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Levy, Peter; van Dijk, Netty; Gray, Alan; Sutton, Mark; Jones, Matthew; Leeson, Sarah; Dise, Nancy; Leith, Ian; Sheppard, Lucy. 2019. **Response of a peat bog vegetation community to long-term experimental addition of nitrogen.** *Journal of Ecology*, 107 (3). 1167-1186, which has been published in final form at <https://doi.org/10.1111/1365-2745.13107>

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## Response of a peat bog vegetation community to long-term experimental addition of nitrogen

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November 19, 2018

### Abstract

1. We report results from a long-term experiment in which additional nitrogen has been deposited on a peat bog in central Scotland for over fourteen years, in three different forms: as ammonia ( $\text{NH}_3$ ) gas, as ammonium ( $\text{NH}_4^+$ ) solution or as nitrate ( $\text{NO}_3^-$ ) solution. The automated experiment was designed to apply nitrogen in such a way that mimics real-world nitrogen deposition. Background nitrogen deposition at the site was  $0.8 \text{ g N m}^{-2} \text{ y}^{-1}$ .
2. Observations of cover for 46 species were made. We analysed the change in six common species in relation to nitrogen dose and form. The responses differed among species and nitrogen forms, but five out of the six species declined, and  $\text{NH}_3$  produced the biggest change in cover per unit of nitrogen addition. The exception was the graminoid sedge *Eriophorum vaginatum*, which increased dramatically in the  $\text{NH}_3$  treatment. Multivariate analyses identified responses

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1365-2745.13107

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to nitrogen dose across treatments which were consistent with the univariate results.

3. We surmised that the larger experimental response to nitrogen observed in the  $\text{NH}_3$  treatment (cf. the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments) was because of the higher nitrogen concentrations at the vegetation surface produced by dry deposition.  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were sprayed in solution, but much of this will enter the peat porewater, and be further diluted. Because  $\text{NH}_3$  deposits directly to the leaf, it stays contained within the small volume of water on and in the leaf, producing a high internal concentration of nitrogen ions.
4. *Synthesis*. Consistent trends with nitrogen were discernible across species. All species showed a decline with  $\text{NH}_3$  treatment, except for *Eriophorum vaginatum* which increased. In the absence of PK, all species declined with  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , except for *Calluna vulgaris* and *Hypnum jutlandicum*. The effect of PK was not consistent across species. Per unit of nitrogen deposited,  $\text{NH}_3$  generally had a larger impact on vegetation composition than  $\text{NH}_4^+$  or  $\text{NO}_3^-$ . However, the actual deposition rate of  $\text{NH}_3$  on UK peat bogs is lower than the other forms. In the case of the most common species of the peat-forming genus *Sphagnum*, we estimate that  $\text{NH}_4^+$  deposition has the largest impact, followed by  $\text{NO}_3^-$  and  $\text{NH}_3$ .

Keywords: Global change ecology, plant community, air pollution, nitrogen deposition, peatlands, *Sphagnum*, ammonia, nitrate, multivariate analysis

## 1 Introduction

Deposition of anthropogenic nitrogen (N) has increased since the Industrial Revolution as a result of fossil fuel burning and agricultural use (Fowler *et al.*, 2005). Peat bog communities are adapted to conditions of very low nitrogen availability, and hence are

likely to be sensitive to additional nitrogen inputs (Bobbink *et al.*, 1998). Western Europe, and particularly the UK, have large areas of peatland in relatively close proximity to emission sources of nitrogen pollution, and hence these areas may be particularly vulnerable.

Demonstrating the true long-term effects of nitrogen pollution on vegetation is difficult. Much debate in the literature concerns the validity of short-term field experiments in extrapolating the long-term response, and the utility of glasshouse experiments for estimating the response of vegetation in the field (e.g. Wiedermann *et al.*, 2009, Armitage *et al.*, 2012, Limpens *et al.*, 2012, Phoenix *et al.*, 2012). Several short-term manipulative experiments have demonstrated rapid loss of *Sphagnum* moss communities when large nitrogen doses are applied over a short time (Limpens *et al.*, 2012). Results are not altogether consistent, and some studies have shown positive responses or no detectable effect on areal cover (Gunnarsson & Rydin, 2000, Saarnio *et al.*, 2003). Few experiments have run for more than five years, but two experiments are of particular note. At Mer Bleue in Canada, treatments of up to  $6.4 \text{ g N m}^{-2} \text{ y}^{-1}$  in  $\text{NH}_4\text{NO}_3$  solution were applied for up to 12 years (Bubier *et al.*, 2007, Larmola *et al.*, 2013)(and are on-going). Fertilization was applied in 2 mm of water every third week from early May to late August, with seven applications per year. Juutinen *et al.* (2010) found that *Sphagnum* cover decreased rapidly in relation to nitrogen addition. Effects were clear after three years, and *Sphagnum* became absent from the higher nitrogen treatments ( $\geq 3.2 \text{ g N m}^{-2} \text{ y}^{-1}$ ) after 5 years. Dwarf shrubs and the moss *Polytrichum strictum* Brid. benefitted from the nitrogen addition (Juutinen *et al.*, 2015). At Degerö Stormyr in Sweden, treatments of  $3 \text{ g N m}^{-2} \text{ y}^{-1}$  of  $\text{NH}_4\text{NO}_3$  solution were applied for eight years (Wiedermann *et al.*, 2007). One third of the seasonal dose was applied directly after snowmelt in May, with four further monthly applications covering the short growing season (Eriksson *et al.*, 2010). No effects were seen on the vegetation in the first four years, but after eight years *Sphagnum* cover was reduced from 100 % to 41 %.

A serious issue in almost all experiments is the application of nitrogen in a few large doses (e.g. in monthly watering treatments), thereby exposing the plants to unrealistically high concentrations of nitrogen in solution on the leaf and root surface (Pitcairn *et al.*, 2006, Pearce & Van der Wal, 2008, Wu & Blodau, 2015). This may have toxic effects, unrepresentative of nitrogen deposition in real ecosystems. In observational studies, where trends in peatland vegetation were studied over time in areas with different nitrogen deposition rates, little sensitivity to nitrogen has been seen (Hájková *et al.*, 2011). In addition, surveys along nitrogen deposition gradients across Europe have suggested lower sensitivity of peatland vegetation to nitrogen deposition than other nutrient-poor and potentially sensitive habitats (Robroek *et al.*, 2017). The above points suggest that, as well as long-term experiments, consideration needs to be given to the nitrogen concentration experienced at the vegetation surface, as well as the total nitrogen deposition per year (see the modelling analysis of Wu & Blodau, 2015). Because of this artefact of high-concentration doses, real-world ecosystems may be less sensitive to nitrogen than experiments suggest.

The experiment at Whim bog in central Scotland (Leith *et al.*, 2004, Sheppard *et al.*, 2004) is a globally unique opportunity to investigate the effect of different forms of nitrogen when applied at realistic rates. Here, nitrogen treatment is applied near-continuously over the year, via automated sprayers mimicking realistic rain events for wet deposition, and as a gas plume for dry deposition. Because the nitrogen is applied as many (>100) small application events per year, the effect of this is to allow manipulation of the nitrogen deposition whilst maintaining realistic nitrogen concentrations in solution at the leaf and soil surface. This paper reports results from this long-term experiment, where 14 years of treatment data are now available. Nitrogen has been deposited in three different forms: as ammonia (NH<sub>3</sub>) gas, as ammonium (NH<sub>4</sub><sup>+</sup>) solution, or as nitrate (NO<sub>3</sub><sup>-</sup>) solution (Sheppard *et al.*, 2004). Ambient nitrogen inputs at the site are relatively low for Europe (0.8 g N m<sup>-2</sup> y<sup>-1</sup>), and the site had not received obvious damage prior to the experiment, so it is reasonably representative of similar

sites across Europe.

Previously, Sheppard *et al.* (2011) showed that very high doses of  $\text{NH}_3$  produced visible damage, mortality and reduced cover of *Calluna vulgaris* (L.) Hull, but could not detect effects of wet-deposited  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . Effects on cover change of other species were largely unclear, although trends could be discerned (Sheppard *et al.*, 2008, 2011, 2014). Here, we build on this work in several ways. Firstly, we present a longer-term analysis, with six additional years of experimental treatment. Secondly, we apply a linear mixed-model approach (Pinheiro & Bates, 2006), allowing us to treat nitrogen deposition as a continuous variate, accounting appropriately for the correlation in residuals which arises from making repeated measurements on the same locations (quadrats nested within plots, nested within blocks). Thirdly, a phosphorus and potassium addition treatment was included in the experiment, but was excluded from most previous analyses. We include this interaction in our analysis. Fourthly, we apply multivariate analyses of species cover (principal response curves, PRC, and partial least squares regression, PLS, Van den Brink & Braak, 1999, Mevik & Wehrens, 2007), which have greater statistical power in detecting changes in community composition.

The aims of this paper were to:

1. quantify the response of the key species to enhanced nitrogen deposition, using appropriate analysis techniques, including the effects of phosphorus and potassium in modulating the response;
2. quantify the time-dependent, community-level effects of nitrogen deposition;
3. relate vegetation change to changes in the below-ground chemical environment;  
and
4. estimate the likely effect of nitrogen deposition in different forms on the key peat-forming *Sphagnum* species across the UK, based on the results from the

long-term experiment.

## 2 Methods

### 2.1 Field site

The experiment was carried out at Whim bog in the Scottish Borders (3° 16 W, 55° 46 N). The site is at a transition between lowland raised bog and blanket bog, on 3-6m of deep peat. Mean temperatures of the air and soil (at 10-cm depth) were 7.9 °C and 7.6 °C respectively (all statistics are for the period 2002-2016). The annual rainfall was 1141 mm (734-1486 mm range). The site was wet, with the average water table at 10 cm below the peat surface. This has remained relatively constant over time, with the exception of the drought year in 2003, when it was reduced to 24 cm below the surface on average. The peat was very acidic, with pH 3.4 (3.27-3.91 in water).

The vegetation was mainly classified as a *Calluna vulgaris* - *Eriophorum vaginatum* blanket mire community (M19 in the UK National Vegetation Classification, Rodwell, 1998). The vegetation was dominated by *Calluna vulgaris* which had not been managed by burning or grazing, and so was of variable age and stature. Replicate plots were highly variable mosaics containing *Calluna vulgaris* and *Sphagnum capillifolium* (Ehrh.) Hedw. hummocks and hollows containing *S. fallax* (H. Klinggr.) H. Klinggr. and *S. papillosum* Lindb. Other common species included *Erica tetralix* L. and the mosses *Hypnum jutlandicum* Holmen & E. Warncke and *Pleurozium schreberi* (Brid.) Mitt.

### 2.2 Experimental Treatments

Nitrogen was applied to the site using two different treatment systems, one for dry deposition of NH<sub>3</sub> gas, and one for wet deposition of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> in solution. Treatments commenced in June 2002 and continued all year round, except when temperatures were near freezing.

NH<sub>3</sub> deposition was manipulated using a free-air release system (Leith *et al.*, 2004). NH<sub>3</sub> was supplied from a cylinder of pure liquid NH<sub>3</sub>, diluted with ambient air and released from a perforated 10-m long pipe, 1 m off the ground. NH<sub>3</sub> was released only when the wind direction was in the south-west, between 180 and 215°, temperatures exceeded freezing and wind speed exceeded 2.5 m s<sup>-1</sup>. This produced a sector downwind wherein NH<sub>3</sub> decreased with distance from the fumigation source. NH<sub>3</sub> concentrations were measured 0.1 m above the vegetation using Adapted Low-cost Passive High Absorption (ALPHA) samplers (Tang *et al.*, 2001) at 8, 12, 16, 20, 24, 32, 48 and 60 m from the source along the transect. A detailed profile was measured to capture the concentration gradients both vertically and horizontally (Leith *et al.*, 2004). NH<sub>3</sub> deposition was calculated from the concentration measurements, using the method of Cape *et al.* (2008). The deposition at the permanent quadrat locations was interpolated using ordinary kriging, assuming the deposition velocity was spatially homogeneous.

Wet deposition of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> was experimentally increased in a number of replicated plots in a randomised block design, using a water sprayer system (Sheppard *et al.*, 2004). Using rainwater collected on a 178 m<sup>2</sup> pitched surface and stored in a 1.25 m<sup>3</sup> reservoir, concentrated solutions of either NH<sub>4</sub>Cl or NaNO<sub>3</sub> were diluted and transferred to each plot via lengths of 16-mm pipe. Each pipe terminated in a central sprayer with a 360° spinning disc that distributed the solution uniformly over the 12.8 m<sup>2</sup> plot. The volume of solution applied to each plot was monitored using a water meter on each supply line. Three treatment levels were applied, aiming to provide total nitrogen deposition rates of 1.6, 3.2 and 6.4 g N m<sup>-2</sup> y<sup>-1</sup> (i.e. background plus experimental addition). A control treatment received only background nitrogen deposition (0.8 g N m<sup>-2</sup> y<sup>-1</sup>). The three treatment levels were achieved by applying either NH<sub>4</sub>Cl or NaNO<sub>3</sub> solution at concentrations of 0.57, 1.71 or 4.0 mmol dm<sup>-3</sup>. In addition, phosphorous and potassium (PK) were added in the form of (K<sub>2</sub>HPO<sub>4</sub>) to the lowest and highest treatment levels, in the ratio 1:14 P:N, following the P:N ratio



of amino acids.  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments increased precipitation amounts by ca. 10%. Control plots receive the additional rainwater without any additional nitrogen. There were four blocks, with eleven combinations of the treatment levels in each (1 control, 3  $\text{NH}_4^+$  levels without PK, 2  $\text{NH}_4^+$  levels with PK, 3  $\text{NO}_3^-$  levels without PK, 2  $\text{NO}_3^-$  levels with PK), to give a total of 44 plots. The sprayer system was automatically triggered every 15 minutes, so long as there was sufficient rainwater in the collection tank, air temperature was above 0 °C and wind speed was above 5 m  $\text{s}^{-1}$ . This produced a realistic pattern of high frequency, extensive nitrogen deposition, with ca. 120 applications  $\text{y}^{-1}$ . The distribution of treatment applications occurred approximately evenly over the whole year except for the middle of winter (Leeson *et al.*, 2017).

Peat porewater samples were extracted from dipwells in all plots, approximately monthly from 2006 onwards. Concentrations of all detectable ions in the porewater were measured by ion chromatography following filtration. The detection limits were 0.014 and 0.062  $\text{mg l}^{-1}$  for  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N respectively.

### 2.3 Vegetation survey

Vegetation species composition was surveyed in all plots over the course of the experiment, usually every two years. In each experimental plot, three permanent quadrats (40 × 40 cm) were established before the start of treatment application in 2002. These were sub-divided into 16 sub-quadrats (10 × 10 cm). At each survey, the percent cover of all species was recorded at the sub-quadrat level. For each species, the 16 sub-quadrat values were averaged to give a mean cover for each quadrat.

Visual assessment of cover is prone to subjective factors which make the different species appear more or less abundant to the human eye - e.g. effects of daylight, survey date in relation to flowering time, etc. To try to account for this, we also analysed the data using an alternative measure of abundance, calculated as the fraction of the 16 sub-quadrats per quadrat in which a species occurred. This should give a more

conservative measure of change, dependent only on estimates of presence/absence rather than scoring cover as a percentage. The results were not markedly different, and only the analysis based on the mean cover is presented here.

We also investigated modelling the proportional changes instead of the absolute changes in cover, as a 2 % change from 5 to 3 % may have more significance than from 95 to 93 %. This was achieved using a natural log transformation of the cover data. Again, although the coefficients differed, this did not substantially change the results or conclusions drawn. More objective quantities, such as shoot length growth, point quadrats or gravimetric measures of biomass, are less prone to such errors (and have shown more sensitivity in other experiments), but are more difficult to carry out on a large scale to provide an adequate sample, covering all the experimental plots.

## 2.4 Statistical analysis

### 2.4.1 Univariate analyses

We chose six common species for univariate analysis, the only species which frequently occurred with more than 5 % cover: *Calluna vulgaris*, *Sphagnum capillifolium*, *Eriophorum vaginatum* L., *Cladonia portentosa* (Dufour) Coem., *Hypnum jutlandicum*, and *Pleurozium schreberi*. (Five other species occurred with at least 5 % cover, but not frequently enough for univariate statistical analysis: *Empetrum nigrum*, *Erica tetralix*, *Sphagnum fallax*, *Sphagnum papillosum*, and *Vaccinium myrtillus*). For each of the six most common species, the change in cover in each quadrat was analysed using a linear mixed-effects model (Pinheiro & Bates, 2006). We fitted fixed-effect terms for  $\text{NH}_3\text{-N}$  deposition rate,  $F_{\text{NH}_3}$ ,  $\text{NH}_4^+\text{-N}$  deposition rate,  $F_{\text{NH}_4}$ ,  $\text{NO}_3^-\text{-N}$  deposition rate,  $F_{\text{NO}_3}$ , PK, and interactions between PK and  $F_{\text{NH}_4}$  and PK and  $F_{\text{NO}_3}$ . Random-effect terms with a design matrix  $Z_{ijk}$  were included to account for the repeated measures on each quadrat location,  $i$ , nested within each plot,  $j$  within each experimental block,  $k$ . The analysis included the interaction effects of time, specifying four two-way inter-

actions between time and  $\text{NH}_3$ ,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and PK, and two three-way interactions, between time,  $\text{NH}_4^+$ , and PK, and between time,  $\text{NO}_3^-$  and PK.

The analysis yields estimates of several parameters for each species. Firstly, there is an intercept term, effectively the mean cover at the start of the experiment (at  $t_y = 0$ ). Four main effect terms are additional intercept terms, giving the mean effect of  $F_{\text{NH}_3}$ ,  $F_{\text{NH}_4}$ ,  $F_{\text{NO}_3}$  and PK on initial cover when  $t_y = 0$ , i.e. before any effect is expected. The time main effect,  $t_y$ , is the inferred change in cover per year when the other main effects are zero. The main focus here is on the interaction between nitrogen deposition and time. For example, the  $t_y:F_{\text{NH}_3}$  term quantifies how the slope of cover against time changes with  $\text{NH}_3$  deposition rate. If  $\text{NH}_3$  is deleterious, we would expect the cover of a species to be reduced more quickly at high doses of  $\text{NH}_3$ , and stay approximately stable at an ambient dose of  $\text{NH}_3$ . This would be reflected in a significant, negative  $t_y:F_{\text{NH}_3}$  coefficient. By extension, the three-way interactions describe whether the addition of PK affects the relationship between nitrogen deposition and time.

When extinctions occurred in quadrats (i.e. the cover of a species was reduced to zero and remained at zero thereafter), there was no further change to detect. This could be accounted for using a “broken-stick” model, but simply removing these post-extinction observations allowed us to use a simple linear model. We analysed the data both with and without post-extinction observations. Tables and Figures show the analysis of the data set including post-extinction observations, but we report wherever this makes a substantial difference.

#### 2.4.2 Multivariate analyses

With ecological data, multivariate methods are commonly used to find those factors that best explain the differences in species composition between samples. Partial Least-Squares regression (PLS) is a multivariate technique, closely related to principal components analysis (PCA) and multiple linear regression which maximizes the covariance between  $\mathbf{X}$  and a multivariate response matrix  $\mathbf{Y}$ . In this context, the

response matrix comprises the plant species cover data, and the independent variables are the porewater chemistry data describing the changes to the physicochemical environment that accompany the nitrogen treatments.

Principal response curves (PRC, Van den Brink & Braak, 1999) are a variant of redundancy analysis (itself a variant of PCA) which focus on the differences between the species compositions of the treatments and that of the control at the corresponding time. PRC provides two sets of coefficients, which can be interpreted graphically. The first set consists of the treatment-time coefficients  $c_{dt}$  estimated for each combination of the treatment levels and time-point.  $c_{dt}$  represents the effect size of treatment  $d$  at time  $t$  relative to the control.  $c_{dt}$  values are depicted in the principal response curves, a line-plot of  $c_{dt}$  against time grouped by treatment. The second set of coefficients are the loadings for the species,  $b_s$ . They represent the resemblance of species  $s$  to the overall response pattern specified by the principal response curves (i.e., the set of  $c_{dt}$  values). For the PRC analysis, quadrats in the  $\text{NH}_3$  treatment were binned in groups matching the nitrogen deposition levels used in the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatment, with an additional group where deposition was  $> 7 \text{ g N m}^{-2} \text{ y}^{-1}$ . Separate PRC analyses were run for dry-deposited ( $\text{NH}_3$ ) and wet-deposited forms ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) because of the difference in experimental design. Further information on these statistical techniques is provided in the online Supporting Information.

### 3 Results

We observed a clear decline in the cover of *Calluna vulgaris* in response to  $\text{NH}_3$ , with the cover reduced to near zero within a few years of treatment commencing at the highest doses, with a similar pattern only slightly delayed in the lower doses. This is reflected in the results of the univariate linear mixed model, with a significant interaction term between time and  $\text{NH}_3$  ( $p = 0.014$ ) in the model (Table 1). The interpretation of this interaction term is that the decline in cover over time was greater

at higher levels of  $\text{NH}_3$  (by  $-0.13 \%$   $(\text{g N m}^{-2} \text{ y}^{-1})^{-1}$ ). The effects of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  are much less clear, although the trends in cover were always negative at the high levels of nitrogen deposition. The interaction between time and  $\text{NO}_3^-$  shows statistical significance ( $p = 0.045$ ) and PK significantly exacerbates this interaction, i.e. the decline over time with  $\text{NO}_3^-$  is greater in the presence of PK. ( $p = 0.018$ , Table 1). The PK had no clear effect in the  $\text{NH}_4^+$  treatment.

There was a clear decline in the dominant moss *Sphagnum capillifolium* in response to  $\text{NH}_3$ , with cover reduced to zero within six years of treatment commencing with all doses, except at the lowest dose (Figure 2). The response to the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments was slower, with considerably more scatter in the data, but there was a very substantial loss of *Sphagnum capillifolium* cover in the higher doses ( $3.2$  and  $6.4 \text{ g N m}^{-2} \text{ y}^{-1}$ ). There was no clear change in cover at the lower doses of  $0.8$  and  $1.6 \text{ g N m}^{-2} \text{ y}^{-1}$  for either  $\text{NH}_4^+$  or  $\text{NO}_3^-$ . Because the effect of  $\text{NH}_3$  was so abrupt, the linear model does not detect the effect of nitrogen in this decline 6; since there was no *Sphagnum capillifolium* after year 6 except in the control plots, there was no further response to nitrogen to detect. If the data are truncated to the years where *Sphagnum capillifolium* was still present in plots, the interaction between  $\text{NH}_3$  and time becomes larger ( $-1 \%$   $(\text{g N m}^{-2} \text{ y}^{-1})^{-1}$ ) and close to statistically significant ( $p = 0.06$ ). With  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , although the data are more scattered, the response was more gradual, and these are picked up as clearer linear effects. Table 2 shows highly significant interactions between time and both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  ( $p < 0.01$ ). Again PK appears to exaggerate the effect, significantly so in the case of  $\text{NH}_4^+$ .

The dominant sedge, *Eriophorum vaginatum*, significantly increased in cover in response to  $\text{NH}_3$  ( $p \ll 0.01$ , Table 3), with cover increasing by up to  $40 \%$  (Figure 3). Cover decreased with nitrogen dose in the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments, though this is only significant in the case of  $\text{NO}_3^-$  ( $p = 0.02$ , Table 3). The effect of PK was to change the decrease with  $\text{NH}_4^+$  and  $\text{NO}_3^-$  into an increase, and this is manifested in the interaction term for time,  $\text{NO}_3^-$  and PK ( $p < 0.01$ , Table 3).

*Cladonia portentosa* (Dufour) Coem. is the most common lichen species at the site, and lichens might be expected to be some of the most sensitive to nitrogen deposition. In two of the treatments ( $\text{NH}_3$ , and  $\text{NO}_3^-$  with PK), *Cladonia* died out almost completely after four years (Figure 4). In the other treatments, the effect was similar but less extreme. However, despite the visually clear results, this effect is not detected by the linear model analysis, for the same reasons as discussed above: the effect of time was not linear, as *Cladonia portentosa* was often already at 0 % within four years, with no subsequent change over the remaining nine years. Also, *Cladonia portentosa* was generally rarer, and there happened to be none in most control plots. The option of truncating the data to plots and years when it was present left too few observations to give a satisfactory model fit. Although it detects effects of time, and the interaction between time and  $\text{NH}_3$  and  $\text{NH}_4^+$ , the univariate linear modelling approach could therefore be misleading, in detecting no other statistically significant effects (Table 4).

The change in cover of *Pleurozium schreberi*, a moss which is common at the site but not usually dominant, is shown in Figure 5. Here, the decline with nitrogen over time was similar across the treatment forms without PK, and is statistically significant in the  $\text{NO}_3^-$  treatment ( $p \ll 0.01$ , Table 5). The more striking result is the response to PK with intermediate nitrogen addition, where the cover is increased (in absolute terms, by around 50 % of the quadrat area on average). *Pleurozium schreberi* declined in the high  $\text{NH}_4^+$  and  $\text{NO}_3^-$  plus PK treatments, though with the nonlinearities noted for *Cladonia portentosa* above. The combination of PK addition without nitrogen addition was not available, so interpolating or extrapolating this relationship is not easy.

Figure 6 shows the change in cover of *Hypnum jutlandicum*, the only other commonly-occurring moss species in the experimental plots. The clearest response this shows is the decrease in cover over time with  $\text{NH}_3$  addition, which the linear modelling shows as statistically significant ( $p \ll 0.01$ , Table 6). Responses across the other treatments

are variable and equivocal.

To summarise the univariate results, consistent trends with nitrogen were discernible across species. All species showed a decline with  $\text{NH}_3$  treatment, except for *Eriophorum vaginatum* which increased. In the absence of PK, all species declined with  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , except for *Calluna vulgaris* and *Hypnum jutlandicum*. The effect of PK was not consistent across species, being unclear in most cases (*Calluna vulgaris*, *Sphagnum capillifolium* and *Cladonia portentosa*) but reversing the decline with nitrogen to become an increase (*Eriophorum vaginatum*), and enhancing growth at low nitrogen whilst having no effect at high nitrogen (*Pleurozium schreberi*).

The multivariate analyses show significant community-level responses. The principal response curves for the treatments show the time-dependent, community-level effects of nitrogen form and dose, with and without addition of PK (Figures 7 and 8). The  $y$  axis shows the treatment-time coefficients  $c_{dt}$  from the first PRC component, representing the multivariate measure of plant species composition which best captures the time-dependent response of the vegetation to treatments.  $c_{dt}$  values are relative to the control, so by definition the control values form a horizontal line on the plot at  $c_{dt} = 0$ . Figure 7 shows a coherent response to the  $\text{NH}_3$  treatment, with a clear decline over time, followed by an apparent recovery phase after 2011 (even though the treatments continued). The responses closely follow the nitrogen dose, except that the second highest dose produces the greatest response. The right-hand panel depicts the loadings,  $b_s$ , for each species. These relate to the correlation between cover of each species and the overall response pattern specified by the principal response curves.  $b_s$  values near zero indicate that the cover of species  $s$  does not differ between treatments or is uncorrelated with the overall response pattern. This shows that the response to  $\text{NH}_3$  over time was characterised by a reduction in *Sphagnum capillifolium*, *Calluna vulgaris*, and *Pleurozium schreberi*, together with an increase in *Eriophorum vaginatum* (in live, dead and litter forms). In the  $\text{NH}_3$  treatment data set, 22 % of the total variance in species cover could be attributed to the treatment groups (including the

interaction with time), and 16 % to effects of time *per se*. Monte Carlo permutation tests indicated that the PRC components were highly significant ( $p < 0.01$ ).

Figure 8 shows similar responses to the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments, with a general decline over time, followed by a possible recovery phase after 2011 in three or more cases. The significant exception is when PK is added at intermediate levels of nitrogen, where community change takes a quite different trajectory. This is dominated by an increase in *Pleurozium schreberi*. Without PK, the response again follows the nitrogen dose, with the smallest response produced by adding the lowest dose of  $1.6 \text{ g N m}^{-2} \text{ y}^{-1}$ , and the largest response with the highest dose ( $6.4 \text{ g N m}^{-2} \text{ y}^{-1}$ ). The species loadings (right-hand panel) share some similarities with the  $\text{NH}_3$  treatment, showing a loss of *Sphagnum capillifolium* and gains of *Eriophorum vaginatum* (live dead and litter), dead *Calluna vulgaris* with nitrogen addition but are somewhat skewed by the PK response of *Pleurozium schreberi*. 18 % of the total variance in species cover could be attributed to the treatment groups, and 13 % to effects of time. The PRC components were again highly significant.

Figure 9 shows the PLS ordination of the samples in relation to the loadings for the plant species (right-hand) and the chemical ions. The first axis is very similar to that identified by PRC on the  $\text{NH}_3$  treatment, and differentiates between the quadrats with high  $\text{NH}_3$  deposition (with increased *Eriophorum vaginatum* (live, dead, and litter) and dead *Sphagnum capillifolium*), and those with no  $\text{NH}_3$  deposition (with increased *Calluna vulgaris*, *Sphagnum capillifolium* and *Pleurozium schreberi*). Negative values on this axis (high  $\text{NH}_3$  deposition, high *Eriophorum vaginatum*) are associated with high  $\text{NO}_3^-$  and  $\text{NH}_4^+$  ion concentrations in the porewater. The second axis appears to differentiate within the  $\text{NH}_4^+$  treatment, showing quadrats with high doses of  $\text{NH}_4\text{Cl}$  and high Cl concentrations on the left. However, the interpretation of this in terms of species composition is not very clear. and the magnitude and variability in  $\text{NO}_3^-$  and  $\text{NH}_4^+$  ion concentrations was much less in the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments. So, PLS can distinguish an axis of vegetation variation which corresponds to high  $\text{NO}_3^-$  and  $\text{NH}_4^+$



ion concentrations in the porewater in the  $\text{NH}_3$  treatment, but species composition change in the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments was not clearly related to porewater chemistry.

## 4 Discussion

The univariate analyses of the six common species showed a clear effect of  $\text{NH}_3$  on all of the species. Importantly, we demonstrated an effect on the main peat-forming species *Sphagnum capillifolium*. The response to  $\text{NH}_3$  was in some cases very drastic, so that extinctions occurred within a plot over the 14 years. We detected a clear effect of either  $\text{NH}_4^+$  or  $\text{NO}_3^-$  on all of the species considered, except *Hypnum jutlandicum* where responses were very variable. We observed a dramatic effect on the lichen species *Cladonia portentosa*, expected to be most sensitive to nitrogen, and this was clearly detectable using a linear model, except in the case of  $\text{NO}_3^-$ . Our results showed that some species gained from nitrogen addition, notably *Hypnum jutlandicum* and *Eriophorum vaginatum*, although this seemed to vary with nitrogen form. The experiment was originally designed, to detect community-level responses, not for univariate responses; quadrats were placed quasi-randomly at the outset of the experiment, not on patches of the species of interest. The initial cover of the species of interest was therefore quite variable across treatments, and was missing altogether in some quadrats. For example, *Sphagnum capillifolium* was already largely absent from the highest  $\text{NH}_3$  treatment plots in 2002. This makes it more difficult to interpret some univariate responses, as all quadrats did not start from the same initial conditions.

The multivariate analyses also demonstrated significant responses to the treatments. PRC produced a component which has a simple interpretation, and confirmed the trends visible in the univariate analyses and informal observation in the field. This was particularly true in the case of the  $\text{NH}_3$  treatment; in the case of the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments, the interpretation was less straightforward, because of the strong

leverage of *Pleurozium schreberi* at one end of the axis, which is an effect of PK rather than nitrogen. In the case of PLS, we established an axis which described the multivariate variation in species composition along the  $\text{NH}_3$  gradient, and related this to the porewater chemistry (mainly the change in  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ). In the  $\text{NH}_4^+$  treatment, although species composition appeared unrelated to  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , PLS did identify a vegetation axis which corresponded with  $\text{Na}^+$  and  $\text{Cl}^-$  in the soil solution as passive tracers for nitrogen deposition in the form of  $\text{NaNO}_3$  and  $\text{NH}_4\text{Cl}$ , though the interpretation of this vegetation axis was less clear. Using univariate analysis, no correspondence between species cover and porewater chemistry could be established. Standard diversity indices were less useful in identifying change (see Supporting Information). There was some evidence for a decrease in Shannon diversity in all treatments, but also in the controls. Species evenness did not change with nitrogen dose, but did have a significant interaction with PK in the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments.

We compare our results with those from the closest comparable experiments in the literature. At Degerö Stormyr, around 50 % of the *Sphagnum capillifolium* cover was lost with a nitrogen deposition rate of  $3 \text{ g N m}^{-2} \text{ y}^{-1}$  after nine years (Wiedermann *et al.*, 2007). At Mer Bleue, 100 % of the *Sphagnum capillifolium* cover was lost with a nitrogen deposition rate of  $6.4 \text{ g N m}^{-2} \text{ y}^{-1}$  (Juutinen *et al.*, 2010). The rate and magnitude of the effects we observed was somewhat slower and smaller, typically 30 % reduction in cover at the highest dose of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  after 14 years (Figure 3). The most likely reason for this is probably the way in which nitrogen was applied in the Whim experiment, in a large number of small doses. Considering the processes in which nitrogen concentration exerts a physiological effect in mosses, these results are entirely plausible (Bridgham, 2002, Fritz *et al.*, 2014). The modelling analysis of Wu & Blodau (2015) discusses this from a more theoretical perspective. They used a process-based model, which included internal dynamics of nitrogen transport from the Hurley pasture model (Thornley, 1998), to simulate the Mer Bleue experiment, applying the same nitrogen doses either in three-weekly or daily applications. The

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results showed that the effects on moss biomass were more severe when applied three-weekly, to the extent that moss died out completely in the highest simulated treatment when applied three-weekly, but maintained a stable biomass of  $120 \text{ g m}^{-2}$  when applied daily. Our results therefore support the idea that supplying nitrogen in a realistic way produces a lesser or slower response than conventional nitrogen addition experiments. Most other experiments may contain a bias towards over-sensitivity because of the artefactual way in which nitrogen is normally applied. However, the experiments do differ in other ways, which complicates the comparison. For example, at Met Bleue at least some of the detrimental effect on Sphagnum was attributable to an increase in shading by the shrub layer, which responded positively to nitrogen addition (Chong *et al.*, 2012). At Whim, the shrub layer was less dense and did not respond positively, whilst at Degerö Stormyr there was no substantial shrub layer.

Similar competitive shifts have been observed in other experiments (e.g. with *Polytrichum strictum* out-competing *Sphagnum capillifolium* at Mer Bleue (Juutinen *et al.*, 2015)). The ramifications of such changes on biodiversity, peat physical structure and accumulation rates (and hence carbon balance) are hard to predict, but potentially far-reaching. Robroek *et al.* (2017) suggest that peatland communities are actually more robust than presupposed. Their analysis suggests that peatland ecosystem function was maintained at different sites across large environmental gradients across Europe, because different species performed similar functional roles. The effect of this may be to increase resilience, because function is not dependent on particular taxa being present. Whether this resilience inferred from their analysis of spatial pattern can be assumed to apply in the time dimension is open to question.

Collating 29 studies (including the Whim site), Limpens *et al.* (2011) found that increased addition of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  tended to decrease *Sphagnum* production, though the effect was not consistent. They performed a meta-analysis of the factors which appear to affect this response, and detected effects of factors including summer temperature, annual rainfall, background nitrogen deposition rate, and foliar nitrogen

concentration. Applying their fitted model to the highest dose of the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments at Whim, based on the foliar nitrogen concentration of around 17 mg N/g dry weight, their simple model predicts a reduction in *Sphagnum* production of 9 % compared to the control (using the coefficients in Table S2 of Limpens *et al.*, 2011)). At the lowest dose, with foliar nitrogen concentration of around 12 mg N/g dry weight, their model predicts an increase in *Sphagnum* production of 6 %. Whilst we cannot exactly equate change in production with change in cover, this approximately tallies with the trends found at Whim, although *Sphagnum* only increased cover in one low nitrogen treatment ( $\text{NH}_4^+$ )

The  $\text{NH}_4^+$  and  $\text{NO}_3^-$  response at Whim sits intermediate within the range of responses found at the other sites collated by Limpens *et al.* (2011). According to the meta-analysis, this outcome is a result of counter-acting effects of high rainfall, low summer temperature, and intermediate foliar nitrogen concentrations. The PK effect is opposite to the wider trend, and PK does not ameliorate the reduction in *Sphagnum* cover, but rather is replaced by *Pleurozium schreberi*.

The mechanism of the responses to nitrogen, and the reasons why the sensitivity to nitrogen may depend on summer temperature and annual precipitation are unclear (Limpens *et al.*, 2011). Although good evidence on physiological stress in *Sphagnum* is scarce, the foliar nitrogen concentrations found are generally below the levels that would be toxic or cause direct damage to the photosynthetic system (Granath *et al.*, 2009). Although not directly toxic, the excess nitrogen may cause an imbalance in plant nutrient status that results in physiological stress and poor growth (Bragazza *et al.*, 2004, Carfrae *et al.*, 2007). This may manifest itself as increased sensitivity to drought, frost, winter desiccation, and pathogen outbreaks (Wiedermann *et al.*, 2007, Sheppard *et al.*, 2008). Alternatively, there is evidence that the effects of nitrogen are mediated through effects on the competitive balance between species. For example in the Mer Bleue experiment, at least some of the detrimental effect on *Sphagnum* is via increased shading by the shrub layer (Chong *et al.*, 2012). A similar effect is not

present at Whim, where the shrub layer has not increased in cover. The potential for such an effect could be investigated in future studies with experimental removal of vascular plants.

In the vascular plants, *Calluna vulgaris* was severely impacted by the NH<sub>3</sub> fumigation and sequentially disappeared over time with doses of 1.6 g N m<sup>-2</sup> y<sup>-1</sup> and above. *Eriophorum vaginatum* was the only species studied that appeared to benefit from the NH<sub>3</sub> fumigation: at the highest dose it approximately doubled in cover. With low and intermediate doses of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>, *Calluna vulgaris* tended to increase, whereas *Eriophorum vaginatum* decreased. At high doses of NH<sub>4</sub><sup>+</sup> NO<sub>3</sub><sup>-</sup>, *Calluna vulgaris* tended to decrease, whereas *Eriophorum vaginatum* increased in the presence of PK.

Several studies (e.g. Carroll *et al.*, 1999, Kool & Heijmans, 2009, van Voorn *et al.*, 2016) have demonstrated a positive response of *Calluna vulgaris* to intermediate levels of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> in the field, at least in the short-term. In a field experiment in Wales with large additions of NH<sub>4</sub><sup>+</sup>NO<sub>3</sub><sup>-</sup> up to 12 g N m<sup>-2</sup> y<sup>-1</sup>, *Calluna vulgaris* showed a rapid increase in shoot extension and canopy height over the first four years, followed by no change in growth (Carroll *et al.*, 1999). After eight years, the higher levels of NH<sub>4</sub><sup>+</sup>NO<sub>3</sub><sup>-</sup> accelerated senescence, with dose-related increases in susceptibility to winter injury and frost damage. This is in contrast to results at Whim which showed no effect of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> on *Calluna* health (Sheppard *et al.*, 2008), though the maximum dose was half that used by Carroll *et al.* (1999). Studies are less clear for *Eriophorum vaginatum*, with some showing no effect and others showing a large positive response to nitrogen addition (reviewed in Kool & Heijmans, 2009). Kool & Heijmans (2009) suggest that P may limit the response to nitrogen in *Eriophorum vaginatum*, with studies in tundra ecosystems showing a stronger positive response than studies in bogs (where P is generally more limiting).

There is also evidence in the literature on the effect of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> on competition between *Calluna vulgaris* and *Eriophorum vaginatum*. Kool & Heijmans (2009)

found that *Calluna vulgaris* rapidly increased shoot growth and leaf area when supplied with  $5 \text{ g N m}^{-2} \text{ y}^{-1}$ , thereby outcompeting *Eriophorum vaginatum* for nutrients. They attributed the more rapid response of *Calluna vulgaris* to a higher phenotypic plasticity of ericoids over graminoids, allowing them to adapt faster to higher nutrient availability. van Voorn *et al.* (2016) also found that *Calluna vulgaris* can persist under intermediate nutrient enrichment in competition with graminoids, as long as its canopy is not damaged. However, opening of the canopy can allow a regime shift from ericoid to graminoid domination under nitrogen enrichment. To our knowledge there are no similar competition experiments between ericoids and graminoids under conditions of enhanced  $\text{NH}_3$  deposition.

Taken together these studies suggest that, in the Whim experiment, *Calluna vulgaris* was able to withstand (and respond positively to) low levels of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  deposition by allocating more resources to shoots and leaves, but that *Calluna vulgaris* was not able to sustain this positive response over time at the high treatment levels. Over a longer term, we would expect accelerated senescence in the high  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatment plots, with potential out-competition by *Eriophorum vaginatum*, especially if P and other nutrient limitations are released, as demonstrated in the PK plots.

Whereas the predominant uptake pathway of wet-deposited nitrogen to vascular plants is via the soil and can be metabolically controlled, the predominant dry-nitrogen deposition pathway cannot be similarly controlled: all gas exchange is a function of the opening and closing of the stomata, which itself is primarily controlled by light, water availability, and temperature (Hurkuck *et al.*, 2015). In this case we might expect that the high stature and leaf surface area of *Calluna vulgaris* would lead to a high interception rate of  $\text{NH}_3$ , ultimately to toxic levels. The rapid decline of *Calluna vulgaris* would have opened the canopy, giving *Eriophorum vaginatum* the competitive edge (van Voorn *et al.*, 2016). Although we are not aware of experiments defining toxicity levels of  $\text{NH}_3$  for *Calluna vulgaris* or *Eriophorum vaginatum*, clearly

the persistence of *Eriophorum vaginatum* even at the highest  $\text{NH}_3$  levels for 14 years suggests a high tolerance.

A major feature of the results to be explained is the greater effect of  $\text{NH}_3$  on vegetation species cover, compared to  $\text{NH}_4^+$  and  $\text{NO}_3^-$  at the equivalent deposition rate. For higher plants, Sheppard *et al.* (2011) previously attributed the greater effect of  $\text{NH}_3$  to direct uptake via the stomata. This explanation cannot apply exactly to *Sphagnum* as they do not have true stomata, and gas exchange mainly takes place across the wet leaf surface. However, the basic proposition probably remains true:  $\text{NH}_3$  deposition results in higher nitrogen concentrations at the vegetation surface and in the leaf apoplast because it deposits directly on the thin film of water on the leaf surface, without any dilution in rain water or porewater. With  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , the ions are dissolved in rain water before being sprayed on, and much of this sprayed water runs off the leaf surface (as it will usually exceed the already-saturated moss canopy interception capacity) to mix with the porewater. Partly this difference comes down to the definition of “deposition rate”. In the case of  $\text{NH}_3$  deposition, we can equate the deposition rate that we estimate to the actual addition of nitrogen to the leaf apoplast, where it has its biological effects. In the case of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , the deposition rate that we estimate adds mainly to nitrogen in the porewater, and the fraction of this which actually ends up elevating concentrations in the leaf apoplast is much smaller. So, in the former case we have the deposition rate to the leaf apoplast, in the latter we have the deposition rate to the whole ecosystem.

To estimate the effects of the three forms in the real world, we need to factor in the magnitude of their relative deposition rates, as well their relative effects per g N deposited. We did this for *Sphagnum capillifolium*, as the major peat-forming species, fundamental to the development of the peat bog system. Estimates of wet and dry deposition of  $\text{NH}_3$ ,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were taken from the Concentration-Based Estimation of Deposition (CBED) model (Smith *et al.*, 2000, Smith & Fowler, 2001), based on observed atmospheric concentrations and in rainfall. The spatial distribution

of peat bogs was taken from the CEH Land Cover Map 2015 (Morton et al. 2017) at 1-km resolution. At each location where peat bogs occur, the linear model coefficients for *Sphagnum capillifolium*, quantifying the interactions between time and  $\text{NH}_3$ ,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (Table 2), were multiplied by the respective deposition of each, to give their separate effects on cover change (Figure 10). The effects are expected to be additive, so the total response to nitrogen deposition is the sum of the three effects, but this suggests that  $\text{NH}_4^+$  deposition has the largest impact on *Sphagnum capillifolium*. This is because  $\text{NH}_4^+$  and  $\text{NO}_3^-$  have similar deposition rates on UK peat bogs, both averaging around  $0.5 \text{ g N m}^{-2} \text{ y}^{-1}$  and both with maxima close to  $1.5 \text{ g N m}^{-2} \text{ y}^{-1}$ , but  $\text{NH}_4^+$  has a greater effect on *Sphagnum capillifolium* cover ( $-0.5$  versus  $-0.3 \%$  ( $\text{g N m}^{-2} \text{ y}^{-1}$ ) $^{-1}$ ). Based on the coefficients in Table 2,  $\text{NH}_3$  has the least impact of the three; the deposition rate is lowest, averaging  $0.16 \text{ g N m}^{-2} \text{ y}^{-1}$  with maximum of  $0.6 \text{ g N m}^{-2} \text{ y}^{-1}$ , and its effect on *Sphagnum capillifolium* cover is similar to  $\text{NO}_3^-$  ( $-0.2 \%$  ( $\text{g N m}^{-2} \text{ y}^{-1}$ ) $^{-1}$ ). When the model fit with zero values removed is used instead, this coefficient is higher ( $-1 \%$  ( $\text{g N m}^{-2} \text{ y}^{-1}$ ) $^{-1}$ ), and has a bigger overall effect than  $\text{NO}_3^-$  (at least over the 14-year time scale of the experiment). Given this level of uncertainty in our estimates, we cannot readily distinguish between the effect sizes of  $\text{NH}_3$  and  $\text{NO}_3^-$  deposition. We acknowledge that this is based on a single-site experiment, albeit a long-term study which mimics realistic conditions, and some caution is needed in this extrapolation of results. However, it is effective in placing the importance of the three nitrogen forms in the context of their likely impacts at national scale. The results come from a 14-year study, but the impacts are potentially different over multiple decades of exposure in the real world. Conceivably there are short-term direct impacts of  $\text{NH}_3$  and long-term indirect impacts of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (if these gradually accumulate in the soil, and the effects are manifested later through different pathways).

The results highlight the importance of nitrogen form and concentration in determining the impact on vegetation in bog ecosystems: with the same nitrogen dose,



the detrimental effects may be greater or lesser, depending on the nitrogen form and concentrations that the plants are exposed to. Weather phenomena (such as dew, fog, and low cloud) and episodic dry deposition may produce events where high concentrations of nitrogen occur on plant tissues and cause damage at low nitrogen loads. Such events are stochastic in nature, and will tend to make the relationship between nitrogen dose and damage rather variable. The background nitrogen deposition levels might also be a factor in determining the response to nitrogen, as previous exposure may have a de-sensitising effect, or result in adaptive change (Nordin *et al.*, 2005). This is unlikely to be a factor in our experiment, where the historical background has been low.

## 5 Conclusions

- After 14 years of treatment, we detected significant reductions in cover in response to nitrogen in the plant species examined. The responses differed among species and nitrogen forms, but all species declined, and  $\text{NH}_3$  produced the biggest change in cover per unit of nitrogen addition. The exception was the graminoid sedge *Eriophorum vaginatum*, which increased dramatically in the  $\text{NH}_3$  treatment.
- Multivariate analyses found significant responses to nitrogen, by combining information across all species. Principal response curves identified coherent, community-level effects of nitrogen deposition, which increased with nitrogen dose. The effects were similar across all nitrogen forms. The significant exception was when PK was added at intermediate levels of nitrogen, where community change took a quite different trajectory.
- Partial least-squares regression identified high levels of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in the porewater as the chemical drivers of this change in the  $\text{NH}_3$  treatment. A clear change in porewater chemistry was not found in the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments.

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- Phosphorus(and/or potassium) could radically alter the response to nitrogen addition, particularly at low-intermediate levels of nitrogen input, but effects were species-specific.
  - We surmised that the larger experimental response to nitrogen observed in the  $\text{NH}_3$  treatment (compared with the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments) was because of the higher nitrogen concentrations at the vegetation surface produced by dry deposition.  $\text{NH}_3$  is deposited as a gas, directly to the water on the leaf surface and through the stomata to the leaf apoplast. Because  $\text{NH}_3$  deposits directly to the leaf, it stays contained within the small volume of water on and in the leaf, producing a high internal concentration of nitrogen ions. Much of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  solution sprayed on will run off to the porewater, and be further diluted and subject to biological regulation of nitrogen uptake. So, the nitrogen ion concentrations produced at the leaf apoplast are inevitably lower, when comparing equivalent nitrogen deposition rates.
  - The same reasoning may explain why the observed experimental responses to nitrogen were smaller than in the closest comparable experiments; at Whim, the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were applied in many small doses, rather than in a few large monthly doses as in most other experiments. In this way, we avoid artificially high nitrogen concentrations at the vegetation surface, and maintain conditions closer to real-world nitrogen deposition.
  - Considering the actual deposition rates of  $\text{NH}_3$ ,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  on UK peat bogs, and the relative magnitude of their effects based on our experimental results, we estimate that  $\text{NH}_4^+$  deposition has the largest impact on the peat-forming species *Sphagnum capillifolium*.  $\text{NO}_3^-$  has a greater impact than  $\text{NH}_3$ , but this difference is within the confidence limits of our estimates.

## 6 Authors' contributions

LJS and MS conceived the ideas and designed the experiment; LJS, IDL, NvD and ND collected the data; LJS, IDL, SL and MJ maintained the experimental treatments; PL and AG analysed the data; PL wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

## 7 Data Accessibility

Data are available from the NERC Environmental Information Data Centre: <https://doi.org/10.5285/65e518-4cf5-85bf-7d93e66fdb96> (van Dijk *et al.*, 2018).

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## 8 Tables

	DF	F.value	p.value	Coefficient
(Intercept)	1144	94.38	0.000	30.2572
$t_y$	1144	0.79	0.373	0.0699
Fnh3	42	13.52	0.001	-1.5421
Fnh4	42	5.96	0.019	1.3042
PK	42	0.88	0.353	-6.6738
Fno3	42	2.47	0.124	-0.0931
$t_y : Fnh3$	1144	6.03	0.014	-0.1304
$t_y : Fnh4$	1144	1.20	0.274	-0.0568
$t_y : PK$	1144	0.53	0.468	1.1655
Fnh4:PK	42	0.17	0.684	1.1707
$t_y : Fno3$	1144	4.03	0.045	-0.0200
PK:Fno3	42	1.81	0.186	0.7825
$t_y : Fnh4 : PK$	1144	0.45	0.502	-0.2013
$t_y : PK : Fno3$	1144	5.58	0.018	-0.3241

Table 1: *Calluna vulgaris* (green) - Results of fitting a linear mixed-effects model to the cover data by maximizing the restricted log-likelihood. Columns show the denominator degrees of freedom, F-values, p-values from Wald tests for each term, and the  $\beta$  coefficients. The interaction terms ( $t_y:F_{\text{NH}_3}$  etc.) give the change in cover per year per g N deposited  $\text{m}^{-2} \text{y}^{-1}$ . Because we include interactions in the model,  $F_{\text{NH}_3}$ ,  $F_{\text{NH}_4}$ ,  $F_{\text{NO}_3}$  and PK are effectively intercept terms, the effects of nitrogen deposition when time,  $t_y = 0$ .

	DF	F.value	p.value	Coefficient
(Intercept)	755	21.95	0.000	38.6593
$t_y$	755	47.33	0.000	0.1208
Fnh3	37	1.49	0.229	-1.6849
Fnh4	37	3.37	0.075	-1.0124
PK	37	0.07	0.795	15.9730
Fno3	37	3.68	0.063	0.7142
$t_y : Fnh3$	755	0.40	0.527	-0.2010
$t_y : Fnh4$	755	9.96	0.002	-0.5312
$t_y : PK$	755	8.32	0.004	-1.7140
Fnh4:PK	37	0.97	0.332	-1.7737
$t_y : Fno3$	755	9.63	0.002	-0.3040
PK:Fno3	37	1.35	0.253	-4.0743
$t_y : Fnh4 : PK$	755	4.10	0.043	0.4448
$t_y : PK : Fno3$	755	0.14	0.706	0.0790

Table 2: *Sphagnum capillifolium* - Results of fitting a linear mixed-effects model to the cover data by maximizing the restricted log-likelihood. Columns show the denominator degrees of freedom, F-values, p-values from Wald tests for each term, and the  $\beta$  coefficients. The interaction terms ( $t_y:F_{\text{NH}_3}$  etc.) give the change in cover per year per g N deposited  $\text{m}^{-2} \text{y}^{-1}$ . Because we include interactions in the model,  $F_{\text{NH}_3}$ ,  $F_{\text{NH}_4}$ ,  $F_{\text{NO}_3}$  and PK are effectively intercept terms, the effects of nitrogen deposition when time,  $t_y = 0$ .

	DF	F.value	p.value	Coefficient
(Intercept)	1121	232.69	0.000	12.4412
$t_y$	1121	21.03	0.000	-0.5162
Fnh3	41	28.39	0.000	0.0636
Fnh4	41	0.26	0.610	0.0024
PK	41	0.28	0.599	-1.0652
Fno3	41	4.44	0.041	0.0735
$t_y : Fnh3$	1121	84.45	0.000	0.2681
$t_y : Fnh4$	1121	1.14	0.287	-0.0121
$t_y : PK$	1121	7.51	0.006	-0.7248
Fnh4:PK	41	0.32	0.575	-0.7056
$t_y : Fno3$	1121	5.51	0.019	-0.0534
PK:Fno3	41	11.91	0.001	0.1040
$t_y : Fnh4 : PK$	1121	1.13	0.288	0.2207
$t_y : PK : Fno3$	1121	29.79	0.000	0.3868

Table 3: Eriophorum vaginatum - Results of fitting a linear mixed-effects model to the cover data by maximizing the restricted log-likelihood. Columns show the denominator degrees of freedom, F-values, p-values from Wald tests for each term, and the  $\beta$  coefficients. The interaction terms ( $t_y:F_{\text{NH}_3}$  etc.) give the change in cover per year per g N deposited  $\text{m}^{-2} \text{y}^{-1}$ . Because we include interactions in the model,  $F_{\text{NH}_3}$ ,  $F_{\text{NH}_4}$ ,  $F_{\text{NO}_3}$  and PK are effectively intercept terms, the effects of nitrogen deposition when time,  $t_y = 0$ .

	DF	F.value	p.value	Coefficient
(Intercept)	363	9.99	0.002	24.4639
$t_y$	363	119.00	0.000	-1.5096
Fnh3	16	0.92	0.351	-1.7369
Fnh4	16	0.17	0.682	0.6652
PK	16	1.32	0.267	-5.7545
Fno3	16	0.69	0.417	-0.2751
$t_y : Fnh3$	363	8.07	0.005	0.1122
$t_y : Fnh4$	363	7.45	0.007	-0.1705
$t_y : PK$	363	0.37	0.541	-0.1442
Fnh4:PK	16	0.38	0.546	2.2568
$t_y : Fno3$	363	0.10	0.754	-0.0886
PK:Fno3	16	0.03	0.870	-2.3700
$t_y : Fnh4 : PK$	363	2.55	0.111	-0.1200
$t_y : PK : Fno3$	363	2.13	0.145	0.2840

Table 4: Cladonia portentosa - Results of fitting a linear mixed-effects model to the cover data by maximizing the restricted log-likelihood. Columns show the denominator degrees of freedom, F-values, p-values from Wald tests for each term, and the  $\beta$  coefficients. The interaction terms ( $t_y:F_{\text{NH}_3}$  etc.) give the change in cover per year per g N deposited  $\text{m}^{-2} \text{y}^{-1}$ . Because we include interactions in the model,  $F_{\text{NH}_3}$ ,  $F_{\text{NH}_4}$ ,  $F_{\text{NO}_3}$  and PK are effectively intercept terms, the effects of nitrogen deposition when time,  $t_y = 0$ .

	DF	F.value	p.value	Coefficient
(Intercept)	834	65.71	0.000	28.5815
$t_y$	834	8.50	0.004	-0.9475
Fnh3	37	4.45	0.042	-1.8030
Fnh4	37	8.17	0.007	-2.4762
PK	37	21.68	0.000	-11.8285
Fno3	37	15.67	0.000	-0.7366
$t_y : Fnh3$	834	3.32	0.069	-0.0371
$t_y : Fnh4$	834	3.25	0.072	-0.0942
$t_y : PK$	834	100.57	0.000	6.6840
Fnh4:PK	37	1.22	0.276	2.9225
$t_y : Fno3$	834	48.63	0.000	-0.2480
PK:Fno3	37	3.39	0.074	2.4872
$t_y : Fnh4 : PK$	834	16.44	0.000	-0.9807
$t_y : PK : Fno3$	834	28.08	0.000	-0.9185

Table 5: *Pleurozium schreberi* - Results of fitting a linear mixed-effects model to the cover data by maximizing the restricted log-likelihood. Columns show the denominator degrees of freedom, F-values, p-values from Wald tests for each term, and the  $\beta$  coefficients. The interaction terms ( $t_y:F_{\text{NH}_3}$  etc.) give the change in cover per year per g N deposited  $\text{m}^{-2} \text{y}^{-1}$ . Because we include interactions in the model,  $F_{\text{NH}_3}$ ,  $F_{\text{NH}_4}$ ,  $F_{\text{NO}_3}$  and PK are effectively intercept terms, the effects of nitrogen deposition when time,  $t_y = 0$ .

	DF	F.value	p.value	Coefficient
(Intercept)	782	192.02	0.000	27.3618
$t_y$	782	16.76	0.000	-0.0616
Fnh3	35	4.56	0.040	-0.1520
Fnh4	35	1.10	0.301	-1.2185
PK	35	0.04	0.843	-4.4116
Fno3	35	5.84	0.021	-0.4110
$t_y : Fnh3$	782	11.47	0.001	-0.2924
$t_y : Fnh4$	782	0.40	0.525	-0.0390
$t_y : PK$	782	18.25	0.000	-2.0456
Fnh4:PK	35	1.05	0.312	1.2468
$t_y : Fno3$	782	0.18	0.673	0.1093
PK:Fno3	35	5.74	0.022	5.2828
$t_y : Fnh4 : PK$	782	4.97	0.026	0.3886
$t_y : PK : Fno3$	782	0.17	0.683	-0.0780

Table 6: *Hypnum jutlandicum* - Results of fitting a linear mixed-effects model to the cover data by maximizing the restricted log-likelihood. Columns show the denominator degrees of freedom, F-values, p-values from Wald tests for each term, and the  $\beta$  coefficients. The interaction terms ( $t_y:F_{\text{NH}_3}$  etc.) give the change in cover per year per g N deposited  $\text{m}^{-2} \text{y}^{-1}$ . Because we include interactions in the model,  $F_{\text{NH}_3}$ ,  $F_{\text{NH}_4}$ ,  $F_{\text{NO}_3}$  and PK are effectively intercept terms, the effects of nitrogen deposition when time,  $t_y = 0$ .

Accepted Article

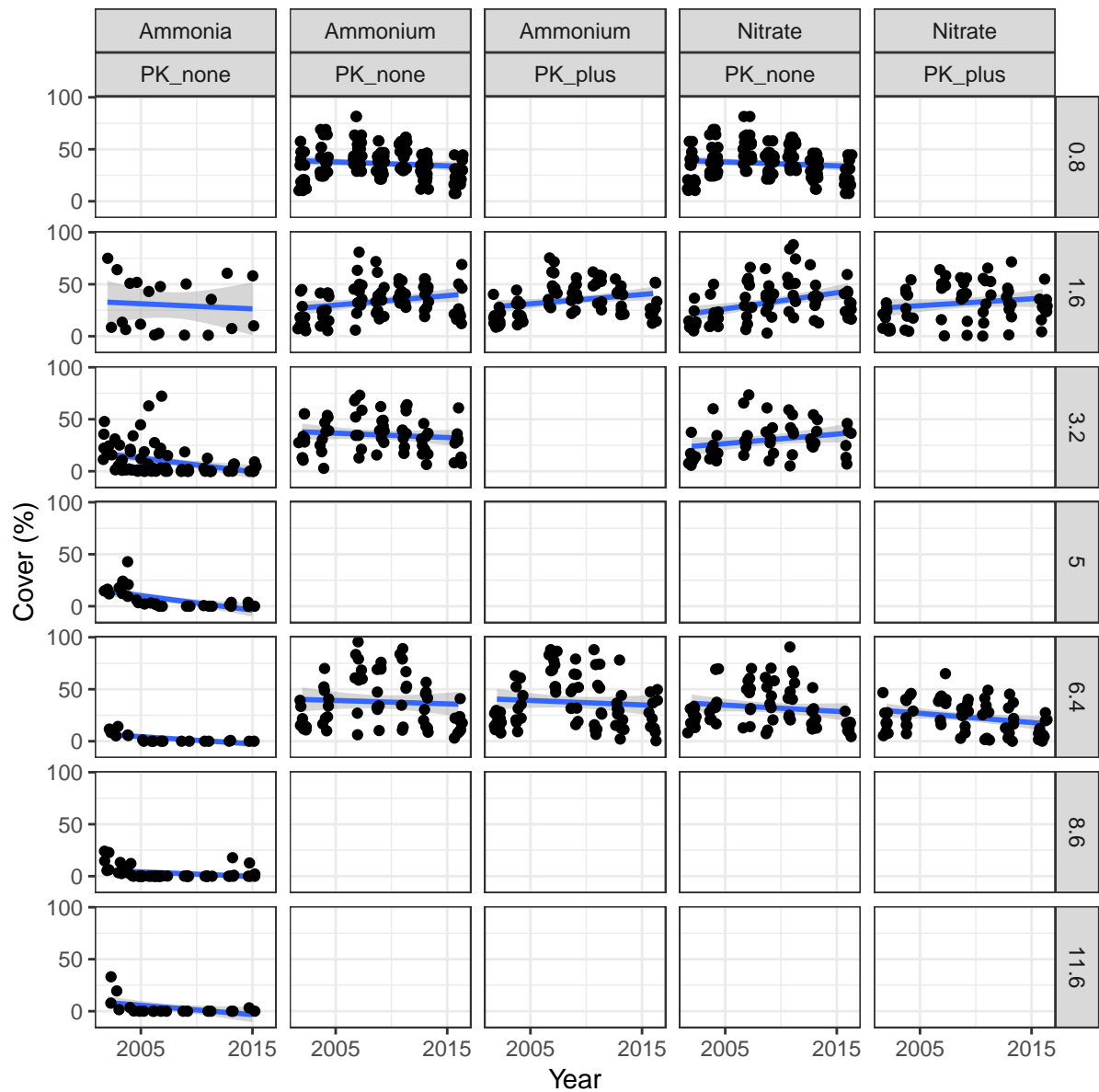


Figure 1: Cover of *Calluna vulgaris*, the most dominant shrub species, over the course of the experiment in relation to the dose of nitrogen added. Panels show the different forms of nitrogen treatment, with and without the addition of PK. Points show the annual mean for each permanent quadrat. Symbol colour denotes the dose of nitrogen in  $\text{g N m}^{-2} \text{y}^{-1}$  for each treatment level. Lines show a linear fit against time for each treatment level. Grey bands around these lines show the 95 % confidence interval for these fits. There was only a single set of control plots for both the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments, with only background nitrogen deposition, and these data are duplicated in the panels for both the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments without PK addition.

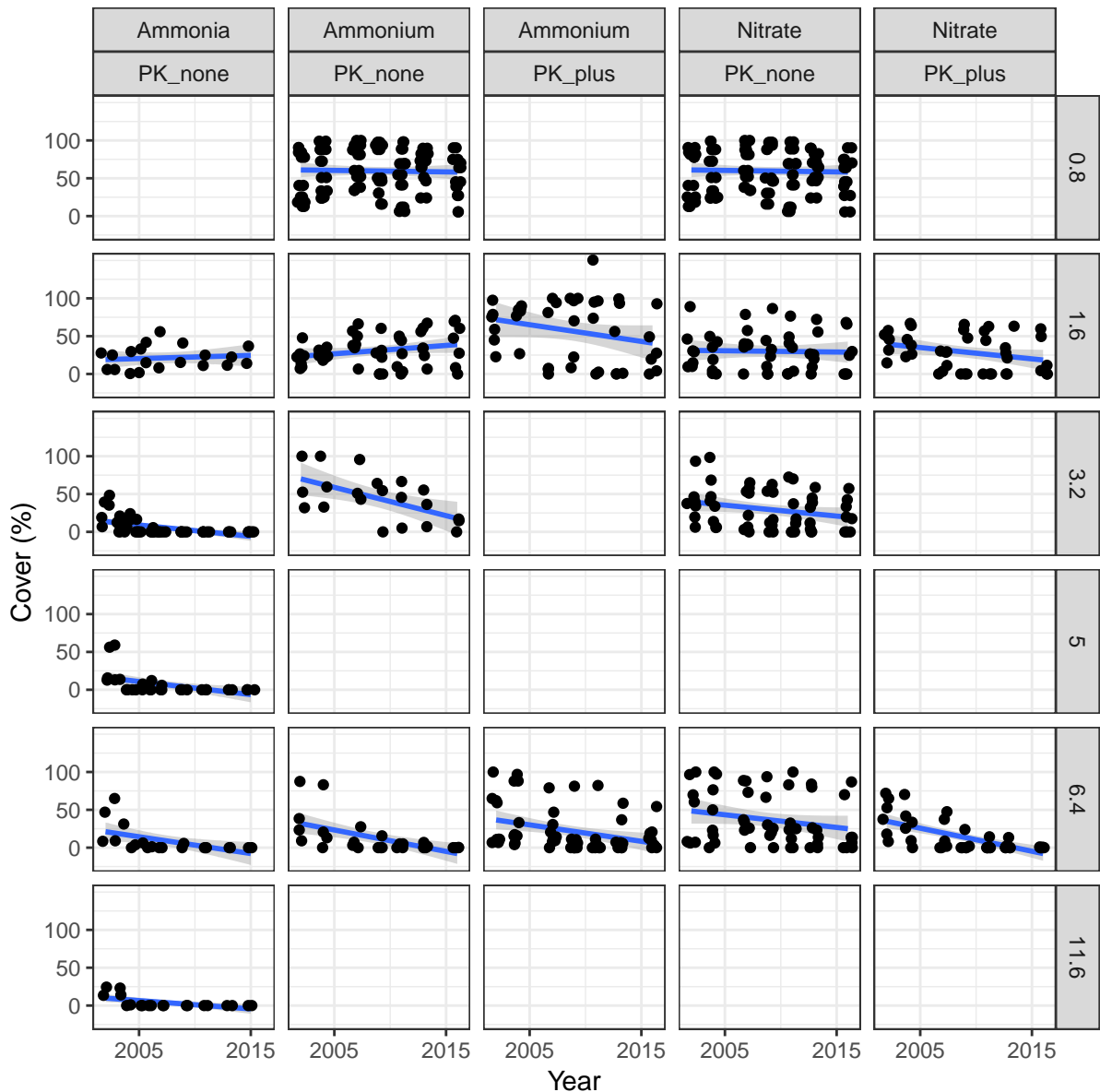


Figure 2: Cover of *Sphagnum capillifolium*, the most common moss species, over the course of the experiment in relation to the dose of nitrogen added. Panels show the different forms of nitrogen treatment, with and without the addition of PK. Points show the annual mean for each permanent quadrat. Symbol colour denotes the dose of nitrogen in  $\text{g N m}^{-2} \text{y}^{-1}$  for each treatment level. Lines show a linear fit against time for each treatment level. Grey bands around these lines show the 95 % confidence interval for these fits. There was only a single set of control plots for both the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments, with only background nitrogen deposition, and these data are duplicated in the panels for both the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments without PK addition.

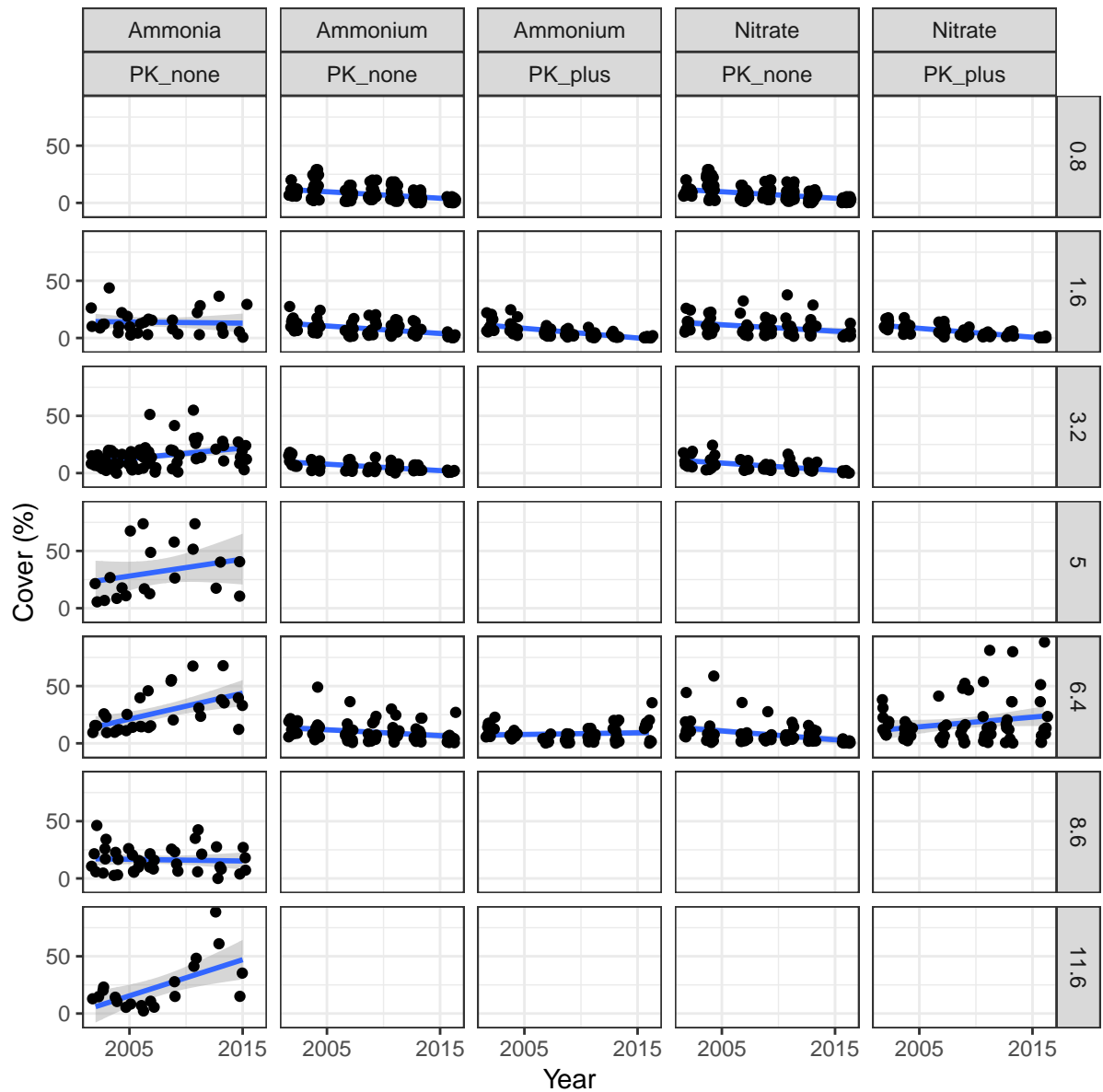


Figure 3: Cover of *Eriophorum vaginatum* over the course of the experiment in relation to the dose of nitrogen added. Panels show the different forms of nitrogen treatment, with and without the addition of PK. Points show the annual mean for each permanent quadrat. Symbol colour denotes the dose of nitrogen in  $\text{g N m}^{-2} \text{y}^{-1}$  for each treatment level. Lines show a linear fit against time for each treatment level. Grey bands around these lines show the 95 % confidence interval for these fits. There was only a single set of control plots for both the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments, with only background nitrogen deposition, and these data are duplicated in the panels for both the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments without PK addition.



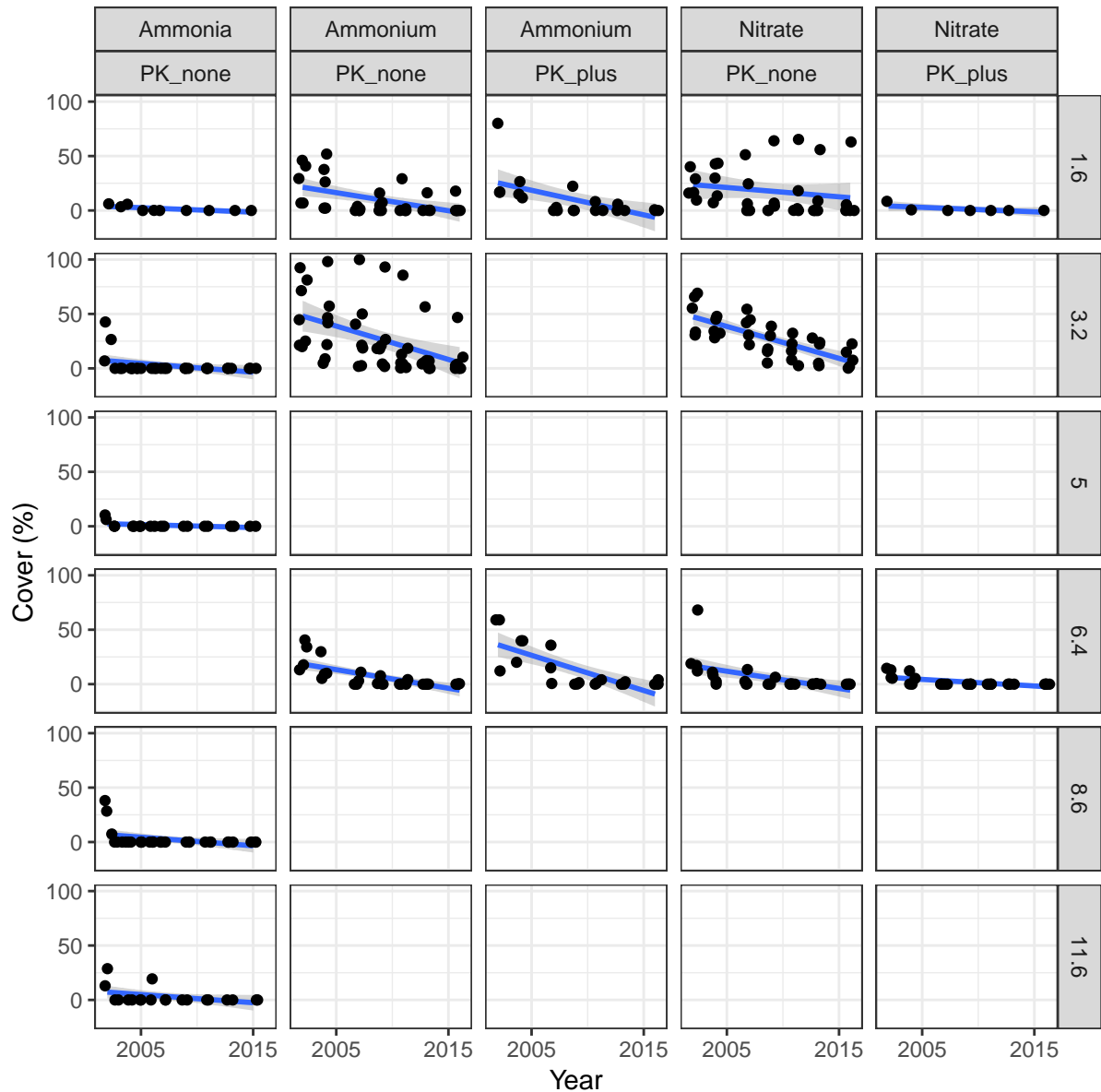


Figure 4: Cover of *Cladonia portentosa* over the course of the experiment in relation to the dose of nitrogen added. Panels show the different forms of nitrogen treatment, with and without the addition of PK. Points show the annual mean for each permanent quadrat. Symbol colour denotes the dose of nitrogen in  $\text{g N m}^{-2} \text{y}^{-1}$  for each treatment level. Lines show a linear fit against time for each treatment level. Grey bands around these lines show the 95 % confidence interval for these fits. There was only a single set of control plots for both the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments, with only background nitrogen deposition, and these data are duplicated in the panels for both the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments without PK addition.

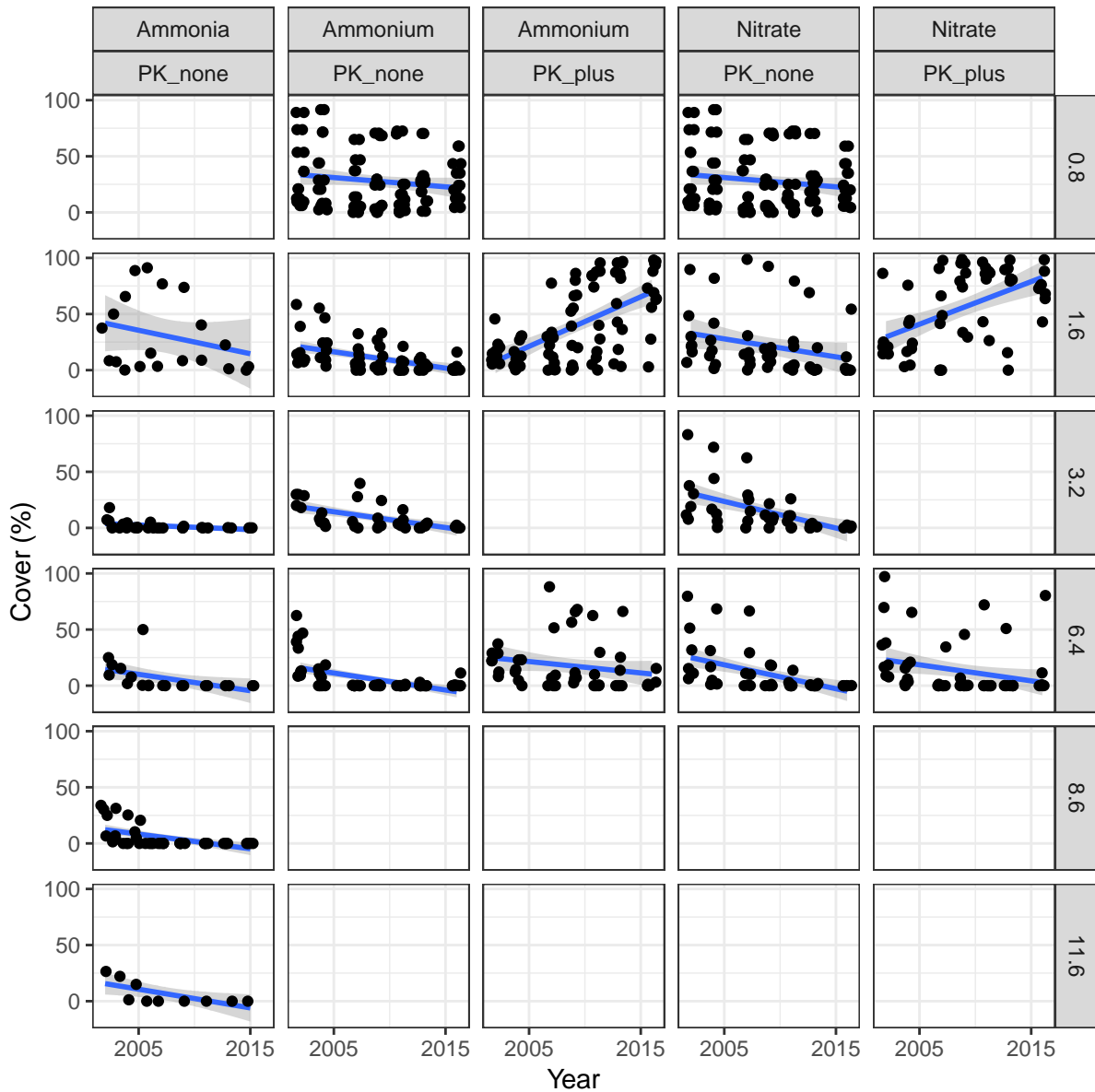


Figure 5: Cover of *Pleurozium schreberi* over the course of the experiment in relation to the dose of nitrogen added. Panels show the different forms of nitrogen treatment, with and without the addition of PK. Points show the annual mean for each permanent quadrat. Symbol colour denotes the dose of nitrogen in  $\text{g N m}^{-2} \text{y}^{-1}$  for each treatment level. Lines show a linear fit against time for each treatment level. Grey bands around these lines show the 95 % confidence interval for these fits. There was only a single set of control plots for both the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments, with only background nitrogen deposition, and these data are duplicated in the panels for both the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments without PK addition.

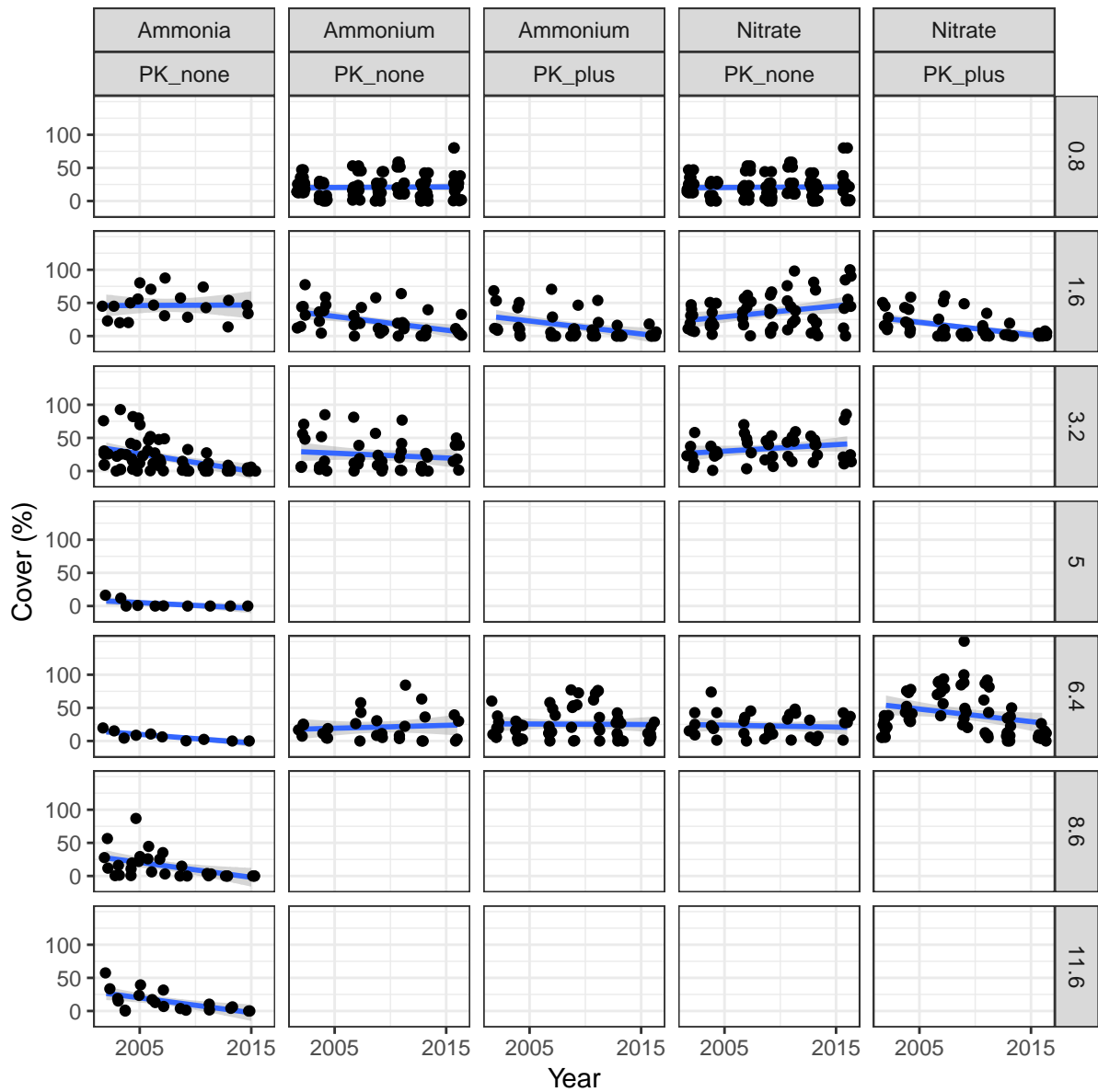


Figure 6: Cover over of *Hypnum jutlandicum* the course of the experiment in relation to the dose of nitrogen added. Panels show the different forms of nitrogen treatment, with and without the addition of PK. Points show the annual mean for each permanent quadrat. Symbol colour denotes the dose of nitrogen in  $\text{g N m}^{-2} \text{y}^{-1}$  for each treatment level. Lines show a linear fit against time for each treatment level. Grey bands around these lines show the 95 % confidence interval for these fits. There was only a single set of control plots for both the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments, with only background nitrogen deposition, and these data are duplicated in the panels for both the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments without PK addition.

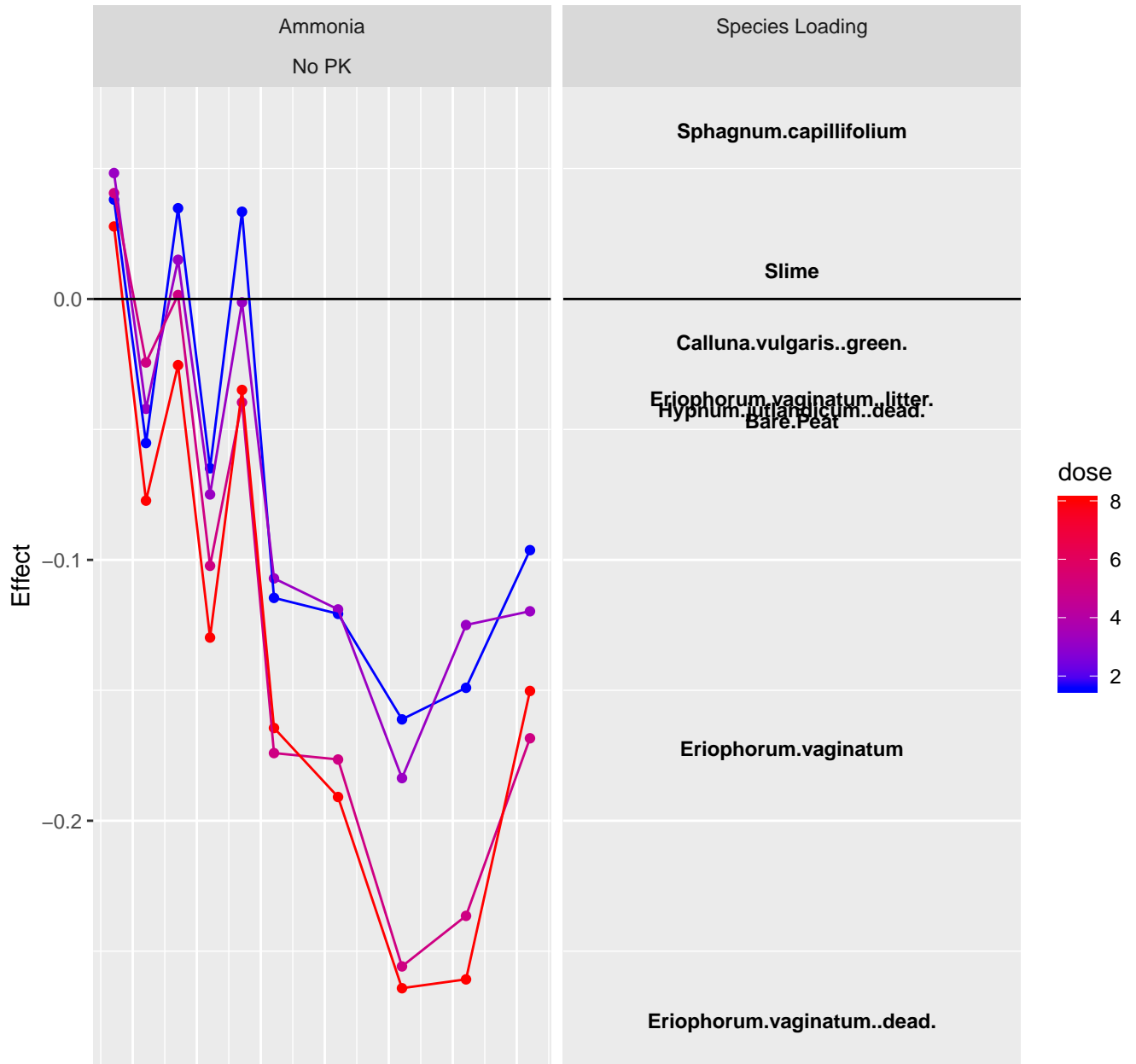


Figure 7: Principal response curves for the  $\text{NH}_3$  treatment, showing the time-dependent, community-level effects of nitrogen form and dose. The  $y$  axis shows the treatment-time coefficients  $c_{dt}$  from the first PRC component, representing the multivariate measure of plant species composition which best captures the time-dependent response of the vegetation to treatments. Lines represent the different levels of nitrogen dose. Control values are shown as a horizontal line on the plot at  $c_{dt} = 0$ . The right-hand panel depicts the species loadings,  $b_s$ . The further these are from zero, the more closely the response pattern for that species correlates with the overall response pattern.  $b_s$  values near zero were omitted from the plot for legibility.

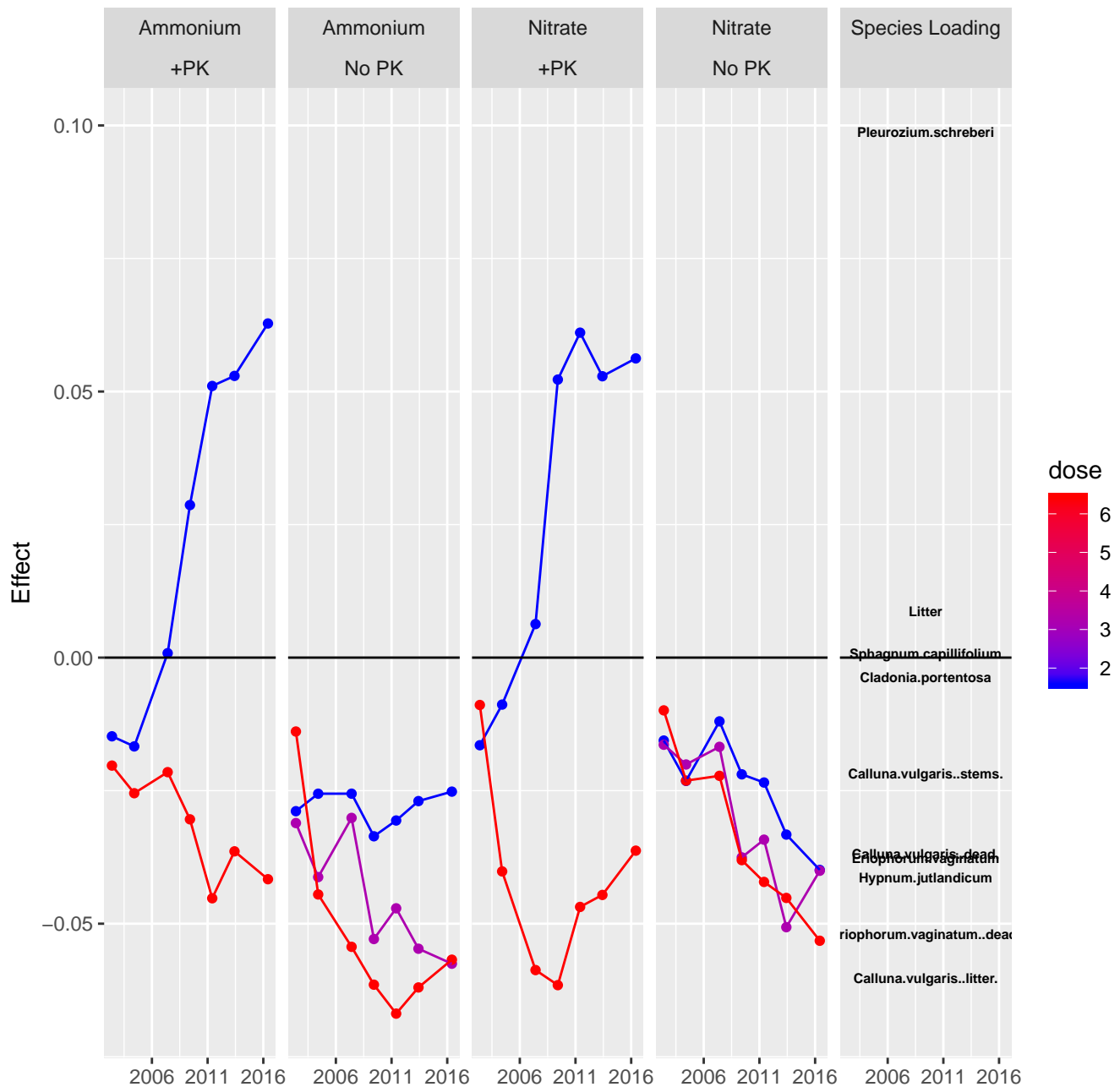
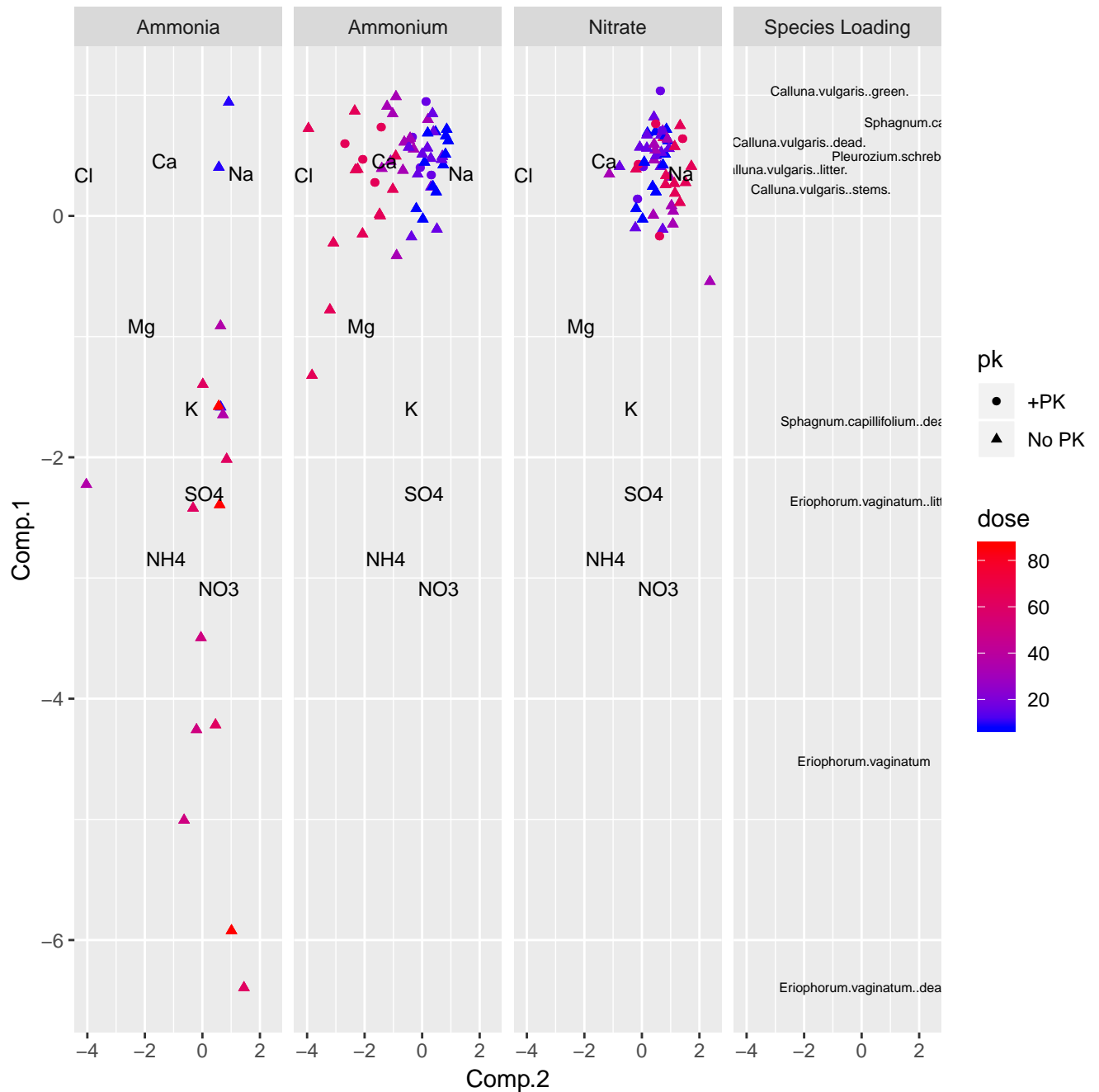


Figure 8: Principal response curves for the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treatments, showing the time-dependent, community-level effects of nitrogen form and dose, with and without addition of PK. The  $y$  axis shows the treatment-time coefficients  $c_{dt}$  from the first PRC component, representing the multivariate measure of plant species composition which best captures the time-dependent response of the vegetation to treatments. Control values are shown as a horizontal line on the plot at  $c_{dt} = 0$ . The right-hand panel depicts the species loadings,  $b_s$ . The further these are from zero, the more closely the response pattern for that species correlates with the overall response pattern.  $b_s$  values near zero were omitted from the plot for legibility.



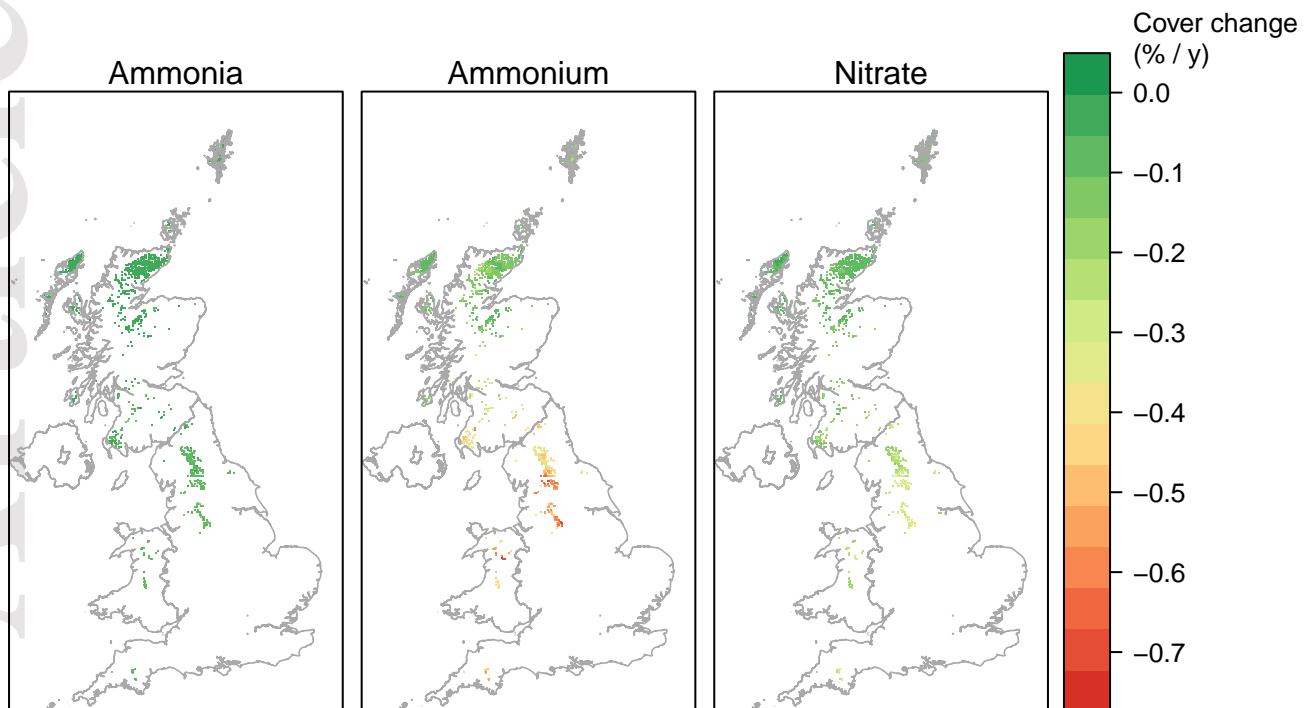


Figure 10: Estimated annual change in cover of *Sphagnum capillifolium* as a result of nitrogen deposition in the form of  $\text{NH}_3$ ,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  across UK peat bogs. Wet and dry deposition was estimated by the CBED model (Smith *et al.*, 2000, Smith & Fowler, 2001), based on observed atmospheric concentrations and in rainfall. The distribution of peat bogs was taken from the CEH Land Cover Map 2015 (Morton *et al.* 2017) at 1-km resolution. At each of these locations, the model coefficients for the interactions between time and  $\text{NH}_3$ ,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (Table 2) were multiplied by the respective deposition of each, to give their separate effects on cover change, as estimated from the responses in the Whim experiment. The effects are expected to be additive, so the total response to nitrogen deposition is the sum of the three effects. The model coefficients are derived from the 14-year Whim experiment, so represent the initial response after a step increase in nitrogen deposition, and may not reflect the current response, particularly in areas which have received high nitrogen deposition over a longer historical period.