



Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Evidence for differential effects of reduced and oxidised nitrogen deposition on vegetation independent of nitrogen load

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ARTICLE INFO

Article history:

Received 2 September 2015

Accepted 4 September 2015

Available online xxx

Keywords:

NH_x:NO_y ratio

N deposition

Countryside survey

Acidification

Grassland

Heathland

Bogs

ABSTRACT

Nitrogen (N) deposition impacts natural and semi-natural ecosystems globally. The responses of vegetation to N deposition may, however, differ strongly between habitats and may be mediated by the form of N. Although much attention has been focused on the impact of total N deposition, the effects of reduced and oxidised N, independent of the total N deposition, have received less attention. In this paper, we present new analyses of national monitoring data in the UK to provide an extensive evaluation of whether there are differences in the effects of reduced and oxidised N deposition across eight habitat types (acid, calcareous and mesotrophic grasslands, upland and lowland heaths, bogs and mires, base-rich mires, woodlands). We analysed data from 6860 plots in the British Countryside Survey 2007 for effects of total N deposition and N form on species richness, Ellenberg N values and grass:forb ratio. Our results provide clear evidence that N deposition affects species richness in all habitats except base-rich mires, after factoring out correlated explanatory variables (climate and sulphur deposition). In addition, the form of N in deposition appears important for the biodiversity of grasslands and woodlands but not mires and heaths. Ellenberg N increased more in relation to NH_x deposition than NO_y deposition in all but one habitat type. Relationships between species richness and N form were habitat-specific: acid and mesotrophic grasslands appear more sensitive to NH_x deposition while calcareous grasslands and woodlands appeared more responsive to NO_y deposition. These relationships are likely driven by the preferences of the component plant species for oxidised or reduced forms of N, rather than by soil acidification.

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1. Introduction

There is widespread evidence across the globe, from both experiments and field surveys, of the significant ecological impacts of nitrogen (N) deposition on semi-natural ecosystems of low nutrient status (e.g. Bobbink et al., 2010), which also carries economic costs (Jones et al., 2014). However, interpretation and quantification of these effects, and predictions of the benefits of emission control policies, need to consider the different components of N deposition

(Brink et al., 2011). There are two main chemical forms – reduced N (ammonia, NH₃ and ammonium, NH₄⁺) emitted primarily from agricultural sources, and oxidised N (nitrogen oxides, NO_x, nitric acid, HNO₃ and nitrate NO₃⁻) emitted primarily from fossil fuel combustion. In addition, N deposition may be in the form of dry deposition of gases and aerosols, which is most important close to sources, and in regions of the world with low rainfall, and as wet deposition as snow, dew, cloud or rainwater, which are important in more remote regions and in areas with high rainfall.

The mechanisms underlying the ecological effects of N deposition include direct toxicity, growth stimulation and competitive exclusion, soil acidification and increased susceptibility to other

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abiotic and biotic stresses (e.g. Bobbink et al., 1998; Roem and Berendse, 2000). There are strong reasons, which have been recently reviewed by Stevens et al. (2011), for expecting that there may be different effects of reduced and oxidised N deposition for each of these mechanisms. For example, foliar uptake of gaseous NH_3 is more likely to be directly toxic than uptake of gaseous nitrogen oxides, while soil NH_4^+ is more likely to be toxic to plant roots than soil NO_3^- (Sheppard et al., 2011, 2014). Plant species also differ strongly in their preference and tolerance for NH_4^+ or NO_3^- uptake from soil solution with species of acidic habitats generally more tolerant of higher soil ammonium (Falkengrengrerup and Lakkenborgkristensen, 1994). The soil $\text{NH}_4^+/\text{NO}_3^-$ ratio is partly a function of the ratio in atmospheric deposition, but also of the degree of nitrification in soils; high rates of nitrification result in a lower soil solution $\text{NH}_4^+/\text{NO}_3^-$ ratio, which may reduce the risk of direct NH_4^+ toxicity but may increase acidification because of the greater oxidation to NO_3^- .

Experimental studies provide some evidence of the differential effects of reduced and oxidised N deposition. For example, van den Berg et al. (2008) showed that higher $\text{NH}_4^+/\text{NO}_3^-$ ratios in deposition to heathland mesocosms had significant adverse effects on acid-sensitive species but not on acid-tolerant species that were also tolerant of high soil $\text{NH}_4^+/\text{NO}_3^-$ ratios. This effect was lost in limed mesocosms, suggesting that acidification at higher $\text{NH}_4^+/\text{NO}_3^-$ ratios was the key driving mechanism. By contrast, in Mediterranean maquis vegetation, the application of both NH_4^+ and NO_3^- increased biomass but not plant diversity, while NH_4^+ alone increased plant diversity but not biomass (Dias et al., 2014); these effects can at least partly be explained by the different responses of individual species to total N inputs or to reduced N deposition specifically.

A combination of targeted field surveys and analysis of nationwide surveillance data over the last decade have provided a strong body of evidence of the impacts of N deposition. Strong negative associations between N deposition and species richness have been reported in acid grasslands (Stevens et al., 2004, 2010a; Duprè et al., 2010; Stevens et al., 2010a), grasslands, heathlands and bogs (Maskell et al., 2010; Caporn et al., 2014; Field et al., 2014) and sand dunes (Jones et al., 2004). In acid grasslands, this negative association is linked to declines in forb species richness and a corresponding increase in graminoids (Maskell et al., 2010) with differential responses of individual forb species to N deposition (Payne et al., 2013). In acid grasslands acidification rather than eutrophication may be the main driver of change (Stevens et al., 2010b), but the relative influence of sulphur versus nitrogen as the driver of acidification has not been separated.

However, in some other habitats; for example in calcareous grasslands, gradient surveys have shown no significant association between N deposition and species richness (Maskell et al., 2010). However, high rates of N deposition have been associated in calcareous grassland plots with an increase in grass:forb ratio (Maskell et al., 2010) and a decline in species diversity and in the frequency of characteristic species (van den Berg et al., 2011). This latter study suggests that, while direct effects of N deposition were responsible for shifts in diversity, effects on herb species number reflect indirect effects of both N and S deposition on soil acidity.

These and other findings from field surveys suggest that the responses to N deposition of vegetation characteristics in different habitats may be at least partly explained by differences in the underlying mechanisms of impact of reduced and oxidised N, mediated by soil pH, with acidification effects prevailing in poorly-buffered habitats and eutrophication effects in well-buffered habitats. Few field surveys have tried to separately evaluate the strength of associations with reduced and oxidised nitrogen but were only able to do so with relatively low number of samples/sites (Caporn et al., 2014; Field et al., 2014). Three studies have showed

adverse changes in vegetation composition that were significantly correlated with reduced N deposition but not with oxidised N deposition: an increase in mean Ellenberg fertility index in semi-natural grassland and heaths/bogs between 1990 and 1998 in UK Countryside Survey data (Smart et al., 2004); a loss of species with a low Ellenberg fertility index in UK national recording data between 1987 and 1999 (McClean et al., 2011); and increases in graminoid cover and decreases in lichen cover in heathlands (Southon et al., 2013). A further study showed effects only of dry deposition of NH_x and no effect of wet reduced or oxidised N on abundance of N sensitive epiphytic lichens (Seed et al., 2013).

However, interpretation of such field surveys is difficult due to problems of spatial autocorrelation, and the confounding effects of other environmental and land use changes. The levels of reduced and oxidised N deposition are often highly correlated (areas of low reduced N usually have low oxidised N, etc); in addition, the range and spatial variability of reduced N deposition is often greater than that of oxidised N deposition, thereby increasing the probability of detecting a statistically significant association with vegetation characteristics (e.g. Smart et al., 2012). In this paper, we present new analyses of national surveillance data in the UK to provide a more rigorous evaluation of whether there are differences in the effects of reduced and oxidised N deposition that are more robust to statistical limitations. The data that are used here provide a greater sample size and spatial scope that includes almost the complete N deposition range in the UK and allows us to evaluate our mechanistic understanding of the differential effects of the two forms of N deposition in different habitats and on different groups of species. In our analysis we focus on species richness of vascular plants as a measure of biodiversity, Ellenberg N as a measure of nutrient status (Diekmann and Falkengren-Grerup, 2002) and grass:forb ratio as a measure of competitive dominance effects. We hypothesise that:

- The form of N (oxidised NO_x , or reduced NH_x) in deposition has an effect on vegetation composition that is independent of, and additional to, that of total N deposition.
- Reduced N deposition has a greater impact on vegetation composition than oxidised N deposition.

2. Methods

2.1. Vegetation data

The effect of N deposition on vegetation was assessed using vegetation data obtained from 6860 plots ($2 \times 2\text{m}$) from the UK Countryside Survey 2007 (Carey et al., 2008). For each plot, total species richness, grass:forb ratio and mean Ellenberg N values were calculated. Species richness was defined as the sum of all vascular plants in each $2 \times 2\text{m}$ plot. Grass to forb ratio was based on the cover of grass species (*Poaceae*) divided by the cover of forb species. Cover-weighted average Ellenberg N numbers (Ellenberg et al., 1991) that were modified for the UK (Hill et al., 2004) were calculated based on the cover per $2 \times 2\text{m}$ plot to obtain strong correlates of species responses to nutrient availability and succession (Vile et al., 2006).

The vegetation in each plot was classified according to the UK National Vegetation Classification (NVC) and plots were pooled into broad groups of similar habitat (see Table in supplementary material). Earlier studies have shown that soil pH or base saturation can explain species richness and can affect the responses of the vegetation and the ecosystem to N deposition (van den Berg et al., 2005; Stevens et al., 2006). Therefore, habitats that belong to a similar broad NVC classification but differ strongly in average soil pH and/or base cation content (heaths, mires and grasslands) were

subdivided according to pH for analysis. The resulting broad habitat types in this study were: bogs and mires (acidic), base rich mires and fens, upland dry heaths, lowland dry heaths, calcicolous grasslands, mesotrophic grasslands, calcifugous grasslands and woodlands. Bogs and mires comprise the NVC classes: M1 to M21 (mires) and H3, H4 and H5 (wet heaths). Base rich mires and fen habitat consist of the NVC classes M22 to M38. Upland dry heaths are NVC classes H10 to H22, lowland dry heaths are H1, H2, H6 to H9, calcicolous grasslands are CG1 to CG14, mesotrophic grasslands are MG1 to MG13, calcifugous grasslands are U1 to U21 and woodlands are W1 to W25. All sub-communities were included.

2.2. Atmospheric deposition and climatic data

Climatic factors such as precipitation and temperature were included in our models as these are known to affect species richness (Cleland et al., 2013). Average annual temperature (°C) and average annual precipitation (mm), calculated over a 5 year period 2000–2005 were obtained from UK Meteorological Office (www.metoffice.co.uk). Sulphur (S) deposition, that peaked in the UK in the 1970s can have an acidifying effect on the soil (Kirk et al., 2010) and may thereby affect species richness (McGovern et al., 2011). Historical data on S deposition was therefore included in our models to account for potential legacy of soil acidification effects due to sulphur. Modelled N deposition data for each plot were obtained from the Centre of Ecology and Hydrology (CEH) for the year 2007; data from 1987 were used for historical S deposition. Climate and pollution data were all at 5 × 5 km resolution. Total N deposition ranged from 5.1 to 54.2 kg N ha⁻¹ yr⁻¹ while S deposition ranged from 5.0 to 43.5 kg S ha⁻¹ yr⁻¹. Oxidised and reduced N were included in our models as the sum of wet and dry NO_y or NH_x deposition and expressed in kg N ha⁻¹ yr⁻¹. NO_y deposition ranged from 2.5 to 25.6 kg N ha⁻¹ yr⁻¹, NH_x deposition ranged from 2.3 to 36.1 kg N ha⁻¹ yr⁻¹. The ranges of N and S deposition for each habitat are different and depend on their geographical distribution.

2.3. Linear models

Multicollinearity is common between variables such as N deposition and the different forms of N in deposition, S deposition and climatic variables. In our dataset, NH_x and NO_y deposition were highly correlated ($r = 0.69$, $p < 0.001$) and could therefore not be analysed simultaneously. In addition, total N deposition and either NH_x or NO_y deposition were highly correlated ($r = 0.95$, $p < 0.001$ and $r = 0.89$, $p < 0.001$ respectively). Analysis to determine the effect of N form was therefore performed using linear models taking two different approaches that each overcome problems typically associated with multicollinearity and that each test specific hypotheses. Linearity of the relationship between the predictor factors and the dependent variables were tested in single linear regressions. If needed, data were transformed to meet assumptions of linearity.

In the first method (models coded with A), the effect of NH_x/NO_y ratio in deposition was tested against the effects of total N deposition. For this analysis, multiple regressions were performed with dependent variables: species richness, cover weighted Ellenberg N (hereafter Ellenberg N) and grass:forb ratio that were regressed on the explanatory variables: total N deposition, NH_x/NO_y ratio, S deposition, precipitation and temperature. All models were at first explicitly tested for spatial autocorrelation in the response variable and residuals by inspection of semi-variograms using generalized linear mixed-effect models (GLMM). In these models, a correlation structure was added to correct for spatial autocorrelation. Correlation structures such as corExp, and corSpher were used with the “form = ~Easting + Northing” argument in the correlation option to

calculate the Euclidean distances (using Pythagoras theorem) between sites with coordinates given by Easting and Northing. When spatial autocorrelation was not present or not severe, multiple linear regression models were used.

In the second method (models coded with B), the additional effects of either NH_x or of NO_y on species richness, Ellenberg N and grass:forb ratio were tested after taking into account the variation explained by S deposition, precipitation, temperature and the other form of N. In this analysis, the residuals of a model that regresses a predictor against NH_x, S deposition, precipitation and temperature were regressed in a second model against NO_y, i.e. the relationship between NO_y deposition and the unexplained variance of the model was tested. The calculation was then repeated for an analysis of NH_x on the residuals of a model that included NO_y. Given that the data cover a substantial range of NO_y and NH_x deposition, and making the assumption that the observed responses with N deposition are linear, any differential effect of reduced and oxidised N deposition is independent of the range of the length of the deposition gradient. In this way, the slope coefficients (effect sizes) that are derived allow a comparison of the independent effects of either NH_x and NO_y, after accounting for other sources of (co-correlated) variation.

Multicollinearity in the models was detected by calculating the Pearson correlation coefficient among pairs of the predictors and by calculating the variance inflation factors (VIF) for each predictor in the model. Predictors with VIF of less than 4 were maintained in the models since these indicate that problems with multicollinearity are not severe (Gujarati, 1995). Predictor variables that were highly correlated (VIF > 4) were not analysed in the same model. In an additional step, multicollinearity was explored by comparing the beta-coefficients of the explanatory variables that were obtained in a multiple regression with the beta coefficients from single regressions of these explanatory variables. In this analysis, major changes in beta coefficient or changes in sign indicate multicollinearity between explanatory variables that needs to be accounted for.

Statistical analysis were performed using the ‘nlme’ package in the ‘R’ (version 2.9.0) statistical and programming environment (R_Development_Core_Team, 2008) and SPSS version 21 (IBM statistics).

3. Results

3.1. N deposition and NH_x/NO_y ratio

Our analysis shows that species richness was negatively affected by total N deposition for all habitats apart from base rich mires (no significant effect) and calcareous grasslands (a significant positive effect) (Tables 1 and 2; Fig. 1). Coefficients were comparable for the habitats mesotrophic grasslands, bogs and mires, woodlands, acidic grasslands and dry upland heaths. The strongest negative coefficient was found for dry lowland heath.

Species richness of all three grassland habitats was negatively related to NH_x/NO_y ratio in deposition when effects of total N deposition were accounted for (Tables 1 and 2; Fig. 2). For woodlands a positive relationship between species richness and NH_x/NO_y ratio was found, while there was no significant effect of N form on the upland and lowland heathlands and the base-rich mires, bogs and mires. Analyses of residuals against NH_x and NO_y (Models B) in all cases were consistent with responses shown by NH_x/NO_y ratio (Models A). These analyses showed that the negative effects on species richness in all three grassland habitats were driven by strong negative effects of NH_x. Species richness in calcareous grasslands was also positively related to NO_y (Table 2). In woodlands in contrast, the negative effects on species richness were

Table 1
Coefficients and their significance for the habitats dry upland heaths, dry lowland heaths, bogs & mires and base rich mires. Results of a multiple regression (A) and regression analysis of the residuals of models containing either NH_x or NO_y with respectively NO_y and NH_x to separate N form (B) (see methods for details). Results are shown for the response variables: species richness, Ellenberg N fertility score and grass:forb ratio. Grass:forb ratio was log transformed. (p < 0.05 = *; p = 0.01 = **; p < 0.01 = ***).

			Sp. rich	Ellenberg N	Grass:Forb
Dry upland heaths	A	Temperature	-0.002	0.017	0.113
		Precipitation	0.014***	-0.001**	0.000
		S deposition	0.103	-0.017	-0.003
	B	Total N deposition	-0.174***	0.006	0.031*
		NH _x :NO _y ratio	0.783	0.475***	0.528
		NO _y deposition	-0.037	-0.007	-0.001
Dry lowland heaths	A	NH _x deposition	-0.019	0.009	0.012
		Temperature	0.474	0.103**	0.035
		Precipitation	0.010	-0.002**	0.005*
	B	S deposition ^a	-0.320	-0.480	1.290
		Total N deposition	-0.266***	-0.022***	0.035*
		NH _x :NO _y ratio	1.148	0.634***	0.342
Bogs and mires	A	NO _y deposition	-0.099	-0.021*	0.001
		NH _x deposition	-0.030	0.006	0.011
		Temperature	-0.226*	0.032*	0.178***
	B	Precipitation	0.011***	0.000	0.002**
		S deposition	-0.033	0.001	-0.030*
		Total N deposition	-0.057***	0.007**	0.028***
Base rich mires	A	NH _x :NO _y ratio	0.797	0.374***	0.312
		NO _y deposition	-0.044	-0.006	0.000
		NH _x deposition	0.004	0.007*	0.009
	B	Temperature	-0.444	0.125**	0.107**
		Precipitation	0.001	-0.004***	-0.003***
		S deposition	0.004	0.023*	0.016
Bogs and mires	A	Total N deposition	-0.011	0.016*	0.005
		NH _x :NO _y ratio	-0.364	-0.046	0.081
		NO _y deposition	0.004	0.004	0.003
	B	NH _x deposition	-0.014	0.008	-0.001

Number of plots: Dry upland heaths (267), dry lowland heaths (182), Bogs and Mires (1136), Base rich Mires (274).

^a S deposition was inverse transformed.

Table 2
Coefficients and their significance for the habitats acidic grasslands, mesotrophic grasslands, calcareous grassland and woodlands. Results of a multiple regression (A) and regression analysis of the residuals of models containing either NH_x or NO_y with respectively NO_y and NH_x to separate N form (B) (see methods for details). Results are shown for the response variables: species richness, Ellenberg N fertility score and grass:forb ratio. Grass:forb ratio was log transformed. (p < 0.05 = *; p = 0.01 = **; p < 0.01 = ***).

			Sp. rich	Ellenberg N	Grass:Forb
Acidic grasslands	A	Temperature	0.097	0.086***	-0.098**
		Precipitation ^a	-0.003***	0.016***	-0.040
		S deposition	-0.113**	0.005	-0.013
	B	Total N deposition	-0.145***	-0.015***	0.030***
		NH _x :NO _y ratio	-1.783***	0.368***	0.455**
		NO _y deposition	-0.011	-0.018***	0.002
Mesotrophic grasslands	A	NH _x deposition	-0.061*	0.006	-0.001
		Temperature	0.457***	0.010	-0.110***
		Precipitation	0.028***	-0.006***	0.000
	B	S deposition	0.006	0.008*	0.000
		Total N deposition	-0.058*	0.008**	-0.001
		NH _x :NO _y ratio	-0.947**	0.172***	0.100
Calcareous grasslands	A	NO _y deposition	0.023	-0.010	-0.001
		NH _x deposition	-0.096***	0.018***	0.003
		Temperature	-0.906***	0.126***	0.015
	B	Precipitation	-0.012***	-0.005***	0.004***
		S deposition	-0.034	0.018*	0.031*
		Total N deposition	0.103**	0.032***	-0.027***
Woodlands	A	NH _x :NO _y ratio	-2.849***	0.618***	0.258*
		NO _y deposition	0.259***	-0.025**	-0.026
		NH _x deposition	-0.109**	0.046***	-0.002
	B	Temperature	-0.103	0.207***	-0.145*
		Precipitation	0.002	-0.006***	0.003
		S deposition	-0.044	0.008	-0.007
Woodlands	A	Total N deposition	-0.121***	0.006	-0.003
		NH _x :NO _y ratio	1.178*	0.271**	-0.116
		NO _y deposition	-0.229***	-0.022	0.017
	B	NH _x deposition	0.019	0.026***	-0.015

Number of plots: Acidic grasslands (1090), Mesotrophic grasslands (1195), Calcareous grasslands (830), Woodlands (514).

^a Precipitation was inverse transformed.

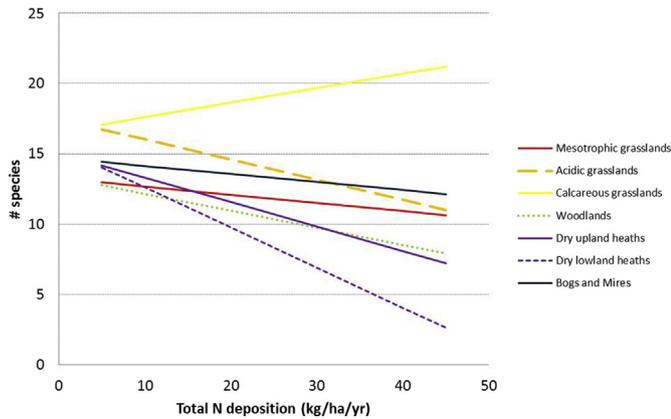


Fig. 1. Effect sizes (slopes) of total N deposition on the number of species for UK habitats. Only significant effects are shown. Actual ranges of N deposition covered by each vegetation type differs but slopes are shown for a range between 5 and 45 kg N ha⁻¹ y⁻¹.

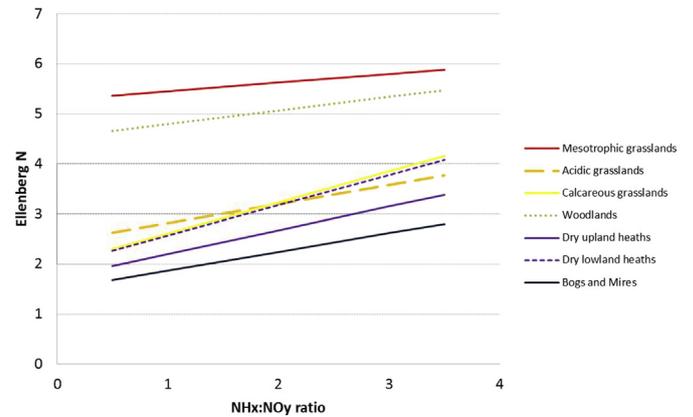


Fig. 4. Effect sizes (slopes) of NH_x:NO_y ratio in deposition on the Ellenberg N number for UK habitats. Only significant effects are shown. Actual ranges of NH_x:NO_y ratio covered by each vegetation type differs but slopes are shown for a range between 0.5 and 3.5.

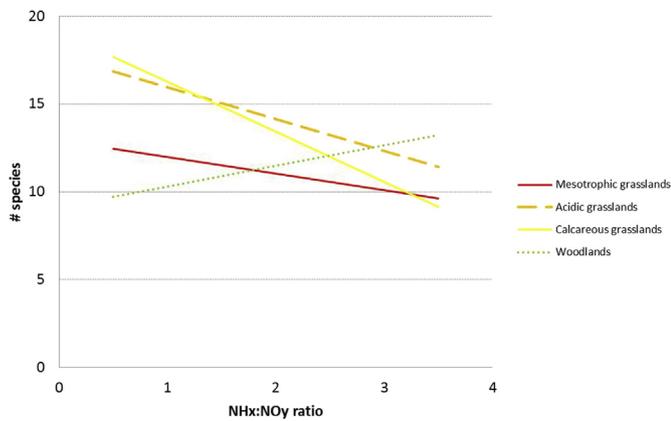


Fig. 2. Effect sizes (slopes) of NH_x:NO_y ratio in deposition on the number of species for UK habitats. Only significant effects are shown. Actual ranges of NH_x:NO_y ratio covered by each vegetation type differs but slopes are shown for a range between 0.5 and 3.5.

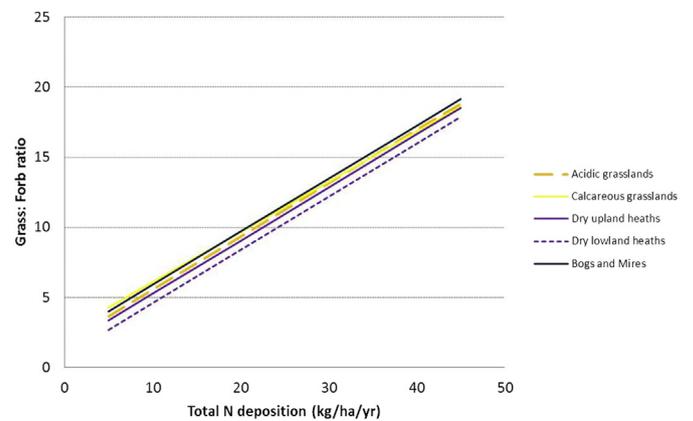


Fig. 5. Effect sizes (slopes) of total N deposition on the Grass:Forb ratio for UK habitats. Only significant effects are shown. Actual ranges of N deposition covered by each vegetation type differs but slopes are shown for a range between 5 and 45 kg N ha⁻¹ y⁻¹.

strongly related to NO_y deposition.

Total N deposition increased Ellenberg fertility index for bogs & mires, base rich mires, mesotrophic grasslands and calcareous grasslands, but decreased fertility index in dry lowland heath and

acidic grasslands (Tables 1 and 2; Fig. 3). There was no significant effect on fertility index in upland heaths or woodlands. In all habitats apart from the base rich mires, there was a significant positive relationship of comparable size between NH_x/NO_y ratio

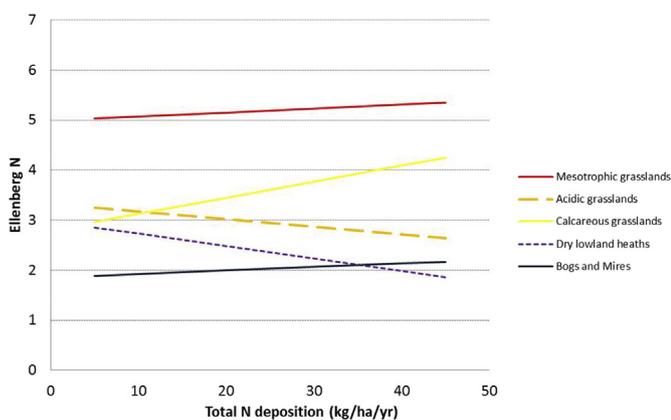


Fig. 3. Effect sizes (slopes) of total N deposition on the Ellenberg N number for UK habitats. Only significant effects are shown. Actual ranges of N deposition covered by each vegetation type differs but slopes are shown for a range between 5 and 45 kg N ha⁻¹ y⁻¹.

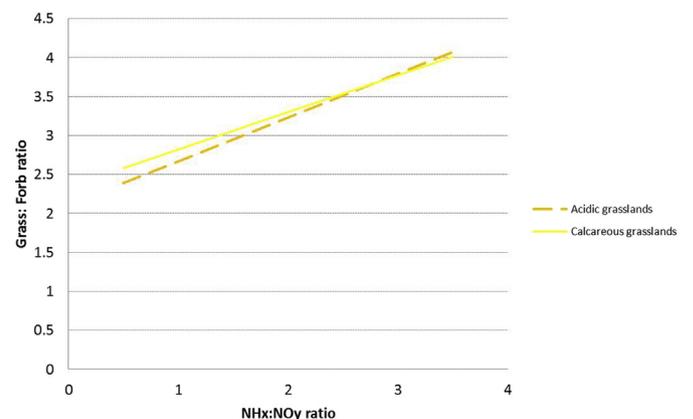


Fig. 6. Effect sizes (slopes) of NH_x:NO_y ratio in deposition on the Grass:Forb ratio for UK habitats. Only significant effects are shown. Actual ranges of NH_x:NO_y ratio covered by each vegetation type differs but slopes are shown for a range between 0.5 and 3.5.

and the Ellenberg fertility index (Fig. 4). However, the form of N responsible and the nature of the relationship differed among the habitats. For dry lowland heath and acidic grassland, this ratio effect was driven by a strong negative relationship with oxidised N, i.e. NO_y reduced fertility index. In the case of the bogs & mires, mesotrophic grasslands and woodlands, the ratio effect was caused by a positive relationship with reduced N, i.e. NH_x increased fertility index. For calcareous grassland there was both a negative relationship for oxidised N and a positive relationship for reduced N with Ellenberg fertility scores.

Grass:forb ratios increased with greater N deposition in upland and lowland heathland, bogs & mires and acidic grasslands (Fig. 5). Only in calcareous grasslands, grass:forb ratio was found to be lower with increased N deposition. There was no effect on grass:forb ratio in base rich mires, mesotrophic grasslands or woodlands. In acidic and calcareous grasslands the increased grass:forb ratio was associated with increased NH_x/NO_y ratio (Table 2, Fig. 6), but separate relationships for either reduced N or oxidised N were not significant and could not be used to infer which form of N was more responsible.

3.2. Additional environmental factors

Climate variables were frequently a significant explanatory variable for total species richness and Ellenberg fertility score (Tables 1 and 2). Precipitation was negatively associated with species richness in the acidic and calcareous grasslands but positively associated with species richness in mesotrophic grasslands, dry upland heath and bogs & mires. A higher precipitation was associated with a lower Ellenberg fertility score in most habitats. Temperature was positively associated with Ellenberg fertility scores. Past sulphur deposition also showed some significant effects. Sulphur deposition showed a significant relationship with species richness in acidic grasslands (negative), Ellenberg fertility scores for mesotrophic and calcareous grasslands (positive) and grass:forb ratio for bogs & mires (negative). This highlights the importance of factoring out these co-correlated variables to genuinely extract any relationships due to N deposition or N form.

4. Discussion

4.1. Total N deposition effects on species richness

Almost all habitats showed a negative relationship between total N deposition and species richness, largely corroborating previous gradient studies for acid grasslands, heathlands and bogs (Stevens et al., 2004; Duprè et al., 2010; Henrys et al., 2011; Caporn et al., 2014). In a previous study using 1998 Countryside Survey data, as opposed to the 2007 data used in our analysis, Maskell et al. (2010) found significant negative relationships for acid grassland and heathlands, but not for calcareous or mesotrophic grassland, a pattern consistent with our findings. Although woodlands showed a negative relationship for species richness in this study, Verheyen et al. (2012) suggest that species richness changes in woodlands are more attributable to management than to N deposition. The positive relationship in calcareous grasslands runs contrary to findings in most other habitats and may reflect differences in the types of grassland included in this category that are due to a combination of glacial history, biogeographical regions, altitude and management. The calcareous grasslands include the species rich CG1 and CG2 and the relative species poor CG10 and CG11. These habitat types are different with respect to species numbers, management and altitude. Separate analysis of the most abundant communities within the dataset (95% of the calcareous grassland records), the relatively species rich communities (UK NVC classes CG1 and CG2) and

relatively species-poor communities (CG10 and CG11) showed no significant relationship with N deposition in either case, which is in line with other surveys in calcareous grasslands showing no effect of N (Bennie et al., 2006; van den Berg et al., 2011) and similar to Maskell et al. (2010) who used a subset of NVC classes (CG2,3,4,6,8,10,11).

A lack of significant relationships with N deposition may be caused by differences in local management that is aimed specifically at the conservation of high species diversity and in which grazing regimes are implemented to prevent the dominance of eutrophic species. In this study, local management was not taken into account. Base rich mires showed no relationship, but there are no other studies in this habitat against which to compare a response. However, negative effects of N on species richness have been shown in calcareous dune grasslands (Jones et al., 2004; Field et al. *in press*), suggesting that base-rich habitats are not immune to N impacts.

The decline in species richness was accompanied by an increased grass:forb ratio in the acidic, open habitats (acidic grasslands, dry upland and lowland heaths and bogs and mires). In contrast, the highly buffered alkaline habitats base rich mires and calcareous grasslands, showed no or even a negative relationship between grass:forb ratio and N deposition. These results are in agreement with previous studies on acidic habitats that showed an increased grass:forb ratio with increasing N deposition due to either loss of forb species richness (Maskell et al., 2010; Payne et al., 2013) or increased grass encroachment (Remke et al., 2009; Friedrich et al., 2011; Provoost et al., 2011). Grass encroachment and a decline in forb species in acidic ecosystems are often attributed to accelerated acidification of the soil leading to a depletion of base cations and increased availability of potential toxic metals such as iron and aluminium (De Graaf et al., 1997; Horswill et al., 2008). Both (historical) deposition of sulphur and N deposition are known causes for acidification (RoTAP, 2012) and N deposition also results in eutrophication. However, our data does not allow us to disentangle the effects of eutrophication and acidification due to N deposition. Note that grass:forb ratio only increased in 4 of the 8 habitats, and therefore is not a consistent indicator of N impact.

Base rich habitats and, to a lesser extent, bogs and mires increased in Ellenberg fertility index with increasing N deposition which was not necessarily accompanied with a loss in species richness suggesting that elevated N deposition in these habitats results in a shift in species composition favouring more nutrient-loving species. In contrast, acid grasslands and lowland heaths show a small decline in fertility index with increasing N deposition. Others have also reported lower Ellenberg N values with higher N deposition in acid grasslands (Maskell et al., 2010) and heathlands (Caporn et al., 2014) and these relationships may be linked to the high correlation between Ellenberg N and Ellenberg R, suggesting mechanisms such as acidification to operate in these systems. The exact mechanisms for these relationships are however not known and need further exploration at the species level of both vascular plants and bryophytes; studies have shown much greater effects of N deposition on bryophyte species richness than vascular plant species richness (e.g. Caporn et al., 2014).

4.2. N form and the relative influence of reduced versus oxidised N

NH_x deposition and NO_y deposition are highly correlated. In addition, NH_x was highly correlated to total N deposition. Separate analysis of NH_x and NO_y effects in models that allowed us to factor out the variance that was explained by either one of the N forms was therefore considered the best method to compare effects of these N forms, after taking account of other variables and the multicollinearity that existed in the datasets. Since both forms are

correlated the variance that is explained by one N form, and which is factored out, is likely to contain some degree of variance that in fact should be attributed to the other form. The method that we employed here is therefore considered conservative in its estimation of effect sizes and significance levels.

The range of NH_x (2.3–36.1 kg $\text{Nha}^{-1} \text{y}^{-1}$) exceeds that of NO_y (2.5–25.6 kg $\text{Nha}^{-1} \text{y}^{-1}$) over all habitats together. Although the gradient length of explanatory variables may affect the outcome of the analysis in small data sets (Smart and Scott, 2004), large datasets such as the CS data capture a good proportion of the relationship (i.e. not just a small segment), even with smaller ranges of the explanatory variable. Our analysis is based on the assumption that the relationships are linear (transformations were applied when necessary) between the response variable and either NH_x or NO_y and estimations of the effect sizes of the relationships are therefore considered relatively unaffected by the length of the gradients. Plots of beta coefficients of the regressions for NO_y and NH_x against N-gradient length confirm that the beta coefficients were indeed not affected by gradient length in the N ranges that we tested (data not shown). In addition, the modelled NH_x data may be more prone to error in predicting the actual NH_x deposition at each site. Although this increased scatter reduces the likelihood of finding a significant relationship with NH_x , the longer gradient length partly offsets this problem. Therefore the effect sizes give a good indication of the relative influence of NH_x or NO_y .

The form of N in deposition independent of N load did affect species richness, but only in certain habitats. N form altered species richness in all grasslands and in woodlands but not in the mires and heaths. The lack of response of species richness and also grass:forb ratio, to N form in heaths and mires may be due to the prevailing acidic conditions, restraining nitrification rates with naturally high soil $\text{NH}_4^+/\text{NO}_3^-$ ratios and low base cation concentrations (e.g. De Graaf et al., 2009). Many species of acidic habitats, such as ericoids, are generally adapted to elevated NH_4^+ concentrations and tolerate high NH_4^+ concentrations (De Graaf et al., 1998; Britto and Kronzucker, 2002; Sheppard et al., 2014).

Where N form was important, in the acidic and mesotrophic grasslands NH_x appeared to be more important than NO_y as a driver of species richness decline, corroborating an experimental study on N form in acid grasslands (Dorland et al., 2013). However, in the woodlands and in calcareous grassland, NO_x was more important than NH_y , having a positive effect on species richness in the grassland but a negative effect in the woodland. Nitrification and mineralisation in woodlands can be very high (Falkengrengrerup and Lakkenborgkristensen, 1994; Falkengren et al., 1998). Atmospheric deposition of oxidised N may therefore favour nitrophilous species such as bramble and nettle that outcompete slow-growing forb and shrub species that are more adapted to ammonium nutrition (such as *Vaccinium myrtillus*), corroborating a recent simulation study (Stevens et al. this volume). The positive impact of NO_y on calcareous grassland species richness may relate to the preference of many calcareous species for available N in oxidised rather than in reduced form.

The question remains why the species richness and composition of only some habitats are sensitive to N form, even though the fertility index of almost all habitats responded to N form. N form may alter species composition through preferences of the component species for oxidised or reduced N, through direct toxicity of NH_3 and NH_4^+ (Britto and Kronzucker, 2002; van den Berg et al., 2005; Sheppard et al., 2011), or through indirect effects mediated by N-induced acidification (e.g. Bobbink et al., 1998; Stevens et al., 2011), which would be more apparent in acidic habitats. The lack of significance of N form in the more acidic habitats suggests that acidification is not the main cause. However, experimental studies have shown that elevated $\text{NH}_4^+/\text{NO}_3^-$ ratios in deposition result in a

decline of acid-sensitive species but not of acid-loving species tolerant of high soil $\text{NH}_4^+/\text{NO}_3^-$ ratios (Paulissen et al., 2004; van den Berg et al., 2008). The response in acidic and mesotrophic grasslands may therefore reflect the abundance of species that are sensitive to reduced N in these neutral to moderately acidic habitats compared with the more strongly acidophile vegetation in heaths, bogs and mires. Clearly responses to N form are habitat-specific, and may be driven by the preference or tolerance of the component species for N in oxidised or reduced forms.

In conclusion, this study has shown that N affects species richness in almost all habitats, after correlating factors such as temperature, rainfall and historical sulphur deposition have been factored out. The form of N is important, with fertility index increasing with NH_x/NO_y ratio in almost all habitats. However, the effects of the ratio on species richness were only found in certain habitats (grasslands and woodland), not in others (mires and heaths). In habitats where there were differential effects of one N form or the other, acidic and mesotrophic grassland were more sensitive to NH_y , while calcareous grassland and woodland were more sensitive to NO_x . This study suggests that, contrary to our original hypothesis, sensitivity to N form is more likely due to the inherent preferences of component species for oxidised or reduced N, rather than linked to soil acidification. However, those preferences are related to soil pH with NH_4^+ -loving species generally more prevalent on acidic soils.

Acknowledgements

We thank Lindsay Maskell for her help with the CS-data and David Fowler for making the N deposition data available.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.envpol.2015.09.017>.

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