ 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 Section 6.2). Gunnarsson et al. (2004) concluded that increased N deposition may transform mires that are dominated by Sphagnum into vascular-plant-dominated mires.

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 4 years, but after 8 years of continuous N addition, the closed *Sphagnum* carpet had been drastically reduced from 100% down to 41% (Figure 6.6). The total vascular plant cover (*Eriophorum vaginatum*, *Andromeda polifolia* and *Vaccinium oxycoccus*) increased from 24% to an average of 70% (Figure 6.6). Wiedermann *et al.* (2007) stress that both bryophytes and vascular plants in boreal mires receiving background levels

Figure 6.6. Time series for the years 1995, 1996, 1997 and 1998 (blue bars) and 2003 (green bars). Data represent cover (mean \pm 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).







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.

The critical load previously recommended for poor fens (D2.2) by Bobbink et al. (2003) (10-20 kg N ha-1 yr-1) was based on (limited) field evidence and experiments and, hence, was judged to be 'quite reliable'. Bobbink et al. (2003) recommended further field experiments in poor fens, with realistic doses of N being added. Since the last update, several publications have appeared on the effects of N on poor fens. The study by Malmer et al. (2003) showed significant effects from additions of 20 kg N ha⁻¹ yr⁻¹ (total input c. 25 kg N ha⁻¹ yr⁻¹) on poor fen vegetation within a 2-year period. In the long term, effects would be even more pronounced. Since Sphagnum mosses function as N filters in poor fens, similar to those in ombrotrophic bogs, we think that the upper range of the current critical load is too high. We propose a modified critical load range for valley mires, poor fens and transition mires (D2) of 10 to 15 kg N ha⁻¹ yr⁻¹ (quite reliable) and still recommend long term field experiments with low N doses (e.g. 5-10 kg N ha⁻¹ yr⁻¹). Because of a lack of field studies, we propose that the lower end of this critical load range is applied for valley mires (D2.1), and urgently recommend that such field studies will be initiated.

6.4 Base-rich fens (D4)

Similar to poor fens, base-rich fens 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. They are largely occupied by calcicolous small sedges and brown moss communities (Davies et al., 2004). Despite the fact that 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. A previous study has set the critical load 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 (D4.1) 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).

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 growthlimiting 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). In another study, biogeochemistry and nutrient cycling was studied in rich fens in a region with high atmospheric N loads (the Netherlands) and in a region with very low N deposition (northern Japan). This study demonstrated that N mineralisation was much higher in the Dutch rich fens, than in the Japanese ones, despite the fact that the sites in the Netherlands were managed through mowing (Nohara et al., 2002). This clearly indicates an acceleration of the cycling of N in Dutch rich fens under high atmospheric N inputs (25-40 kg N ha⁻¹ yr⁻¹; mostly as ammonia/ammonium).

Microcosm experiments with bryophytes (*Sphagnum wanstorfii*) 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, changes in moss growth could be measured, as well as 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 7-10 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 4 years, ammonium significantly reduced the biomass of Scorpidium revolvens although Sphagnum contortum biomass was not

affected by N addition (Paulissen and Bobbink, submitted). This implies that, in the longer term, Sphagnum will profit from high ammonium deposition, while it will negatively affect the typical brown mosses. After 2 years, biomass production and tissue N:P ratio of the vascular vegetation indicated N limitation (Paulissen, 2004). After 4 years, however, total vascular plant cover was significantly increased by nitrate addition (Paulissen and Bobbink, submitted). 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 6-8 kg N ha⁻¹ yr⁻¹), for 5 years (Dorland *et al.*, 2008; Verhoeven *et al.*, in press). 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 Ca; Verhoeven *et al.*, in press). Both N additions hardly resulted in increased N concentrations in the peat water. However, 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 6.7; Dorland *et al.*,2008; Verhoeven *et al.*, in press). 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.

Bergamini and Pauli (2001) studied the effects of N enrichment on bryophytes in rich fens in the Swiss mountains (D4.2). The authors added 100 kg N ha⁻¹ yr⁻¹ to 18 different rich-fens (background deposition of between 15 and 30 kg ha⁻¹ yr⁻¹) for 2 years, and found a significant increase (30%) in vascular plant biomass and a significant decrease (39%) in bryophyte biomass. Using vascular plant biomass as a covariate, they showed that N had a direct negative effect on bryophyte biomass, after taking into account effects associated with increased competition from vascular plants (Bergamini and Pauli, 2001; Pauli et al., 2002). Despite the clear effects on bryophyte biomass, species heterogeneity between sites was too high to find any significant changes in bryophyte community composition (Bergamini and Pauli, 2001). Although the N additions in this experiment were too high to set a critical load, they are an indication that rich-fens in mountainous areas are also sensitive to N.

Figure 6.7. Dry biomass (left) and species number (right) of bryophytes $(20 \times 20 \text{ cm}^2)$ after 5 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 kg ha⁻¹ yr⁻¹ (after Verhoeven *et al.*, in press).



78 | Review and revision of empirical critical loads and dose-response relationships

The critical load for rich fens (D4.1) that was previously recommended by Bobbink *et al.* (2003) (15-35 kg N ha⁻¹ yr⁻¹) was based on limited field evidence and experiments and, hence, was judged as 'expert judgement'. Since the last update, the Irish experiment has provided valuable data that contribute to the validation of this estimated critical load for rich fens. Additions of 35 kg N ha⁻¹ yr⁻¹ under low ambient N depositions (6-8 kg N ha⁻¹ yr⁻¹) resulted in severe negative effects on the bryophyte layer within 5 years. Considering these strong effects within a relatively short period, we propose a new critical load of 15 to 30 kg N ha⁻¹ yr⁻¹ for rich fens, which is considered as 'expert judgement'. The higher end of the range is recommended for managed or non-N-limited systems, while the lower end of the range is recommended for N-limited systems. As no new evidence is available for high latitude systems and rich fens in the mountains (D4.2) (15-25 kg N ha⁻¹ yr⁻¹) (expert judgement), the 2003 range remains unchanged. Longterm fertilisation experiments with ecologically relevant additions of N in temperate regions, northern countries and montane fens should increase the reliability of these figures.

An overview of the empirical critical load ranges of N for mire, bog and fen habitats is presented in Table 6.1.

Table 6.1 Empirical critical loads of N and effects of exceedances on different mire, bog and fen habitats (D). ## reliable, # quite reliable and (#) expert judgement. Changes with respect to 2003 are indicated in bold.								
Ecosystem type	EUNIS code	kg N ha ⁻¹ yr ⁻¹	Reliability	Indication of exceedance				
Raised and blanket bogs	D1ª	5-10	##	Increase in vascular plants, altered growth and species composition of bryophytes, increased N in peat and peat water				
Valley mires, poor fens and transition mires	D2 [⊾]	10-15	#	Increase in sedges and vascular plants, negative effects on bryophytes				
Rich fens	D4.1°	15-30	(#)	Increase in tall graminoids, decrease in bryophytes				
Montane rich fens	D4.2°	15-25	(#)	Increase in vascular plants, decrease in bryophytes				

^{a)} The high end of the range applies to areas with high precipitation and the low end of the range to that with low precipitation; the low end of the range applies to systems with a low water table, and the high end of the range to those with a high water table. Note that water tables can be modified through management.

^{b)} For D2.1 (valley mires), the lower end of the range applies (#).

^{c)} For high latitude systems, the lower end of the range applies.

6.5 References

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Chapter 7

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



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

7.1 Introduction

A large number of grassland ecosystems (class E of the European Nature Information System (EUNIS)) 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). Only a small proportion of these grasslands is of natural origin (e.g., dry steppe grasslands, alpine grasslands), while most of these habitats are covered by semi-natural vegetation. 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; Woodin and Farmer, 1993). 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 characterised by many species of low stature and nutrient-poor soils (Ellenberg jr., 1988). However, some semi-natural meadow communities of high nature conservation value, particularly those on deep alluvial soils in river flood plains subject to periodic inundation or inputs of farmyard 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, artificial fertilisers have to be avoided. It is thus to be expected that several of these species-rich grasslands, especially those of oligotrophic or mesotrophic soils, will be sensitive to increased atmospheric nitrogen (N) inputs. Moreover, several of the most species-rich grasslands are found under weakly buffered or almost neutral conditions, which make them sensitive to soil acidification and very sensitive to 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 E), which are dry or only seasonally wet (with the water table at or above ground level for less than half of the year) have a vegetation cover greater than 30%. The dominant part of the vegetation is grasses and other non-woody plants, including moss-, lichen-, fern- and sedge-dominated communities. An important level of division is based on soil water availability (dry (E1), mesic (E2) and wet (E3) grasslands). Most of the studies on N effects in grassland habitats have been carried out for ecosystems which are classified as dry grasslands (E1). Furthermore, the impacts of N inputs have only been studied in parts of the other major EUNIS categories (E2-E7). Some information exists on mesic grasslands (E2) and wet grasslands (E3), the classification of which particularly relates to present land use or management. The latter group is divided into oligotrophic wet grasslands (E3.5) and mesotrophic to eutrophic wet grasslands (E3.4). Evidence also exists on the impacts of N deposition in alpine and subalpine grasslands (E4).

7.2 Dry grasslands (E1)

7.2.1 Perennial calcareous grassland and basic steppes (E1.2)

Calcareous grasslands (EUNIS category E1.2) are communities on limestone, 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 well-buffered rendzina soils low in phosphorus (P) and N (pH of the top soil: 7-8 with a calcium carbonate content of ca 10%). Here, 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. These grasslands decreased strongly in area during the second half of the last century (e.g., Wolkinger and Plank, 1981; Ratcliffe, 1984). In several European countries, some remnants were turned into nature reserves. 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).

Figure 7.1 Above-ground biomass of Brachypodium pinnatum (g m⁻²) and vascular plant species number (per 50 x 50 cm²) on Dutch perennial calcareous grassland (E1.2) after three years of nutrient additions (N, in the form of ammonium nitrate; 100 kg N ha⁻¹ yr⁻¹) (after Bobbink, 1991). O=control; **: $p \le 0.01$; (*): $p \le 0.10$



A gradual increase in one grass species (Brachypodium pinnatum) was observed in Dutch calcareous grasslands (E1.2.6), by the Nature Conservation Agencies in the late 1970s and early 1980s, although management of these grasslands (hay making in autumn) had not changed since the mid 1950s. 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). The effects of N enrichment, therefore, have been 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 3-year period resulted in a drastic increase in the grass Brachypodium pinnatum, and in a strong reduction in species diversity (including several Dutch Red List species), caused by the change in vertical structure of the grassland vegetation (Figure 7.1). Brachypodium 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 redistributed to the below-ground rhizomes by enhanced growth in the next spring. In this way Brachypodium pinnatum strongly monopolised (>75%) the N storage in both the aboveground and below-ground compartments of the vegetation in response to increasing N availability (Bobbink et al., 1988; 1989; De Kroon and Bobbink, 1997). Besides this decrease in phanerogamic plant species, 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). To date, no relevant data are available on the effects of N loads on the species-rich fauna of calcareous grasslands.

N cycling and accumulation in calcareous grasslands can be significantly influenced by two major outputs: (i) leaching from the soil, and (ii) removal through management regimes. N losses by denitrification in dry calcareous grasslands are low (<1 kg N ha⁻¹ yr⁻¹) (e.g. Mosier *et al.*, 1981). Ammonium and nitrate leaching has been studied in Dutch calcareous grasslands by Van Dam (1990). The observed N leaching from untreated vegetation was very low (0.7 kg N ha⁻¹ yr⁻¹), representing only 2% of total atmospheric N deposition. After two-weekly spraying of ammonium sulphate for a 2-year period (50 kg N ha⁻¹ yr⁻¹), N leaching significantly increased to 3.5 kg N ha⁻¹ yr⁻¹, although this figure is also only a small proportion (4%) of the total N input (Van Dam, 1990). Thus it has become evident that calcareous grassland ecosystems almost fully retain N in their systems, because of a combination of increased plant uptake (Bobbink et al., 1988; Bobbink, 1991) and increased immobilisation in the soil organic matter (Van Dam, 1990).

The most important output of N from calcareous grasslands is through management. From the 1950s to the mid 1980s, almost all of the calcareous grasslands in the Netherlands would be mowed in autumn, and the hay would be subsequently removed. The annual N removed within this hay varied slightly between years and sites, but in general between 17 and 22 kg N ha-1 would be removed from the system under the usual land management (Bobbink, 1991; Bobbink and Willems, 1991). Legume species (Fabaceae) also occur in these grasslands, and provide an additional N input associated with the N-fixing micro-organisms in their root nodules (ca 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 critical long-term immobilisation rate for N of between o 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, 15 to 25 kg N ha-1 yr-1 was established in Lökeberg 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 by far the highest 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, Brachypodium pinnatum was found to have increased, and species diversity to have 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 bordering the agricultural fields (Neitzke, 1998; 2001).

In addition, a 4-year N addition experiment was performed on a calcareous grassland (E 1.26) in Voeren, Belgium (Jacquemyn et al., 2003), only 20 km to the south of the previously mentioned Dutch calcareous region. 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 (commercial fertiliser; added once a year), were examined, in a factorial experiment with two management treatments (high density cattle grazing and mowing of the vegetation after which the hay was removed). The background deposition at the site was ca 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 a decrease in light availability, resulting from increased above-ground productivity of tall grasses and forb species. In contrast to the results in Dutch calcareous grasslands, *Brachypodium pinnatum* was not present on this study site, although other tall grasses and forbs became very abundant.

In studies on calcareous grasslands in England, additions of N only 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, Brachypodium 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. Following a survey of data from a number of conservation sites in southern England, Pitcairn et al. (1991) concluded that Brachypodium pinnatum had expanded in the United Kingdom during the last century. They considered that much of the early spread could be attributed to a decline in pressure from grazing, but that more recent increases in the grass, in some cases, had occurred despite grazing or mowing, and may have been related to N inputs. This was partly confirmed by Hewins and Ling (1998), who assessed the diversity of lower plants and more aggressive grass species on calcareous grassland in those parts of the Cotswold (UK) that receive higher concentrations of ammonium. 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). Brachypodium pinnatum was present in the sward but had not expanded as in the Dutch grasslands, but these field data from British calcareous grasslands are still consistent with the 1992 critical load range of 15 to 25 kg N ha⁻¹ yr⁻¹. In a mesocosm study with plant species of calcareous grasslands (South Downs, south-east England) the biomass of Brachypodium pinnatum significantly increased after a 2-year period of N additions (≥70 kg N ha⁻¹ yr⁻¹), even under a summer defoliation regime of 8 weeks (Bryant, 1998). Unkovich et al. (1998) also found N limitation in their field study in Wytham (UK). That study found more than a doubling in herbage production after weekly additions of 11.5 kg N ha⁻¹ during 6 weeks (total N addition of almost 60 kg N ha-1) independent 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 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).

Long-term effects of ammonium nitrate additions (35, 70 and 140 kg N ha-1 yr-1) were studied between 1990 and 2008, in a calcareous grassland area (Wardlow Hay Cop) with a shallow soil (< 10 cm) on carboniferous limestone bedrock in Derbyshire, in the United Kingdom (E1.26) (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). The highest N addition was also applied in the form of ammonium sulphate. Within the first four years of N addition, no significant decline 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* was able to invade 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, clearly indicating 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 7.2). The lowest NH_4NO_3 additions (35 kg N ha⁻¹ yr⁻¹) produced significant declines in frequency of Hypnum cupressiforme, Campylium

Bryophyte Species

Figure 7.2 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 ha⁻¹ yr⁻¹ (Haworth *et al.*, 2007). Significance between N forms is denoted by *. Different letters indicate significant differences between the treatments.



0 kg N ha⁻¹ yr⁻¹
 140 kg N ha⁻¹ yr⁻¹
 35 kg N ha⁻¹ yr⁻¹
 140 kg N ha⁻¹ yr⁻¹ ((NH₄)₂SO₄)
 70 kg N ha⁻¹ yr⁻¹

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chrysophyllum, and Calliergon cuspidatum. Significant reductions in frequency at higher NH_NO_ application rates were recorded for Pseudoscleropodium purum, Ctenidum molluscum, and Dicranum scoparium. The highest NH₄NO₂ and (NH₄)₃SO₄ additions provided conditions conducive for the establishment of two typical calcifuges - Polytrichum species and Campylopus introflexus, respectively. Substratesurface (o-2.5 cm) pH measurements showed a doserelated reduction in pH with increasing NH, NO, 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 NO, and (NH)_SO_treatments. In conclusion, N enrichment resulted in a decline in the frequency of the indigenous bryophyte species and the establishment of non-indigenous calcifuge species. 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 10 years of N addition (Horswill et al., 2008). They also found significant losses of soil base cations and increases in Al and Mn following the highest N treatments (140 kg N ha⁻¹ yr⁻¹; especially with (NH₂)₂SO₂); clear signs of soil acidification caused by high N loads, which may lead to declines in sensitive calcicolous species.

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 $(\geq 70 \text{ kg N ha}^{-1} \text{ yr}^{-1})$ (Morecroft *et al.*, 1994; Carroll *et al.*, 1997). After 6 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 addition were not significantly different from those of the controls. The nitrification rates in the vegetation treated with the different 140 kg N ha-1 yr-1 additions were clearly related to increased soil acidification (Carroll et al., 2003). Soil microbial activity was not significantly affected by 6 years of ammonium nitrate additions, but root-surface phosphomonoesterase (PEM) 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 (PEM) activities were found for 3 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.

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 increases in output fluxes of N. In general, 80 to 90% of the additional pollutant N was retained in the system; even 65% remained in the plots with the high 140 N ha⁻¹ 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 simulated grazing and leaching of organic N, constituting 90% of leached N under ambient conditions. Leaching of nitrate only contributed significantly to the output flux of N under the highest N treatment, and not at all following additions of 35 kg N ha⁻¹ yr⁻¹. Thus, even in this P-limited grassland, a (very) high fraction of the extra N was accumulated (immobilised) in the soil system.

The naturally occurring N deposition on this study site in the United Kingdom was estimated at 25 kg N ha⁻¹ yr⁻¹. Thus, it was not possible to accurately estimate a critical load below 60 kg N ha⁻¹ yr⁻¹ from the N additions used. For that reason, an N-reduction experiment was set up, under greenhouse conditions, with mesocosms from a neighbouring calcareous grassland site (2, 10, 20 and 55 kg N ha⁻¹ yr⁻¹ added in the form of ammonium nitrate) (Jones and Ashenden, 2000). Two years after the start of this experiment, very few effects from the different N loadings on the vegetation were evident. However, in view of the strong P limitation, it may take a much longer time for any effect to become significant. Leaching of nitrate, however, was already found during both winters following the start of the experiment, in all treatments with 10 kg N ha⁻¹ yr⁻¹ or more, but an indication of a clear dose-related effect could only be observed in the second winter (Jones and Ashenden, 2000).

Negative impacts of N enrichment were evident in addition experiments in the Netherlands, Belgium and the United Kingdom. Unfortunately, all effects were observed in situations with N loads of at least 50 to 60 kg N ha⁻¹ yr⁻¹ (additions plus ambient deposition), thus too high to set a realistic critical load. Therefore, simulations with a dynamic ecosystem model for calcareous grassland were used for setting the critical load in the 2002 update (Bobbink et al., 2003). This calcareous grassland model described the biomass, N and light dynamics at different levels of N deposition (Vergeer and Heil, 1998). The proportion of tall grasses was used as an indicator for the effects of increased N. The model incorporated the growth and competitive interaction between tall grasses and the other three functional groups (other graminoids, perennial forbs, annual forbs), the litter production, decomposition and N mineralisation, soil N leaching, removal through management, N fixation by legumes and atmospheric N deposition. Simulations with different N loads showed a strong increase in tall grass at loads above 20 kg N ha-1 yr-1

(Mouissie and Heil, 1999). Using the percentage of tall grass as an indicator, the outcome of this model clearly confirmed 15 to 25 kg N ha⁻¹ yr⁻¹ as a critical load range for the transition of a species-rich vegetation into a grass-dominated (>50%) sward within 10 to 15 years. In view of all the field and modelling evidence, we propose to maintain the critical load at the level in the Berne paper, that is, 15 to 25 kg N ha⁻¹ yr⁻¹, and qualified as 'reliable'.

Increased N availability is probably of major importance in a number of European calcareous grasslands (E1.2). In N-limited calcareous grasslands, increased availability of N is indicated by increased growth of some 'tall' grasses, especially of stress-tolerant species which have a slightly higher potential growth rate and more efficient N utilisation. The species composition of before the start of the N additions, would obviously influence which of the grass species will increase in response to elevated N inputs. N 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). N mineralisation and nitrification are increased under P limitation, and in N-saturated systems with shallow soils this would result in somewhat high 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 (E1.26), and from sites with relatively high atmospheric N deposition. There is a need for studies in continental regions, and for experiments with low N doses at sites with low ambient atmospheric deposition (< 10 kg N ha⁻¹ Vr⁻¹).

7.2.2 Mediterranean xeric grassland (E1.3)

Many dry grasslands are located in the Mediterranean areas of Europe, but, until the early 2000s, the effects of N enrichment on these grasslands of Europe had not been studied experimentally. Since the 2002 update, a 3-year N addition experiment has been performed in Italy, in dry Mediterranean grassland (Bonamoni et al., 2006). In this experiment, the effects of cutting and N enrichment were investigated in a species-poor dry Mediterranean grassland in central Italy. The site represented a Brachypodium rupestre grassland, 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 kg N ha⁻¹ yr⁻¹. Estimated background deposition was less than 15 kg N ha⁻¹ yr⁻¹.

As for most grasslands without proper management, cutting of the vegetation highly reduced above-ground biomass, compared with the uncut controls. However, N additions significantly increased total above-ground biomass following 3 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 of diversity (H), with diversity increasing particularly after cutting of the vegetation, and to a lesser extent after litter removal. Species diversity was lower following all N-addition treatments, but this effect was only significant in one case: after litter removal. Furthermore, it was evident that the species diversity was highly negatively correlated (R²=0.85) with the living biomass percentage of Brachypodium rupestre, a finding similar to that for Brachypodium pinnnatum in Dutch calcareous grasslands (Bobbink and Willems, 1987). Additional research on N impacts in Mediterranean dry ecosystems has been started in Portugal and Spain, but the duration of the experiments, to date, has been too short (< 2 yr) and results unpublished, for them to be included in the current evaluation in respect of critical loads.

To date, no empirical critical loads have been proposed or evaluated for xeric Mediterranean grasslands (E1.3), because of a lack of empirical evidence. The recent experiment in Italy can be considered a first indication of the possible sensitivity of these dry grasslands, despite of its clear limitation. Significant effects have been obtained in the 3 years of this experiment with additions of over 40 to 45 kg N ha⁻¹ yr⁻¹, but no data from other experiments with lower loads are available for Europe. A first estimate of the critical load for the EUNIS category E1.3, based on expert judgement, is 15 to 25 kg N ha⁻¹ yr⁻¹.

7.2.3 Non-Mediterranean dry acid and neutral closed grassland (E1.7)

This EUNIS category groups all dry, base-deficient grasslands on acidic and neutral 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. Typical phytosociological units are Violion caninae, Nardetalia strictae and Agrostion curtisii (see also EUNIS overview, Davies et al., 2004). Rare species, such as Arnica montana, Antennaria dioica, Thymus vulgaris and Dactylorhhiza maculata, have been observed to disappear from these grasslands before high and dense growing grasses started to dominate the vegetation in the Netherlands (e.g., Bobbink et al., 1996). These rare, 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 easily decreases the acid neutralising capacity (ANC) and subsequently the soil pH in these grasslands, which have weakly buffered soils in the cation exchange range. Furthermore, 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 as a result of N inputs and/or changes in N form within the soil.

The impacts of N loads have been experimentally studied for this grassland type. Tomassen et al. (1999) added N (1, 5, 10, 15, 20, 40, 60 or 80 kg N ha⁻¹ yr⁻¹ in the form of ammonium nitrate) to artificial Nardus communities (E1.7) planted in 1x1 m mesocosms ('pristine' sand taken at 5 to 25 cm depths from sod-cut Nardus grassland) under greenhouse conditions (background deposition in the greenhouse < 1-2 kg N ha⁻¹ yr⁻¹). The mesocosms had received clean water for 15 weeks before N additions were initiated. Within two years Tomassen et al. (1999) found a gradual increase in biomass of Danthonia decumbens and Deschampsia flexuosa following increasing N additions of between 1and 20 kg N ha⁻¹ yr⁻¹. However, there was a much stronger, significant effect on both biomass and cover of grasses (D. decumbens and D. flexuosa) following additions of between 20 and 40 kg N ha⁻¹ yr⁻¹. Nitrate concentrations in the soil pore water were strongly increased by N additions of 40, 60 and 80 kg N ha⁻¹ yr⁻¹, and this indication of high nitrification is in accordance with the observation that the system remained well buffered (no change in soil pH).

Additional, experimental N applications were carried out over 3 years on an *Agrostis capillaris* grassland area and a *Festuca ovina* grassland area (E1.72), 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 area (atmospheric load 13 kg N ha⁻¹ yr⁻¹) (Figure 7.3). 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 3 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 area (E1.72) in Derbyshire, in the United Kingdom (atmospheric deposition ca 25 kg N ha⁻¹ yr⁻¹) (Morecroft *et al.*, 1994; Carroll *et al.*, 1997; 2000; 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). The experiment was started in 1990 and, to date, is still ongoing. Already during the second year of the experiment a significant decline in bryophytes (especially *Rhytidiadelphus*) was found following all levels of N **Figure 7.3** Proportion of graminoids (mean + SE) following 3 years of adding 19 kg N ha⁻¹ yr⁻¹ in two non-Mediterranean dry acid closed grassland sites (E1.7) in southern Sweden (Berlin, 1998). Blue columns: control; green columns: with N addition; M0 = normal mowing; M+ = more intensive management; left part = Agrostis grassland (fertile site); right part: *Festuca ovina* site (less fertile site).



treatment. Although this may partly have been an artefact, resulting from the relatively high N concentration in the nutrient solution that was applied, it nevertheless indicates a high sensitivity to N. Moreover, Rhytidiadelphus stems that were collected from the plots showed an increasingly higher N concentration with each N addition (Carroll et al., 1997; 2000). Despite the fact that N concentrations in bryophyte shoots, 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. By 1999, the vegetation had become grassier in the plots that had received high N additions (Carroll et al., 2003, Lee et al., 2000). 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). As found for the P-limited calcareous grassland (Subsection 7.2.1), N accumulation 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, but with a relatively low number of inputs.

Phosphomonoesterase activity in the soil during the long-term experiment already increased at the lowest N

addition (35 kg N ha⁻¹ yr⁻¹) within 3 to 4 years (Carroll *et al.*, 1997). Additionally, microcosm studies on soils from this field experiment (after 7 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 above. Thus, the increased N addition eventually also affected the P budget in this severely P-limited system. A similar experiment with soils that had received N addition 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).

Furthermore, a targeted 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 (E.1.7; Violion caninae) across the United Kingdom. The authors sampled 68 sites of high nature conservation interest across the United Kingdom, in 2002 and 2003, with naturally occurring 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, explaining more than half of the variation in the number of plant species per plot. A clear negative linear (or negative exponential, see Emmett (2007)) relationship between species richness of these E1.7 grassland communities and N deposition was found (Figure 7.4). Looking in detail at the data, it is obvious that high species richness only occurred at N loads of between 5 and 10 kg N ha⁻¹ yr⁻¹, with species richness clearly lower at deposition rates of above 15 to 20 kg N ha⁻¹ yr⁻¹. This survey thus indicates that the critical load range of N for this acidic grassland type is around 10 to 15 kg N ha⁻¹ yr⁻¹.

In addition to this, Maskell *et al.* (2010) reported results from acidic grasslands, in general, in the United Kingdom, which broadly support these findings, while Stevens *et al.* (2009) provide a direct comparison of results from Stevens *et al.* (2004) and the wider Countryside Survey. 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 (Stevens *et al.*, 2010a). **Figure 7.4** Species richness of acidic grasslands (E1.7) across a gradient of N deposition in the United Kingdom (Stevens *et al.,* 2004).



To investigate if this relationship holds for the whole Atlantic range of these grasslands, and to obtain more information on the lower and higher ends of this range of N depositions, the European Science Foundation (ESF) funded a programme (BEGIN) that carried out a targeted survey identical to the UK study of Stevens *et al.* (2004), in 9 countries, from Norway to the south of France, in the 2007-2009 period (Stevens *et al.*, 2010b). This survey has shown that, also on this scale, species richness is negatively correlated with total N deposition (Figure 7.5).

Figure 7.5 Species richness of acidic grasslands (E1.7) across a gradient of N deposition in the Atlantic region of Europe (Stevens *et al.*, 2010).



These studies indicate that many dry acidic grasslands (E1.7) are also sensitive to N loads. In N-limited systems, there is evidence of a significant increase in grass dominance, leading to increased competition and exclusion of smaller species such as rosette forbs, at 15 to 20 kg N ha-1 yr¹ or more, at relatively low atmospheric N deposition (e.g., Berlin et al., 2000; Tomassen et al., 1999). This is most likely to be increased by a positive feedback to litter quality and rates of soil N mineralisation, but this has not yet been studied. In P-limited systems, the response in the vascular vegetation is much slower, but there is a clear and rapid effect on bryophytes (Carroll et al., 2003). In addition to these effects, N and P economy of the soil is significantly affected, which, among other effects, is likely to result in significant leaching of N (Carroll et al., 2003; Phoenix et al., 2003). Furthermore, the effects of N deposition became very prominent in the targeted N survey in the United Kingdom, clearly indicating lower species richness at above 10 to 15 kg N ha⁻¹ yr⁻¹; these changes were clearly related to soil acidification effects of deposition.

Taking the new UK and European survey results into account, as well as the effects found in Sweden and the Netherlands, the critical load for dry acid grasslands (E1.7) is set at 10 to 15 kg N ha⁻¹ yr⁻¹, qualified as 'reliable'. There is clearly a need for more field studies in different countries, especially experiments with lower N addition on sites with low atmospheric deposition.

7.2.4 Non-Mediterranean dry acid and neutral open grassland, including inland dune grassland (E1.9)

This EUNIS class contains all dry grasslands on acidic and neutral soils with open vegetation, in non-Mediterranean regions. Inland dune grasslands (E1.94 and E1.95), both pioneer and more mature communities, fall within this EUNIS class. Recently, 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 2 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.

Defining a critical load based on one experiment, it is not possible. However, these inland dune grasslands have a species composition and ecological functioning comparable with that of coastal (grey) dune grasslands (see Chapter 4 for details). Because of this similarity, the critical load for inland dune pioneer grasslands (E1.94) and inland dune siliceous grasslands (E1.95) has been set at the same level as that of coastal grey dune grasslands (8-15 kg N ha⁻¹ yr⁻¹). This estimation is based completely on expert judgement and there is thus a significant need for long-term research on these, probably sensitive, systems.

7.3 Mesic grasslands (E2)

7.3.1 Low- and medium-altitude hay meadows (E2.2)

Grasslands situated at low or medium altitudes and which are managed for making hay (E2.2) are the only class of mesic grasslands for which 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). N has been annually applied (single dose) in the form of ammonium sulphate or sodium nitrate (48 kg N ha-1 yr-1) to plots of this mesic, low-altitude hay meadow (E2.2). On N-treated plots, the vegetation has become dominated within 5-10 years 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, has reduced the diversity of higher plant and bryophyte species significantly more than other forms of N within 10 years and this difference was still present after more than 150 years (Goulding et al., 1998; Virtanen et al., 2000; Silvertown et al., 2006).

In addition to an increase in biomass, a 25% reduction in species diversity was observed after long-term (>4 years) additions of N (100 kg N ha⁻¹ 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 8 years, although differences between N treatments and the controls remained significant. Phosphorous additions did not affect the vegetation in this long-term experiment.

The effects of nutrients have been investigated in a long-term (40 years) fertiliser experiment, in a highly productive alluvial meadow (E2.2) in the Czech Republic (Honsova *et al.*, 2007). The vegetation was cut twice a year and, next to having a control plot, on other plots nutrients

were added in combinations of PK, and NPK, the latter including four levels of N addition (50-200 kg N ha⁻¹ yr⁻¹). To gain insight into the effects of N alone, the N_{EO}PK and N₁₀₀PK results were compared with those from the PK-only additions. Although the lack of N-only treatments makes this a sub-optimal experiment in terms of critical loads evaluation, there is an acute shortage of data with which to evaluate responses to N deposition for this system. After long-term N addition (with 50 or 100 kg N ha⁻¹ yr⁻¹) the cover of grasses increased significantly, to 80%, compared with ca 40% in the PK-treated vegetation, whereas the percentage of forbs decreased significantly, from 45 to 20%. Because of the natural high fertility in these alluvial grasslands, the changes in species richness were less pronounced. This is again a clear indication of N loads possibly affecting the abundances of functional groups, and, to a lesser extend, species numbers.

Since 2002, no new evidence has been published from low N-dose studies on habitats in the E2.2 category. Therefore, and because hay management removes N from the system annually, the critical load range for low- and medium-altitude hay meadows in this category will remain at 20 to 30 kg N ha⁻¹ yr⁻¹, based on expert judgement. 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 (E2.3)

Many semi-natural grassland types occur in montane regions across Europe, containing many rare and endangered plant and animal species (e.g., Ellenberg, 1996). It is important to emphasise the effects of N loads also on these montane grasslands (E2.3), because N deposition has certainly increased in mountainous regions in central Europe. However, studies with low doses of N only, are not available for this grassland type, despite the long-term Rengen Grassland Experiment (RGE) with fertiliser treatments in a mountainous hay meadow in Germany (Hejcman *et al.*, 2007). Unfortunately, this German study applied N only in combination with Ca and Mg, thus preventing soil acidification effects of N inputs, but, in this way also preventing any significant impact of N deposition on these acidic meadows.

It light of the above, it is difficult to establish a critical load with any reliability rating for these grasslands, although it may be expected that (i) several of these grassland types are sensitive to N (Ellenberg, 1996), and (ii) their critical load of N is probably lower than for lowland dry or mesic grasslands, because of the shorter vegetation period and/ or the generally nutrient-poorer soils. The lack of information on effects of N on montane grasslands has been identified as a major gap in knowledge (Bobbink *et al.*, 2003). Unfortunately, this gap still exists, and the critical load range (10-20 kg N ha⁻¹ yr⁻¹) for mountain hay meadows (E2.3) is based on expert judgement only.

7.4 Seasonally wet and wet grasslands(E3)

7.4.1 Moist or wet oligotrophic grassland (E3.5)

Moist or wet oligotrophic grassland habitats (E3.5) are characterised by oligotrophic and moist to wet peaty soil conditions. This EUNIS category consists mostly of hay meadows under original agricultural management and that are especially rich in typical plant and animal species. Two subcategories have been distinguished in EUNIS, namely (i) Molinia caerulea meadows (E3.51; 'litter meadows' or 'fen meadows') and, (ii) heath meadows and humid Nardus stricta swards (E3.52). 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 critical load range.

Fortunately, the impacts of N additions on species richness have been quantified in flower-rich, oligotrophic wet hay meadows (E3.51) in Somerset (UK) (Mountford *et al.*, 1994; Tallowin *et al.*, 1994; Kirkham *et al.*, 1996). N 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, an N-manipulation experiment examined the effects of additions of ammonium sulphate (10 and 20 kg N ha⁻¹ yr¹) or sodium nitrate (20 kg N ha⁻¹ yr¹ only) to an upland grass heath in Wales (E3.52) (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 myrtilus and Festuca (E3.52). The treatments were applied to paddocks which had 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 in the 20 kg N ha⁻¹ yr⁻¹ treatment. These high leaching rates suggested that N-levels at this site were already above the critical load for nutrient 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. Root bioassay experiments with *Nardus* indicated an overall relatively low uptake of N and a relatively high uptake of P, which suggested that the site was P rather than N limited, although P-addition experiments did not support this conclusion (Emmett *et al.*, 2001).

The atmospheric deposition at this site was estimated at 20 kg N ha⁻¹ yr⁻¹. Jones and Ashenden (2000), to assess the impacts of lower deposition rates, in a greenhouse experiment, 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. To assess possible interactions with grazing pressure, three levels of simulated grazing (clipping) were also applied. Within 1 to 2 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 kg N ha-1 yr-1 - while for Hypnum jutlandicum this would be around 20 kg N ha⁻¹ yr¹ (Figure 7.6). Although results have shown an increase in fine grass cover and a decrease in Nardus following

Figure 7.6. Cover (mean ± SE) of moss species in Nardus stricta grassland (E3.52) 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. The year denotes when the optimum N level for each species became apparent (Jones *et al.*, 2002). * p≤0.05; ** p≤0.01.



increasing N additions, these effects occurred primarily at between 20 and 55 kg N ha⁻¹ 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).

Several moist or wet oligotrophic grasslands (E3.5) of high conservational value have been shown to be sensitive to N eutrophication. Increases in dominant grasses and decreases in diversity have been observed following increasing levels of N inputs. Furthermore, interactions with P limitation are also apparent. The study of a degraded upland heath meadow (E3.52) has provided evidence of response in bryophyte cover to relatively low levels of N deposition, suggesting increases in leaching and acidification at levels above 20 kg N ha⁻¹ yr⁻¹. In view of these UK studies, the critical load for moist to wet oligotrophic grasslands is set at 10-20 kg N ha-1 yr-1 specifically for Nardus stricta swards (E3.52), and considered to be 'quite reliable'. The critical load for Molinia caerulea meadows (E3.51), however, is based only on expert judgement, and estimated to be somewhat higher (15-25 kg N ha⁻¹ yr⁻¹), because, to date, this habitat has barely been studied. All these values have not changed from those in the 2003 document. Base status has been identified as a significant modifying factor; systems with low base status are likely to be more sensitive, while fluctuations in the water table cause habitats in the E3.52 category to be less sensitive.

7.5 Alpine and subalpine grasslands (E4)

7.5.1 Moss- and lichen-dominated mountain summits, ridges and exposed slopes (E4.2)

Within the EUNIS system, an important sub-category of alpine and subalpine grasslands (E4) are communities without extensive snow cover which are dominated by moss and lichen species. They form the E4.2 category. 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 critical load. However, the only substantive evidence to support a specific critical load is that for Racomitrium 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, recently, evidence from experimental manipulation studies has become available, which suggests that changes in grazing pressure are also likely to be significant. 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 (NO $_{2}^{-}$ and NH $_{4}^{+}$), 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 the low N-addition rates, responding with a raised tissue N content, a severe inhibition of nitrate reductase activity, increased leakage of potassium from the shoots, and shoot growth rates which were less than 50% of those on the control plot. Of all the measurements, only nitrate reductase showed a clear distinction between NO,⁻ and NH⁺ applications. After only two growing seasons, Pearce and Van der Wal (2002) also demonstrated how quickly Racomitrium was replaced by graminoid species; Racomitrium cover following the low treatment was reduced by 31%, while graminoid cover increased by 57%. These results could reflect both a toxic effect and increased competition for light from graminoids, which can utilise excess N.

The high sensitivity of Racomitrium lanuginosum to N deposition is supported by the results from a glasshouse experiment that used monoliths taken from subalpine/

montane grassland in central Wales. In this experiment, N deposition and simulated grazing were manipulated over a 4-year period (Jones, 2005). The applied N treatments were both above and below the source field level of 20 kg ha-1 yr-1. Racomitrium only had a significant presence in monoliths when subjected to heavy simulated grazing, confirming its sensitivity to competition for light with 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 7.7).

Figure 7.7 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).



Racomitrium lanuginosum

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 moss heath in Iceland with a background deposition of about 2 kg ha⁻¹ yr⁻¹, found only small non-significant increases in Racomitrium growth and shoot density. These were associated with increases in N concentrations when reduced N, not when oxidised N, was added. It was suggested that the small response to the low deposition rates used in this experiment was associated with growth limitations due to other factors.

Although experimental evidence is limited to three studies with somewhat contrasting results, the fact that large and rapid changes in growth or cover have been observed in

two of these studies at deposition rates of 10 kg ha⁻¹ yr¹ or lower, suggests that a critical load for moss and lichen dominated mountain summits, ridges and exposed slopes (E4.2) may be set at 5-10 kg N ha⁻¹ yr¹ and be described to be 'quite reliable'. This value is identical to that in the 2003 document.

7.5.2 Alpine and subalpine acid (E4.3) and calcareous (E4.4) grasslands

Many natural grassland types are found in the alpine and subalpine regions of Europe, on both acidic (E4.3) and calcareous (E4.4) soils. These grasslands contain a large proportion of the high biodiversity in these areas (Körner, 1999). It is important to emphasise the consequences of N inputs also in these grasslands, because atmospheric N deposition has started to increase even in these remote regions.

Figure 7.8. Responses of plant biomass (mean \pm SE) of high elevation (>2450 m) grassland and glacier foreland (most left) to 2 to 4 years of nutrient addition (Heer and Körner, 2002; Körner *et al.*, 1997; E. Hiltbrunner, unpublished data). * p<0.05; ** p<0.01.



There is clear evidence of the results of high rates of N addition (>40 kg N ha⁻¹ yr⁻¹). Late successional acidic grassland at a 2500 m elevation in the central Swiss Alps (Furkapass area) showed a rapid and massive response to additions of 40 to 50 kg N ha⁻¹ yr⁻¹ (Körner *et al.*, 1997; Figure 7.8). Although these experiments were conducted with a multi-nutrient fertiliser, circumstantial evidence suggests that the response was mainly driven by N. Biomass was doubled already in the second year (maintained up to the fourth year), with sedges (*Carex curvula*) profiting most. Plants that grow in very cold climates might be assumed to be largely temperature- rather than nutrient-limited, however, results from an experiment on vegetation in an N-exposed glacier foreland proved this notion to be wrong. A 100 kg N ha⁻¹ yr⁻¹ addition converted an open vegetation with cushion plants (<10% cover) into a lush meadow (100% cover) within just two years (Heer and Körner, 2002).

Although such high-dose experiments may be seen to be purely of academic interest, they still prove a basic response by such cold-climate vegetation to N additions. Earlier experiments with additions of 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 4 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, high-elevation vegetation is responsive to a variable nutrient supply. Yet, the lack of information on the impacts of more realistic, low N loads (<25 kg N ha⁻¹ yr⁻¹) in alpine and subalpine grasslands was identified as a major gap in knowledge in the 2002 update (Bobbink et al., 2003).

Since then, fortunately, several long-term studies on N additions have been set up in Switzerland, to quantify the impacts of N loads, even at very low values.

Alpine and subalpine acid grasslands (E4.3)

The effects of N addition on plant production in acidic alpine grasslands (Caricietum curvulae) was studied from 2002 to 2005 in the central Alps (Furka) in Switzerland, at two altitudes (2450 and 2650 metres above sea level) (Hiltbrunner et al., 2005; Hiltbrunner et al., submitted). In the 4th year of this experiment, the addition of 25 kg N ha⁻¹ yr⁻¹, in the form of ammonium nitrate, resulted in a significant increase (27-45%) in the above-ground biomass, compared with the control vegetation. Background deposition at the research site was around 4 to 5 kg N ha⁻¹ yr⁻¹. The experiment clearly showed that plant production in these alpine and subalpine acidic grasslands was limited by N, as alpine acidic grassland is rather sensitive to loads of 25 kg N ha⁻¹ yr⁻¹. Experiments in the Tatra mountains, in an already very acidic alpine habitat, also has demonstrated that depletion of base cations (Ca) and increases in Al and Fe concentrations (indicating soil acidification!) may be of major importance in the decline of these grasslands (Bowman et al., 2008).

In 2004, a long-term N-addition experiment was set up, which is still running today, in a species-rich subalpine acid grassland area (Geo-montani Nardetum), at 2000 metres above sea level, in the central Swiss Alps (Bassin *et al.*, 2007; Bassin *et al.*, 2009). In this experiment, 5, 10, 25 and 50 kg N ha⁻¹ yr⁻¹ are being applied, with an ambient background of ca 4 kg N ha⁻¹ yr⁻¹. The above-ground biomass of the vegetation significantly increased with the added N levels. In addition, the species composition of the vegetation changed in favour of the sedges. Most effects were significant following the two highest additions in the 3rd year of the experiment, and significant changes in the sedge cover were already prominent at addition rates of 10 kg N ha⁻¹ yr⁻¹ and more (Bassin et al., 2007.) In one of the subsequent years, this effect already became significant at additions of 5 kg N ha⁻¹ yr⁻¹ (unpublished data). To date, no effect on species richness has been observed for the treated vegetation. 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 54g C m⁻² per season, from the grassland, compared to the control area (Volk et al., 2010). This implies that under increased N deposition, the ecosystem may act as a CO₂ source to the atmosphere and thus increases the CO₂ related greenhouse effect.

Based on this new evidence, alpine and subalpine acid grasslands (Eq.3) are likely to be very sensitive to N loads. The critical load for these habitats thus clearly is low and has been set at 5 to 10 kg N ha⁻¹ yr⁻¹, which is considered to be 'quite reliable'.

Alpine and subalpine calcareous grasslands (E4.4) Alpine grasslands on calcareous soils are especially species-rich communities. The impacts of N enrichment have been investigated in a calcareous alpine grassland area in Switzerland, at ca 2500 metres above sea level, between 2004 and 2007 (Hiltbrunner *et al.*, submitted). Background deposition at this site was between 3 and 5 kg N ha⁻¹ yr⁻¹, and, in addition to a control area, six N-application levels were applied, with inputs of 2.5, 5.0, 10, 15, 20 and 25 kg N ha⁻¹ yr⁻¹. This long-term experiment was set up with small differences between N-application levels, to come to a detailed evaluation of the critical load for these alpine grasslands. The treatment results from the 4th year (2007) are described here, but the experiment is expected to continue for at least three more years.

No changes in total plant cover were found following the six N additions in 2007, both compared with the control area and with the cover at the start of the experiment. However, significant shifts in species composition were found, especially for the sedge species. Several sedges increased significantly in cover, following treatments of 10 kg N ha⁻¹ yr⁻¹ and more, but, in the 4th year of this field N addition experiment, the most important sedge species, Carex firma, already showed a positive response in the amount of cover following additions of 5 kg N ha⁻¹ yr⁻¹. Among the herb species, the hemiparasite species Bartia alpina enjoyed a particular advantage and increased in abundance at additions of more than 15 kg N ha⁻¹ yr⁻¹, presumably due to the better nutrient supply of the host species. By 2007 (the 4th season) no significant changes in species richness had been found, but monitoring of the

responses still continues, to date, and soon there will be 8 years of data available on this experiment. Total biomass clearly increased, following additions of 15 kg N ha⁻¹ yr⁻¹ and more, and, given the cover responses by *Carex firma* after 4 years, it is expected that an overall biomass response will be seen, in the longer term, for additions of 5 kg N ha⁻¹ yr⁻¹.

Based on this very recent information, alpine and subalpine calcareous grasslands are also likely to be very sensitive to N loads, with significant effects around 10 kg N ha⁻¹ yr⁻¹. The critical load range for alpine and subalpine calcareous grasslands (E4.4), thus, is low and has been set at 5 to 10 kg N ha⁻¹ yr⁻¹, which is considered to be 'quite reliable'. For a summary, see Table 7.1.

Table 7.1 Empirical critical loads of N and effects of exceedances on grassland and tall forb habitats (E). ## reliable, # quite reliable
and (#) expert judgement. Differences with 2003 indicated in bold.

Ecosystem type	EUNIS code	kg N ha-1 yr-1	Reliability	Indication of exceedance
Sub-Atlantic semi-dry	E1.26	15-25	#	Increase in tall grasses, decline in
calcareous grassland				diversity, increased mineralisation, N
				leaching, surface acidification
Mediterranean xeric	E1.3	15-25	(#)	Increased production, dominance by
grasslands				graminoids
Non-Mediterranean dry	E1.7ª	10-15	##	Increase in graminoids, decline of
acid and neutral closed				typical species, decrease in total
grassland				species richness
Inland dune pioneer	E1.94ª	8-15	(#)	Decrease in lichens, increase in
grasslands				biomass
Inland dune siliceous	E1.95ª	8-15	(#)	Decrease in lichens, increase in
grasslands				biomass, increased succession
Low- and medium-altitude	E2.2	20-30	(#)	Increase in tall grasses, decrease in
hay meadows				diversity
Mountain hay meadows	E2.3	10-20	(#)	Increase in nitrophilous graminoids,
				changes in diversity
Moist and wet oligotrophic	E3.5			
grasslands				
 Molinia caerulea meadows 	E3.51	15-25	(#)	Increase in tall graminoids, decreased
				diversity, decrease in bryophytes
 Heath (Juncus) meadows 	E3.52	10-20	#	Increase in tall graminoids, decreased
and humid (Nardus stricta)				diversity, decrease in bryophytes
swards				
Moss and lichen dominated	E4.2	5-10	#	Effects on bryophytes or lichens
mountain summits				
Alpine and subalpine acid	E4.3	5-10	#	Changes in species composition,
grasslands				increase in plant production
Alpine and subalpine	E4.4	5-10	#	Changes in species composition,
calcareous grasslands				increase in plant production

^a Apply the lower end of the range to habitats with a low base availability; and the higher end of the range to those with high base availability.

7.6 References

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Chapter 8

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



Photo p. 107 Strong dominance of grasses in dry inland heath in the central part of the Netherlands as seen in the early 1980s in regions with high N loads (photo R. Bobbink).

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

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 1 metre or less above soil surface. Grasses and forbs may form discontinuous canopies, and there is frequently a ground cover of mosses or lichens (Gimingham et al., 1979; De Smidt, 1979). Heathlands are classified together with scrub and tundra habitats in class F 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 a subcategory of these systems, the vegetation is determined by climate, and succession towards woodland is inhibited by drought, low temperature or length of the frost period (e.g., categories F1 and F2). In contrast, the extensive inland, lowland dwarf-shrub heathlands in Altantic and sub-Atlantic Europe are certainly man-made, although they have existed for several centuries. In these heaths, the development towards woodland has been prevented by mowing, burning, sheep grazing or sod removal. They are widely dominated by some 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 increased N (nitrogen) deposition. Because of their high nature conservation value, many heathlands have become nature reserves, in recent years.

In accordance with the EUNIS habitat classification, this chapter distinguishes the following categories and subcategories. Tundra (F1), Arctic, alpine and subalpine scrub habitats (F2), temperate shrub habitats (F4), with subcategories of wet (F4.1) and dry (F4.2) heaths. In view of their functional differences, wet heaths are subdivided, according to climate, into northern (F4.11) and southern (F4.12) wet heaths. For the last subcategories, no data are available to assign them a critical load. Northern wet heaths were assigned a separate critical load if dominated by Calluna vulgaris (upland Calluna moorlands) or Erica tetralix. Coastal dune heaths have been categorised as coastal habitats (Chapter 4; B1.5), and acidic grasslands with some heather species as grassland habitats (Chapter 7; E1.7). For other EUNIS categories in class F, including other heathland types, no empirical critical loads of N have been determined, due to a lack in data availability.

8.2 Tundra (F1) and Arctic, alpine and subalpine scrub habitats (F2)

Alpine and Arctic habitats have many ecological characteristics in common, although the climatic conditions are more severe in the 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 activity or solifluction. Decomposition of organic matter and nutrient cycling are slow, and the low nutrient availability limits primary production (Robinson and Wookey, 1997). Despite the constraints, there are a number of plant species growing on tundra in the Arctic and sub-Arctic, including low shrubs, sedges, reindeer mosses and other lichens (crustose and foliose), bryophytes (mosses and liverworts), tussock grasses, and approximately 400 herb species.

In classifying these communities under the EUNIS system, it is necessary to distinguish between tundra (F1) and Arctic, alpine and subalpine scrub habitats (F2). Tundra is defined as vegetated land with graminoids, shrubs, mosses or macro-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 and northern Russia. Vegetation of the same species also occurs on boreal mountains and in the low Arctic region, far away from the main permafrost region, notably in Fennoscandia and Iceland. These habitats are listed under alpine and subalpine grasslands (E4), or Arctic, alpine and subalpine scrub habitats (F2) (Davies et al., 2004). The latter occur north of or above the climatic tree line, but outside the permafrost zone, or scrub occurring close to but below the climatic tree line, where trees are suppressed either by late- season snow, wind, or repeated browsing (Davies et al., 2004).

8.2.1 Tundra (F1)

Tundra habitats are divided into two subcategories: shrub tundra (F1.1) and moss and lichen tundra (F1.2) (Davies *et al.*, 2004). Shrub tundras (F1.1) are the tundras of the southernmost tundra belt, characterised by an abundance of medium-small and small shrubs, such as *Alnus fruticosa*, *Salix lanata*, *Betula nana*, *Betula exilis*, *Salix reptans*, *Salix pulchra*, and of dwarf shrubs, in particular, *Vaccinium uliginosum*, *Vaccinium vitis-idaea*, *Ledum decumbens*, *Rubus chamaemorus*, *Empetrum hermaphroditum*, *Empetrum nigrum*, and *Arctostaphylos alpine* (Davies *et al.*, 2004). Moss and lichen tundras (F1.2) are characterised by a thick cover of mosses, formed notably by Hylocomium splendens, Aulacomnium turgidum, Tomentypnum nitens, and Ptilidium ciliare, with dwarf shrubs, particularly, Dryas octopetala, Cassiope tetragona, Salix reptans, and Vaccinium vitis-idaea, and sedges, including the often dominant Carex ensifolia. Drier stands alternate in mosaic fashion with wetter areas dominated by sedges, in particular, Carex stans, Eriophorum angustifolium, and Eriophorum scheuchzeri, and grasses, notably, Arctophila fulva and Dupontia fischeri (Davies et al., 2004). Although several new studies have been published, there are not enough data available to assign a critical load of N to both these tundra subcategories.

Significant increases in the atmospheric deposition of N have been reported in Arctic regions during recent decades (Woodin, 1997). Plant growth and distribution in the Arctic are strongly constrained by the relatively short snow-free period, low air and soil temperatures, low soil moisture and low soil nutrient availability. Thus, anthropogenic sources of N may have direct and indirect effects on this ecosystem, impacting on the local flora and fauna, and the economy and culture of indigenous populations (Press *et al.*, 1998a).

Numerous field studies have been conducted, manipulating tundra ecosystems with nutrients. Most, however, have involved NPK fertiliser additions (e.g., Robinson *et al.*, 1998; Press *et al.*, 1998b; Schmidt *et al.*, 2000), or single large applications of N (e.g., Shaver and Chapin, 1995). Typical of the latter is the experiment by Henry *et al.* (1986), who examined the effect of single N additions of 50 and 250 kg ha⁻¹yr¹, applied to three Canadian tundra communities, namely a wet sedge meadow, a mosaic *Cassiope tetragona* heath, and a dry-mesic *Dryas integrifolia* heath. Although a range of vegetation responses were observed in the third year following additions of 250 kg N ha⁻¹yr⁻¹, no significant effects were observed following applications of 50 kg N ha⁻¹yr⁻¹.

In contrast, in a study of tundra ecosystems with a similar species composition, significant effects have been reported at much lower deposition rates. An experimental study was started in 1991, of mixed tundra heath near Svalbard (Spitsbergen) (Baddeley et al., 1994). In this experiment, plots were chosen in areas with three 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 4 to 5 applications, during summer periods. 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. Ambient atmospheric deposition was not measured, but is expected to have been very low. 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. *Cassiope tetragona* showed no response to the N addition, whilst *Dryas octopetala* showed an intermediate response of the measured variables.

Gordon et al. (2001) re-examined the impacts of N on these plots, with particular attention for the bryophyte communities. Overall bryophyte cover was unaffected by increased N supply, although this was the net result of individual species showing different responses. For example, Polytrichum juniperinum increased in cover, whilst the Dicranum scoparium cover was reduced. Tissue N content increased with increasing supply, demonstrating its close coupling with atmospheric inputs. Importantly, a number of significant persistent effects were observed at additions of 10 kg N ha⁻¹ yr⁻¹. Nitrate reductase activity was inhibited in Polytrichum juniperinum, suggesting N saturation, and the potential for inorganic N to pass through the bryophyte layer, thus becoming available to soil microbes and higher plants. The N addition also increased the proportion of green bryophyte shoots, to a small extent, thus apparently increasing potential total bryophyte productivity. The increased 'greenness' of the bryophyte cover on the fertilised Cassiope heath plots was independent of P treatment and was found five years after N additions had ceased. This suggests that the added N is retained within the bryophyte layer of the Cassiope heath for long periods of time, and implies that any reversal of the effect will be slow. This lack of recovery demonstrates that even small amounts of N deposition, potentially, may cause long-term ecological change (Gordon et al., 2001).

Co-limitation by N and P was clearly demonstrated in this study. For example, in combination with added P, 10 kg N ha⁻¹ yr⁻¹ significantly decreased total lichen cover, increased the cover of Polytrichum juniperinum and changed vegetation composition in the Dryas plots. Hence, critical loads for tundra ecosystems may be dependent on P availability and be influenced by increased nutrient availability due to global warming. Long-term responses in tundra vegetation to NPK fertiliser treatment, in most cases, showed an effect from nutrient addition, with indication of both N and P limitation. Shaver et al. (1998) suggest that mechanistic analysis of the results of fertilisation experiments can identify factors associated with either N or P limitation. They suggest 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 macrolichen and vascular plant abundance in Arctic vegetation ecosystem experiments. These experiments manipulated factors such as temperature and nutrient availability, and concluded that negative correlations were greater at sites in milder climates with a greater aboveground biomass, where increased shading and litter

production is likely to negatively affect the lichens. Hence, it may be possible to identify those tundra communities for which low critical loads are required to prevent changes in species composition.

More recently, Arens et al. (2008) studied the effects of additions of small amounts 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 kg N ha⁻¹ yr⁻¹). They used factorial additions of N and P (25 kg P ha⁻¹ 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 CO₂ exchange appeared to be very sensitive to low rates of N inputs (5 kg ha⁻¹ yr⁻¹). Additions of 5 kg N ha⁻¹yr⁻¹ led to a significant increase in deciduous shrubs, 10 kg N ha⁻¹ yr⁻¹ almost doubled the cover of graminoids, and 50 kg N ha⁻¹ yr⁻¹ resulted in a more than seven-fold increase in forbs (Figure 8.1). The mean NDVI (Normalized Difference Vegetation Index) showed a saturating response to increasing levels of N addition, such that the largest NDVI response occurred at 10 kg N ha⁻¹ yr⁻¹, while no further increase in NDVI was observed at 50 kg N ha⁻¹ yr⁻¹ (Figure 8.2). This decreasing response in NDVI (cover of green biomass), next to decreasing ecosystem respiration and photosynthesis in the plots receiving 50 kg N ha⁻¹yr⁻¹ suggested that the N saturation in the ecosystem or vegetation began to occur between 10 and 50 kg N ha-1 yr⁻¹ (Arens et al., 2008). Combined additions of both N (50 kg N ha⁻¹ yr⁻¹) and P released the system from N saturation and dramatically increased ecosystem photosynthesis and respiration, leading to a drastic increase in the cover of graminoids (especially Festuca brachyphylla; Figure 8.2).







Figure 8.2 Mean NDVI (Normalized 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 g P m⁻² yr⁻¹ + 5.0 g N m⁻² yr⁻¹ Source: Arens *et al.* (2008). Numbers behind type of treatment indicate nutrient load in g m⁻² yr⁻¹.



Aerts (2009) studied the effect of increased soil N availability due to summer warming, in a tundra ecosystem in northern Sweden. In a 7-year experiment the recovery of sub-Arctic Empetrum hermaphroditum was investigated, using a factorial combination of aboveground Empetrum removal (simulating recurrent extreme winter warming) and N addition (40 kg N ha⁻¹ yr⁻¹). N addition led to an increase in abundance of Empetrum at the expense of subordinate species. Only when Empetrum was removed could subordinate species benefit from N addition (Aerts, 2009). Jonasson (1992) also found a positive effect of N addition on the biomass of Empetrum, whereas Press et al. (1998b) did not find any effect. This is in contrast with other studies were N addition had strong negative effects on Empetrum (Brancaleoni and Gerdol, 2006; Nilsson et al., 2002).

Summary

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 kg N ha⁻¹ yr⁻¹. To a large extent, however, the response to atmospheric N within the tundra ecosystem may well depend on other factors, such as P status and temperature. Bobbink *et al.* (2003) proposed a critical load for tundra (F1) of 5 to 10 kg ha⁻¹ yr⁻¹, considered to be 'quite reliable'. This classification of 'quite reliable' was based on only one experiment (Gordon *et al.*, 2001), but was justified because (i) the experiment involved three different plant

communities, (ii) background deposition was very low (<1 kg ha⁻¹ yr⁻¹), (iii) the N treatment (10 kg ha⁻¹ yr⁻¹) was within the critical load range, (iv) effects were rapid and are very persistent, and (v) subsequent unpublished results confirm the persistence of these effects.

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 kg N ha⁻¹ yr⁻¹, this supports adjusting the current critical load of 5 to 10 kg ha⁻¹ yr⁻¹. We therefore propose a new critical load for tundra (F1) of 3 to 5 kg ha⁻¹ yr⁻¹, to be considered as 'quite reliable'.

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 critical load. Thus, higher critical loads should be applied to systems which are limited by P, and lower critical loads to systems which are not.

8.2.2 Arctic, alpine and subalpine scrub habitats (F2)

The EUNIS class of Arctic, alpine and subalpine scrub habitats is subdivided into four categories: sub-Arctic and alpine dwarf willow scrubs (F2.1), evergreen alpine and subalpine heath and scrubs (F2.2), subalpine deciduous scrubs (F2.3), and conifer scrub close to the tree line (F2.4) (Davies et al., 2004). Dwarf willow scrubs (F2.1) are well developed in boreal and Arctic mountains and in sub-Arctic lowlands. Evergreen alpine and subalpine heath and scrubs (F2.2) are small, dwarf or prostrate shrub formations in alpine and subalpine mountainous zones, dominated by ericaceous species, Dryas octopetala, dwarf junipers, brooms or greenweeds. Subalpine deciduous scrubs (F2.3) include the subalpine scrubs of Alnus, Betula, Salix and Rosaceae (Amelanchier, Potentilla, Rubus, Sorbus), which are less than 5 metres tall, often accompanied by tall herbs. The last category of conifer scrubs close to the tree line (F2.4) relates to scrubland with dwarf conifers (krummholz), often with incomplete canopy cover, close to the tree line. The tree species at the Arctic tree line can grow to large stature under favourable conditions. However, Pinus mugo in central and southern Europe is often genetically a shrub (Davies et al., 2004).

Since the last update of empirical critical loads of N (Bobbink *et al.*, 2003), several publications on the effects of N on Arctic, alpine and subalpine scrub habitats have appeared. Most relevant studies involve either NPK fertilisation or very high levels of N addition (e.g., Theodose and Bowman, 1997; Nilsson *et al.*, 2002; Soudzilovskaia and Onipchenko, 2005; Brancaleoni and Gerdol, 2006; Jägerbrand *et al.*, 2009). However, there are a few published studies that are relevant for critical load evaluation.

A study in the Dovre mountains in Norway, at 1000 to 1400 metres above sea level, investigated the effects of three years of fertilisation of a Betula nana dominated community (F2.3) 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 relatively small effects of N 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 species cover (Fremstad et al., 2005)

Nilsson *et al.* (2002) conducted an 8-year experiment in an alpine tundra (F2.2) in northern Sweden (background deposition ca 1-2 kg N ha⁻¹ yr⁻¹). Addition of N (50 kg N ha⁻¹ yr⁻¹) in the form of ammonium nitrate reduced *Empetrum hermaphroditum* cover, while on the same plots *Deschampsia flexuosa* rapidly increased in cover (30%), compared to the vegetation in the control treatment area (5%; see Figure 8.3). N fertilisation decreased the cover of the mosses *Dicranum* species and *Pleurozium schreberi* and the lichen *Cladina* species.

Figure 8.3 Percentage of ground cover of *Deschampsia flexuosa* during the course of the experiment at different nutrient treatments. Relevant symbols: —— = control and —— = ammonium nitrate addition (50 kg N ha⁻¹ yr⁻¹). Source: Nilsson *et al.* (2002). Please note: N addition started in 1989.



Vertical bars represent least significant differences at P = 0.05 following analysis of variance within each year across all treatments

Britton and Fisher (2007a) studied the effects of N deposition on low-alpine Calluna - Cladonia heath (F2.2) 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 5 years Calluna vulgaris shoot extension was stimulated by N additions of 10 kg ha-1 yr⁻¹ (total N input of 20 kg N ha⁻¹ yr⁻¹), indicating that alpine heathlands are very sensitive to low levels of N deposition (Britton and Fisher, 2008). Biodiversity was significantly reduced at additions of more than 10 kg N ha⁻¹ yr⁻¹, primarily through reductions in lichen diversity (Figure 8.4). N addition caused rapid and significant increases in plant tissue 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 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). However, the measured N:P ratios ranged from 10 to 20. Therefore, this statement is rather questionable.

Figure 8.4 Effect on mean lichen species richness of N addition treatments of 10, 20 and 50 kg ha⁻¹ year⁻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).



In the N-addition experiment on low-alpine *Calluna* heath described earlier, N addition was combined with burning and grazing (clipping) management treatments. Burning had a large effect on vegetation diversity and composition, but both were quick to recover. N deposition 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⁻¹treatment (Britton and Fisher, 2007a; 2008). Clipping had no effect on vegetation diversity.

Previously, the critical load for Arctic, alpine and subalpine scrubs had been set at 5 to 15 kg ha⁻¹ yr⁻¹ (Bobbink *et al.*, 2003). Data from the 5-year fertilisation experiment of Britton and Fisher (2007a; 2008) further support this critical load, which was set on the basis of expert judgement. Therefore, we propose to set the critical load at 10 to 15 kg ha⁻¹ yr⁻¹ for Arctic, alpine and subalpine scrub habitats (F2), this time classifying it as 'quite reliable'.

8.3 Temperate shrub habitats: wet and dry heaths (F4.1 and F4.2)

As discussed in Section 8.1, both wet and dry heathlands have been placed within EUNIS class F4 (temperate shrub heathlands), because they occur in the Atlantic climate region and are dominanted by ericoid shrubs. This EUNIS class has been divided into subcategories of wet heaths (F4.1), which are damp and characterised by peat soils, and dry heaths (F4.2). Both upland *Calluna* moorlands and lowland wet heaths dominated by *Erica tetralix* fall within the category of 'northern' wet heaths (F4.11). However, since these communities are clearly ecologically different and have been assigned with different critical loads, it is important that this habitat distinction is retained. Since there is no altitude-based cut-off which can be recommended to distinguish the two habitats, the primary criterion must be that of species dominance. Therefore, national experts will need to provide advice on how to map the two habitats.

8.3.1 'U' Calluna-dominated wet heath (upland moorland) (F4.11)

Bobbink et al. (1996) have suggested that the Calluna vulgaris heaths of the upland areas of Britain and other mountainous parts of Europe (F4.11) are likely to be sensitive to N deposition. These acidic communities are characterised by a dominance of dwarf shrubs (in particular Calluna vulgaris), a high abundance of bryophyte species and peaty soils. Furthermore, abiotic conditions are colder and wetter than in lowland heathlands (F4.2). The effects of N deposition on upland Calluna vulgaris heaths have been studied in the United Kingdom using both field surveys and experiments. The critical load range of 10 to 20 kg N ha⁻¹ yr⁻¹ that was recommended by Bobbink et al. (1996; 2003) for this community, was based on three types of evidence from these UK studies: a) effects on growth and species composition, b) effects on shoot nutrient content, and c) effects on soils and root characteristics.

Since the last update, the number of new relevant studies has been limited. Most 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.

(a) Effects on growth and species composition

The longest running experiment of N manipulation in Calluna vulgaris moorland is that in North Wales, where experimental plots were established in May 1989 on an area of moorland at an altitude of 470 metres (Caporn et al., 1994). This site has been estimated to receive an atmospheric N deposition of approximately 20 kg N ha⁻¹ yr¹, although more recent assessments suggest that the deposition could be somewhat higher. To date, additions of N in the form of ammonium nitrate, at doses of 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 period from 1989 to 1993 was characterised by apparently beneficial effects of N on Calluna vulgaris in terms of improvements in 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, in particular, data showed no effect at all of N on shoot extension (Carroll *et al.*, 1999). One interpretation of the *Calluna vulgaris* growth responses in this experiment is that additions of N have accelerated the natural *Calluna vulgaris* cycle, with earlier ageing and opening of the canopy in the plots that had received the highest doses. To date, this is the only moorland study of such a duration and the results clearly demonstrate the need for caution in interpreting growth increases commonly reported in response to N additions to *Calluna vulgaris* over periods of up to 5 years.

One factor causing increased canopy opening may be greater winter damage 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 the autumn (Caporn *et al.*, 1994). However, field surveys in 1996 and 1998 clearly showed large increases in 'winter browning' of heather shoots, most notably following additions of 80 and 120 kg N ha⁻¹ yr⁻¹ (Figure 8.5) (Carroll *et al.*, 1999; Lee *et al.*, 2000). However, this damage mechanism may have been the result of low temperature desiccation, rather than frost injury.

In the earlier years, both bryophytes and lichens had disappeared from below the Calluna vulgaris canopy following all N addition treatments, although Vaccinium myrtillus maintained its cover (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 architecture and increased litter production resulting in reduced light penetration. A later survey, following ten years of treatment, showed not only that bryophytes had returned to the N treated plots, but also that 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). However, no such response was found in lichen species. These results highlight the need to establish more precisely whether elevated N deposition would have a direct and/or indirect impact on bryophyte and lichen populations, especially as it has been suggested that N deposition is partly responsible for the decline in certain mosses and lichen species in upland Calluna vulgaris heath, over the past decades (Pitcairn et al., 1995).

Figure 8.5 Effects of 7 years of ammonium nitrate additions (kg N ha⁻¹ yr⁻¹) on the frequency (means ± SE) of winter damaged *Calluna vulgaris* shoots in northern wet heath (F4.11) in North Wales (Carroll *et al.*, 1999). Columns sharing a letter are statistically not significantly different.



Pilkington *et al.*, (2007a) treated experimental plots in a mature upland *Calluna* heath with factorial combinations of N (o and 20 kg N ha⁻¹ yr⁻¹) and P (o and 5 kg ha⁻¹ yr⁻¹). They found that lichen cover had virtually disappeared within 4 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 8.6).

Destructive harvesting of the above-mentioned site took place in March 2000, prior to a controlled burn. Subsequent regrowth, primarily from stem bases, was significantly lower in the plots with higher N addition treatments, which may reflect the more mature status of *Calluna vulgaris* in the plots with with these higher N additions (Lee and Caporn, 2001). Pilkington *et al.* (2005a) 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 kg N ha⁻¹ 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.*, 2005a).

In a number of areas in the United Kingdom, losses of *Calluna vulgaris* moorland occurred as a result of invasion by bracken. Werkman and Callaghan (1996) undertook a three-year study in the northern Pennines (estimated atmospheric deposition of 15 kg N ha⁻¹ yr⁻¹) to examine the effects of N additions (50 kg N ha⁻¹ yr⁻¹) to a series of plots in either heather or bracken-dominated areas, or at the heather-bracken interface. There was little effect on stands consisting entirely of heather or bracken, but at the heather-bracken boundary there was a trend of enhanced bracken growth and poorer heather growth. Anderson and Hetherington (1999) showed that decomposition rates

Figure 8.6 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 kg N ha⁻¹ yr¹). N0 = 0 kg N ha⁻¹ yr¹, N1 = 20 kg N ha⁻¹ yr¹, P0 = 0 kg P ha⁻¹ yr¹ and P1 = 5 kg P ha⁻¹ yr¹. The dotted line in figure (a) indicates a separate N addition of 10 kg N ha⁻¹ yr¹. Source: Pilkington *et al.* (2007a).



were faster in mixed litters than in litter of either of the two species alone, and also increased to a greater extent in response to N addition. These results, however, are in contrast to the findings by Gordon et al. (1999), who transplanted turf taken from a site in Scotland to experimental plots and added 50 kg N ha⁻¹ yr⁻¹ over three years, in addition to a number of other treatments. This level of N addition increased the shoot length of heather from the first year of treatment onwards, and also increased shoot growth in spring, implying earlier bud break. In contrast, the response in bracken was slow, showing no aboveground growth stimulation until the third year of treatment, when there was a small, but significant, increase in canopy height. Furthermore, factors such as drought and temperature may play an important role, modifying responses to N. For example, the faster and larger shoot growth stimulation of heather, due to at least 3 years of N additions, may be advantageous when ample water is available, but may be clearly disadvantageous when drought occurs (Gordon et al., 1999).

(b) Effects on shoot nutrient content

Several studies have shown that N content in moorland *Calluna vulgaris* and bryophytes was significantly higher in areas with higher N inputs (> 10-15 kg N ha⁻¹ yr⁻¹), and increased in both heather and mosses, compared with measurements of historical plant material (Pitcairn *et al.*, 1995). The increase in N content of *Calluna vulgaris* following N deposition has also been clearly demonstrated in experimental studies with a range of N treatments (Leith *et al.*, 2001; Carroll *et al.*, 1999). In other studies, increases in N content 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 nutrient content 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). A significant, but smaller absolute effect was apparent in the 2000 harvest following the two highest treatments (Pilkington et al., 2005a). Interestingly, 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, 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 induction of P limitation might explain the loss of 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). However, it would be rather inappropriate to use such a narrow threshold, and confidence in the nature of the limitation could only be reached at ratios of below 10 and above 20 (Güsewell, 2004).

This observation is consistent with the study by Kirkham (2001), who sampled Calluna vulgaris shoots from a number of sites in England and Wales, and analysed them for N and P content. They found a significant positive correlation between NO, but not total N, deposition and N:P ratio. The Calluna vulgaris N:P ratios 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 United Kingdom from N-limited ecosystems into P-limited ones. Kirkham (2001) suggested that this could favour species, such as Molinia, which are better adapted to P limitation, although this would not necessarily be the case if increased N deposition also increased mineralisation rates. The relationship between deposition and N:P ratio could be used for estimating a critical deposition threshold for this switch in nutrient limitation. However, the R² value for the relationship between NO, deposition and N:P ratio as reported by Kirkham (2001) was only 0.1, and analysis of other UK data sets, such as that from the Countryside Survey 2000, did not show the expected increase in N:P ratio with increasing N depositions. Furthermore, data from a national survey of Calluna vulgaris in the United Kingdom showed a negative relationship between N deposition and N:P ratio, suggesting that an improved N supply may stimulate P uptake (Rowe et al., 2008).

(c) Effects on soils 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. However, 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 contrast, 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. The reasons for the differences in findings between these two studies are unclear and could be related either to methodological differences or to site-specific circumstances.

Soil studies at the North Wales site 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., 2005a). Significant increases in weight and total N content of Calluna vulgaris litter that was collected from beneath the canopy, were observed following additions of 80 and 120 kg N ha-1 yr-1, but increases in litter production were also observed at N additions of as low as $40 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Carroll *et al.*, 1999). There was also evidence of increased mineralisation and decreased C:N ratio in litter and rhizosphere peat with increasing N treatments, although the peat effect was not found below 2 centimetres in the soil (Lee and Caporn, 2001). 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. Litter C:N ratios remained above the threshold that was predicted to increase leaching of N in forests (Pilkington et al., 2005b). The maintenance of high C:N ratios with negligible rates of net nitrification was associated with the common presence of ericaceous litter and a mor humus layer in moorland (Pilkington et al., 2005c).

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) during 1 year. The purpose of the study was to determine the fate of ¹⁵N-labelled N inputs over a period of 1 year of 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 wider 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

(d) N deposition and management measures

These upland *Calluna vulgaris* heaths are actively managed, and this may be an important factor in their response to N. Increased frequencies of burning might compensate for the effects of acceleration of the *Calluna vulgaris* cycle in response to increased N deposition, as demonstrated by Carroll *et al.* (1999). Grazing pressure is also a significant factor. For example, Alonso *et al.* (2001) described experiments of the interacting effects of nutrient and grazing regimes on competition between *Calluna vulgaris* and two contrasting grass species on moorland sites in Scotland. Although these experiments involved application of NPK, and hence cannot be used for estimating critical loads of N, their broader implications are important. Only where grazing levels are high and gaps are formed in the *Calluna vulgaris* canopy, the unpalatable and shade intolerant *Nardus stricta* is likely to replace *Calluna vulgaris*. In contrast, the more shade tolerant and more palatable *Deschampsia flexuosa* is more likely to compete effectively with *Calluna vulgaris* at low grazing pressures. In general terms, active management of *Calluna vulgaris* moorlands may reduce the impact of increased N deposition and be associated with higher critical loads.

In the United Kingdom, 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. N additions magnified the effect of burning on leaching losses but lessened this effect on the N pools in the mineral layer. Pilkington et al. (2007b) concluded that burning approximately every 10 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 the Scottish uplands, the effects were studied of 6 years of nutrient additions (N, P and K) and protection from grazing on moorland plant and hemipteran communities (Hartley et al. 2003; Hartley and Mitchell, 2005). Grazing in combination with N addition (75 kg N ha⁻¹ yr⁻¹) caused the greatest decline in Calluna cover, typically by 40 to 50%, but N addition did not cause a significant decline in Calluna on plots protected from grazing. N addition allowed grasses to increase in cover, especially on grazed plots. However, Nardus stricta, Festuca ovina and Agrostis species all declined within fenced areas, but increased on grazed plots, whereas Deschampsia flexuosa and Festuca rubra increased on fenced plots. The impact of N addition on the cover of Calluna and on competing grass species critically depends on the level of grazing (Hartley and Mitchell, 2005). These changes in vegetation also significantly affect abundance, species richness and species composition of moorland invertebrates (Hartley et al., 2003).

Summary

Since the last update, the number of experiments with N addition within the range of the proposed critical load has been limited. Important new data have become available from Pilkington *et al.* (2007a), who found that lichen cover virtually disappeared within 4 years in plots receiving a total N input of approximately 26 kg N ha⁻¹ yr⁻¹. It is recommended that the critical load range of 10 to 20 kg ha⁻¹ yr⁻¹, as proposed by Bobbink *et al.* (1996; 2003), is

retained for northern wet heath dominated by Calluna vulgaris (F4.11), a range that is now considered to be 'quite reliable'. This is partly because there is no clear justification for a higher critical load other than for dry heaths (for which there is a reliable estimate), since the dominant species, the indicators of exceedance and the modifying factors are all comparable. Furthermore, the range is mostly based on extrapolation, as the available experimental data are based on total N inputs (background plus treatments) that are significantly above the range of the proposed critical load. This critical load 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.

8.3.2 'L' Erica tetralix-dominated wet heath (lowland) (F4.11)

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 (F4.11). The lowland wet heathland communities are generally richer in plant species than dry heaths. In recent decades a drastic change in species composition has been observed in Dutch wet heathlands. Nowadays, many wet heathlands, which were originally dominated by Erica tetralix, have become monospecific stands of the grass Molinia caerulea. Alongside Erica tetralix, almost all of the rare plant species have disappeared from the system. It has been hypothesised that this change was caused by atmospheric N eutrophication. Competition experiments using wet heath turfs have clearly shown that Molinia caerulea is a better competitor than Erica tetralix at high N availability. Following two years of applications of N (150 kg N ha⁻¹ yr⁻¹), the relative competitive strength of Molinia caerulea compared with Erica tetralix had doubled (Berendse and Aerts, 1984). A three-year field experiment with N applications in Dutch lowland wet heathland (ca 160 kg N ha-1 yr-1) also indicated that Molinia caerulea was able to outcompete Erica tetralix at high N availabilities (Aerts and Berendse, 1988). In contrast to the competitive relations between Calluna vulgaris and grass species, Molinia caerulea may outcompete Erica tetralix without opening of the dwarf-shrub canopy. This difference is caused by the lower canopy of Erica tetralix (25-35 cm), compared with that of Calluna vulgaris, and the tall growth of Molinia caerulea, which can overgrow and overshadow Erica tetralix if enough N is available. An added important condition, in this respect, is that heather beetle plagues do not occur in these wet heathlands, and until now no frost damage has been observed in this community.

Furthermore, it has been demonstrated that in many Dutch wet heathlands the accumulation of litter and humus has led to increased N mineralisation (100-130 kg N ha⁻¹ yr⁻¹) (Berendse *et al.*, 1987). In the first 10 years following sod removal, the annual N mineralisation is very low, but afterwards it increases strongly to the abovementioned high figures. This accumulation of N may be strongly influenced by increased deposition, as soil N leaching from wet heathlands is extremely low (Berendse, 1990). The observed N availabilities are high enough to change *Erica tetralix*-dominated wet heathlands into mono-specific stands of *Molinia caerulea*.

Berendse (1988) developed a wet heathland model to simulate carbon and N dynamics during secondary succession. In this model, he incorporated the competitive relationship between Erica tetralix and Molinia caerulea, the litter production from both species, soil N accumulation and mineralisation, leaching, atmospheric N deposition and sheep grazing. He simulated the development of lowland wet heathland following sod removal, as almost all of the Dutch communities are already strongly dominated by Molinia caerulea, and changes in this situation are extremely unlikely without drastic management. Using the biomass of Molinia caerulea compared to that of Erica tetralix as an indicator, his results suggested 17 to 22 kg N ha-1 yr-1 as the critical load range for the transition of lowland wet heath towards a grass-dominated sward. This critical load range was also the value recommended by Bobbink et al. (1996).

No new evidence has been published since 1995, with respect to the critical load for Erica tetralix-dominated wet heaths. Because of the lack of natural variation in the modelling approach, the previous range of 17 to 22 kg N ha⁻¹ yr⁻¹ was considered too narrow. In particular, the model by Berendse (1990) had been based on a management regime of sod (turf) cutting every 50 years, in combination with grazing. Allchin et al. (2001) applied a similar model to UK dry heaths, and found that their typical management regimes, such as mowing every 15 years, gave a threshold for changes in species composition which was about 10 kg N ha-1 yr-1 lower than for sod cutting. A similar effect of management regimes is to be expected for wet heaths. Thus, while the upper end of the critical load range may remain comparable to that proposed by Bobbink et al. (1996; 2003), based on the model with sod cutting, the lower end of the critical load range should be reduced to account for the effects of N under less intensive management regimes. There is probably no clear evidence of a differential response in Calluna vulgaris and Erica tetralix to simulated N depositions, and Smart et al. (2004) found comparable spatial relationships between change in cover and N deposition in the United Kingdom for the two species. Therefore, it is

recommended that the lower end of the critical load range should be 10 kg N ha⁻¹ yr⁻¹, the same as that for *Calluna*-dominated wet and dry heaths.

Previously, the critical load for this system was based principally on output from a simulation (empirical) model (Berendse *et al.*, 1998). However, based on an overlap in species and habitat characteristics with both upland wet heaths (species composition, including N-sensitive bryophytes) and lowland dry heaths (oligotrophic mineral soils) the upper end of the critical load has been reduced, to make it equivalent with that for other heathland (F4.11 (upland) and 4.2) habitats. The revised critical load is thus proposed as 10-20 kg N ha⁻¹ yr⁻¹, based on expert judgment.

8.3.3 Dry heaths (F4.2) (mostly sub-Atlantic *Calluna-Genista* heaths (F4.22))

(a) Introduction

Despite the conservation and management efforts in nature reserves, many lowland heaths (F4.2) in western Europe have become dominated by grass species. For instance, an evaluation, using aerial photographs, has demonstrated that more than 35% of Dutch heaths have developed into grasslands, during the 1980s (Van Kootwijk and Van der Voet, 1989). It has been suggested that a strong increase in atmospheric N deposition might have been a significant factor in the observed transition towards grassland. Similar, although not completely consistent patterns were found in the United Kingdom over the past 20 to 50 years. Pitcairn et al. (1991) assessed changes in abundance of Calluna vulgaris at three heathland areas in East Anglia over the past decades. All three heaths showed a decline in Calluna vulgaris and an increase in grasses; the authors concluded that increased N deposition (up to ca 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, too. A wider assessment of heathlands in eastern England showed that in some cases Calluna vulgaris had declined and subsequently been invaded 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 sometimes been linked with the steadily increasing levels of N deposition over the past 30 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.). Unpublished Norwegian studies have indicated that Calluna vulgarisdominated heaths were invaded by grasses after the death of Calluna vulgaris, which seems to correlate with frost injury and plagues of heather beetle, a species recently

found in south-western Norway and which is expanding its range (Hansen, 1991; Fremstad, 1992).

Despite this amount of circumstantial evidence and indications, competition experiments in the Netherlands have shown a significant effect of N addition for *Calluna vulgaris* and grass species only in young (low stature and cover) heaths (Heil and Diemont, 1983; Roelofs, 1986; Heil and Bruggink, 1987; Aerts *et al.*, 1990). Combinations of mesocosm, field and modelling studies across Europe over the last decade have shown that the observed changes can only be explained by increased N deposition as part of a complicated and interacting sequence of events, at different time scales, rather than by a simple change in competitive strength.

(b) 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 (Figure 1.1). Many studies indeed have shown increased plant 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). This clearly 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 Surrey (UK). The experiment was set up in 1989 to assess long-term impacts of realistic N loads on lowland dry heaths (F4.22) 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, atmospheric deposition 8 kg N ha⁻¹ yr⁻¹; Power and Barker, 2003) no negative effects were observed for Calluna vulgaris. Indeed, a significant stimulation of flower production, shoot density, and litter production occurred (15.4 kg N ha⁻¹ yr ⁻¹), and, after 6 to 7 years, the canopy in cases of the highest N treatment was 50% taller 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. N 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, personal communication). In 1998, a parallel experiment was set up at the same site to investigate interactions between N deposition and different forms of habitat management. This experiment involved 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 at this site, 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 system (Barker, 2001, Jones, 2009). Collectively, these experiments demonstrated continued N limitation of this lowland heathland under prolonged N inputs and, given negligible leaching rates even after many years of addition, the experiments also revealed the high immobilisation capacity of the system.

Similar growth stimulation of Calluna vulgaris has been observed in a dry lowland heath in the United Kingdom (Cheshire) and the Netherlands. 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 clearly 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 ca 20 centimetres (Cawley, 2001; Lee and Caporn, 2001). Several nutrient addition experiments in the Netherlands have shown N-limited plant growth (see Bobbink et al. (1992a) for an overview). A survey of UK lowland heathlands (Jones, 2009, Jones and Power, submitted), carried out in 2007, revealed significant relationships between N deposition and both N and P concentrations in Calluna foliage. Interestingly, although foliar N:P ratios were related to N deposition, the relationship was not linear.

c) Nitrogen accumulation and mineralisation

During secondary dry heath succession, an increase was observed in the amount of organic material and N in the soil (Chapman et al., 1975; Gimingham et al., 1979). The accumulation of organic matter and N was quantified, after sod removal, 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. Regression analysis suggested an annual increase in N in the system of ca 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). The build up of organic matter in the soil, following sod removal in which almost all of the soil surface organic matter was removed, was likely to have been accelerated by the enhanced biomass and litter production of the dwarf-shrubs caused by the extra N inputs. Furthermore, N accumulation was accelerated. In 1996, 7 years after the start of the experiment in Surrey (UK), Power et al. (1998a) determined the N budget of the control and N enriched plots, using destructive harvesting. Approximately 15 and 24% of the added N had accumulated in plant material on the plots with low and high N treatments, respectively, while another 10 and 14% of the added N was found in the litter layer.

Almost no N was lost from these systems, as ammonium immobilisation in the soil was high, and hardly any leaching losses to deeper layers were measured in Dutch, British or Danish dry non-coastal heaths (De Boer, 1989; Van der Maas, 1990; Power et al., 1998a; Kristensen and McCarty, 1999; Kristensen, 2001; Nielsen et al., 2000). The N cycling in dry heaths is thus very tight, with hardly any N leaching to deeper soil layers, even following high N inputs, as has also been observed in N manipulation studies in upland Calluna vulgaris moorland (Subsection 8.3.1; F4.11). 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 7 years of 15.4 kg N ha⁻¹ yr ⁻¹ addition applied to Surrey dry heath, while N leaching remained very low (Power et al., 1998a/b; Barker, 2001). Significant relationships between N deposition and concentrations of extractable N and total soil N (in addition to foliar N) were also found in a United Kingdom lowland heath survey (Jones, 2009, Jones and Power, submitted), providing field-based evidence of an accumulation of N in lowland heathlands in response to N inputs. At the Cheshire 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. Only after severe damage to the Calluna vulgaris canopy, caused by heather beetles, significant N leaching to the subsoil was 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 the rates of decomposition and soil N mineralisation. Power et al., (1998a) found that adding only 15.4 kg N ha⁻¹ 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 kg N ha⁻¹ yr⁻¹), and to 6.1 years in plots with high additions of N (15.4 kg N ha⁻¹ yr⁻¹) (Power et al., 1998a). In the Netherlands, during secondary succession after sod cutting, following additions of high loads of N, Berendse (1990) found that soil N mineralisation was low during the first 10 years (ca 10 kg N ha⁻¹ yr⁻¹), but strongly increased over the next 20 years to 50 to 110 kg N ha-1 yr-1 with increased N content in the soil. This suggests a threshold for the amount of N immobilisation in the system, above which net N mineralisation occurs. Thus, apart from direct N inputs from the atmosphere, soil N availability will also finally increase because of the almost complete retention of extra N in the system. Eventually, this will alleviate the N limitation to plant production and may lead to a shift to more nitrophytic species. The strong increase in N availability after an accumulation period of 1 to 2 decades may affect the competitive interactions within the systems, not only for (potential) dominant species, but also for subordinate

vascular species. For lichens and mosses, direct effects might also be of importance. A study on the rate of recovery of the Surrey (UK) heathland following cessation of N additions after a 7-year period of elevated inputs (7.7 or 15.4 kg ha⁻¹ yr⁻¹) indicated that the effects of even relatively small additions would persist for many years, even after N deposition inputs are substantially reduced. Power *et al.* (2006) demonstrated effects of earlier N loading on canopy height, density and flowering of *Calluna*, more than 7 years after a reduction in N inputs. This highlights the slow rate of recovery of lowland heathland from the effects of eutrophication and thus the sensitivity of the system to even very small increases in atmospheric N deposition.

(d) 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 kg N ha⁻¹ yr⁻¹) over 4 years during the 1980s (with additional atmospheric deposition of ca 25-35 N ha⁻¹ yr⁻¹), grasses (*Festuca ovina*) strongly outcompeted *Calluna vulgaris*, if the total cover of the vegetation was still low at the start of the experiment (e.g., Heil and Diemont, 1983). However, if its canopy remains closed, *Calluna vulgaris* clearly is a better competitor than grass species in mature heath vegetation, even at high N loads (Aerts *et al.*, 1990; Aerts, 1993).

Understory species, especially the typical and frequently occurring lichen and moss species, can be negatively affected by increased growth of vascular species. It has been found that, after 7 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 kg N ha⁻¹ yr⁻¹ (atmospheric deposition 8 kg N ha⁻¹ yr⁻¹) (Barker, 2001; Figure 8.7). 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 nitrophytic moss, was also considerably lower following 4 or 5 years of N additions (60 and 120 kg N ha $^{\!\!\!^{-1}}$ yr^{-1}) (Lee and Caporn, 2001).

When the productivity of *Calluna vulgaris* would no longer be primarily limited by N, the growth response would be 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 (atmospheric deposition of 18 kg N ha⁻¹ yr⁻¹), N additions of 15, 35 and 70 **Figure 8.7** Lichen cover (%; mean + SE) following 7 years of N additions applied to dry heath (F4.2) at plots in Thursley, Surrey (UK) with low (7.7 kg N ha⁻¹ yr⁻¹) and high (15.4 kg N ha⁻¹ yr⁻¹) additions of N (redrawn from Barker, 2001). Different letters indicate significant differences between the treatments.



kg N ha⁻¹ yr⁻¹ in the form of ammonium nitrate increased N concentrations in shoots, significantly, within 2 years of the start of the experiment (Johansson, 2000), while there was no significant growth response in the dwarf shrubs (Riis-Nielsen, 1997). Correlative field studies in areas with low and high N inputs, and experimental applications of N to heathlands, showed that high N inputs caused increased concentrations of N in the (green) parts of Calluna vulgaris (e.g., Heil and Bruggink, 1987; Bobbink and Heil, 1993; Pitcairn et al., 1995, Jones and Power, submitted). In forests, a clear relation has been established between increased foliar N concentrations and pathogenic attacks as well as sensitivity secondary stresses (see Chapter 9). Similarly, experiments have at least partly indicated an increased risk to canopy damage for *Calluna vulgaris* under increased N availability. Heather beetle infestations and N-induced secondary stresses (especially winter injury and drought) are probably the main processes, which may open the canopy, and are thus crucial in the dramatic shift in species composition observed in lowland heath in regions with high N loads.

(e) Opening of the Calluna vulgaris canopy: heather beetle infestations

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). 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 or Molinia caerulea. In a rearing experiment, Brunsting and Heil (1985) found that the growth of the beetle larvae increased after foraging on leaves of Calluna vulgaris that had higher N concentrations. Following field additions of ammonium sulphate in a heath vegetation under an experimental roof, the total number or biomass of the first-stage larvae of the beetle was not affected by the N treatments, but the development of subsequent larval stages was significantly accelerated (Van der Eerden et al., 1990). Larval growth rates and adult weights of heather beetles were found to be significantly higher when these insects were reared on Calluna vulgaris plants that had been collected in the British lowland heath experiment (Surrey, UK) after seven years of relatively low N additions (Power et al., 1998b).

Heather beetle larvae were also cultivated on shoots of Calluna vulgaris taken from plants that had been fumigated with ammonia in open top chambers (12 months; 4-105 μ g m⁻³) (Van der Eerden *et al.*, 1991). After 7 days, both the mass and development rate of the larvae had clearly increased at higher ammonia concentrations. In addition, the growth of heather beetles' instars was also significantly stimulated on Calluna vulgaris from UK heaths after ammonia gas fumigation (with relatively high concentrations), probably driven by the enhanced N concentrations in the plant material (Uren 1992). Furthermore, it has also been demonstrated that increased N deposition to upland Calluna vulgaris stands stimulated larval development and growth rates of winter moths (Operophtera brumata) (Kerslake et al., 1998). 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 8 years. In addition, it was observed that Calluna vulgaris plants were 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 United Kingdom (Lee and Caporn, 2001). It is thus likely that enhanced atmospheric N deposition influences the frequency and severity of beetle infestations, although the exact controlling processes needs further quantification.

(f) Opening of the Calluna vulgaris canopy: secondary stresses It has been shown that, in some tree species, frost sensitivity increased at increasing concentrations of air pollutants (e.g., Aronsson, 1980; Dueck *et al.*, 1991). This increased susceptibility is sometimes correlated with enhanced N concentrations in the leaves or needles. Impacts of N deposition on the frost sensitivity of *Calluna vulgaris* is also possible, and it is suggested that the observed die-back of *Calluna vulgaris* shoots in successive severe winters of the mid-1980s in the Netherlands was, at least partly, caused by increased winter injury.

Van der Eerden et al. (1990) studied the effects of ammonium sulphate and ammonia on frost sensitivity in Calluna vulgaris. Fumigation of Calluna vulgaris plants with ammonia in open-top chambers during periods of 4 to 7 months (100 µg m⁻³) revealed that frost sensitivity was not affected by NH_{_} in autumn (September to November), but in February, just before growth started, frost injury increased significantly at -12 °C (Van der Eerden et al., 1991). A similar greenhouse study during the winter period in England also demonstrated increased frost sensitivity of heather plants, and although experimental, concentrations of ammonia were very high (140-280 μg m⁻³) (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 5 months the frost sensitivity of Calluna vulgaris had increased slightly, although 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. The sensitivity to frost decreased again two months later, and no significant effects of the ammonium sulphate application were measured. All these studies used high levels of N deposition. However, the only study on 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.

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. This would suggest that N enrichment increases the sensitivity of Calluna vulgaris to periods of drought, probably because of reduced root growth which affects the development of shoots, or as a result of a decrease in mycorrhizal infection. These effects might have major implications for the capacity of plants to deal with water or nutrient stresses. Similar effects of N addition (30 kg ha⁻¹ yr⁻¹) on the extent of drought damage to Calluna shoots have also been reported in the experiment in Surrey (UK), following the particularly dry summer of 2003 (Green, 2005) The ratio between root and shoot biomass is flexible and mostly determined by nutrient availability and light intensity (e.g., Brouwer, 1962; 1983). It has been shown that most of the plant species studied developed more biomass at their shoots than at their roots at higher nutrient concentrations (e.g., Poorter and Nagel, 2000). This phenomenon

also held for Calluna vulgaris and other heathland species in a pot experiment that was conducted over a two-year period. The root weight ratio (RWR) of Calluna significantly decreased with increasing N additions (Aerts et al., 1991). However, field validations of these lower root to shoot ratios as a response to N enrichment are scarce. An indication of the importance was observed after long-term N applications on the dry lowland heathland in Surry (UK) (Power et al., 1998a). Here, a small reduction in root to shoot ratio was found after seven years of N additions (15.4 kg N ha⁻¹ yr⁻¹). Van der Eerden et al. (1991) applied artificial rain containing ammonium sulphate (3, 6,11, 23, 45 and 91 kg N ha⁻¹ yr⁻¹) during two growing seasons to a dry heath (Assel, the Netherlands), under a partial roof. Measurements of plant characteristics demonstrated decreases in the root:shoot ratios in both Calluna vulgaris and Deschampsia flexuosa. Power et al. (1998b) also found higher water losses for Calluna vulgaris plants from the N-treated vegetation, compared with the control situation, but no differences in water potential of the shoots. Hence, the hypothesis that increased N deposition might lead to severe growth reduction or even to local 'browning' and die-back of this species, in cases of severe drought episodes, is still mostly speculative. A summer drought period (May-September) was experimentally imposed on roofed split plots, in the second year of the study on N-enriched lowland heath plots in Cheshire (UK) (Cawley et al., 1998; Lee and Caporn, 2001). The high N treatments (60 and 120 kg N ha⁻¹ yr⁻¹) clearly increased the vulnerability of Calluna vulgaris to the imposed drought stress. The cover of Deschampsia flexuosa significantly increased on these water-stressed plots, but this was partly attributable to a heather beetle infestation, which was more severe in the plots exposed to experimental drought. However, by 2000, the Calluna vulgaris cover had partly recovered from the 1997 drought period. This indicates the potential importance of the interaction between N enrichment and long dry periods (e.g., Green, 2005).

In addition to changes in root to shoot ratios, changes in the level of ericoid mycorrhizal infection of heather roots could also be influenced by an increase in N load. However, few studies have been conducted on this subject and, as with arbuscular mycorrhizal infection, outcomes are highly variable (Aerts and Bobbink, 1999). Some studies on the effects of increased N availability on ericoid mycorrhizal infection in *Calluna vulgaris* roots, showed no effects (Johansson, 2000). In other studies, N inputs were found to stimulate the infection of mycorrhizas (Caporn *et al.*, 1995), or showed restricted infection after N treatment (Yesmin *et al.*, 1996). At this moment the importance of this phenomenon in the decline of *Calluna vulgaris* and the shift to grass dominance is not at all clear. 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 to be clarified under long-term N applications with repeated drought episodes.

(g) Management measures

Barker et al. (2004) and Power et al. (2001) investigated the impact of four different management treatments (low-intensity mowing, high-intensity mowing, management burning and simulated accidental burning) on the response in Calluna-dominated heath to N addition of 30 kg N ha-1 yr-1 at a lowland heath in Surrey (UK). Postmanagement *Calluna* regeneration, growth and canopy development were significantly affected by both the form of management and by N addition. The effect of N on shoot growth was lower, in absolute terms, in those plots that had undergone more intensive management treatments. Power et al. (2001) also reported an effect of habitat management on microbial responses to N, suggesting that the whole ecosystem response to N inputs is dependent on the type and, in particular, intensity of management activities carried out at heathland sites.

In the long term, the need for maintaining a low-nutrient environment to favour *Calluna* dominance, particularly in the face of elevated N deposition, favours the use of management regimes that result in the export of a significant proportion of the organic N stores. However, further study is required to ensure that initial enhancement of seedling invasion by grass and other species, associated with these more intense management regimes, does not outweigh the long-term benefits of associated nutrient removal (Barker *et al.*, 2004). Furthermore, Green (2005) and Jones (2009) did not find any evidence of increased grass cover in the continued N addition in plots in Surrey (UK).

Härdtle et al. (2006; 2007) also evaluated the effectiveness of different management measures in reducing the impact of ambient atmospheric nutrient loads. They compared the effects of mowing, prescribed burning (low-intensity management) and also sod cutting (high-intensity management), on heathland nutrient budgets in the Lüneburg Heath nature reserve (north-west Germany). The quantities of N removed through sod cutting were equivalent to 89 years of atmospheric input. In contrast, the quantities of N removed through mowing and prescribed burning were equivalent to only 5 years of atmospheric input. Output-input ratios for P exceeded those for N in the mowing and sod-cutting experiments. It is therefore likely that heathlands that today are (co-) limited by N will shift to being more P limited, in the long term. Furthermore, the German study showed that low-intensity management cannot compensate for atmospheric N loads, in the long term (Härdtle et al., 2007).

(h) Modelling

Computer models offer a tool to assess the impacts of N deposition on dry heathlands over decades with varying levels of secondary stresses and under different management regimes. Allchin et al. (2001) modified the CALLUNA model originally developed by Heil and Bobbink (1993) for UK lowland heaths, and examined the effects of different management regimes. Under the less-intensive current UK management systems, compared with traditional sod cutting applied in the Netherlands, the critical load for invasions by grass species was reduced to 10 kg N ha⁻¹ yr⁻¹. Terry et al. (2004) studied the effects of management intensity in more detail. The effect of N deposition depended on grazing pressure and the degree of litter removal through mowing, burning or sod cutting. The benefits of policies on reducing N deposition, in terms of restoring heather dominance, may only be realised after several decades, and active site management may be needed to capture the full benefits of such policies.

(i) Mediterranean montane heath

The effects of fertilisation and experimental cutting were intensively studied in three mountain heathlands in Spain (Marcos et al., 2003; Calvo et al., 2005; 2007; Cuesta et al., 2008). Additions of twice the estimated background N deposition (56 kg N ha⁻¹ yr⁻¹) did not significantly alter soil characteristics. N addition led to increased plant N content with higher concentrations of Calluna than Erica. N addition favoured perennial herbaceous species (e.g., Nardus stricta, Festuca rubra and Deschampsia flexuosa). Calvo et al. (2005) concluded that, in the short term, increased nutrients alone, at twice the estimated current atmospheric deposition for the area, would not significantly alter the composition of the mountain heathlands. However, once stands would reach mature phase, 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 will also benefit from the increased nutrients. In the Calluna vulgaris heathlands studied, cutting plus fertilisation allowed an increase in biodiversity, over time. Cutting patches of heathland is recommended as a mechanism for maintaining high vegetation diversity, when grazing is not possible (Calvo et al., 2007).

Summary

The impacts of increased N inputs to dry inland heaths (F4.2) are complex and occur at different time scales. Firstly, increased N availability stimulates biomass and litter production of *Calluna vulgaris* in most situations. N is strongly retained in the system, gradually leading to higher N mineralisation rates in the soil. However, the species remains the stronger competitor with respect to grasses, even at very high N availability, if the canopy is not opened. 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. After decline in the *Calluna vulgaris* shoots, grasses quickly profit from the increased light intensity, together with the high N availability from N accumulation. Within a few years, this may lead to a drastic increase in grass cover. Because of the stochastic behaviour of several processes (e.g., heather beetle infestations, winter injury and drought) and the many long-term processes that interact with them, it is very difficult to clarify all these relationships without results from long-term (10-20 years) and large-scale experiments.

The critical load range for dry inland heaths has been set at 10 to 20 kg N ha⁻¹ yr⁻¹ (Bobbink et al., 2003). This range, based primarily on a long-term field experiment in Surrey (UK), is 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 empirical critical load. Therefore, we suggest the critical load range for dry heaths be maintained at 10-20 N kg ha-1 yr⁻¹, classified as 'reliable'. It should be stated that most N-addition studies have been conducted on a subcategory of dry heaths and sub-Atlantic Calluna-Genista heaths (F4.22), but it seems reasonable for this critical load to be applied to all habitats in the F4.2 category. However, it is advisable to apply the low end of the range to lichen-rich dry heaths, as shown by the study in Surrey (UK). The relative importance of P availability in some dry heath areas and habitat management as modifiers of dry heath response to increased N deposition, has only partly been investigated.

8.4 Maquis, arborescent, matorral and thermo-Mediterranean brushes (F5)

Maquis, arborescent, matorral and thermo-Mediterranean brushes (F5; in short Mediterranean scrub) are important habitats (in terms of diversity and cover area) in Mediterranean areas of Europe. The first N manipulation field study on these habitats, however, was started not until 2007, in southern Portugal, investigating the effects of N doses (40 and 80 kg N ha⁻¹ yr⁻¹) and forms (ammonium and ammonium nitrate) on maquis vegetation at the Natura 2000 Arrábida/Espichel site. N additions were homogenously applied, in three equal applications throughout the year: in spring, summer and between autumn and winter. Estimated EMEP background deposition was 5.2 kg N ha⁻¹ yr⁻¹ (http://webdab.emep.int).

In contrast to most N-addition studies (see Bobbink et al.,

2010 for worldwide review) the first results suggested that one year of N enrichment had already influenced the soil bacterial community, AMF sporal community and plant composition (Diaz *et al.*, submitted). Changes in plant community occurred through changes in species composition and cover, and were related to soil N availability. This increase in diversity caused by such a short period of N addition corresponded with the coexistence of N 'savers' and the appearance of 'spender' species. This coexistence has been reported for epiphytic lichens (Mitchell *et al.*, 2005; Pinho *et al.*, 2008), but has probably not been observed in many studies on northern temperate ecosystems, since these systems may already have had a higher N status before the start of such a study. In spite of limitations of the Diaz *et al.* (submitted) study, data can be considered as a first indication of the possible sensitivity of these Mediterranean scrub communities. Given the fast response, a first estimate of the critical load range, based on 'expert judgement', for Maquis, arborescent matorral and thermo-Mediterranean brushes (F5) has been set at 20 to 30 kg N ha⁻¹ yr⁻¹.

An overview of the empirical critical loads of N for heathland, scrub and tundra habitats (F) is presented in Table 8.1.

Table 8.1 Empirical critical loads of N and effects of exceedances on heathland, scrub and tundra habitats (F). ## reliable, # quite
reliable and (#) expert judgement. Changes with respect to 2003 are indicated in bold.

Ecosystem type	EUNIS code	kg N ha ⁻¹ yr ⁻¹	Reliability	Indication of exceedances
Tundra	F1	3-5ª	#	Changes in biomass, physiological effects, changes in species composition in bryophyte layer, decrease in lichens
Arctic, alpine and subalpine scrub habitats	F2	5-15ª	#	Decline in lichens, bryophytes and evergreen shrubs
Northern wet heath	F4.11			
'U' Calluna-dominated wet heath (upland moorland)	F4.11 ^{a,b}	10-20	#	Decreased heather dominance, decline in lichens and mosses, increased N leaching
'L' Erica tetralix-dominated wet heath (lowland)	F4.11 ^{a,b}	10-20	(#)	Transition from heather to grass dominance
Dry heaths	F4.2 ^{a,b}	10-20	##	Transition from heather to grass dominance, decline in lichens, changes in plant biochemistry, increased sensitivity to abiotic stress
Maquis, arborescent matorral and thermo-	F5	20-30	(#)	Change in plant species richness and community composition

Mediterranean brushes

^{a)} Apply the high end of the range to situations with high levels of precipitation, and the low end of the range to those with low levels of precipitation. Apply the low end of the range to systems with a low water table, and the high end of the range for those with a high water table. Note, that water tables may be modified by management.

^{b)} Apply towards high end of range to situations where sod cutting has been practiced; apply the lower end of the range to those with low-intensity management.

8.5 References

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Chapter 9

Effects of nitrogen deposition on woodland, forest and other wooded land (EUNIS class G)



9 Effects of nitrogen deposition on woodland, forest and other wooded land (EUNIS class G)

9.1 Introduction

The description of class G of the European Nature Information System (EUNIS), for woodlands and forest habitats and other wooded land (Davies *et al.*, 2004) is: Woodland and recently cleared or burnt land where the dominant vegetation is, or was until very recently, trees with a canopy cover of at least 10%. Trees are defined as woody plants, typically single-stemmed, that can reach a height of at least 5 m at maturity unless stunted by poor climate or soil. Includes lines of trees, coppices, regularly tilled tree nurseries, and tree-crop plantations. Includes [Alnus] and [Populus] swamp woodland and riverine [Salix] woodland. Excludes [Corylus avellana] scrub and [Salix] and [Frangula] carrs. Excludes stands of climatically-limited dwarf trees (krummholz) < 3m high, such as occur at the arctic or alpine tree limit. Excludes parkland and dehesa with canopy less than 10%.

According to the EUNIS classification, woodland and forest habitats (EUNIS level 2; G1, G2, G3, and G4) are separated from other wooded habitats (G5), such as lines of trees, small anthropogenic woodlands (< 0.5 ha), recently felled woodlands, early-stage woodlands and coppice. Forests are characterised in EUNIS by the dominant tree types, which may be mixtures of species within the categories *broadleaved deciduous* (G1), *broadleaved evergreen* (G2), *coniferous* (G3), and *mixed broadleaved and coniferous* (G4). The EUNIS classification emphasises the dominant tree species, soil hydrology and management practices, more so than soil chemistry. For more details, see Davies *et al.* (2004) and the EUNIS website.

As in the previous updating procedure, non-forest ecosystems (Chapters 3 to 8) have been classified and ordered 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 *et al.*, 2003). Empirical critical loads of N were set in 2003 for G1 (broadleaved woodland) and G3 (coniferous woodland), with the latter being divided into boreal and temperate types. In cases where empirical critical loads for broadleaved and coniferous woodland were the same, these values were also applied to G4 (mixed deciduous and coniferous woodland).

One of the main aims of this background document has been to achieve a more detailed differentiation (down to level 3) of the empirical critical loads for forest ecosystems (class G). However, this approach has been restricted by the following major constraints:

- Several studies cannot be classified below level 2 of EUNIS because of a lack of original data or combination of different forest types within data sets;
- Lack of data from N addition studies and/or gradient studies on major habitat types, such as all riparian forests, wet forest types and broadleaved evergreen woodland of the Mediterranean region (G2);

• EUNIS classes referring to the dominant tree species often do not represent the natural communities because the tree species composition is of anthropogenic origin. This problem is especially acute in central Europe, where the native deciduous tree cover often has been replaced by conifers.

To address these constraints, the following structure was adopted in this chapter. First, the overall empirical critical loads of N for European forest ecosystems are discussed in Section 9.2. This summarises the main effects of N deposition on habitats in classes G1 and G3 (G4), without specification down to level-3 categories, as in the 2003 document, but with the inclusion of new studies from the 2003-2010 period, and exclusion of studies on the boreal (taiga) forest zone. An evaluation of impacts of N deposition on this boreal forest zone (Taiga woodlands, G3.A and G3.B) is separately presented in Section 9.3, as sufficient experimental data were now available to distinguish effects on this important subtype of coniferous forests in Europe (G3). Furthermore, an additional differentiation between EUNIS types is proposed in Section 9.4, 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, an additional 6 to 8 level-3 categories could be allocated a critical load value. Finally, an overview of the empirical critical loads for class G are summarised in Section 9.5. As before, studies based only on plantation stands or short rotation forestry (e.g., EUNIS categories G1.C, G1.D, G2.8, G2.9, G3.F and G4.F) were excluded, because critical loads of N for these intensively used systems are obtained via the steady-state mass balance method (UNECE Mapping Manual, 2004).

9.2 Effects of N deposition on broadleaved and temperate coniferous forests (G1, G3.1, G3.4, G3.5, G3.7 and G3.6)

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 nutrition and tree growth. This may alter trees' resistance to abiotic and biotic stress factors. Additionally, forest ecosystems may release NO, N₂ and N₂O into the atmosphere, the last of which contributing to global warming and depletion of the ozone layer. Furthermore, an ecosystem approach requires understanding of the interactions between different compartments that lead to an overall reaction of forest ecosystems. In fact, many studies are of a very mechanistic nature and are limited to investigating relationships between only a few factors. Therefore, future research should be focussed on the derivation of more integrative indicators of the total systems dynamics. The final objective is to find threshold levels for N deposition that correspond to qualitative 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:

Acidification, mineralisation, nitrification, leaching, N trace-gas emission, and litter decomposition.

Trees:

Nutrition, physiology, phenology, recruitment and susceptibility to pest and pathogens.

Biodiversity:

Macrofungi and mycorrhiza, lichens and green algae, ground vegetation, and fauna.

9.2.1 Effects on soil processes

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

- a. 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 demonstrate that a large proportion of incoming N (11-56%) is retained in the forest floor through biotic and abiotic processes within the first two years of N enrichment (Tietema *et al.*, 1998; Emmett *et al.*, 1998). An accumulation of ammonium at the ion exchanger may happen in areas with high deposition of reduced N (Roelofs *et al.*, 1985; Van Dijk and Roelofs, 1988; Schulze *et al.*, 1989; Boxman *et al.*, 1991), although ammonium is usually not detectable in soil solution, as evidenced by data from ICP Forests plots (De Vries *et al.*, 2003).
- b. Nitrate leaching. Nitrate that is not taken up by the plants or incorporated into organic matter is leached. Nitrate leaching is an indicator of ecosystem N status, but depends also on the C:N ratio in the humus (Gundersen *et al.*, 1998a, Augustin *et al.*, 2005). 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).
- c. Soil acidification. This occurs due to nitrification of ammonium and leaching of nitrate. This process leads to accelerated leaching of base cations and, in poorly buffered soils, to increased dissolution of aluminium, which can damage fine root development and mycor-

rhiza, and thus reduce nutrient and water uptake (Ulrich, 1983; Ritter, 1990).

d. 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 (Davidson, 1991; Butterbach-Bahl *et al.*, 1997). Uptake of atmospheric CH₄ by forest soils is also catalysed by soil microorganisms (King and Schnell, 1998; Dunfield *et al.*, 1999). 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 (Fenn *et al.*, 1996; Davidson and Kingerlee, 1997; Gasche and Papen, 1999; Butterbach-Bahl *et al.*, 2002; Jenssen *et al.*, 2002).

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 treated in separate guidelines (e.g., Nilsson and Grennfelt, 1988; Sverdrup and Warfvinge, 1993). 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) (all data from Vigdis (2001)).

Nitrogen mineralisation, nitrification, NO_3^{-1} leaching, NH_a^{+} accumulation

N mineralisation and nitrification rates both may be stimulated by N deposition. In a field study on 600

deciduous forests, in four geographically separate regions of southern Sweden, 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 C:N ratio were also found between areas with 7 and 10 kg N ha⁻¹ yr⁻¹ (Figure 9.1). The effect depended on deposition history. NITREX experiments, for example, did not find effects on mineralisation (Emmett, 1999), and McNulty et al. (1996) observed the highest N mineralisation potential in control plots with spruce-fir stands at high elevation, although the net nitrification potential was highest in N fertilised plots (15.7 kg N ha⁻¹ yr⁻¹).

Organic forms of N (i.e. free amino acids) also serve as important plant N sources in boreal forests (Näsholm *et al.*, 1998; Nordin *et al.*, 2001) and N deposition can disrupt the ratio between organic and mineral N supply in such soils.

In acidic forest soils with raw humus cover and C:N-ratios of above ca 25, nitrification plays a minor role. In these soils, N losses are negligible and N is accumulated in all compartments of the ecosystem. NH₄⁺ accumulation in the soil may shift the ratio between NH₄⁺ and base cations (K, Ca or Mg) in the soil with severe consequences for tree nutrition (see Subsection 9.2.2; Van Dijk and Roelofs, 1988; Roelofs *et al.*, 1985; Boxman *et al.*, 1988), although usually little NH₄⁺ can be detected in the soil solution (De Vries *et*

Figure 9.1 N mineralisation, nitrification and C:N ratio in 10 pH classes (top soil) and three regions with varying N depositions. Means ± SE. From Falkengren-Grerup and Diekmann (2003).





al., 2003). Harmful effects of N accumulation on biodiversity are discussed in Subsection 9.2.3. In forest soils with moder- and mull-like humus and C:N ratios of below ca 25, nitrification plays an important role. In these soils, deposited NH⁺ is nitrified in the topsoil and may leach as NO⁻ together with deposited NO⁻ to deeper soil layers. Various studies on deciduous and coniferous stands have demonstrated that if N loads in throughfall exceed 12 to 15 kg ha⁻¹ yr⁻¹, NO₂⁻ leaching increases exponentially (Westling, 1991; Kölling and Neustifter, 1997; Gundersen et al., 1998a; Nilsson *et al.*, 1998). NO⁻ leaching from coniferous stands is higher at sites with a low C:N ratio in the organic layer (Gundersen et al., 1998a; Dise et al., 1998; De Vries et al., 2001; MacDonald et al., 2002; Borken and Matzner, 2004). Below a C:N ratio of 25, the rate of nitrate leaching increases, endangering groundwater quality (MacDonald et al., 2002; Moldan et al., 2006).

Data from ICP Forests (UNECE, 2005) show a neat correlation between N input and N leaching for sites with a C:N ratio of less than 22 (Figure 9.2) and at input rates of more than or equal to 10 kg N ha⁻¹ yr⁻¹. Whether leaching rates are dependent on the type of tree species is not quite clear. From the same data set, 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. The relationship may depend on stand structure, tree age and the proportion of dry deposition in total N load (with coniferous stands having a higher potential of retaining dry deposition), the forest floor (usually with higher C:N ratios in coniferous stands) and the actual growth rate (which may be higher in the coniferous stands than in deciduous ones, if nothing else limits conifer growth). Therefore, different relationships do not necessarily imply a differentiation between critical loads of N for broadleaved and coniferous forests. In addition, not only NO_leaching but also N accumulation in the forest floor has negative effects on the ecosystem.

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 show a strong increase in NO and N₂O emissions, both related with the humus quality (transition from raw humus to moder, or C:N ratio of humus below 25), and with N deposition varying from 10 to 25 kg N ha⁻¹ yr⁻¹ (throughfall deposition). Furthermore, a drastic decrease in atmospheric CH₄ uptake by forest soils was measured with increasing N deposition. **Figure 9.2** N leaching fluxes (kg N ha⁻¹ yr⁻¹) set against N input in throughfall for sites with C:N < 22. (Source: UNECE, 2005).



In summary, the available data on soil processes suggest a critical load range of 10 to 15 kg N ha⁻¹ yr⁻¹ for mineralisation and nitrification ('quite reliable') and for nitrate leaching in both coniferous and broadleaved forests ('reliable').

Litter decomposition, carbon sequestration, DOC-leaching, CO₂-release

The effect of increased N deposition on litter decomposition seems to depend on the lignin concentration of the substrate (Carreiro *et al.*, 2000; Sinsabaugh *et al.*, 2002, Frey *et al.*, 2004, Knorr *et al.*, 2005) 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 of the late decomposition stages are delayed. Consistent with this general hypothesis, Waldrop *et al.* (2004) reported on an inhibition of litter decomposition by N (80 kg N ha⁻¹ yr⁻¹) in an oak stand and a stimulation in a maple stand.

Carreiro *et al.* (2000) found significant effects of experimental NH_4NO_3 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. 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. Knorr *et al.* (2005) reviewed 900 litter-decomposition studies for a meta-analysis of the effects of N additions on litter decay. The litter decay response to N addition ranged from 38% inhibition to 64% stimulation. In detail, decomposition showed a small, but significant inhibition (-5%) in response to N additions < 75 kg N ha⁻¹ yr⁻¹. Litter decomposition was inhibited by N additions at rates of 2

to 20 times the anthropogenic N deposition level, when ambient N deposition was 5 to 10 kg N ha⁻¹ yr⁻¹, or when litter quality was low (typically high lignin litters). Decomposition was stimulated at field sites exposed to low ambient N deposition (< 5 kg N ha⁻¹ yr⁻¹), at low addition rates (< 5 kg N ha⁻¹ yr⁻¹) and for low-lignin litters. In another 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).

Long-term N fertilisation in northern temperate zones has been estimated to enhance C storage by 0.25 Pg C yr¹ (Nadelhoffer et al., 1999). This estimate does not, however, include effects on soil organic matter (SOM) processes such as the stability increase of soil with long-term elevated N deposition (Swanston et al., 2004, Hyvönen et al., 2008). Concomitant decreases in rates of microbial respiration (the release of CO₂) and C mineralisation in forest soil decreases (Sjöberg et al., 2003, Swanston et al., 2004, Bowden et al., 2004) thereby increasing potential for increased C sequestration. Up to now, no consistent DOC leaching response to N addition is documented. Evans et al. (2008) reviewed 17 field N manipulation studies across northern Europe and northeastern United States and found that DOC concentrations depend on the form of N used for manipulation: increases (9 experiments) were documented with NaNO, additions or gaseous NH, exposure, and decreases (8 experiments) with most NH_a additions. However, although northern temperate forests might now function as significant CO₂ sinks, N deposition only accounts for < 20% of the sink and 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).

Microbial soil biomass

Treseder (2008) published a meta-analyis from 82 field studies on the effect of N on soil microbial biomass in a range of different ecosystems. The results suggest that N enrichment could reduce microbial biomass in many ecosystems, with corresponding declines in soil CO₂ emissions. This is in accordance with the results from Frey *et al.* (2004) who found an altered microbial community and a reduced total biomass in the long-term N-addition experiment at Harvard Forest, Massachusetts, United States (N addition rates of 50 and 150 kg N ha⁻¹ yr⁻¹, background deposition 8 kg N ha⁻¹ yr⁻¹).

In summary, the available data on soil processes suggest a critical load of 10 to15 kg N ha $^{\rm 1}$ yr $^{\rm 1}$ for mineralisation and

nitrification (quite reliable), for nitrate leaching in both coniferous and broadleaved forests (reliable). For litter decomposition, the meta-analysis by Knorr *et al.* (2005) suggests that there are effects at quite low N deposition rates, with a proposed critical load of 5 to10 kg N ha⁻¹ yr⁻¹ (expert judgement).

9.2.2 Effects of nitrogen 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 which time increased N inputs no longer stimulate tree growth, but start to disrupt ecosystem structure and function (Agren, 1983; Aber *et al.*, 1989; Tamm, 1991).

Growth of above-ground plant parts

In temperate European regions, forest growth has increased over the past 40 to 50 years (Spiecker et al., 1996). A pan-European research project concluded that increased N deposition has been the main cause of the observed increases in forest growth (EFI, 2002). Braun et al. (2010) showed a significant correlation between stem increment in beech and modelled levels of N deposition in Switzerland. The increment, however, was restricted to plots with sufficient supply of phosphorus (Figure 9.4). An observed decrease in stem increment in beech and Norway spruce between 1984 and 2006 was paralleled by a drop in foliar P concentrations (average N deposition 24 kg N ha⁻¹ yr⁻¹). In southern Norway, an analysis of increment cores from over 31,000 spruce forest plots showed a stem growth increase between 1960 and 1970, followed by a decline between 1980 and 1990. A growth decrease started in plots 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 9.3; Nellemann and Thomsen, 2001). R. Wright (personal communication) estimated dry deposition in southern Norway to add another 10 to 20% to these deposition rates.

This pattern of initial growth stimulation, followed by a subsequent growth decline, was also observed in an N-addition experiment (10, 20, 40, 80 and 160 kg N ha⁻¹ yr⁻¹) in a young beech stand in Switzerland, on calcareous soil with a modelled atmospheric deposition of 15 kg N ha⁻¹ yr¹ (Flückiger and Braun, 2011). Five years after the start of this experiment, shoot growth had significantly increased

following additions of no more than 10 kg N ha⁻¹ yr¹. After 8 years, growth stimulation had continued at additions of 10 kg. However, a significant decrease in growth was observed following an addition of 160 kg N ha⁻¹ yr¹. In the 9th year, growth stimulation no longer occurred, but a significant growth decrease was found at 80 and 160 kg N ha⁻¹ yr¹. On acidic soil, a growth decrease was only observed at additions of 160 kg N ha⁻¹ yr¹ (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 southern Sweden, a positive relationship between N concentration and branch growth of Norway spruce was found in trees younger than 40 years, whereas growth of older trees seemed to be limited by K and/or P (Thelin, 2000). Also, 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 3 years than those on the control plots, but after 10 years their growth fell below that on the control plot (Jönsson et al., 2004b). As, nowadays, most forests in central and western Europe are probably N saturated, increasing limitation by other nutrients, such as phosphorus or base cations, is likely (Flückiger and Braun, 1999b; Braun et al., 2010).

Figure 9.3 Stem increment of spruce in Norway, grouped in relation to the amount of 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).



Figure 9.4 Stem increment in beech, in Switzerland, in relation to N deposition and foliar P concentration. Bars: 95% confidence intervals (Flückiger and Braun, 2011).



Changed growth rates may also affect the mechanical stability of tree stems. Meyer *et al.* (2008) analysed wood properties of broken and uprooted *Picea abies* after the storm 'Lothar' in Switzerland (in 1999), and compared samples to those of standing trees. They observed that broken stems had a greater fraction of sapwood area, lower non-structural carbohydrates and starch concentrations in the sapwood, and a higher N concentration in the phloem. Such changes may be caused by increased N deposition; modelled N deposition in the study area ranged from 15 to 45 kg N ha⁻¹ yr⁻¹.

Growth of Scots pine, beech and oak forests in the north-east of the German Northern Lowland was investigated for the influence of different rates of N deposition (Anders et al., 2002). For all the investigated tree species, accelerated growth was observed at long-lasting (2-3 decades) deposition rates (bulk deposition) exceeding 10 to 15 kg N ha⁻¹ yr⁻¹. This tendency was most pronounced in oligotrophic Scots pine forests (raw humus, C:N ratio below 25). Here, growth acceleration was accompanied by a change in vegetation type, humus form, and a significant decrease in C:N ratio of top soil. Hypertrophic growth was correlated with an increase in N concentration in lastyear's needles. In addition, tree growth became more sensitive to drought episodes or to enhanced temperatures during the vegetation period. These could be signs of beginning destabilisation, which can be assumed to be amplified with frequent weather extremes as are expected with climate change (see section on drought tolerance). In the vicinity of N emission sources with local deposition

rates exceeding 35 kg N ha⁻¹yr⁻¹ during the 1980s and early 1990s, a significant growth reduction (growth depression) was observed in Scots pine forests. This growth depression was accompanied by increased tree mortality. Depressed growth was correlated with significant nutrient disharmonies within needles (in particular Mg and K), a significant shift in productivity from tree layer to ground vegetation, and massive water stress mainly induced by increased evaporation of dominating nitrophilous grasses, such as *Calamagrostis epigejos* (Hofmann *et al.*, 1990).

Root growth

N may also stimulate root growth (Heinsdorf and Schulzke, 1969; Persson, 1980; Zöttl, 1964) in ecosystems which are not N saturated. However, some studies showed that root growth is inhibited by excessive N supply, and there is strong evidence for 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 kg N ha⁻¹ yr⁻¹ in Ysselsteyn (Scots pine) (Gundersen et al., 1998b), from 36 to less than 5 kg N ha⁻¹ yr⁻¹ in Solling (Douglas-fir, Norway spruce) and from 13 to less than 4 kg N ha⁻¹ yr⁻¹ in Gårdsjön (Norway spruce). Total fine-root biomass of Norway spruce saplings decreased significantly when NO,⁻ and NH_{2}^{+} in soil water was more than 2 mg N l⁻¹ (Figure 9.5; Matzner and Murach, 1995). From the relationship between N deposition and NO₂⁻ concentration as published by De Vries et al. (2001), this concentration may be attributed for coniferous stands to an average N throughfall load of 25 kg N ha⁻¹ yr⁻¹ (range 13-33). Evidence for declining fine-root biomass is also given by Magill et al. (2004) although they found only a trend of a 20 to 25% fine-root biomass reduction in the organic horizons following a 15-year N addition experiment at 50 and 150 kg N ha⁻¹ yr⁻¹, on pine and hardwood stands, respectively (Harvard Forest, Massachusetts (USA), background deposition 8 kg N ha⁻¹ yr⁻¹). Fenn et al. (2008) report a 26% reduction in fine-root biomass of Pinus ponderosa at a 17 kg N ha⁻¹ yr⁻¹ N deposition from throughfall and an NO₂⁻ concentration peak of 10.8 meq l⁻¹ in stream water. Additionally, Braun et al. (2005) observed decreasing fine-root lengths (< 0.25 mm Ø) 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 kg N ha⁻¹ yr⁻¹). Altogether, increased N deposition will lead to a less developed fine-root system, resulting in possibly reduced tree stability, and forests experiencing high N depositions may become more vulnerable to storms.

In a field fertilisation experiment in Switzerland, starch concentration in the fine roots of beech was significantly decreased following 8 years of N additions of ≥ 20 kg N ha⁻¹ yr⁻¹ (Hiltbrunner *et al.*, 2001). The uprooting of mature beech trees in forest observation plots in Switzerland during the 1999 storm 'Lothar' was positively correlated with N concentration in the foliage (atmospheric N deposition 15-35 kg N ha⁻¹ yr⁻¹), and with soils that showed a base saturation of $\le 40\%$ (Braun *et al.*, 2003).

Figure 9.5 Fine-root biomass of Norway spruce in relation to

N in soil solution (Matzner and Murach, 1995).



From the available data on growth, a critical load range of 10 to 15 kg N ha⁻¹ yr⁻¹ can be set for both coniferous and broadleaved temperate forests, and considered as 'quite reliable'. Growth responses seem to be similar on acidic and calcareous soils (Flückiger and Braun, 2011).

Nutrition of trees

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 hand, tend to increase. These changes were found in both field observations and experimental N additions. A decrease in P and Mg concentrations in tree foliage may be caused by (1) reduced uptake as a result of Al toxicity during soil acidification (Haynes, 1982; Godbold, 1991), (2) as a result of competition from NH⁺₄ in the root uptake system (Roelofs et al., 1985), (3) as a result of N impacts on mycorrhizal fungi (see Section 9.2.3), or (4) by a decreased supply rate of nutrients other than N due to leaching and N-induced higher growth rates depleting the resource (Thelin *et al.*, 1998).

In N-addition experiments in the east of the German Northern Lowland, carried out between 1980 and 1987, N concentrations in last year's needles where shown to be closely correlated with productivity and stability of Scots pine forests (Hofmann et al., 1990). N saturation in needles was observed at concentrations approaching 18 mg N per g dry weight. For an oligotrophic Scots pine forest with N deficiency (C:N ratio in top soil about 30, N concentration in last-year's needles about 14 mg N per g dry weight), this saturation threshold was reached after 40 years at a constant N-addition rate of 10 kg N ha⁻¹ yr⁻¹, and after 15 years at a rate of 20 kg N ha⁻¹ yr⁻¹, both with a background deposition of approximately 15 kg N ha⁻¹ yr⁻¹ (20 kg N ha⁻¹ yr¹ below canopy). Beyond this threshold, nutrient deficiencies (Mg, K, Ca), growth reduction and - above N concentrations of 23 mg N g⁻¹ – tree damages were observed (Hofmann et al., 1990).

Rising N concentrations in needles of various conifer species in Europe have been reported over recent decades (Van den Burg, 1990; Sauter, 1991). N concentrations in needles of Scots pine from European ICP Forest plots (De Vries et al., 2003) were found to be correlated with N deposition. Furthermore, plots with a balanced nutrition (ratios between N and the other macronutrients) had a median N deposition of 10 kg N ha⁻¹ yr⁻¹, while plots with an unbalanced nutrition experienced a median N deposition of 21 kg N ha⁻¹ yr⁻¹ (Table 9.1). Foliar mineral concentrations in 118 beech stands in north-eastern France were compared between two sampling periods, viz. between 1969-71 and 1996-97. Between the two sampling periods, foliar N concentrations increased by 12%, whereas a decrease was observed for P (-23%), K (-6%), Mg (-38%) and Ca (-16%), resulting in increased ratios of N:P-, N:Kand N:Mg, of 42%, 19% and 77%, respectively. The positive N trend was independent of soil type, with similar trends for calcareous and acidic soils. The atmospheric N deposition was estimated to be between 20 in the first sampling period and 30 kg N ha⁻¹ yr⁻¹ in the latter (Duquesnay et al., 2000). In Switzerland, significant decreases in P and Mg and, to a lesser extent, N, were observed in the foliage of 60 mature beech and 31 Norway spruce plots between 1984 and 2007. In spite of this, foliar N:P ratios in beech rose significantly, by 13.4% (Braun et al., 2010, modelled N deposition between 19 and 32 kg N ha⁻¹ yr⁻¹). The increase in N:P ratio – as in the French study - was independent of soil type. In pine forests in Brandenburg and in spruce forests in the Mittelgebirge (Germany), N concentration in foliage increased, and Mg decreased significantly, between 1964 and 1988 (approx. N deposition of 15-25 kg N ha⁻¹ yr⁻¹) (Hippeli and Branse, 1992; Nebe, 1991). In Sweden, N addition increased nutritional imbalances in a 120-year-old Fagus forest (Balsberg-Påhlsson, 1992). Mohren et al. (1986) and Houdijk et al. (1993) found a significantly lower P supply, or P deficiency,

in conifers exposed to increased N depositions in the Netherlands. Nihlgård and Olsson (1993) also report an increase in the N:P ratio in conifer needles during recent decades in south Sweden. There appeared to be a positive relationship between nutrient imbalances (increasing N:K and N:Cu) and depositions of N and S (Thelin *et al.*, 1998). P and N are well 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).

Experimental N addition to saplings of beech and Norway spruce in young stands on both acidic and calcareous soils induced nutrient imbalances and deficiencies, significant at added N loads of more than 10 to 20 kg N ha⁻¹ yr⁻¹, following 4 and 6 years of N treatment (modelled atmospheric depositions of 15 and 20 kg N ha⁻¹ yr⁻¹, respectively). On acidic soil, N treatment led to acute Mg deficiency (Figure 9.6), whereas on calcareous soil, K and P became limiting (Flückiger and Braun, 1999a). N concentrations remained unchanged. 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 in the foliage, during 3 years of treatment (Gundersen, 1998).

With high N inputs, concentrations of organic N in needles may increase to levels above the optimum range (Van Dijk and Roelofs, 1988; De Kam et al., 1991). N-rich free amino acids, especially arginine, were found to have increased significantly in needles with high tissue N concentrations (Hällgren and Näsholm, 1988; Van Dijk and Roelofs, 1988; Näsholm and Ericsson, 1990; Balsberg-Påhlsson, 1992; Pietilä et al., 1991). Arginine concentrations in foliage have been suggested to be 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 mmol g dw⁻¹ have been linked to NO₂⁻ leaching, because the arginine accumulation in trees corresponded with decreased uptake rates of NH⁺, leaving NH⁺ ions available for nitrification, which was subsequently followed by NO leaching (Näsholm et al., 1997). In permanent observation plots in Sweden, arginine concentrations in Norway spruce were higher in areas receiving high inputs of N (Ericsson et al., 1995). In Swiss plots, arginine concentrations in spruce foliage, although not in beech leaves, were correlated with modelled N depositions in the range of 14 to 37 kg N ha⁻¹ yr⁻¹ (Quiring et al., 1997; Braun et al., 2010). The strongest correlation, however, was observed between arginine and phosphorus 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 significantly
Figure 9.6 Nutrient concentrations in the foliage of young beech subjected to different N treatments on calcareous soil (Hochwald) and acidic soil (Zugerberg) (averages of 8 samples over a experimental period of 14 years). Bars: 95% confidence intervals. Bold lines: Regression significant at p<0.05, filled points: difference to control significant. Dashed color line: lower limit for optimum nutrient concentrations, according to Stefan *et al.* (1997). Modified from Flückiger and Braun (2011).



decreased (Boxman and Van Dijk, 1994; Boxman *et al.*, 1995). 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).

Based on a critical level of N concentration of 18 mg N g⁻¹ in foliage, De Vries *et al.* (2002) suggest a critical N load for pine species of 14 kg N ha⁻¹ yr⁻¹ and near 20 kg N ha⁻¹ yr⁻¹ for spruce. However, it must noted that such high foliar concentrations are not reached when P is limiting (Braun *et al.*, 2010). In 109 ICP Forest plots, median N deposition for plots with a balanced nutrition (ratio between N and the other macronutrients) was 9.6, and for the unbalanced plots this was 21 kg N ha⁻¹ yr⁻¹ (Table 9.1). Considering all demonstrated effects of N deposition on the nutritional status of trees, we recommend a critical load range for N of 10 to 20 kg N ha⁻¹ yr⁻¹ for deciduous temperate forests, and a range of 5 to 15 kg N ha⁻¹ yr⁻¹ for coniferous temperate forests, both ranges are considered to be '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.

unbalanced ratios of the macronutrients P, K, Ca or Mg to N (De Vries <i>et al.,</i> 2003).								
Element	Unbalanced nutrition				Balanced nutrition			
	No. of plots	N deposition (kg ha ⁻¹ yr ¹)			No. of	N deposition (kg ha ⁻¹ yr ¹)		
		Median	5% Quantile	95% Quantile	piots	Median	5% Quantile	95% Quantile
Р	46	21	6.9	34	63	11	1.5	34
К	15	23	14	37	94	14	1.7	33
Ca	4	28	20	35	105	16	1.9	34
Mg	24	22	11	35	85	13	1.7	33
All	57	21	7.8	34	52	9.6	1.4	32

Table 9.1. Ranges in nitrogen deposition (kg N ha⁻¹ yr⁻¹) at 109 Intensive Monitoring plots of ICP Forests in Europe, with balanced and unbalanced ratios of the macronutrients P, K, Ca or Mg to N (De Vries *et al.*, 2003).

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, following N addition (Klein et al., 1989; DeHayes et al., 1989; L'Hirondelle et al., 1992). Sensitivity to frost drought stress seemed to increase. For example, long-term fertilisation with various loads of N (15.7, 19.8, 25.6 and 31.4 kg N ha⁻¹ yr⁻¹, atmospheric bulk precipitation 5.4 kg N ha⁻¹ 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 kg N ha⁻¹ yr⁻¹, although cold tolerance was not affected and dehardening was decreased by the N treatment (Perkins et al., 2000). Jönsson et al. (2004b) observed an increased frost sensitivity of the inner bark of Norway spruce following 11 years of continuous application of ammonium sulphate (Skogaby experimental plot, 100 kg N ha⁻¹ yr⁻¹). They attributed this observation mainly to nutrient deficiency, with Mg of the N treated trees being 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 the supply of these elements is often decreased by N (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

The effects of N on the fine roots of trees and on mycorrhiza, as well as on nutrition, may have consequences for drought tolerance. Indeed, the vitality of many tree species in Dutch regions with high N depositions was found to be particularly low during the dry years of the mid-1980s, but increased again in subsequent years with normal precipitation levels (Heij et al., 1991). Nilsen (1995) described increased internal water stress in droughted Picea abies following additions of 27 and 82 kg N ha⁻¹ yr⁻¹ (control 5 kg N ha⁻¹ yr⁻¹). He explained this as resulting from increased water use by plants with larger crowns. In a N-addition experiment (25, 50, 100, 200, 400 kg N ha⁻¹ yr⁻¹) in pots, the shoot:root ratio of beech had significantly increased at additions of ≥ 25 kg N ha⁻¹ yr⁻¹, and leaf surface per tree had increased at additions of ≥ 50 kg N ha⁻¹ yr⁻¹, following six years of treatment. Subsequently, this led to

more rapid water loss from the soil (significant at ≥100 kg N ha-1 yr-1) and increased leaf necroses after a period of drought (significant at \geq 200 kg N ha⁻¹ yr⁻¹) (Flückiger and Braun, 1999b). During dry summers, the occurrence of foliar drought-induced necroses of beech leaves was augmented by increasing rates of N additions (Flückiger and Braun, 2011). In this case this was probably mediated by a K shortage, as the drought damage was negatively correlated to foliar K concentrations. At the same time, water use efficiency of the beech trees in the summer of 2003 significantly decreased following N-addition rates of ≥20 kg N ha⁻¹ yr⁻¹ (Figure 9.7) and pre-dawn water potential (used as a measure of water supply) was significantly reduced at N-addition rates of ≥ 40 kg N ha⁻¹ yr⁻¹ (modelled background deposition 15 kg N ha⁻¹ yr⁻¹). A similar observation of increased drought-induced necroses after a short period of drought was reported for N additions of ≥20 kg N ha⁻¹ yr⁻¹ (Thomas *et al*, 2002).

Figure 9.7 Water use efficiency of beech in the N-addition experiment at Hochwald (Switzerland) following 12 years of treatment, calculated from δ^{13} C measurements in the leaves. Differences with the control trees was significant at *p<0.05; ***p<0.001; background deposition 15 kg N ha⁻¹ yr⁻¹ (Flückiger and Braun, 2011). * = outliers



Pests and pathogens

With increasing N deposition, trees' susceptibility to fungal pathogens and insects may change. An increased drought sensitivity in response to N, as outlined in the previous section, may play a role as many pathogens and pests require weakened hosts. Decreased rates of mycorrhizal infection (see Subsection 9.2.3.) may increase susceptibility to root pathogens (Marx, 1969; Branzanti et al., 1999). Furthermore, altered concentrations of phenolic compounds and soluble N compounds, such as free amino acids, may also play a role (Nordin et al., 2005; Balsberg-Påhlsson, 1992; Bolsinger and Flückiger, 1989; McClure, 1980; Huber, 1980). A fertilisation experiment involving additions of 10 kg N ha⁻¹ yr⁻¹ at two Swiss sites (with an atmospheric 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 following seven years of N treatment, with levels mostly decreasing (Tomova et al., 2005). In the same experiment, two fungistatic phenolic compounds in the leaves were negatively correlated 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 ca 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). At the same time, high potassium supply reduced the development of necroses (De Kam et al., 1991).

In permanent beech observation plots in Switzerland (modelled N deposition of 15-35 kg N ha⁻¹ yr⁻¹), as well as in 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 at 25 of 48 studied sites in Scania (south Sweden), and were more frequent at sites with higher N deposition (20-25 kg N ha⁻¹ yr⁻¹) compared to those with lower N deposition (15-20 kg N ha⁻¹ yr⁻¹) (Westling *et al.*, 1992). Bark lesions were positively correlated with the occurrence of the beech scale insect *Cryptococcus fagisuga* and algal cover of *Lecanara fagisuga* (Jönsson, 1998). 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 following treatments of \geq 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, 1999a). 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. These are considered in Subsection 9.2.3.

Insect pests

Many of the insects that 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, also was 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 on pine needles in permanent observation plots in the UK was found to be positively correlated 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 has also been reported (NEGTAP, 2001).

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 a 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 (Avenella) flexuosa increases significantly when N deposition is more than 10 to 15 kg N ha⁻¹ yr⁻¹ (see Section 9.2.3.).

Two long-term fertilisation plots with N additions of 50 and 150 kg N ha⁻¹ yr⁻¹ were established in 1996, in the San Bernadino Mountains (US), at ambient N depositions of 18 and 94 kg N ha⁻¹ yr⁻¹ (Eatough Jones et al., 2004). Incidence of bark beetle activity on pines at the site with low ambient N deposition was 20% higher in stands receiving N additions, while bark beetle activity at the site with high atmospheric N input 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, and it was found that both ozone exposure and N deposition further increased pine susceptibility to beetle attacks. However, an oak herbivore community did not respond to the addition of 150 kg N ha⁻¹ yr⁻¹ at a low pollution site (throughfall deposition of 9 kg N ha⁻¹ yr⁻¹), but did respond at the high pollution site (throughfall deposition of 72 kg N ha⁻¹ yr⁻¹) (Eatough Jones *et al.*, 2008) The authors hypothesised that longer-term fertilisation treatments would be needed at the site with low pollution, before foliar N nutrition would increase sufficiently to affect herbivore communities.

N addition of 10 kg N ha⁻¹ yr⁻¹ (background deposition of 15-20 kg N ha⁻¹ 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 kg N ha⁻¹ yr⁻¹). Taking the importance of drought interactions into account, a critical load range for N of 10 to 15 kg N ha⁻¹ yr⁻¹ is recommended. However, to date, there has not been enough data available to differentiate between forest types. The San Bernadino Mountains study suggests that Mediterranean ecosystems may respond more slowly to N additions.

9.2.3 Effects of nitrogen deposition on biodiversity of forests

Effects on macrofungi and mycorrhiza

It is well known that N fertilisation of forest stands can reduce mycorrhizal development (Wallenda and Kottke, 1998; Treseder, 2004). N deposition can influence ectomycorrhizal fruit body formation, production and distribution of the extramatrical mycelium in the soil, and formation of ectomycorrhiza. Available data from long-term N-deposition studies indicate that the most prominent effects might be those which are discernible aboveground, that is, on the formation of fruit bodies. 'Generalist' species, forming a symbiosis with a wide range of tree species, seem to be less affected by increased N availability than 'specialist' species. Laccaria, Paxillus, Theleophora, 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 following N fertilisation with up to 240 kg N ha⁻¹ yr⁻¹ (Laiho, 1970; Ohenoja, 1988; Hora, 1959), while 35 kg N ha⁻¹ yr⁻¹ in the Gårdsjön experimental plot in Sweden was sufficient to reduce fruit-body production in most mycorrhizal species, especially in *Cortinarius* and *Russula* species (Brandrud and Timmermann, 1998). The easily cultivated species that are used in culture experiments are adapted to higher N concentrations, which 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 abundance of mycorrhizal fungi in forests. 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 of the Salzburg region (Austria). Data collected in 1937 showed 110 and in 1987 48 species of ectomycorrhizal fungi, and 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 found to have remained 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. Schlechte (1986) compared two Picea abies sites in the Göttingen area in Sweden. 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%). In a gradient study with additions from less than 1 to up to 18 kg N ha⁻¹ yr⁻¹ near an industrial ammonia production facility in the United States, 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). However, there is no information about the importance of dry deposition at the investigated site, and thus it is difficult to use these data to derive a critical load.

N experiments have also shown reduced fruit-body production of mycorrhizal fungi. Rühling and Tyler (1991) found that, within 3 to 4 years following N additions of 60 and 180 kg N ha⁻¹ yr⁻¹ in the form of NH₄NO₃ (atmospheric deposition of 15-20 kg N ha⁻¹ yr⁻¹), almost all mycorrhizal species ceased their fruit-body production. Termorshuizen (1990) applied 30 and 60 kg N ha⁻¹ yr⁻¹ over a 3-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. The mycorrhizal frequency and the number of mycorrhiza per unit of soil volume were not affected. It was, therefore, concluded that fruit-body production is much more sensitive to N enrichment than the mycorrhizal formation.

The effect of N additions on the below-ground ectomycorrhizal community may be less dramatic than that on fruit bodies (Wallenda and Kottke, 1998), and several studies stress the lack of correlation between the abundance of ectomycorrhizal fruit bodies and ectomycorrhizal root tips (Dahlberg, 2001; Lilleskov *et al.*, 2002). However, belowground ectomycorrhizal communities are more 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.

Ectomycorrhizal communities at root tips have been studied in experiments and in gradient studies. In a pot experiment with seedlings of Pinus sylvestris and added mycelium of Paxillus involutus or Suillus bovinus, the total number of mycorrhiza per plant dry weight decreased significantly with additions of 50 kg N ha⁻¹ yr⁻¹ ((NH₂)₂SO₂ or NaNO_) (Termorshuizen, 1990). Erland and Taylor (2001) used a gradient from low deposition in northern Europe to higher deposition in southern Europe, finding no 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, and 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%, but the total number of mycorrhiza 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.

Frey *et al.* (2004) found a reduced species diversity and a shift in species composition of mycorrizae in both a pine and hardwood stand at Harvard Forest (US) which had been fertilised with 50 kg N ha⁻¹ yr¹. Along a gradient of N deposition from 1 to 18 kg N ha⁻¹ yr¹ in Alaska, Lilleskov *et al.* (2002) observed a reduction in below-ground richness

of ectomycorrhizal species with increasing N deposition in stands of Picea glauca. The authors hypothesised that N-efficient species which prevail under N-limiting conditions are replaced by species that best function in nutrient-rich soils, and, subsequently, by P-efficient species under high N conditions. Wöllecke et al. (1999) investigated the number of mycorrhizal morphotypes in a stand with low N pollution (bulk deposition between1985 and 1988 of 10 to 20 kg N ha⁻¹ yr⁻¹, in 1996 7.6 kg N ha⁻¹ yr⁻¹, average NH, concentration between 1996 and 1998 was o.5 mg/m³) and in a stand with high N pollution (between 1985 and 1988 >35 kg, in 1996 13.5 kg N ha⁻¹ yr⁻¹, NH concentration between 1996 and 1998 10.3 mg/m³). They found 18 mycorrhizal morphotypes at the site with low N, and 9 mycorrhizal morphotypes and low mycorrhizal frequency at the site with high N. 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 KNO₂ and $(NH_{2})_{SO_{2}}$ was 21 kg N ha⁻¹ yr⁻¹, the ambient deposition ca 7 kg N ha⁻¹ yr⁻¹.

The growth of external ectomycorrhizal mycelium was found to be reduced on the experimental plot of Skogaby, where Picea abies had been treated with 100 kg N ha⁻¹ yr⁻¹ (Nilsson and Wallander, 2003). 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). With increasing N inputs, ectomycorrhizal mycelial growth was similarly reduced in coniferous forests (Nilsson *et al.*, 2005; no N deposition estimates given). In an N-addition plot with young beech, Braun *et al.* (2010) found a significantly lower mycelium density after 16 years of treatment with 40 and 80 kg N ha⁻¹ yr⁻¹ (background deposition of 15 kg N ha⁻¹ yr⁻¹).

Arbuscular mycorrhiza (AM) play a smaller role in forest ecosystems, but effects on this type of mycorrhiza have also been described. Using phospholipid analysis of fine roots, a significant decline in AM fungi was found in 2 out of 4 Acer species stands after 12 years of additions of 30 kg N ha⁻¹ yr⁻¹ (background deposition of 4.8-8.3 kg N ha⁻¹ yr⁻¹) (Van Diepen *et al.*, 2007). In California, Egerton-Warburton and Allen (2000) observed a shift in AM community composition along a gradient in N deposition from 10 to 35 kg N ha⁻¹ yr⁻¹. This study may be representative for Mediterranean ecosystems; N was deposited mainly in the oxidised form, and mostly dry.

Taking the available evidence into account, a critical load of 10 to 20 kg N ha⁻¹ yr⁻¹ is proposed, based on 'expert judgement'. This range is supported by L.O. Nilsson (pers. comm.), who found a tendency of decreased growth of mycelia from ectomycorrhizal fungi in oak-forest soils in a N-deposition gradient (9-17 kg ha⁻¹ yr⁻¹) in southern Sweden.

Effect on ground-living and epiphytic lichens and algae

Since the end of the 18th century, epiphytic or tree-barkinhabiting 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 also could have been an S or acidity effect, but results from fertilisation experiments in northern Sweden with low depositions of both N and S showed that all Cladina species had disappeared following 28 years of N additions (34 kg N ha⁻¹ yr⁻¹), while they were still present on the unfertilised control plots (Strengbom et al., 2001). This indicates that N deposition alone may be responsible for observed reductions in the abundance of ground-living lichens. Fenn et al. (2008) investigated critical loads for lichens in Californian mixed-conifer forests. They suggest that a critical load of 3.1 kg N ha-1 yr-1 would protect the lichen community from a shift in composition (loss in acidophyte dominance). Extirpation of acidophytes was observed at loads of 10 kg N ha⁻¹ yr⁻¹, whereas NO₂⁻ leaching occurred only at N loads of \geq 17 kg N ha⁻¹ yr⁻¹.

In Atlantic oak woods in Scotland and north-western England, epiphytic lichens, such as *Lobaria pulmonaria* and *Lobaria amplissima*, have been shown by Mitchell *et al.* (2003) to be effective indicators of N pollution. 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.

Data on epiphytic lichens, in cork-oak woodlands (*Quercus suber*) in Mediterranean climate, have shown that critical levels for NH_3 should be below 2 μ g m⁻³, and that the critical load range, thus, is below 10 to 15 kg N ha⁻¹ yr⁻¹ (with an estimated background deposition of 10 kg N ha⁻¹ yr⁻¹) (Pinho *et al.*, 2009). More study on Mediterranean woodland ecosystems is needed to confirm other changes in their structure and functioning.

Around 10 percent of all lichen species in the world have blue-green algae as the photobiont (Insarova *et al.*, 1992). These blue-green algae lichens are negatively affected by both acidity and N. In an international survey that stretched from the Netherlands via Denmark to Sweden, the decline in these lichens with blue-green algae were found to correlate significantly with N deposition rates of over 5 to 10 kg N ha⁻¹ yr⁻¹ (Göransson, 1990). However, there is still little information available about the effect of N on blue-green algae lichens, which may be the most sensitive component of some forest ecosystems and thus determine the critical load for these systems (Hallingbäck, 1991). Instead of being caused by N, the negative effects on lichens may have been an indirect effect from competition with N-favouring vascular plants (Cornelisen *et al.*, 2001). Dahlmann *et al.* (2002), in a study on about 500 lichens that both had green algae and cyanobacterias as their photobiont, found that most species seemed to be rather resistant to even high N additions.

Green algae, especially such 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 ca 10 kg N ha⁻¹ yr⁻¹ (Bobbink et al., 1996).

The data on this free-living algae and (epiphytic) lichens suggest a critical load range of 5 to 10 kg N ha⁻¹ yr⁻¹, considered 'reliable'. Although most of these data refer to boreal forests, results from Bobbink *et al.* (1996) confirm this range. Furthermore, these data are in accordance with recent findings in the United States, which show critical loads of 3 to 5 kg N ha⁻¹ yr⁻¹ for lichens in Mediterranean forests, chaparral and oak woodlands in California (Fenn *et al.*, 2008; Fenn *et al.*, in press), and between 3 and 9 kg N ha⁻¹ yr⁻¹ in forests on the west coast of Oregon and Washington states, just north of California (Geiser *et al.*, 2010).

Effect on forest ground vegetation

There is a large number of observations that show an increase in abundance of nitrophilous species in forests, either as time series or within an N deposition gradient. These species include Galeopsis tetrahit, Rubus idaeus, Rubus fruticosum, 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. In parallel, the average N indicator number (according to Ellenberg, 1988) has been shown to rise. A lot of endangered species are plants with a low N indicator value (Ellenberg, 1985), and the number of nitrophilous species has been shown to increase drastically at deposition loads of as low as 10 kg N ha⁻¹ yr⁻¹ (UNECE, 2006) (Figure 9.8). Understory species in deciduous forests respond to N deposition by higher growth rates, a more broadleaved anatomy, and higher N concentration and nitrate reductase activity in the tissue (Falkengren-Grerup and Diekmann, 2003).





A large representative evaluation of species occurrences at 2000 sampling sites, covering ca 90% of the Dutch forests, in the mid 1980s, revealed that, with an N deposition of around 40 kg N ha⁻¹ yr⁻¹, nitrophilous species such as Galeopsis tetrahit, Rubus species, Deschampsia flexuosa, Dryopteris carthusiana, Molinia caerulea, Poa trivialis and Urtica *dioica,* were among the 40 most common plants in forest ecosystems (Dirkse and Van Dobben, 1989; Dirkse, 1993). In Sweden, Quercus robur stands in two geographical areas with different N depositions (6-8 and 12-15 kg N ha⁻¹ yr⁻¹, respectively) were compared, with a special emphasis on N-indicator species (Tyler, 1987). The following species were more common at the most polluted site: Urtica dioica, Epilobium angustifolium, Rubus idaeus, Stellaria media, Galium aparine, Aegopodium podagraria, and Sambucus species. Comparable observations were reported by Falkengren-Grerup (1986; 1995), who examined the changes in soil and vegetation in repeated studies (over 40 years), in deciduous forests in southern Sweden where N deposition had doubled since 1955 to between 15 and 25 kg N ha⁻¹ yr⁻¹. A marked increase in frequency was found for almost 15 species, including Aegopodium podagraria, Epilobium angustifolium, Rubus idaeus, Stellaria nemorum, Stellaria holostea, Dryopteris filix mas and Urtica dioica, all considered to be nitrophilous species, according to Ellenberg (1988). Rosén et al. (1992), over a period of 20 years, found a significant positive correlation between Deschampsia flexuosa-dominated coniferous forests in Sweden and the pattern of N deposition, based on comparisons between ground-vegetation surveys in the Swedish Forest Inventory 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.

The influence of atmospheric N deposition on mixed oak-forest vegetation along a deposition gradient in Sweden, from Skåne (14-20 kg N ha-1 yr-1) via eastern Småland and Öland (7-11 kg N ha-1 yr-1) to lake Mälaren (6-9 kg N ha⁻¹ yr⁻¹), was investigated by Brunet et al. (1998). They found that 20 of 30 field-layer species that were most closely associated with high N deposition increased in frequency, in areas with high N depositions over the past decades. This group of field-layer species included many species generally considered as nitrophilous, but also several acid-tolerant species. A site index calculated from data on N values used by Ellenberg was higher in the most exposed regions, except on soils with a pH of less than 3.5, where soil acidity was probably more important, and with a pH of more than 5.0 (Falkengren-Grerup and Diekmann, 2003). The change in vegetation between 1983 and 1993 was large enough, by 1993, to show Ellenberg N values that were significantly higher in both Skåne and Småland (Diekmann et al., 1999).

In German mixed fir-spruce and Scots pine forests, where N deposition is around 15 to 30 kg N ha⁻¹ yr⁻¹, an increasing abundance of nitrophilous species has been reported, such as Dryopteris dilatata, Dryopteris carthusiana, Rubus idaeus, Rubus fruticosus, Milium effusum, Deschampsia (Avenella) flexuosa, Urtica dioica, and Epilobium angustifolium (Kraft et al., 2000; Rodenkirchen, 1992). However, changes in management regimes may also affect these changes. In Germany, at forest sites with earlier litter raking, the average Ellenberg N indicator values had increased by 0.6 units, at forest sites without litter raking the increase was 0.3 units (Rodenkirchen, 1992). In Switzerland, a significantly increased abundance of nitrophilous species was observed at 17 of 18 forest sites in two regions (northern Switzerland, with modelled N deposition for 1995 of 20-30 kg N ha⁻¹ yr⁻¹, and the Geneva region, with N deposition of 15-20 kg N ha⁻¹ yr⁻¹) after comparing vegetation data from the period 1938-47 with those from the 1984-85 (Kuhn et al., 1987). At 37 forest sites in the Central Plateau of Switzerland, the comparison between two surveys of ground vegetation between 1940/1965 and 1998 revealed a decreased frequency of 241 species and an increased frequency of 44 species, some of them typical nitrophilous species, such as Rubus fruticosus, Rubus caesius, Dryopteris dilatata, Dryopteris filix mas, Sambucus nigra, Hedera helix and Urtica dioica. The N deposition in this region was between 30 and more than 40 kg N ha⁻¹ yr⁻¹ (Walther and Grundmann, 2001). In Belgium, Lameire et al. (2000) also found a significant increase in N-indicator species, in mixed deciduous forests over a 20-year period – between 1977 and 1997 – such as Dryopteris dilatata, Galeopsis tetrahit, Hedera helix, Moehringia trinervia, Sambucus nigra, Stellaria media and Urtica dioica (approximate N deposition 25-30 kg N ha⁻¹ yr⁻¹). In a gradient study, the species composition of ground flora along a 500-metre transect downwind

from livestock buildings showed marked changes within 30 m. *Deschampsia flexuosa, Holcus lanatus, Rubus idaeus* and *Urtica dioica* were abundant, close to the livestock buildings and their percentage of cover decreased sharply away from the buildings. In this study, a critical load range of 15 to 20 kg N ha⁻¹ yr⁻¹ was proposed to protect the ground flora (Pitcairn et al., 1998).

Large-scale changes in vegetation have been observed in Scots pine forests of north-eastern Germany, since the 1970s. N deposition of between 10 to 15 kg N ha⁻¹ yr⁻¹ (bulk deposition) over several decades has led to N accumulation in oligotrophic Scots pine forests, which 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). This shift was accompanied by a narrowing of the C:N ratio of top soil and an increase in N content in needles. A N deposition that exceeded 20 to 25 kg N ha-1 yr-1 (bulk deposition) for several decades in some places, and massive depositions that exceeded 35 to 45 kg N ha⁻¹ yr⁻¹ for several years in other places, led to the establishment of (novel) N-induced forest types that are dominated by tall grasses (Calamagrostis epigejos, Arrhenatherum elatius) and shrubs (Rubus idaeus, Rubus fructicosus, Prunus serotina, Sambucus nigra), even in mesotrophic Scots pine forests. Strong spreading of Calamagrostis epigejos led to increased water stress for trees (Anders et al., 2002). As a result of these area-wide changes in vegetation, the understorey diversity in these German Scots pine forests had decreased, significantly (Hofmann, 1995b; 1995a).

The change in frequencies of forest plant species due to N deposition was also analysed for a region representative of the north-east German young-moraine regions (Jenssen and Hofmann, 2006). The study was based on the statistical analysis of a large set of relevees with more than 1500 plant species in most widespread forest types not influenced by groundwater or surface water. N deposition before 1960 can be assumed to have been below 10 kg N ha⁻¹ yr⁻¹. Between 1970 and 1990, the region was subject to an N deposition of about 10 to 20 kg N ha⁻¹ yr⁻¹. During this period, particularly the following nitrophilous species had increased significantly (p<0.05): Frangula alnus, Prunus serotina, Prunus spinosa, Rubus fructicosus agg., Rubus idaeus, Sambucus nigra and Sorbus aucuparia. Certain herbs and grasses had also increased, such as Arrhenatherum elatius, Calamagrostis epigejos, Calamagrostis arundinacea, Deschampsia flexuosa, Epilobium angustifolium, Fallopia convolvulus, Galeopsis tetrahit, Impatiens parviflora, Moehringia trinerva, Mycelis muralis, Stellaria media and Urtica dioica. At the same time, the frequency of protected plant species with a low N-indicator value significantly decreased. The conclusion

that the input of atmospheric N compounds had been the main driving force of the observed change in vegetation was reached following a comparison with results from an N-fertilisation experiment (Hofmann, 1987; Hofmann *et al.*, 1990; Anders *et al.*, 2002; Jenssen and Hofmann, 2005; Jenssen, 2009).

At two sites near Nancy (Haye forest), in north-eastern France, on calcareous soils with beech, oak and hornbeam, results from 278 floristic surveys from 1972 were re-examined in 1991 (139 surveys at each site). This led to a significant increase observed in N-demanding and acidophilous species. At the first site, 100% of the plots showed eutrophication and 79% acidification; at the second site , eutrophication was evident at 85% of plots and acidification at 74% (N deposition around 15-20 kg N ha⁻¹ yr⁻¹) (Bost, 1991). In 1990, at the intersections of a systematic grid covering a mixed hardwood forest on the Lorraine Plaine in north-eastern France, Thimonier et al. (1992) resampled 221 floristic surveys that had been conducted the first time in 1971 and 1972. This led to three main findings: a) an increase in the frequency of N-demanding species, b) an increase in Ellenberg's ecological N values, and c) an increased nutrient status revealed by trophic level indices. Thinning does not appear to be responsible for the observed changes in trophic level. The Ellenberg N values increased from 4.89 to 5.16, and the light indication values changed from 4.82 to 4.7. Annual N inputs at this mixed hardwood forest were assessed to have been 20 to 30 kg N ha⁻¹ yr⁻¹ at the beginning of the 1970s.

At 47 vegetation plots in the Villey forest near Nancy on the Lorraine Plain in north-eastern France, species were first sampled in 1972 and resampled in 1991. The mean number of species per sample increased from 32 to 39 during this period, and the frequency of nitrophilous species increased throughout the forest. On acidic soils, acidophilic species increased in frequency. Repetition of soil analyses, at the same time interval, on nearby plots, revealed a strong increase in N content. Although the stands had been converted from coppice to high forest, it is suggested that atmospheric N depositions are the most probable cause of eutrophication. Similar eutrophication was found for calcareous and acidic soils (N deposition around 20 kg N ha⁻¹ yr⁻¹) (Thimonier *et al.*, 1994).

In Switzerland, the cover of Rubus fruticosus species increased strongly in forest plots with a modelled N-deposition rate of ≥ 25 kg N ha⁻¹ yr⁻¹ (Flückiger and Braun, 2004, Figure 9.9). 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. The same holds true for Deschampsia flexuosa, which also shows a positive reaction to N (see section on boreal forests).





In Atlantic oakwood stands in Scotland and north-western England, epiphytic bryophytes *Pagiochila atlantica* and *Pagiochila spinalosa* appeared to be more sensitive to N pollution. These epiphytic species were strongly associated with sheltered sites and low N depositions (9.6-17.6 kg N ha⁻¹ yr⁻¹) compared to sites with high N depositions of 11.2 to 53 kg N ha⁻¹ yr⁻¹ (Mitchell *et al.*, 2003).

There is a large number of observations that show an increase in abundance of nitrophilous species, either in time series or within an N-deposition gradient. For temperate forests, many reports show an increasing frequency of nitrophilous species with long-term N depositions of more than 15 to 25 kg N ha⁻¹ yr⁻¹. These reports are confirmed by the relationship, observed in the ICP Forest data set, between N-indicating species and measured N depositions. Hence, on the basis of changes in ground vegetation, a critical N load range of 10 to 15 kg N ha⁻¹ yr⁻¹ is considered 'reliable'.

Fauna

Soil fauna

The lack of data on the effect of increased N input on soil fauna is a serious gap in knowledge and needs more attention in further research. The abundance of *Nematoda*, *Oligochaeta* and microarthropodes, especially *Collembola*, had increased according to some studies and decreased according to others, following applications of high doses of N fertilisers (>150 kg N ha⁻¹ yr⁻¹) (Abrahamsen and Thompson, 1979; Huhta *et al.*, 1983; Vilkamaa and Huhta, 1986). A single application of 100 kg N ha⁻¹ (in the form of NH_aNO₃) in a state forest in eastern Brittany, France,

produced an effect on soil microorganisms that was still significant 23 years later. There were decreases in Oribatida, Gamarida, Collembola, Symphyla (small Myriapoda) and Pseudoscorpionida (Deleporte and Tillier, 1999). The atmospheric deposition at this site was estimated at 10-20 kg N ha⁻¹ yr⁻¹. 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) following 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. The results were explained by a shift in nutrient supply, that is, an N-induced decrease in soil fungi. Collembola are fungal feeders and mycorrhizal grazers and, thus, directly depend on the density of soil fungi. A reduction in N deposition on a Pinus sylvestris stand (NITREX site Ysselsteyn), down to pre-industrial levels, increased species diversity of microarthropods, as dominance of certain species was decreased (Boxman et al., 1995). A significant decrease (66%) in the abundance of earthworms was observed in a young beech stand, following seven years of fertilisation with 20 kg N ha⁻¹ yr⁻¹ (atmospheric deposition of 12 kg N ha⁻¹ yr⁻¹) (Flückiger and Braun, 1999b). However, this may have been the result of soil acidification, as the pH of the upper soil layer (30 cm) decreased from 3.7 to 3.5. 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 (Gärdenfors et al., 1995). However, in this Swedish study, there was a sulphur deposition gradient (soil acidification), as well. Hence, no critical N load could be defined on the basis of these data.

Mammals

Foliage of fertilised spruce seedlings (Picea sitchensis) was most palatable to Orkney voles, Microtus arvalis orcadensis, compared to untreated seedlings. The foliage had higher levels of N (1.6-1.7%, compared with 0.9% in control seedlings) and lower levels of phenolics (2.6%, compared with control seedlings with 5.1%). Although the fertilisation of the spruce seedlings had contained NPK and could not be quantified in weight per unit area, a second experiment with *Calluna* fertilised with 52 kg N ha⁻¹ yr⁻¹ gave similar results (Hartley et al., 1995). In southern Sweden, N concentrations and browsing of young Norway spruce were studied for two clear-cut sites with different N depositions (at Asa, the N deposition was around 12-18 kg; at Tönnersjöheden, this was around 18-29 kg N ha⁻¹ yr⁻¹). At Tönnersjöheden, the site with higher N deposition, the seedlings had darker green needles and higher N concenTable 9.2 Empirical critical loads of N and effects of exceedances on different components of general forest classes. ## reliable; # quite reliable and (#) expert judgement. Bold: changes to version 2003.

Component	kg N ha ⁻¹ yr ⁻¹	Indication of exceedance
Soil processes		
Deciduous & coniferous	10-15 #	Increased N mineralisation, increased nitrification
	10-15 ##	Increased NO ₃ ⁻ leaching
Trees		
Deciduous & coniferous	10-15 #	Nutrient imbalances, increased N and decreased concentrations of
		P, K and Mg in foliage
Temperate forests	10-15 (#)	Increased susceptibility to pathogens and pests, change in
		fungistatic phenolics
Mycorrhiza		
Temperate & boreal forests	10-20 (#)	Reduced sporocarp production, changed or reduced below-ground
		species composition
Ground vegetation		
Temperate forests	10-15 ##	Changed species composition, increase in nitrophilous species,
		increased susceptibility to parasites
Lichens and algae		
Temperate and boreal forests	5-10 #	Decline of lichens, increase in free-living algae

trations in foliage. Frequency of browsing of young trees increased with increasing colour and hence with increasing N concentrations in the needles. Browsing damage was significantly greater at Tönnersjöheden than at Asa. Vegetation cover and density of roe deer in the clear-cut areas were similar at both sites (Bergquist and Örlander, 1998a; 1998b). However, the available data are not strong enough to set a critical load for this fauna.

9.2.4 Summary of empirical critical loads for coniferous and deciduous forests

This section provides an overview of the impacts of N deposition on different components of general forest classes. The empirical critical loads for the different classes are summarised in Table 9.2.

9.3 Effects of N deposition on spruce and pine taiga woodlands (G₃.A and G₃.B)

Together, spruce (G3.A) and pine (G3.B) taiga woodlands constitute the westernmost part of the continuous Eurasian northern taiga belt (Davies *et al.*, 2004). Spruce taiga woodlands (G3.A) include the boreal spruce and spruce-pine forests of Fennoscandia, north-eastern Poland, the Baltic States, Belarus and European Russia. Pine taiga woodlands (G3.B) include the boreal pine forests in the regions mentioned above. Both spruce and pine taiga woodlands are mostly very poor in N, and any increase in N input may influence major soil processes. Because soil responses to increased N input are likely to be similar in both habitat classes, in this section, effects from N deposition on soil processes are presented together, whereas the impacts on biodiversity are treated separately.

9.3.1 Soil processes

A long-term N fertilisation experiment in northern Sweden showed that 34 years of N additions (\geq 34 kg N ha⁻¹ yr⁻¹, background deposition 2-3 kg N ha⁻¹ yr⁻¹) caused 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). Decreased abundance of base cations has been found to occur in response to N additions. 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). 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⁻¹yr⁻¹) was negatively correlated to base saturation in the humus layer, to soil pH, and to Ca:Al ratio, and was positively correlated to Al³⁺ concentration in the mineral soil layer (Solberg et al., 2004). However, Solberg et al. (2004) suggested that geographical patterns and natural processes also may 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 NH⁺, and increased production of organic acids (Kvaalen et al., 2002).

There is much debate over the extent to which N deposition may lead to increased N leaching from coniferous forests. Boreal forest ecosystems have large capacities to retain N (Petrone et al., 2007), and there are empirical and modelling studies that show a positive relationship between N leaching and N deposition, and others that show no such relationship (Kaste et al., 2004; Sjoeng et al., 2009; Futter et al., 2009; Nadelhoffer et al., 1999; Gundersen et al., 1998b). Under undisturbed conditions, boreal forests have a high N-retention capacity. Timber harvest has been shown to remove part of this capacity from the ecosystem, as it causes not only soil disturbance but also removes an important N sink. Problems with loss of N-retention capacity from timber harvest may increase under high N input, and in south Sweden it has been demonstrated that increased N depositions increase the risk of N leaching (Akselsson et al., 2004).

Decreased N deposition may have rapid positive effects on soil processes. In Finland, long-term monitoring of deposition and run-off chemistry over a period of 12 to 25 years, showed decreased $SO_4^{2^{-1}}$ and NO_3^{-1} concentrations in bulk deposition, since the 1980s and 1990s, respectively, which led to a rapid decrease in concentrations of these elements in run-off (Moldan *et al.*, 2001). Ten years after termination of N additions to experimental plots that had been fertilised with 108 kg N ha⁻¹ yr⁻¹ for 20 years, a significant increase in soil pH was observed (Högberg *et al.*, 2006). In addition, after 14 years, N mineralisation rates also were still elevated on these plots (Chen and Högberg, 2006).

Although decomposition of high quality litter, which is low in lignin, may be stimulated following increased N input; for litter with a high lignin concentration, however, the decomposition rate is reduced by N (Knorr et al., 2005). Soil C storage is positively affected by N addition, with low N-addition rates being more effective in increasing soil C storage capacity, than high N addition rates (Hyvönen et al., 2008; De Vries et al., 2009). The increase in soil C following N additions to coniferous forests has been 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). Twenty years of N additions (\geq 34 kg N ha⁻¹ yr⁻¹) to an experimental pine forest in northern Sweden reduced soil respiration by 40%, and may double its soil C storage capacity for the next 100 years (Franklin et al., 2003).

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 of around 31 kg N ha⁻¹yr⁻¹to a Norway spruce stand in south-eastern Finland, showed that the CH₄ uptake of the soil had not been negatively affected by N fertilisation (Saari *et al.*, 2004). N₂O is a greenhouse gas that is 298 times more potent than CO₂. 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 were (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; Papen and Butterbach-Bahl, 1999).

9.3.2 Effects on biodiversity in taiga woodlands

Productivity in spruce- and pine-dominated taiga forests is primarily limited by a cold climate and low N availability (Tamm, 1990). By definition, plant growth will increase when N input increases, in such N-limited ecosystems. In this type of nutrient-poor environment, with low species richness, increased plant biomass is a more common initial response to elevated N input than reduced species richness. In a meta-analysis of data from boreal N addition experiments there was no effect on species richness, but Sorensen's similarity index, compared to the control plots, decreased with increasing N loads (Figure 9.10). This suggests that, as N input increases, plant species that disfavour high N availability are gradually replaced by more N-demanding species, resulting in an altered composition, but more or less constant species richness.





Mycorrhiza

Increased N input alters species composition of mycorrhizal fungi, as measured both in production of fruit bodies (above ground) and in below-ground mycelia (Brandrud, 1995; Lilleskov et al., 2001; 2002; Treseder, 2004). Fruitbody production in species belonging to the genera Cortinarius and Russula decreased, while species such as Paxillus involutus and Lactaria rufus were less sensitive, or even favoured N additions of to 35 kg N ha⁻¹ yr⁻¹, for 1.5 years (background deposition ~13 kg N ha⁻¹ yr⁻¹) (Brandrud, 1995). Strengbom et al. (2003) found production of fruit-bodies in Cortinarius species to be around 300 times higher on control plots than on plots receiving 34 kg N ha⁻¹ yr¹ (background deposition ~2-3 kg N ha⁻¹ yr⁻¹); on the latter, production was very small. Studies focusing on above-ground effects have been criticised for only describing a small part of the responses, as effects on the below-ground part of vegetation may differ substantially from those observed in fruit-body production (Lilleskov et al., 2002). Although there is a lack of studies on belowground effects of N deposition in European boreal forests, studies on above-ground effects do suggest that the ectomycorrhizal fungi community is influenced by N deposition. Mycorrhizal fungi on ericaceous dwarf-shrubs 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, there was no effect from N addition (12.5 and 50 N ha⁻¹ yr⁻¹ for 4 years in pine and 12 years in spruce forests, with background depositions of $\sim 2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$).

Spruce taiga woodlands (G3.A)

Ground vegetation

Responses in ground vegetation in spruce forests have been studied fairly well. A common initial response following 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 (Mäkipää, 1998; Nordin et al., 1998; Strengbom et al., 2002; Nordin et al., 2006; Forsum et al., 2006). Several studies also report that the composition of ground vegetation is sensitive to increased N input. They report altered abundance of commonly occurring dwarf shrubs such as Vaccinium myrtillus and Vaccinium vitis-idaea, and the grass Deschampsia flexuosa. Such changes have been reported to occur already at low or moderate levels of N (Kellner and Redbo-Torstensson, 1995; Strengbom et al., 2003, Nordin et al., 2005; 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) had increased the abundance of Deschampsia flexuosa by around 50% (UNECE, 2007). Additions of 12.5 kg N ha⁻¹yr⁻¹, over five years (background deposition of around 2 kg N ha⁻¹ yr⁻¹), had resulted in a 300% higher abundance of Deschampsia flexuosa, and a 34% lower abundance of Vaccinium myrtillus (Figure 9.11).

In central Sweden (background deposition of 2-6 kg N ha⁻¹ yr⁻¹), shoot density of *Deschampsia flexuosa* increased by 70, 250, 430 and 780%, following four years of additions of 5, 10, 20, and 40 kg N ha⁻¹ yr⁻¹ (Figure 9.12). At the same site, shoots density of *Trientalis europaea* showed significant increases when N additions exceeded 10 kg N ha⁻¹ yr⁻¹.

Figure 9.11 Response in the two dominant plant species of the ground vegetation (the dwarf shrub *Vaccinium myrtillus* and the grass *Deschampsia flexuosa*) in a Swedish boreal forest (G3.A) that had been exposed to N additions corresponding to 12.5 and 50 kg N ha⁻¹ yr⁻¹ for 5 years. The fertilised plots were sized 1000 m² and each treatment was replicated 6 times (n=6). Vertical bars show \pm one S.E. Redrawn from Nordin *et al.* (2005).



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Figure 9.12 Number of shoots of *Deschampsia flexuosa* vs nitrogen supply (kg N ha⁻¹ yr⁻¹), between 1988 and 1992, in a boreal forest (G3.A) located outside Söderhamn, in central Sweden (Kellner and Redbo-Torstensson, 1995).

The above-listed effects following 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 G3.A and G3.B classes), showed that occurrences of *Vaccinium myrtillus* and *Vaccinium vitis-idea* (Figure 9.13) were less frequent in areas were N depositions had been greater than or equal to 6 kg N ha⁻¹ 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 kg N ha⁻¹ 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).

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, effects from the input of high levels of N could become limited by reduced light availability as tree canopy cover increased. In comparison, light limitation for more open experimental sites, such as sites 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), density of Deschampsia flexuosa increased linearly (or exponentially) as N additions increased (Figure 9.12), while 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⁻¹yr⁻¹ ((Figure 9.11). Kellner and Redbo-Torstensson (1995) studied a mixed pinespruce forest, while Nordin et al. (2005) studied a sprucedominated forest, in which light limitation for groundliving species may have limited N responses at high input rates. This illustrates that 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 interpretation of the effects from N addition (Gilliam, 2006; Nordin et al., 2008).

Figure 9.13 Proportion of subplots with *Vaccinium myrtillus* and *Vaccinium vitis-idaea* in forest stands with various N-deposition rates. Green bars represent Scots pine and orange bars represent Norway spruce dominated stands. Vertical bars: mean and confidence intervals (95%). Redrawn from Strengbom *et al.* (2003).



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As in vascular plants, composition of the bryophyte community also often shows large and rapid changes when N input increases. In Finland, biomass of Pleurozium schreberi and Dicranum polysetum were reduced by 59 and 47%, respectively, after 4 years of N additions of 25 kg N ha⁻¹ yr⁻¹ (in the form of ammonium sulphate, $S = 30 \text{ kg ha}^{-1} \text{ yr}^{-1}$, background deposition of 2.5 kg N ha⁻¹ yr⁻¹). Moreover, biomass of the N-favouring Brachytecium and Plagiothecium species increased (Mäkipää, 1995; 1998). In another Finnish study, which also included spruce forests (Mäkipää, 1994), the biomass of both Hylocomium splendens and Pleurozium schreberi were reduced by 75 to 90% following a total N addition of 746 kg N ha⁻¹ yr⁻¹ (82 to 180 kg N ha⁻¹ at 6 occasions over 25 years). In addition, nationwide surveys of ground vegetation in Finland has revealed that the relative abundance of Hylocomium splendens has been reduced since the early 1950s, and that Brachytecium species has increased during the same time period (Mäkipää and Heikkinen, 2003). Although the observed changes may be linked to differences in age structures of the forests and/or intensified forestry, these changes are in keeping with results from N-addition experiments, suggesting that N deposition may be partly responsible for the observed changes.

Bryophytes depend directly on wet and dry deposition of N and are therefore considered to be highly sensitive even to small changes in the supply. Additional N doses of between 12.5 and 50 kg N ha⁻¹, to coniferous forest in northern Sweden, caused arginine concentrations of *Pleurozium schreberi* and *Dicranum majus* to increase (Nordin *et al.*, 1998). This indicates that the mosses were not able 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 to reductions in 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).

Increased sensitivity to pathogens and herbivores Increased concentrations of N in plant tissue following increased N input may result in increased damage from pathogens and herbivores. Foliar pathogens attacking *Vaccinium myrtillus* and *Deschampsia fleuxosa* increased following N additions of 12.5 kg N ha⁻¹ yr⁻¹ during 5-10 years (background deposition of around 2 kg N ha⁻¹ yr⁻¹; Nordin et *al.*, 1998; 2005; 2006). In the absence of pathogen attacks on *Vaccinium myrtillus*, N-induced growth of the grass *Deschampsia flexuosa* was limited by low light availability (Strengbom et al., 2002; 2004). In addition, 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).

Effects from increased N availability on pathogens, for the host plant often depend on interactions with weather conditions. For example, disease incidence by Valdensia heterodoxa infecting Vaccinium myrtillus is positively correlated with summer precipitation, with higher precipitation levels, increasing the effect of added N (Figure 9.14). This means that the effect from 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 9.14 Disease incidence (proportion of leaves diseased) 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. Redrawn from Strengbom *et al.* (2006).



Plant damage caused by insect herbivores may increase, following 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 following 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 population densities of Operopthera larvae (Nordin et al., 2008), 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 effects of N deposition, it is important to consider that plant species exhibit differences in their capacities to utilise NO3⁻. Although the effect of N deposition to an ecosystem is mainly related to the quantity of N deposited, the qualitative aspect also needs to be recognised. To date, few studies have addressed the quantitative differences of N deposition on taiga habitats. Nordin et al. (2006) compared effects of additions of 12.5 and 50 kg N ha-1 yr-1 in the form of NH_NO_, (NH_)_SO_, or KNO,, to a spruce forest in northern Sweden (background deposition of around 2 kg N ha⁻¹ yr⁻¹). Deschampsia flexuosa took up more NO⁻ than NH⁺, and, consequently, NO⁻ addition induced more grass growth than additions of NH^{*}. It was concluded that increased grass growth is less likely to occur when N is deposited as NH⁺₄ than when it is deposited as NO₂⁻.

Green algae

In Finland, a survey of 3009 forest plots revealed a positive correlation between the occurrence of green algae (free living or in symbiosis with the lichen Scoliciosporum chlorococcum) on tree branches and modelled N deposition (Poikolainen et al., 1998). In areas with NO⁻ depositions of less than 2 kg N ha⁻¹ yr⁻¹, green algae were absent from over 90% of the plots; in areas with $NO_{_{\!\!\!\!2}}^{-}$ depositions of zto 3 kg N ha⁻¹ yr⁻¹, green algae occurred in over 50% of the plots, and when deposition of NO,⁻ exceeded 3 kg N ha⁻¹ yr¹, green algae occurred in around 85% of the plots. The trend was similar if occurrences were related to depositions of NH₋. In Sweden, Bråkenhielm and Quinghong (1995) found that colonisation rates and density of epiphytic green algae on spruce needles increased when N deposition exceeded 12 kg ha-1 yr-1. Southernmost sites did not fall within taiga habitat types. For both studies, confounding factors such as climatic differences and changes and/or differences in S deposition complicated interpretation of the results.

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, 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). In addition, 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 indicates 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.

Critical loads

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 abundance and occurrence of Vaccinium myrtillus was lower when N deposition exceeded this level (Strengbom et al., 2003). Additions of 5 kg N ha⁻¹yr⁻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, 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 (Strengbom *et al.*, 2002; Nordin et al., 2005; 2006). The low reversibility of N-induced effects (Strengbom et al., 2001) support the suggestion that spruce taiga woodlands is a habitat class sensitive to N deposition. Based on the results presented above, the critical load range for spruce taiga woodlands (G3.A) we propose to set the critial load range at 5 to 10 kg N ha⁻¹yr⁻¹, which is considered as 'reliable'.

Effects on biodiversity of pine taiga woodlands (G3.B)

Ground vegetation

Response to N deposition may partly depend on initial site productivity. In general, forests dominated by Scots pine, *Pinus sylvestris* (G3.B), tend to be less productive than forests dominated by Norway spruce, *Picea abies* (G3.A). There are indications that ground vegetation in forest 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 (G3.A), 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 forest (G3.B), this effect is seen not until N deposition exceeds 12 kg N ha⁻¹ yr⁻¹ (Figure 9.14; 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 (Strengbom et al., 2004; Hautier et al., 2009). This is especially important in forested systems where the response in understory vegetation often is 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. This implies that such stands will be less sensitive to reduced light availability that results 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 forest may also explain why, in these habitats, Vaccinium myrtillus initially may be unresponsive or may even increase (at the expense of other dwarf-shrubs, such as Vaccinium vitis-idaea) following N addition (Kellner and Redbo-Torstensson, 1995). However, if N input is sufficiently high, or lasts long enough, abundance in 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 species richness of ground vegetation in low-productivity habitats such as boreal forests, may remain unchanged, or initially may even increase, as a response to increased N deposition (Bobbink, 2004). Despite these indications of differences in N sensitivity between spruce- and pinedominated forests, the available data on such differences is scarce, and more data is needed to clarify these differences.

Ground-living lichens often constitute most of the species richness of the ground vegetation in pine forests. Several experiments have identified this group of plants as sensitive and one of the first plant groups to disappear under increased inputs of N (Dirkse and Martakis, 1992; Mäkipää, 1994; 1998; Strengbom et al., 2001; Skrindo and Økland, 2002). In Finland, lichen biomass was reduced by more than 80%, following N additions of 82 to 180 kg N ha⁻¹ per application over 24 years (total N addition was 926 kg N ha⁻¹, background deposition of 2.5 kg N ha⁻¹ yr⁻¹) (Mäkipää, 1994; 1998). 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 (Van Dobben et al., 1999; Strengbom et al., 2001), and in Norway reduced occurrence of lichens were observed following 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 abundance of ground-living lichens is not clearly understood. Mäkipää and Heikkinen (2003) report that the relative abundance of *Peltigera aphtosa*, which has cyanobacteria as its photobiont (and is expected to be N sensitive), decreased in Finland between 1951 and 1986, and again in 1995. Note that the observed changes may not have been solely due to N deposition. Although several studies report that increased N availability may disrupt physiological processes in lichens (Dahlman *et al.*, 2002; Kytöviita and Crittenden, 2007), 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 increased N deposition. There are, however, large differences in responses between species, ranging from strong negative effects to positive effects. In pine forests, decreased abundance of Hylocomium splendens and Pleurozium schreberi, and increased abundance of litterdwelling species, such as Brachythecium and Plagiothecium species, are frequently reported following increased N input (Dirkse and Martakis, 1992; Mäkipää, 1994; Van Dobben et al., 1999; Strengbom et al., 2001; Skrindo and Økland, 2002; Nordin et al., 2005). Strengbom et al. (2001) reported a more than 70% reduction in the abundance of Pleurozium schreberi and increased abundance of Brachythecium species, following 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) report 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, abundance of Pleurozium schreberi has been negatively correlated to 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, 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.

Effects of different N forms

There are no data available on the specific effects on pine forests from the different forms of N, but the responses reported for spruce forests are thought to also be valid for pine forests (see text for G3.A).

Green algae and epiphytic lichens

In Sweden, Bråkenhielm and Quinghong (1995) found decreasing frequency, as well as decreasing total cover and number of N-sensitive lichens growing on stems of Scots pine (*Pinus sylvestris*), following N depositions exceeding 5 to 8 kg N ha⁻¹ yr⁻¹. However, it should be noted that the southernmost and northernmost sites were not located in any of the taiga habitat types. The correlative nature of the study does not exclude the possibility of confounding factors, such as general climatic differences or differences in S deposition. For more information on algae and epiphytic lichens, see spruce taiga woodlands.

Reversibility of N-induced effects

Quist *et al.* (1999) report swift recovery in soil N concentrations following cessation of 20 years of N additions of 108 kg N ha⁻¹yr⁻¹ (background deposition of around 2 kg N ha⁻¹ yr⁻¹). However, data on reversibility of the N-induced effects on biodiversity at this site, suggest that reversibility is a slow process. Strengbom *et al.* (2001) found no recovery of plant species composition or fruit-body production, nine years after N additions had been ceased. And 14 years later, Chen and Högberg (2006) noted that N mineralisation rates were still elevated for the plots that were formerly treated with N.

Critical loads

Compared to spruce taiga woodlands, there are indications that, for pine forests, negative effects on biodiversity from increased N input starts to occur at higher N input rates. However, data that would support the finding that pine forests are less sensitive to increased N input is currently not sufficient to make such a distinction between pine and spruce forests. There are few studies showing effects on biodiversity from N inputs below 10 kg N ha⁻¹ yr⁻¹ in pine forests. With two exceptions: a study by Poikolainen et al. (1998), who found increased occurrence of green algae on tree branches (no distinction between spruce and pine forests) when N inputs exceeded 3 kg N ha⁻¹ yr⁻¹, and a study by Bråkenhielm and Quinghong (1995), who found decreased occurrence of N-sensitive lichens when N depositions exceeded 5 to 8 kg N ha⁻¹ yr⁻¹. Strengbom et al. (2003) found that, in pine forests, Vaccinium vitis-idaea in pine forests had a significantly lower occurrence when N depositions were higher than 6 kg N ha⁻¹yr⁻¹, but 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 large effects on composition of ground vegetation (Dirkse and Martakis, 1992; Van Dobben et al., 1999; Strengbom et al., 2001), while 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 stopped. In summary, these results

suggest that pine taiga woodlands are sensitive to increased N deposition. With the support of gradient studies (3) that revealed effects at N loads of less than 10 kg N ha⁻¹ yr⁻¹ we recommend to set the critical load range for Pine taiga woodlands (G3.B) at 5 to 10 kg N ha⁻¹ yr⁻¹, to be considered as 'quite reliable'.

9.4 Additional differentiations between empirical critical loads for forest ecosystems (EUNIS level 3)

Relevant information on N-addition experiments and gradient studies on forest ecosystems have been summarised in Appendix 2 for non-taiga woodlands, and in Appendix 3 for taiga woodlands. The different studies have been classified, where possible, according to a EUNIS level-3 category, and subsequently ordered in the table under EUNIS type (level 3) to enable an evaluation of sensitivity to N deposition per EUNIS category at this level. Based on significant effects found at various N application rates, in combination with information on background depositions, the empirical critical loads of N were set for the distinguished EUNIS level-3 categories, and compared with the outcome of available gradient studies. In this way, empirical critical loads can be suggested for 8 types of forests in the level 3 category (see Table 9.3). Unfortunately, no specific recommendations can be given for EUNIS classes that have not been investigated, and thus, these are not present in Appendix 2. However, the outcome of Californian studies on N-deposition effects in Pinus ponderosa stands (Fenn et al., 2008) suggests a critical load for EUNIS category 3.7 (Mediterranean Pinus woodland) of 3 to 15 kg N ha⁻¹ yr⁻¹, based on 'expert judgement'.

Table 9.3 Empirical critical loads of nitrogen, per EUNIS categories, based on Appendices 2 and 3.					
EUNIS code		Proposed critical load (kg N ha ⁻¹ yr ⁻¹)	Reliability ^{a)}	Total number of studies	
Level-2	classification (based on Section 9.2)				
G1	Broadleaved deciduous woodland	10-20	##	36	
G3	Coniferous woodland	5-15	##	46	
Level-3 classification (based on Section 9.3)					
G3.A	Spruce taiga woodland	5-10	##	12	
G3.B	Pine taiga woodland	5-10	#	5	
Level-3 classification (based on Appendix 2)					
G1.6	Fagus woodland	10-20	(#)	23	
G1.8	Acidophilous Quercus-dominated woodland	10-15	(#)	7	
G1.A	Mesotrophic and eutrophic Quercus woodland	15-20	(#)	3	
G2.1	Mediterranean evergreen (Quercus) woodland	10-20	(#)	1 ^{b)}	
G3.1	Abies and Picea woodland	10-15	(#)	16	
G3.4	Pinus sylvestris woodland south of the taiga	5-15	#	8	
G3.5	Pinus nigra woodland	15	(#)	1	
G3.7	Mediterranean Pinus woodland c)	3-15	(#)		
G4.2	Mixed taiga woodland with Betula	5-8	(#)	1	
G4.6	Mixed Abies-Picea woodland ^{d)}	10-20	#		

^{a)} Reliablity ranges are: ## reliable; # quite reliable and (#) expert judgement.

^{b)} This critical load is based on one study in Portugal and supported by similar studies in Mediterranean woodlands in California. During the final editing procedure it became clear that the ambient background N deposition in the Portugese study had not been taken into account; therefore, the critical load has been adapted to this value.

^o Critical load range based on studies in Meditteranean forests in California.

Critical load range based on studies from C1.6 and C7.1

 $^{\rm d)}\,$ Critical load range based on studies from G1.6 and G3.1.

However, a problem for the differentiation between empirical critical loads for EUNIS category G-level 3, as proposed in Table 9.3, can be the availability of the number of studies on the recognised types. A second problem can be the difference in which parts of forest ecosystems were investigated in these studies. These matters can complicate a comparison of the inherent variation in sensitivities to atmospheric N deposition between these level-3 categories. Indeed, the more the data set was split into subgroups, the harder it was to reach a certain level of reliability. However, it is also likely, that the found differences in sensitivities, at least in part, were related to real differences in (a)biotic conditions, as has been demonstrated for a wide range of non-forest ecosystems across Europe.

9.5 Summary of empirical critical loads of N for woodland and forest (EUNIS Class G)

In the previous empirical critical load update (Bobbink *et al.*, 2003), overall empirical critical loads have only been distinguished for temperate forests and boreal forests. For the different components of the forest systems, critical loads were differentiated, in almost all cases, for deciduous (i.e. G1) and coniferous (i.e. G3) forests, and in two situations for boreal and temperate forests. In this chapter, it became obvious that the separate treatment of taiga (=boreal) woodland ecosystems (G3.A and G3.B) is reasonable, leading to robust critical loads (Table 9.4).

Table 9.4 Overall empirical critical loads of N and effects of exceedances on woodland and forest habitats (Class G). In bold: changes with the 2003 version. For a more detailed differentiation within categories G1 and G3, see Table 9.3.

Ecosystem type	EUNIS code	kg N ha'¹ yr'¹	Reliability	Indication of exceedance
Broadleaved deciduous	G1 ^{a, b}	10-20	##	Changes in soil processes, nutrient
woodland				imbalance, altered composition
				mycorrhiza and ground vegetation
Coniferous woodland	G3 ^{a, b}	5-15	##	Changes in soil processes, nutrient
				imbalance, altered composition
				mycorrhiza and ground vegetation
Spruce taiga woodland	G3.A ^c	5-10	##	Changes in ground vegetation,
				decrease in mycorrhiza, increase in
				free-living algae
Pine taiga woodland	G3.B ^c	5-10	#	Changes in ground vegetation, in
				mycorrhiza, increased susceptibility
				to parasites, increase in free-living
				algae

^{a)} In 2003 presented as overall value for temperate forests.

^{b)} For application at broad geographical scales.

^{c)} In 2003 presented as overall value for boreal forests.

9.6 References

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Chapter 10

Overview of the relationship between exceedances of N critical loads and plant species diversity at the European scale



Photo p. 173 Compilation of pictures from N-addition experiments: control vegetation (top left) and after N addition (top right) in Dutch calcareous grassland (photos R. Bobbink) and control vegetation (bottom left) and after N addition (bottom right) on the understory of Swedish Taiga woodland (photos A. Nordin & J. Strengbom).

10 Overview of the relationship between exceedances of N critical loads and plant species diversity at the European scale

10.1 Introduction

The emissions of ammonia (NH₂) and nitrogen oxides (NO_) increased strongly, during 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 and industry. 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 nitrogen deposition, nowadays, are central and western Europe, eastern United States and, since the 1990s, Eastern Asia. Atmospheric N deposition rates of 20 to 60 kg N ha⁻¹ yr⁻¹ have been observed in non-forest ecosystems in western Europe, whereas in forest stands in Europe and the United States, values between 20 and 100 kg ha⁻¹ yr⁻¹ were reached, instead of the estimated background inputs of 1 to 2 kg N ha⁻¹ yr⁻¹ of the early 1900s.

The availability of nutrients is one of the most important abiotic factors which determine plant species composition in ecosystems. Nitrogen is the limiting nutrient for plant growth in many natural and semi-natural ecosystems, especially of oligotrophic and mesotrophic habitats. Most of the plant species from such conditions are adapted to

nutrient-poor conditions, and can only survive or compete successfully on soils with low nitrogen availability. In addition, the nitrogen cycle in ecosystems is complex and strongly regulated by biological and microbiological processes, and it is thus likely that, as a result of increased deposition of air-borne N pollutants, many changes will occur in plant growth, interspecific relationships and soil-based processes (see Chapter 1 for an overview). The series of events which occur 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. This explains the observed high variations in sensitivity to atmospheric nitrogen deposition 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 vegetation composition and diversity and in nitrogen cycling. For an overview of the different indicators, see Lökke et al. (2000).

Empirical critical loads of N have been set based on field evidence. These critical loads are especially based on significant changes in the structure and function of specific ecosystems under N enrichment (mostly level 3 of the European Nature Information System (EUNIS)). However, a key question relating to their use for supporting policy development (for deriving national emission ceilings and for biodiversity protection through the UN Convention on Biological Diversity and the European Habitats Directive) is whether there is a link between total N loads and 'observable' biodiversity effects in the field. It is evident that information on these dose-response relationships is needed to demonstrate the causality between increased atmospheric N deposition and the species richness and diversity of sensitive ecosystems.

The aim of this chapter is to show the experimentally derived dose-response relationships between the total rate of N inputs and the change in biodiversity in major groups of European ecosystems (EUNIS classification). Because data is almost only available on vegetation, this chapter was restricted to the observed changes in plant species richness (number of plant species per plot) with respect to increased N inputs, under experimental field conditions.

10.2 Approach

The basis of this evaluation was the extended database of publications containing studies on the empirical critical loads of N (from 1992 to early 2010). Incorporated in this database are peer-reviewed publications, book chapters, nationally published papers and 'grey' reports from various institutes (if available). Studies providing insights into ecosystem reactions to an increase in N load were conducted for a variety of reasons. This has resulted in many different experimental designs. In this study, the outcomes of field addition experiments were only used if they were in accordance with the following criteria:

- independent, sole N treatments;
- no applications of additional nutrients, such as P, K or lime;
- below 150 kg N ha⁻¹ yr⁻¹ experimental addition;
- experimental periods of more than 2 years (thus incorporating at least 3 growing seasons);
- located in Europe (west of 40° longitude);
- containing data on plant species richness (number of plant species per plot)

The number of plant species per control plot and N-treated plot were either directly obtained from the publications, calculated from presented full species tables, or extrapolated from presented figures in the studies. Per used study, data were averaged per N treatment per investigated site (with sometimes more sites per publication), to avoid pseudo-replication and overrepresentation. The amount of experimental N that was applied (kg N ha⁻¹ yr⁻¹) was also noted. The background depositions at the experimental sites were directly obtained from the publications, or, when unavailable, via the EMEP model calculations according to location (longitude/latitude) of the experimental site and experimental period (personal communication J.Slootweg, CCE).

Many studies, even within the same EUNIS class, had used different plot sizes for plant species richness and it was thus necessary to prevent confounding effects from different plots sizes, as area is of major importance for the number of species. To make the outcome of experiments independent of plot size and thus comparable, the species richness ratio (per N treatment) was calculated as follows:

Species richness ratio: $S_N : S_c$

with: $S_{N = \text{species number in N-treated situation}}$ $S_{c = \text{species number in control situation}}$

If this ratio is 1, the number of species after N addition equals that of the control situation; above 1, the number of species following N addition is higher than those on the control plots. If the value of this ratio is below 1, the species number after N enrichment is lower than that in the control situation. This species richness ratio is relatively simple to interpret: a ratio of 0.75 indicates a 25% reduction in species number after N treatment, compared with the control situation. The available data on the species richness ratio were ordered according to EUNIS levels 1 or level 2 (Davies *et al.*, 2004) and analysed with 1x1 regression methods (including line fit procedures) against the total N load (sum of the

Ine fit procedures) against the total N load (sum of the experimental N addition rate plus the background deposition). The changes in species composition of the understory

vegetation in boreal forests could be analysed in detail, because of the availability of a full digital data set on longterm N fertilisation experiments in Swedish forests (kindly provided by Dr Han W. van Dobben, Alterra, Wageningen, The Netherlands). Besides plot species richness, the similarity index of Sorensen could be calculated with MVSP 3.0, based on presence and absence of species in the experimental plots, in comparison with the respective control situation (7 locations across boreal Sweden, n=12). This index quantifies the similarities and dissimilarities between two vegetation samples on a simple scale between 0 - 1, with 1 being full similarity (all species common) and 0 indicating no similarity (no common species).

10.3 Relationships between plant species richness and exceedances

The outcomes of the nutrient addition studies are presented EUNIS habitat classification levels 1 and 2. Unfortunately, the number of experiments that were conducted only provided sufficient data for the following three EUNIS habitat classes: grasslands and tall forbs habitats (E), Arctic, alpine and subalpine scrub habitats (F2) and coniferous boreal woodland (G3.A-C). For all other classes, the data set was much too limited to facilitate a regression analysis (below n=6 per habitat class, level 2).

10.3.1 Grassland and tall forb habitats (E)

The effects of nutrients on (semi-)natural species-rich grasslands have been studied for several European countries. The literature study on the effects of sole N treatments on plant species richness revealed the largest regression data set of this chapter (n=22). Results included those from studies under both dry and wet conditions, or with very different soil pH (acid – calcareous) (E1, E2, E3 and E4) across Europe (6 countries) (Lüdi, 1959; Bobbink, 1991; Tallowin *et al.*, 1994; Berlin, 1998; Jacquemin *et al.*, 2003; Crawley *et al.*, 2005; Bonanomi *et al.*, 2006; Beltman *et al.*, 2007). A significant negative relationship (negative exponential fit) between the species richness ratio and the total N exceedances (Exc-min) was found (Figure 10.1).

Figure 10.1. Relationship between the species richness ratio $(S_N:S_c)$ and N exceedance (N addition plus background deposition minus minimum value of the critical load) in grassland habitats (E) (6 countries; n=22; p<0.001).



10.3.2 Arctic, alpine and subalpine habitats (F2)

In general, plant productivity in Arctic, alpine and (sub) alpine (scrub) ecosystems is highly influenced by N, olbeit within the specific climatic constraints (see chapter 8). Because of the remoteness of most of these ecosystems and thus (still) very low atmospheric N inputs, possible impacts of N additions on the vegetation received attention only recently. Results from some relatively long-term studies on habitats within this EUNIS class (F2; n=11, 4 countries) could be used to obtain a first impression of the relation between the amount of N exceedance and the species richness ratio in Arctic-alpine vegetation (Lüdi, 1959; Gordon et al., 2000, Nilsson et al., 2002; Fremstad et al., 2005; Britton and Fisher, 2007). These results showed a significant negative linear relationship between the species richness ratio and total N load (p < 0.05) and between the species richness ratio and N exceedances (p < 0.05) (Figure 10.2). For example, Figure 10.2 suggests a 20% reduction of plant species richness at an exceedance of 25 kg N ha⁻¹ yr⁻¹. However, the data set is rather limited and should be extended to improve the fit of the regression line.

Figure 10.2 Relationship between the species richness ratio $(S_{_N}:S_{_C})$ and N exceedance (N addition plus background deposition minus minimum value of the critical load) in arctic and (sub)alpine scrub habitats (F2) (4 countries; n=11; p<0.05).



10.3.3 Taiga woodlands (G3 A-C)

Taiga woodlands form one of the largest forest zones of Europe. Over the last decades the number of studies on the impacts of N deposition in this ecozone has increased, but mostly they have determined the consequences for separate species, tree growth or soil processes. In the past two decades the impacts on the understory vegetation have received more attention, but the number of published studies with species numbers per plot or full vegetation lists are, however, scarce and have been restricted to the three Scandinavian countries (Van Dobben, 1993; Kellner, 1993; Mäkipää, 1998; Van Dobben et al., 1999; Shrindo and Okland, 2002). Because of the possibility to analyse the original data set of Van Dobben (n=12), the number of data points could be increase (n=18). The results for this EUNIS habitat type clearly showed that the species richness ratio was not influenced at all by the total N exceedance (Figure 10.3), although many studies reported a sharp decline in lichen diversity, a change in bryophyte composition and lower abundance of typical dwarf shrubs. This result suggests that this decline in typical species is counterbalanced by increases in invasive or non-characteristic species of more nutrient-rich habitats. These data clearly demonstrated that plant species richness is not a good indicator for effects of the N exceedances upon understory vegetation in these taiga woodlands of the boreal zone of Europe. This can be explained, at least partly, by the species-poor nature of these vegetation types which obscured the N effects upon species richness (Soons et al., submitted).

Figure 10.3. Relationship between the species richness ratio $(S_{N}:S_{c})$ and N exceedance (N addition plus background deposition minus minimum value of the critical load) in Taiga woodland (G3.A-C) (3 countries; n= 18; not significant).



However, a detailed comparison of the species composition of the understory in Swedish boreal woodlands (7 locations, n=12) following various long-term N additions, showed that the similarity of the N-treated vegetation significantly decreased (negative exponential curve) with the exceedances of N (Figure 10.4). **Figure 10.4** Sorensen's similarity index of N-treated understory vegetation, compared with the control vegetation in Swedish taiga woodland G.3A-C (7 locations; p <0.01; n=12) against the N exceedance (N addition plus background deposition minus minimum value of the critical load).



These results indicate that with an exceedance of ca. 30 kg N ha⁻¹ yr⁻¹, ca. 30 % of the species has changed whereas above 80 kg N ha⁻¹ yr⁻¹ a 45 to 50 % change in the species composition could be seen. Since species richness was not affected (Figure 10.3), this means that the original species were replaced by others!

10.4 Conclusions

The central aim of this inventorying chapter was to reveal, if possible, the relationship between species richness and N exceedances in the major EUNIS habitat classes based on evidence from experimental studies using quantified total N loads (application plus background deposition). This approach could only be applied to plant species richness, because experimentally quantified data for fauna in relation to applied N doses, is extremely rare. The effects of experimentally increased N on plant species richness was expressed as the species richness ratio, that is. the ratio between the number of plants in the N-treated situation and those in the control situation (S:S). For European grasslands habitats (E) and Arctic, alpine and subalpine habitats (F2), highly significant and significant negative relationships were found between the total N loads and species richness ratio. Remarkably, the quantified relationship between N exceedance across a range of grasslands in Europe strongly reflected the observations in temperate prairie grassland in the mid plains of the United States (e.g., Haddad et al., 2000).
As for grassland habitats, the number of studies on taiga woodlands (G 3 A-C) of the boreal zone was just large enough to come to a quantitative relationship between the species richness ratio and exceedance. The species richness ratio was clearly not related to increasing N in this habitat group. However, because of the availability of a full species data set, it became obvious after studying the similarities between species composition in the N-treated situation and in the control vegetation, that a significant negative relationship could be demonstrated. . This clearly suggests that typically nutrient-poor species were replaced by other plants, such as 'invasive' or 'N-favouring' species, without changing the overall number of species per plot (i.e. species richness). It is very likely that the number of plant species characteristic of unaffected vegetation, for example, those that are typical to nutrient-poor environments, is a more sensitive indicator for the changes in plant diversity in a certain habitat class, with respect to N exceedances. However, in almost all publications, data had been unclassified and counted as such. Because of this fact, it is likely that the slopes of the fitted curves for total plant species richness in the previous sections clearly underestimate the overall change in plant species composition.

In conclusion, it became obvious that for several EUNIS habitat classes (A, B, C and D) hardly any data on species richness were available or only for restricted parts of the class (F and G). Furthermore, for some relatively wellstudied systems (temperate heaths and bogs) the effects on species richness were hardly quantified, and could even be of restricted use because of the species-poor characteristics of these habitats. Hence, although there are limitations and uncertainties in the methods used to derive empirical critical loads, it is evident that exceedances of empirical critical N loads can clearly be linked to reduced plant species richness in some major European ecosystem types.

10.5 References

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Chapter 11

Conclusions and gaps in knowledge

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 steadystate 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, 2010).

Based on observed changes in the structure and function of ecosystems, empirical critical loads of 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 empirical critical loads under the LRTAP Convention was first published by Achermann and Bobbink (2003). Empirical critical loads 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 publication of Achermann and Bobbink (2003) have justified a review and revision of empirical critical loads of which the results have materialised in this report.

The review and revision was conducted using a similar 'empirical approach' as described in Bobbink et al. (2003) and Achermann and Bobbink (2003). For this purpose (see Chapter 2 for more details) we first collected, as complete a number as possible of European publications on the effects of N on natural and semi-natural ecosystems, from the period between 2002 and early 2010. We 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 (Davies et al., 2004). To improve incorporation of the empirical N critical loads 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 from A to G, and empirical N critical loads updated with newly published evidence, when available. Empirical critical loads of N have been revised, if necessary, and summarised in separate tables, per chapter. Previously to the CCE workshop (Noordwijkerhout, 23-25 June 2010), 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.

The text of this background document was intensively discussed and evaluated during the CCE workshop (23-25

June 2010, Noordwijkerhout, The Netherlands), 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 results and all the empirical critical loads of N that were set. For additional information on the outcome of this workshop, see the summary reports of working group 1 (EUNIS class A, B, C and E), working group 2 (EUNIS class D and F) and of working group 3 (EUNIS class G) in Appendices 5, 6 and 7 respectively. Furthermore, a workshop summary¹ has been prepared to have the use of revised empirical critical loads by National Focal Centres be endorsed by the Working Group on Effects (WGE) of the LRTAP Convention at its 29th session (Geneva, 22-24 September 2010). This WGE summary includes the main outcomes of the revision procedure and of the workshop itself, as well as a full list of agreed empirical critical loads of N for European ecosystems (Table 11.1).

http://www.unece.org/env/documents/2010/eb/wge/ece.eb.air. wg.1.2010.14.e.pdf

Table 11.1 Overview of empirical critical loads of nitrogen deposition (kg N ha⁻¹ yr⁻¹) to natural and semi-natural ecosystems (column 1), classified according to EUNIS (column 2), as originally established in 2003 (column 3), and as revised in 2010 (column 4).The reliability is qualitatively indicated by ## reliable; # quite reliable and (#) expert judgement (column 5). Column 6 provides a selection of effects that can occur when critical loads are exceeded. Finally, changes with respect to 2003 values are indicated in bold.

Ecosystem type	EUNIS code	2003 kg N ha ⁻¹ yr ⁻¹ and reliability	2010 kg N ha ⁻¹ yr ⁻¹	2010 reliability	Indication of exceedance
Marine habitats (A)					
Mid-upper salt marshes	A2.53		20-30	(#)	Increase in dominance of graminoids
Pioneer and low-mid salt	A2.54 and	30-40 (#)	20-30	(#)	Increase in late-successional species,
marshes	A2.55				increase in productivity
Coastal habitats (B)					
Shifting coastal dunes	B1.3	10-20 (#)	10-20	(#)	Biomass increase, increased N leaching
Coastal stable dune	B1.4ª	10-20 #	8-15	#	Increase in tall graminoids, decrease in
grasslands (grey dunes)					prostrate plants, increased N leaching, soil acidification, loss of typical lichen species
Coastal dune heaths	B1.5	10-20 (#)	10-20	(#)	Increase in plant production, increased N leaching, accelerated succession
Moist to wet dune slacks	B1.8 [♭]	10-25 (#)	10-20	(#)	Increased biomass of tall graminoids
Inland surface water habit	tats (C) ⁿ				
Permanent oligotrophic	C1.1 ^c	5-10 ##	3-10	##	Change in the species composition of
lakes, ponds and pools					macrophyte communities, increased algal
(including soft-water					productivity and a shift in nutrient limitation
lakes)					of phytoplankton from N to P
Dune slack pools	C1.16	10-20 (#)	10-20	(#)	Increased biomass and rate of succession
(permanent oligotrophic					
waters)					
Permanent dystrophic	C1.4 ^d		3-10	(#)	Increased algal productivity and a shift in
lakes, ponds and pools					nutrient limitation of phytoplankton from N to P
Mire, bog and fen habitat	s (D)				
Raised and blanket bogs	D1°	5-10 ##	5-10	##	Increase in vascular plants, altered growth and species composition of bryophytes, increased N in peat and peat water
Valley mires, poor fens and transition mires	D2 ^f	10-20 #	10-15	#	Increase in sedges and vascular plants, negative effects on broophytes
Rich fens	D4.1 ^g	15-35 (#)	15-30	(#)	Increase in tall graminoids, decrease in bryophytes
Montane rich fens	D4.2 ^g	15-25 (#)	15-25	(#)	Increase in vascular plants, decrease in bryophytes
Grasslands and lands dom	ninated by fo	orbs, mosses and	lichens (E)		
Sub-Atlantic semi-dry	E1.26	15-25 ##	15-25	##	Increase in tall grasses, decline in diversity,
calcareous grasslands					increased mineralisation, N leaching; surface acidification
Mediterranean xeric	E1.3		15-25	(#)	Increased production, dominance by
grasslands					graminoids
Non-Mediterranean dry	E1.7 ^ь	10-20 #	10-15	##	Increase in graminoids, decline in typical
acidic and neutral closed					species, decrease in total species richness
grasslands					
Inland dune pioneer	E1.94 [♭]	10-20 (#)	8-15	(#)	Decrease in lichens, increase in biomass
grasslands					
Inland dune siliceous	E1.95 [♭]	10-20 (#)	8-15	(#)	Decrease in lichens, increase in biomass,
grasslands					increased succession
Low and medium altitude hay meadows	E2.2	20-30 (#)	20-30	(#)	Increase in tall grasses, decrease in diversity

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selection of effects that ca	n occur wher	i citucal loads ale	exceeded. Fill	ally, changes with	rrespect to 2005 values are indicated in bold.
Ecosystem type	EUNIS	2003	2010	2010	Indication of exceedance
	code	kg N ha ⁻¹ yr ⁻¹ and reliability	kg N ha'' yr''	reliability	
Mountain hay meadows	E2.3	10-20 (#)	10-20	(#)	Increase in nitrophilous graminoids, changes
					in diversity
Moist and wet					
oligotrophic grasslands					
Molinia caerulea	E3.51	15-25 (#)	15-25	(#)	Increase in tall graminoids, decreased
meadows					diversity, decrease in bryophytes
Heath (Juncus)	E3.52	10-20 #	10-20	#	Increase in tall graminoids, decreased
meadows and humid					diversity, decrease in bryophytes
(Nardus stricta) swards					
Moss- and lichen-	E4.2	5-10 #	5-10	#	Effects on bryophytes and/or lichens
dominated mountain					
summits					
Alpine and subalpine	E4.3		5-10	#	Changes in species composition; increase in
acidic grasslands					plant production
Alpine and subalpine	E4.4		5-10	#	Changes in species composition; increase in
calcareous grasslands					plant production
Heathland, scrub and tun	dra habitats	(F)			
Tundra	F1	5-10 #	3-5	#	Changes in biomass, physiological effects,
					changes in species composition in bryophyte
					layer, decrease in lichens
Arctic, alpine and	F2	5-15 (#)	5-15	#	Decline in lichens, bryophytes and evergreen
subalpine scrub habitats					shrubs
Northern wet heath	F4.11				
• 'U' Calluna-dominated	F4.11 ^{e,h}	10-20 (#)	10-20	#	Decreased heather dominance, decline in
wet heath (upland					lichens and mosses, increased N leaching
moorland)					
• 'L' Erica tetralix-	F4.11 ^{e,h}	10-25 (#)	10-20	(#)	Transition from heather to grass dominance
dominated wet heath					
(lowland)					
Dry heaths	F4.2 ^{e,h}	10-20 ##	10-20	##	Transition from heather to grass dominance,
					decline in lichens, changes in plant
					biochemistry, increased sensitivity to abiotic
					stress
Mediterranean scrub	F5		20-30	(#)	Change in plant species richness and
					community composition
Woodland, forest and oth	ner wooded	land (G)			
Fagus woodland	G1.6		10-20	(#)	Changes in ground vegetation and
					mycorrhiza, nutrient imbalance, changes in
					soil fauna
Acidophilous Quercus-	G1.8		10-15	(#)	Decrease in mycorrhiza, loss of epiphytic
dominated woodland					lichens and bryophytes, changes in ground
					vegetation
Meso- and eutrophic	G1.A		15-20	(#)	Changes in ground vegetation
Quercus woodland					
Mediterranean evergreen	G2.1 [™]		10-20	(#)	Changes in epiphytic lichens
(Quercus) woodland					

Table 11.1 Overview of empirical critical loads of nitrogen deposition (kg N ha⁻¹ yr⁻¹) to natural and semi-natural ecosystems (column 1), classified according to EUNIS (column 2), as originally established in 2003 (column 3), and as revised in 2010 (column 4). The reliability is qualitatively indicated by ## reliable; # quite reliable and (#) expert judgement (column 5). Column 6 provides a collection of effects that say a court when critical loads are eveneded. Finally, changes with respect to 2003 values are indicated in hold.

selection of effects that ca	n occur when	critical loads are	exceeded. Fin	ally, changes with	respect to 2003 values are indicated in bold.
Ecosystem type	EUNIS	2003	2010	2010	Indication of exceedance
	code	kg N ha ⁻¹ yr ⁻¹	kg N ha ⁻¹	reliability	
		and reliability	yr 1		
Abies and Picea woodland	G3.1		10-15	(#)	Decreased biomass of fine roots, nutrient
					imbalance, decrease in mycorrhiza, changed
					soil fauna
Pinus sylvestris woodland	G3.4		5-15	#	Changes in ground vegetation and
south of the taiga					mycorrhiza, nutrient imbalances, increased
					N ₂ O and NO emissions
Pinus nigra woodland	G3.5		15	(#)	Ammonium accumulation
Mediterranean Pinus	G3.7		3-15	(#)	Reduction in fine-root biomass, shift in
woodland					lichen community
Spruce taiga woodland	G3.A ⁱ	10-20 #	5-10	##	Changes in ground vegetation, decrease in
					mycorrhiza, increase in free-living algae
Pine taiga woodland	G3.B ⁱ	10-20 #	5-10	#	Changes in ground vegetation and in
					mycorrhiza, increase in occurrence of free-
					living algae
Mixed taiga woodland	G4.2		5-8	(#)	Increased algal cover
with Betula					
Mixed Abies-Picea Fagus	G4.6 ^j		10-20	(#)	
woodland					
Overall					
Broadleaved deciduous	G1 ^{k,l}	10-20 #	10-20	##	Changes in soil processes, nutrient
woodland					imbalance, altered composition mycorrhiza
					and ground vegetation
Coniferous woodland	G3 ^{k,I}	10-20 #	5-15	##	Changes in soil processes, nutrient
					imbalance, altered composition mycorrhiza
					and ground vegetation

^{a)} For acidic dunes, the 8 to 10 kg N ha⁻¹ yr⁻¹ range should be applied, for calcareous dunes this range is 10 to 15 kg ha⁻¹ yr⁻¹.

^{b)} Apply the lower end of the range to habitats with a low base availability; and the higher end of the range to those with high base availability.

^o This critical load should only be applied to oligotrophic waters with low alkalinity with no significant agricultural or other human inputs. Apply the lower end of the range to boreal, sub-Arctic and alpine lakes, and the higher end of the range to Atlantic soft waters.

^{d)} This critical load should only be applied to waters with low alkalinity with no significant agricultural or other direct human inputs. Apply the lower end of the range to boreal, sub-Arctic and alpine dystrophic lakes.

^{e)} Apply the high end of the range to areas with high levels of precipitation and the low end of the range to those with low precipitation levels; apply the low end of the range to systems with a low water table, and the high end of the range to those with a high water table. Note that water tables can be modified by management.

^{f)} For EUNIS category D2.1 (valley mires): use the lower end of the range (#).

^{g)} For high-latitude systems, apply the lower end of the range.

^{h)} Apply the high end of the range to areas where sod cutting has been practiced; apply the lower end of the range to areas with low-intensity management.

ⁱ⁾ In 2003 presented as overall value for boreal forests.

¹⁾ Included in studies that were classified under G1.6 and G3.1.

^{k)} In 2003 presented as overall value for temperate forests.

¹⁾ For application at broad geographical scales.

^{m)} This critical load has been based on one European study in Portugal and evidence from studies in Mediterranean woodlands in California. During the final editing procedure of this report it became clear that the ambient background deposition of N in the Portuguese study had not been taken into account; therefore, the critical load was subsequently adapted to this value.

ⁿ⁾ See Appendix 4 for recent findings by the International Cooperative Programme on Waters under the LRTAP Convention.

Modifying factors

It has been suggested to the different countries, where insufficient national data for specific national ecosystems are available, to use the lower, middle or upper part of the ranges of the empirical N critical loads for (semi-)natural ecosystem groups, subject to so-called modifying factors, i.e. general relationships between abiotic factors and critical loads for N as given in Table 11.2 (adapted after Bobbink et al. (2003)). This table was discussed at the workshop in Noordwijkerhout. The modifying factors were treated for each distinguished EUNIS category, separately, by working groups 1 and 2, where possible leading to specific instructions with respect to the application of these ranges (see the working group reports in Appendices 5 and 6 for details; chapters containing summaries of these reports in this background document, and notes in Table 11.1). However, it became clear that, for the various forest EUNIS categories, such an approach currently would not be feasible (see Appendix 7 for the report by working group 3).

In conclusion to the above, additional qualitative information had been assigned to a number of modifying factors, in comparison to recommendations provided in 2003 on how to interpret the agreed ranges of critical loads in specific situations for the various ecosystems. However, short of agreement on how to quantify modifying factors for assessments on broad regional scales, consensus was reached to use the minimum value of the ranges of empirical critical loads in every EUNIS class to enable the comparison of their exceedances between different air pollution abatement scenarios Furthermore, in the United Kingdom and the Netherlands, several new national approaches have been developed to estimate or calculate the critical loads for specific ecosystems that are part of one larger EUNIS class with an agreed range of empirical critical loads (Van Dobben and Van Hinsberg, 2008; Hall and Wadsworth, 2010). Although this is an important development to create a more complete list of critical load values for the large number of ecosystem types, it should be broadened to a more European-based approach.

Most of the earth's biodiversity is present in semi-natural and natural ecosystems. It is thus crucial to control atmospheric N loads, in order to prevent any negative effects on these ecosystems. Fine-resolution maps of the sensitive ecosystems of high conservation value are needed per country, to map critical loads of N for these systems. It is advised to use both the mass balance and empirically derived N critical loads to improve the robustness on broad regional scales of effect based assessments of natural areas at risk of nitrogen deposition scenarios (see Hettelingh *et al.*, 2007). To assess negative impacts of exceedances on broad, regional scales in Europe, it was generally agreed at the workshop that specific dose-response relationships between nitrogen load and relevant indicators (Chapter 10) could be considered, provided that results would only be presented to compare the environmental risk of scenarios in relative terms.

Countries are advised to identify those highly sensitive receptor ecosystems within the EUNIS classification relating to their individual interest. Effort should be directed to produce fine-resolution maps of sensitive ecosystems of high conservation value. The current empirical critical loads of N have been set in values of total atmospheric N (kg N ha⁻¹ yr⁻¹). More information is needed on the relative effects of oxidised and reduced N deposition. It was emphasised during the last two UNECE expert meetings that there is increasing evidence of NH, having a greater effect than NO,. Particularly, lichens and sometimes bryophytes in a number of ecosystems, and several, mostly weakly buffered ecosystems in EUNIS classes F, E, C and B are thought 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, were recently revised, and became much lower for vegetation rich in lichens and bryophytes (Cape et al., 2009; Sutton et al., 2009).

11.2 Gaps in knowledge and research needs

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 2003 and 2010. The following

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.

Action	Temperature/ frost period	Soil wetness	Base cation availability	Management intensity
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

gaps in knowledge have been recognised as most important:

- more research and data are required to establish a critical load for the following ecosystems: steppe grasslands, all Mediterranean vegetation types, wet swamp forests, many mires and fens and several coastal habitats;
- more research is needed on all distinguished EUNIS habitat types with an 'expert' judgement rating, or on which only few studies are available;
- impacts of N enrichment in (sensitive) freshwater and shallow marine ecosystems (including coastal waters) need further research and are sometimes overlooked;
- additional effort is needed to allocated observed N effects to the appropriate EUNIS forest habitat subtypes (level 3);
- an increasing number of gradient (survey) studies with respect to atmospheric N deposition have been either reported or recently initiated. More rigorous guidelines should be identified for evaluation of these studies, covering estimation of deposition rates, quantification of confounding factors and application of methods for statistical analysis.
- possible differential effects of the deposited forms of N (NO_y or NH_x) are not sufficiently known for the setting of critical loads for oxidised and reduced nitrogen separately;
- in order to refine current critical loads, long-term (5-10 years) N-addition experiments using high frequency of N-treatments, between 5 and 50 kg N ha⁻¹ yr⁻¹, in regions with low background depositions, or in mesocosms, are useful. It would increase the reliability of the derived critical loads when the lowest treatment level does not exceed the critical load.

In conclusion, it is crucial to understand the longterm effects of increased N deposition on ecosystem processes for a representative range of ecosystems. It is thus important to quantify the effects of N loads by the manipulation of N inputs in long-term ecosystem studies in both unaffected and affected areas. These data are essential to validate the set critical loads and to develop robust dynamic ecosystem models and/or multiple correlative species models that are reliable enough to use in the calculation of critical loads of N deposition for natural and semi-natural ecosystems, and to predict natural recovery rates for N-affected systems.

11.3 References

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Appendices

Appendix 1

Classification of habitats according to EUNIS and EU Habitats Directive (Natura2000)

This appendix gives the correspondence between ecosystems, classified according to EUNIS (European Nature Information System) (Davies *et al.*, 2004) and the EU Habitats Directive (Annex I).

The third column (A) gives the corresponding Annex I habitat classification related to the European Nature Information System (EUNIS) with empirical critical loads of N, as treated in this background document. This column has been compiled by comparing the actual classification of vegetation from background data used for setting critical loads in this report with the Annex I habitat typology. Comparable or related EU habitats are presented in the fourth column (B). Empirical critical loads of N, as set in this report, can be assigned to EU habitats given in column A, with the same reliability as for the corresponding EUNIS classifications (second column, same line). In addition, we suggest, for the EU Annex I habitats, given in column B, to apply the empirical critical load as set for the EUNIS classes in the second column (same line). This suggestion has been based on expert comparison of the ecological correspondence between the (investigated) systems in columns A and B.

EUNIS habitat type	EUNIS code	EU habitats (A)	EU habitats (B)
Marine habitats (A)			
Mid-upper salt marshes	A2.53	1330	1410
Pioneer and low-mid salt marshes	A2.54 and A2.55	1310, 1320	1420
			1130, 1150
			(1630)
Coastal habitats (B)			
Shifting coastal dunes	B1.3	2110	1640
		2120	
Coastal stable dune grasslands	B1.4	2130	2210, 2220, 2230 2240
(grey dunes)			1220
			21A0
Coastal dune heaths	B1.5	2150	2140
			2250
Moist to wet dune slacks	B1.8	2190	
Inland surface water habitats (C)			
Permanent oligotrophic waters	C1.1	3110, 3130	3120
(Including soft-water lakes; boreal			(3170)
and alpine lakes)	61.16		71.40
Dune slack pools (permanent	C1.16		3140
Dermanent dystrephis lakes, pands	C1 4	7160	
and pools	C1.4	5100	
Mire. bog and fen habitats (D)			
Raised and blanket bogs	D1	7110, 7130	(7120)
Valley mires, poor fens and	D2	7140	
transition mires			
Rich fens	D4.1	7230	7210
Montane rich fens	D4.2	7240	7220
Grasslands and lands dominated by f	forbs, mosses and lichens (E)		
Sub-Atlantic semi-dry calcareous	E1.26	6210	6240, 6250, 6260, 6260
grasslands			
Mediterranean xeric grasslands	E1.3	6220	62A0
Non-Mediterranean dry acid and	E1.7	6230, 6270	
neutral closed grasslands			
Inland dune pioneer grasslands	E1.94	2330	2340
Inland dune siliceous grasslands	E1.95	2330	2340
Low and medium altitude hay	E2.2	6510	6270
meadows	52.7	(5.20	
Mountain hay meadows	E2.3	6520	
Moist and wet oligotrophic			
grasslands			
Molinia caerulea meadows	F3 51	6410	
Heath (Juncus) meadows and	E3.52	-	
humid (Nardus stricta) swards			
Moss- and lichen-dominated	E4.2	-	
mountain summits			

FUNIS habitat type	FUNIS code	FII babitats (A)	FU habitats (B)
Alpine and subalpine acid	F4 3	6150	6140 6160
grasslands	L4.9	0150	8240
grassiallus			6470
	54.4	6170	6450
Alpine and subalpine calcareous	E4.4	6170	
grasslands			
Heathland, scrub and tundra habitat	s (F)		
Tundra	F1		
Arctic, alpine and subalpine scrub	F2	4060	
habitats		4080	
Northern wet heath	F4.11	4010	4020
Dry heaths	F4.2	4030	2310, 2320, 4040
			5130
Maguis, arborescent matorral and	F5	unknown	5210, 5230, 5310, 5330
thermo-Mediterranean brushes			
Forest habitats (G)			
	G1 6	9110 9120 9130 9140	9210 9220 9270 9280
	31.0	0150	5210, 5220, 5210, 5200
Acidophilous Quarcus-dominated	61.8		
Actophilous Quercus-dominated	61.8	9190, 91A0	
woodialid Maaataa bia aa daataa bia Quanna	C1 A	0160 0100	0160
Mesotrophic and eutrophic Quercus	GI.A	9160, 9180	9160
woodland		9170, 9020	
Mediterranean evergreen (Quercus)	G2.1	9330, 9340	
woodland			
Abies and Picea woodland	G3.1	9410	9270, 9510, 9520
Pinus sylvestris woodland south of	G3.4	91C0	
the taiga			
Pinus nigra woodland	G3.5	9530	
Mediterranean Pinus woodland	G3.7	2270	9540
Spruce taiga woodland	G3.A	9010, 9050	
Pine taiga woodland	G3.B	9010	
Mixed taiga woodland with Betula	G4.2	-	
Mixed Abies-Picea Fagus woodland	G4.6	-	

Appendix 2

References for the critical load ranges for non-taiga woodlands

Appendix 2 gives a list of the references for the critical load ranges for non-taiga woodlands given in Table 9.3 in Chapter 9. Appendix 2A includes the references of N-addition experiments (US studies are given separately) and Appendix 2B of gradient and resampling studies (US studies are given separately).

Appendix 2A: N-addition experiments

The structure of the table below is as follows:

Column No.	Comment
1	EUNIS class at level 1
2,3	EUNIS class at level 2. If the study extends
	over more than one EUNIS class, the
	second class is given in column 3.
4	Relevance: rated according treatment
	concentrations (high concentrations or
	high background deposition = low
	relevance) and significance for under-
	standing of mechanisms
5	Location of the experiment
6	Tree species
7	Background atmospheric deposition at
	experimental site
8	Application rates (kg N ha-1 yr-1). Significant
	effects in bold (if indicated)
9	N form applied
10	Duration (years)
11	Observed effects
12	Type: soil, tree physiology or biodiversity
13	Reference

	13. Reference	Flückiger and Braun, 2010	Flückiger and Braun, 1999a	Balsberg-Påhlsson,	1992	Flückiger and Braun, 1999a	Hiltbrunner <i>et al.</i> , 2001	Tomova et al., 2002	Flückiger and Braun, 1999a	Flückiger and Braun, 1999b	Flückiger and Braun, 2010	Rühling and Tyler, 1991	Flückiger and Braun, 2010	Flückiger and Braun, 1999b
	12. Түре	Tree physiology	Tree physiology	Tree	physiology	Tree physiology	Tree physiology	Tree physiology	Tree physiology	Tree physiology	Tree physiology	Biodiversity	Biodiversity	Biodiversity
	10. Duration (years) 11. Observed effects	Growth decrease	Increased disease incidence	Increase in N concentration, decrease of P and Cu, decrease of total phenolic	compounds in leaves. No difference between low and hich treatment	Decrease in Mg, increase in N/Mg	Decreased starch concentration in roots	Changed phenolic composition in fine roots	Increased damage by Apiognomonia errabunda and Phomopsis sp.	Increase in shoot/root ratio	Decreased water use efficiency	Ceased fruit body production of almost all mycorrhizal species	Reduced mycelium density in in-growth mesh bags	Decrease in earthworm abundance by 66%, in parallel to a pH(CaCl ₂) decrease from 3.7 to 3.5
		∞		~	t	4-6	8	7	ß	9	12	3-4	16	~
	9. N form	NH ₄ NO ₃	NH ₄ NO ₃	NH ₄ NO ₃ with	2% Mg and 4% Ca	NH ₄ NO ₃	$\rm NH_4 NO_3$	NH ₄ NO ₃	$\rm NH_4 NO_3$	NH ₄ NO ₃	$\rm NH_4 NO_3$	NH ₄ NO ₃	NH ₄ NO ₃	NH ₄ NO ₃
	8. Application rates (kg N ha ⁻¹ vr ⁻¹)	0, 10, 20, 40, 80, 160	0, 10, 20, 40, 80, 160	0 AE 13E	cc1,c +,0	0, 10, 20, 40, 80, 160	0, 10, 20, 40, 80, 160	0 , 10, 20, 40, 80, 160	0, 10, 20, 40, 80, 160	0, 25, 50, 100, 200, 400	0, 10, 20 , 40, 80, 160	0 , 60,180	0, 20, 40, 80 , 160	0, 10, 20, 40, 80, 160
(7. Atmospheric deposition אר אנשטאריי) (kg N ha ⁻¹ ער ^י)	16	16-20			12-21	12-21	12-21	12-21	14	15	15-20	14	12
ean forest (non-taige	6. Tree species	Fagus sylvatica	Fagus sylvatica	Fagus sylvatica,	1 20 yrs	Fagus sylvatica	Fagus sylvatica	Fagus sylvatica	Fagus sylvatica	Fagus sylvatica (pot experiment)	Fagus sylvatica	Fagus sylvatica	Fagus sylvatica	Fagus sylvatica
experiments in Europe	5. Location of experiment	Switzerland	Switzerland	Couthors Cuodon		Switzerland	Switzerland	Switzerland	Switzerland	Switzerland	Switzerland	Sweden	Switzerland	Switzerland
dition (4. relevance (1=low, 3=high)	-	2	ſ	V	м	2	M	м	2	2	2	2	2
2 A: N-ac	3, EUNIS class?	10	10		0	10	10	10	10	10	10	10	10	10
endix 2		G1.6	G1.6	5		G1.6	G1.6	G1.6	G1.6	G1.6	G1.6	G1.6	G1.6	G1.6
Арр		G	ច	5	5	5	ច	ច	ច	ច	ច	ច	ច	5

	13. Reference	Deleporte and Tillier, 1 999	Murach and Parth, 1999	Persson and Ahlström, 2002	Flückiger and Braun, 1999a	Tomova et al., 2002	Gundersen, 1998	Jönsson et al., 2004	Brandrud, 1995; Brandrud and Timmermann, 1998	Hartley <i>et al.</i> , 1995	Xu et al., 2009	Gundersen <i>et al.</i> , 1998b
	12. Type	Biodiversity	Tree physiology	Tree physiology	Tree physiology	Tree physiology	Tree physiology	Tree physiology	Biodiversity	Biodiversity	Biodiversity	Tree physiology
	11. Observed effects	23 years after the one time application significant decreases of <i>Oribatida</i> , Gamarida, Collembola, Symphyla, Pseudoscorpionida	Increase in root biomass	Increased vitality of fine roots in the roof condition	Increase in N/P	Changed phenolic composition in fine roots	Increased N, decreased P, Mg, unchanged decomposition	Increased frost sensitivity of the bark, decreased growth	Reduction in fruitbody production of most mycorrhizal fungi (not Paxillus involutus and Lactarius rufus)	Increase in palatability to Orkney voles	Decrease of Collembola, disappearance of Tomocerus, Arrhopalites, Smithurus, Neanura	Increase in root biomass, decrease arginine concentration, increase in growth
	10. Duration (years)	-	2-3	0-4	9	2	m	10	1.5	-	13	4
	9. N form	NH ₄ NO ₃	NITREX roof clean	NITREX roof clean and added	NH ₄ NO ₃	NH ₄ NO ₃	NITREX	$(NH_4)_2 SO_4$	NH ₄ NO ₃	NH₄NO ₃	NH ₄ NO ₃	NITREX roof clean
	8. Application rates (kg N ha ⁻¹ yr ¹)	0, 100	reduction from 36 to <5	<4, 13, 48	0, 10 , 20, 40, 80, 160	0, 10, 20, 40, 80, 160	0, 25	0, 100	0, 35	0, 52	0, 25	reduction from 56 to 4
~	noiticodeb دامه مرود (kg N ha ^{r 1}) راده (kg N ha ^{r 1})	10-20			12-21	12-21	15-20	16	13		12	
an forest (non-taiga	6. Tree species	acid beech forest	Pseudotsuga menziesii, Picea abies	Picea abies	Picea abies	Picea abies	Picea abies	Picea abies	Picea abies	Calluna vulgaris	Picea abies	Pinus sylvestris
experiments in Europe	5. Location of experiment	France, Eastern Brittany	Germany (Solling)	Sweden, Gårdsjön	Switzerland	Switzerland	Denmark (Klosterhede)	Skogaby	Sweden (Gårdsjön)	UK	Switzerland	The Netherlands (Ysselsteyn)
addition (3. EUNIS Class2 4. relevance (1=low, 3=high)	7	м	M	m	ю	2	-	N	2	2	2
x 2A: N-a	2. EUNIS dass	9.	1.2	A. ⁸	5.1	1.2	.1	5.1	5.A	5.1	L.2	5.4
Appendi	r level sinus .r	6	33 63	33	33	33	33	33	3	53 G:	33	33 G
-		J	5	5	5	J	5	5	5	5	5	5

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	13. Reference	Termorshuizen, 1990	Termorshuizen, 1990	Boxman et al., 1995	Strengbom et al., 2002	Nordin <i>et al.</i> , 1998	Nilsson and Wallander, 2003	Strengbom <i>et al.</i> , 2001	Strengbom et al., 2002	Nordin <i>et al.</i> , 1998	Nordin <i>et al.</i> , 2009
	12. Tune	Biodiversity	Biodiversity	Biodiversity	Tree physiology	Tree physiology	Biodiversity	Biodiversity	Biodiversity	Biodiversity	Biodiversity
) 11. Observed effects	Reduced fruit body production, especially by the (NH,),SO, treatment	Reduced total number of mycorrhiza per plant dry weight	Increase in species diversity of microarthropods	Increase damage by Valdensia heterodoxa and Podosphaera myrtillina	Increased damage by the moth Orgyia antrana	Reduced extramatrical mycelium	Decrease of fruit body production of the EM fungus Russula still reduced 50 years after termination of N addition, Decrease of the moss Hylocomium splendens	Increase in Deschampsia flexuosa, decrease in Vaccinium myrtillus	Increase of arginine concentrations in the mosses Pleurozium schreberi and Dicranum majus	Increase of infection of Vaccinium myrtillus with insects and fungi, decrease of Varcinium
	10. Duration (vears)	m	m	3(?)	m	-	10	15	м	2	12
	א form .9	NH ₄ NO ₃ or (NH ₄) ₃ SO ₄	(NH ⁴) ₂ SO ⁴ or NaNO ₃	NITREX roof clean	NH ₄ NO ₃		(NH ₄) ₂ SO ₄	NH ₄ NO ₃	NH ₄ NO ₃	NH ₄ NO ₃	NH ₄ NO ₃
	8. Application rates (kø N ha ⁻¹ vr ¹)	0, 30, 60	0, 50	reduction from 56 to 4	0, 12.5, 50	0, 12.5, 50	0, 100	0, 96	0, 12.5, 50 (not tested separately but significant trend)	0, 12.5 , 50	0, 12.5, 50
	ר Atmospheric deposition (kg N haי' איי ^י)				<u>~</u>	ň	5		ň	Ň	<u>ب</u>
an forest (non-taiga)	6. Tree species	Pinus sylvestris	Pinus sylvestris (seedings in pot)	Pinus sylvestris	Vaccinium myrtillus 2	Vaccinium myrtillus 2	Picea abies	Picea abies	Picea abies	Picea abies	Picea abies
xperiments in Europ€	5. Location of experiment	The Netherlands	The Netherlands	The Netherlands (Ysselsteyn)	North Sweden	North Sweden	Sweden (Skogaby)	Sweden Hesselman	Sweden	Sweden	Sweden
ddition e	4. relevance (1=low, 3=high) 3=high)	2	2	2	ю	ю	-	-	2	2	2
2 A: N-ac	Z. FUNIS class?	4	4	4	А	А	_	٩	A	A	٩
pendix	r Ievel Rinua .	G3.	63.	63.	63.	63.	G3.	G3.	G3	<u>6</u> 3	63.
Ą		ß	G	63	63	G	6	63	ß	ß	ß

	rved effects 12. Type 13. Reference	in Deschampsia flexuosa Biodiversity Torstensson, 1995	moss species Brachythecium Biodiversity Martakis, 1992	moss species Pleurozium Hylocomium splendens	Pleurozium schreiberi, Dicranum Biodiversity Mäkipää, 1998	rance of Cladina species, Biodiversity 2001	of the moss <i>Pleurozia schreberi</i> Biodiversity 2001 fter cessation of the treatment
	חס. Duration (years) ב ס	5 Increase	18-20 Increase spp.	18-20 Decrease schreiberi	4 Decrease polysetun	29 Disappe	18 Decrease years a
	9. N form		(0			$\rm NH_4 NO_3$	$\rm NH_4 NO_3$
	8. Application rates (kg N ha ⁻¹ yr')	0, 5, 10, 20	0, 20, 40, 60 (Lisselb	0, 30, 60 (Norrliden)	0, 25, 30	0, 34, 68	0, 108
(a)	۲. Atmospheric deposition (kg N ha ⁻¹ yr ⁻¹)	ۍ.			~.		
an forest (non-taig	6. Tree species	Picea abies, Pinus sylvestris	Pinus sylvestris	Pinus sylvestris	Pinus sylvestris	Pinus sylvestris	Pinus sylvestris
xperiments in Europe	5. Location of experiment	Sweden (Söderhamn)	Sweden (Lisselbo)	Sweden (Norrliden)	Finland	Sweden Norrliden	Sweden Norrliden
dition ey	4. relevance (1=low, 3=high)	m	2	2	м	2	-
ו: N-ad	3. EUNIS class2	G3.B					
ndix 2A	2. EUNIS class1	G3.A	G3.B	G3.B	G3.B	G3.B	G3.B
Apper	T. EUNIS level 7	ß	63	63	63	ß	63

	13. Reference	Carreiro <i>et al.</i> , 2000	McNulty <i>et al.</i> , 1996	Magill and Aber, 1998	Frey et al., 2004	Perkins et al., 2000	:y Avis et al., 2008	y Frey et al., 2004	y zoo7	Egerton- y Warburton and Allen, 2000
	12. Tane	Soil	Soil	Soil	Soil	Tree physiology	Biodiversit	Biodiversit	Biodiversit	Biodiversit
	11. Observed effects	Increase of litter decomposition in low lignin litter, decrease in high lignin litter	Increased net nitrification potential	Decreased litter decomposition	Reduced total microbial biomass	Increased winter injury	20% drop in EM fungi richness	Reduced species diversity of belowground EM mycorrhiza and shift in species composition.	Significant decline of AM fungi in 2 out of 4 stands	Shift in AM community composition
	10. Duration (years)	3.4, 4.0, 4.5 yrs	9	6 yrs		8	M	14	12	-
	9. N form	NH ₄ NO ₃	NH ₄ Cl, NaNO ₃	NH ₄ NO ₃	NH ₄ NO ₃		KNO ₃ and (NH ₄) ₂ SO ₄	NH ₄ NO ₃	NaNO ₃	NH ₄ NO ₃
	8. Application rates Ke N ha ⁻¹ vr ⁻¹	0, 20, 80	0, 15.7, 19.8, 25.6, 31.4	0, 50, 150	0, 50, 150	0, 15.7, 19.8, 25.6, 31.4	0, 21	0, 50	0, 30	0, 60
	ר Atmospheric deposition 7. אנשטאין אר ^{ין} אר ^{ין}) ארי ^ן)	10	5	ø	ω	5.4b	7	ø	4.8- 8.3	са. 10
	6. Tree species	Cornus florida, Acer rubrum, Quercus rubra	Picea rubens	Red pine, red maple, yellow birch and black oak	Red pine, red maple, yellow birch and black oak	Picea rubens	Quercus alba, Quercus rubra	Pinus resinosa, Quercus velutina	Acer saccharum	coastal sage scrub (Artemisia californica, Encelia farinosa, Eriogonum fasciculatum, Salvia
	5. Location of experiment	Louis Calder Center, Armonk, NY	Vermont, US	Harvard forest	Harvard forest	Mount Ascent, Vermont, US	US (Chicago area)	US (Harvard Forest)	US (Michigan)	US (California)
nents in US	3, EUNIS class2 4. relevance (1=low, figh)	m	м	2	2	м	м	-	2	-
ition experir	Caselo SINU3 .2	(E1)	(G3)	(64)	(64)	(G3)	(G1.A)	(64)	(G1.A)	(F3)
N-add	r level 7.									

Appendix 2B: Gradient and resampling studies

The structure of the table below is as follows:

Column No.	Comment
1	EUNIS class at level 1
2,3	EUNIS class at level 2. If the study extends
	over more than one EUNIS class, the
	second class is given in column 3.
4	Relevance: Relevance high for large
	datasets and for clear effects at low
	deposition levels. Relevance low for time
	series as N deposition is just one possible
	explaining variable or for spatial
	correlations with confounding variables
5	Location of field study
6	Tree species
7	Range of atmospheric deposition
	(w=wet, t=throughfall)
8	Effect threshold suggested by the authors
	or derived directly from the data (if
	applicable)
9	Observed effects
10	Type: soil, tree physiology or biodiversity
11	Reference

dix Z	S: Gradien class 2	tand (dgid=8	esampling studie	s in European forests (n	ion-taiga) 7.	8. Suggested			
	SINU3 .5	4. relevai 5 ,wol=1)	5. Location of field studv	6. Tree species	Atmospheric deposition (kg N ha ^{.1} vr ¹)	effect threshold (kg N ha ⁻¹ vr ⁻¹)	9. Observed effects	10. Тире	11. Reference
		m	metaanalysis of 900 studies			<5 <	Increased litter decomposition	Soil	Knorr et al., 2005
		M	metaanalysis of 900 studies			>5-10	Decreased litter decomposition	Soil	Knorr et al., 2005
	101	м	Bayern	ć	8-18	15	Nitrate leaching	Soil	Kölling and Neustifter, 1997
	131	м	Europe	various, C:N < 22	10-55 t	10	Nitrate leaching	Soil	ICP Forests, 2005
		M	Europe	various	<2->37	between 9.6 and 21	Imbalanced nutrition in 109 ICP forest plots	Tree physiology	De Vries et al., 2003
		м	Europe	ICP forest plots	2-60	10	Occurrence of N indicating species	Biodiversity	UNECE, 2006
	. –	_	The Netherlands		20 (1958) vs 40 (1981)		Disappearance of all lichens	Biodiversity	Dirkse and Van Dobben, 1989
		2	The Netherlands, Denmark, Sweden			5-10	Decline of lichens with blue-green algae	Biodiversity	Göransson, 1990
		2	The Netherlands		40		Large number of nitrophilous species	Biodiversity	Dirkse and Van Dobben, 1989
		2	Sweden		3-6 vs 15-25		Decrease of snails (acidification effect!)	Biodiversity	Gärdenfors <i>et al.</i> , 1995
	141	ю	Europe	deciduous	5-40 t	15	Nitrate leaching	Soil	De Vries et al., 2003
		_	France	Fagus sylvatica	20-30		Changes in foliar concentrations of N (increase), P, K, Mg, Ca (decrease) within 27 years	Tree physiology	Duquesnay et al., 2000
		-	Switzerland	Fagus sylvatica, Picea abies	15-35		Changes in foliar concentrations of N, P, Mn within 15 years	Tree physiology	Flückiger and Braun, 1998
		2	Switzerland	Fagus sylvatica	25-50		Increase of stem increment when P supply is sufficient	Tree physiology	Braun et al., 2009
		-	Switzerland	Fagus sylvatica	15-35		Increased windthrow with increasing leaf N concentration	Tree physiology	Braun et al., 2002
	. 0	2	South Sweden (Scania)	Fagus sylvatica	15-20;20-25	20	Increased bark lesions by Nectria coccinea	Tree physiology	Jönsson, 1998
		_	Switzerland	Fagus sylvatica	18-35		Decreasing relative length of fine roots	Tree physiology	Braun et <i>a</i> l., 2005
		2	Sweden	Fagus sylvatica	15-25		Nectria coccinea more frequent at sites with N-deposition 20-25 compared to 15-20 kg N ha ⁻¹ vr ⁻¹	Tree physiology	Westling <i>et al.</i> , 1992

		pue	nann,		4	2004	bne			
	11. Reference	Falkengren-Grerup a Diekmann, 2003	Walther and Grundr 2001	Bost, 1991	Thimonier et al., 199.	Flückiger and Braun,	Falkengren-Grerup a Diekmann, 2003	Nilsson et al., 2007	Mitchell et al., 2003	Tyler, 1987
	10. Type	Biodiversity	Biodiversity	Biodiversity	Biodiversity	Biodiversity	Soil	Biodiversity	Biodiversity	Biodiversity
	9. Observed effects	Ellenberg site index increased in the N exposed regions, 40–80% higher soil N mineralisation rate, 2–90% higher nitrification rate and 10– 25% lower C:N ratio in the region with the highest deposition, Critical loads for the most acid soils lower than less acid soils	Decreased species diversity, increase in nitrophilous species	Eutrophication and acidification	Increase in the frequency of N indicating species	Increase of the cover of <i>Rubus fruticosus</i> at N deposition f >20 kg N ha ⁻¹ yr ⁻¹	Increased mineralisation and nitrification. gradient study	Trend to reduced EM mycelial growth (extramatrical)	Loss of epiphytic lichens, loss of the bryophytes Pagiochila atlantica and P. spinalosa	Increased frequency of nitrophilous species in the higher N deposition region
	8. Suggested effect threshold (kg N ha ⁻¹ yr ⁻¹)	2-10				20	7-10		11-18	
ion-taiga)	7. Atmospheric deposition (kg N ha ⁻¹ yr')	7-17	time series 45 yr; current deposition 30->40	time series 19yr, current deposition 15-20	time series 19 yr; approx. 20 kg N ha ⁻¹ yr ⁻¹	12-38	17 vs 7-10	20 vs 10	9.6-53	6-8 vs 12-15
s in European forests (n	6. Tree species	Quercus robur, Q. petraea		deciduous forest (beech, oak, hornbeam)	deciduous forest (beech, oak, hornbeam)	Fagus sylvatica, Picea abies	Quercus robur/petraea	Quercus robur	Atlantic oakwoods	Quercus robur
resampling studie	5. Location of field study	Sweden	Switzerland	France	France	Switzerland	southern Sweden	Sweden	England, Scotland	Sweden
nt and	4. relevance (1=low, 3=high)	Μ	7	7	2	м	м	2	м	м
radier			1.8	1.A	1.A	3.1			1.83	
2 B: G	S. EUNIS class 2	.e	.9	9.	9	9.	×.	ø.	8	×.
endix	2. EUNIS class1	6	61.	61.	61	15	15	19	15	61
App	I. EUNIS level 7	6	5	19	61	6	5	5	5	6

		986; 395						g		
	11. Reference	Falkengren-Grerup, 1 Falkengren-Grerup, 1	Brunet et al., 1998	Diekmann <i>et al.</i> , 1999	Kuhn et al., 1987	Lameire et al., 2000	Thimonier et al., 1992	Gundersen et al., 1998	Nilsson et al., 1998	De Vries et al., 2003
	10. Type	Biodiversity	Biodiversity	Biodiversity	Biodiversity	Biodiversity	Biodiversity	Soil	Soil	Soil
	9. Observed effects	Increase in frequency of nitrophilous species	Increase of nitrophilous and acid tolerant species in the high N deposition region	Increase of the N Ellenberg between 1983 and 1993, increase of the nitrification ratio	Increase in nitrophilous species	Significant increase of nitrogen indicators, decline of moisture indicators	Increase in Ellenberg's ecological N value	Nitrate leaching	Nitrate leaching	Nitrate leaching
	8. Suggested effect threshold (kg N ha ⁻¹ yr ⁻¹)							10	>15	20
on-taiga)	7. Atmospheric deposition (kg N ha ⁻¹ yr ¹)	7-12 vs 15-25 (time series)	6-9 vs 7-11 vs 14-20	time series, 9-20	time series 43 yr; current deposition 15-30)	time series 20 yr; current deposition 25-30	time series 18 yr; past deposition 20-30			2-45 t
s in European forests (n	6. Tree species	Fagus sylvatica, Quercus robur, Carpinus betulus	mixed oak	Quercus robur, Q. petraea, Fagus sylvatica	Quercus spec.	mixed deciduous forest	mixed hardwood forest	mostly coniferous	coniferous	coniferous
l resampling studie	5. Location of	Sweden	Sweden	Sweden (Småland, Skåne)	Switzerland	Belgium	France	Europe	Sweden	Europe
lient and	4. relevance []=low_3=bigh)	2	m	Μ	Ν	5	2	m	m	m
B: Grad	2. EUNIS class 2	G1.6		G1.6						
andix 2	2. EUNIS class1	G1.8	G1.8	G1.8	G1.A	G1.A	G1.A	63	63	63
Appe	l level SINU3 . l	5	5	5	5	61	6	ß	ß	ß

11. Reference	Matzner and Murach, 1995	Hippeli and Branse, 1992; Nebe, 1991	Quiring et al., 1997	Meyer et al., 2008	Schlechte, 1986	Erland <i>et al.</i> , 1999	Kraft <i>et al.</i> , 2000; Rodenkirchen, 1992	NEGTAP, 2001	Wöllecke <i>et al.</i> , 1999	Strengbom <i>et al.</i> , 2003
10. Type	Tree physiology	Tree physiology	Tree physiology	Tree physiology	Biodiversity	Biodiversity	Biodiversity	Tree physiology	Biodiversity	Biodiversity
9. Observed effects	Decrease of total fine root biomass	Increase N concentration, decrease Mg concentration	Arginine concentrations in needles correlated with N deposition	Increased risk of broken stem with increased N concentration in the phloem	Reduced species diversity of basidiomycetes, especially ectomycorrhiza	Reduced species diversity of ectomycorrhiza, reduced number of mycorrhizal roots	Increasing abundance of nitrophilous species	Insect damage positively correlated with N deposition	Reduced mycorrhizal projection area, decrease in root tips, reduced number of morphotypes	Increase in Deschampsia flexuosa, decrease in Vaccinium myrtillus and V. vitis-idaea
8. Suggested effect threshold (kg N ha¹ yr'l)	N concentration in soil water >2 mg /l (-> throughfall range 13-33 kg N ha ⁻¹ yr ⁻¹)									~ 6
7. Atmospheric deposition (kg N ha ⁻¹ yr')		15-25	14-37	15-45	23 vs 42	15 vs. 27	15-20	7-22m	~12 vs >35	
6. Tree species	Picea abies	pine, spruce	Picea abies	Picea abies	Picea abies	Picea abies	Abies and Picea with Fagus and Pinus	Pinus sylvestris	Pinus sylvestris	Pinus sylvestris
5. Location of field study	Germany	Germany (Brandenberg, Mittelgebirge)	Switzerland	Switzerland	Germany	South Sweden	Germany	Я	Germany	Sweden
4. relevance (1=low, 3=high)	50	_	_	_	~	~	M	~	~	10
2 sselb SINU3 .5		G3.4	·							
Teelo SINUE .2	53.1	53.1	53.1	53.1	53.1	53.1	53.1	53.4	53.4	53.4
l level SINU3 .l	63	E G	G	e	ß	ß	ß	G	ß	e
	1 Suggested 1 Sug	1 3 G3.1 3 Germany R N	I ENVISE class 2 C EEVINE class 45 7. Suggested Amospheric effect effect <t< td=""><td>1. EUNIS I Constructed 1. EUNIS I Constructed 3. Suggested Amospheric 6 ffect. 1. EUNIS I Constructed 4. Field study 6. Trespecies 8. Suggested Amospheric 6 ffect. 1. EUNIS I Constructed 6. Trespecies 6 aposition 8. Namespheric 8. Suggested Amospheric 6 ffect. 1. Ele Dov, 3. Station 6. Trespecies 6 aposition 1. Reshold 10. Type 11. Reference 1. Ele Dov, 3. Station 1. Reshold 1. Sold water > 2 9. Observed effets 10. Type 11. Reference 1. Ele Dov, 3. Station 1. Sold water > 2 1. Sold water > 2 9. Observed effets 10. Type 11. Reference 1. Ele Dov, 3. Station 1. Sold water > 2 1. Sold water > 2 10. Type 11. Reference 1. Ele Dov, 3. Station 1. Sold water > 2 1. Sold water > 2 10. Type 11. Reference 1. Ele Dov, 3. Station 1. Sold water > 2 1. Sold water > 2 10. Type 11. Reference 1. Ele Dov, 3. Station 1. Ele Dov, 3. Station 1. Ele Dov, 3. Station 1. Rephysiolog 1. Rephysiolog 1. Ele Dov, 3. Station 1. Sold water > 2 1. Sold water > 2 <</td><td>Image: Image: Image:</td><td>Independent T. Subsected Apposition T. Subsected Apposition T. Subsected Apposition Subsected Apposidion Subsected Apposition</td><td>Indication Indication Indicataned Indicataned Indic</td><td>International conditional control Consistent control</td><td>I convision Autospheric denoting interstol Autospheric denoting denoting Autospheric denoting Autospheric denoting Autospheric denoting G1 3 6 remote denoting 5. reesocies (ganstim) (restation denoting 1. relevance denoting 1. relevance G3 3 6 emany denoting (restation denoting 0. observed effect. (o. type 1. relevance G6 3.1 9 6 emany denoting (restation denoting 0. observed effect. (o. type 1. relevance G6 3.1 1 8 (restation denoting 1. restation denoting 1. restation denoting 1. restation G6 3.1 1 Sutterland Protochics 0. restation 1. restation G6 3.1 1 Sutterland Protochics 0. restation 1. restation G6 3.1 1 Sutterland Protochics 0. restation 1. restation G6 3.1 1 Sutterland Protochics 0. restation 1. restation G6 3.</td><td>Image: Section of the secting of the secting of the sectio</td></t<>	1. EUNIS I Constructed 1. EUNIS I Constructed 3. Suggested Amospheric 6 ffect. 1. EUNIS I Constructed 4. Field study 6. Trespecies 8. Suggested Amospheric 6 ffect. 1. EUNIS I Constructed 6. Trespecies 6 aposition 8. Namespheric 8. Suggested Amospheric 6 ffect. 1. Ele Dov, 3. Station 6. Trespecies 6 aposition 1. Reshold 10. Type 11. Reference 1. Ele Dov, 3. Station 1. Reshold 1. Sold water > 2 9. Observed effets 10. Type 11. Reference 1. Ele Dov, 3. Station 1. Sold water > 2 1. Sold water > 2 9. Observed effets 10. Type 11. Reference 1. Ele Dov, 3. Station 1. Sold water > 2 1. Sold water > 2 10. Type 11. Reference 1. Ele Dov, 3. Station 1. Sold water > 2 1. Sold water > 2 10. Type 11. Reference 1. Ele Dov, 3. Station 1. Sold water > 2 1. Sold water > 2 10. Type 11. Reference 1. Ele Dov, 3. Station 1. Ele Dov, 3. Station 1. Ele Dov, 3. Station 1. Rephysiolog 1. Rephysiolog 1. Ele Dov, 3. Station 1. Sold water > 2 1. Sold water > 2 <	Image:	Independent T. Subsected Apposition T. Subsected Apposition T. Subsected Apposition Subsected Apposidion Subsected Apposition	Indication Indicataned Indicataned Indic	International conditional control Consistent control	I convision Autospheric denoting interstol Autospheric denoting denoting Autospheric denoting Autospheric denoting Autospheric denoting G1 3 6 remote denoting 5. reesocies (ganstim) (restation denoting 1. relevance denoting 1. relevance G3 3 6 emany denoting (restation denoting 0. observed effect. (o. type 1. relevance G6 3.1 9 6 emany denoting (restation denoting 0. observed effect. (o. type 1. relevance G6 3.1 1 8 (restation denoting 1. restation denoting 1. restation denoting 1. restation G6 3.1 1 Sutterland Protochics 0. restation 1. restation G6 3.1 1 Sutterland Protochics 0. restation 1. restation G6 3.1 1 Sutterland Protochics 0. restation 1. restation G6 3.1 1 Sutterland Protochics 0. restation 1. restation G6 3.	Image: Section of the secting of the secting of the sectio

	11. Reference	Pitcairn et al., 1998	Roelofs et al., 1985	Nellemann and Thomsen, 2001	Bergquist and Örlander, 1998b; Bergquist and Örlander, 1998a	Bråkenhielm and Quinghong, 1995	Bråkenhielm, 1991; Thomsen, 1992	Poikolainen <i>et al.</i> , 1998	Rosén et al., 1992	Strengbom et al., 2003	Bråkenhielm and Quinghong, 1995
	10. Type	Biodiversity	Soil	Tree physiology	Biodiversity	Biodiversity	Biodiversity	Biodiversity	Biodiversity	Biodiversity	Biodiversity
	9. Observed effects	Increase of nitrophilous species along a transect close to livestock units	Ammonium accumulation, gradient study	Increase and subsequent decline of stem increment	Increased frequency of browsed young trees	Increased weighted mean sensitivity (WMS) of lichens	Colonisation of spruce needles with green algae, mainly <i>Pleurococcus viridis</i>	Increase abundance of the green alga Scoliciosporum chlorococcum on conifers	Correlation between Deschampsia flexuosa cover and nitrogen deposition, decrease of Vaccinium myrtillus in the high N deposition region	Increase in Deschampsia flexuosa, decrease in Vaccinium myrtillus and V. vitis-idaea	Increased algal cover
	8. Suggested effect threshold (kg N ha ⁻¹ yr ⁻¹)	15-20	10-15	7-8.5		6-8	>5 (throughfall)	3-4	7-11	>6	5-8
ion-taiga)	7. Atmospheric deposition (kg N ha- ¹ yr')	5-80		<7; 7-15; >15 (w) + 10-20% dry deposition	12-18 vs 18-29						
es in European forests (r	6. Tree species	Pinus sylvestris, Fagus sylvatica, coniferous shelter belts''	Pinus nigra	Picea abies	Picea abies	Pinus sylvestris			coniferous forests	Picea abies	Betula pendula
d resampling studie	ر المراجع ال المراجع المراجع	лк	The Netherlands	Southern Norway	Sweden	Sweden	Norway, Sweden	Finland	Sweden	Sweden	
entan	4. relevance	м	2	Μ	2	2	2	2	м	Μ	2
Gradi	S. EUNIS class 2	G1.6									
ndix 2B:	Ceaselo SINUE .2	G3.4	G3.5	G3.A	G3.A	G3.B	G3.B	G3.B	G3.B	G3.B	G4.2
Apper	l ləvəl SINUƏ .r	63	G3	63	G3	G3	G3	G3	63	G3	64

			ס		
	11. Reference	Fenn et al., 2008	Egerton-Warburton an Allen, 2000	Fenn <i>et al.</i> , 2008	Lilleskov et al., 2002
	10. Type	Tree physiology	Biodiversity	Biodiversity	Biodiversity
	9. Observed effects	26% reduction in fine root biomass at a throughfall N deposition of 17 kg N ha ⁻¹ yr ⁻¹ and streamwater peak NO ₅ concentration of 10.8 meq l ⁻¹	Shift in AM community composition	Shift in lichen community	Reduced species diversity of ectomycorrhiza sporocarps in high deposition sites
	8. Suggested effect threshold (kg N ha ⁻¹ yr ⁻¹)			3.1	<7
	7. Atmospheric deposition (kg N ha ⁻¹ yr ¹)	1.2-1.1	10-35	1.2-71	<1 -18 (bulk)
	6. Tree species	Pinus ponderosa	coastal sage scrub (Artemisia californica, Encelia farinosa, Eriogonum fasciculatum, Salvia apiana)	Pinus ponderosa	Picea glauca, Betula kenaica, Populus tremuloides, P. balsamifera
resampling studies in US	5. Location of field study	US (California)	US (California)	US (California)	Alaska
ent and	4. relevance (1=low, 3=high)	N	~	2	Ν
3: Gradi	S 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	0		~	~
indix 2E	2. EUNIS class1	(G3.7)	(F3)	(G3.7)	(G3.A
Appe	L I9V9I SINU3 . L				

Appendix 3

References for the critical load ranges for taiga woodlands

Appendix 3 gives a list of the references for the critical load ranges for taiga woodlands given in Table 9.3 in Chapter 9. Appendix 3A includes the references of N-addition experiments and Appendix 3B of gradient and resampling studies.

Appendix 3A: N-addition experiments

The structure of the table below is as follows:

Column No.	Comment
1	EUNIS class at level 1
2, 3	EUNIS class at level 2. If the study extends over more than one EUNIS class, the
4	Relevance: rated according treatment concentrations (high concentrations or high background deposition = low relevance) and significance for understan- ding of mechanisms
E	Location of the experiment
6	Tree species
7	Background atmospheric deposition at experimental site
8	Application rates (kg N ha ⁻¹ yr ⁻¹). Significant effects in bold (if indicated)
9	N form applied
10	Duration (years)
11	Observed effects
12	Type: soil, tree physiology or biodiversity

Reference 13

	3. Reference	JN/FCE report. Recent results ind updating of scientific and echnical knowledge. Workshop an effects of low-level nitrogen leposition. UN report ECE/EB.AIR WG.1/2007/15 (http://www. carp.se/news /startsidenyheter/ itrogencriticalloadsforterrestrial cosystemsinlowdepositionareas 5.6579ab601 1d9b20740f8 0021606.html)	cellner and Redbo-Torstensson, 995	Jordin et al., 2005	itrengbom et al., 2002	Jordin et al., 1998	itrengbom <i>et al.</i> , 2001
	12. Type	Biodiversity	Biodiversity 1	2	Biodiversity	Ecophysiology/ _N biodiversity	Biodiversity S
	11. Observed effects	Increase in Deschampsia flexuosa	Increased density of D. flexuosa at 5 kg N ha ⁻¹ yr ⁻¹ , increased density of T. europaea at 10 kg N ha ⁻¹ yr ⁻¹		Increase in D <i>eschampsia</i> flexuosa, increase damage by Valdensia heterodoxa	Increased damage by Lepidoptera larvae and two parasitic fungus, altered composition of amoni acids	Decrease of fruit body production of the EM fungus Russula still reduced 50 years after termination of N addition, decrease of the moss Hylocomium splendens
	10. Duration (years)	4	ы		м	F	15
	9. N form	HN S S S S S S S S S S S S S S S S S S S		NH₄NO ₃	NH ₄ NO ₃	NH ₄ NO ₃	NH ⁴ NO ₃
	8. Application rates (kg N ha' ¹ yr')	ک ٥	0, 5, 10, 20		0, 12.5, 50 (not tested separately but significant trend)	0.5, 12.5, 25, 50	0, 96-180
	7. Atmospheric deposition (kg N ha' ¹ yr ¹)	* ~	2-6	2*->12	2*	2*	2-3*
a woodland	6. Tree species	Picea abies	Picea abies/ Pinus sylvestris		Picea abies	Picea abies	Picea abies
xperiments taig	5. Location of	North Sweden (Svartberget)	Sweden (Söderhamn)		N Sweden (Svartberget)	North Sweden (Svartberget)	N Sweden, Hesselman
dition e	4. Relevance (faid=5 ,wol=1)	M	ю		ю	5	7
A: N-ad	3. EUNIS class2						
andix 3/	2. EUNIS class1	G3.A	G3.A	G3.A	G3.A	G3.A	G3.A
Appe	T. EUNIS level 7	63	63	ß	63	63	63

	13. Reference	Brandrud, 1995; Brandrud and Timmermann, 1998	Nordin et al., 2006	Nordin et al., 2009	Mäkipää, 1998	Manninen <i>et al.</i> , 2009	Forsum et al., 2006	Bobbink, 2004	Dirkse and Martakis, 1992
	12. Type	Biodiversity	Ecophysiology	Biodiversity	Biodiversity	Biodiversity	Ecophysiology	Biodiversity	Biodiversity
	11. Observed effects	Reduction in fruit body production of most mycorrhizal fungi (not Paxillus involutus and Lactarius rufus)	Effect of N addition differ depending on N form	Increase of infection of Vaccinium myrtillus with insects and fungi, decrease of Vaccinium	Decreased abundance of Pleurozium schreberi and Dicranum polysetum	Reduced V. myrtillus and increased D. flexuosa	N uptake by mosses similar to control N treatment after 3 yrs	No overall negative effect on species richness (except for ground living lichens), but effects on composition increases as N input increases.	Increase moss species Brachythecium spp.
	10. Duration (years)	1.5	4	12	4	Ŀ			18-20
	9. N form	NH ₄ NO ₃	0,12.5 and 50 added as NH ₄ NO ₃ (NH ₄) ₂ SO ₄ , or KNO ₃	NH ₄ NO ₃		Urea			
	8. Application rates (kg N ha ⁻¹ yr ⁻¹)	0, 35	0,12.5,50	0, 12.5, 50	0, 25(+305)	0, 20, 40, 80	0,50+ recovery from 50 (3 yrs)	0-300	0, 20, 40, 60 (Lisselbo)
	7. Atmospheric deposition (kg N ha ^{.1} yr ¹)	13	۲* ۲	× 2	2.5	2?	2*		10
a woodland	6. Tree species	Picea abies	Picea abies	Picea abies	Picea abies	Picea abies	Picea abies		Pinus sylvestris
xperiments taig	5. Location of experiment	Sweden (Gårdsjön)	N Sweden (Svartberget)	N Sweden (Svartberget)	S Finland	NE Finland	N Sweden (Svartberget)	Review report of other studies from boreal forests	Sweden (Lisselbo)
addition ϵ	4. Relevance (1=low, 3=high)	5	2	7	-	2	-	5	2
3 A: N-			-	~	7	-	_	A G3.E	~
pendix		G3.A	G3.A	G3.A	G3.A	G3.A	G3.A	G3.A	G3.E
App		63	63	63	G	G	G3	63	G

		2; Van gbom,					
	13. Reference	Dirkse and Martakis, 199; Dobben <i>et al.</i> , 1999; Stren <i>et al.</i> 2001	Mäkipää, 1998	Chen and Högberg, 2006	Högberg et al., 2006	Skrindo & Økland, 2002	position of N in the area is
	12. Type	Biodiversity	Biodiversity	Soil chemistry	Soil and plant chemical composition	Biodiversity) ₃ ha ⁻¹ yr ⁻¹ . Dry de
	11. Observed effects	Decrease moss species Pleurozium schreiberi, Hylocomium splendens, Disappearance of Cladina species, altered mychorrizal community composition. No recovery after 9 yrs	Decrease Pleurozium schreiberi, Dicranum polysetum	Increased N mineralisation rates, also after cessation.	Increased N accumulation, increased N concentration of pine needles, no effect on pH in moor layer, decreased pH in mineral soil, decreased concentrations of base cations in mineral soil	Reduced occurrence of some mosses and lichens	g NH $_4^+$ ha $^{-1}$ yr $^{-1}$ and 0.7–1.7 kg NC
	10. Duration (years)	18-29	4	30	20	7	0.4-0.9 kg
	9. N form	NH4 NO3		NH ₄ NO ₃	NH ₄ NO ₃	NH ₄ NO ₃ + Mg	osition of N o
	8. Application rates (kg N ha '' yr')	0, 34, 68, 108 ceased for 9 yrs (Norrliden)	0, 25, 30	0, 34,68, +108 ceased for 10 yrs	0,34,68, +108 ceased for 10 yrs	0, 30, 90	tackground wet dep
	7. Atmospheric deposition (kg N ha ⁻¹ yr ¹)	2-3*	۰.	2-3*	2-3*	5-8	in et al., 2006). E
ga woodland	6. Tree species	Pinus sylvestris	Pinus sylvestris	Pinus sylvestris	Pinus sylvestris	Pinus sylvestris	position (Nord 5 kg N ha ⁻¹ vr ⁻¹ .
xperiments taig	5. Location of experiment	Sweden (Norrliden)	Finland	N Sweden, (Norrliden)	N Sweden, (Norrliden)	Norway	alues of wet de
ddition e	4. Relevance (1=low, 3=high)	7	2	2	~	2	easured v stal N dei
3 A: N-ac	3. EUNIS class2						rom me t. i.e. to
endix 3	2. EUNIS class1	G3.B	G3.B	G3.B	G3.B	G3.B	erived fr inifican
App	<u>l ləvəl SINUƏ .</u>	63	63	63	G	63	* De insie
Appendix 3B: Gradient and resampling studies

The structure of the table below is as follows:

Column No.	Comment
1	EUNIS class at level 1
2,3	EUNIS class at level 2. If the study extends
	over more than one EUNIS class, the
	second class is given in column 3.
4	Relevance: Relevance high for large
	datasets and for clear effects at low
	deposition levels. Relevance low for time
	series as N deposition is just one possible
	explaining variable or for spatial correlati-
	ons with confounding variables
5	Location of field study
6	Tree species
7	Range of atmospheric deposition
8	Effect threshold suggested by the authors
	or derived directly from the data (if
	applicable)
9	Observed effects
10	Type: soil, tree physiology or biodiversity
11	Reference

					1005	1221 (B)		en, 1992								98b;	98a
		11. Reference	Strengbom et al., 2003		Bråhanhord Ouinachan	biakeiiiieiiii allu Quiligiioi		Bråkenhielm, 1991; Thoms			Poikolainen <i>et al.</i> , 1998			Rosén et al., 1992		Bergquist and Örlander, 19	Bergquist and Örlander, 19
	:	10. Type	Biodiversity		Diodivorrity	פוטטועפואוע		Biodiversity			Biodiversity			Biodiversity		Biodiversity	DIOUIVEI SILY
		9. Observed effects	Decrease in Vaccinium myrtillus and V. vitis-idaea, increase in the parasitic	fungus Valdensia heterodoxa	Decreased cover and frequency of	N sensitive lichens	Colonization of spruce needles with	green algae, mainly Pleurococcus	viridis	Increase abundance of the green	alga Scoliciosporum chlorococcum on	conifers	Correlation between Deschampsia	flexuosa cover and exceedance of	critical load.	Increased frequency of browsed	young trees
	8. Suggested effect threshold	(kg N ha'' yr')	>6		0	0-1		>5 (throughfall)			3-4			11-2			
	7. Atmospheric deposition	(kg N ha'' yr')	3-12													12-18 vs	18-29
ga woodland		6. Tree species	Picea abies and Pinus	SIJVESUIJS	Dianc cultoctric	ci ijcavije culija								coniferous forests		Direa akiec	r icea anieo
l resampling studies ta	=low, 3=high) - roc - cation - cation	r 🖯 held study	Sweden		Curoton	סאפתפון		Norway, Sweden			Finland			Sweden		Cureden	
sht ang	2 SSEID CINIO3 .	ע ק	M		ſ	J		2			2			2		ſ	J
Gradieı		2	G3.B											G3.A			
ndix 3B:	F22615 2INU3 .	Z	53.A			D.CD		G3.B			G3.B			G3.B		V 23	
Apper	r ləvəl sınuə .	L	ß		r J	9		G3			G3			G3		r U	6

216 | Review and revision of empirical critical loads and dose-response relationships

Proposed new empirical critical loads of nutrient N for fresh waters, based on ICP Waters report

The critical loads of nutrient N for fresh waters, as proposed in this appendix, are based on a recent literature review (De Wit and Lindholm, 2010) carried out under the international Cooperative Programme on Assessment and Monitoring Effects of Air Pollution on Rivers and Lakes (ICP Waters, www.icp-waters.no).

The critical loads proposed in the ICP Waters report (see table below) are slightly different from those agreed on in the Noordwijkerhout critical-load workshop in June 2010, where the background document by Bobbink *et al.* (2010) was used as a basis. That had the following reasons.

In the ICP Waters report, paleolimnological (lake sediment) data were included, contrary to the Bobbink *et al.* (2010) report. The paleolimnological studies originated predominately from Arctic, sub-Arctic and alpine lakes, which extended the regional coverage of the ICP Waters report (in contrast to the Bobbink *et al.* (2010) report). This also led to the inclusion of more studies on regions with areas of low N deposition. Additionally, the ICP Waters report had a stronger focus on catchment type rather than on EUNIS habitat type, because nitrogen retention capacity of catchment areas is a major indicator for ecosystem sensitivity to N deposition.

For future updates of critical loads of N, we recommend to include paleolimnological studies, as they include areas of low N deposition and because of the increase in regional coverage by relevant studies. More importantly, paleolimnological studies present a unique possibility to detect historical ecosystem responses to drivers of change and are an extremely useful supplement to insights obtained from experimental and monitoring studies.

Table A4.1 Proposed new empirical critical loads of nutrient N for fresh waters, based on this review. N deposition in kg N ha⁻¹ yr⁻¹. Table numbers refer to tables in De Wit and Lindholm (2010). In *italics*, critical loads that were suggested in other reviews.

EUNIS	Description	Catchment type	Regions	Response	Critical load
C1.1	Oligotrophic soft-water lakes	Arctic	Europe, Canada, Greenland	1. Phytoplankton community shift at N deposition <1-1.5 (Table 1)	1
		Alpine, boreal	USA, Europe	 Phytoplankton community shift at N deposition 3-5 (Table 1) Higher phytoplankton productivity at N deposition < 5 (Table 3) 	3-5
		Temperate, boreal	Canada, USA, UK, Scandinavia, Netherlands	 Phytoplankton community shift at N deposition 2-9 (Table 1) Higher phytoplankton productivity at N deposition < 5 (Tables 2 and 3) Shift of N to P limitation of benthic algae at N deposition 2-12 (Tables 2 and 4) Productivity of benthic algae increases at N deposition 2-12 (Table 4) Macrophytes: loss of key isoetid species, increase in species such as Juncus bulbosus and Sphagnum (Bobbink and Roelofs, 1995) 	5-10
		Dunes	Netherlands	1. Increased biomass and rate of succession (Bobbink et al., 2003)	10-20
C1.4	Dystrophic lakes	Temperate, boreal	Sweden, Canada	 Higher phytoplankton productivity, especially at N deposition 5 (Table 3) 	3-5

Reference

De Wit, H.A. and Lindholm, M. (2010). Nutrient enrichment effects of atmospheric N deposition on biology in oligotrophic surface waters – a review. ICP Waters report 101/2010. NIVA report 6007 – 2010. Oslo, Norway. ISBN 978-82-577-5742-7.

Report of Working Group 1: Marine habitats (A), Coastal habitats (B), Inland surface waters (C) and Grasslands and lands dominated by forbs, mosses or lichens (E)

Chair: Jan Roelofs (NL)

Rapporteur: Mike Ashmore (UK)

Participants: Seraina Bassin (CH), Shaheen Begum (UK), Leon van den Berg (NL), Roland Bobbink (NL), Helene Evstafyeva (UA), Anne Christine le Gall (FR), Laurence Jones (UK), Berit Kvaeven (NO), Tomasz Pecka (PL), Max Posch (NL), Wil Prins (NL), Eva Remke (NL), Muhammad Riaz (UK), Angela Schutlow (DE), Wim de Vries (NL), Clare Whitfield (UK), Heleen de Wit (NO).

Working Group 1 began by discussing the proposals in the background document for each EUNIS class (European Nature Information System). The discussion was focussed on the background document in combination with additional information and data that was presented to the group; this summary only focuses on the new data and on the basis for the specific recommendations made by the group. Generic issues and modifying factors were discussed as they arose, with a final summary at the end of this report of working group 1.

A Marine Habitats

A2.54 and 2.55. Group members with knowledge of these systems suggested that, for these categories, the criticalload range applied was too high to prevent adverse effects. New information from long-term monitoring (over 25 years) of vegetation on one of the Dutch islands showed a trend towards more eutrophic vegetation in both grazed and ungrazed salt marshes as well as in dune regions (Dijkema et al., 2005). This study was based primarily on mid-successional salt marshes; in early-successional salt marshes the trend was less pronounced, partly because of the low number of species. The total deposition range in the study was estimated to be 15 to 20 kg ha⁻¹ yr⁻¹. On the basis of expert judgement and the evidence from this one study, the group recommended a new critical load range of 20 to 30 kg ha⁻¹ yr⁻¹ as a conservative estimate. The group suggested that this new critical load be extended to mid and upper successional salt marshes A2.53. For this EUNIS class and for many others, the group emphasised the need for new experiments with a range of N additions in regions with low N deposition.

B Coastal Habitats

B1.3. The group agreed with the proposal that, for this class, the critical load should not be changed. There were views that this range should perhaps be lower, but there was no evidence to support this. The working group

recommended that the study by Jones *et al.* (2008) be included in the background document. This study describes a chronosequence at one site in the United Kingdom where rates of soil development are related to both N deposition and temperature (these variables are co-correlated so cannot be separated). The study supports the suggested range. No information was available on modifying factors.

B1.4. The group agreed with the recommendation, for this class, to reduce the critical load to 8 to 15 kg ha⁻¹ yr⁻¹. This range is quite reliable. The group discussed the data from the gradient study on the Baltic region (Remke et al., 2009a; 2009b; Remke, 2010), which was used in the background document, and discussed the threshold for significant ecological change. The group agreed to using the N content and deposition values at which large changes in species composition occurred at some sites, to identify thresholds from the scatter plots in the study, rather than formal statistical analysis based on fitted curves. It was agreed that the use of *Cladonia* biomonitor data in this study and the conversion of wet to total deposition were acceptable. The working group recommended that the loss of typical lichen species be included to the indication of exceedance in the summary table.

New data presented from a gradient study covering 5 European countries showed a significant relationship between species richness and N deposition that provided further support for the revised critical level. In addition, unpublished experimental data from the United Kingdom (Jones *et al.*) showed that N leaching increased with N deposition, from a background of 11.3 to 18.8 kg ha⁻¹ yr⁻¹, which further supported the new critical load range. However, B1.4 covers a range of dune systems. The main modifying factor identified was base status. For acidic dunes, the range should be 8 to 10 kg ha⁻¹ yr⁻¹, for calcareous dunes it should be 10 to 15 kg ha⁻¹ yr⁻¹. The working group recommended that the footnote from the Berne report on P be deleted.

B1.5 The group agreed with the proposal that this critical load should not be changed, as no new evidence was available. Some concern was expressed about the critical load needing to be lower than that for F4.2, but given the lack of relevant evidence no change could be supported. No information was available on modifying factors.

B1.8. Based on expert judgement, the group proposed a reduction in the critical load range to 10 to 20 kg ha⁻¹ yr⁻¹, rather than 15 to 25 kg ha⁻¹ yr⁻¹ as proposed in background document. The group identified relevant information from Plassman *et al.* (2009), who showed positive effects on germination in greenhouse experiments, adding 15 kg ha⁻¹ yr⁻¹ to columns taken from the field. These effects were

also visible from the fact that in areas of the Netherlands with a deposition of 20 kg ha⁻¹ yr⁻¹, there is a need to remove sludge every 20 years (A.P. Grootjans, pers. comm). However it should be noted that gradient studies in the United Kingdom show no effects for the newly proposed critical load range. Based on expert judgement, the lower end of the range should be used for habitats with low base availability and the higher end for those with high base availability. Surface water fluctuation was identified as an important modifying factor - low fluctuations lead to greater sensitivity. The group raised concerns about how to apply critical loads to systems such as those in the B1.8 category when there were N inputs from other pollution sources in inundation and groundwater. The working group recommended identifying the habitats affected in the background document, and including generic advice on how to apply the critical load ranges in Chapter 11.

C Inland surface water habitats

C1.1 (except C1.16). This class covers shallow soft-water lakes as well as boreal sub-Arctic and alpine lakes. Different critical loads have been proposed for these systems although they are both within the C1.1 class. There is no way of distinguishing these two types of systems using one EUNIS code. Therefore, the working group recommended that more information be included in the background document and summary table, to clarify which types of systems are being referred to, and why they may have different critical loads. The group also noted that the critical load for Atlantic soft-water lakes was based on experiments, while, for boreal sub-Arctic and alpine lakes, this was based on surveys or gradient studies. Although some of the individual gradient studies were perhaps unable to account for confounding factors in formal statistical analysis, the use of bioassays for N limitation, the fact that several independent gradient studies were involved, and the fact that similar effects were reported from several regions in Europe and North America all support the interpretation that N deposition is the causal factor. The working group recommended that relevant information from the report by De Wit (2010) be added to the background document.

The working group concluded that it would be clearer to provide a single critical load range for the C1.1 class. On this basis the group agreed a single critical load range of 3 to 10 kg ha⁻¹ yr⁻¹, which could be described as reliable. An important limitation of this EUNIS class is that it does not categorise according to alkalinity. The working group recommended that in the background document and summary table be emphasised that this critical load should only be applied to oligotrophic waters with low alkalinity with no significant agricultural or other direct human inputs. We recommended that the tables indicate that the lower end of the range is to be used for boreal sub-Arctic and alpine lakes, and the upper end of range for Atlantic soft waters. The working group suggested that guidance be provided as to which part of the range is to be applied to which types of systems. The group recommended that indicators of exceedance for the two types of systems be separated in the background document but not in the summary table. The working group recommended that the indicators in the table be re-worded to: change in the species composition of macrophyte communities, increased algal productivity, and a shift in nutrient limitation of phytoplankton from N to P.

C1.16 The working group agreed that, for this class, there was no new evidence available, and that therefore the critical load range should not be changed. Surface water fluctuations were identified as the most important modifying factor – low fluctuations lead to greater sensitivity.

C1.4. The working group found that the lack of critical loads for this class was an omission in the background document, as there is evidence summarised by De Wit (2010) that dystrophic lakes may be sensitive to N deposition. There was insufficient basis for assessing the sensitivity of the C1.4 class, relative to that of C1.1. Based on expert judgement, the critical load range of 3to 10 kg ha⁻¹ yr⁻¹ should be applied to these systems, with the lower end of the range to be applied to boreal sub-Arctic and alpine dystrophic lakes. The critical load should only be applied to waters with low alkalinity and no significant agricultural or other direct human inputs.

E Grasslands and tall forb habitats

E1.26. The critical load for this class is stated in the background document to be 'quite reliable', although the Berne document stated it to be 'reliable'. Given that new information was available, this difference was not supported by the group. New, as yet unpublished data on the United Kingdom (Van den Berg et al., in review) in a gradient study showed the loss of rare and scarce species, including other confounding factors, at critical loads of above 25 kg ha⁻¹ yr⁻¹. The study's UK sites had been intensively managed to maintain high species diversity. This would support the proposed critical load range. Further support is provided by Maskell et al. (2010), from a national survey in the United Kingdom, in which the vegetation was found to be more eutrophic in areas with high N deposition. Unpublished data from the BEGIN study (Stevens et al., 2010), which covers an area from southern France to Norway, provides no clear evidence of effects of N depositions across Europe, but this is confounded by the different plant community types across

Europe. The working group recommended that this new information be added to the background document. The group agreed that the critical load range should be 15 to 25 kg ha⁻¹ yr⁻¹, but that this range should be classified as 'reliable' instead of 'quite reliable'. The working group suggested adding loss in rare and scare species to the indicators of exceedance. Management was identified as an important modifying factor. More intense mowing (and to a lesser extent grazing) decreases the sensitivity of habitats in the E1.26 class.

E1.3 The working group recommended that the table value for this class' critical load range be 15to 25 kg ha⁻¹ yr⁻¹ for consistency with the background document. The group agreed that this new critical load range, which was based on one experiment on a former agricultural site, should be adopted based on expert judgement. In the group some considered that these systems were likely to be very sensitive to N deposition, but a lack of evidence meant that a lower critical load range could not be recommended. In addition, no assessment could be made of modifying factors.

E1.7 The group discussed the proposal for a reduction in the critical load ranges from 10-20 to 10-15 kg ha⁻¹ yr⁻¹. This proposed reduction was based on results from a gradient study in the United Kingdom, which had been subject to detailed statistical analysis taking a range of confounding factors into account. The working group agreed that a new critical load range of 10 to 15 kg ha⁻¹ yr⁻¹ should be adopted. The working group recommended that the indicators of exceedance be modified to include an increase in graminoid abundance as well as a decrease in total species richness. Supporting evidence is available from a wider national survey of acidic grasslands in the United Kingdom (Maskell et al., 2010) and from the BEGIN study (Stevens et al., 2010), which covers a geographical range from southern France to Norway. The group recommended that information from these two studies be included in the background paper. Given the additional evidence and results from gradient studies, time series and experimental studies, the group agreed that this new critical load could be labelled as 'reliable' rather than 'quite reliable'. In terms of modifying factors, there was evidence that many of the effects were driven by acidification. Therefore, the lower end of the range should be applied to sites with a low base status.

E1.94 and 1.95. At the Berne workshop, it was agreed that the same critical load range should be applied to these systems as those applied to the systems in the B1.4 category, for which more data were available. Accordingly, and based on expert judgement, the working group recommended that the critical load range for E1.94 and E1.95 also be altered to 8 to 15 kg ha⁻¹ yr⁻¹. Modifying

factors were identified as base status for B1.4. Since the habitats in the E1.94 and E1.95 classes are acidic only, the lower end of the critical load range should be applied.

E2.2 and E2.3. The group agreed with the recommendations provided in the background document that the critical load range should not be changed. No new data were identified by the group for the E2.2 and 2.3 categories.

E3.51 and E3.52. The group agreed with the recommendation provided in the background document that the critical load range should not be changed. No new data were identified by the group. Base status was identified as a significant modifying factor; systems with low base status would be likely to be more sensitive. Fluctuations in the water table cause the habitats in the E3.52 category to be less sensitive. There is also information available from an experimental study in Wales, where 10 kg ha⁻¹ yr⁻¹ was added to a background of 20 kg ha⁻¹ yr⁻¹ over a period of 15 years. In the experiment, there was evidence of interactions with grazing, but this was not sufficient to recommend a modifying factor. The working group recommended that information from this study be added to the background document.

Eq.2. The group agreed with the recommendation provided in the background document that the critical load range for this system should not be changed. Additional evidence for this critical load range was provided by a gradient study in the United Kingdom (PhD thesis by Armitage (2010) and associated draft papers). The working group recommended that information from this study be included to the background document. Concerns were raised about an experimental study (Pearce and Van der Wal, 2002) that used very infrequent applications with high concentrations that were likely to have caused direct toxic effects. However, even if this experiment would be ignored, there would still be sufficient evidence to support the proposed critical load range. Interactions with grazing are likely, but the available evidence was not consistent enough to warrant recommendations on modifying factors.

E4.3. Important new data have become available from an experiment in the Swiss Alps (Bassin *et al.*, 2007; 2009). Published data for the four years up to 2007 were considered in the background document, but new data for 2008 and 2009 (both of which were relatively cool and wet years) were presented and discussed. The study on the Swiss Alps involved a range of N additions to a background of 4 ha⁻¹ yr⁻¹. In 2007, 2008 and 2009, there was a significant effect on sedge or legume cover at an addition of 10 kg ha⁻¹ yr⁻¹, but only in 2007 were significant effects found at an addition of 5 kg ha⁻¹ yr⁻¹. The group agreed that these

data supported the proposed critical load range of 5-10 kg ha⁻¹ yr⁻¹. This new critical load range could be labelled 'quite reliable' because it originated from a well-designed experiment with a wide range of N additions at a site with very low background deposition. As yet unpublished data from 10 sites in the eastern Swiss Alps over a period of 2 years of N addition at 50 kg ha⁻¹ yr⁻¹ showed the same trends, which would suggest that the effects observed at the experimental site are not unique to that location. In future cases, results from these studies could be used to identify modifying factors. The working group recommended that this additional information be included in the background document.

E4.4. Important new data have become available from an experiment in the Swiss Alps (Hiltbrunner et al., submitted). These data that have not been published as yet, were provided to the working group by the study team and in a presentation at the CCE workshop in 2008. The study involves a range of N additions to a background of 3 kg ha⁻¹ yr⁻¹. In the study, a first significant effect on total biomass was found at an addition of 10 kg ha⁻¹ yr⁻¹ and, on total biomass of Carex species, at an addition of 5 kg ha⁻¹ yr⁻¹. The working group agreed that these data supported the proposed critical load range of 5 to 10 kg ha⁻¹ yr⁻¹. This new range could be labelled as 'quite reliable', even though this concerned only a single experiment, because the experiment was well-designed, with a wide range of N additions at a site with very low background deposition. No judgement could be made on modifying factors.

Other EUNIS classes and high-level EUNIS classes

In EUNIS classes A, B, C and E, there are many categories for which critical loads could not be defined. Since the background document focuses on EUNIS classes which are known to be N limited, many of the sensitive ecosystems in northern and western Europe should be covered by the recommended critical load ranges. However, some important EUNIS classes, especially in southern and eastern Europe (e.g. steppes, dehesa), may be sensitive to N deposition, but do not have critical loads defined. The working group agreed that no critical load range could be recommended for these classes because of the lack of relevant data.

The working group considered the proposal of the organisation to provide a default critical load range for all grasslands, all coastal habitats or all inland surface waters, for European scale mapping. However, the group found this to be inappropriate, especially for grasslands, because of the large variety in ecological systems within these categories, each with their own sensitivity.

Summary of Modifying Factors

The table below provides a summary of the modifying factors that were identified for each EUNIS class.

EUNIS code	Modifying factor	Low (kg ha ⁻¹ yr ⁻¹ when stated)	High (kg ha'¹ yr'¹ when stated)
B1.4	Base status	8-10	10-15
B1.8	Water table fluctuations	Low range	High range
C1.1	Region	Boreal, alpine, sub-Arctic 3-6	Atlantic
			5-10
C1.4	Region	Boreal, alpine, sub-Arctic	Atlantic
		3-6	5-10
C1.16	Water table fluctuations	Low range	High range
E1.7	Base status	Low range	High range
E1.94, E1.95	Base status	8-10	10-15
E3.51, E3.52	Base status	Low range	High range
E3.51	Water table fluctuations	Low range	High range

Some of this information could only be used for local-scale applications, for example, water table fluctuations. For applications on a European scale, the most important factor identified was that of base status. However, the group warned that in the proposed generic application for European mapping the fact would be ignored that for many of the EUNIS categories, base status may not be a significant modifying factor. The level of base status within each relevant EUNIS class needs to be considered when applying this modifying factor.

For grasslands, management is an important local modifying factor, but this cannot be simply represented in a summary table, because of the wide range of management types of different EUNIS classes in various regions of Europe. The working group recommended that the importance of this factor be described more clearly and elaborately, for each of the EUNIS classes, in the background document.

Knowledge gaps and future research

Strong recommendations were made during the Berne workshop, which stated that, for almost all sensitive EUNIS classes, new well-designed experiments would be needed with a wide range of N additions at sites with low background deposition. It is very disappointing that, eight years later, only two studies that had followed this recommendation were available for use. This key knowledge gap is clearly identified in the background document and was strongly endorsed by the working group; for many sensitive EUNIS classes there are no experimental studies that meet the requirements needed to identify critical loads. Many more well-designed long-term studies are urgently needed, if additional and more significant

progress is to be made in defining and improving critical loads. In many cases, the lack of such data will lead to overestimation of critical loads, and subsequent lack of protection of important European habitats. There are increasing numbers of gradient studies being reported or initiated, and when these were judged to be well designed and rigorously analysed, they were used by the group to provide important new information for identifying critical loads. However, the group recognised that more rigorous guidelines should be identified for evaluation of these studies, covering the estimation of deposition rates, the quantification of confounding factors, and the methods of statistical analysis. Because of the large scatter in data, particularly in regional gradient studies, it would be more appropriate to identify thresholds from a reduction in the range of values rather than from formal curve fitting and statistical analysis. In order to better identify priorities for a Europe-wide evaluation of critical loads, it would be valuable to rank all EUNIS classes that are likely to be N limited by the area of Europe covered, alongside their critical load range and reliability. Priority, in terms of support of European policy, could then be assigned to those classes of greatest significance on which only minimal information is available.

For fresh waters, new experimental studies on added nitrate would be valuable to support the data on critical loads that are based on gradient studies. In addition, these would need to be linked to an assessment of the contribution of N deposition from direct input and from the catchment area.

Report of Working Group 2: Mires, Bogs and Fens (D) and Heathland, Scrub and Tundra (F)

Chair: Sarah Woodin (UK) Rapporteur: Sally Power (UK) Participants: Julian Aherne (CA, IE), Teresa Diaz (PT), Thomas Gauger (DE), Jane Hall (UK), Ed Rowe (UK), Gudrun Schuetze (DE), Lucy Sheppard (UK), Jaap Slootweg (NL), Ian Strachan (UK), Hilde Tomassen (NL)

General issues relating to estimation of critical loads

The group unanimously decided to include background deposition in all critical load estimates. In addition, particular weight was awarded to studies with low ambient background deposition (ABD) and treatment levels, as well as with good levels of resolution between treatments and realistic application regimes. Although surveys (gradient studies) have issues of co-correlation with other drivers (e.g., S and climate), they may add valuable information if they have been subject to rigorous (multivariate) statistical analyses and/or if the role of N can be clearly demonstrated. These surveys also generally cover a wider range of habitat types and deposition histories (and site conditions) than are included in manipulation experiments. It was concluded, therefore, that survey evidence of N effects and thresholds should be included, but treated on a case-by-case basis. The many different variables that change in temporal re-surveys, made it difficult to attribute change to shifts in N deposition. Nevertheless, such surveys were considered useful as supporting information next to other available studies. The rationale for a critical load range (rather than a single value) was discussed and the group recommends that this is clearly defined in the background document. For future revisions of critical loads, the group suggests treating the uncertainty associated with deposition estimates in a different way (e.g., through formal uncertainty analysis?).

D1. Raised and blanket bogs

There is a strong evidence base for effects of N at low deposition rates, with many studies using low inputs at sites with a low ambient background deposition (e.g., Redbo-Torstensen *et al.*, 1994; Lütke-Twenhöven *et al.*, 1992; Nordbakken *et al.*, 2003). Therefore, the group accepted the proposed critical load range of 5 to 10 kg N ha⁻¹ yr⁻¹, a range that was judged to be 'reliable'.

Modifying factors: Water tables are judged to be an important modifying factor, with evidence of greater sensitivity to N in drier systems¹. It should be noted that management-related factors, especially those of drainage

Water tables can be modified through management – especially drainage and scrub encroachment

and scrub encroachment, may modify the water table. The concentration-dependence of many bryophyte species' response to N (Pearce and Van der Wal, 2002) indicates that the high end of the critical load range can be applied to areas receiving high precipitation levels (i.e. low ion concentrations).

Modifying factors	LOW END OF	HIGH END OF	
	RANGE	RANGE	
Precipitation	Low levels	High levels	
Water table *	Low (dry)	High (wet)	
P limitation	Conflicting mechanisms		

D2. Valley mires, poor fens and transition mires

Based on the observed N accumulation in soil in response to additions of 15 kg (ambient background deposition of 2 kg) (Granberg *et al.*, 2001; Gunnarsson *et al.*, 2004) and effects on *Sphagnum* growth at N additions of 12 kg (ambient background deposition of 12 kg) (Hogg *et al.*, 1995), lowering of the upper end of the critical load range to 15 kg N ha⁻¹ yr⁻¹ is recommended. Many of the species in poor fen habitats are likely to overlap with those in bog habitats (D1), although the slightly more minerotrophic nature of these habitats is likely to make them less sensitive to N, compared to bogs. Overall, there is sufficient evidence to support a critical load range of 10 to 15 kg N ha⁻¹ yr⁻¹ for this EUNIS category, a range judged to be 'quite reliable'.

Given that systems with a closed N cycle (quaking bogs and transition mires) can be expected to be relatively sensitive to N, the working group recommends that the lower end of the range be applied to the D2.1 category. However, since mires that receive groundwater inputs may experience (potentially considerable) additional N via this route, the group was unable to determine whether valley mires and poor fens (D2.3) would be more or less sensitive. Therefore, for these habitats, no specific recommendation could be given for the appropriate end of the range for these systems.

For closed systems (D2.1 category), the role of precipitation is addressed by placing them at the low end of the critical load range; precipitation is not suggested as a modifier for habitats in the D2.3 category, because of groundwater influence.

D4.1. Rich fens

Based on new data showing a large and rapid effect of N additions of 35 kg (ambient background deposition of 6 to 8 kg) on bryophyte biomass and species diversity (Robat *et al.*, 2008), lowering of the upper end of the critical load is recommended, as suggested in the background document. The working group agreed the proposed critical load of 15 to 30 kg N ha⁻¹ yr⁻¹ (#) for this system. In terms of potential modifying factors, the working group recommended application of the high end of the range to managed (cut) fens and the low end to high-latitude systems. However, since it is not possible to assign a latitudinal cut off, the group suggested that climatic zones be used for distinguishing more sensitive (lower temperature) base-rich fens. The working group judged that Dutch base-rich fens' sensitivity to N to be greater in areas with low water-table fluctuations.

Modifying factors	LOW END	HIGH END
Management	Lacking	Cutting
Latitude (climatic region)	High latitude	-
Water table	Low range	High range

D4.2 Montane rich fens

In the absence of new evidence, and from the results of a single experiment (at high N loads), the critical load range of 15-25 kg N ha⁻¹ yr⁻¹ (#) for this system should remain unchanged.

Modifying factors	LOW END	HIGH END
Latitude (climatic region)	High latitude	-

F1. Tundra

Evidence from Arens *et al.* (2008) suggests a significant effect of N additions of 5 kg (ambient background deposition of 1 kg) on grass and shrub cover. On this basis, the critical load range is recommended to be lowered to 3 to 5 kg N ha⁻¹ yr⁻¹. This range is supported by earlier studies, and thus judged to be 'quite reliable'. Because of the complications of strong seasonal variations in the amount of precipitation and because different forms of precipitation (rain, snow) have different interactions with N, precipitation was not suggested as a modifier of N response.

Modifying factors	LOW END	HIGH END
Precipitation	N/A (depending	g on form (snow or rain)
	and timing)	

F2. Arctic, alpine and subalpine scrub habitats

Experiments by Mols *et al.* (2007) and Fremstad *et al.* (2005) shows significant effects of N additions of 7 kg (2-4 kg ambient background deposition), therefore, the working group proposes that the lower end of the critical load range be reduced. However, lack of response to high inputs found in another study support retaining the upper end of the range; the working group proposed a revised critical load of 5 to 15 kg N ha⁻¹ yr⁻¹, which is judged to be 'quite reliable'. Modifying factors are judged to be the same as those for tundra systems.

Modifying factors	LOW END	HIGH END
Precipitation	N/A (depending	g on form (snow or rain)
	and timing)	

F4.11. Northern wet heath

As EUNIS classes make no distinction between upland and lowland wet heaths, the two sub-categories perhaps could be combined. However, in recognition of the strong differences in soil characteristics between upland and lowland systems, the working group would choose to retain the division, and recommends that a detailed description of the differences in these systems and rationale for this division be included in the background document.

F4.11. Calluna-dominated (upland) wet heathland

A new experiment shows evidence of effects of N additions of 10 kg (ambient background deposition of 16 kg), supporting the value of the upper end of the critical load range. However, no new evidence has been provided to warrant a change in the lower end value, therefore values remain between 10 and 20 kg N ha⁻¹ yr⁻¹, a range that is judged to be 'quite reliable'.

Regular use of management methods that remove nutrients (e.g., burning, mowing, sod cutting) favour the application of the high end of the critical load range. The working group has made no recommendations for the use of grazing, as its effects are more complicated than nutrient export alone. For areas with low levels of precipitation, the low end of the range is recommended (based on increased sensitivity to drought with N addition), and the high end for areas with high precipitation levels.

LOW END	HIGH END	
-	High nutrient export (e.g.,	
	from burning, mowing	
	with biomass removal) ²	
Low levels	High levels	
	LOW END - Low levels	

F4.11. Erica tetralix-dominated (lowland) wet heathland Previously, the critical load for this system had been based principally on output from a simulation (empirical) model (Berendse *et al.*, 1998). However, based on an overlap in species and habitat characteristics between upland wet heaths (species composition, including N-sensitive bryophytes) and lowland dry heaths (oligotrophic mineral soils), the working group proposes to reduce the upper end of the critical load range, to the same level of that for other heathland (F4.11 (upland) and F4.2) habitats. The proposed revised critical load range, thus, is 10 to 20 kg N ha⁻¹ yr⁻¹, based on expert judgement.

Modifying factors	LOW END	HIGH END
Management	-	High nutrient export (e.g.,
		from mowing with
		biomass removal, sod
		cutting)
Precipitation	Low levels	High levels

F4.2 Dry heaths

In the absence of new data, the previously set critical load range of 10 to 20 kg N ha⁻¹ yr⁻¹ is recommended to be retained, and is judged to be 'reliable'. However, given the response in both higher and lower plants to N inputs below the upper end of this range, as indicated in studies by Power et al. (1998) and Barker (2001), the lower end of the range is recommended for lichen-rich and/or very nutrient-poor heaths. Management typically involves export of nitrogen. Both empirical and modelling studies show that this affects nutrient budgets, and it is thus suggested that the higher end of the critical load range applies to systems managed through regular burning, mowing or sod-cutting. A demonstrated increased sensitivity to drought following N addition suggests that, to areas of low precipitation, the lower end of the range should be applied.

Modifying factors	LOW END	HIGH END
Habitat type	Lichen-rich and/or	-
	nutrient-poor	
	heaths	
Management	-	High nutrient
		export, through
		burning, mowing
		with biomass
		removal, sod
		cutting
Precipitation	Low levels	High levels

F5. Mediterranean scrub

A new study by Diaz *et al.* (submitted) shows rapid effects of N additions of 40 kg (ambient background deposition of 5 to 10 kg) on plant species richness and community composition, as well as on microbial community. Although this is the only study on this habitat type, the speed and magnitude of response has led the working group to recommend a new critical load range of 20 to 30 kg N ha⁻¹ yr⁻¹, based on expert judgement. However, no information can be given on modifying factors at this time.

Other issues concerning the use of modifying factors

The use of base-cation availability as a potential modifying factor was discussed for all systems, and discarded as a) virtually all are strongly acidic, with species adapted to these conditions and base-cation availability being generally low, and b) because the working group considered that, whilst this may modify the acidifying effects of

² Grazing management has complicated effects, so should not be included as a modifying factor

N, the nutrient effects would remain.

Phosphorus availability was considered at length in the working group. P limitation has been seen to reduce species compositional responses to N, however, it may increase N accumulation and leaching in response to N. The working group, therefore, concluded that P limitation would not protect ecosystems from (potentially) detrimental effects of N, and thus should not be used as a modifying factor.

Summary of critical load ranges for EUNIS classes D and F										
Ecosystem type	EUNIS code	kg N ha ⁻¹ yr ⁻¹	Reliability	Indication of exceedance						
Raised and blanket bogs	D1	5-10	##	Increase in vascular plants, altered growth and species composition of bryophytes, increased N in peat and peat water						
Poor fens	D2	10-15	#	Increase in sedges and vascular plants, negative effects on bryophytes						
Rich fens	D4.1	15-30	(#)	Increase in tall graminoids, decrease in bryophytes						
Montane rich fens	D4.2	15-25	(#)	Increase in vascular plants, decrease in bryophytes						
Tundra	F1	3-5	#	Changes in biomass, physiological effects, changes in species composition in bryophyte layer, decrease in lichens						
Arctic, alpine and subalpine scrub habitats	F2	5-15	#	Decline in lichens, bryophytes and evergreen shrubs						
Northern wet heath F4.11										
'U' Calluna dominated wet heath (upland moorland)	F4.11	10-20	#	Decreased heather dominance, decline in lichens and mosses, N leaching						
'L' Erica tetralix dominated wet heath (lowland)	F4.11	10-20	(#)	Transition from heather to grass dominance						
Dry heaths	F4.2	10-20	##	Decline in lichens, changes in plant biochemistry, increased sensitivity to abiotic stress, transition from heather to grass dominance						
Mediterranean scrub	F5	20-30	(#)	Change in plant species richness and community composition						

Report of Working Group 3: Forest and woodland habitats (G)

Chairs: Joachim Strengbom (SE) and Martin Jenssen (DE) **Rapporteur:** Mark Fenn (US)

Participants: Beat Achermann (CH), Rocio Alonso (ES), Sabine Augustin (CH), Estelle Bortuluzzi (FR), Christina Branquinho (PT), Sabine Braun (CH), Han van Dobben (NL), Harry Harmens (UK), Jean-Paul Hettelingh (NL), Arjen van Hinsberg (NL), Carmen Iacoban (RO), Linda Pardo (US), Jesus Miguel Santamaria (ES), Thomas Scheuschner (DE), Kirsten Schütz (CH), Irena Skorepova (CZ), Harald Sverdrup (SE), Maaike Weijters (NL)

Introduction

The working group on forest and woodland habitats (class G of the European Nature Information System (EUNIS)) has provided recommendations for critical load ranges (CLs) for the EUNIS classes presented in Table 1. The more specific EUNIS categories are listed first, followed by critical load ranges for the broad categories of broadleaf deciduous woodlands and coniferous woodlands. Table 2 shows the EUNIS woodland categories for which no critical load ranges have been set because of a lack of data, or for which critical load ranges were set based on very limited data or data from outside Europe, based on expert judgement (EUNIS categories G2.1 and G3.7).

The group began by discussing a general working method for valuing the studies that were used for setting the critical load ranges. The working group agreed that, to protect all forests, critical loads be set to protect the most sensitive ecosystem component. This means that critical loads be set at the lowest level of N input for which any part of the ecosystem shows a response to. This implies that study results would not influence the recommended critical load range if no response was found for a particular indicator at a specified level of N input. It was assumed that, in such cases, the forest and the specified indicator would respond given a longer period with N input above the critical load or that changes had already occurred before the study was done. This procedure also implies that the response variables used for setting critical loads may vary between EUNIS categories.

The group also agreed that when data from N-addition experiments would be used to set critical loads, the background deposition at the experimental site would need to be added to the doses used. This also implies that the critical load derived from an N-addition experiment, per definition, could not be set to a lower value than the background deposition at the site.

An important question in regard to N-addition experiments is whether the particular ecosystem is responding to a change in N input or to the total input of N? There may be unknown lag effects caused by the background deposition that could be missed in N-addition experiments. If setting critical loads is to be based on N effects, then high background depositions may make it difficult to determine whether the response is due to N or to other factors. As this issue also pertains to other EUNIS classes, the working group agreed that this be discussed further in regard to other habitat types. In some cases it may be necessary to give more weight to gradient studies in regions with high background deposition. But gradients are gradients of other factors as well (e.g., climate), so they would need to be used in combination with other approaches, in support of experimental studies.

Procedure for assessing the recommended critical load

The background document suggests a critical load range for each habitat class. In most cases a number of studies were indicated in support of this range. Before setting a critical load the working group evaluated the different studies and special attention was given to the following criteria:

- Is the suggested critical load in accordance with the effects found in the studies?
- Has the background deposition been included and is the given value robust?
- Are there any important confounding factors, such as S deposition and climatic variation along gradients?
- Are there other studies that would need to be included?

The range given for a particular critical load represents the range from studies that show significant effects. For experiments, the lowest N treatment plus background deposition was used to set the critical load. Long-term experimental studies in regions with low N deposition were given a high value. In cases where the lowest N treatment in an experiment was high, but produced large effects (e.g., 50% changes in abundance of a species), the critical load was allowed to be lower than the lowest N treatment. Gradient studies or studies comparing changes over time were primarily used as supportive studies. A critical load was set to be 'reliable' (##) when several geographically separated studies showed similar results, or when experimental studies were supported by gradient studies. A critical load was set as 'quite reliable' (#) when only one or two studies supported the range given. The critical load was set, based on 'expert judgement' in cases where results from experimental studies were lacking.

Given the above stated definitions, a critical load cannot be set at a value below that of the background deposition. This makes it difficult to set meaningful critical load ranges for habitats in regions with high N depositions (e.g., temperate forests). For these habitats, therefore, retrospective or gradient studies were used to support the setting of lower critical loads than their background depositions. In such cases the reliability was set as 'expert judgement'. The working group agreed that for regions with a high N load it would be better to conduct studies that focus on recovery following reduced N input, than to attempt to set the critical load based on N addition experiments.

Presentations

Han van Dobben (Netherlands) presented an analysis of data from the ICP Forests network on the effects of N on ground vegetation. Nitrate deposition based on EMEP simulated deposition and throughfall N deposition, were both related to an increase in nitrophilous species and Ellenberg N indicator values.

Christina Branquinho (Portugal) presented studies of epiphytic lichens in response to atmospheric ammonia in cork oak woodland in southern Portugal with a Mediterranean climate. Critical levels for ammonia were discussed and a critical load was derived from these data and supported by studies in woodlands in California, the United States. As a result, a critical load for Mediterranean evergreen (Quercus) woodland (G2.1) was established based on expert judgement. Because of their high sensitivity (critical load range of 3 to 7 kg N ha⁻¹ yr⁻¹ for this habitat type), lichens can also be considered as early warning systems for further effects on terrestrial ecosystems. Furthermore, in Mediterranean systems, it can sometimes be difficult to establish N response patterns and to determine critical load ranges because of extreme variability in water availability from year to year. Lichen responses to N allow for more consistent and repeatable observations.

Irena Skorepova (Czech Republic) and Carmen Iacoban (Romania) presented data on nitrate leaching in support of critical load ranges for deciduous and coniferous forests (G1, G3).

Martin Jenssen (Germany) presented findings mainly from Scots Pine forests in east Germany. Data was presented on trace gas exchange (N_2O , NO, CH_4), tree growth and nutrition and ground vegetation that corresponds to thresholds indicative of qualitative changes of ecosystem structure and function. These data support the critical load for Pinus sylvestris woodland south of the Taiga (G3.4).

Gaps in knowledge

Although a number of studies on the effects of N deposition in forest ecosystems have been published since the last update (2003) of empirical critical loads, there is still a lack of data for several forest types (see Table 2). This is most obvious for Mediterranean forests, on which very few published studies in Europe could be found. In order to set critical load ranges that will protect European forests from the effects of N deposition, data on the response in these ecosystems are needed. More data from manipulation experiments with low levels of N input (from areas with low background deposition) are also needed for several of the indicators for which the working group has made a recommendation for the critical load.

Recommendations

For many EUNIS classes there is still an urgent need for more and better data. Although the situation has improved, data on N sensitivity is still lacking for large areas of European woodlands (see Table 2). For many habitat classes critical loads were set as 'quite reliable' or as 'based on expert judgement' because the available studies were few in number or because only gradient studies were available. Hence, the recommendation from the working group is to encourage new experiments in areas for which available data are insufficient.

Because of the difficulty in setting critical loads for areas with a high background deposition, it was recommended that more weight be given to other kinds of studies in these areas, preferably those using a combination of approaches. These could include: gradient studies, such as local gradients near point emission sources, thus reducing confounding factors that occur in regional gradients; retrospective or correlative studies; mesocosms; and manipulative studies in which N deposition is reduced.

It was concluded that the critical load ranges of level-2 EUNIS categories (G_x) cover the full critical load range for the subcategories that they include. When no data is available for a subcategory, the critical load range for the level-2 EUNIS category should be applied. The working group recommends in these cases to apply the lower end of the range.

In Table 9.4 of the background document the G3 subcategories are presented together, cancelling the separation of woodlands with and without Taiga woodland.

The critical loads for two Mediterranean habitats (G2.1 and G3.7) that are included in Table 1 are based on results from Portugal and California. Despite the severe lack of European data on these habitats, the working group concluded that it would still be worthwhile to set their critical loads based on the scarce, available data from Europe and using data from North America. These critical loads were given a reliability rating of 'expert judgement'.

Many or perhaps even all of the modifying factors given in Table 11.2 are likely to be important. However, the working group concluded that there was not enough information available to make recommendations about how to apply these factors. The factors presented are qualitative, and the working group agreed that further work would be needed to develop quantitative methods for applying modifying factors. Potentially, this could be done through modelling. To date, no such models are available, and the suggestion was that, if modification factors are to be used, effort should be made to develop such models.

Suggestions for future updates

Further work would be needed to improve some of the methods used for setting critical loads. For example, how to use gradient studies and control for potentially confounding factors. Development of such methods could be the topic of a separate workshop.

If modification factors would be used, these would need to be further developed. Until then, the precautionary principle is recommended, that is, to use the low end of the range for EUNIS categories without a set critical load.

The working group recommends that a map is developed, showing the experimental sites from which data was used to set the critical loads presented in the background document.

Table 1	 Recommended critical load ranges 	s for EUNIS categories wit	h available data	a on N sensitivi	ty
EUNIS	code	Proposed critical	Reliability ^a	Total	
				studies	Indication of exceedance
G1.6	Fagus woodland	10-20	(#)	23	Changes in ground vegetation and mycorrhiza, nutrient imbalance, changes in soil fauna
G1.8	Acidophilous Quercus- dominated woodland	10-15	(#)	7	Decrease in mycorrhiza, loss of epiphytic lichens and bryophytes, changes in ground vegetation
G1.A	Mesotrophic and eutrophic Quercus woodland	15-20	(#)	3	Changes in ground vegetation
G2.1	Mediterranean evergreen (Quercus) woodland	3-7	(#)	1 ^b	Changes in epiphytic lichens
G3.1	Abies and Picea woodland	10-15	(#)	16	Decreased biomass of fine roots, nutrient imbalance, decrease in mycorrhiza, changed soil fauna
G3.4	Pinus sylvestris woodland south of the taiga	5-15	#	8	Changes in ground vegetation and mycorrhiza, nutrient imbalances, increased N ₂ O and NO emissions
G3.5	Pinus nigra woodland	15	(#)	1	Ammonium accumulation
G3.7	Mediterranean Pinus woodland	3-15 ^c	(#)		Reduction in fine-root biomass, shift in lichen community
G3.A	Spruce taiga woodland	5-10	##		Changes in ground vegetation, decrease in mycorrhiza, increase in free algae
G3.B	Pine taiga woodland	5-10	#		Changes in ground vegetation and in mycorrhiza, increased occurrence of free algae
G4.2	Mixed taiga woodland with Betula	5-8	(#)	1	Increased algal cover
G4.6	Mixed Abies-Picea Fagus woodland ^d	10-20	(#)		
G1	Broadleaved deciduous woodland	10-20	##	36	Changes in soil processes, nutrient imbalance, altered composition mycorrhiza and ground vegetation
G3	Coniferous woodland	5-15	##	46	Changes in soil processes, nutrient imbalance, altered composition mycorrhiza and ground vegetation

^a Reliability ratings are: ## reliable; # quite reliable and (#) expert judgement.

^b This critical load range is based on one study in Portugal and supported by similar studies in Mediterranean woodlands in California. ^c Critical load range based on studies in Mediterranean forests in California.

 $^{\rm d}$ Critical load range based on studies on habitats in the G1.6 and G3.1 categories

Table 2. EUNIS categories for which no critical load ranges were set because data on N sensitivity was lacking, and for Mediterranean
forests for which critical load ranges were set based on limited data or data from outside of Europe ^a .

EUNIS Code	Description	Range	Reliability
G2.1	Mediterranean evergreen (Quercus) woodland	3-7 ^b	(#)
G3.2	Alpine larix pinus cembra woodland		
G3.6	Subalpine pinus woodland		
G3.7	Mediterranean pinus woodland	3-15	(#) ^c
G4.4	Mixed Pinus silvestris Betula woodland		
G4.3	Mixed subtaiga-taiga woodland with acidophilous Quercus		
G4.1	Mixed swamp woodland		
G4.E	Mixed Mediterranean pine evergreen oak woodland		
G4.7	Mixed Pinus silvestris acidophilous Quercus woodland		
G4.C	Mixed Pinus silvestris thermophilous Quercus woodland		
G1.1	Riparian Salix, Alnus and Betula woodland		
G4.B	Mixed Mediterranean Pinus thermophilous Quercus woodland		

^a This applies to EUNIS categories G2.1 and G3.7.

^b Based on transferred values from critical levels for NH₃ for epiphytic lichens in Portugal, and for critical load ranges for lichens of Mediterranean habitats in the United States.

^cThis critical load range is based on Mediterranean forests in California. Reliability is quite reliable (##) for California Mediterranean forests, but extrapolation to European forests results in an 'expert judgement' reliability rating for this EUNIS category.

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List of Participants

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Agenda of the Workshop on the Review and Revision of Empirical Critical Loads and Dose-response Relationships

Under the UN/ECE Convention on Long-range Transboundary Air Pollution at the NH Conference Centre Leeuwenhorst, Langelaan 3, 2211 XT Noordwijkerhout, The Netherlands, 23-25 June 2010.

Agenda

Tuesday, 22 June 2010

Arrival of Participants

Wednesday, 23 June 2010 Chairperson: Jean-Paul Hettelingh

08.00 – 09.00	Registration
09.00 – 09.15	Welcome Address ; Ms. Drs. M.G. van Empel, Director, Climate and Air Quality Directorate of
	the Ministry of Infrastructure and the Environment
09.15 – 09.20	Objectives and Organizational Information
	Jean-Paul Hettelingh, Coordination Centre for Effects (CCE)
	of the Int. Cooperative Programme on Modelling and Mapping, Convention on Long-range
	Transboundary Air Pollution (LRTAP)

09.20 - 09.25	Status of the UN/ECE Convention on LRTAP Air Pollution Secretariat of the UN/ECE LRTAP Convention
Presentation of scientific backgr Chairperson: Beat Achermann	ound information
09.25 - 9.45	Introduction to the background paper and to the updating and review approach of the empirical critical loads for nitrogen Roland Bobbink
9.45 - 10.15	Empirical critical loads for nitrogen for marine habitats, coastal habitats, inland surface waters and grassland habitats (EUNIS class A, B, C & E) Roland Bobbink & Maaike Weijters
10.15 – 10.40	Coffee Break
10.40 - 11.10	Empirical critical loads for nitrogen for mire, bog and fen habitats and heathland, scrub and tundra habitats (EUNIS class D & F) Hilde Tomassen, Roland Bobbink & Maaike Weijters
11.10 – 11.50	Empirical critical loads for nitrogen for forest and woodland habitats (EUNIS Class G) Sabine Braun, Annika Nordin, Kirsten Schütz & Joachim Strengbom
11.50 – 12.30	N deposition affects biology in nutrient-poor waters: a review of N effects on phytoplankton, benthic algae and macrophytes Heleen de Wit
12.30 - 14.00	Lunch (at the Conference Center)
14.00 - 14.30	Integration of empirical and simulated critical loads Han van Dobben en Arjen van Hinsberg
14.30 – 15.00	Relationships between N exceedances and plant diversity at the European scale <i>Roland Bobbink</i>
15.00 – 15.20	The use of Empirical critical loads and D-R relationships in Integrated Assessment Modelling Jean-Paul Hettelingh
15.20 - 15.30	Short discussion for clarification of the "Instructions"
15.30 – 15.50	Coffee Break
15.50 – 17.20	Working Groups 1 - 3
	Working Group 1: Marine habitats, coastal habitats, inland surface waters and grassland habitats (EUNIS class A, B, C & E) Chairperson: Jan Roelofs Rapporteur: Mike Ashmore
	Working Group 2: Mire, bog and fen habitats and heathland, scrub and tundra habitats (EUNIS class D & F) Chairperson: Sarah Woodin Rapporteur: Sally Power

Working Group 3: Forest and woodland habitats (EUNIS Class G) Chairperson:Joachim Strengbom & Martin Jenssen Rapporteur: Mark Fenn

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Adjourn

Workshop Dinner

Thursday, 24 June 201

09.00 – 10.15	Working Groups 1 – 3
10.15 - 10.45	Coffee Break
10.45 - 12.15	Working Groups 1 – 3
12.30 - 14.00	Lunch (at the Conference Center)

Plenary Session: Reporting of Working Groups

Chairperson: Roland Bobbink

15.00 – 16.00	Working Groups 1 – 3
16.00 – 16.20	Coffee Break
16.20 – 17.20	Working Groups 1 – 3
17.20	Adjourn
	Guided excursion in the dunes

Friday, 25 June 2010

09.00 – 10.00	Final Discussions in Working Groups 1 – 3
10.00 – 10.30	Coffee Break
Plenary Session Chairperson: Jean-Paul Hettelingh	
10.30-12.15	Presentation of Working Groups 1 - 3
12.15 - 12.30	Conclusions and Recommendations
12.30 - 13.30	Lunch (at the Conference Center)
13.30	Adjourn Departure of Participants

R. Bobbink | J-P. Hettelingh (eds)

Report 680359002 / 2011

Empirical critical loads for nitrogen were reviewed, revised and finally agreed by consensus at a UNECE workshop held under the auspices of the Convention on Long-range Transboundary Air Pollution in Noordwijkerhout, The Netherlands (23-25 June 2010). Results are presented and summarized in this report. These show that in many cases the outer ranges of the empirical critical loads have decreased. The outcome of this report is of major importance for the protection of N-sensitive natural and semi-natural ecosystems across Europe.



National Institute for Public Health and the Environment Ministry of Health, Welfare and Sport





This is a publication of:

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March 2011

