

REVIEW

Impacts of atmospheric nitrogen deposition: responses of multiple plant and soil parameters across contrasting ecosystems in long-term field experiments

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Abstract

Atmospheric nitrogen (N) deposition is a global and increasing threat to biodiversity and ecosystem function. Much of our current understanding of N deposition impacts comes from field manipulation studies, although interpretation may need caution where simulations of N deposition (in terms of dose, application rate and N form) have limited realism. Here, we review responses to simulated N deposition from the UKREATE network, a group of nine experimental sites across the UK in a diversity of heathland, grassland, bog and dune ecosystems which include studies with a high level of realism and where many are also the longest running globally on their ecosystem type. Clear responses were seen across the sites with the greatest sensitivity shown in cover and species richness of bryophytes and lichens. Productivity was also increased at sites where N was the limiting nutrient, while flowering also showed high sensitivity, with increases and declines seen in dominant shrub and forb species, respectively. Critically, these parameters were responsive to some of the lowest additional loadings of N (7.7–10 kg ha⁻¹ yr⁻¹) showing potential for impacts by deposition rates seen in even remote and ‘unpolluted’ regions of Europe. Other parameters were less sensitive, but nevertheless showed response to higher doses. These included increases in soil %N and ‘plant available’ KCl extractable N, N cycling rates and acid–base status. Furthermore, an analysis of accumulated dose that quantified response against the total N input over time suggested that N impacts can ‘build up’ within an ecosystem such that even relatively low N deposition rates can result in ecological responses if continued for long enough. Given the responses have important implications for ecosystem structure, function, and recovery from N loading, the clear evidence for impacts at relatively low N deposition rates across a wide range of habitats is of considerable concern.

Keywords: biodiversity, bog, dune, ecosystem services, grassland, heathland, pollution, soil

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Introduction

Increased atmospheric nitrogen (N) deposition is considered one of the most important components of global change, threatening both the structure and functioning of ecosystems (Bobbink *et al.*, 1998, 2010; Sala *et al.*, 2000). Anthropogenic activity now dominates the global N cycle (Galloway *et al.*, 2004), and both emissions and deposition rates of N are predicted to double from current values by 2050, greatly increas-

ing the number of regions receiving potentially damaging levels of N inputs (Galloway *et al.*, 2004; Phoenix *et al.*, 2006; Bobbink *et al.*, 2010). Understanding ecosystem responses, and the mechanisms driving those responses, continues therefore to be of major importance for the conservation of natural and seminatural ecosystems, the preservation of biodiversity and the sustainability of ecosystem services.

Concerns about the impacts of N deposition have led to a considerable expansion of studies over the last 2 decades, particularly those simulating increased N deposition through applications of N to experimental plots (e.g. Van der Eerden *et al.*, 1991; Caporn *et al.*,

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1994; Morecroft *et al.*, 1994; Power *et al.*, 1998a,b). These studies, combined with survey studies evaluating patterns of ecosystem response along gradients of N deposition in space or time (e.g. Jones *et al.*, 2004; Stevens *et al.*, 2004; Duprè *et al.*, 2010; Maskell *et al.*, 2010; Van den Berg *et al.*, 2010), have revealed diverse impacts on ecosystem structure and function. Broadly speaking, the major types of impact are: (i) N accumulation causing declines in biodiversity via the expansion of nitrophilous species and competitive exclusion of others (Bobbink & Willems, 1987; Bobbink *et al.*, 1988); (ii) accumulation of NH_4^+ ions leading to toxic effects on sensitive species in ecosystems where NO_3^- is usually the dominant N form (Roelofs *et al.*, 1996; Kleijn *et al.*, 2008; Stevens *et al.*, 2011); (iii) soil acidification, base cation depletion and enhanced availability of toxic metals (e.g. Al^{3+} , Fe^{3+}) which can reduce plant health and productivity, alter community composition, and cause declines in species richness (Roem & Berendse, 2000; Bowman *et al.*, 2008; Horswill *et al.*, 2008); and (iv) increased susceptibility of plants to secondary stresses including increased herbivory, reduced resistance to pathogen attack or increased susceptibility to drought or freezing damage (Power *et al.*, 1998b; Carroll *et al.*, 1999; Sheppard *et al.*, 2008a).

The role of N deposition as a driver of biodiversity loss has been reviewed recently (Bobbink *et al.*, 2010; Dise *et al.*, 2011), with mechanistic understanding drawing strongly on results of field manipulation experiments. It has become increasingly apparent, however, that the manipulation experiments that underpin much of current understanding can have several potential limitations. These can include: (i) a short time scale that may inadequately predict the long-term consequences of elevated deposition, (ii) high N loadings that may overestimate N deposition impacts [including where response is driven by the high concentration of treatment applications, rather than dose (Pearce & van der Wal, 2008)]; (iii) single or few applications of (sometimes solid) N fertilizer, which may not adequately simulate N deposition, (iv) different ambient deposition (or management) histories which affect ecosystem sensitivity to experimental N inputs (Emmett, 2007) and (v) lack of separation of the impacts of wet and dry, and oxidized and reduced N deposition, a major shortfall if we are to reliably predict deposition impacts and design mitigation strategies (Sheppard *et al.*, 2008b).

Assessment of the N deposition threat can be facilitated by analysis of long-term experiments, across multiple ecosystems, employing realistic N doses and application techniques that adequately simulate N deposition, and where the same response variables have been measured over similar timescales. The strength of experimental networking has been demon-

strated in forest systems (not covered in this review) where the European NITREX network, for instance, has made major contributions to the testing of the N saturation concept, model testing and understanding N limitations on carbon sequestration (Boxman *et al.*, 1998; Emmett *et al.*, 1998; Gundersen *et al.*, 1998; Nadelhoffer *et al.*, 1999). Similarly, there have been a number of valuable analyses of N enrichment impacts on ecosystem productivity and diversity across the LTER sites in the USA (e.g. Gough *et al.*, 2000; Suding *et al.*, 2005; Clark *et al.*, 2007; Chalcraft *et al.*, 2008), although the form and dose of N inputs used in the LTER analyses generally do not simulate atmospheric N deposition (Clark *et al.*, 2007).

A network of field experiments that provides an excellent opportunity for assessing N deposition impacts is that run by 'UK Research on the Eutrophication and Acidification of Terrestrial Ecosystems' (UKREATE, 2008; <http://ukreate.defra.gov.uk/>). UKREATE combines a large number of field studies across multiple habitats, which include modest treatment doses and avoid single dose or solid form applications. Some of these studies have run for over 20 years, uncovering truly long-term responses, and in some cases are the only long-term N manipulation studies worldwide for their particular habitat types. The network also includes some of the most recent investigations to distinguish impacts of reduced and oxidized, and wet and dry N deposition.

Here, we present a synthesis of results from the UKREATE field experiments to identify both general and ecosystem-specific responses to simulated N deposition, and discuss these in relation to current understanding of N deposition impacts on seminatural ecosystems. We evaluate multiple responses in both plants and soils, including impacts on floristic diversity, plant growth, tissue chemistry, flowering, phenology, N cycling rates, N pools and fluxes, acid-base status and interactions with management. We also consider which responses only become clear at unrealistically high N treatment levels.

Furthermore, we quantify the extent of change in commonly measured parameters in response to accumulated dose (N input \times duration of experiment). Accumulated dose is a recently developed approach (e.g. Payne *et al.*, 2011), and is best applied where long-term datasets are available. Evaluating responses in terms of cumulative N may provide novel insight into how damage may develop over time under enhanced deposition rates, thereby identifying sensitivity to long-term inputs. Since it integrates time, N input rates and ambient deposition, accumulated dose facilitates comparison of responses between sites that (i) have used contrasting treatment doses (whilst still

taking advantage of different treatment levels within sites), (ii) have treated plots for very different lengths of time, and (iii) are located in areas of contrasting background N deposition. This approach should improve our understanding of the changes to be anticipated over the decadal timescales associated with chronic N deposition.

The network of experimental sites

The UKREATE network consists of 14 experiments across nine sites representative of grassland, heath, bog and dune ecosystems, all of which are widely distributed in northern Europe (Table 1, Table S1 and Fig. 1). In addition to their high conservation and amenity value, these ecosystems were selected for study because they are considered threatened by N-deposition as a result of their low nutrient status. Most sites were the first N deposition experiments to be established on their ecosystem type, and they include the world's longest running experiments simulating atmospheric N deposition on shrub and grassland ecosystems [Thursley lowland heath (TLH); Ruabon upland heath (RUH); Wardlow acidic grassland (WAG); and Wardlow calcareous grassland (WCG)]. The ecosystems cover a range of soil types, climates and ambient N deposition rates (Table 1).

Most sites use regular (2–4 weekly) applications of N solution applied as either a fine mist using a back-pack sprayer, or through a watering can. The exception is Whim bog (WBO) that uses the most sophisticated and realistic N application method, where automated sprinklers supply dilute N solution coinciding with rain events (>120 per year), and dry NH₃ deposition is provided through a free-air release system (Leith *et al.*, 2004; Sheppard *et al.*, 2004a). This site, therefore, uniquely allows direct comparison of the impacts of wet oxidized (NO₃⁻), wet reduced (NH₄⁺) and dry reduced (NH₃) N deposition. Wet reduced and wet oxidized deposition are also compared at Pwllpeiran acidic grassland (PAG).

Responses to N deposition in experimental sites

Productivity

Impacts of atmospheric N deposition on primary productivity are of considerable interest, as increased productivity can drive species loss by promoting competition from nitrophilous species (Suding *et al.*, 2005; Bobbink *et al.*, 2010). Impacts on productivity are also an important means by which perturbation of the N cycle can impact the C cycle, where more productive ecosystems, for instance, may have greater draw-down

of atmospheric CO₂ (van Groenigen *et al.*, 2006; Reich *et al.*, 2006).

Increased plant productivity with N treatment is observed at six of the nine UKREATE sites (Table 2 – which shows the lowest treatment dose at which a statistically significant effect occurs). All heathland and bog sites showed greater productivity when measured directly as shoot growth or indirectly as canopy cover, of the dominant shrub *Calluna vulgaris* (Power *et al.*, 1995, 1998a; Uren *et al.*, 1997; Wilson, 2003; Pilkington *et al.*, 2005b; Britton & Fisher, 2008), with productivity increases occurring at treatment applications as low as 7.7–10 kg N ha⁻¹ yr⁻¹ (TLH, CAH) – a level of N deposition that is typical of 'unpolluted' European regions such as central Scotland or southern Finland.

Increased productivity may be associated with longer periods of physiological activity as seen in the earlier spring bud burst observed at heathland sites (Power *et al.*, 1998b; Cawley, 2000; Pilkington, 2003), and is consistent with mainland European studies (e.g. Heil & Diemont, 1983; Aerts, 1993; Marcos *et al.*, 2003). As warned previously by Cleland *et al.* (2006), the often well documented phenological shifts observed in nature should not only be considered attributable to warming (Fitter & Fitter, 2002; Root *et al.*, 2005).

Productivity responses can, however, depend on growth stage and cumulative N loading, with increases at RUH and BLH heaths halted once *C. vulgaris* reached its degenerate phase (Carroll *et al.*, 1999; Ray, 2007). Interactions between management and N deposition can also be important. For instance, re-growth following management burns or cutting was reduced under loads of 40 (RUH) and 20 kg N ha⁻¹ yr⁻¹ (BLH) (Pilkington *et al.*, 2007a; Ray, 2007) while in contrast, high N treatments following management burns have resulted in increased growth at TLH and CAH heaths (Barker *et al.*, 2004; Britton & Fisher, 2008). Differences in these cases are likely to reflect much higher cumulative N loadings prior to management at the former two sites compared to the latter two. Differences in N form may also influence outcomes: in the bog ecosystem, dry NH₃ deposition ≥ 24 kg N ha⁻¹ yr⁻¹ significantly reduced the cover of *C. vulgaris*, in contrast to the positive (significant for reduced N at 56 kg NH₄-N ha⁻¹ yr⁻¹) effects of wet deposition (Sheppard *et al.*, 2011). However, the subsequent replacement of *C. vulgaris* under dry deposition with the cotton grass, *Eriophorum vaginatum*, means long-term primary productivity changes are unclear.

Where N is the limiting nutrient, clear increases in grassland productivity are also apparent. The rapid increase of biomass production in the dune grassland after only 1 year (Plassmann *et al.*, 2009) is analogous to rapid productivity responses observed in Dutch

Table 1 Vegetation type, soil, simulated N deposition treatments and background atmospheric N deposition rates for the nine UKREATE sites

Site name (Abbreviated code)	Vegetation type: NVC classification	Soil type	N treatment rates (kg N ha ⁻¹ yr ⁻¹)	N form (as solution unless stated)	How applied	Year started	Duration of N treatments – years to date or until ceased	Modelled N dep. at site (kg N ha ⁻¹ yr ⁻¹)
Ruabon (RUH)	Upland heath: H12 <i>Calluna – Vaccinium</i>	Peaty podzol	0, 40, 80, 120 0, 10, 20, 40, 120	NH ₄ NO ₃	Watering can	1989 1998 ²	22 13 ²	25
Budworth (BLH)	Lowland heath: H9 <i>Calluna – Deschampsia</i>	Humo ferric podzol	0, 20, 60, 120	NH ₄ NO ₃	Watering can	1996	15	28
Thursley (TLH)	Lowland heath: H2 <i>Calluna – Ulex minor</i>	Podzol, over lower greensand	0, 7.7, 15.4 0, 30	(NH ₄) ₂ SO ₄	BPS; fine mist – weekly ¹ BPS; fine mist – fortnightly	1989–1996 ¹ 1998	7 ¹ 13	20
Culardoch (CAH)	Low Alpine Heath: H13 <i>Calluna – Cladonia</i>	Sub-alpine podzol	0, 10, 20, 50	NH ₄ NO ₃	Monthly with Watering can (fine spray)	2000	11	11
Whim (WBO)	Ombrotrophic bog: M19, <i>Calluna – Eriophorum</i>	<i>Sphagnum</i> peat	0, 8, 24, 56 for wet dep. NH ₃ transect 4–70	NH ₄ Cl NaNO ₃ NH ₃ gaseous	Rainwater as fine droplets Gas plume in air	2002 2002	9 9	10
Pwllpeiran (PAG)	Upland acid grassland	Shallow ferric stagnopodzol	0, 10, 20	NaNO ₃ (NH ₄) ₂ SO ₄	Fortnightly with watering can	1996	15	17
Wardlow acid (WAG)	Acid grassland: U4e <i>Festuca – Agrostis – Galium</i>	Paleo-argillic	0, 35, 70, 140 0, 35, 140	NH ₄ NO ₃	BPS: fine mist, monthly	1990 ¹ 1995 ²	12 ¹ 16 ²	34
Wardlow calcareous (WCG)	Calcareous grassland: CG2d <i>Festuca – Avenula</i>	Rendzina	0, 35, 70, 140 0, 35, 140	NH ₄ NO ₃	BPS: fine mist, monthly	1990 ¹ 1995 ²	12 ¹ 16 ²	34
Newborough (NDG)	Fixed sand dune grassland: SD8 <i>Festuca – Galium</i>	Para-rendzina	0, 7.5, 15	NH ₄ NO ₃	Watering can – fine drops, bar extension	2003	8	11

NVC, National Vegetation Classification (UK); BPS, backpack sprayer.

¹experimental treatments now ceased to follow recovery;²treated plots now split in half with one side no longer treated to follow recovery.

Location of experiments are shown in Fig. 2.

chalk grassland and alpine grassland (Bobbink, 1991; Bassin *et al.*, 2007). In contrast, at UKREATE acidic and calcareous grassland sites few or no productivity responses have been observed, which is thought to be indicative of limitation by nutrients other than N, or co-N limitation (WAG, WCG, PAG) (Morecroft *et al.*, 1994; Carroll *et al.*, 2003; Emmett *et al.*, 2004). Given that up to half the world's vegetation may be limited or co-limited by nutrients other than N, absence of a productivity response may well be expected in more ecosystems globally (Elser *et al.*, 2007), though other evidence suggests that stimulation of productivity by N should be the most common response (LeBauer & Treseder, 2008).

In summary, wet N deposition increases productivity in all ecosystems where N is considered to be the primary limiting nutrient, with clear responses apparent at low N deposition rates for the most sensitive sites. Limitation by nutrients other than N (or co-N limitation) is probably the main reason for restriction of productivity responses to even large N loads at some sites.

Floristic change

Ecosystems are considered more sensitive to N deposition if soils have low pH buffering capacity, the vegetation is strongly N limited, and the climate is relatively harsh, providing a greater chance of secondary stress (Clark *et al.*, 2007). The mechanisms driving changes in community composition may act sequentially, with loss of rare or highly sensitive species (such as bryophytes and lichens) as a result of eutrophication or toxicity occurring first, and acidification-driven species change developing later (Suding *et al.*, 2005; Emmett, 2007; Stevens *et al.*, 2011).

Vascular plants. At the heathland sites (RUH, CAH, BLH, TLH), there has been little change in floristic composition, except where increased productivity has increased cover of the already dominant *C. vulgaris*. Grasses have yet to invade and establish, with only *Deschampsia flexuosa* showing a temporary expansion at the lowland heath sites (BLH, TLH) following management burn or cutting (Barker, 2001; Ray, 2007). These absent or transitory changes contrast with significant changes in Dutch heathlands following long-term N treatments (Heil & Diemont, 1983; Aerts *et al.*, 1990). The reasons for this contrast are unclear, though at some sites the lack of establishment by new species may be due to limited availability of propagules (e.g. at the low alpine heath, the nearest grassland is 1500 m away, down-slope). The bog system (WBO) does, however, appear rather more sensitive to community change (though note this has not resulted in a significant change in species richness, Table 2). Here, *Eriophorum vaginatum* and

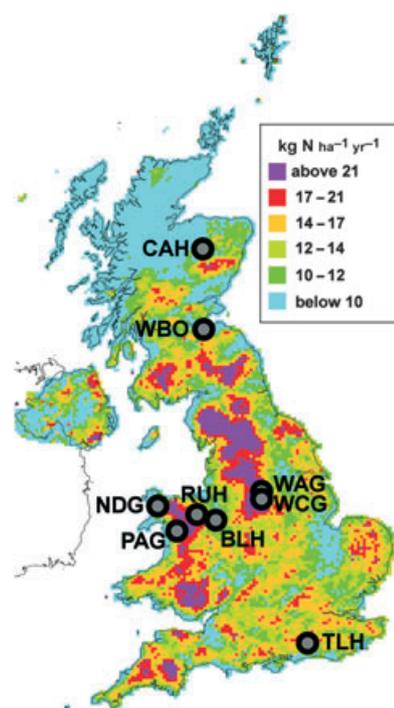


Fig. 1 UKREATE site locations. Map shows total N deposition ($\text{kg N ha}^{-1} \text{yr}^{-1}$) modelled for 2008 (RoTAP, in press). Site codes as for Table 1.

the shrubs *Erica tetralix* (initially), *Vaccinium myrtillus* and *Empetrum nigrum* have expanded concurrently with the decline in *C. vulgaris* under dry NH_3 deposition, and therefore probably as a result of the canopy opening and reduced competition from this dominant species. Again, the differing impacts of wet and dry deposition are highlighted since the wet N treatments result in mainly nonsignificant increases in dwarf shrub cover.

The grassland sites have also shown only limited changes in floristic composition. At PAG acidic grassland, the dwarf shrub *Vaccinium myrtillus* has declined, perhaps driven by an increase in the sedge *Carex pilulifera*, but only under a light grazing regime and the oxidized (not reduced) N treatment. This suggests N form is important when determining competitive advantage of some species (as with WBO), and also that the effects of heavy grazing mask the effects of N, and so switches the control of vegetation composition from bottom up (i.e. nutrient controlled) to top down (consumption by herbivores) (Emmett *et al.*, 2004, 2007). This importance of top down maintenance of biodiversity by herbivores in the face of environmental pressures that drive biodiversity down has similarities in other grasslands, for instance in tallgrass prairie where grazing by bison (or its surrogate – mowing) negates the decline of diversity driven by nutrient enrichment or frequent burning (Collins *et al.*, 1998).

Table 2 Minimum treatment deposition rate ($\text{kg N ha}^{-1} \text{yr}^{-1}$) at which a statistically significant response was observed for a diversity of plant and soil parameters at each experimental site. Responses obtained from review of existing publications and site databases (see citations in main text). Statistical approaches vary and are those most suitable for each site (see cited literature for details) – no new analyses have been undertaken

Response	Ruabon (RUH)	Budworth (BLH)	Thursley (TLH)	Culardoch (CAH)	Whim (WBO)		Wardlow acidic (WAG)	Wardlow Calc (WCG)	Pwllpeiran (PAG)	Newborough (NDG)
					Wet ^{R-O}	Dry				
Productivity ¹	↓20 ² ↓40 ³	↓60 ² ↓20 ³	↑7.7	↑10 ⁴	56 _R	↓56	↑35 ⁵	↑140 ⁵	0	↑7.5
Higher plant richness	0	0	0	0	0	0	0	0	0	0
Lichen abundance	↓20	–	↓7.7	↓10	↓24 _{R,O}	↓8	–	–	↓10	–
Bryophyte abundance	↓20 ⁶ ↓120 ⁶	0	–	–	↓24 _{R,O}	↓24	↓35	↓140	–	↑15
Foliar [N] higher plants	↓40	↓60	↑15.4	↑20	↑56 ⁷ _{R,O}	↑24	↑140 ⁸	↑140 ⁸	↑20 ⁹	0
Foliar [N] lower plants	↓40	↓20	–	–	↑24 _R	↑24	↑140	–	↑20	↑15
Flowering	↓20	↓60	↑7.7	↑10	–	–	↓140	↓35	↑20	–
Secondary stress	↓180 (winter damage)	↓160 (beetle damage)	↑30 (drought damage)	↑20 (winter damage)	↑56 _{R,O} (pathogen damage)	↑20 (pathogen damage)	–	–	0 (pathogen infection)	–
Soil %N	↓40	↑120	↑15.4	0	0	0	0	0	0	0
Soil NO ₃ ⁻ and NH ₄ ⁺	↓180 (NH ₄ ⁺) ↓140 (NO ₃ ⁻)	↑120 (NH ₄ ⁺) ↑120 (NO ₃ ⁻)	↑30 (NH ₄ ⁺) ↑30 (NO ₃ ⁻)	↑10 (NH ₄ ⁺) ↑10 (NO ₃ ⁻)	0 0	0 0	↑140 (NH ₄ ⁺) ↑140 (NO ₃ ⁻)	↑140 (NH ₄ ⁺) ↑140 (NO ₃ ⁻)	0 0	0 0
Mineralization	↓140	0	↑30	↑50	0	0	↑70 ¹⁰	↑35 ¹⁰	0	–
Nitrification	↓120	0	0	0	0	↑56	0	↑35	0	–
Denitrification	↓120	–	0	–	0	↑56	0	0	0	–
Leaching of NH ₄ ⁺ and NO ₃ ⁻	↓180 (NO ₃ ⁻) 0 (NH ₄ ⁺)	↓160 (NO ₃ ⁻) 0 (NH ₄ ⁺)	0	↑50 (NO ₃ ⁻) ↑10 (NH ₄ ⁺)	–	–	↑140 (NO ₃ ⁻) ↑140 (NH ₄ ⁺)	↑140 (NO ₃ ⁻) 0 (NH ₄ ⁺)	↑20 ¹¹ (NO ₃ ⁻) 0 (NH ₄ ⁺)	– –

Table 2 (continued)

Response	Ruabon (RUH)	Budworth (BLH)	Thursley (TLH)	Culardoch (CAH)	Whim (WBO)		Wardlow acidic (WAG)	Wardlow Calc (WCG)	Pwllpeiran (PAG)	Newborough (NDG)
					Wet ^{R-O}	Dry				
pH	↓80	0	↓15.4	0	↑56 _O	↑56	↓140	↓70	↓10 ¹²	0
Acid-base status change indicators	↓40 (Ca : Al ratio)	0	0	-	-	-	0 ¹³	↓140 ¹³ (base cation : Al ratio)	20 ¹⁵ ↓ (buffering capacity)	0

↑increase in response to N deposition at stated dose; ↓decrease in response; 0, no statistically significant change; -, not measured. Subscripts refer to exceptions in, or particular circumstances of, the response: [R-O] indicates whether the lowest deposition response was seen under reduced (R) or oxidized (O) forms of wet N deposition at WBO;

¹productivity of vegetation or growth of dominant species;

²productivity increases were reversed during degenerate phase of *C. vulgaris*;

³RUH postfire and BLH postcut re-growth slowed by N treatments;

⁴in combination with burning treatment, and significant response observed at 20 kg N ha⁻¹ yr⁻¹ without burning;

⁵response not typically observed but seen in one assessment;

⁶dynamic response – bryophyte cover was enhanced by low deposition rates and reduced at higher rates;

⁷response only occasionally seen, not typical;

⁸highly species specific and many species do not show significant change;

⁹significant effect only with reduced N [(NH₄)₂SO₄] treatment, and varying across years;

¹⁰initial positive response was not sustained after 7 years of treatment;

¹¹effect observed only with oxidized N treatment;

¹²reduced N [(NH₄)₂SO₄] treatment only, oxidized N showed a nonsignificant increase in pH;

¹³most base cations also show large declines, though changes were not statistically significant;

¹⁴reduced N [(NH₄)₂SO₄] treatment only.

Correction added after online publication (30 Nov 2011): Headers for Dry, Wardlow acidic (WAG), Wardlow Calc (WCG), Pwllpeiran (PAG) and Newborough (NDG) in Table 2 has been corrected.

Similarly, little change in floristic composition has occurred at the dune grassland, except for a decrease in the cover of the wood rush *Luzula campestris*, while at the WAG and WCG acidic and calcareous grasslands few significant plant community changes occurred in the first 6 years (Morecroft *et al.*, 1994; Carroll *et al.*, 2003). At these latter two sites, longer-term monitoring has revealed an overall decline in forb cover, with an increase in graminoids (Horswill, 2004), but without causing a change in species richness (Table 2). These minimal and/or slow changes in the grass-dominated systems are in contrast to the more rapid and considerable change in plant community composition seen in Dutch chalk grasslands, other dune grasslands and sub-alpine grassland experiments (Bobbink, 1991; Van den Berg *et al.*, 2005; Bassin *et al.*, 2007) and possibly reflects the limitation, or co-limitation, by nutrients other than N in the UK grassland experiments (as also suggested by their minimal or absent productivity responses). Some responses to N deposition may therefore be over-estimated if only N-limited ecosystems are studied. It should also be acknowledged that species composition may show little change in the more N-polluted sites due to a previous loss of sensitive species (see accumulated dose section below).

To summarize, the UKREATE sites show only limited evidence of N-driven shifts in the composition of vascular plant communities, especially at realistic N deposition rates. This suggests either that soil chemical changes have not been sufficient to shift competitive balances or permit invasive species to establish, or that the long history of N inputs at some sites and possible previous loss of sensitive species lessens responses to experimental N treatments.

Bryophytes and lichens. Bryophytes and lichens are considered sensitive to N deposition, since the lack of a well-developed cuticle means they can absorb pollutants across their surface area, and also because productivity increases in vascular plants may lead to increased shading and reduction in understorey species (Cornelissen *et al.*, 2001; Bates, 2002; Arróniz-Crespo *et al.*, 2008). Cryptogam declines are not only of concern in relation to conservation of biodiversity, but also because bryophytes and lichens can be important components of ecosystem function. This includes both carbon uptake and, more directly of concern in relation to N deposition, their function as 'N filters' that trap N deposition and may ultimately reduce N leaching to ground-waters (Lamers *et al.*, 2000; Curtis *et al.*, 2005; Emmett, 2007).

Lichen abundance has shown particular sensitivity across UKREATE sites, with declines occurring at application rates of only 7.7–10 kg N ha⁻¹ yr⁻¹ (Barker,

2001; Power *et al.*, 2004, 2006; Sheppard *et al.*, 2004a,b; Sheppard *et al.*, 2011; Britton & Fisher, 2007; Pilkington *et al.*, 2007a), rates equivalent to those seen in some of the least polluted areas of Europe.

Bryophytes were also sensitive to N deposition, but less consistently so than lichens. Among the most sensitive was *Sphagnum capillifolium* at the bog site (WBO), where significant declines were detected at 24 kg N ha⁻¹ yr⁻¹ after 3 and 5 years (dry NH₃ and wet deposition respectively, Sheppard *et al.*, 2011). In acidic grassland (WAG), a considerable 84% loss of the dominant *Rhytidiadelphus squarrosus* has been seen at treatment additions of 35 kg N ha⁻¹ yr⁻¹ (Arróniz-Crespo *et al.*, 2008). In the upland and lowland heaths (RUH, BLH) and bog sites, intermediate N deposition rates can sometimes increase bryophyte cover *et al.*, while the highest N loadings cause declines (Carroll *et al.*, 1999; Wilson, 2003; Pilkington *et al.*, 2007b; Sheppard *et al.*, 2011). This is consistent with the potential for positive growth effects at lower N concentrations which can result in expansion of nitrophilous bryophytes, as seen for *Hypnum jutlandicum* at the RUH and BLH heath sites (Wilson, 2003; Pilkington *et al.*, 2007b).

Overall, across sites, cryptogams are clearly the group most sensitive to N deposition in terms of both abundance and community composition. Given that floristic change amongst higher plants at realistic N deposition rates can be modest or absent, the tendency to focus on vascular plant diversity in many studies may miss much greater impacts on lower plant communities. This oversight should be avoided, given the role cryptogams play in many ecosystem functions, particularly N and C retention (Lamers *et al.*, 2000; Curtis *et al.*, 2005).

In summary, cryptogams show considerable sensitivity to N deposition. Lichens especially were detrimentally affected by even low rates of N loading. Bryophytes were also sensitive, but intermediate doses could stimulate growth of some species and nitrophilous bryophytes can benefit from N loading.

Flowering

Nitrogen deposition effects on flowering have often been overlooked, despite the potential long-term impacts on community composition and subsequent effects on seed banks. Nitrogen can alter seed bank composition through differential stimulation of seed germination (Plassmann *et al.*, 2008), while communities with short-lived seed banks such as calcareous grasslands are especially vulnerable from depletion of viable seed stocks resulting from declines in seed production (Bossuyt *et al.*, 2005).

Flowering rates appear sensitive to N treatments across a range of UKREATE sites. Doses of 7.7–60 kg N ha⁻¹ yr⁻¹ have increased *C. vulgaris* flowering at heathland sites (TLH, CAH, RUH and BLH; Power *et al.*, 1995; Uren *et al.*, 1997; Cawley, 2000; Pilkington, 2003; Britton & Fisher, 2008) and the magnitude of responses can be considerable. For instance, a 2-fold increase in *C. vulgaris* flowering under a modest 15.4 kg N ha⁻¹ yr⁻¹ was observed at the lowland heath (THL; Power *et al.*, 1995), and up to a 3-fold increase was observed at 50 kg N ha⁻¹ yr⁻¹ in low alpine heath (CAH; Britton & Fisher, 2008). Earlier extension of flowering buds of the ericaceous dwarf shrub *Vaccinium myrtilloides* has also been observed under 20 kg N ha⁻¹ yr⁻¹ in acidic grassland (PAG) and under 60 kg N ha⁻¹ yr⁻¹ at the BLH heathland (Cawley, 2000). These latter responses are again examples of advancement of phenology similar to the earlier growth initiation seen in *C. vulgaris* in heathland sites under elevated N deposition (described above).

In contrast, forbs show reduced flowering rates at the WAG and WCG grasslands (at 35 and 140 kg N ha⁻¹ yr⁻¹ respectively) with, for example, an 89% decline in *Gentianella amarella* and a 97% reduction seen in *Potentilla erecta* (O'Sullivan, 2008). These considerable declines can be larger in magnitude than declines in cover (Horswill, 2004; O'Sullivan, 2008) and so flower numbers may be a more sensitive indicator of plant response to N deposition than assessment of cover change. Indeed, flowering shifts may indicate underlying changes in allometry and/or greater shifts in competitive balance between species than may be apparent from vegetative cover or growth estimates (Cleland *et al.*, 2006). The greater sensitivity of flowering compared to vegetative cover seen in some forbs, for instance, may be indicative of a shift in allocation away from flowering to growth as a response against the competitive pressure from grasses in N enriched grasslands.

The reported impacts on flowering also indicate the potential for changes in seed supply and a depletion of the seed bank in grasslands that may limit regeneration capacity of affected systems. In grasslands, there may also be reduced rates of genetic recombination that could impact on genetic diversity. Furthermore, changes in flower abundance will have knock-on effects on the amenity value of seminatural ecosystems and on pollinators, potentially driving changes in ecosystem services.

To summarize, N deposition can suppress forb flowering in grasslands, and stimulate flowering of the dominant dwarf shrub, *C. vulgaris*, in a wide range of heathland ecosystems – in the latter case even at low rates of N loading. These changes may have important consequences for pollinators and regeneration from seedbanks of affected communities.

Foliar chemistry

Higher plants. Changes in foliar chemistry, particularly N concentration, are considered to be among the most sensitive indicators of N deposition impacts, especially since changes may be expected in response to both increased soil N availability and direct foliar uptake. Such changes may also be the first indicators of ecosystem eutrophication (Arróniz-Crespo *et al.*, 2008), though growth dilution may serve to limit increases in foliar N. Understanding the nature of changes in foliar chemistry is important because increases in tissue N can increase the likelihood of damage from biotic and abiotic stress (Power *et al.*, 1998b; Sheppard *et al.*, 2008a,b), and increases in N : P ratios may indicate increasing P limitation to growth, which can be important for understanding the mechanisms driving species and productivity changes (Phoenix *et al.*, 2004a).

Among higher plants, changes in foliar N and N : P ratios have been observed at all sites to varying degrees. These changes include increases in foliar N : P ratios of the dominant *C. vulgaris* at RUH, TLH and CAH heaths, and reduced foliar C : N ratios at BLH and CAH heaths (Wilson, 2003; Green, 2005; Britton *et al.*, 2008; Edmondson *et al.*, 2010). At the bog site (WBO), *C. vulgaris* shoot N concentrations have increased under wet reduced-N in some years (Sheppard *et al.*, 2008b), but much clearer responses have been observed under dry deposition (NH₃), with dose-related (exponential) increases in foliar N above 24 kg N ha⁻¹ yr⁻¹ (Sheppard *et al.*, 2008a).

Changes in foliar chemistry are generally less apparent in the grassland ecosystems. At the WCG and WAG grasslands, only some species respond to even the largest N treatment of 140 kg N ha⁻¹ yr⁻¹ (Horswill, 2004; Horswill *et al.*, 2008; O'Sullivan, 2008). At the PAG grassland, N-driven increases in foliar N (and resulting increases in N : P ratios) have been dominated by inter-annual variability and effects of grazing treatments (Emmett *et al.*, 2004, 2007). Furthermore, no changes have occurred in above-ground higher plant foliar N in the dune grassland (Plassmann *et al.*, 2009), although N storage increased in *Carex arenaria* rhizomes (Hodges, 2006).

In summary, N concentrations appear more responsive to N deposition in the heathland and bog sites. In the grassland systems and when considering the more realistic dose levels, foliar N is not a broadly reliable response.

Lower plants. Bryophytes may show greater changes in tissue N than vascular plants as they absorb nutrients over their entire surface area (Bates, 2002), though among the UKREATE sites, only the BLH heathland

and the dune grassland support this (Wilson, 2003; Plassmann *et al.*, 2009). When treatments include reduced N forms, dose rates that result in significant changes in tissue N are generally low (15–40 kg N ha⁻¹ yr⁻¹ for NDG, BLH, PAG, WBO and RUH) (Wilson, 2003; Plassmann *et al.*, 2009), though at the WAG acidic grassland, significant increases in bryophyte tissue N only occurred under 140 kg N ha⁻¹ yr⁻¹ (Arróniz-Crespo *et al.*, 2008). Oxidized N caused smaller increases than reduced N in *Hypnum jutlandicum* and *Sphagnum capillifolium* at the bog site (Carfrae, 2006) but there was no consistent difference between N forms or treatments at the PAG acidic grassland (Emmett *et al.*, 2004; Emmett, 2007). Furthermore, at the bog site, the threshold for oxidized-N effects was higher than for reduced-N (56 compared to 24 kg N ha⁻¹ yr⁻¹; Carfrae, 2006; Kivimäki *et al.*, 2008). Dry NH₃ deposition at this site tends to cause variable responses in bryophytes, as tissue damage and leakage partly counteract N accumulation (L.J. Sheppard, unpublished data).

In summary, tissue N in lower plants often increases at low to intermediate N loading, though the expected greater sensitivity of lower plants compared to higher plants is only seen at two sites. Species-specific changes in tissue chemistry of lower plants also appear dependent on the N form deposited.

Interactions with abiotic and biotic stress (climate, herbivory and pathogen damage)

Nitrogen/stress interactions can provide important triggers responsible for major shifts in vegetation composition. This has been clearly documented, for instance, in Dutch heathlands, where opening up of the *C. vulgaris* canopy by heather beetle outbreaks facilitated the invasion and subsequent rapid expansion of grasses (Berdowski, 1987, 1993) and has been a major driver of heathland loss (Bobbink & Lamers, 2002). It is of concern, therefore, that enhanced N deposition at most UKREATE sites has led to signs of increased stress, ranging from injury in response to climatic events (drought, severe frosts) to increased rates of pathogen and herbivore damage.

Greater levels of winter desiccation damage to shoots of *C. vulgaris* have been observed at upland and low alpine heath sites (RUH, CAH) (Carroll *et al.*, 1999; Britton & Fisher, 2007) and at the bog (WBO) (Sheppard *et al.*, 2008a). In the latter, the greater damage from dry NH₃ deposition was again apparent with wet reduced N causing significant damage at 56 kg N ha⁻¹ yr⁻¹ compared to 24 kg N ha⁻¹ yr⁻¹ for dry deposition (Sheppard *et al.*, 2011).

At one lowland heath site (TLH), exacerbation of secondary stress damage (shoot browning) of *C. vulgaris*

has been observed in association with summer drought (Green, 2005) and is consistent with reduction of shoot growth at the other lowland heath (BLH) under enhanced N deposition when combined with experimentally applied summer drought (Cawley, 2000). Similarly, a drought year at the bog site resulted in the only occasion of lower *C. vulgaris* shoot extension under wet N deposition (at 56 kg N ha⁻¹ yr⁻¹; Carfrae, 2006).

N deposition may also exacerbate pathogen damage as seen at the bog site, where dry NH₃ deposition increased infection of *C. vulgaris* by *Botrytis* and *Phytophthora* pathogens at doses as low as 20 kg N ha⁻¹ yr⁻¹ (Sheppard *et al.*, 2008a), though at the PAG acidic grassland the frequency of brown leaf spots from fungal infection was not affected by N deposition treatments. Enhanced N deposition has also been shown to lead to increased herbivory damage, as previously seen in Dutch and Danish heathlands (Heil & Diemont, 1983). At the BLH heath, N treatments of 60 kg N ha⁻¹ yr⁻¹ resulted in greater damage from heather beetle larvae (Wilson, 2003) while in laboratory studies, heather beetle larvae feeding on *C. vulgaris* from the TLH field plots showed greater relative growth rates and greater adult mass, with impacts seen at doses of 15.4 kg N ha⁻¹ yr⁻¹ (Power *et al.*, 1998b).

In summary, there is clear evidence that N deposition can exacerbate secondary abiotic and biotic stresses in heathland and bog systems, increasing the likelihood of ecosystem damage. Responses are apparent at modest (though not necessarily the lowest) N deposition rates. Given future climate scenarios of more extreme weather patterns, or climate change that may result in greater pathogen or herbivore damage, these stresses likely represent increasing threats to semi-natural systems receiving greater rates of N deposition.

Soil N pools and cycling

Changes in soil chemistry can drive ecosystem responses to N deposition, through acidification and eutrophication. Increasing N availability is a key driver of altered competitive balance between species (Bobbink *et al.*, 2010), while N-driven increases in soil acidity can lead to nutrient imbalances through base cation depletion and increased solubilization of toxic metals such as aluminium and iron (Tietema *et al.*, 1998; Van der Eerden *et al.*, 1998; Roem & Berendse, 2000; Bowman *et al.*, 2008; Van den Berg *et al.*, 2008).

At the lowland and upland heaths (TLH, BLH and RUH) significant increases in soil %N have been observed in response to doses of 15.4–120 kg N ha⁻¹ yr⁻¹ (Power *et al.*, 1998a; Field, 2010). In contrast, at all four grassland sites and the bog (NDG, PAG, WAG, WCG and WBO), no increases in %N have been

recorded, even under the long-term very high N doses used at WAG/WCG (Emmett *et al.*, 2004, 2007; O'Sullivan, 2008).

Significant increases in KCl extractable (plant available) soil NO_3^- and NH_4^+ have been detected at a range of grassland and heathland sites (THL, CAH, RUH, WAG and WCG) in response to treatments of 30–140 kg N $\text{ha}^{-1} \text{yr}^{-1}$ (Green, 2005; Pilkington *et al.*, 2005c; Horswill *et al.*, 2008; O'Sullivan, 2008; Papanikolaou, 2008). At the BLH lowland heath, however, only increases in NH_4^+ (not NO_3^-) have been observed (Wilson, 2003), while at the PAG and NDG grasslands and the bog, no change has been detected. Overall, heathlands appear to be the most sensitive in terms of KCl extractable N, since all other sites either show no response, or need very high treatment rates to induce a response.

The impact of N deposition on N cycling ultimately determines the long-term availability and fate of the element in ecosystems. The amounts of N retained in soil and vegetation, and the forms in which it accumulates, is released to plants, or is lost from soil through gaseous fluxes and leaching, can all affect the duration and strength of the influence of N as a driver of change in ecosystem functioning. Furthermore, a sequential response of (at first) reduced NO_3^- immobilization, then increased NH_4^+ production followed by an increase in net nitrification may represent progressive stages of increasing N saturation (Aber *et al.*, 1989; Emmett, 2007), and hence changes in these parameters can provide informative indicators of the development of N deposition impact and N saturation.

N loading has stimulated N mineralization at three heath sites (TLH, RUH and CAH), and two grassland sites (WCG, WAG), under treatments of 30–70 kg N $\text{ha}^{-1} \text{yr}^{-1}$, with the greatest effects occurring in spring and/or summer (Carroll *et al.*, 2003; Green, 2005; Pilkington *et al.*, 2005c; Papanikolaou, 2008). Similarly, mineralizable N is positively correlated with N dose in the dune grassland (L. Jones, unpublished data). The highest N inputs lead to considerable 2.5-, 3- and 6-fold increases in mineralization rates at TLH, CAH and WAG (Morecroft *et al.*, 1994; Green, 2005; Papanikolaou, 2008). This positive feedback can enhance ecosystem eutrophication by increasing the mineralization of organic N returned to the soil through plants, animals and microorganisms. However, enhanced mineralization is not a universal response and has not been observed at the bog (assessed under the wet deposition treatments; Field, 2010) or at the PAG acid grassland, while at the WAG and WCG grasslands, increases reported in the early years were not sustained after 7 years of treatment (Emmett *et al.*, 2004, 2007; Macdonald, 2004). The lack of significant changes in

mineralization rates in some sites may be due to high spatial heterogeneity.

Nitrification rates increased in the bog (dry NH_3 deposition only, based on soil water nitrate concentrations) and the WCG calcareous grassland, but more commonly, either small increases or no clear impacts were observed at the other heathlands and grasslands (Emmett *et al.*, 2004, 2007; Green, 2005; Pilkington *et al.*, 2005c; Papanikolaou, 2008).

In summary, enhanced N deposition tends to increase plant available (KCl extractable) soil N concentrations, and some mineralization processes, with increases in bulk soil %N also seen in the heathland but not grassland sites. Responses are much clearer at the highest N deposition rates, possibly a result of the large spatial variability in these parameters confounding detection of subtle changes.

Losses and retention of pollutant N

An understanding of N deposition impacts on ecosystem N retention and leaching rates is important because an increase in NO_3^- leaching is one of the first indicators of the onset of N saturation within an ecosystem (Aber *et al.*, 1989). However, this may occur much later than many biodiversity and internal N cycle changes (Emmett, 2007). N retention is an important ecosystem service that limits pollution of aquatic ecosystems and groundwater (Stoddard, 1994; Phoenix *et al.*, 2003).

NO_3^- is sensitive to N loading showing enhanced leaching rates in most heathland and grassland sites where assessed (RUH, BLH, CAH, WAG, WCG, PAG, but not THL). In contrast, NH_4^+ leaching more often does not respond to N loading, but has been seen to increase at the CAH heath and WAG grassland (at 10 and 140 kg N $\text{ha}^{-1} \text{yr}^{-1}$) (Phoenix *et al.*, 2003; Pilkington *et al.*, 2005a; Field, 2010) while increases in dissolved organic N (DON, although much less frequently assessed) have occurred at the low alpine heath and calcareous grassland (CAH, WCG) under 10 and 140 kg N $\text{ha}^{-1} \text{yr}^{-1}$ respectively (Phoenix *et al.*, 2003; Helliwell *et al.*, 2010).

Contrasting impacts on N leaching of different forms of N deposition can also be seen. At the PAG acidic grassland, NaNO_3 but not $(\text{NH}_4)_2\text{SO}_4$ at 20 kg N $\text{ha}^{-1} \text{yr}^{-1}$ increased NO_3^- leaching, while at the bog (although leaching was not directly measured) wet deposited NH_4^+ increased NH_4^+ concentrations in surface soil water samplers (under 56 kg N $\text{ha}^{-1} \text{yr}^{-1}$ and greater) without affecting NO_3^- (Field, 2010). As seen in many other responses at the bog site, dry NH_3 deposition caused the greatest response, with >10-fold increases in NH_4^+ and NO_3^- at treatment rates of 56 kg N $\text{ha}^{-1} \text{yr}^{-1}$ (Field, 2010).

While most sites show increases in N leaching, it should be noted that small or no increases often occur under treatments at the lower (and most realistic) range of N loadings. These habitats may, therefore, have considerable capacity to act as N sinks under typical ambient levels of pollutant N deposition and highlights their important role in the ecosystem service of clean groundwater provision (Phoenix *et al.*, 2003). Further evidence for pollutant N storage can be seen in N budget studies undertaken at some sites. At the TLH lowland heath, microbial biomass plays a major role in N retention, with this pool representing two-thirds of the total ecosystem N store, probably explaining the negligible N leaching rates at all N treatment levels there (Green, 2005). At the WAG and WCG grasslands, the bulk soil and plant pools appear to play an equal role in pollutant N retention (Phoenix *et al.*, 2004b) and in fact the WCG grassland was shown to retain a considerable 65% of deposited N, even after 8 years of the very high 140 kg N ha⁻¹ yr⁻¹ treatment (this retention was only 15% at the neighbouring WAG grassland; Phoenix *et al.*, 2003, Phoenix *et al.*, 2004b). At the upland heath site, around 60–90% of N input is retained within the soil and litter pool (Pilkington *et al.*, 2005a) while at the BLH lowland heath, N accumulates not only in the soil but a large fraction also appears in the understorey moss (*Hypnum jutlandicum*) (Ray, 2007); only at the higher N treatments at BLH, when the soil pool saturates and the moss growth is inhibited, does N leaching become significant. Similarly, in the dune grassland moss biomass is also a significant store of added N (Plassmann *et al.*, 2009) while a ¹⁵N tracer study in the PAG grassland indicated an equivalent sink strength for the labelled tracer by the soil, higher plants and lower plants (Emmett *et al.*, 2004). These findings are consistent with the proposed important role of cryptogam vegetation in influencing N leaching (Curtis *et al.*, 2005).

Having highlighted N retention, it should also be noted that some systems such as the low alpine heath, have low biological demand and relatively small soil pools, and so can be very sensitive to leaching. This can be an issue for water quality in the headwaters of river systems where these systems predominate.

N losses can also occur through denitrification but this has been measured less often and impacts are either not apparent or only clear at relatively high doses.

In summary, while increases in N leaching have occurred at most sites (and at some sites, increases in denitrification also), responses are often only apparent at relatively high N loadings or when N is applied as nitrate rather than ammonium. Overall therefore, levels of N retention – particularly at realistic N deposition

rates – are typically high showing the significant capacity of these systems for pollutant N storage

Soil pH, base cation depletion, Ca : Al ratios

Changes in soil acidity, base cation status and Ca : Al molar ratios are important indicators of damage to the soil and can explain shifts in plant community composition (Stevens *et al.*, 2011). Close correlations with pH and species richness show that species loss can occur as a direct response to acidification (Johnson *et al.*, 1986; Roem & Berendse, 2000). Furthermore, associated mobilization and depletion of base cations can lead to nutrient imbalance in tissues while mobilization of potentially toxic ions such as Al³⁺ and Fe³⁺ can cause direct plant damage (Bowman *et al.*, 2008). Therefore, high Ca : Al ratios are often considered a good indicator of soil health, and a decrease in this ratio can be an indicator of potentially damaging soil conditions (Emmett *et al.*, 1998). Overall, these changes can take many years to develop and would not necessarily be apparent in shorter-term studies, but may be indicators of an ecosystem that has been considerably impacted by N deposition (Bowman *et al.*, 2008).

Many of the UKREATE sites show evidence of such changes. Significant acidification has been observed in the soils at the heathlands of RUH (0.1 pH units) and TLH (0.5 units), and the grasslands of PAG (0.2 units), WAG (0.2 unit) and WCG (0.5 units) (Pilkington *et al.*, 2005a; Power *et al.*, 2006; Horswill *et al.*, 2008). Such acidification may contribute to changes in plant community composition, particularly at sites with lower buffering capacity which may be more prone to the impacts of acidification (Clark *et al.*, 2007; Stevens *et al.*, 2011). In contrast, no changes in soil pH have been observed at the low alpine and BLH lowland heaths or the dune grassland (Plassmann *et al.*, 2009; A. J. Britton and S. J. M. Caporn unpublished data). In the dune grassland, the absence of change is perhaps unsurprising given the modest doses and the considerable buffering capacity of this site.

Again, contrasting effects of N forms have also been observed. At the bog, both dry NH₃ and wet oxidized treatments increased peat pH by 0.5 and 0.3 units respectively, whereas wet reduced N additions have not changed pH (Sheppard *et al.*, 2008b). At the PAG acid grassland, the (NH₄)₂SO₄ treatments reduced soil pH (0.2 units), while the NaNO₃ treatments resulted in a slight (nonsignificant) increase in pH (Emmett *et al.*, 2004).

Associated changes in soil base cations and anions have also been measured at some sites (though this has not frequently been assessed). In the upland heath (RUH), N treatments have resulted in a decline in Ca²⁺ concentration in the mineral horizon soil solution,

resulting in a decrease in soil Ca : Al ratios (Pilkington *et al.*, 2005a), though clearly not yet to the detriment of the dominant *C. vulgaris* that shows greater productivity under high N treatments. In the PAG acidic grassland, shifts in charge balance alkalinity (sum of base cations – sum of acid ions) indicates that the buffering capacity of this grassland has been significantly decreased by the $(\text{NH}_4)_2\text{SO}_4$ treatments (though this increased in response to the NaNO_3 treatments due to the addition of sodium; Emmett *et al.*, 2004). Such loss of buffering capacity could in future exacerbate acidification impacts and enhance plant community change driven by this process (Emmett, 2007).

Similar changes have occurred at the WAG and WCG grasslands, and although these changes have not been statistically significant, almost all base cations have shown relatively large declines (~25% and 35% reduction on average for WAG and WCG respectively), providing a strong indication of the onset of base cation depletion (Horswill *et al.*, 2008). Given that P-limitation in these two grasslands (see floristic change section) means species change is unlikely to result from N-driven productivity responses and competitive exclusion, instead base cation depletion, altered Al availability and pH changes may be the main drivers of N-induced species change in these systems.

These changes, however, are not universal across UKREATE sites. At the two lowland heathlands, the absence of treatment effects on leaching rates of H^+ , Ca^{2+} , Na^+ , Mg^{2+} , K^+ , Al^{3+} , Cl^- (BLH) or base cation status (TLH) indicates that soil cation retention is not significantly affected by N addition in sandy lowland soils (Wilson, 2003; S. A. Power, unpublished data).

In summary, changes in soil pH and base cation status were variable between sites, reflecting the diversity in soil chemistry, potential buffering capacity, organic matter content and initial soil pH. Nevertheless, the responses corroborate established literature on the subject and suggest that N-driven effects on acid–base status may have important ecological consequences at some sites.

Accumulated dose responses – comparison of ecosystem sensitivities and rate of response

Our review above – in common with past reviews of N deposition impacts in experimental plot studies (e.g. Bobbink *et al.*, 1998, 2010; Lee & Caporn, 1998; Bobbink & Lamers, 2002) – registers impacts when a statistically significant effect has occurred in enhanced N deposition treatment plots compared to control plots. While adhering to statistically significant changes is essential in ensuring robust assessment of responses, such an approach does not consider how long an impact may

have taken to become apparent, whether the rate or direction of response changes over time (i.e. how the impacts of pollution may build up with time and continued N loading) and confounds comparison of sensitivity between sites with different treatment levels or duration of experiments.

To address these issues we have undertaken an analysis of some key plant and soil response parameters measured across most or all UKREATE sites, and determined the extent of change in those parameters with accumulated dose (i.e. N deposition rate \times number of years of deposition). This allows both the rate of deposition and the duration of deposition to be accounted for in the response. Furthermore, accumulated dose includes both current and historic (since 1945) ambient deposition. This was done to ensure that responses were (i) determined against the total N input (treatment plus ambient) into the plots, and (ii) allows separation of sites with historically greater or lesser ambient N deposition. We chose to start accumulated dose in 1945 since this represents the time from which the greatest increases in N emissions in the UK have occurred (Fowler *et al.*, 2004).

Much of the oldest data on which this analysis relies has been archived as treatment level means (i.e. the average of replicate plots), rather than individual plot values. Therefore, the accumulated dose response calculations use treatment level means rather than individual plot values. For each data point, accumulated N dose was calculated as the amount of ambient N deposited since 1945 estimated from the Centre for Ecology and Hydrology CBED-model (Smith *et al.*, 2000) with national historical scaling factors (Fowler *et al.*, 2004) plus any additional N received through N manipulation treatments (summed from the start of the treatment period to the time point at which the data point measurement was made). Amount of change in the ecological parameter was determined as the change in its value compared to a control value. The control value was calculated for each experimental site, as the average of the two data values with the lowest cumulative N (i.e. generally the first two times a measurement was made on control plots). We used an average of two values as the control to provide a more stable analysis (i.e. to reduce the chance of an unusual single control value altering the entire accumulated dose response relationship). Amount of change was then expressed as the responsiveness index L .

$$L = \ln \frac{(X_a + 1)}{(X_c + 1)}$$

where X_a is the value of the ecological parameter measured and X_c is the respective control value. Use of

the logarithm has the advantage of linearizing the L indices since L also equals $[(\ln X_a + 1) - (\ln X_c + 1)]$.

L was plotted against accumulated dose for each site, for lower plant cover, richness of lichens, bryophytes and higher plants, soil %N and pH. Linear, inverse, asymptotic and exponential decline models describing the relationship between L and accumulated dose were fitted separately for each site using the nls procedure of R, version 2.13.0 (R Development Core Team, 2007), and assessed with F -tests for variance reduction. Relationships were not plotted where there was no significant reduction in variance from the null model. These plots therefore (i) allow visualization of the rate of change with accumulated dose (i.e. the line gradient), (ii) provide insight into whether sensitivity changes with increasing accumulated dose (i.e. whether the line steepens or become more shallow), and also (iii) separates out sites of contrasting ambient deposition, with sites of low historic rates appearing on the left of the graphs (i.e. low initial accumulated dose values) and more polluted sites on the right.

Lower plant cover showed very clear and significant declines with accumulated dose across eight of nine sites (Fig. 2a, Table 3), emphasizing the considerable sensitivity of lower plants to N deposition across a diversity of habitat types of contrasting community composition, edaphic and climatic conditions, and ambient deposition rates. This is also the first evidence that accumulated dose can be used to readily describe the trajectory of an ecological response in N deposition field simulation experiments. The rates of change with accumulated dose (line gradient) at each site also appear to divide into two main groups with those sites of lower historic ambient deposition (WBO, CAH, NDG, PAG and THL) having much steeper responses compared to those with higher ambient deposition histories (BLH, RUH and WAG) (indeed, note that the responses of these latter sites are shallower than visualized on the graph due to the contracting of the axis by the log scale for accumulated dose). There is also some evidence of a lessening of response at higher accumulated dose at WBO, CAH and WAG, where response gradients become shallower as N load accumulates.

Declines in bryophyte richness with accumulated dose were only found at WBO, but also showed a lower response rate as N load accumulated (Fig. 2b). More consistent responses were seen for lichen richness (Fig. 2c) with WBO, CAH and PAG all showing significant declines. Here again, responses at all three sites were best described by curves that lessened in response as dose accumulated, consistent with decreasing sensitivity with increasing accumulated dose.

The separation of sensitivity with historic deposition (Fig. 2a) appears unrelated to ecosystem type (e.g.

grasslands and heaths appear in both low and high historic deposition groups) and suggests the most polluted sites may have seen the greatest declines in cover, or shifted towards a more nitrophilous assemblages before the experiments began. Similarly, the steeper declines in richness of lichens and bryophytes at some sites at lower accumulated doses (Fig. 2b and c) is consistent with the hypothesis that the greatest changes in species composition occurs in the early stages of enhanced N loading, when the sensitive species of low abundance are rapidly lost. This has been seen in analyses of USA-LTER sites, prairie grassland and European grasslands (Suding *et al.*, 2005; Clark & Tilman, 2008; Bobbink *et al.*, 2010). The trajectories of the accumulated dose responses also suggest that even low rates of N deposition may impact an ecosystem if continued for long enough. This approach, therefore, supports the downward revision of critical loads that often occurs as evidence of impact becomes apparent at lower N deposition rates over longer time scales.

In contrast, higher plant richness showed little consistent pattern of change with accumulated dose (Fig. 2d). The increases at CAH and BLH were the only statistically significant changes (Table 3), but neither represent major vegetation shifts. In the former, the increased richness results from the tendency for N treated plots to have five species rather than four, while in the latter, the increase results from the relatively transitory response of the appearance of *Rumex acetosella* and *Erica cinerea* which had established (at the times of the vegetation surveys) due to N treatment induced delayed regrowth of *C. vulgaris* after a management cut (see Floristic Change section above). It should also be noted that changes in plant community structure observed at the UKREATE sites do not necessarily demonstrate complete loss of a species, but are often instead seen as important and large declines in abundance of sensitive species. Heathland habitats also typically have low vascular plant diversity, with responses expressed principally in terms of the structure and biology of the dominant plant, *C. vulgaris*. It is therefore unsurprising that accumulated dose does not show pronounced changes in higher plant richness across the UKREATE sites.

Only NDG and WCG display a significant relationship between soil pH and accumulated dose (Fig. 3a, Table 3), and responses across sites do not necessarily follow understanding gained from comparison of treatment with control plots (review above and Table 2). For instance, at NDG, comparison between treatment levels indicates no impact on pH (Table 2) and instead the positive accumulated dose response appears to be an artefact of a shift in pH data between just two sampling years. The heathlands at TLH and RUH both show significant acidification when comparing

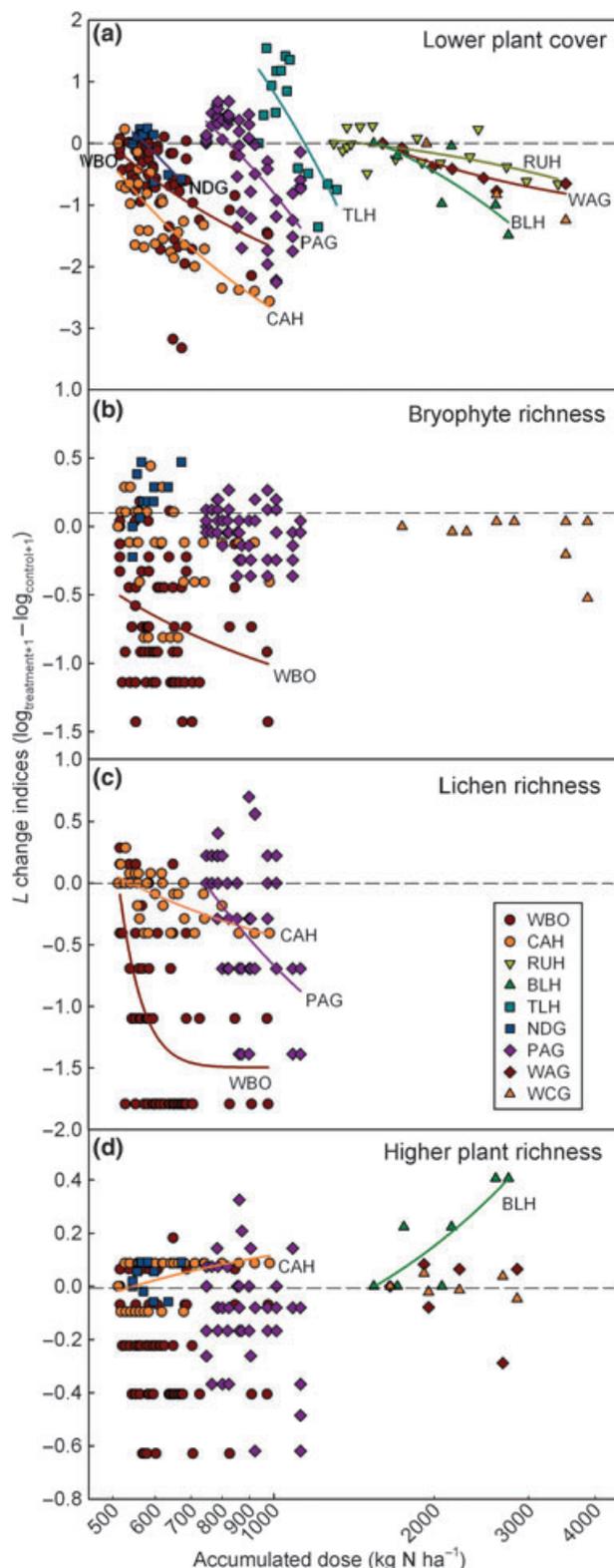


Fig. 2 Change in (a) lower plant cover, (b) bryophyte richness, (c) lichen richness and (d) higher plant richness with accumulated N dose. Change is expressed as the *L* indices. Site codes are for Table 1. Lines were only plotted where an *F*-test showed a significant reduction in variance compared with the null model (see Table 3). A log scale for accumulated dose was used to allow clear presentation on the same graph of sites with contrasting N loading, but note this contracts the scale a higher N values and so response gradients of sites on the right of the graph visually appear steeper than in reality. For clarity and comparison with other sites, responses at WBO and PAG are for all N forms combined. Separate oxidized and reduced N form lines are similar for PAG. For WBO, dry deposition results in steeper gradients and more negative *L* values than the wet N forms.

data sets are small and/or where data are spread across relatively few years in which there may have been large inter-annual variation.

The two heathland sites of TLH and RUH showed significant relationships between soil %N and accumulated dose, with these being notable for their steep response relationships (Fig. 3b, Table 3). Other sites do not show accumulated dose responses in soil %N, which largely agrees with understanding gained from comparison of treatment plots (review above and Table 2; BLH has few data points hence limiting the chance of an accumulated dose response in %N).

Overall, accumulated N dose can provide additional insight into the trajectories of responses and allow comparison of both response and sensitivity between experimental sites that differ in treatment levels, treatment duration and ambient deposition. Lower plant cover proved a particularly powerful parameter, indicating (i) much lower sensitivity to accumulated N loading in sites with historically high N deposition, (ii) some evidence of lessening response within sites as N load accumulates (i.e. greater sensitivity at first), and (iii) the possibility that even relatively low N loadings can result in community change. These are benefits that are not necessarily readily attainable from the traditional approach of directly comparing treatment and control plots. However, accumulated dose proves less useful where data sets are limited, where inter-annual variation may override N deposition impacts or where responses may be relatively subtle. In such cases, traditional comparisons of treatment plots against controls can provide better insight into the response of ecosystems to N deposition.

Conclusion

The UKREATE sites show clear and consistent evidence of the impacts of N deposition across nine sites of contrasting vegetation, soils, climate and ambient N deposition. Clear responses were seen with increased

treatment plots with controls, but these changes do not come through in the accumulated dose responses, probably due to a low number of data points. The accumulated dose approach appears to have limitations where

Table 3 Regression analysis of the relationship between *L* change indices and accumulated N dose for the response parameters shown in Figs 2a–d and 3a and b

Site	Line type	r^2	P	$P(b)$	$P(c)$
Lower plant cover					
WBO	$a + b/x$	0.257	<0.001	0.000	
CAH	$a + b/x$	0.667	<0.001	0.000	
RUH	$a + bx$	0.394	<0.01	0.039	
BLH	$a + bx$	0.714	<0.05	0.050	
TLH	$a + bx$	0.512	<0.01	0.002	
NDG	$a + bx$	0.533	<0.05	0.012	
PAG	$a + bx$	0.419	<0.001	0.000	
WAG	$a + b/x$	0.852	<0.05	0.001	
WCG	Null				
Bryophyte richness					
WBO	$a + b/x$	0.074	<0.05	0.000	
CAH	Null				
NDG	Null				
PAG	Null				
WCG	Null				
Lichen richness					
WBO	$a+b \cdot e^{-cx}$	0.366	<0.001	0.795	0.011
CAH	$a + b/x$	0.397	<0.001	0.000	
PAG	$a + b/x$	0.212	<0.001	0.000	
Higher plant richness					
WBO	Null				
CAH	$a + b/x$	0.185	<0.05	0.007	
BLH	$a + bx$	0.706	<0.05	0.053	
NDG	Null				
PAG	Null				
Soil pH					
WBO	Null				
CAH	Null				
RUH	Null				
TLH	Null				
NDG	$b(1 - e^{-c(x-a)})$	0.951	<0.05	0.358	0.038
PAG	Null				
WAG	Null				
WCG	$a + b/x$	0.502	<0.01	0.001	
Soil%N					
WBO	Null				
CAH	Null				
RUH	$a + b/x$	0.857	<0.001	0.000	
BLH	Null				
TLH	$a + b/x$	1.000	<0.01	0.001	
NDG	Null				
PAG	Null				
WAG	Null				
WCG	Null				

Line type = model form fitted, where different from the null model i.e. mean fitted only. r^2 , proportion of variance explained; P , probability of no significant difference from null model; $P(b)$, F -probability for slope term; $P(c)$, F -probability for exponent term.

productivity (except where sites were not N limited), declines in cover and diversity of lower plants, both

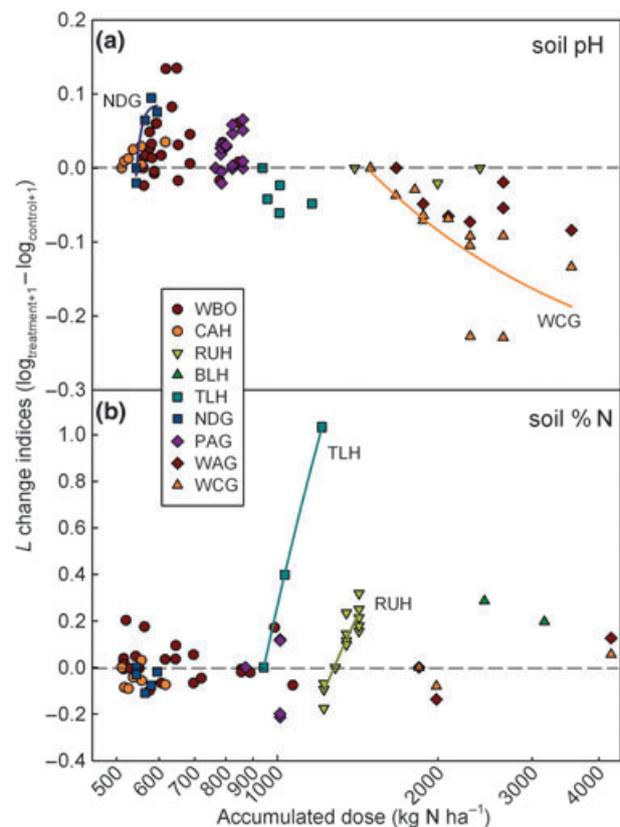


Fig. 3 Change in (a) soil pH, and (b) soil%N with accumulated N dose. Change is expressed as the *L* indices. Site codes as for Table 1. Lines were only plotted where an F -test showed a significant reduction in variance compared with the null model (see Table 3). Note log scale for accumulated dose as for Fig. 2. For clarity and comparison with other sites, responses at WBO and PAG are for all N forms combined. For WBO, separate plotting of dry deposition results in a significant positive response for *L* with accumulated dose (Fig. 3a).

stimulation (*C. vulgaris*) and declines (forbs) in flowering, and exacerbation of biotic and abiotic secondary stresses. Further impacts were apparent in soil nutrient status and cycling, and acid–base status, with some sites showing a considerable capacity to store pollutant N. Less clear responses were seen in higher plant richness, though the nature of higher plant changes at the UKREATE sites (i.e. changes in abundance without necessarily a loss of species) is consistent with this and shows that N deposition still has important impacts on higher plant community structure. All of these responses will have significant consequences for the structure and function of ecosystems, the provision of ecosystem services, and the capacity of ecosystems to recover once N deposition rates decline following successful legislation.

N deposition field experiments can be criticized where they risk over-estimating responses through

use of very high N inputs, single high dose applications or solid fertilizer inputs. The UKREATE sites largely avoid these concerns, showing sensitivity in a diversity of parameters to low N deposition rates using frequent, low input, applications. Similarly, the accumulated dose analysis suggests that some sensitive ecological responses (such as lower plant cover) may be affected by even very low rates of N loading if continued for long enough. This analysis, at least in terms of lower plant species, also supports suggestions that sites which have historically been in receipt of high N loads, may already have lost their most sensitive species, and so now appear less sensitive to further N loading.

Future policy development should take note that there is clear evidence that modest N loading can have significant impacts on seminatural ecosystems and even low N inputs may induce effects over time through accumulated loading.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Soil, precipitation and temperature summaries for UKREATE sites.

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