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How to cite:

Stevens, Carly J.; Manning, Pete; Van den Berg, Leon J. L.; De Graaf, Maaike C. C.; Wamelink, G. W.; Weiger; Boxman, Andries W.; Bleeker, Albert; Vergeer, Philippine; Arroniz-Crespo, Maria; Limpens, Juul; Lamers, Leon P. M.; Bobbink, Roland and Dorland, Edu (2011). Ecosystem responses to reduced and oxidised nitrogen inputs in European terrestrial habitats. Environmental Pollution, 159(3) pp. 665–676.

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Ecosystem responses to reduced and oxidised nitrogen inputs in European terrestrial habitats

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While it is well established that ecosystems display strong responses to elevated nitrogen deposition, the importance of the ratio between the dominant forms of deposited nitrogen (NH$_x$ and NO$_y$) in determining ecosystem response is poorly understood. As large changes in the ratio of oxidised and reduced nitrogen inputs are occurring, this oversight requires attention. One reason for this knowledge gap is that the NH$_x$:NO$_y$ ratio which plants are exposed to differs to that in deposition as inputs are modified by soil transformations, mediated by soil pH. Consequently species of
neutral and alkaline habitats are less likely to encounter high $\text{NH}_4^+$ concentrations than species from acid soils. We suggest that the response of vascular plant species to changing ratios of $\text{NH}_x$:$\text{NO}_y$ deposits will be driven primarily by a combination of soil pH and nitrification rates. Testing this hypothesis requires a combination of experimental and survey work in a range of systems.

**Capsule:** Changing ratios of $\text{NH}_x$ and $\text{NO}_y$ in deposition have important consequences for ecosystem function.

**Keywords:** Ammonium toxicity, atmospheric nitrogen deposition, $\text{NH}_x$:$\text{NO}_y$ ratio, mitigation, nitrogen cycling, nitrification, plant communities, soil acidification.
Nitrogen (N) is essential for life as a component of amino acids, proteins, and DNA. Much N is present in the Earth’s atmosphere, but almost all is in the unreactive form ($N_2$, gaseous N). The reactive forms of N utilised by organisms (Nr) naturally enter ecosystems via biological N fixation (by legumes, cyanolichens or free-living bacteria), biomass burning, volcanic activity and lightning but human activities over the last century have more than doubled the inputs of Nr into the World’s ecosystems. The result has been widespread changes to the global N cycle (e.g. Galloway et al., 2008). Most of this rise in atmospherically deposited N has been in the form of wet deposition, N which enters ecosystems via precipitation, and dry deposition, which is the direct input of atmospheric N gasses and aerosols by wind and gravity. These inputs take two main forms, reduced ($NH_x$ – ammonia and ammonium) and oxidised ($NO_y$ – nitrogen oxides, nitric acid and particulate nitrate) both of which can be deposited as wet or dry deposition depending on climate. For example, wet areas such as Scandinavia will be dominated by wet deposition whereas dry areas such as the Mediterranean will be dominated by dry deposition. In Europe and parts of North America, the deposition of $NH_x$ and $NO_y$ strongly increased in the second half of the 20th century, primarily due to increasing agricultural and industrial activities (Fowler et al., 2004; Fenn et al., 2003). Since the 1990s N deposition has also started increasing in eastern Asia and India and it is expected that this dramatic rise will continue in the coming decades (Galloway et al., 2008; Dentener et al., 2006). Increased deposition of N can increase net primary productivity in some terrestrial ecosystems (LeBauer and Treseder, 2008) but it has also been demonstrated in both
experimental (e.g. Mountford et al., 1993; Wilson et al., 1995; Bobbink et al., 1998) and observational studies (e.g. Stevens et al., 2004; Maskell et al., 2010; Dupré et al., 2010) that increasing N deposition reduces plant species richness, with lichens, bryophytes, low growing forbs and legumes being particularly sensitive (Bobbink & Lamers 2002; Suding et al., 2005). The mechanisms underlying these changes are yet to be fully understood and, while light exclusion has been demonstrated to be the cause of N induced species losses in mesotrophic grasslands (Hautier et al. 2009), N inputs may also decrease diversity through factors such as nutrient imbalance, soil acidification, ammonium toxicity, and increased susceptibility to pests and frost (see Bobbink et al., 1998; 2010 and references herein).

There have been previous calls that both the amount (load and dose) and form of N inputs need to be considered when considering N deposition effects on ecosystems (e.g. Bobbink & Roelofs 1995; Bobbink et al., 2003) but in almost all ecological studies of N deposition reduced and oxidised N are considered equivalent, with the total amount of N deposition assumed to determine ecosystem response (e.g. Stevens et al., 2004; Manning et al., 2006). Very few studies have investigated the impact of changing ratios of NH$_4$ and NO$_3$ inputs although a number of studies have investigated the differential effects of reduced and oxidised N on vegetation (e.g. Sanchez-Hoyos and Manrique, 1995; Pearce et al., 2003; Paulissen et al., 2004; van den Berg et al., 2008; Hogan et al., 2010) with some studies showing different impacts for different N forms. In this paper we argue that the form of N deposition inputs have important consequences for ecosystem response to N enrichment, and that viewing N deposition loads as total inorganic N input may be insufficient to understand ecosystem responses to N deposition.
Firstly, we explore the spatial and temporal pattern of NH$_x$ and NO$_y$ inputs (section 1), the effects of changing NH$_x$ and NO$_y$ inputs and NH$_x$:NO$_y$ ratios on soil processes and biogeochemical cycling, and discuss how these changes influence the NH$_x$:NO$_y$ ratio which plants are exposed to in European terrestrial habitats (section 2). We synthesise findings on plant species differences in their preference and tolerance of these two N forms (section 3), identify situations where NH$_x$:NO$_y$ ratio needs to be considered when explaining community-level responses to N deposition, and make predictions regarding these community responses (section 4). Finally, we discuss the science required to confirm these predictions and the implications of changing NH$_x$:NO$_y$ ratios for biodiversity conservation and habitat management (section 5).

This review will focus on the impacts of the changing inputs and ratios of N deposition in European terrestrial habitats because they have been most intensively studied. While we do not expect the mechanistic basis to change, translating our hypotheses to areas of the world not covered by our data (e.g. South America, East Asia and East Africa) must be treated with caution.

1. The cause of differences in N input ratios

There are considerable differences in the temporal and spatial patterns of NH$_x$ and NO$_y$ emissions (Holland et al., 2005), and there is evidence that ratios of reduced to oxidised N deposition are changing at regional scales as a result of land use, technology and policy changes.
Nitrogen oxides (NO\textsubscript{y}) are emitted mainly from stationary combustion sources (industrial processes and power plants) and transport activities (road and ship traffic), while reduced N (NH\textsubscript{x}) emissions are mostly a result of agricultural activities such as animal husbandry (livestock wastes) and fertiliser application (e.g. Asman et al., 1998; Holland et al., 2005). Because of the differences in sources and their distribution, atmospheric transformations and deposition velocities, clear regional N deposition patterns have been observed. In general, total N deposition is spatially highly variable, with the highest deposition rates often dominated by dry deposition of NH\textsubscript{3} close to its source, and declining rapidly with distance (see Stevens and Tilman (2010) for an example). In contrast, wet deposition of both NH\textsubscript{x} and NO\textsubscript{y} and dry deposition of NO\textsubscript{y} display less spatial variability, as a result of the diffuse and point source origins of these two forms of pollutant. The result of this variability is spatial heterogeneity in the pattern of NO\textsubscript{y} and NH\textsubscript{x} deposition at a local scale (Sutton et al., 2008).

This local variability is nested within large scale patterns of N deposition. In western-Europe, the highest values were observed in the 1980s and 1990s, with hot spots of NH\textsubscript{x} deposition (>70% of total deposition) in intensively farmed regions such as Netherlands, Belgium, Denmark, north-west Germany and Italy’s Po Valley. Contrary to this, NO\textsubscript{y} deposition peaked in the 1980s in parts of Eastern Europe (Van Leeuwen et al. 1996). Over Europe as a whole NO\textsubscript{y} deposition decreased between 1990 and 2005 as a result of a change from coal burning to gas or nuclear power, while NH\textsubscript{x} deposition stabilised after 1995, resulting in an increased NH\textsubscript{x}:NO\textsubscript{y} ratio (Fagerli and Aas 2008). Significant differences in the input of NO\textsubscript{y} and NH\textsubscript{x} are even seen at the continental scale. In the 1990s the NH\textsubscript{x}:NO\textsubscript{y} ratio for the USA was 0.64, in contrast to
1.02 in Europe and 2.48 in Asia as a result of different energy production, farming and waste management practices (Galloway et al., 2004; Fig. 1).

Galloway et al. (2004) predict that by 2050 terrestrial NO\textsubscript{y} deposits will have increased by up to 70% from levels in the 1990s, while NH\textsubscript{x} deposits will increase by 133% over the same period primarily due to increased demand for food. This trend towards increased NH\textsubscript{x} deposits relative to those of NO\textsubscript{y} is already being observed in Europe (Fagerli and Aas 2008). However, in the Netherlands NH\textsubscript{x} deposition has decreased considerably through legislation, which significantly reduced NH\textsubscript{x} emissions. Detailed measurements from a Dutch pine forest have shown that NH\textsubscript{4}\textsuperscript{+} deposition in throughfall decreased by 40% since 1984, while NO\textsubscript{3}\textsuperscript{-} deposition increased, resulting in significant decrease in throughfall NH\textsubscript{4}\textsuperscript{+}:NO\textsubscript{3}\textsuperscript{-} ratio. At the same time, the NH\textsubscript{4}\textsuperscript{+}: NO\textsubscript{3}\textsuperscript{-} ratio in the mineral soil solution at 10 cm depth also decreased (Fig. 2; Boxman et al, 2008), indicating that changes in the ratio of deposits may be experienced by plant roots.

2. Factors controlling plant responses

After deposition, N is affected by a number of factors, influencing the ratio of reduced and oxidised N that plants are exposed to (Figure 3). Where plants take up N via their leaves, the NH\textsubscript{4}:NO\textsubscript{3} ratio they experience will often be similar to atmospheric N deposits. In contrast, N deposits to the soil may be strongly modified by chemical and biological transformations in the heterogeneous soil environment, and so soil NH\textsubscript{4}:NO\textsubscript{3} ratios may be very different to those of N deposits (Figure 3).
2.1. Stratification by vegetation

Spatial patterns of reduced and oxidised N deposition, and the relative abundances of these two forms, will be modified at local scales by vegetation structure (e.g. Fangmeier et al., 1994) and soil conditions. For example, dry deposition of N onto vegetation with a rough canopy structure, e.g. coniferous forest, is considerably higher (>50%) than that onto smooth surfaces, e.g. a calcareous grassland. Moreover, the roughness of the vegetation may also influence the NH$_4$:<NO$_3$> ratio of deposited N. In coniferous and deciduous forests NH$_4$::<NO$_3$> ratios in open field precipitation and throughfall are approximately of the same order of magnitude (Berger et al., 2008). In (grass-dominated) heathlands there are indications that the NH$_4$::<NO$_3$> ratio in throughfall is much lower than that of open field precipitation. The latter is solely due to lower NH$_4$ concentrations, with NO$_3$ concentrations remaining unaltered, indicating preferential foliar uptake of NH$_4$ (De Schrijver et al., 2008). Modification of NH$_4$::<NO$_3$> ratios also occurs in some moss-dominated ecosystems, where the preferential uptake and sequestration of NH$_4^+$ in the moss carpet (Wiedermann et al., 2009) regulates the soil NH$_4$:<NO$_3$> ratio that is experienced in the rhizosphere of vascular plants (Lamers et al., 2000; Limpens et al., 2003).

2.2 Soil processes affecting NH$_4$::<NO$_3$> ratio

In the soil, gaseous forms of NH$_4$ and NO$_3$ dissolve readily to form NH$_4^+$ and NO$_3^-$.

Typically most NO$_3^-$ is either taken up by plants and microbes, with a small portion denitrified. Unlike NO$_3^-$, NH$_4^+$ can bind strongly to soil cation exchange sites,
resulting in soil NH$_4^+$ accumulation (Fig. 3). In the soil solution, however, plant roots will experience changes in the NH$_3$:NO$_3^-$ ratio that will influence plant and soil properties, including transformations of N within the soil. All of these effects will be modified by the existing properties of the soil (derived primarily from the type of bedrock), such as its buffer capacity, pH, redox potential, microbial community composition, and climate. However, due to complex interactions between these soil properties it may prove difficult to predict general effects of changing NH$_3$:NO$_3^-$ ratios on soil N availability.

While the effect of NH$_3$:NO$_3^-$ ratio on decomposition and mineralisation is poorly known, both direct effects on the activity and abundance of dominant decomposer groups and indirect effects through litter quality are to be expected. For example, increased N input most often stimulates the initial stages of litter decomposition, in which easily degradable materials are consumed by microbes, but suppresses later decomposition stages, in which recalcitrant C is ‘mined’ for nitrogen (Berg & Matzner, 1997; Knorr et al., 2005); however, exceptions to occur (e.g. Keeler et al., 2009 found no or negative effects on overall rates of litter and organic matter decomposition). The strength of such effects may differ depending on the form of deposited N. DeForest et al. (2004) found chronic NO$_3^-$ additions did not alter the microbial community composition but broadly suppressed all microbial groups which may also impact soil processes. Indirect effects may also occur through changes in plant litter quality (see section 3.1). The resulting changes to decomposition and N mineralisation rates may subsequently lead to differences in the amount and form of N available to plants. Initial evidence for such effects is provided by Currey et al. (2010), who found that potential mineralisation of labile carbon was higher under
NH$_4^+$ addition than under NO$_3^-$ addition in peat soils. In the same study it was also shown that consumption of the amino acid glutamic acid was higher under NH$_4^+$ than NO$_3^-$ addition.

**Nitrification & Denitrification**

The ratio of NH$_4^+$ and NO$_3^-$ in soil is strongly influenced by the activity of nitrifying, ammonia oxidizing bacteria (AOB). Nitrification itself is also affected by several soil factors including availability of NH$_4^+$ (the sole energy source for autotrophic nitrifying bacteria, Booth et al., 2005), pH, O$_2$ availability (and factors influencing O$_2$ diffusion through soil such as soil structure), temperature, soil moisture, C:N ratio (e.g. Vestgarden & Kjønaas, 2003) and DOC:NO$_3^-$ ratios (Taylor and Townsend, 2010). It is very likely that soil nitrifying activity will increase with increased NH$_4^+$ availability (in absolute or relative terms), but the magnitude of this increase may depend on soil pH and soil aeration, which are often correlated. In various ecosystems, such as grasslands and heathlands, nitrification is inhibited at low soil pH, resulting in NH$_4^+$ accumulation (e.g. Roelofs et al., 1985; Dorland et al., 2004). Since nitrification yields protons, it reduces pH and so constitutes a negative feedback, reducing nitrification. As a result high NH$_4^+$ inputs can acidify the soil and limit future nitrification. In other ecosystems such as forests, nitrification may depend less on soil pH (Booth et al., 2005) or can even be high despite low soil pH, as has been reported for some mature forests (Houdijk et al., 1993). Nitrification also depends on the presence and abundance of the initial bacterial community structure. For example, elevated NH$_3$ concentrations result in increased nitrification rates in the presence of NH$_3$-tolerant AOB, whereas nitrification will be delayed (or even
inhibited) when NH$_4^+$-sensitive AOB dominate (Prosser, 1989; Webster et al., 2005).

In summary, higher NH$_4^+$:NO$_3^-$ ratios in N deposition will only lead to increased ratios of plant-available NH$_4^+$:NO$_3^-$ ratios in soils with low nitrification rates. These soils are characterized by initially low NH$_4^+$ and low O$_2$ availability, low pH, and very low or very high soil moisture levels (Booth et al., 2005). In soils with high nitrification rates a high ratio of NH$_4$:NO$_3$ deposits is less likely to be retained in the soils. This conversion of NH$_3$ to NO$_3^-$ lessens the threat of NH$_3$ toxicity to plants (see below) but poses a greater risk of problems associated with acidification, and NO$_3^-$ pollution in groundwater and runoff.

In general, the anaerobic processes denitrification (the conversion of NO$_3^-$ to NO, N$_2$O and N$_2$) and DNRA (Dissimilatory Nitrate Reduction to Ammonium, i.e. the conversion of NO$_3^-$ into NO$_2$ and subsequently to NH$_4^+$) play a minor role in well-aerated soils (e.g. De Kleijn and van Logtestijn, 1996; Stevens et al., 1998). Consequently, plant-available NH$_4^+$:NO$_3^-$ ratios in soils of temperate European grasslands, heathland and forests will be largely unaffected by these microbial processes. In wetlands however, denitrification and DNRA may play a major role in removing excess NO$_3^-$ from the soil. Once the supply of NO$_3^-$ from either atmosphere or nitrification decreases, denitrification and DNRA rates drop (Paavolainen and Smolander, 1998; Silver et al., 2001; Sánchez-Martin et al., 2008), resulting in accumulation of NH$_4^+$ and high NH$_4^+$:NO$_3^-$ ratios. We hypothesize that in wetlands with low nitrification rates (i.e. with low soil redox potential and low availability of carbon and O$_2$), increasing NH$_4$:NO$_3$ ratios in N deposition lead to lower denitrification and DRNA activity and accumulation of NH$_4^+$. 
2.3. Interaction with other elements and acidification

Besides differential effects on microbial processes, the dominant form of inorganic soil N also affects several other soil properties affecting plant performance. Increased NH₄⁺ deposition may directly and indirectly affect the concentration of essential base cations, such as K⁺, Mg²⁺ and Ca²⁺, by replacing them on the cation exchange sites in the soil, or by depressing base cation uptake by plants, particularly at low pH (Boxman et al., 1991; Gloser and Gloser, 2000). As a result, base cations are readily leached to deeper soil layers, potentially leading to base cation depletion or trace-element shortages.

Additionally, increased NH₄ deposition may lead to soil acidification via a number of pathways including nitrification, with two H⁺ produced per mol N, exchange of NH₄⁺ with H⁺ at the soil complex, or by plant uptake of NH₄⁺ (Fig. 3), particularly in weakly buffered soils. This acidification can in turn, reduce plant available N and N-uptake by plants and their symbionts through changes in the availability of heavy metals and aluminum (e.g. Lazof et al., 1994; Ghnaya et al., 2007).

3. Plant responses

3.1 Root and foliar N uptake

Foliar (including bryophyte shoot system) N uptake (Fig. 3) can be significant in vascular plants, bryophytes and lichens, particularly in N limited systems (e.g. Pérez-Soba & Van der Eerden, 1993; Cape et al., 2009) and during periods of rapid growth.
when stomatal conductance is high (e.g. Wilson, 1992; Teklemariam & Sparks, 2004).

Although many lichens capture both nitrate and ammonium equally under ambient concentrations (Hauck et al., 2010), in general, in vascular plants foliar uptake of NH₄⁺ exceeds that of NO₃⁻ (e.g. Garten & Hanson, 1990; Wilson, 1992; Lumme, 1994; Palmqvist and Dahlman; 2006). In an N-limited spruce forest, foliar N uptake was ~90% for NH₄⁺ and ~75% for NO₃⁻ (e.g. Sievering et al., 2007). Foliar NH₄⁺ uptake is usually accompanied by an increase in base cation concentration in stem flow and throughput, implying the loss of base cations from the foliage and potential risk of deficiencies of ions such as K⁺, Mg²⁺, Ca²⁺ and Mn²⁺ (Roelofs et al., 1987; Garten & Hanson, 1990; Bobbink et al., 1992; De Schrijver et al., 2008). In addition, foliar NH₄⁺ uptake affects cell pH regulation as ions (e.g. H⁺) are exchanged with the cell apoplasts, potentially resulting in cell acidification and problems relating to cell elongation and growth (Peuke et al., 1998; Paulissen et al., 2004; Van den Berg et al., 2005). In contrast, uptake and assimilation of reduced N by roots is usually accompanied by H⁺ exchange with the rhizosphere (Fig. 3) preventing cell acidification, unless pH is low. Under such conditions, root uptake of NH₄⁺ may lead to cell acidification in non-adapted plant species, as these plants are not able to exude H⁺ to the acid rhizosphere (Lucassen et al., 2002, Van den Berg et al., 2005). In contrast, uptake of NO₃⁻ by either shoots or roots has no consequences for cell pH.

The rate and form of inorganic N uptake depends heavily upon both plant species and soil properties. Plants adapted to soils with low nitrification rates e.g. many slow growing and perennial groups including Ericaceae and conifers, tend to prefer NH₄⁺ as their main N source. They can tolerate high NH₄⁺ concentrations and often possess a reduced capacity to utilise NO₃⁻ (Forde and Clarkson, 1999; Britto and Kronsucker,
A lack of nitrate reductase synthesis may be one physiological explanation for poor NO$_3^-$ utilisation (Koyama & Tokuchi, 2003). In contrast, species of less acidic or calcareous, open and disturbed soils (e.g. after fire (Cruz et al., 2008)), which tend to have faster nitrification rates (e.g. fast growing annuals including agricultural crops and pioneer trees), generally prefer NO$_3^-$ or a mixture of both NH$_4^+$ and NO$_3^-$ (e.g. Pearson and Stewart, 1993; Falkengren-Grerup, 1995; Pearson and Soares, 1995).

As an illustration we use data detailing the presence or absence of 98 plant species in 520 vegetation relevés in which soil NH$_4^+$:NO$_3^-$ ratio was also measured to determine the relationship between species abundance and soil NH$_4^+$:NO$_3^-$ ratio (see Wamelink et al. (2005) for details of survey methods). Species associated with high NH$_4^+$:NO$_3^-$ ratios or independent in their response include some trees (e.g. Quercus robur), (dwarf) shrubs and generalist species like Molinia caerulea (Fig. 4a,b). Species associated with low NH$_4^+$:NO$_3^-$ ratios include many species of (wet) grasslands, like Ranunculus repens (Fig. 4c). Other variables such as soil moisture have not been taken into account in these analyses so co-variation cannot be ruled out.

Although plants may have clear preferences in terms of N form, uptake rates do not necessarily reflect this. In the presence of both inorganic N forms, NH$_4^+$ is more readily taken up by most plants, because its reduced state makes its uptake less energetically costly than that of NO$_3^-$ (Bloom et al., 1992). Even in plants that can suffer from NH$_4^+$ toxicity, NH$_4^+$ uptake often exceeds that of NO$_3^-$ (De Graaf et al., 1998).
The form of N taken up or experienced by a plant may affect its health and performance. There is considerable evidence that sole NH$_4^+$-N nutrition and high NH$_4^+$:NO$_3^-$ ratios are toxic to plants (e.g. Dijk and Eck, 1995, De Graaf et al., 1998, Van den Berg et al., 2005). NH$_4^+$ toxicity is manifested by leaf chlorosis, stunted root growth, increased shoot : root ratio, decreased fine : coarse root ratio, growth retardation and, in extreme cases, plant death (Britto & Kronzucker 2002, Van den Berg et al., 2005, Boxman et al., 1991). Several non-exclusive mechanisms have been postulated as the cause of this toxicity. These are: 1) NH$_4^+$ uptake reduces the uptake of important cations such as Ca$^{2+}$, Mg$^{2+}$ and K$^+$ (Marschner, 1995); 2) NH$_4^+$ is metabolised immediately to N-rich amides and amino acids within plant cells (Raven and Smith, 1976), diverting resources away from plant growth (Schortemeyer et al., 1997); 3) NH$_4^+$ uptake and assimilation imposes homeostatic energetic costs on the plant to maintain cytosol pH, potentially retarding growth (Bligny et al., 1997, Gerendas and Ratcliffe, 2000).

3.2 Variation in plant tolerance to NH$_x$

Regardless of the mechanism by which NH$_x$ impairs plant performance, it is clear that plant species differ strongly in their tolerance of high NH$_x$ concentrations. For species of originally weakly acid soils (4.5 < pH < 6.5) NH$_4^+$ toxicity occurs at external concentrations of 100 µmol l$^{-1}$ or less (Dijk & Eck, 1995, De Graaf et al., 1998, Van den Berg et al., 2005), but in species from acid soils it is only manifested at much higher concentrations (Boxman et al., 1991; De Graaf et al., 1998). Underlying these patterns are physiological differences between species. Many plants, including bryophytes, respond to NH$_4^+$ uptake by synthesising specific amino acids and amides,
particularly amino acids with a low C:N ratio (e.g. Lucassen et al. 2002; Limpens & Berendse, 2003), and this may help to prevent NH$_4^+$ toxicity. Bryophyte and herb species have shown increases in the fraction of N-rich free amino acids when grown at increased N supply, with NH$_4^+$ as the dominant N form, in both greenhouse and field experiments (Fig. 5).

Differences in the sensitivity of plant species to reductions in their cytoplasmic pH (acidosis) and in the capacity of species to buffer their cytoplasm, may also explain species-specific responses to NH$_4$ deposition (Pearson & Stewart, 1993). For appropriate cell functioning, the pH of the cytosol should be maintained within an appropriate range. Plants which predominantly take up and assimilate NO$_3^-$ generate bases and thereby buffering capacity, either through OH$^-$ release or by base cation uptake. This reduces their sensitivity to cytoplasmic acidosis. In contrast, with NH$_4^-$ uptake plants generate acidity instead of OH$^-$. Though it has been shown that NH$_4^+$ nutrition does not always lead to decreased cytosol pH (Bligny et al. 1997, Gerendas and Radcliffe 2000), maintaining optimal cytosol pH is an energy demanding process. This energy is not available for plant maintenance or growth and may therefore, at least partly explain the retarded growth of many species grown on NH$_4^+$ nutrition (Britto & Kronzucker, 2002).

Light may also play a role in modifying species responses to NH$_4$ as its toxicity is less pronounced at low light intensity (Britto & Kronzucker, 2002). This may mean that understory species are less likely to be sensitive to increases in the ratio of NH$_4$:NO$_3$ inputs but more research is needed to test this. Plants susceptible to NH$_4^+$ toxicity might be afflicted by reduced rates of net photosynthesis, and thus, are in lower need
of N. However, understory plants are most likely well adapted to these low-light conditions, with ‘normal’ photosynthesis rates in shaded conditions.

In addition to these effects on mature plants NH₄⁺:NO₃⁻ ratio may also influence germination and early seedling establishment. Both N forms are known to break seed dormancy but germination effects will be species specific as not all species are stimulated, some only respond to NO₃⁻ or NH₄⁺, and responses may be both positive and negative (e.g. Singh and Amritphale, 1992, Dorland et al., 2003, Luna and Moreno, 2009).

Plant species sensitivity to NH₄⁺:NO₃⁻ ratio may also depend upon the preferences and sensitivities of their associated symbionts. A meta-analysis of N addition effects on mycorrhizal fungi found negative effects of N addition but no significant impact of N form (Treseder, 2004). However, some individual studies have found an impact of N form, for example, Yoshida and Allen, 2001 found a large mycorrhizal response to ammonium addition but not nitrate addition in costal sage scrub species in California. Ectomyorrhizal plants appear to prefer NH₄⁺ (e.g. Cumming 1990), whereas arbuscular mycorrhizal (AM) plants seem to prefer NO₃⁻ (e.g. Cuenca & Azcon, 1994; Persson et al., 2003) although this is not always the case (e.g. Yoshida and Allen, 2001). These preferences are consistent with the natural patterns of N availability and plant uptake, and mycorrhizal distribution (Nordin et al., 2001). This implies that AM plant species may decline when the NH₄⁺:NOx ratio in the soil is high or increasing.

4. Community and ecosystem responses to changing NH₄⁺:NO₃⁻
Despite strong evidence that NH\textsubscript{4}\textsuperscript{+}:NO\textsubscript{3} ratio affects plant species differentially with respect to germination, growth and survival it is still not known how important the form of N is relative to the amount of N being deposited in determining community responses to N enrichment. Where ratio effects are important the mechanism is likely to differ depending on soil NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3} concentration as determined by the factors and processes explained above, and by their differential effects (toxicity, growth etc) on species performance.

Based on the preceding review we hypothesise that there is a gradient of NH\textsubscript{4}\textsuperscript{+}: NO\textsubscript{3} ratio in the soil, driven primarily by soil pH and subsequently nitrification rates, with plant species life history strategies evolving to specialise at different points on this gradient and thus displaying a range of inherent sensitivities to NH\textsubscript{4}\textsuperscript{+} (Fig. 6). We also predict that the relationship between NH\textsubscript{4}\textsuperscript{+}: NO\textsubscript{3} ratios in the soil and plant species is general, with these patterns being found in a wide range of habitats. Such a natural gradient generates context dependency in the effects of NH\textsubscript{4}:NO\textsubscript{3} deposits and makes the prediction of responses to changing NH\textsubscript{4}:NO\textsubscript{3} input ratios challenging.

In acid environments (pH < 4.5) where nitrification rates are frequently low, N will accumulate as NH\textsubscript{4}\textsuperscript{+}, potentially reaching toxic concentrations. In such cases species differences in sensitivity to high NH\textsubscript{4}\textsuperscript{+} concentration will determine community response (Fig. 6).

Acidification processes related to high NH\textsubscript{4}\textsuperscript{+} concentrations result in the mobilisation of metals and an increase in metal concentrations such as aluminium (Al\textsuperscript{3+}) and iron (Fe\textsuperscript{3+}) (Stevens et al., 2009). In addition base cation (Ca\textsuperscript{2+}, Mg\textsuperscript{2+}) concentrations
Tolerance to free metal concentrations may also be important in determining community response (e.g. De Graaf et al., 1994). While some species found at these sites may tolerate these increases in NH$_4^+$ and metal ion concentrations, species growth rates and thresholds for tolerance may differ within a community (Kleijn et al., 2008). For any given community it is likely to be those species with the lowest tolerance to NH$_4^+$ and metals and strongest preference for NO$_3^-$ that will decline when the ratio of NH$_4$:NO$_3$ inputs increases. Evidence for this has been found in dry heaths (pH 3.8-4.9), where soil NH$_4^+$ concentrations were strongly negatively correlated with the number of plant species and a better predictor for plant diversity in this ecosystem than pH or Al$^{3+}$ (De Graaf et al., 2009).

As the initial soil pH increases and nitrification rates increase a greater proportion of NH$_4$ deposition inputs will be converted into the non-toxic NO$_3^-$ form. We predict that in soil of intermediate pH (4.5-6.0) nitrification rates will be sufficient to convert a large proportion of the deposited NH$_4$ into NO$_3$. However, if associated pH decline is large, and soil pH approaches 4.5, then deposition of NH$_4$ may cause species loss via Al$^{3+}$ or H$^+$ toxicity, and shift conditions towards low pH conditions in which NH$_4^+$ accumulates (Fig. 6). Species that are adapted to very acid conditions will not suffer from NH$_4^+$ toxicity. For them, the enhanced N-availability will lead to increased productivity and enhanced competitive ability. In wet heathlands this has resulted in vegetation changes from dwarf shrub dominated heaths to domination of grasses such as Molinia caerulea and Deschampsia flexuosa. We therefore hypothesize that in soil of intermediate pH species typically found on soils with pH 4.5-6 will decrease in abundance with increasing NH$_4$:NO$_3$ inputs and that species typical of soils of pH <4.5, or insensitive species e.g. M. caerulea, will increase in abundance. In heathlands
and grasslands on weakly buffered soils, it has been shown that soil acidification was the main factor in explaining plant species richness declines, thereby supporting the current hypothesis (De Graaf et al. 2009). In summary, we predict that in soils of low and intermediate pH the species with the highest pH and lowest NH$_4^+$:NO$_3^-$ optima are most at risk if the system experiences an increase in NH$_4$:NO$_3$ input ratios.

In more neutral soils (pH 6.0-7.5) an increase in the NH$_4$:NO$_3$ ratio of inputs will be mitigated by rapid nitrification of NH$_4^+$ to NO$_3^-$, thereby preventing ammonium toxicity from occurring. The high initial soil pH will also mean that the soils are initially well buffered against the effects of acidification. In these soils with a high NH$_4^+$ turnover, N deposition is more likely to have a fertilising effect and it may be the degree of N limitation and the total amount of N deposited, rather than its form that is important to plant community dynamics. In such cases community response to N is most likely driven by increases in plant productivity (Clark et al., 2007), with tall, rapidly growing and canopy forming species casting a shade which excludes smaller plants (Bobbink, 1991; Manning et al., 2009; Hautier et al., 2009) and legumes, which lose their N fixing advantage and are outcompeted at high N availability (Suding et al., 2005).

Further research is required to confirm the relationship we suggest between soil NH$_4^+$:NO$_3^-$ ratios and plant species composition. A number of modifying factors need to be assessed including the habitat specific threshold of soil conditions above which high nitrification rate renders the form of N inputs unimportant, and the degree of generality in the relationship between pH and nitrification rates should be evaluated. The potential for acidification is also likely to vary between sites depending on their
soil buffering system. We therefore need to identify the positions on natural environmental gradients above which plant community responses to N addition are no longer predominantly determined by NH₃ sensitivity mechanisms, but by acidification and light or other nutrient competition. Although this hypothesis is community dependent in its details, and there are likely to be exceptions to the hypothesized trends, its principles are general and so it could be tested with a combination of experimental and survey work across a range of systems, adopting an approach similar to that of Kleijn et al. (2008).

5. Assessment and management of the ecosystem impacts of changing NH₄⁺:NO₃⁻ ratios

While the synthesis presented here suggests an important role of N form in determining ecosystem responses to N deposition there is great difficulty in assessing these effects. One such hurdle is that the largest values of N deposition are always dominated by gaseous NH₃ deposition. As a result it is difficult to distinguish the effects of high NH₄⁺:NO₃⁻ ratio and high total N load. The observational data of Stevens et al. (2004) and Kleijn et al. (2008) for instance, show correlations (Pearson’s r) of 0.42 and 0.35 (P <0.001, 0.07) between total N load and NH₄⁺:NO₃⁻ ratio respectively (data taken from species level means from Table 1 of Kleijn et al. 2008). The correlation between NH₄⁺ deposition and total N deposition is high (r =0.98 and 1.00; P <0.001 in both cases), making it difficult to distinguish whether fertilization, acidification or NH₄⁺ toxicity, or a combination of these mechanisms, may be responsible for compositional change of ecosystems. Their relative importance can only be found by acquiring data from ecosystems in which the NH₄⁺ N deposition
load and NH₄:NOₓ ratio correlations are absent, in combination with experimental work which removes this correlation (e.g. van den Berg 2008). Experimental work in mesocosms or in low N input regions may overcome this problem but measurements must distinguish between the acidifying and toxic effects of NH₄⁺. Here, plant community data may be combined with physiological measures of plant health, e.g. amino acid concentrations, pigment content and chlorophyll fluorescence, that may indicate which stresses are the causes of species loss.

Using conservation management to mitigate N deposition impacts is challenging. Many of the available techniques for removing N also involve the removal of carbon and other nutrients, thus potentially altering a range of ecosystem functions. As a result the only truly effective way of managing for N deposition is the reduction of N inputs to the ecosystem by the reduction of N emissions. Critical loads for N deposition are currently based on total reactive N deposition but devising habitat specific critical loads for the components of deposition and NH₄:NOₓ ratio could be beneficial in some ecosystems. This still leaves the problem of potentially high amounts of NH₄⁺ accumulated in the soil and slow recovery. Suggestions for deposition management on a local scale have been made, such as the use of tree shelter belts to capture NH₃ (Theobald et al., 2001), but generally, the reduction of N deposition requires action at a national or international level. Unfortunately, the formulation and delivery of policy at these levels is a slow process and more immediate action is often required if sites of conservation importance are to be protected and restored. An understanding of the mechanisms underlying ecosystem responses to different ratios N input should allow for the development of management and mitigation options.
563 The most appropriate form of management to apply in a habitat will depend on the
564 vegetation community and its position along the pH gradient described above and in
565 Fig. 6. In low pH soils (to the left of figure 6), where NH$_4^+$ and heavy metal toxicity
566 are likely to be the key processes associated with changes in species composition
567 caused by N enrichment, management must change soil properties to restore low NH$_4$
568 concentration and reduce the NH$_4$;NO$_3$ ratio (Kleijn et al., 2008). One of the most
569 effective ways to do this is by turf cutting and liming. Turf (or sod) cutting removes
570 excess nutrients and restores damaged ecosystems. It has been used successfully in a
571 range of habitats (e.g. heathland: Dorland et al. 2005; De Graaf et al. 1998, grassland:
572 Jansen and Roelofs, 1996, fens: Beltman et al., 2001, pine forest: Boxman and
573 Roelofs, 2006), especially in the Netherlands. It may, however, decrease the
574 nitrification potential of acidified soils and lead to a rapid increase in soil NH$_4^+$
575 concentrations (Dorland et al., 2003). The addition of lime after cutting has been
576 found to successfully prevent NH$_4^+$ accumulation by increasing nitrification (Dorland
577 et al., 2004; De Graaf et al., 1998; van den Berg et al., 2003). While a powerful tool,
578 turf cutting is a dramatic and expensive form of management with a number of
579 potential problems (see Klap and Schmidt, 1995; van den Berg et al., 2003; Dorland et
580 al., 2004; Vergeer et al., 2006).
581
582 An alternative and emerging option for removing reactive N from soils is the addition
583 of carbon (C). The addition of organic C to the soil increases the C:N ratio and could
584 be expected to induce microbial communities to immobilize N. Several studies have
585 reported successful reduction of soil N availability and a reduction in nitrophilous
586 species as a result of addition of C supplements (e.g. Török et al., 2000; Eschen et al.,
While there are a limited number of C addition studies on soils that are likely to have naturally low nitrification rates, results to date suggest that carbon addition may be more effective in reducing soil NO$_3^-$ concentrations than those of NH$_4^+$ (Török et al., 2000; Blumenthal et al., 2003; Prober et al., 2005; Eschen et al., 2007). It may therefore be more suitable for systems with naturally high nitrification rates (the right of figure 6).

At slightly higher pH, moving towards the centre of figure 6, where acidification rather than the toxicity is the main driver of compositional changes resulting from N enrichment, liming is an effective management option. Liming has been widely used in many terrestrial habitats to counteract acidification from atmospheric pollutants (e.g. forests: Löfgren et al., 2009, heathland: Dorland et al. 2005a; grassland: Blake et al., 1999; bogs: Sanger et al., 1993). Increasing the pH also has the potential to increase nitrification, thus reducing the NH$_4^+$:NO$_3^-$ ratio. In areas fed by base-rich groundwater restoration of hydrology by blocking drainage ditches has been used as an alternative method to reduce acidification in combination with sod cutting (Roelofs et al., 1987). Increasing the pH has the potential to increase nitrification reducing the NH$_4^+$:NO$_3^-$ ratio.

In high pH and nitrification rate systems, where most species have a preference for NO$_3^-$ (i.e. those found at the far right of Figure 6) canopy closure and light limitation are likely to be the most important mechanisms driving species changes as a result of N deposition. Here measures can be taken to reduce competition for light. This could include an increase in cutting frequency or grazing intensity, especially in ecosystems dominated by herbaceous vegetation (e.g. Bobbink and Willems, 1988).
levels can also be reduced when these methods are combined with the removal of cut vegetation or the folding of livestock (Ausden, 2007). This method is commonly used in the restoration of grasslands on former agricultural land (e.g. Walker et al., 2003) and can change species composition, reducing the cover of productive grasses promoted by N enrichment.

Conclusion

Future changes in the ratio of NH$_y$ and NO$_x$ inputs have the potential to alter numerous soil processes, plant N uptake, community composition and ecosystem functioning. This has important implications for plant community composition as, depending on soil type and habitat, some species may be favoured by the changing ratio to the expense of others. Further research is needed to test the hypothesis that there is a natural spectrum of NH$_x$:NO$_y$ ratios in the soil, driven primarily by soil pH and buffer capacity and subsequently nitrification rates. The response of plants to NH$_x$:NO$_y$ ratios at different points on this gradient also needs to be investigated, as does its generality to a wide range of ecosystems as it is in many poorly studies regions of the world, e.g. South America, East Africa and East Asia where NH$_x$:NO$_y$ ratios are expected to show the greatest increases. If this hypothesis is supported, and found to be general across a range of ecosystems it may enable us to predict ecosystem responses to changing NH$_x$:NO$_y$ ratios, increasing the effectiveness of the management and mitigation of N deposition impacts.

Acknowledgements
We are grateful to the British Council and the Netherlands Organization for scientific research for funding the workshop at which this review was initiated. Dina Koulama and Rodolphe Bernard assisted with figure preparation.

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Figure legends

Figure 1: Ratio between modelled NH$_x$ and NO$_y$ deposits (by mass NH$_4$-N : NO$_3$-N) for 1860, 1993 and 2050. Data were taken from the modelled output of Dentener et al. 2006.

Figure 2: Ground measurements of NH$_x$:NO$_y$ ratio in throughfall, and in the mineral soil at 10 cm depth [eq/eq] in a Dutch Pine forest. Original data source: Boxman et al, 2008.

Figure 3. Processes influencing the availability and ratio of oxidised (NO$_y$) and reduced (NH$_x$) nitrogen forms which plants are exposed to. DNRA: dissimilatory nitrate reduction to ammonium, anammox: anaerobic ammonium oxidation.

Figure 4. Response curves and 95% confidence intervals (broken lines) of three plant species across a NH$_4$+:NO$_3$- ratio gradient. The curves represent species with (a) a positive association with high NH$_4$+:NO$_3$- ratios, (b) abundance independent of NH$_4$+:NO$_3$- ratio, and (c) a species associated with low NH$_4$+:NO$_3$- ratios. Open circles are those in which the probability of occurrence is calculated from ≤10 relevés, filled circles were drawn from 11-25 relevés and triangles 26-50 relevés. See Wamelink et al., (2005) for methods, soils were collected in the field, extracted using 0.01 M CaCl$_2$ solution and analysed using an autoanalyser.

Figure 5. The ratio between the concentrations of the three main N-rich free amino acids (arginine, glutamine and asparagine) and all free amino acids (g g$^{-1}$) in
bryophytes from N-poor acidic ecosystems (a *Sphagnum magellanicum*, b *Sphagnum rubellum*) and in two perennial herbs from N-poor, weakly buffered ecosystems (c *Gentiana pneumonanthe*, d *Arnica montana*) subjected to different NH$_4^+$:NO$_3^-$ ratios and N supply. Reduced or oxidised N treatments were applied to the bryophytes and plants in the greenhouse for three months (a), in the field for three years at NH$_4$:NO$_3$ ratios of 1:9 and 9:1 (b) or in a climate chamber for three months at NH$_4$:NO$_3$ ratios of 4:1, 1:1 and 1:4 (c, d). Different capital letters indicate significant effects of N load (ANOVA, p<0.05), different lower case letters indicate effects of N form (paired t-test). Data sources: a) J. Limpens (unpublished) and b-d) L.J.L. van den Berg (unpublished).

Figure 6. Hypothetical relationships between the background soil pH and the mechanisms regulating plant community composition responses to N addition. The performance of different species, as represented by r, with each hump equivalent to a species' realised niche, differs across the pH gradient and so any shift in conditions results in compositional change. In low pH systems high NH$_4$:NO$_3$ inputs move the community to the left and low NH$_4$:NO$_3$ inputs shift the community to the right. At higher pH (probably ~5.5) most NH$_4^+$ is nitrified and the form of N addition becomes less important in predicting plant community response. The hatched area represents the region of uncertainty in which high nitrification rates and buffering capacity render the form of N inputs unimportant.
Figure 2

![Graph showing the concentration of NH₄⁺ and NO₃⁻ in throughfall and soil solution at 10 cm depth over time from 1984 to 2008.](image-url)
Figure 4

(a) Molinia caerulea

(b) Quercus robur
Figure 5

- **a) S. magellanicum**
- **b) S. rubellum**
- **c) G. pneumonanthe**
- **d) A. montana**

**N deposition rate (g m\(^{-2}\) yr\(^{-1}\))**

- **NH\(_4\) only**
- **NO\(_3\) only**
- **NH\(_4\) dominant**
- **NO\(_3\) dominant**
- **NH\(_4\)NO\(_3\)**

**N-rich free aminoacids: total**

- **0.0**
- **0.3**
- **0.5**
- **0.8**
- **1.0**
- **1.3**
- **1.5**

- **a**
- **b**
- **ab**
Figure 6