Empirical nitrogen critical loads for natural and semi-natural ecosystems: 2002 update

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1. Introduction

The emissions of ammonia (NH₃) and nitrogen oxides (NOₓ) have strongly increased in Europe in the second half of the 20th century. Ammonia is volatilized 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 are nowadays central and western Europe, eastern USA and, since the 1990s, Eastern Asia (e.g. Galloway and Cowling, 2002). Atmospheric N deposition rates of 20-60 kg N ha⁻¹ yr⁻¹ have been observed in non-forest ecosystems in Western Europe, whereas in forest stands in Europe or the USA values between 20-100 kg ha⁻¹ yr⁻¹ have been reached, instead of the estimated background inputs of 1-3 kg N ha⁻¹ yr⁻¹ in the early 1900s (e.g. Galloway, 1995; Asman et al., 1998; Fowler, 2002).

The availability of nutrients is one of the most important abiotic factors which determines the 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 (e.g. Tamm, 1991; Bobbink et al., 1998; Aerts and Chapin, 2000). In addition, the nitrogen cycle in ecosystems is complex and strongly regulated by biological and microbiological processes, and it is thus likely that many changes can occur in plant growth, inter-specific relationships and soil-based processes as a result of increased deposition of air-borne nitrogen pollutants.

The series of events which occurs when nitrogen 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. The severity of the impacts of atmospheric nitrogen deposition depends on a number of factors, of which the most important are: (1) the duration and total amount of the increased inputs; (2) the chemical and physical form of the airborne nitrogen input; (3) the intrinsic sensitivity of the plant and animal species present; (4) the abiotic conditions in the ecosystem; and (5) the past and present land use or management. Acid neutralising capacity (ANC), soil nutrient availability, and soil factors which influence the nitrification potential, nitrogen immobilisation and denitrification rates, are especially important. As a consequence, high variations in sensitivity to atmospheric nitrogen deposition have been observed between and within different natural and semi-natural ecosystems.

Despite this highly diverse sequence of events, the following main effect “categories” can be recognised. A schematic overview of the possible sequence of events is given in Figure 1.1. We emphasise that this is a rather simplified outline of possible events, which will not necessarily be followed in any specific ecosystem.
Figure 1.1. Scheme of the main impacts of enhanced nitrogen deposition on ecosystems. ↑ indicates increase; ↓ decrease; small arrow: effect will occur in the short term (< 5 yrs); wide arrow: indicates long-term effect. (+): positive feedback; (-) negative feedback (Bobbink and Lamers, 2002). In this figure stress is considered to occur when external constraints limit the rate of dry matter production of (part of) the vegetation, whereas disturbance consists of mechanisms which affect plant biomass by causing its partial or total destruction.

(a) Direct toxicity of nitrogen gases and aerosols to individual species

An important effect of dry and wet nitrogen deposition can be direct toxicity to the above-ground parts of individual plants. These effects have been mostly studied for crops and young trees, but studies with native herbaceous or dwarf shrub species in open top chambers (OTC) have also demonstrated changes in physiology and reductions in growth at high concentrations of airborne N pollutants (e.g. Pearson and Stewart, 1993). Direct toxicity effects of pollutant gases are not treated in this background document, as they are part of the critical level approach. Direct effects of wet deposited nitrogen on bryophytes and lichens are, however, considered, especially in organic soil systems with high abundance of these groups.
(b) Accumulation of nitrogen compounds, resulting in changes of species composition

Enhanced nitrogen loads result in an increase in the availability of inorganic nitrogen in the topsoil in the short term. In the first step this leads to an increase in plant productivity in nitrogen-limited vegetation and thus to higher annual litter production. Because of this, nitrogen mineralisation will gradually increase, which may cause an extra increase in plant productivity. This is a positive feedback, because higher nitrogen mineralisation gives higher nitrogen uptake, etc. In the longer term, competitive exclusion of characteristic species of oligotrophic or mesotrophic habitats by relatively fast-growing nitrophilous species is to be expected. The rate of nitrogen cycling in the ecosystem is thus clearly enhanced in this situation, although the response time to enhanced nitrogen inputs can be long in this respect in highly organic soils with their high C:N ratio, or, perhaps in any soil with large potential nitrogen sinks. When the nitrogen deficiencies in the ecosystem are no longer limiting, plant growth becomes restricted by other resources, such as phosphorus (P). In this situation, the productivity of the vegetation will not further increase. Nitrogen concentrations in the plants will, however, tend to increase, which may affect the palatability of the vegetation for herbivores or the sensitivity to pathogens. In addition, the first-stage decomposability of litter becomes higher, because of the decreased C:N ratios in the litter. Finally, the ecosystem becomes “nitrogen-saturated”, which leads to an increased nitrogen (e.g. nitrate) leaching from the soil to the deeper groundwater.

(c) Long-term negative effect of ammonium and ammonia

In many regions with a relatively high rate of nitrogen deposition, a very high proportion of the deposited nitrogen originates from ammonia and ammonium (e.g. Asman et al., 1998; Fowler, 2002). This may cause a change in the dominant nitrogen form in the soil from nitrate to ammonium, especially in habitats with moderately acidic conditions (pH: 4.5-6.5). The response of sensitive plant species can be significantly affected by this change. Species of calcareous or somewhat acidic soils are able to use nitrate, or a combination of nitrate and ammonium, as the nitrogen source, whereas early studies showed that species of acid 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). Recent laboratory and field studies demonstrated that most forest understory species are favoured when both ammonium and nitrate can be taken up (Falkengren-Grerup, 1998; Olsson and Falkengren-Grerup, 2000). One of the impacts of increased ammonium uptake is the reduced uptake of base cations (K⁺, Ca²⁺ and Mg²⁺). Ultimately this can lead to severe nutritional imbalances, which are considered to be the main factor in the decline in tree growth in areas with high ammonia/ammonium deposition (Nihlgård, 1985; Van Dijk et al., 1990; see also section 3.3.2 of this background document). High ammonium concentrations in the soil or water layer are also toxic to the roots of sensitive plant species, causing very poor root development, and finally, inhibition of shoot growth. Strong evidence exists that several endangered grassland and heathland species are very intolerant to high ammonium concentrations and high NH₄⁺/NO₃⁻ ratios (Roelofs et al., 1996; De Graaf et al., 1998).
(d) Soil-mediated effects of acidification

Soil acidification, caused by inputs of acidifying compounds, is characterized by a wide variety of long-term effects. It is defined as the loss of buffering capacity (ANC) and may lead to a decrease in pH. Decreases in pH are dependent on the buffering capacity of the soil (e.g., Ulrich, 1983; 1991). Acidifying inputs, deposited on calcareous soils, will at first not give a change in acidity. In these soils, which overly limestones, HCO₃⁻ and Ca⁡²⁺ ions leach from the system because of the acidifying pollutants, but the pH remains the same until most 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 (clay minerals and humus particles). In this situation, protons are exchanged with Ca⁡²⁺ and Mg⁡²⁺ of the adsorption complex, and these cations are leached from the soil together with anions, especially nitrate. Because of the restricted capacity of this buffering system, soil pH will soon start to decrease, together with losses of several base cations. In mineral soils with a large cation exchange capacity and high base saturation, this buffering may, however, hold for several hundred years, even at relatively high inputs. At low pH (<5.0), clay minerals are broken down and hydrous oxides of several metals are dissolved. This causes a strong increase of the concentration of toxic Al³⁺ and other metals in the soil solution. As a consequence of the decrease in pH, nitrification is strongly hampered or even completely absent in most soils. This may lead to accumulation of ammonium, whereas nitrate decreases to almost zero at these or lower pH values (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 complex of changes, growth of plant species and the species composition of the vegetation can be seriously affected; acid-resistant plant species will gradually become dominant, and several species typical of intermediate pH disappear.

(e) Increased susceptibility to secondary stress and disturbance factors such as drought, frost, pathogens or herbivores.

The sensitivity of plants to stress or disturbance factors may be significantly affected by deposition of nitrogen pollutants. The resistance to plant pathogens can be lowered because of a lower vitality of individual plants as a consequence of the different impacts of pollutants, or by decreased formation of secondary compounds such as phenolics, whereas increased organic nitrogen contents of plants can also result in increased herbivory. Furthermore, nitrogen-related changes in plant physiology, biomass allocation (root/shoot ratios) and mycorrhizal infection can also influence the sensitivity of plant species to drought or frost stress.

Under the Gothenburg Protocol, significant decreases in emissions of nitrogen oxides and smaller decreases in emissions of reduced nitrogen are expected by 2010. The issue of how quickly the changes described above can be reversed if nitrogen deposition falls, has thus become an important issue (e.g., Galloway and Cowling, 2002). Recent experimental evidence, and practical field experience in ecosystem restoration, suggests that, once the process of altered species composition and increased nitrogen mineralisation described in (b) above has occurred, recovery of the original vegetation may occur only over very long timescales, or with very active management intervention to decrease nitrogen status and cycling (e.g., Roelofs et al., 1996). This emphasises the need for caution in setting critical loads at which these major changes in vegetation composition and nitrogen cycling do not occur.
Aims of the document

Within the Convention on Long-range Transboundary Air Pollution covering the UNECE region, procedures have been developed to set and to map critical loads for airborne nitrogen deposition. Both the steady-state mass balance method and the empirical approach have been used within this Convention. Empirical critical loads of nitrogen for natural and semi-natural terrestrial ecosystems and wetland ecosystems were firstly presented in a background document for the 1992 workshop on critical loads held under the Convention at Lökeberg (Sweden) (Bobbink et al., 1992). After detailed discussion before and during the meeting, the proposed values were set at that meeting (Grennfelt and Thörnelöf, 1992). Additional information from the period 1992-1995 was evaluated and summarised in an updated background paper and published as Annex III in the Convention’s Manual on Methodologies and Criteria for Mapping Critical Levels/Loads (Bobbink et al., 1996). The updated nitrogen critical loads were discussed and set by full consensus at the December 1995 expert meeting held under the Convention in Geneva (Switzerland). They were also used for the development of the second edition of the Air Quality Guidelines for Europe of the World Health Organisation Regional Office for Europe (WHO, 2000). More recently, it was recognised (workshop at York held under the Convention, 2001) that considerable new insights into, and data on, the impacts of nitrogen deposition on natural and semi-natural vegetation have become available since the compilation of the last background paper in the mid-1990s. On the basis of the availability of new scientific evidence for many nitrogen-sensitive ecosystems an update of the background material used for the 1996 Convention’s Mapping Manual is thus clearly needed.

The aims of this background document are:

- to collect and to evaluate the new scientific data from the period 1996 to 2002;
- to formulate an extended and updated table of empirical nitrogen critical loads for semi-natural and natural ecosystems;
- to update the Convention’s Mapping Manual for empirical nitrogen critical loads;
- to provide more guidance regarding the classification and mapping of nitrogen-sensitive ecosystems.

In this background document, new information from the period 1996-2002 on the impacts of increased nitrogen deposition on the structure and function of natural and semi-natural ecosystems is given. The information is summarised by groups of ecosystems in more or less the same order as in the 1996 document. The approach used to set empirical critical loads is described in Chapter 2, while the information and proposals for updated nitrogen critical loads by groups of ecosystems is presented in Chapters 3 to 9 (from forest and woodland habitats to marine habitats). Finally, a fully updated table of empirical nitrogen critical loads is presented in the last chapter of this document, Chapter 10.
2. Updating procedure for empirical nitrogen critical loads

2.1 Updating procedure

Based on observed changes in the structure and function of ecosystems, reported in a range of publications, empirical critical loads for nitrogen were evaluated for specific receptor groups of natural and semi-natural ecosystems in both 1992 and 1996 (Bobbink et al., 1992a; 1996). In this updating procedure we have used a similar “empirical approach” as for the earlier background documents. For this purpose, we have firstly collected, as completely as possible, European publications on the effects of nitrogen in natural and semi-natural ecosystems from the period 1996 to mid 2002. We used peer-reviewed publications, book chapters, nationally published papers and “grey” reports of institutes or organisations, if available by request. In principle, only European studies have been used as the basis for the assessment of nitrogen critical loads. However, when no or very few studies were available, non-European (mostly USA) literature has been used to make an expert judgement as reliable as possible.

Studies providing insights into ecosystem reactions to an increase in nitrogen input or availability have been conducted for a variety of reasons. This has resulted in many different designs, from correlative or retrospective field studies, experimental studies in pots and mesocosms to field addition experiments. In addition, dynamic ecosystem modelling is also relevant in this respect. We especially use statistically and biologically significant outcomes of field addition experiments and mesocosm studies for the assessment of empirical nitrogen critical loads. Only studies which have independent nitrogen treatments and realistic nitrogen loads and durations (below 100 kg N ha\(^{-1}\) yr\(^{-1}\); more than 1 yr) have been used for the updating and setting of the critical load values. Studies with higher nitrogen additions or shorter experimental periods have only been interpreted with respect to the possible nitrogen 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.

In addition, the results from correlative or retrospective field studies have been used as the basis for estimates of critical loads, but only as additional evidence either to complement the results from experimental nitrogen addition studies, or as an indication for expert judgement. If available, the outcome of dynamic ecosystem models has served to fill gaps where empirical data of appropriate time scales are missing, or to provide 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 have not been accepted for the setting of critical loads, except for bryophyte layer studies. However, the outcome of these studies is in some selected cases used as an indication of the nitrogen sensitivity of the most important plant species of an ecosystem.
2.2 Ecosystem classification

In this background document, the groups of natural and semi-natural ecosystems have now been re-classified and ordered according the EUNIS habitat classification for Europe (Davies and Moss, 2002). For a general description of the EUNIS classification and an introduction to its use with respect to nitrogen critical loads, see the separate background document of Hall et al. (2003). In general, the ecosystems described in this document have been classified down to level 2 or 3 of the EUNIS hierarchy and the EUNIS-code is given in the text and tables in brackets, e.g. raised and blanket bogs (D1). Because of the use of the EUNIS classification, some ecosystems have been treated in different sections to those used in Bobbink et al. (1996). Specifically:

- soft water lakes and permanent dune pools are treated in a separate Chapter 7 (inland surface water habitats; C);
- the short items on dune grasslands, dune heaths and slacks are now described in a separate Chapter 8 on coastal habitats (B);
- salt marshes are discussed in a short section (Chapter 9) on marine habitats (A).

Generally, a good agreement exists between the vegetation classification used by Bobbink et al. (1996) and the EUNIS classification which is now adopted, although in some cases the EUNIS classification is somewhat incomplete (e.g. no gradient from soft to hard water in class C). However, the EUNIS classification of grasslands and tall forb habitats (E) has had considerable consequences for the structure of the grassland chapter. An overview of the old and new classification has been given in Table 2.1 to facilitate the shift to this newly used EUNIS classification.

Furthermore, it has to be mentioned that for forest ecosystems the text has been structured as in the 1996 background document, and thus not structured as in EUNIS. However, in the introduction of Chapter 3 the classification used in this document has been compared with the forest classification of EUNIS (forests and woodland habitats; G). As before, studies based on pure plantation stands are not accepted in the forest section, because the nitrogen critical loads of these intensively used systems are obtained via the steady-state mass balance method (Mapping Manual, 1996). Lichens and bryophytes have been, as before, incorporated in the sections of the ecosystems where they are found as an integral part of their biodiversity.
Table 2.1. Cross-comparison between the ecosystem classification used in the 2002 empirical N critical load setting (according to the EUNIS system) and the classification previously used (Bobbink et al., 1996); with n.d. = not distinguished.

<table>
<thead>
<tr>
<th>Ecosystem classification 2002</th>
<th>EUNIS</th>
<th>Ecosystem classification 1996</th>
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</thead>
<tbody>
<tr>
<td>Heathland, scrub and tundra habitats</td>
<td>F</td>
<td>Heathlands</td>
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<tr>
<td>Tundra</td>
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<td>Arctic, alpine and subalpine scrub</td>
<td>F2</td>
<td>Arctic and Alpine heaths</td>
</tr>
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<td>Northern wet heaths</td>
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<td>Upland Calluna heath</td>
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<td>• ‘U’ Calluna dominated wet heath</td>
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<td>Lowland wet heathlands</td>
</tr>
<tr>
<td>• ‘L’ Erica tetralix dominated wet heath</td>
<td>F4.11</td>
<td>Lowland dry heathlands</td>
</tr>
<tr>
<td>Dry Heaths</td>
<td>F4.2</td>
<td>Lowland dry heathlands</td>
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<td><strong>Grasslands and tall forb habitats</strong></td>
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<td>Specie-rich grassland</td>
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<td>Calcareous grassland</td>
</tr>
<tr>
<td>Non-mediterranean dry acid and neutral closed grasslands</td>
<td>E1.7</td>
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<td><strong>Mire, bog and fen habitats</strong></td>
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<td><strong>Inland surface water habitats</strong></td>
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<td>Shallow softwater bodies</td>
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<td>• Dune slack pools</td>
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<td><strong>Marine habitats</strong></td>
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</tr>
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</table>
Ranges and reliability

As in the 1992 and 1996 documents, the empirical nitrogen critical loads have been established within a range for each ecosystem class, because of: (i) real intra-ecosystem variation between different regions and sites where an ecosystem has been investigated; (ii) the intervals between experimental additions of nitrogen; and (iii) uncertainties in presented total atmospheric deposition values, although the latter have been checked by local specialists on atmospheric nitrogen deposition. In chapter 10 some additional information has been given on how to interpret this range in specific situations for an ecosystem. For every group of ecosystems (Chapter 3-9), the updated empirical nitrogen critical loads are, as before, summarized in a table, together with an indication of exceedance and of their reliability.

The reliability of the presented nitrogen critical loads figures is indicated as in the 1996 document (Bobbink et al., 1996):

- reliable ##: when a number of published papers of various studies show comparable results;
- quite reliable #: when the results of some studies are comparable;
- expert judgement (#): when no data are available for this type of ecosystem. The nitrogen critical load is then based upon expert judgement and knowledge of ecosystems, which are likely to be more or less comparable with this ecosystem.
3. Effects of nitrogen inputs in forest habitats (EUNIS class G)

3.1 Introduction
Empirical critical loads were established for trees (deciduous and coniferous), for ground flora in forest stands, and for epiphytic lichens and algae, on acidic and on calcareous soils by Bobbink et al. (1996). The introduction of the EUNIS classification emphasizes the dominant tree species, soil hydrology and management practices more than soil chemistry. Furthermore, according to the review of new data, the differentiation into calcareous and acidic forests seems not to be necessary except for acidity related processes, as a range of changes such as effects on growth, nutrition, parasite infestation and changes in ground flora have been found at similar nitrogen loads. Critical load values can be set for EUNIS class G1 (broad-leafed woodland), and G3 (coniferous woodland), with the latter being divided into boreal and temperate, but often without clear differentiation between these classes and subgroups. When the critical load is the same for broad-leafed and coniferous woodland, values can also be applied to G4 (mixed deciduous and coniferous woodland). Moreover, a differentiation according to hydrology is not possible because of missing results for fluvial, riparian or swamp woodland. There are no studies available for G2 (broad-leafed evergreen). Highly artificial forest plantations (G1.C, G1.D, G3.F and G4.F) are excluded from setting empirical critical loads.

Forest ecosystems consist of different compartments which may be affected differently by increased nitrogen deposition. The soil may be acidified or eutrophied, both processes having consequences for microbiology, soil fauna and vegetation. Nutrition and growth of trees are changed by acidification and/or eutrophication. This may alter their resistance to abiotic and biotic stress factors. The species composition of the ground vegetation and of the mycorrhizal fungi may also be affected, with nitrogen demanding or acid tolerant species increasing and other species decreasing. Thus, the biodiversity may be altered by nitrogen deposition.

3.2 Effects on soil processes
The soil plays an important role in mediating nitrogen effects to the whole forest ecosystem. The following processes are important:

a) soil acidification, due to nitrification of ammonium and to 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 mycorrhiza, and thus reduce nutrient uptake (Ulrich, 1983; Ritter, 1990).

b) eutrophication. A surplus of nitrate, originating from deposition or enhanced nitrification due to accumulated nitrogen in the soil, will lead to eutrophication. Field-based $^{15}$N studies demonstrate that a large proportion of incoming nitrogen (11-56%) is retained in the forest floor through biotic and abiotic processes within the first two years (Tietema et al., 1998; Emmet et al., 1998). An accumulation of ammonium at the ion exchanger may happen in areas with high NH$_4^+$-deposition (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 shown by the data from the ICP forest plots (De Vries et al., 2003).
3.2.1 Soil acidification

Soil acidification per se is only briefly reviewed in this document, because critical loads for acidity, set on the basis of base cation to aluminium ratio and tree growth, are well established (e.g. Nilsson and Grennfelt, 1988; Sverdrup and Warfvinge, 1993). However, the significance of nitrogen compounds in acidification is increasing in parallel to sulphur reduction. In Western Europe, their contribution increased from 53% (1990) to 72% (1999). Regions with high acid loads have a high proportion of nitrogen and the significance of nitrogen in them is increasing (all data from Tarrason et al., 2001).

3.2.2 Mineralization, nitrification, NO\textsubscript{3}\textsuperscript{–}-leaching, NH\textsubscript{4}\textsuperscript{+} accumulation

Nitrogen mineralization and nitrification may both be stimulated by nitrogen deposition. In a field study in 600 deciduous forests in four geographically separate regions of southern Sweden, nitrogen mineralization and nitrification were by far the highest in the region with the highest nitrogen deposition (17 kg N ha\textsuperscript{-1} yr\textsuperscript{-1}), especially in the most acid soils. Soil nitrogen mineralization rate was increased by 40-80%, nitrification rate increased by 20-90% and C:N ratio decreased by 10-25% compared to the region with 7-10 kg N ha\textsuperscript{-1} yr\textsuperscript{-1} (Falkengren-Grerup et al., 1998; Falkengren-Grerup and Diekmann, 2003). Differences were also found between 7 and 10 kg N ha\textsuperscript{-1} yr\textsuperscript{-1} (Fig. 3.1). The effects depend on the deposition history. NITREX experiments, e.g., did not find effects on mineralization (Emmet, 1999), and McNulty et al. (1996) observed the highest nitrogen mineralization potential in control plots of a spruce-fir stand at high elevation but the net nitrification potential was highest in nitrogen fertilized plots (15.7 kg N ha\textsuperscript{-1} yr\textsuperscript{-1}).

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

In acid forest soils which have little or no nitrification, ammonium is accumulating (Van Dijk and Roelofs, 1988). As a reliable critical load, 10-15 kg NH\textsubscript{4}\textsuperscript{+}-N ha\textsuperscript{-1} yr\textsuperscript{-1} has been proposed to prevent critical NH\textsubscript{4}\textsuperscript{+} accumulation and nutrient imbalances caused by high ratios between NH\textsubscript{4}\textsuperscript{+} and K, Ca or Mg (Roelofs et al., 1985; Boxman et al., 1988) although usually no NH\textsubscript{4}\textsuperscript{+} can be detected in the soil solution (De Vries et al., 2003). However, in a large number of cases, NH\textsubscript{4}\textsuperscript{+} is nitrified and may leach together with deposited NO\textsubscript{3}\textsuperscript{–} from the soil. Various investigations in
deciduous and coniferous stands demonstrate that if nitrogen loads in throughfall exceed >12-15 kg N ha\(^{-1}\) yr\(^{-1}\), NO\(_3^-\) leaching increases exponentially (Westling, 1991; Kölling and Neustifter, 1997; Gundersen et al., 1998a; Nilsson et al., 1998). NO\(_3^-\) leaching in coniferous stands is higher at sites with low C/N ratio in the organic layer (Dise et al., 1998; Gundersen et al., 1998a; De Vries et al., 2001; 2002). In broad-leaved stands, the relationship between soil solution NO\(_3^-\) concentration and throughfall is steeper than in conifer stands (Fig. 3.2) indicating that NO\(_3^-\) concentration in soil solution under deciduous forests will respond much more to deposition changes than under conifers. The difference in relationship between soil solution nitrate and throughfall nitrogen between broad-leaved and conifers could be due to a higher potential nitrogen retention capacity in the forest floor or in the conifer trees because of a higher potential growth rate. The former has to be avoided because of subsequent negative effects on nutrition, and the latter will only work as long as nothing else becomes limiting to conifer growth. Therefore, the different relationship does not necessarily imply a differentiation between nitrogen critical loads for broad-leaved and coniferous forests but it has consequences for setting the reliability of the critical load.

Figure 3.2. Scatter plots of the leaching of total nitrogen against the total nitrogen deposition for conifers (C) and deciduous trees (D) with a polynomial fit through the data (data from ICP Forests, De Vries et al., 2003).

3.2.3 Litter decomposition

The effect of increased nitrogen deposition on litter decomposition seems to depend on the lignin concentration of the substrate (Carreiro et al., 2000) and on the decomposition stage (Magill and Aber, 1998). The activity of phenol oxidases which decompose lignin is decreased by nitrogen while cellulase activity is stimulated. Thus, the decomposition of lignin-rich litter and of the late decomposition stages with lignin enrichment is delayed. Significant effects of experimental NH\(_4\)NO\(_3\) application on litter decomposition of dogwood (Cornus florida) and oak (Quercus rubra) were found at nitrogen 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 (Carreiro et al., 2000). In contrast, Gundersen (1998) found no response of decomposition rates of spruce litter to an addition of 25 kg N ha\(^{-1}\) yr\(^{-1}\) (atmospheric nitrogen deposition 15-20 kg). The observed responses may lead to increased accumulation of soil organic matter in stands with a higher nitrogen deposition (Magill and Aber, 1998), causing a fixation of base cations in this organic matter and
thus enhancing the risk of soil acidification. Decomposition is going on until the remaining organic matter reaches a limit value at which total mass loss virtually stops. At such a stage the remaining litter is close to that of stabilized soil organic matter. In 106 long-term studies on litter decomposition, enclosing 21 litter types, limit values were significantly negatively related to nitrogen concentrations. The higher the nitrogen concentration in the litter (i.e. the lower the C/N ratio), the more organic matter was left when the litter reached its limit value (Berg and Meentemeyer, 2002).

In summary, the available data on soil processes suggest a critical load of $10-15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for mineralization and nitrification (quite reliable), for nitrate leaching in coniferous forests (reliable) and for nitrate leaching in broad-leaved forests (expert judgement). For litter decomposition, the existing data did not allow to define a separate critical load.

### 3.3 Effects of nitrogen deposition on growth, nutrition, physiology and parasite attacks of trees

The growth of a vast majority of the forest tree species in the Northern hemisphere was until recently limited by nitrogen (Tamm, 1991). An increase in the supply of any essential nutrient, including nitrogen, will stimulate tree growth as long as growth is not limited by other factors; the initial impact of enhanced nitrogen deposition will, therefore, most often be a fertilizer effect. However, chronic nitrogen deposition may result in 'nitrogen saturation', when enhanced nitrogen inputs no longer stimulate tree growth, but start to disrupt ecosystem structure and function (Agren, 1983; Aber et al., 1989; Tamm, 1991).

#### 3.3.1 Growth

**Growth of above-ground biomass**

In temperate European regions, forest growth has increased in the past 40-50 years (Spiecker et al., 1996). Recently a pan-European research project concluded that the increased nitrogen deposition is the main cause for the observed increases in forest growth (EFI, 2002). There are many publications showing that nitrogen fertilization increased growth or had no effect (e.g. Alcubilla et al., 1976; Spiecker, 1991; Nilsson and Wiklund, 1992; Emmet, 1999). Braun et al. (1999) showed a significant correlation between stem increment of beech and modelled nitrogen deposition in Switzerland. It was, however, restricted to plots with sufficient phosphorus supply. The largest growth dataset comes from southern Norway, where data from increment cores of >31,000 spruce forest plots were analysed for the time period 1954-1996. Increases in growth during 1960-1970, followed by a subsequent decline in the 1980-1990, were observed in the plots with modelled wet nitrogen deposition >15-25 kg N ha$^{-1}$ yr$^{-1}$. A growth decrease started in plots with wet nitrogen deposition of 7-15 kg N ha$^{-1}$ yr$^{-1}$ in the 1990s. No change was observed in plots with wet nitrogen deposition <7 kg N ha$^{-1}$ yr$^{-1}$ (Fig. 3.3; Nellermann and Thomsen, 2001). However, nitrogen deposition might be higher as dry nitrogen deposition was not included in the model. Also, an additional acidification effect of codepositing sulfur cannot be excluded. This pattern of initial growth stimulation, followed by subsequent growth decline, was also observed in a nitrogen addition experiment in a young beech stand in Switzerland on calcareous soil with a modelled atmospheric deposition of 16 kg N ha$^{-1}$ yr$^{-1}$. Five years after the start of the experiment,
shoot growth was significantly increased by addition of 10 kg N ha\(^{-1}\) yr\(^{-1}\), not by higher nitrogen loads. After 8 years, there was still a growth stimulation at 10 kg, but a significant growth decrease at 160 kg N ha\(^{-1}\) yr\(^{-1}\). In the 9th year, growth stimulation no longer occurred, but a significant growth decrease was found at 80 and 160 kg N ha\(^{-1}\) yr\(^{-1}\). On acidic soil, a growth decrease was only observed at 160 kg N ha\(^{-1}\) yr\(^{-1}\) (Braun and Flückiger, 2002). It was suggested that the reason for a changed response to nitrogen addition is the limitation of other nutrients such as phosphorus or base cations (Flückiger and Braun, 1999b).

Figure 3.3. Stem increment of spruce in Norway from 31,606 increment cores grouped according to modelled wet nitrogen deposition. Growth increase in the highest deposition class as well as the decrease in the two highest classes are significant at p<0.01 (Nellemann and Thomsen, 2001).

A growth reduction by nitrogen was also shown in the NITREX experimental plot in the Netherlands where ambient nitrogen deposition was reduced from 56 to 4 kg N ha\(^{-1}\) yr\(^{-1}\); trees in the roof clean condition grew better than in the roof control (Boxman et al., 1998). In southern Sweden, a positive relationship between nitrogen concentration and branch growth of Norway spruce was found in trees which were younger than 40 years, whereas older trees seemed to be limited by K and/or phosphorus (Thelin, 2000).
Nitrogen may also stimulate root growth (Zöttl, 1964; Heinsdorf and Schulzke, 1969; Persson, 1980) in ecosystems which are not nitrogen saturated. However, some studies showed that root growth is inhibited by excessive nitrogen supply, and there is strong evidence for increased nitrogen deposition causing reduced fine root biomass and root length. Boxman et al. (1995), Murach and Parth (1999) and Persson and Ahlström (2002) reported increasing root biomass and root vitality in Scots pine, Douglas fir and Norway spruce respectively when trees were protected from nitrogen deposition in the NITREX roof experiments. The corresponding treatments were a reduction of nitrogen deposition from 56 to 4 kg N ha\(^{-1}\) yr\(^{-1}\) in Ysselsteyn (Scots pine) (Gundersen et al., 1998b), from 36 to <5 kg N ha\(^{-1}\) yr\(^{-1}\) in Solling (Douglas fir, Norway spruce) and from 13 to <4 kg N ha\(^{-1}\) yr\(^{-1}\) in Gårdsjön (Norway spruce). Total fine root biomass of Norway spruce saplings decreased significantly when NO\(_3^-\) + NH\(_4^+\) in soil water was >2 mg N l\(^{-1}\) (Fig. 3.4; Matzner and Murach, 1995). From the relationship between nitrogen deposition and NO\(_3^-\) concentration published by De Vries et al. (2001), this concentration can be attributed in coniferous stands to an average nitrogen throughfall load of 25 (range 13-33) kg N ha\(^{-1}\) yr\(^{-1}\).

![Figure 3.4. Fine root biomass of spruce in relation to nitrogen in soil solution (Matzner and Murach, 1995).](image_url)

### 3.3.2 Nutrition of trees

Increased nitrogen deposition may change the nutrition of trees by increasing the nitrogen concentration in the foliage. Usually, P concentrations and - depending on the soil - also K and/or Mg concentrations decrease simultaneously. Thus, the ratios between nitrogen on the one hand and P, K and Mg on the other hand tend to increase. These changes have been shown in both field observations and experimental nitrogen additions. A decrease of P and Mg concentration in tree foliage may be caused by reduced uptake as a result of Al toxicity and hence soil acidification (Haynes, 1982; Godbold, 1991), as a result of competition with NH\(_4^+\) in the soil (Roelofs et al., 1985), as a result of nitrogen impacts on mycorrhizal fungi (see section 3.4), or by a decreased supply rate of nutrients other than nitrogen due to leaching and nitrogen induced high growth rates depleting the resource (Thelin et al., 1998).
Rising nitrogen concentrations in the needles of various conifer species in Europe were reported in recent decades (Van den Burg, 1990; Sauter, 1991). Nitrogen concentrations in needles of Scots pine from the ICP forest plots (De Vries et al., 2003) and of Norway spruce from Switzerland (Flückiger and Braun, 1998) were found to be correlated with nitrogen deposition. Based on a critical level of nitrogen concentration of 18 mg N g\(^{-1}\) in foliage, a critical nitrogen load for pine of 14 kg N ha\(^{-1}\) yr\(^{-1}\) and near 20 kg N ha\(^{-1}\) yr\(^{-1}\) for spruce was suggested by De Vries et al. (2003). In northeastern France, foliar mineral concentrations in 118 beech stands were compared between 1969-1971 and 1996-1997. Between the two sampling periods, foliar nitrogen concentrations increased by 12% whereas a decrease was observed for P (-23%), K (-6%), Mg (-38%) and Ca (-16%). The N/P ratio increased by 42%, N/K by 19% and N/Mg by 77%. The positive nitrogen trend did not depend on soil type, with similar trends on calcareous or acid soils. The atmospheric nitrogen deposition was estimated to be between 20-30 kg N ha\(^{-1}\) yr\(^{-1}\) (Duquesnay et al., 2000). In Switzerland, foliar P concentration in beech (49 plots) decreased between 1984 and 1999 by 26%, leading to an increase in N/P ratios from 18.1 (1984) to 25.4 (1999) (+40%), in N/K ratios from 2.58 to 3.66 (+42%) and of N/Mg ratios from 16.3 to 18.3 (+12%) – as in the French study independently from the soil type. At the same time, P concentrations in Norway spruce (18 plots) dropped by 15%, with N/P ratios increasing from 14.0 to 16.3. Modelled nitrogen deposition was in the range of 15-35 kg N ha\(^{-1}\) yr\(^{-1}\) (Flückiger and Braun, 1998). In pine forests in Brandenburg and in spruce forests in the Mittelgebirge (Germany), nitrogen concentration in foliage increased, and Mg decreased, significantly within 25 years, between 1964 and 1988 (approx. nitrogen deposition of 15-25 kg N ha\(^{-1}\) yr\(^{-1}\)) (Nebe, 1991; Hippeli and Branse, 1992). In Sweden, addition of nitrogen enhanced nutritional imbalances in a 120 year old Fagus forest (Balsberg-Påhlsson, 1992). Mohren et al. (1986) and Houdijk and Roelofs (1993) found significantly lower phosphorus supply, or phosphorus deficiency, in conifers in forests exposed to raised nitrogen deposition in the Netherlands. Nihlgård and Olsson (1993) also report an increase of 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 deposition of nitrogen and sulphur (Thelin et al., 1998). Phosphorus and nitrogen are well known to be limiting nutrients in forests (Johnson and Taylor, 1989). However, the increasing nitrogen deposition during the last decades could have aggravated phosphorus deficiency in forest ecosystems low in phosphorus supply (Tamm, 1991).

Experimental nitrogen 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 nitrogen loads of ≥10-20 kg N ha\(^{-1}\) yr\(^{-1}\) after 4-6 years of nitrogen treatment (modelled atmospheric deposition 12-20 kg N ha\(^{-1}\) yr\(^{-1}\)). On acidic soil, nitrogen treatment led to acute Mg deficiency (Fig. 3.5) whereas on calcareous soil K and P became limiting (Flückiger and Braun, 1999a). Addition of 35 kg N ha\(^{-1}\) yr\(^{-1}\) to an ambient nitrogen input of 15-20 kg N ha\(^{-1}\) yr\(^{-1}\) in a spruce forest at Klosterhede led to increased needle nitrogen concentrations and decreased P and Mg concentrations in the foliage during the 3 years of treatment (Gundersen, 1998).
Insight into the possible impact of nitrogen on foliar nutrition can also be gained from results from 109 level II plots of ICP Forests with information on both the chemical composition of the foliage and the total nitrogen input by deposition. In plots with “adequate ratios” of K, Ca, Mg or P to N, the median nitrogen deposition was 9.6 kg N ha\(^{-1}\) yr\(^{-1}\), whereas in plots with unbalanced nutrition it was 21 kg N ha\(^{-1}\) yr\(^{-1}\) (De Vries et al., 2003, Tab. 3.1). The results, however, depend on the critical nutrient ratios used for the evaluation (Flückiger and Braun, 2003).

Table 3.1. Ranges in nitrogen deposition (kg N ha\(^{-1}\) yr\(^{-1}\)) at 109 Intensive Monitoring plots of ICP Forests in Europe with a balanced and unbalanced ratio of the macronutrients P, K, Ca or Mg to N. (De Vries et al., 2003).

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<th>Element</th>
<th>Unbalanced nutrition</th>
<th>Balanced nutrition</th>
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<td>Nr of plots</td>
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<tr>
<td>P</td>
<td>46</td>
<td>21 6.9 34</td>
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<tr>
<td>K</td>
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<td>All</td>
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With high nitrogen inputs, the concentrations of organic nitrogen in needles can increase to high levels above the optimum range (Van Dijk and Roelofs, 1988; De Kam et al., 1991). Nitrogen-rich free amino acids, especially arginine, significantly increased in the needles with high nitrogen concentration (Hällgren and Näsholm, 1988; Van Dijk and Roelofs, 1988; Näsholm and Ericsson, 1990; Pietilä et al., 1991; Balsberg-Pählsson, 1992). Arginine concentrations in foliage are suggested to be a sensitive indicator for nitrogen input (Edfast et al., 1990; Huhn and Schulz,
In Sweden, arginine concentrations of coniferous foliage >5 µmol g dw⁻¹ has been linked to forest stands susceptible to NO₃⁻ leaching (Näsholm et al., 1997), because arginine accumulation in trees corresponds to decreased uptake rates of NH₄⁺, leaving NH₄⁺ ions available for nitrification followed by NO₃⁻ leaching (Näsholm et al., 1997). In permanent observation plots in Sweden, arginine concentrations in Norway spruce were higher in areas receiving high inputs of nitrogen (Ericsson et al., 1995). In Swiss plots, arginine concentrations in spruce foliage were strongly correlated with modelled nitrogen deposition in the range of 14-37 kg N ha⁻¹ yr⁻¹ (Quiring et al., 1997). By decreasing nitrogen deposition in the NITREX roof experiment, arginine concentrations in needles of Scots pine significantly decreased (Boxman and Van Dijk, 1994; Boxman et al., 1995). Similarly, halted nitrogen 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).

Considering all demonstrated effects of nitrogen deposition on the nutritional status of trees, a reliable critical load for nitrogen for both deciduous and coniferous temperate forests of 15-20 kg N ha⁻¹ yr⁻¹ can be recommended. Calcareous and acidic soils may differ in the type of nutrient imbalance but not fundamentally in the sensitivity of the response.

### 3.3.3 Tree physiology

#### Winter injury

Winter injury may be caused either by low temperatures or by drought stress. Whereas sensitivity to low temperatures is usually decreased by nitrogen addition (DeHayes et al., 1989; Klein et al., 1989; L'Hirondelle et al., 1992), sensitivity to frost droughts seems to be increased. For example, long term nitrogen fertilization (0, 15.7, 19.8, 25.6, 31.4 kg N ha⁻¹ yr⁻¹, atmospheric bulk precipitation 5.4 kg N ha⁻¹ yr⁻¹) from 1988 to 1995/96 in the eastern US increased winter injury on montane red spruce (*Picea rubens*) foliage significantly at ≥15.7 kg N ha⁻¹ yr⁻¹, although cold tolerance was not affected and dehardening was decreased by the nitrogen treatment (Perkins et al., 2000). Moreover, in field fertilization experiments it is often observed that tree growth starts earlier in the season, which may increase damage by late frost.

#### Drought tolerance

The effects of nitrogen on fine roots of trees and on mycorrhiza may have consequences for drought tolerance. Indeed, the vitality of many tree species in the regions with high nitrogen deposition in the Netherlands was particularly low in the dry years in the mid 1980s, but vitality increased again in the following normal years (Heij et al., 1991). In a nitrogen addition experiment (0, 25, 50, 100, 200, 400 kg N ha⁻¹ yr⁻¹) in pots, the shoot/root ratio of beech was significantly increased at ≥ 25 kg N ha⁻¹ yr⁻¹ and the leaf surface per tree at ≥ 50 kg N ha⁻¹ yr⁻¹ after six year’s treatment. This led to more rapid water loss, as shown by a negative correlation between water content of the soil one day after watering and nitrogen fertilization (significant at ≥ 100 kg N ha⁻¹ yr⁻¹). It also led to increased leaf necroses after a drought period (significant at ≥ 200 kg N ha⁻¹ yr⁻¹) (Flückiger and Braun, 1999b). Planted beech saplings in another nitrogen fertilization experiment showed significantly increased leaf necroses after short drought periods in the second and third treatment year at ≥20 kg N ha⁻¹ yr⁻¹ (Fig. 3.6; Thomas et al., 2002).
Figure 3.6. Leaf necroses in beech caused by drought in a nitrogen addition experiment. Differences to control significant at *p<0.05, ***p<0.001, general linear trend p<0.001 (Thomas et al., 2002).

Root physiology, roots and soil properties
In a field fertilization experiment in Switzerland, starch concentration in fine roots of beech was significantly decreased at ≥ 20 kg N ha\(^{-1}\) yr\(^{-1}\) after 8 years of nitrogen application (Hiltbrunner et al., 2001). Uprooting of mature beech in forest observation plots in Switzerland by the storm ‘Lothar’ in 1999 was positively correlated with nitrogen concentration in the foliage (atmospheric N-deposition 15-35 kg N ha\(^{-1}\) yr\(^{-1}\)), and with soils showing a base saturation ≤ 40% (Braun et al., 2003).

Mycorrhizal fungi are crucial for nutrient and water uptake of forest trees and for resistance of roots against pathogens. Their species diversity, the formation of extramatrical mycelium and the production of fruitbodies are affected by nitrogen deposition. These effects are discussed in section 3.4.1.

3.3.4 Parasites
With increasing nitrogen deposition, the susceptibility to fungal pathogens and attacks by herbivores can change. This is probably due to altered concentrations of phenolic compounds and soluble nitrogen compounds such as free amino acids (Huber, 1980; McClure, 1980; Bolsinger and Flückiger, 1989; Balsberg-Pålsson, 1992). A fertilization experiment with only 10 kg N ha\(^{-1}\) yr\(^{-1}\) in two sites (atmospheric deposition 12 and 20 kg N ha\(^{-1}\) yr\(^{-1}\)) was enough to alter the concentrations of fungistatic phenolic compounds in fine roots of young beech and spruce after seven years of nitrogen treatment, with most of them decreasing (Tomova et al., submitted). 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 decreased by more than 30% after fertilization with ca. 45 kg N ha\(^{-1}\) yr\(^{-1}\) over 4 years, compared with the control (Balsberg-Pålsson, 1992).
**Fungal pathogens**

In the Netherlands, an epidemic outbreak 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 Netherlands with high levels of air-borne nitrogen deposition (Roelofs *et al.*, 1985). Attacked trees in the infested stand had significantly higher foliar nitrogen concentrations, occurring together with higher soil ammonium levels, than uninfected trees. Most of the additional nitrogen 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 nitrogen supply and water stress increased the susceptibility of trees to attacks from *Sphaeropsis sapinea* (Blodgett *et al.*, 1997). High potassium supply at the same time reduced the development of necroses (De Kam *et al.*, 1991).

In permanent beech observation plots in Switzerland (modelled nitrogen deposition 15-35 kg N ha\(^{-1}\) yr\(^{-1}\)), as well as in nitrogen fertilization experiments, a significant positive correlation was found between the nitrogen/potassium ratio in leaves and the necroses caused by the beech cancer *Nectria ditissima* (Flückiger *et al.*, 1986; Flückiger and Braun, 1998). 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 nitrogen deposition (20-25 kg N ha\(^{-1}\) yr\(^{-1}\)) compared to sites with lower nitrogen deposition (15-20 kg N ha\(^{-1}\) yr\(^{-1}\)) (Westling *et al.*, 1992). Bark lesions were positively correlated to the occurrence of the beech scale *Cryptococcus fagisuga* and algal cover of *Lecanara fagisuga* (Jönsson, 1998).

In two nitrogen fertilization experiments with 0, 10, 20, 40, 80 and 160 kg N ha\(^{-1}\) yr\(^{-1}\) on acid and on calcareous soils, respectively, the damage of young beech by the pathogenic fungi *Apiognomonia errabunda* and *Phomopsis* sp., was significantly increased at treatments ≥10 kg N ha\(^{-1}\) yr\(^{-1}\) (atmospheric nitrogen deposition 20 and 16 kg N ha\(^{-1}\) yr\(^{-1}\), respectively). There was also a strong positive correlation of the extent of twig necroses with N/P and N/K ratios in leaves (Flückiger and Braun, 1999a). The effect was similar on acidic and on calcareous soils.

Effects of nitrogen on the pathogen and insect infestation have also been observed in understorey shrubs. They are treated in chapter 3.4.3.

**Insect pests**

Most insects which have been found to react to nitrogen fertilization feed by sucking. The infestation of beech aphid *Phyllaphis fagi* in a nitrogen fertilization experiment increased significantly with increasing nitrogen concentration in leaves and N/P ratios respectively (Flückiger and Braun, 1998). The attack of beech nuts in Swiss permanent observation plots by the tortricid *Cydia amplana* also increased significantly with increasing N/P ratio in leaves (N-deposition 15-60 kg N ha\(^{-1}\) yr\(^{-1}\)) (Braun and Flückiger, 2002).

The occurrence of insect damage to pine needles in permanent observation plots in the UK was found to be positively correlated with modelled nitrogen deposition (range 7-22 kg N ha\(^{-1}\) yr\(^{-1}\)), but only within Scotland itself. This relationship was associated with a negative relationship between the years of needles retained and modelled nitrogen deposition (NEGTAP, 2001).
Whereas the better performance of the insects listed above may be explained by a better nutrition with soluble nitrogen compounds, the situation is more complicated in the case of the red-black pine bug *Haematoloma dorsatum*, originating from the Mediterranean region, which causes severe needle damage to pine stands in the Netherlands. The nymphs of the bugs suck on the roots or basal stem parts of *Deschampsia flexuosa* exclusively. Only the adults cause damage in the trees. *D. flexuosa* seems to be crucial for nymphal development, because it is a wintergreen grass and hence suitable for sucking in wintertime and early spring. The abundance of this grass in pine forests is thereby an important ecological factor for pest development of *H. dorsatum* (Moraal, 1996). It has been shown by various authors that the abundance of *D. (Avenella) flexuosa* increases significantly when nitrogen deposition is >10-15 kg N ha\(^{-1}\) yr\(^{-1}\) (see section 3.4).

Considering the detrimental effects of pathogens and insect pests upon tree health with increasing nitrogen deposition, a critical load for nitrogen of 15-20 kg N ha\(^{-1}\) yr\(^{-1}\) is recommended (expert judgement). However, there is not enough data available to differentiate between forest types. No data are available for boreal forests; thus no separate critical load can be defined.

### 3.4 Effects of nitrogen deposition on biodiversity of forests

#### 3.4.1 Effects on macrofungi and mycorrhiza

Nitrogen deposition can influence fruit body formation by ectomycorrhizal fungi, the production and distribution of the extramatrical mycelium in the soil and the formation of ectomycorrhiza. Available data from long-term nitrogen deposition studies indicate that the most prominent effects might be those which are discernible above-ground (i.e. on the formation of fruit bodies) (Wallenda and Kottke, 1998). ‘Generalist’ species, forming a symbiosis with a wide range of tree species, seem to be less affected by increased nitrogen availability than ‘specialist’ species. *Laccaria, Paxillus, Thelephora, Scleroderma* and *Lactarius* are examples of the less sensitive group whereas *Tricholoma, Cortinarius* and *Suillus* are more sensitive (Arnolds, 1991). Thus, *Paxillus involutus, Lactarius rufus, Laccaria bicolor* increased formation of fruitbodies after nitrogen fertilization with up to 240 kg N ha\(^{-1}\) yr\(^{-1}\) (Hora, 1959; Laiho, 1970; Ohenoja, 1988) while 35 kg N ha\(^{-1}\) yr\(^{-1}\) was sufficient to reduce fruit body production of most mycorrhizal species, especially *Cortinarius* spp. and *Russula* spp. (Brandrud, 1995). The easily cultivated species which are used in culture experiments are adapted to higher nitrogen 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 between 1912-1954 and 1973-1983 from 71 to 38, while saprophytic and parasitic fungi colonizing wood 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, while the species number of saprophytes and parasites colonizing wood 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 the data collected between 1918 and 1942. From the 236 species found in the first period only 137 were found in the second period, which corresponds to a loss of 99 species including many ectomycorrhizal fungi. Termorshuizen and Schaffers (1987) found a negative correlation between the total nitrogen 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. He found a negative relationship between nitrogen deposition and ectomycorrhizal species: at the site with nitrogen deposition of 23 kg N ha\(^{-1}\) yr\(^{-1}\), 85 basidiomycetes including 21 ectomycorrhiza (25%) were found, at the site with 42 kg N ha\(^{-1}\) yr\(^{-1}\) 55 basidiomycetes including 3 ectomycorrhiza (5%). In a gradient study from <1 to up to 18 kg N ha\(^{-1}\) yr\(^{-1}\) bulk deposition in the neighbourhood of an industrial ammonia production facility which had operated for almost 30 years, sporocarps of 14 mycorrhizal fungi species were found at the six plots with highest nitrogen load compared to 144 mycorrhizal species at the six plots with lowest nitrogen load (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.

Reduced fruit body production of mycorrhizal fungi was also observed in experiments. Rühling and Tyler (1991) found that within 3 to 4 years after application of 60 and 180 kg N ha\(^{-1}\) yr\(^{-1}\) as NH\(_4\)NO\(_3\) (atmospheric deposition 15-20 kg N ha\(^{-1}\) yr\(^{-1}\)) almost all mycorrhizal species ceased fruitbody production. Brandrud (1995) observed in a *Picea abies* stand in the Swedish NITREX site a decrease in fruit body production of mycorrhizal species at a nitrogen application of 35 kg N ha\(^{-1}\) yr\(^{-1}\) (NH\(_4\)NO\(_3\)) within 1.5 years. Termorshuizen (1990) applied 0, 30 and 60 kg N ha\(^{-1}\) yr\(^{-1}\) to young *Pinus sylvestris* stands as (NH\(_4\))\(_2\)SO\(_4\) or NH\(_4\)NO\(_3\). In general, fruit body production was more negatively influenced by the higher ammonium level than by the ammonium-nitrate mixture. The mycorrhizal frequency and the number of mycorrhiza per unit of soil volume were not affected. It was concluded that fruit body production is much more sensitive to nitrogen enrichment than mycorrhizal formation. Effects on fruit body formation may be long lasting even though the nitrogen deposition is reduced or terminated. In an old forest fertilization experiment in northern Sweden the fruit body production of nitrogen-sensitive mycorrhizal fungi such as *Russula* species was still lower in formerly fertilized plots than in controls nearly 50 years after the nitrogen additions was terminated (Strengbom et al., 2001).

The effect of nitrogen additions on the belowground ectomycorrhizal (EM) community may be less dramatic than that on fruit bodies (Wallenda and Kottke, 1998) and several studies stress the lack of correspondence between the abundance of EM fruit bodies and EM root tips (Dahlberg, 2001; Lilleskov et al., 2002). The effect of reduced diversity and reduced external EM biomass may both have a substantial effect on nutrient uptake.

Several studies report responses in belowground EM communities after nitrogen addition, especially in long-term experiments. 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 50 kg N ha\(^{-1}\) yr\(^{-1}\) ((NH\(_4\))\(_2\)SO\(_4\) or NaNO\(_3\)) (Termorshuizen, 1990). Erland and Taylor (2001) used a gradient of low deposition in northern Europe to higher deposition in southern Europe. There were no apparent negative effects on EM fungal diversity in beech forests. EM root tips in spruce forests appeared to be more sensitive to high levels of nitrogen deposition and the diversity of root morphotypes decreased from north to south.
Interestingly, the proportion of species that can take up organic nitrogen declined as mineral nitrogen availability increased. Lilleskov et al. (2002) found a similar reduction in belowground richness of EM species with increasing nitrogen deposition in a gradient in Alaska. The authors hypothesize that N-efficient species under nitrogen limiting conditions are followed by species with best function in nutrient rich soils and thereafter by P-efficient species under high nitrogen conditions. In two similar 60 year old Picea abies forests in south Sweden with different rates of nitrogen deposition (Vedby with 14-15 kg N ha⁻¹ yr⁻¹ and Skrylle with 24-29 kg N ha⁻¹ yr⁻¹), the level of mycorrhizal colonization was almost 100%, but the total number of mycorrhiza was 30-42% higher at the low N-deposition site. Data show 13 ectomycorrhizal taxa at Vedby and 7 at Skrylle. The total number of mycorrhizal roots was also significantly lower at Skrylle than at Vedby. Six morphotypes were distinguished at Vedby and four at Skrylle (Erland et al., 1999). Wöllecke et al. (1999) investigated the number of mycorrhizal morphotypes in a stand with low nitrogen pollution (bulk deposition 1985-1988 10-20, 1996 7.6 kg N ha⁻¹ yr⁻¹, average NH₃ concentration during 1996-1998 0.5 µg/m³) and with high nitrogen pollution (1985-1988 >35 kg, 1996 13.5 kg N ha⁻¹ yr⁻¹, NH₃ concentration 1996-1998 10.3 µg/m³). They found 18 mycorrhizal morphotypes in the low nitrogen site and 9 mycorrhizal morphotypes and low mycorrhizal frequency at the high nitrogen site.

Considering the observed nitrogen effects on mycorrhizal fungi, a critical nitrogen load of 10-20 kg N ha⁻¹ yr⁻¹ is recommended (expert judgment).

3.4.2 Effects on ground-living and epiphytic lichens and algae

In the Netherlands, the forest vegetation of one Scots pine stand in the central part of the country with a deposition of approx. 20 kg N ha⁻¹ yr⁻¹ was investigated in 1958 and then re-investigated in 1981 when the deposition was approx. 40 kg N ha⁻¹ yr⁻¹. During this period, all lichens disappeared (Dirkse and Van Dobben, 1989). This could be also a sulphur effect, but results from fertilization experiments in northern Sweden with low deposition of both nitrogen and sulphur shows that all Cladina species had disappeared following 28 years of nitrogen addition (34 kg N ha⁻¹ yr⁻¹), while they were still present in the unfertilized control plots (Strengbom et al., 2001). This indicates that nitrogen deposition alone may be responsible for observed reductions in the abundance of ground-living lichens. Bräkenhielm and Quinghong (1995) found along a geographical gradient with 14 plots in Sweden a significantly increased weighted mean sensitivity (WMS) of lichens from south to north and from west to east. WMS drops with >6-8 kg N ha⁻¹ yr⁻¹.

In Atlantic oakwoods in Scotland and NW England, epiphytic lichens such as Lobaria pulmonaria and L. amplissima have been shown by Mitchell et al. (2003) to be effective indicators for nitrogen pollution. Comparing sites with low nitrogen deposition (9.6-17.6 kg N ha⁻¹ yr⁻¹) and high nitrogen deposition (11.2-53 kg N ha⁻¹ yr⁻¹), these epiphytes were only found at low nitrogen sites, suggesting a critical load of 11-18 kg N ha⁻¹ yr⁻¹.

Ca. 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 acidity as well as by nitrogen. In an international survey from the Netherlands via Denmark to Sweden, the decline of these lichens with blue-green algae correlated significantly with nitrogen deposition rates above 5-10 kg N ha⁻¹ yr⁻¹ (Göransson, 1990). However, there is still little information about
the effect of nitrogen 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). The negative effects on lichens may instead be an indirect effect from competition with nitrogen-favoured vascular plants (Cornelissen et al., 2001). Dahlmann et al. (2002) found that among about 500 lichens which both have green algae and cyanobacteria as their photobiont most species seem to be rather resistant towards even high nitrogen additions.

Green algae, especially such of the genus *Pleurococcus* (syn. *Protococcus*, *Desmococcus*) are strongly stimulated by enhanced nitrogen deposition. They cover outdoor surfaces which are not subject to frequent desiccation in regions with high nitrogen deposition, above 10-15 kg N ha\(^{-1}\) yr\(^{-1}\) (Bobbink et al., 1996). Results from the Swedish Environment Monitoring Programme and from Norway show that these algae, mainly *Pleurococcus viridis*, do not colonize spruce needles in regions with a throughfall nitrogen deposition lower than 5 kg N ha\(^{-1}\) yr\(^{-1}\) (Bråkenhielm, 1990; Thomsen, 1992). However, the results may be confounded by sulfur and temperature. In a 10 year monitoring period in Sweden, patterns for sensitive lichens were opposite to those observed for algae. WMS (weighted mean sensitivity) of lichens and algal colony thickness (15 areas) were positively correlated with growing season, nitrogen and sulphur deposition. The colonization rate demonstrated a clear trend along a geographical gradient decreasing from south to north and from west to east, showing an increasing algal cover with nitrogen deposition >5-8 kg N ha\(^{-1}\) yr\(^{-1}\) (Bråkenhielm and Quinghong, 1995). Poikolainen et al. (1998) investigated the abundance of the epiphytic lichen *Scoliciosporum chlorococcum* on conifers on 3009 permanent Finnish forest plots between 1985 and 1995. There was an increasing abundance of this lichen where nitrogen deposition reached ≥3-4 kg N ha\(^{-1}\) yr\(^{-1}\). However, climate factors might be involved as well.

The data for this *freeliving algae and epiphytic lichens* suggest a *reliable* critical load of 10-15 kg N ha\(^{-1}\) yr\(^{-1}\). All data presented refer to *boreal* forests, and there is no information available to set the critical load for temperate forests.

### 3.4.3 Effects on forest ground vegetation

There is a large number of observations showing an increase in abundance of nitrophilous species in forests, either as time series or within a nitrogen deposition gradient. These species include *Galeopsis tetrahit*, *Rubus idaeus*, *R. fruticosus*, *Deschampsia flexuosa*, *Molinia caerulea*, *Poa trivialis*, *Milium effusum*, *Urtica dioica*, *Epilobium angustifolium*, *Galium aparine*, *Aegopodium podagraria*, *Sambucus nigra*, *Stellaria media*, *S. nemorum*, *S. holostea*, *Dryopteris filix mas*, *D. dilatata* and *D. carthusiana*. In parallel, the average N indicator number (after Ellenberg, 1988) has been shown to rise. A lot of endangered species are plants with a low N indicator value (Ellenberg, 1985).

A large representative evaluation of species occurrence at 2000 sampling sites covering ca. 90% of the Dutch forests revealed in the mid 1980s that, with a nitrogen deposition of approx. 40 kg N ha\(^{-1}\) yr\(^{-1}\), nitrophilous species such as *Galeopsis tetrahit*, *Rubus spp*, *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 nitrogen deposition (6-8 and 12-15 kg N ha\(^{-1}\) yr\(^{-1}\), respectively) were compared with special emphasis on nitrogen
indicator species (Tyler, 1987). The following species were more common in the most polluted site: *Urtica dioica*, *Epilobium angustifolium*, *Rubus idaeus*, *Stellaria media*, *Galium aparine*, *Aegopodium podagraria*, *Sambucus* spp.. Comparable observations were reported by Falkengren-Grerup (1986; 1995) who examined the changes in soil and vegetation in repeated studies (10-40 years) in deciduous forests in southern Sweden where nitrogen deposition has doubled since 1955 to 15-25 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*, *S. holostea*, *Dryopteris filix mas*, *Urtica dioica*, all considered to be nitrophilous species after Ellenberg (1988). Rosén et al. (1992) found a significant positive correlation between *Deschampsia flexuosa* dominated coniferous forests in the past 20 years in Sweden and the pattern of nitrogen deposition, based upon comparisons of ground vegetation surveys in the Swedish Forest Inventory between 1973/77 and 1983/87. *Deschampsia flexuosa* increased significantly in this period. These changes occurred above a nitrogen deposition of 7-11 kg N ha\(^{-1}\) yr\(^{-1}\).

The influence of atmospheric nitrogen deposition on mixed oak forest vegetation along a deposition gradient 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). 20 of 30 field layer species that are most closely associated with high nitrogen deposition, increased in frequency in areas with high nitrogen deposition during recent decades. This group of field layer species includes many species generally considered as nitrophilous but also several acid tolerant species. A site index calculated on Ellenberg N-values was higher in the most exposed regions except on soils with pH <3.5, where soil acidity was probably more important, and with pH >5.0 (Falkengren-Grerup and Diekmann, 2003). The number of species was about 20% lower in the most exposed regions within the same soil pH interval. The change in vegetation between 1983 and 1993 was large enough to give significantly higher Ellenberg N-values in 1993 in both Skåne and Småland (Diekmann et al., 1999).

In Germany, too, an increasing abundance of nitrophilous species (such as *Dryopteris dilatata*, *D. carthusiana*, *Rubus idaeus*, *R. fruticosus*, *Milium effusum*, *Deschampsia* (Avenella) *flexuosa*, *Urtica dioica*, *Epilobium angustifolium*) in mixed fir/spruce and in Scots pine forests is reported, where nitrogen deposition is approx. 15-30 kg N ha\(^{-1}\) yr\(^{-1}\) (Rodenkirchen, 1992; Kraft et al., 2000). However, changes in management practice may also affect these changes. In forest sites in Germany with earlier litter raking, the average nitrogen indicator values after Ellenberg (Ellenberg, 1988) increased by 0.6 units, in forest sites without litter raking by 0.3 units (Rodenkirchen, 1992). In Switzerland, a significantly increased abundance of nitrophilous species in 17 of 18 forest sites was observed in two regions (northern Switzerland, modelled N-deposition for 1995 20-30 kg N ha\(^{-1}\) yr\(^{-1}\), and Geneva region, nitrogen deposition 15-20 kg N ha\(^{-1}\) yr\(^{-1}\)) between 1938/1942/1947 and 1984/1985 (Kuhn et al., 1987). At 37 forest sites in the central plateau of Switzerland, the comparison between two surveys of the 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*, *R. caesius*, *Dryopteris dilatata*, *D. filix mas*, *Sambucus nigra*, *Hedera helix* and *Urtica dioica*. The nitrogen deposition in this region is approx. 30-40 kg N ha\(^{-1}\) yr\(^{-1}\) (Walther and Grundmann, 2001). In Belgium also, Lameire et al. (2000) found a significant increase of nitrogen indicator species in mixed deciduous forests during the past 20 years - between 1977/80 and 1997/98 - such as *Dryopteris dilatata*, *Galeopsis tetrahit*, *Hedera helix*, *Moehringia trinervia*, *Sambucus*.
nigra, Stellaria media and Urtica dioica (approx. nitrogen deposition 25-30 kg N ha\(^{-1}\) yr\(^{-1}\)). In a gradient study, the species composition of ground flora along a 500 m transect from livestock buildings showed marked changes within 30 m downwind. Deschampsia flexuosa, Holcus lanatus, Rubus idaeus and Urtica dioica were abundant close to livestock units and their percentage cover decreased rapidly with distance from source. In this study, 15-20 kg N ha\(^{-1}\) yr\(^{-1}\) was proposed to protect the ground flora (Pitcairn et al., 1998).

At two sites near Nancy (Haye forest) in northeastern France on calcareous soils with beech, oak and hornbeam, 278 floristic surveys from 1972 were reexamined in 1991 (139 at each site). A significant increase in nitrogen demanding and acidophilous species was observed. In site 1 (carré permanente), 100% of the plots showed an eutrophication and 79% an acidification, in site 2 (Villey), 85% of the plots showed an eutrophication and 74% an acidification (nitrogen deposition approx. 15-20 kg N ha\(^{-1}\) yr\(^{-1}\)) (Bost, 1991). In 1990, Thimonier et al. (1992) resampled at the intersections of a systematic grid covering a mixed hardwood forest in the Amance forest (Menthe et Moselle) in the Lorraine Plaine, northeastern France, 221 floristic plots sampled the first time in 1971 and 1972. Three main features were noted: a) an increase in the frequency of nitrogen-demanding species, b) an increase in Ellenberg’s ecological N value and c) an enrichment of the site revealed by trophic level indices. Thinning does not appear to be responsible for the changes in the trophic level observed. While the N values increased from 4.89 to 5.16, the light values changed from 4.82 to 4.7. Annual nitrogen inputs in Amance forest were assessed to be 20-30 kg N ha\(^{-1}\) yr\(^{-1}\) in the beginning of the 1970s.

At 47 vegetation plots in the Villey forest near Nancy of the Lorraine Plain, northeast 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, acidophilous species increased in frequency. Repetition of soil analysis at the same time interval on nearby plots revealed a strong increase in nitrogen content. Although the stands have been converted from coppice to high forest, it is suggested that atmospheric nitrogen deposition is the most probable cause of eutrophication. Eutrophication was found equally on calcareous and on acidic soils (nitrogen deposition approx. 20 kg N ha\(^{-1}\) yr\(^{-1}\)) (Thimonier et al., 1994).

Although D. flexuosa is not a typical nitrophilous species, it shows a positive reaction to nitrogen. After 5 years of treatment, 5 kg N ha\(^{-1}\) yr\(^{-1}\) was enough to increase the abundance of this grass species in a coniferous forest in Sweden (Fig. 3.7; Kellner and Redbo-Torstensson, 1995).
In a field survey in 557 coniferous stands in Sweden, the occurrence of *Vaccinium myrtillus*, *V. vitis-idaea* and *Deschampsia flexuosa* was investigated. In areas with increased nitrogen deposition (≥ 6 kg N ha\(^{-1}\) yr\(^{-1}\)), *V. myrtillus* was less frequent and more susceptible to the leaf pathogen *Valdensia heterodoxa* than in areas with lower levels of nitrogen deposition. The occurrence of *V. vitis idaea* was also strongly negatively correlated with increasing nitrogen deposition but no such trend was observed for *D. flexuosa* (Strengbom et al., 2003). These relationships were also shown experimentally. In a nitrogen fertilization experiment with 0, 12.5 and 50 kg N ha\(^{-1}\) yr\(^{-1}\) (atmospheric deposition 2-3 kg N ha\(^{-1}\) yr\(^{-1}\)), the abundance of *D. flexuosa* increased significantly after 3 years with 12.5 kg N ha\(^{-1}\) yr\(^{-1}\) while the abundance of *V. myrtillus* decreased. Disease incidence by *Valdensia heterodoxa* was more than twice as high in plots receiving 12.5 kg N ha\(^{-1}\) yr\(^{-1}\) and more than three times as high in plots receiving 50 kg N ha\(^{-1}\) yr\(^{-1}\) compared to controls (Fig. 3.8). The abundance of the mildew fungus *Podosphaera myrtillina* on *Vaccinium* was increased ten-fold by 25 kg N ha\(^{-1}\) yr\(^{-1}\) compared to 0.5 kg N ha\(^{-1}\) yr\(^{-1}\). As a consequence, *Vaccinium* density decreased, and *Deschampsia* cover increased (Strengbom et al., 2002). In addition, shoots of *Vaccinium myrtillus* were significantly more damaged by larvae such as the moth *Orgyia antrana* after addition of 12.5 kg N ha\(^{-1}\) yr\(^{-1}\) in the first year of treatment (Nordin et al., 1998). On the other hand, nitrogen fertilization with 30 kg N ha\(^{-1}\) yr\(^{-1}\) (atmospheric nitrogen deposition 12 kg N ha\(^{-1}\) yr\(^{-1}\)) did not change the ground vegetation in a montane forest within three years (Schleppi et al., 1999).
Understorey species in deciduous forests respond to nitrogen deposition by higher growth rate, a more broad-leafed anatomy and higher nitrogen concentration and nitrate reductase activity in the tissue (Falkengren-Grerup and Diekmann, 2003).

Bryophytes depend directly on wet and dry deposition of nitrogen and are therefore considered to be highly sensitive even to small changes in the supply. Addition of nitrogen doses 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 nitrogen additions by increased growth, and instead nitrogen was accumulated in the form of arginine. High amino acid concentrations may be harmful to bryophytes, and correlated to reductions in length growth of *Sphagnum* (Nordin and Gunnarsson, 2000). Some bryophytes are very sensitive to nitrogen deposition, while others are not. Effects of nitrogen on bryophytes were studied in a nitrogen field fertilization experiment in two southern Swedish Scots pine stands (Lisselbo and Norrilden). *Brachythecium oedipodium*, *B. reflexum* and *B. starkei* increased significantly at fertilization levels up to 60 kg N ha\(^{-1}\) yr\(^{-1}\). At higher doses these species tended to decline. In contrast, *Hylocomium splendens* and *Pleurozium schreberi* declined strongly at doses of 30 to 60 kg N ha\(^{-1}\) yr\(^{-1}\) (Dirkse and Martakis, 1992). However, the biomass of the mosses *Pleurozium schreberi* and *Dicranum polysetum* was reduced by 60% and 78%, respectively, when the mosses were fertilized with only 25 and 30 kg N ha\(^{-1}\) yr\(^{-1}\), respectively, after four years in a spruce stand in Finland with a low atmospheric deposition rate (Mäkipää, 1998). In two pine forest stands in central and northern Sweden (122 plots in total) which had been treated with N, P, K, lime and sulphuric acid for 15 years, nitrogen additions (20 and 60 kg N ha\(^{-1}\) yr\(^{-1}\)) had by far the strongest effect causing a shift in dominance from lichens and bryophytes such as *Pleurozium schreberi* and *Hylocomium splendens* and Ericaceae towards *Deschampsia flexuosa* and ruderal species (Van Dobben et al., 1999). In an old fertilization experiment in northern Sweden the abundance of *H. splendens* was still lower in formerly nitrogen-treated plots nearly 50 years after termination of nitrogen additions, whereas
nitrogen favoured bryophytes such as *B. reflexum* and *Plagiothecium denticulatum* were more abundant in formerly nitrogen-treated plots than in control plots. This shows that the negative effects on bryophyte communities may be long lasting although the nitrogen input is reduced and there are good opportunities for colonization from the surrounding non-affected vegetation (Strengbom *et al.*, 2001).

In an Atlantic oakwood stand in Scotland and NW England, the epiphytic bryophytes *Plagiochila atlantica* and *P. spinalosa* appear to be sensitive to nitrogen pollution. These epiphytic species were strongly associated with sheltered sites and low nitrogen deposition (9.6-17.6 kg N ha\(^{-1}\) yr\(^{-1}\)), compared to high nitrogen sites with 11.2-53 kg N ha\(^{-1}\) yr\(^{-1}\) (Mitchell *et al.*, 2003).

Most studies presented above suggest a **reliable** critical load of **10-15 kg N ha\(^{-1}\) yr\(^{-1}\)** for the protection of **ground flora** in **temperate forests**. The lack of low-dose experiments, uncertainties about background deposition and confounding factors in gradient studies do not allow to set a lower critical nitrogen load for **boreal forests** at the moment. Hence, the recommended critical load for boreal forests is set as an **expert judgment** to **10-15 kg N ha\(^{-1}\) yr\(^{-1}\)**.

### 3.4.4 Fauna

**Soil fauna**

The effect of enhanced nitrogen 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 in some studies, but decreased in others after application of high doses of nitrogen fertilizers (>150 kg N ha\(^{-1}\) yr\(^{-1}\)) (Abrahamsen and Thompson, 1979; Huhta *et al.*, 1983; Vilkamaa and Huhta, 1986). A one-time application of 100 kg N ha\(^{-1}\) yr\(^{-1}\) (as NH\(_4\)NO\(_3\)) in the Fontères state forest in Eastern Brittany, France, produced an effect on soil microorganisms which was still significant after 23 years. There were decreases in *Oribatida*, *Camarida*, *Collembola*, *Symphyta* (small *Myriapoda*) and *Pseudoscorpionida*; the atmospheric deposition at this site was estimated as 10-20 kg N ha\(^{-1}\) yr\(^{-1}\) (Deleporte and Tillier, 1999). A reduction in the nitrogen deposition in a *Pinus sylvestris* stand (NITREX site Ysselsteyn) to preindustrial levels increased the species diversity of microarthropodes due to a decreased dominance of some species (Boxman *et al.*, 1995). A significant decrease by 66% of the abundance of earthworms was observed after seven years of fertilization in a young beech stand with 20 kg N ha\(^{-1}\) yr\(^{-1}\) (atmospheric deposition 12 kg N ha\(^{-1}\) yr\(^{-1}\)) (Flückiger and Braun, 1999b). Simultaneously, the pH of the upper soil layer (30 cm) decreased from 3.7 to 3.5. In Sweden, a significant decrease of snails over 14-46 years in areas with nitrogen deposition of 15-25 kg N ha\(^{-1}\) yr\(^{-1}\) was observed, while in areas with nitrogen deposition of 3-6 kg N ha\(^{-1}\) yr\(^{-1}\) no significant changes were found (Gärdenfors *et al.*, 1995). However, in the same area there was a sulfur deposition gradient (soil acidification) as well. Hence, no critical nitrogen load can be defined based on these data.
Mammals
Foliage of fertilized spruce seedlings (*Picea sitchensis*) was most palatable to Orkney voles, *Microtus arvalis orcadensis*. Foliage had higher levels of nitrogen (1.6-1.7% compared with 0.9% in control) and lower levels of phenolics (2.6%, control 5.1%). Whereas the fertilization of the spruce seedlings contained NPK and cannot be quantified in weight per unit area, a second experiment with *Calluna* fertilized with 52 kg N ha\(^{-1}\) yr\(^{-1}\) gave similar results (Hartley *et al*., 1995). In southern Sweden, nitrogen concentrations and browsing of young Norway spruce was compared in two clear cut sites with different nitrogen deposition (Asa, N-deposition approx. 12-18 kg, and Tönnersjöheden, N-deposition approx. 18-29 kg N ha\(^{-1}\) yr\(^{-1}\)). At the site with higher nitrogen deposition (Tönnersjöheden), the seedlings had darker green needles and higher nitrogen concentrations in foliage. Frequency of browsed seedlings increased with increasing color and hence with increasing nitrogen concentration in the needles. Browsing damage was significantly higher at Tönnersjöheden compared to Asa. Vegetation cover in the clear cuts and density of roe deer around the clearcut were not different at the two sites (Bergquist and Örlander, 1998a; 1998b). However, the present data are not strong enough to set a critical nitrogen load for the fauna at the moment.

3.5 Summary of empirical critical loads for forests

3.5.1 Effects on soil processes
Considering the results presented in par. 3.2., the critical load for soil processes such as mineralization, nitrification, NH\(_4^+\) accumulation, NO\(_3^-\) leaching can be set at 10-15 kg N ha\(^{-1}\) yr\(^{-1}\) for boreal and temperate forests.

3.5.2 Effects on trees
*Growth*: Growth effects to nitrogen may be positive, neutral or negative depending on the ecosystem status. Because nutrition changes will precede growth changes, no separate critical load is set for growth.

*Nutrition*: Long term observations in France, Sweden and Switzerland show a significant increase of N/P, N/K and/or N/Mg ratio in the foliage of beech and Norway spruce with nitrogen deposition between 20-35 kg N ha\(^{-1}\) yr\(^{-1}\). Fertilization experiments also demonstrate a significant change in the N/P or N/Mg ratios when nitrogen fertilization is ≥10 kg N ha\(^{-1}\) yr\(^{-1}\) (atmospheric nitrogen deposition 12-20 kg N ha\(^{-1}\) yr\(^{-1}\)). Data from ICP Forests (De Vries *et al*., 2003) show a median nitrogen deposition of 9.6 kg N ha\(^{-1}\) yr\(^{-1}\) for forest plots with a balanced nutrition and of 21 kg N ha\(^{-1}\) yr\(^{-1}\) for plots with an unbalanced nutrition. Hence, a nutrition based critical load of 15-20 kg N ha\(^{-1}\) yr\(^{-1}\) for coniferous and deciduous trees can be proposed and considered as quite reliable.

*Physiology*: The physiological data presented here are too weak to set a separate critical load for physiology.
Parasites: In a long-term fertilization study in young stands, beech showed significantly increased attacks by *Apiognomonia errabunda* and *Phomopsis* sp. with a nitrogen fertilization of ≥10 kg N ha⁻¹ yr⁻¹ (atmospheric deposition 20 and 16 kg N ha⁻¹ yr⁻¹, respectively). A critical load of 15-20 kg N ha⁻¹ yr⁻¹ can be set as an expert judgment because the effect at the treatment of 10 kg N ha⁻¹ yr⁻¹ (+21 kg N ha⁻¹ yr⁻¹ atmospheric deposition) was quite strong. In 1996, no critical load was set on the basis of parasite sensitivity.

### 3.5.3 Effects on mycorrhizal fungi and ground flora

**Mycorrhizal fungi**

Strengbom *et al.* (2001) found large effects of a nitrogen fertilization with 34 kg N ha⁻¹ yr⁻¹ on sporocarp abundance even 9 years after termination of the treatment. This study was the basis to set a critical load of 10-20 kg N ha⁻¹ yr⁻¹ as an expert judgment. Also, the tendency of decreased growth of mycelia by ectomycorrhizal fungi in oak forest soils in a nitrogen deposition gradient (9-17 kg N ha⁻¹ yr⁻¹) in southern Sweden (L.O. Nilsson, pers. comm.) support the proposed range. In 1996, the critical load set on the basis of effects on mycorrhiza (together with ground flora) was 7-20 kg N ha⁻¹ yr⁻¹ for coniferous and 10-20 kg N ha⁻¹ yr⁻¹ for deciduous forests.

**Ground-living and epiphytic lichens and algae**

The gradient studies by Göransson (1990), Bräkenhielm and Quinghong (1995) on epiphytic algae and of Poikolainen *et al.* (1998) on lichens which would support a low critical load have uncertainties on dry deposition and on climate and sulfur as confounding factors. Therefore, a critical load can be set to 10-15 kg N ha⁻¹ a⁻¹ as an expert judgment. This is higher than the critical load of 5-10 kg N ha⁻¹ yr⁻¹ set in 1996.

**Ground vegetation**

There is a large number of observations showing an increase in abundance of nitrophilous species either as time series or within a nitrogen deposition gradient. In boreal forests, changes of ground vegetation, e.g. decreasing *Vaccinium myrtillus*, occurred above ≥6 kg N ha⁻¹ yr⁻¹ (Strengbom *et al.*, 2003) and increased growth of *Deschampsia flexuosa* with ≥5 kg N ha⁻¹ yr⁻¹ (Kellner and Redbo-Torstensson, 1995). *Vaccinium myrtillus* showed an increased attack by parasites when nitrogen fertilization was ≥12.5 kg N ha⁻¹ yr⁻¹ (Strengbom *et al.*, 2002; Nordin *et al.*, 1998). Bryophyte species are also sensitive; the biomass of the mosses *Pleurozium schreberi* and *Dicranum polysetum* fertilized with 25 and 30 kg N ha⁻¹ yr⁻¹ respectively was reduced by 60% and 78% respectively after four years (Mäkipää, 1998). In temperate forests, many reports show an increasing frequency of nitrophilous species such as *Dryopteris filix-mas*, *Rubus idaeus*, *Epilobium angustifolium*, *Urtica dioica*, *Sambucus nigra* and others with nitrogen deposition >15-25 kg N ha⁻¹ yr⁻¹ in the long term. Hence, a critical nitrogen load on the basis of ground vegetation changes of 10-15 kg N ha⁻¹ yr⁻¹ is quite reliable. Together with effects on mycorrhiza, this critical load was set in 1996 to 7-20 kg N ha⁻¹ yr⁻¹ for acidic coniferous forests, to 10-20 kg N ha⁻¹ yr⁻¹ for acidic deciduous forests, to 15-20 kg N ha⁻¹ yr⁻¹ for calcareous forests and to 7-15 kg N ha⁻¹ yr⁻¹ for acidic unmanaged forests. Table 3.2. summarises these values. Given the range of responses found above these loads in field experiments and in field observations, we can
consider $10-20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ as overall empirical nitrogen critical load for both temperate and boreal forests as quite reliable.

Table 3.2. Empirical critical loads for the effects of excess nitrogen upon trees and different forest ecosystems. ## reliable; # quite reliable and (#) expert judgement.

<table>
<thead>
<tr>
<th>Ecosystem type</th>
<th>EUNIS-code</th>
<th>kg N ha$^{-1}$ yr$^{-1}$</th>
<th>Reliability</th>
<th>Indication of exceedance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forest habitats (G)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil processes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deciduous &amp; coniferous</td>
<td></td>
<td>10-15</td>
<td>#</td>
<td>Increased N mineralization, nitrification</td>
</tr>
<tr>
<td>Coniferous forests</td>
<td></td>
<td>10-15</td>
<td>##</td>
<td>Increased nitrate leaching</td>
</tr>
<tr>
<td>Deciduous forests</td>
<td></td>
<td>10-15</td>
<td>(#)</td>
<td>Increased nitrate leaching</td>
</tr>
<tr>
<td>Trees</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deciduous &amp; coniferous</td>
<td></td>
<td>15-20</td>
<td>#</td>
<td>Changed N/macro nutrients ratios, decreased P, K, Mg and increased N concentrations in foliar tissue</td>
</tr>
<tr>
<td>Temperate forests</td>
<td></td>
<td>15-20</td>
<td>(#)</td>
<td>Increased susceptibility to pathogens and pests, change in fungistic phenolics</td>
</tr>
<tr>
<td>Mycorrhiza</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperate and boreal forests</td>
<td></td>
<td>10-20</td>
<td>(#)</td>
<td>Reduced sporocarp production, changed/reduced below-ground species composition</td>
</tr>
<tr>
<td>Ground vegetation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperate and boreal forests</td>
<td></td>
<td>10-15</td>
<td>#</td>
<td>Changed species composition, increase of nitrophilous species, increased susceptibility to parasites</td>
</tr>
<tr>
<td>Lichens and algae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperate and boreal forests</td>
<td></td>
<td>10-15</td>
<td>(#)</td>
<td>Increase of algae, decrease of lichens</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperate forests</td>
<td></td>
<td>10-20</td>
<td>#</td>
<td>Changes in soil processes, ground vegetation mycorrhiza and increased risk of nutrient imbalances and susceptibility to parasites</td>
</tr>
<tr>
<td>Boreal forests</td>
<td></td>
<td>10-20</td>
<td>#</td>
<td>Changes in soil processes, ground vegetation mycorrhiza and increased risk of nutrient imbalances and susceptibility to parasites, increase in free algae</td>
</tr>
</tbody>
</table>
4. Effects of nitrogen deposition on heathland, scrub and tundra habitats (EUNIS class F)

4.1 Introduction

Heathlands have for a long time played an important part in the West European landscape. Various types of plant communities have been described as heath, but the term is applied here to plant communities where the dominant life form is that of the small-leaved dwarf-shrubs forming a canopy of 1 m or less above soil surface. Grasses and forbs may form discontinuous strata, and there is frequently a ground layer of mosses or lichens (Gimingham et al., 1979; De Smidt, 1979). Heathlands are classified together with scrub and tundra habitats in EUNIS class F. This category includes all dry or seasonally wet inland vegetation (cover > 30%) that is dominated by (dwarf-) shrubs or scrub (Davies and Moss, 2002). In part 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., F1 and F2). In contrast, the extensive inland lowland dwarf-shrub heathlands in 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 and Erica tetralix in the wet heathlands, or Erica cinerea in the western Atlantic heathlands (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 both to eutrophication and to acidification effects of increased nitrogen deposition. Because of their conservational importance, many lowland heathlands have become nature reserves in recent years.

In previous versions of the empirical critical load approach, dwarf-shrub heathlands were divided into five categories according to broad differences in habitat: (1) dry heathlands; (2) wet heathlands; (3) species-rich heaths and acidic grasslands; (4) upland Calluna moorland; and (5) arctic-alpine heathlands (Bobbink et al., 1992a; 1996). Following the EUNIS habitat classification, in this document we distinguish the following classes. Tundra (F1) which is combined with arctic, alpine and subalpine scrub habitats (F2) and replaces (5) arctic-alpine heathlands. Temperate shrub habitats (F4) are divided into wet (F4.1) and dry (F4.2) heaths. Category F4.2 correspond to (1) dry heathlands in the former classification. In view of their functional differences, wet heaths are divided on a climatic basis into northern (F4.11) and southern (F4.12) wet heaths. For the latter, no data are available to assign a critical load. Northern wet heaths were assigned a different critical load if dominated by Calluna vulgaris, corresponding broadly to (4) upland Calluna moorlands in the previous division, and if dominated by Erica tetralix, which broadly correspond to the original (2) wet heathlands category. In contrast to previous versions, coastal dune heaths are treated in coastal habitats (Chapter 8; B1.5). The section (3) species-rich heaths and acidic grasslands is now (at least partly) included in the grassland section (Chapter 5; E1.7 & E3.5)). For other EUNIS categories in class F, including other heathland types, no data are available to determine an empirical nitrogen critical load.
4.2 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 (class F1) and arctic, alpine and subalpine scrub habitats (class F2). The key feature within EUNIS which distinguishes tundra is the presence of permafrost, which prevents root penetration and often keeps the ground waterlogged in summer. Hence, in assessing the need for critical load revision within the EUNIS system it is important to assign studies to these two distinct classes and to assess whether differentiated critical loads may be required.

4.2.1 Tundra (F1)

Significant increases in the atmospheric deposition of nitrogen in arctic regions have been reported during recent decades (Woodin, 1997). Plant growth and distribution in the Arctic are strongly constrained by the duration of the snow-free period, low air and soil temperatures, low soil moisture and low soil nutrient availability. Thus, anthropogenic sources of nitrogen 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).

There have been numerous field manipulation studies with nutrients in tundra ecosystems; however, most have involved NPK fertilizer additions (e.g. Robinson et al., 1998; Press et al., 1998b; Schmidt et al., 2000) or single large applications of nitrogen (e.g. Shaver and Chapin, 1995). Typical of the latter is the experiment of Henry et al. (1986), who examined the effect of a single nitrogen addition of 0, 50 and 250 kg ha\(^{-1}\) yr\(^{-1}\) to three Canadian tundra communities; 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 after the addition of 250 kg N ha\(^{-1}\) yr\(^{-1}\), no significant effects were observed after the application of 50 kg N ha\(^{-1}\) yr\(^{-1}\).

In contrast, in a study of tundra ecosystems with a similar species complement, significant effects have been reported at much lower deposition rates. The experimental site was established in mixed tundra heath near Svalbard in 1991. Plots were located in each of three tundra heath vegetation types, which received factorial combinations of nitrogen (10 and 50 kg N ha\(^{-1}\) yr\(^{-1}\)) and phosphorus (5 kg P ha\(^{-1}\) yr\(^{-1}\)) as 4-5 applications in the summer period. Plots 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 only. Current atmospheric
deposition was not measured, but is expected to be very low. Baddeley et al. (1994) reported early responses to the nitrogen treatments. *Salix polaris* had increased levels of foliar nitrogen, increased leaf biomass and increased photosynthetic rate. *Cassiope tetragona* showed no response to nitrogen addition, whilst *Dryas octopetala* showed an intermediate response of the measured variables.

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

Co-limitation by nitrogen and phosphorus was clearly demonstrated by this study. For example, with, but not without, added phosphorus, 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 phosphorus availability and may be influenced by increased nutrient availability from global warming. Long-term responses of tundra vegetation to NPK fertiliser treatment show in most cases an effect of nutrient addition, with indication of both nitrogen and phosphorus limitation. Shaver et al. (1998) suggest that mechanistic analysis of the results of fertilization experiments can identify factors associated with either nitrogen or phosphorus limitation. They suggest that wet tundra sites are more likely to be phosphorus limited than moist sites with a thinner peat layer, while dry tundra deserts are primarily nitrogen limited. Cornelissen et al. (2001) examined relationships between macrolichen and vascular plant abundance across arctic vegetation ecosystem experiments which manipulated factors such as temperature and nutrient availability and concluded that negative correlations were greater at milder sites with a greater above-ground 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.
Summary

Despite the limited number of long-term experiments, a clear picture is now emerging of the potential impact of long-term nitrogen deposition on tundra ecosystems. Ecosystem response to nitrogen has been observed at deposition rates as low as 10 kg N ha\(^{-1}\) yr\(^{-1}\). To a large extent, however, the response to atmospheric nitrogen within the tundra ecosystem may well depend on other factors, such as phosphorus status and temperature. Expert judgement was used by Bobbink et al. (1996), in the absence of empirical evidence, to propose a critical load for arctic and alpine heaths that was lower than that for lowland heathland and was set at 5-15 kg N ha\(^{-1}\) yr\(^{-1}\). Since then, many more experimental manipulation studies have been reported but very few use nitrogen at deposition rates which are useful in defining critical loads.

Nevertheless, the tundra study showing significant effects of adding 10 kg N ha\(^{-1}\) yr\(^{-1}\) to a low current atmospheric deposition provides strong support for a lower critical load, especially since significant effects were observed rapidly and do not appear to be readily reversible. We therefore propose a critical load for tundra (F1) of 5-10 kg N ha\(^{-1}\) yr\(^{-1}\), as quite reliable. Although this classification as quite reliable is based on only one experiment, it can be justified because: (i) the experiment involved three different plant communities; (ii) background deposition was very low (<1 kg N ha\(^{-1}\) yr\(^{-1}\)); (iii) the N treatment (10 kg N ha\(^{-1}\) yr\(^{-1}\)) was within the critical load range; (iv) the effects were seen rapidly and are very persistent; and (v) subsequent unpublished results confirm the persistence of the effects.

Inclusion of a phosphorus treatment in this experiment produced unequivocal evidence of co-limitation by N and P, with greater responses to N when phosphorus was also applied. Phosphorus supply is thus identified as an important modifier of the critical load, with higher critical loads applying to systems, which are limited by P, and lower critical loads to systems which are not phosphorus limited.

4.2.2 Arctic, alpine and subalpine scrub habitats (F2)

In contrast to tundra, there is no new empirical evidence to support a revised critical load for arctic, alpine and subalpine scrub habitats, with most relevant studies involving either NPK fertilization or very high levels of nitrogen deposition (e.g. Theodose and Bowman, 1997). However, there are two Norwegian studies which have been reported since 1995 which are relevant.

A study in the Dovre mountains in Norway, at 1000-1400 m a.s.l., investigated the effects of three years fertilization of a Betula nana dominated community with 12 and 61 kg N ha\(^{-1}\) yr\(^{-1}\), at a site with an estimated background deposition of 2-4 kg N ha\(^{-1}\) yr\(^{-1}\) (Paal et al., 1996). There was no significant effect on plant growth, and no evidence of increased N contents in vegetation or soils. In a second study, two lichen-dominated communities were investigated, one low-alpine and the other middle-alpine. After three years of application of 7, 35 and 70 kg N ha\(^{-1}\) yr\(^{-1}\), there was evidence of a negative response of the lichens Cladonia coccifera, Cladonia mitis, and Cetraria nivalis (Mols et al., 2001). However, the statistical analysis presented in this paper does not allow the threshold deposition for effects to be identified. Some indication of a possible threshold is given by unpublished results for ten years of treatment of these plots, which suggest
that significant negative effects on lichen cover are found at 35 kg N ha\(^{-1}\) yr\(^{-1}\), but not at 7 kg N ha\(^{-1}\) yr\(^{-1}\).

Thus, in contrast to tundra, there is no new evidence to support a change in the previous critical load for arctic and alpine heaths of 5-15 kg N ha\(^{-1}\) yr\(^{-1}\), as recommended by Bobbink et al. (1996). Given the lack of supporting evidence for any change for alpine systems, we recommend a critical load of 5-15 kg N ha\(^{-1}\) yr\(^{-1}\), on the basis of expert judgement for arctic, alpine and subalpine scrub habitats (F2).

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

As discussed in section 4.1, both wet and dry heathlands have to be placed within the class F4 (temperate shrub heathland), because of their climatic condition and their dominance by ericoid shrubs. The use of level 3 of the EUNIS system allows the class to be split into wet heaths (F4.1), which are damp and characterized 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 (class F4.11). However, since these communities are clearly ecologically different and 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 based on species dominance. National experts will need therefore to provide advice to the mapping community on how to map the two habitats.

4.3.1 ‘U’ Calluna-dominated wet heath (upland moorland) (F4.11)

It was suggested by Bobbink et al. (1996) that the Calluna vulgaris heaths of the upland areas of Britain and other mountainous parts of Europe are expected to be sensitive to nitrogen deposition. These acidic communities are characterized by a dominance of dwarf shrubs (in particular C. vulgaris), a high abundance of bryophyte species and peaty soils. Furthermore, the abiotic conditions are colder and wetter than in lowland heathlands (F4.2). The effects of nitrogen deposition on upland C. vulgaris heaths have been studied in the UK using both field surveys and experiments. The critical load of 10-20 kg N ha\(^{-1}\) yr\(^{-1}\) recommended by Bobbink et al. (1996) for this community was based on three types of evidence from these UK studies, which are considered in turn below in the light of more recent literature.

(a) Effects on growth and species composition

The longest C. vulgaris moorland nitrogen manipulation experiment is in North Wales where experimental plots were established in May 1989 on an area of moorland at an altitude of 470 m a.s.l.. This site has been estimated to receive a current atmospheric nitrogen deposition of approximately 20 kg N ha\(^{-1}\) yr\(^{-1}\), although more recent assessments suggest that the deposition is somewhat higher. Additions of nitrogen in the form of ammonium nitrate at doses of 0, 40, 80 and 120 kg N ha\(^{-1}\) yr\(^{-1}\) have been applied since 1989 at monthly intervals and continue to date (Lee and Caporn, 1998; Carroll et al., 1999). The period from 1989 to 1993 was characterized by apparently beneficial effects of nitrogen on C. vulgaris in terms of improvements in shoot growth, nitrogen 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 nitrogen inputs. The 1996 data in particular showed no effect of nitrogen on shoot extension at all (Carroll et al., 1999). One interpretation of the *C. vulgaris* growth responses in this experiment is that addition of N has accelerated the natural *C. vulgaris* cycle, with earlier ageing and opening of the canopy in the highest treatment plots. This is the only moorland study of such a duration and the results clearly demonstrate the need for caution in interpreting the growth increases commonly reported in response to nitrogen addition to *C. vulgaris* over periods of 1-5 years.

One factor causing increased canopy opening may be greater winter damage to *C. vulgaris* shoots. Detailed experimental studies of frost tolerance in *C. vulgaris* shoots collected in the early years of the study (1989-1994) demonstrated that nitrogen addition actually improved frost tolerance in autumn (Caporn et al., 1994). However, field surveys in 1996 and 1998 clearly showed large increases in ‘winter browning’ of heather shoots, most notably in the 80 and 120 kg N ha$^{-1}$ yr$^{-1}$ treatments (Fig. 4.1) (Carroll et al., 1999; Lee et al., 2000). This damage mechanism may be the result of low temperature desiccation, rather than frost damage.

In the earlier years, both bryophytes and lichens had disappeared from below the *C. vulgaris* canopy in all nitrogen addition treatments, although *Vaccinium myrtillus* maintained its cover (Carroll et al., 1999). It is not certain, however, whether this was a response to direct effects of nitrogen addition or through changes in *C. vulgaris* canopy architecture and increased litter production resulting in reduced light penetration. A later survey, after ten years of treatment, showed not only that bryophytes have returned to the nitrogen treated plots, but that their cover actually increased with increasing nitrogen deposition, probably as a result of the accelerated ageing and opening up of the *C. vulgaris* canopy (Lee et al., 2000). However, no such response was found for lichen species. These results highlight the need to establish more precisely whether elevated nitrogen deposition is having a direct and/or indirect impact on bryophyte and lichen populations, especially as it has been suggested that nitrogen deposition is partly responsible for the decline of certain mosses and lichen species in upland *C. vulgaris* heath over the past decades (Pitcairn et al., 1995).
Figure 4.1. Effects of 7 years of ammonium nitrate additions (kg N ha\(^{-1}\) yr\(^{-1}\)) on the frequency (means ± SE) of winter damaged *C. vulgaris* shoots in northern wet heath (F4.11) in N. Wales (Carroll *et al.*, 1999). Columns sharing a letter are not significantly different.

A destructive harvest of the site took place in March 2000, prior to a controlled burn. Subsequent regrowth, primarily from stem bases, has been significantly lower in the higher nitrogen addition treatments, which may reflect the more mature status of *C. vulgaris* in the higher nitrogen addition plots (Lee and Caporn, 2001). Pilkington *et al.* (submitted) have completed a nitrogen budget for the site based on the harvest data. Significant increases in green tissue, wood and litter biomass, and litter depth were found in all nitrogen treatments. Although most of the added nitrogen in the 40 kg N ha\(^{-1}\) yr\(^{-1}\) treatment was found in green tissue and litter, increasing amounts of added nitrogen were found in the peat horizons in the higher nitrogen treatments.

Loss of *C. vulgaris* moorland in a number of UK areas has occurred as a result of invasion by bracken. Werkman and Callaghan (1996) undertook a three-year study in the northern Pennines (estimated current atmospheric deposition: 15 kg N ha\(^{-1}\) yr\(^{-1}\)) to examine the effect of nitrogen addition (50 kg N ha\(^{-1}\) yr\(^{-1}\)) to a series of plots set up on 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 for enhanced bracken growth and poorer heather growth. Anderson and Hetherington (1999) showed that decomposition rates of mixed litters were faster than those of litter of either of the two species alone, and also increased to a greater extent in response to nitrogen addition. These results, however, are in contrast to the findings of Gordon *et al.* (1999), who transplanted turf taken from a site in Scotland into experimental plots and added 50 kg N ha\(^{-1}\) yr\(^{-1}\) over three years, as part of a number of other treatments. This level of nitrogen addition increased the leading shoot length of heather from the first year of treatment onwards, and also increased shoot growth in spring, implying earlier budbreak. In contrast, the response of bracken was slow, showing no above-ground growth stimulation until the third year of treatment when there was a small, but significant, increase in canopy height. The responses of a mix of the two species were also examined in this three-year factorial experiment, but, unfortunately, yet not published (S.J. Woodin, pers. comm.). Furthermore, both experiments show that factors such as drought and
temperature play an important role in modifying responses to nitrogen. For example, the faster and larger shoot growth stimulation of heather than bracken by nitrogen, at least over 3 years, may be advantageous when water is not limiting but may be clearly disadvantageous when drought occurs (Gordon et al., 1999).

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

(b) Effects on shoot nutrient content

It has been shown in several studies that the nitrogen contents in moorland C. vulgaris and in bryophytes was significantly higher in areas with higher nitrogen inputs (>10-15 kg N ha⁻¹ yr⁻¹), and increased in both the heather and mosses, compared with measurements of historical plant material (Pitcairn et al., 1995). The increase in nitrogen content of C. vulgaris with nitrogen deposition has also been clearly demonstrated in experimental studies with a range of nitrogen treatments (Leith et al., 2001; Carroll et al., 1999). Increases in shoot nitrogen content have been linked in other studies to increased performance of winter moth (Operophtera brumata), outbreaks of which have led to extensive damage to heather moorland 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 nitrogen concentration with increasing nitrogen dose, and measurements taken in 1996 still showed a significant increase in response to nitrogen addition (Carroll et al., 1999). A significant, but smaller absolute, effect was apparent in the two highest treatments in the 2000 harvest (Pilkington et al., submitted). Interestingly, N:P ratios were clearly increased as a result of the treatments by 1996, with values of 16:1 in the controls compared with 23:1 in the plots with the highest nitrogen treatment (Carroll et al., 1999). Carroll et al. (1999) compared these values with the critical threshold of N:P ratio of 14-16:1 proposed by Koerselman and Meuleman (1996) to indicate a switch from nitrogen to phosphorus limitation, and suggested that the induction of phosphorus limitation might explain the loss of response of shoot extension to nitrogen which occurred at this stage of the experiment. This interpretation is supported by evidence of increased phosphomonoesterase activity in peat and root surface (Johnson et al., 1998; Lee et al., 2000).

This observation is consistent with the study of Kirkham (2001), who sampled C. vulgaris shoots from a number of sites in England and Wales and analysed these for nitrogen and phosphorus
content, and found a significant positive correlation between NO\textsubscript{x}, but not total nitrogen, deposition and N:P ratio. The \textit{C. vulgaris} N:P ratios were above 16:1 at about half the sampled sites, suggesting that nitrogen deposition has changed a substantial proportion of \textit{Calluna}-dominated uplands in the UK from nitrogen-limited ecosystems into phosphorus-limited ones. Kirkham (2001) suggested that this could favour species such as \textit{Molinia} that are better adapted to phosphorus limitation, although this would not necessarily be the case if increased nitrogen deposition increased mineralisation rates. The relationship between deposition and N:P ratio could be used to estimate a critical deposition threshold for this switch. However, the R\textsuperscript{2} value for the relationship between NO\textsubscript{x} deposition and N:P ratio reported by Kirkham (2001) was only 0.1, and analysis of other unpublished UK datasets, such as that from the Countryside Survey 2000 (S.A. Power, pers. comm.) does not show the expected increase in N:P ratio with increasing N deposition. Furthermore, data from a national survey of \textit{Hylocomium splendens} in the UK show a weaker relationship between N deposition and N:P ratio than for N content (S.J. Woodin, pers. comm.).

(c) \textit{Effects on soils and root characteristics}

\textit{C. vulgaris} roots characteristically exhibit a substantial degree of ericoid mycorrhizal infection (Yesmin \textit{et al.}, 1996), which is important for the degradation of complex organic substances in order to give plants access to nitrogen sources, which would be otherwise unavailable. The nitrogen addition experiment in North Wales has consistently shown little effect on mycorrhizal infection levels (Caporn \textit{et al.}, 1995; Lee \textit{et al.}, 2000), using either the ergosterol method or visual assessment. However, Yesmin \textit{et al.} (1996) reported a negative correlation between nitrogen deposition and mycorrhizal infection rate at five remote Scottish sites with total deposition in the range 2-10 kg N ha\textsuperscript{-1} yr\textsuperscript{-1}, while 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\textsuperscript{-1} yr\textsuperscript{-1} for one year. The reasons for the differences in findings between the two studies are unclear and could relate either to methodological differences or the nature of the sites.

Soil studies at the North Wales site have found a number of responses with nitrogen addition. Leaching rates, although showing a small response to the higher nitrogen addition treatments, account for only a very small percentage of the added nitrogen. Significant increases in weight and total nitrogen content of \textit{C. vulgaris} litter, collected beneath the canopy, were observed at 80 and 120 kg N ha\textsuperscript{-1} yr\textsuperscript{-1}, but increases in litter production were also observed at values as low as 40 kg N ha\textsuperscript{-1} yr\textsuperscript{-1} (Carroll \textit{et al.}, 1999). There was also evidence of increased mineralization and decreased C:N ratio of litter and rhizosphere peat with increasing nitrogen treatment, although the peat effect was not found below 2 cm (Lee and Caporn, 2001). Overall, the results imply a high retention of the added nitrate and ammonium, probably through microbial immobilisation; Johnson \textit{et al.} (1998) demonstrated that the long-term application of nitrogen at the site increased soil microbial biomass.
Summary
The long-term experimental study of a moorland ecosystem has significantly increased understanding of the key long-term mechanisms associated with vegetation change. However, the lack of studies at relatively low rates of nitrogen deposition is a major barrier to accurate definition of critical loads, which in turn may be highly dependent on management practices, while correlation studies based on a limited number of field sites are difficult to interpret with any confidence. It is recommended that the critical load of 10-20 kg N ha\(^{-1}\) yr\(^{-1}\) for northern wet heath dominated by Calluna vulgaris (F4.11) as proposed by Bobbink et al. (1996), is retained. This is because there is no clear justification for a higher critical load than for dry heaths (which have a reliable estimate), as the dominant species, the indicators of exceedance, and the modifying factors are comparable. This is expert judgement, based on extrapolation, as the only available experimental evidence uses total N inputs (background plus treatments) significantly above the range of the proposed critical load.

4.3.2 ‘L’ Erica tetralix dominated wet heath (lowland)
The west European lowland heathlands of wet habitats are dominated by the dwarf-shrub Erica tetralix (Ellenberg, 1988) and classified in 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 of Dutch wet heathlands has been observed. Nowadays, many wet heathlands, which were originally dominated by E. tetralix, have become mono-stands of the grass Molinia caerulea. Together with E. tetralix, almost all of the rare plant species have disappeared from the system. It has been hypothesized that this change has been caused by atmospheric nitrogen eutrophication. Competition experiments using wet heath turfs have clearly shown that M. caerulea is a better competitor than E. tetralix at high nitrogen availability; after two years of application of nitrogen (150 kg N ha\(^{-1}\) yr\(^{-1}\)) the relative competitive strength of M. caerulea compared with E. tetralix was doubled (Berendse and Aerts, 1984). A 3-year field experiment with nitrogen application in Dutch lowland wet heathland (ca. 160 kg N ha\(^{-1}\) yr\(^{-1}\)) also indicated that M. caerulea is able to outcompete E. tetralix at high nitrogen availabilities (Aerts and Berendse, 1988). In contrast to the competitive relations between C. vulgaris and grass species, M. caerulea can outcompete E. tetralix without opening of the dwarf-shrub canopy. This difference is caused by the lower canopy of E. tetralix (25-35 cm), compared with C. vulgaris and the tall growth form of M. caerulea, which can overgrow and shade E. tetralix if enough nitrogen is available. It is in this respect also important that heather beetle plagues do not occur in wet heathlands, and until now no frost damage has been observed in this community.

Furthermore, it has been demonstrated in many Dutch wet heathlands that the accumulation of litter and humus has led to increased nitrogen mineralization (100-130 kg N ha\(^{-1}\) yr\(^{-1}\)) (Berendse et al., 1987). In the first 10 years after sod removal, the annual nitrogen mineralization is very low, but afterwards it increases strongly to the above-mentioned high figures. This accumulation of nitrogen can be strongly influenced by increased deposition, because soil nitrogen leaching from wet heathlands is extremely low (Berendse, 1990). The observed nitrogen availabilities are high enough to change E. tetralix-dominated wet heathlands into mono-stands of M. caerulea.
Berendse (1988) developed a wet-heathland model to simulate carbon and nitrogen dynamics during secondary succession. He incorporated in this model the competitive relationships between *E. tetralix* and *M. caerulea*, the litter production from both species, soil nitrogen accumulation and mineralization, leaching, atmospheric nitrogen deposition and sheep grazing. He simulated the development of lowland wet-heathland after sod removal, because almost all of the Dutch communities are already strongly dominated by *M. caerulea* and it is impossible to expect changes in this situation without drastic management. Using the biomass of *M. caerulea* with respect to *E. tetralix* as an indicator, his results suggested 17-22 kg N ha$^{-1}$ yr$^{-1}$ as critical load for the transition of lowland wet-heath into a grass-dominated sward. This was the value recommended by Bobbink *et al.* (1996) as the critical load.

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 17-22 kg N ha$^{-1}$ yr$^{-1}$ was too narrow. In particular, the model of Berendse (1990) is based on management using sod (turf) cutting every 50 years, together with grazing. Allchin *et al.* (2001) applied a similar model to UK dry heaths, and found that typical management regimes in the UK, 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 in wet heaths. Thus, while the upper end of the critical load range may remain comparable to that proposed by Bobbink *et al.* (1996), based on the model with sod cutting, the lower end of the critical load range should be reduced to account for the effects of nitrogen under less intensive management regimes. There is no clear evidence of a differential response of *Calluna vulgaris* and *Erica tetralix* to simulated nitrogen deposition, and S. Smart (pers. comm.) found comparable spatial relationships between change in cover and nitrogen deposition in the UK for the two species. Therefore, it is recommended that the lower end of the critical load range should be the same as that for *Calluna*-dominated wet and dry heaths, i.e. 10 kg N ha$^{-1}$ yr$^{-1}$.

In summary, a critical load range of 10-25 kg N ha$^{-1}$ yr$^{-1}$ is recommended for *Erica tetralix*-dominated northern wet heath (F4.11). This is expert judgement, as the effects of management and the validity of comparisons between *Calluna* and *Erica tetralix* are not supported by experimental data. The upper end of the range should be applied to systems with high intensity management, such as sod-cutting, and the lower end of the range to systems with low intensity management.

### 4.3.3 Dry heaths (F4.2; mostly Sub-Atlantic *Calluna-Genista* heaths F4.22)

**(a) Introduction**

Despite the conservation efforts and the adapted management in nature reserves, many lowland heaths (F4.2) have become dominated by grass species in Western Europe. An evaluation, using aerial photographs, has for instance demonstrated that more than 35% of Dutch heaths have been altered into grassland in the 1980s (Van Kootwijk and Van der Voet, 1989). It has been suggested that the strong increase in atmospheric nitrogen deposition might be a significant factor in the observed transition to grasslands. Similar, although not completely consistent, patterns were found in the UK over the past 20 to 50 years. Pitcairn *et al.* (1991) assessed changes in abundance of *Calluna vulgaris* at three heaths in East Anglia over the past decades.
All three heaths showed a decline in *C. vulgaris* and an increase in grasses; the authors concluded that increases in nitrogen deposition (to ca. 30-40 kg N ha\(^{-1}\) yr\(^{-1}\)) was at least partly responsible for the changes, but also noted that the management had changed, too. A wider assessment of heathlands in SE England showed that in some cases *C. vulgaris* had declined and subsequently been invaded by grasses while other areas where 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 nitrogen deposition in the past 30 years. Furthermore it has been hypothesized that, besides important changes in land use, increased nitrogen deposition is an additional cause of the decline of heaths in the southern parts of the Nordic countries (e.g. Fremstad, 1992; Tybirk *et al*., 1995). Unpublished Norwegian studies indicated that *C. vulgaris* dominated heaths have been invaded by grasses after death of *C. vulgaris*, which seems to correlate with frost injury and plagues of the heather beetle, a species recently found in SW Norway and expanding its territory (Hansen, 1991; Fremstad, 1992).

Despite this range of circumstantial evidence and indications, competition experiments in the Netherlands have shown a significant effect of nitrogen addition between *C. 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). A combination of mesocosm, field and modelling studies across Europe has made it clear over the last decade that the observed changes can only be explained by increased nitrogen 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 nitrogen-limited systems, one of the first effects of increased nitrogen availability through atmospheric deposition is an increase in biomass production of the vegetation (e.g. see Fig. 1.1). Many studies indeed show increased plant productivity of dwarf shrubs after experimental nitrogen enrichment in dry heathlands in several NW European countries (e.g. Heil and Diemont, 1983; Van der Eerden *et al*., 1991; Aerts and Heil, 1993; Power *et al*., 1995; Lee and Caporn, 2001). This clearly indicates that most of these lowland dry heath ecosystems are primarily limited by nitrogen, although some inland dry heaths are limited by phosphorus (Riis-Nielsen, 1997; Nielsen *et al*., 2000). An illustrative example of the growth stimulation of *C. vulgaris* has been found in a field experiment in Surrey, UK. It was set up in 1989 to assess the long-term impacts of realistic nitrogen loads on a lowland dry heath (F4.22) in southern Britain (Uren, 1992; Uren *et al*., 1997; Power *et al*., 1995; 1998a; 2001). After seven years, application of ammonium sulphate (7.7 & 15.4 kg N ha\(^{-1}\) yr\(^{-1}\), current atmospheric deposition 8 kg N ha\(^{-1}\) yr\(^{-1}\); Power and Barker, 2003) has not resulted in any negative effects upon *C. vulgaris*. Indeed, a significant stimulation of flower production, shoot density, and litter production occurred (15.4 kg N ha\(^{-1}\) yr\(^{-1}\)) and the canopy in the highest nitrogen treatment was 50% taller than in the control plots after 6 or 7 years (Power *et al*., 1995; 1998a). The increased shoot growth in the nitrogen-treated vegetation is not reflected in root growth, and an increased shoot:root ratio has been inferred. Shoot nitrogen concentrations increased somewhat, too, but only significantly at the start of the experiment (yrs 1 and 3) or in current year’s shoots. Clearly, this site remained nitrogen-limited over the whole experimental period of 7 years despite the additional nitrogen input of 7.7 or 15.4 kg N ha\(^{-1}\) yr\(^{-1}\). This is consistent with the observation that the studied dry heath site in Surrey is probably one of the most nutrient-limited lowland heaths in the UK.
(Power et al., 1998a). However, similar growth stimulation of *C. vulgaris* has been found in a dry lowland heath in Cheshire, UK and the Netherlands. Nitrogen was applied as ammonium nitrate (0, 20, 60 and 120 kg N ha\(^{-1}\) yr\(^{-1}\)) in Cheshire since 1996; the current atmospheric deposition at this site is estimated to be 20 kg N ha\(^{-1}\) yr\(^{-1}\). Shoot growth and flowering in *C. vulgaris* clearly increased at the two highest nitrogen addition rates within two years; after 5 years of nitrogen addition these effects were maintained in terms of canopy density, while the canopy height of *C. vulgaris* was ca. 20 cm higher (Cawley, 2001; Lee and Caporn, 2001). Several nutrient addition experiments have shown nitrogen-limited plant growth in the Netherlands (see Bobbink et al. (1992a) for overview).

c) Nitrogen accumulation and mineralization

During secondary dry heath succession there is an increase in the amount of organic material and nitrogen in the soil (Chapman et al., 1975; Gimingham et al., 1979). The accumulation of organic matter and of nitrogen has been quantified after sod removal in dry heaths in the Netherlands by Berendse (1990). He found a large increase in plant biomass, soil organic matter and total nitrogen storage in the first 20-30 years of succession. Regression analysis suggested an annual increase of nitrogen in the system of ca. 33 kg N ha\(^{-1}\) yr\(^{-1}\). These values are in good agreement with the measured nitrogen inputs in Dutch heathlands (Bobbink et al., 1992b). The build up of organic matter in the soil after sod removal, which removed almost all of the soil surface organic matter, is likely to be accelerated by the enhanced biomass and litter production of the dwarf-shrubs caused by the extra nitrogen inputs. Furthermore, nitrogen accumulation will be accelerated. Using destructive harvesting in 1996, 7 years after the start of the Surrey-UK experiment, Power et al. (1998a) determined the nitrogen budget of the control and nitrogen enriched plots. Approximately 15 and 24% of the added nitrogen had been accumulated in plant material for the low and high nitrogen treatments respectively, while another 10 and 14% of the added nitrogen was found in the litter layer. Almost no nitrogen is lost from these systems, as ammonium immobilization in the soil is high and hardly any leaching losses to deeper layers have been measured in Dutch, British or Danish dry non-coastal heaths (De Boer, 1989; Van der Maas, 1990; Power et al., 1998a; Kristensen and McCarty, 1999; Kristensen, 2001; Nielsen et al., 2000). The nitrogen cycling in dry heaths is thus very tight and hardly any nitrogen leached to deeper soil layers, even at high nitrogen inputs, as has also observed in nitrogen manipulation studies in upland *C. vulgaris* moorland (par. 4.3.1; F4.11). Indeed, the nitrogen content of the soil (upper 10 cm) significantly increased from 35.0 g N m\(^{-2}\) to 45.5 g N m\(^{-2}\) after 7 years of 1.54 g N m\(^{-2}\) yr\(^{-1}\) addition in the Surrey dry heath, and nitrogen leaching remained very low too (Power et al., 1998a/b; Barker, 2001). At the Cheshire site, Caporn et al. (2002) reported leaching only at 120 kg N ha\(^{-1}\) yr\(^{-1}\) addition, constituting less than 10% of the added nitrogen. Only after severe damage of the *C. vulgaris* canopy by heather beetles, significant nitrogen leaching to the subsoil has been observed at lower rates of deposition (Van der Maas, 1990; Nielsen et al., 2000).

The accumulation of nitrogen in the soil and litter layers affects the rates of decomposition and soil nitrogen mineralization. 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 controls to 6.3 years in low nitrogen plots (7.7 kg N ha\(^{-1}\) yr\(^{-1}\) added) and 6.1 years in
high nitrogen plots (15.4 kg N ha\(^{-1}\) yr\(^{-1}\) added) (Power et al., 1998a). During secondary succession after sod cutting in the Netherlands, under high nitrogen loads, Berendse (1990) found that soil nitrogen mineralization was low in the first 10 years (ca. 10 kg N ha\(^{-1}\) yr\(^{-1}\)), but strongly increased in the next 20 years to 50-110 kg N ha\(^{-1}\) yr\(^{-1}\) with increased nitrogen content in the soil. This suggests a threshold for the amount of nitrogen immobilisation in the system, above which net nitrogen mineralization occurs. Thus, apart from direct nitrogen inputs from the atmosphere, soil nitrogen availability will also finally increase because of the almost complete retention of extra nitrogen in the system. Eventually, this will alleviate the nitrogen limitation to plant production and may lead to a shift to more nitrophilous species. The strong increase in nitrogen availability after an accumulation period of 1-2 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 be also of importance in this respect.

(d) Changes in species composition

Competition experiments in containers and in the field have clearly demonstrated an important effect of increased nitrogen availability on the competitive interactions between C. vulgaris and grasses in the early phase of secondary succession in dry lowland heath. After experimental nitrogen additions (0, 7 and 28 kg N ha\(^{-1}\) yr\(^{-1}\)) during 4 years in the 1980s in the Netherlands (with additional atmospheric deposition ca. 25-35 N ha\(^{-1}\) yr\(^{-1}\)), grasses (Festuca ovina) strongly outcompeted C. vulgaris, if the total cover of the vegetation was still low at the start of the experiment (e.g. Heil and Diemont, 1983). However, C. vulgaris clearly is a better competitor than grass species in mature heath vegetation even at high nitrogen loads if its canopy remains closed (Aerts et al., 1990; Aerts, 1993).

Understorey species, especially the typical and frequent lichen and moss species, can be negatively affected by the increased growth of the vascular species. It has been found that after 7 years of nitrogen addition at Surrey (UK) the cover of the lichens and the lichen diversity (Cladonia spp; Parmelia) significantly decreased after additions of 7.7 & 15.4 kg N ha\(^{-1}\) yr\(^{-1}\) (current atmospheric deposition 8 kg N ha\(^{-1}\) yr\(^{-1}\)) (Barker, 2001; Fig. 4.2). Because of the weekly application of the extra nitrogen, this decline is certainly not caused by the direct toxic effects of nitrogen, but probably by increased shading through the greater canopy density of C. vulgaris. This has been confirmed by experimental removal of the shoots, which caused rapid recovery of the lichens (Barker, 2001). The same has been found for the moss understorey in the Cheshire-UK nitrogen experiment. The cover of Hypnum spp., a nitrophilous moss, was also considerably lower after 4 or 5 years of nitrogen additions (60 & 120 kg N ha\(^{-1}\) yr\(^{-1}\)) (Lee and Caporn, 2001). Recently, a strong decline in the lichen biomass (especially Cladonia) has been measured after 3 ½ year of addition of nitrogen (0, 20, 40 and 80 kg N ha\(^{-1}\) yr\(^{-1}\)) to a dry C. vulgaris heath at Clara, Ireland (current atmospheric 8-10 kg N ha\(^{-1}\) yr\(^{-1}\)). The cover of lichens has already been significantly reduced to ca. 10%, compared with ca. 30% in the control vegetation in the treatment with 20 kg N ha\(^{-1}\) yr\(^{-1}\), while in the high nitrogen plots the lichens almost disappeared (Tomassen et al., 2002).
When the productivity of *C. vulgaris* is no longer primarily limited by nitrogen, the growth response will be less or absent, and luxury consumption will lead to increased nitrogen concentrations in the plant. In a phosphorus-limited heathland in Denmark (current atmospheric deposition 18 kg N ha\(^{-1}\) yr\(^{-1}\)), nitrogen addition of 15, 35 and 70 kg N ha\(^{-1}\) yr\(^{-1}\) as ammonium nitrate increased shoot nitrogen concentrations significantly within 2 years of the start of the experiment (Johansson, 2000), while there was no significant growth response of the dwarf shrubs (Riis-Nielsen, 1997). Correlative field studies in low and high nitrogen inputs areas and experimental applications of nitrogen to heathlands have shown increased concentrations of nitrogen in the (green) parts of *C. vulgaris* at high nitrogen fluxes (e.g. Heil and Bruggink, 1987; Bobbink and Heil, 1993; Pitcairn et al., 1995). In forests, a clear relation between increased foliar nitrogen concentrations and pathogenic attacks as well as sensitivity secondary stresses has been established (see chapter 3). Similarly, experiments have at least partly indicated an increased risk to canopy damage for *C. vulgaris* under increased nitrogen availability. Heather beetle outbreaks and nitrogen-induced secondary stresses (especially winter injury and drought) are probably the main processes, which can open the canopy, and are thus crucial for the dramatic shift in species composition observed in lowland heath in high nitrogen load regions.

(e) Opening of the *C. vulgaris* canopy: heather beetle outbreaks

Outbreaks of heather beetle (*Lochmaea suturalis*), a chrysomelid beetle, can occur frequently in dry lowland heaths. It forages exclusively on the green parts of *C. vulgaris*. Insect herbivory is generally affected by the nutritive value of the plant material, with nitrogen concentration especially important (e.g. Crawley, 1983). Outbreaks of the beetle can lead to the opening of closed *C. vulgaris* canopy over large areas, greatly reducing light interception (Berdowski, 1987; 1993) and leading to enhanced growth of understorey grasses, such as *Deschampsia flexuosa* or *Molinia caerulea*. Brunsting and Heil (1985) found that the growth of the larvae increased after
foraging on the leaves of *C. vulgaris* with higher nitrogen concentrations in a rearing experiment. After field additions of ammonium sulphate in a roofed heath experiment, total number or biomass of the 1st stage larvae of the beetle was not affected by the treatments, but the development of subsequent larval stages was significantly accelerated (Van der Eerden et al., 1990). Larval growth rates and adult weight of heather beetles were found to be significantly higher when these insects were reared on *C. vulgaris* plants, which were collected in the British lowland heath experiment, after seven years of relatively low nitrogen addition (Power et al., 1998b).

Heather beetle larvae were also cultivated on shoots of *C. vulgaris* taken from plants, which had been fumigated with ammonia in open top chambers (12 months; 4-105 µg m⁻³) (Van der Eerden et al., 1991). After 7 days, both the mass and development rate of the larvae were clearly increased at higher ammonia concentrations. In addition, the growth of heather beetles instars was also significantly stimulated on *C. vulgaris* from UK heaths after ammonia fumigation, with relatively high concentrations, probably caused by the enhanced nitrogen concentrations in the plant material (Uren, 1992). It has also been demonstrated that increased nitrogen deposition to upland *C. vulgaris* stands stimulated larval development and growth rate of winter moth (*Operophtera brumata*) (Kerslake et al., 1998). It is thus likely that the frequency and the intensity of insect outbreaks can be stimulated by increased atmospheric nitrogen loads. This is supported by the observations of Blankwaardt (1977), who reported that from 1915 onwards heather beetle outbreaks occurred at ca. 20 year intervals in the Netherlands, whereas in the last 15 years of the observation period, the interval has been less than 8 years. In addition, it was observed that *C. vulgaris* plants were more severely damaged in nitrogen-fertilised vegetation during a heather beetle outbreak, both in the Netherlands (Heil and Diemont, 1983), in Denmark (Tybirk et al., 1995; Riis-Nielsen, 1997) and in the UK (Lee and Caporn, 2001). It is thus likely that enhanced atmospheric nitrogen deposition influences the frequency and severity of beetle outbreaks, although the exact controlling processes need further quantification.

*(f) Opening of the *C. vulgaris* canopy: secondary stresses*

It has been shown that frost sensitivity increased in some tree species with increasing concentrations of air pollutants (e.g. Aronsson, 1980; Dueck et al., 1991). This increased susceptibility is sometimes correlated with the enhanced nitrogen concentrations in the leaves or needles. Impacts of nitrogen deposition on the frost sensitivity of *C. vulgaris* could be possible, and it is suggested that the observed die-back of the *C. vulgaris* shoots in the 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 upon frost sensitivity in *C. vulgaris*. Fumigation with ammonia of *C. vulgaris* plants in open-top chambers over 4-7 month periods (100 µg m⁻³) revealed that frost sensitivity was not affected in autumn (September or November), but in February, just before growth started, frost injury increased significantly at -12 °C (Van der Eerden et al., 1991). A similar study in open-top chambers during the winter period in England also demonstrated increased frost sensitivity of heather plants, but the applied concentrations of ammonia were very high (140-280 µg m⁻³) (Uren, 1992). Van der Eerden et al. (1991) studied the frost sensitivity of *C. vulgaris* vegetation artificially sprayed with different levels of ammonium sulphate (3-91 kg N ha⁻¹ yr⁻¹). After 5 months the
frost sensitivity of *C. vulgaris* increased slightly, although significantly, compared with the control in vegetation treated with the highest level of ammonium sulphate (400 µmol l⁻¹; 91 kg N ha⁻¹ yr⁻¹). The sensitivity decreased again two months later and no significant effects of the ammonium sulphate application upon frost hardiness were measured at that time. All these studies used high levels of nitrogen deposition; however, the only study of the effects of low levels of nitrogen addition (7.7 & 15.4 kg N ha⁻¹ yr⁻¹) on frost sensitivity of *C. vulgaris* showed no significant effects after seven years of treatment (Power *et al.*, 1998b). Hence, the significance of increased frost sensitivity at ambient nitrogen loads is very uncertain and application of ammonium nitrate may even reduce frost sensitivity.

Summer 'browning' of the *C. vulgaris* canopies was frequently seen in dry summers in the 1980s in the Netherlands - the decade with the highest nitrogen loads. This suggested that nitrogen enrichment stimulated the sensitivity of *C. vulgaris* to periods of drought, probably by reduced root growth with respect to the development of the shoot or by a decrease in mycorrhizal infection. These effects might have major implications for the capacity of plants to deal with water or nutrient stress. The partitioning of biomass is very plastic and mostly determined by nutrient availability and light intensity (e.g. Brouwer, 1962; 1983). It has been shown that most plant species studied allocated more biomass to the shoots than to the roots at higher nutrient concentrations (e.g. Poorter and Nagel, 2000). This also held for *C. vulgaris* and other heathland species in a pot experiment over two years; its root weight ratio (RWR) significantly decreased with increasing nitrogen additions (Aerts *et al.*, 1991). However, field validation of these lower root to shoot ratios as a response to nitrogen enrichment is scarce. An indication of its importance was observed after long-term nitrogen applications in the Surrey-UK dry lowland heathland (Power *et al.*, 1998a). They found a small reduction in root to shoot ratio after seven years of nitrogen addition (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 characteristic demonstrated decreases in the root:shoot ratios in both *C. vulgaris* and *Deschampsia flexuosa*. Power *et al.* (1998b) also found higher water losses from *C. vulgaris* plants from the nitrogen-treated vegetation, compared with the control situation, but no differences in water potential of the shoots. Hence the hypothesis that increased nitrogen deposition might lead to severe growth reduction or even local ‘browning’ and die-back of this species in cases of severe drought episodes is still mostly speculative. Recently, a summer drought period (May-September) was experimentally implied under roofs to half of the plots in the second year of the nitrogen-enriched lowland heath plots in the Cheshire-UK study (Cawley *et al.*, 1998; Lee and Caporn, 2001). The high nitrogen treatments (60 & 120 kg N ha⁻¹ yr⁻¹) clearly increased the vulnerability of *C. vulgaris* to this drought stress. The cover of *D. flexuosa* significantly increased in these water-stressed plots, but this was partly attributable to a heather beetle outbreak, which was more severe in the droughted plots. However, *C. vulgaris* cover has partly recovered by 2000 from the 1997 drought period. This experiment is the only one to indicate the potential importance of the interaction between nitrogen enrichment and long dry periods.

Besides the changes in root to shoot ratios, ericoid mycorrhizal infection of the roots of the heathers could also be influenced by an increase in nitrogen load. However, few studies have been conducted and the outcomes are highly variable, as for AM mycorrhizal infection (Aerts and Bobbink, 1999). Some studies on the effects of increased nitrogen availability on ericoid
mycorrhizal infection of *C. vulgaris* root showed no effects (Johansson, 2000), in others nitrogen inputs stimulated the infection of mycorrhizas (Caporn et al., 1995), whereas other studies showed restricted infection after nitrogen treatment (Yesmin et al., 1996). At this moment the importance of this phenomenon in the decline of *C. vulgaris* and the shift to grass dominance is not at all clear. It is obvious that the sensitivity of *C. vulgaris* to drought stress might be increased by the 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 nitrogen applications with repeated drought episodes.

**(g) Modelling**

Computer models offer a tool to assess the impacts of nitrogen deposition on dry heathlands over decades with varying levels of secondary stresses and under different management regimes. Allechin 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 used in the Netherlands, the critical load for invasion of grass species was reduced to 10 kg N ha$^{-1}$ yr$^{-1}$. This implies that a reduction in the lower limit of the critical load range may be needed to cover all the management regimes used for dry heaths.

**Summary**

The impacts of increased nitrogen inputs to dry inland heaths (F4.2) are complex and occur at different time scales. Firstly, increased nitrogen availability stimulates biomass and litter production of *C. vulgaris* in most situations. Nitrogen is strongly retained in the system, gradually leading to higher nitrogen mineralization rates in the soil. However, the species remains the stronger competitor with respect to grasses, even at very high nitrogen availability, if the canopy is not opened. The shift from dwarf shrub to grass dominance is clearly triggered by opening of the canopy caused by heather beetle attacks, winter injury or drought. After decline of the *C. vulgaris* shoots, grasses quickly profit from the increased light intensity, together with the high nitrogen availability because of nitrogen 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 outbreaks, winter injury and drought) and the many long-term processes, which interact with them, it is very difficult to clarify experimentally all these relationships without long-term (10-20 years) and large-scale experiments.

It is finally concluded that it is necessary to modify the established critical load of 15-20 kg N ha$^{-1}$ yr$^{-1}$ for dry heaths which was recommended by Bobbink et al. (1992a; 1996). A key factor in this decision is the range of new evidence of impacts from the Surrey (UK) long-term experiment, which has applied relatively low rates of nitrogen deposition. Of particular importance is a re-evaluation of the background deposition at this site by Power and Barker (2003), who based their estimate on site measurements of NO$_2$ and NH$_3$ concentrations and of wet deposition of nitrate and ammonium. This provided a site-specific estimate of 8 kg N ha$^{-1}$ yr$^{-1}$, rather than the previous estimate of 10-15 kg N ha$^{-1}$ yr$^{-1}$. However, neither estimate accounts for the input from aerosol sources and nitric acid, which may add another 4 kg N ha$^{-1}$ yr$^{-1}$ at this site (M. Sutton, pers. comm.). Significant effects were observed in this experiment at application
rates of 7.7 kg N ha\(^{-1}\) yr\(^{-1}\), i.e. at a total deposition of 16-20 kg N ha\(^{-1}\) yr\(^{-1}\), the previously recommended critical load range for dry heaths.

It is therefore recommended that the lower end of the critical load range is lowered from 15 kg N ha\(^{-1}\) yr\(^{-1}\) to 10 kg N ha\(^{-1}\) yr\(^{-1}\). This value, based primarily on a long-term field experiment, is also supported by the results of simulation modelling using low intensity management regimes. Hence the new recommended **critical load of 10-20 kg N ha\(^{-1}\) yr\(^{-1}\)** can be classified as **reliable**. It should be stated that most nitrogen-addition studies have been done in a subcategory of dry heaths (F4.22), but it seems reasonable that this critical load can be applied across F4.2. However, it is advisable to use the low end of the range in lichen-rich dry heaths, as shown in the Surrey-UK study. The relative importance of phosphorus availability in some dry heath areas, and habitat management, as modifiers of dry heath response to increased nitrogen deposition have to be quantified. However, in general terms, the lower end of the range should be used with systems which have less intensive management and which are not phosphorus-limited.

Table 4.1. Empirical critical loads for the effects of excess nitrogen on heathland, scrub and tundra habitats (F). ## reliable, # quite reliable and (#) expert judgement.

<table>
<thead>
<tr>
<th>Ecosystem type</th>
<th>EUNIS-code</th>
<th>kg N ha(^{-1}) yr(^{-1})</th>
<th>Reliability</th>
<th>Indication of exceedance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tundra</td>
<td>F1</td>
<td>5-10(^a)</td>
<td>#</td>
<td>Changes in biomass, physiological effects, changes in species composition in moss layer, decrease in lichens</td>
</tr>
<tr>
<td>Arctic, alpine and subalpine scrub habitats</td>
<td>F2</td>
<td>5-15(^a)</td>
<td>(#)</td>
<td>Decline in lichens mosses and evergreen shrubs</td>
</tr>
<tr>
<td>Northern wet heath</td>
<td>F4.11</td>
<td>10-20(^a)</td>
<td>(#)</td>
<td>Decreased heather dominance, decline in lichens and mosses</td>
</tr>
<tr>
<td>• ‘U’ Calluna-dominated wet heath (upland moorland)</td>
<td>F4.11</td>
<td>10-20(^a)</td>
<td>(#)</td>
<td>Transition heather to grass</td>
</tr>
<tr>
<td>• ‘L’ Erica tetralix dominated wet heath (lowland)</td>
<td>F4.11</td>
<td>10-25(^a,b)</td>
<td>(#)</td>
<td>Transition heather to grass, decline in lichens</td>
</tr>
<tr>
<td>Dry heaths</td>
<td>F4.2</td>
<td>10-20(^a,b)</td>
<td>##</td>
<td>Transition heather to grass, decline in lichens</td>
</tr>
</tbody>
</table>

\(^a\) use towards high end of range at phosphorus limitation, and towards lower end if phosphorus is not limiting;
\(^b\) use towards high end of range when sod cutting has been practiced, use towards lower end of range with low intensity management.
5. Effects of nitrogen inputs in grassland and tall forb habitats (EUNIS class E)

5.1 Introduction

An overwhelming range of grassland ecosystems (EUNIS class E) are found across Europe, from very dry to wet habitats, acid to alkaline conditions, inland saline soils, heavy metal vegetation or under very different climatic regimes (e.g. Ellenberg, 1988; Davies and Moss, 2002). Only a restricted part 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 in 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, conservational grasslands are generally nutrient-poor, because of the long agricultural use with low levels of manure and with removal of plant parts by grazing or hay making. The vegetation is characterized by many species of low stature and of nutrient-poor soil status (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 specimens than for example oligotrophic acid or calcareous grasslands. To maintain high species diversity, artificial fertilisers have, of course, to be avoided. It is thus to be expected that several of these species-rich grasslands, especially of oligotrophic or mesotrophic soils, can be affected by increased atmospheric nitrogen inputs (e.g. Wellburn, 1988; Liljelund and Torstensson, 1988; Ellenberg jr., 1988). Moreover, some of the most species-rich grasslands are found under weakly buffered or almost neutral conditions, which make them sensitive to acidification and very sensitive to negative impacts of ammonium accumulation in case of high deposition of reduced nitrogen.

Empirical nitrogen critical loads were established for a restricted range of grassland habitats, i.e. calcareous and neutral to acidic grasslands, in the two previous reports on empirical nitrogen critical loads because of the lack of studies in almost all other grassland ecosystems. Very nutrient-poor acid grasslands were combined with species-rich heaths in the heathland section (Bobbink et al., 1992a; 1996). In this report, the EUNIS-habitat cataloguing has been fully adopted, leading to some rearrangement of the former classification. Grassland and tall forb habitats (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. The first level of division with relevance for the empirical approach, is based on soil water availability (dry-mesic-wet grasslands). Most of the nitrogen effect studies (both those used to set previous critical loads, as well as those from the 1996-2002 period) have been done in ecosystems which have to be classified as dry grasslands (E1). This subcategory is also, unfortunately one of the few, with a subsequent and necessary division into base-rich systems (perennial calcareous grassland and basic steppes, E1.2) and into
base-deficient grassland systems (non-Mediterranean dry acid and neutral grasslands, \textbf{E1.7}) (Davies and Moss, 2002). Thus these categories are more or less comparable with the 1996-groupings of calcareous grasslands (which are all dry grasslands) and the acidic/neutral grasslands (with some exceptions and additions, discussed in section 5.3). However, for the other sub-categories of E1, there is no relevant information.

The impacts of nitrogen inputs are, furthermore, only studied in a very small section of the other major EUNIS types (E2-E7). Some information exists for mesic grasslands (\textbf{E2}), which are especially classified on the basis of the present land use or management, and wet grasslands (\textbf{E3}). The latter group is split into oligotrophic wet grasslands (\textbf{E3.5}) and mesotrophic to eutrophic wet grasslands (\textbf{E3.4}). As this last division is of major importance for the setting of the critical loads, we tried to determine the critical load value for all grasslands at the third hierarchy level of EUNIS, despite the low overall number of experimental studies for mesic and wet grasslands. A new category within the grassland section is the impacts of nitrogen deposition in alpine and subalpine grasslands (\textbf{E4}).

\textbf{5.2 Dry grasslands (E1)}

\textit{5.2.1 Perennial calcareous grassland and basic steppes (E1.2)}

Calcareous grasslands (EUNIS code E1.2) are communities on limestone, which are wide-spread 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 and nitrogen (A/C-profiles; pH of the top soil: 7-8 with calcium carbonate content of ca. 10%). Plant productivity is low and calcareous grasslands are among the most species-rich plant communities in Europe and contain a large number of rare and endangered species. These grasslands decreased strongly in area during the second half of this century (e.g. Wolkinger and Plank, 1981; Ratcliffe, 1984). Some remnants became nature reserve in several European countries. To maintain the characteristic calcareous vegetation a specific management is needed in most situations to prevent their natural succession towards woodland (Wells, 1974; Dierschke, 1985).

A gradual increase of one grass species (\textit{Brachypodium pinnatum}) has been observed by the Nature Conservation Agencies in Dutch calcareous grasslands in the late 1970s/early 1980s, although the management (hay making in autumn) had not changed since the mid 1950s. It has been hypothesized that the increased atmospheric deposition of nitrogen (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 nitrogen enrichment have, therefore, 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\(^{-1}\) + ambient load of 30-35 kg N ha\(^{-1}\) yr\(^{-1}\)) over 3 years resulted in a drastic increase of the grass \textit{B. pinnatum} and a strong reduction in species diversity (including several Dutch Red List species), caused by the change in vertical structure of the grassland vegetation (Fig. 5.1). \textit{B. pinnatum} proves to have a very efficient nitrogen acquisition and a very efficient withdrawal from its senescent shoots into its well-developed rhizome system. It benefits from the extra nitrogen redistributed to the below-ground
rhizomes by enhanced growth in the next spring. In this way *B. pinnatum* strongly monopolized (>75%) the nitrogen storage in both the above-ground and below-ground compartments of the vegetation with increasing nitrogen 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 in recent years from the calcareous grasslands (During and Willems, 1986). This is partly caused by the (indirect) effects of extra nitrogen inputs, as experimentally shown by Van Tooren *et al.* (1990). Until now, data on the effects of nitrogen eutrophication on the species-rich fauna of calcareous grassland are not available.

Nitrogen cycling and accumulation in calcareous grassland can be significantly influenced by two major outputs: (i) leaching from the soil, and (ii) removal with management regimes.

![Figure 5.1. Above-ground biomass of *Brachypodium pinnatum* (g m⁻²) and number of vascular plant species (per 50 x 50 cm) in Dutch perennial calcareous grassland (E1.2) after three years of nitrogen application as ammonium nitrate (kg N ha⁻¹ yr⁻¹) (adapted from Bobbink, 1991).](image)

Nitrogen 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 nitrogen leaching from untreated vegetation is very low (0.7 kg N ha⁻¹ yr⁻¹), and only 2% of total atmospheric nitrogen deposition. After two-weekly spraying of ammonium sulphate for 2 years (50 kg N ha⁻¹ yr⁻¹), nitrogen 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 nitrogen inputs (Van Dam, 1990). It is thus evident that the studied calcareous grassland ecosystems almost completely retain nitrogen in the system, caused by a combination of enhanced plant uptake (Bobbink *et al.*, 1988; Bobbink, 1991) and increased immobilization in the soil organic matter (Van Dam *et al.*, 1990).

The most important output of nitrogen from calcareous grasslands is by exploitation or management. From the 1950s to mid 1980s, almost all of the calcareous grasslands in the Netherlands were mown in autumn with removal of the hay. The annual nitrogen removal in the hay varies slightly between years and sites, but in general between 17-22 kg N ha⁻¹ is removed from the system with the usual management (Bobbink, 1991; Bobbink and Willems, 1991). Legume species (*Fabaceae*) also occur in calcareous vegetation, and provide an additional
nitrogen input with the nitrogen-fixing microorganisms in their root nodules (ca. 5 kg N ha\(^{-1}\) yr\(^{-1}\)). The nitrogen mass balance of Dutch calcareous grasslands was summarized in the Lökeberg paper (Bobbink et al., 1992a), and a critical nitrogen load was estimated with a steady-state mass balance model (e.g. De Vries, 1994), because of the lack of long-term addition experiments with low nitrogen loads. Assuming a critical long-term immobilization rate for N of 0-6 kg N ha\(^{-1}\) yr\(^{-1}\), the critical nitrogen load can be derived by adding the nitrogen fluxes due to net uptake, denitrification and leaching, corrected for the nitrogen input by fixation. In this way, 15-25 kg N ha\(^{-1}\) yr\(^{-1}\) has been established in Lökeberg as nitrogen critical load. These values are in close accordance with the results found by Neitzke (1998; 2001) in a calcareous grassland in the East Eifel in southwest Germany. In a gradient of nutrient enrichment extending from an agricultural field, she found that nitrogen mineralization explained by far the highest variation in species composition and species degradation from the nutrient enriched border zone to the intact central chalk grassland (principal components analysis, correlation of phosphorus only with 3\(^{rd}\) axis). Comparing the soil nitrogen mineralization rates of the undisturbed chalk grasslands and the plots with significantly altered species composition, she found that \textit{B. pinnatum} had increased, and species diversity had decreased when nitrogen mineralization increased from 6-10 kg N ha\(^{-1}\) yr\(^{-1}\) in the unaffected parts to 35-55 kg N ha\(^{-1}\) yr\(^{-1}\) in the area bordering the agricultural fields (Neitzke, 1998; 2001).

In calcareous grassland in England, addition of nitrogen hardly stimulated dominance of grasses (Smith et al., 1971; Jeffrey and Pigott, 1973). In these studies, with application of 50-100 kg N ha\(^{-1}\) yr\(^{-1}\) and a further addition of phosphorus, a strong dominance of the grasses \textit{Festuca rubra}, \textit{F. ovina} or \textit{Agrostis stolonifera} was observed. However, \textit{B. pinnatum} or \textit{Bromus erectus}, the most frequent species in continental calcareous grassland, were absent from these British sites, so the data are not comparable in this respect. Following a survey of data from a number of conservation sites in southern England, Pitcairn et al. (1991) concluded that \textit{B. pinnatum} had expanded in the UK during the last century. They considered that much of the early spread could be attributed to a decline in grazing pressure but that more recent increases in the grass had, in some cases, taken place despite grazing or mowing, and could be related to nitrogen inputs. This was partly confirmed by Hewins and Ling (1998), who observed lower plant diversity and more aggressive grass species in calcareous grassland in those parts of the Cotswold (UK), receiving higher ammonium concentrations. A retrospective study of a heavily grazed chalk grassland at Parsonage Downs (UK) has, however, shown no substantial change in species composition over the twenty years between 1970 and 1990, a period when nitrogen deposition is thought to have increased to 15-20 kg N ha\(^{-1}\) yr\(^{-1}\) (Wells et al., 1993). \textit{B. 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 range of the 1992 critical load. In a study with chalk grassland species in mesocosms (South Downs, southeast England) the biomass of \textit{B. pinnatum} significantly increased after 2 years of nitrogen addition (≥70 kg N ha\(^{-1}\) yr\(^{-1}\)), even under a 8-weekly summer defoliation regime (Bryant, 1998). Unkovich et al. (1998) also found nitrogen limitation in their field study in Wytham, UK. These authors have found more than a doubling of herbage production with 6-weekly additions of 11.5 kg N ha\(^{-1}\) (total nitrogen addition of almost 60 kg N ha\(^{-1}\)) independent of nitrogen form (ammonium or nitrate) and no response to phosphorus or any other nutrient. Adding the nitrogen in the form of ammonium sulphate or ammonium nitrate increased nitrogen concentrations significantly compared with the controls and plots where nitrogen was added as potassium nitrate. Plants took up more than 40% of added
nitrogen, but still most of the added nitrogen (almost 60%) was immobilised in the soil (Unkovich et al., 1998).

The long-term effects of ammonium nitrate additions (35, 70 and 140 kg N ha\(^{-1}\) yr\(^{-1}\) since 1990) have been studied in a calcareous grassland on carboniferous limestone in Derbyshire in the UK (E1.26) (Morecroft et al., 1994; Carroll et al., 1997; Lee and Caporn, 1999; Carroll et al., 2000). Within the first 4 years, they did not observe a significant decline in species composition of vascular plants caused by nitrogen. From the sixth year on, there has been a marked and significant dose-related decline in vascular plant cover with increased nitrogen addition (Carroll et al., 1997; Lee and Caporn, 1999; Carroll et al., 2003). In 1999 there was an increase in grass cover, and a strong dose-related decrease in herbs, legumes, geophytes and monocarpic plants from the lowest nitrogen treatment on (35 kg N ha\(^{-1}\) yr\(^{-1}\)). The species that were negatively affected by nitrogen included a range of typical subordinate species of calcareous grasslands, while on the other hand *Hypochaeris radicata* was able to invade in nitrogen-enriched plots only (Carroll et al., 1997, Lee and Caporn, 1999). Significant changes in bryophyte species composition have also been observed in response to nitrogen addition, many of which reflect an acidification effect. Vegetation responses were overall slow, and significance for individual species cover was found mainly at higher levels of nitrogen addition, though the same trends could be witnessed from the lowest treatment on. Recent results from a labelling experiment with mesocosms from this site suggest an effect of long-term nitrogen application on increasing carbon allocation to shoots and decreasing it to roots. An additional nitrogen x phosphorus factorial field experiment, which was started in 1995 (35-140 kg N ha\(^{-1}\) yr\(^{-1}\)) at the same site, showed similar effects on species composition for the sole nitrogen treatment in the sixth growing season (Lee and Caporn, 2001).

In contrast to the slow vegetation responses, shoot nitrogen concentrations, nitrate reductase activities, and soil nitrogen mineralization and nitrification rates significantly increased during the first years with enhanced inputs of nitrogen (≥70 kg N ha\(^{-1}\) yr\(^{-1}\)) (Morecroft et al., 1994; Carroll et al., 1997). By 1995 soil pH values also had decreased significantly in response to increased nitrogen (≥70 kg N ha\(^{-1}\) yr\(^{-1}\)), most probably as a consequence of enhanced nitrification rates with its associated proton production. This acidification effect was stronger if ammonium sulphate (only included at the highest treatment (140 kg N ha\(^{-1}\) yr\(^{-1}\))) was added. Soil microbial activity was not significantly affected by ammonium nitrate additions but phosphomonoesterase activity increased significantly with the addition of ammonium sulphate (Johnson et al., 1998). Despite high additions of nitrogen, analyses of the soil for total nitrogen gave no indication for nitrogen accumulation in these thin soils. As the site is strongly phosphorus limited (Carroll et al., 1997), the vegetation could not take up extra nitrogen to a level that could explain the lack of nitrogen accumulation in the soil. Calculating the annual budget on the basis of nitrogen mineralization, fixation and addition as inputs and plant uptake as output, there is a substantial amount of nitrogen missing from the system. This calculated missing nitrogen has most probably leached out, thereby exceeding the accepted leaching losses for these kind of grassland systems (≥ 2 kg N ha\(^{-1}\) yr\(^{-1}\)) already at an addition of 35 kg N ha\(^{-1}\) yr\(^{-1}\).
The current nitrogen deposition in this area of the UK is estimated to be (19)-25/30 kg N ha\(^{-1}\) yr\(^{-1}\). Thus, it is not possible to accurately estimate a critical load from the nitrogen additions used. For that reason, a reduction experiment in greenhouse conditions has been set up with mesocosms from a neighbouring calcareous grassland site (2, 10, 20 and 55 kg N ha\(^{-1}\) yr\(^{-1}\) added as ammonium nitrate) (Jones and Ashenden, 2000). Two years after the initiation of this experiment, very few effects of differential nitrogen loadings on the vegetation were evident. However, in view of the strong phosphorus limitation, it may take a much longer time for any effect to become significant. Leaching of nitrate, however, was already found in both winters since the start of the experiment in all treatments receiving 10 kg N ha\(^{-1}\) yr\(^{-1}\) or more, but an indication of a clear dose-related effect was only observed in the second winter (Jones and Ashenden, 2000).

In 1996 the values from the Lökeberg paper were adapted to 15-35 kg N ha\(^{-1}\) yr\(^{-1}\) to allow for the lack in vegetation response in case of phosphorus limitation in combination with adequate management. It was suggested to use the low end of the range for nitrogen-limited, and the high end of the range for phosphorus-limited calcareous grasslands (Bobbink et al., 1996). However, since 1996 it has become clear that even phosphorus-limited systems may be vulnerable to species change in response to increased nitrogen loading, as illustrated in the UK long-term experiment. In phosphorus-limited systems, however, the general decrease in plant cover suggests some kind of toxicity effects, instead of the increased light competition found in Dutch chalk grasslands, as most important mechanism to species loss (Carroll et al., 1997). Additionally, the lack in growth response and associated nitrogen uptake in phosphorus-limited conditions lead to increased nitrogen leaching, as nitrification rates are high in these well-buffered systems. Thus, there is no longer evidence to allow a higher critical load for phosphorus-limited systems.

Simulations with a dynamic ecosystem model for calcareous grassland have also confirmed the original Lökeberg-critical load. This calcareous grassland model describes the biomass, nitrogen and light dynamics at different levels of nitrogen deposition (Vergeer and Heil, 1998). As an indicator for the effects of enhanced nitrogen the proportion of tall grass (>50%) is used. In the model the growth and competitive interaction between tall grass (i.e. in this case B. pinnatum) and the other three functional groups (other graminoids, perennial forbs, annual forbs), the litter production, decomposition and nitrogen mineralization, soil nitrogen leaching, removal with management, nitrogen fixation by legumes and atmospheric nitrogen deposition are incorporated. Simulations with different nitrogen loads deposition has shown a strong increase in tall grass 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 affirm 15-25 kg N ha\(^{-1}\) yr\(^{-1}\) as a critical load for the transition of a species-rich vegetation into a grass-dominated (>50%) sward within 10-15 years. In view of all this evidence, the critical load is set back at the level of the Lökeberg paper, i.e. **15-25 kg N ha\(^{-1}\) yr\(^{-1}\)**, and qualified as **reliable**.

Increased nitrogen availability is probably of major importance in a number of European calcareous grasslands (E1.2). In nitrogen-limited calcareous grasslands, an increased availability of nitrogen is indicated by an enhanced growth of some 'tall' grasses, especially of stress-tolerant species which have a slightly higher potential growth rate and efficient nitrogen utilization. The original species composition, obviously influences which of the grass species will increase at enhanced nitrogen inputs. The nitrogen retention in the system is very high with hardly any
leaching and nitrogen mineralization could be enhanced by nitrogen inputs. On the other hand, in phosphorus-limited conditions, vegetation responses are slow and loss of species is most probably associated with some kind of negative effects of the changed soil conditions. Nitrogen mineralization and nitrification are increased, and in these nitrogen-saturated systems with shallow soils this will most likely result in high leaching losses. Most data from calcareous grasslands are from studies conducted in temperate, sub-atlantic calcareous grasslands (E1.26), and sites with relatively high atmospheric nitrogen deposition. There is a need for more studies from continental regions, and for experiments with low nitrogen doses from sites with low atmospheric deposition.

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

The EUNIS class E1.7 groups all dry grasslands on acid and neutral soils with closed vegetation, in Atlantic or sub-Atlantic regions. This includes *Nardus stricta* swards (E1.71) that were combined with species-rich heaths in Bobbink *et al.* (1996), as well as *Agrostis-Festuca* (E1.72), *Deschampsia flexuosa* (E1.73), *Calamagrostis epigejos* (E1.74) and *Carex arenaria* (E1.75) grasslands, which all were classified together as neutral-acidic to acid grasslands in Bobbink *et al.* (1996). The critical load for dry species-rich *Nardus stricta* swards has been set to 10-15 kg N ha\(^{-1}\) yr\(^{-1}\), and this was based mainly on the observation that rare species disappeared from these grasslands before high and dense growing grasses started to dominate over the vegetation (Bobbink *et al.*, 1996). These endangered species are extremely sensitive to acidification and ammonium accumulation (Roelofs *et al.*, 1996; De Graaf *et al.*, 1998). The input of acidifying nitrogenous deposition easily decreases the ANC and later on soil-pH in these grasslands with their weakly buffered soils in the cation exchange range, while 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 mainly associated with soil acidification by nitrogen inputs, and the critical load for acidity is most probably below the critical load for nitrogen eutrophication effects.

Tomassen *et al.* (1999) have added nitrogen (1, 5, 10, 15, 20, 40, 60 or 80 kg N ha\(^{-1}\) yr\(^{-1}\) as ammonium nitrate) to planted mesocosms (“pristine” sand taken from 5-25 cm depth of sod-cut *Nardus* grassland) in greenhouse conditions. The mesocosms had received clean water for 15 weeks before treatments were initiated. Within two years they found a gradual increase in biomass of *Danthonia decumbens* and *Deschampsia flexuosa* with increasing nitrogen between the lowest nitrogen level and addition of 20 kg N ha\(^{-1}\) yr\(^{-1}\). However, there was a much stronger effect on both biomass and cover of grasses (*D. decumbens* and *D. flexuosa*) between 20 and 40 kg N ha\(^{-1}\) yr\(^{-1}\). Nitrate concentrations in the soil pore water were strongly increased by nitrogen additions of 40, 60 and 80 kg N ha\(^{-1}\) yr\(^{-1}\), and this indication for high nitrification is in accordance with the observation that the system was still buffered (no change in soil pH).

Berlin *et al.* (2000) compared species composition of grasslands between 1965 (background deposition 5-7 kg N ha\(^{-1}\) yr\(^{-1}\)) and 1990 (10-15 kg N ha\(^{-1}\) yr\(^{-1}\)) in 8 semi-permanent plots in Smaland, Southern Sweden, from which they could ascertain that no changes in the traditional management (summer mowing and aftermath grazing, no fertilizer use) had occurred during that period. They found very little changes on species richness and diversity, and no directional change in species composition. The only indications for the effects of increased nitrogen inputs
were a significant increase in graminoid cover at the expense of forb cover and a decrease in number of forb species. As among forbs, rosette species decreased most, there may be an indication for increased light competition. Additionally, experimental nitrogen applications were carried out for 3 years in an *Agrostis capillaris* grassland and a *Festuca ovina* grassland (both E1.72) differing in initial fertility in the just-mentioned province of southern Sweden. This resulted in an increased above-ground biomass as well as proportional more graminoid biomass with an addition of 19 kg N ha\(^{-1}\) yr\(^{-1}\) for the *Festuca ovina* grassland, with lowest initial productivity (atmospheric load 13 kg N ha\(^{-1}\) yr\(^{-1}\)) (Fig. 5.2). No significant response to addition of 37 kg N ha\(^{-1}\) yr\(^{-1}\) for the *Agrostis capillaris* grassland (with current atmospheric deposition of 15 kg N ha\(^{-1}\) yr\(^{-1}\)) was found within 3 years (Berlin, 1998).

The effects of nitrogen additions (35-70-140 kg N ha\(^{-1}\) yr\(^{-1}\) as ammonium nitrate) have been studied in a *Festuca-Agrostis-Galium* grassland (E1.72) in Derbyshire in the UK (current atmospheric deposition (19-25/30 kg N ha\(^{-1}\) yr\(^{-1}\)) (Morecroft *et al*., 1994; Carroll *et al*., 1997; 2000; Johnson *et al*., 1999; Lee *et al*., 2000). The experiment was initiated in 1990 and is still running up to day. Already during the second year of treatment a significant decline of bryophytes (especially *Rhytidiadelphus*) was found in all nitrogen treatments. This may partly have been an artefact of the relatively high nitrogen concentrations of the nutrient solution that was applied, but still indicates a high sensitivity to nitrogen. Moreover, *Rhytidiadelphus* stems collected from the plots showed an increasingly higher nitrogen concentration with nitrogen addition (Carroll *et al*., 1997; 2000). Despite the fact that shoot nitrogen concentrations, nitrate reductase activities and soil nitrogen mineralization rates clearly increased with enhanced inputs of nitrogen within three year (≥35 kg N ha\(^{-1}\) yr\(^{-1}\)), this did not significantly alter cover of higher plant species, diversity or species composition during the first 4 years (Morecroft *et al*., 1994; Carroll *et al*., 1997). From 1995 (sixth year of treatment) on, there was a clear tendency to decreased overall cover of the vegetation as well as decreased herb cover with increasing nitrogen addition. By 1999, the vegetation had became more grassy in the high nitrogen treatments (Carroll *et al*., 1997, Lee *et al*., 2000). In 1995 an additional experiment with a factorial nitrogen x phosphorus design was initiated with nitrogen additions of 35 and 140 kg N ha\(^{-1}\) yr\(^{-1}\). The results from the nitrogen only treatments corroborate the results found in the longer term experiment, with a clear decrease of *Festuca ovina*, *Luzula campestris* and *Potentilla erecta*, and an increase of *Nardus stricta* (Lee and Caporn, 2001). This experiment also confirmed that these grasslands were strongly limited by phosphorus rather than by nitrogen (Lee and Caporn, 2001). As found in the phosphorus-limited calcareous grassland (section 5.2.1), nitrogen accumulation was not significant for any of the treatments, and calculated nitrogen leaching was significant at addition rates ≥35 kg N ha\(^{-1}\) yr\(^{-1}\).
Figure 5.2. Proportion of graminoids after 3 years of adding 19 kg N ha$^{-1}$ yr$^{-1}$ in two Non-Mediterranean dry acid closed grassland sites (E1.7) in S. Sweden (Berlin, 1998). Hatched columns: with nitrogen addition; M0 = normal mowing; M+ = more intensive management; left part = Agrostis grassland (fertile site); right part: Festuca ovina site (less fertile site).

Phosphomonoesterase activity in the soil of the long-term experiment was enhanced already at the lowest nitrogen addition (35 kg N ha$^{-1}$ yr$^{-1}$) (Carroll et al., 1997). Additionally, microcosm studies with soils from this field experiment (after 7 years of nitrogen addition) showed increased root-surface phosphomonoesterase activity on the roots of Agrostis stolonifera seedlings already from 35 kg N ha$^{-1}$ yr$^{-1}$ on. Thus the increased nitrogen addition eventually also affected the phosphorus budget/economy in these severely phosphorus-limited system. A similar experiment with soils that had received nitrogen 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).

These studies indicate that many of these dry acid grasslands are also sensitive to nitrogen eutrophication. In nitrogen-limited systems, there is evidence for a significant increase in grass dominance leading to increased competition and exclusion of smaller species like rosette forbs from 15-20 kg N ha$^{-1}$ yr$^{-1}$ or higher at relatively low atmospheric nitrogen deposition (e.g. Berlin et al., 2000; Tomassen et al., 1999). Most probably, this is enhanced by a positive feedback to litter quality and soil nitrogen mineralization, but this has not been studied yet. In phosphorus-limited systems, the response of the vascular vegetation is much slower, but there is a clear and rapid effect on bryophytes (Carroll et al., 2000). On top of these effects, soil nitrogen and phosphorus economy is significantly impacted, which among other effects most probably results in significant leaching of nitrogen (Carroll et al., 1997; Johnson et al., 1999).

Taking into account that the UK results may be influenced by the already high deposition (19-25/30 kg N ha$^{-1}$ yr$^{-1}$), and the effects found in Sweden, the critical load for dry acid grasslands is set at 10-20 kg N ha$^{-1}$ yr$^{-1}$ and qualified as quite reliable. There is clearly a need for more
field studies in different countries, especially experiments with lower nitrogen addition in sites with low atmospheric deposition.

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

The EUNIS class E1.9 contains all dry grasslands on acid and neutral soils with open vegetation, in non-Mediterranean regions. Grasslands of this class were not treated in Bobbink et al. (1996), because of lack of data. However, inland dune grasslands (E1.94 & E1.95), both pioneer and more mature communities, are also part of this EUNIS class. These acidic inland dune grasslands are in species composition and ecological functioning comparable with coastal (grey) dune grasslands (see par. 8.2.1 for details). Because of this similarity, the critical load for inland dune pioneer (E1.94) and inland dune siliceous grasslands (E1.95) has been set at the same level as that of coastal grey dune grasslands (10-20 kg N ha$^{-1}$ yr$^{-1}$). This estimation is completely done on the basis of expert judgement and there is thus a significant need for research in these systems.

5.3 Mesic grasslands (E2)

5.3.1 Low and medium altitude hay meadows (E2.2)

Grasslands situated at low or medium altitude and with a management of hay making (E2.2) are the only class of mesic grasslands for which field experiments with nitrogen only treatments in realistic doses have been conducted. The Park Grass experiment at Rothamsted, UK, has been in existence since 1856 (Williams, 1978; Dodd et al., 1994). Nitrogen has been applied as ammonium sulphate or sodium nitrate (48 kg N ha$^{-1}$ yr$^{-1}$) to plots in this mesic low altitude hay meadow (E2.2). In nitrogen-treated plots, the vegetation has become dominated by a few grasses such as Alopecurus pratensis, Arrhenatherum elatius, Holcus lanatus or Agrostis ssp. Species diversity was correlated negatively 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 nitrogen forms (Goulding et al., 1998; Virtanen et al., 2000). In addition, Beltman and Barendreft (2002) clearly showed an increase in biomass and a 25% reduction in species diversity after long-term (>4 yrs) additions of nitrogen (100 kg N ha$^{-1}$ yr$^{-1}$) in a hay meadow along the river Rhine in the Netherlands. Formerly, these grasslands were combined with other neutral-acidic grasslands (e.g. dune grasslands, wet oligotrophic stands) and the critical load has been set at 20-30 kg N ha$^{-1}$ yr$^{-1}$ and considered as quite reliable (Bobbink et al., 1996). Because of the split of this group of grasslands under the EUNIS system, and the very few studies with low doses in E2.2, we suggest the critical load for low and medium altitude hay meadows (E2.2) is set at 20-30 kg N ha$^{-1}$ yr$^{-1}$, based upon expert judgement, because of the hay management which yearly removes nitrogen from the system. There is thus a strong need for field addition studies in different countries, especially in regions with low atmospheric deposition.
5.3.2 Mountain hay meadows (E2.3)

Many semi-natural grassland types occur in the montain regions across Europe, containing many rare and endangered plant and animal species (e.g. Ellenberg, 1996). It is important to emphasize the effects of nitrogen eutrophication in these mountain grasslands (E2.3), too, because nitrogen deposition has certainly increased in mountainous regions in central Europe (e.g. Hesterberg et al., 1996; Rihm, 1996). However, relevant studies are not available to establish a critical load with any reliability for these grasslands, although it may be expected that: (i) several of these grassland types are sensitive to nitrogen (Ellenberg, 1996), and (ii) the critical load for nitrogen is probably lower than for lowland dry or mesic grasslands, because of the shorter vegetation period or the generally poorer soils. The effects of nitrogen in montane grasslands has been identified as a major gap in knowledge (Bobbink et al., 1992a; Grennfelt and Thörnelöf, 1992; Hornung et al., 1995). Unfortunately, this gap still exists and the critical load (10-20 kg N ha$^{-1}$ yr$^{-1}$) for mountain hay meadows (E2.3) is based upon expert judgement only (Tab. 5.1).

5.4 Seasonally wet and wet grasslands (E3)

5.4.1 Moist or wet oligotrophic grassland (E3.5)

Moist or wet oligotrophic grassland habitats (E3.5) are characterized by oligotrophic and moist to wet peaty soil conditions. This class consists mostly of hay meadows under original agricultural management and 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 meadow”) and, (ii) heath meadows and humid Nardus stricta swards (E3.52). Because of their long traditional land-use with low additional inputs of nutrients, it is likely that these grassland communities are sensitive to extra nutrient inputs. Several fertilization experiments have demonstrated both nitrogen, phosphorus or even co-limitation of these elements in these wet oligotrophic grasslands (e.g. Vermeer, 1986; Egloff, 1987; Spink et al., 1998; Van Duren et al., 1998; Olde Venterink et al., 2001). In the case of nitrogen limitation, productivity of the grasses, especially the dominant M. caerulea, had increased and species diversity declined (e.g. Vermeer, 1986). However, almost all of the studies performed in moist or wet oligotrophic grasslands have been done with high to very high loads (>100 kg N ha$^{-1}$ yr$^{-1}$) or on a too short time span with respect to the critical load approach.

Fortunately, the impacts of nitrogen 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). Nitrogen additions of 25 kg N ha$^{-1}$ yr$^{-1}$ or higher (with estimated additional 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, H. lanatus and Bromus hordeaceus). The number of forbs, characteristic of these old meadows, declined sharply and some, for example, Cirsium dissectum, Lychnis flos-cuculi and Lotus pedunculatus disappeared from nitrogen-treated plots.

More recently, a nitrogen manipulation experiment has examined the effects of ammonium sulphate (10 or 20 kg N ha$^{-1}$ yr$^{-1}$) or sodium nitrate (20 kg N ha$^{-1}$ yr$^{-1}$ only) addition to an upland heath meadow in Wales (E3.52) (Emmett et al., 2001). This site, at 600 m a.s.l., was overgrazed
in the 1970s and 1980s, resulting in degradation of Calluna moorland to a sward dominated by Nardus stricta, Vaccinium myrtillus and Festuca (E3.52). The treatments were applied to paddocks which had different rates of experimental sheep grazing from 1989 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 cover of Festuca cover in the lightly grazed paddock, and greater frost injury (in nitrate treatment only) to Vaccinium, which may be linked to earlier budbreak in the spring. The lack of response observed in palatable grasses in the heavily grazed paddock may reflect selective grazing by sheep. However, there was a clear decline in moss cover in response to increased nitrogen addition, with Rhytidiadelphus loreus and Pleurozium schreberi being the most response species, and of lichen cover in the lightly grazed paddocks.

Measurements of soil water chemistry at this site showed significant leaching losses in the control plots which represented 25% of inorganic inputs, and which were increased from 5 to 7 kg N ha⁻¹ yr⁻¹ in the 20 kg N ha⁻¹ yr⁻¹ treatment. These high leaching rates suggested that the site is already above the critical load for nitrogen as a nutrient. In the ammonium treatments only, there was also increased base cation leaching and decreased pH. However, there were no significant treatment effects on mineralization or nitrification rates. Root bioassay experiments with Nardus indicated relatively low overall rates of nitrogen uptake and relatively high rates of phosphorus uptake overall, suggesting the site is phosphorus rather than nitrogen-limited, although phosphorus addition experiments do not support this conclusion (Emmett et al., 2001).

The current atmospheric deposition at this site is estimated to be 20 kg N ha⁻¹ yr⁻¹. To assess the impacts of lower deposition rates, Jones and Ashenden (2000) applied a range of deposition rates above and below the site estimate (2, 10, 20 and 55 kg N ha⁻¹ yr⁻¹ as ammonium nitrate) to mesocosms taken from the site in a greenhouse experiment. To assess possible interactions with grazing pressure, simulated clipping was also applied at three levels. Within 1-2 years, there were strong effects of nitrogen treatments below 20 kg N ha⁻¹ yr⁻¹ in increasing the cover of certain moss and lichen species, but only with heavy clipping, presumably because of the lower competition from vascular plants. Subsequent data (Jones et al., 2002a) show the emergence of different optima for bryophyte species – that for Racemutrum lanuginosum and Polytrichum juniperinum lying below 10 kg N ha⁻¹ yr⁻¹, while that for Hypnum jutlandicum lies around 20 kg N ha⁻¹ yr⁻¹ (Fig. 5.3). Although the results show an increase in fine grass cover and a decrease of Nardus with increasing nitrogen addition, these effects occur primarily between 20 and 55 kg N ha⁻¹ yr⁻¹. In contrast to the calcareous mesocosms in the parallel experiment and the 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., 2002a).
Figure 5.3. Cover of moss species in *Nardus stricta* grassland (E3.52) mesocosms exposed since 1997 to four nitrogen 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 nitrogen level for each species became apparent (Jones et al., 2002a).

It is concluded that several moist or wet oligotrophic grasslands (E3.5) of high conservational value are sensitive to nitrogen eutrophication. Increases in dominant grasses and decreases in diversity have been observed with increased levels of nitrogen inputs. Furthermore, interactions with phosphorus limitation are obvious. The study of a degraded upland heath meadow (E3.52) provides evidence of response of bryophyte cover to relatively low levels of N deposition, and suggests increases in leaching and acidification above 20 kg N ha\(^{-1}\) yr\(^{-1}\). In view of these UK studies, the critical load of moist to wet oligotrophic grasslands is set at 10-20 kg N ha\(^{-1}\) yr\(^{-1}\) for *Nardus stricta* swards (E3.52) and considered as quite reliable. However, the critical load of the hardly studied *Molinia caerulea* meadows (E3.51) is estimated as somewhat higher (15-25 kg N ha\(^{-1}\) yr\(^{-1}\)), but till present only based upon expert judgement.

5.5 Alpine and subalpine grasslands (E4)

5.5.1 Alpine and subalpine acid or calcareous grasslands (E4.3 & E4.4)

Many natural grassland types occur in the alpine and subalpine regions of European mountains, containing a large proportion of the biodiversity of that area. It is important to emphasize the effects of nitrogen inputs in these grasslands, too, because nitrogen deposition starts to increase in these remote regions. However, the impacts of nitrogen deposition are hardly studied in these grasslands, although it may be expected that; (i) several of these grassland types are sensitive to nitrogen and (ii) critical load for nitrogen is probably lower (see table 10.2) than for lowland dry grasslands (E1), because of the very nutrient-poor and thin soils. The impacts of nitrogen in alpine and subalpine grasslands has been identified as a major gap in knowledge (Hornung et al., 1995; Bobbink et al., 1996). Only one experiment with low-dose nitrogen treatments has been performed in this important group of grasslands. It has been shown that an alpine grassland in Switzerland significantly responded to nitrogen additions (4 years; 20 kg N ha\(^{-1}\) yr\(^{-1}\)); the biomass of the vegetation and the leaf area index (LAI) were both doubled or more after nitrogen application compared with the control situation. Furthermore, no effects of enhanced CO\(_2\) were
found (Körner, 1999). This experiment clearly showed the sensitivity of alpine and subalpine grassland to nitrogen, but data are too scarce to establish a quite reliable critical load. The critical load for alpine and subalpine acid or calcareous grasslands (E4.3 and E4.4) is thus still based upon expert judgement and set as 10-15 kg N ha\(^{-1}\) yr\(^{-1}\).

5.5.2 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. This constitutes class E4.2. Since these communities are nutrient limited and many moss and lichen species are highly responsive to increased nitrogen 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 for Racomitrium heath, which is found on mountain summits in Britain and in montane areas of arctic and sub-arctic zones.

In the UK, there has been a serious decline in the distribution of Racomitrium heath in recent decades, with replacement by grass communities. Increasing rates of nitrogen deposition may be one of the main factors involved in this deterioration of Racomitrium heath (Thompson and Baddeley, 1991), although changes in grazing pressure are also likely to be a significant factor. However, only recently has evidence from experimental manipulation studies become available to support this interpretation. Pearce and Van der Wal (2002) set up an experiment in the northeast Scottish Highlands within montane Racomitrium lanuginosum-Carex bigelowii heath in which plots on the summit were subject to experimentally increased low (10 kg N ha\(^{-1}\) yr\(^{-1}\)) and high (40 kg N ha\(^{-1}\) yr\(^{-1}\)) nitrogen input in both forms (NO\(_3^-\) and NH\(_4^+\)) for a period of two summer seasons. Current deposition was estimated to be 15-18 kg N ha\(^{-1}\) yr\(^{-1}\). Racomitrium was shown to be extremely sensitive to even the low rates of nitrogen deposition, responding with a raised tissue nitrogen 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 the control. Of all the measurements, only nitrate reductase showed a clear distinction between NO\(_3^-\) and NH\(_4^+\) application. After only two growing seasons, Pearce and Van der Wal (2002) also demonstrated how quickly Racomitrium is replaced by graminoid species; Racomitrium cover in the low treatment was reduced by 31%, while graminoid cover increased by 57%. The results may reflect both a toxic effect and increased competition for light from graminoids, which can utilise the excess nitrogen.

The high sensitivity of Racomitrium lanuginosum to nitrogen deposition is supported by the results of a glasshouse experiment using monoliths taken from a subalpine/montane grassland in central Wales, in which nitrogen deposition and simulated grazing have been manipulated over 4 years (Jones et al., 2002a). The nitrogen treatments applied were both above and below the 20 kg N ha\(^{-1}\) yr\(^{-1}\) at the source field site. Racomitrium only had a significant presence in monoliths subjected to heavy simulated grazing, confirming its sensitivity to competition for light by grasses. In these monoliths, there was a significant effect of nitrogen application within one year, with the cover of Racomitrium falling from 10% at 2 kg N ha\(^{-1}\) yr\(^{-1}\) to 2% or less at 10 kg N ha\(^{-1}\) yr\(^{-1}\) and above. However, a three-year manipulation study by Jónsdóttir et al. (1995), in which low levels of nitrogen 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 N ha\(^{-1}\) yr\(^{-1}\) over a period of three years, found only small non-significant increases in Racomitrium growth and shoot density.
These were associated with increases in nitrogen concentrations when reduced, but not oxidised, nitrogen was added. It was suggested that the small response to the low deposition rates used in this experiment was associated with growth limitation by other factors.

Although experimental evidence is limited to three studies with 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 N ha\(^{-1}\) yr\(^{-1}\) or lower suggests that a critical load for moss and lichen dominated mountain summits, ridges and exposed slopes (E4.2) should be set at 5-10 kg N ha\(^{-1}\) yr\(^{-1}\) and described as quite reliable. A summary of the empirical critical loads for grassland and tall forb habitats is given in table 5.1.

Table 5.1. Empirical critical loads for the effects of excess nitrogen upon grassland and tall forb habitats (EUNIS class E). ## reliable, # quite reliable and (#) expert judgement.

<table>
<thead>
<tr>
<th>Ecosystem type</th>
<th>EUNIS-code</th>
<th>kg N ha(^{-1}) yr(^{-1})</th>
<th>Reliability</th>
<th>Indication of exceedance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-atlantic semi-dry calcareous grassland</td>
<td>E1.26</td>
<td>15-25</td>
<td>##</td>
<td>Increase tall grasses, decline in diversity, increased mineralization, N leaching</td>
</tr>
<tr>
<td>Non-mediterranean dry acid and neutral closed grassland</td>
<td>E1.7</td>
<td>10-20</td>
<td>#</td>
<td>Increase in graminoids, decline typical species</td>
</tr>
<tr>
<td>Inland dune pioneer grasslands</td>
<td>E1.94</td>
<td>10-20</td>
<td>(#)</td>
<td>Decrease in lichens, increase biomass</td>
</tr>
<tr>
<td>Inland dune siliceous grasslands</td>
<td>E1.95</td>
<td>10-20</td>
<td>(#)</td>
<td>Decrease in lichens, increase biomass, increased succession</td>
</tr>
<tr>
<td>Low and medium altitude hay meadows</td>
<td>E2.2</td>
<td>20-30</td>
<td>(#)</td>
<td>Increase in tall grasses, decrease in diversity</td>
</tr>
<tr>
<td>Mountain hay meadows</td>
<td>E2.3</td>
<td>10-20</td>
<td>(#)</td>
<td>Increase in nitrophilous graminoids, changes in diversity</td>
</tr>
<tr>
<td>Moist and wet oligotrophic grasslands</td>
<td>E3.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Molinia caerulea meadows</td>
<td>E3.51</td>
<td>15-25</td>
<td>(#)</td>
<td>Increase in tall graminoids, decreased diversity, decrease of bryophytes</td>
</tr>
<tr>
<td>• Heath (Juncus) meadows and humid (Nardus stricta) swards</td>
<td>E3.52</td>
<td>10-20</td>
<td>#</td>
<td>Increase in tall graminoids, decreased diversity, decrease of bryophytes</td>
</tr>
<tr>
<td>Alpine and subalpine grasslands</td>
<td>E4.3 and E4.4</td>
<td>10-15</td>
<td>(#)</td>
<td>Increase in nitrophilous graminoids, biodiversity change</td>
</tr>
<tr>
<td>Moss and lichen dominated mountain summits</td>
<td>E4.2</td>
<td>5-10</td>
<td>#</td>
<td>Effects upon bryophytes or lichens</td>
</tr>
</tbody>
</table>
6. Effects of nitrogen deposition on mire, bog and fen habitats (EUNIS class D)

6.1 Introduction

The EUNIS class D includes a wide range of wetland systems that have their water table at or above soil level for at least half of the year, dominated by either herbaceous or ericoid vegetation (Davies and Moss, 2002). Nutrient budgets in wetland ecosystems are characterized by inputs and outputs of nutrients via groundwater and surface water, and tightly linked with local hydrology. The extent to which these systems receive and lose nutrients with in- and out-flowing water determines for a large part their sensitivity to excess nitrogen. Very little or little effects of nitrogen enrichment is to be expected in several open wetland systems, like (riverine) reed marshes or sedge beds (EUNIS D5 and D6) (e.g. Morris, 1991). A larger impact of atmospheric nitrogen deposition is expected in systems with a closed nitrogen cycle. This is, of course, especially clear in the case of ombrotrophic raised bogs, which receive all their major nutrients from the atmosphere.

Several wetland types (e.g. D1, D2, D4) are characterized by an (partly) incomplete decomposition of the plant litter, resulting in peat formation (Mitsch and Gosselink, 2000; Malmer et al., in press). The capacity for peat formation is a major component in the nutrient balance of these wetland systems, and is in many situations strongly linked to the presence of peat mosses (*Sphagnum* spp.) (Van Breemen, 1995). These peat mosses are capable of efficiently accumulating nitrogen 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) in the top layer of the peat, nitrogen is conserved in the peat. During peat accumulation, mineral storage actively removes nitrogen and other nutrients from the moss layer and rhizosphere, and is thus not available for plant life (e.g. Mitsch and Gosselink, 2000; Malmer and Wallén, in press).

In recent years, the concern over high atmospheric CO₂ levels has generated an increasing attention to 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, mainly bryophyte, material (Risager, 1998; Mitsch and Gosselink, 2000). Both increased carbon and nitrogen availability may increase primary production in pristine (nutrient-poor) conditions, while higher availability of carbon and nitrogen should have opposing effects on the C:N ratio of litter and thus hypothetically also on peat decomposition rates. However, the peat mosses (*Sphagnum* spp.), like mosses in other systems (e.g. grasslands, forests), have proved to be sensitive to increasing nitrogen availability, and react with decreased growth to high nitrogen doses. Thus, the interactions between carbon and nitrogen under expected global change are still not clear.

This sensitivity of peat mosses may also have implications for other systems that are characterized by a large bryophyte component, but which lack reliable evidence for a critical load up to date.
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 nitrogen loads. These bogs are systems of acidic, wet areas and are very common in the boreal and temperate parts of Europe. Because of the abiotic conditions, decomposition rates are slow, favouring the development of peat. In Western Europe and high 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 are represented by class D1 (raised and blanket bogs) for which the criterion is that the water supply is continuously or primarily rainwater. This is entirely consistent with the original description of these plant communities by Bobbink et al. (1996). For the purpose of critical load definition, there is no basis on which to differentiate raised and blanket bogs.

b) Effects on moss growth and species composition

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

The effects of atmospheric nitrogen deposition on ombrotrophic bogs have also been intensively studied in Britain (e.g. Lee and Studholme, 1992). Many characteristic Sphagnum species have become largely absent from affected ombrotrophic bog areas in Britain, such as the southern Pennines of England, where atmospheric nitrogen deposition has increased to ca. 30 kg N ha⁻¹ yr⁻¹. Several studies in British bogs have shown that increased supplies of nitrogen are rapidly absorbed and utilized by bog-mosses (Sphagnum), reflecting the importance of nitrogen as a nutrient and its scarcity in unpolluted regions. The high nitrogen loadings are, however, supraoptimal 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 nitrogen loads such as the Pennines the growth of Sphagnum is in general 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 nitrogen content from ca. 12 to 20 mg g⁻¹ dry weight was observed (Press et al., 1988). Furthermore, a large increase in nitrogen-containing amino acids (arginine) in the shoots of these bog-mosses was found after application of nitrogen, indicating a nutritional imbalance of the species.

Although these, and other, studies strongly indicate the detrimental effects of high nitrogen deposition rates on the development of the bog-forming Sphagnum species, there is also evidence of growth stimulation in response to small increments in nitrogen 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 growth four-fold following addition of nitrogen (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
nitrogen deposition during the decades before the experiment, the growth of Sphagnum at the site with high atmospheric nitrogen deposition rates had become phosphorus limited (Malmer, 1990).

The importance of taking ambient atmospheric deposition into account when interpreting the results of field manipulation experiments is also demonstrated by Berendse et al. (2001), who analysed the results of 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 growing seasons (50 kg N ha\(^{-1}\) yr\(^{-1}\) in the Netherlands) increased Sphagnum nitrogen concentrations at all sites. However, this treatment decreased Sphagnum production only at the two sites with the highest atmospheric deposition (Switzerland and the Netherlands, with 18 and 39 kg N ha\(^{-1}\) yr\(^{-1}\) atmospheric deposition, respectively), and these sites also had the highest nitrogen concentrations at the start of the experiment.

In another Swedish study (Gunnarsson and Rydin, 2000; Nordin and Gunnarsson, 2000), a three-year field manipulation experiment used a range of nitrogen addition rates at two mires with differing current atmospheric loads. N:P ratios and experimental results confirmed the idea that Sphagnum growth at the site in northern Sweden, with very low ambient deposition, was nitrogen limited, while at the southern site it was phosphorus limited (Gunnarsson and Rydin, 2000). After two years, the experimental addition of nitrogen 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 above. At these low nitrogen deposition rates (lower than 7-11 kg N ha\(^{-1}\) yr\(^{-1}\)), however, there was no correlation observed between Sphagnum total amino-acid nitrogen 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 nitrogen 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 this bog community below 10 kg N ha\(^{-1}\) yr\(^{-1}\).

Differential effects on the growth of different Sphagnum species have also been demonstrated in several experimental studies. For example, in a recent controlled environment experiment, Risager (1998) examined the growth responses of Sphagnum fallax to different forms of nitrogen (NO\(_3\)\(^{-}\), NH\(_4\)\(^{+}\) or NH\(_4\)NO\(_3\)) at low addition rates (0, 5, 10 and 20 kg N ha\(^{-1}\) yr\(^{-1}\)). Growth of S. fallax was significantly stimulated by application of NH\(_4\)\(^{+}\) (especially with 5 and 10 kg NH\(_4\)N ha\(^{-1}\) yr\(^{-1}\)), but this growth stimulation was, logically, not reflected in the tissue nitrogen concentration. Sole NO\(_3\)\(^{-}\) additions did not influence the growth of this species in this study. In addition, she investigated the responses of S. magellanicum after addition of NH\(_4\)NO\(_3\)-N in a comparable study with respect to the nitrogen levels, but without the differentiation between reduced or oxidised nitrogen. S. magellanicum showed no increase in length, but addition of nitrogen decreased the production density of capitula. In contrast to S. fallax, the tissue nitrogen concentration of S. magellanicum increased with increasing addition of nitrogen. In both cases, uptake of NO\(_3\)\(^{-}\) was considerably lower than for NH\(_4\)\(^{+}\). Risager (1998) concluded that the form of nitrogen is also important in species response, and that increased nitrogen availability may cause shifts in species composition in favour of S. fallax.
A German nitrogen addition experiment has shown 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 nitrogen type applied, depending on micro-habitat (soil moisture content). *S. 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\(^{-1}\)) in bog hollows, but only partly on the bog lawns. This resulted in *S. fallax* outcompeting *S. magellanicum* in the hollows and, if 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. The results of Gunnarsson and Rydin (2000) also suggest that lawn communities are less vulnerable to increased nitrogen deposition than are hummock communities. Because of the differences in vegetation structure, the rate of supply of nitrogen to a hummock community dominated by dwarf shrubs is about 40% greater than to a lawn community (Bobbink *et al*., 1992b; Malmer and Wallén, 1999).

The importance of competition between moss species is also indicated by the recent study of Mitchell *et al.* (2002) at rather higher rates of nitrogen deposition. This experiment examined the effect of addition of 30 kg N ha\(^{-1}\) yr\(^{-1}\) *in situ* to a cutover bog in the Jura mountains of Switzerland, where ambient deposition is estimated to be 15 kg N ha\(^{-1}\) yr\(^{-1}\). The normal pattern of succession in restoration of these sites is that keystone species such as the moss *Polytrichum strictum* create favourable microenvironments for establishment of *S. fallax* and thence of the typical bog vegetation. However, three years of study showed a cumulative differential height growth in response to added nitrogen deposition between overgrowing *P. strictum* and *Sphagnum capitula*, which might prevent this typical regeneration process in central European bogs. Thus, biomass production of *P. strictum* was almost doubled because of nitrogen addition, whereas production of *S. fallax* decreased with almost 50% in the study period.

c) Effects on competition between mosses and vascular species

A national survey of Danish ombrotrophic bogs has shown a decline of the original bog vegetation together with an increase of more nitrogen demanding grass species (such as *Molinia caerulea* and *Deschampsia flexuosa*) and trees (such as *Betula pubescens*) in areas with wet ammonium (NH\(_4^+\)) deposition loads higher than 10-15 kg N ha\(^{-1}\) yr\(^{-1}\) (Aaby, 1994), together with dry ammonia (NH\(_3\)) deposition, which is rapidly deposited to ombrotrophic mires. An increased growth of trees, particularly *Pinus sylvestris*, has also been demonstrated for many ombrotrophic sites in southern Sweden (Åberg, 1992; Gunnarsson *et al.*, 2002), as well as a reduced *Sphagnum* cover (Malmer and Wallén, 1999). For a bog in southern Sweden in a region with an ambient nitrogen deposition of 7-9 kg N ha\(^{-1}\) yr\(^{-1}\), Gunnarsson *et al.* (2002) showed that the total number of species (vascular plants + mosses) per plot did not change much from 1954 to 1997. However, there were large changes in species composition, which were indicative particularly of a drier mire surface and an increased availability of nitrogen. The increase in trees may also have triggered further changes in the plant cover. Also, on a mire in central Sweden in a region with lower nitrogen deposition, species numbers decreased over a similar period (Gunnarsson *et al.*, 2000). However, this decrease was mainly found in sites with pH>5.0. On sites with lower pH, at the site in southern Sweden, species numbers hardly decreased at all.

Such observations suggest that enhanced nitrogen deposition can influence the competitive relationships between mosses and vascular plants in nutrient-deficient vegetation such as bogs.
For light the competitive interactions between *Sphagnum* and vascular plants are symmetric, while for mineral nutrients they are partly asymmetric, as *Sphagnum* mosses rely on atmospheric supply only while the vascular plants also rely on mineralization (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 N and P (20 and 4 kg ha$^{-1}$ yr$^{-1}$, respectively) only affected the growth of the mosses if it was supplied to the surface, and only affected the vascular plants if supplied below the moss surface. The mosses were obviously nitrogen saturated and the target vascular plant, *Narthecium ossifragum*, had a N/P-ratio as high as about 40 (Malmer *et al*., in press).

These responses were also demonstrated experimentally by Heijmans *et al*. (2001), who studied the effect of added nitrogen 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 phosphorus limitation, which corresponded with the observed lack in growth response to nitrogen addition. The mosses were still able to capture a large part of the deposited nitrogen (Heijmans, 2000) and three years after the start of the experiment, all species showed increased nitrogen concentrations. The mosses showed decreased length increment, but no changes in dry matter production, indicating an effect on moss morphology (Heijmans *et al*., 2001). The fertilized mesocosms showed a significantly higher biomass production of vascular plants, but only the cover of *Vaccinium oxyccocus* was significantly increased, due to high experimental variability. There was a negative relationship between litter (also increased by nitrogen) and vascular plant cover on one hand and *Sphagnum* on the other hand (Heijmans *et al*., 2001), suggesting that *Sphagnum* growth might also be limited by increased shading.

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

The effects of the supply of extra nitrogen on the population ecology of *Drosera rotundifolia* were studied in a 4-year fertilization 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 NH$_4$NO$_3$ at an ambient deposition of 5 kg N ha$^{-1}$ yr$^{-1}$) significantly reduced the survivorship of the plants after 4 years, while additions of 20 and 40 kg N ha$^{-1}$ yr$^{-1}$ had this effect after one year, and also negatively affected flowering after two years (Fig. 6.1). The decrease in the density of the population of the characteristic bog species *Drosera* was associated with increased density of tall species such as *Eriophorum* and *Andromeda*, which resulted in enhanced competition for light.
d) Effects on peat and peat water chemistry as evidence of nitrogen saturation

The influence of nitrogen deposition entering the moss layer on nitrogen dynamics in the underlying peat was investigated in experiments on a raised bog, the 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 colonise contrasting sites; *S. capillifolium* is a hummock-forming species whereas *S. recurvum* occupies hollows and pools. The addition of 30 kg N ha\(^{-1}\) yr\(^{-1}\) increased the total nitrogen concentration in the mosses. Dissolved organic nitrogen in the moss water associated with *S. capillifolium* was proportional to the quantity of added nitrogen, suggesting an accumulation of amino acids in the mosses receiving nitrogen. This relationship was undetectable with *S. recurvum*, but this may be a consequence of its pool habitat, where surface waters in winter could dilute the water associated with the moss. Three seasons of nitrogen fertilization (30 kg N ha\(^{-1}\) yr\(^{-1}\)) had few effects on phosphorus cycling in the peat layer. The main effect was an increase in the C:P ratio of 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 nitrogen loads to *Sphagnum magellanicum* carpets at four contrasting sites, in Finland, Estonia and France, in addition to the Moidach More site in the UK. These experiments were only of four months duration, and hence of little value in terms of assessing critical loads, but showed clear contrasts in response between the sites, in terms of growth, increases in nitrogen content of the mosses, and in ammonium concentrations in the peat at 30 cm depth. The results suggest that both phosphorus limitation and a deep water table may decrease the retention of deposited inorganic nitrogen, and Williams et al. (1999a) suggest that peat drainage may reduce retention of deposited nitrogen by *Sphagnum* carpets.

Figure 6.1. Numbers of individuals of *Drosera rotundifolia* in an ombrotrophic raised bog (D1) near Stockholm (Sweden) during 4 years of nitrogen additions (Redbo-Torstensson, 1994).
The significance of nitrogen saturation of the *Sphagnum* layer, and increased availability of nitrogen in peat and in peat waters, was investigated by Tomassen *et al.* (2000; 2002), who treated transplanted turfs from an ombrotrophic floating bog in the Netherlands with a range of nitrogen deposition rates in the laboratory over 3 years, and also added *Betula pubescens* and *Molinia caerulea* plants. Tomassen *et al.* (2000) hypothesised that the birch and grass would only respond positively if nitrogen concentrations in the peat water below the *Sphagnum* mat increased. There was a significant and sometimes large increase in nitrogen-rich amino acid concentrations in the plant material of *Sphagnum recurvum* in the treatments above 5 kg N ha\(^{-1}\) yr\(^{-1}\). However, after three years significantly increased ammonium concentrations in peat water were only measured in the 40 kg N ha\(^{-1}\) yr\(^{-1}\) treatment (ca. 25 µmol l\(^{-1}\); a common value in Dutch ombrotrophic bogs), compared with 5-10 µmol l\(^{-1}\) in all other treatments, because of the very high nitrogen retention in the peat mosses. Nevertheless, after two years, there was evidence of increased birch extension growth with increasing nitrogen addition, and after 3 years, total biomass of both *Betula pubescens* and *M. caerulea* plants was significantly higher in the turfs which received 40 kg N ha\(^{-1}\) yr\(^{-1}\) (Tomassen *et al.*, 2002).

Lamers *et al.* (2000) used field data from Ireland and the Netherlands to produce an assessment of the capacity of the moss layer to restrict the amount of nitrogen deposition reaching the rhizosphere and hence enhancing the growth of competitive graminoids and other species (Fig. 6.2). The estimates were based on field data for *Sphagnum* from sites covering a range of values of N deposition. Below 10 kg N ha\(^{-1}\) yr\(^{-1}\), Lamers *et al.* (2000) suggest that growth stimulation would absorb the additional nitrogen inputs, while between 10-20 kg N ha\(^{-1}\) yr\(^{-1}\), an increase in nitrogen content of the *Sphagnum*, primarily from accumulation of amino acids, might assimilate the further increase in nitrogen deposition. Other nutrients, especially phosphorus, may however become secondarily limiting to plant growth when nitrogen inputs reach a threshold (Aerts *et al.*, 1992). Above 20 kg N ha\(^{-1}\) yr\(^{-1}\), it is suggested that this ‘natural filter’ would fail, and that nitrogen availability in the rhizosphere would increase, as also observed in high deposition regions (Fig. 6.2). It is important to note that Lamers *et al.* (2000) estimate total deposition as twice measured bulk deposition in constructing Figure 6.2. However, at the remote sites with low deposition, dry deposition is likely to be very low, and this method would significantly overestimate the actual deposition inputs; this would thus reduce the threshold nitrogen deposition rate for response in this figure.
Figure 6.2. The nitrogen concentration (mg g dry weight$^{-1}$) of raised bog (D1) Sphagnum species (apical parts) in Europe and USA in relation to total atmospheric nitrogen inputs (estimated as twice wet deposition) (Lamers et al., 2000).

e) Effects on decomposition and nutrient cycling

Aerts et al. (1992) concluded, based on the studies on the two Swedish sites discussed above, that high atmospheric nitrogen supply may affect the carbon balance of ombrotrophic bogs, because productivity under these circumstances is phosphorus, rather than nitrogen limited, but decomposition is probably increased by the high nitrogen loads. This hypothesis is supported by the results of the short-term experiments of Williams et al. (1999a) and Williams and Silcock (2000) (for details, see above). The addition of 30 kg N ha$^{-1}$ yr$^{-1}$ resulted in a decrease in peat C:N ratios (Williams et al., 1999a), which could enhance rates of first-stage organic matter decomposition and nitrogen mineralization (Aerts et al., 1992). Furthermore, additions of nitrogen to cores of S. capillifolium and S. recurvum, although captured mainly by the moss, had significant effects on the 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 nitrogen deposition (Hogg et al., 1994) indicated that the decomposition rate of Sphagnum peat was more influenced by the phosphorus content of the material, than by nitrogen. This finding that phosphorus content is significant is consistent with the results of a further study on the same two Swedish sites by Aerts et al. (2001), which involved 4 years of fertilization with 5 or 10 kg N ha$^{-1}$ yr$^{-1}$. These treatments had no significant effects at either site on potential decay rates, measured by taking litter formed in the first 3 years of the experiment and monitoring time-courses of CO$_2$ evolution in the laboratory. There was a significant relationship between potential decay rate and litter nutrient concentrations, but the effects of litter nitrogen and phosphorus content were comparable, and these relationships were primarily governed by the differences between the sites rather than treatments. Moreover, Tomassen et al. (2002) determined the decay rates, by
measuring both CH\textsubscript{4} and CO\textsubscript{2} evolution, of the peat turfs, which had been treated with 8 levels of nitrogen deposition during 3 years (see previous section). Despite significant differences in C:N ratios, carbon mineralization rates were not at all affected by nitrogen treatments.

In summary, these results do indicate that effects of elevated nitrogen deposition on \textit{Sphagnum} growth and litter chemistry, and on microbial biomass, are likely to affect the decomposition process, but this process is certainly not only regulated by the C:N ratio of the peat. However, further evidence is clearly necessary to properly evaluate the long-term effects of enhanced nitrogen supply on the decomposition of \textit{Sphagnum} peat and nutrient cycling.

**Summary**

Even though there are a limited number of really long-term (above 3-4 years) nitrogen manipulation experiments in bog ecosystems, a clearer picture is now emerging of the potential impact of elevated nitrogen deposition on bog habitats. Responses to nitrogen addition as low as 10 kg N ha\textsuperscript{-1} yr\textsuperscript{-1} have been observed in a number of bog species (in terms of survivorship, flowering, and density). Bryophyte species, in particular \textit{Sphagnum} spp., appear to be susceptible to the rise in anthropogenic nitrogen pollution, showing a decline in favour of grass and other competitive species, changes in competition between \textit{Sphagnum} species, and changes in physiological and biochemical characteristics. The concept of a limited capacity for retention of nitrogen deposition in the moss layer, above which nitrogen availability in the rhizosphere increases, offers a tool for assessment of factors which may modify the critical loads. Initial estimates using this method are consistent with a long-term response threshold of 10 kg N ha\textsuperscript{-1} yr\textsuperscript{-1}.

The critical load previously recommended by Bobbink \textit{et al.} (1996) (5-10 kg N ha\textsuperscript{-1} yr\textsuperscript{-1}) was based on a considerable body of field and experimental evidence and hence was judged to be quite reliable. Further studies since then in general provide results, which further support this value and it is therefore proposed that the critical load for \textbf{bog ecosystems (D1)} remains at 5-10 kg N ha\textsuperscript{-1} yr\textsuperscript{-1}, and that this critical load is now described as \textbf{reliable}. Expert judgement, based on observations that response to nitrogen is less in wetter areas of a bog, is that bogs receiving high effective precipititation (e.g. N&W UK, Norway) are less sensitive to nitrogen than those in drier areas (e.g. the Netherlands, Sweden). Precipitation is thus, besides phosphorus limitation, recommended to be a modifying factor to be taken into account when assigning critical loads to individual sites. However, additional long-term studies with enhanced nitrogen deposition (both nitrogen oxides and ammonia/ammonium) are necessary to further validate this figure, especially in relation to the long-term effects upon nitrogen and carbon mineralization, and to provide a stronger basis for identifying variation in response within this range.

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

Valley mires, poor fens and transition mires all have permanently waterlogged soils, with ground water 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 calcifuge small sedges and some brown moss communities, 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 critical load. However, the scarcity of studies does not allow a further distinction, and at present the little information that is available comes mainly from poor-fen systems (D2.2). Based on the generalisation from Morris (1991) on the link between nitrogen sensitivity and hydrology (see introduction par 6.1), it is then to be expected that valley mires are slightly less sensitive, and quaking bogs and transition mires more sensitive to excess nitrogen than poor fens.

In the northern part of Belgium, with a high deposition (about 30 kg N ha\(^{-1}\) yr\(^{-1}\)), Van Duren et al. (1997) found that biomass production in a poor fen was still mostly nitrogen limited. Adding 200 kg N ha\(^{-1}\) yr\(^{-1}\) for 3 years almost doubled biomass at cutting, and stimulated mainly the growth of *Filipendula ulmaria*, a high growing forb (Van Duren et al., 1997). In a more realistic experiment in northern Sweden, Granberg et al. (2001) have simulated increased nitrogen deposition by spraying treatments of 15 and 30 kg N ha\(^{-1}\) yr\(^{-1}\) for three growing seasons, at a ambient deposition of 2 kg N ha\(^{-1}\) yr\(^{-1}\). The authors were interested in possible effects of climate change on CH\(_4\) emission, and they expected (and confirmed) sedges to be an important pathway for CH\(_4\) release into the atmosphere. Similar to effects found in ombrotrophic bogs (see section 6.2), sedge cover was significantly increased with an increasing amount of nitrogen addition (Granberg et al., 2001). Unfortunately, the regression analysis used did not permit a distinction between effects found at 15 or at 30 kg N ha\(^{-1}\) yr\(^{-1}\). When sedge cover was accounted for, the effect of nitrogen addition on CH\(_4\) emission changed over the two years of addition 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 nitrogen 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). This accumulation corroborated the results found by Francez and Loiseau (1999), who added 5 kg N ha\(^{-1}\) yr\(^{-1}\) labelled with \(^{15}\)N to a poor fen in Côte de Braveix, Central France (background deposition 10 kg N ha\(^{-1}\) yr\(^{-1}\)). All nitrogen, added in June or August, remained in the system until October. Most of this nitrogen (55-65%) had accumulated in the *Sphagnum* layer. The upper peat layer between 0-10 cm accumulated about 15-30% of added N, and in all other compartments less than 5% of the added nitrogen was retrieved (Francez and Loiseau, 1999). These results indicate that *Sphagna* have a similar function as a nitrogen filter in poor fens as in ombrotrophic bogs (Lamers et al., 2000). Microcosm studies with *Sphagnum magellanicum*, a poor fen *Sphagnum* in Scandinavia, have shown significant negative effect of nitrogen additions (30 kg N ha\(^{-1}\) yr\(^{-1}\)) on concentrations of nutrients like phosphorus, potassium and calcium in mosses after 3 months (Jauhiainen et al., 1998b).

These studies indicate that a critical load of 20-35 kg N ha\(^{-1}\) yr\(^{-1}\) for mesotrophic fens (Bobbink et al., 1996) is too high for poor fens. Experimental addition in the field of 15 and 30 kg N ha\(^{-1}\) yr\(^{-1}\), with a background deposition of 2 kg N ha\(^{-1}\) yr\(^{-1}\), caused significant changes in species composition (increase in sedge cover) within three years. Furthermore, there are strong indications that at least some *Sphagnum* species found in poor fens are very sensitive to increased nitrogen. Therefore, we propose a new critical load of 10-20 kg N ha\(^{-1}\) yr\(^{-1}\) for poor fens (D2.2) as quite reliable and recommend the initiation of field experiments with realistic doses of nitrogen addition. As long as field studies are lacking, we propose to use the higher range of this critical load interval for valley mires (D2.1) and the lower range of this interval for quaking bogs and transition mires (D2.3) and urgently recommend field studies in these systems.
6.4 Base-rich fens (D4)

Like poor fens, rich fens have developed on permanently waterlogged soils, but in these systems there is a base-rich, nutrient-poor, often calcareous water supply buffering the system. They are largely occupied by calciphile small sedges and brown moss communities. Despite the fact that rich fens are the habitat of a range of specialized and rare species, very few field experiments have been conducted with ecologically relevant doses of nitrogen enrichment to determine the effects of increased nitrogen deposition. Previous work has set the critical load for mesotrophic fen ecosystems at 20-35 kg N ha\(^{-1}\) yr\(^{-1}\) (Bobbink et al., 1996). This was based mainly on nutrient budget studies done in rich fens (D4.1) in the Netherlands by Koerselman and Verhoeven (1992), assuming that the input of nitrogen should be counterbalanced by the output of nitrogen through usual management (mowing). Exceedance of this critical load results in a considerable increase of tall graminoids and a subsequent decrease in diversity of the subordinate plant species (Vermeer, 1986; Verhoeven and Schmitz, 1991). Though in some fens the strong phosphorus limitation inhibits changes in diversity with increased nitrogen, it is expected that such a situation leads to increased losses of inorganic nitrogen to the surface or ground water, thus leading to a similar critical load (Bobbink et al., 1996).

Between 1996 and 2002, some additional field experiments have been conducted, but these were all with (very) high nitrogen additions (>100 kg N ha\(^{-1}\) yr\(^{-1}\)) (e.g. Beltman et al., 1996; Boeye et al., 1997; Wassen et al., 1998). Five locations, in Belgium, Ireland and Poland, were used in factorial fertilization experiments with both nitrogen and phosphorus. Two sites (in the northeastern part of Belgium and in Poland) gave clear evidence of nitrogen as most important growth-limiting factor, while the other three sites were strongly limited by phosphorus (Beltman et al., 1996; Boeye et al., 1997; Wassen et al., 1998). Furthermore, the biogeochemistry and cycling of nutrients was recently studied in rich fens in a region with typical high atmospheric nitrogen loads (the Netherlands) and in a region with very low nitrogen deposition (northern Japan). This study demonstrated that nitrogen mineralization was much higher in the Dutch rich fens, than in the Japanese ones, despite the fact that the sites in the Netherlands were managed by mowing (Nohara et al., 2002). This clearly indicates the acceleration of the cycling of nitrogen in Dutch rich fens under high atmospheric nitrogen inputs (25-40 kg N ha\(^{-1}\) yr\(^{-1}\); mostly as ammonia/ammonium) since the early 1980s.

Microcosm experiments with bryophytes (Sphagnum wanstorfii) or vascular plants (Carex rostrata) collected from rich fens of higher latitudes indicate sensitivity to ammonium nitrate additions of 30 kg N ha\(^{-1}\) yr\(^{-1}\) and higher. Within three months, changes were measurable in moss growth as well as in nitrogen concentration and the concentration of free amino acids of both mosses and vascular plants, both of which are clear indications for nitrogen enrichment effects (Jauhiainen, 1998a; Saarinen, 1998). Though the conditions in these experiments were too artificial to justify by themselves a decrease of the present critical load, they do indicate that the moss species in particular, which are an important and prominent part of biodiversity in rich fens, may be very sensitive to increased nitrogen deposition.

Preliminary results after the two first years of long-term nitrogen addition in a rich fen in Central Ireland (ambient load 7-10 kg N ha\(^{-1}\) yr\(^{-1}\)) clearly indicate that the typical species-rich brown moss vegetation is affected by the form of nitrogen enrichment (Paulissen et al., 2003). Nitrate
application (50 kg N ha\(^{-1}\) yr\(^{-1}\)) significantly stimulated phosphomonoesterase (PMEase) activity in the typical brown moss *Scorpidium revolvens*, whereas ammonium addition (50 kg N ha\(^{-1}\) yr\(^{-1}\)) did the same in the invasive *Sphagnum contortum* within a two-year experimental period (Fig. 6.3). PMEase is an enzyme that enables plants to take up organically bound phosphates that are otherwise unavailable. This enables the plant to increase phosphorus uptake under conditions of high nitrogen availability. This suggests that, in the longer term, *Sphagnum* will profit of high ammonium deposition (comparable with Dutch levels), while brown mosses are negatively affected by it. The first results of the changes in moss cover suggest an increase in *Sphagnum* and a decline in brown mosses after two years of ammonium addition, but this experiment will be continued in the near future to validate this conclusion.

Bergamini and Pauli (2001) studied the effects of nitrogen enrichment on bryophytes in montane rich-fens (D4.2). These authors added 100 kg N ha\(^{-1}\) yr\(^{-1}\) to 18 different montane rich-fens (background deposition between 15 and 30 kg N ha\(^{-1}\) yr\(^{-1}\)) for 2 years, and found a significant 30% increase in vascular plant plant biomass and a significant 39% decrease in bryophyte biomass. Using vascular plant biomass as a covariable, they showed that nitrogen had a direct negative effect on bryophyte biomass, excluding the effects through increased competition by vascular plants (Bergamini and Pauli, 2001; Pauli et al., 2002). Despite the clear effects on bryophyte biomass, species heterogeneity between the sites was too high to find any significant changes in bryophyte community composition (Bergamini and Pauli, 2001). Although the nitrogen additions in this experiment are too high to set a critical load, they are an indication that montane rich fens are also sensitive to nitrogen.

The critical load for rich fens (D4.1) thus becomes 15-35 kg N ha\(^{-1}\) yr\(^{-1}\), with the higher end of the range recommended for managed or non-nitrogen-limited systems, and the lower end of the range recommended for nitrogen-limited systems but also for all high latitude systems and montane rich-fens (D4.2) (15-25 kg N ha\(^{-1}\) yr\(^{-1}\)) (expert judgement). The latter have longer frost periods, on average a colder climate, and more shallow soils, which are all factors that increase sensitivity to nitrogen (Bobbink et al., 1996). Long-term fertilization experiments with ecologically relevant additions of nitrogen, both in temperate regions, in northern countries and
in montane fens should increase the reliability of these figures. An overview of the empirical nitrogen critical loads for mire, bogs and fen habitats is presented in table 6.1.

Table 6.1. Empirical critical loads for the effects of excess nitrogen upon different mire, bog and fen habitats (Eunis class E). ## reliable, # quite reliable and (#) expert judgement.

<table>
<thead>
<tr>
<th>Ecosystem type</th>
<th>EUNIS-code</th>
<th>kg N ha(^{-1}) yr(^{-1})</th>
<th>Reliability</th>
<th>Indication of exceedance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raised and blanket bogs</td>
<td>D1(^{a,b})</td>
<td>5-10</td>
<td>##</td>
<td>Increase vascular plants, decrease bryophytes, altered growth and species composition of mosses, increased N in peat and peat water</td>
</tr>
<tr>
<td>Poor fens</td>
<td>D2.2(^{c})</td>
<td>10-20</td>
<td>#</td>
<td>Increase sedges and vascular plants, negative effects on peat mosses</td>
</tr>
<tr>
<td>Rich fens</td>
<td>D4.1(^{d})</td>
<td>15-35</td>
<td>(#)</td>
<td>Increase tall graminoids, decrease diversity</td>
</tr>
<tr>
<td>Montane rich fens</td>
<td>D4.2</td>
<td>15-25</td>
<td>(#)</td>
<td>Increase vascular plants, decrease bryophytes</td>
</tr>
</tbody>
</table>

\(^{a}\) use towards high end of range at phosphorus limitation, and towards lower end if phosphorus is not limiting;
\(^{b}\) use towards high end of range with high precipitation and towards low end of range with low precipitation;
\(^{c}\) For D2.1 (quaking fens and transition mires): use lower end of range (#)
For D2.3 (valley mires): use higher end of range (#)
\(^{d}\) For high latitude or nitrogen-limited systems: use lower end of range (#)
7. Effects of nitrogen deposition on inland surface water habitats (EUNIS class C)

7.1 Introduction

In this chapter, the effects of atmospheric nitrogen deposition on freshwater ecosystems (inland surface water habitats; EUNIS class C) are evaluated. Inland surface water habitats are non-coastal open fresh or brackish waterbodies (e.g. lakes and pools, rivers, streams and springs), including their littoral zones. Class C, however, also includes dune slack pools (Davies and Moss, 2002). Previously, the consequences of nitrogen enrichment upon soft water lakes were treated in the wetland section (Bobbink et al., 1996). However, in the EUNIS system, these ecosystems are classified in standing surface waters as class 1.1: permanent oligotrophic lakes, pools and ponds. They are thus treated in this background document in a separate chapter. Surprisingly, the subdivision of standing waters is only based on the trophic status of the waters, ignoring 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). This chapter summarises field and experimental evidence to establish critical loads for nitrogen deposition with respect to eutrophication or adverse ammonium effects. The acidifying effects of airborne nitrogen compounds to surface waters are only briefly summarized, as critical loads for acidity, including nitrogen, are well established for most waters (Henriksen, 1988; Kämärä et al., 1992; Henriksen and Posch, 2001).

7.2 Permanent oligotrophic lakes, pools and ponds (C1)

7.2.1 Permanent oligotrophic lakes, pools and ponds (softwater lakes; C1.1)

In the lowlands of Western Europe, many soft waters are found on sandy soils, which are poor in calcium carbonate or almost devoid of it. The waters are poorly buffered and the concentrations of calcium in the water layer are very low; they are shallow and fully mixed water bodies, with periodically fluctuating water levels, and are mainly fed by rain water, and thus oligotrophic. In the EUNIS approach, these waters have to be classified as part of permanent oligotrophic lakes, pools and ponds (C1.1). These softwater ecosystems are characterized by plant communities from the phytosociological alliance LITTORELLETEA (Schoof-van Pelt, 1973; Wittig, 1982; Arts, 1990; 2002) and have been well studied with respect to the effects of nitrogen deposition. The stands of these communities are characterized by the presence of rare and endangered isoetids (with the growth form of *Isoetes*), such as *Littorella uniflora*, *Lobelia dortmanna*, *Isoetes lacustris*, *I. echinospora*, *Echinodorus* species, *Luronium natans* and many other softwater macrophytes. These soft waters are nowadays almost all within nature reserves and have become very rare in Western Europe. This decline may be illustrated by the fact that *Littorella uniflora* was known from more than 230 sites in the Netherlands in the early 1950s, of which only ca. 40 still existed at the end of the 1980s. Furthermore, a strong decline in amphibians has been observed in these soft waters (Leuven et al., 1986).

The effects of nitrogen pollutants on these soft waters 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 these macrophytes were still abundant in the early 1980s were poorly buffered (alkalinity 50-500 meq l⁻¹), circumneutral (pH 5-6) and very poor in nitrogen (Roelofs, 1983; Arts et al., 1990). The softwater sites where these plant species had disappeared could be divided into two groups. In 12 of the 53 softwater sites eutrophication, resulting from inlet 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 of the 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) another development had taken place: the isoetid species were replaced by dense stands of Juncus bulbosus or aquatic mosses such as Sphagnum cuspidatum or Drepanocladus fluitans. This clearly indicates acidification of these soft waters in recent decades, probably caused by enhanced atmospheric deposition. In the same field study it was shown that the nitrogen levels of the water layer were higher in ecosystems where the natural vegetation had disappeared, compared with ecosystems where the isoetid stands were still present (Roelofs, 1983). This strongly suggests the detrimental effects of atmospheric nitrogen deposition in these softwater lakes.

A number of ecophysiological studies have revealed the importance of (i) inorganic carbon status of the water as a result of intermediate levels of alkalinity, and (ii) low nitrogen concentrations, for the growth of the endangered isoetid macrophytes. Furthermore, almost all of the typical softwater plants had a relatively low potential growth rate. Increased acidity and higher concentrations of ammonium in the water layer clearly stimulated the development of Juncus bulbosus and submerged mosses such as Sphagnum and Drepanocladus species (Roelofs et al., 1984; Den Hartog, 1986). It has also been shown in cultivation experiments that the nitrogen species involved (ammonium or nitrate) differentially influenced the growth of the studied species of water plants. Almost all of the characteristic softwater isoetids developed better with nitrate instead of ammonium addition, whereas Juncus bulbosus and aquatic mosses (Sphagnum and Drepanocladus) were clearly stimulated by ammonium nutrition (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 have been studied in softwater mesocosms during a 2-year treatment with different artificial rainwaters. Acidification due to sulphuric acid, without nitrogen inputs, has not resulted in an increased mass growth of Juncus bulbosus and a diverse isoetid vegetation remains present. However, after increasing the nitrogen concentration in the precipitation (19 kg N ha⁻¹ yr⁻¹ or higher as ammonium sulphate; control <2 kg N ha⁻¹ yr⁻¹), similar changes in floristic composition as under field conditions have been observed: a dramatic increase in dominance of Juncus bulbosus, of submerged aquatic mosses and of Agrostis canina (Schuurkes et al., 1987). These results demonstrate 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 stimulated directly the growth of plants such as Juncus bulbosus, whereas the surplus of the extra ammonium will be nitrified in these waters (pH<4.0). During this nitrification process H⁺-ions are produced, which increase the acidity of the system. The results of this study clearly demonstrated that large changes already occurred after 2-year treatment with 19 kg N ha⁻¹ yr⁻¹ or higher. In addition, it has been observed that the strongest decline in the species composition of the macrophytes of the Dutch softwater
Communities has coincided with areas with atmospheric nitrogen loads of ca. 10-13 kg N ha\(^{-1}\) yr\(^{-1}\) (Arts, 1990). Furthermore, Brouwer et al. (1997) showed that after 10 years of clean-rain treatment the recovery in the same softwater mesocosms, which had been treated with ammonium sulphate, is only partial and the vegetation is still dominated by *Juncus* and *Molinia*.

The nitrogen critical load for these **permanent oligotrophic waters (shallow softwater bodies, C1.1)** was set in 1996 at **5-10 kg N ha\(^{-1}\) yr\(^{-1}\)** (Bobbink et al., 1996). Since 1996 no new experimental evidence has been published on the impacts of nitrogen deposition in softwater lakes, which necessitate a change of the set critical load. A clear support to the established critical load is the outcome of a dynamic ecosystem model of small soft water lakes in the Netherlands, including sediment-water interactions and different groups of water plants (Wortelboer, 1998). Simulations with this model over a 30-year period confirmed the empirically derived nitrogen critical load (Albers et al., 2001), which is thus to be considered as **reliable**.

### 7.2.2 Permanent oligotrophic lakes, pools and ponds (C1.16; dune slack pools)

Another category in the EUNIS class of permanent oligotrophic waters are dune slack pools (C1.16). These relatively small, but permanent clear water pools are found in the coastal dune areas across Europe. Despite their location in the coastal dunes, they are classified in inland surface water habitats in EUNIS. The water layer is nutrient poor and these dune slack pools are mostly insensitive to acidification, because of their higher alkalinity (>1000 µeq l\(^{-1}\)), and characterized by clear water and a diverse submerged macrophyte vegetation (e.g. *Potamogeton* spp., *Chara* spp. and littoral isoetids). Eutrophication in these dune slack pools is more likely caused by atmospheric inputs or by high density of waterfowl than by the inflow of enriched surface water, because of their hydrological isolation. Very few experimental data exist on the sensitivity of these dune slack pools with respect to critical load setting, despite the generally well-known nitrogen limitation of dune slack wetlands (Lammerts and Grootjans, 1997). However, the impacts of atmospheric nitrogen deposition have been quantified in dune slack pool mesocosms (diameter ca. 2 m) during a 2-year treatment with different nitrogen loads (1, 20, 40 and 120 kg N ha\(^{-1}\) yr\(^{-1}\)) (Brouwer et al., 1996). No acidification of the water layer was found within 2 years, but total biomass of water plants and helophytes strongly increased above 20 kg N ha\(^{-1}\) yr\(^{-1}\). Nitrogen additions have clearly caused an increased succession rate in these dune slack mesocosms, leading to more helophytes and less open water. This phenomenon is also observed in many dune slack pools in the Netherlands, but experimental field studies are missing. Therefore, we propose a new critical load of **10-20 kg N ha\(^{-1}\) yr\(^{-1}\)** for **dune slack pools (C1.16)** as expert judgement and recommend the initiation of long-term field experiments with realistic doses of nitrogen additions to these and other shallow oligotrophic waters, especially in regions with a low current deposition.
7.3 Effects of nitrogen deposition on lakes and streams (C1-partly- and C2)

There is ample evidence that an increase of acidic and acidifying compounds in atmospheric deposition had resulted in recent acidification of lakes and streams in geologically sensitive regions of Scandinavia, western Europe, Canada and the USA (e.g. Hultberg, 1988; Muniz, 1991). This acidification is characterized by a decrease in pH and ANC, and by increases in concentrations of sulphate, aluminium, and sometimes nitrate and ammonium. It has already been shown since the 1970s with different approaches (field surveys, laboratory studies, whole-lake experiments) that these changes had dramatic consequences for plant and animal species (macrofauna, fishes) and for the functioning of these aquatic ecosystems. However, due to the strong reduction of sulphur deposition in 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).

The critical loads of acidity for aquatic ecosystems have been published under the Convention on Long-range Transboundary Air Pollution in 1988, 1992 and 1996 based on steady state water chemistry models. These recent models incorporate both acidity from both sulphur and nitrogen 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 thus site specific (sensitive geological regions or not) and also depend on the local hydrology and precipitation. For full details see Henriksen (1988), Kämäri et al. (1992) and Henriksen and Posch (2001). The critical loads for acidifying effects of nitrogen compounds, based upon nitrogen as the only cause of acidification, for the most sensitive lakes and streams are:

\[
\text{kg N ha}^{-1}\text{yr}^{-1}
\]

<table>
<thead>
<tr>
<th>Ecosystem Type</th>
<th>Critical Load</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scandinavian waters (C1.1)</td>
<td>1.4-4.2</td>
</tr>
<tr>
<td>Alpine lakes (C1.1)</td>
<td>3.5-6.1</td>
</tr>
<tr>
<td>Humic moorland pools (C1.4)</td>
<td>3.5-4.5</td>
</tr>
</tbody>
</table>

In many areas with moderate to high alkalinity in the waters and/or high base-cation deposition, the values of the critical loads for acidity from nitrogen deposition are much higher than those for the sensitive fresh waters. At this moment the possible effects of eutrophication by ammonia/ammonium or nitrate deposition are hardly incorporated in the establishment of critical loads of nitrogen for waters, except for permanent oligotrophic water bodies (C1.1; see section 7.2). This is because primary production in almost all surface waters is limited by phosphorus, and thus nitrogen enrichment has been considered unimportant in this respect (e.g. Moss, 1988). This certainly holds for those aquatic ecosystems, which are sensitive to acidification, for which the critical loads for acidifying effects (such as those listed above) are very low and thus certainly provide enough protection for the effects of nitrogen eutrophication.

It is, however, likely that some inland surface waters, which are relatively well buffered against acidification, are sensitive to nitrogen eutrophication, especially in shallow oligotrophic or
mesotrophic clear water habitats, including their littoral zone (Kämäri et al., 1992). The effects of nitrogen eutrophication (including reduced nitrogen forms) in these waters need further research, especially in areas with high phosphorus inputs, and should be incorporated into critical loads for nitrogen in future. At this moment, it is not possible to set critical loads of nitrogen eutrophication for these kinds of surface waters. The recommended critical loads of nitrogen with respect to eutrophication or adverse ammonia/ammonium effects to inlands surface water habitats (C1.1 & C1.16) are summarized in the following table (Table 7.1).

Table 7.1. Empirical critical loads for the effects of excess nitrogen upon surface water habitats (C1)*. ## reliable, # quite reliable and (#) expert judgement.

<table>
<thead>
<tr>
<th>Ecosystem type</th>
<th>EUNIS-code</th>
<th>kg N ha⁻¹ yr⁻¹</th>
<th>Reliability</th>
<th>Indication of exceedance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Softwater lakes (permanent oligotrophic waters)</td>
<td>C1.1</td>
<td>5-10</td>
<td>##</td>
<td>Isoetid species negatively affected</td>
</tr>
<tr>
<td>Dune slack pools (permanent oligotrophic waters)</td>
<td>C1.16</td>
<td>10-20</td>
<td>(#)</td>
<td>Increased biomass and rate of succession</td>
</tr>
</tbody>
</table>

*: for waters sensitive to acidification, use also critical loads for acidity.
8. Effects of nitrogen deposition on coastal habitats (EUNIS class B)

8.1 Introduction

In this chapter the impacts of atmospheric nitrogen deposition on coastal habitats (EUNIS class B) are evaluated with respect to the setting of nitrogen critical loads. Coastal habitats are those above the spring high tide limit (or above mean water level in non-tidal waters) occupying coastal features and characterized by their proximity to the sea. They include coastal dunes (dry grasslands, wet to moist dune slacks and wooded dunes), beaches and cliffs (Davies and Moss, 2002). Dune slack pools are considered in the previous chapter, 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. The first division within class B in EUNIS is based upon underlying substrates, i.e. sand, shingle or rock, but data to support proposals for critical loads are only available for sand substrates. Bobbink et al. (1996) considered the consequences of increased atmospheric nitrogen loads on coastal grasslands and heaths briefly in the grassland and heathland sections, when relevant data were available. In this background document, separate critical loads for nitrogen deposition are set for coastal dune and sand habitats (B1), using these older studies and updating the values with new information.

8.2 Coastal dune and sand habitats (B1)

Many dune ecosystems in the coastal areas of Europe are almost of natural origin and rich in typical plant and animal life. They are found on sandy, nutrient-poor soils, and considered to be sensitive to the impacts of eutrophication and acidification (e.g. Ellenberg, 1988; Wellburn, 1988; De Vries et al., 1994). Evidence exists with respect to the setting of nitrogen critical loads in coastal dune and sand habitats only for some EUNIS categories, especially shifting coastal dunes (B1.3), coastal stable dune grasslands (B1.4), coastal dune heaths (B1.5) and moist and wet dune slacks (B1.8).

8.2.1 Coastal stable dune grasslands (grey dunes) and shifting coastal dunes (B1.4 & B1.3)

A number of species-rich stable dune grasslands occur across the dunes of Europe. However, tall grasses have increased in many Dutch dry dune grasslands over recent decades, a period with higher nitrogen loading (20-30 kg N ha\(^{-1}\) yr\(^{-1}\)). In the primarily non-calcareous dunes, *Ammophila arenaria* is now 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 its diversity. In the past, tall graminoids were usually not dominant on these low nutrient sandy dune soils in the Netherlands. A recent survey in dry dune grasslands along the Dutch coast revealed that non-calcareous, iron-poor dry dune ecosystems are nitrogen-limited, but that in calcareous, iron-rich dunes there is co-limitation of nitrogen and phosphorus. (Kooijman et al., 1998; Kooijman and Besse, 2002). Kooijman et al. (1998) concluded that atmospheric nitrogen deposition may cause tall grass dominance encroachment in non-calcareous ecosystems.
dunes, but probably only accelerates the process in calcareous dune grasslands. Yet, a strong negative correlation between the percentage open dunes and total nitrogen deposition, especially above 15 kg N ha\(^{-1}\) yr\(^{-1}\), has recently been found 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 the increased atmospheric nitrogen deposition, is also supported by the fact that in many coastal areas of Britain, with relatively low nitrogen deposition (ca. 10 kg N ha\(^{-1}\) yr\(^{-1}\)), coastal stable dune grasslands are still species-rich (Jones et al., 2002b).

Additional field evidence is now available from a survey in the coastal dune areas across England and Wales in a region with much lower nitrogen depositions than the Netherlands (Jones et al., 2002d; 2003). Eleven sand dunes sites were surveyed with a range of atmospheric nitrogen inputs from 10-30 kg N ha\(^{-1}\) yr\(^{-1}\). In the mobile and semi-fixed dunes there were significant positive relationships between nitrogen inputs and biomass or sward heights. In the stable dune grasslands, combined nitrogen loads showed a negative relationship with species density and, less clearly, a positive correlation with sward height. In addition, a strong positive relationship between dissolved organic nitrogen in groundwater and nitrogen deposition in these stable grasslands was found. In general, the mentioned effects start to become apparent in the range 10-20 kg N ha\(^{-1}\) yr\(^{-1}\) and higher.

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 for two years and complete NPK fertilization 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 nitrogen, phosphorus and potassium were also investigated and nitrogen (>100 kg N ha\(^{-1}\) yr\(^{-1}\)) proved to be more limiting for plant growth than phosphorus. Although the changes in the vegetation were clearly less profound than after complete fertilization, reduction of species numbers (especially annual species, lichens and mosses) was observed in the treatments with nitrogen (Willis, 1963). Boorman and Fuller (1982) examined the effects of nutrient additions on the species composition of rabbit grazed dune grassland in Norfolk, UK over five years. The grazing prevented F. rubra becoming dominant, but several species (especially annuals, mosses and lichens) declined, while two species (Carex arenaria and Calystegia soldanella) increased in all treatments containing nitrogen (80 kg N ha\(^{-1}\) yr\(^{-1}\)). No evidence was found in this study for reduced diversity in +PK-plots. In a one-year experiment with additions of nitrogen (20, 40, 80 and 160 kg N ha\(^{-1}\) yr\(^{-1}\); atmospheric load 15 kg N ha\(^{-1}\) yr\(^{-1}\)) or phosphorus, the above-ground biomass of a stable dune grassland at the Dutch Wadden island of Schiermonnikoog proved to be strongly nitrogen limited; the 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). The effects of nitrogen have also been studied during two years on the coastal dunes of Iceland (probably EUNIS B1.3, shifting coastal dunes) in a region with very low atmospheric nitrogen deposition (Greipsson and Davy, 1997). The number of flowering spikes and total seed weight of the tall dune grass Leymus arenarius strongly increased within one year after application of 50 or 100 kg N ha\(^{-1}\). In summary, these short-term experiments with relatively high nitrogen applications clearly indicate the importance of nitrogen limitation in several shifting dunes and stable dune grasslands, but in some cases co-limitation with phosphorus has been observed, or rabbit grazing has prevented dominance of tall grasses.
Only two nitrogen addition experiments have been carried out in stable dune grasslands with the specific objective to examine the effects of nitrogen deposition. The effects of nitrogen 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 nitrogen additions (25 kg N ha\(^{-1}\) yr\(^{-1}\) as ammonium nitrate pellets, twice a year; current deposition ca. 23 kg N ha\(^{-1}\) yr\(^{-1}\) ) there were no significant changes in species composition in either the grazed or the ungrazed situation. Exclusion of grazing by rabbits and horses, through the use of enclosures, resulted in graminoid dominance (\textit{Festuca rubra}, \textit{F. ovina} and \textit{Poa pratensis}), especially where nitrogen additions were made, suggesting that grazing can 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\(^{-1}\) ) in the last one and a half year of the experiment. In this period, it had become obvious that ≥80% of the total nitrogen inputs in the nitrogen-treated vegetation had leached from the soil as nitrate due to nitrification at the calcareous sites, while 70% of nitrogen input leached even from the control grazed plots at these sites. At the partly decalcified site, nitrate leaching was substantially smaller, but still between 7-40 % (4-11 kg N ha\(^{-1}\) yr\(^{-1}\) ) of the experimental nitrogen inputs (Ten Harkel et al., 1998). It is thus clear that in these stable dune grasslands experimental nitrogen enrichment had no effect on species composition. This may be related to a shift to phosphorus limitation after the long period of high atmospheric nitrogen inputs in the Netherlands and the relatively high rabbit grazing pressure in that period. The high nitrogen leaching from the control vegetation is also a strong indication of nitrogen saturation of these dune grasslands, probably because of the high nitrogen deposition rates (>20 kg N ha\(^{-1}\) yr\(^{-1}\) ) over several decades.

Nitrogen manipulation experiments in stable dune grasslands have not been carried out to date in regions with low nitrogen deposition. The effects of elevated nitrogen loads starting at natural background deposition (<5 kg N ha\(^{-1}\) yr\(^{-1}\) ) have, however, been studied during 2 to 3 years in dry dune calcareous grassland mesocosms (1x1 m) (Tomassen et al., 1999; Van den Berg et al., 2003). After a pre-treatment period of 2 months with clean rain water that removed the excess of nitrate in the soil, nitrogen was added as ammonium nitrate (1, 5, 10, 15, 20, 40, 60 and 80 kg N ha\(^{-1}\) yr\(^{-1}\) ) and supplied twice a week. The effects on soil pore water chemistry and on two characteristic graminoid species and two endangered herbaceous species have been followed. Within one year of nitrogen additions, a clear difference was found in the amount of algae growing on the surface of the sand. The amounts of green algae increased in the treatments 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\(^{-1}\). The strong increase of algae on the soil top layer due to nitrogen deposition may have important implications as they prevent sand drift due to wind action. ‘Blowouts’ are important for renewed vegetation succession, and biodiversity will decrease when young successional stages decline (Tomassen et al., 1999). The concentrations of nitrate in the soil pore water have shown a strong seasonal fluctuation. During the first winter period an increase of nitrate was measured for the treatments ≥40 kg N ha\(^{-1}\) yr\(^{-1}\). During spring the nitrate concentration rapidly decreased. During the second and third winter, an increase in nitrate could only be observed in the highest nitrogen addition level. The ammonium concentration remained constantly very low (<5 µmol l\(^{-1}\) ).
Differences in plant growth have been observed after one year and have become obvious after two years or three years. The total cover of the vegetation increased with elevated nitrogen inputs. This increase could almost completely be attributed to *Calamagrostis epigejos*. The shoot and root biomass of this tall grass species increased with higher nitrogen additions, significantly above 20 or 15 kg N ha\(^{-1}\) yr\(^{-1}\), respectively (Fig 8.1).

![Figure 8.1](image.png)

**Figure 8.1.** Above- and belowground biomass (g m\(^{-2}\)) of the aggressive dune grass *Calamagrostis epigejos* after 2 years of nitrogen application in coastal stable dune grassland (B1.4) mesocosms (Tomassen et al., 1999). In the control treatment (C) nitrogen input was ca. 1 kg N ha\(^{-1}\) yr\(^{-1}\).

No clear effects of elevated nitrogen deposition rates on the two herbaceous species *Galium verum* and *Carlina vulgaris* was measured within two years, although in the third year, the number and weight of *G. verum* decreased above 40 kg N ha\(^{-1}\) yr\(^{-1}\) (Van den Berg et al., 2003). After two years of treatment, the total amount of nitrogen stored in the vegetation has been strongly elevated due to increased nitrogen deposition (Tomassen et al., 1999). These results are consistent with those of Mohd-Said (1999), who exposed mesocosms to a range of nitrogen deposition rates in the range 2-55 kg N ha\(^{-1}\) yr\(^{-1}\). At 10 kg N ha\(^{-1}\) yr\(^{-1}\) and above, 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 nitrogen load (1 kg N ha\(^{-1}\) yr\(^{-1}\)) have demonstrated that even in calcareous dune grasslands nitrogen loads ≥15 kg N ha\(^{-1}\) yr\(^{-1}\) changed the algae and grass cover while high loads (when not grazed) led to dominance of graminoid species within a few years.

Previously, stable dune grasslands have been considered in the grassland section (neutral and acid grasslands) with a critical load (quite reliable) of 20-30 kg N ha\(^{-1}\) yr\(^{-1}\) (Bobbink et al., 1996).
Based upon the observed effects over a relatively short period in the mesocosm and field study (grass increase ≥ 15 kg N ha\(^{-1}\) yr\(^{-1}\); strong nitrate leaching with 25-50 kg N ha\(^{-1}\) yr\(^{-1}\)), it is realistic to set the nitrogen critical load for **coastal stable dune grasslands (B1.4)** and **shifting coastal dunes (B1.3)** at 10-20 kg N ha\(^{-1}\) yr\(^{-1}\) (quite reliable, and expert judgement, respectively). Although the impacts of nitrogen additions have now been studied in some coastal dune grasslands, especially in the Netherlands, ‘long-term’ studies with realistic nitrogen loads and low current deposition are strongly needed to validate this critical load value.

### 8.2.2 Coastal dune heaths (B1.5)

Besides dry dune grasslands, heathland vegetation is also present in the coastal dunes in north-west Europe (Gimingham et al., 1979; Ellenberg, 1988). These natural coastal dune heaths are mostly dominated by the typical dwarf shrub *Empetrum nigrum*, while *Calluna vulgaris* is less common. Their podsolized soils are less developed than under inland heaths (Chapter 6) and poor in essential nutrients. Bobbink et al. (1996) did not consider critical loads separately for this type of heath, but incorporated it in the lowland dry heathland section. Within EUNIS, coastal heaths (B1.5) are classified as a subdivision of coastal dune and sand habitats.

Only one nitrogen manipulation experiment has been performed in coastal heaths, namely within the Danish HEATH experiment (Riis-Nielsen, 1997; Nielsen et al., 2000), in a coastal heath at Lodbjerg, Denmark. This coastal heath, dominated by *Empetrum nigrum* and *Ammophila arenaria*, is present in a ca. 250-300 years old-dune area at the coast of Jutland, with relatively low current 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 N ha\(^{-1}\) yr\(^{-1}\)) was made over 2 years. As a response to nitrogen additions, the vascular plant species increased in cover, whereas the lichens and bryophytes showed a very slight and insignificant decrease within 2 years (Riis-Nielsen, 1997). *Hypnum cupressiforme*, a typical heathland moss, declined linearly with the applications of nitrogen, whereas the cover of *Empetrum* and *Carex arenaria* increased linearly. Thus, plant productivity in this coastal heath system was obviously controlled by nitrogen limitation. However, drought effects upon *Empetrum* were clearly not related to nitrogen inputs (Tybirk et al., 2000). The leaching of both nitrate and ammonium has also been quantified in this coastal Danish heath. In the control and plots with 15 kg N ha\(^{-1}\) yr\(^{-1}\), no or hardly any nitrogen has leached to the subsoil. However, with higher N additions, especially 70 kg N ha\(^{-1}\) yr\(^{-1}\), a considerable part of the nitrogen has leached as nitrate from the B horizon, accompanied by aluminium, leading to acidification of the soil (Nielsen et al., 2000). Johansson (2000) found no effects of nitrogen additions on ericoid mycorrhizal infection of *Calluna vulgaris* in this coastal heath experiment. It is concluded that coastal heaths are affected by moderate nitrogen additions (≥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, the leaching data indicate a higher sensitivity of coastal heaths than inland dry heaths to extra nitrogen inputs. This is in accordance with the shallow organic layer in these coastal heaths. Moreover, these dune heaths are generally unmanaged, and thus have low nitrogen removal from the system. Because of this, the empirical nitrogen critical load for **coastal heath (B1.5)** has been established at 10-20 kg N ha\(^{-1}\) yr\(^{-1}\) (expert judgement), a lower value than that for inland dry heaths. It is evident that more long-term information is needed to verify this value.
8.2.3 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 areas around Europe. They are characterized 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 the ground water level is considerably lower in these dune slacks. Nowadays, many dune slacks have become part of nature reserves, and these are sometimes maintained via management as hay making or sod cutting (e.g. Ellenberg, 1988; Lammerts and Grootjans, 1997; Davies and Moss, 2002). 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, because of 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 has been recently reviewed by Lammerts and Grootjans (1997). Factorial fertilization experiments have shown that in almost all studied moist to wet, primary or secondary dune slacks in the UK, USA or the Netherlands, the above-ground biomass is limited by nitrogen availability. Primary phosphorus limitation was found only once, in a dune slack where sod cutting had been applied very recently. Single nitrogen additions have led to increased dominance of Carex and Juncus species, as well of tall grasses as Agrostis stolonifera and Calamagrostis epigejos. In some studies, typical forb species had declined in such situations (for an overview and the references, see Lammerts and Grootjans, 1997). Unfortunately, none of the studies have been done with low nitrogen additions (<100 N kg ha⁻¹ yr⁻¹; >1 year), and thus they are not adequate for setting a nitrogen critical load. In addition, a preliminary field study in the UK showed no differences in dune slack vegetation attributable to N inputs between the sites where these communities occurred (range: 10-16 kg N ha⁻¹ yr⁻¹) (Jones et al., 2003). It is, however, clear that most of these dune slacks are sensitive to nitrogen enrichment. The critical load for these moist to wet dune slacks (B1.8), is estimated by expert judgement as 15-25 kg N ha⁻¹ yr⁻¹, because of the resemblance of these slacks with wet, oligotrophic grasslands and base-rich fens. Of course, field validation of this value is certainly necessary. The empirical nitrogen critical loads for coastal habitats (B) are summarized in the following table (Table 8.1).
Table 8.1. Empirical critical loads for the effects of excess nitrogen upon coastal habitats (B). ## reliable, # quite reliable and (#) expert judgement

<table>
<thead>
<tr>
<th>Ecosystem type</th>
<th>EUNIS-code</th>
<th>kg N ha(^{-1}) yr(^{-1})</th>
<th>Reliability</th>
<th>Indication of exceedance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shifting coastal dunes</td>
<td>B1.3</td>
<td>10-20</td>
<td>(#)</td>
<td>Biomass increase, increase N leaching</td>
</tr>
<tr>
<td>Coastal stable dune grasslands</td>
<td>B1.4</td>
<td>10-20</td>
<td>#</td>
<td>Increase tall grasses, decrease prostrate plants, increased N leaching</td>
</tr>
<tr>
<td>Coastal dune heaths</td>
<td>B1.5</td>
<td>10-20</td>
<td>(#)</td>
<td>Increase plant production, increase N leaching, accelerated succession</td>
</tr>
<tr>
<td>Moist to wet dune slacks</td>
<td>B1.8</td>
<td>10-25</td>
<td>(#)</td>
<td>Increased biomass tall graminoids</td>
</tr>
</tbody>
</table>
9. Effects of nitrogen deposition on marine habitats (EUNIS class A)

9.1 Introduction
Marine habitats, as defined by EUNIS class A, are distinguished from other ecosystems by their direct connection to the ocean. Most of these systems are either not covered by plants or fully aquatic, and therefore out of the scope of this paper. Salt marshes were considered by Bobbink et al. (1996) in the wetland section, although at that time it was not considered necessary to set a critical load for these systems with very open nutrient cycles. However, recent evidence has indicated the need to reconsider this.

9.2 Coastal salt marshes and saline reedbeds (A2.6)
Salt marshes have developed on the shores of sheltered coasts or in estuaries, and are typically intertidal, i.e. they are located between lowest and highest tide, and are periodically covered by salt water. They are characterized by an open nutrient cycle, receiving large amounts of nutrients from surface water, and exporting similarly large amounts of nutrients through surface water and denitrification (for nitrogen). This has led to the conclusion, in accordance with Morris (1991), that these systems would not be vulnerable to effects of increased atmospheric nitrogen deposition, at least not at current deposition rates. However, nitrogen limitation has been demonstrated in salt marshes at the island of Schiermonnikoog, the Netherlands (Kiehl et al., 1997) and in Norfolk, UK (Jefferies and Perkins, 1977). During salt marsh succession, nitrogen accumulates in organic material, and nitrogen mineralization increases with marsh age as shown by Olff et al. (1993) and Van Wijnen et al. (1999). This accumulation of nitrogen 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 code A2.64) and a 100-year old salt marsh (EUNIS code A2.63) in the Netherlands (current atmospheric deposition 15-20 kg N ha⁻¹ yr⁻¹). Biomass increased significantly from the first growing season on in the young salt marsh, and continued to be higher in all three years (Fig 9.1). However, in the studied older salt marsh the addition of 50 kg N ha⁻¹ yr⁻¹ had no significant effect on biomass, although the response to a much higher nitrogen application (250 kg N ha⁻¹ yr⁻¹) showed that the vegetation was at least partly nitrogen limited (Van Wijnen and Bakker, 1999). Fertilization 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 nitrogen (250 kg N ha⁻¹ yr⁻¹) and high phosphorus treatment, compared with the control situation. Thus the effect of nitrogen on species composition could not be isolated from the effect of phosphorus. However, as the effects of added phosphorus on biomass were either non significant or quite small compared with the effects of nitrogen, there is a clear indication that increased nitrogen 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 nitrogen deposition might decrease the
surface of early successional vegetation on the marsh and thereby the foraging area that is suitable for these migratory birds.

**Young marsh**

![Figure 9.1](image)

Figure 9.1. Above-ground biomass of young salt marsh vegetation (A2.64) in the Netherlands after 1-year addition of differential nutrients; small n = 50 kg N ha\(^{-1}\) yr\(^{-1}\), capital N = 250 kg N ha\(^{-1}\) yr\(^{-1}\), small p = 20 kg P ha\(^{-1}\) yr\(^{-1}\), capital P = 100 kg P ha\(^{-1}\) yr\(^{-1}\) (adapted from Van Wijnen and Bakker, 1999).

During primary succession nitrogen accumulates in organic material in the soil of the salt marsh. The increasing nitrogen availability is one of the main driving forces for succession. Increased nitrogen 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 of salt marshes of a low successional age (EUNIS codes A2.65 and A2.64). Addition of 50 kg N ha\(^{-1}\) yr\(^{-1}\) to a current deposition of 15-20 kg N ha\(^{-1}\) yr\(^{-1}\) significantly increased biomass production each year over a 3-year period. Furthermore, no change in species composition or diversity was observed in salt marshes of NW Germany and the Netherlands, despite the present atmospheric N loads of 15-25 kg N ha\(^{-1}\) yr\(^{-1}\). Therefore we propose as expert judgement a critical load for *salt marsh systems* (A2.6) of 30-40 kg N ha\(^{-1}\) yr\(^{-1}\). However, field experiments with lower nitrogen additions over an equal or preferably longer period of time are necessary to improve the reliability for this estimate (Table 9.1).

<table>
<thead>
<tr>
<th>Ecosystem type</th>
<th>EUNIS-code</th>
<th>kg N ha(^{-1}) yr(^{-1})</th>
<th>Reliability</th>
<th>Indication of exceedance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early successional</td>
<td>A2.64</td>
<td>30-40</td>
<td>(#)</td>
<td>Increase late-successional species, increase productivity and A2.65</td>
</tr>
</tbody>
</table>
10. Conclusions and gaps in knowledge

Within the Convention on Long-range Transboundary Air Pollution covering the UNECE region, procedures have been developed to set and to map critical loads for airborne nitrogen deposition. Both the steady-state mass balance method and the empirical approach have been used within this Convention. Based on observed changes in the structure and function of ecosystems, reported in a range of publications, empirical critical loads for nitrogen were evaluated for specific receptor groups of natural and semi-natural ecosystems in both 1992 and 1996 (Bobbink et al., 1992a; 1996). They were also used for the development of the second edition of the Air Quality Guidelines for Europe of the World Health Organisation Regional Office for Europe (WHO, 2000). More recently, it was recognised that considerable new insights into, and data on, the impacts of nitrogen deposition on natural and semi-natural ecosystems vegetation have become available since the compilation of the last background paper in the mid 1990s.

In this updating procedure we have used a similar “empirical approach” as for the earlier background documents (see Chapter 2). For this purpose, we have firstly collected, as completely as possible, European publications on the effects of nitrogen in natural and semi-natural ecosystems from the period 1996 to mid 2002. We used peer-reviewed publications, book chapters, nationally published papers and “grey” reports of institutes or organisations, if available by request. In this background document, the groups of natural and semi-natural ecosystems have been re-classified (table 2.1) to facilitate the mapping procedure and ordered according the EUNIS habitat classification for Europe (Davies and Moss, 2002; see the separate background document of Hall et al., 2003 for details). In Chapter 3 to 9 the effects of nitrogen enrichment have been evaluated per EUNIS class and the empirical nitrogen critical loads updated with newly published evidence. The empirical critical loads for nitrogen have been reformulated (if necessary) and are summarized in separate tables per chapter. The text of this background document was discussed and optimized at the November 2002 expert workshop held in Berne (Switzerland) under the Convention on Long-range Transboundary Air Pollution. At the end of this meeting, the updated list of empirical N critical loads for natural and semi-natural ecosystems was approved by full consensus (table 10.1).
Table 10.1. Overview of empirical critical loads for nitrogen deposition (kg N ha$^{-1}$ yr$^{-1}$) to natural and semi-natural group of ecosystems. Classification of habitats according to EUNIS (except for forests).

## reliable; # quite reliable and (#) expert judgement.

<table>
<thead>
<tr>
<th>Ecosystem type</th>
<th>EUNIS-code</th>
<th>kg N ha$^{-1}$ yr$^{-1}$</th>
<th>Reliability</th>
<th>Indication of exceedance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Forest habitats (G)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil processes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deciduous and coniferous</td>
<td></td>
<td>10-15</td>
<td>#</td>
<td>Increased N mineralization, nitrification</td>
</tr>
<tr>
<td>Coniferous forests</td>
<td></td>
<td>10-15</td>
<td>##</td>
<td>Increased nitrate leaching</td>
</tr>
<tr>
<td>Deciduous forests</td>
<td></td>
<td>10-15</td>
<td>(#)</td>
<td>Increased nitrate leaching</td>
</tr>
<tr>
<td><strong>Trees</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deciduous and coniferous</td>
<td></td>
<td>15-20</td>
<td>#</td>
<td>Changed N/macro nutrients ratios, decreased P, K, Mg and increased N concentrations in foliar tissue</td>
</tr>
<tr>
<td>Temperate forests</td>
<td></td>
<td>15-20</td>
<td>(#)</td>
<td>Increased susceptibility to pathogens and pests, change in fungistatic phenolics</td>
</tr>
<tr>
<td><strong>Mycorrhiza</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperate and boreal forests</td>
<td></td>
<td>10-20</td>
<td>(#)</td>
<td>Reduced sporocarp production, changed/reduced below-ground species composition</td>
</tr>
<tr>
<td><strong>Ground vegetation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperate and boreal forests</td>
<td></td>
<td>10-15</td>
<td>#</td>
<td>Changed species composition, increase of nitrophilous species, increased susceptibility to parasites</td>
</tr>
<tr>
<td><strong>Lichens and algae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperate and boreal forests</td>
<td></td>
<td>10-15</td>
<td>(#)</td>
<td>Increase of algae, decrease of lichens</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperate forests</td>
<td></td>
<td>10-20</td>
<td>#</td>
<td>Changes in soil processes, ground vegetation, mycorrhiza, increased risk of nutrient imbalances and susceptibility to parasites</td>
</tr>
<tr>
<td>Boreal forests</td>
<td></td>
<td>10-20</td>
<td>#</td>
<td>Changes in soil processes, ground vegetation, mycorrhiza, increased risk of nutrient imbalances and susceptibility to parasites</td>
</tr>
<tr>
<td><strong>Heathland, scrub and tundra habitats (F)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tundra</td>
<td>F1</td>
<td>5-10$^a$</td>
<td>#</td>
<td>Changes in biomass, physiological effects, changes in species composition in moss layer, decrease in lichens</td>
</tr>
<tr>
<td>Arctic, alpine and subalpine scrub habitats</td>
<td>F2</td>
<td>5-15$^a$</td>
<td>(#)</td>
<td>Decline in lichens, mosses and evergreen shrubs</td>
</tr>
<tr>
<td>Northern wet heath</td>
<td>F4.11</td>
<td>10-20$^a$</td>
<td>(#)</td>
<td>Decreased heather dominance, decline in lichens and mosses</td>
</tr>
<tr>
<td>• ‘U’ Calluna-dominated wet heath (upland moorland)</td>
<td>F4.11trefoil</td>
<td>10-20$^a$</td>
<td>(#)</td>
<td>Transition heather to grass</td>
</tr>
<tr>
<td>• ‘L’ Erica tetralix dominated wet heath</td>
<td>F4.11</td>
<td>10-25$^{a,b}$</td>
<td>(#)</td>
<td>Transition heather to grass, decline in lichens</td>
</tr>
<tr>
<td>Dry heaths</td>
<td>F4.2</td>
<td>10-20$^{a,b}$</td>
<td>##</td>
<td></td>
</tr>
</tbody>
</table>
### Grasslands and tall forb habitats (E)

<table>
<thead>
<tr>
<th>Habitat Type</th>
<th>Code</th>
<th>Range</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-atlantic semi-dry calcareous grassland</td>
<td>E1.26</td>
<td>15-25</td>
<td>Increase tall grasses, decline in diversity, increased mineralization, N leaching</td>
</tr>
<tr>
<td>Non-mediterranean dry acid and neutral closed grassland</td>
<td>E1.7</td>
<td>10-20</td>
<td>Increase in graminoids, decline typical species</td>
</tr>
<tr>
<td>Inland dune pioneer grasslands</td>
<td>E1.94</td>
<td>10-20</td>
<td>Decrease in lichens, increase biomass</td>
</tr>
<tr>
<td>Inland dune siliceous grasslands</td>
<td>E1.95</td>
<td>10-20</td>
<td>Decrease in lichens, increase biomass, increased succession</td>
</tr>
<tr>
<td>Low and medium altitude hay meadows</td>
<td>E2.2</td>
<td>20-30</td>
<td>Increase in tall grasses, decrease in diversity</td>
</tr>
<tr>
<td>Mountain hay meadows</td>
<td>E2.3</td>
<td>10-20</td>
<td>Increase in nitrophilous graminoids, changes in diversity</td>
</tr>
<tr>
<td>Moist and wet oligotrophic grasslands</td>
<td>E3.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• <em>Molinia caerulea</em> meadows</td>
<td>E3.51</td>
<td>15-25</td>
<td>Increase in tall graminoids, decreased diversity, decrease of bryophytes</td>
</tr>
<tr>
<td>• Heath (<em>Juncus</em>) meadows and humid (<em>Nardus stricta</em>) swards</td>
<td>E3.52</td>
<td>10-20</td>
<td>Increase in tall graminoids, decreased diversity, decrease of bryophytes</td>
</tr>
<tr>
<td>Alpine and subalpine grasslands</td>
<td>E4.3 and E4.4</td>
<td>10-15</td>
<td>Increase in nitrophilous graminoids, biodiversity change</td>
</tr>
<tr>
<td>Moss and lichen dominated mountain summits</td>
<td>E4.2</td>
<td>5-10</td>
<td>Effects upon bryophytes or lichens</td>
</tr>
</tbody>
</table>

### Mire, bog and fen habitats (D)

<table>
<thead>
<tr>
<th>Habitat Type</th>
<th>Code</th>
<th>Range</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raised and blanket bogs</td>
<td>D1</td>
<td>5-10&lt;sup&gt;xc&lt;/sup&gt;</td>
<td>Change in species composition, N saturation of <em>Sphagnum</em></td>
</tr>
<tr>
<td>Poor fens</td>
<td>D2.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10-20</td>
<td>Increase sedges and vascular plants, negative effects on peat mosses</td>
</tr>
<tr>
<td>Rich fens</td>
<td>D4.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>15-35</td>
<td>Increase tall graminoids, decrease diversity, decrease of characteristic mosses</td>
</tr>
<tr>
<td>Mountain rich fens</td>
<td>D4.2</td>
<td>15-25</td>
<td>Increase vascular plants, decrease bryophytes</td>
</tr>
</tbody>
</table>

### Inland surface water habitats (C)

<table>
<thead>
<tr>
<th>Habitat Type</th>
<th>Code</th>
<th>Range</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permanent oligotrophic waters</td>
<td>C1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Softwater lakes</td>
<td>C1.1</td>
<td>5-10</td>
<td>Isoetid species negatively affected</td>
</tr>
<tr>
<td>• Dune slack pools</td>
<td>C1.16</td>
<td>10-20</td>
<td>Increased biomass and rate of succession</td>
</tr>
</tbody>
</table>

### Coastal habitat (B)

<table>
<thead>
<tr>
<th>Habitat Type</th>
<th>Code</th>
<th>Range</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shifting coastal dunes</td>
<td>B1.3</td>
<td>10-20</td>
<td>Biomass increase, increase N leaching</td>
</tr>
<tr>
<td>Coastal stable dune grasslands</td>
<td>B1.4</td>
<td>10-20</td>
<td>Increase tall grasses, decrease prostrate plants, increased N leaching</td>
</tr>
<tr>
<td>Coastal dune heaths</td>
<td>B1.5</td>
<td>10-20</td>
<td>Increase plant production, increase N leaching, accelerated succession</td>
</tr>
<tr>
<td>Moist to wet dune slacks</td>
<td>B1.8</td>
<td>10-25</td>
<td>Increased biomass tall graminoids</td>
</tr>
</tbody>
</table>

### Marine habitats (A)

<table>
<thead>
<tr>
<th>Habitat Type</th>
<th>Code</th>
<th>Range</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pioneer and low-mid salt marshes</td>
<td>A2.64 and A2.65</td>
<td>30-40</td>
<td>Increase late-successional species, increase productivity</td>
</tr>
</tbody>
</table>

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*a* use towards high end of range at phosphorus limitation, and towards lower end if phosphorus is not limiting;  
*b* use towards high end of range when sod cutting has been practiced, use towards lower end of range with low intensity management;  
*c* use towards high end of range with high precipitation and towards low end of range with low precipitation;  
*d* for D2.1 (quaking fens and transition mires): use lower end of range (#) and for D2.3 (valley mires): use higher end of range (#);  
*e* for high latitude or N-limited systems: use lower end of range.
Furthermore, it is 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 nitrogen critical loads for (semi-)natural ecosystem groups according to the general relationships between abiotic factors and critical loads for nitrogen as given in table 10.2.

Table 10.2. Suggestions to use lower, middle or upper part of the set critical loads of terrestrial ecosystems (excluding wetlands), if national data are insufficient.

<table>
<thead>
<tr>
<th>Action</th>
<th>Temperature/Frost period</th>
<th>Soil wetness</th>
<th>Base cation availability</th>
<th>P limitation</th>
<th>Management intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Move to lower part</td>
<td>COLD/LONG</td>
<td>DRY</td>
<td>LOW</td>
<td>N-LIMITED</td>
<td>LOW</td>
</tr>
<tr>
<td>Use middle part</td>
<td>INTERMED</td>
<td>NORMAL</td>
<td>INTERMED</td>
<td>UNKNOWN</td>
<td>USUAL</td>
</tr>
<tr>
<td>Move to higher part</td>
<td>HOT/NONE</td>
<td>WET</td>
<td>HIGH</td>
<td>P-LIMITED</td>
<td>HIGH</td>
</tr>
</tbody>
</table>

Most of the earth’s biodiversity is present in semi-natural and natural ecosystems. It is thus crucial to control the atmospheric nitrogen loads, in order to prevent negative effects on these semi-natural and natural systems. Fine resolution maps of the sensitive ecosystems of high conservation value are needed per country to map nitrogen critical loads for these systems. It is advised to use both the mass balance and empirically derived nitrogen critical loads for forest ecosystems and other ecosystems for which data needed for the application of steady state models is available. If the two approaches yield different values, the one with the lowest values should be used until the background for this difference has been clarified.

Countries are advised to identify those receptor ecosystems of high sensitivity within the mentioned EUNIS classification relating to their individual interest. Effort should be directed to produce fine resolution maps of sensitive ecosystems of high conservation value. At this moment the empirical nitrogen critical loads have been set in values of total atmospheric nitrogen (kg N ha\(^{-1}\) yr\(^{-1}\)). More information is needed on the relative effects of oxidized and reduced nitrogen deposition. It was emphasized during the last two expert meetings held under the Convention that there is increasing evidence of NH\(_x\) having greater impact than NO\(_y\). Particularly, bryophytes and lichens in a number of ecosystems, and several, mostly weakly buffered, ecosystems of EUNIS class F, E, C and B are (probably) more sensitive to deposition of reduced N. It is, however, at present not possible to set critical loads for both forms of N, separately.

Serious gaps in knowledge exist on the effects of enhanced nitrogen deposition (NO\(_y\) & NH\(_x\)) on semi-natural and natural ecosystems, although considerable progress has been made in several habitat groups from 1996 to 2002. The following gaps in knowledge have been recognized as most important:

- research/data collection is required to establish a critical load for the following ecosystems: steppe grasslands, all Mediterranean vegetation types, wet-swamp forests, many mire & fens, several coastal habitats and high altitude systems;
- more research is needed in all distinguished EUNIS items with expert judgement or few research;
• impacts of nitrogen enrichment in (sensitive) freshwater and shallow marine ecosystems needs further research and are sometimes overlooked;
• additional effort is needed to allocated observed nitrogen effects to the appropriate EUNIS forest subtypes (division 2 & 3);
• the EUNIS classification needs clarification/adjustment with respect to some grasslands groups, Nordic bogs and mires and surface water habitats;
• the possible differential effects of the deposited nitrogen species (NO\textsubscript{y} or NH\textsubscript{x}) are insufficiently known to make a differentiation between these nitrogen species for critical load establishment;
• in order to refine current critical loads, long-term (>3-5 yrs) nitrogen addition experiments with a high resolution of treatments between 5 and 50 kg N ha\textsuperscript{-1} yr\textsuperscript{-1} at low background regions or in mesocosms are useful. This would increase the certainty of deriving critical loads when the lowest treatment level considerably exceeds the critical load.

In conclusion, it is crucial to understand the long-term effects of increased nitrogen deposition on ecosystem processes in a representative range of ecosystems. It is thus very important to quantify the effects of nitrogen loads by manipulation of nitrogen inputs in long-term ecosystem studies in 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, which are reliable enough to calculate critical loads for nitrogen deposition in (semi-)natural ecosystems and to predict (natural) recovery rates for nitrogen-affected systems.

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References


