The ability of contrasting ericaceous ecosystems to buffer nitrogen leaching

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SUMMARY

Much attention has been given to the carbon balance of peatland and heathland ecosystems and their role as global carbon stores. They are also important as buffers for atmospheric nitrogen (N) pollution, locking N into the soil and vegetation through tight nutrient cycling and preventing the leaching of soluble N into freshwater ecosystems. We compared mean annual soil exchangeable N, mineralisation and soil solution nitrogen at three contrasting ericaceous-dominated ecosystems: a lowland heath, an upland heath and an ombrotrophic raised bog at intermediate altitude, all of which were sites of long-term N-manipulation experiments. We expected that soil leachate N would be associated with soil C/N and total soil C, and that sites with higher C % and soil C/N would have greater ability to buffer N deposition before N saturation and leaching began. However, although soil solution N responded to N deposition at all the sites, we found that only the heathland sites were consistent with this expectation. The bog, with the highest C/N and largest C pool, was not the most strongly buffered. The upland heath was most effective at retaining N (extractable NH_4^+-N +3900 % from control) compared to the lowland heath (extractable NH_4^+-N +370 % from control) and the bog (extractable NH_4^+-N, +140–240 % from control). We concluded that the absence of a definable *Calluna* litter layer at the lowland heath and the bog, and the anoxic conditions at the bog, explained the earlier onset of leaching and that carbon and nitrogen cycles appeared more closely coupled in the heathlands but became decoupled at the bog due to the strong controlling effect of hydrology.

KEY WORDS: bog; deposition; lowland heath; mineralisation; sequestration; upland heath

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INTRODUCTION

Ecosystems on organic rich soils are often populated, to differing degrees, by a mosaic of dwarf ericaceous shrubs including heather (*Calluna vulgaris*), bilberry (*Vaccinium myrtillus*) and cross leaved heath (*Erica tetralix*) in addition to grasses, sedges and mosses such as *Hypnum jutlandicum* or, in the case of bogs, *Sphagnum* species. They cover large altitudinal ranges over different soil types encompassing drier lowland heaths, well-drained upland moors and ombrotrophic bogs holding deep reserves of water-saturated peat. Pollution from reactive nitrogen (N) is acknowledged as a significant contributor to ecosystem change throughout the world (Bobbink *et al*. 2010). Increases in N deposition can affect an ecosystem in different ways. N has been shown to increase the growth of ericaceous plants such as *Calluna vulgaris* across a number of ecosystem types including upland heath (Caporn *et al*. 1995, Carroll *et al.* 1999), low-alpine dwarf-shrub heathland (Britton & Fisher 2007), lowland heath (Power *et al*. 1995, Lageard *et al*. 2005) and ombrotrophic bog (Bubier *et al.* 2007). This increase in growth of higher plants can cause competitive exclusion of slower-growing species and lower plants including lichens and mosses, with consequent reductions in biodiversity (Carroll *et al.* 1999, Bobbink *et al.* 2010).

Aside from changing above-ground vegetation, increased N supply alters the biogeochemical functioning of an ecosystem. When soluble N exceeds plant and microbial requirements, excess nitrate and ammonium may leach from the system, especially nitrate which as a mobile anion is poorly bound in most soils. Previous studies have found that nitrate leaching is responsive to N deposition in forest soils (Wilson & Emmett 1999), whereas some ericaceous ecosystems show strong ability to buffer leachate against N additions—an ability that is linked to prevalence in the soil of recalcitrant humic compounds capable of immobilising N (Pilkington *et al.* 2005a). Increased N deposition can also increase mineralisation as decomposer organisms are often limited by N and must absorb dissolved N to grow. In this case added N will stimulate mineralisation through reduction of soil C/N (Kristensen & Henriksen 1998, Emmett *et al*. 1998). However, as N deposition continues to increase and soil C/N reduces further, the system may become C-limited and N availability may exceed both plant and microbial abilities to remove it from solution. When this happens, any solutes not immobilised or held on exchange sites within the soil will be leached from the system (Dise & Wright 1995, Emmett *et al*. 1998). Leached N compounds can subsequently enter freshwater systems and cause acidification and eutrophication of these habitats (Allott *et al*. 1995). In their experiment studying moorland systems subject to different N inputs, Curtis *et al*. (2004) found that mineralisation and nitrification increased, respectively, below soil C/N thresholds of 30 and 25–30. They observed that soil C/N tends to respond to N deposition and can indicate potential N saturation, and that leachate concentration appears to be related to C/N. However, one site (a blanket bog) behaved differently, and the authors suggested that the cause was one or more of acidity, metal toxicity and poor litter quality from recalcitrant shrub vegetation. Pilkington *et al.* (2005a) studied responses of moorland to N addition and also found that C/N could be a predictor of N leaching, with increased leaching occurring at C/N below 31–32. They commented that large stocks of C in soil organic matter (SOM) seemed to provide an effective sink for N. There is further evidence that soil carbon pool size can determine nitrate leaching in both heathland (Evans *et al*. 2006) and forests (Dise *et al*. 1998). More recently, Dise *et al*. (2009) examined a database of plot- and landscape-scale N studies from 248 forest sites and found that soil C/N was one of the most consistent indicators of N leaching.

The aim of this study was to explore the relationship between soil C/N, leachate N and N deposition for three contrasting ecosystems on organic soils. We expected that bog, with the greatest reserves of carbon, the highest C % and C/N, should buffer the greatest N deposition before N saturation and leaching began.

METHODS

Study sites

The study sites were: two heathlands, Budworth Common (lowland) and Ruabon Moor (upland); and Whim Moss, an ombrotrophic bog at intermediate altitude. All three are the sites of long-term N addition experiments which began in 1996 at Budworth, 1998 at Ruabon and 2002 at Whim. Locations, meteorological variables, site ambient N deposition and N additions are shown in Table 1.

Budworth Common

Budworth Common is a lowland heath at an altitude of 70 m a.s.l. in north-west England. It is dominated by patchy *Calluna vulgaris* interspersed with clumps of *Deschampsia flexuosa*, patches of *Hypnum jutlandicum* and bare ground. Soils are sandy humo-ferric podzols covered with a shallow organic layer. Nitrogen, as NH_4NO_3, is applied monthly to rectangular (1 × 2 m) plots at N loads of 0, 20, 60 and 120 kg N $ha^{-1}y^{-1}$ using a watering can. N additions began in 1996.

Ruabon Moor

Ruabon Moor is an upland heath at 480 m a.s.l. in north Wales. The vegetation canopy is dominated by *Calluna vulgaris* interspersed with *Vaccinium myrtillus*, and the under-storey consists mainly of the moss *Hypnum jutlandicum*. The soil is an iron-pan stagnopodzol consisting of a 5 cm *Calluna* litter layer over a 10 cm peaty organic surface horizon. Nitrogen, as NH_4NO_3, is watered monthly onto rectangular (1 × 2 m) plots at N loads of 0, 10, 20, 40 and 120 kg N $ha^{-1}y^{-1}$. N additions began in 1989 (Caporn *et al.* 1995), although the current research was carried out on newer plots established in 1998.

Whim Moss

Whim Moss is a raised bog at 282 m a.s.l. in south-east Scotland. The vegetation consists of repeating mosaics of *Calluna vulgaris*, clumps of *Eriophorum vaginatum* and large patches of *Sphagnum* species (predominantly *S. capillifolium* and *S. papillosum*). The soil consists of 400–600 cm of acid-peat with a water table that is often close to the surface. Nitrogen additions began in 2002 and are split between wet-reduced (Red-N: NH_4Cl) and wet-oxidised (Oxi-N: $NaNO_3$) forms, with an additional ‘dry’ NH_3 deposition transect (Leith *et al.* 2004). Wet N additions at Whim provided 0, 8, 24 and 56 kg N ha^{-1} yr^{-1} to circular plots of 4 m diameter equipped with a central sprayer (Sheppard *et al.* 2004). The system was automated and applications were coupled to rainfall. Dry N was emitted from a pipe when the wind was in the appropriate sector and speed exceeded 2 m s^{-1}. This provided an exponentially declining deposition gradient from 56–70 kg down to ~ 8 kg N ha^{-1} yr^{-1} at the end of the 60 m transect (Leith *et al.* 2004, Sheppard *et al.* 2011).

Sampling strategy

At each site there are four blocks, each containing a replicate of each N-addition treatment. The locations of treatments within blocks are randomised. All blocks and treatments were sampled for this study. On the dry-NH_3 transect at Whim, eight samples were taken from different distances along the transect and a further ‘ambient’ sample was taken from alongside the transect.

Table 1. Location and summary meteorological and soil temperature data for each site together with background N deposition, N treatment regime and control plot soil C % and C/N.

Site		Budworth	Ruabon	Whim
Location	latitude / longitude British National Grid	53.19 °N / 2.62 °W SJ 584 658	53.03 °N / 3.15 °W SJ 224 491	55.76 °N / 3.27 °W NT 203 531
Total (annual) rainfall in 2007 (mm)		780	987	1032
Air temperature 2008–2009 (^{o}C)	max. mean min.	31.28 8.83 -11.05	19.47 9.75 -0.02	28.20 7.60 -9.70
Mean soil temperature at 10 cm depth 2008–2009 (^{o}C)	max. mean min.	17.60 9.25 1.50	14.05 6.91 1.10	14.40 7.60 1.30
Ambient N deposition (kg N ha^{-1} yr^{-1})		26.9*	23.1*	8–11**
N form and addition rates (kg N ha^{-1} yr^{-1})		NH_4NO_3 0, 20, 60, 120	NH_4NO_3 0, 10, 20, 40, 120	NH_4Cl (wet) 0, 8, 24, 56 $NaNO_3$ (wet) 0, 8, 24, 56 NH_3 (dry) 44→8
Control plot soil C (%) Control plot soil C/N	mean	17.3 32.0	43.6 34.6	45.6 40.6

* Modelled N deposition, APIS (2010); ** Measured N deposition, Sheppard *et al.* (2004).

Soil solution sampling and analysis

Soil solution samples were collected monthly for at least 18 months at each site: at Budworth from June 2006 to April 2008; at Ruabon from November 2006 to May 2008; and at Whim from May 2006 to October 2007. The collection method differed slightly between sites. At Ruabon and Whim, 'mini-rhizon' suction samplers (Van Walt Ltd., Surrey, UK) were used. The rhizon sampler consists of a porous hydrophilic polymer tube, with a pore diameter of 0.1 µm, extended with a polyvinyl chloride sampling tube. At Budworth, which was too dry for use of the 'mini-rhizon' samplers, zero-tension lysimeters were used. At all sites the soil solution was collected at 10 cm depth, just below the main rooting and microbially active zones. At Ruabon and Whim, turfs were placed over each sampler to exclude light. At Budworth and Ruabon the sampling location was below the organic soil horizons so that each sample could be considered a 'true' leachate. At Whim, however, large peat reserves lay beneath the sample zones and a more complex hydrological regime meant that the sample consisted of soil solution that was not necessarily leachate. Sample pH was measured on collection and the rest of the sample was filtered through 25 mm Whatman cellulose nitrate membrane filters (0.2 µm pore size) then refrigerated. Analyses were made within one month for NO_3^- and NH_4^+ using a Dionex ICS 2000 ion chromatograph. Each monthly plot replicate sample was analysed separately (four each month per N addition treatment except for the transect at Whim) and the annual mean concentration for each set of replicate plots (four values per N addition treatment) is presented here. Seasonal variations are described in the text.

Extractable N, mineralisation and nitrification

Availability of extractable N in the soil was measured seasonally (every three months) between November 2007 and November 2008 at Budworth, and between May 2006 and May 2007 at Ruabon and Whim. One soil core (6 cm diameter × 10 cm deep) was taken from each replicate, refrigerated upon collection, then frozen for later analysis. For Budworth and Ruabon, *Calluna* litter, OH1 (top 1 cm of organic soil) and OH2 (next 1 cm of organic soil) profiles of each core were analysed individually for each replicate (4 × 4 per sampling time). We report, however, the mean for each soil core. The lower mineral horizons of each core were not used. At Whim, no litter layer was present and the core consisted of decomposed *Sphagnum* peat which was homogenised prior to analysis. In all cases roots were removed and the sample was passed through a 2 mm sieve before analysis.

At all three sites NO_3^- and NH_4^+ were extracted from 5 g of soil using 20 ml of 1M KCl solution. In addition, at Budworth and Ruabon, NO_3^- and NH_4^+ were extracted from sub-samples of 2.5 g *Calluna* litter. In all cases the suspension was shaken for 30 minutes using an orbital shaker then centrifuged at 5000 rpm for 4 minutes. The liquid was then passed through a disposable 0.2 µm syringe filter and analysed for NO_3^- and NH_4^+ using a Dionex ICS 2000 ion chromatograph.

Mineralisation and nitrification rates were estimated using a second core for each replicate, collected adjacent to and at the same time as the first core. This second core was placed in a polythene bag, returned to the soil and incubated for approximately three months using a methodology developed from Kristensen & Henriksen (1998). The bags were not sealed at the top, thus allowing aeration, but they were folded over to restrict further atmospheric or treatment deposition and any leaching. After incubation, N was extracted and analysed as before, and mineralisation (NH_4^+) and nitrification (NO_3^-) rates calculated by subtracting the ion concentration in the second core at the end of incubation from that in the first core at the start of incubation, and expressing the result as change per day. This process was repeated for each 3-month season and rates of change were calculated per replicate (4 per treatment × 4 per year), but here we present only the mean annual rate for each treatment.

Soil C, N and C/N

Three soil cores (3 cm diameter × 10 cm deep) were collected using a thin-walled steel corer from each of the four blocks at each of the three field sites. This gave 12 replicates per N addition treatment. For Budworth and Ruabon the soil organic matter content changed through the core profile and for comparison purposes only the top 1 cm of organic matter was used in this study (although full profiles were analysed). At Whim, the core was uniform peat and the whole top 10 cm was homogenised and used for analysis. Soil samples were dried at 80 °C for 24 hours then ground using an adapted coffee grinder. A 100 µg sub-sample was then analysed for C % and N % using a LECO Truspec Carbon and Nitrogen Analyser.

Data analysis

Best-fit curves between leachate and soil C/N, C % and N %; and between leachate extractable N, mineralisation/nitrification rates, pH and N deposition were developed on non-transformed data using curve-fitting in IBM SPSS Statistics 19.0.0. These (mostly curved) lines were then transformed to straight lines as appropriate (none, $\log_e$ or sqrt) and linear regression models fitted in SPSS Statistics 19.0.0. Graphs were produced using Microsoft Excel 2007.

RESULTS

Soil solution nitrogen concentrations

The highest concentrations of NH_4^+ and NO_3^- in soil solution were measured in samples from Budworth (lowland heath) and along the NH_3 transect at Whim (bog) (Figure 1). The lowest concentrations were measured in samples from Ruabon (upland heath) where only plots receiving the highest dose of N deposition had measurable concentrations of N in the soil solution. Responses in the wet-N plots at Whim were of similar magnitude to those at Budworth at similar N deposition rates.

Multiple linear regressions showed increasing soil solution concentrations of NH_4^+ and NO_3^- to be consistently related to nitrogen deposition at all three sites (Figure 1 and Table 2), with more rapid responses at Budworth and Whim; the latter showing NO_3^- and NH_4^+ responses in line with respective Oxi-N and Red-N treatments.

Soil solution N was also related to soil N % (Figure 1) at Budworth (soil solution NO_3^-, $R^2 = 0.30$, $P < 0.01$), at Ruabon (soil solution NO_3^-, $R^2 = 0.67$, $P < 0.001$; soil solution NH_4^+, $R^2 = 0.46$, $P < 0.001$), and at the NH_3 transect at Whim (soil solution NO_3^-, $R^2 = 0.55$, $P < 0.001$). These increases in soil N % were accompanied by concurrent reductions in soil C/N. This was not the case, however, for the wet-N addition plots, suggesting that N was poorly retained in the peat. At Budworth, Ruabon and the NH_3 transect at Whim, the increased soil N % lowered soil C/N and this was also associated with increasing leachate N, showing a weaker relationship than that between N deposition and leachate N. However, this was not applicable to the wet plots at Whim (Figure 1). Regression relationships between leachate N and soil C/N are: Budworth $R^2 = 0.51$, $P = 0.002$ (NO_3^-); Ruabon

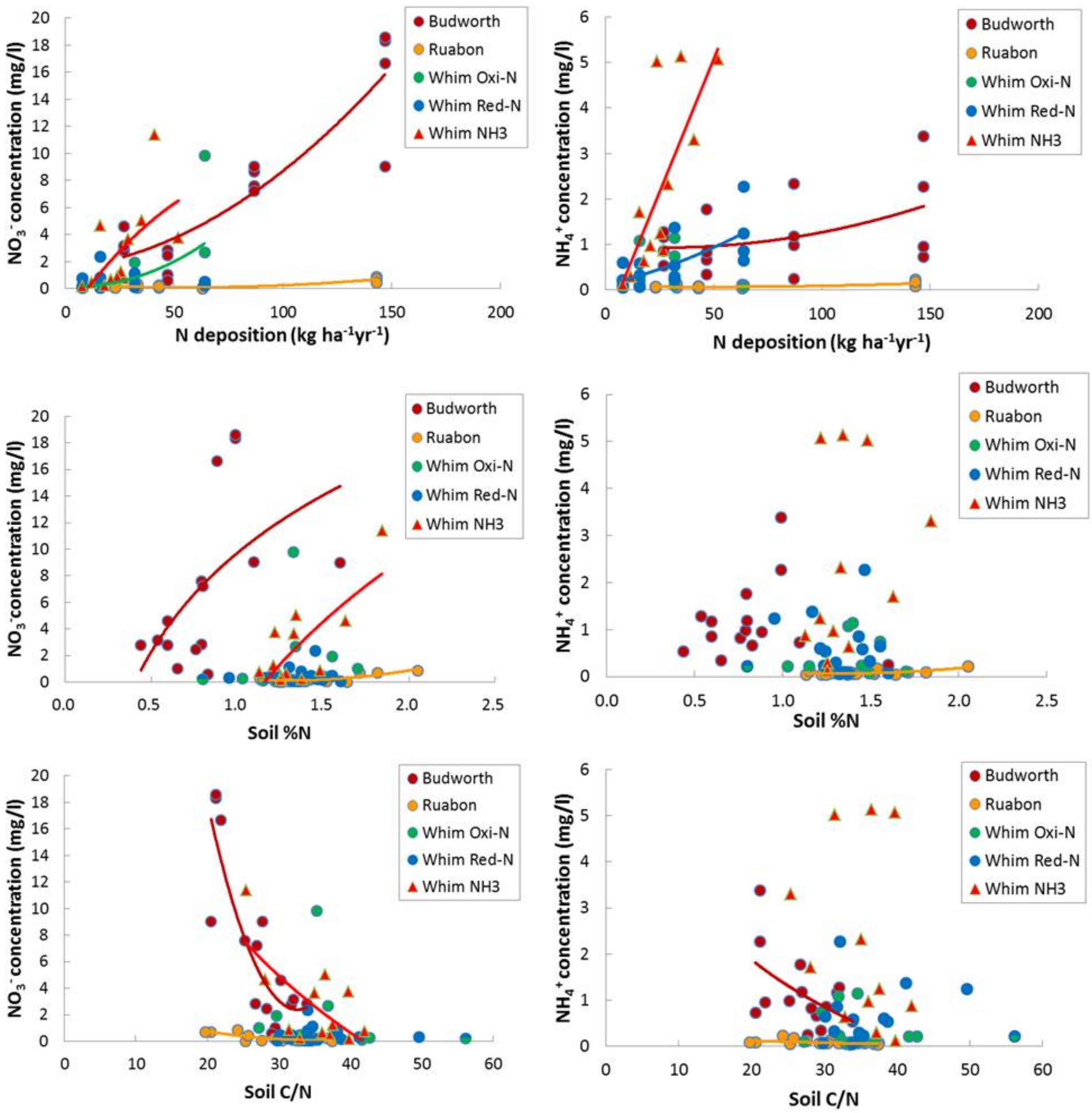

Figure 1: Relationships between concentration in soil solution of NO_3^- and NH_4^+ in relation to nitrogen deposition, soil N % and soil C/N. Each dependent (y-axis) soil solution data point is the annual mean concentration for the plot based on 12 separate monthly measurements. Independent (x-axis) soil N % and soil C/N are means of three replicates per plot. Lines are fitted regressions (see text).

$R^2 = 0.61$, $P < 0.001$ (NO_3^-); and Whim $R^2 = 0.24$, $P = 0.028$ (NH_4^+). Thresholds below which leachate N appeared to increase sharply were at soil C/N around 32–34 at Budworth and 25 at Ruabon, respectively. At Whim, however, with the exception of soil C/N and leachate NO_3^- along the NH_3 transect ($R^2 = 0.46$, $P = 0.016$), there were no other relationships between soil C/N (top 10 cm of the soil homogenised) and leachate concentration of NO_3^- and NH_4^+ on the NH_3 transect or the wet-N addition plots. At all sites, the concentration of NH_4^+ in the leachate was generally lower than that of the mobile NO_3^- anion.

Seasonal responses were apparent at all sites. Soil solution (leachate) N was highest in the summer at Budworth (although concentrations here were consistently high throughout the year and the increase in summer was less marked), and in the spring and summer at Ruabon. At Whim, the greatest NH_4^+ concentrations were in spring 2007 (a notably warm period), and no other significant differences between seasons existed.

Table 2. The effect of N on mean soil leachate NO_3^- and NH_4^+, mean extractable NO_3^- and NH_4^+ and mean mineralisation and nitrification rates, at all sites. The minimum to maximum range together with the % change from control/ambient N deposition at each site, and any statistically significant regression relationships between the variable and increasing N deposition, are shown. ns: not significant.

	Leachate NO_3^- (mg l^{-1})		Leachate NH_4^+ (mg l^{-1})		Extractable NO_3^- (µg g^{-1} dry soil)		Extractable NH_4^+ (µg g^{-1} dry soil)		Mineralisation (µg g^{-1} dry soil day^{-1})	
Site	Range (% ± control)	Regression with N	Range (% ± control)	Regression with N	Range (% ± control)	Regression with N	Range (% ± control)	Regression with N	Range (% ± control)	Regression with N
Budworth	1.7–15.6 (+369)	$R^2 = 0.83$ $P < 0.001$	0.9–1.8 (+42)	ns	3.3–5.3 (+10)	ns	23–106 (+367)	$R^2 = 0.58$ $P < 0.001$	0.01–0.31 (+852)	limited but ns
Ruabon	0.12–0.68 (+438)	$R^2 = 0.68$ $P < 0.001$	0.06–0.13 (+134)	$R^2 = 0.44$ $P = 0.008$	0.1–0.16 (+6)	$R^2 = 0.49$ $P = 0.006$	12–460 (+3900)	$R^2 = 0.72$ $P < 0.001$	0.01–0.31 (+852)	$R^2 = 0.68$ $P < 0.001$
Whim Oxi-N (wet)	0.1–0.9 (+817)	$R^2 = 0.29$ $P < 0.001$	0.2–0.27 (+35)	ns	0.4–0.61 (+53)	$R^2 = 0.32$ $P = 0.021$	28–111 (+166)	summer only	0.97–1.22 (+25)	ns
Whim Red-N (wet)	0.1–0.78 (+658)	ns	0.2–0.87 (+327)	$R^2 = 0.49$ $P = 0.008$	0.4–0.61 (+53)	summer only	28–98 (+138)	summer only	0–1.02 (-100)	summer only
Whim NH_3 (dry)	0.15–11.4 (+7478)	$R^2 = 0.37$ $P < 0.036$	0.1–5 (+4312)	$R^2 = 0.63$ $P = 0.002$	0.4–4.7 (+358)	ns	26–109 (+241)	$R^2 = 0.47$ $P = 0.014$	0.5–3.2 (+572)	ns

There was no relationship between soil C % and soil solution N, suggesting that the organic content of the soil did not directly affect soil solution N. Treatment-related changes in leachate pH were found at Whim (not shown). The wet-oxidised $NaNO_3$ and particularly the dry NH_3 treatments tended to increase pH from control values of around pH 4 up to 4.6 and 4.9 respectively, whereas the wet-reduced treatment tended to lower pH to a numerical minimum of 3.7. These changes were more marked in the summer months, but they were not directly associated with changes in soil solution N.

Extractable N, mineralisation and nitrification

Mean soil extractable N increased with N deposition (Figure 2) at both Budworth (NH_4^+: $R^2 = 0.58$, $P < 0.001$; NO_3^-: ns) and Ruabon (NH_4^+: $R^2 = 0.72$, $P < 0.001$; NO_3^-: $R^2 = 0.49$, $P < 0.001$). At Whim, extractable inorganic N was increased by N addition along the NH_3 transect (NH_4^+ only: $R^2 = 0.47$, $P = 0.014$). It also generally increased in the wet-N plots although plot heterogeneity, particularly in moisture, produced differing responses and mean values were not statistically different. However, measurements from summer 2006 were typically increased by N addition with the greatest concentrations of NO_3^- and NH_4^+ found in the Oxi-N and Red-N treatments respectively. Allowing for the smaller high-N treatment at Whim, extractable N in the wet-N plots lay between the values for Budworth and Ruabon.

Annual net mineralisation was lowest at Budworth (limited non-significant relationship with N) and greatest at Ruabon where strong responses to N addition were seen ($R^2 = 0.68$, $P < 0.001$). Mineralisation at Whim was greater than at Budworth but smaller than at Ruabon. At Whim it remained insensitive to N for the wet plots, with the exception of summer 2006 where statistically significant increases existed in the Red-N plots only ($R^2 = 0.25$, $P < 0.05$). On the NH_3 transect at Whim some N-driven increases were apparent, but variability in the data meant this was not statistically significant. Annual net nitrification was similar and very low at all sites (not shown).

Table 2 summarises the effect of N on soil leachate, extractable N, and mineralisation and nitrification rates at the sites.

Extractable NH_4^+-N and mineralisation were also affected by season, with N addition tending to produce stronger responses in spring and summer when soil temperatures were highest. At Ruabon the greatest extractable N was found in August 2006, and mineralisation was faster between May and August 2006 as well as during the warm spring (February to May) of 2007, compared with the cooler autumn and winter months. At Budworth, mineralisation rates across all seasons were low. At Whim there were no seasonal differences in soil extractable NO_3^- for either the wet plots or the NH_3 transect and the only statistically significant increases in mineralisation were during the summer of 2006 in the wet Red-N plots ($R^2 = 0.25$, $P < 0.05$).

DISCUSSION

Contrasting soil and soil solution N between sites

Despite several years of N addition, all the soils buffered increases in N well, particularly Ruabon upland heath which showed very limited changes in soil solution N as deposition increased. Low leachate losses and high N retention in peaty moorland soils are not uncommon due to the relative abundance of an organic horizon, rich in negatively-charged exchange sites (Nielsen *et al.* 1999, Curtis *et al.* (2004), Pilkington *et al.* (2005a). However, responses at Whim bog are of a similar size to those from the sandy-podzol soil at Budworth lowland heath, despite much greater reserves of organic matter and a higher soil C/N. Additions of N also began several years later at Whim—in 2002, compared to 1996 at Budworth and 1998 at Ruabon; cumulative N deposition to the plots was therefore much lower than the relative differences in treatments suggest. Increases in soil solution N were particularly high along the gaseous-ammonia transect, although the cause of this is unclear. The NH_3 treatment on the transect has had a catastrophic effect on vegetation including dieback of *Calluna vulgaris*, *Sphagnum* moss and other bryophytes, removing the ability of the system to take up N into live plants (Sheppard *et al.* 2008, 2011, 2013). It is also possible that the increases in soil solution pH eased constraints on mineralisation and nitrification and whilst differences in these variables were not found in the study, the high concentrations of NO_3^- found in soil solution and the increased extractable NO_3^- suggest that some N transformations have taken place (see below).

The immobilisation and retention of nutrients suggested by the leachate data is supported by the soil-extractable N and mineralisation results. At Budworth and especially at Ruabon, exchangeable N increased positively with N addition, particularly at the highest N deposition. Increasing mineralisation in response to N addition is common in moorland soils, as are low net nitrification rates (microbial immobilisation and plant uptake removes NH_4^+ from the soil before nitrification can take place), low pH and low average site temperatures

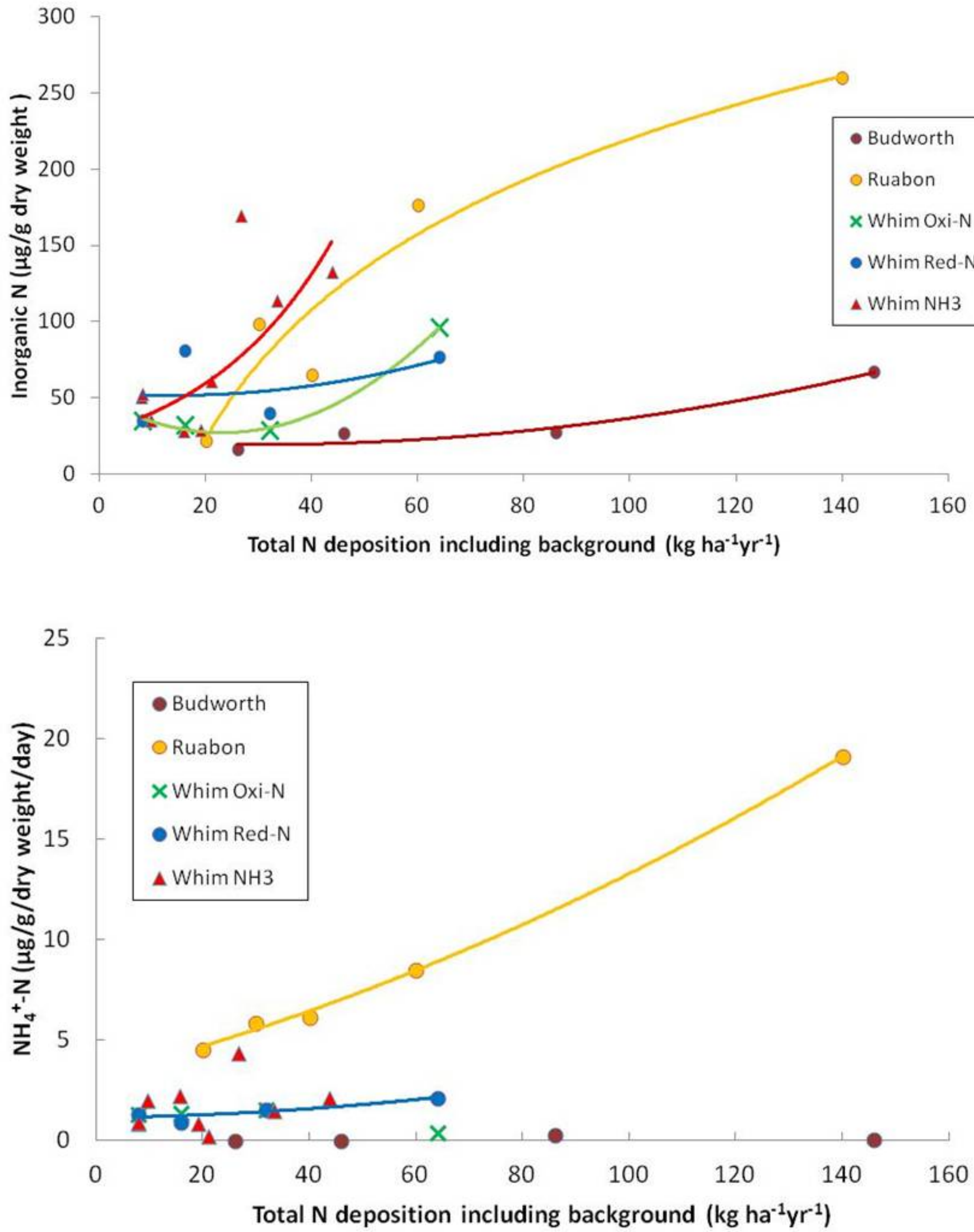

Figure 2. Relationships between N deposition (treatment plus ambient) and total KCl-extractable N (total of NO_3^- and NH_4^+) and with N mineralisation/ammonification rate. Each point represents a treatment mean concentration or rate based on quarterly measurements per plot averaged by treatment. Statistics are calculated on plot means. Regression lines were fitted when a statistically significant relationship existed, either seasonally or overall (Table 2).

(Curtis *et al.* 2004, Pilkington *et al.* 2005b, Emmett 2007). At Whim, exchangeable NH_4^+-N and NO_3^--N were generally slightly lower than in the wet-N plots at Budworth and very low compared with Ruabon, and were not greatly increased by N addition. The NH_3 transect at Whim again proved an exception, with large increases relative to the control. At Ruabon only, mineralisation was positively linked to a falling soil C/N in response to N addition.

Does soil C/N mediate leachate?

Regression relationships between soil solution N and soil C/N existed at both heath sites and along the dry NH_3 transect at Whim. At Budworth, soil solution concentrations increased below a soil C/N threshold of 2–34, reflecting the low organic content of the sandy podzol. At Ruabon, soil solution concentrations were undetectable above a soil C/N of around 25, indicating a high degree of immobilisation within the system either through plant or microbial uptake or by bonding onto soil exchange sites, as demonstrated by increases in exchangeable ammonium approaching +4000 % from the control.

At Whim the threshold appeared much higher, at a soil C/N of around 40. Whilst observations at both Budworth and Ruabon are consistent with the hypothesis of increasing inorganic N in soil solution as soil C/N falls, the site with the most SOM and a high soil C/N, Whim—a peat bog—responded differently and, with the exception of soil C/N and leachate NO_3^- along the NH_3 transect, no relationships between soil C/N and soil solution N existed. The reasons for this are unclear. Curtis *et al*. (2004) suggested that the polluted blanket bogs of the Peak District (England) contradicted this hypothesis due to poor litter quality, acidity limiting microbial activity or metal toxicity. Metal toxicity is not thought to be a problem at Whim as background pollutant deposition from oxidised sources is low (4.8 μg m^{-3} NO_2 and 0.6 μg m^{-3} SO_2) (APIS 2010) and we are not aware of any local smelting, either current or historical. The treatment regimes at Whim have produced changes in pH, with the wet Oxi-N and dry NH_3 treatments reducing acidity (control pH 4.1, treatment pH values 4.6 and 4.9) and the wet Red-N increasing acidity (treatment pH 3.7). But these were not directly linked to changes in soil solution N. Furthermore, the soil water recorded by Curtis *et al*. (2004) was very acidic at around pH 3, whereas the lowest recorded during this study at Whim was 3.7 with a mean of 4.2. The highest soil solution pH at Whim (4.9) was recorded on the NH_3 transect, but no changes in nitrification were observed, suggesting that acidity alone was not constraining this activity. However, leachate NO_3^- on the NH_3 transect did increase markedly from the no-N-addition control indicating that nitrification had occurred. Sheppard *et al.* (2013) found that, at high N deposition, N_2O fluxes from soil along the NH_3 transect increased suggesting that denitrification was also occurring. If the soil sample used in the incubation experiment was waterlogged then this would have inhibited significant nitrification and may have led to denitrification, which was not directly measured.

What drives the contrasting responses?

Soil solution N was strongly linked to N deposition across all sites, with the most rapid responses seen at Budworth (lowland heath) and Whim (bog). On the other hand, soil N % was linked to soil solution N only at Budworth, Ruabon (upland heath), and the dry NH_3 transect at Whim, suggesting that N retention at Whim was generally poor. We suggest that the contrasting responses at Whim may be attributed to other differences that exist between the sites. At Whim, few growth responses to N are seen across the shrub and moss vegetation (Sheppard *et al*. 2013), suggesting that the site may be limited by factors other than N availability, such as hydrology or supply of potassium or phosphorus (Carfrae *et al.* 2007) and that these may restrict shrub growth. By contrast, in the heathland sites, particularly Ruabon, N addition has led to increased growth of the shrub *Calluna vulgaris* (Carroll *et al*. 1999, Ray 2007). Ruabon alone possesses a uniform *Calluna* canopy which has produced a thick litter layer (up to 15 cm) which is almost entirely absent at Budworth and Whim. Given the remarkable ability of *Calluna* litter to buffer N addition (Pilkington *et al*. 2005a), its lack at Budworth and Whim could in itself be reducing N immobilisation potential, as a result of the absence of a labile carbon source and of cation exchange sites. Furthermore, only Whim has a large proportion of *Sphagnum* moss. Lamers *et al*. (2000) compared the N % of *Sphagnum* across a number of European bogs and found that above 18 kg N ha^{-1} yr^{-1} *Sphagnum* became saturated with N, was unable to take up additional N, and would start to leak any further N. These authors suggested that saturation would occur at a tissue C/N of 40–50, which is higher than the respective thresholds of 32–34 and 25 suggested by the Budworth and Ruabon data.

The hydrology of each site is very different, both heaths being much more aerobic and free-draining than Whim Bog, which is the only site where the water table is frequently near the surface. It is well documented that a high water table is capable of slowing soil turnover processes such as decomposition (Freeman *et al.* 2004), mineralisation and nitrification (Blodau *et al.* 2004), and this may further prevent microbial immobilisation of N due to a lack of oxygen in the upper layers of peat. Indeed, during the warm summer of 2006 there was a pulse of mineralisation, which may have been associated with water table drawdown and increased soil temperature, and this was accompanied by statistically significant increases in mineralisation as N addition increased.

These contrasting results suggest that it is not just the amount of organic matter, C pool size and current soil N status that determines N saturation and the onset of leaching (Emmett 2007, Evans *et al.* 2006) but, rather, that the availability of a labile C pool suitable for mineralisation and the oxic conditions that enable this process are also necessary. Rowe *et al.* (2006) studied the N responses of different ecosystems with respect to C/N at the onset of leaching. They attributed differences in leachate N to greater recalcitrance of organic matter in some ecosystems and the difficulty that microbes had in breaking this down.

The absence of a definable litter layer at both Budworth and Whim, and the anaerobic conditions at Whim, could therefore explain the absence of a mineralisation response to falling C/N and the earlier onset of increased soil solution N. Carbon and nitrogen cycles appear more closely coupled in the heathlands but are decoupled at Whim, potentially due to the strong controlling force of hydrology and the restriction that a high water table places on microbial activity.

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