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Whole Ecosystem Nitrogen Manipulation: An Updated Review

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Foreword

This document reviews research undertaken in the UK and across Europe involving nitrogen manipulation experiments in natural and semi-natural ecosystems, updating an original review report from 1997. The experiments are essential to demonstrate and to improve our understanding of the ecological effects of nitrogen deposition, arising from the air pollutants nitrogen oxides and ammonia. Since the initial review two major pieces of work, the Countryside Survey 2000, and the New Plant Atlas of British and Irish Flora (2002), have revealed growing evidence that nitrogen deposition is contributing to a range of ecological changes in semi-natural habitats across the UK. Also since the review, the National Expert Group on Transboundary Air Pollution (NEG-TAP 2001) has provided a comprehensive update regarding emissions, deposition and impacts of nitrogen oxides and ammonia, including an assessment of prospects in 2010.

Between 1990 and 2000, emissions of ammonia fell by about 5% and nitrogen oxides by 40%. Deposition decreased by 16%, reflecting the dominance of UK deposited nitrogen derived from ammonia. Further emissions reductions are planned for 2010, under the UNECE Gothenburg Protocol and the EC National Emission Ceilings Directive. However, despite progress in cutting emissions, nitrogen deposition will continue to be a threat to significant areas of ecosystems. It is estimated that by 2010 critical loads for eutrophication will be exceeded in 20% of UK 1 km² grid squares with sensitive grassland and 40% with heathland (NEG-TAP 2001).

Clearly, the issue of nitrogen deposition continues to raise serious concerns for the foreseeable future. In light of this, it was considered an opportune time to update the nitrogen manipulation review of 1997. The report provides a comprehensive updated synthesis of the experimental work in this area. It should prove invaluable for those in the research field, for conservation managers and for those responsible for developing and implementing nitrogen reduction policies.

The original study was funded by English Nature in 1997 with the update sponsored by Department for Environment, Food and Rural Affairs and published by the Joint Nature Conservation Committee as a collaborative undertaking.

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and

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Executive Summary

1. Over the past two decades, concern over the effects of atmospheric nitrogen deposition on terrestrial ecosystems has led to the instigation of a number of field manipulation studies. These were reviewed in an earlier report, commissioned by English Nature. This review updates and revises that earlier report, based on a comprehensive review of all relevant published literature since 1997.
2. The design of experimental manipulation studies has been carefully evaluated, and only studies which meet specific criteria have been considered in this review.
3. This revision includes an interpretation of new data from a number of important long term field experiments in the UK, as well as additional information on interactions between nitrogen deposition and habitat management. Furthermore, in light of recent assessments that UK nitrogen emissions have stabilised and, in the case of nitrogen oxides, even begun to decline, this updated review also considers studies which have looked at rates and indicators of ecosystem recovery.
4. Nitrogen manipulation experiments in forest ecosystems have shown a relationship between deposition inputs and a range of effects. However, the effects of different manipulation studies are highly variable, depending on factors such as soil type, deposition history, stand age, and various biotic interactions.
5. Reported responses include both increases and decreases in tree growth, improvements or imbalances in foliar nutrition, increased incidence of pests and pathogens, and changes in the root system. Soil biology and chemistry are also affected in forested ecosystems, with functional changes in nutrient cycling widely reported; acidification and eutrophication lead in several studies to increased leaching of base cations and increased availability of aluminium and hydrogen cations.
6. Changes in the species composition of the ground flora, mycorrhizae and macro-fungi have been found in several studies, with a general increase in more nitrophilous species.
7. Despite differences in both soil type and climate, responses of *Calluna*-dominated heathland and moorland ecosystems to N addition in the UK have been fairly consistent. Early responses include increased *Calluna* shoot growth, canopy height, canopy density, flowering and litter production, whereas prolonged exposure results in reduced root:shoot ratios and an acceleration of the *Calluna* life cycle.
8. In both UK and continental European experiments, increased nitrogen deposition has been related to increased vulnerability to biotic and abiotic stresses, such as frost, drought and herbivory. Effects on mycorrhizae are varied, with both increases, decreases and no change reported in response to nitrogen. Observed increases in soil microbial activity and biomass, and consequent effects on decomposition and mineralisation rates, have implications for the turnover and

availability of both nitrogen and phosphorus. Differences between immobilisation and mineralisation rates following N addition appear to reflect the degree of nitrogen saturation of a heathland.

9. In grassland ecosystems, nitrogen additions over 5-10 years have resulted in changes in community composition. Lichens, bryophytes forbs and dwarf shrubs are frequently decreased, while grasses typically increase in dominance. These changes are generally associated with a reduction in species richness and diversity of grassland communities following nitrogen addition.
10. Evidence that nutrient cycling may be affected by increased nitrogen availability has also been found for grasslands: Nitrogen mineralisation rates and soil bacterial biomass and activity have increased in response to nitrogen addition. Whilst many similarities in response exist between acidic and calcareous grasslands, the former are typically more vulnerable to the acidifying effects of nitrogen (particularly reduced N) inputs, while interactions with phosphorus availability are important in the latter.
11. In bog ecosystems, nitrogen addition improves growth and survivorship of some moss species, and decreases that of others, with consequent shifts in bryophyte species dominance. In the longer term, it may also cause a reduction in diversity as bryophytes and other low-growing plants are out-shaded by taller species, including grasses. These changes may be related to penetration of nitrogen through the moss layer into the rooting zone, as the capacity to immobilise inputs is exceeded.
12. The few data available on fen ecosystems indicate that high levels of nitrogen deposition cause changes in community composition, with a reduction in species diversity and bryophyte biomass. Changes in species composition and tissue chemistry are also reported for tundra ecosystems.
13. Overall, there are some similarities in response between different ecosystem types, as well as some common mechanisms underlying the observed changes. Nitrogen deposition typically alters the competitive ability of many plant species, resulting in a shift towards more N-tolerant species. The response of a number of species groups is similar across a range of ecosystems, with a general pattern emerging for a reduction in forbs and dwarf shrubs and an increase in grasses.
14. Bryophytes and lichens appear to be particularly sensitive components of most ecosystems.
15. Although not all experiments have reported shifts in community composition in response to nitrogen addition, results consistently suggest a disruption of normal plant and microbial physiology and/or function. Changes in, for example, foliar chemistry or microbial activity may thus be early indicators of the potential for deleterious responses at the community or ecosystem level, in the longer term.
16. The importance of other limiting nutrients, particularly phosphorus, and interactions with habitat management, are major issues for both grasslands and heathlands.

17. Although there are relatively few experiments which have aimed to assess the rate of recovery from ecosystem eutrophication, there is convincing evidence that many ecosystem effects may persist for many years, with recovery only occurring over time scales of many decades, if at all. Indeed, the loss of species from more sensitive ecosystems (such as bogs or tundra systems) may prevent their re-establishment over any realistic time scale, in the absence of active re-introduction and restoration measures.
18. The evidence from nitrogen manipulation experiments demonstrates a significant cause for concern over the impacts of nitrogenous pollutants on sites of nature conservation value. Given the very slow natural rates of recovery once changes in species composition have occurred, and the costs of management interventions to accelerate this process, there is a clear need for a precautionary approach to minimise the risk to sensitive communities across the UK.
19. This review has identified a number of gaps in current knowledge and highlights the need for further research to improve understanding of the responses of the diverse range of (semi-)natural ecosystems in the UK to nitrogen, and to provide a more informed basis for assessment of appropriate critical loads to prevent long-term effects.

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1. Introduction

1.1. Nitrogen Emissions and Deposition

The earth's atmosphere is approximately 80% nitrogen. This gaseous form (N_2) is inert and unavailable for use directly by most organisms. Nitrogen in its reduced (*e.g.* ammonia) or oxidised (*e.g.* nitrate) forms is a nutrient of great importance to plants, forming vital amino acids and proteins. The availability of usable forms of nitrogen often limits plant growth, and in many natural ecosystems, plants are adapted to relatively low levels of available nitrogen. In the last few decades, large increases in the quantity of reactive nitrogen in the atmosphere have occurred over Europe. This atmospheric nitrogen may be deposited to vegetation or other surfaces as dry deposition of gases or wet deposition of rain, cloud and mist. Total nitrogen deposition comprises both natural and anthropogenic emissions of a number of nitrogen containing gases including, nitric oxide (NO), nitrogen dioxide (NO_2), ammonia (NH_3) and nitrous oxide (N_2O). The two main sources of deposition are: -

1. The increased combustion of fossil fuels, by motor vehicles and industry, leading to the increased emission of oxides of nitrogen (NO_x).
2. Agricultural activities, including nitrogen fertilisation, cultivation of nitrogen fixing crops and, most importantly, intensive livestock farming, leading to increased emission of ammonia (NH_3).

1.1.1. Nitrogen Emissions

In Europe the importance of man-made sources of nitrogen oxides is reflected in a belt of high emission density running from the UK through The Netherlands and Germany into central Europe. In these areas, emission densities are often greater than $2 \text{ t N km}^{-2} \text{ yr}^{-1}$, in contrast to levels over most of Scandinavia, Scotland and western Ireland where emission densities are less than $0.1 \text{ t N km}^{-2} \text{ yr}^{-1}$ (NEG-TAP 2001). The principal sources of nitrogen oxides are vehicle combustion engines and high temperature combustion processes used in power production. UK emissions in 1999 amounted to 488 kt N. This figure has decreased significantly from the early 1990s, where emissions were estimated to be approximately 846 kt N (INDITE 1994). It has been forecast that, due to the penetration of catalysts into the road vehicle fleet, emissions from road vehicles will reduce by 74% between 1998 and 2010. The UK emissions of NO_x for 1998 are shown in Figure 1.1. The map was compiled from the NAEI 1 km x 1 km emission map (NEG-TAP 2001; Goodwin *et al.* 1997). It has been concluded that approximately 30% of total emissions are concentrated in a few grid squares that contain point sources. Another 33% have been attributed to emissions derived from major road links, since vehicles travelling at high speeds contribute a higher amount of NO_x .

With regards to policies for NO_x emissions reductions, both the UN CLRTAP Nitrogen Oxide Protocol Target and the Fifth Environmental Action Programme targets have been achieved by the UK, and emissions have continued to decline since 1990 (NEG-TAP 2001). This also meets the emissions reductions targets for the UK under the Gothenburg protocol, which requires a reduction of 56% from 1990 emissions by 2010.

Emissions of ammonia are from a broad range of agricultural sources (Misselbrook *et al.* 2000) accounting for about 242 kt-N annually; an additional 46 kt-N comes from non-agricultural sources (Sutton *et al.* 2000a). However, it should be noted that due to the difficulties associated with quantifying ammonia emissions, estimates are treated with caution. Figure 1.2 depicts UK ammonia emissions on a 5 km x 5 km grid, produced by the CEH Edinburgh/University of Edinburgh AENEID model and shows that high emissions are centred on areas with considerable cattle farming, i.e. W and NW England and Northern Ireland, as well as in pig and poultry farming areas such as the Scottish Highlands. Policies surrounding the emissions of ammonia are contained within the Gothenburg protocol and the National Emission Ceiling Directive. It is anticipated that the target will consist of a modest reduction of 11% by 2010 from emissions in 1990 and from a forecast of changes in agricultural activity, it is anticipated that this will be met (NEG-TAP 2001).

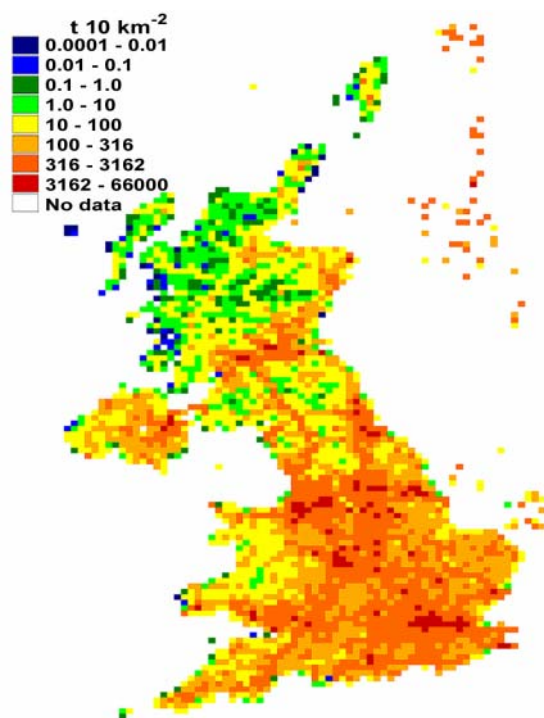


Figure 1.1 1998 UK emissions of NO_x at 10 km x 10 km.
Source NEG-TAP 2001

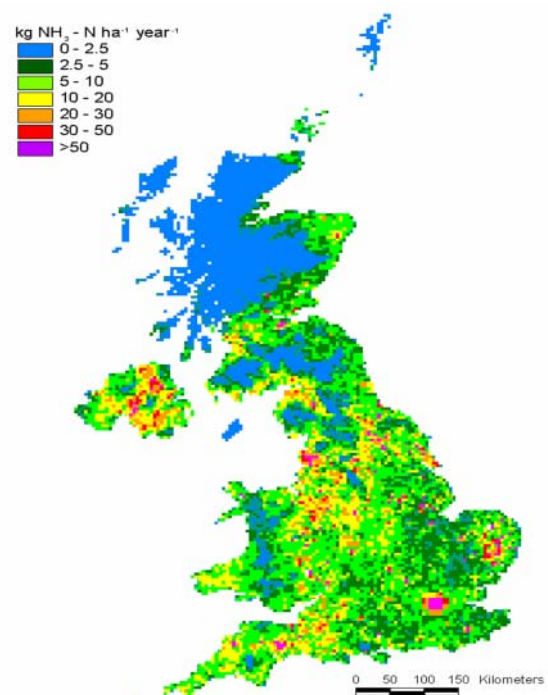


Figure 1.2. Figure 2: UK Spatial Emissions of NH_3 at 5 km x 5 km. Source NEG-TAP 2001

1.1.2. Nitrogen Deposition

Quantifying atmospheric input is a prerequisite in order to understand the adverse effects of air pollutants at the ecosystem level. The amount of bulk precipitation, i.e. the sum of wet deposition and sedimentation of heavy aerosols, is hardly influenced by the characteristics of the surfaces on which they are deposited. The quantity of dry deposition resulting from absorption of gases and particles directly from the atmosphere is, however, strongly determined by the roughness of the deposition surface (Metcalf *et al.* 1999). Fowler (1998) calculated, using data from 1992-1994, 40% of the UK deposition of fixed nitrogen (NO_y and NH_x) was dry and 60% was wet deposition.

The fixed nitrogen compounds in the atmosphere include the reactive gases NO, NO₂, HONO, HNO₃ and NH₃ and aerosols containing NO₃⁻ and NH₄⁺. Some of the gases are soluble and may be removed from the atmosphere by falling precipitation, or incorporated into cloud or rain within cloud processes. The annual wet deposition is, in principle, obtained as the product of the mapped concentration and the annual precipitation (Metcalf *et al.* 1999). A long time series recording wet deposition of nitrogenous compounds at Rothamsted (England) illustrates a dramatic rise in atmospheric deposition, with levels of 4.1-5.0 kg N ha⁻¹yr⁻¹ recorded in the period from 1888 to 1913, compared with levels of 15.1-18.2 kg N ha⁻¹yr⁻¹ from 1969 to 1978 (Tamm 1991). The current amount of wet deposition (NH₄⁺ and NO₃⁻) in Europe has changed little over the last two decades (NETGAP 2001) and ranges from 5 to about 30 kg N ha⁻¹yr⁻¹ (INDITE 1994), with the total wet and cloud deposition in the UK estimated as approximately 110 kt N yr⁻¹ as NO₃⁻ and 120 kt N yr⁻¹ as NH₄⁺ (Fowler 1998).

Different gaseous nitrogen compounds differ in their reactivity with natural surfaces and solubility in water. Thus, the rates at which these gases deposit range from the very reactive nitric acid, whose deposition rate is determined by turbulent atmospheric transport, to nitric oxide, which deposits at a very limited rate on to vegetation, soil or water (NEGTA 2001). NH₃ and NO₂ are the dominant components of the reduced and oxidised pollutant nitrogen respectively and are deposited onto natural surfaces at significant rates; rates of dry deposition in the UK have been estimated as approximately 40 kt N yr⁻¹ for NO₂ and 120 kt N yr⁻¹ for NH₃ (Fowler 1998).

The total current annual deposition of nitrogen in the UK of 380 kt is divided into 43% oxidised N and 57% reduced N, even though emissions of reduced nitrogen are considerably smaller (287 kt) than those of oxidised nitrogen (560 kt) (NEGTA, 2001). The figure for annual total deposition averages to approximately 17 kg N ha⁻¹ yr⁻¹ throughout the country (NEGTA 2001), although this varies slightly according to ecosystem type (Table 1.1).

	FOREST	MOORLAND	GRASSLAND	ARABLE
Total N deposition, kt	60	109	86	109
% reduced N	69%	58%	50%	58%
Area, x 10 ⁶ ha	2	7.9	6.5	7.9
Mean deposition kg N ha ⁻¹	33	16	15	16

Table 1.1: Partitioning of total N deposition to the UK by land class (1995-1997). Source NEGTA (2001)

The frequency distribution of UK nitrogen deposition (Figure 1.3) shows that the majority of grid squares in the UK receive substantially greater rates of nitrogen deposition than the remote areas of northern Europe ($<10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) and that some areas receive in excess of $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (NEGTA 2001).

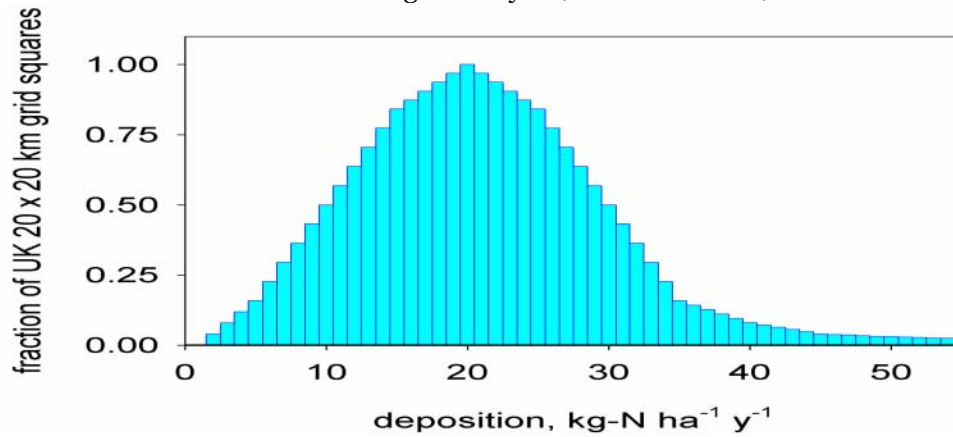
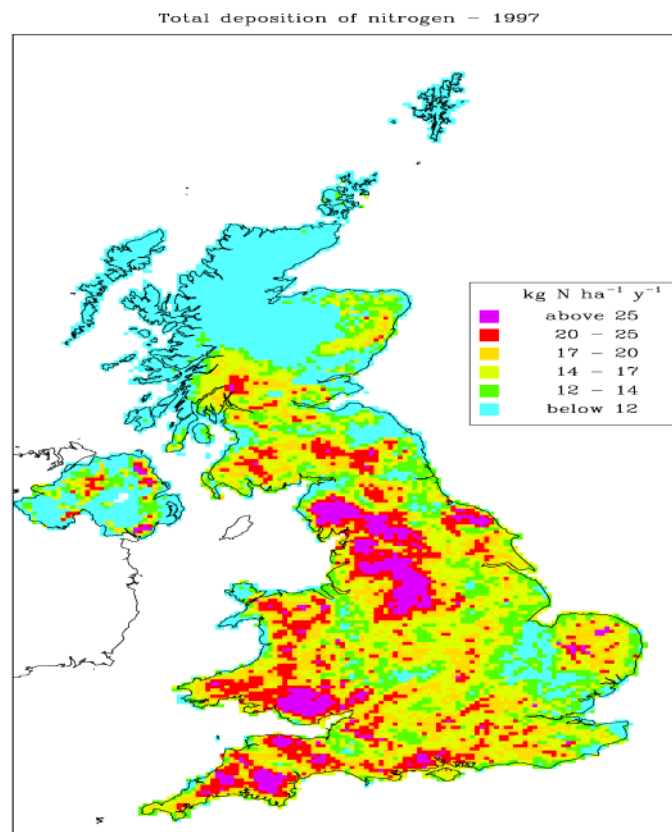
**Figure 1.3** Relative frequency distribution of N deposition to the UK 20 x 20 km grid squares

Figure 1.4 shows the total N deposition in the UK for 1997. It can be seen from the map that the areas of high deposition include much of the uplands of northern England, Wales and parts of western Scotland. There are also substantial areas of south west England and East Anglia which are subject to high deposition inputs of nitrogen.

**Figure 1.4** Total N deposition to the UK for 1997, calculated as the average deposition to each 20 x 20 km grid square accounting for different land cover types. Source NEGTA 2001

1.2. Ecological Impacts of Nitrogen Deposition

Increased airborne nitrogen pollution is one of the major threats to the structure and functioning of natural and semi-natural ecosystems (Matson *et al.* 2002, Bobbink *et al.* 1996, 1998). In Europe, increased deposition of reactive nitrogen has been linked with changes in a wide range of natural and semi-natural ecosystems, most notably the conversion of heathland to acid grassland, shifts in community composition in calcareous grasslands and changes in the nutrient dynamics of forest systems. A variety of other ecosystem changes have also been associated with high levels of atmospheric nitrogen deposition; these include reduced species diversity (especially of fungi, lichens and bryophytes), increases in nitrophilous species in the ground flora of many European forests and alterations in the nitrogen cycle in certain ecosystems. Other factors, especially changes in land use or land management techniques, may have also played an important role in these observed changes, through disturbance of nitrogen cycling within a particular ecosystem. For example, rates of organic matter breakdown and nitrogen mineralisation can be modified through drainage and additions of lime or fertilisers, which alter the soil's physical and chemical state.

Many factors influence ecosystem response to atmospheric deposition. These include: a) the form of deposited nitrogen i.e. wet or dry deposition, oxidised or reduced N; b) the amount of nitrogen being deposited; c) the innate sensitivity of the ecosystem and d) interactions with the environment and habitat management. For example, arctic ecosystems with their short growing season and tightly controlled nutrient cycle appear to be relatively sensitive to increased inputs of nitrogen. Furthermore, the use of habitat management practices, for example to increase nutrient export, has the potential to reduce the impact of atmospheric inputs on the nitrogen cycle in semi-natural ecosystems. The time scale over which ecosystems respond to increased nitrogen availability will also vary in a similar way. Relatively small changes in the performance of one species may be enough to lead to rapid changes in community composition in some circumstances. In contrast, subtle effects, for example on microbial populations may, over time, lead to profound changes in the way nutrients are cycled within an ecosystem, with longer term consequences for the structure and stability of the plant and microbial communities. It is important therefore to take into account the longer term ecosystem consequences when extrapolating from what are generally relatively short term manipulation experiments. Figure 1.5 illustrates a simplified schematic of the mechanisms driving vegetation response to nitrogen deposition.

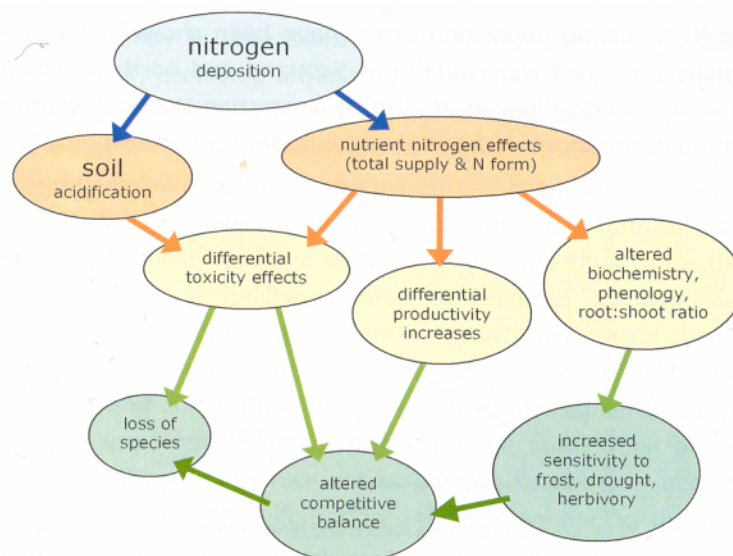


Figure 1.5. Schematic representation of the mechanisms driving vegetation response to nitrogen deposition.
Source: NEGTA, 2001.

1.3. Critical Loads

In recent years, a new way of looking at the impacts of atmospheric deposition of pollution has been developed through the quantification of environmental sensitivity thresholds. Thresholds of this type, which delimit the rate of pollutant deposition below which adverse effects will not appear in an ecosystem, are termed *critical loads*. Nilsson and Grennfelt (1988) define a critical load as "*the quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur, according to present knowledge*". Critical loads have been set on the basis of observed and predicted changes in the structure and function of different ecosystems. Changes in species composition or dominance and changes in ecosystem function, such as nitrogen leaching or accumulation, have been most commonly used as measures of harm in deriving critical loads (Bobbink *et al.* 1996). Critical loads are estimated using information from a variety of sources, including historical observations, laboratory experiments and modelling techniques. However, whole ecosystem nitrogen manipulation experiments provide one of the most important sources of information, aiding our understanding of the effects of increased atmospheric nitrogen deposition on ecosystem structure and processes, and defining thresholds for adverse effects which can be used to estimate critical loads.

Critical loads are used within the United Nations Economic Commission for Europe Convention on Long-Range Trans-Boundary Air Pollution (UN/ECE CLRTAP) as the basis for scientific evaluation of the ecological benefits of measures to reduce pollutant emissions across Europe. A summary of the critical loads of nitrogen deposition currently used within UN/ECE CLRTAP is given in Table 1.1; these are referred to as 'empirical' critical loads, as they are based on observed responses in the field or in experiments, rather than calculated from mathematical models of the environmental fates of deposited nitrogen. The values are defined as ranges, to reflect the fact that the effects of nitrogen deposition depend on many other factors, such as management, climate and phosphorus availability. Bobbink *et al.* (1996) also provided an indication of the level of uncertainty of these values, as indicated in Table 1.2.

Table 1.2. Summary of empirical critical loads for nitrogen deposition ($\text{kg N ha}^{-1}\text{yr}^{-1}$) to natural and semi-natural, freshwater and terrestrial ecosystems. Key: ## = reliable; # = quite reliable; (#) = expert judgement; * = unmanaged, natural systems; 1 = use low end of the range for N limited, high end of the range for P limited calcareous ecosystems. Taken from Bobbink *et al.* 1996.

Ecosystem Type	Critical Load ($\text{kg N ha}^{-1}\text{yr}^{-1}$)	Indication of Exceedance
Tree and Forest ecosystems		
Coniferous trees (acidic)	10-15##	Nutrient imbalance (low nitrification rate).
Coniferous trees (acidic)	20-30#	Nutrient imbalance (moderate to high nitrification rate).
Deciduous trees	15-20#	Nutrient imbalance; increased shoot/root ratio.
Acidic coniferous forests	7-20##	Changes in ground flora and mycorrhizae; increased leaching. Changes in ground flora and mycorrhizae.
Acidic deciduous forests	10-20#	Changes in ground flora.
Calcareous forests	15-20(#)	Changes in ground flora and leaching.
Acidic forests*	7-15(#)	Decline of lichens and increased free living algae.
Forests in humid climates	5-10(#)	
Heathlands		
Lowland dry heathlands	15-20##	Transition of heather to grass; functional change (litter production; flowering; N accumulation).
Lowland wet heathlands	17-22#	Transition of heather to grass.
Species-rich	10-15#	Decline of sensitive species.
heaths/grasslands	10-20(#)	Decline of heather dominance and mosses and lichens; N accumulation.
Upland <i>Calluna</i> heaths	5-15(#)	Decline of mosses, lichens and evergreen dwarf shrubs.
Arctic and alpine heaths*		
Species-rich grasslands		
Calcareous grasslands ¹	15-35#	Increased mineralisation, N accumulation and leaching; Increased tall graminoids; change in diversity.
Neutral-acid grasslands	20-30#	Increased tall graminoids; change in diversity.
Montane-subalpine grasslands	10-15(#)	Increased tall graminoids; change in diversity.
Wetlands		
Mesotrophic fens	20-35#	Increase in tall graminoids; reduced species diversity.
Ombrotrophic bogs*	5-10#	Decrease of typical mosses; increase in tall graminoids; N accumulation.
Shallow soft-water lakes*	5-10##	Decline of isoetid species.

These values are based on studies published up to 1995, and do not incorporate more recent work. The implications of the more recent experimental studies described in this report in terms of critical loads are considered further in Section 3.3. These values were also reviewed at a workshop held in York in March 2001 (Hall, *et al.*), at which it was concluded that a detailed scientific review of new evidence was needed. Furthermore, it was suggested that provisional new critical load ranges should be applied for calcareous grassland ($15\text{--}25 \text{ kg N ha}^{-1}$) and arctic heathlands ($5\text{--}10 \text{ kg N ha}^{-1}$). This process of scientific review is currently in progress, and new critical loads based on it will be adopted by UN/ECE CLRTAP at a workshop to be held in Bern in November 2002. It is therefore likely that Table 1.2 will be substantially revised within the next year. The assessment of new data from nitrogen manipulation experiments in this report will contribute to this review process.

The critical load values adopted by UN/ECE are also used by DEFRA in national risk assessment for nitrogen deposition, by producing maps of critical loads and their exceedance by current and predicted future nitrogen deposition. This requires adopting a single critical load value for mapping, the approach being to use the middle of the range in Table 1.2, unless there is specific UK evidence to suggest a different value. The critical loads currently used in UK mapping, which take account of the modifications proposed in the report of Hall *et al.* (2001), are summarised in Table 1.3.

Table 1.3. Summary of empirical critical loads to prevent changes in species composition recommended for use in the UK (from NETCEN, 2001)

Ecosystem	Critical load (kg N ha⁻¹ yr⁻¹)
Forests	
Deciduous forests	17
Coniferous forests	13
Grasslands	
Acid/neutral grassland	25
Calcareous grassland	25
Montane grassland	12
Heathland/moorland	
Lowland heaths	17
Upland Calluna heaths	15
Arctic/alpine heaths	7.5
Ombrotrophic bogs	10

1.4. Nitrogen Manipulation Experiments: Aims and Scope of this Review

A number of methods are used in the assessment of ecological responses to increased atmospheric nitrogen deposition. Laboratory or small plot experiments can provide important information concerning individual species and soil responses to increased nitrogen availability, although these are typically relatively short term studies. The monitoring of species and/or ecosystems through time or across transects receiving different deposition loads also provides useful comparative information. However, the biological complexity of the nitrogen cycle and the influence of human activities upon it make it difficult to accurately determine ecosystem responses to increased nitrogen deposition from these methods. One of the most appropriate methods for studying the responses to nitrogen deposition is that of whole ecosystem manipulation, which allows cause and effect relationships to be determined. Jenkins (1995) notes that, in terms of the temporal scale, no alternative to manipulation experiments exists as ecosystems tend to change only slowly in response to external stress or environmental change. As feedback mechanisms and processes are scale dependent, whole ecosystem manipulations also provide the most suitable framework for regional prediction of future ecosystem responses.

A large number of nitrogen manipulation experiments have been undertaken for a variety of different reasons. The majority of these experiments have involved nutrient additions in glasshouse or small plot experiments, using individual plants or artificial species mixtures (Di Tommaso and Aarssen 1989). However, the way in which increased levels of atmospheric nitrogen deposition affect ecosystems is difficult to extrapolate from the results of these experiments. Greenhouse or small core/mesocosm studies are therefore considered outside of the scope of this review. The review, therefore focuses on studies in the UK and Europe where field manipulation of nitrogen inputs has been undertaken in natural or semi-natural ecosystems. Since this review also focuses on British/European ecosystems, it draws primarily on literature from these areas, including non-European studies only where they were carried out on relevant ecosystems for which insufficient data otherwise exist.

A wide range of methodologies have been employed in the whole ecosystem nitrogen manipulation approach, and the benefits and limitations of each are outlined below.

Biological and chemical responses to nitrogen manipulation are discussed, both in terms of vegetation and microorganisms, and the consistency of these responses across experiments and ecosystems, is also assessed. Whilst this review is not aimed specifically at verifying or updating critical loads for the different ecosystem types, a brief evaluation of response thresholds is made, in respect to current understanding of critical loads for nutrient nitrogen and policies for both pollutant emissions control and habitat conservation.

This revised review of nitrogen manipulation experiments represents a comprehensive update of all relevant published literature which has emerged since the original review was carried out in 1997. As such, it is not intended to provide a re-interpretation of papers included in the original review, but rather a re-evaluation of the results in the context of new developments, both from ongoing experiments and from new studies which have started since 1997. Whilst many of the gaps in knowledge and areas requiring further study identified in the original review still remain, some important work has emerged during the past 4-5 years to address these research needs. In particular, there are now longer term data from field experiments which have been continued and some information concerning interactions between nitrogen deposition and habitat management. Furthermore, in light of recent assessments that UK nitrogen emissions have stabilised and, in the case of nitrogen oxides, even begun to decline, it is important to consider ecosystem recovery from eutrophication. Several studies have begun to look rates and indicators of recovery and this is an important new area covered in the revised review.

1.5. Nitrogen Manipulation Experiments: Methodology

Nitrogen manipulation experiments in natural and semi-natural ecosystems have been undertaken for a variety of reasons. In the past two decades, a proportion of these studies have specifically examined the response of ecosystems to increased atmospheric nitrogen deposition. In other experiments, the aim has been mainly to examine either competitive responses to limiting resources or single species growth and development responses to fertilisation. The differing aims of nitrogen manipulation experiments mean that the methodologies used have been highly variable. It is important that the methodology used in experiments is taken into account when ecosystem responses to nitrogen additions are interpreted and compared; for instance, the experimental design is rarely representative of the way in which atmospheric nitrogen is actually deposited to an ecosystem, but can vary considerably between experiments on the same ecosystem. The most important factors that may vary between experiments and which require careful interpretation when assessing ecosystem responses include :

1. *The form of the nitrogen application.* Firstly, the nitrogen types applied have varied. The chemical forms used have included ammonium nitrate, ammonium sulphate, sodium nitrate, urea or a number of other lesser used forms. The form of nitrogen used can influence the response observed. Secondly, applications of nitrogen may be applied as dry or wet dressings. Application of dry nitrogen fertiliser has been used extensively (e.g. Kellner and Redbo-Torstensson (1995) [powder]; Falkengren-Grerup (1993) [granules]). However, high application rates of dry nitrogen forms may be responsible for direct toxic effects, especially to bryophytes, lichens and some sensitive forb species. In order to make nitrogen application more representative of actual deposition, hand-spraying, resulting in an even fine mist application, has been used in a number of experiments (e.g.

Power *et al.* 1998a,b [heathland], Gundersen and Rasmussen 1995 [forest]), ensuring that the additional nitrogen is distributed in a realistic manner. Most typically, an intermediate degree of realism has been adopted through the use of either sprinkler or watering can applications of nitrogen in solution (*e.g.* Carroll *et al.* 1999, Johnson *et al.* 1994).

2. *The frequency and timing of nitrogen additions.* Applications of nitrogen vary from being single, once only, additions to weekly dressings of additional nitrogen; frequent dressings are obviously more representative since actual atmospheric nitrogen deposition is a continuous occurrence, not an infrequent event. Vegetation and microbial response to nitrogen inputs will depend on the time of year. The potential for plant uptake or microbial immobilisation can thus be expected to be greatest during the period of active plant growth. Furthermore, since biological, and particularly microbial, activity are both temperature and moisture dependent, nitrogen additions outside of the spring/summer period, particularly if applied during times of high rainfall, are more likely to be lost from the system in leachate. On a similar note, infrequent additions of relatively high nitrogen loads may exceed the capacity of microorganisms and plants to immobilise inputs, compared to more frequent applications at lower concentrations, resulting in either increased leaching losses, denitrification, or both.
3. *The nitrogen application rate.* Nitrogen addition experiments initiated to study optimum forest nutrition or nutrient limitation in a variety of ecosystems have often used very large application rates ($>100 \text{ kg ha}^{-1}\text{yr}^{-1}$), although studies initiated in recent years designed specifically to examine ecosystem responses to increased atmospheric nitrogen deposition have generally used smaller additions. This review focuses on these more realistic studies, specifically where $80 \text{ kg N ha}^{-1}\text{yr}^{-1}$ or less has been applied in forest ecosystems and $50 \text{ kg N ha}^{-1}\text{yr}^{-1}$ or less in other ecosystem manipulations. These levels of nitrogen deposition represent the top end of the range of nitrogen deposition currently found in the UK, although it must be remembered that experimental additions are always made on top of background deposition rates. Even some more recent experiments have included unrealistically high rates of nitrogen addition. Where these experiments also involve treatment levels which fall within the above criteria, they have been included, although with the focus generally on responses to additions at the lower end of the range used.
4. *Soil versus foliar nitrogen application.* Dry nitrogen applications are always made to the ground surface. Wet nitrogen applications may be applied to the ground surface or direct to low growing vegetation. Whilst the latter method is more representative of wet deposition in ecosystems with short vegetation, in forests the experimental applications always bypass the foliage of the trees, where important changes in chemistry can occur (Gunderson 1995). The mode of application will affect the potential for direct canopy uptake, as opposed to root uptake via the soil. This may in turn affect both the magnitude of impact (including direct effects of high ion concentrations on foliage) and the total amount of nitrogen assimilated, particularly in soils where microbial immobilisation of incoming nitrogen dominates the cycling of this nutrient. When comparing the effects observed in experiments in forests with national deposition estimates, it is important to remember that the tree canopy may significantly modify the composition of the deposition to the forest floor.

5. *The background atmospheric nitrogen deposition rate.* Nitrogen manipulation experiments have been undertaken in locations receiving very different background levels of nitrogen deposition. For instance, the UK heathland experiment of Power *et al.* (1995, 1998a,b) has background inputs estimated at 10-15 kg ha⁻¹yr⁻¹, whereas the heathland experiment of van Vuuren and van der Eerden (1992) in The Netherlands received a background deposition rate of 35-50 kg ha⁻¹yr⁻¹. Clearly, ecosystems will be responding to the total deposition load, so accurate quantification of background deposition, and its likely ecosystem effects before the start of the experiment, is an important consideration in the interpretation of ecosystem manipulation experiments.
6. *Soil nutrient status of the site.* The soil nutrient status of the habitats in which experiments are undertaken will vary. In most natural and semi-natural ecosystems, plant growth is nitrogen limited, whilst in some co-limitation with phosphorus may occur and this may influence the outcome of nitrogen addition experiments.
7. *Duration of experiment.* There can be little doubt that the complexity of (semi)natural ecosystems makes it difficult to extrapolate long term responses from the relatively short term effects seen in the time scale of most manipulation experiments. The length of experiments may be of great importance in terms of both the magnitude and direction of responses observed. In the short-term, nitrogen addition may be apparently beneficial, whilst long-term effects may be detrimental. The duration of the study needed to determine the ecosystem response to nitrogen will also vary with the species and ecosystem processes under consideration. The updated review now includes several examples of the way in which early responses have changed, sometimes dramatically, as the experiment has progressed over timescales of 5-20 years. Whilst short term responses can provide useful indicators of change, long term experiments are particularly valuable in evaluating likely ecosystems response over biologically meaningful time scales.
8. *Scale of experiment.* The spatial scale of experiments has varied tremendously, from small plots to experiments where whole, small water catchments are compared, depending on the ecosystem processes and type in question. For instance, the study of Dirkse and Martakis (1992) in a Swedish coniferous forest consisted of 40 plots, each of 30 m², whereas, Boorman and Fuller (1982) used a similar number of 2 m² plots for their study in a dune grassland. Indeed, nearly half of the community ecology studies analysed by Kareiva and Andersen (1988) were carried out using plot sizes of less than 1m diameter. The larger the scale of the experiment, the more important environmental heterogeneities, such as soil type and aspect, become in the interpretation of results.
9. *Interactions with management.* Most semi-natural ecosystems are actively managed in some way, to maintain a low nutrient environment and prevent changes in species composition. Typical management regimes include, for example, hay cutting/grazing in grasslands and grazing/burning/mowing/turf cutting in heathlands. These management activities export a varying proportion of the nitrogen stored in plant and soil pools, through the removal of above ground biomass and litter from a site. Furthermore, grazing involves not only a net export of nutrients from a site, but also a re-distribution, with local patches of nutrient enrichment. Since grazing usually involves an element of selectivity it is an important factor in a variety of ecosystems

and has large effects on the observed species composition and biomass. Grazing pressure may vary between experiments, and the erection of fences in experiments examining the interaction between grazing and nitrogen addition may itself result in substantial ecosystem change. Ecosystem responses to nitrogen can be expected to depend, at least to some extent, on the form and frequency of habitat management, as a result of effects on both vegetation and the microbial community.

10. *Specific experimental design features.* Roof exclusion experiments are few in number but have been undertaken in forests in several different European countries to study the effect of reduced inputs of atmospheric deposition (see Table 2.1.). These experiments typically consist of a transparent roof located 3-5 m above the forest floor, but beneath the tree canopy. The size of these roofed areas is variable; for instance at Gårdsjön (Sweden) the roof covers a 6300 m² catchment. Precipitation falling onto the roof is intercepted and underneath the roof equal volumes of 'clean rain' are applied with sprinklers. Other approaches using roofs at a smaller scale include heathland experiments such that carried out by Cawley (2001), investigating interactions between nitrogen deposition and drought. Introducing a roof into an ecosystem is a major manipulation and, as well as reducing atmospheric deposition, may also affect a number of other important factors. Gundersen (1995) summarises these for NITREX forest experiment as including changes in climate (e.g. light, soil temperature and humidity), water input (e.g. event frequency, event size, rain intensity, droplet size, timing), and element cycling (e.g. litter fall timing, litter fall spatial pattern). Although there is rarely a practicable alternative to the use of roofs in reducing deposition inputs or carrying out droughting experiments in field situations, these limitations must be kept in mind when interpreting the results of such experiments.

All of the above factors must be given careful consideration when interpreting the results of the large number of nitrogen manipulation studies which are summarised, by ecosystem, in the following sections.

2. Review of Nitrogen Manipulation Studies

In the following sections nitrogen manipulation experiments are reviewed by ecosystem type. All the studies reviewed involve the manipulation of nitrogen alone in the field, since studies with potted plant material or in microcosms/greenhouses, or where nitrogen was manipulated alongside other nutrients are not within the scope of this review. The review focuses chiefly on studies in the UK and Europe where nitrogen addition rates are in the region of current levels of atmospheric deposition: 80 kg N ha⁻¹ yr⁻¹ or less for forest ecosystems and 50 kg N ha⁻¹ yr⁻¹ or less for other ecosystems. These rates of addition are comparable to current maximum atmospheric nitrogen deposition levels in the UK.

2.1. Forest Ecosystems

Forests are the most intensively studied of all (semi-) natural ecosystems, with fertiliser addition experiments aimed at optimising timber production, having been carried out for many decades. However, effects on other components of the forest ecosystem have received relatively little attention until much more recently. For the purposes of this review, results from the large number of ecosystem manipulation studies are divided into effects on trees, ground flora, soil processes and fungal communities.

Forest ecosystems are able to accumulate considerable amounts of nitrogen in biomass and soil organic matter and growth in most forests has until recently been considered to be nitrogen limited. However, there is accumulating evidence which suggests nitrogen availability in certain forest ecosystems is in excess of plant and microbial demand (Aber *et al.* 1989). These forest ecosystems have been defined as *nitrogen saturated* (Skeffington and Wilson 1988). Bobbink *et al.* (1996) note that the nitrogen input at which saturation occurs will depend on a number of factors, including the amount of deposition, vegetation and soil type, as well as management history. Excess nitrogen input, in many European conifer forests, has led to reduced tree growth, nutritional imbalances in the needles and a deterioration of the root systems. In addition, compositional changes have been noted in the ground flora, mycorrhizae and macro-fungi, with species adapted to the usually nitrogen poor environment being replaced by nitrophilous species. Soil biology and chemistry may also be affected; acidification and eutrophication lead to increased leaching of base cations and increased availability of aluminium and hydrogen ions.

The majority of nitrogen manipulation experiments in forest ecosystems have been undertaken in conifer plantations (Table 2.2). A great number of nutrient manipulation experiments were initiated to examine tree nutrition and productivity in nutrient poor soils (*e.g.* Tamm *et al.* 1995), often applying large doses of nitrogen to the soil surface on one occasion (Table 2.1). However, there are a number of studies which examine the effects of relatively low rates of nitrogen addition (<80 kg ha⁻¹ yr⁻¹), over many years, on soil chemistry/biology, ground flora composition and tree health. The results of these experiments are listed in Table 2.2. These include a variety of co-ordinated European research programmes, which have sites in a number of countries. The largest of these research programmes is the NITREX (NITrogen saturation EXperiments) project, which

has included both nitrogen addition experiments and nitrogen reduction experiments, the latter through the building of large roofed areas, 3-5 m above the forest floor, and the application of clean, artificial throughfall water below this roof (and below the tree canopy foliage). The experiment involved eight sites along a gradient of background atmospheric nitrogen deposition loads. In the following sections, the results of the many nitrogen manipulation experiments in forest ecosystems are discussed.

2.1.1. Tree Health

Atmospheric nitrogen deposition may be responsible for increasing the susceptibility of trees to pathogens, frost and drought stress. The mechanisms of damage are numerous; they include direct toxicity to high levels of pollutants, soil-mediated nutritional imbalances, water stress through reduced root growth or increasing foliar demand, frost damage through early bud-burst or late winter-hardening, and increased pathogen attack or herbivory resulting from changes in plant chemistry (Aber *et al.* 1989, Nihlgård 1985).

Tree foliage responses

The response of tree foliage to nitrogen addition has been studied in a number of experiments. A lack of response of coniferous foliage to nitrogen addition has been observed in a number of these:

1. Investigations in a Sitka spruce plantation, at the Aber (UK) NITREX site, by Emmett *et al.* (1995b) found no differences in foliar nitrogen content in response to additions of 35 and 75 kg N ha⁻¹yr⁻¹ to a background deposition of 12 kg N ha⁻¹yr⁻¹, during five years of study.
2. No difference in foliar nitrogen content was reported by Wright and Tietima (1995) from a study in a Norway spruce forest at Sognal (Norway, NITREX), after additions of 8, 10 and 24 kg N ha⁻¹yr⁻¹ to a background deposition of 5-7 kg N ha⁻¹yr⁻¹.
3. No change in Norway spruce needle nutrient status was found at Klosterhede (Denmark), part of the EXMAN (EXperimental MANipulations of forest ecosystems in Europe) programme after the addition of 30 kg N ha⁻¹yr⁻¹ (Beier *et al.* 1998, Rasmussen *et al.* 1995) to a background of 23 kg N ha⁻¹yr⁻¹, over a 6 year period.

Changes in foliar nutrient status have, however, been observed in a number of studies. Näsholm and Ericson (1989) found a variety of responses to a medium (60 kg N ha⁻¹yr⁻¹) and a high level (90 kg N ha⁻¹yr⁻¹) of nitrogen addition to a low background of 2.5-5 kg N ha⁻¹yr⁻¹. Scots pine foliar nitrogen concentrations were significantly higher in treated plots than controls. Interestingly, arginine, an amino acid indicator of plant stress usually found in low concentrations in healthy plants was the most abundant amino acid in trees in nitrogen treated plots, but was found only in very low concentrations in control trees. Total protein content in all fertilised trees was higher than controls.

Nutritional imbalances have been observed in Scots pine forests in southern Finland at nitrogen addition rates of 30 and 90 kg N ha⁻¹yr⁻¹ to a background deposition of 6 kg N ha⁻¹yr⁻¹ (Nilsen and Abrahamsen 1995). Trees in nitrogen treated plots had a reduced magnesium content, but no visible sign of nutrient deficiency, after five years of nitrogen additions.

At a NITREX Norway spruce stand in Klosterhede, western Denmark, similar nutrient imbalances have been detected after application of ammonium nitrate at $35 \text{ kg N ha}^{-1}\text{yr}^{-1}$ for 4 years (Gundersen 1998). N foliar concentration increased in the third year of the treatment and the concentrations of phosphorus recorded were below the nutritional threshold for which growth was found to be decreased by $\sim 10\%$, by Rosengren-Brink and Nihlgård (1995). Foliar concentration of Mg was also reduced after the third year. Potassium and calcium concentrations also decreased, although this was not statistically significant. As a consequence of these changes there were imbalances in the ratios of nitrogen to other nutrients, with N:K and N:P ratios reduced to levels potentially damaging for tree growth. These changes in foliar nutrient concentrations were reflected in changes in litter composition, with the nitrogen content being elevated from the first year of the experiment. The increase in litter nitrogen content preceded the same response in live needles by 2 years, suggesting that the trees react to nitrogen loading by reducing retranslocation.

At another NITREX Norway spruce stand in Gårdsjön, south west Sweden, nitrogen additions of $35 \text{ kg N ha}^{-1}\text{yr}^{-1}$ for five years produced a change in litter quality: litter N concentration was increased despite a lack of effect on live needle concentrations or on the amount of litterfall (Kjønaas *et al.* 1998). As at the Klosterhede site, the trees reduced retranslocation of nitrogen as a reaction to N addition. In the last year of the experiment, increases in foliar N:P and N:Ca ratios were recorded.

Nitrogen additions have also resulted in changes in foliar nutritional status in a deciduous forest; Balsberg Pålsson (1992) found that the total leaf nitrogen content of beech trees increased with nitrogen addition (approximately 41 and $122 \text{ kg N ha}^{-1}\text{yr}^{-1}$). Nitrate concentrations in particular increased by as much as 65% in some trees following nitrogen addition. Other changes occurring after fertilisation included a two- to four-fold increase in amide concentration, decreased foliar content of potassium and copper and a decrease in concentrations of phenolic compounds. Balsberg Pålsson (1992) suggested the latter may be responsible for the greater susceptibility of the fertilised trees to pathogen attack.

Nitrogen loading was also correlated with increased attacks by pests and parasites in a study by Flückiger and Braun (1999). In seven beech and Norway spruce stands in the Alps and Lower Alps, Switzerland, ammonium nitrate was added at rates of 10-20-40-80 and $160 \text{ kg N ha}^{-1}\text{yr}^{-1}$ for 6 years. N application resulted in nutrient imbalances in both tree species. Leaf N concentration increased significantly (from $40 \text{ kg N ha}^{-1}\text{yr}^{-1}$ treatment), as did the N:P ratio (from $10 \text{ kg N ha}^{-1}\text{yr}^{-1}$), followed by increases in the N:K and N:Mg ratios (from $80 \text{ kg N ha}^{-1}\text{yr}^{-1}$). These imbalances were associated with increased attack from the fungi *Apiognomonia errabunda* and *Phomopsis* sp. and the aphid *Phyllaphis fagi* in beech. Norway spruce also suffered from increased attack by the fungus *Botrytis cinerea* and the insects *Sacchiphantes abietis* and *Cinara pilicornis*. Most severe attacks were recorded in sites with poor acid soils, where N addition caused the first and most distinct changes in nutrient status (Flückiger and Braun 1999).

Other changes in plant biochemistry include changes in hormonal status, specifically a rise in foliar cytokinin concentrations, which have been recorded after addition of $48 \text{ kg N ha}^{-1}\text{yr}^{-1}$ for 5 years in a young Sitka spruce stand in upland Britain (Crossley *et al.* 2001). In this stand litterfall was also significantly higher (+50%) in N treated plots than control levels.

The NITREX programme also included a number of reduction experiments, where, by means of a transparent roof installed below the canopy, nitrogen input levels were

reduced to pre-industrial levels. Such experiments allow us to understand if and how the ecosystem damage in response to chronic nitrogen addition can be reversed.

The clean-rain roof experiment at Ysselsteyn (The Netherlands; NITREX) resulted in a quick reduction on the levels of the free amino acid arginine in Scots pine needles after one year of reducing deposition to pre-industrial nitrogen levels (from 55-60 kg N ha⁻¹yr⁻¹ to less than 5 kg N ha⁻¹yr⁻¹) (Boxman *et al.* 1998 b, Boxman 1994). This may be an early indication of tree recovery. Nitrogen concentration in the needles started to decline after 3 years, but after 6 years was still high (over 2%). However this decline led to an improvement in the nutritional balance. Concentrations of K and Mg in the needles increased significantly: K concentrations increased to a level above deficiency for the species, although the trees were still very deficient in Mg.

A less significant response to reduction in N inputs was reported at the Klosterhede site, where seven years of reduced N input (background deposition 25 kg N ha⁻¹yr⁻¹ reduced to less than 5 kg N ha⁻¹yr⁻¹) caused a minor, non-significant decrease in needle N content (Beier *et al.* 1998).

Tree growth responses

A number of studies have reported increased tree growth with various rates of nitrogen addition ranging from 20-180 kg N ha⁻¹yr⁻¹ (Crossley *et al.* 2001, Nilsen and Abrahamsen 1995, Erikson *et al.* 1992, Näsholm and Ericsson 1989).

Interestingly, results from the NITREX clean rain roof experiment, at Ysselsteyn have shown that tree growth is greater in plots where nitrogen and sulphur inputs have been reduced at ground level to pre-industrial levels for five years than in control plots receiving background inputs of 55-60 kg N ha⁻¹yr⁻¹ (Boxman *et al.* 1998b). At the Solling site, where background deposition has been estimated as 38 kg N ha⁻¹yr⁻¹, the trees under the roof treatment showed a steady but not significant increase in growth (Beier *et al.* 1998, Bredemeier *et al.* 1998b).

Root system responses

Below-ground biomass has often been observed to decrease with addition of nitrogen. The amount of fine roots has been suggested as a direct estimate of tree vitality (Clemensson-Lindell and Persson 1995).

Experiments by Clemensson-Lindell and Persson (1995) and Persson *et al.* (1998) at the Gårdsjön site (Sweden, NITREX) found that, from the start of the study, there was a decline in fine root growth in untreated catchment areas, receiving background levels of 12 kg N ha⁻¹yr⁻¹ and in the nitrogen addition catchment area (35 kg N ha⁻¹yr⁻¹). However, in the clean-rain, roofed catchment area there was a stabilisation and gradual increase in fine root growth, which was accompanied by an improvement in fine-root nutritional balance, with reduction in the root N:P, N:Ca and N:K ratios. Fine roots also showed a higher width:length ratio in the roofed treatment than in control and fertilisation treatments, which may be an indication of increased mycorrhizal infection. Increases in fine root biomass were also seen at the Speuld (Netherlands) and Solling NITREX sites in the roof treatments (Bredemeier *et al.* 1998b).

Contrasting results were however found by Crossley *et al.* (2001) where 5 years of ammonium nitrate additions at 48 kg N ha⁻¹yr⁻¹ resulted in an increase in fine root production of 60% relative to unfertilised controls.

2.1.2. Ground and Field Layer Flora

In northern Europe, forest soils are typically poor in nitrogen and the understorey vegetation is adapted to growth under this limitation. Increases in nitrogen availability resulting from enhanced atmospheric deposition are therefore likely to result in compositional shifts of the forest ground flora in favour of more nitrophilous species. Such shifts, combined with a general reduction in species diversity and abundance, have been observed in a number of European countries (*e.g.* Tybirk and Strandberg 1999, Thimonier *et al.* 1992, Houdijk and Roelofs 1991). Nitrogen manipulation experiments have been undertaken to clarify the implications of increased atmospheric nitrogen deposition on the growth and community composition of ground flora species. Ground and field layer species composition has been shown to have the potential to be used as an indicator of excess nitrogen deposition, since changes in the vegetation can be observed before other ecosystem processes are evidently affected (van Dobben *et al.* 1999, Mäkipää 1998).

Extensive studies of the response of the herb and moss layer in a Scots pine forest (Lisselbo, Sweden) have been undertaken as part of the Swedish Optimum Nutrition Experiment (van Dobben *et al.* 1999, Dirkse and Martakis 1992, Dirkse *et al.* 1991, Dirkse and van Dobben 1989). The plots in this study received, from 1969, a range of nitrogen addition rates, which were on average approximately 30, 60 and 90 kg N ha⁻¹yr⁻¹, in addition to a background deposition of 5-7 kg N ha⁻¹yr⁻¹. After *ca.* 19 years a number of changes in botanical composition had occurred in the nitrogen addition plots, generally characterised by a shift in dominance from cryptogams and Ericaceae towards *Deschampsia flexuosa* and ruderal plant species. These changes are summarised as follows:

1. Bryophyte abundance changed with fertilisation: the abundance of *Brachythecium oedipodium*, *B. reflexum* and *B. starkei* all increased with fertilisation treatments less than 60 kg N ha⁻¹yr⁻¹; *Hylocomium splendens*, *Pleurozium schreberi* and *Dicranum scoparium* declined strongly at 30-60 kg N ha⁻¹yr⁻¹, whilst *Dicranum polysetum* and *Cetraria islandica* decreased at all levels of nitrogen addition;
2. The growth of the dwarf shrubs *Vaccinium vitis-idaea* and *Calluna vulgaris* was decreased at all levels of nitrogen addition, whilst *Vaccinium myrtillus* growth was favoured at 30 kg N ha⁻¹yr⁻¹, although not at higher levels;
3. The growth of a number of species was favoured by higher levels of nitrogen addition (60-90 kg N ha⁻¹yr⁻¹); these were *Rubus idaeus*, *Dryopteris carthusiana*, *Chamerion angustifolium* and *Plagiothecium laetum*. *Deschampsia flexuosa* was strongly favoured by N at a low dosage (30 kg N ha⁻¹yr⁻¹), but no further stimulation was found at higher N levels.

Dirkse and Martakis (1992) found the production of litter by *D. flexuosa*, ferns and *Rubus idaeus* at the Lisselbo site was increased by the application of nitrogen. This may be an important factor affecting species composition as the bryophyte species that increased abundance in this experiment were all litter dwelling species.

Kellner and Redbo-Torstensson (1995) also found that a number of ground flora species increased with the addition of nitrogen (5, 10, 20 and 40 kg N ha⁻¹yr⁻¹) to a mixed coniferous forest in central Sweden. The magnitude of increases in shoot density of the

grass *D. flexuosa* was closely related to nitrogen addition (increases of 1.7, 3.5, 5.3 and 8.7 times the control plots with 5, 10, 20 and 40 kg N ha⁻¹yr⁻¹, respectively). The ramet density of the forb *Trientalis europaea* increased 2-fold in the two lowest nitrogen addition treatments and 5-fold in the two highest treatments. However, no growth effects in response to nitrogen addition were observed for *Vaccinium myrtillus*, *V. vitis-idaea*, *Linnaea borealis* and *Luzula pilosa*.

Similar results to these studies were also found in a nitrogen manipulation experiment in a coniferous forest in Finland (Mäkipää 1995a,b, 1994, Mälkönen 1990). Variable nitrogen additions, which averaged 25 kg N ha⁻¹yr⁻¹ or less, were made in an area with a background deposition of 10 kg N ha⁻¹yr⁻¹ for 35 years. In treated plots this resulted in a decrease in ground vegetation biomass. The response of the ericoid dwarf shrubs was variable; *Calluna vulgaris* decreased in biomass with nitrogen additions, whilst the response of *Vaccinium myrtillus* and *V. vitis-idaea* depended on soil type. On nutrient poor sites, growth was increased, whilst on mesic sites their biomass was decreased. The bramble, *Rubus idaeus*, was found only on plots receiving nitrogen additions. As with the two previous studies there was also an increase in *D. flexuosa* biomass.

Mäkipää (1998) undertook a similar, short-term study of the effects of enhanced nitrogen deposition in the ground vegetation of a 60-year-old Norway spruce stand in southern Finland. 25 kg ha⁻¹yr⁻¹ of ammonium sulphate was applied once a year for 4 years. At the beginning of the experiment, bryophytes were a major component of the understorey vegetation. The dominant moss species were *Pleurozium schreberi* and *Dicranum polysetum*. The biomass of these mosses was significantly decreased by 60% and 78% respectively at the end of the 4 years. The responses of vascular plants were not significant, and therefore competition cannot explain bryophyte biomass reduction. Nitrogen concentrations in bryophytes and grasses increased significantly. Since bryophytes lack a cuticle and absorb water very rapidly, this may explain why they react more promptly to an increase in nitrogen and sulphur deposition. Therefore, the author proposes that bryophytes are good indicators for deposition of N and S.

The plant chemistry of ground flora species was investigated by Nordin *et al.* (1998, 1996) in a one year study in a Norway spruce forest (background: 2 kg N ha⁻¹yr⁻¹). One off additions of ¹⁵N double labelled NH₄NO₃ (0.5, 12.5, 25 and 50 kg N ha⁻¹yr⁻¹) were made to a ground flora consisting of *Vaccinium myrtillus* (75% of biomass), *Deschampsia flexuosa* (4% of biomass) and the mosses *Dicranum majus* and *Pleurozium schreberi* (20% of biomass together). The moss species took up a disproportionate share of the additional nitrogen (77% at the lowest nitrogen dose and 58% at the highest dose). In *V. myrtillus* and *D. flexuosa* the labelled nitrogen was found mainly in above ground organs as increased amino acid content. Fertilisation resulted in increased attacks of two parasitic fungi, *Valdensia heterodoxa* and *Podosphaera myrtillina* on *V. myrtillus*. This effect was significant at 12.5 kg N ha⁻¹yr⁻¹ and 25 kg N ha⁻¹yr⁻¹, respectively, for the two fungal species. Additionally *V. myrtillus* shoots were significantly more damaged by butterfly larvae on plots receiving 12.5 kg N ha⁻¹yr⁻¹ or higher (Nordin *et al.* 1998, 1996).

A similar increase in parasitic fungal infection with increased nitrogen addition was found by Strengbom *et al.* (2002) in an area of low background deposition (2-3 kg N ha⁻¹yr⁻¹) - the Svartberget Experimental Forest in northern Sweden. In this experiment, nitrogen was added as NH₄NO₃, at 12.5 and 50 kg ha⁻¹yr⁻¹ rates, in one yearly application, for three years. At the end of the three years there was a marked increase in the dominance of the grass *D. flexuosa*, with abundance more than doubled in the 50 kg N ha⁻¹yr⁻¹ treatment; this grass was more abundant in patches in which *V. myrtillus* was

severely affected by fungal infection. *V. myrtillus* abundance tended to decrease although this trend was not statistically significant (Strengbom *et al.* 2002).

The study of leaf amino acid responses to increased nitrogen deposition and respective fungal responses indicated a possible mechanism for this increase in abundance of *D. flexuosa*. The increase in N supply produced an increase in leaf glutamine levels of *V. myrtillus*, which was doubled over the three years in the 50 kg N ha⁻¹year⁻¹ treatment. This increase in glutamine correlated with an increase in the incidence of the parasitic fungus *V. heterodoxa*. In a separate experiment, artificial application of a glutamine coating on the surface of *V. myrtillus* leaves produced a significant increase in the levels of infection by *V. heterodoxa*. This strongly implicates the increase in foliar amino acid concentrations in *V. myrtillus* as the cause for the increase in the incidence of the parasitic fungus (Strengbom *et al.* 2002). This fungus causes leaf loss in the host which in turn allows the grass *D. flexuosa* to spread by increasing the amount of light that passes through the canopy: in a different experiment, Strengbom (2002) showed that nitrogen addition (50 kg NH₄NO₃ ha⁻¹year⁻¹) *per se* had a minor effect on the increase of abundance of *D. flexuosa*, and that the amount of light received by this grass was the biggest determinant of its abundance.

In this study, the parasitic fungus appears to mediate the observed vegetation change, possibly due to an increase in amino acid concentration following N addition (Strengbom *et al.* 2002, Strengbom 2002). This may complicate the interpretation of experimental results on the environmental impact of nitrogen deposition, as both the abundance of natural enemies and the light regime at a site are determined by a large number of abiotic and biotic factors. The above two examples indicate that enhanced nitrogen availability alters the susceptibility of plants to pathogens and herbivores, and that extrapolation of data from manipulation experiments must be carried out with care, to take into account possible biotic interactions.

At the Klosterhede NITREX site in Denmark, addition of 35 kg ha⁻¹yr⁻¹ ammonium nitrate to a background deposition of 15-20 kg N ha⁻¹yr⁻¹ resulted in significant changes in cover (Gundersen 1998). Before the onset of the experiment, *D. flexuosa* was the only vascular plant species in the ground flora of the site, together with several species of mosses. After 4 years there was a significant decrease in moss cover in the N addition plot compared to the control (57% less) and the pre-experimental status of the site (44% less).

Studies of ground flora responses to nitrogen addition in deciduous forests are few. A five year study in Scania (south Sweden) by Falkengren-Grerup (1993), where 60 and 180 kg N ha⁻¹yr⁻¹ were added to a beech forest floor, found little effect on species composition. However, some changes in cover and biomass of common species were observed: *Impatiens parviflora* increased, whilst *Anemone nemorosa*, *Maianthemum bifolium*, *Oxalis acetosa*, *Poa nemoralis* and *Viola riviniana* all decreased in cover and biomass.

In the highly nitrogen-saturated Scots pine stand at the Ysselsteyn site (NITREX), the initial background atmospheric nitrogen deposition level of 60 kg N ha⁻¹yr⁻¹ was reduced to less than 5 kg N ha⁻¹yr⁻¹ (Boxman *et al.* 1998 b). The pre-roof vegetation consisted of nitrophilous species such as brambles (*Rubus* spp.) and broad buckler fern (*Dryopteris dilatata*). After six years of decreased nitrogen inputs a change in the ground flora was observed, with both brambles (*Rubus* sp.) and *D. dilatata* being reduced in number. The return of less nitrophilous plant species has not yet been reported, but there has been an increase in fruitbodies of mycorrhizal fungi (Boxman *et al.* 1998 b).

Strengbom *et al.* (2001) undertook an ecosystem recovery study in two forests where fertilisation had stopped 9 and 47 years earlier. After 9 years of reduction in N input, forest understorey did not show signs of recovery. 47 years after the cessation of fertiliser additions, N-favoured bryophytes such as *Brachythecium reflexum*, *Plagiothecium denticulatum*, and the parasitic fungus *Valdensia heterodoxa*, were still more abundant in the formerly N treated plots than in the controls. In contrast, *Hylocomium splendens* was less abundant in plots that had previously received N additions, although no difference in species composition was reported for vascular plants relative to unfertilised controls. These results disagree with other studies that have interpreted a reduction in N leakage and in tree nutrients soon after the end of fertilisation as symptoms of recovery. From this study Strengbom *et al.* (2001) suggest that, at least in some areas, ecosystem recovery after nitrogen loading will be a very slow process.

2.1.3. Mycorrhizae and Macrofungi in Forest Ecosystems

A number of studies have indicated that increased levels of atmospheric nitrogen deposition may lead to reduced vitality, damage or elimination of mycorrhizal fungi in forest ecosystems (Brandrud 1995). It has been suggested that the sensitivity of mycorrhizal fungi to their environment may make them an important indicator of nitrogen deposition levels in forest ecosystems. Based on several N enrichment studies, Brandrud and Timmermann (1998) divided the sequence of effects of nitrogen loading on the mycorrhizal flora into three phases: 1) above-ground decrease in species diversity and fruit body production, with the exception of a few stress-tolerant species, mostly belonging to the genus *Lactarius*; no below-ground changes; 2) severe above-ground reduction, or even disappearance, of N-sensitive species-rich groups, and retreat of more stress-tolerant groups; variable below-ground responses; 3) complete, or almost complete, disappearance of sporocarp production and decreased density and vitality of below-ground mycorrhiza and fine roots. This sequence of effects can be used when evaluating the levels of N deposition at a particular forest ecosystem. Below we review the manipulation experiments that have assessed the effect of nitrogen on the growth, abundance and diversity of mycorrhizal/ fine root systems.

Brandrud and Timmermann (1998) assessed above- and below-ground species diversity and fruit body production at the NITREX Gårdsjön site over 5 years. The experiment, which began in 1991, consists of three catchment areas; (1) an untreated reference (background: 13 kg N ha⁻¹yr⁻¹), (2) an addition of 35 kg N ha⁻¹yr⁻¹ applied in weekly dressings and (3) a clean rain roof experiment.

In the catchment area which received the nitrogen addition there was a rapid and substantial decrease in species diversity and fruit body production of most species. After 1.5 years of nitrogen additions, there was a significantly lower number of above-ground mycorrhizal species, and after 4.5 years diversity was reduced by 60-70%. Stress-intolerant groups, such as the genus *Cortinarius*, which was dominant before the experiment began, were almost absent after 5 years of N addition (fruit body production reduced from 1065 to 19). Other genera had a more gradual decrease, such as *Russula*. *Lactarius* species increased fruit body production initially but this stopped after the first three years of nitrogen additions. Only one dominant species, *Cantharellus tubaeformis*, increased fruit body production significantly with N addition: after 4 years the proportion of total production it represented increased from 20% to 80-90%. This increase was significantly higher than in the control plots and accounted for the maintenance of total fruit body production in N addition plots throughout the experiment.

Total fruit body production under nitrogen addition was also higher than that in the control plots (Brandrud and Timmermann 1998). However, identification of species as RFLP taxa (Jonsson *et al.* 2000) showed that 4 years of moderate nitrogen additions at Gårdsjön did not affect mycorrhizal species richness and diversity. Below-ground *Cenococcum* was the most abundant group in both plots, and *Piloderma* was significantly more abundant in the nitrogen treatment. Comparison of above and below-ground species richness and abundance show that sporocarp production does not reflect the below-ground situation, and fruiting species constitute a mere 1-3.6% of the total mycorrhizal abundance.

A number of other studies have also found that fruit body production is more sensitive to nitrogen enrichment than the below-ground mycorrhiza. Termorshuizen (1990) applied ammonium sulphate and ammonium nitrate at rates of 0, 30, and 60 kg N ha⁻¹yr⁻¹ to a young Scots pine stand. High ammonium levels were responsible for greater reductions in fruit body production than high nitrate levels. Below-ground investigations found there was no difference in the frequency or density of mycorrhiza among treatments. Rühling and Tyler (1991) studied the response of macrofungi in a Swedish beech forest to 4 years of 12-36 kg ha⁻¹yr⁻¹ nitrogen additions. Within 3-4 years, all mycorrhizal species (49 species) almost ceased production of fruiting bodies, even at the lower treatment rate, which corresponded to about three times the ambient deposition rate. The last mycorrhizal species to disappear were *Lactarius subdulcis* and *Xerocomus chrysenteron*. Wästerlund (1982) examined mycorrhizal diversity and abundance with nitrogen addition at Lisselbo (Northern Sweden), as part of the Swedish Optimum Nutrition Experiments. Mycorrhizal diversity and fruit body abundance were both reduced by low levels of nitrogen addition (20-60 kg ha⁻¹yr⁻¹).

The only UK forest study to include a component on mycorrhiza has been undertaken by Crossley and collaborators (2001) at a 10 year-old Sitka spruce stand, in upland Britain, which receives a low background deposition of 8 kg N ha⁻¹yr⁻¹. In this experiment, which is part of a larger study involving separate acidity treatments, 48 kg N ha⁻¹yr⁻¹ were added as ammonium nitrate, from 1996 to 2000. Again, nitrogen addition caused a significant decrease in fruit body production compared with controls (33% of the control levels) and significant reduction in diversity of mycorrhiza. A shift from a mycorrhizal flora characteristic of organic N to a nitrophilic flora was also reported. This was reflected by reduced ectomycorrhizal diversity, favouring expansion by *Tylospora fibrillosa* (which does not produce fruit bodies and therefore has a low C demand) (non-significant increase) at the expense of *Lactarius rufus* (which uses organic N).

Studies on non-mycorrhizal macrofungi are few. Rühling and Tyler's (1991) nitrogen manipulation experiment (12-36 kg N ha⁻¹yr⁻¹), in a Swedish beech forest, found that a number of leaf litter and humus decomposing fungi species increased the number of fruiting bodies they produced after nitrogen addition. Particularly favoured were *Clitocybe* spp., *Mycena* spp. and *Lepista inversa* and to a lesser extent *Agaricus* spp. and *Lycoperdon* spp. However, most of the common wood decomposer species did not respond to nitrogen additions (Rühling and Tyler 1991). The Crossley *et al.* (2001) study described above also reported results on saprophytic species. After five years of nitrogen additions (48 kg N ha⁻¹yr⁻¹), there were significant reductions in total saprophyte fruit bodies as well as a significant reduction in saprophyte diversity.

As the fungal flora is a good indicator of nitrogen deposition, it also has the potential to be an indicator of recovery of an ecosystem after chronic nitrogen loading. At Gårdsjön (NITREX) the clean rain roof treatment initially showed a very high increase in total fruit body production, which lasted for the first 3 years of the treatment. However, below ground investigations found that mycorrhizal fine root density and diversity did not respond to the treatments, and all fine roots had developed mycorrhiza (Brandrud and Timmermann 1998). Other clean-rain roof experiments in The Netherlands, also within the NITREX program, at Ysselsteyn (Boxman *et al.* 1995, 1998 a,b) and Speuld (Boxman *et al.* 1995), also found an increase in the number of mycorrhizal species and fruiting bodies after some years of reducing nitrogen and sulphur inputs to pre-industrial levels. Before the experiment started, the above-ground fungal community at this Scots pine site was very poor, with a survey detecting only 13 basidiomycete species, none of them mycorrhizal. Van Dijk *et al.* (1992) found, after a year of reduced nitrogen and sulphur inputs, a significant increase in the litter-decaying fungus *Hygrophoropsis aurantiaca*, while the first mycorrhizal fruit bodies were found after two years of nitrogen reduction (Boxmann *et al.* 1998 b). Mycorrhizal fruit bodies belonging to the species *Lactarius hepaticus* were first recorded after four years of nitrogen reduction and, after six years, the presence of a second species, *Collybia butyracea*, was recorded (Boxmann *et al.* 1998 b).

In a recovery study undertaken by Strengbom *et al.* (2001) in two boreal forests recovery was also restricted. Nine years after fertilisation ceased, sporocarp production of mycorrhizal fungi showed differences from production in both control plots and plots still receiving fertiliser. Abundance of *Lactarius rufus* sporocarps was significantly higher than both in controls and fertilised plots, and the abundance of *Cortinarius* spp. was significantly lower than that recorded for the control plots. In a second forest, where fertilisation had stopped 47 years previously, sporocarps of *Russula*, which is an N-sensitive group, were still significantly more abundant in control plots than in the formerly fertilised plots.

The above results on myco-flora recovery after reduction of nitrogen inputs are somewhat mixed. Whilst a rapid above-ground response to a decrease in N deposition has been reported in several studies, a persistent effect is still apparent. Recovery through the stages suggested by Brandrud and Timmermann (1998) in the beginning of this section has not gone beyond the second phase in any of the studies documented so far. Not even in the Strengbom *et al.* (2001) study, where the reduced presence of *Russula* in formerly fertilised plots indicates persistent effects on the mycorrhizal community, several decades after additions ceased.

2.1.4. Soil Processes

In the absence of fertilisation or enhanced atmospheric nitrogen deposition, northern forest ecosystems have an efficient internal cycling and little loss of nitrogen through leaching. Increased nitrogen deposition is thought to be responsible for both soil acidification, through nitrification of ammonium, and eutrophication. Comparison of soil processes along an atmospheric nitrogen deposition gradient as part of the NITREX programme was undertaken by Dise and Wright (1995). They found that throughfall inputs of approximately 10 kg N ha⁻¹yr⁻¹ did not stimulate nitrification beyond the capacity of the forest ecosystem. At deposition levels of 10-25 kg N ha⁻¹yr⁻¹, both nitrification and soil acidification were stimulated, leading eventually to nitrate leakage and at higher levels of deposition (>25 kg N ha⁻¹yr⁻¹) all sites leaked significant quantities of nitrogen.

Investigations of changes in soil processes following the addition of nitrogen (30, 35 and 61 kg N ha⁻¹yr⁻¹) are numerous. At a number of sites (Gårdsjön, Klosterhede, Alptal and

Aber) in the NITREX program the addition of nitrogen resulted in increased nitrate leaching; generally, levels of leaching at these sites were low in the growing season but much enhanced in the winter months. At the Gårdsjön site, nitrate leaching was also high in the summer after four years of N loading. Increased levels of nitrate/ammonium leaching have also been observed in other experiments (Hagedorn *et al.* 2001, Carnal *et al.* 1996, Tamm *et al.* 1995, Nilsson *et al.* 1988). Additionally, nitrogen supplements of between 35-113 kg N ha⁻¹yr⁻¹ have resulted in increased aluminium concentrations in the soil and increased ammonium to base cation ratios (Kjønåas *et al.* 1998, Boxman *et al.* 1995, Rasmussen *et al.* 1995, Emmett *et al.* 1995b, Eriksson *et al.* 1992).

Changes in nitrogen cycling in response to changes in deposition inputs have also been documented. At the Klosterhede NITREX site, net mineralisation increased by 85% compared to the control. Nitrification and denitrification were unaffected by N addition as were microbial activity and decomposition rates (Gundersen 1998). At Gårdsjön there was also an increase in net mineralisation and nitrification rates after addition of 35 kg N ha⁻¹yr⁻¹ (Kjønåas *et al.* 1998) and at Alptal there was an increase in denitrification after adding 30 kg N ha⁻¹yr⁻¹ (Hagedorn *et al.* 2001).

At all roofed sites within the NITREX and EXMAN programmes decreases in the ammonium to base cation ratio were observed and nitrate leaching was much reduced. For instance, at the Solling (Germany) site within three years of roof treatment nitrate output via leaching was reduced from 22 to 5.5 kg N ha⁻¹yr⁻¹ (Beier *et al.* 1998).

Other factors which may affect nitrogen cycling in forest soils include litter production and quality, and soil fauna. Increased litter production with increasing nitrogen loading has been noted in a number of studies (Emmett *et al.* 1995b, Dirkse and Martakis 1992) with nitrogen additions of between 20 and 180 kg N ha⁻¹yr⁻¹. Increased litter production can influence not only nutrient cycling, but may also directly affect the species composition of the ground flora, such as been found in Lissebo, where there was a shift in bryophyte species composition towards litter dwelling species (Dirkse and Martakis 1992). Microarthropods play an important role in organic matter decomposition. In the clean-rain roof experiments at Ysselsteyn, Boxman *et al.* (1995) found an increase in the diversity of Collembola and Oribatei.

Summary

Nitrogen manipulation experiments in forest ecosystems have shown a relationship between enhanced nitrogen deposition and a range of detrimental effects at all levels of the forest. They have also shown that the consequences of similar manipulations are highly variable, and depend upon a variety of factors such as soil type, age of the stand and various biotic interactions. Deleterious effects on the ecosystem include reduced tree growth, nutritional imbalances in the needles and deterioration of the root system. In addition, compositional changes have been noted in the ground flora, mycorrhizae and macro-fungi, with species adapted to a nitrogen-limited environment being replaced by more nitrophilous species. Soil biology and chemistry are also affected: acidification and eutrophication lead to increased leaching of base cations and increased availability of aluminium and hydrogen cations. These results establish a causal link between the forest decline that is being observed in northern Europe and increased levels of atmospheric nitrogen deposition. Furthermore, nitrogen reduction experiments and studies on ecosystem recovery after fertilisation, have shown that effects of chronic nitrogen deposition persist in the ecosystem for a long, and so far undetermined, time, as full recovery has never been recorded.

Table 2.1. Forest nitrogen manipulation experiments undertaken in Europe

Country	Location	N form	Dose (KgN/ha/yr)	Frequency of application (times/year)	Duration (years)	Other treatments	Background deposition (KgN/ha/yr)	Authors
NITREX project. Sites A-H: Listed individually below							<i>Summary Papers.</i>	- Tietema <i>et al.</i> 1998 - Bredemeier <i>et al.</i> 1998 - Boxman <i>et al.</i> 1998a - Gundersen <i>et al.</i> 1998 - Gundersen <i>et al.</i> 1996 - Wright <i>et al.</i> 1995a
A. UK	Aber	[1] NaNO ₃ [2] NH ₄ NO ₃	[1] 35/75 [2] 35	52	6		12	- Emmet <i>et al.</i> 1998 - Emmett <i>et al.</i> 1996 - Emmett <i>et al.</i> 1995a,b - Emmett <i>et al.</i> 1994
B. Sweden	Gårdsjön	NH ₄ NO ₃	[1] 35 [2] Roof experiment	52	5		12	- Jonsson <i>et al.</i> 2000 - Stuanes & Kjønnaas 1998 - Moldan & Wright 1998 - Kjønnaas <i>et al.</i> 1998 - Brandrud & Timmermann 1998 - Persson <i>et al.</i> 1998 - Clemensson-Lindell & Persson 1995 - Wright <i>et al.</i> 1995b - Moldan <i>et al.</i> 1995 - Stuanes <i>et al.</i> 1995 - Brandrud 1995
C. Norway	Sognal	HNO ₃	8/10/24	6	13		2	- Wright & Tietema 1995
D. Denmark	Klosterhede	NH ₄ NO ₃	[1] 35/61 [2] Roof experiment	12	4		23	- Gundersen 1998 - Gundersen & Rasmussen 1995
E. Germany	Solling		Roof experiment	Precipitation restored to pre-industrial N and S levels (12 kg N ha ⁻¹ yr ⁻¹).	5		38	- Holscher <i>et al.</i> 1996 - Wiedey <i>et al.</i> 1995

Country	Location	N form	Dose (KgN/ha/yr)	Frequency of application (times/year)	Duration (years)	Other treatments	Background deposition (KgN/ha/yr)	Authors
F. The Netherlands	Ysselsteyn	[1] - [2] NH ₄ NO ₃	[1] Roof experiment. [2] 5	[1] Precipitation restored to pre-industrial N and S levels. [2] 12	7		55-60	- Boxman <i>et al.</i> 1998 ^{a,b} - Boxman <i>et al.</i> 1995 - Boxman <i>et al.</i> 1994 - Van Dijk <i>et al.</i> 1992
G. The Netherlands	Speuld		Roof experiment	Precipitation restored to pre-industrial N and S levels.	7		42	- Koopmans <i>et al.</i> 1998 - Koopmans <i>et al.</i> 1996 - Koopmans <i>et al.</i> 1995 - Boxman <i>et al.</i> 1995
H. Switzerland	Alptal	NH ₄ NO ₃	30	Applied chronically as a spray.	3		12-18	- Hagedorn <i>et al.</i> 2001 - Schleppi <i>et al.</i> 1996
EXMAN project. Denmark Germany Germany The Netherlands The Netherlands Ireland	Klosterhede Solling Hoglwald Harderwijk Kootwijk Ballyhooly	NH ₄ NO ₃	[1] 30 [2] Roof experiment	12	7	Mg, S, K, P, Fe, B, Mn, Co, Mo, Zn, Cu.	23	- Beier <i>et al.</i> 1998 - Tietema & Beier 1995 - Beier <i>et al.</i> 1995 - Gundersen & Rasmussen 1995 - Rasmussen <i>et al.</i> 1995
CORE project. Ireland UK UK France The Netherlands Germany	Kilkenny Haldon Grizedale Fontainbleau Wekerom Solling	(NH ₄) ₂ SO ₄	75	26	4	Soil cores transplanted between sites.	9.2 15.9 13.7 12 49.7 39	- Carnal <i>et al.</i> 1996 - Carnal <i>et al.</i> 1995 - Berg <i>et al.</i> 1995
Swedish Optimum Nutrition Experiment. Northern Sweden	[1] Lisselbo [2] Norrliden	NH ₄ NO ₃	[1] 0/20/40/60/80/120/180 [2] 0/30/60/90	1	22	NPK, PK.	2.5-7	- van Dobben <i>et al.</i> 1999 - Tamm <i>et al.</i> 1995 - Hogberg <i>et al.</i> 1992 - Dirkse & Martakis 1992 - Dirkse <i>et al.</i> 1991 - Dirkse & van Dobben 1989 - Näsholm & Ericsson 1989 - Wästerlund 1982

Country	Location	N form	Dose (KgN/ha/yr)	Frequency of application (times/year)	Duration (years)	Other treatments	Background deposition (KgN/ha/yr)	Authors
Northern Sweden	-	NH ₄ NO ₃	0.5/12.5/25/50 *	1	1		2	- Nordin <i>et al.</i> 1996
Southern Sweden	Scania	NH ₄ NO ₃	51/153	1985: 2 1986-1989 : 5	4		20	- Balsberg Pålsson 1992
Southern Sweden	Scania	NH ₄ NO ₃	12-36	5.5	4		20	- Rühling & Tyler 1991
Southern Sweden	Scania	NH ₄ NO ₃	60/180	5	5		20	- Falkengren-Grerup 1993
Central Sweden	Grimskar	NH ₄ NO ₃	0/5/10/20/40	5	4			- Kellner & Redbo-Torstensson 1995
South-central Sweden	Strasan	NH ₄ NO ₃	35/75/113	1	22	P, Ca.	10	- Nilsson <i>et al.</i> 1988 - Eriksson <i>et al.</i> 1992
The Netherlands	-	NH ₄ NO ₃ (NH ₄) ₂ SO ₄	0/30/60	1				- Termorshuizen 1990
Southern Finland	6 Sites	(NH ₄) ₂ SO ₄ NH ₄ NO ₃	1959-90: 18 1990>: 25	1959-90: Dressed 5-7 times in 30 years with 80-150 kg N ha ⁻¹ . 1990>: 1	35	Urea,Lime.	10	- Mäkipää 1995 <i>a,b</i> - Mäkipää 1994 - Mäkönen 1990
Southern Finland		(NH ₄) ₂ SO ₄	25	1	4		10	- Mäkipää 1998
Southern Finland	Amlä	NH ₄ NO ₃	0/30/90	1	6	S, Mg, P.	10-15	- Abrahamsen & Erstad 1995 - Nilsen & Abrahamsen 1995 □
Northern Sweden	Svartberget Experimental Forest	NH ₄ NO ₃	12.5 - 50	1	3	Glutamine	2-3	- Strengbom <i>et al.</i> 2002a - Strengbom <i>et al.</i> 2002b
UK	Deepsyke, Scotland	NH ₄ NO ₃	48	? Sprayed from April to November	4	H ₂ SO ₄	8	- Crossley <i>et al.</i> 2001
Switzerland	7 sites at the Alps and Lower Alps	NH ₄ NO ₃	10-20-40-80-160	3	6		18-33	- Flückiger & Braun 1999

Table 2.2. Results of forest nitrogen manipulation experiments undertaken in Europe.

Habitat type	Tree Health	Ground Flora/Mycorrhiza	Soil Biology/Chemistry	Authors
NITREX A. Sitka spruce.	- No change in tree growth/nutritional parameters during five years of treatment. - Decreased fine root biomass.		- Eutrophication and acidification of soil water. - Increased soil water aluminium concentration. - Increase in nitrate leaching. - Slightly increased litter production in year 3.	- Emmett <i>et al.</i> 1998 - Emmett <i>et al.</i> 1996 - Emmett <i>et al.</i> 1995a,b - Emmett <i>et al.</i> 1994
NITREX B. Norway spruce / Scots pine.	- Decline in fine root growth in control and addition treatments but stabilisation and gradual recovery in roofed treatment. - in the clean rain roof treatment there was an improvement of fine-root nutritional balance - Increase in above-ground biomass. - litter N concentration was increased despite a lack of effect on live needle nor on the amount of litterfall . In the last year of the experiment, increases in the N:P and N:Ca ratios were recorded.	- Rapid and substantial decrease in fungal diversity and fruit body production - Reduced abundance of the dominant genera (<i>Cortinarius</i> and <i>Russula</i>) - <i>Lactarius</i> genus increased fruit body production in first 3 yrs. - Dramatic increase in <i>Cantharellus tubaeformis</i> fruit-body production - RFLP showed there was no change in below ground mycorrhizal diversity. - In the clean rain roof treatment there was great increase of fruit body production in the first 3 yrs.	- Elevated nitrate runoff occurred during the first 2 weeks of treatment (April) then levels were low in the growing season. Nitrate appeared in runoff again in November. - Nitrate leaching was also high in the summer after four years of N loading. - Major changes in runoff chemistry under roof area. - Increase in net mineralisation and nitrification rates - Increased aluminium concentrations in the soil and increased ammonium to base cation ratios.	- Jonsson <i>et al.</i> 2000 - Moldan & Wright 1998 - Stuanes & Kjonaas 1998 - Kjonaas <i>et al.</i> 1998 - Brandrud & Timmermann 1998 - Persson <i>et al.</i> 1998 - Clemensson-Lindell & Persson 1995 - Wright <i>et al.</i> 1995b - Moldan <i>et al.</i> 1995 - Stuanes <i>et al.</i> 1995 - Brandrud 1995
NITREX C. Mixed: birch, juniper, willow.	- Measurements of foliar N content do not show any difference from controls.		- During the 9 year treatment 90% of NO₃ retained by the catchment, a similar proportion to the untreated catchment. - No change in N mineralisation rates. - Reduced decomposition rates in treated plots.	- Wright & Tietema 1995
NITREX D. Norway spruce.	- Nutrient imbalances: N foliar concentration increased in the third year of the treatment and P and Mg concentrations were reduced	- significant decrease in moss cover	- Elevated nitrate leaching - Soil chemistry only slightly altered. - Net mineralisation increased by 85% compared to the control.	- Gundersen 1998 - Gundersen & Rasmussen 1995

Habitat type	Tree Health	Ground Flora/Mycorrhiza	Soil Biology/Chemistry	Authors
NITREX E. Norway spruce.	- No effects on tree growth - Increase in fine root biomass		- Ammonium in top 10cm of the soil reduced to below detection levels and nitrate fell below input levels within 1 year in roof treatment. - Within 3 years of roof treatment N-output via leaching was reduced from 22 kg ha ⁻¹ yr ⁻¹ (pre-roof) to 5.5 kg ha ⁻¹ yr ⁻¹ .	- Bredemeier <i>et al.</i> 1998 - Wiedey <i>et al.</i> 1995 - Holscher <i>et al.</i> 1996
NITREX F. Scots pine.	- Improved nutritional balance in needles: quick reduction on the levels of arginine; slower decline of N concentration and increase in K and Mg. - 2-3 years of pre-industrial N and S levels increased tree and fine root growth; this tendency was kept for the following three years	- After 2-3 years of pre-industrial N levels there was a decrease in nitrophilous understorey species. - 2-3 years of pre-industrial N levels increased number of mycorrhizal species and fruiting bodies of <i>Lactarius hepaticus</i> . - After 6 years of pre-industrial N levels fruit bodies of a second species <i>Collybia butyracea</i> appeared.	- The presence of the roof caused a reduction in the nitrate levels leaving the ecosystem at 90cm depth to below the target values for ground and drinking water. - N fluxes via litter fall decreased with roof.	- Boxman <i>et al.</i> 1998b - Boxman <i>et al.</i> 1995 - Boxman <i>et al.</i> 1994 - Van Dijk <i>et al.</i> 1992
NITREX G. Douglas fir.	- Increased fine root biomass in roofed plots.		- Leaching of nitrate in treated plots, in roofed plots there was reduced leaching of base cations and decrease in ammonium to base cation ratio. - Increased microarthropod diversity under roof.	- Koopmans <i>et al.</i> 1998 - Koopmans <i>et al.</i> 1996 - Koopmans <i>et al.</i> 1995 - Boxman <i>et al.</i> 1995
NITREX H. Norway spruce.			- Significant increase in nitrogen losses through denitrification and nitrate leaching	- Hagedorn <i>et al.</i> 2001 - Schleppi <i>et al.</i> 1996
EXMAN Norway spruce.	- No change in needle nutrient status. Increased tree growth in fertilised and irrigated plots.		- Soil chemistry changes - Within three years of roof treatment nitrate output via leaching was reduced from 22 to 5.5 kg N ha ⁻¹ yr ⁻¹	- Beier <i>et al.</i> 1998 - Tietema & Beier 1995 - Beier <i>et al.</i> 1995 - Gundersen & Rasmussen 1995 - Rasmussen <i>et al.</i> 1995
CORE Norway spruce /Scots pine. •	- No effect on tree roots.		- Soils varied in N storage capacity; in some sites there was increased ammonium leaching, whilst in others a high ammonium retention. - Precipitation influenced leaching.	- Carnal <i>et al.</i> 1996 - Carnal <i>et al.</i> 1995 - Berg <i>et al.</i> 1995

Habitat type	Tree Health	Ground Flora/Mycorrhiza	Soil Biology/Chemistry	Authors
Swedish Optimum Nutrition Experiment. 30 year old Scots pine/ Norway spruce and 40 yr old Scots pine.	- Increased tree growth with N addition. - Needle concentrations of most amino acids, except glutamic acid, increased with N fertilisation. - Apart from arginine, the amino acid composition of proteins was similar in all treatments•	- Bryophyte abundance changed with fertilisation; abundance of <i>Brachythecium oedipodium</i>, <i>B. reflexum</i>, <i>B. starkei</i> and <i>Plagiothecium laetum</i> all increased with fertilisation treatments less than 60 kg N ha⁻¹ yr⁻¹. - The mosses <i>Hylocomium splendens</i>, <i>Dicranum scoparium</i> and <i>Pleurozium schreberi</i> declined strongly at 30-60 kg N ha⁻¹ yr⁻¹. - <i>Vaccinium vitis-idaea</i>, <i>Calluna vulgaris</i>, <i>Cetraria islandica</i> and <i>Dicranum polysetum</i> were all reduced by any N addition. <i>Vaccinium myrtillus</i> favoured at 20 kg N ha⁻¹ yr⁻¹ but not at higher levels. - Species favoured by high levels of N (40-60 kg N ha⁻¹ yr⁻¹) were <i>Rubus idaeus</i>, <i>Dryopteris carthusiana</i>, <i>Chamerion angustifolium</i> and <i>Deschampsia flexuosa</i>. - Diversity, biomass and fruiting of mycorrhiza reduced by N application.	- Increased leaching with high N input. Fertilisation promotes loss of N from the forest stands. - Increased litter production by <i>D. flexuosa</i>, ferns and <i>R. idaeus</i>.	- van Dobben <i>et al.</i> 1999 - Tamm <i>et al.</i> 1995 - Hogberg <i>et al.</i> 1992 - Dirkse & Martakis 1992 - Dirkse <i>et al.</i> 1991 - Dirkse & van Dobben 1989 - Näsholm & Ericsson 1989 - Wästerlund 1982
Norway spruce forest floor (75% <i>V. myrtillus</i> , 4% <i>D. flexuosa</i> , 20% mosses).•		- Mosses took up a disproportionate share of N (77% at lowest N, 58% at highest). - In <i>V. myrtillus</i> and <i>D. flexuosa</i> labelled N found mainly in above ground organs as increased amino acid content. - Higher fungal attack by <i>Valdensia</i> and <i>heterodoxa</i> and <i>Podosphaera myrtillina</i> on <i>V. myrtillus</i>. - Increased insect damage to <i>V. myrtillus</i> on higher N treatments.		- Nordin <i>et al.</i> 1996•- - Nordin <i>et al.</i> 1998

Habitat type	Tree Health	Ground Flora/Mycorrhiza	Soil Biology/Chemistry	Authors
120 yr old beech forest.	- Increased foliar N concentrations. - Decreased foliar P and Cu concentrations. - No change in soluble carbohydrate in response to N fertilisation. - Reduced phenolic concentration in response to N fertilisation.		- In high N treatment NO_3^- concentrations were ten-fold NH_4^+ concentrations.	- Balsberg Pålsson 1992•
120 yr old beech forest		- Within 3-4 years all mycorrhizal species practically ceased production of fruiting bodies even at the lower treatment rate, which corresponded to about 3 times the ambient deposition rate.	- Several macrofungal decomposer taxa increased their sporophore production in the treated plots. - Macrofungal wood decomposers no change.	- Rühling & Tyler 1991
120 yr old beech forest	- Beech seedlings more numerous in N treated plots, not caused by a larger seed setting but soil conditions.	- Little effect on species composition. - Some changes in cover and biomass of common species; increases in <i>Impatiens parviflora</i>, decreases in <i>Anemone nemorosa</i>, <i>Maianthemum bifolium</i>, <i>Oxalis acetosa</i>, <i>Poa nemoralis</i> and <i>Viola riviniana</i>.		- Falkengren-Grerup 1993
Scots pine		- Frequency and density of mycorrhizae not affected. - High levels of ammonium negatively affected fruit-body production more than high nitrate levels.		- Termorshuizen 1990

Habitat type	Tree Health	Ground Flora/Mycorrhiza	Soil Biology/Chemistry	Authors
29 yr old Norway spruce. •	- Increased stem-wood growth.		- Decrease in soil pH and increase in Al. - Increased soil ionic strength. - Increased N mineralisation with N addition. - Higher N doses cause considerable NO ₃ ⁻ leaching. - Soil pools of Mg, Mn and K decrease with increasing N dose but system totals do not vary between treatments.	- Eriksson <i>et al.</i> 1992 - Nilsson <i>et al.</i> 1988
60-year old Norway spruce		- Biomass of two dominant moss species, <i>Pleurozium schreberi</i> and <i>Dicranum polysetum</i> , on forest-floor decreased by 60% and 78% respectively. - N concentration in bryophytes and grasses increased significantly		- Mäkipää 1998
36-63 yr old Scots pine/ Norway spruce.	- Increases in stem volume with fertilisation. •	- Decrease in ground vegetation biomass. - Increase in grass biomass. - <i>Rubus idaeus</i> only found on N treated plots.	- Increase in total nutrients (N,P,K,Ca,Mg) bound by the humus layer due to increase in organic matter. - No increase in soil acidity. - Increased carbon in humus layer (increase of 14-87%) and mineral soil (15-167%), accumulation of organic matter on fertilised plots.	- Mäkipää 1995a,b - Mäkipää 1994 - Mäkönen 1990
25-35 yr old Scots pine.	- Significant tree growth increase in N treatments; 53 and 102% on controls for 30 and 90 kgNha ⁻¹ yr ⁻¹). - No sign of deficiency after five years, but decrease in Mg concentrations in needles in treated plots.		- N most limiting nutrient; after 5 years no sign of nitrogen saturation.	- Abrahamsen & Erstad 1995 - Nilsen & Abrahamsen 1995
Boreal late successional Norway spruce forest of <i>Vaccinium myrtillus</i> type		- increase in abundance of the grass <i>Deschampsia flexuosa</i> , which was more than doubled in the 50 kgN/ha.yr treatment - non-significant trend for decrease in the abundance of <i>V. myrtillus</i> - Great increase in glutamine levels in the leaves of <i>V. myrtillus</i> - Increase in the incidence of infection of <i>V. myrtillus</i> by the parasitic fungus <i>Valdensia heterodoxa</i>		- Strengbom <i>et al.</i> 2002a - Strengbom <i>et al.</i> 2002b

Habitat type	Tree Health	Ground Flora/Mycorrhiza	Soil Biology/Chemistry	Authors
10 yr old Sitka spruce	- Raise in foliar cytokinin concentrations - Increase in tree growth and fine root production	- Decrease in mycorrhizal and saprophyte fruitbody production. - Fungal species composition shift to more N-tolerant species. - Reduced mycorrhizal diversity.		- Crossley <i>et al.</i> 2001
Beech and Norway spruce stands	-N application resulted in nutrient imbalances in both tree species: leaf N concentration increased significantly, as did the N:P ratio, the N:K and N:Mg ratios - Increased attacks from parasitic fungus and insects; most severe attacks in sites with poor acid soils, where N addition caused higher changes in the nutrient status			- Flückiger & Braun 1999

2.2. Lowland Heathland Ecosystems

A lowland heath habitat has been defined as an open landscape generally occurring on nutrient poor, acidic sandy soils below 300 metres in altitude (Gimingham 1972) and is characterised by the presence of dwarf shrubs of the heather family, notably *Calluna vulgaris*, *Erica cinerea* and *Erica tetralix*. Heathland habitat in European terms is restricted by climate, as lowland heath requires the 'oceanic climate' of the western seaboard of the continent (Aerts 1993). Consequently, lowland heath habitats are found in the British Isles, southern Scandinavia, Denmark, north-west Germany, The Netherlands, Belgium, western France and the north and west of the Iberian Peninsula.

There is clear evidence of a loss of *Calluna vulgaris* and *Erica tetralix* dominated dry and wet lowland heath in both the UK and other European countries over the last few decades (Pitcairn *et al.* 1995, Bunce 1989). In the UK lowland dry heath is becoming increasingly rare and is of considerable conservation importance. The transition of many areas of heathland to grassland cannot be explained solely by moves away from the traditional management regimes of burning, grazing and sod cutting (Bobbink and Roelofs 1995, Pitcairn *et al.* 1995). It is believed that increases in nitrogen deposition, combined with stress and disturbance factors, are the trigger for the change of lowland heath ecosystems into grasslands, as a result of the opening up of the *Calluna* canopy. Nitrogen manipulation experiments can provide evidence of whether current levels of atmospheric nitrogen deposition can be held responsible for the observed transition of heather-dominated to grass-dominated vegetation. A large number of heathland nutrient manipulation experiments have been undertaken, especially in The Netherlands and Denmark, but with few in the UK. Those studies in which nitrogen was the only nutrient manipulated have their experimental details listed in Table 2.3. Detailed descriptions of the results of those experiments which applied nitrogen at a rate representative of current atmospheric deposition levels ($<50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) are given in Table 2.4.

UK studies

In the UK, lowland heath nitrogen manipulation experiments have been undertaken at two locations, Thursley Common in Surrey and Budworth Common in Cheshire. Thursley Common has been an active nitrogen manipulation experimental site since 1989 and receives an estimated background deposition of $10\text{--}15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Power, unpublished data). To date, three investigations have been undertaken at Thursley, the latter two of which are ongoing:

- Nitrogen addition from 1989 – 1996 at a rate of 0, 7.7, 15.4, or $0/15.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (in alternate years).
- Nitrogen addition (0 or $30 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) following management, since 1998.
- A recovery experiment following cessation of nitrogen additions in 1996.

The Budworth Common lowland heath site was established in 1996. The experimental site was dominated by building phase *C. vulgaris*. Nitrogen additions, in the form of ammonium nitrate, have been applied at two week intervals at 0, 20, 60 and $120 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. The main aim of the investigation was to examine the impacts of elevated nitrogen deposition and drought stress on young, actively growing plants.

No changes in species composition were observed in the *C. vulgaris*-dominated experimental site at Thursley Common following seven years of nitrogen additions (Power *et al.* 1998a, 1995), nor even after opening of the dwarf shrub canopy following management after nitrogen additions ceased. However, changes in the growth, flowering and phenology of *C. vulgaris* were numerous. The canopy height and density were both increased with nitrogen addition, canopy height in the highest nitrogen addition treatment being as much as 50% taller than control plots after six years. At the highest nitrogen addition ($15.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) increases in shoot length were as much as 90% greater than control plot shoots. The increased shoot growth was not reflected in root growth, and a reduction in the root:shoot ratio was inferred. Increases in canopy height and density were also seen in studies carried out in The Netherlands, where the canopy was not opened (Bobbink *et al.* 1996). A destructive harvest carried out at the end of the seven year experiment revealed significant treatment effects on the biomass and chemistry of both above-ground vegetation and litter (Power *et al.* 1998a). Furthermore, a nitrogen budget for the site showed that most of the experimentally added nitrogen had accumulated in the litter and humus layers of the soil, suggesting the potential for long term, persistent effects of the accumulated nitrogen.

An additional study carried out at Thursley Common examined whether nitrogen addition could result in increased sensitivity of *C. vulgaris* to abiotic and biotic stress (Power *et al.* 1998b). In plots which received $15.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, there was a trend towards higher foliar nitrogen concentration; in some years this trend was statistically significant. *C. vulgaris* shoots from the highest nitrogen addition plots showed signs of decreased resistance to frost injury in April, although not in November. However, this was at temperatures much below those normally experienced at the field site.

Enhanced foliar nitrogen concentrations have also been noted during the current investigation at Thursley Common. Significant differences between nitrogen addition and control plots were observed in October 1998 and August 1999. Investigations of soil chemistry at Thursley Common by Barker (2001) and Power *et al.* (1995, 1998a) found that soil pH was significantly reduced from 4.0 to 3.8 in the top 5 cm of ammonium sulphate treated plots in comparison to the control plots. Despite these changes, the concentration of ammonium and nitrate leached from nitrogen addition plots did not differ from that of control plots, and there was no difference in cation (Ca, Mg, K and Na) leaching. Litter production and degradation were also significantly increased following post management nitrogen additions. Such increases may have a long term effect on nutrient cycling and accumulation in these ecosystems (Berendse 1990).

The ongoing nitrogen addition study at Thursley Common enables the effect of different management regimes on heathland response to enhanced atmospheric deposition to be determined. The managements consisted of a low intensity mow, a management burn, a high intensity mow and a simulated accidental burn, representing a gradient of increasing intensity (and N removal) from the system. Full details of the managements are provided in Barker (2001). The results of previous investigations at this site have shown that experimental nitrogen additions have been mainly retained in the soil (Power *et al.* 1998a). The use of different management techniques was therefore designed to produce a gradient of nitrogen removal from the heath. As seen in several other nitrogen manipulation experiments, shoot growth, flowering, litter production and foliar nitrogen concentrations were increased in response to nitrogen addition. There was some evidence of an interaction between nitrogen and management treatments; the intensively managed plots (high mow and simulated accidental burn) responded proportionally more to atmospheric inputs of nitrogen (Power *et al.* 2001). A tentative conclusion was that, during the investigation, higher intensity managements which removed a larger

proportion of stored nitrogen from the heathland may be more effective at reducing absolute plant growth responses to nitrogen. However, further investigation is being undertaken to ascertain the persistence of this effect.

Any form of management opens up the *C. vulgaris* canopy which may enable grass species to become established; however a conflict exists between adequate nutrient removal and the formation of gaps which facilitate grass invasion. As would be expected, the management which resulted in the greatest damage to *C. vulgaris* root stocks, i.e. the simulated accidental burn, resulted in the highest invasion of grass species. These plots also had the greatest increase in seedling invasion in response to nitrogen addition (Power *et al.* 2001), showing a further significant interaction between nitrogen and management treatments. During the investigation both nitrogen addition and habitat management significantly affected rates of decomposition. Rates were elevated in the low intensity mow and management burn, in comparison to the more intensive managements, and the fastest rate was observed in the low intensity mow which received additional nitrogen (Barker 2001, Power *et al.* 2001). Since the quality of the litter used was uniform, the effects seen must have been mediated via the soil environment (Power *et al.* 2001). Therefore, the more intensive managements which removed litter/humus seemed to be more effective at maintaining a lower nutrient environment during the three years of subsequent nitrogen additions.

The present study at Thursley Common also incorporates a recovery experiment, to monitor ecosystem recovery following the cessation of nitrogen addition. The investigation showed that historically high levels of atmospheric nitrogen deposition can continue to have a significant impact on lowland heath for some time following a reduction in deposition (Barker 2001). For example, the former high nitrogen plots had significantly increased shoot growth and total soil nitrogen content, as well as a lower pH than the control plots three years after cessation of nitrogen additions. There was also evidence to suggest that management can have a substantial impact on heathland recovery following a reduction in deposition inputs. The management treatments which removed a greater proportion of the organic nitrogen stores from the system were shown to reduce the magnitude of the carry over effect of former nitrogen applications. For example, shoot growth and height of the *C. vulgaris* canopy in plots which previously received additional nitrogen were lower following the simulated accidental burn than following the low intensity mowing management.

The nitrogen manipulation experiment at Budworth Common in Cheshire began in 1996. Nitrogen was applied as ammonium nitrate at rates of 0, 20, 60 and 120 kg N ha⁻¹ yr⁻¹. A drought was imposed on half of the plots from spring to autumn 1997. Shoot nitrogen content was found to vary with season, however, there were highly significant differences between the nitrogen contents of shoots in the early stages of the experiment, prior to the start of the drought treatment. After ten months of N additions the shoot nitrogen concentrations were directly proportional to application rates, varying between 15 to 20 mg g⁻¹ in the lowest and highest nitrogen treatments respectively (Cawley 2001). However, there was not always a stimulation in shoot nitrogen concentration as significant differences were observed only on three of five occasions. Shoot growth and flowering in *C. vulgaris* were stimulated by nitrogen additions in proportion to application rates during the early years of the experiment. In the low and control treatments applicable to this review, no difference in canopy height was recorded in either nitrogen or drought treatments.

Measurements of soil pH at Budworth Common in June 1996 and June 1998 did not appear to show any significant reduction as a result of nitrogen application. The effect of

elevated nitrogen treatments, plus drought, on the density of *Deschampsia flexuosa* at Budworth Common showed a trend towards an increase of *Deschampsia* in proportion to nitrogen treatment. However, further monitoring at this site by Wilson (unpublished data) has shown that, from 1999 onwards *D. flexuosa* density decreased significantly as the *C. vulgaris* canopy recovered. From the results of this study, the author suggested that increases of nitrogen in excess of 20 kg N ha⁻¹ yr⁻¹ above ambient inputs may have the ability to disrupt *C. vulgaris*. However, the relatively short duration of this study (3 years) must be taken into account when attempting to extrapolate results from the above statement to other lowland heath sites.

Cawley (2001) demonstrated how resilient *C. vulgaris* appears to be under conditions of elevated nitrogen and short to medium term drought episodes. However, under conditions of severe drought, plant water potentials did appear to fall in proportion to increasing nitrogen supply. Examination of drought sensitivity at Thursley Common showed no effect of nitrogen addition on *C. vulgaris* shoot water potentials in the field (Power *et al.* 1998a). However, calculation of shoot drying curves showed that *C. vulgaris* plants from control plots lost water significantly more slowly than those plants which received additional nitrogen. Plants exposed to nitrogen additions may therefore become more stressed under drought conditions.

The simulated drought conditions at Budworth Common (May – September 1997) revealed a marked reduction in root mass for droughted plants in all treatment plots. Above ground growth ceased at a much earlier date in droughted plants in comparison to fully watered plants and growth continued in the fully watered plants for several weeks, with greater growth also in high nitrogen plots. It is considered that the implications of such luxuriant growth combined with increased foliar nitrogen content would lead to a pre-deposition to late summer drought, a high risk of winter injury and an increased risk of attack by herbivores and fungal pathogens (Cawley 2001). Indeed, during the third year of this experiment, a spontaneous heather beetle attack occurred. As seen in other biotic stress sensitivity experiments, a distinct preference for enriched nitrogen foliage was shown. Power *et al.* (1998b) investigated whether nitrogen additions affected heather beetle growth and performance. The relative growth rate of larvae were faster for beetles feeding on shoots from the highest nitrogen addition plots and these larvae grew into significantly larger pupae and adults. A number of other studies have also shown that nitrogen addition increases heather beetle growth and reduces its time to maturity, thereby increasing its damage capabilities and the potential for particularly damaging outbreaks of this insect herbivore (Berdowski 1993, van der Eerden *et al.* 1991, McNeill *et al.* 1988, Brunsting and Heil 1985).

European Studies

The Danish Heath project is located on several lowland heath sites in Denmark. Nitrogen manipulation studies began at Hjelm Hede, north-west Denmark, in 1993 and involved a range of application rates from 0 – 70 kg N ha⁻¹ yr⁻¹. The total annual deposition for the area is estimated to be 18 kg N ha⁻¹ yr⁻¹. An investigation into the response of heathland vegetation to nitrogen addition was carried out by Riis-Nielsen (1997). The study involved six sites, representing different stages of succession. Ammonium nitrate was applied six times per annum at rates of 0, 15, 35 and 70 kg N ha⁻¹ yr⁻¹. No obvious signs of increased (or decreased) growth of any species or increased flowering due to nitrogen fertilisation were found. This absence of growth response could be due to the presence of limiting factors, other than nitrogen. Further investigation on Hjelm Hede has found the site to be limited by phosphorous. It has been suggested that, where phosphorous limits plant growth, heathlands may still be

susceptible to nitrogen deposition, but that they are more likely to be resistant to vegetation changes than nitrogen-limited heathlands. In the summer of 1994, a heather beetle attack occurred at two of the study sites, providing an opportunity to study the response of the heather beetle to nitrogen additions. The largest densities of beetles were found at intermediate nitrogen additions ($15 - 35 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). The damage to *Calluna* vegetation was found to increase with increasing levels of nitrogen (Riis-Nielsen 1997), providing strong support for the link between nitrogen deposition and heather beetle outbreaks.

An investigation into mineralisation and immobilisation rates of nitrogen in heathland soils, using ^{15}N isotope techniques in laboratory incubations of field collected cores, was undertaken by Kristensen and McCarty (1999). Soil samples were taken from under intact and virtually dead *C. vulgaris* vegetation in November 1999. The death of the vegetation had been caused by a naturally occurring heather beetle infestation in the summer of 1994. The heathland had previously been fertilised at rates of 0, 15 or $35 \text{ kg NH}_4\text{NO}_3 \text{ ha}^{-1} \text{ yr}^{-1}$ since 1993. The study found rapid immobilisation of added NH_4^+ under intact *C. vulgaris* vegetation suggesting that mineralised NH_4^+ may be re-immobilised in the soil immediately after being released. However, samples taken from under dead *C. vulgaris* vegetation showed that the tight nitrogen cycling in the mineralisation - immobilisation phases was greatly influenced by heather beetle attack, substantially increasing availability of inorganic nitrogen in the soil. These results may point to a unique influence of the *C. vulgaris* vegetation on microbial cycling of nitrogen within the ecosystem to favour low nitrogen availability (Kristensen and McCarty 1999). This study demonstrated that heather beetle infestations have the ability to change the balance of nitrogen cycling within the ecosystem, resulting in substantial increases in net mineralisation in the soil. Consequently, large accumulations of NH_4^+ occur in soil under *C. vulgaris* vegetation damaged by heather beetle attack and, together, these influences may increase the ability of grasses to gain dominance at heathland sites (Kristensen and McCarty 1999).

Nielson *et al.* (2000) reported on the biochemical cycling of inorganic and organic compounds in a lowland heath environment (N additions as in Riis-Nielsen 1997) as part of the HEATH project. The results from this investigation show that the ability to retain experimental additions as well as background/naturally deposited nitrogen requires the presence and the integrity of a humified H sub-horizon. As discussed previously in Kristensen and McCarty (1999), as long as the heath functions are undisturbed, elemental cycling is characterised by a strong internal cycle. However, one year after a heather beetle infestation, the mor layer started to decompose, resulting in a large translocation of organic compounds down the soil profile, to the B-horizon. Nutrients were not lost via leaching processes, but were instead re-distributed in the upper soil, with the podzolic B horizon functioning as a nutrient trap to the mor layer. This experiment forms part of an ongoing investigation, and although not yet reported, the re-establishment of *Calluna* vegetation is being monitored.

The Danish HEATH project incorporated an investigation into the influence of ammonium nitrate on root growth and ericoid mycorrhizal colonisation of *C. vulgaris* by Johansson (1999). This experiment was relatively short term as ammonium nitrate was applied at rates of 0, 35 and $50 \text{ N ha}^{-1} \text{ yr}^{-1}$ for two years only. However, this work is still of interest in the context of this review since the majority of investigations into the effect of nitrogen of mycorrhizal fungi have been conducted under laboratory conditions. Results showed that ericoid mycorrhizal colonisation of *C. vulgaris* remained unchanged after two years of fertilisation with ammonium nitrate, contrary to expectations based on findings under laboratory conditions, *e.g.* Moore-Parkhurst & Onglander 1982 and

Stribley & Read 1976. Johansson (1999) concluded that, based on the two year experiment at Hjelm Hede, the effect of nitrogen on the mycorrhizal colonisation of *C. vulgaris* is unlikely to provide an explanation for heather decline under enhanced nitrogen input. An earlier experiment by Johansson (1995) also showed no significant changes to mycorrhizal colonisation. The influence of ammonium nitrate on the composition of the saprotrophic fungi in *C. vulgaris* heathland soil was also investigated by Johansson (2001). No measurable changes in specific composition of saprotrophic soil fungal groups due to nitrogen treatment were apparent. Consequently, the author concluded that a direct impact on the saprotrophic fungal community is unlikely under enhanced nitrogen input, more that changes would result from secondary effects, for example, changes in vegetation, soil and litter quality (Carroll *et al.* 1999, Power *et al.* 1998a, Nornbery *et al.* 1993, Nielsen *et al.* 1987a,b,) under sustained deposition of nitrogen (Johansson 2001).

Prins *et al.* (1991) and Van der Eerden *et al.* (1990) examined the effect of nitrogen additions on the maintenance of *C. vulgaris* vegetation in The Netherlands, in an area where background atmospheric nitrogen deposition levels were 35-40 kg N ha⁻¹yr⁻¹. Ammonium sulphate was supplied in 10 annual soil dressings (10, 50, 90 kg N ha⁻¹yr⁻¹). Soil chemistry was affected by the nitrogen treatments: cation (K⁺, Ca²⁺, Mg²⁺, Al³⁺) and NH₄⁺ concentrations showing a clear increase with nitrogen dose. The soil became increasingly more acid with increasing ammonium sulphate addition. An investigation of *C. vulgaris* characteristics found there were no significant differences between treatments in plant, root, green shoot or dead wood dry weights between treatments after three years. Levels of nitrogen were, however, significantly higher in *C. vulgaris* green parts from plots receiving the highest nitrogen additions (90 kg ha⁻¹yr⁻¹).

No vegetation composition changes were found in closed canopy *C. vulgaris* stands. However, where frost damage had occurred prior to commencement of nitrogen additions, there was a vegetative expansion of *Molinia caerulea* and *Deschampsia flexuosa* in response to nitrogen. The number of tillers and biomass of *D. flexuosa* in damaged *C. vulgaris* stands was significantly greater than control plots at all nitrogen application rates; for *M. caerulea* this was the case only at 50 and 90 kg N ha⁻¹yr⁻¹ additions. In this experiment Bobbink *et al.* (1992) observed more than a doubling in base cation leaching (Mg, Ca, K) from *C. vulgaris* shoots at the high nitrogen application rates compared to under the control heather vegetation. However, during measurement, no deficiencies of these ions in the shoots of *C. vulgaris* were found and furthermore a significant uptake of NH₄⁺ by *C. vulgaris* shoots was observed.

Investigations by Heil and Diemont (1983), in which a range of low nitrogen application rates (0, 1.75, 7 and 28 kg ha⁻¹ yr⁻¹) were applied as a single dose or as an annual soil dressing for four years, found dramatic floristic changes to heathland in The Netherlands. The floristic composition of the plots was analysed nine years after the cessation of the nitrogen addition regime. The addition of 28 kg N ha⁻¹yr⁻¹ resulted in a dramatic replacement of *C. vulgaris* by *Festuca ovina*; this was almost complete in plots which had been attacked by heather beetles. No effect was observed in the plots which only received the nitrogen additions on one occasion. An interaction with management was also noted; in older stands of heather (burned 16 years before) no increase in *F. ovina* cover was found in plots which received low nitrogen doses, but the changes in grass-heather dominance were increasingly evident in the more heavily N-dressed plots. In younger plots (1 year old) application of nitrogen, at all doses, had an immediate stimulatory effect on *F. ovina* cover, which was stronger as the amount of nitrogen increased.

The opening up of the heather canopy seems to be a key factor in the transition of heathland to grassland. In plots where there had been considerable heather beetle damage, *F. ovina* had almost completely replaced *C. vulgaris*; no relationship between heather beetle attack and nitrogen application dose was noted (but see also Brunsting and Heil 1985). In the phosphorous-limited Danish heathlands, the probability of vegetation change to grassland after heather beetle attack is suggested to depend largely on the soil phosphorous-availability (Riis-Nielsen 1997). Heil and Diemont (1983) also showed how additional nitrogen fertilisation could occur from heather beetle infestation. Heather beetle faeces and corpses, during a heavy infestation, were responsible for an additional input in the order of 7 kg N ha⁻¹ in some sites; additionally mineralisation of the *C. vulgaris* parts, killed by the heather beetle, can also lead to further nitrogen fertilisation.

Van der Eerden *et al.* (1991) applied, under a partial roof, artificial rain containing ammonium sulphate (2.9, 5.7, 11.3, 22.7, 45.3 and 90.7 kg N ha⁻¹yr⁻¹) every fortnight to the Asselse Heide lowland dry heath in The Netherlands. Measurements of plant characteristics showed decreases in the root to shoot ratios in both *C. vulgaris* and *D. flexuosa* with increasing nitrogen additions. Investigations of litter decomposition rates found those of *C. vulgaris* and *Molinia caerulea* to be unchanged by the addition of nitrogen, whilst the decomposition rate of *D. flexuosa* litter increased at the highest nitrogen doses. These decomposition rates in all species seemed to be limited by the availability of usable carbon in the different litter types. Chemical analysis of the litter types found there to be significant increases in the nitrogen content of *M. caerulea* leaf litter with increasing addition of nitrogen, whilst no increases were found for *D. flexuosa* or *C. vulgaris* leaf litter. Increases in frost sensitivity were also noted in the highest nitrogen addition plots five months after treatment began, but not at seven months (van der Eerden and Dueck 1992). Despite the short duration of this study the results indicate that there could be potential changes in the competitive balance, botanical composition and nutrient cycling patterns in the study heathland. Van Vurren and van der Eerden (1992) also studied the effect of low level nitrogen addition on lowland heath ecosystems. The duration of the study was short (less than two years) and no changes in botanical composition were observed, although smaller changes in soil and plant chemistry were found.

All other whole ecosystem nitrogen manipulations on lowland heathland have involved large additions of nitrogen, at deposition rates ranging from 80 to 200 kg N ha⁻¹yr⁻¹. Heathland subjected to higher levels of nitrogen addition has shown a variety of responses depending on the form of nitrogen addition (Persson 1981). In liquid fertilised plots, there was a change in the ground cover, from almost exclusively lichen to domination by the bryophyte species *Pohlia nutans* and *Pleurozium schreberi*. Where nitrogen was applied in a solid form, there were however, decreases of some bryophyte species (*Dicranum polysetum* and *P. schreberi*). This highlights the problem in interpreting many nitrogen manipulation experiments as the responses observed vary with the experimental procedure employed and the duration of the experiment.

Summary

The effect of increased nitrogen deposition on lowland heath ecosystems has been reasonably well studied and a number of experiments involving relatively low additions of nitrogen have been undertaken. The responses of *Calluna vulgaris*, the main component of these ecosystems, have included increased shoot growth, canopy height and density, flowering and litter production, all with nitrogen additions as low as 15.4 kg N ha⁻¹yr⁻¹. Changes in *C. vulgaris* shoot chemistry and vulnerability to cold and

drought stresses have also been noted. A number of studies have reported increased damage by heather beetles with increasing nitrogen addition, and decreased larval development times. The ability of heather beetle infestations to bring about changes to the microbial community has also been observed. The opening up of the *C. vulgaris* canopy through frost, drought or insect damage is considered to be of key importance in the conversion of heathland to grasslands with increased nitrogen addition, with a more open canopy favouring the growth of the grasses *Molinia caerulea* and *Deschampsia flexuosa*. The study by Power *et al.* (1995, 1998a;b, 2001) emphasises the need for long term studies of ecosystem responses to increased rates of nitrogen deposition, as some responses were observed only after a number of years of nitrogen addition. Furthermore, the importance of P availability and habitat management as modifiers of heathland response to nitrogen deposition have been suggested.

TABLE 2.3. Heathland nitrogen manipulation experiments undertaken in the UK and Europe. The location of the experiment, the nitrogen species and dose added are shown, along with the frequency of application, the number of years the experimental additions were made, any additional treatments and the background atmospheric nitrogen deposition in the experimental location where known

Country	Location	N-Type	Dose (kg N ha ⁻¹ yr ⁻¹)	Frequency of Application	Duration (years)	Other Treatments	Background Deposition (kg N ha ⁻¹ yr ⁻¹)	Authors
UK	Thursley	(NH ₄) ₂ SO ₄	0/7.7/15.4 / alternating 0 or 15.4	Applied as 15 l solutions in fine mist 42 occasions per year.	7		13-18	- Uren. 1992 - Uren <i>et al.</i> 1997 - Power <i>et al.</i> 1995 - Power <i>et al.</i> 1998a - Power <i>et al.</i> 1998b
UK	Thursley	(NH ₄) ₂ SO ₄	30	Applied as 15 l solutions in fine mist 25 occasions per year.	4		13-18	- Barker 2001 - Power <i>et al.</i> 2001
UK	Budworth	NH ₄ NO ₃	0/20/60/120	Applied as 3 l solutions per plot via a fine rose watering can 25 occasions per year.	6	Simulated drought	20.5	-Cawley 2001 -Wilson (unpublished)
HEATH project. Denmark	Lodbjerg Hjelm Hede	NH ₄ NO ₃	0/15/35/70	Applied four annual dressings in solution.	2	P, K.	12	- Riis-Nielson 1994 - Johansson & Sochting 1994 - Johansson 1995 - Kristensen & McCarty 1996 - Nielsen <i>et al.</i> 2000
HEATH project. Denmark	Lodbjerg Hjelm Hede	NH ₄ NO ₃	0/35/50	Applied four times annually in solution.	2		18	- Johansson 1999
HEATH project. Denmark	Lodbjerg Hjelm Hede	NH ₄ NO ₃	0/15/35	Applied in six annual dressings via a 2.5 m crop sprayer.	2		18	- Kristensen and McCarty 1999
HEATH project. Denmark	Lodbjerg Hjelm Hede	NH ₄ NO ₃	0/35/50/70	Applied four times annually in solution.	2		18	- Johansson 2001

Country	Location	N-Type	Dose (kg N ha ⁻¹ yr ⁻¹)	Frequency of Application	Duration (years)	Other Treatments	Background Deposition (kg N ha ⁻¹ yr ⁻¹)	Authors
The Netherlan ds	Asselse Heide	(NH ₄) ₂ SO ₄	2.9/5.7/11.3/ 22.7/45.3/90.7	Applied fortnightly as artificial rain under open roof.	1.1		30-35 (10-15 under roof)	- van Vuuren & van der Eerden 1992 - van der Eerden & Dueck 1992 - Van der Eerden <i>et al.</i> 1990, 1991
The Netherlan ds	Asselse Heide	(NH ₄) ₂ SO ₄	0/10/50/90	Applied in 15 l solutions in 10 dressings annually.	3		30-35	- Prins <i>et al.</i> 1991 - Bobbink <i>et al.</i> 1992
The Netherlan ds	Westerheid e.	NH ₄ NO ₃	0/1.75/7/28	Applied only once or as an annual dressing.	4	P	30-35 (estimate)	- Heil & Diemont 1983

TABLE 2.4. Results of heathland nitrogen manipulation experiments (additions <50kg N ha⁻¹yr⁻¹) undertaken in the UK and Europe.

Habitat Type	Effects on Floristic Composition/Plant Growth	Effects on Plant Chemistry/Stresses/Herbivory	Effects on Soil Chemistry/Biology	Authors
Lowland 20yr. old <i>Calluna</i> stand on an acidic greensand podsol.	- No changes in composition. - <i>Calluna</i> shoot growth increased in all plots receiving additional N, and was significantly greater in the high N plots where increases in shoot length were 35-90% greater than control plots. Growth generally beginning earlier in plots receiving N additions. - Canopy height increased significantly from 22 cm to 30 cm in high N treatment plots. - Evidence of a reduction in root:shoot ratio. - The proportion of flowering shoots was significantly greater than control plots at all N addition doses, with almost 90% more flowering shoots than control plots in the highest N addition plots - Litter production was significantly increased during the early stages of nitrogen additions in the high N plots.	- Trend towards increasing shoot N, and was significantly on several occasions during the study (yr. 1, yr. 3 and yr 7). - No effect of N addition was found on plant water potential. Excised <i>Calluna</i> leaves from control plots lost significantly less water than those from plants which received additional N. - Measurement of frost injury (electrolyte leakage) in November indicated no significant effect of N addition. However, there were indications of reduced frost injury in the November measurement and increased frost injury was significant in April in highest N treatments but only at temperatures considerably below those experienced in the field (-32°C). - Investigation of heather beetle growth found faster larval growth and greater pupal and adult weights were in the highest N treatment.	- Soil pH was significantly reduced in the top 5 cm of ammonium sulphate treated plots after 7 years of nitrogen application. - Concentration of ammonium and nitrate leached from nitrogen addition plots did not differ from that of control plots. - There was no observed difference in cation (Ca, Mg, K and Na) leaching, with the exception of Na in one month (March, 1994). - Nitrogen additions significantly increased the total nitrogen content of the top 10cm of the soil. - Measurements of N₂O and methane fluxes showed a trend towards increased N₂O and decreased methane emissions from high N plots, however, these were not significant. - microbial activity was shown to increase with nitrogen additions, as shown by faster rates of cotton strip degradation.	- Power <i>et al.</i> 1995 - Uren <i>et al.</i> 1997 - Power <i>et al.</i> 1998a,b

Habitat Type	Effects on Floristic Composition/Plant Growth	Effects on Plant Chemistry/Stresses/Herbivory	Effects on Soil Chemistry/Biology	Authors
Lowland 0-4 yr old <i>Calluna</i> stand on acidic greensand podsol.	- No overall changes in species composition. However, plots receiving nitrogen had significantly more <i>Deschampsia</i> seedlings than the controls following management. - <i>Calluna</i> shoot growth increased in nitrogen treatments and low intensity managements, in comparison to high intensity managements. - Nitrogen significantly increased litter production. - The high intensity management regimes also lead to an decrease in litter production. - In the recovery experiment historically high levels were shown to have significant impact on <i>Calluna</i> vegetation for some time following a reduction in nitrogen deposition.	- Increased foliar nitrogen content in nitrogen treated plots. - Nitrogen and management significantly affected rates of decomposition. - Trend towards faster rates of litter degradation in plots receiving nitrogen addition and low intensity managements.	- Soil pH was significantly reduced from 4.0 in the control plots to 3.8 in nitrogen treated plots. - No obvious effect of nitrogen addition or management on ion concentration in leachate. - Nitrogen additions significantly increased the total nitrogen content of the top 10cm of the soil, but no significant impact on soil inorganic nitrogen. - Management had a significant effect on total and inorganic soil nitrogen content of the top 10cm of the soil.	- Barker 2001 - Power <i>et al.</i> 2001
<i>Empetrum nigrum</i> dominated coastal heath (on shallow weekly developed young soil) and <i>Calluna</i> dominated inland heaths on sandy meltwater podsol sediments.	- No changes in species composition or dominance after two years of N addition. - No change in number of flowering <i>Deschampsia</i> shoots following two years of nitrogen treatments. - No changes in root biomass even with highest N addition. - No major effects on the competition between heather and grasses observed in four years.	- Significant increase in <i>Calluna</i> tissue N with addition of nitrogen. - Damage to <i>Calluna</i> vegetation was found to increase with increasing levels of nitrogen. - <i>Lochmaea</i> attack limited to high N treatments at some sites. In other sites all green parts of <i>Calluna</i> removed by the beetles irrespective of N addition. Highest beetle post-pupation emergence densities were found at 15-35 kg ha⁻¹yr⁻¹ and lowest densities at 70 kg ha⁻¹yr⁻¹.	- Mycorrhizal colonisation unaffected by treatments. - No vertical difference seen in mycorrhizal infection. - Saprotrophic fungi composition unaffected by nitrogen treatments. - Difference in cation mobilization with soil types. - One year after a heather beetle attack mor layer was shown to begin to decompose. - Large translocation of organic compounds down the soil profile to the B horizon (not lost to leaching). - Under intact <i>Calluna</i> NH₄⁺ pool in the soil maintained at a very low level, and net immobilisation was greater than gross mineralization.	- Riis-Nielson 1994 - Johansson & Sochting 1994 - Johansson 1995 - Kristensen & McCarty 1996 - Riis-Nielson 1997 - Kristensen & McCarty 1999 - Johansson 1999 - Nielsen <i>et al.</i> 2000 - Johansson 2001

Habitat Type	Effects on Floristic Composition/Plant Growth	Effects on Plant Chemistry/Stresses/Herbivory	Effects on Soil Chemistry/Biology	Authors
			- Heather beetle attack was shown to have significant influence the tight cycling of nitrogen leading to an increased availability of inorganic nitrogen in the soil. - Microbial decomposition processes were shown to dominate following a heather beetle attack, leading to net mineralization.	
Dry heathland: <i>Calluna</i> , <i>Molinia</i> and <i>Deschampsia</i> .	- No change in root or shoot dry weight in undamaged <i>Calluna</i> plants. - Increased tillering by grasses <i>D. flexuosa</i> (all N doses) and <i>M. caerulea</i> (50 and 90 kg N ha⁻¹yr⁻¹) in frost damaged heather sward.	- Increased N content in green, woody and flowering <i>Calluna</i> parts in highest N treatments. - No increase in the N content of roots with increasing N dose. - Increased Mg, Ca and K leaching from N-treated <i>Calluna</i> shoots. - Above ground uptake of NH_x.	- Greater cation (K, Ca, Mg, Al) concentrations in upper 5cm of soil with increasing N doses. - No litter effects.	- Prins <i>et al.</i> 1991 - Bobbink <i>et al.</i> 1992
Inland, <i>Calluna</i> dominated, heath on a brown podzolic soil.	- Replacement of <i>Calluna</i> with <i>Festuca ovina</i> in plots receiving 28 kg ha⁻¹yr⁻¹ or damaged by heather beetles. No effect in the plots which only received the treatments on one occasion. - The effect of N addition varied with <i>Calluna</i> age. In young stands (1 yr. old) N addition stimulated increases in <i>Festuca</i> cover immediately and the effect was stronger with size of N addition. In 7 yr. old plots no effect on <i>Festuca</i> cover at low N additions but effect was increasingly evident in heavily N-dressed plots.	- Increased heather beetle damage in high N plots.	- N fertilization of the soil by heather beetle faeces and corpses during a heavy infestation calculated as being in the order of 7 kg N ha⁻¹. - Mineralization of the killed <i>Calluna</i> parts can also lead to further N fertilization.	- Heil & Diemont 1983

2.3. Moorland Ecosystems

Upland heathland or moorland is defined by the UK Biodiversity Action Plan (2001) as lying below the alpine or montane zone (at about 600-750 m) and usually above the upper edge of enclosed agricultural land (generally at around 250-400 m, but descending to near sea-level in northern Scotland). Moorlands of the UK are typically characterised by low nutrient availability, the presence of dwarf shrubs at a cover of at least 25%, and are typically dominated by *Calluna vulgaris*, *Vaccinium myrtillus*, *Empetrum nigrum*, *Erica cinerea*, and, in the south and west, *Ulex gallii*. In northern areas, *Juniperus communis* is occasionally seen above a heath understorey. Bryophyte species are typically in high abundance throughout UK moorlands.

Moorland encompasses a range of National Vegetation Classification (NVC) plant communities, the distribution of which is influenced by climate, altitude, aspect, slope, maritime influences and management practices including grazing and burning. This habitat type is present on an estimated 270,000 ha in England, 80,000 ha in Wales, up to 69,500 ha in Northern Ireland and between 1,700,000 and 2,500,000 ha in Scotland (UK BAP 2001). The total upland heath resource in the UK thus amounts to between 2 and 3 million hectares. Upland *Calluna vulgaris* heathlands are of economic importance, providing an important food source for grazers including sheep (Caporn *et al.* 2000); of international importance because they are largely confined within Europe to the British Isles and the western seaboard of mainland Europe (UK BAP 2001); and of nature conservation importance - the bird assemblage associated with heather moorland is one of outstanding conservation value (Thompson *et al.* 1995).

Studies carried out over the last 50 years have shown a progressive loss of upland heather moorland, with large-scale replacement of shrub vegetation with more competitive grasses (Pitcairn *et al.* 1995, Marrs 1993). For example, 27% of heather moorland is estimated to have been lost in England and Wales between 1947 and 1980 (UK BAP 2001). On the Berwyn mountains in north-east Wales there was a 44% decline in the extent of heather-dominated vegetation between 1946 and 1984, whereas other upland sites in Wales have shown much smaller losses over similar periods. An estimated 18% was lost in Scotland between the 1940s and 1970s and the trend continued throughout the 1980s with a further estimated loss of 5% (UK BAP 2001). Much of this loss is attributed to agricultural land improvements, heavy grazing by sheep (and, in certain areas, red deer and cattle), afforestation (UK BAP 2001), and increased atmospheric nitrogen inputs (Lee *et al.* 2000). Indeed, Kirkham (2001) has suggested that N deposition has apparently changed a substantial proportion of the upland ecosystems dominated by *Calluna* in England and Wales from those where growth was limited by N into ones where P is now the limiting factor, with the consequences being a change in species composition. The role of increased levels of atmospheric nitrogen deposition in the decline of *C. vulgaris* dominated moorland in the UK has been examined through a number of nitrogen manipulation experiments. The experimental details and results are listed in Tables 2.5 and 2.6 respectively.

The longest moorland nitrogen manipulation experiment is at Ruabon, North Wales where experimental plots were established in May 1989 on an area of moorland at an altitude of 470 m. This site has been estimated to receive a background 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 (Caporn *et al.* 1995a, Lee *et al.* 1992). The period from 1989 to 1993 was characterised 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 (Carroll *et al.* 1999). 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 (Carroll *et al.* 1999). The 1996 data in particular showed no effect of nitrogen on shoot extension at all (Carroll *et al.* 1999).

Analysis of foliar nutrient content between 1989 and 1992 showed increased nitrogen and free amino-acid concentration with increasing nitrogen dose, and measurements taken in 1996 still showed a significant increase in response to nitrogen addition (Carroll *et al.* 1999). Increases in shoot nitrogen content and associated changes in nutrient ratios have been linked in other studies to increased severity of insect herbivore attacks, such as from *Operophtera brumata*, leading to a loss of *C. vulgaris* cover. For example, Kerslake *et al.* (1998) treated *Calluna* plants taken from Scottish moorland with ammonium nitrate (average 52.5 kg N ha⁻¹ yr⁻¹) and showed that it improved the quality of *C. vulgaris* as a host for *O. brumata*, and hence the performance of the insect. Measurements of shoot potassium and phosphorus content taken at various stages throughout the study period at Ruabon have failed to show any consistent trend, with no significant reductions in the concentrations of these elements in response to the nitrogen additions (Carroll *et al.* 1999). The N:P ratios, however, were clearly increased as a result of the treatments, with measurements taken in July 1996 showing values of 16:1 in the controls compared with 23:1 in the plots with the highest nitrogen treatment (Carroll *et al.* 1999).

This observation is consistent with the study by Kirkham (2001), who sampled plant material from a number of sites in England and Wales and analysed these for N content, annual accumulated nitrogen per hectare and N:P ratio. Kirkham (2001) associated increased N deposition with an increase in N:P ratio, surmising that N accumulation has changed a substantial proportion of *Calluna*-dominated uplands from N-limited ecosystems into P-limited ones, which could favour species such as *Molinia* that are better adapted to P limitation. Carroll *et al.* (1999) also surmise that high-dose nitrogen treatment leads to a loss of primary nitrogen limitation on shoot extension in *C. vulgaris*, although a question must be raised over how realistic these doses are (at approximately four to six times the estimated background N deposition at the Ruabon site).

Calluna roots characteristically exhibit a substantial degree of ericoid mycorrhizal infection (Yesmin *et al.* 1996) which is important for the degradation of complex organic substances in order to give plants access to N sources which would be otherwise unavailable. Mycorrhizal symbiosis may therefore play a part in altering the competitive balance between species and hence may influence community structure (Aerts 1999). Up to 1993, the nitrogen additions at Ruabon appeared to have had little effect on mycorrhizal infection levels (Caporn *et al.* 1995a) and the most recent survey by Pilkington (2002) demonstrated no effect of continued N addition on mycorrhizae. However, in a separate greenhouse experiment, Yesmin *et al.* (1996) subjected *Calluna* moorland peat mesocosms to NH₄⁺ and NO₃⁻ at one, two and six times the ambient value of 12 kg N ha⁻¹ yr⁻¹ (where the samples were taken from). 'Strong circumstantial evidence' was provided that acidifying pollution deposition results, in the long term, in a decrease in mycorrhizal infection of *Calluna* roots. It is suggested that the difference in results reflects the greater losses from leaching in the field experiments. Moreover, more NH₄⁺ will be incorporated into organic matter between the less frequent additions of the Caporn *et al.* (1995a) field study and the frequent additions of the Yesmin *et al.* (1996)

laboratory study, resulting in low concentrations of exchangeable NH_4^+ in soil solution for much of the year (Yesmin *et al.* 1996). It is also possible that ergosterol measurements (as employed at Ruabon), may over-estimate mycorrhizal infection, as these measurements would include non-mycorrhizal fungi (Yesmin *et al.* 1996).

The loss of mycorrhizal infection of *Calluna* roots is important when assessing the impact on competitive advantage over encroaching grasses such as *Nardus*. In a greenhouse experiment by Genney *et al.* (2000), *Nardus* was grown alone, or in competition with *Calluna* in a layered organic/ sand substrate with and without inoculation with the ericoid mycorrhizal endophyte *Hymenoscyphus ericae*, and with and without the addition of nitrogen. This study demonstrated that *Nardus* roots were less able to exploit the organic layer when *Calluna* was mycorrhizal and that the maintenance of this mycorrhizal colonisation of *Calluna* may be one mechanism by which *Calluna* dominated swards are maintained in moorland ecosystems.

In the earlier years at Ruabon, both bryophytes and lichens had disappeared from below the *C. vulgaris* canopy in all nitrogen addition treatments. It is not certain, however, whether this was a response to direct effects of ammonium nitrate addition or through changes in *Calluna* canopy architecture and increased litter production resulting in reduced light penetration to the ground flora. Later surveys showed that bryophytes have returned to the nitrogen treated plots and that their abundance has increased, probably as a result of the ageing *Calluna* canopy opening up (Lee *et al.* 2000). One interpretation of the *Calluna* growth responses in this experiment is that addition of N has accelerated the natural *Calluna* cycle, with earlier ageing and opening of the canopy in the highest treatment plots. This study by Lee *et al.* (2000) highlights, together with previous studies, the need to establish more precisely whether elevated nitrogen deposition is in fact having a direct impact on the bryophyte and lichen populations within this habitat, especially as recent studies in other habitats, such as Barker (2001), have highlighted that even relatively small increases in nitrogen deposition can lead to a large-scale decline in the cover of lichens in heathlands, and that this decline may be linked to increased growth of *Calluna vulgaris* with nitrogen additions.

Detailed studies of frost tolerance in shoots of *C. vulgaris* collected in the early years of the study (1989-1994) demonstrated results that varied with the season. Ammonium nitrate additions did not reduce frost hardiness in the first five years of the Ruabon experiment, but rather improved frost tolerance in autumn (Carroll *et al.* 1999). For example, in October 1991 the LT_{50} (lethal freezing temperature to kill 50% of a sample of shoots) was lowered by *c.* 2°C in comparison with the controls (Caporn *et al.* 1994). However, Lee and Caporn (1998) showed increased injury caused by climatic conditions in late winter; acute 'winter browning' of heather shoots was observed, most notably in the 80 and 120 kg N ha⁻¹ yr⁻¹ addition treatments. This damage mechanism may be the result of low temperature desiccation, rather than frost damage, although, in the earlier years of the experiment, de-hardening of shoots was reported by Caporn *et al.* (1994) at the higher nitrogen concentrations.

Soil studies at the Ruabon site found a number of responses with nitrogen addition:

1. The most recent data, Pilkington (unpublished data), showed that:
 - 80% of background atmospherically deposited nitrogen (30 kg N ha⁻¹ yr⁻¹) was retained by the organic layer and that the application of additional N treatments up to a maximum rate of 80 kg N ha⁻¹ yr⁻¹ stimulated increasingly higher rates of retention.

- There was further retention of inorganic N by the mineral layer, mainly as ammonium but also as nitrate. Treatment effects in this layer mirrored those of the overlying organic layer.
 - Small increases in the output of nitrate, in response to the lower levels of addition from the organic layer, was the result of increased rates of nitrification, although in winter and at higher levels of addition, direct leaching of both ammonium and nitrate may have occurred.
 - There was no significant effect on acidification or cation levels from either layer, except for a significant lowering of calcium and calcium/ aluminium ratios in the mineral layer solution, the latter in response to the lowest treatment.
2. In respect to the nitrogen budget, the treatments were shown to have little effect on leaching, which accounted for only 1.3% of the added nitrogen, even in the 120 kg ha⁻¹ treatment. In absolute terms, there was a relative small increase in the total N stored in vegetation, with most of additional nitrogen accumulated in the surface peat (Pilkington, unpublished data).
 3. Johnson *et al.* (1996) examined the soil bacterial component; summer soil samples were found to have increased bacterial biomass in nitrogen-treated plots. In addition, analysis revealed an increased use of bacterial amino-acids, amides and carbohydrates in treated plots. This is backed up by Johnson *et al.* (1998) who demonstrated that the long-term application of N at Ruabon increased soil microbial biomass. Direct incubation of soil suspensions from the heathland in BIOLOG microtitre plates mirrored these results, as there was a significant increase in rates of C utilisation. The level of microbial activity appears to correspond with plant cover and it may be the case that one of the main determinants of the microbial response to pollutant N is the productivity of the plant communities since this determines the C input into the soils (Johnson *et al.*, 1998). However, Pilkington (pers. comm.) suggests a possible increasing toxicity for microbes responsible for the conversion of inorganic to organic forms of nitrogen. In both organic and mineral layers, a departure from an increasing linear production of protons at 80 kg N ha⁻¹ yr⁻¹, due to an increase of ammonium fluxing from the organic horizon and an increase of nitrate fluxing from the mineral horizon, suggests a collapse in the ability of microbes to immobilise incoming N.
 4. Significant increases in weight and total nitrogen content of *C. vulgaris* litter, collected beneath the canopy, were observed at 80, 120 and 200 kg ha⁻¹ yr⁻¹ but increases in litter production were also observed as low as 40 kg ha⁻¹ yr⁻¹ (Carroll *et al.* 1999).
 5. Evidence of increased mineralisation and decreased C:N ratio of litter and rhizosphere peat with increasing N treatment, although the peat effect was not found below 2 cm.

Pilkington (unpublished data) demonstrated that regular burn management may remove a large percentage (95% in this study) of nitrogen, but that this is not entirely removed from the system as a whole. Immediately after the burn, the litter layer was shown to increase its N content (possibly as a result of ash), and six months after the burn there

were further increases in the percentage N content of the litter, possibly as a result of directly absorbing the treatment N. There was some indication of an increase of mineralisation of both the litter and the peat layer with increasing treatment and this was potentially related to the increased amounts of ash, with higher N content, with increasing treatment. Overall the effect of the burn on the nitrogen content of the system as a whole was minimal.

Loss of 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 North Pennines (estimated background deposition: $15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) to examine the effect of nitrogen addition on moorlands at the bracken and heather interface. Additional nitrogen was added at $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, with a control dose of $0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, to a series of plots set up on either bracken-dominated areas or at the heather-bracken interface. Nitrogen additions of $50 \text{ kg ha}^{-1} \text{ yr}^{-1}$ had little effect on stands consisting entirely of heather or bracken; however, at the heather-bracken boundary there was a trend for enhanced bracken growth. This enhanced bracken growth resulted in poorer growth of the heather in these areas.

These results, however, are in contrast to the findings of experiments by Gordon *et al.* (1999). The latter study used turf taken from a site in Scotland, transplanted into experimental plots, and watered once a fortnight from March to November at a rate of $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (as part of a number of other factorial combinations of treatments). This same level of nitrogen addition, as applied by Werkman and Callaghan (1996), increased the competitive ability of heather in the building phase relative to that of bracken in the absence of drought. Nitrogen addition was found to increase the leading shoot length of heather from the first year of treatment onwards, and also increased shoot growth in spring, implying earlier bud break, and greater flower numbers. In contrast, the response of bracken was slow, showing no above-ground growth stimulation, other than a slight advancement of crozier emergence, until the third year of treatment, at which time there was a small (7%), but significant, increase in canopy height.

However, predicting the consequences of increased nitrogen availability on the interactions of bracken and heather in moorlands is complex and other factors such as drought and temperature will play an important part. For example, whereas heather shows a faster and larger shoot growth stimulation than bracken, at least over 3 years, this response is advantageous when water is not limiting but is disadvantageous if drought occurs (Gordon *et al.* 1999). Gordon *et al.* (1999) surmise that climatic events might therefore be more important triggers for change in the relative success of heather and bracken on moorlands than long-term environmental changes, such as increased nitrogen deposition. This is backed up by Werkman *et al.* (1996) who found that simulating an increase in temperature in line with climate predictions would give a growth advantage to the bracken at the heather-bracken interface. There is a need, however, for further investigation into the impact of elevated nitrogen on susceptibility to secondary stresses, such as drought. While some studies show an association between foliar N concentration and susceptibility to stress caused by drought (Power *et al.* 1998b), others, such as the open-top study of Leith *et al.* (2001), suggest that exposure to large doses of wet NH_4^+ deposition may actually prevent drought stress by reducing water loss through reduced stomatal conductance.

Summary

Nitrogen manipulation experiments in moorland have shown responses to nitrogen additions as low as $40 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ with implications for the conservation and economic importance of moorlands throughout the UK. Early beneficial effects of nitrogen addition such as increased growth, nitrogen concentration, flowering, and improved autumn frost tolerance are being out-weighted by the observed adverse effects such as loss of primary nitrogen limitation on shoot extension, increased winter injury, increased chance of attack from insect herbivores, reduced species diversity of habitat-specific fauna, loss of *Calluna* cover and thus changes in species composition. It should be noted, however, that whereas important detrimental effects are occurring below realistic levels of N addition ($<50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) many of these observations occur, or are more apparent, at much higher N addition levels. However, early loss of lichen and bryophyte abundance appears to have been reversed with recovery evident throughout the nitrogen treatment plots, although this may well be the result of loss of *Calluna* cover and, thus increased light penetration. Evidence from laboratory, rather than field manipulation, experiments suggest that increased nitrogen addition may result in the loss of mycorrhizal infection of *Calluna* roots giving an advantage to encroaching grasses such as *Nardus*, especially if there are gaps in the *Calluna* canopy. The fact that earlier studies at Ruabon suggest little effect on mycorrhizal infection emphasises the need for further and longer-term studies of these effects and further nitrogen manipulation studies on the impact upon the lichen and bryophyte flora.

Table 2.5. Moorland nitrogen manipulation experiments undertaken in the UK and Europe. The location of the experiment, the nitrogen species and dose added are shown, along with the frequency of application, the number of years the experimental additions were made, any additional treatments and the background atmospheric nitrogen deposition in the experimental location where known.

Country	Location	N form	Dose (kgNha ⁻¹ yr ⁻¹)	Frequency of application (times/year)	Duration (yrs)	Other treatments	Management	Background deposition (kgNha ⁻¹ yr ⁻¹)	Authors
UK	Cronkley Fell, Upper Teesdale NNR	NH ₄ NO ₃	50	Applied annually.	6	Increased air temperatur e.	Controlled burning; Grazed by sheep.	15	Anderson and Hetherington (1999) Werkman, Callaghan and Welker (1996) Werkman and Callaghan (1996)
UK	Ruabon, Clwyd	NH ₄ NO ₃	0/40/80/120/2 00	Applications in solution were made every 2 weeks up until 1992, and thereafter on a monthly basis.	13	-	Controlled burning.	20	Caporn <i>et al.</i> (1994) Caporn <i>et al.</i> (1995a) Caporn <i>et al.</i> (1995b) Caporn <i>et al.</i> (2000) Carroll <i>et al.</i> (1999) Johnson <i>et al.</i> (1996) Johnson <i>et al.</i> (1998) Lee and Caporn (1998) Lee <i>et al.</i> (2000) Pilkington (2002)

Table 2.6 Results of moorland nitrogen manipulation experiments (additions <50 kg N ha⁻¹ yr⁻¹) undertaken in the UK and Europe.

Habitat type	Effects on floristic composition	Effects on plant chemistry/growth	Effects on soil chemistry/ Fungal infection/ Microbial activity	Management Interactions	Authors
Heather-bracken moorland.	- Relatively little effect in complete heather or bracken stands.	- Enhanced bracken growth at heather/ bracken boundary.	- The qualities of heather and bracken litters were similar with 1.5% N, 22.3% cellulose and 40.6% lignin in heather, and 1.32% N, 28.9% cellulose and 49.0% lignin in bracken. There were qualitative differences in the constituents of the lignins and hemicelluloses. - The decomposition rates of heather and bracken litters were about 10% higher in the N-fertilized plots while the mixtures showed nearly 15% higher mass losses.		-Anderson and Hetherington (1999) -Werkman <i>et al.</i> (1996) -Werkman and Callaghan (1996)
<i>Calluna</i> heath with some <i>Vaccinium myrtillus</i>	- Loss of lichens and bryophytes below <i>Calluna</i> canopy in all levels of treatment has given way to a recovery in the treated plots.	- Early stages of the experiment (1990-94) have shown marked and dose-related increases in shoot extension, flower production and canopy height. Results since 1994, however, show little increase in shoot extension in response to the nitrogen treatments, with no clear dose response to increasing levels of addition. - Increased frost hardness in the October of the first four years, but increased 'winter browning' after four years of highest N treatments. - Increased foliar concentration of N and amino acids (1989-92), levels of P in 1995 significantly decreased with increased N dose, leading to increased N:P ratios. - Accelerated ageing and opening of canopy.	- Litter production stimulated by N. - Litter nitrogen concentrations significantly increased at higher N concentrations, with a doubling of the total return of N to the litter layer over the experimental period. - Increased mycorrhizal biomass in the top 0-15 cm in plots receiving 80 kg ha ⁻¹ N (though a recent study suggest no effect on mycorrhizae). Increased microbial biomass with N addition. Increased bacterial use of amino acids, amides and carbohydrate in N enriched plots. - N additions increased soil microbial biomass C. Phosphomonoesterase activity increased with N addition.* - N treatments caused a 3-fold increase in the utilisation rate of C and N substrates in BIOLOG plates. P utilisation rose 10-fold at 80 kg N ha ⁻¹ and 18-fold at 120 kg N ha ⁻¹ - A recent survey suggests a possible toxicity for microbes, and a collapse in the ability of microbes to immobilise incoming N at higher N treatments. - Increasingly higher rates of N retention in organic and mineral layers with increased N up to max. 80 kg N ha ⁻¹ - Small increases in the output of nitrate from the organic layer with low levels of N addition – due to increased rates of nitrification. - Lowering of calcium and calcium/ aluminium ratios in the mineral layer solution, the latter in response to the lowest N treatment. - There was a small absolute increase in total N store in the above-ground vegetation in response to N addition, with most of the added N accumulating in the soil, mainly the surface peat layer. -C:N ratio of litter and rhizosphere peat decreased with increasing treatment, but not below 2cm depth. -Mineralisation of litter and rhizosphere peat with increasing treatment.	- Management burn, reducing above ground biomass by 90%, reduced the N content of the above-ground vegetation by 95%. - Litter layer, however, increased its N content immediately after the burn. - Further increases in N content of litter layer six months after burn. - Some indication of an increase in the mineralisation of litter and peat layers with increasing treatment – possibly related to increased amounts of ash with increasing N. - Overall burn effect on N content was minimal.	-Caporn <i>et al.</i> (1994) -Caporn <i>et al.</i> (1995a) -Caporn <i>et al.</i> (1995b) -Caporn <i>et al.</i> (2000) -Carroll <i>et al.</i> (1999) -Johnson <i>et al.</i> (1996) -Johnson <i>et al.</i> (1998) -Lee and Caporn (1998) -Lee <i>et al.</i> (2000) -Pilkington (2002)

			-Evidence of increased rates of phosphatase activity.		
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2.4. Grassland Ecosystems

Semi-natural grasslands with traditional agricultural use and low nutrient inputs are an important part of the UK landscape; they are also of high conservation value and botanical interest. For instance, grasslands growing on shallow nutrient-poor calcareous soils can typically maintain 30-40 phanerogamic species per m², many of which are rare or endangered (Wilson *et al.* 1995). Studies in The Netherlands have linked the increase of *Brachypodium pinnatum* in several Dutch calcareous grasslands with increased nitrogen deposition. The dominance of this grass is negatively correlated with species-richness and poses a threat to the diversity of these ecosystems (Bobbink and Willems 1987). In a number of sites of nature conservation interest in southern England, invasions of *B. pinnatum* have also occurred despite grazing management (Woodin and Farmer 1993). Despite these observations few experiments have been established in the UK with the objective of observing grassland ecosystem response to realistic deposition inputs.

Nitrogen manipulation experiments on grasslands have been undertaken for a variety of reasons. Most have been concerned with maximising sward yields in permanent pasture or meadows (*e.g.* Williams 1978) or assessing the importance of a range of nutrients on the diversity of the grassland's botanical composition (*e.g.* Kirkham *et al.* 1996). As such, most have used nitrogen fertilisations at high doses, applied once annually. Those studies in which nitrogen was the only nutrient manipulated at a rate realistic of current atmospheric deposition levels ($\leq 50 \text{ Kg ha}^{-1}\text{yr}^{-1}$) have their experimental details listed in Table 2.7. Detailed descriptions of the results of those experiments are given in Table 2.8.

The experiment undertaken in the Peak District by Lee and his collaborators (Lee and Caporn 2001, Johnson *et al.* 1998, Lee and Caporn 1998, Johnson *et al.* 1996, Lee *et al.* 1996, Morecroft *et al.* 1994) is the most comprehensive European study on the effects of long-term atmospheric nitrogen deposition in grassland ecosystems. The experiment, which has been running since 1990, involves three nitrogen deposition levels: 35, 70 and 140 kg ha⁻¹yr⁻¹ as ammonium nitrate, and 140 kg ha⁻¹yr⁻¹ as ammonium sulphate. Although this review is mainly concerned with nitrogen additions that are representative of realistic deposition levels (*i.e.* $\leq 50 \text{ Kg ha}^{-1}\text{yr}^{-1}$), some results for higher deposition rates obtained from this experiment will be discussed as they may provide an insight into longer term effects of more realistic nitrogen loads to this system. Two neighbouring grasslands were chosen for this study: a calcareous *Festuca-Avenula* type grassland and an acidic *Festuca-Agrostis-Galium* type grassland, both with background nitrogen deposition estimated as 19 kg ha⁻¹yr⁻¹.

After the first three years of the study only a number of relatively minor responses were observed (Morecroft *et al.* 1994). Foliar nitrogen concentration was found to increase with increasing nitrogen addition in a number of species. These increases were significant for *Hieracium pilosella*, *Thymus praecox* and *Briza media*, for which foliar nitrogen concentrations were nearly double the control concentrations with the addition of 140 kg ha⁻¹yr⁻¹ of ammonium nitrate (Morecroft *et al.* 1994). Also three years into this experiment, nitrate reductase activity in most vascular plants showed a general increase following ammonium nitrate addition, and activity in the highest treatment (140 kg ha⁻¹yr⁻¹ NH₄NO₃) was more than double that of the controls for a number of species (*Galium saxatile*, *Potentilla erecta*, *Helianthemum nummularium*). In contrast, in the moss

Rhytidiadelphus squarrosus there was decreased nitrate reductase activity and induceability with increasing ammonium nitrate addition (Morecroft *et al.* 1994).

Three years into this experiment, the only changes in botanical composition were the ones observed for the ammonium sulphate treatment ($140 \text{ kg ha}^{-1}\text{yr}^{-1}$) at the acidic grassland, where there was a marked decline in cover of the moss *R. squarrosus*. This may have been due to the rapid acidifying effect of ammonium sulphate (Morecroft *et al.* 1994). In the following years of the experiment, more substantial effects of nitrogen addition on mosses were registered, with a general decrease in the abundance of bryophytes in both grasslands, which was significant for all treatments at the acidic site (Lee and Caporn 2001).

Also in the first three years of the study there were no effects of addition of either ammonium nitrate or ammonium sulphate on leaf and shoot growth (Morecroft *et al.* 1994). However, six years after the experiment was started, there was a marked decrease in total vegetation cover, which was significant for all treatments in both grasslands. The most dramatic decline in vegetation cover (34%) was observed at the calcareous grassland with the $140 \text{ Kg (NH}_4\text{)}_2\text{SO}_4 \text{ ha}^{-1} \text{ yr}^{-1}$ treatment (Johnson *et al.* 1998). Further on, ten years into the experiment, there was an increase in the dominance of grasses, such as *Koeleria macrantha* and *Festuca ovina* at the calcareous site, with the increase being significant for the latter species (Lee and Caporn 2001). Additionally, a decrease in the abundance of some forb species was reported in both grasslands, most noticeably for *Thymus polytrichus* and *Helianthemum nummularium* at the calcareous site, and *Galium saxatile* and *Potentilla erecta* at the acidic site (Lee and Caporn 2001).

At the calcareous site, in the first three years, plant growth (measured as sward height) appeared to be co-limited by phosphorus, since combined addition of nitrogen and phosphorus increased sward height (Morecroft *et al.* 1994). This tendency was sustained in the following seven years (Lee and Caporn 2001). However, at the acidic site, interaction with phosphorus resulted in a trend for reduction in grass growth as well as for the forb *Rumex acetosa* (Lee and Caporn 2001). Various effects were also observed in terms of soil chemistry and bacterial and fungal activities:

1. Mineralisation rates increased with nitrogen input in a highly significant linear relationship in both grasslands in the first three years of this experiment. There were no consistent differences in mineralisation rates between the ammonium sulphate treatment and the ammonium nitrate treatment applied at the same load ($140 \text{ kg N ha}^{-1}\text{yr}^{-1}$) (Morecroft *et al.* 1994). This result was sustained in the subsequent three years (Johnson *et al.* 1998). At the calcareous site, nitrification rates also increased with nitrogen addition, but at the acidic site nitrification rates were negligible (Morecroft *et al.* 1994).
2. Increasing nitrogen supply resulted in a reduction in the percentage of *Plantago lanceolata* seedlings infected with mycorrhizal arbuscules and this change was associated with an increase in plant nitrogen content (Lee and Caporn 2001).
3. PME activity per weight of microbial biomass carbon (C_{mic}) increased with increasing N deposition; this activity was doubled in the acidic grassland at the lowest treatment ($35 \text{ kg NH}_4\text{NO}_3 \text{ ha}^{-1}\text{yr}^{-1}$) (Johnson *et al.* 1998).
4. Soil bacterial activity was different between sites: at the acid site, bacterial activity was stimulated with ammonium nitrate additions of $35 \text{ kg ha}^{-1}\text{yr}^{-1}$, but was significantly depressed at $140 \text{ kg ha}^{-1}\text{yr}^{-1}$; at the calcareous site nitrogen addition

had no effect on microbial activity. However, in both grasslands nitrogen addition resulted in an increase in the use of amino and carboxylic acids by bacteria, suggesting that nitrogen treatments may have persistent effects on nitrogen cycling in the soil (Lee *et al.* 1996).

5. In the calcareous grassland there was a trend for increasing PME activity with increasing N application, but this effect was only significant at the 140 kg ha⁻¹yr⁻¹ ammonium sulphate treatment where the enzyme activity increased by 69%. In the acidic grassland the only significant effect was at the 140 kg ha⁻¹yr⁻¹ ammonium nitrate treatment (Johnson *et al.* 1998).
6. In the third year of the study, ammonium sulphate was found to lower soil pH at the calcareous site (Morecroft *et al.* 1994). This result was magnified in later years. Six years after the start of the experiment, both nitrogen forms produced an acidifying effect on the soil of the calcareous grassland, although this effect was still more marked with the ammonium sulphate treatment (Johnson *et al.* 1998). Addition of ammonium nitrate resulted in a highly significant trend for increasing soil acidification, which was statistically significant for the individual treatments from 70 kg N ha⁻¹yr⁻¹. There was no significant change in soil pH at the acidic site (Johnson *et al.* 1998).

This study emphasises the need for long term ecosystem manipulation experiments in order for the effects of nitrogen deposition to become apparent. Few changes were observed in the first three years, and plant growth was only affected at high deposition levels of ammonium sulphate (140 kg ha⁻¹yr⁻¹), and that was for only a single moss species. Three years later, six years into the experiment, a number of statistically significant trends with increasing nitrogen deposition were observed in both moss and higher plant species composition.

Several other studies have also included nitrogen additions at rates below 50 kg ha⁻¹yr⁻¹. The Park Grass experiment at Rothamsted, England which was initiated in 1856 as an agricultural trial, is the longest running nutrient addition experiment (Dodd *et al.* 1995, 1994 a,b, Williams 1978). In this experiment, nitrogen was applied in one annual dressing, not as regular applications, such as were used in the Peak District experiment described above. Nitrogen additions of 48, 96 and 144 kg N ha⁻¹yr⁻¹ to these neutral grassland plots has resulted in the development of species-poor, graminoid dominated grassland, dominated by tall, aggressive grass species such as *Arrhenatherum elatius* and *Alopecurus pratensis*. In the Park Grass plots, species diversity is correlated directly with soil acidity and biomass; low numbers of species are found in acid and high biomass plots. As with the Peak District experiment, the addition of ammonium sulphate, through its acidifying affect, caused greater botanical change than the addition of other nitrogen types.

In the Park Grass experiment, bryophyte biomass was significantly reduced in N-addition treatments, although it did not respond differently to different rates of nitrogen addition (Virtanen *et al.* 2000). Additionally, adding nitrogen at the same rate as ammonium nitrate or as ammonium sulphate caused similar responses in bryophyte biomass, species richness and abundance of common species. Different moss species showed specific responses to nitrogen addition: *Bryum subapiculatum*, an ephemeral colonist species, showed no response; the abundance of *Brachythecium rutabulum* decreased linearly with increasing nitrogen deposition rate, being absent from the highest treated plots (144 kg ha⁻¹yr⁻¹); *Eurhynchium praelongum* also declined with increasing nitrogen, but was still present in the plots with the highest nitrogen loads.

Cessation of the fertiliser treatments for eight years lead to a dramatic increase in bryophyte biomass. Besides the direct effect of reduced nitrogen input, this increase is most likely due to a reduction in vascular plant biomass, and consequent increase in light and space, which was recorded in some of these plots.

Nitrogen addition experiments in the Somerset Levels flower-rich meadows (Kirkham *et al.* 1996) have examined whether higher agricultural output can be achieved through the addition of fertiliser with the maintenance of floristic diversity. Nitrogen additions of only 25 kg ha⁻¹yr⁻¹ significantly reduced the species richness of the hay meadows within six years. Nitrogen fertilisation caused increased dominance of the grasses *Bromus hordeaceus*, *Lolium perenne* and *Holcus lanatus*, and reduction in biomass of the original dominants (*Agrostis* spp., *Anthoxanthum odoratum*, *Cynosurus cristatus* and *Festuca rubra*) (Mountford *et al.* 1994). The number of flowering plants of many of the most attractive species of these old wet meadows declined in response to fertilisation. Species that disappeared from all nitrogen treated plots included *Cirsium dissectum*, *Lynchnis flos-cuculi*, *Cardamine pratensis*, *Lotus pedunculatus* and *Filipendula ulmaria* (Tallowin *et al.* 1994).

In a two-year study of Dutch calcareous grasslands, van Dam (1990) found that 96% of additions of 50 kg N ha⁻¹yr⁻¹ applied as a two-weekly spray of ammonium sulphate was retained in the ecosystem through enhanced plant uptake and immobilisation in soil organic matter. Changes in the management regime of grasslands could, therefore, potentially influence both the cycling and accumulation of atmospheric nitrogen deposition. The study of Kirkham *et al.* (1996) examined whether changes in the management regime of the Somerset Levels hay meadows could increase species diversity and reduce graminoid dominance after cessation of nitrogen additions. Three years after nitrogen additions had stopped in a subset of the plots, species richness had recovered to previous levels (*ca.* 30 species/m²) only in the plots which had received 25 kg N ha⁻¹yr⁻¹; in plots which had received 50 kg N ha⁻¹yr⁻¹ or more, significant differences from control plots were still found. Changing the hay cutting date caused no significant increase in species diversity over a two year period in these previously fertilised meadows. Domination of grasses in previously fertilised sites was either maintained or enhanced by early cutting (May) while later cutting (August or later) resulted in an increased quantity of viable grass seed being shed. Experimental disturbance and gap creation in the grass dominated vegetation of these previously fertilised meadows also failed to enhance botanical diversity (Tallowin *et al.* 1994).

In an upland acidic grassland at the ADAS Pwllpeiran research station, Mid Wales, Emmett and collaborators (Emmett *et al.* 2001, Parekh *et al.* 2001) undertook a nitrogen addition experiment in controlled grazing paddocks to investigate the response under different levels of grazing. The dominant plants at this site were *Nardus stricta*, *Vaccinium myrtillus* and *Festuca ovina*. The experiment, which was started in 1997, consisted of two paddocks under different grazing intensities (3 and 6 months/year), where (NH₄)₂SO₄ (10-20 kg ha⁻¹yr⁻¹) and NaNO₃ (20 kg ha⁻¹yr⁻¹) were applied twice a month for 4-5 years (background deposition: 15-26 kg ha⁻¹yr⁻¹). Phosphorus was added once, at 20 kg ha⁻¹ to the 10 kg (NH₄)₂SO₄ ha⁻¹yr⁻¹ treatment. The effects of nitrogen deposition on the various components of the ecosystem were:

1. *Effects on plant tissue chemistry.* A significant increase in tissue N concentration was registered after 15 months in bryophytes, which were particularly sensitive to (NH₄)₂SO₄, specially *Hypnum jutlandicum* and *Rhytidiadelphus loreus*. Addition of NaNO₃ affected only the N:P ratio of *H. jutlandicum* on the second year of the treatment. Tissue chemistry of vascular plants took longer to respond to nitrogen

enhancement, and the responses were less consistent than the ones observed for mosses. *D. flexuosa*, besides showing a response to $(\text{NH}_4)_2\text{SO}_4$, was the only higher plant to show a response to NaNO_3 addition and this appeared to be greater in the heavily grazed paddock. *V. myrtillus* and *N. stricta* did not show consistent changes in tissue chemistry with any form of N addition, in contrast to findings from other experiments (Strengbom *et al.* 2002, Hartley and Amos 1999). The authors conclude that competitive plant interactions can mediate responses of some plants to N inputs and that this will make these species unreliable indicators of N loading.

2. *Effects on vegetation abundance.* Only moss cover approached a significant response to either N form under heavy grazing. A non-significant reduction in abundance was found in *R. loreus*, *Pleurozium schreberi*, *Dicranum* sp. and *Polytrichum formosum* in the heavily grazed paddock, and *Racomitrium lanuginosum*, *P. schreberi*, *Hylocomnium splendens* and *Dicranum* sp in the lightly grazed paddock. In contrast, the mosses *H. jutlandicum* and *P. formosum* showed a trend for increased cover in the lightly grazed paddock. Lichens and *V. myrtillus* showed a tendency for cover reduction under light grazing with the addition of sodium nitrate at $20 \text{ kg ha}^{-1}\text{yr}^{-1}$.
3. *Effects of the interaction with phosphorus.* P addition in combination with ammonium sulphate resulted in increased P uptake by the plants, but there were no significant effects on plant growth or cover. Therefore there is no evidence that the response of the vegetation to N is P limited, although the quick uptake of P is an indication that the system may not be far from becoming P limited, and suggests that there might be growth responses in the longer term.
4. *Effects of the interaction with grazing.* Up until year four, species were generally more sensitive to N enhancement under low grazing, and only in the final year of the study did the heavily grazed paddock start to respond to N addition. This is possibly due to the fact that under heavy grazing, the more N-responsive species also seemed to be the more grazing-sensitive, and were thus absent to start with. Grazing did not affect the rates of internal N cycling or N losses from the system.
5. *Soil water chemistry and soil processes.* There was an increase in nitrate concentrations in both paddocks after one year of N additions. Nitrate leaching increased significantly in NaNO_3 treated plots, which was also higher than in controls. This effect was more marked under light grazing. There were no significant effects of N on net mineralisation and nitrification in either paddock, contrary to findings in the Peak District experiment by Johnson *et al.* (1998). No changes in decomposition rate were observed. $(\text{NH}_4)_2\text{SO}_4$ additions significantly increased sulphate and base cation leaching in the lightly grazed paddock and lowered leachate pH from the upper soil horizons. This, in the longer term, will have implications for acid sensitive plant species, soil nutritional status and drainage water quality.

To summarise the main findings from this study, after five years no major changes in vegetation composition have been recorded following application of 10 and $20 \text{ kg N ha}^{-1}\text{yr}^{-1}$. The first responses were observed after two years of treatment, and both the dose and form of N affected the response. Mosses and lichens were the most affected with a dose dependant decrease in cover. Vegetation favoured N uptake as ammonium rather than nitrate. NaNO_3 addition was detrimental to several species including *Vaccinium myrtillus*. The timescale of responses to nitrogen loading by the different components of

the vegetation was suggested to provide a sequential indicator of increased nitrogen deposition (Emmett *et al.* 2001):

1. Moss chemistry – 1.5 years
2. Damage to *V. myrtillus* with 20 kg NaNO₃ ha⁻¹ yr⁻¹ – 2 years
3. Moss cover - 3-4 years
4. Grass chemistry - 4-5 years.

Results from soil water chemistry analysis indicate that the site was already receiving N in excess of local biota requirements before the fertilisation experiments took place. Nitrate losses were observed within a few months of the start of the treatments, being therefore a good early indicator of increased N deposition in saturated systems. The observed base cation leaching resulted in acidification, as well as a loss of nutrients from the organic horizon. Since this is the main rooting zone, the fall in pH may, in the longer term, cause plant composition changes at the Pwllpeiran site. Leaching losses and changes in plant chemistry appear to be relatively sensitive indicators of N deposition when N is applied at realistic rates.

Summary

Nitrogen manipulation experiments in grasslands have shown a variety of results with important implications for the conservation value of these ecosystems. Changes in species composition, microbial activity and plant and soil chemistry have been found at nitrogen application rates as low as 20 kg ha⁻¹ yr⁻¹ (Emmett *et al.* 2001), 25 kg ha⁻¹ yr⁻¹ (Kirkham *et al.* 1996) and 35 kg ha⁻¹ yr⁻¹ (Johnson *et al.* 1998, Morecroft *et al.* 1994). The composition changes that have been observed at nitrogen deposition rates ≤ 50 kg N ha⁻¹ yr⁻¹ include: decrease in plant diversity, decrease in vegetation cover, reduced lichen and bryophyte abundance, decreased abundance of a number of forbs, dwarf shrubs and non-aggressive grass species, and increased abundance of more aggressive grass species, such as *Arrhenatherum elatius* or *Bromus hordeaceus*. Evidence that nutrient cycling may be affected by the raised levels of nitrogen availability has also been observed; nitrogen mineralisation rates and soil bacterial biomass were both increased by nitrogen inputs. The Peak District study of Lee and collaborators emphasises the need for longer term studies on the effects of increasing rates of atmospheric nitrogen deposition, as changes in plant composition only became apparent after the first six years of the study.

Table 2.7. Grassland nitrogen manipulation experiments undertaken in Europe.

Country	Location	N form	Dose (KgNha ⁻¹ yr ⁻¹)	Frequency of application (times/year)	Duration (yrs)	Other treatment s	Management	Background deposition (KgNha ⁻¹ yr ⁻¹)	Authors
UK	Peak district	(1) NH ₄ NO ₃ (2) (NH ₄) ₂ SO ₄	(1) 35-70-140 (2) 140	26 (1 st year) 12 (other years)	11	Glucose, P	Grazed by: cows: May-Dec sheep: May-June	19	Morecroft <i>et al.</i> 1994 Johnson <i>et al.</i> 1996 Lee & Caporn 1998 Johnson <i>et al.</i> 1998 Lee and Caporn 2001
UK	Rothamst ead (Park Grass)	(1) (NH ₄) ₂ SO ₄ (2) NaNO ₃	(1) 48-96-144 (2) 48-96	1	140	Lime, P, K, Mg, Na	1865-1874: one hay cut in June and afterwards grazed by sheep; 1874-present: hay cut summer/autumn	Variable: 10-45	Williams 1978 Dodd <i>et al.</i> 1994 a,b Dodd <i>et al.</i> 1995
UK	Somerset	NH ₄ NO ₃	25-50	4	6	NPK, P, K, Grazing	Cut and grazed		Mountford <i>et al.</i> 1993 Tallowin <i>et al.</i> 1994 Tallowin & Smith 1994 Mountford <i>et al.</i> 1994 Kirkham <i>et al.</i> 1996
UK	Mid Wales Pwllpeira n	(1) (NH ₄) ₂ SO ₄ (2) NaNO ₃	(1) 10-20 (2) 20	24	5	P, Grazing	Factorial design with two paddocks of different grazing intensity: 3 and 6 months/yr	15-26	Emmett <i>et al.</i> 2001 Parekh <i>et al.</i> 2001
Netherland s	Limburg	NH ₄ NO ₃	1984: 120 1985-86: 50	1	3		Mown annually in autumn and mowings removed	30-40	Bobbink <i>et al.</i> 1988 Bobbink <i>et al.</i> 1991
Netherland s		(NH ₄) ₂ SO ₄	50	104	2			35	Van Dam 1990

Table 2.8. Results of grassland nitrogen manipulation experiments undertaken in Europe. PME – Phosphomonoesterase; NR - Nitrogen reductase; C_{mic} – microbial biomass carbon. In the first study listed, results from the two grassland types are discriminated as: A - results from the calcareous grassland, and B - results from the acidic grassland.

Habitat type	Effects on floristic composition	Effects on plant chemistry/growth	Effects on soil processes/ Fungal infection/ Microbial activity	Management interactions	Authors
(A) Shallow calcareous <i>Festuca-Avenula</i> grassland (B) Acidic <i>Festuca-Agrostis-Galuim</i> grassland	With increasing levels of N addition, the following trends were observed: -marked decrease in total % cover, which was significant for all treatments (A,B); - increase in the dominance of grasses, which was significant for <i>Festuca ovina</i> (A); - decrease in abundance of bryophytes and some forb species (A,B).	- Plant N concentration and NR activity increased in most vascular plants with increasing N addition; NR activity reduced in the moss <i>Rhytidiadelphus squarrosus</i> (A,B) - Interaction with P increased growth of grasses in A (with the exception of <i>Danthonia decumbens</i>), but - Interaction with P shows trend for decrease in grass growth and the herb <i>Rumex acetosa</i> in B.	The following trends corresponded to an increase in N deposition: -reduction in the % of <i>Plantago lanceolata</i> roots with arbuscular mycorrhizal infection (A,B); - increase in PME activity/mg C _{mic} , which doubled control levels in the lowest treatment (B); - highly significant trend for soil acidification; pH reduction significant for $\geq 70\text{KgNH}_4\text{NO}_3\text{ha}^{-1}\text{yr}^{-1}$; most marked effect with $140\text{Kg}(\text{NH}_4)_2\text{SO}_4\text{ha}^{-1}\text{yr}^{-1}$ (A); - highly significant trend for increase in mineralisation rates (A,B); in A nitrification rates followed the same trend, but in B nitrification was not changed; - increase in the use of amino acids and carboxylic acids by bacteria (A, B).		Morecroft <i>et al.</i> 1994 Johnson <i>et al.</i> 1996 Lee & Caporn 1998 Johnson <i>et al.</i> 1998 Lee and Caporn 2001
Neutral-acidic species rich grassland	- Nitrogen application increases biomass and decreases species diversity - Grasses dominate N fertilised plots, especially <i>Arrhenatherum elatius</i> - Bryophyte biomass was reduced by either N-form at all rates of application; <i>Brachythecium rutabulum</i> was most affected, being absent from high-N plots - 8 years after cessation of N-addition lead to great increase in bryophyte biomass		- $(\text{NH}_4)_2\text{SO}_4$ application resulted in acidification		Williams 1978 Dodd <i>et al.</i> 1994 a,b Dodd <i>et al.</i> 1995

Habitat type	Effects on floristic composition	Effects on plant chemistry/growth	Effects on soil chemistry/ Fungal infection/ Microbial activity	Management interactions	Authors
Moorland meadow: <i>Cynosurus cristatus</i> – <i>Centaurea nigra</i> meadow, and <i>Cynosurus cristatus</i> - <i>Caltha palustris</i> flood pasture	- N addition resulted in a species poor sward composed mainly of a few aggressive grasses. - Species richness significantly reduced by 25 kg N ha⁻¹yr⁻¹ treatment - <i>Agrostis canina</i> became dominant in plots receiving 200 kg N ha⁻¹yr⁻¹ - Cessation of N addition caused increases in forbs and grass species after 3 years			- <i>Lolium perenne</i> was not increased by additions of N in the absence of aftermath grazing (grazing after hay cutting) but dominated fertilized plots where aftermath grazing was maintained. - Changing cutting time did not alter species richness recovery rates.	Mountford <i>et al.</i> 1993 Tallowin <i>et al.</i> 1994 Tallowin & Smith 1994 Mountford <i>et al.</i> 1994 Kirkham <i>et al.</i> 1996
Upland acidic <i>Nardus</i> - <i>Vaccinium</i> - <i>Festuca</i> grassland	- Non-significant decrease in moss and lichen cover, as well as for the shrub <i>Vaccinium myrtillus</i>	- Increase of tissue %N in mosses; less consistent response from vascular plants, except for <i>Deschampsia flexuosa</i>	- Increase in soil nitrate concentrations - Nitrate leaching increased significantly in NaNO₃ treated plots, which was also higher than in controls. - (NH₄)₂SO₄ additions significantly increased sulphate and base cation leaching in the lightly grazed paddock and lowered leachate pH from the upper soil horizons.	-Plants were generally more sensitive to N enhancement under low grazing, and only in the final year of the study did the heavily grazed paddock start to respond to N addition -Nitrate leaching was more pronounced in the lightly grazed paddock	Emmett <i>et al.</i> 2001 Parekh <i>et al.</i> 2001

Habitat type	Effects on floristic composition	Effects on plant chemistry/growth	Effects on soil chemistry/ Fungal infection/ Microbial activity	Management interactions	Authors
Calcareous <i>Festuco-Brometea</i> grassland	- Increase in <i>Brachypodium pinnatum</i> possible without enlarged storage of phosphorous in the vegetation - Vertical structure of the vegetation was affected by N addition resulting in reduced light penetration - Decrease in species number	- N:P ratios in <i>B. pinnatum</i> became very high.			Bobbink <i>et al.</i> 1988 Bobbink <i>et al.</i> 1991
Calcareous <i>Festuco-Brometea</i> 70% <i>Brachypodioum pinnatum</i>			- Almost all of SO_4^{2-} applied leached - Leaching of N increased from 2% to 4% of total inputs resulting in N accumulation in the system		Van Dam 1990

2.5. Dune Ecosystems

Dune ecosystems are species rich environments. In many Dutch dune ecosystems which received a nitrogen deposition load of 20-30 kg N ha⁻¹yr⁻¹, grasses have become dominant, and the observed decline in species richness has been attributed to increased nitrogen availability (Bobbink *et al.* 1996). Only one nitrogen manipulation experiment has been carried out in a dune ecosystem specifically to examine the effect of nitrogen deposition (Tables 2.9 and 2.10). This experiment (ten Harkel *et al.* 1998, ten Harkel and van der Meulen 1995) examined, in a factorial design, the effect of nitrogen addition and the interaction with rabbit grazing. After a 4 year study, it was reported that the addition of 25 kg N ha⁻¹yr⁻¹ had no effect on the composition of the dune vegetation when grazed by rabbits. Grazing was found to be the most important factor in determining the botanical composition of the dune ecosystem. The cessation of grazing by horses and rabbits, through the use of enclosures, resulted in graminoid dominance (*Festuca rubra*, *F. ovina* and *Poa pratensis*), especially where nitrogen additions had been made. However, the re-introduction of grazing to these areas increased the species diversity of perennial herbs and reduce graminoid dominance in dune ecosystems after only six months. The lack of effect of nitrogen addition in the foredune was attributed by the authors to the high levels of leaching following application (70%). However, in the innerdune, leaching was relatively low (13%) and therefore cannot explain the lack of effects of fertilisation. The role of phosphorus as the major growth-limiting nutrient in this system, and the short duration of the study were proposed by the authors to explain the lack of N effect.

The effect of nitrogen addition has been examined in the dry and wet dune grasslands of Branton Burrows, UK (Willis 1963); however, the dosage of nitrogen administered was high (127 and 490 kg ha⁻¹yr⁻¹). The high levels of nitrogen addition used by Willis confirm the observations of ten Harkel and van der Meulen (1995), with graminoid dominance being promoted in N treated plots. In Branton Burrows, the grasses *Festuca rubra* and *Poa pratensis* became dominant in the dry dune pasture, whilst *Agrostis stolonifera* became dominant in the dune slacks. Reductions in rosette forms and the elimination of bryophytes and lichens were also observed at these rates of nitrogen addition; this could be a result of initial toxicity to the high level of nitrogen addition. Boorman and Fuller (1982) examined the effects of nutrient addition on the species composition of rabbit grazed dune swards in Holkham, Norfolk. The experiment followed the same protocol as Willis (1963) but with a lower nitrogen dose (80 kg N ha⁻¹yr⁻¹). As with the experiment of Ten Harkel and Van der Meulen (1995), rabbit grazing prevented *Festuca rubra* becoming dominant. In addition, the nitrogen supplements reduced the density of a number of annual species.

Summary

Nitrogen manipulation experiments in dune ecosystems are few and the results of nitrogen addition varied. From these experiments it can be determined that grazing has a large effect on the species composition of these ecosystems. Also, factors like variable levels of leaching and phosphorus limitation may confound the few results available in the literature. The response of dune ecosystems to low levels of atmospheric nitrogen deposition cannot be determined from these experimental data sets.

TABLE 2.9. Dune nitrogen manipulation experiments undertaken in the UK and Europe.

Country	Location	N form	Dose (kg N ha ⁻¹ yr ⁻¹)	Frequency of Application	Time (yrs)	Other Treatments	Background Deposition (kg N ha ⁻¹ yr ⁻¹)	Authors
UK	Braunton Burrows	[1] (NH ₄) ₂ SO ₄ [2] NaNO ₃	[1] 127 [2] 490	Applied in three annual dressings .	3	P, K, Mg.		Willis 1963
UK	Holkham	[1] (NH ₄) ₂ SO ₄ [2] NaNO ₃	80	Applied in three annual dressings .	3	PK, NK, NPK.		Boorman & Fuller 1982
Netherlands	Meijendel	NH ₄ NO ₃	25/50	Added in a spring and autumn dressing.	4	Rabbit/horse exclusions	10	ten Harkel & van der Meulen 1995 ten Harkel <i>et al.</i> 1998

TABLE 2.10. Results of dune nitrogen manipulation experiments (additions <50kg N ha⁻¹yr⁻¹) undertaken in the UK and Europe.

Habitat Type	Effects on floristic composition/Growth	Authors
Coastal dune grasslands (one short species-rich, one short species-poor, one dominated by tall graminoids.	No significant effect of nitrogen addition in the these three grasslands. When grazing is not present the relatively high availability of nitrogen gives graminoids the opportunity to become dominant.	ten Harkel & van der Meulen 1995 ten Harkel <i>et al.</i> 1998

2.6 Bog Ecosystems

Both blanket bog and lowland raised bog are characterised by their peat formation (UK BAP 2001) and their distinctive suite of vegetation types such as *Sphagnum* spp. as well as vascular plants adapted to waterlogged conditions (UK BAP 2001). UK bog ecosystems contain a number of National Vegetation Classification (NVC) types, together with their intermediates (UK BAP 2001).

Ombrotrophic raised bogs receive all of their nutrients from the atmosphere (Bobbink *et al.* 1996), and are traditionally seen as nitrogen limited. They therefore probably belong to the systems most sensitive to nitrogen enrichment (Tomassen *et al.* (2000). The trend for increasing N deposition has important implications for peatland ecosystems (INDITE 1994). Indeed, in some areas, productivity of *Sphagnum* might have changed from being nitrogen limited to phosphorus limited (Aerts *et al.* 1992). Indications of nitrogen eutrophication have been seen in Danish ombrotrophic bogs with a decline of ombrotrophic vegetation and an increase in 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 $\text{ha}^{-1} \text{yr}^{-1}$ (Aaby 1994), together with dry ammonia (NH_3) deposition, which is rapidly deposited to ombrotrophic mires. A number of nitrogen manipulation experiments have been undertaken in bog ecosystems, with the aim of increasing knowledge of their responses to increased levels of atmospheric nitrogen deposition. The experimental details and results are listed in Tables 2.11 and 2.12 respectively.

All of the experiments identified simulate realistic levels of nitrogen deposition, with frequent applications at low application rates ($<50 \text{ kg N ha}^{-1} \text{yr}^{-1}$). The four-year experiment of Redbo-Torstensson (1994), undertaken in Sweden, used a range of nitrogen doses, with the lowest comparable to the background deposition levels at the site (5, 10, 20 and 40 kg N $\text{ha}^{-1} \text{yr}^{-1}$). The demography of *Drosera rotundifolia* was studied, and increased mortality of *D. rotundifolia* was found with increasing nitrogen deposition. After four years, this reduced survivorship was significantly different from controls at nitrogen additions of only 10 kg N $\text{ha}^{-1} \text{yr}^{-1}$, whilst at higher doses (20 to 40 kg N $\text{ha}^{-1} \text{yr}^{-1}$) it was significantly different from controls after only one year of treatment. The higher nitrogen treatments ($>10 \text{ kg N ha}^{-1} \text{yr}^{-1}$) also negatively affected *D. rotundifolia* flowering after two years of treatment. The effect of nitrogen addition on a number of other bog species was noted, with both *Andromeda polifolia* and *Eriophorum vaginatum* increasing in density significantly with increased nitrogen. The increased density of these tall species may influence the survival of *D. rotundifolia*, through increased competition for light and decreased opportunities for seedling establishment.

The decline of *Sphagnum* moss species has been observed in a number of bog ecosystems in the UK and Europe. A study undertaken by Hogg *et al.* (1995) in a small valley mire near York in the UK used a factorial design to investigate the effect of both nitrogen addition and the cutting of the dominant grass species *Molinia caerulea* on the growth of *Sphagnum* species. The growth of the mosses *Sphagnum palustre* and *Sphagnum fimbriatum* was reduced by 50% by the addition of 12 kg N $\text{ha}^{-1} \text{yr}^{-1}$; levels considerably below those entering the site as atmospheric deposition. Where *Sphagnum* was growing poorly and *M. caerulea* was abundant, adding nitrogen had no effect, but cutting *M. caerulea* in the summer was beneficial to *Sphagnum*, re-invigorating its growth.

A German nitrogen addition experiment also resulted in negative effects on the established *Sphagnum* community (Lütke Twenhöven 1992). In this study rainwater was applied to the field site with additional ammonium or nitrate. 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 ($15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) 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. On hummocks in the bog, nitrate and, to a lesser extent, ammonium reduced the growth of both these species.

The Swedish study of Aerts *et al.* (1992) also noted *Sphagnum* growth responses to nitrogen addition; *S. balticum* increased growth (four-fold) following addition of nitrogen (20 and $40 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) to sites with low background deposition levels ($0.6\text{--}2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), whilst no effect was found at sites with high background deposition levels ($7\text{--}9 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). The *Sphagnum* species at the site with high background atmospheric nitrogen deposition rates were phosphorus limited. Aerts *et al.* (1992) conclude that high atmospheric nitrogen supply may affect the carbon balance of ombrotrophic bogs, because productivity under these circumstances is not nitrogen limited, but decomposition is probably increased by the high nitrogen loads. These systems may eventually become carbon-emitting systems, rather than carbon accumulating systems.

In another Swedish study (Nordin & Gunnarsson 2000), a two-year field manipulation experiment was set up at Åkhultmyren and Luttumyren mires in the summer of 1996, in which N availability for *Sphagnum* was manipulated by the addition of NH_4NO_3 ($0, 10, 30, 50$ and $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) to background depositions which differed between the two mires. The aims were to test (a) that there is an upper limit of *Sphagnum* amino acid concentrations above which negative effects of *Sphagnum* growth may occur and (b) that *Sphagnum* amino acid concentrations can be used to indicate current levels of N deposition. The experimental addition of nitrogen increased free amino acid concentrations in *Sphagnum capitula*, whereas it decreased *Sphagnum* extension growth. At natural N deposition rates (lower than $7\text{--}11 \text{ kg ha}^{-1} \text{ yr}^{-1}$), however, there was no correlation observed with *Sphagnum* total amino-acid N concentrations. In general, when *Sphagnum* amino acid N concentrations exceeded $2.0 \text{ mg amino acid N g}^{-1} \text{ dry mass}$, *Sphagnum* extension growth was reduced (mainly when the moss was treated with 50 and $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). However, the decreased growth did not fully explain the variation in amino acid concentrations; hence increased *Sphagnum* N assimilation in N treated plots was most likely to be the factor causing tissue amino acid concentrations to increase. There were no significant differences in *Sphagnum* total amino acid concentrations between control plots at the two mires, suggesting that this parameter is not sensitive enough to reflect differences in N deposition rates when they are below $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Nordin & Gunnarsson 2000).

Recently a series of experiments were conducted in Scotland to investigate: (a) whether atmospheric N deposition that enters the moss vegetation influences N dynamics in the underlying peat (Williams *et al.* 1999); (b) N deposition impacts on the peat beneath the vegetation, via the plants, by examining the impact on microbial biomass C and N and extractable dissolved organic matter (Williams and Silcock 2000); and (c) whether nitrogen addition to raised bogs influences peat phosphorus pools (Williams and Silcock 2001). For these experiments nitrogen manipulation plots were set up in June 1994 on a raised bog, the Moidach More, in the north-east of Scotland (further details from Williams *et al.* 1999). The bryophytes *Sphagnum capillifolium* and *Sphagnum recurvum* were chosen for all three experiments because they colonised contrasting sites; *S.*

capillifolium is a hummock-forming species whereas *S. recurvum* occupies hollows and pools. Treatment plots were set up for each species with half the plots receiving fortnightly doses of deionised water and half receiving 30 kg N ha⁻¹ yr⁻¹ in the form of NH₄NO₃. Two weeks before each harvest the plots received labelled N in the form of ¹⁵NH₄¹⁵NO₃.

The addition of nitrogen increased the total N concentration in the mosses, resulting in a decrease in C:N ratios (Williams *et al.* 1999), which could enhance rates of organic matter decomposition and N mineralisation (Aerts *et al.* 1992). The labelled N was shown to disappear rapidly (a matter of days); the proportion of added ¹⁵N taken up by the mosses two weeks after each addition averaged 72% and ranged between 11% and 100%. Dissolved organic nitrogen in the moss water associated with *S. capillifolium* was proportional to the quantity of added N, suggesting an accumulation of amino acids in the mosses receiving N. 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.

The use of ¹⁵N demonstrates the temporal and spatial variations in the capacity of bog vegetation to assimilate atmospherically derived NH₄NO₃ and to release it as dissolved organic N (Williams *et al.* 1999). Additions of NH₄NO₃ to cores of *S. capillifolium* and *S. recurvum*, although captured mainly by the moss, had significant effects on the microbial biomass C and N values in the underlying peat. These effects did not involve the production of new biomass, but appeared to increase its stability during the autumn following increased rainfall and rising water table levels. Fluctuations in the C:N ratios of the dissolved organic matter indicated that during the summer months low molecular weight amino acids contributed to the dissolved organic matter pool, which became less rich in N and qualitatively different during the winter. There were no obvious relationships between the elevated levels of microbial N and C and the dynamics of C and N in the dissolved organic matter pool (Williams & Silcock 2000).

The addition of NH₄NO₃ was shown to both increase and decrease the amounts of extractable total dissolved phosphate in the water hollows of a raised peat bog, but had less impact on drier sites. The increase may have been the result of increasing phosphatase activity associated with *Sphagnum*, although increased microbial immobilisation of P may have caused the decrease in total dissolved P. Organic P was an important component of the extractable total dissolved P and the C:P ratio differed between the sites and decreased in response to N addition. Microbial biomass P was a significant pool of P at both sites, but showed little influence of N addition or *Sphagnum* species (Williams and Silcock 2001).

Although not strictly within the remit of this review, i.e., *in-situ* ecosystem nitrogen manipulation, an important study by Risager (1998) examined growth responses of two *Sphagnum* species to different forms of nitrogen (NO₃⁻, NH₄⁺ or NH₄NO₃) at very low doses (0, 5, 10 and 20 kg N ha⁻¹ yr⁻¹). Plant material was collected from a raised bog and transplanted into 40 cylinders, which were placed in two controlled-environment growth cabinets. NO₃-N was supplied as HNO₃; NH₄-N was supplied as NH₄Cl and NH₄NO₃-N was supplied as NH₄NO₃. Treatments were applied on a weekly basis for fifteen weeks. Even with very low amounts of nitrogen supplied in this experiment there were significant effects on growth in length and tissue nitrogen concentration of *Sphagnum fallax*. Growth was primarily influenced in *S. fallax* by application of NH₄⁺ with increases at 5 and 10 kg NH₄-N ha⁻¹ yr⁻¹ but this was not reflected in the tissue nitrogen concentration.

Risager (1998) observed that tissue nitrogen concentration of *S. fallax* declined after addition of nitrogen rather than increase, possibly because the plants received more nitrogen in the field prior to the experiment and possibly because of growth dilution. *S. magellanicum* showed no increase in growth in length after addition of $\text{NH}_4\text{NO}_3\text{-N}$, but addition of nitrogen decreased the production density in the capitulum. 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) concludes that both type and amount of nitrogen is important where species response is concerned, and that increased nitrogen availability may cause shifts in species composition in favour of *S. fallax*.

Summary

Even though there are a limited number of long-term nitrogen manipulation experiments in bog ecosystems, a clear picture is now emerging of the potential impact of elevated nitrogen deposition on bog habitats. Positive responses to nitrogen addition as low as $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ have been observed in a number of bog species (in terms of survivorship, flowering, and density). However, the bryophyte communities, in particular *Sphagnum* spp., appear to be susceptible to the rise in anthropogenic nitrogen pollution, showing a decline in favour of grass species, changes in the established *Sphagnum* community, and changes in physiological and biochemical characteristics. It is also clear that N dynamics and microbial biomass are also affected by elevated nitrogen deposition with important consequences for nutrient cycling within bog ecosystems. Further long-term nitrogen manipulation studies, however, are essential since more comprehensive data are required for a detailed assessment of the implications of increased nitrogen deposition for these ecosystems.

Table 2.11. Bog nitrogen manipulation experiments undertaken. The location of the experiment, the nitrogen species and dose added are shown, along with the frequency of application, the number of years the experimental additions were made, any additional treatments and the background atmospheric nitrogen deposition in the experimental location where known.

Country	Location	N form	Dose (kgNha ⁻¹ yr ⁻¹)	Frequency of application	Duration (yrs)	Other treatments	Management	Background deposition (kgNha ⁻¹ yr ⁻¹)	Authors
UK	Yorkshire	NH ₄ NO ₃	12	Applied in two-monthly dressings as a spray.	2	Cutting of <i>Molinia</i> .	-	-	Hogg <i>et al.</i> (1995)
Sweden	Tullgarn	NH ₄ NO ₃	5/10/20/40	Applied as five dressings annually	4	-	-	5	Redbo-Torstensson (1994)
(a) Southern Sweden (b) Northern Sweden	(a) Akhult mire (b) Stordalen mire	NH ₄ NO ₃	20/40	Applies as four (b) or eight (a) dressings a year in solution.	1	P	-	(a) 7-9 (b) 0.6-2	Aerts <i>et al.</i> (1992)
Germany	Felmer Moor	NH ₄ ⁺ /NO ₃ ⁻	[1] NO ₃ ⁻ : 10.5, NH ₄ ⁺ : 4.5 [2] NO ₃ ⁻ : 2.0, NH ₄ ⁺ : 13.0	Solutions added to deionised rain water.	2	-	-	-	Lütke Twenhöven (1992)
UK	Moidach More, north-east Scotland	NH ₄ NO ₃	0 (deionised water)/30	Applied at fortnightly intervals in 200cm ³ aliquots using a syringe.	7	¹⁵ N labelled NH ₄ NO ₃	Vascular plants are removed from each core.	-	Williams <i>et al.</i> (1999) Williams & Silcock (2000) Williams & Silcock (2001)
Sweden	[1] Åkhultmyren and [2]Luttumyren mires.	NH ₄ NO ₃	0/10/30/50/100	In solution with distilled water applied on five different occasions between June and September.	2	-	-	[1] 7-11 [2] 3-4	Nordin & Gunnarsson (2000)

Table 2.12 Results of bog nitrogen manipulation experiments.

Habitat type	Effects on floristic composition/ plant growth	Effects on plant chemistry	Effects on soil chemistry/ Fungal infection/ Microbial activity	Authors
Ombrotrophic <i>Sphagnum-Molinia</i> bog.	- <i>Sphagnum</i> was inhibited by N addition. Where <i>Sphagnum</i> was growing poorly and <i>Molinia</i> abundant, adding N had no effect, but cutting <i>Molinia</i> was beneficial to the <i>Sphagnum</i> .	-	-	Hogg <i>et al.</i> (1995)
Ombrotrophic raised bog. <i>Drosera rotundifolia</i> growing on hummocks of <i>Sphagnum fuscum</i> and <i>S. rubellum</i> with <i>Eriophorum vaginatum</i> , <i>Andromeda polifolia</i> and <i>Vaccinium oxycoccus</i> .	- In plots treated with >10 kg N ha ⁻¹ decreases in the <i>D. rotundifolia</i> population size, caused partly by increased competition for light by tall species (<i>Eriophorum</i> and <i>Andromeda</i>). The proportion of flowering <i>D. rotundifolia</i> plants increased in the second year of treatment and then gradually decreased compared to control plots. Recruitment of <i>D. rotundifolia</i> was also reduced when N additions were greater than 10 kg N ha ⁻¹ .	-	-	Redbo-Torstensson (1994)
Two <i>Sphagnum</i> dominated ombrotrophic raised bogs [1] high and [2] low deposition.	- Four-fold increase in <i>Sphagnum</i> productivity at low background N site after N addition but no phosphorous effect. At the high background N site no nitrogen addition effect, but P effects.	-	-	Aerts <i>et al.</i> (1992)
Ombrotrophic <i>Erico-Sphagnum</i> mire.	- <i>Sphagnum magellanicum</i> : No ammonium treatment effect. Decrease in abundance with nitrate addition. - <i>S. fallax</i> : Increase in abundance with ammonium, decrease with nitrate.	-	-	Lütke Twenhöven (1992)

Habitat type	Effects on floristic composition/ plant growth	Effects on plant chemistry	Effects on soil chemistry/ Fungal infection/ Microbial activity	Authors
Sphagnum-dominated Raised bog: [1] <i>S. capillifolium</i> -forming hummocks [2] <i>S. recurvum</i> in hollows and pools.	-	- On average 72% of applied N taken up by <i>Sphagnum</i> . - Both mosses showed slightly reduced C:N ratio.	- 0.03% of applied N detected as DON in moss waters (smaller in winter). - Fluctuations in the C:N ratios of the DOM from low molecular weight amino acids contributing to the DOM pool in the summer. - Additional N both increased the amount of TDP (possibly from increased phosphatase activity of <i>Sphagnum</i>) and decreased amount of TDP (possibly from increased microbial immobilization of P) in the wetter hollows. - C:P ratio decreased in response to N addition.	Williams <i>et al.</i> (1999) Williams & Silcock (2000) Williams & Silcock (2001)
<i>Sphagnum</i> -dominated mires.	- <i>Sphagnum</i> growth decreased with nitrogen addition. - It was found that when amino acid N concentrations in <i>Sphagnum</i> exceeded 2.0 mg amino acid N g ⁻¹ dry mass, <i>Sphagnum</i> length growth was reduced.	- Nitrogen additions increased amino acid concentrations in <i>Sphagnum capitula</i> , most likely due to increased N assimilation.	-	Nordin & Gunnarsson (2000)

2.7. Fen Ecosystems

Although the two only published nitrogen manipulation experiments in fen ecosystems report responses to nitrogen application rates above $50 \text{ kg ha}^{-1}\text{yr}^{-1}$, they are nonetheless included in this review, since they provide the only indication of effects of nitrogen in these ecosystems.

Vermeer (1986) carried out an experiment involving the addition of a very high dosage of nitrogen ($450 \text{ kg ha}^{-1}\text{yr}^{-1}$) for a relatively short duration (1 year). The ecosystem response to this high level of nitrogen application included a variety of compositional changes, a general reduction in diversity and an increase in vegetation biomass (Table 2.14.). Bergamini and Pauli (2001) reported a significant decrease in bryophyte biomass (38%) and species diversity (9%) after adding $100 \text{ kg N ha}^{-1}\text{yr}^{-1}$ to 18 calcareous fen (nutrient poor) sites in Switzerland for two years. This result may be partly due to the strong increase in vascular plant biomass in fertilised plots and associated shading of the bryophyte layer, particularly since only the shade intolerant *Bryum preusotriquetrum* declined significantly (Bergamini and Pauli 2001).

TABLE 2.13. Fen nitrogen manipulation experiments undertaken in the UK and Europe. The location of the experiment, the nitrogen species and dose added are shown, along with the frequency of application, the number of years the experimental additions were made, any additional treatments and the background atmospheric nitrogen deposition in the experimental location where known.

Country	Location	N form	Dose (kg N ha ⁻¹ yr ⁻¹)	Frequency of Application (times/yr)	Duration (yrs)	Other Treatments	Background Deposition (kg N ha ⁻¹ yr ⁻¹)	Authors
The Netherlands	Vechtplassen	NH ₄ NO ₃	450	1	1			- Vermeer 1986
Switzerland	18 sites in north-eastern and central Switzerland	NH ₄ NO ₃	100	1	2	P,K	12-30	- Bergamini & Pauli 2001

TABLE 2.14. Results of fen nitrogen manipulation experiments undertaken in the UK and Europe.

Habitat Type	Results	Authors
Fen. Dominant species include <i>Equisetum fluviatile</i> , <i>Pedicularis palustris</i> , <i>Carex diandra</i> and <i>C. lasiocarpa</i> .	-Increase in above-ground biomass when fertilized. -Increase in the proportion of the grasses <i>Holcus lanatus</i> and <i>Agrostis stolonifera</i> , increase in the sedges <i>Carex diandra</i> and <i>C. lasiocarpa</i> biomass. Decrease in <i>Equisetum fluviatile</i> biomass. -Overall decrease in species diversity.	- Vermeer 1986
Calcareous, nutrient poor fen	-Significant decrease in bryophyte biomass (38%) and species density (9%), most marked for the shade intolerant <i>Bryum preusotriquetrum</i> -Increase in vascular plant biomass in fertilised plots	- Bergamini & Pauli 2001

2.8 Tundra Ecosystems

Tundra ecosystems are the coldest of all the biomes and are characterised by short growing seasons, low precipitation, low nutrient levels (dead organic material functions as the main nutrient pool) and extremely low temperatures. These ecosystems are typically nitrogen deficient (Robinson and Wookey 1997, Chapin 1996), with the major inorganic form of this element being ammonium (Michelsen *et al.* 1999, Robinson and Wookey 1997), although arctic species have been shown to grow successfully on nitrate as well as ammonium, e.g., *Dryas integrifolia* and *Oxyria digyna* (Atkin and Cummins 1994). Despite the constraints, there are a number of varieties of plants 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 varieties of flowers.

Tundra is separated into two types:

1. Arctic Tundra - located in the northern hemisphere, encircling the North Pole and extending south to the coniferous forests of the taiga. Permafrost prevents root penetration, and often keeps ground water-logged in summer (and so this biome grades into those found in wetlands); much organic matter is present in the ground because decomposition is slow;
2. Alpine Tundra - Alpine tundra is located on mountains throughout the world at high altitude where trees cannot grow and, unlike the arctic tundra, the soil is usually well drained. It is typically hot and dry in summer (grading into desert biome) or wet at mid-levels (grading into 'wet Heathland' biome).

Large 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). A summary of experiments in tundra ecosystems and their results are given in Tables 2.15 and 2.16 respectively. There have been numerous field manipulation studies with nutrients in tundra ecosystems; however, most have involved NPK fertilizer additions (e.g. Press *et al.* 1998b, Robinson *et al.* 1998, Parsons *et al.* 1994, Havström *et al.* 1993) or single large applications of nitrogen (e.g. Shaver and Chapin 1995).

The experiment of Henry *et al.* (1986) examined the effect of nitrogen addition to three Canadian tundra communities; wet sedge meadow, a mosaic *Cassiope tetragona* heath, and a dry-mesic *Dryas integrifolia* heath. Nitrogen was applied as a once only, single application of ammonium nitrate at doses of 0, 50 and 250 kg ha⁻¹. In the initial post-treatment growing season, flower phenology and density were not affected by nitrogen addition. However, flowering of major forb species (*Cerastium alpinum*, *Draba* spp., *Oxyria digyna* and *Papaver lapponicum*) was 5-7 days earlier and continued longer with the addition of 250 kg ha⁻¹ in the second and third growing season. Dwarf shrub and graminoid flowering was not affected by any treatments. In the mesic *C. tetragona* heath, a decrease in the green and total *C. tetragona* standing crop occurred with additions of 250 kg ha⁻¹ as a result of the death of individual plants. The density of *Eriophorum angustifolium* in the wet sedge meadow increased significantly in the third year after the

addition of 250 kg ha⁻¹. No ecosystem responses were observed by Henry *et al.* (1986) after the application of 50 kg ha⁻¹.

In contrast, in a Swedish study of tundra ecosystems with a similar species compliment, Baddeley *et al.* (1994) observed ecosystem responses at lower nitrogen application rates. With the addition of 10 or 50 kg N ha⁻¹ yr⁻¹, applied as five annual dressings of ammonium nitrate, *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 increased nitrogen supply on these plots in high Arctic heath, with particular attention to the bryophyte communities. The experimental site was located in mixed tundra heath near Ny-Ålesund, Svalbard (Sweden) and had been established in 1991. Plots were located in each of three tundra heath vegetation types, each dominated by one of the dwarf shrubs; *Dryas octopetala* L., *Salix polaris* Wahlenb. or *Cassiope tetragona* (L.) D. Don. Heath communities received factorial combinations of nitrogen (0, 10 and 50 kg N ha⁻¹ yr⁻¹). *Dryas* plots received nitrogen treatments every year from 1991 to 1998, *Salix* plots from 1991 to 1997 and *Cassiope* plots from 1991 to 1993 only. Treatments were watered on in four or five applications each summer, fortnightly, from mid-late June.

The main observations for bryophyte communities, recorded for nitrogen addition levels as low as 10kg N ha⁻¹ yr⁻¹, were as follows:

- 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.
- However, nitrate reductase activity was inhibited in *Polytrichum juniperinum* by both the low and high N treatments, whilst that of *Dicranum scoparium* was reduced by the high N treatment only, suggesting that the bryophytes are becoming nitrogen saturated. This saturation has the potential for inorganic nitrogen to pass through the bryophyte layer, thus becoming available for soil microbes and higher plants, and potentially being lost from the system through leaching and N₂O emission.
- Nitrogen, to a small extent, increased the proportion of green bryophyte shoots, thus apparently increasing potential total bryophyte productivity.
- The increased sucrose content of species such as *Polytrichum juniperinum* provides evidence for increased carbon assimilation.
- Persistence of increased 'greenness' of the bryophyte cover in the fertilised *Cassiope* heath plots 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 N and P was demonstrated by Gordon *et al.* (2001), illustrated by the changes in *P. juniperinum* cover and the ordination of the species cover data for the *Dryas* heath plots which showed a distinct separation of those plots receiving both nitrogen and phosphorus. The availability of both nutrients, as indicated by bryophyte tissue contents, was strongly related to the main axis of variation. Species number was also increased only by the combination of low nitrogen levels and phosphorus (Gordon *et al.* 2001).

In terms of other impacts, nitrogen addition had no significant effect on the cover of live vegetation in the *Dryas* heath. The amount of plant litter was increased, although this may have been the result of an unusually warm wet autumn in 1993-94. Lichens showed a significant response to nitrogen, suffering a considerable loss of cover when nitrogen was added. Although nitrogen may have a directly damaging effect on the lichen, fertilized lichens may be preferentially grazed by reindeer. Lichens account for a high percentage of the reindeer's winter food and this experimental site is subject to grazing (Gordon *et al.* 2001).

In the UK, there has been a serious decline in the bryophyte cover of *Racomitrium* heath in recent decades (Pearce and van der Wal 2002). Increasing rates of nitrogen deposition as a result of increased anthropogenic emissions may be one of the main factors involved in this deterioration of *Racomitrium* heath (Thompson and Baddeley, 1991), although the trend for nitrogen deposition across Europe is currently downward. However, a long-term manipulation study by Jónsdóttir *et al.* (1995), where low levels of nitrogen addition ($4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) were applied over a period of three years, found no reduction in growth of *Racomitrium*.

Pearce and van der Wal (2002) set up an experiment in the north-east Scottish Highlands within montane *Racomitrium lanuginosum* – *Carex bigelowii* heath whereby plots on the summit were subject to experimentally increased low ($10 \text{ kg ha}^{-1} \text{ yr}^{-1}$) and high ($40 \text{ kg ha}^{-1} \text{ yr}^{-1}$) nitrogen input in both reactive forms (NO_3^- and NH_4^+) for a period of two summer seasons. *Racomitrium* was shown to be extremely sensitive to even low doses of N deposition. When subjected to the low dose it responded with a raised tissue N content and severely reduced shoot growth. Higher doses caused even stronger growth reduction. A decrease of at least 58% in the treated plots and a severe inhibition of nitrate reductase activity relative to the controls demonstrated that increases in atmospheric N are likely to have deleterious effects on annual growth. Of all the measurements, only nitrate reductase showed a distinction between NO_3^- and NH_4^+ application. It was also observed that at the end of the growing season, tissue N content was greater in the low dose plots than in the high dose plots. This may reflect physiological damage caused by the raised N supply. After only two growing seasons, Pearce and van der Wal (2002) also demonstrated how quickly *Racomitrium* is replaced by graminoid species, which can utilise the excess nitrogen - having consequences for interspecific competition, particularly through competition for light. Jonasson *et al.* (1999) demonstrated a similar increase in graminoids at the expense of bryophytes. This change in species is likely to have further consequences for nutrient cycling. As stems of moss with elevated N levels eventually decompose, an even greater supply of nitrogen may then become available to higher plants (Aerts, 1999), further favouring graminoids, and also attracting herbivores, adding grazing pressure and further fertilization.

An extensive series of high dose ($250 \text{ kg ha}^{-1} \text{ yr}^{-1}$), one application, nitrogen manipulations have been undertaken in northern and central Alaska (Shaver & Chapin, 1995). A variety of responses were found, depending on site and species. Increases in foliar nitrogen content, shoot and tiller mass, and flowering were observed in the

dominant sedge species (*Eriophorum vaginatum*, *E. angustifolium* and *Carex aquatilis*). The studies examined both wet sedge and moist tussock tundra sites; however, no consistent differences in ecosystem responses between wet and moist sites were observed. Shaver and Chapin (1995) describe a common sequence of events; increased N content in leaves in year 1, increased tiller number in year 2 and increased inflorescence number in year 3.

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 $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Changes in plant community composition (favouring graminoids), plant growth, physiology and chemistry have been observed, as has a decrease in lichen cover. A further range of responses have been observed within the bryophyte communities, such as changes in the relative abundance of different species, tissue nutrient status, and nitrate assimilation capacity, which have significant consequences for nutrient cycling within the whole ecosystem. Further research is required on these responses, since a) bryophytes exhibit such dominance in the Arctic; b) *Racomitrium* heath is a threatened community in Britain (Ratcliffe and Thompson, 1998); c) the recovery potential of bryophytes could be slow, and d) bryophytes have important ecological functions within the tundra ecosystem (Gordon *et al.*, 2001). In particular, the impact of nitrogen deposition on the responses of individual bryophyte and lichen species should be further examined to ascertain why, even though some species may benefit from increased nitrogen supply, many suffer a decline. To a large extent, however, the response to atmospheric nitrogen within the tundra ecosystem may well depend on other factors such as the phosphorus status of the community due to the occurrence of co-limitation (Gordon *et al.*, 2001) of these two nutrients. There is also a knowledge gap in the understanding of the effects of nitrogen cycling and soil processes within the ecosystem, which requires further research.

Table 2.15. Tundra nitrogen manipulation experiments undertaken. The location of the experiment, the nitrogen species and dose added are shown, along with the frequency of application, the number of years the experimental additions were made, any additional treatments and the background atmospheric nitrogen deposition in the experimental location where known.

Country	Location	N form	Dose (kgNha ⁻¹ yr ⁻¹)	Frequency of application (times/year)	Duration (yrs)	Other treatments	Management	Background deposition (kgNha ⁻¹ yr ⁻¹)	Authors
Sweden	Ny Ålesund	NH ₄ NO ₃	0/10/50 Unwatered controls	Applied in five dressings per season at fortnightly intervals.	7	P and K	-	-	Baddeley <i>et al.</i> (1994) Gordon <i>et al.</i> (2001)
Canada	Alexandra Fiord	NH ₄ NO ₃	0/50/250	Applied once (1980) in solution.	3	NPK, Irrigation.	-	-	Henry <i>et al.</i> (1986)
USA	Eagle Creek, Brooks Range, Northern and central Alaska.	NH ₄ NO ₃	250	Applied once only.	20	NPK, Lime, Sugar.	-	-	Shaver and Chapin (1980) Chapin and Shaver (1985) Shaver and Chapin (1995)
UK	Glas Maol, Grampian Mountains, Scotland	KNO ₃ and NH ₄ CL	0/10/40	Applied three (1998) or four (1999) times in June and July.	Two summer seasons	-	Grazed predominantly by mountain hares throughout the year and by sheep and red deer in summer.	12	Pearce and van der Wal (2002)
Iceland	Thingvellir National Park	NH ₄ Cl, NaNO ₃ or NH ₄ NO ₃	0/4	Applied 7-12 times during a growing season.	Three growing seasons (1989-1991)	-	-	0.68 – 6.10	Jónsdóttir <i>et al.</i> (1995)

Table 2.16. Results of tundra nitrogen manipulation experiments undertaken in the UK and Europe.

Habitat type	Effects on floristic composition	Effects on plant chemistry/growth	Effects on soil chemistry/ Fungal infection/ Microbial activity	Authors
Mixed tundra heath.	- Net bryophyte cover was unaffected – may be the result of individual species reacting in different ways. - Bryophyte flora became dominated by <i>Polytrichum juniperinum</i> and <i>Pohlia wahlengbergii</i>, whilst cover of the other dominant species, <i>Dicranum scoparium</i>, was reduced. - Considerable loss of lichen cover with increasing nitrogen supply.	- Increase in <i>Salix polaris</i> leaf N content, biomass and photosynthetic rate. No effect on <i>Cassiope tetragonal</i>, whilst <i>Dryas octopetala</i> response was intermediate. - Increased plant litter (faster turnover of plant material). - Increased proportion of green bryophyte shoots, thus increased potential bryophyte productivity. - Extended growing season. - Increased C assimilation in <i>P. juniperinum</i>. Slight increase in <i>D. scoparium</i>. - Increase in tissue N concentration of both <i>D. scoparium</i> and <i>P. juniperinum</i>. - Nitrate reductase activity was inhibited by both high and low N treatments in <i>P. juniperinum</i> and by the high N treatments in <i>D. scoparium</i>. - Physiological N saturation is suggested at just 10 kg N ha⁻¹ yr⁻¹ for both these bryophyte species.	- N saturation observed in bryophytes, <i>P. juniperinum</i> and <i>D. scoparium</i> means that inorganic N is likely to pass through the bryophyte vegetation becoming available for soil microbes.	Baddeley <i>et al.</i> (1994) Gordon <i>et al.</i> (2001)
Three tundra communities (wet sedge meadow, mesic <i>Cassiope</i> heath and dry-mesic <i>Dryas</i> community)	-	- Response to N greatest in driest community resulting in increased inflorescence density in dicots and some graminoids, increased tiller density in wet sedge species and increased net productivity of graminoids and forbs at highest N application.	-	Henry <i>et al.</i> (1986)
Wet sedge and moist tussock grass tundra.	-	- Increases in foliar nitrogen content, shoot and tiller mass, and flowering in the dominant sedge species (<i>Eriophorum vaginatum</i>, <i>E. angustifolium</i>, and <i>Carex aquatilis</i>).	-	Shaver and Chapin (1995)

Habitat type	Effects on floristic composition	Effects on plant chemistry/growth	Effects on soil chemistry/ Fungal infection/ Microbial activity	Authors
Montane heath dominated by <i>Racomitrium lanuginosum</i> .	- Reduction in the percentage cover of <i>Racomitrium</i>. - Graminoids significantly increased at the expense of <i>Racomitrium</i>.	- <i>Racomitrium</i> responded to the low N dose with a raised tissue N content and severely reduced shoot growth. Higher doses of N caused an even stronger growth reduction. - At the end of the growing season, <i>Racomitrium</i> tissue N in low dose plots was greater than in high dose plots – possibly reflecting physiological damage caused by raised N supply. - Greater uptake of reduced over oxidised form of N causing inhibition of the nitrate reductase enzyme.	-	Pearce and van der Wal (2002)
Habitat type	Effects on floristic composition	Effects on plant chemistry/growth	Effects on soil chemistry/ Fungal infection/ Microbial activity	Authors
Well drained, post glacial lava field. A <i>Racomitrium lanuginosum</i> – <i>Carex bigelowii</i> moss-heath.	-	- Only slight response of the moss to the N treatments in terms of growth in the first period. Significant differences were found in two later periods. - Shoot density in control plots was significantly lower than the mean for all plots with N additions. - N concentrations of the uppermost 2 cm of moss shoots were greater in the plots receiving NH_4^+ - N and NH_4NO_3 treatments but not in those with the NO_3^- - N treatment. - Evidence that moss growth is limited by factor other than N.	- Very low levels of N found in the soils for all treatments. Available form was mainly NH_4^+. NO_3^- was almost absent. Suggests that after three years, the moss was still effective in immobilizing atmospheric N.	Jónsdóttir <i>et al.</i> (1995)

3. Discussion and Conclusions

3.1. Experimental Design

The importance of the results obtained from the nitrogen manipulation experiments reviewed in this report are two-fold: first, they allow us to establish a causal link between the current levels of anthropogenic deposition and a range of deleterious effects on ecosystems which have been observed in the past three decades; and second, they allow us to make predictions concerning the effects that future deposition scenarios will have on the natural environment. Experimental design is a particularly important consideration in the latter case, since not only are no two systems exactly the same, but many factors can potentially complicate the extrapolation of results to similar systems, or over longer (biologically realistic) time scales.

From the experimental manipulations documented in this review, the duration of the study stands out as a major feature with the potential to influence the outcome of an experiment. Long-term studies have shown that many of the consequences of chronic nitrogen deposition are not detectable in the first few years of the treatments (*e.g.* Boxman *et al.* 1998 [forest], Lee and Caporn 2001 [acid and calcareous grassland, moorland and heathland]). Short-term studies provide relatively limited information, not only because it may take some time for the effects to become apparent, but also because responses have been shown to change in magnitude and even direction as an experiment progresses. Long term studies, particularly those using realistic rates of N addition, provide a greater insight into ecosystem response over biologically meaningful timescales and there is clearly value in extending short term experiments, where practicable.

The method, rates and frequency of nitrogen addition all vary between experiments and in how well they simulate atmospheric deposition processes. Small and frequent dressings of nitrogen, which more realistically represent natural atmospheric deposition of nitrogen, have been used in only a small number of valuable long-term experiments (*e.g.* Brandrud and Timmermann 1998 [forest], Power *et al.* 1995, 1998a [heathland], Lee and Caporn 2001 [moorland, heathland, acid and calcareous grassland]). Experiments using a single, or only a few, annual dressings may produce artificial results; for instance, dramatic reductions in species diversity, especially the very rapid loss of sensitive taxa such as mycorrhizae, bryophytes and lichens, may result from short-term exposure to toxic levels of nitrogen. Furthermore, frequent applications might be expected to result in a greater retention of added N within the system than infrequent dressings at a much higher rate, since the capacity of the system to immobilise inputs will depend upon both the timing and magnitude of experimental additions, particularly in relation to rainfall events.

Application methods of the different experiments also vary. Only in a limited number of experiments has nitrogen been applied as a mist or fine spray to the vegetation canopy, a method which is perhaps most representative of actual deposition, whilst addition of powder or granule forms to the soil surface have been used in a large number of studies. Application of nitrogen as a spray or a mist will result in a greater proportion of direct canopy uptake by the vegetation, relative to soil based applications, with consequences in terms of the competition between plants and soil microorganisms for uptake of

additional N. The choice of which nitrogen compound to use in an experiment will also determine ecosystem responses to nitrogen addition. For instance, ammonium sulphate has been found to acidify soils more readily than ammonium nitrate (Johnson *et al.* 1998, Morecroft *et al.* 1994, Williams 1978). Furthermore, several experiments have shown different responses depending on whether nitrogen was added in the reduced or oxidised form (e.g. Lee & Caporn 2001, Lutke-Twenhöven 1992).

One of the studies described in this review, which tested the effect of experimental plot size on the incidence of a pathogen, draws our attention to an aspect of experimental design which has been largely overlooked – that of spatial scale (Strengbom 2002). In this study, which considered a wide range of areas (from 1 to 5000 m²), plot size was shown to affect the response of key biotic interactions. There is therefore good evidence to suggest that results from small-plot experiments should be extrapolated to the whole ecosystem level only with extreme caution. This could be a serious limitation to the interpretation of experimental data if the pattern found in this study, where the N-related damage caused by the pathogen was greater in larger plot sizes, is a general phenomenon. All of these features of the experimental design may influence the results of a particular study and should be taken into account when extrapolating the results into a wider context. Differences in experimental design may also explain inconsistencies found between otherwise similar experiments.

Although not strictly related to experimental design, reference to a number of key factors and mechanisms that might relate to the varied responses between experiments cannot be left out of this review. These include:

1. *Age of the stand.* New stands in an exponential growth phase will have a higher nitrogen demand and may therefore show a more positive growth response to nitrogen enhancement (Miller and Miller 1988). This effect could perhaps contribute to an explanation of the differences in the results from Crossley *et al.* (2001) in a 10 year old Sitka spruce stand and ones from a 30 year old stand reported by Emmett *et al.* (1995), although other factors, such as soil type (see below) also have contributed;
2. *Soil type.* Growth responses in peat soils tend to be limited by K and P, whereas in mineral soils growth is often also limited by Mg and Ca. The relative impact of nitrogen addition will also depend upon the availability of nitrogen within a site; vegetation in nutrient poor soils generally responds faster to nitrogen enrichment than at sites 'naturally' rich in nitrogen. Different responses may also be expected for acidic versus calcareous soils, as a result of wide difference in pH and nutrient availability. However, whilst species-level effects clearly vary, some overall similarities in response have nevertheless been reported by Flückiger & Braun 1999 [Forests] and Lee & Caporn 2001 [grasslands].
3. *Interactions with other nutrients.* The stimulation frequently seen in response to nitrogen addition increases plant demands for other nutrients. Increased uptake may result, but frequently nutrient imbalances are seen. Depletion of soil supplies of readily available nutrients may therefore result in reduced soil nutrient availability, particularly of relatively immobile ions, such as phosphorus. Comparatively little is known about the response of P-limited or N,P co-limited ecosystems to nitrogen addition, although there is evidence of some systems being pushed towards P-limitation following sustained elevated inputs of nitrogen (Carroll *et al.* 1999). Clearly soil type and nutrient availability are

important determinants of ecosystem response to nitrogen and will have a substantial impact on both the magnitude and timing of response.

4. *Biotic interactions.* N addition has been related to increased vulnerability to attacks by pathogens and herbivores, and therefore the effects of nitrogen addition will depend upon the presence and response of these agents. Also, the species composition at a site will determine the competitive environment for an individual species, and hence its response to nitrogen addition. Other biotic interactions, for example with large herbivores, may also complicate interpretation of the impact of nitrogen addition on an ecosystem. For example, Emmett *et al.* (2001) reported that in a grassland grazing/nitrogen addition experiment, sheep preferentially grazed fertilised plots.
5. *Abiotic interactions.* Climatic stresses such as drought and frost have been identified as drivers of community level response to nitrogen addition. Temperature and soil moisture deficit have a highly significant effect on microbial activity, with important consequences for nutrient cycling and, therefore, the availability of growth-limiting nutrients to the plant community. Light availability can also mediate plant responses to nitrogen addition, with damage to the plant canopy providing greater illumination to, and frequently expansion of, the understorey (*e.g.* Strengbom 2002, Cawley 2001).

3.2. Ecosystem Responses to Nitrogen Addition

This review summarises results from a large number of nitrogen manipulation experiments that have been undertaken in natural and semi-natural terrestrial ecosystems in the UK and Europe. In this section, we summarise the major trends observed for each ecosystem type and the key biological processes and mechanisms underlying responses to nitrogen deposition. Effects on bryophytes and lichens, which have been reported for many different ecosystems, are considered separately in Section 3.4, while the wider implications of the findings are considered in Sections 3.3 (critical loads), 3.5 (ecosystem recovery), 3.6 (interactions with habitat management) and 3.8 (policy implications).

Forests. Several reports on the response of forest ecosystems have found nitrogen addition to cause changes in tree nutritional status and both a reduction and a stimulation in root and tree growth, depending on dose, duration and location (*e.g.* Crossley 2001, Persson *et al.* 1998, Balsberg Pålsson 1992). The species diversity of forest ground flora (bryophytes and higher plants) has been observed to decrease in a number of experiments (*e.g.* Mäkipää 1995a,b, 1998, Kellner and Redbo-Torstensson 1995; Dirkse and Martakis 1992) as has the abundance and diversity of mycorrhizal fruiting bodies (*e.g.* Boxman *et al.* 1998; Brandrud and Timmermann 1998; Rühling and Tyler 1991). In a few cases, there was an increase in the growth and abundance of nitrophilous ground flora species, particularly grasses (Strengbom *et al.* 2002, van Dobben *et al.* 1999, Boxman *et al.* 1998, Kellner and Redbo-Torstensson 1995, Mäkipää 1995 a,b).

A number of other responses to nitrogen addition have been noted in individual studies, including increased fungal pathogen and insect attack of both the ground flora and trees (Strengbom *et al.* 2002, Flückiger and Braun 1999, Nordin *et al.* 1998, 1996) and increases in litter production (Dirkse and Martakis 1992). Changes in soil chemistry and processes have also resulted from nitrogen addition, including increased leaching of

nitrate, ammonium and base cations and increased aluminium concentrations in the soil solution (e.g. Emmett *et al.* 1998, Gundersen 1998). Many of the above studies have taken place in boreal forest systems are therefore of relatively limited relevance to UK forests. Large differences in climate, nutrient availability and, to some extent, also species composition between these systems makes it uncertain whether such results can be applied more widely to forests in either Britain or elsewhere in Europe.

Heathland and Moorland. Despite differences in both soil type and climate, responses of *Calluna*-dominated heathland and moorland ecosystems have been fairly consistent, and there is a strong representation of UK studies in the literature. Early responses include increased *Calluna* shoot growth, canopy height, canopy density, flowering and litter production, whereas prolonged exposure results in reduced root extension and an acceleration of the *Calluna* life cycle (Power *et al.* 2001, Cawley 2001, Carroll *et al.* 1999). Responses of mycorrhizae are unclear, but could be of major significance in modifying nutrition and competition (Johansson 1999, Yesmin *et al.* 1996). In both UK and European experiments, increased nitrogen deposition has been related to increased vulnerability to biotic and abiotic stresses, such as frost, drought and herbivory, with consequent damage and break down of the canopy (Cawley 2001, Power *et al.* 1998b, Caporn *et al.* 1995c). In turn, the opening of the *Calluna* canopy favours the growth of grasses, such as *Festuca ovina*, *Molinia caerulea* and *Deschampsia flexuosa*, which may inhibit *Calluna* regeneration (Cawley 2001, Bobbink *et al.* 1992, Heil and Diemont 1983, Wilson unpublished data).

These observations strongly implicate nitrogen deposition in the conversion of heathland/moorland to acid grassland which is being observed in the UK and Europe, although it must be remembered that changes in land use and habitat management practices also play an important role in this change. Long-term nitrogen manipulation experiments, in both these ecosystems, have found the duration of the study to be an important factor in the responses observed, with short-term benefits of nitrogen addition eventually giving way to adverse effects and/or resulting in a build up of nutrient stores, with potential long term consequences for the ecosystem.

In contrast to forests, there is little evidence of increased nitrogen leaching from heathland/moorland systems, even in response to relatively high rates of nitrogen addition (Power *et al.* 1998a; Pilkington unpublished data); much of the incoming nitrogen appears to be immobilised in the surface horizons. The role of heather beetle outbreaks in disrupting the normally highly conserved nitrogen cycle at heathland sites has been illustrated in Denmark, suggesting the importance of not only canopy opening, but also mobilisation of stored (organic) nitrogen as mechanisms driving changes in community composition at heathlands. The availability of inorganic nitrogen for plant uptake also appears to be an important driver of change in *Calluna*-dominated heathland; differences between rates of mineralisation and immobilisation are apparent between those systems which have undergone dramatic changes in species composition and those where changes have been more subtle. This again emphasises the importance of time scale, since the ratio of immobilisation to mineralisation appears to reflect the degree of nitrogen saturation of a heathland, and will thus depend on the magnitude and duration of elevated nitrogen inputs.

Grasslands. In grassland ecosystems, nitrogen additions have resulted in a number of responses on both acidic and calcareous soils which have included changes in species composition, as well as plant and soil chemistry (e.g. Lee and Caporn 2001, Emmett *et al.* 2001, Kirkham *et al.* 1996). Although there are few studies in the literature, some valuable long-term studies have been conducted in the UK. The compositional changes

include reduced lichen and bryophyte abundance, decreased abundance of a number of forbs, dwarf shrubs and non-aggressive grass species, and increased abundance of grasses typical of acid grasslands such as *Festuca ovina* and *Arrhenatherum elatius*. These changes in species composition have frequently led to a reduction in species richness and diversity. Evidence that nutrient cycling may be affected by the raised levels of nitrogen availability has also been observed; nitrogen mineralisation rates and soil bacterial biomass and activity have been increased following nitrogen addition.

Two major differences were found in the responses to nitrogen addition between acidic and calcareous grasslands: acidic grasslands are naturally more vulnerable to the acidifying effect of nitrogen compounds, particularly reduced N; calcareous grasslands, which are typically P-limited, respond to combined addition of N and P (more than to N alone) with a pronounced increase in grass growth. The duration of manipulation experiments in grassland ecosystems was, again, found to be an important factor in the types of responses observed, with changes in species composition only being found after several years. Management interactions, and particularly the effects of grazing, are a major issue for grasslands and are considered in more detail in Section 3.6.

Bogs, Tundra and Fens. A relatively small number of studies have been undertaken in bog, tundra and fen ecosystems. In bog ecosystems, which are typically nutrient poor, nitrogen addition improves growth and survivorship in some moss species, and has the reverse effect on others, with consequent shifts in dominance (Redbo-Torstensson 1994, Lütke Twenhöven 1992). In the longer term, it may also cause a reduction in diversity as bryophytes give way to grasses with increasing levels of nitrogen deposition (Hogg *et al.* 1995). In tundra ecosystems, nitrogen addition resulted in changes in community composition with increased abundance of grasses and reduction of lichen cover (Pearce and van der Wal 2002, Gordon *et al.* 2001, Jonasson *et al.* 1999). Bryophytes responded with changes in species composition and tissue chemistry (Gordon *et al.* 2001, Shaver and Chapin 1995). The little data available on fen ecosystems indicates that high levels of nitrogen deposition cause changes in community composition, with a reduction in species diversity and bryophyte biomass (Bergamini and Pauli 2001, Vermeer 1986). In contrast to forests, heathlands and grasslands few field manipulation experiments have studied responses of soil chemistry and nitrogen cycling in these systems.

From this review of nitrogen manipulation experiments, it is clear that there are some similarities in response between different ecosystem types, as well as some common mechanisms underlying the observed changes. Tables 3.1 and 3.2 summarise, by ecosystem, those studies where responses to nitrogen addition have been found for higher plants, lichens and mosses. In addition, Table 3.3 lists those studies which have reported responses in the fungal and microbial communities, or changes in soil chemistry and nutrient cycling, following nitrogen addition.

It is clear from Table 3.1 that nitrogen deposition can reduce species diversity and alter the competitive ability of many plant species, resulting in composition shifts towards more N-tolerant species. The response of a number of species groups is similar across a range of ecosystems, with a general pattern emerging for a reduction in forbs and dwarf shrubs and an increase in grasses. Bryophytes and lichens appear to be particularly sensitive components of most ecosystems, and are considered in detail below. Changes in species composition are brought about either directly as a result of a suppression (e.g. Redbo-Torstensen, 1994) or stimulation (e.g. Bobbink, 1991) of individual species growth, or indirectly, through changes in either sensitivity to biotic or abiotic stress, or changes in nutrient cycling. Although not all experiments have reported shifts in community composition in response to nitrogen addition, the studies summarised in

Tables 3.2 and 3.3 show that vegetation or microbial responses, which suggest a disruption of normal physiology and/or function, have been found consistently. Changes in, for example, foliar chemistry or microbial activity may thus be early indicators of the potential for deleterious responses at the community or ecosystem level, in the longer term.

Table 3.1. Nitrogen manipulation experiments by ecosystem, where responses in abundance and composition were identified for different plant groups.

	Herbaceous species.	Grasses	Shrubs	Lichens	Bryophytes
Forest	van Dobben <i>et al.</i> 1999 Boxman <i>et al.</i> 1998b Mäkipää 1995a Falkengren-Grerup 1993 Dirkse & Martakis 1992	Strengbom <i>et al.</i> 2002a van Dobben <i>et al.</i> 1999 Kellner & Redbo-Torstensson 1995 Dirkse & Martakis 1992	van Dobben <i>et al.</i> 1999 Strengbom <i>et al.</i> 2002a Mäkipää 1995a		Gundersen 1998 Mäkipää 1998
Grassland	Lee & Caporn 2001 Dodd <i>et al.</i> 1995 Tallowin <i>et al.</i> 1994	Lee & Caporn 2001 Kirkham <i>et al.</i> 1996 Dodd <i>et al.</i> 1995 Mountford <i>et al.</i> 1994 Bobbink <i>et al.</i> 1988	Emmett <i>et al.</i> 2001	Emmett <i>et al.</i> 2001	Emmett <i>et al.</i> 2001 Virtanen <i>et al.</i> 2000
Heathland		Cawley 2001 Prins <i>et al.</i> 1991 van der Eerden <i>et al.</i> 1990 Heil & Diemont 1983	Cawley 2001 Heil & Diemont 1983	Barker 2001	
Moorland			Carroll <i>et al.</i> 1999 Werkman & Callaghan 1996	Lee <i>et al.</i> 2000	Lee <i>et al.</i> 2000
Bog	Hogg <i>et al.</i> 1995 Redbo-Torstensson 1994				Hogg <i>et al.</i> 1995 Lütke Twenhöven, 1992
Tundra	Baddeley <i>et al.</i> 1994	Pearce & van der Wal 2002 Jonasson <i>et al.</i> 1999 Henry <i>et al.</i> 1986	Henry <i>et al.</i> 1986		Pearce & van der Wal 2002 Gordon <i>et al.</i> 2001 Jonasson <i>et al.</i> 1999

Table 3.2 Nitrogen manipulation experiments which reported changes in plant growth (excluding those growth responses which resulted in changes in species composition, listed in Table 3.1) and nutritional balance

	Herbaceous sp.	Grasses	Shrubs	Lichens	Bryophytes	Trees
Forest	Falkengren-Grerup 1993	Strengbom <i>et al.</i> 2002a	Strengbom <i>et al.</i> 2002a Nordin <i>et al.</i> 1996,1998		Mäkipää 1998	Crossley <i>et al.</i> 2001 Flückiger & Braun 1999 Gundersen 1998 Clemensson-Lindell & Persson 1995 Nilsen & Abrahamsen 1995 Balsberg Pålsson 1992 Erikson <i>et al.</i> 1992 Näsholm & Ericsson 1989 Any missing NITEREX expts ?
Grassland	Morecroft <i>et al.</i> 1994	Emmett <i>et al.</i> 2001 Morecroft <i>et al.</i> 1994			Emmett <i>et al.</i> 2001 Morecroft <i>et al.</i> 1994	
Heathland		Van der Eerden <i>et al.</i> 1991	Cawley 2001 Riis-Nielsen 1997 Bobbink <i>et al.</i> 1996 Power <i>et al.</i> 1995, 1998a,b Barker 2001 Van der Eerden <i>et al.</i> 1991			
Moorland			Lee <i>et al.</i> 2000 Carroll <i>et al.</i> 1999 Kerslake <i>et al.</i> 1998			
Bog	Redbo-Torstensson 1994				Williams & Silcock 2001 Nordin & Gunnarsson 2000 Hogg <i>et al.</i> 1995	
Tundra	Baddeley <i>et al.</i> 1994			Gordon <i>et al.</i> 2001	Pearce & van der Wal 2002 Gordon <i>et al.</i> 2001	

Table 3.3. Summary of studies reporting effects of nitrogen addition on soil and microbial processes, and on mycorrhizal fungi abundance/diversity .

	Soil chemistry and N-cycling	Microorganisms	Mycorrhizae
Forest	Hagedorn <i>et al.</i> 2001 Kjønaas <i>et al.</i> 1998 Gundersen 1998 Moldan & Wright 1998 Nilsson <i>et al.</i> 1988 Stuanes & Kjønaas 1998 Emmett <i>et al.</i> 1998 Koopmans <i>et al.</i> 1998 Carnal <i>et al.</i> 1996 Tamm <i>et al.</i> 1995 Boxman <i>et al.</i> 1995 Rasmussen <i>et al.</i> 1995 Emmett <i>et al.</i> 1995a,b Moldan <i>et al.</i> 1995 Eriksson <i>et al.</i> 1992 Wright <i>et al.</i> 1995 Wright & Tietema 1995 Bredemeier <i>et al.</i> 1995 Gundersen & Rasmussen 1995		Crossley <i>et al.</i> 2001 Brandrud & Timmermann 1998 Majdi & Nylund 1996 Wright <i>et al.</i> 1995b Brandrud 1995 Rühling & Tyler 1991 Termorshuizen 1990 Arnebrant & Soderstrom 1989 Wästerlund 1982 Alexander & Fairley 1983 Menge & Grand 1978 Ritter & Tolle 1978 Hora 1959
Grassland	Emmett <i>et al.</i> 2001 Johnson <i>et al.</i> 1998 Dodd <i>et al.</i> 1995 Dodd <i>et al.</i> 1994a,b Morecroft <i>et al.</i> 1994 van Dam 1990 Williams 1978	Johnson <i>et al.</i> 1998 Lee <i>et al.</i> 1996	Lee & Caporn 2001
Heathland	Barker 2001 Nielson <i>et al.</i> 2000 Kristensen & McCarty 1999 Power <i>et al.</i> 1995, 1998a van Vurren & van der Eerden 1992 Prins <i>et al.</i> 1991 van der Eerden <i>et al.</i> 1990	Lee & Caporn 1998	Johansson & Sochting 1994
Moorland	Yesmin <i>et al.</i> 1996 Pilkington 2002	Johnson <i>et al.</i> 1996, 1998	Caporn <i>et al.</i> 1995a
Bog	Williams & Silcock 2001	Williams & Silcock 2001	

3.3. Ecosystem Manipulation Experiments and Critical Loads

The results of ecosystem manipulation experiments are of considerable value in developing a critical load approach for nitrogen deposition. However, this review has shown that the number of nitrogen manipulation experiments involving the application of appropriately low doses of nitrogen is limited. Furthermore, most experiments are of short duration, while for some ecosystems there are no appropriate experiments. It is also important to recall (cf. Section 1.3) that critical loads are defined as the deposition rate below which significant harmful effects *do not* occur. Most field manipulation experiments use a limited range of treatments; often, significant effects are found at the lowest nitrogen deposition, and it is impossible to identify the application rate at which there is no adverse effect. Therefore, it is more valuable to use the experiments which have been conducted to identify rates of deposition at which significant harmful effects have been shown to occur. These are the threshold depositions for biological responses derived from whole ecosystem nitrogen manipulations which are given in Table 3.4. It should be noted that, in some cases, there are experiments described in the earlier sections which provide different results, with no effect observed at higher rates of deposition; the information in Table 3.4 summarises the lowest deposition rates at which significant adverse effects have been reported in an individual study. Interpretation of these findings in the context of critical loads also depends on the background deposition value, which is not well defined in some studies.

For coniferous forests, there are a number of studies which demonstrate adverse effects of adding nitrogen in the range 10-20 kg N ha⁻¹ yr⁻¹; many of these are studies in Scandinavia, where the experimental sites have a low background deposition. Given that adverse effects can be demonstrated at these deposition rates in a number of studies, all of limited duration compared with forest growth cycles, it appears that the current critical load of 7-20 kg N ha⁻¹ yr⁻¹ should be reduced. However, it is unclear how far these studies in Scandinavia reflect responses to N deposition in the U.K. and other parts of Europe. Furthermore, they are not intended for application to the production forests which dominate in the U.K. In contrast, ecosystem manipulation studies provide very little evidence to demonstrate adverse effects of critical load exceedance for deciduous forests. The most relevant evidence comes from the extensive studies of Flückiger & Braun (1999) on both beech and spruce, over six years, which suggest a similar effect of the addition of 10 kg ha⁻¹ yr⁻¹, and hence that comparable critical loads might apply to coniferous and deciduous species. However, their sites have a relatively high background deposition.

In lowland heath, the significant changes in *Calluna vulgaris* growth and phenology found with applications of 7.7 kg N ha⁻¹ (Power *et al.* 2001, 1998a,b, 1995) to a background of 10-15 kg N ha⁻¹ yr⁻¹ suggest that at least the lower end of the current critical load range may need to be reduced. In upland moorland, significant *C. vulgaris* responses were seen in the only long-term experiment when 40 kg N ha⁻¹ yr⁻¹ was added to a background of 30 kg N ha⁻¹ yr⁻¹, but since this was the lowest nitrogen dose applied it is difficult to establish where the threshold lies. The two tundra studies showing significant effects of adding 10 kg N ha⁻¹ yr⁻¹ to relatively low background deposition provide strong support for a lower critical load than the current value of 5-15 kg ha⁻¹ yr⁻¹ for tundra, especially since significant effects were observed rapidly and do not appear to be readily reversible.

TABLE 3.4. Summary of experiments showing the lowest nitrogen addition (+ background deposition) at which an ecosystem response was noted, the type of response and author of the research. The critical loads for these ecosystems are reproduced from Bobbink *et al.* (1996). See tables in Section 2 for a more detailed description of responses.

Ecosystem	Response Dose (Background) kg N ha ⁻¹ yr ⁻¹	Response Type	Author	Critical load kg N ha ⁻¹ yr ⁻¹
Forest ecosystems				
Acidic coniferous	30 (2.5-5) 10 (18-33) 5 (3) 12.5 (3) 12.5 (3) 20 (2.5-5)	Species composition Nutrient imbalance; Fungal/aphid attack Ground flora growth Species composition Fungal attack species composition Mycorrhizal diversity	Dirkse & Martakis (1991) Fluckiger & Braun (1999) Kellner & Redbo-Torstensson (1995) Strengbom <i>et al.</i> (2002) Nordin <i>et al.</i> (1998)	7-20
Acidic deciduous	25 (10) 10 (18-33) 12 (4)	Species composition Nutrient imbalance; fungal/aphid attack Mycorrhizal fruiting body production -	Makipaa (1995a, 1998) Fluckiger & Braun (1999) Ruhling & Tyler (1991)	10-20
Calcareous forest	-	-	-	15-20
Heathlands				
Lowland dry heath	7.7 (10-15) 20 (?) 15 (18)	Growth, grass seedling invasion Growth; grass invasion Heather beetle outbreak	Power <i>et al.</i> (1998a); Power <i>et al.</i> (2001) Cawley (2001) Riis-Nielsen (1997)	15-20
Moorland	40 (30)	Growth, accelerated ageing, microbial activity Growth, bryophyte species composition	Carroll <i>et al.</i> (1999); Pilkington (pers.comm)	10-20
Tundra	10 (<5) 10 (12)	Growth, species composition	Gordon <i>et al.</i> (2001) Pearce & van der Wal (2002)	5-15
Grasslands				
Calcareous	35 (19)	Species composition	Morecroft <i>et al.</i> (1996); Lee & Caporn (2001)	15-35
Neutral-acid	35 (19) 25 (?)	Microbial activity; Species composition Species composition	Johnson <i>et al.</i> (1998); Lee & Caporn (2001) Kirkham <i>et al.</i> (1996)	20-30
Acid grassland	10 (15-26)	Bryophyte species composition	Emmett <i>et al.</i> (2001)	?
Wetlands				
Mesotrophic fens	-	-	-	
Ombrotrophic bogs	10 (5) 12(12)	Species composition Growth	Redbo-Torstensson (1994) Hogg <i>et al.</i> (1995)	20-35 5-10

Studies in grasslands provide clear evidence of changes in species composition at deposition rates above the current critical load, but the range of treatments in the relevant experiments provide no basis for assessing the validity of these values. No critical load value is set for dune grassland, but the responses observed by Ten Harkel & van der Meulen, (1995) at 25 kg N ha⁻¹yr⁻¹ after four years may provide the basis for recommending a value. Similarly, no critical load is currently available for upland species-poor acid grassland, although the evidence of Emmett *et al.* (2001) suggests that

to prevent changes in bryophyte species composition, a value below that currently used for neutral-acid grassland may be required.

Changes in species composition have been found in bog ecosystems with nitrogen additions of 12 and 10 kg N ha⁻¹yr⁻¹ by Hogg *et al.* (1995) and Redbo-Torstensson (1994), respectively. The latter experiment was at a site with a background deposition of only 5 kg N ha⁻¹yr⁻¹. These results provide support for the current critical loads.

In several of these ecosystems, either the only or the earliest effects of the lowest nitrogen addition was on the lower plant component. This raises the question of whether further development of critical loads should focus more on bryophytes and lichens, an issue addressed in a wider context in Section 3.4 below.

In summary, current findings from field manipulation studies provide evidence which is relevant to defining the critical load for some ecosystems and not for others. Of course, few, if any, of these experiments were specifically designed for critical load validation and Bobbink *et al.* (1996) state that, for this purpose, there is a need for nitrogen addition experiments with a high resolution of treatments between 5 and 40 kg N ha⁻¹yr⁻¹. To increase the possibility of validating proposed critical loads, it is essential that the lowest treatment dose is below the critical load. However, because current background deposition is relatively high in many areas, this restricts the locations in which such experiments can be conducted, and hence their value in supporting critical loads for national and international application.

3.4. Bryophytes and Lichens

Although an increasing number of field manipulation studies are providing detailed data on the responses of the bryophyte and lichen cover, many report only total cover of these groups, or report only effects on the major species. Despite these limitations, the field experiments reviewed in this document clearly demonstrate that, in many cases, the bryophyte component of the ecosystem is the first to respond or is the most responsive to low rates of nitrogen application. It is therefore worthwhile to consider the implications of these findings.

Firstly, the mechanism by which increased nitrogen deposition causes adverse effects is much less clear than it is for the higher plant component. Some studies (*e.g.* the long-term moorland experiment at Ruabon (Carroll *et al.* 1999) suggest that the *Calluna* canopy density is a key factor controlling the response of bryophyte and lichen cover, with an initial decline when the cover increased in response to nitrogen being reversed when the canopy opened up. In contrast, other studies appear to demonstrate changes in cover in the absence of major effects on the higher plant cover. Other studies show changes in species composition within the bryophyte component, for example between *Sphagnum* species in bog ecosystems (*e.g.* Lutke Twenhoven 1992) and between *Polytrichum juniperum* and *Dicranum scoparium* in the study of Gordon *et al.* (2001). The mechanisms which determine the differential positive and negative responses of different species to nitrogen deposition are poorly understood, as is whether the toxicity of nitrate differs from that of ammonium. Such shifts in species composition may provide early indications of a change from N limitation to P limitation of an ecosystem through changes in the dominance of indicator species.

The limited available data also suggest that changes in bryophyte species composition may persist for many years after nitrogen deposition has ceased (*e.g.* Gordon *et al.* 2001, Strengbom *et al.* 2001). The mechanisms underlying this persistence of response is

unclear, but it is presumably not due to greater nitrogen availability in the soil, and further investigations might further illuminate the factors controlling the dynamics of response to decreased nitrogen deposition. It is also important to emphasise that no experimental manipulation experiments have directly assessed changes in the species composition of lichen and bryophyte communities on trees and rocks, despite their conservation importance in the UK, and this is a gap in knowledge which needs to be addressed.

In conclusion, the sensitivity of bryophyte responses, in particular, to relatively low rates of nitrogen deposition, is an important common finding from manipulation studies in a range of ecosystems. These responses are important for two reasons. Firstly, in tundra and bog ecosystems, and in some woodland ecosystems, these species are a vital component of the overall community and of the food chain, and are a major element in the nitrogen cycle. For example, the capacity of the moss ground cover in bogs and tundra to assimilate inorganic nitrogen and release it as organic nitrogen may play a crucial role in controlling the availability of inorganic nitrogen to higher plants (Gordon *et al.* 2001, Williams *et al.* 1999). Hence, the concept of nitrogen saturation is one which could usefully be extended to this component of the ecosystem. Secondly, the bryophyte and lichen flora of the UK is of global significance and there is an urgent need to assess the scale of the threat posed by current levels of nitrogen deposition to the conservation of this flora.

3.5. Ecosystem Recovery

Since recent European policies controlling emissions of nitrogenous pollutants are likely to result in a reduction in deposition rates across Europe, it is of particular interest to evaluate the potential and timescale for ecosystem recovery in the future. This can be achieved by monitoring biological and chemical changes following either a) the cessation of an earlier nutrient addition experiment or b) the use of artificial roofs to intercept deposition and the subsequent application of "clean" rain. A number of new results have emerged since the original review was completed, illustrating qualitative and quantitative changes in above ground vegetation, roots and mycorrhizae, fungi and soil chemistry, following a reduction in nitrogen inputs.

The NITREX roof experiments in coniferous forests at five European sites have provided valuable information on the response of forest ecosystems, although with greater consistency between sites in terms of below- (as opposed to above-) ground responses. A reduction in nitrogen inputs to pre-industrial levels (c. 5 kg ha⁻¹ yr⁻¹) generally improved foliar nutrition; for example, at Ysselsteyn (The Netherlands), there was a rapid decrease in needle arginine and total nitrogen concentrations and increased calcium and magnesium concentrations (Boxman *et al.* 1998b). Tree growth did not show a consistent response to reduced inputs, although there was evidence of improved growth at Ysselsteyn, the site which had previously received the highest background rates of nitrogen deposition (55-60 kg ha⁻¹ yr⁻¹; Boxman *et al.* 1998b). Responses in terms of increased fine root growth and greater abundance and diversity of fungal fruiting bodies were seen at several sites, including the Swedish site (Gårdsjön) which had previously received relatively low (12 kg N ha⁻¹ yr⁻¹) deposition inputs (Persson *et al.* 1997, 1998). A consistent, relatively rapid reduction in nitrate leaching was also seen across the roofed sites (Beier *et al.* 1998, Boxman *et al.* 1998a, Bredemeier *et al.* 1998a).

On the basis of the NITREX experiments, it is tempting to suggest that recovery may begin to occur more rapidly at sites which have previously received higher deposition

inputs over the past several decades. However, the study by Strengbom *et al.* (2001) indicates that effects of earlier, relatively high rates of N fertilisation on ground flora and fungal species composition were still apparent 9 or 47 years after nutrient additions ceased. Interestingly, in this study, bryophytes and a leaf parasitic fungus exhibited the most persistent effects of earlier N addition, suggesting that, whilst changes in foliar chemistry may occur quite rapidly, lower plants and fungi might be relatively slow to recover following ecosystem eutrophication.

A limited number of experiments in other ecosystems can also shed some light on the issue of how the magnitude and duration of historically elevated inputs affects the speed of recovery under conditions of lower deposition. Recovery of pre-fertilisation species richness was faster at lower rates of nitrogen addition ($25 \text{ kg ha}^{-1} \text{ yr}^{-1}$) in a grassland experiment in the Somerset levels (Kirkham *et al.* 1996). Furthermore, whilst foliar chemistry recovered relatively rapidly from small increases in deposition inputs at a heathland site in Surrey (Barker, 2001; Power *et al.* 2001), effects on soil chemistry and shoot growth were still apparent several years after the cessation of nitrogen additions, even following the management removal of nitrogen stores from the system.

Although there are still relatively few experiments which are aimed at assessing the rate of recovery from ecosystem eutrophication, it appears that some responses may be seen relatively rapidly. However, it must be remembered that the return of a particular ecosystem characteristic (e.g. fungal fruiting body abundance) does not in itself constitute recovery of the entire ecosystem. There is convincing evidence that ecosystem effects may persist for many years, with recovery only occurring over time scales of many decades, if at all. Indeed, the loss of species from more sensitive ecosystems (such as bogs or tundra systems) may in fact prevent its re-establishment over any realistic time scale, in the absence of active re-introduction and restoration measures. This is particularly the case if species which have increased in abundance have higher rates of decomposition and/or mineralisation, leading to more rapid nutrient cycling and availability. Whilst this is a subject which has seen a small increase in the number of studies during the past five years, there is clearly the need for a much greater research effort in this area.

3.6. Interactions with Management

A number of semi-natural ecosystems (grasslands, heathlands and moorlands) are managed to maintain or enhance their conservation value. Methods traditionally used include burning, grazing and cutting, but these management regimes vary between habitat types and location. Precise management aims also vary according to ecosystem type and conservation objectives. However, key aims of habitat management for semi-natural ecosystems are 1) to maintain a low nutrient environment to prevent natural species succession through to woodland and 2) to prevent dominance of a limited number of species and maintain ecosystem biodiversity. The increase of atmospheric nitrogen deposition has coincided in many areas with widespread changes in traditional management practices making it difficult to attribute observed vegetation change to either factor. However, management clearly has the potential to alter ecosystem response to nitrogen inputs, by reducing internal nitrogen pools and minimising individual species' responses to nitrogen.

Relatively few studies have examined the interaction between enhanced nitrogen availability and management practices. One example is the moorland study of Hartley (1997) which used nitrogen additions of $75 \text{ kg ha}^{-1} \text{ yr}^{-1}$. When fencing and fertiliser

treatments were applied to a fine-scale mosaic of *Calluna vulgaris* and grasses, including *Nardus stricta*, nutrient addition only favoured *N. stricta* in the unfenced (grazed) areas; where sheep and deer grazing was excluded using fences, nitrogen addition resulted in an increase in *C. vulgaris* ground cover. The shade intolerant *N. stricta* declined in the absence of grazing due to the increased shade of the *C. vulgaris* canopy. Hartley (1997) concluded that reductions in grazing pressure in upland heathland would increase heather cover and prevent the transition to *N. stricta* acid grassland, which is favoured by nitrogen addition if the *C. vulgaris* canopy is damaged.

A similar experiment, incorporating different grazing intensities, has also been carried out at an upland grassland site in Wales (Emmett *et al.* 2001; Parekh *et al.* 2001). Results from this study indicate that, in general, species were more sensitive to nitrogen additions in lightly grazed, as opposed to heavily grazed plots. There was an apparent preference amongst the grazing animals for grasses which had received higher nitrogen additions which may have resulted in an overall reduction in the more nitrogen-responsive grasses. However, in heavily grazed plots, there was a greater increase in the grass *Deschampsia flexuosa*, as well as a greater decrease in moss cover, in response to nitrogen addition, compared to those plots which were lightly grazed. This experiment suggests that although there are important interactions between grazing and nitrogen availability, the apparent benefits of grazing, in terms of reducing species response to nitrogen, are not straightforward due to the competing effects of different processes.

Another grassland study, by Kirkham *et al.* (1996) examined whether changes in the management regime of the Somerset Levels hay meadows could increase species diversity and reduce graminoid dominance after cessation of nitrogen additions. Changing the hay cutting date caused no significant increase in species diversity over a two year period in these previously fertilised meadows. Domination of grasses in previously fertilised sites was either maintained or enhanced by early cutting (May) while later cutting (August or later) resulted in an increased quantity of viable grass seed being shed. Experimental disturbance and gap creation in the grass-dominated vegetation of these previously fertilised meadows also failed to enhance botanical diversity (Tallowin *et al.* 1994). In dune grasslands, Ten Harkel and Van der Meulen (1995) examined, the effect of nitrogen addition and interactions with grazing. Grazing was found to be the most important factor in determining the botanical composition of the dune ecosystem. The cessation of grazing by horses and rabbits, through the use of enclosures, resulted in graminoid dominance (*Festuca rubra*, *F. ovina* and *Poa pratensis*), especially where nitrogen additions had been made. However, the re-introduction of grazing to these areas increased the species diversity of perennial herbs and reduced graminoid dominance in dune ecosystems after only six months.

Different management regimes may be more or less effective at promoting ecosystem recovery following a reduction in deposition. A recent study at a lowland heath in Surrey investigated the effect of a range of different management techniques (low intensity mow, management burn, high intensity mow and a high temperature burn), representing a scale of increasing intensity of removal, on heathland response to nitrogen addition (Barker 2001, Power *et al.* 2001). Results from this study indicate that managements which remove a greater proportion of organic material from the system (i.e. high intensity mow or high temperature burn) can reduce the magnitude of plant and microbial response to nitrogen additions. For example, shoot growth of *Calluna vulgaris* following nitrogen addition at a rate of 30 kg ha⁻¹ yr⁻¹ was lower in plots which experienced a high temperature burn or mowing with litter removal, compared with those which underwent just the removal of above ground plant biomass (low intensity mow or management burn). A similar interaction was also reported for decomposition

rates, suggesting that more intensive managements may in fact slow down the rate of nutrient cycling, as a result of both direct and indirect perturbations of the microbial community. A further study at this heathland site also indicates that ecosystem recovery from previous nitrogen additions may be accelerated through the use of more intensive management regimes, particularly those which remove litter (and humus) nutrient stores (Barker 2001, Power *et al.* 2001).

Whilst there are still relatively few studies which have explicitly investigated interactions between nitrogen deposition and habitat management, there is now some evidence to suggest that ecosystem response to nitrogen depends greatly on the form and intensity of management practice. Results from field experiments are also supported by modelling studies which further illustrate that the frequency of habitat management may also be an important factor in determining community level response to enhanced deposition. The possibility of using habitat management as a tool to promote ecosystem recovery from the effects of enhanced nitrogen deposition has been suggested by Power *et al.* (2001) and there is clearly a need for further studies into the effect of different management regimes on a wider range of ecosystem types.

3.7. Gaps in Knowledge

This review has highlighted the value of long-term whole ecosystem nitrogen manipulation experiments in natural and semi-natural ecosystems. It also identifies that medium- or long-term manipulation experiments using low levels of nitrogen addition and realistic application methods are still comparatively rare, and that there are considerable gaps in our knowledge of ecosystem responses to enhanced nitrogen deposition. Bobbink *et al.* (1996) identified a number of key gaps in knowledge in the specific context of developing critical loads for pan-European application, but a wider range of issues, some of them specific to the UK, can be identified on the basis of this review:

- Nitrogen addition experiments with a high resolution of treatments between 5 and 40 kg N ha⁻¹ are needed in most ecosystem types to better define response thresholds.
- Understanding of the effects of nitrogen addition is very limited in a number of ecosystems. These include (a) neutral/calcareous forests, and particularly ground flora (b) montane and tundra communities, (c) species-rich grasslands, (d) dune communities, and (e) fens. Even for those ecosystems which have been studied in ecosystem manipulation experiments, information is available only from very few experiments in a limited number of sites.
- The requirements of the Habitats Directive mean that the impacts of nitrogen deposition needs to be evaluated experimentally on a range of more specific communities of high conservation value in the UK. Examples of which are likely to be relatively sensitive to nitrogen deposition include montane screes, limestone pavement, bog woodland, dune heaths and machair. This work also needs to be extended to individual species identified under the Habitats Directive.
- Many studies have only considered total bryophyte and lichen cover, but there is evidence of significant shifts in species composition within bryophyte communities at relatively low rates of N deposition. Given the global significance of the UK's bryophyte and lichen flora, more work is needed on species-specific responses and

on experimental designs which allow direct effects on bryophytes to be distinguished from indirect effects through changes in canopy density.

- The long-term effects of enhanced atmospheric nitrogen deposition in grassland, heathland and moorland under different management regimes need to be better defined. This would allow identification of management techniques which might minimise the impacts of current nitrogen deposition.
- The significance of the timescale over which chemical and biological responses develop needs to be defined for different ecosystems, and the significance of immobilisation as a short- or long-term factor constraining responses to N deposition needs to be better quantified.
- More experiments are needed to assess the dynamics of recovery of systems as N deposition decreases. These experiments should clearly distinguish systems with primarily chemical impacts from those in which major changes in species composition have already been induced, and also need to address the role of different management techniques in accelerating recovery processes.
- The significance of N deposition in pushing systems towards limitation or co-limitation by other nutrients, particularly phosphorus, needs further investigation.
- Few experiments address the effects of enhanced nitrogen deposition on fauna in any ecosystem types, although such responses may be a major factor determining ecosystem response.
- The differential effects of the deposited nitrogen species (NO_x or NH_x) have only been distinguished in a few experimental studies. Future emphasis on the specific effects of NH_x is needed as it will become an even more significant component of total deposition in the UK in the future.
- Interpretation of experimental manipulation studies of a duration of 5-20 years would benefit from the application of dynamic models to infer the possible longer-term consequences of the observed responses, and possible multiple interaction effects which cannot be readily addressed in standard field manipulation experiment designs.
- Interactions with climate and the carbon cycle are important for assessing the longer-term impacts of N deposition and thus also need more attention in experimental studies.
- The methods used so far to assess mycorrhizal diversity may not accurately reflect below-ground diversity. Recent developments in the use of molecular techniques will allow an improved evaluation of the effects of N deposition on fungal and microbial communities.

3.8. Policy Implications

This review has highlighted a number of studies in a variety of ecosystem types in which addition of relatively low levels of nitrogen has resulted in impacts of concern to

nature conservation. Ecosystem responses have included changes in higher plant composition, but more importantly losses of moss, lichen and mycorrhizal species, all with applications of nitrogen at levels exceeded by current deposition in some areas of the UK. Additionally, there is strong evidence to suggest that nutrient cycling and patterns of nitrogen accumulation are changed dramatically at low levels of nitrogen addition. However, the results of ecosystem manipulation experiments should be viewed with caution as often the methodology used in experiments fails to realistically represent natural nitrogen deposition processes. In the few studies where representative application methods and rates of nitrogen addition are used, the duration of the study is extremely important to the ecosystem responses observed. In view of this, a continued commitment in the UK to the support of existing long-term field experiments which apply low levels of nitrogen in a manner realistic of natural deposition is of the utmost importance.

Nitrogen oxides and ammonia differ in the trends of emissions over the past decade and those anticipated by 2010 (NEGTAP, 2001). Annual UK emissions of nitrogen oxides peaked in the late 1980s at about 850 kt-N and had declined to less than 500 kt N by 1999; they are predicted to decline further to about 360 kt N by 2010. This is consistent with the UK objective of a 50% decrease in nitrogen oxide emissions from 1990 to 2010, under the Gothenburg Protocol. Annual UK emissions of ammonia are also thought to have peaked at about 300kt N, in the late 1980s but, in contrast to nitrogen oxides, the UK commitment is only for a 12% reduction from 1990 to 2010 under the Gothenburg Protocol.

Despite these falls in national emissions, the latest assessment for the UK Government (NEGTAP, 2001) suggests that nitrogen deposition will remain a significant ecological concern in 2010. This assessment is based on a comparison of modelled deposition with the critical load values summarised in Table 1.2 of this report, which suggests that in 2010 critical loads for woodlands will be exceeded over all of England, Wales and southern Scotland, while those for moorlands and heathlands will be exceeded over most of England and a significant proportion of Wales. There will be also be some areas of exceedance of critical loads for grasslands. While there is considerable uncertainty over this assessment in terms of both the modelled deposition and the estimated critical load, it does suggest that there remains a significant concern about the future effects of deposition of reduced nitrogen in particular on species composition. Given the very slow natural rates of recovery from the effects of nitrogen deposition once changes in species composition have occurred which is suggested by recent evidence (see Section 3.5), and the costs of management interventions to accelerate this process, there is a clear need for a precautionary approach which minimises the risk of significant changes in species composition in sensitive communities across the UK. This situation highlights the need for more research, including nitrogen manipulation experiments, to provide a better assessment of the risk of species change in sensitive ecosystems and to provide a more informed basis for assessment of appropriate critical load values for application in the UK.

Even in regions of the UK where mean nitrogen deposition is below the critical load, there may still be local concerns over specific developments which may cause localised increases in nitrogen deposition, for example through construction of roads for nitrogen oxides or the operation of intensive animal units for ammonia. Furthermore, the implementation of the Habitats Directive means that there is a requirement to protect individual species or habitats from the adverse effects of nitrogen deposition. The evidence from nitrogen manipulation experiments reviewed in this report, taken overall, demonstrates a general cause for concern over the impacts of local developments or local

sources on sites of conservation value. However, this evidence is not adequate to define the impacts of specific increases in nitrogen deposition on particular habitats. Furthermore, the national critical loads relate to broad ecosystem categories, although recently an initial attempt has been made to extend them to a wider range of habitats and species in the context of the Habitats Directive (Hall *et al.* 2002). It is clear that decision making in these cases would benefit from a much larger number of field manipulation studies applying realistic levels of nitrogen to a much wider range of sensitive habitats over periods of 5-10 years.

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