REPORT

Functional Relationships with N Deposition Differ According to Stand Maturity in *Calluna*-Dominated Heathland

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Abstract Plant and soil bio(chemical) indicators are increasingly used to provide information on N deposition inputs and effects in a wide range of ecosystem types. However, many factors, including climate and site management history, have the potential to influence bioindicator relationships with N due to nutrient export and changing vegetation nutrient demands. We surveyed 33 heathlands in England, along a gradient of background N deposition ($7.2\text{--}24.5\text{ kg ha}^{-1}\text{ year}^{-1}$), using *Calluna vulgaris* growth phase as a proxy for time since last management. Our survey confirmed soil nutrient accumulation with increasing time since management. Foliar N and phosphorus (P) concentrations in pioneer- and mature-phase vegetation significantly increased with N deposition. Significant interactions between climate and N deposition were also evident with, for example, higher foliar P concentrations in pioneer-phase vegetation at sites with higher temperatures and N deposition rates. Although oxidized N appeared more significant than reduced N, overall there were more, stronger relationships with total N deposition; suggesting efforts to control all emissions of N (i.e., both oxidized and reduced forms) will have ecological benefits.

Keywords Habitat management · Oxidized N · Reduced N · Lowland heathland · *Calluna* growth phase · Bioindicators

INTRODUCTION

Nitrogen (N) deposition remains a significant influence on semi-natural managed heathland plant communities distributed across highly populated and industrialized regions of north-western Europe (Phoenix et al. 2012). These globally important habitats are restricted to a narrow area defined by climate and soil type, and considered to be of high conservation value (JNCC 2010). Eutrophication of lowland heathlands by N deposition in the Netherlands has driven the conversion of *Calluna vulgaris* (L.) Hull (henceforth referred to as *Calluna*) dominated heathlands, to graminoid communities over the last 25 years (Aerts and Berendse 1988) and N eutrophication remains an important risk factor for heathland across Europe (Bobbink et al. 2010). Management of heathland exports N by removing biomass and litter, typically via mowing, grazing, or burning, and is considered an important tool to help minimize impacts from accumulated N deposition (Power et al. 2001; Terry et al. 2004; Hardtle et al. 2006). With increasing time since management, N accumulates in vegetation biomass, litter, and soil (Pilkington et al. 2005; Power et al. 2006), enhancing overall ecosystem nutrient status and increasing the risk of habitat degradation. Inadequate frequencies and/or intensities of management enable N inputs to accumulate and are likely to have contributed strongly to the scrub-invasion that has occurred in some heathlands in the UK (Price 2003).

N deposition inputs may alter the rate of vegetation development. For instance, recently managed *Calluna* stands have been found to respond strongly to N inputs, compared with unmanaged vegetation (Britton and Fisher 2008). Management also directly influences the physical structure and growth rates of regenerating plants, thereby modifying N cycling within heathland systems.

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Gimingham (1972) describes four clearly defined growth phases within the *Calluna* life-cycle with different traits. Initially, regenerating stands of juvenile vegetation grow rapidly during the “pioneer” phase, where greater demand for nutrients exists (per unit biomass) than at any other point (Barclay-Estrup 1970). As *Calluna* stands develop from “building-” and into “mature-” phases, growth rates decline and nutrients accumulate in litter and soil. Similar patterns are also seen in other managed ecosystems, such as temperate forests subject to periodic harvesting, where nutrient accumulation in soils is also associated with greater N deposition impacts over time (Aber 1992).

To date, N deposition experiments manipulating N in heathlands have typically focused on building-, or mature-phase *Calluna* (e.g., Johnson et al. 1998; Power et al. 1998). Survey studies along gradients of N deposition have also generally targeted building- (Rowe et al. 2008; Jones and Power 2012) or mature-phase (Pitcairn and Fowler 1995; Southon et al. 2013) vegetation, with surveys often having less control over stand age. It is not currently known how age affects the response of *Calluna*—a keystone, indicator species within heathland ecosystems—to N deposition. However, with increasing use of bioindicator approaches to evaluate both N deposition inputs and effects, involving foliar- or soil nutrient concentration, plant community composition, and soil enzyme activities (Jones and Power 2012; Payne et al. 2013; Southon et al. 2013), it is important to understand how these may be modified by vegetation age and/or management history. Our study presents novel information on bioindicator relationships with N deposition, by examining combined plant and soil characteristics for heathland vegetation from contrasting vegetation phases, and from sites with different geologies, across a moderate gradient of N deposition.

Evidence from other semi-natural ecosystems suggests that management frequency directly alters N deposition impacts. For example, Jacquemyn et al. (2003) concluded that regular, high-intensity management maintained forb, and grass diversity in a calcareous grassland subject to elevated N inputs, while Payne et al. (2011) found that the effects of N addition depended strongly on grazing intensity in a UK grassland. Furthermore, in temperate forests, the effects of chronic N saturation in soils and biomass have been shown to be alleviated by harvesting, particularly in more mature forests (Fenn et al. 1998). Although examples are limited, such studies collectively demonstrate that the effects of chronic N inputs can be alleviated by an appropriate management regime, reducing the nutrient accumulation that results from prolonged atmospheric N inputs, particularly in areas where deposition loads are high.

The overarching aim of our study was to establish whether relationships between common bio(chemical) indicators and ambient N deposition are affected by time

since management in a range of heathland sites across the UK. We used a suite of previously validated biochemical indices for detecting N deposition impacts within *Calluna* foliage and heathland soil (Jones and Power 2012), and the distinct developmental phases of *C. vulgaris* growth of individual stands as a proxy for time since management. *Calluna* growth phases have been clearly defined according to the structural characteristics of vegetation stands, and although their developmental rates may be influenced to some extent by climate and nutrient availability, the relationship of growth phases with time is well established (Gimingham 1972). We hypothesized that the influence of N deposition would be strongest in pioneer-phase vegetation, because of higher growth rates and a high demand for nutrients in this phase. Foliar nutrient uptake and productivity in mature-phase vegetation were expected to be less strongly related to atmospheric N inputs than in younger vegetation, given slower growth rates and reduced nutrient demand in this phase, although soil nutrient accumulation was expected to be most strongly related with N deposition in these older stands.

MATERIALS AND METHODS

Survey Sites and Sampling

Thirty-three lowland heathlands in southern England were selected according to information available in the literature and soil and geology maps (Soil Survey of England and Wales 1980; Chapman et al. 1989; Marrs and Britton 2000; British Geological Survey 2005) (Fig. S1, Electronic Supplementary Material). We chose sites across a modest N deposition gradient ($7.2\text{--}24.5\text{ kg ha}^{-1}\text{ year}^{-1}$), maximizing both the range of soil types and geologies, and the geographical extent of the survey (Table S1, Electronic Supplementary Material). The survey was conducted during a 2-week period from 21st July–5th August 2005, with the short sampling window minimizing variations in tissue nutrient levels from growth dilution during the growing season (Aerts 1993; Barker et al. 2004). At each of the 33 sites, clearly delineated areas dominated by a combination of either pioneer- ($n = 26$), building- ($n = 29$), or mature-phase ($n = 30$) *Calluna* (sensu Gimingham 1972) were located where present. Given the generally strong relationship between time and *Calluna* growth phase (Gimingham 1972), pioneer-phase stands were considered to be relatively recently managed (<5 years), building-phase stands were deemed to be 5–15 years old and mature-phase vegetation was estimated to be around 15–25 years old. Observations during the survey clearly indicated that mowing had been used as the principle management technique in all heathland reserves selected for this study (i.e., distinct stands of

evidently mown vegetation were present). A 25×25 m quadrat was positioned randomly in each *Calluna*-dominated stand and samples collected at 25 evenly distributed points within the sampling area. Soil samples (0–5 cm depth) were collected at each of these points and bulked to form a single sample for each quadrat. A representative sample of the current year's *Calluna* shoots was collected from 100 plants, also following this sampling pattern. 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 database (British Geological Survey 2005). The soil type for each site was determined using mapping data by the Soil Survey of England and Wales (1980). On this basis, sites were assigned to one of four categories according to common geology types: chalk, gravel, sand, and sandstone. Similarly, soil types were categorized into seven groups: loam-sand, loam-clay, loam-silt, clay, sand, sand-clay, and organic-sand.

Sample Preparation and Analysis

Fresh soil samples were sieved (<2 mm) to homogenize material and remove any small stones and larger root matter. Soils used in biochemical assays used material at field moisture levels and were analyzed immediately. The remaining soil and *Calluna* shoots were dried at 60 °C for 48 h. Dried samples were then ball milled (Glen Creston, England) to produce a homogenous fine powder.

Leaf and soil samples were digested following the total Kjeldahl digestion method of Allen et al. (1974). A Kjeltac digester block (Model 2020 Digester; Tecator AB, Hoganas, Sweden) was used to digest soil and foliar material in 3 ml of H_2SO_4 + Se catalyst tablet per 200 mg sample at 400 °C for 2 (leaf) or 3 (soil) hours. Ammonium (NH_4^+) and phosphate (PO_4^-) concentrations of digests were analyzed using a Skalar “San-Flow” automated segmented flow colorimetric analyser (Skalar UK (Ltd), York, UK).

Concentrations of extractable ammonium and nitrate in soils were also determined by Skalar colorimetric analysis, following extraction in 1 M KCl (Allen et al. 1974). Extractable phosphate concentrations were measured in the same way, following extraction in 0.01 M H_2SO_4 using the Truog's method (Allen et al. 1974). Measures of soil phosphomonoesterase (PME) enzyme activity were obtained following the method of Tabatabai and Bremner (1969), which quantifies enzymatic decomposition of an organic phosphate reagent, *para*-nitrophenyl phosphate (PNP-P), to *para*-nitrophenol (PNP) over time. The pigment density of PNP was measured by absorption with a Perkin Elmer Lambda 3 UV spectrophotometer (Perkin Elmer, USA) at 400 nm and compared with known

standards. Soil organic matter (SOM) content was measured by mass loss, following the complete combustion of dried soil to ash at 400 °C for 8 h (Allen et al. 1974). The Skalar autoanalyser was calibrated by fitting a standard curve from solutions of known concentration ($R^2 > 0.99$) and re-calibrated using the middle standard after each batch of 20 samples. Standard reference materials (SRMs) were used for all digest runs (soil: GBW-07406; foliage: GBW-07603, China National Standard Materials). In all cases, our analyses reported values to within 90 % of the certified value. Analytical precision was confirmed by inclusion of 10 % duplicate samples.

Statistical Analyses

Modeled annual mean N deposition values (2004–2005) at a resolution of 5 km² were provided by the Centre for Ecology and Hydrology, Edinburgh (Rognvald Smith, *pers. comm.*). Climate data originated from the UKCP09 2005 monthly gridded data sets, which are available from the UK Meteorological Office (Met Office, Exeter, UK). These comprised the mean annual precipitation amount (mm) and mean July air temperature (°C) for between 2001 and 2005 at the 5 km² grid square resolution. Climatic variables were included in the modeling analysis because a previous spatial survey of heathlands (Jones and Power 2012) indicated climatic influences can modify the nature of relationships with N deposition. All statistical analyses used the R statistical package version 2.12.2 (R Development Core Team 2011). Relationships between continuous variables were tested using a combination of uni- and multivariate generalized linear models (GLM), or generalized linear mixed effects models (GLMER). GLMER was used to analyze the combined (all phases) dataset ($n = 85$) (henceforth: “all phase dataset”), and “phase” was used as a nested random effect in the model, to account for co-occurring samples (from different phases) at the same site. For linear regressions variables were log transformed in order to meet assumptions of normality and homogeneity of variance. By contrast, the GLMs or GLMERs used untransformed data and applied error correction terms to the model, when necessary, to overcome model overdispersion. Likelihood ratio deletion tests were used to refine the minimum adequate model (Crawley 2007). For each response variable, three GLMs or GLMERs were fitted: (1) with total N deposition, (2) with oxidized N deposition, and (3) reduced N deposition, with temperature and rainfall fitted as potential explanatory covariates in each of the models. As total N deposition is the product of oxidized and reduced N, these three variables could not be combined in the same model. The distinct modeling approaches allowed relationships with total N deposition rates to be compared alongside the relative

Fig. 1 Mean values for soil N (a) and soil P (b) concentration according to *Calluna* growth phase. Error bars indicate ± 1 standard error of the mean. *F* values given, with significance, for GLM analysis of the effect of phase on soil nutrient status. * $p < 0.05$

strengths of relationships with oxidized or reduced forms. In every case, delta AIC < 2 (i.e., the difference in AIC values between candidate models) was used to define the minimum adequate model. GLMs or GLMERs (for the all phase data) were used to individually test the significance of differences associated with geology (four levels), soil type (seven levels), or *Calluna* growth phase (three levels). Six sites were excluded from geology, and four from soil, analyses as they were the sole representatives of their types and differed from other categories to such an extent that they could not be combined with other categories.

RESULTS

Effect of *Calluna* Phase on Soil and Plant Nutrient Status

Soil N and P concentrations differed significantly between *Calluna* growth phases, with higher values associated with more mature phases (and, hence, greater time since management) (Fig. 1; Table 1). Soil N concentrations ranged from $18.4 \pm 4.0 \text{ mg g}^{-1}$ (pioneer-phase) to $34.9 \pm 5.9 \text{ mg g}^{-1}$ (mature-phase), while Soil P concentrations ranged from $1.12 \pm 0.17 \text{ mg g}^{-1}$ (pioneer-phase) to $1.95 \pm 0.27 \text{ mg g}^{-1}$ (mature-phase). Foliar nutrient concentrations for N ($149.2 \pm 5.0 \text{ mg g}^{-1}$) or P

($11.5 \pm 0.4 \text{ mg g}^{-1}$) were not significantly related to *Calluna* growth phase.

Relationships Between N Deposition, *Calluna* Tissue Chemistry, and Vegetation Characteristics

GLM analysis revealed significant positive relationships between *Calluna* foliar N concentration and both total and oxidized N deposition (Table 2) for pioneer-phase vegetation. This relationship was also significant for total N deposition and foliar N in mature-phase vegetation ($p = 0.05$), but not for the building-phase (Fig. 2a–c). Foliar P concentrations were similarly related to total and oxidized N deposition for mature-phase vegetation; there was also a significant relationship between foliar P and total N deposition, and an interaction between N and temperature in pioneer-phase stands. The strongest relationships were apparent for total N; the highest foliar P concentrations were at sites experiencing high deposition loads, combined with a warmer climate for pioneer-phase stands (Fig. 3). Foliar N:P ratios were not related to any of the N deposition or climatic variables in pioneer- or building-phase, but were significantly higher in mature-phase vegetation at sites with high total N deposition inputs. Although there were no significant relationships between foliar chemistry and environmental variables for building-phase vegetation, canopy height was significantly higher in this phase at sites receiving the

Table 1 Mean values for foliar nutrient chemistry, soil chemistry, litter depth, and SOM (0–5 cm, excluding litter) according to phase, with ± 1 standard error of the mean. *F* values given with significance for GLM analysis of the effect of growth phase on vegetation and soil characteristics. * $p < 0.05$, *** $p < 0.001$

Variable	Pioneer <i>n</i> = 26	Building <i>n</i> = 29	Mature <i>n</i> = 30	Significance of “growth phase” df = 82
Foliar N (mg g ⁻¹)	152.8 ± 7.2	148.5 ± 10.9	146.9 ± 7.0	ns
Foliar P (mg g ⁻¹)	11.8 ± 0.6	11.3 ± 0.6	11.4 ± 0.9	ns
Foliar N:P ratio	12.0 ± 0.6	13.3 ± 0.4	13.4 ± 0.5	ns
Total soil N (mg g ⁻¹)	18.4 ± 4.0	24.6 ± 5.2	34.9 ± 5.9	<i>F</i> = 3.2*
Total soil P (mg g ⁻¹)	1.12 ± 0.17	1.46 ± 0.19	1.95 ± 0.27	<i>F</i> = 3.5*
Litter depth (mm)	16 ± 2.1	45 ± 4.2	72 ± 6.2	<i>F</i> = 35.4***
Soil Organic Matter (SOM) (%)	26.9 ± 3.6	28.4 ± 3.5	28.5 ± 3.7	ns

Table 2 Statistical summary table showing outputs for modeled relationships with total, oxidized, and reduced N deposition from GLMs for each of the growth phases (and GLMER models of the all phase dataset), testing response variables (and interacting climatic co-variables). The optimum likelihood-ratio model, according to the Akaike information criterion (AIC), is highlighted by italics for each variable, with separate bold and italic *P* values for other significant models. *F* value and *P* significance: * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001, non-significant: n/s. For brevity, some non-significant outputs for pioneer-, building-, and mature-phase, and weaker alternative models for the all phase dataset, are not shown

	df	Interactions with N	AIC	<i>F</i>	<i>P</i>	AIC	<i>F</i>	<i>P</i>	AIC	<i>F</i>	<i>P</i>
Pioneer-phase											
<i>Calluna</i> Foliar N	24	N-only	2.8	6.32	0.02*	4.3	4.63	0.04*	8.2	0.88	0.36
	22	N*Temp	6.2	0.34	0.56	7.9	0.15	0.71	8.4	1.55	0.23
	22	N*Rain	6.4	0.32	0.58	7.9	0.37	0.55	11.9	0.00	0.98
<i>Calluna</i> Foliar P	24	N-only	3.9	10.4	<0.01**	8.2	0.04	0.84	12.5	1.48	0.24
	22	N*Temp	<i>1.3</i>	<i>6.27</i>	0.02*	5.7	3.36	0.08	14.1	1.40	0.25
	22	N*Rain	6.5	1.16	0.29	8.0	1.14	0.29	16.3	0.16	0.70
Building-phase											
Canopy height	27	N-only	<i>24.3</i>	<i>17.7</i>	<0.001***	23.7	18.8	<0.001***	35.9	3.02	0.09
	25	N*Temp	27.6	3.52	0.07	27.7	3.80	0.06	37.1	2.94	0.10
	25	N*Rain	32.2	0.03	0.87	33.5	0.01	0.91	39.5	1.17	0.29
Litter depth	27	N-only	51.6	20.3	<0.001***	58.2	10.6	<0.01**	57.6	11.4	<0.01**
	25	N*Temp	<i>43.0</i>	<i>6.23</i>	0.02*	49.2	5.82	0.02*	55.6	2.93	0.10
	25	N*Rain	54.7	0.50	0.45	61.7	0.16	0.69	54.8	6.45	0.02*
pH	27	N-only	<i>21.5</i>	<i>16.8</i>	<0.001***	25.3	11.3	<0.01**	23.5	13.9	<0.001***
	25	N*Temp	25.0	3.37	0.08	29.3	3.61	0.07	27.6	1.05	0.32
	25	N*Rain	29.1	0.41	0.53	34.3	0.26	0.62	26.4	2.20	0.15
Mature-phase											
<i>Calluna</i> Foliar N	28	N-only	<i>30.6</i>	<i>4.13</i>	0.05*	31.2	1.38	0.25	37.7	0.15	0.70
	26	N*Temp	32.1	2.11	0.16	32.9	1.40	0.25	37.6	0.82	0.37
	26	N*Rain	34.3	0.31	0.58	34.3	0.42	0.52	38.5	0.09	0.76
<i>Calluna</i> Foliar P	28	N-only	<i>26.8</i>	<i>11.9</i>	<0.01**	28.4	9.87	<0.01**	36.6	0.77	0.39
	26	N*Temp	28.9	1.63	0.21	31.1	0.63	0.43	38.8	1.06	0.31
	26	N*Rain	30.8	0.04	0.84	32.3	0.05	0.82	40.5	0.14	0.71
<i>Calluna</i> Foliar N:P	28	N-only	<i>25.5</i>	<i>5.45</i>	0.02*	27.0	3.78	0.06	29.5	1.24	0.28
	26	N*Temp	28.9	0.05	0.82	29.7	0.87	0.36	33.0	0.04	0.85
	26	N*Rain	25.7	2.40	0.13	28.1	1.80	0.19	31.4	0.42	0.52
All phases combined											
<i>Calluna</i> Foliar N	81	N-only	<i>95.2</i>	<i>6.16</i>	0.02*	96.4	5.71	0.02*	100.4	0.46	0.50
<i>Calluna</i> Foliar P	81	N-only	<i>98.3</i>	<i>7.73</i>	<0.01**	99.2	7.60	<0.01**	105.1	0.26	0.61
Canopy height	81	N-only	99.3	5.56	0.02*	100	5.71	0.02*	103.4	0.97	0.33
	79	N*Temp	<i>89.5</i>	<i>4.80</i>	0.03*	91.5	3.45	0.07	92.7	6.27	0.01*
Litter depth		N-only	153.9	23.4	<0.001***	157.6	19.7	<0.001***	167.7	6.72	0.01*
	79	N*Temp	<i>136.4</i>	<i>5.22</i>	0.03*	139.8	4.80	0.03*	156.6	3.30	0.07
pH	81	N-only	<i>51.3</i>	<i>10.9</i>	<0.01**	56.0	6.57	0.01*	51.5	10.3	<0.01**
Extractable N	81	N-only	170.9	0.19	0.66	171.7	0.16	0.69	170.7	0.03	0.86
Extractable P	81	N-only	111.1	3.61	0.06	113.0	2.52	0.11	112.7	1.67	0.19
PME activity	81	N-only	154.5	3.12	0.08	155.9	2.44	0.12	156.6	0.62	0.43
Soil organic matter	81	N-only	194.9	2.01	0.16	196.0	1.74	0.19	196.2	0.34	0.56

Fig. 2 Relationships between total N deposition and foliar N concentration for **a** pioneer-phase, **b** building-phase (no significant relationship), and **c** mature-phase *Calluna*

greatest inputs of total and oxidized N deposition (Table 2). When all phases were considered together, there was a significant positive relationship between total N deposition for both foliar N and P.

Relationships Between N Deposition and Chemical and Physical Characteristics of Litter and Soil

GLM analysis of soil nutrient data did not reveal any statistically significant relationships between N deposition and total soil N or P in any of the growth phases. Similarly,

Fig. 3 Relationship between *Calluna* foliar P concentration and total N deposition, showing co-variate relationship with temperature in pioneer-phase

there were no significant relationships between N deposition and extractable soil N (NO₃ and NH₄) or P (PO₄), PME activity, or SOM content. Soil pH was, however, significantly lower in building-phase stands receiving higher total and reduced N deposition inputs and in the all phase dataset with reduced N (Table 2). An interaction between N deposition and temperature was significant for litter depth in building-phase stands, with deeper litter layers at sites experiencing the warmest temperatures and highest inputs of total or oxidized N deposition.

Relationships with Geology and Soil Type

Geology and soil type were both found to have a significant influence on the nutrient status of heathland vegetation (Table 3). The highest foliar concentrations of N and P were found at sites overlying chalk and at those with sandy soil types. However, there were no significant interactions between N deposition and either underlying geology or soil type.

DISCUSSION

The Effects of Time Since Management on Relationships with N Deposition

A large degree of variability is expected to exist in field-scale survey data, compared to experimental manipulation approaches, and this is reflected by the limited number of significant relationships that were detected in our survey. Despite this, our investigation indicates that the strength of relationships with N deposition, and interactions with climate, differ according to *Calluna* growth

Table 3 Summary table of means ($\pm$ SEs) and significance of GLMER analyses, with no accounting for N deposition rates, on the all phase dataset to examine the effects of geology type and soil type on foliar and soil nutrient concentrations. *F* values and significances of model given according to * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, with phase nested as a random effect within the model

Geology ($n = 79$) df = 73	Foliar N (mg g ⁻¹) <i>F</i> = 2.6*	Foliar P (mg g ⁻¹) <i>F</i> = 4.4***	Soil N (mg g ⁻¹) n/s	Soil P (mg g ⁻¹) n/s
Chalk ($n = 13$)	17.2 $\pm$ 1.0	1.52 $\pm$ 0.07	1.5 $\pm$ 0.4	0.15 $\pm$ 0.04
Gravel ($n = 21$)	15.4 $\pm$ 1.0	1.11 $\pm$ 0.06	2.6 $\pm$ 0.6	0.17 $\pm$ 0.03
Sandstone ($n = 22$)	15.0 $\pm$ 1.0	1.13 $\pm$ 0.07	4.0 $\pm$ 0.9	0.18 $\pm$ 0.03
Sand ($n = 23$)	12.5 $\pm$ 1.0	0.89 $\pm$ 0.07	2.3 $\pm$ 0.4	0.12 $\pm$ 0.02
Soil type ($n = 82$) df = 76	Foliar N (mg g ⁻¹) <i>F</i> = 5.7**	Foliar P (mg g ⁻¹) <i>F</i> = 10.2***	Soil N (mg g ⁻¹) n/s	Soil P (mg g ⁻¹) n/s
Sand ($n = 28$)	17.0 $\pm$ 0.7	1.40 $\pm$ 0.06	1.7 $\pm$ 0.3	0.15 $\pm$ 0.02
Loam ($n = 29$)	15.0 $\pm$ 0.8	1.10 $\pm$ 0.06	3.8 $\pm$ 0.7	0.15 $\pm$ 0.02
Organic-sand ($n = 16$)	12.6 $\pm$ 0.9	0.93 $\pm$ 0.06	2.0 $\pm$ 0.4	0.13 $\pm$ 0.03
Sand-clay ($n = 9$)	11.1 $\pm$ 0.9	0.75 $\pm$ 0.05	2.9 $\pm$ 1.0	0.21 $\pm$ 0.04

phase. Pioneer-phase *Calluna* was shown to be sensitive to atmospheric N inputs, as evidenced by the strongest relationships with foliar N (and P) amongst the different phases (Fig. 2a). This supports work by Britton and Fisher (2008) who also highlighted the sensitivity of younger heathland vegetation. Pioneer-phase stands, with less standing biomass, more active green shoots and faster growing tissue, contrast with the large amounts of relatively inert woody material present in older phases (Gimingham 1972). These characteristics generate a high demand for nutrients and a rapid potential for nutrient uptake in the pioneer-phase. The significant relationship between N deposition and foliar N concentrations in mature-phase vegetation may reflect an increasing limitation by other nutrients or possibly water, restricting shoot growth dilution of N (Barker et al. 2004) where luxury uptake has occurred. However, any such limitation is unlikely to be driven by P since we also found decreasing N:P ratios (and increasing foliar P) in mature-phase foliage with increasing N deposition inputs. Similar evidence of higher levels of P uptake at heathland sites experiencing high N deposition inputs has also been reported in surveys elsewhere (Rowe et al. 2008; Power and Collins 2010; Jones and Power 2012), suggesting that N deposition has an influence on rates of P cycling and availability.

The lack of significant relationships between N deposition and foliar nutrient concentrations in building-phase plants indicates that N inputs may have been used to support relatively high rates of biomass accumulation, rather than accumulating in plant tissues. This is supported by evidence of greater building-phase canopy height (and litter depth) with N deposition. The building-phase of the *Calluna* life-cycle is defined by a closed-canopy which invests in woody tissue to increase above ground biomass (Gimingham 1972). A fertilizing effect from additional N

input to building-phase *Calluna* has, however, been demonstrated in previous field experiments (e.g., Barker et al. 2004). Importantly, the evidence we provide of increased productivity of building-phase *Calluna* at sites receiving higher N inputs has implications for understorey vegetation, especially bryophytes and lichens. Negative relationships between the abundance of these organisms and N deposition have been found in studies focusing on building-phase vegetation, where it has been suggested that lower light penetration under a rapidly developing *Calluna* canopy and/or direct toxicity associated with reduced forms of N is detrimental (Southon et al. 2013).

The varying strengths of N deposition relationships detected within different phases of the heathland management cycle has not been demonstrated previously. These impacts on either the tissue chemistry or productivity of *Calluna* at all developmental stages (and hence time since management), across a variety of widely distributed sites, suggest the intensity and/or frequency of previous management has been inadequate to counter the effects of sustained high inputs of atmospheric N. The use of more intense management regimes (e.g., mowing with litter removal or turf cutting) that are associated with a high level of nutrient export has been suggested as important to counter the effects of elevated N deposition, both in modeling studies (Terry et al. 2004) and manipulation experiments (Barker et al. 2004). Sites surveyed in this study had been mown, in line with the predominant form of heathland management undertaken in the UK (Symes and Day 2003). This relatively low intensity management is associated with lower levels of N export than burning or turf cutting, although a modeling study by Britton et al. (2001) concluded that regular mowing (e.g., every 10–25 years) could effectively reduce the influence of elevated N. Higher intensity managements such as frequent

burning (e.g., on a 10- to 15-year cycle) can export up to 90 % of accumulated atmospheric N inputs (Niemeyer et al. 2005), whereas turf cutting can export the equivalent of up to 90 years of deposition inputs in a single management cycle (Hardtle et al. 2006).

Soil pH in the building-phase and all phase datasets was strongly and significantly related to deposition rates for reduced N and this was in fact the only significant relationship with reduced N identified in this study. The acidifying effects of ammonium inputs are well known (Pearson and Stewart 1993) and, given the lack of buffering capacity in acidic heathland soils, reduced N inputs can have significantly greater impacts on soil pH than in less acidic ecosystems. Soil acidification by reduced N is considered a major contributing factor driving species declines across a wide range of heathland sites (Maskell et al. 2010; Southon et al. 2013). The lack of significant relationships between soil nutrients and N deposition in our study contrasts with similar studies in a range of habitats. For example, Southon et al. (2013) reported a strong positive relationship between soil N and oxidized N inputs across 52 heathland sites spanning deposition inputs from 5.9 to 32.4 kg N ha⁻¹ year⁻¹, while N deposition has been associated with higher levels of extractable or total N in the soils of heathland (von Oheimb et al. 2010), temperate forest (Lovett and Rueth 1999), and grassland (Stevens et al. 2006). The relatively narrow range of N deposition in this survey (7.2–24.5 kg N ha⁻¹ year⁻¹) and focus on only the upper soil horizon (0–5 cm depth) may offer a partial explanation for the lack of significant relationships with soil nutrient concentrations in the current study.

Effects of Climate on Relationships with N Deposition

The large number of interactions with temperature suggest that this is an important modifier of bioindicator relationships with N deposition. Climatic influences on the strength of N deposition relationships have also been identified in modeling studies by Britton et al. (2001) as well as in previous surveys of lowland and upland heathlands (Jones and Power 2012; Southon et al. 2013), although rainfall, rather than temperature, was found to be important in previous studies. Temperature has a strong influence on vegetation productivity (Larsen et al. 2011) and will also affect soil microbial activity (Selsted et al. 2012) and thus rates of organic matter turnover and nutrient cycling within soils. A positive effect of higher temperatures on *Calluna* shoot growth has been shown in a gradient study of heathland vegetation in northern Europe (Prieto et al. 2009) and also indirectly in an altitudinal gradient study by Hicks

et al. (2000). Temperature- (and precipitation-)driven increases in shoot growth can dilute tissue nutrient concentrations (Hicks et al. 2000) and, therefore, should be considered in surveys that rely on biochemical indicators of N deposition loads and/or its effects.

Overall Implications of N Deposition Relationships

Significant relationships with N deposition in the all phase dataset were largely found for total N, with the analysis in Model 2 further suggesting oxidized N contributed more strongly to relationships with foliar N and P compared to reduced N. Overall, the relative lack of significant relationships with reduced N in our study contrasts with findings from earlier surveys (Stevens et al. 2006; van den Berg et al. 2008) that have identified reduced N as a stronger driver of impacts than oxidized forms. In part this may be due to the smaller range of reduced N deposition values (4.3–10.0 kg ha⁻¹ year⁻¹), compared with oxidized N (2.9–18.4 kg ha⁻¹ year⁻¹), in our study (Table S1). However, other recently published studies along moderate gradients of N deposition have considered oxidized N to be a stronger influence on *Calluna*-dominated ecosystems (Jones and Power 2012) than reduced N, or have identified similar strengths of relationship with both forms of N (Southon et al. 2013). Our results suggest that oxidized N may be a more important driver of relationships with foliar chemistry than reduced N. However, the consistently greater significance of relationships with total N deposition within single-phase and all phase analyses suggest that total N deposition is the most reliable metric when considering N deposition impacts in the field.

It is notable that foliar P concentrations were significantly increased by N deposition in the all phase dataset (as well as in pioneer- and mature-phase datasets). This type of relationship with N deposition has not been reported widely, although a number of recent studies have found similar increases in tissue P concentrations along N deposition gradients (Rowe et al. 2008; Jones and Power 2012). Our findings support earlier interpretations that N deposition increases the demand for and uptake of P, which may account for relatively poor relationships with foliar N:P ratios, although the mechanism behind this is unclear. Potentially, increased P acquisition could be mediated by increased activity of P-mobilizing enzymes such as acid-phosphatase (PME), in response to increased plant (and microbial) P demand. Other studies have reported positive relationships between N deposition and PME activity (e.g., Phoenix et al. 2003), although this was not found in our survey. PME activity is highly sensitive to differences in substrate pH above and below an optimum pH of 4.5 (Leprince and Quiquampoix 1996) and, in the present study, reduced N deposition significantly lowered soil pH

(soil pH range in the survey 3.5–5.4), a phenomenon that has been observed in studies elsewhere (Stevens et al. 2004; Power et al. 2006). The influence of soil pH may, therefore, have confounded potential relationships between PME activity and N deposition. Foliar P concentrations were also significantly higher on chalk, which was the underlying geology at four of the survey sites, all within the Breckland heathlands of East Anglia. Chalk occurs at shallow depths in the Breckland area, due to thin soils developed from wind-blown sand (Watt 1936). Ancient marine sediments such as chalk are relatively rich in P (White 1997) but, despite this, the chalk geology did not influence total soil P concentrations in the upper (0–5 cm) soil horizon (Table 3).

CONCLUSION

Foliar nutrient concentrations in the youngest (pioneer-phase) and oldest (mature-phase) vegetation stands had the strongest relationships with N deposition and, therefore, appear to be the most suitable phases for use as indicators of N deposition inputs. In building-phase vegetation, the lack of significant relationships between foliar nutrient concentrations and N inputs may reflect N-driven increases in productivity and associated growth dilution. However, the clear link between climate and plant growth rates precludes the use of biomass metrics as indicators of N deposition loads. Relationships between N inputs, tissue chemistry, canopy height, and litter depth were also generally influenced by prevailing temperature, highlighting the link between climate, plant growth, and organic matter turnover. The influence of climate and management history should, therefore, be taken into account when evaluating N deposition effects.

With the exception of soil pH, very few relationships between N deposition and soil chemistry were found in this study. However, the phase-specific approach used here confirmed the expectation that soil nutrients accumulate strongly with increasing time since management. This highlights the requirement for regular management export of litter, and ideally upper soil horizons, to maintain the low nutrient environment that favors slow growing dwarf shrubs and the associated assemblages of plants and animals they support.

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