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**Inertia in an ombrotrophic bog ecosystem in response to nine years'
realistic wet N perturbation, separated by form**

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Running head: Resilience of bog vegetation to wet N inputs

Research paper

Abstract

Wet N deposition occurs in oxidised (nitrate) and reduced (ammonium) forms, in proportions that vary spatially with source and topography. Whether one form drives vegetation change more than the other is widely debated, as we lack corroboratory field evidence. We have manipulated N form in wet deposition to an ombrotrophic bog, Whim, for nine years. Ammonium and nitrate were provided in rainwater spray as NH_4Cl or NaNO_3 at 8, 24 or 56 $\text{kg N ha}^{-1} \text{ y}^{-1}$, plus a rainwater only control, via an automated system coupled to site meteorology. Cover of key species fluctuated considerably, displaying temporal increases, declines or both, independent of N. Detrimental N effects were observed in sensitive non-vascular plant species, with higher cumulative N loads leading to more damage at lower annual doses, but overall the effect on moss cover was small. Cover responses to N, both form and dose were species specific, and mostly dependent on N dose. Some species were generally indifferent to N form and dose, *Eriophorum vaginatum*, *Erica tetralix*, while others: *Pleurozium schreberi* > *Cladonia portentosa* > *Sphagnum capillifolium* were dose sensitive. *Calluna vulgaris* showed a preference for higher N as reduced N and *Hypnum jutlandicum* for oxidised N. However, after nine years, the magnitude of change from wet deposited N on overall species cover (HOF model) is small, indicating only a slow decline in key species. Differences in soil N availability were similarly muted and rarely, directly related to species cover. Ammonium caused most N accumulation and damage to sensitive species at lower N loads, but toxic effects also occurred with nitrate. Generic N form effects were absent, making ecosystem specific critical load separation by form problematic. However, we recommend implementing the lowest value of the critical load range where communities include sensitive non-vascular plants and ammonium dominates wet deposition chemistry.

Key words: ammonium, cover, *Calluna*, chronology, critical loads, growth, nitrate, peatland, *Sphagnum capillifolium*.

67 Introduction

68 Globally, evidence exists of detrimental effects of enhanced reactive N deposition on plant
69 species composition (Clark & Tilman, 2008; Bobbink *et al.*, 2010). Although reactive N is a
70 key plant nutrient (Marschner, 1995) its availability varies across different habitats. Plants
71 have evolved different, specialized strategies and traits appropriate to their natural N
72 environments (Raven *et al.*, 1992). Increasing N availability causes species that have evolved
73 traits or strategies to exploit an enriched N supply to replace N conservative N users (Aerts,
74 1990; Steubing & Fangmeier, 1991). Such species replacements can lead to loss of
75 specialized communities and ecosystems, e.g. heathland transformed into grassland in the
76 Netherlands (Bobbink & Heil, 1993). However, reactive N deposition is not simply N, but
77 comprises two chemically different forms, oxidised and reduced N.

78 Enhanced reactive nitrogen (N) deposition is a consequence of rising global emissions of
79 nitrogen oxides NO and NO₂, (NO_y) from fossil fuel combustion (Dignon & Hameed, 1989)
80 and reduced N (NH_x) from agriculture to meet the growing demand for energy and food.
81 Reactions in the atmosphere produce nitric acid and particulate and aqueous NO₃⁻, the main
82 NO_y components of wet deposition. Wet deposition of reduced N comprises fine particulate
83 ammonium (NH₄⁺) salts or aerosols of acidic gases which have a relatively long atmospheric
84 residence time, 4 to 15 days, and when removed by precipitation contribute to N deposition in
85 remote ecosystems, after long-range transport (Asman *et al.*, 1998). Proportions of NH₄⁺ and
86 NO₃⁻ ions in precipitation, like the ecosystems they deposit to, vary spatially reflecting the
87 non-uniform distribution of their sources and topography (RoTAP, 2011). Evidence of the
88 respective effects of reduced and oxidised nitrogen to (semi) natural habitats is urgently
89 needed in order to support targeted emission control in either fields of agriculture and energy
90 sectors of both (Bobbink & Hettelingh, 2011, RoTAP, 2011). Similarly, there is a pressing
91 need to establish whether N critical loads (CL_N) should be separated by form, as we lack

comparable evidence on which to judge whether one or other form is more damaging and under what circumstances (Bobbink & Hettelingh, 2011, RoTAP, 2011). Very few studies have addressed the importance of the form of reactive N in wet deposition (e.g. Paulissen *et al.*, 2004; van den Berg *et al.*, 2008) and none have studied these effects in the field at low background N deposition and for an extended (>5 y) period. Due to the fact that many species show unimodal response curves to nutrient additions it is necessary for field N-manipulation studies to: 1. Start from a clearly defined point on the response curve, 2. Be maintained long enough to fully capture the N response and 3. Avoid sites with background N loads that may have already pushed the ecosystems onto the falling side of the curve (Sverdrup *et al.*, 2013). Ours is an ongoing study of the effects of N form on species cover that has been running for more than a decade at a relatively N clean site.

Semi-natural ecosystems that rely on atmospheric inputs for most of their nutrient supply e.g. ombrotrophic bogs and peatlands are considered to be particularly at risk from enhanced N deposition (Bobbink & Hettelingh, 2011). Much of the component vegetation e.g. mosses, *Sphagnum* species and lichens are described as nitrophobes (N sensitive) and their presence has formed the basis for setting CL_N in many ecosystems (Bobbink & Hettelingh, 2011). There is a significant literature (Berendse *et al.*, 2001; Limpens *et al.*, 2003; Bragazza *et al.*, 2004; Gunnarsson *et al.*, 2004, 2008; Paulissen *et al.*, 2004; Bobbink & Hettelingh, 2011) detailing adverse effects of enhanced N deposition on *Sphagnum* species, the main peat forming moss (van Breeman, 1995) which can change the nature and function of bogs. There is a pressing need to establish the extent of N driven changes in species cover on peatlands because of their importance for the global C balance, the link between vegetation and greenhouse gas emissions and the implications for up-scaling (Joabsson *et al.*, 1999; Gray *et al.*, 2013). Thus, we manipulated wet N deposition to an ombrotrophic bog, expecting to rapidly initiate contrasting effects on the cover of key species, provide corroboratory

evidence on the importance of N form and a long term assessment of N effects on a much valued ecosystem.

Our experiment addressed some key concerns on field N manipulation experiments, namely: 1) background N (ca. 8 kg N ha⁻¹ y⁻¹) deposition at the Scottish site is relatively low by European standards (apart from northern latitudes); 2) the simulation of wet deposition is highly realistic, being coupled to meteorology (Phoenix *et al.*, 2011); 3) the duration of the experiment has been sufficient to characterise the N response curves. Monitoring control plots enabled us to put N driven changes into the context of the underlying natural dynamics of species cover change, and the role of climate in determining what is present when observations are made, an influence that is very difficult to account for in one off surveys.

Here we report the results of nine years' application of oxidised and reduced N using a unique and highly realistic delivery system on the cover of key peatland species growing on an ombrotrophic peatland.

Aims

1. Demonstrate the long-term consequences of different N forms in wet deposition on the cover of key components of an ombrotrophic bog, peatland, at a site with relatively low ambient N deposition, using a 'real world' treatment regime.
2. Establish whether changes in the proportions of different species can be related to cumulative (averaged over the most recent assessment period) treatment effects on soil pH and N availability, soil water nitrate and ammonium.
3. Address the significance of N form in N deposition and implications for separating CL_N by form.

Methods

Site

Whim bog is located in the Scottish Borders, at 282 m a.s.l, 3°16' W, 55°46' N, on 3-6 m deep peat. No active management for at least 70 years has left a very uneven aged *Calluna vulgaris* stand (hereafter shortened to *Calluna*) with plants ranging from mature to degenerate (Gimingham, 1972). The peat is very acid, with a pH ca. 3.4 range 3.27-3.91 (H₂O), 10 % base saturation and low in available P and K (Table S1). The vegetation conforms best (Mizunuma, 2008) to NVC M19 and repeats in heterogeneous mosaics with hummocks dominated by *Calluna* and *Sphagnum capillifolium*, hollows with *Sphagnum fallax* and *Sphagnum papillosum* and cyperaceous graminoid species of *Eriophorum*. The most common species on this bog, *Calluna*, *E. vaginatum*, *S. capillifolium*, *Hypnum jutlandicum*, *Pleurozium schreberi* and *Cladonia portentosa* occur widely on similar habitats through the northern hemisphere (Gore, 1983).

Treatments

The treatments, replicated in 4 plots, are supplied at three doses of N: 8, 24 and 56 kg N ha⁻¹ y⁻¹, in addition to the total ambient deposition of ca.8 kg N ha⁻¹ y⁻¹, as either oxidised N in NaNO₃ or reduced N in NH₄Cl, referred to as NoxY and NredY respectively, where Y represents the annual dose applied excluding ambient deposition, e.g. Nox56. Treatments provide 10% additional rainwater, collected on site (Sheppard *et al.*, 2004a, 2008), which dilutes the N concentrate to 0.57, 1.71 or 4.0 mM corresponding to 8, 24 and 56 kg N ha⁻¹ y⁻¹. The treatment is pumped through 100 m of pipe to a spinning disc sprayer at the centre of each 12.8 m² plot. Application is automatic when weather conditions permit: sufficient rainfall, air temperature > 0 °C and wind speed < 5 m s⁻¹, coupling application to real world conditions with a realistic frequency, ca. 120 applications y⁻¹. Six metre buffer strips separate

the plots. Treatment commenced in June 2002 and has been maintained throughout the year when temperatures exceed those likely to cause freezing.

Meteorological variables are measured at 1 or 15 minute frequencies (Sheppard *et al.*, 2004a; Leith *et al.*, 2004). Annual wet-only N deposition varies with rainfall amount in the range 4.6 to 6.2 kg N ha⁻¹y⁻¹ with dry deposited NH₃ < 3 kg N ha⁻¹ y⁻¹. Rainfall, temperature and water table data (Table 2S) are presented for the periods, 2002-2004, 2004-2007, 2007-2009, 2009-2011 between species cover assessments.

Cover assessments

The protocol for estimating species cover change was detailed in Sheppard *et al.*, (2011). Three permanent quadrats (0.25 m²) in each plot included the most common species and, where possible, represented most species in the plot, although often in different proportions. Percent cover data for these three quadrats were averaged to provide a plot value. The same two recorders made all the assessments. In most cases the sum of the cover exceeded 100%, due to the multi-layering of the vegetation. *Calluna* was separated into green shoot cover (*Calluna* green), dead shoot cover and fallen litter. In 2011, cover was also estimated for all species in the whole-plot (12.8 m²) by the same two recorders.

Growth, N concentrations and vitality assessments

Shoot growth of *Calluna* was measured in 2011, post growing season, on 24 shoots per plot, removed at three positions on eight compass points for up to five years, retrospectively. Ten capitulum of *S. capillifolium*, per plot, were removed in both October 2006 and November 2011, cleaned, dried and weighed for comparison. Extension growth of *H. jutlandicum* and *P. schreberi* was measured between March 2008 and March 2009. Three rectangles of plastic mesh were placed over areas of the respective mosses and anchored down. These were

removed after one year by cutting below the mesh. The area covered by the moss was estimated and 10 moss stems were measured, dried and weighed. The remaining moss was dried and weighed. N was measured on the dried samples using a CN analyser. Length and weight per cm in 2009 are presented. N was also measured in cleaned, dried, ground samples of current year *Calluna* and *E. tetralix* shoots, apices of *C. portentosa* and green leaves of *E. vaginatum* harvested at the end of the summer in 2009. Visible damage to non-vascular plants was assessed subjectively (Sheppard *et al.*, 2011) in all plots. Winter damage to *Calluna* following the cold winter and hot dry spring in 2009/2010 was scored in October 2010 as the percentage of dead grey shoots (Sheppard *et al.*, 2011) per plot.

Soil pH and soil water nitrate and ammonium

In each plot, pH was measured in a 2/1 (v/v) mixture (1 h) of deionised water and peat originating from one peat sample per plot, cored down 10 cm below a shallow layer of pleurocarpous moss. Measurements started in 2006 and between 7 and 16 measurements were made between quadrat assessments: 2006-2007, 2007-2009, 2009-2011. Soil pore water nutrients (NH_4^+ and NO_3^-) were sampled using rhizon suction samplers (Eijkelkamp, The Netherlands), comprising a 6 cm reinforced porous tube, incorporating a 0.45 μm filter, at 0-10 cm depth, again below pleurocarpous moss. NO_3^- and NH_4^+ concentrations were measured by ion chromatography, with a detection limit of 0.018 mg N L⁻¹.

Data analysis

Cover and growth data were tested for normality (Bartlett's test and residual plots) and appropriate transformations (\log_{10} , arcsin, square-root) were made if required. A two-way generalized model was used to separate the effects of N dose (both forms combined) and form (all doses combined), with post hoc tests (Tukey) to separate the interactions (N form x dose) where justified. Separate comparisons were made for individual years. All analyses

were undertaken with Genstat (GenStat Release 12.1, ©2009; VSN International Ltd, Harpenden, UK). Significance is indicated when p values were < 0.05 . Statistically significant chronological changes are summarised in Table 4S. Because of the between plot, treatment variability in species cover at the start of the experiment, the number of treatments, in order to demonstrate chronology and simplify presentation, the data are represented as % cover with trend lines fitted, linear or polynomial ($R^2 > 0.7$) (Fig. 1S) and difference from the start with error bars. HOF models (Huisman *et al.*, 2003), which show individual plot values, were used to analyse the time response. These were fitted to express the shape of the species cover response over the 9 years of the experiment. Our expectation was that, if they occurred, responses would be either monotonic changes in cover or a form of hump back response. Hence fitted models varied from a horizontal line to a unimodal skewed response and comprised five forms of increasing complexity and number of parameters. Models were fitted using the R package ‘gravy’ (Oksanen, 2004; 2005). Assessment of the best model fit was based on AIC values and permutation testing (see Table 5S). We applied HOF model fitting to the untransformed cover data for each species and each N treatment including controls.

Quantifying the effects of N treatment on soil chemistry and species cover change

N treatment could drive change in species cover via changes in soil chemistry, altering the favourability of conditions for each species where cover would decline or increase through direct effects on growth or indirectly via competition with neighbours. In addition it is possible that N deposition might directly affect the growth of a species because of physiological effects not mediated via change in soil chemistry. Foliar uptake by mosses and ericaceous shrubs can be quite significant (Bates *et al.*, 2002; Bobbink & Heil, 1993). Path analysis was used to quantify the direct effect of each N treatment on species cover as well as simultaneously estimating any indirect effects via change in soil chemistry and highlight changes in soil chemistry not so far linked to cover change but with the potential to do so. A

model specifying all possible directed relationships was tested for the Nox plots and separately for the Nred plots (Fig. 1). The same control data was used in each model. Each species with sufficient observations was tested separately. Analyses were applied to two cover-derived response variables; plant species cover in 2011, and also to the linear slope of change in cover over nine years. We used the average of 2009-2011 pH and soil solution N chemistry data as being the most appropriate to represent the cumulative effect of N load. Slope coefficients were based on arcsin square root transformed cover data then centred to zero and standardised to unit standard deviation within each treatment across years. Cover data in 2011 were also centred to zero mean and unit standard deviation across all treatments and plots. The resulting standardised regression coefficients and path coefficients can be interpreted as effect sizes of the magnitude of change in terms of standard deviational units following Cohen (1988) where values >0.2 and <0.8 are medium effects and >0.8 are considered large effects.

The path analysis model was written in the Bayesian analytical software OpenBUGS software version 3.2.1. (<http://www.openbugs.info/w/>). A Bayesian treatment was adopted as this allowed us to estimate all regression parameters and indirect effects in one analysis while also coping with relatively small sample sizes. Two data values were missing and the Bayesian approach allows these to be estimated by drawing from the posterior predictive distribution of each covariate in the model (Gajewski *et al.*, 2006).

Estimating indirect effects tested the hypothesis that species cover response was linked to N load via change in soil chemistry. As is normal in path analysis, indirect effects were computed as the product of the standardised regression coefficients for each component path (Grace, 2000). Hence if β_1 is the standardised regression coefficient expressing the effect of N load on soil pH and β_2 is the coefficient for the effect of soil pH on species cover then the indirect effect of N load on species cover is the product of β_1 and β_2 . The

significance of this term can be readily tested by repeatedly sampling its value from the posterior distribution and counting how many times the estimate is greater or less than zero. The mean of the repeated draws of these binary values gives the required exact probability value for the data. Significance tests of regression parameters were performed in the same manner (Table S6).

Results

Changes in species cover

Pre treatment cover was highly variable with seven-fold differences in non-vascular plant cover (Fig. 1S). With no N addition, species cover changed by up to 100% over the nine years, exhibiting mostly increases, *Calluna*, *H. jutlandicum*, declines, *P. schreberi*, *S. capillifolium*, *E. vaginatum* or both, *E. tetralix* (plateaued 2007). Up to 2007, conditions appeared to favour the two most common ericoids and most species except *P. schreberi*. N additions tended to amplify the direction of these responses, except for *S. capillifolium* which decreased its cover at N doses above N8, irrespective of N form (Fig. 1S). The two pleurocarpous mosses that dominated non *Sphagnum* moss cover, showed opposite responses to N: *H. jutlandicum* increased with N dose and Nox; *P. schreberi* decreased with N dose, especially Nred. *C. portentosa* cover declined with N addition, but because control values were so low (Fig. 1S) it was difficult to assess whether N was the only cause. Only *Calluna* cover changed significantly as a result of N inputs, but only with Nred in 2007. Increases in dead *Calluna* foliage and litter were significant from 2004 (Fig. 1S). Dead shoot cover increased in N56 treatments and litter cover especially showed significant increases with Nred, all doses, but smaller, non significant increases with Nox.

Vaccinium oxycoccus, *V. myrtillus* and *Empetrum nigrum* occurred in too few plots for statistical analysis. High N56 increased cover in all three ericoids. *E. tetralix* which

represented < 1% to 10% cover, showed a short –term positive N response, most noticeable with Nred, 3-4 fold increases significant in 2007 (Fig. 1S). *E. vaginatum*, green leaves, comprised ca. 25% of the cover, were relatively unresponsive to N form and dose although cover tended to decline, the opposite of *Calluna* (Fig. 1S). Dead and green leaves of *E. vaginatum* and *Calluna* dominated the higher plant cover (40-68%).

Cover fluctuations from the start are shown in Fig. 2. Several *Sphagnum* species grow on site: *S. capillifolium*, *S. russowii*, *S. papillosum* and *S. fallax*, but only *S. capillifolium*, sometimes in combination with *S. russowii* and ‘lumped’ as *S. capillifolium*, occurred in all plots. In control plots *S. capillifolium* cover showed small increases which were reversed after 5 years (Fig. 2). N56 had significantly reduced *S. capillifolium* cover within 5 and N24 within 7 years (Figs 1S & 2), although Nred8 continues to outperform controls (Fig. 2). Over time the differences between N doses became less significant, as the reductions in cover at lower doses ‘caught up’ with higher doses.

H. jutlandicum cover fluctuated in control plots (Fig. 2), but within two years of treatment, there was a positive Nox effect ($p = 0.054$). By 2011 these increases represented 92%, 61%, 189% for Nox 8, 24 and 56 respectively, but only 35%, 21%, 103% for equivalent Nred additions. *P. schreberi* displayed a rapid, acute sensitivity to N: within 2 years the linear decline with Nred56 in *P. schreberi* was significant, compared with 9 years for No56 (Fig. 2). Effects of ≤ 24 kg Nred were not significant, despite the 9 year cumulative dose for Nred24 exceeding the 2 year Nred56 dose. In the N treatments, especially Nred *P. schreberi* cover in 2004 was relatively low by comparison with subsequent years (Fig. 1S). *C. portentosa* cover varied hugely across treatment plots, being quite extensive on the drier hummocks but sparse in wetter plots and absent from 3 sets of control quadrats, compromising the statistics. By 2011 *C. portentosa* had almost disappeared from the Nred56 and Nox56 treatments (Fig. 1S).

Percent species cover was quantified at the plot level, 12.8 m² in 2011 to corroborate the quadrat data (Fig. 3). Among the ericoids: *Calluna*, *E. nigrum*, *V. oxycoccus* and *V. myrtillus* covers were significantly ($p=0.042$) lower with N56 but showed no significant effect of N form ($p= 0.414$) (Fig. 3). Neither N dose nor form affected graminoid cover, predominantly green and dead leaves of *E. vaginatum*. Moss cover was significantly higher in the N8 and Nox plots (Fig. 3), largely reflecting *H. jutlandicum*. Cover of *Sphagnum* (all species) and *S. capillifolium* alone, was lower the higher N dose, where there was also more dead, and with Nred but not significantly so. *C. portentosa* cover reflected how much of the plot sat above the water table, some plots had very high initial covers of 20-25 %, masking the treatment effects. The N56 plots started with more typical cover (2-5 %) and *C. portentosa* has disappeared from three of the Nred56 plots and two of the Nox56 plots.

Quantifying the shape of the species' cover response curves – HOF model

Most treatments induced no change in species cover and no deviation from control plots (Fig. 4). Hence the best fitting model in 21 out of 42 possible responses was an intercept-only horizontal line (Table 5S). There were too few positive non-zero values to fit a model to *P. schreberi* and results summarise those already described: Nred56 rapid decline with consistent declines in Nred24 and Nred8, but only Nox56 (Fig. 4). *E. vaginatum* also declined over time in all three Nred treatments and in the Nox8 treatment. *H. jutlandicum* increased significantly over time with Nox8 and Nox56 but no significant changes with Nred (Fig. 4). In all three Nred treatments *E. tetralix* showed a significant but small hump, peaking ca. 5 y. *Calluna* showed positive responses in all treatments but the magnitude was not significantly different from the controls (Fig. 4). These HOF models confirm the relatively small magnitude of the cover changes over 9 years.

Nox versus Nred effects on difference in cover

Comparing cover increases or decreases relative to the start on a 1 : 1 plot of Nox versus Nred, $R^2 = 0.69$; Nred change $R^2 = 0.85$ * Nox change $R^2 = 0.50$ (Fig. 5) illustrates the relative effects of N form. Relatively, form and dose effects are quite small, < 20% difference in species cover, except for *Calluna*, up to + 40 %. Despite nine years elapsing, the magnitude of N driven cover change has been small, except for *P. schreberi* which has gone from the Nred56 quadrats.

Cumulative dose effects

Where N dose significantly changed species cover, *e.g.* for *S. capillifolium* and *P. schreberi* (Table S2) relationships with cumulative N load were investigated (Fig. 6). Once the cumulative Nred load exceeded 200 kg N, *S. capillifolium* cover decreased, but the rate of decrease was small. Nred load explained 42% of variation in cover ($p = 0.02$). There was no relationship between change in cover and cumulative Nox load ($p = 0.46$) although the effect of Nox was generally negative. *P. schreberi* was negatively affected by N at all N doses, appearing more N sensitive than *S. capillifolium* and halving its cover for a smaller N load (Fig. 6). Both cumulative Nox and Nred loads explained significant amounts of the cover changes in *P. schreberi*: Nox = 78%, $p = 0.0001$ and Nred = 53%, $p = 0.007$. The form effects were significantly different ($p = 0.05$) with Nred causing the largest cover reductions.

Shoot extension

Shoot elongation was measured to indicate whether cover changes were related to growth. In 2011 there was a significant positive dose response and effect of Nred on *Calluna* shoot extension (Table S3). Nred56 increased shoot extension significantly more than Nox56 (post hoc tests). In 2010, N addition also increased shoot extension ($p = 0.067$), especially as Nred ($p = 0.075$) but there were no significant treatment effects in 2009, 2008 and 2007. Shoot extension suggests Nred56 is still favouring *Calluna* growth. *H. jutlandicum* growth data

contradicted the cover changes: N dose reduced shoot length weight ($p = 0.03$) and there was no N form effect (Table S3). For *P. schreberi* N dose significantly reduced both weight per unit length and shoot extension. Neither N form nor N dose significantly affected *S. capillifolium* capitulum weights in 2006 or 2011 (Table S3). In 2006, capitulum weights were larger with $\leq 24 \text{ kg N ha}^{-1} \text{ y}^{-1}$, but by 2011 only the N8 addition maintained that positive trend.

Visible damage

In October 2010, 30% of *Calluna* shoots were grey and dead (Sheppard *et al.*, 2011) in N56 plots, with a significant ($p = 0.04$) effect of N dose (Table S3). A subjective visual scoring of health (Sheppard *et al.*, 2011) showed that *P. schreberi* and *H. jutlandicum* were not visibly damaged, scoring 5 = no visible damage, in all but one treatment Nred56 (4.7). Some damage was seen on *S. capillifolium* but the scores exceeded 4. *C. portentosa* showed most damage in the N56 plots, especially with Nred.

Foliar N

The N status of all species responded positively and significantly to N dose. Mostly % N was higher with Nred (Fig. 7, most points above the 1 : 1 line). The difference between Nred and Nox was largest at the highest N dose. Among the vascular plants the increases in %N were modest, the largest increases in % N occurred among the non-vascular plants which were more N sensitive.

Meteorology, soil pH and soil water nitrate and ammonium

Between 2002 and 2011 mean air temperature got colder, reflecting two very cold winters (10/11, 11/12) down to 7 °C. RH has remained relatively constant, between 88 and 91 %, likewise the water table at 10 cm below the surface, once recovered from the 2003 drought

and cumulative rainfall. Pre treatment pH values measured in all plots varied by 0.2 pH units (3.45 and 3.65). By 2011 pH's in the Nox plots, were up to 0.4 units higher depending on the N dose, whereas pH's in Nred remained similar to control plots but ca. 0.1 units less acid than 2002. Between 2009 and 2011 NO₃-N concentrations were relatively low, < 0.15 mg L⁻¹ but exceeded control values. N additions significantly increased soil water NO₃-N concentrations, but there was no distinguishable trend with time. Differences in the form of N applied had no significant effect on NO₃-N concentrations. In control plots mean soil water NH₄-N concentrations were low ca. 0.7 mg L⁻¹. Nox treatments increased soil water NH₄-N concentrations, but not in relation to dose, whereas Nred treatments increased NH₄-N concentrations in proportion to dose. NH₄-N concentrations with Nox were two-threefold smaller than with Nred and were lowest in controls and highest with Nred56.

Relating N treatment effects on soil chemistry and species cover using Path analysis

Direct effects on species covers: Medium sized positive and negative effects of increasing loads of Nox were detected for *H. jutlandicum* and *P. schreberi* covers in 2011 respectively (Fig. 8). Medium sized negative effects of Nred load were seen for cover of *H. jutlandicum*, *P. schreberi* and *S. capillifolium*. Increasing Nred load had a significant positive effect on final *Calluna* cover (Fig. 8). For linear cover change between 2002 and 2011 (Fig. 9), there was only one direct effect: *E. tetralix*, positive for Nox. **Direct effects on soil chemistry with no transmitted effect on species cover:** The only direct effect on soil water chemistry that was not subsequently linked to species cover was a positive relationship between increased NH₄⁺ concentrations and Nred dose (Fig. 8). **Indirect effects on final cover in 2011:** Nox load increased pH, positive effect, which decreased *P. schreberi* cover, large negative effect (Fig. 8). By contrast, increasing Nox had a large positive effect on final *H. jutlandicum* cover via a positive effect on soil pH (Fig. 8). Increasing Nox load also had a medium sized negative effect on *P. schreberi* cover in 2011 via its' positive effect on soil

water NH_4^+ (Fig. 8). An indirect negative effect of increased Nred on *E. vaginatum* cover was also detected via its' positive effect on NH_4^+ concentrations, but the exact Bayes p-value indicated a 90% rather than 95% chance of a credible relationship (Fig. 9).

Significant effects of soil chemistry variation on species cover were also found that were not in turn associated with N treatment. These can either reflect lack of statistical power given the noise in the data compared to signal or, they highlight background relationships related to ecological variation in the habitat over time that were independent of the experimental treatments (Figs 8 & 9). Path analysis confirmed that significant differences in the relatively small cover changes could be attributed to changing amounts of applied N. Moreover it demonstrated that both Nox and Nred caused detectable effects, and that most of the strong negative effects were linked to Nred.

Discussion

Importance of this long-term study at Whim bog: capturing a time series to detect resilience

By tracking cover change at ca. two year intervals we have demonstrated considerable amplitude within direction of change trends in cover, highlighting the uncertainties that can affect 'one off' evaluations. The results highlight difficulties in demonstrating whether an N response is increasing, stagnating or declining in the absence of a chronology of measurements e.g. the hump back response *E. tetralix*. Combining assessments of growth, cover change and foliar N concentrations attributes change to N directly and from growth measurements understand what, if any, physiological processes are being affected or whether cover changes just reflect competition for resources. For the mosses increases in cover were sometimes associated with morphological changes that implied reduced C assimilation and a decrease in overall mass, which could become a problem for the future (Armitage *et al.*, 2012). The study also highlights the lack of generic responses to both N dose and form e.g.

Hypnum moss increasing whereas *Pleurozium* declined rapidly and *Sphagnum* in a constrained way, the former to Nox and the latter to Nred but also Nox. Through path analysis we have clearly demonstrated that N can induce change but based on the HOF models we see that the magnitude of change over nine years is small by comparison with the control. Effects of N form were also clear among sensitive species, with the strongest negative effects associated with Nred which caused highest foliar N concentrations. However, given that the keystone *Sphagnum* was also negatively impacted by Nox, both would need to be controlled to protect peatlands.

It appears that a hallmark of the subtlety of realistically applied N deposition effects is that impacts are played out largely via adjustment of the competitive relations of the mix of species present. N effects on *Calluna*, especially litter fall and associated moss species suggest a key role for the dominant species in responding to elevated N and in turn shaping conditions for subordinate species.

In terms of understanding the mechanisms of damage our results caution against over-interpreting short term, high dose studies that elicit damage but where such impacts do not represent realistic ecosystem trajectories over realistic time scales. Although our 2011 whole plot assessment did not take into account differences in species cover at the start, the trends supported the permanent quadrat data, and in reality the influence of start cover was not significant after five years. Despite the halving of cover, most plots remain > 10 % covered in *S. capillifolium* indicating slow rates of change and inferring this keystone species is relatively resilient to N impacts. Significant form effects on change in species cover were rare except for *P. schreberi* where Nred was most detrimental.

Consequences of different N forms in wet deposition on the cover of key components of an ombrotrophic bog

Assuming that this ombrotrophic bog relies on deposition for nutrients, we had expected N deposition to change species proportions within the vegetation. The non-vascular plant components have no cuticle and take up nutrient ions over their entire surface, rendering them both vulnerable but also effective competitors for N deposition until they saturate (Bates, 2002). Saturation of this moss filter in peat bogs (Lamers *et al.*, 2000; Curtis *et al.*, 2005) allows mineral N to leak into pore water and soil, where it can be accessed by vascular plant roots. Use of this additional N by the vascular over-storey to increase its cover has caused the disappearance of moss and *Sphagnum* (Cornelissen *et al.*, 2001; Nilsson *et al.*, 2002; Bubier *et al.*, 2007). But our data show this is not the only N induced scenario, especially in the absence of competition for light.

In an earlier paper (Sheppard *et al.*, 2011) we reported highly detrimental, visible effects of elevated N deposition as dry deposited ammonia (NH₃) at equivalent N doses to those used in this paper, which eradicated *S. capillifolium* and also the over-storey, arguably through NH₃ toxicity (Krupa, 2003). We suggested that some of the reported N sensitivity of *Sphagnum* species, such as *S. capillifolium*, could reflect exposure to elevated NH₃ concentrations, especially in areas where agriculture dominates the landscape. The data reported here support that idea indicating that, in the absence of N induced eutrophication and ensuing competition effects, the sustainability of *S. capillifolium* is more resilient to wet N deposition than previously inferred and, based on the curvilinear relationship with cumulative N load, that *S. capillifolium* can adjust to high wet N inputs. The relative proportions of species in this peatland plant community dominated by unmanaged degenerate *Calluna*, *E. vaginatum* and under-storey moss have yet to be destabilised by realistic applications of wet N deposition separated by form.

Why is Whim vegetation resilient to enhanced wet N deposition nine years on? There are characteristics of this study that could have modified the responses: 1. Method of treatment

application: Unlike many N manipulation studies (Phoenix *et al.*, 2012) N additions at Whim were automated, enabling them to be coupled to rainfall, facilitating frequent small N inputs at concentrations more closely resembling those in wet deposition to relatively large plots (less edge effect). We would not expect this treatment scenario to cause artefacts associated with high concentrations / doses (Pearce & van der Wal, 2008). 2. Recent manipulation studies applied Nox and Nred combined as NH_4NO_3 , which may cancel out or restrict some effects we have seen since they affect pH differently both within the plant and the soil (Raven, 1988), and have no associated counter ions to influence the impact (Evans *et al.*, 2008). However, on balance, one might expect NH_4NO_3 deposition studies to be less, not more damaging. 3. Our study has run three times longer than most and has thus been impacted by the potential for climate interactions e.g. 2003 drought (Carfrae *et al.*, 2007) which can exacerbate N effects (Carroll *et al.*, 1999; Sheppard *et al.*, 2008). But this would be expected to reduce, not increase resilience. 4. The vegetation composition at Whim is typical of NVC M19 (Rodwell, 1991), but the *Calluna* is old and unmanaged, and may not be capable of significantly growing its' over-storey and depleting light to the under-storey sufficiently to exclude photosynthesis, as happened with Labrador tea (*Ledum groenlandicum*) at Mer Bleue (Bubier *et al.*, 2007; Juutinen *et al.*, 2010). Also, *Calluna* only covered 40 ~ 60 % of the plots so even the significant increase in fallen litter may not have impacted all areas occupied by *Sphagnum* and the bottom 30 cm or so of stems from > 60 % of *Calluna* plants were devoid of greenery.

Implications

This long-term study has demonstrated that an intuitively N sensitive ecosystem can resist destabilising changes from significant N inputs, such that the cumulative impacts of N deposition have yet to be fully played out. The flattening of the response of *S. capillifolium* to cumulative N load implies some sensitivity but much more tolerance than has been implied

from surveys (Smart *et al.*, 2010). Our results suggest that once the initial effects of Nox have manifest themselves additional N makes very little difference, whereas for Nred increasing the N load perpetuates the slow decline. However, even nearly a decade may not be sufficient time to capture response curves given between species interactions. Generally among responders, species level impacts represented slight, negative net effects. Our results reinforce the importance of running manipulations for realistically long time scales to show whether negative N responses are linear or curvilinear and when, if, positive responders become negative. In the absence of these response curves our predictions of N impacts on bogs may be over stated.

The results challenge the value of short term experiments or where unrealistic concentrations are applied, since they may either generate responses that do not represent long term impacts in resistant ecosystems or fail to find effects because insufficient time has elapsed. However, these effects should be seen in the context of the 150 years or more of heightened N loading prevalent across much of Britain (Fowler *et al.*, 2004). Thus the experiment tries to replicate the start of the trajectory of perturbation but even here we must acknowledge background deposition and so cannot rule out effects present prior to the start of the experiment that have predisposed the site to respond in the way it has. The possibility of non-linear change occurring in the future is highlighted by the two positive impacts of Nox and Nred on NH_4^+ . These changes in soil water chemistry were linked with subsequent negative impacts on *P. schreberi* cover in 2011 and in *E. vaginatum* cover change between 2002 and 2011, but it is possible that further species-level impacts have yet to happen. In this respect, soil chemical change may be the fore runner of further changes in community structure. Similarly, the positive direct effect of Nred on *Calluna* growth could lead to earlier realisation of degenerate phases and subsequent gap formation. Such gaps can then trigger the expansion of

nitrophilous grasses favoured by elevated N load and a better lit ground layer although expansion of *E. vaginatum*, given its sensitivity to NH_4^+ , might depend on nitrification rates (Heil & Diemont, 1983). Replicating these cascade effects under realistic scenarios requires long-term experiments that enable such subtle interactions to play out. As we have seen, changes in dwarf shrub dominance and the unnatural acceleration of their growth cycles, leaf fall will also impact lower storey plants.

Were effects driven by changes in soil chemistry?

Biomass assessments, together with nutrient concentrations in 2009, inferred most N should be in the soil (Sheppard *et al.*, 2013) and we used soil pore water chemistry as the sensitive indicator (Sheppard *et al.*, 2008) for the path analysis. Both soil water N chemistry and soil pH varied spatially and temporally, representing the difference between deposition and the biological activity of the various sink strengths: mosaics of plant assemblages of which no two are identical. Interestingly, although NO_3^- concentrations were dose dependent in the Nox treatments, the concentrations appeared to vary too little for this to come through in the path analysis unlike NH_4^+ concentrations in the Nred treatments. Overall, changes in pore water N chemistry appear to have been too small to strongly affect cover: intuitively we would expect direct effects on vascular plant cover only, in reality no relationships were identified for *Calluna*, or *E. tetralix* that were mediated via soil water N or even pH. There was a positive effect of Nred on NH_4^+ concentrations that was related to the decline in *E. vaginatum*. However, we suggest this is implemented through enhanced competition from *Calluna* in the Nred treatments (Marschner, 1995; Nordin *et al.* 2006). Pretreatment cover of green *E. vaginatum* was negatively related ($r^2 = -0.86^*$) to *Calluna* cover.

Non-vascular plant cover did however, relate to soil chemistry: 2011 cover of *H. jutlandicum* and *P. schreberi* in Nox plots with soil pH (+ve) or soil water NH_4^+ (-ve) respectively,

although the link with soil water NH_4^+ is counter intuitive. This ion was not included in the Nox treatment: perhaps N transformations (ammonification, nitrification) are occurring within or on the vegetation, producing ions in proportions that mirror those in soil water? N fixation and methanotroph activity is known to occur within this layer (DeLuca *et al.*, 2002; Raghoebarsing *et al.*, 2005; Larmola *et al.*, 2010). *P. schreberi* is sensitive to NH_4^+ (Solga *et al.*, 2005). However, mosses have no roots and these mosses occupy niches above the water table, so would not come into direct contact with soil water N concentrations. Possibly the linkage reflects a proxy, working through competition effects which we did not explicitly test for. However, although *Calluna* cover and litter increased with N addition, possibly lowering PAR below that needed for *P. schreberi* to survive under *Calluna* (Scandrett & Gimingham, 1989), and the moss was often buried under litter and ‘hidden’ from view, this effect was most pronounced in Nred plots. *P. schreberi* decline was shown clearly in the HOF model for Nox56 and path analysis did indicate medium negative treatment effects not mediated directly via soil water chemistry, the mechanism however, remains unclear.

Manninen *et al.* (2011) reported effects of the wet N treatments on *S. capillifolium* capitulum pH at Whim, and we have similar (unpublished data) for *H. jutlandicum* and *P. schreberi*, replicating those described here for soil, *ie.* Nox increased pH. Given pH controls many physiological processes (Raven, 1988; Marschner, 2005) we suggest that the positive relationship with pH for *H. jutlandicum* reflects a more alkaline physiological optimum.

We have not measured large increases in soil N availability over this 9 year period which is consistent with the absence destabilising changes in proportions of the key component species (Sheppard *et al.*, 2012).

Sensitivity to N form in non-vascular plants

574 Nordin *et al.*, (2006) concluded, from a similar manipulation of N form in boreal forest, that
 575 Nred caused moss to decline through N accumulation, which didn't occur with Nox. Tissue N
 576 data (see also Solga *et al.*, 2005) suggests *P. schreberi* cannot tolerate large (> 30%)
 577 increases in its N status; in the Nox56, Nred24 and Nred56 plots, percent N increased by 55,
 578 49 and 109 % over the control, respectively. Taking into account growth and leaf N
 579 concentrations, and the response to the cumulative N load our results show *P. schreberi* is
 580 damaged by both N forms, but via different mechanisms: Nox via a combination of increased
 581 pH and N accumulation and Nred through greater N accumulation. Nred56 caused damage
 582 earlier and for a lower cumulative dose than Nox56, but for long-term protection of N
 583 sensitive bryophytes both N forms should be controlled.

584 Lichens take up and use both N forms equally efficiently (Crittenden, 1989; 1998). Visible
 585 damage was most conspicuous in *C. portentosa* with Nred56, which consistently caused
 586 elevated N concentrations (Hogan *et al.*, 2010) and its' disappearance from 3 of the 4 Nred56
 587 plots. Such damage fits the classic phytotoxicity chain response: reduced growth, NH_4^+
 588 accumulation and ensuing cellular injury e.g. leaky membranes (Krupa, 2003). However, Nox
 589 was also toxic (Hauck, 2010). NO_3^- has to be converted to NO_2^- then NH_4^+ , both potentially
 590 toxic ions, in order to be assimilated (Glime, 2007). If there is insufficient energy, C
 591 skeletons or enzyme activity available, these ions can be toxic (Hauck, 2010). Bleaching
 592 commensurate with usnic acid breakdown and loss of protection from light damage (Munzi *et*
 593 *al.*, 2009) was recorded in both Nred and Nox plots, together with lower Fv/Fm values
 594 indicating reduced photosystem II activity (Sheppard *et al.*, 2004b). In addition, prior to
 595 bleaching, algal colonization was observed, reducing light to the phycobiont impairing C
 596 assimilation (James, 1973; Hauck, 2010).

597 *S. capillifolium* prefers acid bogs (Brown & Bates, 1990), where NH_4^+ dominate pore water
 598 (Hemond, 1983) and is used more effectively than NO_3^- for growth, because of the energy

needed to move excess NO_3^- into the vacuole to avoid toxicity (Touffet, 1971). Both N forms providing N24 or N56 decreased *S. capillifolium* cover, and were associated with significant increases in amino acids in 2006 (de Lange I. unpub), indicating excess NH_4^+ (Ohlson *et al.*, 1995; Nordin *et al.*, 2000). Twenhoven (1992) also reports low NH_4^+ concentrations stimulating *S. fallax* growth, whereas NO_3^- decreased the abundance of both *S. fallax* and *S. magellanicum*. Nox56 may also have compromised the acid base balance, increasing *S. capillifolium* capitulum pH by 0.5 units (4.57 control, 5.04 Nox56, 4.51 Nred56) (Kivimäki, 2011; Manninen *et al.*, 2011). Not all negative effects of Nox on *S. capillifolium* were direct, stimulation of *H. jutlandicum* cover (+50%) meant that some *S. capillifolium* was overgrown and shaded by *H. jutlandicum*. For this peatland community the increase in plant pH associated with Nox appears to be quite deleterious and its impacts continue. In 2011 the negative effect of Nox on *S. capillifolium* was almost significant ($p=0.096$) corresponding to a medium size effect (-0.4 compared with -0.62 for Nred).

N form and CL_N setting

Overall Nred was more damaging to nitrophobic non-vascular plants than Nox. But this study shows that Nox can also damage such plants. However, increasing N deposition, as NH_4^+ or NO_3^- , to the canopy of this acid peatland vegetation caused few significant changes in species cover, after 9 years, over and above the evolving changes in control plots. In the NH_4^+ sensitive species, *P. schreberi*, the annual 56 kg Nred ha^{-1} significantly decreased cover before the equivalent Nox input. In *S. capillifolium* and *Cladonia* NH_4^+ also decreased cover faster than NO_3^- , but not significantly. *Calluna* preferred Nred and *H. jutlandicum* Nox but other species were insensitive to N form. Thus the main components of this single ecosystem differed in their responses to N form, but CL_N 's are applied at the ecosystem level, making it difficult to apply form based CL_N 's.

Knowledge of potential eutrophication responses of species growing at a given site to either Nox or Nred could indicate an additional risk from N deposition reflecting the dominant N form. N form could be used as a moderator for establishing which end of the CL_N range is appropriate for a site. It would be prudent to set the CL_N at the low end of the published range of N deposition when site contains sensitive non-vascular plants, the input is predominantly in the damaging form, or where ecosystems contain vascular plants with nitrophilic characteristics and Nox dominates deposition.

A further aspect of the N form CL debate to be considered concerns the interaction between N form and soil chemistry. Post deposition these N forms are interchangeable, both in the plant and in the soil through enzyme mediated conversions that depend heavily on pH (Stevens *et al.*, 2011). The significance of these transformations will depend on the ecosystem and the ratio of reduced to oxidised N in precipitation but require further evaluation (Stevens *et al.*, 2011).

Other drivers influence species cover

Species cover in the control plots did not remain static. Species responses in control plots indicate other factors *e.g.* the environment or just the age dynamics and competitive balance, homeostasis, within the community are actively shaping vegetation cover at Whim, which has not been managed for at least 60 years. To what extent these factors have exacerbated or suppressed treatment responses is not clear.

In 2009, the cover of most species irrespective of treatment dipped, coinciding with 25% higher rainfall the preceding year. The higher rainfall may have reduced CO₂ diffusion, and PAR was ca. 15 % lower than in other years. By 2011 cover had recovered. In 2003, Whim bog, like most in Europe (Gerdol *et al.*, 2008), experienced an exceptional, prolonged drought, which caused short-lived adverse effects in *S. capillifolium* (Carfrae *et al.*, 2007), reversed by the following wet autumn / winter, and undetectable in 2004 cover. *P. schreberi*

too appears to prefer damp conditions judging from 2004 cover values, post 2003 drought, which were lower than expected, but recovered as rainfall increased through the course of the study. Climatic driven changes appear to be more transient, than those associated with N deposition.

Conservation Implications: indicator species

P. schreberi and *C. portentosa* appear to be strongly nitrophobic and unlikely to be present, or of very limited occurrence, at sites where ambient inputs exceed $24 \text{ kg N ha}^{-1}\text{y}^{-1}$. Thus their absence from environmentally suitable sites could indicate high N deposition levels. In peatlands however, water table height also determines species presence and absence (Lindsay, 2010) so such environmental factors must also be accommodated within N deposition indicators. Non responding species, tolerant of or indifferent to N deposition and its effects without long-term or significant cover changes were also identified. Some ericoids fell into this category e.g. *E. tetralix*, *V. oxycoccus* and *E. nigrum*. None of the species in the wet deposition plots, from either the graminoids or ericoids can be described as true nitrophiles. However, we saw (Sheppard *et al.*, 2011) that once competition from *Calluna* was removed *E. vaginatum* displayed its nitrophilic characteristics (Redbo-Torstensson, 1994; Wiedermann *et al.*, 2007). Many grasses, even though they have low Ellenberg values, behave as nitrophiles and up regulate their growth capability in eutrophicated environments at the expense of small forbs with similar Ellenberg values e.g. *Molinia caerulea* (van den Berg unpub.). *M. caerulea* readily capitalizes on increased NH_4^+ e.g. the Netherlands during the 1990s, where it replaced *E. tetralix*, transforming wet *Ericion tetralicis* heathland into grassland (Bobbink *et al.*, 1998; Aerts & Bobbink, 1999). However, though present nearby, it has not moved into plots at Whim. Although graminoids exist naturally as low N index plants, a significant cover of such species should be seen as cause for concern given their capacity to respond to N eutrophication.

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935 *Supplementary information legends*

936 Table S1 Soil and meteorological characteristics at Whim

937 Table S2 Mean climatic variables at Whim

938 Table S3 Effects of N on growth and vitality responses

939 Table S4 Summary of responses of common plant species growing on Whim bog to wet N

940 deposition, and the year of response, ns = $p > 0.05$, * = $p < 0.05$, where Nox = nitrate and

941 Nred = ammonium.

942 Table S5 Model parameters a-d from HOF curve fitting by the R package 'gravy'.

943 Table S6 Descriptive statistics of path model parameters based on summarising 20000 values

944 from the posterior distributions of each.

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958 *Figure headings*

959 Fig.1 Path diagram illustrating possible causal/correlative relationships between experimental
 960 treatments, soil chemistry and species abundance at Whim Bog.

961 Fig. 2 Difference in mean cover, +/- standard error, from mean plot quadrats pretreatment
 962 cover (2002), after 2y (2004), 5y (2007), 7y (2009), 9y (2011) of *Calluna*, *Erica tetralix*,
 963 *Eriophorum vaginatum*, *Sphagnum capillifolium*, *Hypnum jutlandicum*, *Pleurozium schreberi*
 964 and *Cladonia portentosa* in response to wet deposited oxidised N, nitrate (NaNO_3) or
 965 reduced N, ammonium (NH_4Cl) at 8, 24 or 56 kg N $\text{ha}^{-1}\text{y}^{-1}$ or wet only (control) = Nox8,
 966 Nox24, Nox56 or Nred8, Nred24 or Nred56 respectively.

967 Fig. 3 Percent cover of functional groups: ericoids, graminoids, moss excluding *Sphagnum*,
 968 *Sphagnum* species and sensitive species: *S. capillifolium* and *C. portentosa* in response to N
 969 dose 8, 24 or 56 kg N $\text{ha}^{-1}\text{y}^{-1}$ or wet only (control) and form : oxidised N, nitrate (NaNO_3) or
 970 reduced N, ammonium (NH_4Cl) in whole plots assessed in September 2011. Dose was
 971 significant for ericoids, moss and *C. portentosa*, $p=0.042$, 0.014 and 0.008 respectively; form
 972 was significant only for moss $p=0.009$.

973 Fig. 4 HOF model response curves for the major species present in experimental plots. Plant
 974 cover change over time was fitted to wet deposited oxidised N, nitrate (NaNO_3) or reduced
 975 N, ammonium (NH_4Cl) at 8, 24 or 56 kg N $\text{ha}^{-1}\text{y}^{-1}$ = nox8, nox24, nox56 or nred8, nred24 or
 976 nred56 respectively or wet only = con. On the x axis the time gradient 1 to 5 refer to
 977 assessments in 2002, 2005, 2007, 2009 and 2011 respectively. The y axis, response, 0.2 is
 978 equivalent to 20 % cover etc. The colour of the fitted response refers to the different model
 979 fits outlined in the text. Cv = *Calluna*, Et = *Erica tetralix*, Ev = *Eriophorum vaginatum*, Scap
 980 = *Sphagnum capillifolium*, Hj = *Hypnum jutlandicum* and Ps = *Pleurozium schreberi*.

981 Fig. 5 Difference in cover from the start (2002) for the most common species growing on
 982 Whim bog receiving oxidised N (Nox) or reduced N (Nred) plotted against each other. The
 983 closer points are to the 1:1 solid line the more similar the response to reduced and oxidised N.

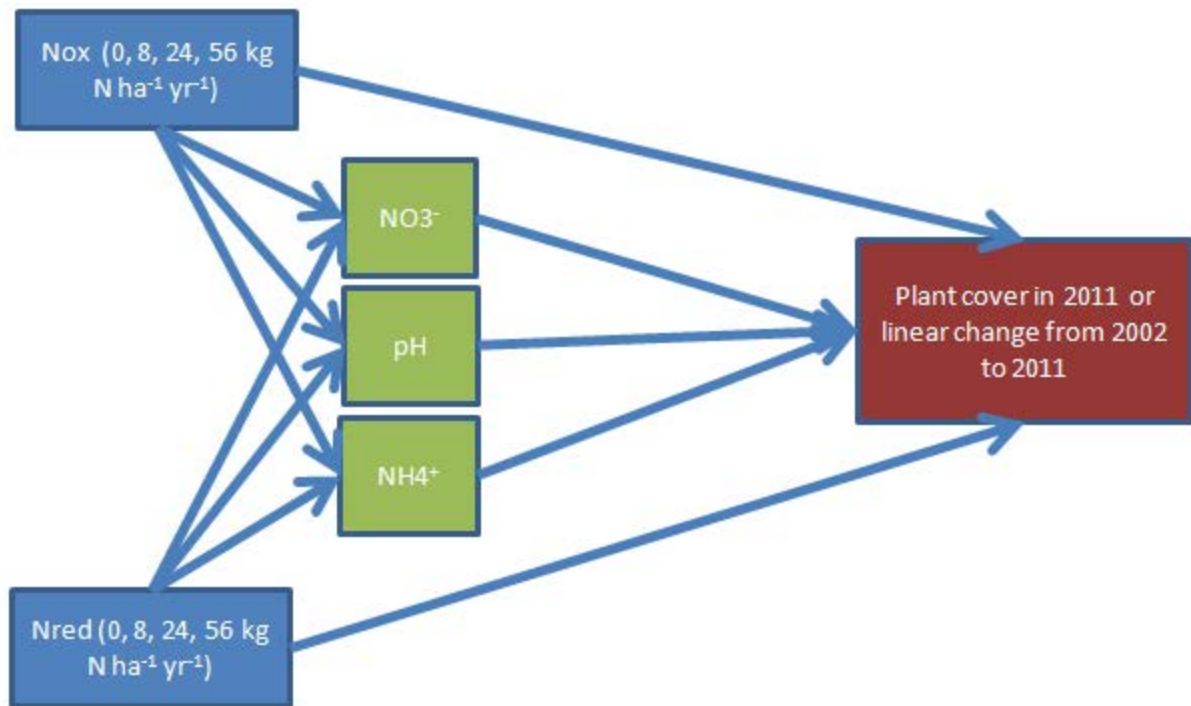
984 Fig. 6 Relationship between the cumulative N load and the relative cover change from the
 985 start in 2002, for *Pleurozium schreberi* (Ps black squares) and *Sphagnum capillifolium* (Sc
 986 grey triangles), the species showing only negative responses to N deposition as Nox (solid
 987 symbols) and Nred (open symbols). Nred and Nox both caused large reductions in *S.*
 988 *capillifolium* (grey lines) cover but the effect of Nox was worse than Nred at low loads < 280
 989 kg N whereas at higher loads Nred caused larger reductions, however the but for *P. schreberi*
 990 (black lines) the effects of Nred were consistently ($p < 0.05$) worse than those of Nox.

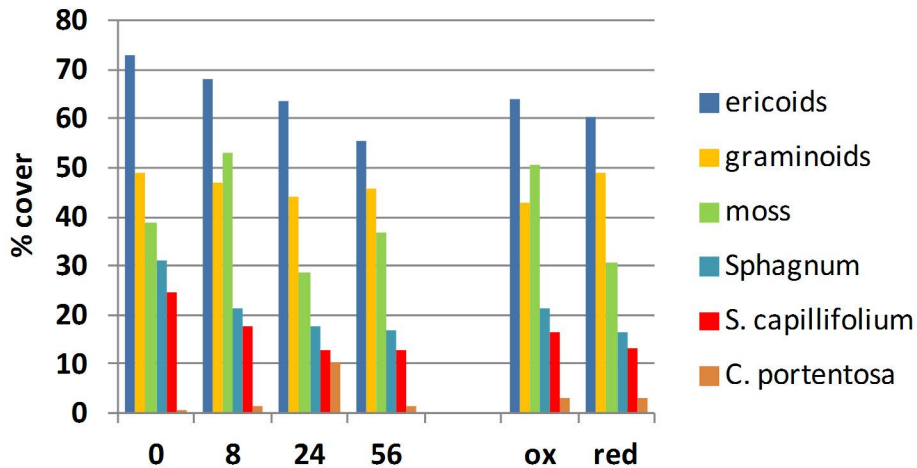
991 Fig. 7 Percent N in current years' growth of lower plants, Hj = *Hypnum jutlandicum*, Sc =
 992 *Sphagnum capillifolium*, Cp = *Cladonia portentosa* and Ps = *Pleurozium schreberi* receiving
 993 N, as either Nox or Nred. %N values sitting above the one to one line indicate higher
 994 concentrations occur when the N is applied as Nred.

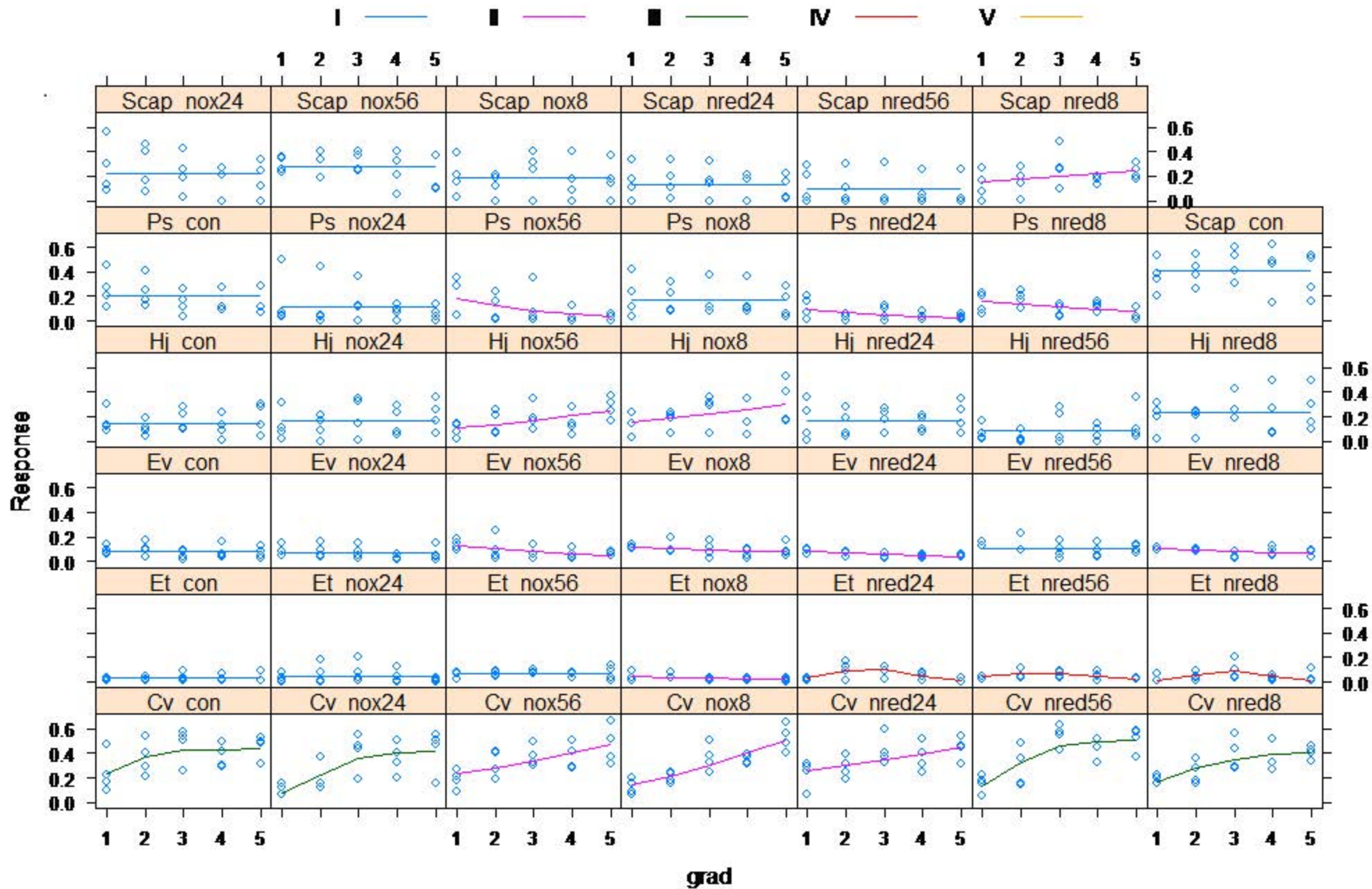
995 Fig. 8 Path diagram showing statistically significant relationships between Nox and Nred
 996 treatments, soil chemistry averaged over 2009-2011 and final species cover measured in
 997 2011. Numbers on the vertices are regression coefficients based on analysis of centred and
 998 standardised data and can be interpreted as effect sizes.

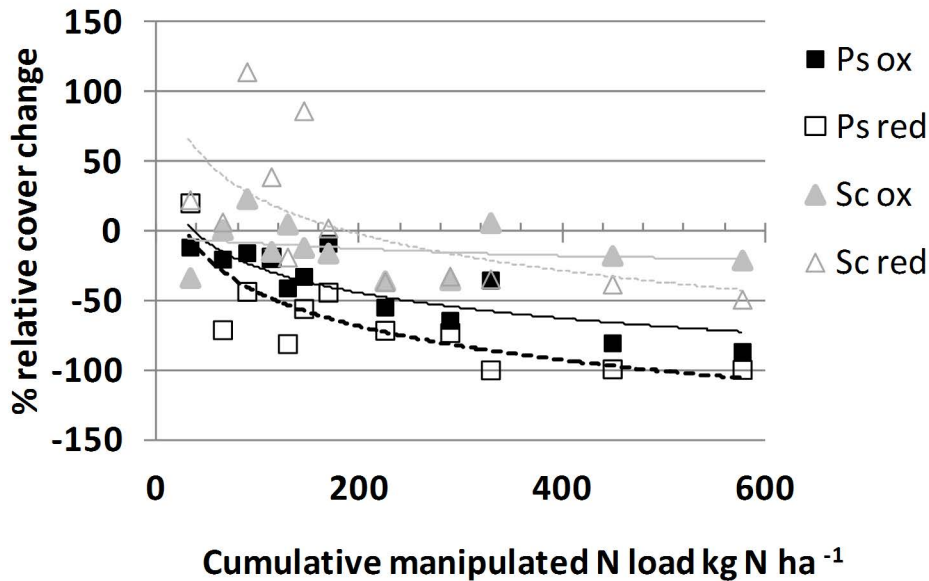
999 Fig. 9 Path diagram showing statistically significant relationships between Nox and Nred
 1000 treatments, soil chemistry averaged over 2009-2011 and cover change between 2002 and
 1001 2011 measured as the linear slope of cover versus year of recording. Numbers on the vertices
 1002 are regression coefficients based on analysis of centred and standardised data and can be
 1003 interpreted as effect sizes.

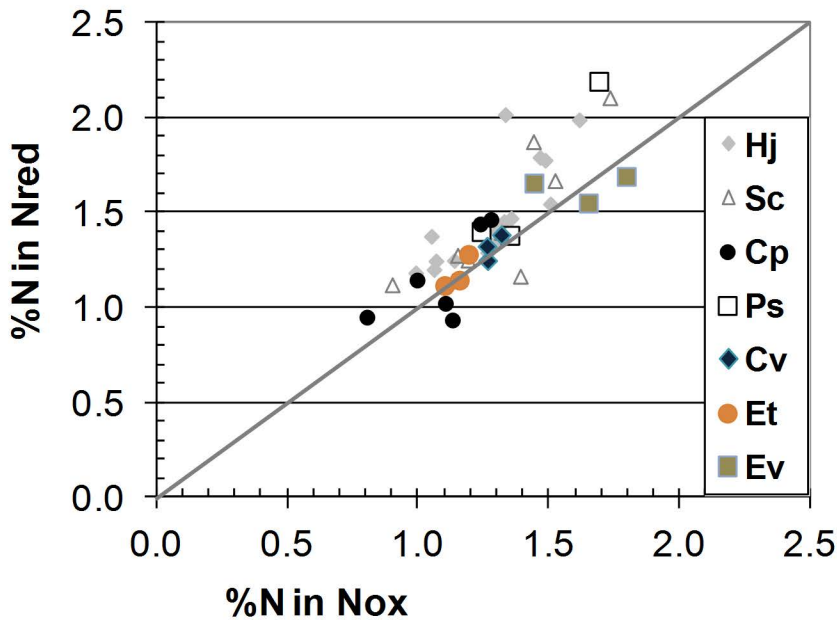
1004 Fig. S1 Percent cover responses of key species in permanent quadrats since 2002
 1005 (pretreatment) up to 2011, growing on Whim bog and treated with wet deposited nitrate
 1006 (NaNO_3) or ammonium (NH_4Cl) at 8, 24 or 56 $\text{kg N ha}^{-1}\text{y}^{-1}$ or wet only (control) = Nox8,
 1007 Nox24, Nox56 or Nred8, Nred24 or Nred56 respectively. (Cv gr = *Calluna* green shoots; Cv
 1008 dea = *Calluna* dead brown shoots; *Calluna* lit = *Calluna* litter; En = *Empetrum nigrum*; Vo =
 1009 *Vaccinium oxycoccus*; Et = *Erica tetralix*; Vm = *V. myrtillus*; Sc = *Sphagnum capillifolium*;
 1010 Hj = *Hypnum jutlandicum*; Ps = *Pleurozium schreberi*; Ev = *Eriophorum vaginatum*; Cp =
 1011 *Cladonia portentosa*). Linear or polynomial (unimodal) trendlines describe the response.

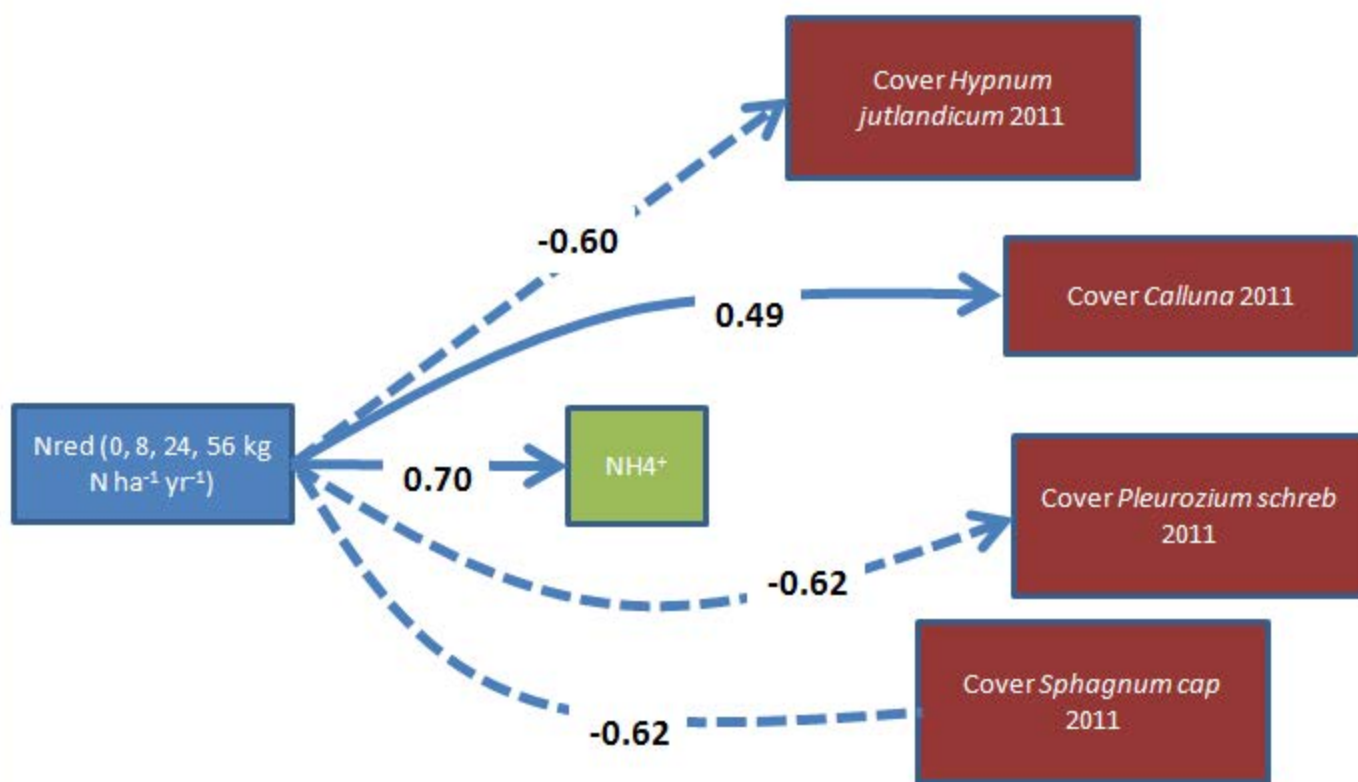
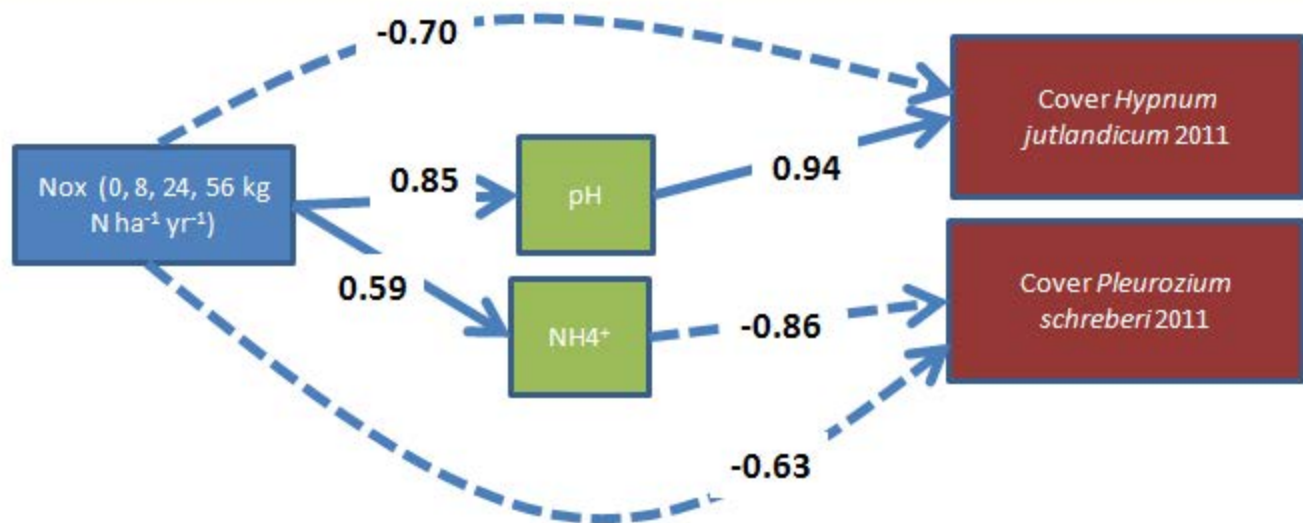












Negative relationship; as driving variable increases, response variable decreases

Positive relationship; as driving variable increases, response variable increases

