

# Field-scale evaluation of effects of nitrogen deposition on the functioning of heathland ecosystems

Alan G. Jones† and Sally A. Power\*

Division of Biology, Imperial College London, Silwood Park, Ascot, Berkshire SL5 7PY, UK

## Summary

1. Plant communities that are adapted to low levels of nutrient availability are particularly sensitive to the effects of elevated nitrogen (N) deposition. Although site-level manipulation experiments have been used widely to examine the effects of N deposition on (semi-)natural ecosystems under controlled conditions, relatively few studies have investigated field-scale impacts of elevated ambient N deposition on native plant communities and ecosystems.

2. This study investigates the relationship between atmospheric N deposition, plant and soil nutrient status and microbial enzyme activities at 32 lowland heathland sites across the UK. Sites were chosen to cover a range of N deposition rates (13.3–30.8 kg N ha<sup>-1</sup> year<sup>-1</sup>), geographical areas and different geologies.

3. Significant relationships were found between rates of N deposition (total, reduced and oxidized) and concentrations of N and phosphorus (P) in *Calluna* shoots, litter and soil; relationships were generally stronger with total and reduced forms of N, compared with oxidized N. Litter and humus layers were deeper at sites receiving higher atmospheric N inputs, suggesting increased rates of soil carbon and N accumulation, despite higher levels of phenol-oxidase activity (implying faster rates of organic matter decomposition) at these sites.

4. The combination of elevated plant and soil P concentrations at sites receiving greater N inputs suggests strongly that N is increasing the availability and uptake rates of P, in addition to N. Furthermore, significant interactions between temperature and N deposition on indices relating to productivity and the turnover and uptake of nutrients highlight the influence of climate on ecosystem response to N deposition.

5. *Synthesis*: Field-scale evidence of changes in rates of nutrient cycling, organic matter accumulation and plant biochemistry suggests that ambient levels of N deposition are affecting the functioning of many heathland ecosystems and that the magnitude of these effects is also influenced by climate. Since such changes are known to be associated with reduced resistance to environmental stress and loss of plant diversity, current (and predicted future) levels of N deposition are likely to have important implications for the conservation and long-term sustainability of nutrient-poor ecosystems, particularly in the face of climate change.

**Key-words:** bioindicators, *Calluna*, climate change, foliar N/P ratio, oxidized and reduced nitrogen, P limitation, phenol-oxidase, phosphomonoesterase, plant–soil (below-ground) interactions

## Introduction

Elevated nitrogen (N) deposition is a major driver of change in plant communities and the fungal and bacterial assemblages that support them (Treseder 2008). Ecosystem-level effects of elevated N deposition include biodiversity loss (Bobbink *et al.*

2010; Duprè *et al.* 2010) and changes in ecosystem function (Galloway *et al.* 2003), for example, changes in the biogeochemical cycling of carbon (C) and N, with associated increases in primary productivity and greenhouse gas emissions (Treseder 2008; Thomas *et al.* 2010). Globally, deposition rates of N are predicted to almost double by 2050 (Galloway *et al.* 2004; Dentener *et al.* 2006), threatening those plant communities that are adapted to low levels of N availability. Within Europe and North America, temperate forests, grasslands, dunes, bogs, fen, tundra and heathland ecosystems

\*Correspondence author. E-mail: s.power@imperial.ac.uk

†Present address: Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Ceredigion SY23 3DA, UK.

are known to be affected by current levels of N deposition (Aber *et al.* 2003; Clark & Tilman 2008; Bobbink *et al.* 2010). Indeed, field-scale manipulation experiments have shown that plant growth, biochemistry and phenology are altered by elevated N inputs, often resulting in increased sensitivity to biotic and abiotic stresses and changes in competitive dynamics of plant communities (e.g. Bobbink 1998; Bobbink, Hornung & Roelofs 1998; Power, Ashmore & Cousins 1998a; Power *et al.* 1998b; Pilkington *et al.* 2005a).

Soil, litter and plant nutrient concentrations, Ellenberg N indices and rates of soil and litter enzyme activity can each serve as potential indicators of ecosystem N status and/or of bio(geo)chemical responses to N deposition (Sutton, Pitcairn & Whitfield 2004). For example, experimental N addition has been shown to increase tissue N concentrations in a wide range of ecosystems, including temperate forest (Pitcairn *et al.* 1998; Aber *et al.* 2003), oligotrophic bogs (Bragazza *et al.* 2004), *Calluna* heath (Hicks *et al.* 2000; Rowe *et al.* 2008; Power & Collins 2010), coastal sand dunes (Jones *et al.* 2004) and grasslands (Stevens *et al.* 2004, 2006). Furthermore, faster rates of litter accumulation (Power, Ashmore & Cousins 1998a; Crossley *et al.* 2001) and decomposition (Barker 2001), as well as increased phosphomonoesterase (PME) enzyme activities (Phoenix *et al.* 2003; Pilkington *et al.* 2005b) and higher levels of extractable nutrients (van der Eerden *et al.* 1991), have been reported for heathland and grassland habitats in response to experimental N inputs. However, although these responses have been consistently found in N manipulation field experiments, they represent only a relatively small number of sites on a limited range of geologies and soil types. A key question is therefore whether similar relationships exist at larger spatial scales, across a wide range of climatic and edaphic conditions, and thus whether there is evidence of impacts of N deposition on ecosystem functioning in the wider field environment.

Field surveys along gradients of N deposition have demonstrated a negative relationship between N inputs and plant diversity in nutrient-poor grassland and heathland systems (e.g. Stevens *et al.* 2006; Maskell *et al.* 2010). However, surveys have only rarely looked at changes in bio(geo)chemical indicators that relate to the cycling and availability of nutrients responsible for driving observed changes in plant communities. Notable exceptions include the study by Stevens *et al.* (2006) which reported increases in soil extractable  $\text{NH}_4$  in UK grasslands receiving higher N inputs that reflected changes in biodiversity and plant community composition. Increases in soil extractable N and changes in the arbuscular mycorrhizal community have also been reported along a deposition gradient in a survey of coastal sage scrub vegetation in southern California (Allen *et al.* 1998; Egerton-Warburton & Allen 2000). Whilst enzyme assays have not been used widely to assess the levels of nutrient demand in field surveys, Edmondson *et al.* (2010) showed that litter phenol-oxidase enzyme activity (as well as exchangeable N) decreased along a gradient of N deposition in a recent survey of UK upland moorlands.

In this paper, we report the results of a wide-scale survey of British lowland heathlands along a gradient of ambient N deposition to evaluate the wider impacts of N deposition on

the functioning of heathland ecosystems. The aims of this study were to (i) test whether biochemical indicators identified from manipulation experiments show similar patterns in relation to N deposition in the field, across a range of geologies and soil types, and (ii) determine the relationship between ambient N deposition and soil/litter enzyme activities as an indication of nutrient demand and mobilization rates across a wide range of heathland sites throughout the UK.

## Materials and methods

### SURVEY SITES AND SAMPLING

Samples of plant tissue, litter and soil were collected from 32 heathland sites (Fig. 1) along an N deposition gradient ranging from 13.3 to  $30.8 \text{ kg ha}^{-1} \text{ year}^{-1}$ . Sites were selected to maximize the range of N deposition inputs and to include a number of different geologies and soil types within the survey. At each site, a  $25 \times 25 \text{ m}$  quadrat was positioned randomly in a heathland patch dominated by building-phase (*sensu* Gimingham 1972) *Calluna vulgaris* (L.) Hull (henceforth referred to as *Calluna*), characterized by vigorous growth and a closed canopy. Sample collection took place in late October 2007 to avoid growth dilution effects that could influence tissue nutrient concentrations (Sutton, Pitcairn & Whitfield 2004). The length of current year's *Calluna* shoots and depth of the litter layer were measured at 25 regularly distributed points within the ( $25 \times 25 \text{ m}$ ) sampling area. Soil samples (0–2 and 2–5 cm deep) were also collected at these 25 locations and bulked to form a single sample for each depth, per site. During soil sampling, proximate samples of the litter layer and the

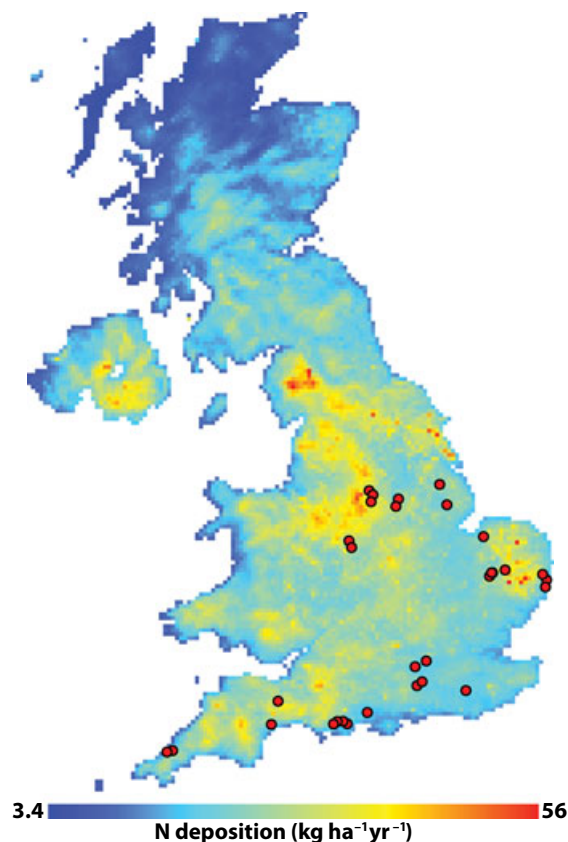


Fig. 1. Location of the 32 lowland heathland sample sites in relation to total N deposition.

bryophyte *Hypnum jutlandicum* were also taken, where present. At each site, a representative, current year shoot sample was collected from 100 plants, along a regular grid pattern within the 25 × 25 m sampling area. Humus and soil profile measurements were made from a single, central soil pit. All samples were stored in zip-lock bags and kept in a cooler box during transit back to the laboratory where they were refrigerated at 4 °C prior to analysis.

Geological information was obtained from the British Geological Society (BGS, 2007). The soil type for each site was determined using mapping data by the Soil Survey of England & Wales (1980) and verified using soil profile observations from the field. Sites were assigned to one of six categories for geology: chalk, clay, gravel, gritstone, sand and sandstone. Soil types were categorized into groups defined as organic-sand, organic and sand; seven sites were excluded from the soil type analysis due to their being either from poorly defined soils or the sole representatives of widely differing soil type categories.

#### SAMPLE PREPARATION AND ANALYSIS

Fresh soil samples were sieved (<2 mm) to homogenize material and remove any small stones and larger root matter. The 0–2 cm depth sample and the fresh litter were used straight away (typically within 64 h of field collection) for biochemical assays that require material at field moisture levels. The remaining soil, litter, *Calluna* shoot and bryophyte samples were dried at 60 °C for 48 h. Dried samples were then ball-milled to produce a homogeneous fine powder.

Leaf and soil samples were digested using 3 mL H<sub>2</sub>SO<sub>4</sub> + Se catalyst tablet per 200 mg sample at 400 °C for 2 (leaf) or 3 (soil) h in a Kjeltac Digester block (Model 2020 Digester; Tecator AB, Hoganas, Sweden), following the Kjeldahl N or P digestion method of Allen *et al.* (1974). The digests were then analysed for ammonium (NH<sub>4</sub><sup>+</sup>) and phosphate (HPO<sub>4</sub><sup>3-</sup>) concentrations using a Skalar 'San-Flow' automated segmented flow colorimetric analyser [Skalar UK (Ltd), York, UK].

Concentrations of extractable ammonium and nitrate in soils and litter were determined by Skalar colorimetric analysis, following extraction in 1 mol L<sup>-1</sup> KCl (Allen *et al.* 1974). Phosphate concentrations were measured by Skalar analysis following extraction in 0.01 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> using the Truog's method (Allen *et al.* 1974). Soil and litter PME enzyme activities were quantified using the method of Tabatabai & Bremner (1969), which measures the enzymic decomposition of the organic phosphate source, *para*-nitrophenyl phosphate (PNP-P) to *para*-nitrophenol (PNP). The pigment density of PNP was measured using a Perkin Elmer Lambda 3 UV spectrophotometer (Perkin Elmer, MA, USA) at 400 nm. Soil and litter phenol-oxidase enzyme activities were assayed using the method of Pind, Freeman & Lock (1994). This measures the rate of formation of 2-carboxy-2, 3-dihydroindole-5,6-quinone (dicq), the absorbance of which was measured at 460 nm using the spectrophotometer described above. Soil organic matter (SOM) content was determined by the combustion of dried soil to ash at 400 °C for 8 h (Allen *et al.* 1974).

#### STATISTICAL ANALYSES

The annual mean N deposition values (2004–06), modelled at a resolution of 5 km<sup>2</sup>, were provided by the Centre for Ecology & Hydrology, Edinburgh (Rognvald Smith, pers. comm.). Climate data were taken from the UKCP09 2006 monthly gridded data sets available on request from the UK Meteorological Office (Met Office, Exeter, UK). The data used were mean monthly precipitation amount (mm)

and mean monthly air temperatures (°C). All statistical analyses were carried out using the R statistical package version 2.12.2 (R Development Core Team, 2011). Outliers were initially removed from the data set using Dixon's Q test for outliers, if significant at the 99% confidence interval (Ellison, Barwick & Duguid Farrant 2009). Relationships between continuous variables were tested using a combination of univariate linear and multivariate generalized linear models (GLMs). For linear regressions, the entire data set was log-transformed to meet the assumptions of normality and homogeneity of variance. GLMs used untransformed data with error correction terms applied to the model to ensure that models were not overdispersed. Likelihood ratio deletion tests were used to derive the minimum adequate model (Crawley 2007). For each response variable, two GLMs were fitted: the first using temperature, rainfall and total N deposition and the second using temperature, rainfall, oxidized and reduced N deposition as explanatory variables. Since total N deposition is the sum of oxidized and reduced N, these three variables could not all be included in the same model. Use of these two GLM approaches allowed the evaluation of relationships with overall rates of N deposition as well as the assessment of the relative strengths of relationships with oxidized and reduced forms of N deposition. ANOVA was used to test the strength of relationships between continuous data and categorical variables (i.e. geology and soil type).

#### Results

Significant relationships between climatic variables, N deposition and plant, litter and soil characteristics are summarized in Tables 1 and 2. Most of the relationships were in a positive direction, with a greater number of (generally more significant) relationships seen for total N and reduced N deposition than for oxidized N.

#### PLANT TISSUE CHEMISTRY AND GROWTH

Analysis using generalized linear models revealed a significant relationship between *Calluna* foliar N concentration and total N deposition (Table 1, Fig. 2a), while foliar P concentrations were significantly related to both total and reduced N (Fig. 2b). In both cases, foliar concentrations were higher at sites with higher N deposition inputs. The optimal model accounting for variation in foliar N/P ratios included the effects of temperature (positive), total N (negative) and a significant interaction between these two variables (Table 1). Foliar N/P ratios were lowest at sites with high rates of total N deposition and low annual mean temperatures (Fig. 2c). A similar relationship between N/P ratios, reduced N and temperature was also apparent; interactions between these two were statistically significant, despite non-significant relationships with either variable on their own (Table 1). There were no significant relationships between bryophyte (*H. jutlandicum*) tissue N or P concentrations and either N deposition or climate variables (Fig. 2d).

Field-based measurements of *Calluna* shoot length and canopy height were related to N deposition and climatic variables, although neither explanatory variable was significant on its own and the most appropriate GLMs involved interactions between these in all cases. For canopy height and total N depo-

**Table 1.** Summary table of optimal GLMs for plant variables. Model 1 – total N deposition with climate; Model 2 – oxidized and reduced N with climate explanatory variables

Variable	Model 1: Total N deposition & climate				Model 2: Oxidized/reduced N & climate			
	d.f.	Optimal model	<i>F</i>	<i>P</i>	d.f.	Optimal model	<i>F</i>	<i>P</i>
<i>Calluna</i> foliar N	30	Total N	5.9	< 0.05	–	–	–	n/s
<i>Calluna</i> foliar P	30	Total N	8.7	< 0.01	30	Reduced N	9.0	< 0.01
<i>Calluna</i> foliar N/P	30	Temperature	1.4	n/s	29	Temperature	1.4	n/s
	29	Total N	2.1	n/s	28	Reduced N	1.7	n/s
	28	Temperature × total N	6.6	< 0.05	27	Temperature × reduced N	6.1	< 0.05
<i>Hypnum jutlandicum</i> tissue N	–	–	–	n/s	–	–	–	n/s
<i>H. jutlandicum</i> tissue P	–	–	–	n/s	–	–	–	n/s
Shoot length	28	Temperature	1.5	n/s	28	Temperature	1.7	n/s
	27	Total N	0.1	n/s	27	Oxidized N	0.01	n/s
	26	Temperature × total N	4.4	< 0.05	26	Temperature × oxidized N	7.6	< 0.05
Canopy height	28	Temperature	5.6	< 0.05	28	Temperature	5.1	< 0.05
	27	Rainfall	3.4	n/s	27	Oxidized N	0.0	n/s
	26	Total N	0.3	n/s	26	Temperature × oxidized N	6.1	< 0.05
	22	Temperature × rainfall × total N	5.4	< 0.05				

GLM, generalized linear models.

**Table 2.** Summary table of optimal GLMs for litter and soil variables: Model 1 – total N deposition with climate and Model 2 – oxidized and reduced N with climate explanatory variables

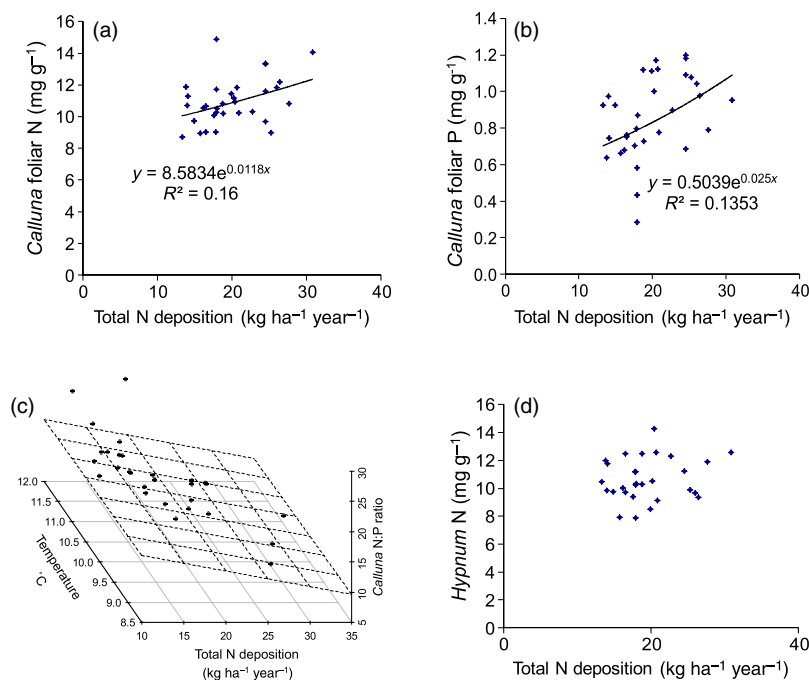
Variable	Model 1: Total N deposition & climate				Model 2: Oxidized/reduced N & climate			
	d.f.	Optimal model	<i>F</i>	<i>P</i>	d.f.	Optimal model	<i>F</i>	<i>P</i>
Litter N	27	Total N	6.8	< 0.05	27	Reduced N	4.8	< 0.05
Litter extractable NH <sub>4</sub>	–	–	–	n/s	–	–	–	n/s
Litter extractable NO <sub>3</sub>	–	–	–	n/s	–	–	–	n/s
Litter P	27	Total N	5.6	< 0.05	27	Temperature	2.0	n/s
					26	Oxidized N	0.7	n/s
					25	Temperature × oxidized N	4.7	< 0.05
Litter extractable PO <sub>4</sub>	–	–	–	n/s	–	–	–	n/s
Litter PME activity	–	–	–	n/s	27	Reduced N	8.2	< 0.01
Litter Phenox activity	27	Temperature	3.2	n/s	27	Temperature	4.6	< 0.05
	26	Total N	1.4	n/s	26	Oxidized N	1.6	n/s
	25	Temperature × total N	8.8	< 0.01	25	Temperature × oxidized N	24.1	< 0.001
Soil N	–	–	–	n/s	29	Temperature	3.4	n/s
					28	Oxidized N	0.6	n/s
					27	Temperature × oxidized N	4.3	< 0.05
Soil extractable NH <sub>4</sub>	–	–	–	n/s	–	–	–	n/s
Soil extractable NO <sub>3</sub>	–	–	–	n/s	–	Reduced N	5.3	< 0.05
Soil P	30	Total N	27.0	< 0.001	30	Reduced N	14.1	< 0.001
Soil extractable PO <sub>4</sub>	27	Total N	5.0	< 0.05	27	Oxidized N	6.4	< 0.05
Soil PME activity	–	–	–	–	–	–	–	n/s
Soil Phenox activity	–	–	–	–	29	Oxidized N	7.9	< 0.001
Litter depth	28	Total N	6.1	< 0.05	28	Reduced N	6.8	< 0.05
Humus depth	24	Total N	9.9	< 0.01	24	Temperature	5.8	< 0.05
					23	Reduced N	1.1	n/s
					22	Temperature × reduced N	5.7	< 0.05

GLM, generalized linear models; PME, phosphomonoesterase.

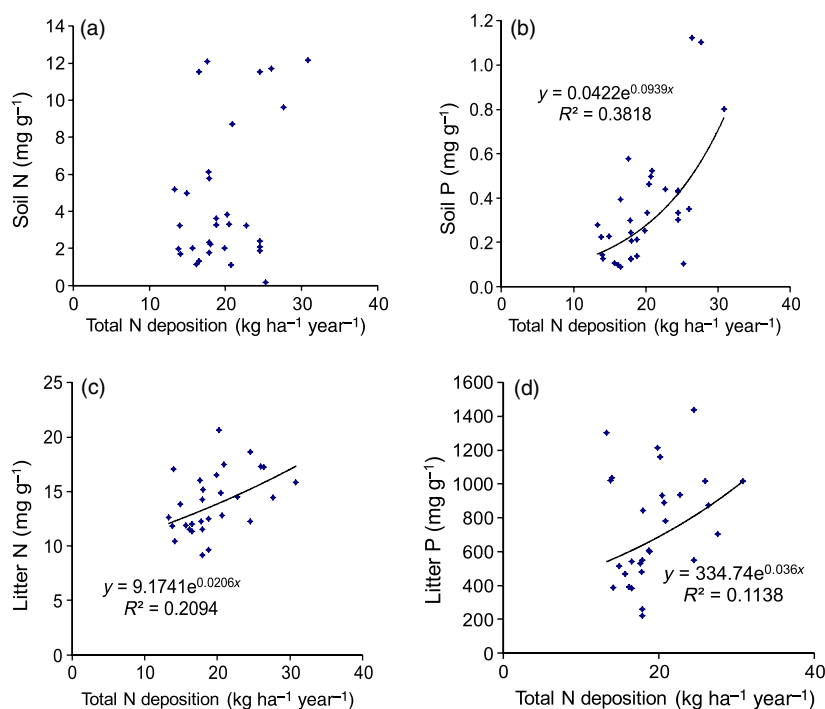
sition (model 1), the optimal model included interactions with temperature (negative) and rainfall (positive), with the smallest canopies in areas of high N deposition, low rainfall and high temperature. For shoot length, a similar interaction between total N and temperature was seen, with the lowest values

recorded at warmer sites receiving high deposition inputs. Model 2 GLMs indicated significant interactions between oxidized N deposition and temperature, with the highest values for both shoot length and canopy height at cooler sites receiving lower oxidized N inputs (Table 1).





**Fig. 2.** Relationships between total N deposition and plant tissue chemistry: (a) *Calluna* foliar N, (b) *Calluna* foliar P, (c) *Calluna* foliar N/P ratios (significant interaction with temperature) and (d) *Hypnum jutlandicum* tissue N (no significant relationship).



**Fig. 3.** Relationships between total N deposition and concentrations of (a) soil N (no significant relationship), (b) soil P, (c) litter N and (d) litter P.

#### CHEMICAL AND PHYSICAL CHARACTERISTICS OF LITTER AND SOIL

Generalized linear models analysis revealed statistically significant interactions between oxidized N inputs and temperature for total soil N, although the relationships with N deposition were not significant on their own (Fig. 3a). The highest soil N concentrations were found at cooler sites receiving higher deposition inputs (Table 2). Soil P concentrations were significantly (positively) related to both total N and reduced N depo-

sition (Table 2, Fig. 3b), as was also the case for litter N concentrations (Table 2, Fig. 3c). The best-fit model for litter P concentrations involved total N deposition (Table 2, Fig. 3d). However, a model involving oxidized N deposition with temperature as an interacting term was also significant (Table 2), indicating that the highest litter P concentrations were found at warmer sites receiving higher oxidized N inputs. There were no significant relationships between N deposition and concentrations of extractable NO<sub>3</sub>, NH<sub>4</sub> or PO<sub>4</sub> in litter samples. Soil extracts provided some evidence of an influence

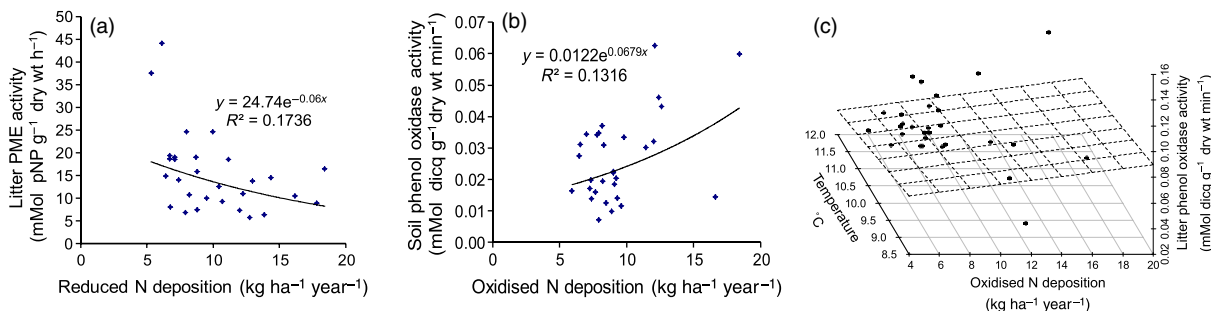


Fig. 4. Scatter plots of the relationship between enzyme activities and N deposition: (a) litter phosphomonoesterase enzyme (PME) activity and reduced N, (b) soil phenol-oxidase activity and oxidized N, (c) litter phenol-oxidase and oxidized N (significant interaction with temperature).

of N deposition on nutrient availability, with lower concentrations of  $\text{NO}_3$  at sites receiving greater inputs of reduced N, and higher soil concentrations of  $\text{PO}_4$  at sites in areas of high oxidized N deposition (Table 2).

Litter depth was greater at sites with higher rates of both total N (model 1) and reduced N (model 2) deposition. The humus layer was also deeper at sites with higher inputs of total N. In the second model, although reduced N was not significant on its own, there was a significant interaction between reduced N and temperature, with smallest humus layer depths at warmer sites with low reduced N inputs (Table 2).

#### SOIL AND LITTER ENZYMES

Phosphomonoesterase enzyme activity in litter samples was significantly lower at sites with higher inputs of reduced N (Table 2, Fig. 4a); similar patterns in enzyme activity were seen in soil samples, although these were not statistically significant. Soil phenol-oxidase activity was significantly higher at sites receiving high rates of oxidized N deposition ( $P < 0.001$ , Fig. 4b). The best-fit models from GLM analysis of litter phenol-oxidase activity suggest positive interactions between temperature and total N (model 1), and temperature and oxidized N (model 2, Table 2), although the model with oxidized N was stronger overall. Model outputs indicated that the highest rates of litter enzyme activity occurred at cooler sites receiving higher N inputs (Fig. 4c).

#### RELATIONSHIPS BETWEEN SOIL/LITTER ENZYMES AND SOIL/PLANT CHEMISTRY

Table 3 summarizes statistical analyses of relationships between enzyme activities and soil, litter and plant chemistry. Soil and litter PME activities were both lower at sites where *Calluna* tissue P concentrations were highest [ $P < 0.01$  (soil),  $P < 0.001$  (litter)]. Strong, positive relationships were also found between soil PME activity and both extractable ( $P < 0.001$ ) and total ( $P < 0.01$ ) N concentrations in the soil. However, no relationship was apparent between soil PME activity and either extractable or total soil P concentrations.

Soil phenol-oxidase activity was higher at sites with greater concentrations of (total) soil P ( $P < 0.01$ ) and N

( $P < 0.001$ ), as well as at those sites with greater levels of extractable soil N ( $P < 0.01$ ). Similar patterns were seen for litter phenol-oxidase activity; enzyme activity was higher at sites with high litter concentrations of N ( $P < 0.001$ ) and P ( $P < 0.001$ ), as well as those where *Calluna* foliar P concentrations were greatest ( $P < 0.05$ ). As might be expected, activities of phenol-oxidase ( $P < 0.001$ ) and PME enzymes in soil samples ( $P < 0.01$ ) were strongly (positively) related to SOM content.

#### RELATIONSHIPS WITH GEOLOGY & SOIL TYPE

Foliar P ( $P < 0.01$ ), foliar N ( $P < 0.05$ ) and soil total P ( $P < 0.001$ ) concentrations were all significantly related to geology (Table 4). The highest mean foliar P concentration was found at sites over chalk ( $n = 6$ ), while the highest mean value for soil P was at sites on gritstone ( $n = 3$ , Table 4). Soil type also had a significant influence on soil N ( $P < 0.01$ ) and phenol-oxidase activity ( $P < 0.05$ ) with the highest values of both found in organic soils. No other indices of soil or plant chemistry were significantly related to soil type, although soil N and P concentrations and enzyme activities were generally highest in organic soils.

#### Discussion

Despite the large degree of inter-site variability that is inherent in surveys of this type, highly significant relationships were found between N deposition and multiple (bio)chemical indices in soil, litter and plant material. Most of the observed relationships with N deposition were positive, suggesting that increased N availability accelerates ecosystem uptake and turnover rates of both N and P. There were more, generally stronger, relationships with total N deposition than with reduced N, with oxidized N having fewer significant relationships overall. This trend is consistent with other studies that have explicitly contrasted responses to oxidized and reduced N (e.g. Stevens *et al.* 2006; van den Berg *et al.* 2008). The greater strength of relationships with reduced N may be explained in part by the additional acidifying effects of this form of N, compared with oxidized inputs. In common with many grassland, bog and dune systems, heathlands typically have nutrient-poor, acidic soils. Additional  $\text{NH}_4$ -derived acidity will increase

**Table 3.** Summary of relationships between enzyme activities and soil, litter and *Calluna* chemistry

	Soil phenol-oxidase <i>n</i> = 30	Litter phenol-oxidase <i>n</i> = 29	Soil PME <i>n</i> = 30	Litter PME <i>n</i> = 29	<i>Calluna</i> foliar N <i>n</i> = 30	<i>Calluna</i> foliar P <i>n</i> = 30
<i>Calluna</i> foliar N	n/s	n/s	n/s	n/s		
<i>Calluna</i> foliar P	n/s	+ <i>F</i> = 7.3 <i>R</i> <sup>2</sup> = 0.21	– – <i>F</i> = 17.2 <i>R</i> <sup>2</sup> = 0.38	– – – <i>F</i> = 18.8 <i>R</i> <sup>2</sup> = 0.41		
<i>Calluna</i> foliar N/P	n/s	n/s	n/s	n/s		
Litter extractable N	n/s	++ <i>F</i> = 13.4 <i>R</i> <sup>2</sup> = 0.21	n/s	n/s	n/s	n/s
Litter extractable P	n/s	++ <i>F</i> = 8.6 <i>R</i> <sup>2</sup> = 0.18	n/s	n/s	n/s	n/s
Soil extractable N	++ <i>F</i> = 12.0 <i>R</i> <sup>2</sup> = 0.33	n/s	++ <i>F</i> = 20.5 <i>R</i> <sup>2</sup> = 0.41	+++ <i>F</i> = 16.6 <i>R</i> <sup>2</sup> = 0.38	n/s	– <i>F</i> = 5.4 <i>R</i> <sup>2</sup> = 0.16
Soil extractable P	n/s	n/s	n/s	n/s	n/s	n/s
Soil total N	+++ <i>F</i> = 19.9 <i>R</i> <sup>2</sup> = 0.30			++ <i>F</i> = 13.5 <i>R</i> <sup>2</sup> = 0.16	n/s	n/s
Soil total P	++ <i>F</i> = 11.0 <i>R</i> <sup>2</sup> = 0.15				n/s	n/s

PME, phosphomonoesterase.

Significance and direction of relationships as follows: +/– *P* < 0.05, ++/– – *P* < 0.01, +++/– – – *P* < 0.001, n/s, non-significant. Shaded cells represent variable combinations where analyses of relationships were not appropriate.**Table 4.** ANOVA summary for geology and soil type, and category mean values (±SEM), for soil and foliar nutrient concentrations, and enzyme activities

	Soil N (mg kg <sup>–1</sup> )	Soil P (mg kg <sup>–1</sup> )	Foliar N (mg g <sup>–1</sup> )	Foliar P (mg g <sup>–1</sup> )	Soil PME (mMol PNP g <sup>–1</sup> dwt h <sup>–1</sup> )	Soil phenol-oxidase (mMol dicq g <sup>–1</sup> dwt min <sup>–1</sup> )
Geology ( <i>n</i> = 31)	n/s	*** <i>F</i> = 5.6	* <i>F</i> = 2.6	** <i>F</i> = 4.4	n/s	* <i>F</i> = 2.9
Chalk ( <i>n</i> = 6)	2477.7 ± 132.4	351.4 ± 40.6	12.01 ± 1.15	1.11 ± 0.17	14.1 ± 0.97	0.0230 ± 0.001
Clay ( <i>n</i> = 5)	4220.1 ± 221.7	235.8 ± 33.7	11.26 ± 2.05	0.9 ± 0.64	5.5 ± 1.33	0.0172 ± 0.005
Gravel ( <i>n</i> = 4)	2966.2 ± 134.4	166.2 ± 45.2	10.70 ± 1.5	0.7 ± 0.55	11.7 ± 2.89	0.0170 ± 0.003
Gritstone ( <i>n</i> = 3)	10889.7 ± 356.6	1008.5 ± 103.8	12.35 ± 2.30	0.90 ± 0.99	16.3 ± 1.23	0.0463 ± 0.003
Sand ( <i>n</i> = 8)	5082.2 ± 58.1	218.4 ± 47.0	10.16 ± 1.43	0.7 ± 0.19	23.8 ± 1.76	0.0343 ± 0.004
Sandstone ( <i>n</i> = 5)	5690.6 ± 62.5	419.3 ± 84.2	10.42 ± 1.98	0.8 ± 0.77	12.1 ± 1.51	0.0244 ± 0.005
	**					*
Soil type ( <i>n</i> = 25)	<i>F</i> = 5.1	n/s	n/s	n/s	n/s	<i>F</i> = 3.2
Organic-sand ( <i>n</i> = 7)	4282.3 ± 99.8	272.6 ± 45.3	10.79 ± 1.91	8.36 ± 0.98	17.7 ± 1.61	0.0290 ± 0.006
Organic ( <i>n</i> = 3)	11724.8 ± 123.4	467.7 ± 49.8	10.14 ± 2.34	7.14 ± 0.87	27.4 ± 3.89	0.0403 ± 0.004
Sandy ( <i>n</i> = 15)	4731.9 ± 73.8	250.1 ± 31.7	10.78 ± 1.87	8.96 ± 0.57	7.9 ± 3.33	0.0182 ± 0.005

PME, phosphomonoesterase; PNP, *para*-nitrophenol.Significance of relationships: \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001, n/s, non-significant.

the already relatively high level of H<sup>+</sup> and Al<sup>3+</sup> ion stress, and low availability of nutrient cations, with potentially detrimental effects on the rate of organic matter turnover and nutrient uptake. Plants such as *Calluna* are particularly well adapted to NH<sub>4</sub><sup>+</sup> uptake (de Graaf *et al.* 1998), so the dual acidifying and direct fertilizing effect of NH<sub>4</sub><sup>+</sup> may explain why slightly more, generally stronger relationships were found with reduced N, compared with oxidized N.

Although temperature was a significant factor when combined with N in several cases, and rainfall was signifi-

cant on one occasion (in combination with both N and temperature), there were no models where either temperature or rainfall was the only significant explanatory variable. The lack of significant relationships with rainfall indicates that this is not a major driver of process rates in the typically dry, sandy heaths covered in this survey. The appearance of temperature as a significant interaction term in several models does, however, indicate that climate modifies relationships with N, particularly for responses relating to productivity and enzyme activity.

Such relationships are known from the literature and are discussed alongside N effects in the relevant sections below.

#### PLANT GROWTH AND TISSUE CHEMISTRY

The strong positive relationship between *Calluna* N concentrations and N deposition reflects earlier findings from N manipulation experiments (Iason & Hester 1993; Bobbink, Hornung & Roelofs 1998; Power, Ashmore & Cousins 1998a; Carroll *et al.* 2003; Power *et al.* 2004; Britton, Helliwell & Fisher 2008) and field surveys along N deposition gradients (Hicks *et al.* 2000; Pitcairn *et al.* 2001; Rowe *et al.* 2008; Power & Collins 2010). Species-level increase in tissue N concentrations with increasing N deposition/addition is a phenomenon reported in many temperate ecosystems where this relationship has been investigated (Gilliam 2006). Elevated foliar N concentrations are also associated with increased sensitivity to biotic and abiotic stresses (e.g. van der Eerden *et al.* 1991; Nordin, Näsholm & Ericson 1998; Power *et al.* 1998b; Perkins *et al.* 2000; Green 2005), which implies detrimental effects of ambient N deposition at many of the sites surveyed in this study.

The interactions between temperature, rainfall and N deposition in the model for *Calluna* canopy height and (without rainfall) shoot length indicate that multiple environmental drivers contribute to the differences in plant performance observed between sites and that the impacts of N deposition on productivity depend on climatic conditions. The relationship between litter chemistry and N inputs reported here is not well documented in the literature. Higher shoot N and P concentrations will in part explain the patterns observed in litter, while the generally slow rate of decomposition and nutrient loss from recalcitrant *Calluna* litter (Gimingham 1972), combined with potentially retarded decomposition of lignin under high N conditions (e.g. Berg 2000; Carreiro *et al.* 2000; Moorhead & Sinsabaugh 2006), may also contribute to the observed patterns. Elevated litter N concentrations have been found in response to experimental N addition in upland and lowland heathland (Power *et al.* 1998b; Pilkington *et al.* 2005a), mires (Pancotto *et al.* 2010), grassland (Clark & Tilman 2008) and forest (McNulty *et al.* 2006) systems, as well as in a regional survey of UK moorlands (Edmondson *et al.* 2010), suggesting that percentage of N in litter is a fairly consistent indicator of N inputs across ecosystem types.

The lack of relationship between bryophyte tissue N and ambient N deposition in the current study is slightly surprising as strong correlations have been reported in earlier field transect studies along deposition gradients (e.g. Pitcairn, Fowler & Grace 1995; Pitcairn *et al.* 1998, 2003; Bragazza *et al.* 2004). Whilst there is evidence that bryophytes may become constrained by P limitation when N inputs are high (Phuyal *et al.* 2008), it is possible that confounding factors such as (micro)climatic effects on bryophyte growth (and associated nutrient dilution) and/or interception of deposited N by the (overstorey) *Calluna* canopy may have obscured relationships with N deposition.

Despite the modest range of N deposition at the survey sites (13.3–30.8 kg ha<sup>-1</sup> year<sup>-1</sup>), the relationships reported here are likely to apply to vegetation receiving inputs beyond this range. Impacts of N at both lower and higher rates of deposition are evident in the literature. For example, Yesmin, Gammack & Cresser (1996) found reduced levels of ericoid mycorrhizal infection along a deposition gradient of 2–10 kg N ha<sup>-1</sup> year<sup>-1</sup>, while Strengbom *et al.* (2003) reported a decline in the understorey ericaceous shrub community of a Swedish boreal forest along a deposition gradient of < 3–12 kg N ha<sup>-1</sup> year<sup>-1</sup>. These studies illustrate how even relatively low rates of ambient N deposition can lead to a significant change in community composition and species abundances. While the mechanisms driving compositional changes have not been explicitly investigated in most of the reported studies, it is reasonable to assume that biogeochemical components, similar to those reported by our study, underlie many of the observed responses.

At the other end of the scale, sites that have experienced elevated N inputs over prolonged periods typically have an impoverished flora, with most studies showing lower community-level responses to changes in deposition inputs at higher N loads (e.g. Stevens *et al.* 2006; Clark & Tilman 2008). Where reductions in plant and fungal diversity have been reported in response to high rates of experimental N addition (e.g. Eger-ton-Warburton & Allen 2000; Strengbom *et al.* 2001), these studies have typically been at sites with relatively low ambient rates of deposition and which may therefore have a more diverse existing flora to respond to changes in deposition inputs. At sites that have experienced high ambient inputs of N over prolonged periods, a lack of plant community response to experimental N addition can be attributed to pre-existing N saturation of soils (e.g. Gilliam 2006), with community-level responses to N appearing to be a function of site deposition histories (Hurd, Brach & Raynal 1998). However, although changes in plant community composition and, in particular, diversity appear to saturate at modest deposition inputs, there is evidence from field surveys that both higher and lower plants continue to accumulate N in foliage (and, by extension, litter) at rates of up to c. 50 kg ha<sup>-1</sup> year<sup>-1</sup> (Pitcairn *et al.* 2003) or even c. 100 kg ha<sup>-1</sup> year<sup>-1</sup> (Pitcairn *et al.* 2006). Furthermore, experiments involving high rates of N addition have shown elevated tissue N concentrations, increased rates of enzyme activity and changes in nutrient cycling (Carreiro *et al.* 2000; Pilkington *et al.* 2005b; Allison *et al.* 2009), which imply that the biogeochemical responses observed in the current field survey would still be apparent at sites receiving the upper end of ambient rates of deposition across Europe.

The highest *Calluna* P concentrations were associated with chalk geology. Chalk is an ancient marine substrate relatively rich in P (White 1997), the presence of which appears to increase plant-available P, despite the generally low phosphorus sorption capacity of the well-drained, sandy soils that are typical of lowland heaths and many acidic grasslands (Chapman, Rose & Basanta 1989). Although the association between N deposition and foliar N concentrations is well-established from field experiments and surveys, positive relationships



between N deposition and foliar P concentrations have only recently begun to emerge in the literature. For example, Rowe *et al.* (2008) and Power & Collins (2010) reported significantly higher *Calluna* P concentrations at sites receiving higher N inputs in recent surveys along national and regional deposition gradients. Bethers *et al.* (2009) also found evidence of elevated foliar P concentrations in maple trees from a forest catchment receiving experimental ammonium sulphate additions (*c.* 25 kg N ha<sup>-1</sup> year<sup>-1</sup>), although they noted that soil acidification associated with this form of N input could also have contributed to the observed response. Accelerated rates of organic matter turnover and/or increased mycorrhizal activity have been proposed as mechanisms promoting enhanced uptake of P under elevated N inputs, and it seems likely that similar mechanisms may explain the observed relationships in the current survey.

Interestingly, foliar N/P ratios were not related to N deposition in this survey, although there were significant interactions between temperature and both total and reduced N. These suggest that the rate of increase in foliar P compared with N under higher rates of N deposition may be slower at warmer sites. Although N/P ratios of *Calluna* have been shown to increase with N addition in manipulation experiments (e.g. Carroll *et al.* 1999; von Oheimb *et al.* 2010) and with NO<sub>x</sub> deposition in a field survey (Kirkham 2001), the results from the study by Rowe *et al.* (2008) and Power & Collins (2010), and findings from the current study, suggest that N/P ratios are not consistent indicators of inputs of, or responses to, N deposition at the wider scale.

#### EFFECTS OF N DEPOSITION ON SOIL NUTRIENT AVAILABILITIES AND ENZYME ACTIVITIES

The lack of any consistent relationship between N deposition and extractable (plant-available) N concentrations in the current study contrasts with surveys elsewhere in temperate forest (e.g. Magill *et al.* 1997; Lovett & Rueth 1999), grassland (Stevens *et al.* 2006) and moorland (Edmondson *et al.* 2010) habitats. Soil nutrient availability is, however, dependent on seasonal variations in plant and microbial immobilization rates (Taylor, De-Felice & Havill 1982), the composition and activity of the soil microbial community (Muruganandam, Israel & Robarge 2009) and litter/substrate quality (McClaugherty, Pastor & Aber 1985). The high temporal and spatial variability in nutrient availability both within and between sites means that this measure is not a robust indicator of effects of N deposition at the ecosystem level and that other, more integrated responses (e.g. plant and litter nutrient concentrations) may provide a better indication of long-term effects on the functioning of (semi-)natural ecosystems.

Freeman, Ostle & Kang (2001) suggested that phenol-oxidase is the biochemical 'latch' facilitating lignin decomposition and mobilizing nutrients from dead plant matter. The current survey suggests that elevated N inputs are accelerating phenol-oxidase-mediated organic matter (and therefore nutrient) turnover in the field, but that this stimulation is reduced under warmer conditions. The relationship with N deposition

contrasts with findings from a recent survey of UK moorland ecosystems (Edmondson *et al.* 2010) and observations of reduced litter turnover rates in forest ecosystems with increased N inputs (Carreiro *et al.* 2000; Sinsabaugh, Carreiro & Report 2002; DeForest *et al.* 2004). A meta-analysis by Knoor, Frey & Curtis (2005) highlighted the highly variable effects of N on rates of litter decomposition, suggesting that N stimulates decomposition only at very low ambient deposition rates (< 5 kg ha<sup>-1</sup> year<sup>-1</sup>) and where litter quality is high (i.e. low lignin content). Given the high lignin content of *Calluna* litter and the N deposition range (13.3–30.8 kg ha<sup>-1</sup> year<sup>-1</sup>) in the current survey, our findings contrast with expectations based on the meta-analysis by Knoor, Frey & Curtis (2005). This may in part be explained by the fact that, in the current study, samples were taken from the top 2 cm of soil and litter layers and may not be representative of the entire pools. For litter phenol-oxidase activity, the significant interaction with temperature in the current study suggests that controls on decomposition are complex and that N-driven stimulation in turnover rates of organic matter would be suppressed under the warmer conditions associated with climate change predictions.

The positive relationships between N deposition, foliar P, litter P and soil extractable P suggest that N increases the availability and uptake of P. Although this is not supported by higher rates of PME activity in the current survey, the observed negative relationship between litter PME activity and reduced, but not oxidized or total, N deposition suggests that acidity associated with reduced N inputs (now the dominant form of deposited N in many parts of Europe) may in fact suppress enzyme activity. N addition experiments that have reported a stimulation in phosphatase activity have typically added N in the oxidized (NaNO<sub>3</sub>) or balanced (NH<sub>4</sub>NO<sub>3</sub>) form (e.g. Johnson *et al.* 1998; Phoenix *et al.* 2003; Pilkington *et al.* 2005b; Gress *et al.* 2007), which has the opposite (oxidized) or no (balanced) net effect on soil acidity.

Considered together, the combination of enhanced plant, litter and soil N and P indices provides compelling evidence that elevated ambient N deposition (i) drives biochemically mediated increases in nutrient availability, leading to (ii) enhanced rates of plant uptake and (iii) faster turnover rates of N and P. Similar interpretations have been suggested by Rowe *et al.* (2008) in a survey of UK moorlands and also by Perring *et al.* (2008). Despite the often cited phenomenon of progressive P limitation in response to prolonged N loading (e.g. Phuyal *et al.* 2008; Braun *et al.* 2009), there is little evidence that ambient rates of N deposition are increasing P limitation at the sites in this national-scale survey.

#### Conclusions

This survey suggests a number of sensitive biogeochemical indicators of, and responses to, N deposition within a modest ambient deposition range (13.3–30.8 kg N ha<sup>-1</sup> year<sup>-1</sup>). These include N and P concentrations in *Calluna* shoots, litter and (to a lesser extent) soil, as well as litter enzyme activities. Many of these responses correspond with those reported from

manipulation experiments and surveys of ecosystems elsewhere. Surprisingly, however, indices relating to the availability and uptake of P are amongst the most significant responses found along a national-scale gradient of N deposition. Although there is now evidence that P-limited heathland and grassland ecosystems can respond negatively to N deposition (Green 2005; Horswill *et al.* 2008), the survey data presented here imply that elevated levels of N deposition facilitate increased mobilization, availability and uptake of P, regardless of the nature of nutrient limitation.

Taken together, the results of this survey suggest that despite differences in underlying geology, soil type and climate, ambient rates of N deposition are having substantial effects on biogeochemical processes and the functioning of nutrient-poor heathland ecosystems. Given that N deposition rates in Europe and the USA are unlikely to decline significantly in the next few decades (Galloway *et al.* 2004), this has implications for habitat conservation and the preservation of important ecosystem services, not only in heathlands, but also potentially in similar sensitive ecosystems throughout the northern hemisphere. Furthermore, significant interactions between N deposition and temperature found in the current study suggest that the effects of N on many ecosystem processes will also be modified by predicted future changes in climate.

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