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NEW SCIENCE ON THE EFFECTS OF NITROGEN DEPOSITION AND CONCENTRATIONS ON NATURA 2000 SITES (THEME 3)

5.1 Background document

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Summary

This background paper summarizes established and new science on the effects of nitrogen (N) deposition on ecosystems and considers the potential for improved assessment of N deposition impacts on Natura 2000 sites. The key aspects covered are N deposition effects on biodiversity and on biogeochemistry, links to ecosystem services, the importance of N form, N deposition indicators, management practices and ecosystem reversibility following decreased N input.

The paper shows that:

- Evidence of N impacts to different vegetation types in Europe exists, but that it is important that it is translated meaningfully to the target habitats listed under the Habitats Directive. Evidence for N deposition effects to important ecosystem services, such as carbon sequestration, also exists but the cause and effect relationships underlying them are often complex and not sufficiently understood.
- Chemical N form can influence both the rate of ecosystem change, and the extent of impacts on the short and long-term. Evidence is presented for ammonia causing detrimental plant physiological effects, probably on a majority of species, whilst ammonium and nitrate effects will depend on plant species present.
- Plant species composition as well as plant biochemical parameters may be useful bioindicators for assessment of N deposition effects in Natura 2000 sites, however "baseline" data is mostly not available for rare species. Ecosystem specific indicators, that are predictive of further damage, rather than a consequence of already existing adverse effects (i.e., early warning indicators) are needed.
- Site level management practices can be useful to reduce the impact if N deposition but they will certainly not be able to mitigate all the impacts of enhanced N deposition and enhanced

N concentration on Natura 2000 habitats. More knowledge is needed to better understand where and if management intervention is appropriate to mitigate N effects.
- Studies on the reversibility of N impacts show that some ecosystem parameters may revert quickly, while other components may show strong inertia. In some cases reversion to the original state may however be impossible.
- Climatic factors interfere with ecosystem effects of N deposition. It is clear that climate both can emphasize and mitigate effects of N deposition. Current climate and expected climatic changes must be included in assessments and predictions of N deposition effects on ecosystems.

The aim is to provide a broad picture of scientific advancement within the field of N deposition research, and to provide a starting point for workshop discussions. Workshop discussions will address the relevance of new science in assessing N deposition impacts on Natura 2000 sites, and identify when there is potential to make management adjustments to mitigate such effects.

5.1.1   Introduction

In response to rising world demand for food and energy anthropogenic nitrogen (N) emissions are now around the same order of magnitude as N input from natural sources, which means that the N pool available to terrestrial organisms has more than doubled in less than a century (Vitousek *et al*., 1997). The sources and sinks for biologically reactive N have become decoupled, as N released into the atmosphere from agricultural sources and combustion processes is subject to short and long range atmospheric transport (Galloway *et al*., 2008). Biologically, reactive N can be redistributed from emission "hot-spots" (i.e. agricultural and densely populated regions) to remote regions with undisturbed ecosystems naturally adapted to very low N inputs and availability.

Nitrogen is the second most important plant nutrient behind carbon and the productivity of terrestrial ecosystems is most often limited by the N supply (Tamm, 1991). Hence, increasing N deposition will be expected to exert a large impact on ecosystem biodiversity, biogeochemical cycling of N and ecosystem functioning and service provision. Nitrogen loads to European ecosystems have increased substantially over the last century. At the same time as N deposition loads have increased, substantial alterations in land-use have taken place. In addition, there are on-going climatic changes. Thus, it is difficult to estimate exactly how important N deposition *per se* has been for ecosystem changes in Europe. In many cases useful historic data on ecosystem structure and function prior to the time period of substantial N deposition does not exist. Nevertheless, experimental manipulation studies along with extensive environmental monitoring efforts suggest that N deposition effects on many habitat types have been substantial (see for example Bobbink *et al*., 1992, 1996, 1998, 2003, 2009).

In this background paper we discuss current understanding and recent scientific findings on N deposition effects on European ecosystems in relation to the requirements of implementing the Habitats Directive. In particular, how to assess potential for significant effects affecting the integrity of a site for the assessment of 'plans and projects' under Article 6.3 (see Background Document 1) and assess whether N deposition is a 'pressure' or a 'threat' to conservation status under Article 17 (see Background Document 2). Key considerations discussed below are:

- How does N deposition affect habitat structure and function of different habitat types?
- How does current scientific understanding map onto Annex 1 habitats?
- Is the chemical form of N deposition (reduced N versus oxidized N) or type of deposition (wet versus dry) important?
- What potential is there for the use of bioindicators to assess N deposition effects in Natura 2000 habitats?

- How reversible are N deposition effects?
- What is the potential for the use of on-site management practices for maintaining favourable status?

5.1.2 Effects of N deposition on structure and function of different habitat types

The concept of empirical critical load

The relationship between pollutant dose and resultant environmental effect forms the basis for the critical load concept. The critical loads is defined as "a quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge" (Nilsson and Grennfelt, 1988).

Empirical critical N loads are based on field evidence with respect to increased N inputs. In Europe, empirical critical loads have been assessed since the early 1990s for use within the Convention on Long-Range Transboundary Air Pollution (LRTAP) for impacts on biodiversity in natural and semi-natural systems in Europe (Bobbink *et al*., 1992, Bobbink *et al*., 1996, Bobbink *et al*., 2003). Empirical critical N loads are fully based on observed changes in the structure and functioning of ecosystems, primarily in a) species abundance, composition and/or diversity (structure) or in b) mineralization rate, decomposition or N leaching (functioning). The effects are evaluated for specific receptor groups of ecosystems.

Studies providing insights into ecosystem reactions to an increase in N input or availability have been conducted for a variety of reasons. This has resulted in many different designs, from correlative or retrospective field studies, experimental studies in pots and mesocosms to field addition experiments. Especially statistically and biologically significant outcomes of field addition experiments and mesocosm studies have been used for the assessment of empirical N critical loads. Changes in species abundance, composition and/or diversity is used as the dominant indicator. Only studies which have independent N treatments and realistic N loads (below 100 kg N ha^{-1} $^{-1}$) and two yr or more duration have been used for the setting of the critical load values. The methods used in these studies have been carefully scrutinised to identify factors related to the experimental design or data analysis, which may constrain their use in assessing critical loads. This includes evaluation of the precision of the estimated values of background N deposition at the experimental site. In addition, the results from correlative or retrospective field studies have been used as the basis for estimates of critical loads, but only as additional evidence either to complement of the results from experimental N addition studies, or as an indication for expert judgement. The most recent overview of the European empirical N critical loads are given in Bobbink *et al*., (2003), but an European update procedure will be performed in the period 2009 – 2010.

Critical loads of N can be compared to past, present or future deposition rates in order to establish the amount of excess deposition, also called exceedance. Exceedances of empirical critical loads have been used in European pollution abatement policy for defining emission reduction targets. However, a key question in their use to support policy development is whether there is a link between the exceedance of critical N loads and effects on biodiversity, such as species richness. A recent synthesis of results of European N addition experiments in grasslands, wetlands, (sub) Arctic and alpine vegetation showed a clear negative-log relationship between exceedance of empirical N critical loads and plant species richness (Bobbink *et al*., 2009). Hence, although there are methodological limitations and scientific uncertainties in the methods used to derive empirical critical loads, exceedance of these values is clearly linked to reduced plant species richness in a broad range of European ecosystems.

Examples of N induced biodiversity loss for some European ecosystems

In a recent synthesis article Bobbink *et al.*, (2009) described N deposition effects on terrestrial plant diversity across a latitudinal range of main categories of ecosystems. In this workshop background paper, we briefly summarize the N deposition effects on plant diversity according to this article for some of the ecosystem types represented in the Natura 2000 biogeographical regions.

Arctic and Alpine ecosystems

Common responses to increased N input in arctic and alpine ecosystems include decreased abundance of bryophytes and lichens and increased growth of graminoids. In the harshest habitats (polar deserts and arctic heaths) plant growth is often co-limited by N and P, and increased N input *per se* has in short-term studies not been found to cause significant vegetation changes.

Boreal forest

Increased N input causes major changes in ground vegetation species composition, but often no decline in overall plant species richness. Bryophyte, lichen and dwarf-shrub species are sensitive to elevated N inputs, while many graminoids and herbs with faster growth rates and higher N demand benefit and proliferate. Changes in biotic interactions (increased pathogen damage to plants), or physical disturbance of the ecosystem (for example tree harvest) may reinforce N induced vegetation changes.

Temperate forest

The vegetation response to increased N input in temperate forests include an initial increase in plant cover, a decrease in richness due to loss of N efficient species, a decrease in species evenness from increasing dominance of few nitrophilic species and loss of diversity due to decreases in species richness and evenness.

Temperate heathlands and grasslands

Most of these ecosystems in Europe have evolved under long-term, low-intensity agricultural management. Continued management is thus a prerequisite to sustain them. Natural temperate grasslands (steppe or prairie) with no tree growth due to climatic constraints are relatively rare in Europe. For heathlands in central Europe and UK dwarf-shrub growth is enhanced by elevated N inputs, while bryophytes and lichens often are negatively affected. Biotic, abiotic and climate stresses (for example herbivore damage to dwarf-shrubs and winter desiccation) and some management regimes may however trigger vegetation change from dwarf-shrub to grass dominance under high N input. For grasslands N induced species loss has been observed with more detrimental effects on rare than on common species. For acidic grasslands in the UK, species loss has been shown to occur as a function of cumulative N deposition.

Mediterranean vegetation

N effects on Mediterranean vegetation in Europe have been very little studied. One Italian study supported by several Californian ones, indicate that invasive grasses increase with N input and as a consequence species richness of native vegetation declines. In addition studies have shown that Mediterranean lichen communities are very sensitive to N deposition and major shifts in lichen communities occur at relatively low N input.

Such changes need to be considered in further detail at the workshop in relation to the target habitats listed under the Habitats Directive.

N induced ecosystem functionality changes

For many European ecosystem types, studies have concluded that N deposition results in loss of species richness. Species loss may lead to changes or even loss of key ecosystem functions

and the ability of ecosystems to provide valuable ecosystem services. Due to the assumption of more effective utilization of available ecosystem niches at high than at low biodiversity, a positive relation between species richness and ecosystem functionality has been proposed (van Ruijven *et al*., 2005, Fornara and Tilman, 2009). Important ecosystem functions that may be affected by N deposition effects on biodiversity include productivity, carbon sequestration, N cycling and N retention. There is therefore potential for the consideration of positive and negative N impacts on ecosystem services provided by Natura 2000 sites to further promote the importance of habitat preservation in policy development (See Background Document 5). However, the cause and effect relationships underlying important ecosystem services are often complex and not sufficiently understood as discussed below.

Peatland ecosystems provide an example of how species replacement, resulting from N deposition, may alter ecosystem functionality. On a global scale peatlands store huge amounts of carbon and usually function as active carbon sinks. However, several studies have indicated that the carbon sequestration capacity of ombrotrophic bog ecosystems decreases when subjected to elevated N inputs. Plant growth on ombrotrophic bogs is under low N deposition strictly N limited, as the ecosystem only receives water and nutrients from precipitation. Raised N deposition has a negative impact on the Sphagnum (peatmoss) productivity (see for example Gunnarsson and Rydin, 2000). In addition, increased N input may make Sphagnum shoots more easily decomposable (Limpens and Berendse 2003, Bragazza *et al*., 2006). It has been suggested that reduced polyphenol concentration may contribute to increased Sphagnum decomposability under high N input (Bragazza *et al*., 2006, Bragazza and Freeman, 2007). Nitrogen input also causes vegetation shifts from bogs dominated by Sphagnum to domination by vascular plants (mainly Cyperaceae and Ericaceae species; Gunnarsson *et al*., 2002, 2008, Wiedermann *et al*., 2007, 2009a, Heijmans *et al*., 2008). This shift can have several effects. Cyperaceae and Ericaceae species usually have higher growth rates and nutrient demands and are more easily decomposed than Sphagnum (Limpens and Berendse 2003, Breeuwer *et al*., 2008). Increased abundance of vascular species may also cause the groundwater table on bogs to lower. Taken together, these N induced alterations of plant species composition and chemistry are likely to reduce the ability of bogs to sequester carbon at elevated N inputs.

5.1.3   Nitrogen deposition interferes with ecosystem biogeochemistry

Is the chemical form and type of N deposition important for ecosystem response?

Deposition of reactive N (all species except for unreactive N_2 gas) occurs in several chemical forms. Nitric oxide and nitrogen dioxide (collectively termed NO_x) are eventually oxidized to form nitrate (NO_3-) in aerosols as well as gaseous nitric acid (HNO_3). The combination of oxidized N forms (collectively NO_y) originates from combustion processes (using fossil or bio-fuels) and can be transported long distances in the atmosphere. Farmyard manure and emissions from intensive animal rearing units are the main emission sources of ammonia (NH_3) which forms ammonium (NH_4+) in aerosols and precipitation. Organic N forms occur mainly in the form of amine N (R-NH_2). Reduced N forms (collectively NH_x) are generally transported more regionally/locally than NO_y Atmospheric N inputs (in the form of NO_y and/or NH_x) to an ecosystem can occur both via wet (with precipitation in the form of rain, cloud and snow) and dry (with particle or gaseous) deposition.

Currently, all forms of reactive N deposition are treated as equal with regard to their ecosystem effects when using the critical loads approach. However, their chemical and physical properties and their spatial and temporal deposition are very different. The deposition of wet, dry, oxidized and reduced N species for Natura 2000 sites and their individual effects are therefore a key consideration. The different chemical forms of reactive N have considerable different effects on plant and soil properties and in the following we have briefly summarized the main differences.

Some of these differences between pollutant form may be treated by the 'critical levels' approach for air concentration effects, however, there may also be differences in impacts of deposition between N forms.

Ammonia emitted from farmyards can easily occur in potentially phytotoxic concentrations (Krupa, 2003). It deposits as a 'dry' gas, which is alkaline and highly reactive, and is taken up by plants through open stomata, directly into leaves in response to physical and chemical laws rather than biological demand. By contrast the deposition of ammonium and nitrate is in solution, as these ions are 'washed out' of the atmosphere in precipitation, be it rain, cloud or snow. For higher plants ionic concentrations in precipitation are rarely phytotoxic, with minimal uptake through the cuticle, although much higher concentration exposure can occur as a result of exposure to polluted cloudwater by vegetation. Most ionic N is instead absorbed from the soil via plant roots or mycorrhizal fungi and is thereby subject to biological control as higher plants have physiological mechanisms regulating their root N uptake (Miller *et al*., 2008). For lower plants (bryophytes and lichens), factors influencing uptake of the different N forms are less well understood. Their high surface to volume ratio, the lack of cuticle and low (acidic) tissue surface pH will enhance ammonia deposition and uptake (Jones *et al*., 2007). Also all ionic N forms in solution are efficiently taken up by bryophytes and lichens (Dahlman *et al*., 2004, Turetsky 2006, Forsum *et al*., 2006), although there are studies indicating that ammonium is more readily absorbed that nitrate (Dahlman *et al*., 2004, Nordin *et al*., 2006, Palmqvist and Dahlman 2006, Wiedermann *et al*., 2009b). It has been shown that mosses subjected to high N input (which accumulate abnormally high internal N concentrations) still do not down-regulate N uptake (Forsum *et al*., 2006). In the long term (> 30 years of elevated N input) there is some evidence that at least Sphagnum mosses may be able to adjust their N uptake to high N supply (Press *et al*., 1986, Limpens and Berendse 2003, Wiedermann *et al*., 2009b).

In soils, ammonium, due to its positive charge, can accumulate adsorbed to minerals and organic matter. Hence it may compete with other cations (like for example potassium, K+) that are important plant nutrients, for uptake by roots (Marschner, 1995). If not taken up by plant roots or soil microbes, ammonium can be nitrified, a soil acidifying process which can also increase the risk of plant root damage from elevated Al_3+ toxicity, in mineral soils (see references in van den Berg *et al*., 2008). Nitrate is negatively charged and does not accumulate in soils, rather, if not taken up by plants or soil microbes, it will be leached into water courses taking with it base cations (the mobile anion effect) or it can denitrified to N_2 and/or N_2O, potentially adding to the greenhouse effect (N_2O is 298 times more potent than CO_2 as a greenhouse gas). Plant and soil microbe utilisation of nitrate can increase soil pH, unless base cations are lost through the mobile anion effect.

It should not be overlooked however, that our different ecosystems and the biota they sustain have evolved to deal with the properties associated with the different N forms which are inextricably linked to soil pH and other inherent soil chemical properties. Plant communities characteristic of acid conditions tend to 'prefer' ammonium while those inhabiting more alkaline soils are better adapted to use nitrate (Gigon and Rorison, 1972). The composition of N deposition in precipitation can change the balance of reduced to oxidised N in the soil solution, decoupling it from pH, as well as providing a supply of N for foliar uptake. This means that ecosystems that have evolved on more alkaline nitrate dominated soils may now be challenged by the deposition of ammonium and vice-versa.

Evidence of effects of the different N forms on ecosystem form and function

Field N manipulations offer the most objective approach to separating the effects of the different N forms, especially if the treatment scenario is coupled to real world environmental drivers like precipitation and wind direction. In a globally unique experiment, ammonia, ammonium and nitrate

have been applied since 2002 to an ombrotrophic bog, Whim, in the Scottish Borders, with both historically and currently low (in UK terms) ambient N deposition. The treatments realistically simulate deposition conditions for gaseous ammonia and wet ammonium (NH$_4$Cl) and nitrate (NaNO$_3$), using high application frequencies coupled to meteorology and low ionic concentrations (maximum 4mM) at a range of N doses (+ eight kg N ha^{-1} $^{-1}$ to 56 kg N ha^{-1} $^{-1}$). Measured ammonia concentrations along the release transect have been converted to deposition using a model based on findings from carefully controlled flux chamber studies with the same bog vegetation (Jones *et al*., 2007, Cape *et al*., 2008). This experiment has provided confirmatory evidence that effects observed in controlled experiments (see Krupa, 2003) can be replicated in the field.

Ammonia was shown to be the most damaging N form, effects occurred faster and thus at lower accumulated N doses than with wet deposited oxidised or reduced N (Sheppard *et al*., 2008). Sensitive plants (e.g. Calluna vulgaris, Sphagnum capillifolium and Cladonia portentosa) have a significantly lower tolerance threshold to N deposited as ammonia compared to ionic N deposited in precipitation. Similar N doses as ammonium or nitrate increased the growth of Calluna and had no adverse effects on its tolerance of abiotic or biotic stress to date (Sheppard *et al*., 2008). Exposure to ammonia caused acute responses, probably indicative of higher exposure concentrations, than typically occur with rain or cloud water ion uptake, whereas, the effects of wet deposition were of a less severe but more chronic nature. The effects of oxidised versus reduced N in precipitation were restricted to the bryophytes and lichens: Sphagnum capillifolium, Hypnum jutlandicum and Pleurozium schreberi all showed that ammonium deposition increased N concentrations significantly more than nitrate at higher doses > 24 kg N ha^{-1} $^{-1}$ when accumulation became exponential. In S. capillifolium the resulting high concentrations of soluble toxic ammonium at 56 kg N ha^{-1} $^{-1}$ has contributed to reduced growth, loss of cover and breakdown of the capitulum.

N form may also affect soil mediated plant N responses. In a mesocosm study, the acidification effect associated with the nitrification of ammonium which resulted in, high soil solution concentrations of toxic metal ions, together with the potential for ammonium adsorption on soil cation exchange sites and reduced base cation uptake, were all seen as contributory factors causing the decline in sensitive species seen with high ammonium inputs (van den Berg *et al., 2008*). By contrast detrimental effects of nitrate additions appear to be associated with the growth promoting effects of nitrate additions on some selected plants and thereby increased competition. Studies have demonstrated that many N conservative dwarf-shrubs and herbs have only limited capacity to utilize nitrate (Chapin *et al*., 1993, Kronzucker *et al*., 1997, Nordin *et al*., 2001). In contrast, plant species adapted to N rich habitats (some of them invasive), often exhibit high capacities to take up nitrate (Nordin *et al*., 2001, 2006). In the context of increasing graminoid cover in response to nitrate rather than ammonium, Pearce and van der Wal (2002) recorded the opposite, with slightly more graminoids occurring with ammonium on a Racomitrium heath. However, in this situation all the plants preferred ammonium and the response was explained by the occurrence of nutrient leakage from Racomitrium in response to ammonium toxicity.

5.1.4   Indicators of N deposition

To be able to evaluate N deposition effects in Natura 2000 sites and to be able to adjust the management of affected sites, easily identified indicators of N deposition appear useful. If we are to protect rare species and ecosystems and maintain ecosystem function and services it will be important to establish relationships between changes in soil chemistry, plant metabolites and species composition. There is a need to have a clear remit for N indicators, whether they are to indicate N effects already brought about by N, or provide an early warning of potential effects. Indicators of empirical critical loads for N have been discussed during previous workshops (see Lokke *et al*., 2000 for a report from a critical load workshop held in Copenhagen in 1999). Also in

the UK there have been a series of detailed reports evaluating ecosystem characteristics that could serve as bioindicators of elevated N effects (Sutton *et al*., 2004, Leith *et al*., 2005).

In the following, we briefly summarize information on some potential N indicators;

- N indices for plant species have been suggested as one indicator of N deposition on vegetation. The idea is that by defining species according to their N requirements, one can assess the N status of a habitat by an inventory of its flora. The most frequently used index is Ellenberg's indicator values that have been assigned to a great number of European vascular, bryophyte and lichen species. The Ellenberg index characterizes a species according to a range of variables of which soil fertility at the site where the species is normally found is one of the more important. Another index is FNIS that characterize a species according to its occurrence in relation to soil ammonification and nitrification (Diekmann and Falkengren-Grerup, 1998). A limitation with both these indices is that they are developed explicitly for temperate ecosystems. The Ellenberg index is not specific to N as it denotes soil fertility (including all soil nutrients) rather than just N availability. Also it is only possible to assess changes that have already occurred, and the monitoring results cannot predict future changes.
- Another bioindicator that may be useful for assessing effects of N deposition on vegetation is measuring amino acid concentrations of plant tissues (Pitcairn *et al*., 2003, Wiedermann *et al*., 2009b). According to this idea elevated amino acid concentrations in tissues of a plant would denote that N uptake exceeded the plants' capacity to convert N to growth. This would indicate a risk for others species (with a better capacity to convert N to growth) to take over the habitat. An advantage with the method would be that instead of just assessing changes that already occurred, predictions of future changes may be possible to make as amino acid accumulation in plant tissues is an immediate response to N enrichment preceding any vegetational changes (Nordin *et al*., 1998).
- Total tissue N per cent may be a simpler measure of plant N accumulation than amino acid N concentrations. However, Sheppard *et al*., (2008) found that statistically significant increases in the N concentration in Calluna shoots did not correlate with loss of cover, unless the increase in N per cent was large, as happened when the N deposition was in the form of ammonia. Obviously, by only measuring plant tissue N per cent it is difficult to evaluate the size of the signal and scale of threat N deposition poses to an ecosystem. Although there exists a large literature on the effects of N on plant tissue N per cent there is no central database or major compilation of available data in Europe. Nevertheless, foliar N thresholds may be established for different ecosystem indicator plants which could be calibrated to supply a metric for predicting ecosystem sensitivity.
- The most sensitive N indicators, especially when the N form is ammonia, have been shown to be epiphytic lichen communities. Changes in the proportion of acidophiles to nitrophiles can indicate increasing exposure to ammonia. This suggests that this lichen community response could provide a reliable robust indicator for enhanced ammonia concentrations which is also relatively cheap once field workers have been trained in identification (Leith *et al*., 2005, Wolseley *et al*., 2009). By contrast, such lichens appear to be much sensitive to ammonia than total N deposition, and while lichens may give an indication of the latter, more work is needed to distinguish between the ammonia and overall N effects.

Bioindicators of N deposition need to capture the chain of events (N accumulation cascading through the various ecosystem compartments) that ultimately leads to altered ecological structure and/or function of an ecosystem. Capturing this chain of events may require a cocktail of bioindicator tools rather one specific, as discussed in detail by Sutton *et al*., (2005), especially since other environmental factors and management can also influence indicators. Probably, a combination of

the bioindicators presented above will best report on the state on Natura 2000 sites. Moreover, the applicability of the presented N deposition bioindicators has still to be tested for all vulnerable ecosystems. Likewise for many important plant species, we lack data for many threatened species, i.e. their "baseline" state and have no estimate of acceptable variability, tolerance range or seasonal variability in the majority of bioindicators. In the UK and Europe, many similar issues have been considered with respect to freshwater ecosystems since 1970s. Research into methods for assessing the biological quality of running waters was initiated in response to the perceived need by scientists and water quality managers for a greater understanding of the ecology of running water sites and their macroinvertebrate commununities. This resulted in the development of RIVPACS (River InVertebrate Prediction And Classification Scheme) by the FBA and CEH. It worked on the basis of classifying unpolluted running water sites based on their macroinvertebrate fauna and determining the composition of a macroinvertebrate community at specific sites in response to physical and chemical features. This concept of assessing 'reference condition' (now based on 500 sites) and making comparisons with the observed fauna at sites of interest, directly influenced the drafting of the European Union Water Framework Directive (WFD) (European Commission, 2000). The core concept of the WFD, that an ecological status target is set for each site, is essentially derived from the RIVPACS type approach. A similar approach may be used for terrestrial habitats.

5.1.5 Reversibility of N deposition effects

A key conservation question is whether, and to what extent, N induced changes are reversible, if N deposition levels are reduced. Related to this, it needs to be known over what timescale any recovery might operate for different effects and receptor ecosystems. From a scientific point of view, we have a reasonable knowledge concerning effects of increased N input, while the reversibility of N-induced effects is largely unknown. A small, but growing literature dealing with this topic is, however, emerging (e.g. Strengbom *et al*., 2001, Mitchell *et al*., 2004, Power *et al*., 2006, Limpens and Heijmans 2008, Clark and Tilman, 2008). From such studies it is apparent that some ecosystem parameters may revert quickly, while other components may show strong inertia.

Although several studies have shown that N leakage or exchangeable N in the soil may return to control conditions within a few years following cessation of external N input (Bredemeier *et al*., 1998, Högberg *et al*., 2006, Oulehle *et al*., 2006), changes in plant species composition may be slow. Strengbom *et al*., (2001) found no, or only small signs of recovery in boreal ground vegetation nine years after cessation of N addition (c. 100 kg N ha^{-1} for 20 years). Nearly 50 years after cessation of N addition, the abundance of bryophytes sensitive to N addition was still lower (e.g., Hylocomium splendens), while bryophytes favoured by N addition were still higher (e.g., Brachytecium spp.) (Strengbom *et al*., 2001). In a study using controlled mesocosms, containing an aquatic habitat similar to that which can be found in shallow soft-water lakes, a two-year treatment with ammonium resulted in substantially altered plant species composition (Brouwer *et al*., 1997). Despite a 10-year treatment with clean rain water following the cessation of the ammonium treatment, only minor recovery of the plant species composition was observed. In grasslands, here exemplified by prairie system from North America, low levels of elevated N input (6 kg N ha^{-1} $^{-1}$ above a background deposition of 4 kg N ha^{-1} $^{-1}$) reduce species richness and alter relative abundances of plant species (Clark and Tilman, 2008). A decade after cessation of the N treatment, plant species richness (on plot level) had returned to control level, but the relative abundance of component species still differed. This suggest that in several habitat types, once altered by elevated N input, the species composition if reversible, requires substantial time to revert to a state comparable to that prevailing under low N input.

Other habitats, or important parts of habitats, may revert more quickly to the low N input state. For example tissue N concentration in Sphagnum species in raised bogs may return to pre-treatment concentrations within 15 months after cessation of N addition (Limpens and Heijmans, 2008). This

suggests that, as long as high N input has not caused the peat forming Sphagnum species to die, the ecosystem service of peat accumulation may be restored rather quickly following reduced N input (Limpens and Heijmans, 2008). Similarly, in a reciprocal transplant experiment, Mitchell *et al*., (2004) were able to show recovery of tissue N concentrations and growth rates within a year of transplanting ephiphytic bryophytes to a cleaner location.

In some cases reversion to the original state may however be impossible. Species may locally or regionally have become very rare or even gone extinct providing no propagule source for the original species. Moreover, new internal or external factors may have emerged in the ecosystem, to hinder reversion. For example, changed precipitation patterns have proven reversion of N altered plant communities difficult or impossible (Choi *et al.,* 2006). In addition, internal feed-backs on nutrient turnover-rates (Bowman and Steltzer 1998, Chen and Högberg 2006, Power *et al*., 2006), may have increased the persistence of the N induced state, and made new alternative states of the system possible (Suding *et al*., 2003).

5.1.6 The use of management practices for maintaining favourable status

Decreasing N deposition would, of course, be the preferred way to protect Natura 2000 sites from N induced ecosystem changes. However, management methods that remove N from a habitat can be useful in mitigating N deposition effects on ecosystems. From semi-natural habitats, such as grasslands and heathlands, which require an active management regime for their maintenance, intensified use of methods causing biomass removal by mowing or prescribed burning may at least partly mitigate N induced alterations (Mountford *et al*., 1996, Barker *et al*., 2004).

For Dutch calcareous grasslands Willems (2001) suggests that N deposition effects can be decreased by mowing in early August. The mowing suppresses the N favored grass Brachypodium pinnatum (Tor grass) and promotes the original species-rich grassland vegetation. Also for heathlands, originally dominated by Calluna vulgaris, active management involving prescribed burning and mowing can mitigate effects of N deposition (Barker *et al*., 2004). Active management may be a promising alternative for many semi-natural habitats, and often the N management can be incorporated in the management that is already imposed to maintain the conservational value of the habitat. For other habitats there is no, or very little, available information on management strategies mitigating effects of N enrichment. For forest ecosystems, timber harvest and/or forest fires can remove large quantities of N. If timber harvesting is not combined with burning of the clear-cut area, it is necessary to remove also needles/leaves and branches (beside the timber) to achieve a significant N removal from the ecosystem. Moreover, we should be reminded that the physical disturbance caused by timber harvest may under some circumstances reinforce N effects on the ground vegetation, thus counteracting the potential positive effect of removing N from the ecosystem (Strengbom and Nordin, 2008). In forest types where re-occurring forest fires have been part of a natural disturbance regime, prescribed burning seems like an efficient management strategy for mitigating effects of N deposition, as it both reduces the amount of N stored in the forest floor vegetation and in the uppermost humus layer while also restoring the natural disturbance regime. However, such an approach could be considered controversial, due to the need to more fully understand other interactions, including the fate and impact of the reactive N emitted in such fires.

It should be added that realistic site level management practices will certainly not be able to mitigate all the impacts of enhanced N deposition and enhanced N concentration on Natura 2000 habitats. For example, the loss of epiphytic flora would be very difficult to deal with by on site management practices.

5.1.7 How can we use current understanding of N impacts to protect Natura 2000 sites from N deposition?

As already concluded, a majority of European Natura 2000 sites are affected by historic and present patterns of N deposition. It is scientifically well established that the effects of N deposition on plant community structure and function depend on the ecosystem type and the size of the cumulative N deposition load. Bobbink *et al*., (2003) provides the most recent compilation of data indicating sizes of N loads causing significant changes in various ecosystem types. In addition, recent scientific findings point out that:

- Chemical N form can influence both the rate of ecosystem change and possibly even whether N impacts will occur, at least in the short-term.

- Where the N source is agricultural and local, the effects will be more damaging and occur at lower N doses, mainly due to detrimental plant physiological effects of ammonia.

- Because plant species vary in their ability to use nitrate, nitrate effects will depend on present plant species and the likely risk from species invasion.

- Climatic factors interfere with ecosystem effects of N deposition and it is clear that climate both can emphasize and mitigate effects of N deposition.

- Following decreased ecosystem N input, some ecosystem parameters may revert quickly, while other components may show strong inertia. In some cases reversion to the original state may be impossible.

- More knowledge is needed to better understand where and if management intervention is appropriate to mitigate N effects.

Acknowledgements

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5.2 Working group report

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5.2.1   Conclusions and recommendations of group discussions

• It was concluded that the latest science supports and strengthens the already established empirical critical loads approach, encouraging their use in environmental decision making.

• The workshop concluded that there are no acceptable exceedances above a critical load or critical level. Discussions regarding "acceptable exceedances" are not a science issue and should be addressed at a policy level. In order to improve the situation, one should aim at reducing nitrogen deposition below the critical loads and levels.

• New data has strengthened the view that it is important to consider different nitrogen forms when evaluating effects of nitrogen deposition. It was concluded that evidence of responses for the different nitrogen forms is consistent across ecosystems and species. Moreover, because the effects from nitrogen deposition differ between different nitrogen forms (dry/ wet deposition and oxidized/reduced nitrogen) it is important to evaluate their effects independently. Hence several types of critical loads/levels for a particular habitat type are

needed. For example, the critical level for ammonia may be well below the critical load set for total nitrogen deposition. Hence it is important that both critical loads and levels are used.

- Important new data from Southern Europe have emerged over the last five years, for example, during the workshop results from experiments and surveys conducted in Portugal and Spain were presented. These should inform future revisions of critical loads for nitrogen

- The workshop concluded that improved conditions following reduction in nitrogen deposition are only relevant when nitrogen deposition is reduced below the critical load/level. Reduction of exceedance will only improve the situation in the sense that it reduces the risk of further worsening of the effects. Information about the effects on recovery time following reductions below critical loads/levels is still largely lacking. Available data suggests that the rate of improvement will differ depending on type of function/species studied, and is often site specific.

- It was concluded that management to reduce the impact of nitrogen deposition will only work in combination with reductions in nitrogen deposition and should not be seen as an alternative to reducing the nitrogen deposition. For semi-natural habitats, positive effects from reducing the nitrogen inputs will only be possible in combination with appropriate management.

- The workshop agreed that there are important interactive effects between nitrogen deposition and climatic factors. Therefore a changing climate may also influence the effects of nitrogen deposition. Currently, the knowledge of such interactive effects, and how they may change with a changing climate is, however, poorly understood. The climatic factors most important for interactive effects with nitrogen are also the most uncertain in climate change modelling (e.g. precipitation), making predictions of future interactions between nitrogen deposition and climate change difficult.

- It is recommended that future research should prioritize the assessment of relative impacts of different nitrogen forms in relation to critical thresholds and dose response relationships, the relationships between nitrogen dose and site- and landscape-level management practices as a basis for minimizing adverse effects on ecosystem integrity, and the quantification of the interactive effects between climate change and nitrogen deposition.

5.2.2   Introduction

Actions to manage the Natura 2000 network and to assess conservation status must be based on a sound scientific understanding of how reactive nitrogen deposition causes impacts on sensitive habitats The working group reviewed the latest science on the effects of nitrogen (N) deposition and concentrations on Natura 2000 sites, including the use of bio-indicators, effects of N-form (e.g. NH_x vs NO_y) and the relationships between critical thresholds and biodiversity loss.

Nordin et al., (this volume) summarizes established and new science on the effects of nitrogen deposition on ecosystems and considers the potential for improved assessment of N deposition impacts on Natura 2000 sites. The working group discussion was organised around five key issues identified from Nordin *et al*., (this volume):

- How does N deposition affect the structure and function of different habitat types?
- Is the chemical form of N deposition important?
- What is the potential for use of on-site management for improving conservation status?
- Interactions between N deposition and climate and climate change
- How reversible are N deposition effects?

Group members were given the opportunity to give presentations concerning the topic. Some of the points and conclusions from the presentations are referred to in this summary. However, more detailed descriptions can be found in the papers in this volume (see sections 5.3 to 5.11).

5.2.3 Highlights of discussion and views expressed

Key issue 1: How does N deposition affect the structure and function of different habitat types?

The impacts of nitrogen deposition on structure and function were summarised as:

- direct toxicity of gases and aerosols,
- eutrophication, resulting in changes of species composition (more nitrophytic species) and sometimes reduction in species richness,
- soil-mediated effects of acidification (more acid-resistant species),
- increased sensitivity to stresses and disturbances (drought, frost, pathogens, herbivores).

The impacts are very complex, have many interactions, and are working on different timescales. Discussion of this key issue centered on the following topics which had been highlighted by other working groups:

- What are the strengths and limitations of the critical load/level approach?
- What is the minimum detectable effect above a critical load/level?
- What indicators/biomonitors can be used?

Critical load/level

Empirical critical loads for nutrient nitrogen are based on total nitrogen deposition and do not consider different nitrogen forms separately. Conversely, there are separate critical levels for ammonia and oxides of nitrogen. Critical levels for NO_x (30ugm⁻³) were established in 1992 (UBA, 2004). Areas most at risk of exceedance of the NO_x critical level are those in or close to urban or industrial areas or close to major roads. Exceedance of ammonia critical levels is more widespread in Natura 2000 sites (which tend to be located in rural areas). New critical levels for ammonia were approved by the UNECE in 2007. They are considerably lower than the former critical level, and incorporate an element of long-term protection cf critical loads. The 'long-term' annual average critical level is now one µg NH_3 m⁻³ for lichens and bryophytes and ecosystems where lichens and bryophytes are a key part of the ecosystem integrity. The 'long-term' annual average critical level for higher plants (e.g. heathland, grassland and forest ground flora and their habitats) is three µg NH_3 m⁻³, with an uncertainty range of 2–4 µg NH_3 m⁻³. The monthly mean value is 23 µg NH_3 m⁻³ to address the possibility of high peak emissions during periods of manure spreading. The impact of peak concentrations of ammonia is not well researched. Other nitrogen forms were discussed by the group. No critical levels exist for HNO_2 (evidence was provided of effects on Scots pine at very low concentrations, Cape, pers comm.), PAN and HNO_3.

The empirical N critical loads are based on results from published experiments or surveys (see Nordin et al., this volume) The most useful data is those derived from long-term experiments (5-10 years or longer) using realistic N doses, conducted in areas with low N-deposition, with good estimate of background deposition. .

Critical loads encompass dry and wet deposition of several reduced and oxidized nitrogen compounds. However, in most monitoring programmes only inorganic nitrogen is measured. However, organic N contributes accounts for around 30 per cent of UK wet N deposition (Cape, pers comm.). Presently, the origin of the organic nitrogen is unknown. Although spatially associated with NH_4+ in rain, its concentrations have a different seasonal pattern. Organic N is currently not

included in the assessment of eutrophication and critical loads. Further knowledge of its origin and its potential importance are needed before it can be evaluated whether this N form should be included in assessment of eutrophication and critical loads.

There has not been a validation of critical loads/levels against effects on invertebrates. This may be important in a biodiversity perspective, because invertebrates are a species rich group that often significantly contribute to the overall biodiversity of a habitat.

Indicators/biomonitors
The working group concluded that the Annex 3 (Ecological indicators) from the report made at a workshop on critical load in 1999 in Copenhagen (Arbejdsrapport DMU nr. 121) remained relevant and this issue was only covered briefly during the discussion. This key issue was, however, indirectly covered in several presentations, as these included case studies with different types of organism. Presentations covered:

- The use of butterflies as indicators of nitrogen deposition impact (see Feest, this volume)
- Measurements of nitrogen content of mosses across Europe and comparison with modeled data (see Harmens *et al.*, this volume)
- Species richness in calcareous grassland and correlations with nitrogen deposition (see Alard *et al.,* this volume)
- Lichen functional diversity and nitrogen content (see Pinho *et al.,* this volume)
- C/N ratio as an indicator of N leaching (Forsius, pers comm.).

What is the minimum detectable effect above and below a critical load/level?
This question had been put to the group to inform discussions under Theme 1: assessment of impacts on Natura 2000 sites under Article 6.3 of the Habitats Directive (see Section 3.2). The working group agreed that the detection level is a matter of resource availability. More resources imply that detection levels can be improved. Better replicated experiments will increase the statistical power, which enables detection of smaller effects. When the critical load or level is exceeded, it was concluded by the group that discussing minimum detectable effects is irrelevant. Once the N input reaches the critical load/level any further increase will, by definition, lead to an increased risk of negative effects. The more the critical load/level is exceeded the greater the risk of negative effects. Because the critical load/level describes an increased probability of negative effects when the load/level is exceeded, the exact response among sites will vary. Furthermore, for an individual site, negative effects may occur even though the N deposition is below the critical load/level, while for another site no negative effects may be apparent when the critical load/level is exceeded. However, once the critical load/level is exceeded, 95 per cent of the sites will show negative effects. Acceptance of exceedance above the set critical loads/levels is thus a political rather than a scientific issue: Science can only provide the evidence to help inform policy makers' decisions. In order to ensure protection of Natura 2000 sites from elevated N input deposition needs to be less than the critical load. The question is therefore, how can you best achieve this? Data were presented from field studies with N-addition experiments conducted in grassland and arctic/alpine areas. The data show that the more the critical load is exceeded the greater is the reduction in species richness (Bobbink, 2008). Furthermore, the datasets suggest that the established value for the empirical critical loads remains well supported by recent experimental data.

The working group also agreed that the timescale, geographical and spatial dependence need to be considered when the likelihood of detecting effects of elevated N input are discussed. The empirical critical load/level concept is designed to protect an ecosystem over a time–period of ~ 30 years. This implies that there often is a time lag between when the critical load/level is exceeded and when the negative effects of the exceedance become detectable. Further, effects are not necessarily linear

related to N deposition, as they may depend on interactions with other factors e.g. drought and pathogens. This implies that negative effects from exceedance of the critical load/level may not be seen until such an event/episode occurs, potentially a further time lag. The effect of N deposition will also depend on the history of the site (cumulated deposition, management history), which may cause variation in the response to exceedance of the critical load/level.

A potential problem with using the concept of critical loads for protecting Natura 2000 sites is that critical loads are not designed to protect individual species, but rather to ensure that the N input is below the level responsible for negative effects on the habitat/ecosystem. To ensure protection to all species and functions one possibility is to use the most N sensitive (according to current knowledge) of "characteristic" species or the most N sensitive functions of the habitat to define the critical load or level for a habitat. By ensuring that such target species/functions are protected from the negative effects of N deposition, other parts of the system will be automatically protected. It was also stated that it is important to consider both critical loads and levels, since even if the critical load (based on total N deposition) is not exceeded the critical level of e.g. ammonia may still be exceeded for highly sensitive species.

Key issue 2: Is the chemical form of N deposition important?
The working group focused on the following key questions:

- How do effects from reduced and oxidized N forms differ?
- What are the differences between wet and dry N deposition?

There is clear evidence that N effects depend on the form in which N is deposited (see Nordin et al., this volume). Dry deposition of gaseous ammonia, per unit N deposited, causes more damage than the equivalent amount of wet deposited ammonium, which again is more damaging than the equivalent dose of wet deposited nitrate in most instances. The effects of ammonium on sensitive lichens and mosses are more detrimental than those of nitrate. Effects of wet deposited ammonium and nitrate on higher plants depend strongly on soil pH at the site. Currently, there is insufficient data to establish separate critical loads for NO_y and NH_x. However, during the discussion it was noted, that the relative importance of NH_x compared to NO_y is increasing due to greater reduction of emissions of NO_x relative to NH_x.

In an experiment in Mediterranean ecosystems in Portugal, nitrogen was added as oxidized and reduced N in combination, or only in the reduced form (Dias *et al.*, this volume). After one year of N treatment, effects were seen on species richness for both types of treatment. Diversity, expressed as Shannon diversity index, increased when oxidized and reduced nitrogen was added in combination, but decreased when N was added in reduced form only. The effect of wet deposited ammonium and nitrate on higher plants depends strongly on soil pH and can be summarized as follows:

- mineral soil pH 4.5-5: (Ca/Al buffering range) uptake of NH_4+ reduces pH leading to increased risk of Al-toxicity, and potentially base cation deficiency.
- soils above pH 5:Acidification effect of NH_4+ is not so pronounced, the effect is rather a change in species composition through eutrophication. Competitive relationship between species will shift, resulting in changed composition and loss of species, e.g. such that are unable to exploit increased N availability due to nitrification. However, if nitrification of the ammonium fails in these soils, due to the acidity generated, ammonium can accumulate to toxic levels.
- acidic soils pH < 4: In general, plants have a greater tolerance to NH_4+. However if N input is high, nitrification may occur (normally less important in such soils), which may induce changes in plant species composition as it will favor a relative few number of species with good capacity to utilize nitrate.

• Insufficient data is available for calcareous soils

Key issue 3: How reversible are N deposition effects?

The working group focused on the following key questions:

• What is the baseline?
• How can we measure improvement (i.e. effects of reduced N input above and below critical loads/levels)?
• Time horizons for improvement?

Baseline
It is difficult to define a baseline for most of the sites within the Natura 2000 network. Many Natura 2000 sites have a long history of high N deposition and negative effects such as loss of species and changed species composition have probably already occurred, a long time ago. For many sites the full extent to which N deposition has affected species richness and diversity is unknown, and will probably never be fully understood. During the discussion it was concluded that it was impossible to reach a consensus on a common definition on what the baseline should be

It is important to distinguish between the situation above and below the critical load/level. If the critical load/level is already exceeded, a reduced N input will result in a decreased risk of a worsening of the effects rather than recovery. Only when the N input is reduced to below the critical load/level will recovery in the real sense be possible. The available literature on improved conditions/recovery from N induced effect is limited (see Nordin *et al.*, this volume). It is evident that the rate of improvement will differ between different components of the ecosystem and differ between different sites depending on geographical location, climatic conditions, N deposition history, and in some cases also on site management, and management history.

A wide range of parameters may be used to assess improvement from lowered N deposition. Indicators that can be used are: increased species richness and increased occurrence of N sensitive species, as well as recovery of the original species composition (in cases where this is known and species have not been lost) or changes into a composition that resembles more pristine conditions. Use of the Ellenberg index will be restricted to some areas in Europe and cannot be used for all Natura 2000 sites, as Ellenberg index values are lacking for many species outside central/Western Europe. Chemical characteristics can also be used to measure improvement. For example reduced

Table 5.1: Characteristics of the different depositing nitrogen compounds (Sheppard *et al.*, this volume)

Ammonia (NH₃)	Ammonium (NH₄+)	Nitrate (NO₃-)
Deposits close to source	Naturally occurring in soil (mineralization)	No reports of direct damage to plants
Highly reactive and alkaline	Dominates in acid soils, as pH increases more will be nitrified	Effects soil mediated through eutrophication, acidification and competition
Effects most likely mediated above ground	Reduces growth and survival in sensitive species	
Effects are concentration driven	High concentration potentially toxic	
Close relationship between effects and proximity to sources	Soil mediated effects through acidification and eutrophication	

N leakage, reduced N mineralization rate, reduced exchangeable soil N, reduced concentrations of N rich amino acids in plant tissue are all potential indicators of improved conditions.

Time horizon
If N input is reduced below the critical load/level, recovery time from direct N effects and soil mediated indirect N effects can be assumed to differ between ecosystems. When the affected species are still present, recovery from direct effects is, in general, assumed to occur faster than recovery from soil mediated effects. It is important to note that improvement from N induced effects under conditions with lowered N input may not necessarily show the same dose response relationship as when the N load was increasing.This implies that some N induced effects will not necessarily recover when the N deposition is restored to the 'original' level. In some situations improvement or recovery may be difficult, or not possible, because key functions or key species may have been lost from the habitat. The time horizon before improvements show depends on the type of effect (e.g. acidification, leaching, species composition etc.). In some cases the improvement will also be dependent on management regimes (e.g. moving, grazing). Some ecosystem parameters may show rather rapid improvement following reduction of the N input. For example, improved conditions for exchangeable soil N or N concentration in Sphagnum mosses growing on bogs can be rapid, whereas other parameters such as re-establishment of species or recovery of original species composition are slow processes.

Key issue 4: What is the potential for use of on-site management for improving conservation status?

During the discussion it was concluded that active management, that removes nitrogen from the system, should not be seen as an alternative to lowering the N deposition at a site. Likewise it was concluded that intensified management cannot justify increased N deposition at a site. It was also concluded that management strategies are more or less confined to semi-natural habitats, and for many Natura 2000 sites there are no available management strategies today that will help to improve the situation. It is important to distinguish between management for "restoration" and management as a means of maintaining function and form of semi-natural habitats such as heathland and grassland (i.e. non-climax vegetation types that are man-made). In such semi-natural habitats active management like mowing, burning and/or grazing is necessary to maintain the desired species composition and function of the system. Such management will result in removal of nitrogen, which helps the system to maintain its nitrogen limitation. For wetlands many habitats have been drained, and here restoration of the hydrological conditions may be a prerequisite before benefits can be expected to come from lowered N deposition. It was also noted that mismanagement of water is a very important threat to Mediterranean ecosystems (Tsiouris, pers. comm.).

Key issue 5: Interactions between N deposition and climate and climate change

The working group concluded that there are undoubtedly important interactions between N deposition and climate, and that a changed climate will interact with N deposition and these need to be addressed in discussions of the effects of N deposition. It was also concluded that there is a large gap in knowledge concerning such interactive effects. It is evident that N effects interact with climate factors such as drought, frost, precipitation and temperature. Hence, alteration of such climatic factors will likely alter the effect of N deposition. The climatic factors that probably are most important for interactive effect with N are also the factors that are most uncertain in climate change modeling (e.g. changed precipitation pattern), making predictions of future interactions between N deposition and climate change difficult. Habitats already exposed to N deposition will, regardless of future climate, still be nitrogen rich systems. Such systems may be sensitive to establishment of invasive or new species.

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5.3 Defining a biodiversity damage metric and threshold using Habitat Directive criteria

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Abstract

- Changes in the environmental suitability of a site for particular plant species in response to nitrogen (N) load can be predicted fairly objectively using model chains such as MAGIC-GBMOVE.
- Lists of positive and negative indicator plant species, such as those in UK Common Standards Monitoring Guidance, provide operational definitions of habitat quality and damage.
- Whilst N-sensitive species can provide early warnings of change, they may not be representative of the desirable features of the habitat.
- A metric of habitat quality is proposed, based on predicted environmental suitability for positive and negative indicator species.
- This metric allows assessment of the impact of N pollution on habitat quality as defined independently of the N effects research community.

5.3.1   Introduction

National emissions limits of reactive nitrogen (N) are established under the UNECE Convention on Long-Range Transboundary Air Pollution (LRTAP) using the critical load approach to reduce damage to habitats. This approach requires operational definitions of a metric which can be used to assess habitat quality, and a threshold above which damage can be said to have occurred. Metrics such as soil pH and soil total C/N ratio have been used, but it is increasingly recognised that changes in these abiotic measurements may not be synchronised with significant changes such as species loss and invasion. There is thus a need for damage definition criteria that are explicit and repeatable (Nicholson *et al*., 2009) and that can be related directly to habitat quality as defined by conservation experts.

The Habitats Directive aims to maintain biodiversity in Europe. It defines habitats and species of Community importance and provides protection measures. (EEC, 1992). Comparatively few species are listed in the annexes, and these are rather rare. By contrast, some of the habitats listed are extensive and thus the Annex I definitions and criteria are applicable to a larger set of sites. Member States are required to designate Special Areas of Conservation (SACs) to protect habitats and species listed in Annex I and II respectively (see Whitfield and Strachan, this volume), although responsibility for these habitats and species also extends beyond these areas. Assessments must be made of plans or projects likely to have a significant effect on special areas of conservation in view of their conservation objectives. For habitats, conservation status is defined as the sum of the influences that may affect long-term natural distribution, structure and functions as well as survival of typical species. However, structure and function are not easily defined without reference to particular species. Plant species assemblages and their phytosociological names are primary definitions for terrestrial habitats within the Habitats Directive. These classifications can usually be related to lists of typical species, for example using the National Vegetation Classification (Rodwell, 1991-2000) in the UK. The quality of vegetation on a site is typically assessed in terms of conformity to descriptions of a typical assemblage of species, the presence of rare or typical species, and/or the absence of untypical species. In the UK, assessment of the condition of interest features on special areas of conservation includes a standard procedure known as Common Standards Monitoring (CSM), which lists positive and negative indicator species for many habitats.

This emphasis on species is useful not only to derive concrete and measurable criteria for site assessment, but because responses of individual species to reactive N pollution can be predicted empirically. Niches for individual plant species have been defined in relation to environmental factors in models such as MOVE (Latour and Reiling, 1993; Wamelink *et al*., 2009), VEG (Belyazid *et al*., 2006) and GBMOVE (Smart *et al*., 2010). By solving these models with respect to time series of abiotic factors generated by process models, the likely occurrence of individual species can be derived for different scenarios (de Vries *et al*., 2010).

For environmental suitability predictions for a set of species to be interpreted in terms of conservation status, it is necessary to refer to lists of desirable and undesirable species. The loss of N-sensitive species is a major basis for defining empirical critical loads (Bobbink *et al*., 2003), and N-sensitive species are useful in providing early warnings of change. However, N-sensitive species may not be important components of a particular habitat, and there is a worrying circularity in defining damage from reactive nitrogen as damage to N-sensitive components. A more objective approach would be to use existing lists of desirable and undesirable species defined beyond the N effects research community. Desirable and undesirable species for particular habitats have already been identified in many cases. Where such lists are not yet available they could be derived from information about species scarcity such as the IUCN Red List (Mace at al., 2008), by indicator species analysis (e.g. Ejrnaes *et al*., 2004) and/or by consultation with habitat experts and the public.

An assessment of a site's conservation status can be derived from a list of desirable and undesirable species, if indicators are available of the actual or likely occurrence of these species on the site. Models derived from floristic survey data can predict likelihood of occurrence, but many other factors such as dispersal traits and local presence govern actual occurrence on a particular site so such predictions are best interpreted as the suitability of the site for the species. Different statistics can be derived from site floristics, such as the presence / absence, abundance or frequency of each species. Such data could in principle be converted into a habitat quality metric using the approach advocated here, but this is beyond the scope of the current paper. Rather, we propose a method for weighting and aggregating predictions of environmental suitability for individual species, for use in interpreting the outputs from models of effects of N pollution on plant species. This simply uses

weightings of +1 for species listed as positive indicators, -1 for species listed as negative indicators, and 0 for species not listed for a given habitat.

5.3.2   Aims and objectives

- To summarise habitat suitability predictions for a large set of species into a single metric of habitat quality
- To base this metric on existing definitions of site condition .
- To explore the use of this metric in predicting habitat damage for critical load exceedance calculations.

5.3.3   Results and discussion

Historic deposition sequences of N and sulphur derived from the FRAME model (Fowler *et al.*, 2005) and Gothenburg scenario projections of future deposition were used to generate time series of soil C/N ratio and pH for blanket bog at the Moor House long-term monitoring site, using the MAGIC model (Cosby *et al.*, 2001) (Figure 5.11a). These time series of abiotic variables were used to calculate probabilities of occurrence for species regarded as positive (Figure 5.1b) and negative (Figure 5.1c) indicator species for UK blanket bog in the UK Common Standards Monitoring guidance (JNCC, 2006). An overall habitat quality index Q was calculated as

$$Q = \sum_{i=1}^{p} \frac{\left(\dfrac{P_i}{P\max_i} \right)}{p} - \sum_{j=1}^{n} \frac{\left(\dfrac{P_j}{P\max_j} \right)}{n}$$

where P_i is the probability of occurrence of positive indicator species i and P_j is the probability of occurrence of negative indicator species j (Figure 5.1d).

These results illustrate the potential of the approach. Even though species were not selected for inclusion in the indicator calculation on the basis of their nitrogen-sensitivity, there is a tendency for probability of occurrence of positive CSM indicators for this habitat to decline with an increase in N saturation. The probability of occurrence for negative indicator species did not clearly increase with an increase in N saturation, but nevertheless the overall habitat quality index showed a clear and continuing decline under this N emission scenario.

The proposed metric has the advantage of using the entire set of species named in previous condition assessment, reducing the danger of bias towards well-studied and charismatic species (Sitas *et al.*, 2009). The metric will be influenced by the number of indicator species included, and it would therefore be useful to standardise the number of positive and negative indicator species. Another issue is the relative weighting of positive and negative indicators and other species; giving a greater weighting to more rare or typical positive indicators, and a more strongly negative weighting to particularly negative indicators, would likely give a more responsive metric. This advantage should be assessed against the increased subjectivity involved with assigning different weightings to different species.

If it can be agreed that such a habitat quality index at least partially reflects conservation status, the next issue to be resolved is how to set a threshold level of Q below which the habitat can be said to be damaged. Empirical critical loads as currently defined (Bobbink *et al.*, 2003) provide a basis for calculating threshold values for indicator species' probabilities of occurrence. In ongoing
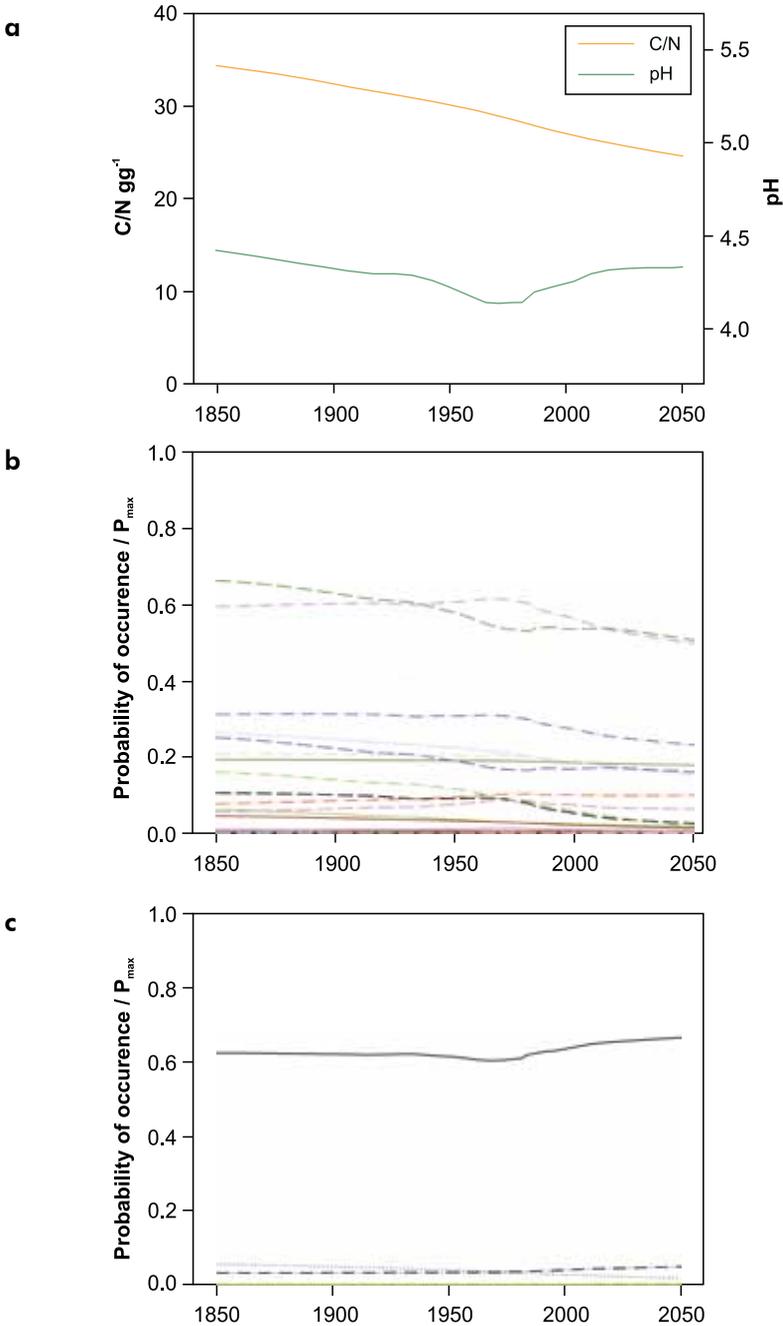
Figure 5.1: Simulated changes in blanket bog at Moor House long-term monitoring site, Cumbria, UK, under the Gothenberg emission scenario: (a) Soil pH and C/N ratio simulated using the MAGIC soil chemistry model; probabilities of occurrence for (b) positive and (c) negative Common Standards Monitoring indicator species for blanket bog obtained from the GBMOVE species niche model, rescaled to Pmax, the maximum probability of occurrence for the species (soil water content and canopy height were assumed to be constant).
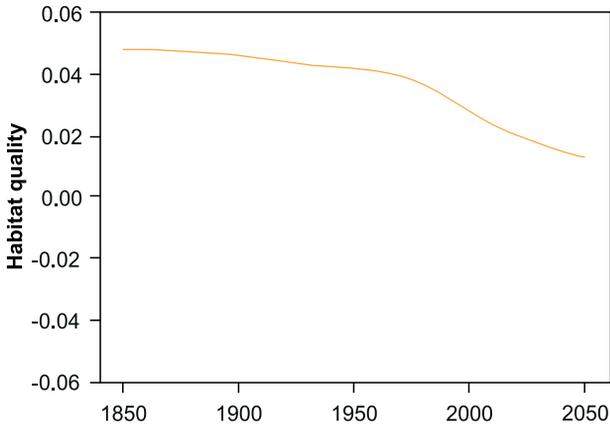
Figure 5.1 (d): Simulated changes in blanket bog at Moor House long-term monitoring site, Cumbria, UK, under the Gothenberg emission scenario: (d) overall habitat quality Q.

work, we aim to extend the approach outlined here to define threshold levels of habitat quality for different habitats.

5.3.4 Conclusions

- Operational definitions of conservation status and habitat quality can be derived from the occurrence of positive and negative indicator species as defined by conservation experts.
- Habitat suitability predictions for positive and negative indicator species can be summarised into a habitat quality metric.
- This habitat quality metric is a direct and useful link between N pollution and nature conservation policy mechanisms.

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Acknowledgements

5.4 Influence of nitrogen deposition on plant biodiversity at Natura 2000 sites in Spain

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Abstract

• Worldwide scientific evidence has been accumulated showing that anthropogenic emissions of nitrogen compounds to the atmosphere are affecting terrestrial ecosystems, thus threatening the world's biodiversity.

• This problem may be relevant in Spain, where contrary to other European countries, NO_x emissions have significantly increased since 1990 until present.

- An occurrence index for nitrophilous species of herbaceous plants, mosses and lichens was calculated to check the potential influence of nitrogen emissions on plant biodiversity.
- Data from the latest eleven decades suggested an increasing trend in the reported occurrence and relative richness of nitrophilous species throughout Spain.
- The proposed methodology is useful for the detection of temporal and spatial changes in plant biodiversity, allowing the identification of potential threats to sensitive biotopes within the Natura 2000 Network.

5.4.1   Introduction

An acceleration of biodiversity loss has occurred worldwide, linked to the over-exploitation of natural resources, habitat destruction and climate change (Sala *et al*., 2000, Thuiller *et al*., 2005). Furthermore, with the intensification of agricultural and industrial activities over the past several decades, large amount of N compounds have been released to the atmosphere (Galloway *et al*, 2003), resulting in elevated N deposition in terrestrial and aquatic ecosystems (Asner *et al*., 2001; Matson *et al*., 2002). In this regard, several experimental studies have reported that nitrogen (N) enrichment reduces plant diversity, leading to the conclusion that anthropogenic N deposition is a threat to global biodiversity (Stevens *et al*., 2004; Davies *et al*., 2007; Xiankai *et al*., 2008).

One of the main actions that have been undertaken at the European level to contribute to the maintenance of biodiversity is the establishment of the Natura 2000 network. The network is composed of Special Areas of Conservation (SAC), designated under the Habitats Directive (Council Directive 92/43/EEC), and of Special Protection Areas (SPAs), designated under the Birds Directive (Council Directive 79/409/EEC). Spain has designated approximately 1400 SACs and 600 SPAs, representing almost 30 per cent of Spanish territory.

Since nitrogen enrichment has been considered one of the most important anthropogenic factors influencing ecosystem structure and function, the European Union has implemented several measures to abate the emissions of nitrogen compounds. As a result, the majority of EEA-32 countries have reported lower emissions of NO_x and NH_3 in 2007 compared to 1990. An opposite trend has occurred in Spain, as the emissions of these compounds increased by 19.9 per cent and 24.5 per cent, respectively, in the same period. This increasing trend determined that 2007 emissions of NO_x and NH_3 exceeded by 39 per cent and 20 per cent, respectively, the Spanish levels included in the National Emission Ceilings Directive (NECD).

In order to meet Spanish obligations with the NECD and Gothenburg Protocol Spain approved in December 2007 the second National Emission Reduction Programme (II PNRE) aiming to achieve significant reductions in NO_x and NH_3 emissions from 2010 onwards. According to this Programme, several measures are being implemented, including the development of new strategies for sustainable mobility, the reduction of NO_x emissions in stationary natural gas engines and the improvement of the national statistics in agriculture (MARM, 2009).

5.4.2   Aims and objectives

In Spain the number of studies dealing with long-term nitrogen deposition on a widespread area is almost negligible. Thus, it is very difficult to estimate whether the above-mentioned increasing trend in nitrogen emissions has resulted in the nitrogen enrichment of Spanish ecosystems.

Taking into account this lack of information, we aimed at assessing trends in nitrophilous plants and nitrogen emissions in the last decades by:

- Developing a large-scale approach to map and quantify temporal biodiversity changes related with nitrogen emissions.

- Calculating a bioindicator index from the occurrence records of nitrophilous herbaceous plants, mosses and lichens collected in Spain and currently indexed at the Global Biodiversity Information Facility (GBIF).
- Detecting the areas in Spain that may be at risk of recording structural changes in vegetation induced by nitrogen deposition (hotspots), including the Natura 2000 sites.

5.4.3   Material and methods

GBIF (www.gbif.org/) provides access to millions of scientific data records supplied by a wide range of institutions and organizations from all over the world. The strength of the data shared by the GBIF network (species occurrence records and names and classifications of organisms) relies on their potential to be represented geospatially, as geographical coordinates of the records are usually provided. In this work we have used the GBIF database to extract information about the spatial and temporal evolution of nitrophilous herbaceous plants (186 taxa), mosses (44 taxa) and lichens (78 taxa) in Spain from 1900 to 2008.
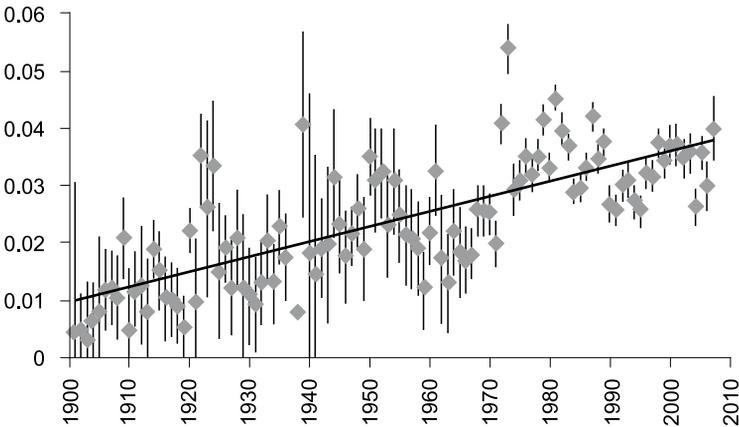
Figure 5.2:   Variation of the plant nitrophilous index through 20th and 21st centuries in Spain
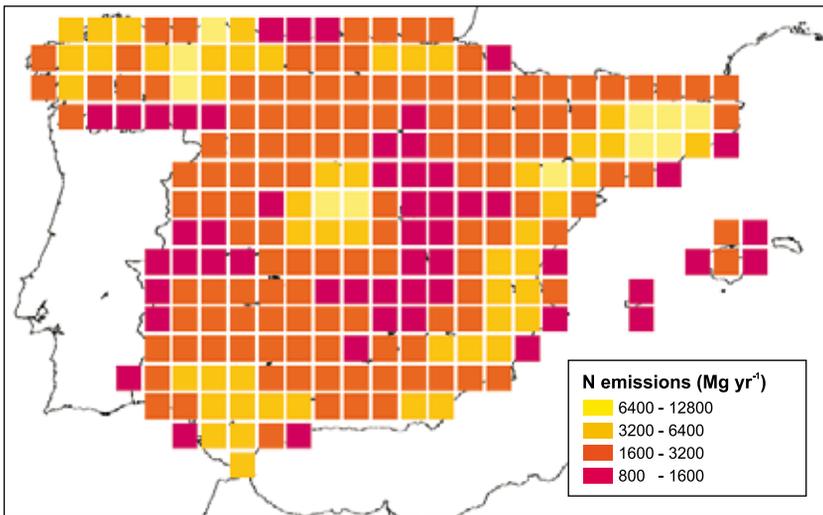
Figure 5.3a: Map of total N emissions from Spain according to the EMEP model for the period 1990-2006 (data from EMEP reduced to half-degree cell).

A nitrophilous index was calculated using over 750,000 occurrence records of plants that had been indexed at GBIF for the Iberian Peninsula by March, 2009. To construct this index, the annual number of records of nitrophilous taxa was obtained for the period 1900-2008. Next, the obtained value was divided by the total number of plant records documented every year, obtaining a nondimensional index representing the proportion of nitrophilous plants for each year of study.

These data, along with the latitude and longitude values, were used to elaborate nitrophilous index anomaly maps for the whole twentieth century. Data from recent years (1990-2008) was contrasted against the 1900-1989 baseline of previous records. The records were pooled into centered half-degree cells. Grid cells were coloured as a function of the nitrophilous index or the increase or decrease in percentage of nitrophilous plants with respect to the baseline.

The emission data model for NO_x and NH_3 for the period 1990-2006 was downloaded from the EMEP home page (www.emep.int/). In this case, longitude/latitude coordinates from EMEP database grid were also converted to centered square half-degree cells. The model's time precision is yearly, from 1990 onwards.

Finally, the index data were used to predict the effect on the SPAs and SACs included within the Natura 2000 network. The georeferenced site list was obtained from the EUNIS database (www.eea.europa.eu/data-and-maps/data/natura-2000-eunis-database) and overlayed to the index anomaly maps, selecting the grid cells that included SPAs.

5.4.4   Results and discussion

The bioindicator index developed by the study showed a continuous increase of nitrophilous plants for the period 1900-2008 (Figure 5.2), thus suggesting a change in biodiversity composition related to nitrogen enrichment in ecosystems. This increase seemed to peak in the 1970 and 1980 decades, decreasing slightly in the last decade of the XXth century, and increasing again in the first decade of the XXIst century.
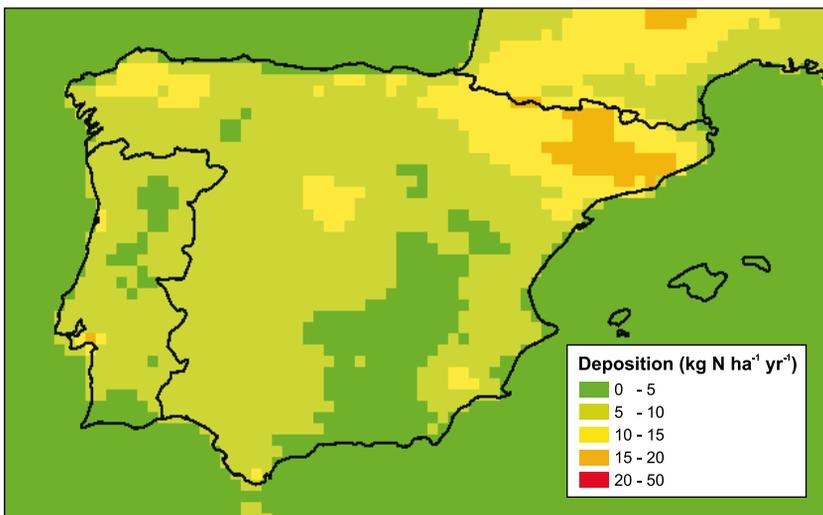

Figure 5.3 b: Map of predicted deposition within the modelling domain for oxidised and reduced nitrogen in the Iberian Peninsula (adapted from Theobald *et al.*, 2009).

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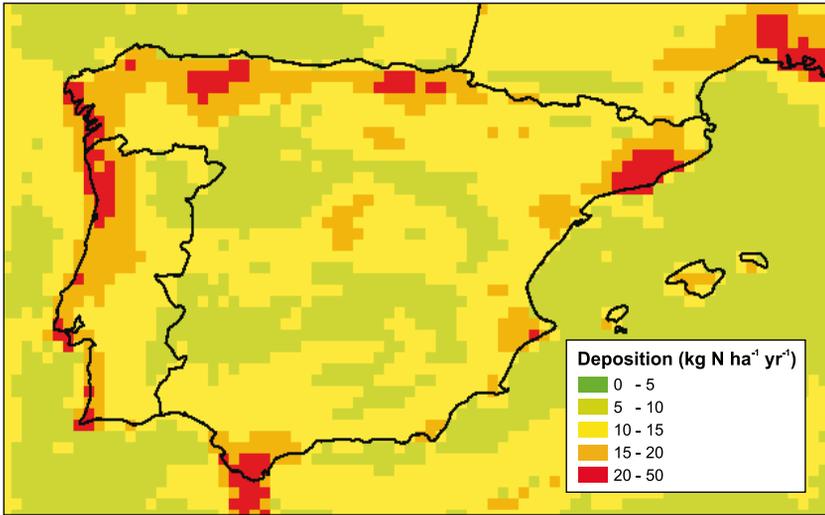
Figure 5.3 c: Map of predicted deposition within the modelling domain for oxidised and reduced nitrogen in the Iberian Peninsula (adapted from Theobald *et al.*, 2009).

Both the N emission data model from EMEP and the more recent CHIMERE deposition model by Theobald *et al.*, (2009) indicate an uneven distribution of anthropogenic N throughout Spain (Figure 5.3), with higher concentrations in the northeast, the northwest, and the south of the Peninsula. There are also disagreements. According to Theobald *et al.*, most of N deposited in Spain is in the oxidized form, predominantly as nitric acid, as reduced nitrogen only contributes about 25 per cent to the total (in contrast with EMEP model ,which predicts a 52 per cent of reduced N deposition). Although the CHIMERE model has not been validated for N deposition (this would explain the different prediction of both models), these results can be used to qualitatively identify sites at risk, if not to predict absolute deposition rates.

When the index was plotted, data indicated also an uneven distribution but did not seem to agree with the emission and deposition models (Figure 5.4). Therefore, the static value of the index would not appear to support the hypothesis of the effect of N on biodiversity.

However, when the index is plotted against the baseline of previous records (Figure 5.3b), there is a general agreement between the change in the occurrence index and the deposition models. Most increases in the index seem to occur in the northeast and northwest, and south of the Iberian Peninsula.

Therefore, these results seem to confirm that it is species richness (i.e. more nitrophilous species present as opposed to just the composition of existing species changing) that is affected by the changes in the N deposition.

Of the SPA and SAC site list in the Natura 2000 Network in Spain, 54 per cent experienced an increase in the nitrophilous index, whereas 44 per cent showed decrease and 2 per cent did not change. This suggests that a large number of sites would be suffering N-induced shifts in natural vegetation. Under these conditions, the Favorable Conservation Status (FCS) of a habitat is considered to be endangered, as the natural range of the species seems to be changing for the foreseeable future.
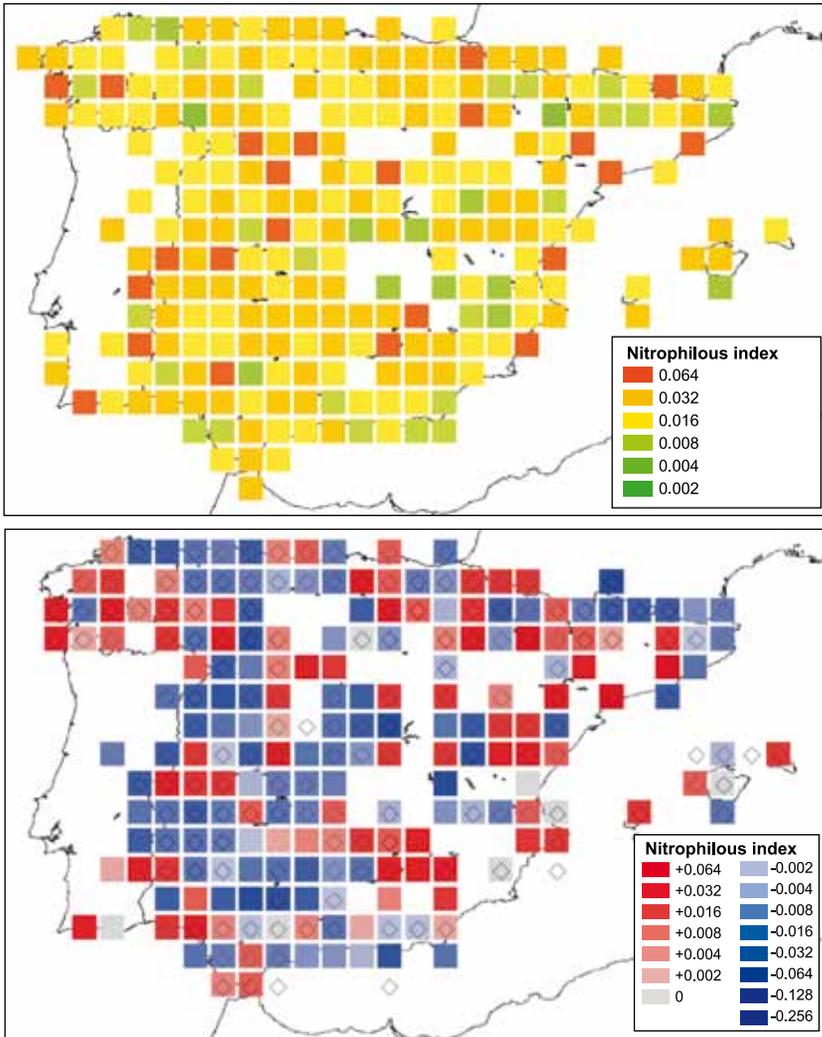
Figure 5.4: A: Map of nitrophilous index for the period 1990-2008. B: Map of anomaly for the same period against the 1900-1989 baseline. Increased index in red, decreased in blue. Half-degree cells containing one or more SPAs are marked with a diamond.

The proposed methodology could be used to derive empirical critical loads in the Spanish territory by relating both the time series of N deposition values and the temporal variations in structural changes in Spanish vegetation.

5.4.5   Conclusions

- The calculation of a bioindicator index from records of nitrophilous plants indexed at the GBIF has shown to be a useful method for the detection of temporal and spatial changes in plant biodiversity.

- A continuous increase of nitrophilous plants was detected in the Iberian Peninsula for the period 1900-2008, identifying numerous threats to biodiversity hotspots in the Natura 2000 Network.
- The obtained results suggest the need for developing more in-depth studies in order to confirm if the observed tendencies are related to nitrogen deposition.
- This methodology could be used in the future for the potential calculation of N critical loads.

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Acknowledgement:

5.5 Mosses as biomonitors of atmospheric nitrogen deposition - potential application at Natura 2000 sites

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Abstract

- In 2005/6, carpet forming ectohydric mosses were tested for the first time as monitors of atmospheric N deposition at the European scale in 16 countries.
- The lowest total N concentrations in mosses were observed in northern Finland and northern parts of the UK, the highest concentrations were found in parts of Western, Central and Eastern Europe.
- The spatial distribution of the N concentration in mosses was similar to that of the total N deposition modelled by EMEP[1], except that the N deposition tended to be relatively lower in Eastern Europe.
- The total N concentration in mosses showed the highest significant correlations with EMEP modelled deposition of different N forms, followed by urban and agricultural land use and population and livestock density.
- The total N concentration in mosses can potentially be used as a high spatial resolution tool in identifying Natura 2000 sites at risk from atmospheric N deposition.

5.5.1   Introduction

The European moss biomonitoring network was originally established in 1990 to estimate atmospheric heavy metal deposition at the European scale (Rühling, 1994). The moss technique is based on the fact that carpet forming ectohydric mosses obtain most trace elements and nutrients directly from precipitation and dry deposition with little uptake from the substrate. The technique provides a complementary, time-integrated measure of metal deposition from the atmosphere to terrestrial ecosystems. It is easier and cheaper than conventional precipitation analysis as it avoids the need for deploying large numbers of precipitation collectors with an associated long-term

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1   Co-operative programme for monitoring and evalution of the long-range transmission of air pollutants in Europe

programme of routine sample collection and analysis. Therefore, a much higher sampling density can be achieved than with conventional precipitation analysis.

The European moss survey has been repeated at five-yearly intervals and has been coordinated since 2001 by the ICP Vegetation[2], a subsidiary body of the UNECE[3] Long-range Transboundary Air Pollution Convention (Harmens *et al*., 2008a; 2010). The most recent European moss survey was conducted in 2005/6; for the first time, 16 countries also determined the total N concentration in mosses at a total of almost 3,000 sites (Harmens *et al*., 2008b). A pilot study in selected Scandinavian countries had shown that there was a good linear relationship between the total N concentration in mosses and EMEP modelled atmospheric N deposition rates (Harmens *et al*., 2005). The potential of mosses as monitors of atmospheric N deposition and its limitations has been described in a number of studies (e.g. Solga *et al*., 2005; Pitcairn *et al*., 2006; Pesch *et al*., 2008; Salemaa *et al*., 2008; Zechmeister *et al*., 2008; Poikolainen *et al*., 2009). The 2005/6 survey was the first attempt to establish whether mosses can be used as biomonitors of atmospheric N deposition at the European scale.

5.5.2   Aims and objectives

- To establish whether mosses can be used as biomonitors of atmospheric N deposition at the European scale, including Natura 2000 sites.
- To provide, in the form of maps, spatial information on the distribution of total N concentrations in mosses across Europe and identify hotspots of N pollution.
- To identify factors contributing to the spatial variation of the total N concentration in mosses.

5.5.3   Results and discussion

Pleurozium schreberi (Brid.) Mitt was the most frequently sampled species, accounting for 41.3 per cent of the samples, followed by *Hylocomium splendens* (Hedw.) B.S.G. (19.0 per cent), *Hypnum cupressiforme* Hedw. (18.1 per cent), and *Pseudoscleropodium purum* (Hedw.) M. Fleisch (15.5 per cent). Other moss species constituted only 6.1 per cent of the mosses sampled. The sampling density varied between countries and in some countries mosses were only sampled in selected regions. Further details of applied methodologies have been described elsewhere (Harmens *et al*., 2008b; 2010).

The lowest total N concentrations in mosses were generally observed in northern Finland and northern parts of the UK (Figure 5.5a). In Finland there was a clear north-south gradient which continued into the Baltic States. In the UK, locally high concentrations were found in the Midlands and South-East. The highest concentrations were found in parts of Western, Central and Eastern Europe, in particular in Belgium, Germany, Slovakia, Slovenia and parts of Bulgaria and France. However, considerable regional variations in the total N concentration in mosses were observed in each country.

The spatial distribution of the N concentration in mosses was similar to the one of the total N deposition modelled by EMEP for 2004 (Figure 5.5b), except that the N deposition tended to be relatively lower in Eastern Europe. However, the relationship between total N concentration in mosses and modelled total N deposition, based on averaging all sampling site values within any one EMEP grid square, showed considerable scatter (Harmens *et al*., 2008b). Some of scatter can be explained by relating site-specific N concentrations in mosses with N depositions averaged per 50 x 50 km^2 EMEP grid. Actual deposition values vary considerably within each EMEP grid

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2   The International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops

3   United Nations Economic Commission for Europe

cell due to for example topography, vegetation, local pollution sources and climate. The apparent asymptotic relationship shows saturation of the total N in mosses above a N deposition rate of approximately 10 kg ha^{-1} $^{-1}$. In Switzerland, however, the relationship was significantly linear (R2 = 0.91) when based on measured site-specific bulk N deposition rates (Figure 5.6; Thöni *et al*., 2008). This suggests that the relationship is more robust when based on measured site-specific N deposition rather than modelled N deposition averaged over a larger area. There is a need to measure atmospheric N deposition at selected moss sampling sites in other countries too in order to further investigate the robustness of the relationship with total N concentration in mosses.

Bivariate Spearman rank correlation coefficients (rs) were computed to investigate the strength and direction of the statistical relationship between the total N concentrations in mosses and i) EMEP modelled N depositions and ii) additional factors that were expected to influence the total N concentration in mosses (Schröder *et al*., 2010; Table 5.2). Moderate correlation coefficients (i.e. 0. $5 \leq rs < 0.7$) were observed for EMEP modelled N depositions ($0.55 \leq rs \leq 0.65$), independent of N form. Regarding regional land characteristics, the ratio of urban land uses in a radius of 100 km and agricultural land uses in a radius of 50 km around the monitoring sites (Corine Land Cover 2000 data) showed the highest correlations with the total N concentration in mosses (rs = 0.55 and rs = 0.54, respectively). Lower correlations (rs < 0.5) were observed for population and livestock density (Eurostat data), precipitation (Global Climate Dataset), distance to sea and altitude. In general, the total N concentration in mosses appears to mirror land use-related atmospheric N depositions.

In a pilot study in Germany, the moss sites where overlayed with the locations of Sites of Community Importance (SCI's). This allowed identifying SCI's with the highest total N concentration in mosses in Germany and therefore most at risk from high atmospheric N depositions (Figure 5.7). Such an analysis should be extended to other countries to establish whether the total N concentration in mosses can be used for identifying Natura 2000 sites at risk from high atmospheric N depositions.

Factors potentially influencing the relationship between total N deposition and N concentration in mosses require further investigation in order to establish the robustness of the application of mosses as biomonitors of atmospheric N deposition at Natura 2000 sites. Such factors include for example i) effects of N and climate on moss growth, ii) species-specific responses to N deposition, iii) the role of N speciation and iv) altitude (Harmens *et al*., 2008b). It might be that other N parameters, e.g. amino acid concentration or soluble ammonium concentration in mosses, are better indicators for potential effects of N deposition on Natura 2000 sites (Strengbom *et al*., report WG 3). The disadvantage of these measures is that they are more complicated to analyse and more expensive. Most likely, a combination of the bioindicators/biomonitors will best describe the state on Natura 2000 sites (Nordin *et al*., background document WG 3).

5.5.4   Conclusions

- The total N concentration in mosses can potentially be used in identifying Natura 2000 sites at risk from enhanced N deposition at a high spatial resolution.
- Measurements of site-specific N deposition and other site-specific characteristics are required to establish the robustness of the relationship between total N deposition and total N concentration in mosses.
- It is unclear yet whether the total N concentration in mosses can be used as early warning for N impacts on Natura 2000 habitats or as part of an integrated assessment of the state of habitats.

Table 5.2:   Spearman rank correlation coefficients (rs) between N concentration in mosses and i) EMEP modelled deposition (50 x 50 km²) of different N forms and ii) other site-specific or regional characteristics. All coefficients were significant at p=0.001, except for livestock density with p=0.01.

EMEP deposition	r_s	Other predictors	r_s
Wet oxidised	0.65	Ratio urban land use (100km radius)	0.55
Total (wet + dry)	0.64	Ratio agricultural land use (50km radius)	0.53
Total wet	0.64	Population density	0.48
Dry oxidised	0.64	Livestock density	0.42
Wet reduced	0.62	Precipitation	0.25
Total dry	0.59	Distance to sea	0.25
Dry reduced	0.55	Altitude	-0.10
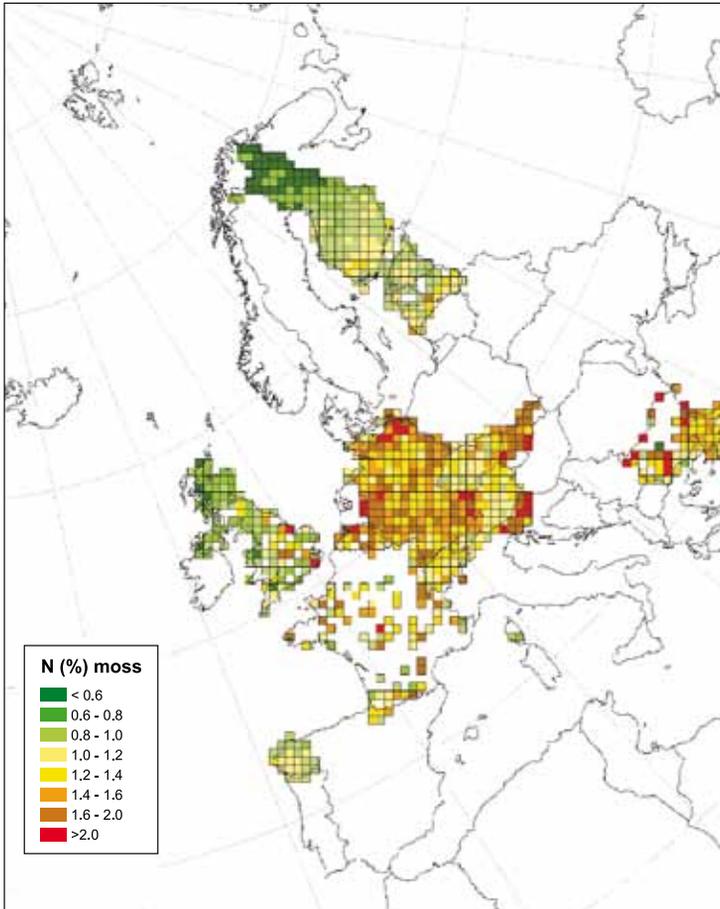
Figure 5.5a: Mean total N concentration in mosses per EMEP grid square in 2005/6
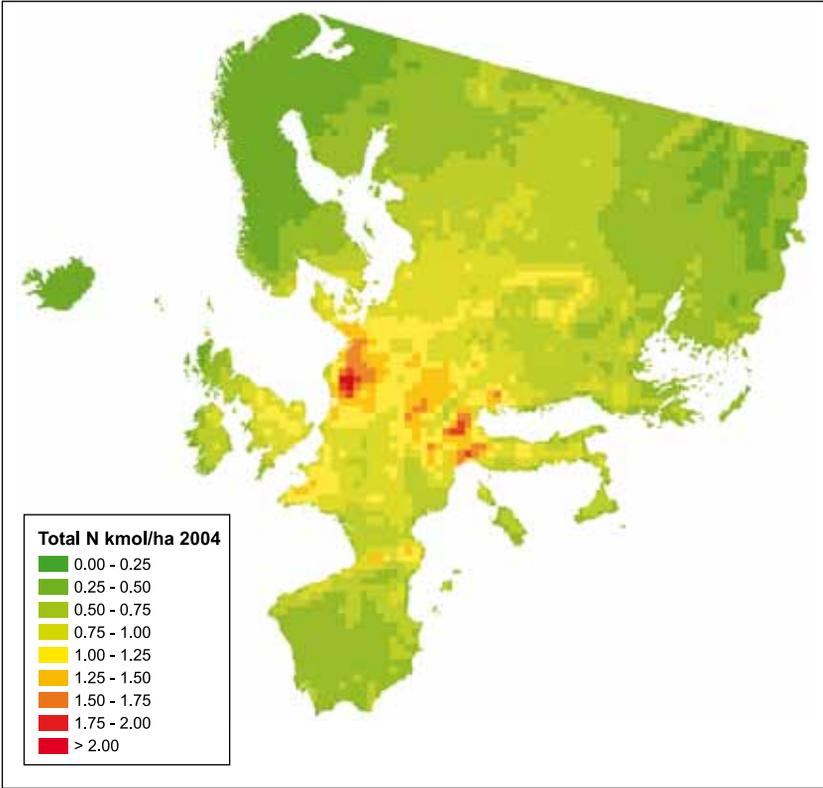
Figure 5.5b: N deposition per EMEP grid square in 2004

$$y = 0.70 + 0.055x$$
$$R^2 = 0.91 \ (P < 0.001)$$
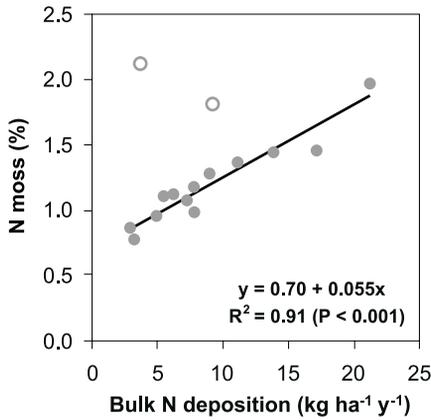
Figure 5.6: Relationship between measured bulk N depostion rate and N concentration in mosses at selected sites in Switzerland; the open symbols (samples possibly contaminated with faeces) were excluded from the regression (Thoni *et al.*, 2008)
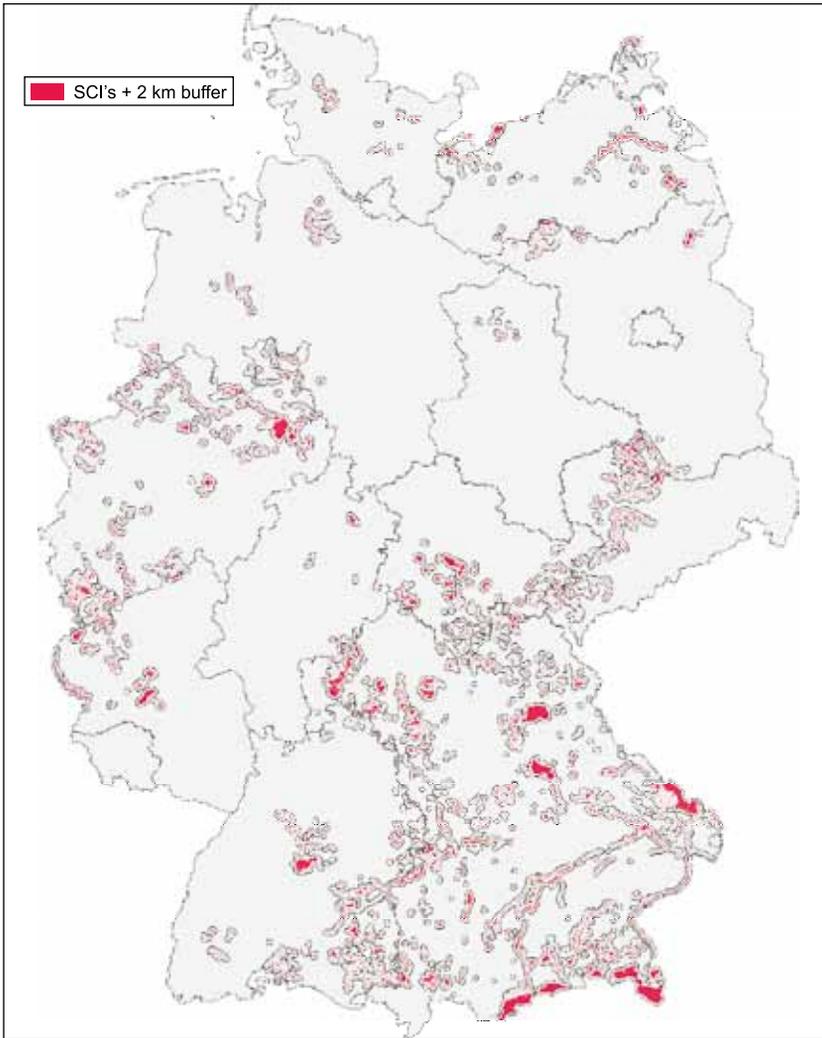
Figure 5.7: Sites of Community Importance (SCI's) in Germany with a total N concentration in mosses above the 75th percentile (1.36 per cent, calculated from the raster). The map of total N concentration in mosses was overlayed with the location of SCI's using a two km buffer around the SCI's. This maps identifies SCI's potentially most at risk from adverse effects of N deposition, however, SCI's might be at risk at concentrations below 1.36 per cent. Calculations by: W. Schröder, R. Pesch, M. Holy (University of Vechta, Germany), source of data on locations of SCI's: Federal Agency of Nature Conservation, Germany.

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5.6 Impact of nitrogen deposition on species richness of calcareous grasslands in Europe - some preliminary results

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Abstract
- This paper seeks to determine whether N-deposition has a negative impact on the species richness of calcareous grasslands at a European wide scale.
- 100 calcareous grasslands across the Atlantic region of Europe were sampled in one season. Species composition and richness of vegetation communities were compared to some key environmental drivers (climate and N deposition) indirectly estimated via surrogates (latitude, longitude, N concentration in bryophyte tissue).
- There are marked differences in species composition across the calcareous grasslands of the Atlantic biogeographic zone within Europe. Contrasts in mean species richness between regions are also detectable at a European wide scale. These natural gradients may mask any footprint of N deposition on vegetation at a European-wide scale.
- For grasslands located along the western range of distribution, there are indications of a decline in species richness as N concentration in moss increases. This suggests that N deposition may be reducing biodiversity in calcareous grasslands at a wide scale, but that this impact can only be detected at the regional, rather than cross-European, level.
- Further research is needed to investigate the impact of N deposition on calcareous grasslands, particularly through the direct assessment of potential drivers as well as the characterisation of variations in species pools at the European scale.

5.6.1   Introduction

The increase of atmospheric deposition of nitrogen (N) in recent decades, due to fertilizer application and fuel consumption, represent nowadays a major threat for biodiversity in ecosystems (Langan 1999, Phoenix *et al.*, 2006). In Europe, where this trend has been particularly documented, the

effects of N deposition have been studied through empirical or experimental approaches (Bobbink 1998, Stevens *et al.*, 2004). Among several effects, N deposition affects nutrient availability, which is a major driver of plant community composition and species richness (Tilman and Pacala, 1993). As a consequence, the impact of N deposition is of major concern for those species-rich ecosystems which are strictly associated with nutrient-poor soils such as Natura 2000 grasslands and heathlands. Recent comparative studies, based on either spatial gradients or on time series analyses, have shown clear evidences of an impact of N deposition on acidic grasslands, leading to a decrease of plant species richness and a loss of species associated with less fertile conditions (Dupré *et al.*, 2009, Stevens *et al.*, 2004). Such evidence is also found, at local or national level, for other species-rich habitats such as calcareous grasslands or heathlands (Maskell *et al.*, 2009), or from experimental results (Bobbink, 1991, Willems and van Nieuwstadt, 1996). Whether such results are generally applicable is of particular importance because calcareous grasslands are of major interest for the conservation of biodiversity in Europe as they support communities of exceptional diversity and many rare and endangered species of plants, insects and birds (WalliesDeVries *et al.*, 2002).

The lack of knowledge at a broader scale has motivated the BEGIN project (Biodiversity of European Grasslands – the Impact of Atmospheric Nitrogen Deposition) which seeks to determine whether N-deposition is impacting the species richness of grasslands on a European wide scale . Different approaches have been used to assess the loss of biodiversity associated with N deposition in acidic grasslands: historical analysis (Dupré *et al.*, 2009), experimental and comparative surveys (Stevens *et al.*, 2004, 2010). Another objective of BEGIN was to investigate whether a similar decrease in biodiversity is occurring in a contrasting grassland system. The most important grassland type across Europe in terms of biodiversity are the calcareous grasslands of the Mesobromion alliance (Koch, 1926). Compared to acid grasslands, these habitats have a much greater species richness and larger number of rare species, and are also presumed to be sensitive to N deposition through increasing nutrient availability. We thus hypothesised that N deposition may be significantly impacting these grasslands.

5.6.2   Aims and objectives

- We aim to determine whether any significant variability in plant species richness in calcareous grasslands across Western Europe could be detected and related to any regional-scale evaluation of N atmospheric deposition.
- In 2008, we surveyed 100 calcareous grasslands belonging to the Mesobromion alliance on a transect across the Atlantic biogeographic zone of Europe. Site selection was performed through a composition criterion (required presence of five species among a predefined list of target species) and a management criterion, in order to avoid abandoned grasslands. For each site, five 1 m × 1 m replicates were recorded. In each square meter, the cover of all occurring plant species (vascular plants and bryophytes) was visually estimated. Each site is therefore characterised by a list of species with average abundance (calculated from the five quadrats) and an average richness per plot (n=5 replicates).
- In this preliminary study, we only used environmental surrogates to account for the major environmental drivers we identified. A major predictor of large scale species richness is latitude (Hillebrand, 2004). We used latitude and longitude as aggregate variables integrating distinct climate factors i.e. mainly temperature (North-South) and precipitation (West-East) gradients. Total nitrogen concentration in bryophytes was used as a surrogate to estimate total N deposition at a high resolution (Harmens *et al.*, 2008). Because species-specific differences are expected, we considered only the sites where the same moss species (i.e. Ctenidium molluscum) was collected and analysed. This accounted for about half of the sampled sites (51 sites for the 100 sites of this study).

- We performed a correspondence analysis (CA) and a hierarchical clustering of the [100 sites x 161 species] data table in order to provide an ordination and classification of plant communities. The table was obtained after removal of species occurring in less than 5 per cent of the records in the initial table [100 sites x 225 spp]. Similar analysis was performed on the sub-set of 51 sites where some environmental surrogates were available. Simple regressions were performed, after data normality was tested (Shapiro-Wilk test), to assess correlations between environmental surrogates and species richness (i.e. mean species number for the five replicates) and species composition (floristic gradients from the CA). All analyses were performed with R free software (2007).

5.6.3 Results and discussion

The Correspondence Analysis shows that gradients in species composition are well correlated to the geographical distribution of the sites (Figure 5.8a,b). Latitude and longitude are highly significantly correlated with respectively axis one (n=100, r²=0.52, p<0.001), and axis two (r²=0.63, p<0.001) of the CA. A hierarchical clustering (Ward method) performed on the output of this CA (Figure 5.8c)

gives a very similar result to clusters defined on a national basis. Three main types of plant communities can be defined (Table 5.3), related to sites from France (FR), United Kingdom and Eire (UK+IRL), and Germany (GER). Sites from north central Europe (Belgium, Netherlands, Denmark) are distributed within these three clusters, while Norway sites form a specific sub-cluster.

Looking for pattern of variation of community species richness along these floristic/geographic gradients of the CA, we found no evidence of a correlation with species richness for any of the CA axes. However, when considering the different clusters from the CA, species richness appear significantly different between some regions (Figure 5.9 - one way ANOVA; F= 3.01, df=99, p<0.05). As we avoided abandoned sites, these differences could not be due to management contrasts but rather to differences in species pool size, depending on regional specificity (soil, climate, history).

We analysed the sub-set of 51 sites to test whether species composition gradients and species richness variations could be correlated to N deposition, estimated via the N surrogate (N per cent in the moss C. molluscum). The 51 sites were distributed in the three main clusters-regions: 27/27 sites of the South-West (SW) of Europe (CL2), 13/36 sites of the North-West (NW) of Europe (CL3) and 11/32 sites of the Est (E) of Europe (CL1). These sites were also regularly distributed along CA axes. We then performed a new Correspondence Analysis (CA2) on these 51 sites, to built floristic gradients on this specific data set. Patterns were similar to the first CA, the CA2 axes being even more correlated to latitude and longitude (n=51, r²=0.56, p<0.001 for axis 1; r²=0.82, p<0.001 for axis 2). N per cent in bryophyte tissue was correlated only with the axis three of this second correspondence analysis (r²=0.10, p<0.05), suggesting at least that N deposition could be correlated to gradient of species composition in this data sub-set. We did not find any correlation

Table 5.3:

| Cluster	Region	Countries	Some differential species
CL1	E	GER, (B), (NL)	*Silene vulgaris, Inula conyza, Poa angustifolia*
(CL1bis)	E	Norway	*Viola canina, Deschampsia flexuosa, Alchemilla filicaulis*
CL2	SW	FR, (B)	*Teucrium montanum, Gaudinia fragilis, Seseli montanum, Thesium humifusum*
CL3	NW	UK, IRL, DK, (B), (NL)	*Carex humilis, Festuca arundinacea, Ranunculus repens*
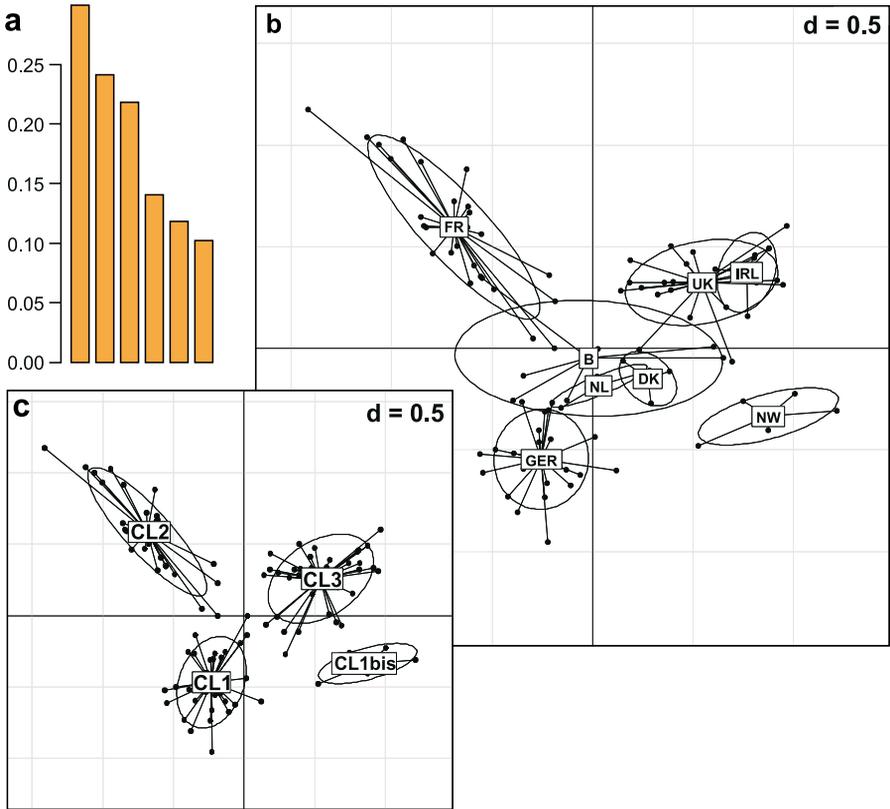
Figure5.8: Correspondence analysis of the [100 relevés x 161 species] data table for the analysis of composition gradients in calcareous grasslands. a) Eigenvalues; b) F1×F2 plane showing distributions of relevés in the national surveys, c) F1×F2 plane with clusters performed from hierarchical clustering (Ward method) on relevés coordinates.
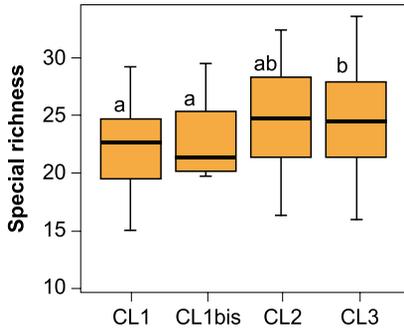

Fig.5.9: Box plots showing species richness (mean SR.m-²; n=5 replicates) variations in the four clusters from the CA. see text for details. Boxes sharing the same letter are not statistically different (P < 0.05, Tukey's HSD comparing all clusters).

between N surrogate and species richness of plant communities at a broad scale (Figure5.10a; n=51, p=0.18). However, when we performed regressions at the regional level (Figure 5.10 b,c,d), correlation was significant for SW region (Cluster 2, n=27, r²=0.20, p<0.05), while no trend was detected for other

clusters. When significant, correlation shows a decline of species richness at the highest levels of N concentration (deposition). The processes responsible for this decline may be found in the effects of N enrichment, resulting in changes in vegetation structure and species interactions to the benefit of competitive tall grasses (Bobbink, 1991, Liancourt *et al*., 2005).

From our data, composition gradients in calcareous grasslands are marked at the European scale, even though we removed the less frequent species in the data set (i.e. with occurrence less than 5 per cent) which should attenuate contrasts between countries. This species turn-over is shown in phytosociological works (Royer 1985, Willems, 1982). Our survey confirms that climate gradients are likely to be the most important drivers of species turn-over in calcareous grasslands in Europe, as climate variables such as temperature and rainfall are known to be correlated with latitute and longitude (Ozenda 1994, Duckwoth *et al*., 2000). Similarly, our data suggest that regions in Europe could be characterised by species pools of different sizes. However, this has to be confirmed with species pool studies (e.g. Dupré, 2000), based on more complete phytosociological datasets.

Because of the strong climate-driven variation in species composition and richness, it is difficult to detect a separate signal of N deposition as a potential driver of calcareous grassland diversity on a cross-Europe scale. The use of N concentration in moss as a surrogate for N deposition can also introduce some potential artefacts. Besides differing among different species, this relationship might also depend on other factors such as N speciation, the ratio of wet/dry deposition in N deposition, and local climate (Harmens *et al*., 2008). Despite these limitations, there are some indications of an N-deposition signal on species composition at a European wide scale and on species richness for calcareous grasslands located at the western range of their distribution.
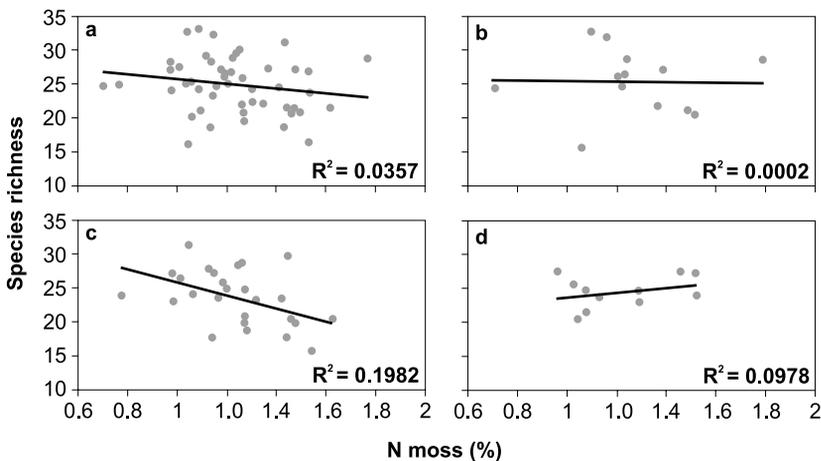

Fig.5.10: Regression plots between nitrogen concentration in the moss Ctenidium molluscum (N per cent dry weight) and species richness (mean SR.m-²; n=5 replicates) in calcareous grasslands according to different geographic ranges: a) subset covering the whole geographic range i.e. Atlantic Europe; n=51; b) subset from NW Atlantic Europe i.e. Cluster 3; c) subset from

5.6.4   Conclusions

- There are strong gradients of species composition in calcareous grasslands in western Europe. Contrasts in mean species richness between regions are also detectable at a European-wide scale. These gradients and contrasts appear to be driven primarily by climate.
- Because of these strong environmental responses, it is difficult to detect a clear influence of N deposition on species richness and composition at the European scale.
- However, when filtering the data at the regional to national scale, there are indications of an N-deposition signal on species richness for grasslands sampled in western regions (Atlantic coast). If real, regressions suggest a fairly strong decline in diversity with increasing N deposition for these sites
- These intimations of a N impact on calcareous grassland diversity strongly point to a need for targeted research, particularly through the direct assessment of potential drivers as well as the characterisation of natural variations in species pools at the European scale.
- The above have strong implications for conservation and pollution mitigation actions for management of calcareous grasslands (Calciura and Spinelli, 2008).

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Acknowledgements

5.7 Nitrogen critical load and butterflies

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Abstract

The biodiversity effect of nitrogen deposition is now well recorded and explored for vegetation but the effect on invertebrates representing most of biodiversity (by number of species) is unknown.

Biodiversity is defined and used as the product of a number of qualitative indices of a group of organisms (in this case butterflies).

Meta data analysis of seventeen years of survey data of butterflies in a wide range of habitats in the Netherlands is analysed along with nitrogen critical load exceedence (CLE) data calculated for the same survey sites.

The Netherlands is in recovery from high nitrogen CLE and yet nitrophilic species are still replacing nitrophobic species so the situation is complex due to the data still showing exceedence.

Species Richness is not indicative of nitrogen CLE. Other biodiversity indices have a better indication relationship with CLE.

The importance of nitrogen CLE in the set of biodiversity indicators accepted by the European Environment Agency in the SEBI 2010 assessment shows it to be a critical factor and related to butterfly populations.

5.7.1   Introduction

Almost all of the work on the ecological effect of nitrogen deposition has been related to plant biochemistry and distribution (in particular grasses, forbs, bryophytes and lichens). These habitat structural components do not in terms of species number represent more than a small part of biodiversity. Since the plant structural component effect of nitrogen deposition is now clearly established it becomes necessary to determine whether other components of biodiversity are also affected and whether they are more sensitive or less sensitive than the present set of bioindicators.

In terms of species number, ubiquity and sometimes biomass of invertebrates are the largest component of biodiversity. There are three mechanisms by which they might be affected by nitrogen deposition:

*   They may be directly affected by the chemistry of the nitrogen (in particular that part due to NH+);
*   They may be affected by a change in the food-plant chemistry. We know that, for example, some butterflies are very sensitive to the nutrient status of the plants upon which they lay their eggs;
*   They may respond to a change in the habitat due to plant responses to nitrogen deposition.

The latter of these hypotheses is the basis of the paper by Wallis de Vries and van Swaay (2006) where they hypothesize that increased nitrogen-induced grass growth in the spring causes a slower increase in ground temperature and thus eggs and larvae are delayed in emergence from overwintering diapause.

Butterflies are good exemplar invertebrates since a) they have been the subject of extensive standardized recording in a number of countries b) they are popular c) they are obvious when flying and therefore relatively easy to record and d) they are found in some habitats that are clearly affected by nitrogen deposition. Difficulties result from their non-ubiquity and presence only in low numbers of individuals or species in some habitats.

5.7.2   Aims and objectives

The aim of this research was to triangulate butterfly biodiversity against nitrogen deposition to determine if indeed butterfly biodiversity was affected by nitrogen deposition. This (and the methodologies utilised) could then be a starting point for estimation of the effect of nitrogen deposition on a wider element of biodiversity than is currently established.

In order to understand more clearly the possible effect of nitrogen deposition on butterfly populations a refinement of the definition of biodiversity is used (Feest, 2006; Feest *et al*., 2010) to cover its functional qualities (Hooper *et al*., 2005) supplemented by an Ellenberg scale of nitrogen sensitivity (Oostermeijer & van Swaay, 1998), known as theSpecies Nitrogen Value Index (SNVI).

The butterfly population biodiversity quality elements were calculated using the Fungib programme (ecosulis ltd.: https://www.ecosulis.co.uk) as follows:

- Species Nitrogen Value Index (SNVI)
- Species Conservation Value index (SCVI)
- Species Richness
- Simpson Biodiversity Index
- Population Index
- Biomass Index

For derivation of indices 2-6 see Feest (2006) and Feest *et al*., (2010).

These data were then statistically analysed for relationships with nitrogen CLE. The butterfly data were supplied by Chris van Swaay of de Vlinderstichting and the nitrogen CLE for the identified butterfly sites was supplied by Arjen van Hinsberg of MPN. Since the data set covered a spread of 17 years and butterfly data is subject to considerable annual variations the data were smoothed by aggregation into three groups representing six, six and five years.

The advantages of this approach are:

- Data produced can be easily tested statistically for significance
- Other groups e.g. bryophytes, beetles, spiders, macrofungi etc. could be assessed in the same way
- Baseline biodiversity quality indices/values can be set and revisited at any time in the future and tested for statistical significance of differences
- The taxonomy of butterflies is not difficult

Table 5.4:   Typical results for the biodiversity of Dutch butterflies for consecutive six, six and five years. SNVI= Species Nitrogen Value Index; SR= Species Richness; SCVI= Species Conservation Value Index; nCLE= nitrogen critical load Exceedence

Habitat	SNVI	SR	SCVI	Population	Biomass	nCLE	n=
Grassland period 1	5.8	15.86	2.83	720	15698	1765	
Grassland period 2	5.74	14.9	2.78	638	12778	641	57
Grassland period 3	6.22	16.48	2.72	527	11352	572	54
Heathland period 1	4.01	18.22	3.23	569	10407	1626	12
Heathland period 2	4.22	15.9	3.2	437	7423	1071	20

Table 5.5:   Principal Component Analysis of Woodland Butterflies 2001-2006. PC1= Principal Component 1; PC2= Principal Component 2. Other abbreviations as for Table 5.4.

|	PC1(0.532)	PC2(0.247) (Σ=0.779)
SNVI	0.281	0.415
Sp Rich	-0.490	0.040
Simpson	-0.406	-0.109
SCVI	-0.433	-0.298
Pop	-0.341	0.549
Biomass	-0.324	0.571
nCLE	-0.327	-0.313
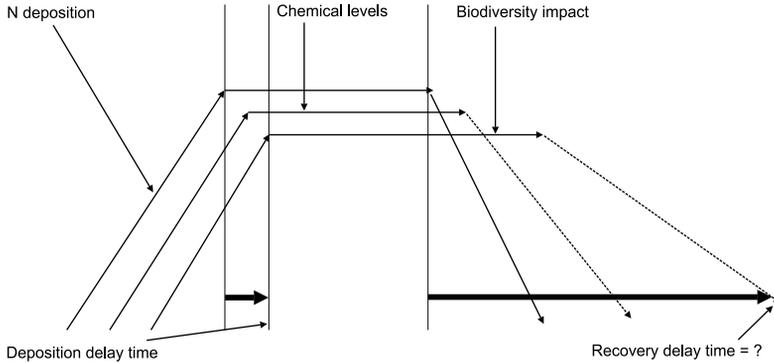
Figure 5.11: The disjunction between the impact nitrogen deposition, its effects and the recovery.
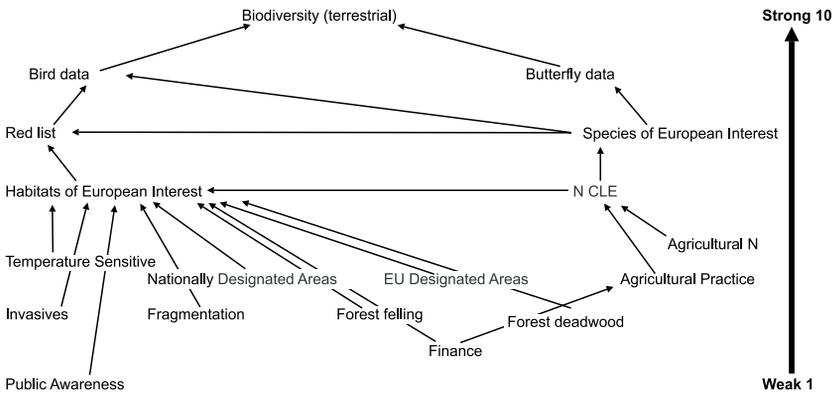

Figure 5.12: Linkages between the SEBI 2010 indicators and their relationship with biodiversity

5.7.3   Results and discussion

Table 5.4 gives the data for two habitat types in the Netherlands. The data shows clear apparent trends for all indices except Species Richness.

To add statistical strength to this picture the data were further analysed by principle component analysis by habitat and year aggregation. Table 5.5 gives the result of the first two component axes for an example of Woodlands in the Netherlands for the years 2001-6. The picture revealed in this assessment was one typically found for other habitats and year aggregations.

In component axis one representing 53.2 per cent or the variation all indices have a similar strength and relationship with the exception of SNVI. Looking at the coarse data this will be seen to indicate that all of the indices are in decline except the one indicating the nitrophilic/nitrophobic balance of the butterfly population which is moving to a more nitrophilic status. Whilst this shows a clear relationship between CLE and other factors this is not very helpful since it fails to differentiate the indices. In component axis two representing 24.7 per cent of the variation a radically different picture emerges that allows some understanding of the mechanisms operating. Firstly, Species Richness is of low significance (nitrophobic species being replaced by nitrophilic); SNVI,

population/biomass are associated (the more nitrophilic the species the larger the populations/biomass) and Simpson, SCVI and CLE are associated, but less strongly (the rarity, CLE and evenness of populations are in decline). The analysis also reveals the problematic situation in the Netherlands where over the period of the surveys the nitrogen CLE has been in a steady decline throughout although still registering positive results of exceedance. Figure 5.11 shows the area of uncertainty that has prevailed during the survey period in that there is a recovery delay as Nitrogen CLE declines of unknown duration (whereas deposition has a much more immediate effect). It is hypothesized that this relates to slow vegetation change and differential colonization effects (Poyry *et al*., 2009).

5.7.4 Conclusions

This paper shows that there is a clear relationship between nitrogen deposition and butterfly biodiversity quality and that this may be complex during a period of recovery of populations in response to reduced nitrogen deposition. This is important for future biodiversity protection since this work has not determined whether this effect extends to other invertebrate groups (or indeed other taxa such as macrofungi) and how sensitive butterflies are -are they more or less sensitive than plants?

The overall success of biodiversity protection in the European Union is being registered by the Streamlining European Biodiversity Indicators 2010 (SEBI, 2010) process. SEBI 2010 has determined a set of 26 indicators and a putative relationship of the terrestrial indicators is presented in Figure 5.12. Note that only two groups of organisms representing organism group biodiversity are included in the scheme (birds and butterflies). It is also clear that two elements are of importance to the whole indicator set and they are: 1. Habitats of European Interest and 2. Nitrogen CLE. This work is the first that has validated the relationship of two of the SEBI 2010 indicators and shows the importance of nitrogen CLE for more than vegetation characteristics.

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5.8 Selecting critical areas for monitoring the impact of ammonia on biodiversity

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Abstract

- The main impact of atmospheric ammonia (NH_3) is known to occur at short distances (less than 500 m). Therefore, for successful development and implementation of policies aiming at preventing the impacts of NH_3 on biodiversity of Natura 2000 sites, it is necessary to make use of a high spatial resolution mapping. However the available information of NH_3 emission and deposition is clearly of an insufficient resolution for this purpose, at least in Portugal.

- Our objective was to provide a practical method for selecting critical areas for monitoring the impact of NH_3 in plant and animal biodiversity within Natura 2000 sites. Lichen functional-diversity is a good indicator of NH_3 impact at small spatial scales, when a single source of disturbance is present. However, because Natura 2000 sites are large areas and contain multiple sources of disturbance, lichen-functional diversity may not be the most appropriate indicator since it responds to other factors besides NH_3. Therefore, we propose to use total nitrogen concentration in lichens, [N], as a method for selecting critical areas of NH_3 impact in Natura 2000 sites.

- A first question that was addressed was: what are the [N] concentration in lichens reflecting? For that we tested if: i.) [N] was reflecting atmospheric NH_3 deposition (by relating it with agriculture land-use); ii.) was [N] reflecting NO_x (by relating it to industrial and urban land-uses).

- The [N] in lichens was shown to be very significantly related to agriculture areas and not to urban or industrial areas thus showing that N concentration in lichens is most probably reflecting the NH_3 emissions. In this way we propose here to apply the N concentration in lichens as a detailed ecological indicator for selecting critical areas for the impact of NH_3 on biodiversity.

- Furthermore, we applied this indicator to two Natura 2000 sites by mapping [N] in lichens. By doing so we could select the critical areas for the assessment of the impact of atmospheric NH_3 deposition on plant diversity in Mediterranean Natura 2000 sites. Further studies on the impact of plant diversity can now be focus in high NH_3 deposition areas.

5.8.1   Introduction

In Mediterranean landscapes a large variety of land-cover types occur in small areas (Blondel and Aronson, 1999). Besides, areas dedicated to Nature Protection are surrounded by centuries old human-matrix. Among important sources of disturbance in Natura 2000 sites we can find agriculture. Those activities are major sources of atmospheric NH_3 in Europe (EPER, 2004; Galloway *et al*., 2003). Moreover, the deposition of N is related to biodiversity loss (Phoenix *et al*., 2006; Suding *et al*., 2005) and is considered not only a major threat to global biodiversity but also one of those threats that are expected to increase worldwide (SCBD, 2006). Thus, biodiversity within protected areas might be highly threatened by atmospheric NH_3. However in Portugal the spatial resolution of the available emission and deposition mapping is clearly insufficient to allow an adequate selection of the areas under the greater risk of NH_3 impact (Martins-Loução, this volume). Therefore the main goal of this work was to provide a practical method for selecting critical areas for monitoring the impact of NH_3 on biodiversity within Natura 2000 sites. To do so we used lichens,

poikilohydric organisms resulting from the symbiosis of a fungus and a photosynthetic partner. Lichens are considered one of the most sensitive communities of organisms in the ecosystems, due to its particular physiological characteristics such as the absence of a protective cuticle. Lichens are the most sensitive group of organisms to N and its functional biodiversity changes with NH_3 atmospheric deposition (Pinho *et al*., 2009). However the application of lichen diversity in this case is problematic, since it responds to a series of other factors. In fact, lichens have been shown to be sensitive to a large number of factors including pollutants, with both human and natural origin and microclimate changes (Geiser and Neitlich, 2007; Giordani *et al*., 2002; Pinho *et al*., 2004; Pinho *et al*., 2008a) and therefore have been used as biomonitors of complex environmental changes such as habitat fragmentation, habitat stability and influence of forest management (Coxson and Stevenson, 2007; Edman *et al*., 2008; Nascimbene *et al*., 2007; Ranius *et al*., 2008). More specifically, we propose to use lichens as nitrogen biomonitors (Gaio-Oliveira *et al*., 2001). This is so because total N in lichens has been shown to increase in NH_3 rich areas (Pinho *et al*., 2008b) and increased N concentration in lichens and plant tissues has been associated to higher N availability (Adrizal *et al*., 2008; Boggs *et al*., 2005; Fluckiger and Braun, 1998; Gaio-Oliveira *et al*., 2001; Pocewicz *et al*., 2007).

5.8.2 Aims and objectives

- The aim of this work was to provide a method with high spatial resolution for selecting critical areas for monitoring the impact of NH_3 in plant and animal biodiversity within Natura 2000 sites.
- For that we proposed to use total N concentration in lichens. We firstly tested if [N] was related to the area occupied by agriculture (a source of atmospheric NH_3) and artificial zones (a source of NO_x). Secondly we mapped [N] within the Natura 2000 sites.

5.8.3 Results and discussion

We found that [N] in lichens can be used as an ecological indicator to reflect NH_3 deposition in Natura 2000 sites with a high spatial resolution. The hypothesis was that this indicator would be useful for mapping critical areas of potential impact of NH_3 pollution, and to test this we first determined if [N] in lichens was related to human activities in neighboring areas, considering agriculture (emitting NH_3), industry and traffic (emitting NO_2).

In small areas, with a single source of disturbance, lichen biodiversity has been shown to respond accurately to NH_3, leading to a change in lichen functional-diversity (Pinho *et al*., 2009). However, when dealing with Natura 2000 sites, normally occupying a regional area, we must consider the possible existence of multiple disturbance sources, many of which may be diffuse. In such areas, and more specifically in the studied area (Figure 5.13), nitrophytic and oligotrophic lichen species have been shown to respond to a large number of factors, including natural (such as the sea) and anthropogenic ones (such as industrial areas) (Pinho *et al*., 2008a; Pinho *et al*., 2008b). These may impede the use of biodiversity as an indicator of NH_3 pollution. In order to avoid the interference by other factors we used [N] in lichens to determine the critical areas of potential impact of nitrogen pollution, the usefulness of the use of nitrogen concentration as a biomonitoring tool having already been suggested by a preliminary work (Gaio-Oliveira *et al*., 2005). In this work, [N] was determined in the lichen species Parmotrema hypoleucinum (J.Steiner) Hale, collected from 104 cork-oak woodland sites. First we determined if [N] was related to neighboring sources of atmospheric nitrogen, by performing a local correlation analysis (Figure 5.14). We considered neighborhood areas around sampling sites, with radius ranging from 50 to 6400 m (Pinho *et al*., 2008a). A local analysis was made using these areas, by relating [N] and area of agriculture and artificial land-cover, considering sites located at less than 10 km distance. This local correlation was preferred to a regional analysis because NH_3 pollution is known to be short-range (Pinho *et al*., 2009; Sutton *et al*., 1998). The local correlation analysis was plotted for the study area
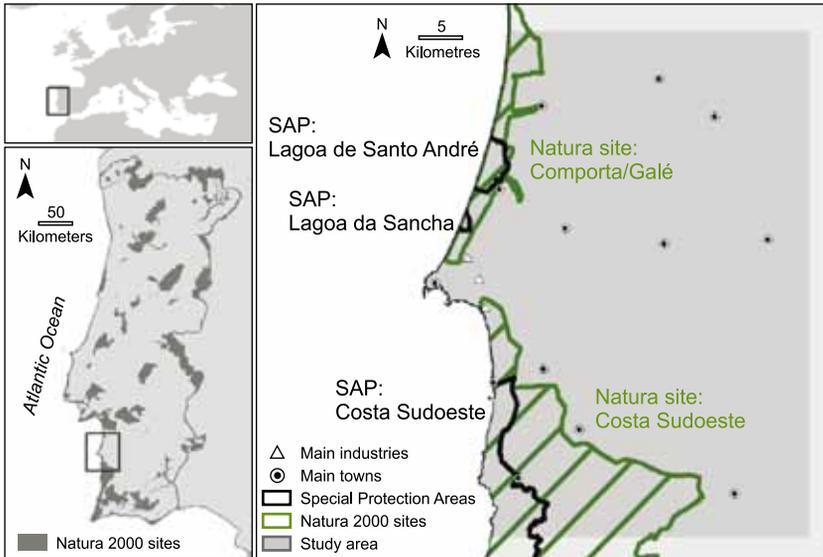
Figure 5.13: Location of Natura 2000 sites in Portugal and of the studied region in SW Portugal. The labeled Natura 2000 sites are the ones included in this work. Main industrial areas are part of Sines industrial complex and include a power-plant, an oil refinery and other petrochemical industries.

(Figure 5.14), showing many very significant correlations between [N] in lichens and the area occupied by annual agriculture (mainly rice fields and cereals), as well as heterogeneous and other permanent agriculture (mainly small farms and orchards) (Figure 5.14). The results also showed that there was no significant correlation between [N] in lichens and artificial areas (mainly roads, urban and industrial areas) (data not shown), excluding NO_x emissions as contributing to the [N] in lichens. Moreover the distance of influence, that for which the maximum correlation was observed (Pinho *et al*., 2008b), was found to be on average 1600 m for annual cultures and 1200 m for heterogeneous and other permanent agriculture (average values of all sites). This distance is in agreement with other studies that have shown that most nitrogen deposition and its effects on biodiversity occur less than one km from sources (Pinho *et al*., 2009; Sutton *et al*., 1998). Taken together, these results have shown that [N] in lichens can be used to map areas under the impact of agriculture NH_3, even if other sources of disturbance are present.

Once we had determined that agricultural areas are the most likely cause for increased nitrogen concentration in lichens, we mapped [N] in the study region. This variable was analyzed by geostatistical techniques (CERENA, 2000), namely variogram interpretation used to interpolate [N] for the region using ordinary kriging (ESRI, 2008). By focusing on the Natura 2000 sites (Figure 5.14) we could observe that it presents a patchy distribution, highlighting the short-range nature of nitrogen pollution. Moreover this mapping also provides an efficient way to map the critical areas probably affected by nitrogen pollution, and should be considered critical areas for monitoring biodiversity. In this way we reduce cost focusing the plant and animal diversity monitoring in high impact NH_3 deposition areas.

In the Natura 2000 site "Costa Sudoeste" (Figure 5.13, right) most nitrogen is probably being emitted by vegetables, as well as by grain cultivation sites located in the nitrogen hot-spot areas (Figure 3, right). Of special concern is the Natura 2000 site "Comporta/Galé" (Figure 5.13, left),
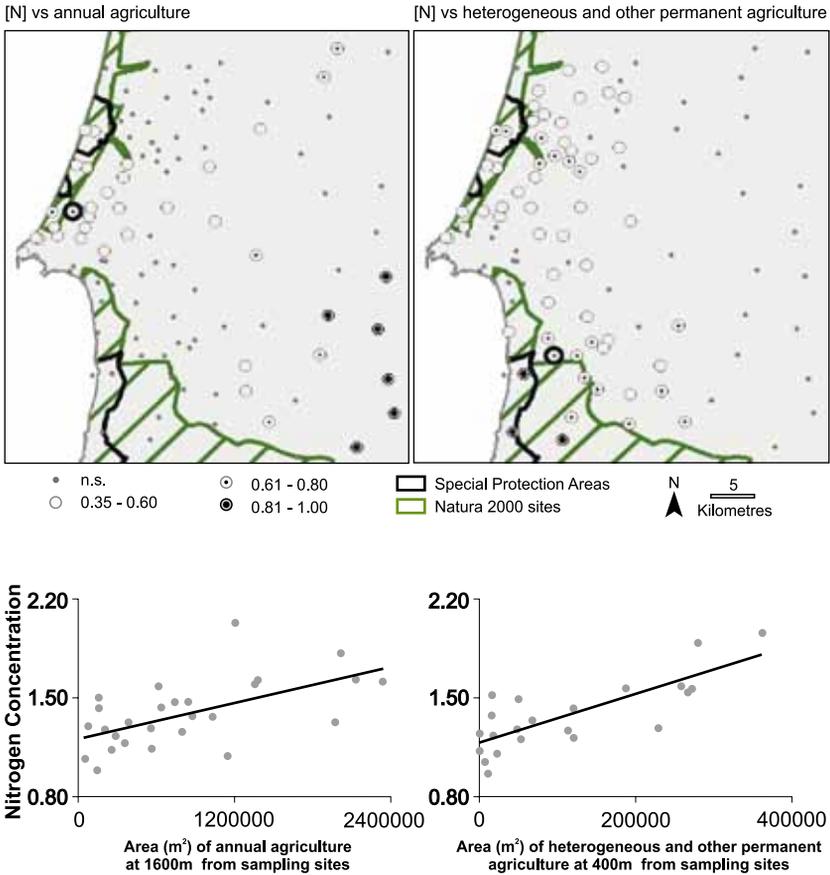
Figure 5.14: Local correlation analysis between [N] in lichens and: i.) neighboring annual agriculture (left) and ii.) neighboring heterogeneous and other permanent agriculture (right).
This is the result of a moving window analysis that correlates two variables using as samples all sites at a distance of 10km from each sampling site. A significant correlation indicates that, within a 10km radius neighborhood, the two variables are significant correlated. In the maps not-significant correlations (n.s.) are marked with small dots, significant ones with larger circles. The magnitude of the correlation (R) is given on the legend, and varied between 0.35 and c. 1.00. The two biplots are example of correlation for two sites, marked with a darker symbol in the maps above. More details on this type of analysis can be found in (Pinho et al., 2008a).

characterized by costal dunes habitats and costal lagoons. Up-stream of those lagoons there are rice cultures, known to be important sources of NH_3 (Yan *et al.*, 2003). Rice fields are the probable source of N leading to accumulation in lichens (Figure 5.14). Within this Natura 2000 area there are two Special Protection Areas (Birds Directive), one of which, "Lagoa de Santo André", is located in the area with higher Nitrogen concentration (Figure 5.13). This area is likely to be under strong nitrogen-pollution and should be particularly monitored for its impacts.

Aiming in the future at establishing precise boundaries for the critical areas outlined in Figure 5.15, we aim at calibrating [N] in lichens with a legal-bounded variable, namely i.) loss of biodiversity (e.g. loss of endangered plant species) or ii.) exceedance of critical levels of measured NH_3. Within the legal limits the criteria for protection level given by the chosen boundaries is a matter of decision makers choice.
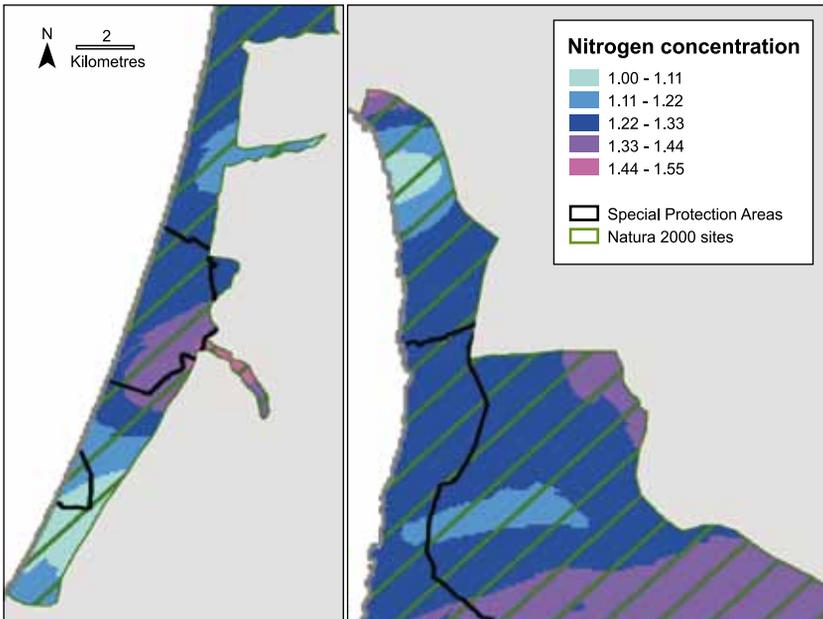
Figure 5.15: Mapping of the interpolation of Nitrogen concentration (per cent dry weight) measured in Parmotrema hypoleucinum within the two Natura 2000 sites located in the study area. The areas with the higher concentration are the ones under greater threat from N pollution, and are the ones that should be monitored for biodiversity loss.

5.8.4 Conclusions

- Although lichen-diversity is a good ecological indicator of NH_3 impact on ecosystems, in areas with multiple sources of disturbance, it responds also to factors other than NH_3, such as industrial and urban pollution. Therefore in such areas lichen diversity is not a good indicator of atmospheric NH_3.
- Nitrogen concentration in lichens was shown to be highly significant related to agricultural areas, and not related to artificial ones, being therefore a good measure of NH_3 atmospheric deposition, even in areas with multiple disturbance sources.
- By mapping nitrogen concentration in lichens, we could provide criteria for selecting critical areas with potential risk for biodiversity from NH_3 pollution within Natura 2000 sites.

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Acknowledgements

5.9 Effects of increased N availability on biodiversity of Mediterranean-type ecosystems: a case study in a Natura 2000 site in Portugal.

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Abstract
*   Although Mediterranean-type ecosystems are biodiversity hotspots, very little is known about the effects of increased N availability in these systems;
*   This paper describes an integrated field study on the effects of increased N availability in a Mediterranean-type ecosystem in a Natura 2000 site in Portugal;
*   The ecosystem was highly N responsive: visible changes were seen within one year; N additions created new and distinct seasonal patterns of soil N availability; plant and soil bacterial diversity together with plant cover were increased;
*   The effects of increased N availability appeared to depend on N form - plant evenness; N dose - plant species richness; and on both N form and dose - species cover and soil bacterial richness.

5.9.1   Introduction
Global biodiversity is changing at an unprecedented rate (Pimm *et al*., 1995) as a complex response to anthropogenic-derived changes at the global scale (Sala *et al*., 2000). The magnitude of this biodiversity change is so large that it constitutes a threat to the sustainability of human societies and natural systems (Galloway *et al*., 2008). But, what is biodiversity and how can we study it in

order to preserve it? Biodiversity is a complex term that includes taxonomic, functional, spatial and temporal aspects of organism diversity, with species richness (the number of species) and evenness (their relative abundance) considered among the most important measures (Wilsey and Potvin, 2000). Worldwide, many have focused on biodiversity loss. Sala *et al*., (2000) developed biodiversity change scenarios in terrestrial ecosystems, ranking increased N deposition as its third (out of five) main driver. Subsequent works, inferred that N deposition constitutes a threat to biodiversity (Phoenix *et al*., 2006, Clarisse *et al*., 2009). Mediterranean-type ecosystems are biodiversity hotspots (Phoenix *et al*., 2006), and could be experiencing the greatest proportional change in biodiversity (Sala *et al*., 2000). However, very little is known about the effects of increased N availability in these systems (Phoenix *et al*., 2006). Apart from being nutrient-poor (Cruz *et al*., 2003, Cruz *et al*., 2008) Mediterranean-type ecosystems also have distinct seasonal resource availability (water, nutrients and temperature). Enhanced N availability is likely to create new patterns of N availability that will allow new species to appear and others to disappear. It is also possible that N form, especially ammonium, could influence the system's response. The seasonal differences in N availability mean that it will be difficult to extrapolate from northern European to Mediterranean ecosystems.

5.9.2   Aims and objectives
- Study the effects of short-term increased N availability in a Mediterranean-type ecosystem; and
- Understand the effect of N doses and forms in the biodiversity of above- and below-ground communities.

5.9.3   Methods
The study site (38º29' N - 9º 01' W) is in Serra da Arrábida in the Arrábida Natural Park, south of Lisbon, Portugal (a Natura 2000 site - PTCON0010 Arrábida/Espichel). N availability (dose and forms) at the site has been modified by the addition of 40 and 80 kg N ha^{-1}yr^{-1} as NH_4NO_3 or 40 kg N-NH_4^+ha^{-1}yr^{-1} (control plots are not fertilized) since January 2007. N is added in three equal applications throughout the year, correlating with distinct biological activities (spring, summer and middle autumn/winter). Each treatment has three replicates (400 m² experimental plots).

5.9.4   Results and discussion
Aboveground community change
The standing plant community (Eunis class F5.2 – Mediterranean maquis) is in an early stage of a post-fire succession. The fire occurred in 2003, four years before the beginning of the N additions. Assessment of the plant communities in two consecutive springs (the first and second springs after beginning N fertilization) identified 80 plant species belonging to 27 families (Table 5.6). At the beginning of the N manipulations (first spring) the plots were homogeneous for species richness, evenness and plant cover. However, after one year of N additions treatment differences were found (Table5.7):

1) Non-fertilized plots exhibited a decrease in species richness (Table 5.7) that is characteristic of communities in similar stages of succession in Mediterranean-type ecosystems (Thompson, 2005) whereas adding N appeared to have prevented the natural decrease in species richness. This N-induced effect was dose-dependent and form-independent.

2) Plant evenness decreased in all treatments (Table 5.7). If this trend is sustained, plant communities would become more uneven, a characteristic of natural ecosystems (Naeem, 2009). Ammonium fertilization caused the greatest decline in plant evenness, possibly due to low ammonium tolerance of some plant species. We would expect these plots to become dominated by plant species that

are more tolerant to ammonium. Changes in plant evenness were N-form dependent and dose-independent.

In ecology, it is widely accepted that 'nutrient limitation' occurs when there are differences between fertilized and unfertilized samples (Vitousek and Howarth, 1991). Similarly, ammonium toxicity would occur when there are differences in response to the same N dose but supplied in different N forms. Since only fertilization with 40 Kg N-NH$_4$NO$_3$ha^{-1}yr^{-1} led to an increase in plant cover relatively to the control (Table 5.7), it may again be related with plant ammonium toxicity (Güsewell, 2004). Throughout succession the predominant form of available N changes (Cruz *et al.*, 2003). As a consequence, early successional species prefer nitrate while late successional are ammonium tolerant (Cruz *et al.*, 2003, Kronzucker *et al.*, 2003). The standing plant community is in an early phase of succession and therefore dominated by species less ammonium tolerant making it therefore more ammonium sensitive. If the site had been in a latter stage of succession the response to ammonium may well be different, with the effect being less detrimental (Cruz *et al.*, 2003).

First-year effects of fertilization are often determined by the original dominant species' responses, but in following years, subordinate or even new species may reach dominance (Stöcklin *et al.*, 1998). Dittrichia viscosa (L.) W. Greuter was the only plant species that significantly changed (increased) its cover in response to N additions (Table 5.6). However, there is growing literature suggesting that focusing on functional traits rather than species (McGill *et al.*, 2006) is more relevant for ecosystem functioning (Naeem, 2009) and a more practical approach for biodiversity hotspots like Mediterranean-type ecosystems. Therefore, plant species were grouped (Table 5.6) according to their functionality (Barradas *et al.*, 1999) or their common habitat. Increased N availability appears to promote the appearance of new herbaceous maquia species and the maintenance of ruderals. Changes in plant cover were analysed on a plant group basis highlighting the effects of increased N availability (Figure 5.17). Plant groups could be viewed as: (i) benefiting from increased N availability - ruderals and herbaceous maquia species; (ii) benefiting from increased N availability as long as there was no ammonium toxicity - ericaceous, legume shrubs and grasses; and (iii) affected by increased N availability especially in the form of ammonium - summer semi-deciduous.

Effects on soil microorganisms

Aboveground communities may also be influenced by changes in soil microorganisms and vice-versa (Klironomos 2002, Brooker, 2006). Therefore, Temperature Gradient Gel Electrophoresis (TGGE) fingerprinting was applied to monitor the impact of N addition in the soil bacterial communities structure. Numerical analysis of TGGE profiles and the corresponding dendrogram (Figure 5.18) indicated that, for the N doses used, only one year of addition was needed to induce changes also in soil bacterial communities. The three main clusters observed in the dendrogram presented high similarity levels and included: (i) control plots and one of the 40 kg N-NH$_4^+$ha^{-1}yr^{-1} fertilized plots (84 per cent similarity), (ii) all the remaining 40 kg N ha^{-1}yr^{-1} and one of the 80 kg N-NH$_4$NO$_3$ha^{-1}yr^{-1} and (iii) the remaining two plots receiving 80 kg N-NH$_4$NO$_3$ha^{-1}yr^{-1} (both with 100 per cent similarity). These results suggest a community shift mainly in response to the amount of N added rather than to the N form. TGGE outputs also allowed the estimation of bacterial diversity using the number and intensity of the TGGE separated bands. Accordingly plots fertilized with 40 kg NH$_4$NO$_3$ha^{-1}yr^{-1} displayed the highest bacterial band richness, while non-fertilized plots had the lowest. The remaining treatments showed intermediate values (Table 5.7). These data support the previously invoked ammonium toxicity as a mechanism that could account for the lack of stimulation of plant cover (Table 5.7 and Figure 5.18) by increased N availability. Soil bacterial band richness depended on both N-form and dose. Bacterial band evenness showed no differences between treatments (Table 5.7).

Table 5.6: Grouping of plants according to their functionality or common habitat

Plant Group	Species	Family	Ambient N depostion	.+40kg N-NH$_4$$^+$ha^{-1}yr^{-1}	.+40kg N-NH$_4$NO$_3$ ha^{-1}yr^{-1}	.+80kg N-NH$_4$NO$_3$ ha^{-1}yr^{-1}
Summer Semi-deciduous	Helichrysum stoechas	Asteraceae	D			
	cf Halimium halimifolium	Cistaceae				
	Cistus crispus	Cistaceae			D	
	Cistus ladanifer	Cistaceae				
	Cistus monspeliensis	Cistaceae				A
	Cistus salvifolius	Cistaceae				
	Lavandula stoechas ssp lusieri	Lamiaceae				0
	Rosmarinus officinalis	Lamiaceae				
Ericaceous	Calluna vulgaris	Ericaceae				
	Erica arborea	Ericaceae				
	Erica scoparia	Ericaceae				
	Erica umbellata	Ericaceae				
Legume Shrubs	Genista triacanthos	Fabaceae				
	Ulex densus	Fabaceae				
Grasses	Carex flacca	Cyperaceae				
	Agrositis sp	Poaceae	A			
	Avenula sp	Poaceae	A			
	Brachypodium phoenicoides	Poaceae		0		
	Briza maxima	Poaceae			A	A
	Briza minima	Poaceae		D		D
	Briza minor	Poaceae			0	A
	cf Arrhenatherum album	Poaceae				D
	cf Dactylis glomerata ssp lusitania	Poaceae				A
	Poaceae	Poaceae	0			0
	Vulpia sp	Poaceae	D	D		D
Herbaceous maquia sp	Apiaceae	Apiaceae	A			A
	Asphodelus ramosus	Asphodelaceae	D			A
	Asteraceae	Asteraceae	D	D	A	
	Cynara sp	Asteraceae	D			
	Leontondon taraxacoides	Asteraceae				0
	Phagnalon saxatile	Asteraceae				D
	Pulicaria odora	Asteraceae			D	A
	Lithodora prostrata	Boraginaceae	0			A
	cf Anthyllis vulneraria	Fabaceae				A
	Fabaceae	Fabaceae				D
	Blackstonia perfoliata	Gentianaceae			D	
	Centaurium erythraea	Gentianaceae			D	
	Gladiolus illyricus ssp reuteri	Iridaceae		A	A	A
	Iris xiphium	Iridaceae				A
	Stachys arvensis	Lamiaceae	A	A	A	
	Liliaceae	Liliaceae	A		A	
	Urginea maritima	Liliatae		A		D
	cf Orobanche latisquama	Orobancheae	A			
	Anemone palmata	Ranunculaceae	A			
	Sanguisorba hybridia	Rosaceae			D	D
	Galium sp	Rubeaceae			A	A
	Rubia peregrina	Rubeaceae				A
	Rubeaceae	Rubeaceae				A
Ruderals	Daucus carota	Apiaceae			D	
	Carlina corymbosa	Asteraceae			A	
	cf Andryala ragusina	Asteraceae				
	cf Centaurea melitensis	Asteraceae	A			
	cf Evax pygmaea ssp ramosissima	Asteraceae				A
	cf Filago minima	Asteraceae		D	A	D
	Chrysanthemum coronarium	Asteraceae		A		
	Crepis capillaris	Asteraceae		A		
	Dittrichia viscosa	Asteraceae	a	b	b	b
	Galactites tomentosa	Asteraceae		D	D	D
	Matricaria recutita	Asteraceae	D	D	D	
	Picris echoides	Asteraceae		D		
	Senecio jacobaea	Asteraceae	A		D	
	Sonchus sp	Asteraceae	D		D	A
	Campanula rapunculus	Campanulaceae			D	
	Lotus sp.	Fabaceae	A	A		
	Trifolium sp.	Fabaceae	D		D	D
	Vicia sp	Fabaceae		A		
	Hypericum sp.	Hypericaceae				

Table 5.7: Estimation of bacterial diversity using the number and intensity of temperature gradient gel electrophoresis separated bands

Properties	Ambient N deposition	.+40kg N-NH$_4$+ ha^{-1}yr^{-1}	.+40kg N-NH$_4$NO$_3$ ha^{-1}yr^{-1}	.+80kg N-NH$_4$NO$_3$ ha^{-1}yr^{-1}
Vascular plants community				
Species richness in 2007 (S$_{2007}$)	18.67 $\pm$ 1.2	18.00 $\pm$ 1.53	20.00 $\pm$ 1.53	20.33 $\pm$ 1.45
Species richness in 2008 (S$_{2008}$)	16.33 $\pm$ 1.76	18.33 $\pm$ 1.3	20.33 $\pm$ 2.4	24.00 $\pm$ 2
Weighted $\Delta_{(S2008-S2007)}$ (per cent)	14 $\pm$ 6	2$\pm$ 4	1 $\pm$ 9	16 $\pm$ 3
Weighted Gain (per cent)	. $\pm$ 5a	16 $\pm$ 4ab	15 $\pm$ 6ab	31 $\pm$ 4b
Evenness in 2007 (E$_{H2007}$)	0.78 $\pm$ 0.01	0.84 $\pm$ 0.03	0.80 $\pm$ 0.01	0.81 $\pm$ 0.01
Evenness in 2008 (E$_{H2008}$)	0.67 $\pm$ 0.03	0.61 $\pm$ 0.08	0.77 $\pm$ 0.03	0.77 $\pm$ 0.05
Weighted $\Delta_{(E2008-E2007)}$ (per cent)	-16 $\pm$ 4	-32 $\pm$ 15	-4$\pm$ 4	-6 $\pm$ 8
Weighted Gain (per cent)	. $\pm$ 2ab	-17 $\pm$ 8a	12 $\pm$ 3b	8$\pm$ 4b
Plant Cover in 2007 (per cent)	157.70 $\pm$ 18.27	141.37 $\pm$ 18.47	130.38 $\pm$ 9.67	156.03 $\pm$ 6.67
Plant Cover in 2008 (per cent)	219.06 $\pm$ 32.35	199.40 $\pm$ 8.29	257.37 $\pm$ 20.25	213.72 $\pm$ 16.94
Weighted $\Delta_{(per cent2008- per cent2007)}$ (per cent)	31 $\pm$ 17	35$\pm$ 16	65 $\pm$ 13	31 $\pm$ 4
Weighted Gain (per cent)	.- $\pm$ 12ns	4 $\pm$ 12ns	34 $\pm$ 11ns	0 $\pm$ 8ns
Soil Bacterial Community				
Band richness in 2008 (S$_{2008}$)	5.67 $\pm$ 0.33	8.33 $\pm$ 0.33	9.67 $\pm$ 0.33	7.67 $\pm$ 0.33
Absolute Gain (per cent)	. $\pm$ 24a	267 $\pm$ 24b	400 $\pm$ 24c	200$\pm$ 24b
Band evenness in 2008 (S$_{2008}$)	0.93 $\pm$ 0.01	0.93 $\pm$ 0	0.89 $\pm$ 0.01	0.90 $\pm$ 0.04
Absolute Gain (per cent)	.- $\pm$ 1ns	0$\pm$ 0ns	-4 $\pm$ 1ns	-4 $\pm$ 2ns
Soil				
Soil (inrogN) (ppm) *	8.93 $\pm$ 1.05	9.45 $\pm$ 1.85	9.21 $\pm$ 2.25	11.51 $\pm$ 4.8
Yearly average (sd) **	7.18 (4.27)	10.26 (4.43)	12.13 (8.90)	18.74 (2.76)
Gain in annual mean (per cent)	.- $\pm$ 32a	308 $\pm$ 28b	495 $\pm$ 40b	1156$\pm$ 14c
Gain in annual sd (per cent)	.- $\pm$ 37a	17$\pm$ 26a	463$\pm$ 147ab	697 $\pm$ 249b

Soil inorganic N availability

Because plants and soil biota evolved under specific nitrogenous environments they show preferences for specific patterns of N availability (Cruz *et al*., 2003, Gallardo *et al*., 2005, Cruz *et al*., 2008). Can the observed biotic changes be explained by changes in patterns of soil inorganic N availability? In spite of the levels of N applied to the system, soil inorganic N concentration, did not change between treatments after one year of fertilization (Table 5.7). Although, based on the seasonal means, there were significant changes in the annual pattern of soil inorganic N concentration: adding N significantly increased annual mean availability and annual variation. After fertilization, N accumulates in these soils until it is washed away by strong rain events, characteristic of the Mediterranean climate. The final value corresponds to the Mediterranean spring when strong rains (Figure 5.16) and intense biological activity occur (Sardans and Peñuelas, 2005), these factors combined explain why soil inorganic N concentrations were similar in all treatments. However more research is needed to identify periods of simultaneous increased soil N availability and biological activity (strongly limited by water) because biota (plants and microorganisms) that are active during these periods will directly 'win' or 'lose' from increased N availability.

Increased N availability in a Mediterranean-type ecosystem

In general our data suggests that short-term N fertilizations increase plant and soil bacterial diversity (richness and evenness), which seems to contradict most of the worldwide studies published so far (see Bobbink *et al*., 2010 for review). However, current knowledge suggests that the effects of N enrichment are dependent on the initial N status of the system: on highly productive sites, there is a potential for biodiversity loss and vice versa (Emmett 2007, Chalcraft *et al*., 2008). In fact,


Figure 5.16 a) General location of the studied site; b) landscape view; c) surface soil view; d) mean monthly temperature (dotted line), total monthly precipitation (grey) and times of N additions (arrows) and soil sampling (*) from Spring 2007 to Spring 2008; and e) examples of some of the existing plant species at the study site. *Photos © T. Dias*

Figure 5.17 Changes in plant cover according to plant groups. Changes were calculated as in Table 5.6. Plant species were grouped according to (i) functional groups (Barradas *et al.*, 1999) - * - or (ii) its common habitat. Trees and evergreen sclerophyllous were not considered on this analysis because at this stage of succession they are not dominant and their study requires other methods. Horizontal bars: control; Triangle 40 kg N-NH$_4$$^+$; Diamonds- 40 N-NH$_4NO_3$; and Circles 80 N-NH$_4NO_3$ha^{-1}yr^{-1}. Different letters refer to statistically significant differences between treatments (ANOVA followed by a Bonferroni test). Dark grey shadows correspond to a decrease; light grey shadows correspond to an increase (n = 3 experimental plots per treatment).
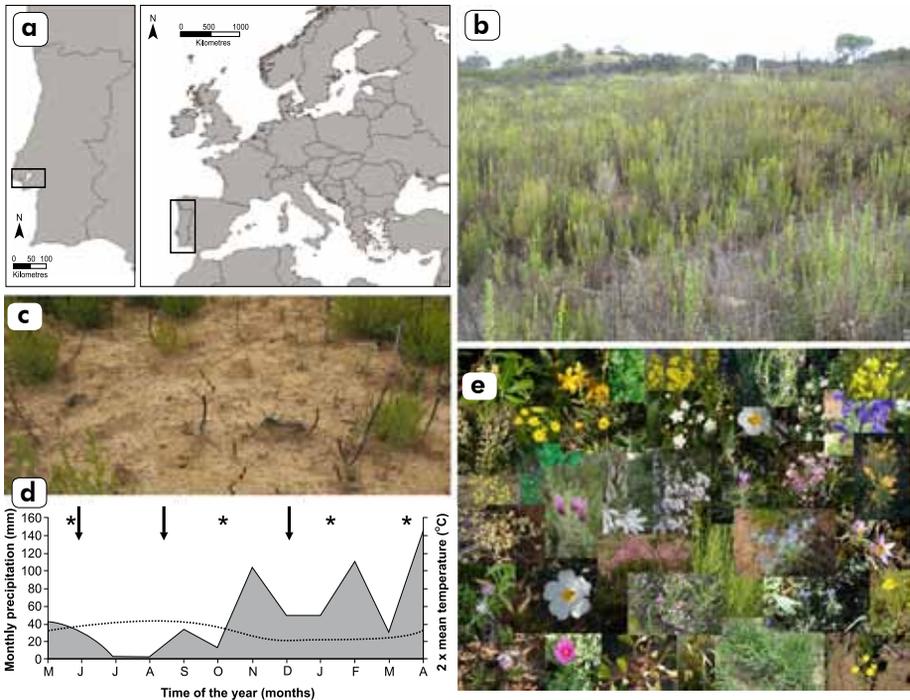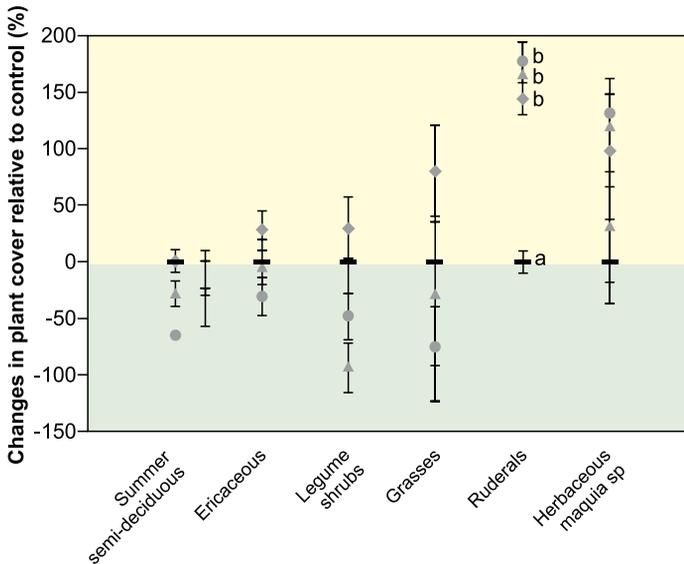
many studies have been performed in systems no longer N-limited, so that the stage of N-induced increases in biodiversity are not detectable. Data reported here refer to the initial changes in an ecosystem that has historically been subjected to low N deposition, with the initial increase in species richness probably representing an alleviation of the N limitation imposed on communities. Similar results have been reported for lichen community diversity in cork-oak woodland (Pinho *et al.*, 2009) and other systems under similar circumstances (e.g. Calvo *et al.*, 2007).

Adding N significantly changed the pattern of soil inorganic N availability. But the different N treatments appear to be differentially targeting distinct plant species (Table 5.6) and groups (Figure 5.17) and therefore distinct ecosystem functions (Hooper and Vitousek, 1997). These community changes may have been driven by altered patterns of soil inorganic N thus suggesting a key role for N in shaping Mediterranean-type ecosystems. Considering the reactivity of Mediterranean-type ecosystems to N, maintaining these systems within favourable conservation status constitutes a scientific, social and political challenge.

5.9.5   Conclusions
- The ecosystem was very responsive since only one year of N fertilizations was enough to induce 'visible' changes in both biotic and abiotic compartments: N fertilizations created new and distinct annual patterns of soil inorganic availability; N induced an increase in plant and soil bacterial biodiversity and plant cover.
- Ecosystem responses depended on the N-form (plant evenness), N-dose (plant richness) or both (plant cover and soil bacterial richness).
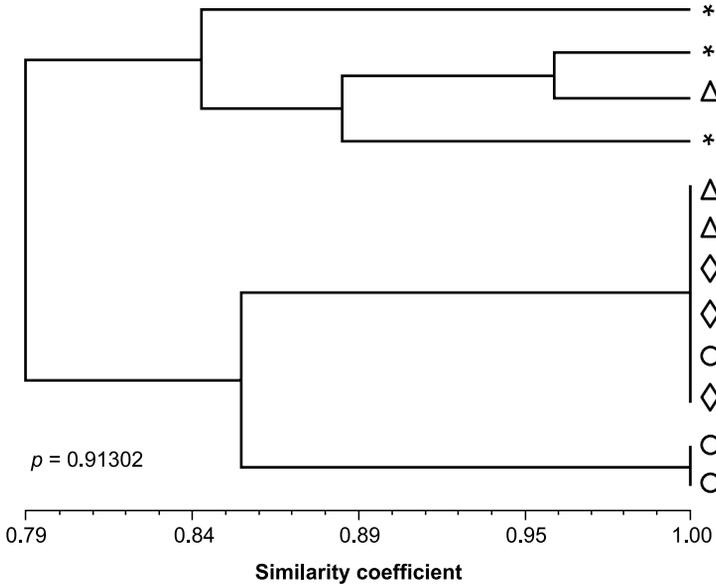
Figure 5.18: Dendogram obtained from numerical analysis of TGGE fingerprints of soil bacterial communities evaluated in Spring 2008. Asterisk: control; Triangle 40 kg N-NH$_4$+; Diamonds- 40 N-NH$_4$NO$_3$; and Circles 80 N-NH$_4$NO$_3$ha^{-1}yr^{-1}.

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5.10 All forms of reactive nitrogen deposition to Natura 2000 sites should not be treated equally: effects of wet versus dry and reduced versus oxidised nitrogen deposition.

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Abstract

• Atmospheric nitrogen deposition occurs in several different forms, including wet deposition of ammonium and nitrate, and dry deposition of ammonia. Each of these inputs occurs intermittently, according to patterns of precipitation, long range pollutant transport and local ammonia dispersion from point sources.

• Evidence is presented from a nitrogen manipulation study, undertaken using 'real world' treatment scenarios, on an ombrotrophic bog where the effects of gaseous ammonia are compared with wet deposited nitrogen, as ammonium or nitrate.

• Per unit N deposited, ammonia is found to be much more damaging to nitrogen sensitive plant species than wet deposited ammonium, which, in turn, is found to be more damaging than wet deposited nitrate.

• Damage is related to the likelihood of nitrogen accumulation in the plant tissue, which is greater with ammonia > ammonium > nitrate.

• Ammonia effects on lower plants are thought to be related to physiological damage associated with the intermittent high ammonia concentrations.

• Thresholds for damage effects from ammonia reduce logarithmically with the logarithm of time, indicating a memory effect.

• Ammonia damage to Calluna vulgaris appeared to be mediated indirectly through interaction with stress, winter desiccation, pests and pathogens.

• Wet ammonium deposition at N doses > 24 kg N ha^{-1-1} significantly increases N accumulation in lower plants leading to reduced growth in the pleurocarpous mosses Hypnum jutlandicum and Pleurozium schreberi.

• By comparison no significant effects of nitrate have been detected except in Sphagnum capillifolium, which is sensitive to N dose.

• The results clearly demonstrate that the form of nitrogen deposition affects the impacts on a sensitive habitat, with adverse effects per unit N input in the order: dry ammonia >>> wet ammonium > wet nitrate. These differences need to be recognized the development of air pollution policies.

5.10.1 Introduction

The effects of enhanced nitrogen deposition on semi-natural systems: elevated N concentrations, increased incidence of pests, pathogens and stress (Bobbink *et al.*, 2009) and reductions in species richness and change in function (Stevens *et al.*, 2006) are well described. However, the contributions of the main forms of N deposition to these N responses are much less well understood, but may be crucial to predicting N impacts on Natura 2000 sites. A key question for conservation policy and regulation is, are all forms of N equally damaging per unit N deposited, and if not, what are the implications for biodiversity and ecosystem services?

This paper briefly summarises what is known about the N forms most likely to affect semi-natural ecosystems, i.e. ammonia as dry deposition and ammonium and nitrate in wet deposition. Field evidence of effects of these three forms, based on a seven year, ongoing experiment on an ombrotrophic bog (Whim Bog, Sheppard *et al.*, 2004; Leith *et al.*, 20004) is discussed in relation to the different effects of these three forms of nitrogen deposition. Observations from this experiment on Whim Bog are particularly relevant for Natura 2000 sites because they represent the only comparison between these three N forms on the same site and where the application of N is consistent with 'natural' N deposition.

It should be recognized that other forms of reactive nitrogen contribute to dry deposition, including nitric acid, nitrous acid, nitrogen oxides, organic nitrogen forms (including PAN, amines, etc) and nitrogen containing aerosol. Although there may be differential toxicity of these many different forms, in most cases each of these forms provides only a small additional contribution to total nitrogen deposition. The major individual forms are ammonia, wet deposited nitrate and wet deposited ammonium, as investigated here.

The aims of this paper are:

*   to show that the effects of different N forms are not equal per unit N deposited to on an acid bog ecosystem and are species specific;
*   to show that field effects are generally consistent with current understanding of the mode of action of the different N forms.

5.10.2 The Whim Bog experiment

Wet deposition exposure frequency is coupled to precipitation and concentrations are relatively low, though individual rain events can contain higher ammonium and nitrate concentrations. By contrast, gaseous ammonia may deposit with a much higher variation, especially in sites near point sources, such as intensive pig and poultry farms. The Whim Experiment is designed to reproduce these differences in delivering the different N deposition inputs to the experimental treatments.

For the dry nitrogen treatment, the ammonia gas is released only in a 'downwind' direction from the source. In the free air release, ammonia is mixed with air and dispersed from a line source, providing an exponential concentration gradient declining down to ambient concentrations (0.3-0.4 $\mu g\ m^{-3}$) ~ 100 m from the source. The release simulates a range of ammonia concentrations typical of those measured downwind of agricultural sources, intermittent high concentrations (1000-2000 $\mu g\ m^{-3}$) for short (<h) periods with ambient concentrations dominating for ~85 per cent of the time (Leith *et al.,* 2004).

For the wet nitrogen treatment, all salts of sodium nitrate or ammonium chloride, combined with rainwater, are used to provide wet deposited oxidised N and reduced N inputs, respectively (Sheppard *et al.*, 2004). Ammonium sulphate salt was not used, because sulphate strongly influences soil pH and aluminium concentrations and can be phytotoxic (Sheppard *et al.*, 1994;

Silvertown *et al.,* 2006). Treatment was automated and all year round, except when the temperature fell below 0 °C. As with dry deposition, N was deposited at a range of concentrations, at least 100 times more concentrated than average rain concentrations, in > 100 events. The N deposition loads supplied 8, 24 or 56 kg N ha^{-1}, over and above the ambient eight kg N ha^{-1} deposition to four ~13 m^2 replicate plots (Sheppard *et al.*, 2004b). These N doses were replicated at different distances along the ammonia transect (Cape *et al.*, 2008).

5.10.3 Results and discussion

Observed effects of the different nitrogen forms relative to the damage they cause: dry versus wet deposition of nitrogen.
Ammonia is a highly reactive alkaline gas that deposits to acidic surfaces especially when wet. Acid ombrotrophic bogs support many lower plants that have large acidic surfaces, e.g. Sphagnum and Cladonia species (Jones, 2007). Such species have evolved to obtain their nutrients from atmospheric deposition, i.e. maximising their uptake surface (area to mass) rather than developing features to exclude ions/gases, such as cuticles.

Sphagnum capillifolium and Cladonia portentosa growing along the gaseous ammonia transect at Whim were both found to be highly sensitive to ammonia, with detrimental effects observed very quickly (< three months) in C. portentosa. The lichen was first bleached, then 'turned' green and slimey. Membrane damage was assumed based on the much lower potassium concentrations recorded in the apical parts of the lichens for the transect zone receiving > 24 kg N ha^{-1-1} (Sheppard *et al.*, 2004c).

Over subsequent years, the zone of species loss has extended to areas receiving > eight kg NH$_3$-N ha^{-1-1} of dry deposition, indicating that the impact of gaseous ammonia increases with time. It appears that continuous exposure gradually weakens sensitive plants, causing the damage threshold concentration to fall (Sheppard *et al.*, 2009).

Equivalent N doses (56 kg N ha^{-1-1}) applied as either wet deposited ammonium or nitrate have caused limited damage, which has developed slowly over the years. Damage has been confined to small patches within a clump, rather than causing complete eradication of C. portentosa from the plot (Figures 5.19, 5.20).

The detrimental effects of gaseous ammonia on the bog moss S. capillifolium took longer to develop than the effects on sensitive lichens, i.e. required a larger accumulated NH$_3$-N dose and were restricted to the green pigmented form of the moss. (The red pigmented form of S. capillifolium is found to be less sensitive to ammonia.) The speed of response of S. capillifolium to wet N deposition was more similar to that seen with C. portentosa, except that detrimental effects were more pronounced with ammonium than with nitrate.

Implications of the form of nitrogen: dry versus wet deposition.
The implications from this field comparison are that sensitive lower plants can tolerate >5 times the N load, when the N is deposited wet in precipitation than when it is supplied by ammonia dry deposition. One explanation for this may be related to the high gaseous concentrations associated with ammonia deposition, when the wind is blowing from an ammonia source. In the field in source regions, ammonia concentrations are locally highly variable (Asman *et al.*, 1998) responding to wind direction, meteorological conditions and the distribution of ammonia sources. Also, due to its reactive nature, up to 50 per cent of ammonia emissions are deposited within a five km radius of their source (Sutton *et al.*, 1998). In rural areas dominated by livestock production, sites downwind of ammonia sources will experience intermittent very high, ammonia concentrations, > 600 μg m^{-3}

(Esther Vogt pers comm.). Such intermittent spikes in ammonia concentrations are characteristic of this pollutant, even in areas remote from sources. In the Whim free air release the average concentrations of these spikes can be estimated from the mean monthly concentration because the duration of the ammonia release is known. During the six years the experiment has been running the prevailing wind direction has permitted release for < 14 per cent of the month, implying that the ammonia concentrations during the exposure period to be on average seven times higher than the monthly mean air concentration measured with passive samplers, and potentially phytotoxic (Van der Erden, 1982; Krupa, 2003). Exchange of this gaseous ammonia concentration with plants is dominated by the solubility of ammonia in water, how these air concentrations compare with concentrations of ammonium in precipitation, wet deposition, have still to be demonstrated for the vegetation at Whim when ammonia is being released, but we expect them to significantly exceed those measured in rain.

Concentrations of ammonium and nitrate in precipitation are generally quite low: highly skewed to the low concentrations, median 20-25 µM max ~ 320 µM, even in cloudwater where they can be up to 10 times higher. Although high concentrations can occur, these tend to be restricted to occasional pollution events associated with long range transported air pollution. In the Whim manipulation study considerably higher concentrations, maximum 4000 µM, with a high application frequency are used to deliver the N dose, interspersed amongst the low N concentrations in rainfall. But, despite these relatively high concentrations, visible damage has been minimal, even to N sensitive species (Figure 5.19). Our results suggest that the effects of ammonia on vegetation may be mediated aboveground through foliar uptake of high concentrations that exceed the capacities, especially those of lower plants, to detoxify it. This hypothesis is supported by the significantly higher N concentrations (+>45 per cent) measured in plants treated with ammonia, compared with those treated with the equivalent wet N deposition (+ 10-15 per cent) (Sheppard *et al*., 2008). In addition, effects may be partly mediated by pH interactions, where gaseous ammonia can increase the pH of leaf surfaces. The role of this mechanism remains to be explored in more detail.

As a gas, ammonia has more potential uptake pathways than wet deposited N, which is taken up through the roots, which are often mycorrhizal, and for some plants, e.g. Calluna also via the shoots (Bobbink *et al*., 1992). Gases are taken up through stomata in higher plants, when they are open. The stomatal pathway is controlled through a combination of physical and biological processes (see Dragosits *et al*., 2008) and is not as effectively regulated as root N uptake, which can be adjusted in relation to demand (see Sheppard and Wallander 2004a).

Loss of Calluna cover was recorded at > 10 kg N after five years and significant losses, > 85 per cent cover loss were observed when the NH_3-N deposition exceeded 24 kg N. The loss of Calluna cover coincided with an almost 100 per cent increase in N content and was accompanied by significantly increased damage from abiotic and biotic stress (Sheppard *et al*., 2008). The combination of ammonia exposure and drying winter conditions led to the loss of Calluna cover. These observations suggest that much of the damage/loss of heathlands in the Netherlands during the late 80s early 90s was caused by the dry deposited gaseous ammonia form of nitrogen. Exposure to ammonia can double foliar N status significantly increasing the likelihood of death from abiotic stressors such as drought, freezing injury and pests and pathogen attacks, as were recorded in the Netherlands (van der Eerden *et al*., 1991). The potency of ammonia and its memory effects are now acknowledged in the revised critical levels (Sutton *et al.,* 2009). The Cyperaceae (Eriphorum spp.) and other ericoids on site, Erica tetralix, Empetrum nigrum, Vaccinium oxycoccus and V. myrtillus were not apparently sensitive to ammonia-N.
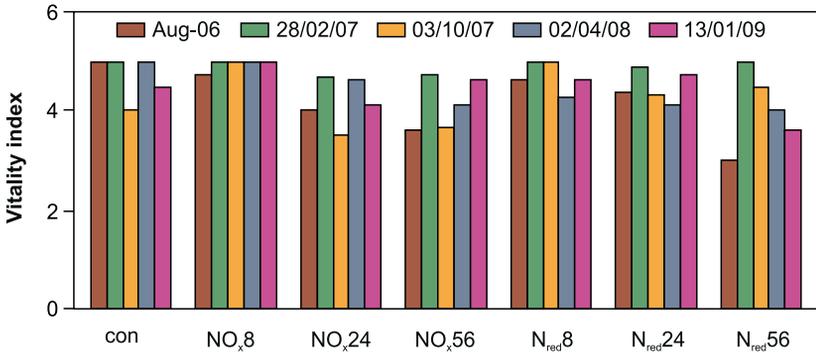
Figure 5.19: Vitality Indices, subjective scoring of visible damage where a maximum score of five constitutes healthy, for Cladonia portentosa and Sphagnum capillifolium treated with sodium nitrate (NOₓ) or ammonium chloride (Nred) in rainfall at 8, 24 or 56 kg N ha⁻¹ ⁻¹.

Differential effects of reduced versus oxidised nitrogen in precipitation

By contrast to the effect of gaseous ammonia in raising surface pH, accumulation of ammonium in plant tissue can lead to acidification, accompanied by toxicity, membrane damage and nutrient imbalance with negative impacts on growth (Krupa, 2003). In addition, foliar uptake of cations exceeds that of negatively charged anions such as nitrate, so that ammonium uptake from atmospheric deposition is thought to be more difficult to regulate. By contrast no such effects have been reported for nitrate, its assimilation is not associated with acidification (rather the reverse) and it can be accumulated safely in the vacuole. Little field evidence, by way of comparisons, exists to support the greater potential for ammonium to cause detrimental effects compared with nitrate. However, high ammonium concentrations in the soil or water layer have been shown to be toxic, restricting root development in sensitive plant species especially when the ratio of ammonium to nitrate is high (Roelofs *et al*.1996). Evidence from field surveys, in the UK at least, is confounded by the differences in range of reduced versus oxidised N deposition.

The Whim experiment on an ombrotrophic bog is the longest running, with the most realistic treatment scenario, to examine the significance of reduced versus oxidised nitrogen.

Significant changes in the cover of the major species in response to wet deposition of either ammonium or nitrate have not been detected after six years, although the vitality of N sensitive plant species does appear to be poorer with ammonium (Figure 5.20). Foliar N content was always found to be higher in the mosses and Sphagnum (Figure 5.21) when treated with ammonium than with nitrate, although the effects were rarely significant below 24 kg N ha⁻¹⁻¹. No significant enhancement, 'memory effect' has been detected in six years of per centN data for Sphagnum capillifolium, although significant differences between oxidised and reduced N were found (p<0.05) (larger effect with ammonium than with nitrate).

Significant reductions in annual growth of -30 per cent were measured in Hypnum jutlandicum a common pleurocarpous moss in response to wet ammonium inputs of > 24 kg N ha⁻¹⁻¹. Both N forms significantly (p=0.1) reduced plant mass in Pleurozium schreberi (-3 per cent with nitrate and -21 per cent with ammonium). In Sphagnum capillifolium both N forms also reduced growth and again the effects were more pronounced with ammonium at all N doses (Kivimaki *et al*., 2008). In Hypnum jutlandicum the N content was increased by +59 per cent with ammonium compared with by +29 per cent with nitrate, with similar increases being recorded for Pleurozium schreberi (+66 per cent and 31 per cent, respectively).
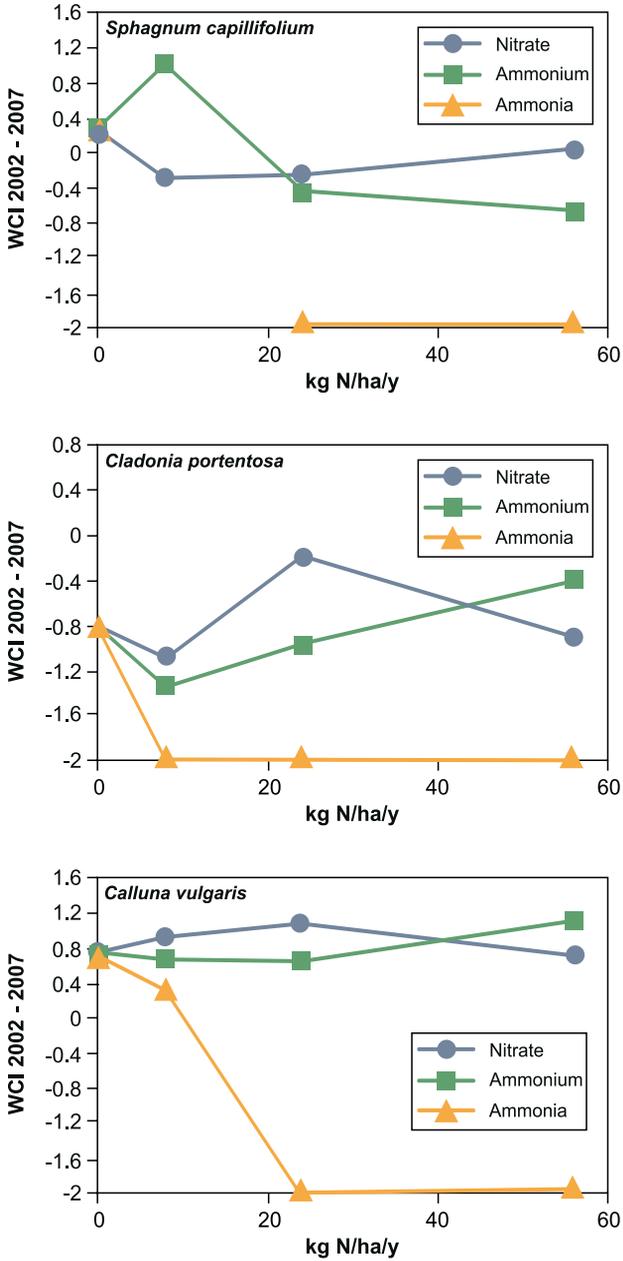
Figure 5.20a,b,c: Change in the cover index, cover after five years of treatment in relation to the start cover, of N sensitive species Sphagum capillifolium, Cladonia portentosa and Calluna over five years of treatment with nitrate, ammonium or ammonia
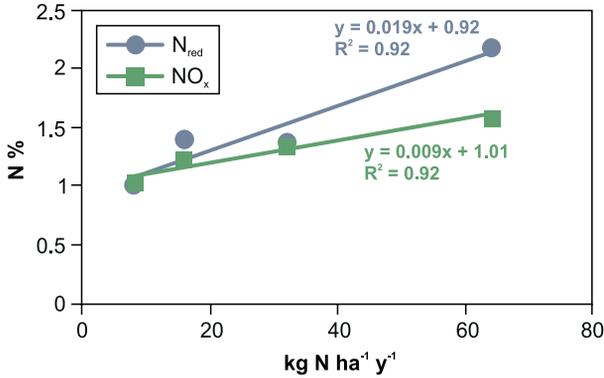
Figure 5.21: Nitrogen concentrations (per cent dry wt) in *Sphagnum capillifolium* treated with 56 kg N ha^{-1} of nitrate (NO$_x$) or ammonium (Nre) and the control between 2002 pre treatment and the end of 2008 and b *Pleurozium schreberi* in March 2009 in relation to N dose in precipitation.

Both ammonium and nitrate increased the Calluna cover, only enhanceing the foliar N concentration by a modest 15 per cent, significant (p < 0.05). Exposure to equivalent wet N doses continues to enhance the growth of Calluna, helping to restrict the accumulation of N. No significant effects of N form were found.

In this manipulation experiment effects associated with wet deposited oxidised N remain barely detectable with respect to changes in vitality, growth and cover of the N sensitive species.

5.10.4 Conclusions

Our results from a field manipulation experiment comparing dry ammonia with wet nitrate and ammonium inputs confirm a clear pattern of much greater N accumulation in response to ammonium addition than to nitrate addition, with the largest accumulation and effects being observed following treatment with gaseous ammonia. The results suggest that the greater the accumulation of N the greater the risk of damage. Thus the implications for Natura 2000 sites are that habitats in the vicinity of point sources of ammonia are expected to be subject to much larger impacts per unit N deposited, than sites where the nitrogen is primarily received by wet deposition. This differential must be recognized in the development of policies to protect the Natura 2000 network from nitrogen deposition. In particular, there would be a case to locate ammonia emission point sources as far from sensitive Natura 2000 sites as possible. With respect to wet deposited N, ammonium is found to be more damaging than nitrate because it accumulates with toxic effects.

Acknowledgements

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5.11 Evaluation of nitrogen indicators on ombrotrophic acid bogs: observations from a nitrogen manipulation study.

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Abstract
*   Among Natura 2000 ecosystems, ombrotrophic bogs/peatlands are likely to be one of the most sensitive to reactive N deposition.
*   This paper briefly examines methods of evaluating species, growing on an ombrotrophic bog, to different N forms using a case study from Whim bog to illustrate peatland vegetation response to wet and dry N deposition.

- Vegetation cover data is used to examine typical N indicators in terms of species richness, diversity, functional group response, Ellenberg indicator values, species cover and National Vegetation Classification (NVC).
- In addition an ordination technique is applied to the data and principal response curves (PRC) generated to examine temporal community response to the N deposition.
- The N indicators tested here of species richness, diversity, Ellenberg indicator values and NVC were insensitive to the apparent effects of the N deposition treatments. This is despite significant responses at both the species and community levels detected using a weighted cover index and PRC.
- In addition responses varied with N form and particular functional groups e.g. species from the ericoids or Sphagnum show differential responses: Calluna responds negatively to ammonia whereas Vaccinium myrtillus and Empetrum nigrum have responded positively, the hummock forming S.capillifolium is negatively affected by N while the wetter loving S. fallax increased in response to N additions.
- The results emphasise that indicators represent a range of sensitivities and a considered approach to the choice of indicator needs to be applied with reference to the ecosystem under question and form of nitrogen applied.
- Future research efforts should concentrate on developing predictive indicators with sufficient sensitivity to show species level change that can be used to detect and predict loss of important peatland species or groups.

5.11.1 Introduction

In the UK, many of the remaining lowland ombrotrophic peat bogs are designated as Natura 2000 sites. Atmospheric reactive nitrogen (N) deposition, largely as a consequence of activities associated with the intensification of agriculture, threatens the sustainability of naturally nutrient poor ecosystems such as ombrotrophic peatlands (Bobbink *et al*., 2009). This is because the organisms that inhabit these ecosystems have evolved under nutrient limitation and, until recently, have been maintained in this state. The threat posed by N deposition can also be compounded by changes to land-use practices such as grazing and burning. Once effects are detected there are high labour costs associated with mitigating N deposition via removal and management. Globally, nitrogen deposition has been ranked as the third most important driver behind land use and climate change, but regionally, in northern temperate biomes, N deposition is expected to be the major driver of biodiversity change (Sala *et al*., 2000).

Evaluating the effects of enhanced N deposition is a complex area of study, but several indicators of the effects have been proposed. Ideally, indicators of enhanced N deposition need to be specific to N, robust, widely applicable and preferably cheap and simple to apply (Sutton *et al*., 2004). A further challenge is to identify sensitive approaches that reliably point as 'early warning' indicators towards the initial onset on nitrogen impacts, before changes become irreversible. More recently, the need to take account of N effects with respect to ecosystem functioning and the provision of ecosystem services has been widely recognised, raising the importance of ecosystem composition at the functional group and species levels.

In ombrotrophic peatlands, non-vascular plants, in particular Sphagnum mosses are crucial, being described as the 'engineers' of these ecosystems (van Breemen, 1995). This genus is arguably largely responsible for the worlds largest terrestrial carbon store mostly found in northern peatlands (Gorham, 1991). However, non-vascular species include mosses, lichens and liverworts, groups which are often overlooked in vegetation surveys. Yet globally, these groups are widespread, present in most major ecosystems, and make substantial contributions to biodiversity, biomass and biogeochemical cycling (Cornelissen *et al*., 2007).

Of the 38 main mire communities recognized in the UKs' National Vegetation Classification (NVC), 11 are specifically defined by occurrence of Sphagnum species (Rodwell, 1991). In a recent survey of 15 Scottish lowland raised bogs, we found that Sphagnum spp. dominated, with up to 11 different species, which together with other mosses and Cladonia spp., made up > 66 per cent of the species recorded, with vascular plant species being in the minority.

Non-vascular species tend to derive almost all their nutrients from atmospheric deposition. They have mostly evolved to maximise the uptake of these atmospherically derived nutrients. For example, they have relatively large surface area to mass, with no means of excluding or restricting uptake, i.e., they have no cuticle. This tight coupling to atmospheric deposition means these plants are often the most sensitive components of the vegetation and importantly their responses can provide a potential monitoring tool for following the effects of N deposition (Leith *et al*., 2005).

The question arises of how best to quantify the response of ombrotrophic peatland species in order to evaluate effects of reactive N deposition?

The most common approach is to look at total species richness; this simply refers to the number of species recorded in a given area, the greater the number the greater the richness. The approach is widely used in conservation studies to determine the sensitivity of ecosystems and their resident species. A long-term decrease in plant species richness in the UK since 1990 has also coincided with the decline in abundance of farmland butterflies and birds over the same period (Countryside Survey UK headline messages from 2007). However, the species richness approach ignores that some species may be more important than others i.e. fails to prioritise between species. With respect to conservation value, ecosystem function and sustainability, all plant species are not equal e.g. the case of Sphagnum spp. detailed above, this genera exerts a pivotal influence over both the existence and sustainability of peatland ecosystems.

Ellenberg N indicator values represent another approach that was initially considered as a general indicator of soil fertility rather than specifically N availability (Hill *et al*., 1999), but has been widely used to assess habitat change with respect to eutrophication (e.g., Countryside survey UK). The value of the Ellenberg approach is that it is easy to score, based on species occurrence and abundance, with the method relying on a derived 'average' species response based on the habitats in which the species is typically found.

This paper examines some of the tools available for assessing the impact of nitrogen in the context of an ombrotrophic bog, one of the ecosystems expected to show the greatest response to increasing nitrogen deposition in terms of change in biodiversity (Sala *et al*., 2000). We use a field nitrogen manipulation study (Whim Moss) established in 2002 (see this volume; Leith *et al*., 2004; Sheppard *et al*., 2004), comparing the three nitrogen forms most likely to affect ombrotrophic bogs (ammonia gas, wet deposited ammonium and nitrate). Specifically, we address the significance of our evaluation methods for the sustainability and functioning of a semi-natural ecosystem. We consider whether all species be treated equally, and discuss how to evaluate species with respect to the functions they serve within an ecosystem.

Whim Moss in the Scottish Borders is a relatively acid site, pH ~3.6 in H_2O, and shows substantial differentiation in micro-habitat. In hollows, the water table is at the surface for most of the year, whereas in hummocks, which can be up to 0.5 m above the hollows, the water table almost never reaches the surface. The bog is not actively managed at present and is subject to some grazing by rabbits. The vegetation, classified as NVC M19 Calluna vulgaris – Eriophorum vaginatum blanket mire dominated by Calluna vulgaris (L.) Hull, Eriophorum vaginatum L., Sphagnum capillifolium (Ehrh.) Hedw. and Cladonia portentosa (Dufour) Coem., which make up >85 per cent of the cover.

The Calluna is either mature phase or in places degenerate. Permanent quadrats were established in the plots, with species cover recorded since 2002. We examine the data collected in terms of species (vascular and non-vascular), functional group and community responses, analysing in terms of richness and diversity using common statistical and multivariate ordination techniques. Changes in species cover were also evaluated through the computer programme ComKey (C. Legg, unpublished).

5.11.2 Results and discussion

Species richness and diversity
No differences in overall species richness were detected reflecting the widely observed trend for species swapping in response to reactive N deposition: N sensitive species are replaced by more N loving, N tolerant species, so that the overall richness remained unchanged.

Species diversity is generally a much more useful metric than species richness because abundance is included rather than just presence/absence. However, the Shannon Weiner Index, Equitability and Simpson's Index likewise remained unaffected by the N treatments at ~ 2.6, 0.55 and 0.8 respectively. The appearance and/or disappearance of species within any single area can be caused by a number of extraneous or inter-related factors. Both richness and diversity suffer from the loss of identity in their metric and thus it is not immediately obvious how changes in these metrics can be related to N deposition effects or to ecosystem function without reference to the actual species. This would suggest that the techniques may be somewhat redundant in low diversity systems.

Ellenberg Indicator Values
In response to the increases in N deposition in this study we might expect to observe an increase in the Ellenberg N indicator score. After five years of N additions equivalent to > five times the critical N load for ombrotrophic bogs, no change in Ellenberg N was detected. There may be three reasons for such an observation. Firstly Whim bog, like many such ombrotrophic bogs, is co-limited by P availability and not just N. Secondly, the Ellenberg indicator N values are derived from the species abundance data. In order for a significant difference to be detected a shift from dominance of, for example, species with an indicator value of one (indicator of extremely infertile sites) to those with a value of three (indicator of more or less infertile sites) would be required. However, given the species pool at Whim, it is more likely that dominance will shift between species that have similarly low Ellenberg N scores or bare peat (which by definition has no Ellenberg value) rather than a large shift to more fertile species. Lastly, there are likely to be temporal effects (see Principal Response Curves below) and it may be that not enough time has elapsed for a shift to more fertile species to have occurred.

We therefore urge caution in the use of Ellenberg indicator values for the detection of N deposition effects as, for ombrotrophic bogs at least, Ellenberg indicator values may not have sufficient sensitivity. Given the significant changes in species composition observed at the Whim site, our results point to the need to develop more suitable indicators for ombrotrophic bogs.

2.3 Change in cover of important peatland species, functional groups
As indicated in Sheppard *et al*., (this volume), exposure to ammonia resulted in large reductions in the cover of three major species/groups, Calluna, S. capillifolium and Cladonia portentosa, which are functionally, key components of this peatland. The effects of exposure to ammonia are captured in the photographs (Figs 5.22, 5.23) and quantified as change in cover after five years in relation to the pre-treatment cover (weighted cover index) (Figure 5.24), for the most common bog plants at Whim. Positive values indicate an increase in cover while negative values indicate cover has been

reduced, with -1 and -2 indicating a 67 per cent and 100 per cent cover loss (the latter indicating complete eradication).

 If we examine functional groups, among the ericoids Calluna has disappeared close to the gaseous ammonia source, whereas Erica tetralix L. and Empetrum nigrum L. and Vaccinium myrtillus L. (data not shown) have increased in the area within 32 m of the ammonia source. Of the graminoids at Whim, Eriophorum spp, (Cyperaceae) are the main components. Graminoids are generally considered to be effective at utilising increased levels of N, so unsurprisingly, E. vaginatum L. has increased its cover. The cover of non-vascular plants has generally decreased, as expected, but there are big differences in sensitivity: among the pleurocarpous mosses Hypnum jutlandicum Holmen & Warncke has generally increased its cover whereas its' cohabiting species Pleurozium schreberi (Willd. ex Brid.) Mitt. has declined. Close to the source, S. capillifolium, and C. portentosa have completely disappeared.

The addition of wet deposited nitrate or ammonium has produced much more subtle changes in species cover (Figure 5.25). Responses to oxidised N, nitrate tend to be non-linear showing a positive response to N additions, < 24 kg N ha^{-1-1} (total deposition, including background <32 kg N ha^{-1} yr-1) which continue after five years to alleviate N limitation in the ericoids, Calluna and E. tetralix. The higher N addition of oxidized nitrogen (64 kg N ha^{-1} yr-1) has reduced cover indicating the capacity of the vegetation to use N has been exceeded. H. jutlandicum has shown the most consistent positive response to oxidised N, irrespective of dose. Different responses were observed for reduced N (Figure 5.25). Calluna appears to show a preference for wet deposited reduced N over wet deposited oxidised N, which may reflect the low activity and inducibility of nitrate reductase in its foliage (Smirnoff *et al.*, 1984), and the increased potential for soil acidification from ammonium deposition. *S. capillifolium* has shown consistent reductions in cover in response to both N forms, though not on the scale caused by equivalent N doses as ammonia. *E. vaginatum* has not responded positively to the wet deposited nitrogen treatments, probably reflecting the increase in Calluna. In the wet deposition treatments, the cover response appears to be mediated through competition rather than phytotoxicity. As expected, the most N-sensitive species are again the non-vascular plants, *C. portentosa and S. capillifolium*.

Anecdotal observations on non-vascular species that are less frequent at the field site suggest that *Cladonia rangiferina* (L.) Weber ex F.H.Wigg. and *C. chloropheae* (Flörke ex Sommerf.) Sprengel are less sensitive than *C. portentosa*. Similarly, among the less frequent Sphagnum species present, limited data suggest that *S. fallax* H. Klinggr. and S. *papillosum* Lindb. are much less N-sensitive than *S. capillifolium*.

Recording the cover of vascular and non-vascular plants from prior to treatment to the present has provided a significant insight into the response of some environmentally important plant species to N deposition, which is clearly visible in the changing character of the site (Figs 5.22 and 5.23). Such a detailed approach appears to work well for experiments, informing us of the relative sensitivities of different species, and enabling us to predict which species are most likely to be adversely affected by additional atmospheric N deposition. However, species composition/cover fails as an 'early warning indicator' because, by the time the effects are expressed, the damage has already been done.

National Vegetation Classification (NVC)

The National Vegetation Classification (NVC) is a standardised method for community classification in the UK. Interestingly it has also been used to demonstrate community level effects in relation to hydrological disturbance in fens (e.g. Fojt and Harding, 1995). However, no direct differences between the treatments at Whim were detected in terms of the NVC classification, either

Figure 5.22: Photograph (taken on 27/08/09) looking downwind of the 60m ammonia free air release transect at Whim Bog, a lowland raised bog in the Scottish Borders. The photo shows the undamaged vegetation upwind of the 6m release pipe and the change in vegetation with the loss of Calluna vulgaris, Sphagnum spp. and Cladonia portentosa up to 24m downwind of the NH$_3$ release point, which is now dominated by Eriophorum vaginatum. *© Ian Leith*

Figure 5.23: Impacts of NH$_3$ on vegetation at Whim Bog showing death of *Calluna vulgaris,* blackening of *Eriophorum vaginatum* shoots and the loss of vegetation cover (mostly *Sphagnum* and lichen spp.) resulting in the exposure of bare peat. The droppings reflect the presence of rabbits across the site.
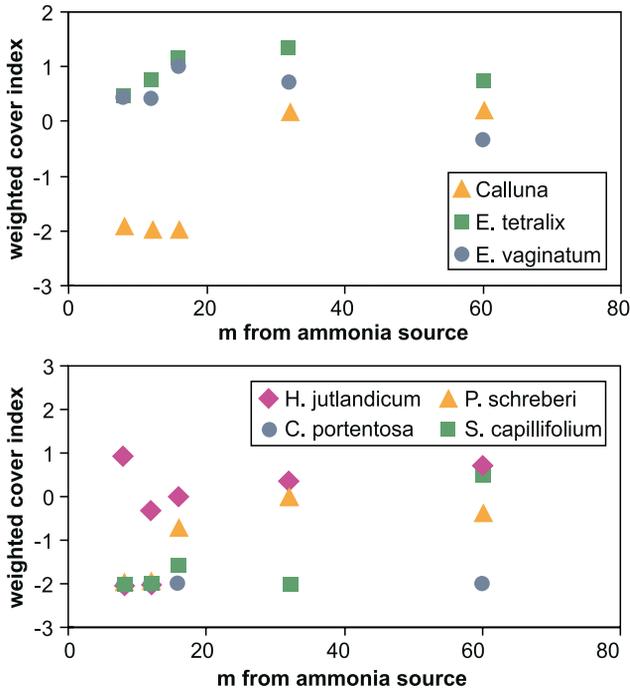
Figure 5.24 Change in the weighted cover index (value based on start value and after five years exposure) for vascular plants growing at different distances from the ammonia source and changes in the non vascular plant index. The exponential decrease in ammonia concentrations along the transect produces NH$_3$-N doses equivalent to the 56, 24 and eight kg N ha^{-1} wet deposition treatments at ~ 8-12m, 24m and 32-40m from the line source, depending on the year.

at community or sub-community levels. All treatments were most closely classified within M19, with the weighted match (the number of species in the sample that also occur in the community weighted according to species fidelity to a community) remaining above 88 per cent for both the wet and dry N treatments.

The lack of detection of community difference using the NVC may be due to several reasons, but is probably mostly related to scale. Firstly, the NVC describes community at a national level from a conglomerate of UK data, so that even when using 'typical' community data, the fit of any individual site is usually not exact. Secondly, the detection of change in the Whim experiment is at a local level from one community type, where differences are likely to be small in relation to differences between distinct communities. Finally, plot replication in expensive field manipulation studies on semi-natural communities can rarely match the scale and level of replication that can be achieved in vegetation surveys. At Whim, logistical reasons dictated the layout of treatment plots which meant that the initial species composition was not precisely replicated in each plot or quadrat. For many of the non- vascular plants occurrence was often limited to a few plots or a few treatments. In the case of species only occasionally occurring at the site, this reduces the quantitative value of individual species observations to anecdotal evidence. Rodwell (1991) suggests that two or four m^2 plots are appropriate for assessing mire NVC communities; thus NVC matching is probably inappropriate for the smaller experimental quadrats (total area of three per plot = 0.75 m^2) used here.
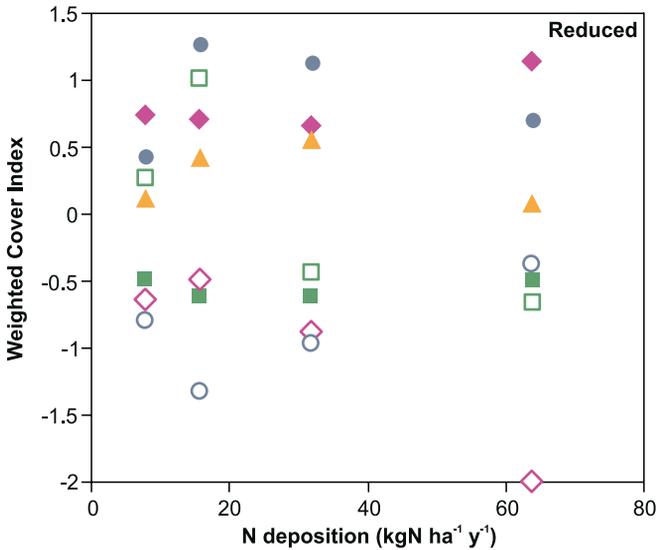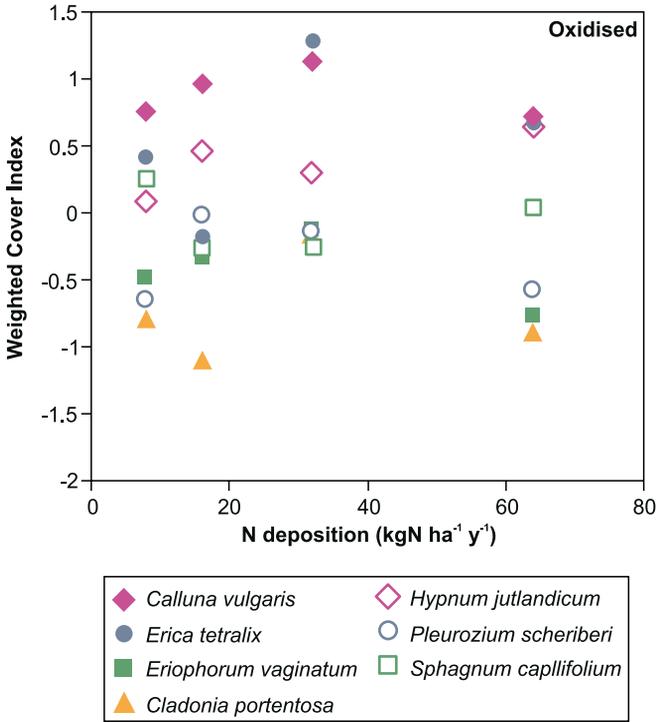
Figure 5.25: Change in the weighted cover index (value based on start value and after five years exposure) for vascular and non vascular plants treated with wet oxidised and wet reduced nitrogen as NaNO₃ or NH₄Cl, respectively. The species represented are the most common species growing at the experimental site an ombrotrophic bog, Whim in Southern Scotland. Values above the bold line represent an increase in cover and vice versa. Cover at 8 kg N ha⁻¹⁻¹ represents the control response, receiving no additional N.

Principal Response Curves

Principal Response Curves analysis (PRC) is a means of analysing repeated measurement designs and testing and displaying optimal treatment effects that change across time (see van Brink and Ter Braak, 1999). It is based on a redundancy analysis (RDA) that is adjusted for changes across time in the control treatment. Thus the treatment effects are expressed as deviations from the control and PRC gives simple representation of how treatment effects develop over time at the community assemblage level. Interpretation of species response can be assessed with reference to the additional species score for the first RDA axis (shown to the right of Figures 5.26a and 5.26b).
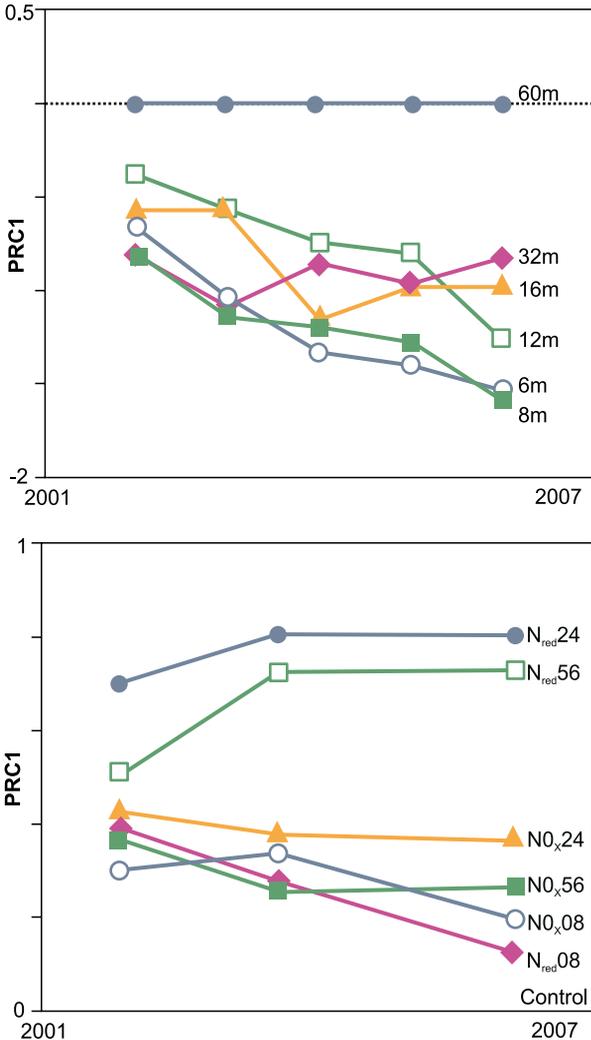

Figure 5.26: Principal Response Curves (PRC) based on redundancy analysis (RDA) of the vegetation cover data from dry and wet N deposition treatments at Whim Moss. (a) PRC for dry N treatment vegetation. (b) PRC for wet N treatment vegetation. The curves represent the temporal trajectory of community composition for each of the experimental treatments

Species with higher values are generally those that are increasing in abundance, those with negative values are decreasing with time, and species with near zero values show no response to the treatments. The significance of the PRC can also be tested using Monte Carlo permutation tests where the significance of the first RDA axis is tested. The dry manipulation was not tested for significance due to insufficient replication, but the first axis of the wet treatment RDA was significant ($p < 0.01$) showing apparent community assemblage change over time in response to N addition (Figures 5.24a,b); i.e. there are clear developments in the deviations from control assemblages over time. All the assemblages are seen to respond in the same direction for the dry treatment, although those closer to the ammonia source are further removed from the control response. The response of the communities in relation to the wet treatments is dependant on the form of N, reduced or oxidised, and dose. It can also be seen that the species responses are similar to those discussed above and illustrated in Figure 5.25.

5.11.3 Conclusions

This paper has exposed a range of issues facing the science community interested in evaluating N effects on semi-natural systems, particularly those in which non-vascular species are pivotal to the delivery of key ecosystem services e.g. the sustainability of our peatlands and their ability to sequester and store carbon. In particular it is recognized that:

* All species are not equal with regard to ecosystem function. In peatlands, indicators of N deposition in ombrotrophic ecosystems must account for non- vascular plants.
* Species richness and/or diversity suffer from the loss of species identity (not clear what you mean by that) and thus attach no value to species role within the ecosystem, ultimately failing to convey the damaging effects of N impacts. They may also be inappropriate measures in species poor ecosystems.
* Ellenberg indicators are not direct N indicators and in peatlands can be insufficiently sensitive to pick up real change, especially in ecosystems which are generally less favourable for vascular plant growth, i.e. are acid and often waterlogged.
* Assessment of community-level N effects can be aided with the use of multivariate techniques such as PRC, which have the ability to assess both community assemblage change and species level information.
* Spatial scale is important when using national classification systems, such as the NVC; in the present study the NVC was not an appropriate tool, as significant species changes occurred, without altering the NVC category.
* Basing assessments of N deposition effects on species composition has the benefit of revealing the important consequences, i.e. it provides evidence to support N pollutant emission regulation.
* Until we can identify the plant/ecosystem equivalent of the 'canary', for conservation purposes, we need to focus the search for 'early warning indicators', on chemical indicators either within the plants themselves or in the soil.
* Research must be directed at identifying threshold values for plant chemical or physiological traits that respond to reactive N, indicating the likelihood of detrimental effects leading to loss of cover / species of conservation value (Sutton *et al*., 2005).

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