Understanding of nitrogen deposition impacts: Topic 3

New science on the effects of N deposition and concentrations on Natura 2000 sites, including bio-indicators, effects of N-form (e.g., NHx vs NOy), and the relationships between critical thresholds and biodiversity loss

Background Document for the 'Nitrogen Deposition and Natura 2000: Science & practice in determining environmental impacts' Workshop at the Bedford Hotel and Conference Centre, Brussels, 18th – 20th May, 2009

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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 on important ecosystem services (such as carbon sequestration) also exists, but the cause-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 biochemical parameters may be a 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 of 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 of this document 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. These 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.

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. 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:

1. How does N deposition affect habitat structure and function of different habitat types and how can significant effects be meaningfully assessed?
2. How does current scientific understanding map onto Annex 1 habitats?
3. Is the chemical form of N deposition (reduced N versus oxidized N) or type of deposition (wet versus dry) important?
4. Strengths and limitations of the critical load/level approaches; can the relationships between concentration/dose, thresholds and biodiversity loss be improved?
5. How can significant effects be meaningfully assessed, what potential is there for the use of biomonitors?
6. How reversible are N deposition effects?
7. What is the potential for the use of on-site management practices for maintaining favourable status?
8. What is the extent of interaction between nitrogen deposition and climate change effects?

2. Effects of N deposition on habitat structure and function of different habitat types

2.1 Nitrogen induced biodiversity loss for European ecosystems

In a recent review article Bobbink et al. (2009) assessed 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 described by Bobbink et al. (2009) for 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.

2.2 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 & Rydin 2000). In addition, increased N input may make *Sphagnum* shoots more easily decomposable (Limpens & 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 & Freeman 2007). N 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 & 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.

3. Nitrogen deposition interferes with ecosystem biogeochemistry

3.1 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 emssions 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 ($R\text{-}NH_2$). 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 nitrogen 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 & 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 & 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 & 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.

3.2 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_4Cl) and nitrate ($NaNO_3$), using high application frequencies coupled to meteorology and low ionic concentrations (maximum 4mM) at a range of N doses (+ 8 kg N ha^{-1} y^{-1} to 56 kg N ha^{-1} y^{-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}y^{-1} when accumulation became exponential. In *S. capillifolium* the resulting high concentrations of soluble toxic ammonium at 56 kg N ha^{-1}y^{-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.

4. Bioindicators 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 bioindicators 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. 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, Morecroft et al. 2008). There is a need to have a clear remit for N bioindicators, whether they are to indicate N effects already brought about by N, or provide an early warning of potential effects. In the following, three potential bioindicators are presented.

1. N indices for plant species have been suggested as one bioindicator 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 & 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.

2. Another bioindicator that may be useful for assessing effects of N deposition on vegetation is measuring amino acid concentrations of plant tissues (Näsholm et al. 1994, 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). However, in perennial vascular species amino acids are also used for seasonal N storage (supporting rapid spring growth at the time of year when soil N

supply is not sufficient to meet plant N demand) (Ohlson et al. 1995, Nordin & Näsholm 1997). Thus it appears difficult to interpret whether amino acid accumulation in a plant occurs in response to excessive N uptake, or just in response to the seasonal N storage cycle.

3. Total tissue N% 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% was large, as happened when the N deposition was in the form of ammonia. Obviously, by only measuring plant tissue N% it is difficult to evaluate the size of the signal and scale of threat N deposition poses to an ecosystem. Although a large literature exists on the effects of N on plant tissue N% 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.

4. 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 nitrogen deposition, and while lichens may give an indication of the latter, more work is needed to distinguish between the ammonia and overall nitrogen 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 (Wright et al. 2000). 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).

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 & 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 9 years after cessation of N addition (c. 100 kg N ha^{-1} yr^{-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} y^{-1} above a background deposition of 4 kg N ha^{-1} y^{-1}) reduce species richness and alter relative abundances of plant species (Clark & 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 & 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 & Heijmans 2008). Similarly, in a reciprocal transplant experiment, Mitchell et al. (2004) were able to show recovery of tissue nitrogen 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 feedbacks on nutrient turnover-rates (Bowman & 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).

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 & 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 nitrogen 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 nitrogen deposition and enhanced nitrogen concentration on Natura 2000 habitats. For example, the loss of epiphytic flora would be very difficult to deal with by on site management practices.

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. (2009) 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.
- Plant biochemical parameters may be a useful bioindicators for assessment of N deposition effects in Natura 2000 sites. However "baseline" data are mostly not available for rare species. Also we need to identify more ecosystem specific indicators that are predictive of further damage, rather than a consequence of already existing adverse effects (i.e., early warning indicators).
- 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.
- More knowledge is needed to better understand where and if management intervention is appropriate to mitigate N effects.

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