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Nitrogen deposition and climate effects on soil nitrogen availability: influences of habitat type and soil characteristics.

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The amount of plant-available nitrogen (N) in soil is an important indicator of eutrophication of semi-natural habitats, but previous studies have shown contrasting effects of N deposition on mineralisable N in different habitats. The stock of readily mineralisable N ($N_{rm}$) was measured in 665 locations across Britain from a range of intensively and extensively managed habitats, allowing N availability to be studied in relation to soil and vegetation type, and also to variation in climate and in reactive N deposition from the atmosphere. Mineralisable N contents were correlated with deposition in extensively managed habitats but not in intensively managed habitats. The following statements apply only to extensively managed habitats. All habitats showed a similar increase in $N_{rm}$ with N deposition. However, soil characteristics affected the relationship, and soil carbon content in particular was a major control on mineralisation. The $N_{rm}$ stock increased more with N deposition in organic than in mineral soils. The nitrate proportion of $N_{rm}$ also increased with N deposition but, conversely, this increase was greater in mineral than in organic soils. The measurements could be used as indicators of eutrophication, e.g. deposition rates of over 20 kg N ha$^{-1}$ y$^{-1}$ are associated with nitrate proportions of > 41% in a mineral soil (2% carbon), and with $N_{rm}$ stocks of over 4.8 kg N ha$^{-1}$ in an organic soil (55% carbon). Both $N_{rm}$ and nitrate proportion increased with mean annual temperature of the sampling location, despite consistent incubation temperature, suggesting that increasing temperatures are likely to increase the eutrophying effects of N pollution on semi-natural ecosystems.

Keywords: deposition; eutrophication; mineralization; nitrate; nutrient; pollution; production
1. Introduction

The progressive eutrophication of terrestrial ecosystems by reactive nitrogen (N) from fertilisers and atmospheric pollution has been implicated in widespread changes in productivity (Hungate et al., 2003), losses of biodiversity (Phoenix et al., 2006; Bobbink et al., 2010) and declines in water quality (Magee, 1982). There is strong evidence that floristic change towards more eutrophic assemblages is occurring (Braithwaite et al., 2006; Maskell et al., 2010), but these changes have not been easy to ascribe to N pollution, in part due to lack of clear evidence that the availability of N in soil has increased. Studies of effects of N deposition rate on soil mineralisable N have shown inconsistent effects in similar habitats (e.g. Rao et al., 2009; Vourlitis et al., 2007). We used a simple measure of soil mineralisable N to investigate patterns of N availability across Britain, in different soil and habitat types, and related these patterns to rates of atmospheric N deposition.

Large amounts of available or readily-mineralisable N in soil reflect increased plant exposure to mineral N, which is likely to increase productivity in many terrestrial habitats (LeBauer and Treseder, 2008). This increased productivity is beneficial for ecosystem services such as agricultural or forest production, and is likely to increase net carbon (C) storage, at least in the short term (Wamelink et al., 2009). However, increased productivity in semi-natural habitats is likely to decrease ground-level light-availability and lead to the loss of low-growing plants and associated invertebrate species (Bobbink et al., 2010; Hautier et al., 2009; Wallisdevries and Van Swaay, 2006), reducing biodiversity value at a landscape scale. Such changes will be more pronounced where N is the main limiting factor, for example in higher-precipitation regions (Lee et al., 2010). Large proportions of nitrate in mineralisable N are also associated with floristic change (Diekmann and Falkengren-Grerup, 1998). Increased mineral N contents, particularly when combined with high rates of nitrification, are also likely to increase N leaching and reduce downstream water quality (Gundersen et al., 2006). Measurements of N availability are therefore useful for several areas of research and policy.
Predictions made by mechanistic models of C and N cycling used at ecosystem and global scales need to be tested against measures of medium-term N fluxes (Finzi et al., 2011; Magid et al., 1997). Agronomic researchers require measures of N availability to predict productivity (Ros et al., 2011). In semi-natural systems, niche occupancy models that predict likely species occurrence in relation to changing environmental factors (Latour and Reiling, 1993; Smart et al., 2010; Sverdrup et al., 2007) sometimes require abiotic indicators of eutrophication; such models are used to inform pollution abatement policy such as the Convention on Long-Range Transboundary Air Pollution (de Vries et al., 2010).

Plant-available N is not straightforward to define or measure, and is thus a major source of uncertainty in current ecosystem models (Wamelink et al., 2002). Soil total C / N ratio has been used as an indicator of N availability, as it provides some indication of the degree to which the capacity of an ecosystem to absorb excess N has been exhausted (Gundersen et al., 1998). However, variation in the reactive proportion of soil N means that this ratio has limited capacity to predict the onset of N leaching (Rowe et al., 2006). Direct measurements of soluble N in lysimeters or by KCl extraction provide a snapshot measurement of plant-available N, but such measurements are inherently variable due to short-term variations in the rate of efflux (leaching and uptake) from the soluble pool. Measurements of potentially mineralisable N seem likely to be more robust indicators of N availability (Ros et al., 2011), for reasons explained below.

Mineralisation is the conversion of organic residues into mineral forms, initially to ammonium, and then when conditions allow nitrification, to nitrate. The rate of mineralisation of N and the total stock of mineralisable N in soil are likely to be affected by factors such as soil pH, temperature, moisture, stocks of C and N, and the recalcitrance of this organic matter. In a study of 31 widely differing soils incubated under standard conditions, the stock of potentially mineralisable N was found to be highly variable, although the proportion of this stock that was mineralised per week was similar (Stanford and Smith, 1972). Studies on single soil types have shown that incubation temperature exerts greater control over N mineralisation than water content, over ranges from 10 to 25 °C and from –30 to –1700
kPa (Sierra, 1997), and that increasing pH leads to decreased ammonification, but an increase in nitrification (Pietri and Brookes, 2008). However, while incubation temperature effects on soil organic matter mineralisation have been widely studied (von Lutzow and Kogel-Knabner, 2009), effects of the typical temperature of the sampling location are less well understood.

Plants are able to take up N even from soils with no net mineralisation flux (Dyck et al., 1987), since they can intercept available N before it can be re-immobilised (Schimel and Bennett, 2004). Net mineralisation measurements therefore probably underestimate plant-available N, at least in low-N systems. Gross mineralisation fluxes can be measured using isotopic dilution or by adsorption onto strong ion-exchange resins, but these measurements probably overestimate plant-available N (Fierer et al., 2001). Soluble organic forms of N may also be produced during the decomposition of organic matter, and may themselves be significant sources of N for plant nutrition (Chapin et al., 1993; Hill et al., 2011; Schimel and Chapin, 1996). Plant growth can also decrease or more commonly increase mineralisation, with plant cultivation changing mineralisation rates to 70 – 500 % of rates in controls without plants (Kuzyakov, 2002). This implies that there can be no definitive measure of plant-available N, but net mineralisation measurements remain useful to distinguish soils across a range of N availability (Schimel and Bennett, 2004). Net mineralisation flux has most commonly been measured by comparing the amounts of extractable nitrate and ammonium before and after a period of incubation, using paired soil samples (e.g. Keeney, 1980; Waring and Bremner, 1964). Disturbance can change mineralisation and immobilisation rates, so in situ or intact core methods are preferred (Raison et al., 1987). As well as introducing some error due to analysis of two spatially separated cores, the paired core method for measuring net mineralisation may be unsuitable when cores cannot be transferred rapidly to the laboratory under controlled conditions, since mineralisation in transit is likely to lead to large variation in mineral N contents on arrival at the laboratory. In the current study we therefore used a single-extraction method, in which soils were flushed through before incubation with approximately four pore-volumes of an artificial rain solution, to remove any accumulation of mineral N during transit. Mineralisable N measured using this method helped explain the occurrence of plant species in associated plots, predicting a component of the variation in mean Ellenberg “N”
score (Ellenberg, 1974) that was largely orthogonal to soil properties such as pH, moisture content and total N/C ratio (Rowe et al., 2011).

Different plant species may be adapted to use oxidised, reduced or dissolved organic N (Miller and Bowman, 2003). Dissolved organic N uptake may become less prevalent and nitrate uptake more prevalent in more productive systems (Nordin et al., 2001), perhaps because competitively superior species are able to take up whichever is currently the most abundant form (Ashton et al., 2010). The availability of nitrate may be more important than total available N for explaining species occurrence (Andrianarisoa et al., 2009; Bengtson et al., 2006; Wilson et al., 2005). Soil nitrate concentrations tend to increase with N enrichment (Corre et al., 2007), perhaps because nitrate immobilisation is inhibited by greater ammonium concentrations (Bradley, 2001). High nitrate concentrations are also associated with greater N leaching losses (MacDonald et al., 2002).

The effects of atmospheric N deposition on N mineralisation rates have been studied in selected ecosystems, with varying results. In a study of sixteen sites in Californian deserts, N deposition was found to be correlated with soil total C and total N, but mineralisation rates were unrelated to N deposition (Rao et al., 2009). However, a similar study of semiarid Californian shrublands found that N mineralisation increased linearly with N deposition rate (Vourlitis et al., 2007). Observed relationships between mineralisable N and N deposition were positive in southern Swedish deciduous forests (Falkengren-Grerup et al., 1998), Appalachian deciduous forests (Boggs et al., 2005) and pine stands in Nanchang, China (Chen et al., 2010); negative in sugar maple stands in Ontario (Watmough, 2010); unrelated in pine stands in Alberta (Laxton et al., 2010); and unimodal for spruce stands across Germany (Corre et al., 2007). A study of forest plots across the northeastern US showed a positive relationship between mineralisable N and N deposition in maple stands, but no relationship in beech stands (Lovett and Rueth, 1999). This variation suggests the need for more studies in which the same survey and analytical techniques are used across different habitats, to clarify whether there are indeed differences in responses to N deposition, and to explore potential reasons for these differences (Nave et al., 2009).
The aim of the current study was to examine variation in soil net N mineralisation and net nitrification across a range of British habitats in relation to soil properties, habitat type, temperature of the sampling site, and the gradient of N deposition, to address the hypothesis that increased N deposition leads to increases in available N and in the nitrate proportion of this available N.

2. Methods

Soil cores for analysis were taken in summer 2007 during the UK Countryside Survey, a large stratified random survey of 1 km² squares across Britain, i.e. England, Wales and Scotland (Firbank et al., 2003). The stratification is based on 32 land use classes, each sampled using eight squares, giving a total of 256 squares. The survey has been repeated five times since 1978, and has expanded, but the current study was restricted to the original set of squares for which there is a long history of repeat measurements. Samples for mineralisable N were taken from three of the five randomly located main plots in each square. Access to some sites was restricted, however, and of the planned 768 analyses only 665 were carried out, from plots located within 237 of the squares. In the Countryside Survey, the squares were mapped in terms of “Broad Habitat” on the basis of floristic and structural characteristics (Maskell et al., 2008), meaning that each sample could be related to a specific Broad Habitat (Table 1).
Table 1. Number of mineralisable nitrogen analyses carried out per Broad Habitat. I = habitat assumed to be intensively managed; E = habitat assumed to be extensively managed.

<table>
<thead>
<tr>
<th>Broad Habitat</th>
<th>N</th>
<th>Broad Habitat</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improved Grassland (I)</td>
<td>149</td>
<td>Fen, Marsh and Swamp (E)</td>
<td>12</td>
</tr>
<tr>
<td>Arable and Horticulture (I)</td>
<td>148</td>
<td>Bracken (E)</td>
<td>6</td>
</tr>
<tr>
<td>Bog (E)</td>
<td>78</td>
<td>Urban (E)</td>
<td>4</td>
</tr>
<tr>
<td>Neutral Grassland (I)</td>
<td>76</td>
<td>Littoral Sediment (E)</td>
<td>3</td>
</tr>
<tr>
<td>Acid Grassland (E)</td>
<td>56</td>
<td>Calcareous Grass (E)</td>
<td>2</td>
</tr>
<tr>
<td>Coniferous Woodland (E)</td>
<td>48</td>
<td>Supralittoral Rock (E)</td>
<td>2</td>
</tr>
<tr>
<td>Dwarf Shrub Heath (E)</td>
<td>42</td>
<td>Supralittoral Sediment (E)</td>
<td>2</td>
</tr>
<tr>
<td>Broadleaff, Mixed and Yew Woodland (E)</td>
<td>37</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Coarse litter was removed before sampling, and soil cores were taken by pressing a 5 cm diameter by 15 cm long plastic pipe into the soil until the end was level with the soil surface. The plastic tube was carefully extracted, and cores were returned to the laboratory by normal post, taking 1-5 days. Cores were kept at 4 °C for a further 1-5 days until sufficient cores had been received for an analytical batch. Mineralisable N analyses were carried out after first flushing out soil solution by laying the core horizontally on a perforated rack and repeatedly spraying with a dilute salts solution, then incubating for 28 days at 10 °C, by extracting mineral N from the incubated core using 1M KCl. This procedure was designed to reduce variability in initial mineral N concentrations due to pre-sampling rain events and uncertain conditions during transfer to the lab, and was described in detail in Rowe et al. (2011), except that the flushing solution was based on concentrations of major ions except ammonium and nitrate in average UK rain in 2007 as estimated using the FRAME model (Rognvald Smith, CEH Edinburgh, pers com.). These concentrations were: 17.6 μeq Ca²⁺ L⁻¹; 30.1 μeq Mg²⁺ L⁻¹; 125 μeq Na⁺
\[ L^{-1}; 140 \ \mu\text{eq Cl}^{-1} \ \text{and} \ 57.2 \ \mu\text{eq SO}_{4}^{2-} \ \text{L}^{-1}, \ \text{resulting in a solution with a pH of approximately 4.6. The} \]

\[ \text{total net mineral N production during the incubation (N}_{mn} \ \text{was expressed as kg N ha}^{-1} \ \text{in the top 15} \]

\[ \text{cm of soil, using bulk density measurements made on soil cores taken from adjacent locations. This} \]

\[ \text{unit was chosen for two reasons. Firstly, the rate of mineralisation of N in a given sample declines} \]

\[ \text{with time (Stanford and Smith, 1972), so a single measurement cannot be used to calculate flux} \]

\[ \text{during shorter or longer periods of time, but is better viewed as an indicator of the stock of readily} \]

\[ \text{mineralisable N. Secondly, since soils vary widely in their organic C content, expressing} \]

\[ \text{mineralisable N concentrations per g soil or per g organic matter gives the impression of high} \]

\[ \text{availability on mineral or organic soils, respectively. The stock of available N in the top 15 cm of soil,} \]

\[ \text{by contrast, is a measure of N availability within the plant rooting zone that is comparable across a} \]

\[ \text{variety of habitats.} \]

\[ \text{Nitrification was calculated as the net nitrate production during the incubation, and was expressed as a} \]

\[ \text{proportion of N}_{mn} \ \text{rather than as a total amount, to separate this signal from that of the overall quantity} \]

\[ \text{of mineralisable N. After incubation, a subsample was analysed for total C content by mass loss on} \]

\[ \text{ignition (375 °C for 16 hours) using a ratio of 0.55, which was the mean ratio of elementally analysed} \]

\[ \text{C to loss-on-ignition in the main Countryside Survey dataset (Emmett et al., 2010). Soil pH was} \]

\[ \text{measured in samples from adjacent soil cores, in a slurry of 10 g fresh soil with 25 ml de-ionised} \]

\[ \text{water. Soil moisture content was measured gravimetrically in samples from adjacent soil cores and} \]

\[ \text{expressed as % of fresh weight.} \]

\[ \text{Estimates of atmospheric N deposition fluxes were obtained using the CBED model (Smith et al.,} \]

\[ 2000), \ \text{which predicts fluxes based on atmospheric concentrations, fertiliser application rates, and the} \]

\[ \text{interception characteristics of vegetation. Deposition estimates for woodland were used for woodland} \]

\[ \text{habitats, and deposition estimates for open moorland were used for all other habitats. Effects of N} \]

\[ \text{deposition were not examined within habitats as defined the Countryside Survey (Maskell et al.,} \]

\[ 2008) \ \text{that were considered to be intensively managed (Improved grassland, Neutral grassland, and} \]

\[ \text{Arable), but only within extensively managed habitats where little or no N fertiliser is likely to have} \]
been applied and where more than 10 analyses were carried out, i.e., for samples from: Broadleaf, mixed and yew woodland; Coniferous woodland; Acid grassland; Dwarf Shrub Heath, Fen, marsh and swamp, and Bog. Mean annual temperature for each Countryside Survey square was estimated as the average of monthly average air temperatures in the years preceding the survey, 2001-2006 (Met Office, 2009).

Correlations between variables were analysed using Spearman’s rank-correlation test. Linear mixed-effects models were fitted to $N_{m}$ stock and nitrate proportion data by maximum likelihood (ML) using the lme procedure of R (Pinheiro & Bates 2004; R Development Core Team, 2007). The Countryside Survey square was included as a random effect. Effects of Broad Habitat and N deposition rate on $N_{m}$ stock and nitrate proportion were examined by fitting these two explanatory variables and the interaction between them as fixed effects. Effects of continuous variables (N deposition, annual mean temperature, soil C content and soil pH) on $N_{m}$ stock and nitrate proportion were examined by fitting these variables and interactions among them as fixed effects. In both cases, a maximal model including all interactions was fitted, and terms were then removed in ascending order of influence on model likelihood, until further simplification caused an increase in Akaike’s information criterion (AIC). To reduce heteroscedasticity, stock data were log transformed before analysis, first adding half the detection limit to zero values, and nitrate proportion was logit transformed, first adding half the detection limit to zero values and subtracting half the detection limit from values of one. Nitrate proportions could not be calculated for samples with no detectable $N_{m}$. Back-transformed means and standard errors are presented.

3. **Results**

3.1 **Mineralisable N stock and nitrate proportion**
The log-average N\textsubscript{m} stock measured across all British soils was 8.8 kg ha\textsuperscript{-1} in 0-15 cm depth soil. The distribution of N\textsubscript{m} by Broad Habitat is shown in Figure 1a. The measurement clearly distinguished habitats that are considered fertile and productive from those considered unproductive, although variability was greater for the ‘Broadleaf, mixed and yew woodland’ and ‘Fen, marsh and swamp’ habitats, both of which can occur on a wide range of soil types in Britain. The intensively managed habitats ‘Arable and Horticulture’ and ‘Improved Grassland’ had consistently large N\textsubscript{m} stocks, and Bog and ‘Dwarf shrub heath’ had consistently small stocks.

The mean proportion of nitrate in N\textsubscript{m} across all British soils was 0.52 g NO\textsubscript{3}-N g\textsuperscript{-1} total mineralisable N, and there was considerable variation in nitrate proportion among Broad Habitats (Figure 1b). The greatest proportion of nitrate was in the Arable and Horticulture habitat, and there were small nitrate proportions in less fertile habitats such as Bog, Acid Grassland and Dwarf Shrub Heath.
Figure 1: Mean (+/- one standard error) values for: a) stock of total readily-mineralisable nitrogen (kg N ha$^{-1}$); and b) nitrate proportion of total readily-mineralisable nitrogen, in the top 15 cm of soil in different Broad Habitats across Britain: Broadleaf = Broadleaf, mixed and yew woodland; Conifer = Coniferous woodland; Arable = Arable and horticulture; Improved grassland; Neutral grassland; Acid grassland; Heath = Dwarf shrub heath; Marsh = Fen, marsh and swamp; Bog.

a)

![Graph showing mineralisable nitrogen kg N ha$^{-1}$ for different Habitats]

b)

![Graph showing nitrate-N proportion for different Habitats]
3.2 Factors affecting mineralisable N

Correlation analysis in extensively managed habitats showed a close association between Nrm and N deposition (Table 2a). The stock of Nrm was also strongly correlated with soil C content, moisture content at sampling, and mean annual temperature. Significant correlations also illustrated spatial associations, for example between higher temperatures towards the south of Britain and greater N deposition rates and lower soil C contents. The proportion of nitrate in mineralisable N was positively correlated with N deposition rate, mean annual temperature and soil pH, and negatively correlated with soil moisture and C contents. Within intensively managed habitats, Nrm was not correlated with N deposition rate (Table 2b). Neither Nrm nor nitrate proportion were correlated with mean annual temperature in intensively managed habitats. The Nrm stock in intensively managed habitats was also not correlated with intrinsic soil properties, but nitrate proportion still tended to be greater with greater Nrm. Nitrate proportion in intensively managed habitats also increased with N deposition rate and soil pH, and decreased with greater soil moisture and C contents. Since soil C content was very strongly associated with soil moisture in both extensively managed and intensively managed habitats (Spearman’s rho = 0.881 and 0.811, respectively), and soil C content was expected to have a more direct effect on Nrm, soil moisture was left out of subsequent regression analyses.
Table 2. Spearman’s rank correlation coefficients among readily-mineralisable N (N$_{rm}$), proportion nitrate in mineralisable N (pNO3), N deposition (N$_{dep}$), soil total carbon (C$_{tot}$), soil moisture, soil pH and mean annual temperature (Temp), in: a) extensively managed habitats (N = 290); and b) intensively managed habitats (N = 375). *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; ns = $P > 0.05$.

<table>
<thead>
<tr>
<th></th>
<th>N$_{rm}$</th>
<th>pNO3</th>
<th>N$_{dep}$</th>
<th>C$_{tot}$</th>
<th>Moisture</th>
<th>pH</th>
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<tbody>
<tr>
<td><strong>a) extensively managed habitats</strong></td>
<td></td>
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<tr>
<td>pNO3</td>
<td>0.296***</td>
<td></td>
<td></td>
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<tr>
<td>N$_{dep}$</td>
<td>0.604***</td>
<td>0.280***</td>
<td></td>
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<tr>
<td>C$_{tot}$</td>
<td>-0.502***</td>
<td>-0.402***</td>
<td>-0.489***</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Moisture</td>
<td>-0.613***</td>
<td>-0.390***</td>
<td>-0.577***</td>
<td>0.881***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>0.110 ns</td>
<td>0.214***</td>
<td>-0.087 ns</td>
<td>-0.426***</td>
<td>-0.273***</td>
<td></td>
</tr>
<tr>
<td>Temp</td>
<td>0.477***</td>
<td>0.327***</td>
<td>0.741***</td>
<td>-0.475***</td>
<td>-0.482***</td>
<td>-0.024 ns</td>
</tr>
<tr>
<td><strong>b) intensively managed habitats</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pNO3</td>
<td>0.259***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N$_{dep}$</td>
<td>0.001 ns</td>
<td>-0.148**</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C$_{tot}$</td>
<td>0.016 ns</td>
<td>-0.298***</td>
<td>0.117*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>-0.093 ns</td>
<td>-0.380***</td>
<td>0.118*</td>
<td>0.811***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>-0.054 ns</td>
<td>0.274***</td>
<td>0.071 ns</td>
<td>-0.395***</td>
<td>-0.405***</td>
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</tr>
<tr>
<td>Temp</td>
<td>0.000 ns</td>
<td>0.045 ns</td>
<td>0.246***</td>
<td>-0.041 ns</td>
<td>-0.053 ns</td>
<td>0.240***</td>
</tr>
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</table>

Within extensively managed habitats, there was an increase in the stock of N$_{rm}$ with more N deposition ($P < 0.001$; Figure 2). Neither the intercept nor the slope of the fitted relationship differed among habitats ($P > 0.05$). The nitrate proportion in N$_{rm}$ also increased with total N deposition ($P < 0.001$), and there were significant differences among habitats in the intercept ($P < 0.05$), but not the slope ($P > 0.05$) of this relationship (Figure 3).
Figure 2. Response of total readily-mineralisable N stock to total N deposition.
Figure 3. Responses of the proportion of nitrate in total readily-mineralisable N stock to total N deposition in selected extensively managed Broad Habitats: Broadleaf = Broadleaf, mixed and yew woodland; Conifer = Coniferous Woodland; Acid Grass = Acid Grassland; Heath = Dwarf Shrub Heath; Marsh = Fen, Marsh and Swamp; Bog = Bog. Lines are from a linear mixed model fit to logit-transformed data, with different intercepts for different Broad Habitats.

Potential explanatory variables for variation in N_{rm} were analysed for the subset of plots from extensively managed habitats that were included in the current study. The extensive Broad Habitats differed in their mean soil C content ($P < 0.001$; Figure 4a), soil pH ($P < 0.001$; Figure 4b), soil moisture content at sampling ($P < 0.001$; Figure 4c), N deposition rate ($P < 0.001$; Figure 4d) and annual mean temperature ($P < 0.001$; Figure 4e). The best model for N_{rm} based on continuous measurements (rather than habitat category) is given in Table 3, and illustrated in Figure 5. The main explanatory factors for N_{rm} were soil C ($P < 0.001$), mean annual temperature ($P < 0.001$), and N deposition ($P < 0.001$). Interactions between soil C and N deposition ($P = 0.062$; Figure 6a) and between soil C and soil pH ($P = 0.252$; Figure 6b) were retained in the model, since removal of these terms increased AIC. The nitrate proportion of N_{rm} was best predicted (Table 4) by soil C ($P < 0.001$), mean annual temperature ($P < 0.001$), and interactions between soil C and total N deposition ($P < 0.05$; Figure 7a) and between soil C and mean annual temperature ($P < 0.05$; Figure 7b).
Figure 4. Soil properties, N deposition and temperature for plots from extensively-managed broad habitats included in the current study: a) soil total carbon content; b) soil pH; c) soil moisture content; d) total N deposition; and e) mean annual air temperature. Broadleaf = Broadleaf, mixed and yew woodland; Conifer = Coniferous Woodland; Acid Grass = Acid Grassland; Heath = Dwarf Shrub Heath; Marsh = Fen, Marsh and Swamp; Bog = Bog.
Table 3. ANOVA table for fixed effects in a linear mixed-effects model predicting log_{10}(readily-mineralisable N, kg ha^{-1} in 0-15 cm soil) in extensively managed habitats, from soil total carbon content (C_{tot}, g C 100 g^{-1} dry soil), soil pH, nitrogen deposition rate (N_{dep}, kg ha^{-1} y^{-1}) and mean annual temperature (Temperature, °C). F- and p- values computed for Type I (sequential) sums-of-squares; numDF = numerator degrees of freedom, denDF = denominator degrees of freedom.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Value</th>
<th>numDF</th>
<th>denDF</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
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<td>1</td>
<td>141</td>
<td>244.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C_{tot}</td>
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<tr>
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<td>N_{dep}</td>
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<td>N_{dep} : C_{tot}</td>
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<td>141</td>
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<td>0.062</td>
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Table 4. ANOVA table for fixed effects in a linear mixed-effects model predicting logit(proportion nitrate in mineralisable N) in extensively managed habitats, from soil total carbon content ($C_{\text{tot}}$, g C 100 g$^{-1}$ dry soil), nitrogen deposition rate ($N_{\text{dep}}$) and mean annual temperature (Temperature, °C). F- and p- values computed for Type I (sequential) sums-of-squares; numDF = numerator degrees of freedom, denDF = denominator degrees of freedom.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Value</th>
<th>numDF</th>
<th>denDF</th>
<th>F-value</th>
<th>p-value</th>
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<td>6.4</td>
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</table>
Figure 5. Comparison of observed total readily-mineralisable N stock with within-group fitted values from a mixed-effects model with fixed effects: $\log_{10}(\text{total readily-mineralisable N stock } + 0.07, \text{ kg N ha}^{-1} \text{ yr}^{-1}) = -0.135 - 0.0329 \times \text{soil C } (\%) + 0.0213 \times \text{soil pH} + 0.0574 \times \text{mean annual temperature (°C)}$

$+ 0.0155 \times \text{total N deposition (kg N ha}^{-1} \text{ yr}^{-1}) + 0.00477 \times \text{soil C} \times \text{soil pH} + 0.000396 \times \text{total N deposition} \times \text{soil C}$, and Countryside Survey 1 km² square as a random effect.
Figure 6. Fitted models for readily-mineralisable N stock in extensively managed habitats, in relation to: a) soil total carbon, and total nitrogen deposition, at the mean values for pH (4.82) and annual mean temperature (9.1 °C) within the dataset; and b) soil total carbon and soil pH at the mean values for total nitrogen deposition (16.9 kg N ha⁻¹ y⁻¹) and mean annual temperature within the dataset.
Figure 7. Fitted models for nitrate proportion of mineralisable N in extensively managed habitats, in relation to: a) soil total carbon and total nitrogen deposition, at the mean value for annual mean temperature (9.1 °C) within the dataset; and b) soil total carbon and temperature, at the mean value for total nitrogen deposition (16.9 kg N ha\(^{-1}\) y\(^{-1}\)) within the dataset.

4. Discussion

The variation among habitats in mineralisable N that was revealed in the current study is consistent with the picture of greater N availability and a greater proportion of nitrate in more intensively managed agricultural habitats, due to inherent soil properties, climatic differences and/or direct effects of more intensive management. However, \(N_{\text{m}}\) stocks in extensively managed habitats were of comparable magnitude, particularly in the woodland and ‘Fen, Marsh and Swamp’ habitats. There was considerable variability in both \(N_{\text{m}}\) stock and nitrate proportion within individual habitats. While extensively managed habitats differed significantly in their relationships between N deposition and
Nrm (Figure 2) and between N deposition and nitrate proportion (Figure 3), much unexplained variance remained in these relationships.

The continuous environmental variables examined in the study were considerably more useful than categorical differences among habitats for explaining variation in Nrm. In extensively managed habitats in the current study, variation in Nrm was clearly related to soil characteristics, but was strongly affected by N deposition rate. The Nrm stock increased with total N deposition, and there was an interaction with soil C content. Increasing N deposition also increased Nrm in more mineral soils, but in completely organic soils was associated with a greater increase in Nrm across the observed range of N deposition. Larger values of Nrm were also associated with greater sampling location temperature, implying that any increase in mean annual temperature is likely to increase N availability, whether directly or by increasing the proportion of plant species with rapid growth rates and more decomposable litter. Nitrate proportions were also greater in soils from warmer locations. A significant negative interaction with soil C content suggests that temperature effects on nitrate proportion will be more pronounced in more mineral soils.

In a meta-analysis of experimental N addition studies in north temperate forest, Nave et al. (2009) found no differences between mineral and organic horizons in the response of mineralisable N to N deposition, but did find differences in this response between different biogeographical regions, and highlighted the importance of the proportions of recalcitrant and labile pools in soil organic matter. In contrast to the current study, Booth et al. (2005) found in a meta-analysis covering a wide range of ecosystems that mineralisable N was correlated with substrate concentrations of organic matter. The negative correlation of Nrm with soil total C found in the current study may differ because many of the soils had large organic matter contents (mean C content for the extensively managed habitats included was 27%). The greater effect of N deposition flux on N availability in organic soils than in more mineral soils shown in the current study may be because a larger proportion of the organic matter is recalcitrant in the very organic soils that were included. In soils from a temperature gradient in the Great Plains region, Barrett and Burke (2000) found that while C mineralisation increased with soil
organic matter content, gross N immobilisation also increased; a similar result to that found in the current study. While the overall effect of increasing soil C content was a decrease in mineralisable N stock in our study, an interaction between C content and N deposition rate suggests that this N immobilisation flux may become saturated under chronically elevated N deposition. However, Hartley and Mitchell (2005) found that experimental N additions increased mineralisable N more in a more mineral soil (20% organic matter) than in a more organic soil (70% organic matter). This suggests that there may be differences between effects observed after short-term additions and after chronic high N deposition rates.

Several explanations are possible for the greater increase in N_{rm} with N deposition rate in more organic soils. Proposed effects of increased N deposition include productivity stimulation (LeBauer and Treseder, 2008) and inhibition of litter decomposition, at least on sites that are not greatly N-limited (Craine et al., 2007; Knorr et al., 2005), either of which might increase the stock of readily-mineralisable organic matter. Productivity stimulation by N may have been greater in more organic soils that are generally less water-limited than mineral soils.

The proportion of nitrate in N_{rm} was strongly affected by soil C content and N deposition rate, and was only large in soils with low C content and a large rate of N deposition. Nitrification is affected by aeration (Sahrawat, 2008), and the texture of the soil on fine scales (e.g. clay, silt and sand fractions, or the degree of humification of organic matter) and medium scales (e.g. porosity and aggregation) undoubtedly affected the diffusion of air into the soil core during the incubation. However, both organic and mineral soils can vary considerably in aggregation development and porosity, and hence the increase in nitrate proportion with decreasing organic matter content (where there is a large rate of N deposition) may not be related to effects of soil structure. The large-scale spatial pattern of nitrate proportion suggests little influence of soil texture, which varies at a smaller scale. Nitrification has been found in previous studies to be correlated with total N mineralised (Booth et al., 2005) and with soil pH (Andrianarisoa et al., 2009; Sahrawat, 2008; Ste-Marie and Pare, 1999). We also found evidence of correlation between nitrate proportion and both total N_{rm} and soil pH ($P < 0.001$ for both
correlations, in extensively and intensively managed habitats; Table 2). Nitrification rates may also indicate the size of the nitrifying bacteria population, and hence greater nitrate proportions may be related to a history of elevated N inputs. The strong increase in nitrate proportion with N deposition in more mineral soils suggests that N deposition has increased nitrifier activity in these soils, whereas factors such as limited aeration may have prevented an increase in nitrifiers in more organic soils.

The N_{rm} measurement reflects an amount of N that was insoluble at the start of the study but was readily mineralised during the incubation. The net N mineralisation during an equivalent period under field conditions would likely have been different, due to differences in disturbance, temperature, aeration, interactions with plant roots, and other factors. The measurement nevertheless provides some indication of the rate of N release from soil organic matter into the soil solution, whence it may be available for plant uptake, or may be leached. Chen et al. (2006) found that gross N mineralisation remained elevated 14 years after cessation of N additions, despite recovery of mineral N concentrations and leaching rates. Although large amounts of N in readily-mineralisable organic matter are not as immediate a cause for concern (in semi-natural systems susceptible to eutrophication) as are large mineral N concentrations in soil solution, they reflect a pool of N that is likely to lead to long-term increases in plant production and/or increased leaching of mineral N.

5. Conclusions

In extensively managed habitats, mineralisable N stock and nitrate proportion of mineral N were both strongly influenced by N deposition rate, and by interactions with soil C content. Habitats varied in mean mineralisable N stock, but did not show evidence of differential effects of N deposition, perhaps due to variation in soil type within each habitat. The effect of N deposition on mineralisable N stock was more apparent in more organic soils, whereas the effect on nitrate proportion was more apparent in more mineral soils. With the proviso that responses also depend on soil C content and site temperature, the study supports the use of both mineralisable N and nitrate proportion as indicators of...
ecosystem eutrophication due to N pollution. The increase in mineralisable N stock with temperature implies that climate change and N deposition are likely to have synergistic effects, accelerating the change of semi-natural habitats to a more eutrophic state.

6. Acknowledgements

We are grateful to the surveyors who participated in the Countryside Survey in 2007 (http://www.countrysidesurvey.org.uk/) and supplied soil samples. The Countryside Survey in 2007 was funded by the Natural Environment Research Council (NERC) and a consortium of UK Government departments and agencies headed by DEFRA (Contract no. CR0360).
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