Response to comments by Referee 1 - Tim Moore

Peter E. Levy

October 13, 2016

1 Referee 1 - Tim Moore

We thank the referee for their thorough reading of the manuscript. We address their points *(shown in italics)* below.

A large data set of N2O chamber fluxes (the exact number is not stated ...

The total number is now stated in section 2.4.

Specific comments: The study is devoid of specific mechanisms for N2O production and emission, being concentrated (quite reasonably) on the relationships between treatments, environmental drivers and observed flux. The general argument, as I read it, is as long as vegetation is there, it will take up the deposited N, resulting in no significant emission. Given the work done at Whim, perhaps this could be fleshed out a bit. What is the annual N uptake rate at Whim? This might be cacluated from the C budget (about which quite a bit is known) and some assumptions of C:N ratio. An unknown is N2 fixation, as well as fluvial N losses, though you have DOC export and most N will be in the organic form. Given your addition rates of up to 60 kg/ha/yr of NH4 or NO3 and fluvial N losses (perhaps 3 kg/ha/yr, more if the elevated solution N forms get leached out), can these be accounted for in vegetation uptake (given your vegetation data) or peat storage? Can you add anything more to the Sheppard et al. (2013) Figure 7, based on data in 2009/10, whereas your results are based on fluxes through 2015?

We expand on this in an extra paragraph in the Discussion, but we err on the side of caution, as many of the terms listed are only poorly estimated. Unfortunately, we don't have data to update the N budget shown in Sheppard et al. (2013) Figure 7. Also, estimating fluxes from the change in stocks of N is very prone to errors in bulk density, C & N concentrations and sampling error. The analysis presented in Sheppard et al. (2013) Figure 7 implies that N accumulation in the peat and vegetation has been rather larger than the known N addition. This is implausible, and we think must be due to sampling error, so we have to be cautious in drawing conclusions from this data.

The sources of N2O remain a black box, a story unto itself. We need to get the N into N2O through either nitrification of denitrification (competing against plant uptake). Does the peat have a high nitrification and denitrification potential? Given the low pH, I suspect that nitrification of NH4 to NO3 will be slow, and it may be that natural rates of denitrification are also slow. But does addition of NO3 speed up denitrification rates, or has the soil pH been raised by NH3 to stimulate the microbial population? At the Mer Bleue peatland we examined denitrification rates which were small naturally but when we added NO3 and a labile carbon source, there was substantial N2O production. We also observed no significant N2O emission from fertilized plots, with up to 64 kg N (as NH4NO3)/ha/yr. Perhaps there are no data to draw upon, but it would be worthwhile commenting on how these microbial processes may explain your observed result.

We agree this is an interesting topic, which we discuss only cursorily because of lack of data; unfortunately, we don't have any measurements of nitrification and denitrification rates or potentials. However, we now add some further discussion of how these underlying microbial processes may explain our results.

Finally, it is interesting that a substantial proportion of the N2O flux measurements suggested a consumption, though many had errors which overlapped zero. A few years ago, Chapuis-Lardy et al. (2007) drew attention that the process may occur but scientists had dismissed it as error. Since then, there has been some examination of the possibility of N2O consumption (essentially denitrification to N2) and under what conditions. Our work (Frasier et al. 2010) suggested that N2O consumption can occur, but mainly under anoxic conditions with a large N2O pool and very little NO3. Although I realise it is not part of your remit for this paper, it would be interesting to know under what conditions N2O consumption occured. Chapuis-Lardy L, Wrage N, Metay A, Chotte JL, Bernoux M. 2007. Soils, a sink for N2O? A review. Global Change Biol 13:117. Frasier, R., S. Ullah and T.R. Moore 2010. Nitrous oxide consumption potentials of well-drained forest soils of southern Quebec, Canada. Geomicrobiology 27: 53-60.

We did look for a pattern explaining N_2O consumption in the data, but there is nothing very clear. The main problem is that the negative fluxes are so small compared to the measurement error, we can't be sure they are real. We now make reference to the Chapuis-Lardy et al. (2007) and Frasier et al. (2010) work, but we think it is pushing the limit of our observations to say much more about this.

Technical comments: I felt that the manuscript could have been clearer if some aspects were better described and more careful proof-reading had been done. I have annotated the pdf with comments and suggestions to address this.

We thank the referee for the very careful proof-reading, and have made all

the changes suggested. These are listed below.

Page 1. Typos corrected.
Page 2. 1. Point accepted.
Page 2. 2. No, the 1 % is used in the IPCC methodology for all indirect emissions of anthropogenic N which is deposited then re-emitted.
Page 2. 3. Sign convention for water table now made explicit.
Page 3. 1. "south-west" added.
Page 3. 2. Dip-well location now explicit.
Page 3. 3. Now made clear that vegetation was measured in the chamber collar itself.
Page 3. 4. Corrected. The chambers were initially shown as green symbols, but removed because they made the figure too cluttered.
Page 4. 1. Monthly frequency of sampling now stated.
Page 4. 2. Fundamentally, it is a molar quantity that is measured by the chamber method, and this is the unit relevant to plant physiology and biochemistry. However, the community is familiar with nitrogen application rates in non-SI mass units of kg N per ha, and no one has a feeling for these values in molar units. I don't see a better solution, unfortunately.
Page 4. 3. Now made explicit that negative values indicate depth below the surface.
Page 5. 1. Yes, we assume deposition velocity was spatially homogeneous; we now say this in section 2.2.
Page 5. 2. North arrow added to the figure.
Page 5. 3. Typo corrected.
Page 5. 4. Yes, wet treatment measurements only in ambient & 60.
Page 5. 5. Respectively removed. Uptake of N2O discussed in main response.
Page 6. 1. Typo corrected.
Page 6. 2. Yes, the reviewer is correct - S. capifollium is indeed a hummock species. We have corrected the text.
Page 6. 3. Typo corrected here and elsewhere.
Page 6. 4. Sentences merged as suggested, with a semi-colon.
Page 8. 1. Units corrected.
Page 8. 2. "N2O" added for clarity.
Page 15. 1. Yes, the solid lines shows the fitted response from the general additive mixed model. This is now added to the caption. And yes, wet treatment measurements were only in ambient & 60 levels.
Page 18. 1. Yes, the solid line shows the 1:1 line. This is now added to the caption.

2 Referee 2 - D. Li

Response to comments by Referee 2

This manuscript presents N2O emissions from a peatbog following 13 years of simulated wet or dry N deposition. Compared to most studies in which very high doses of N were applied, this study adopted much mild N does. The estimation or prediction of N2O emission is a challenge largely due to notoriously high spatial and temporal variation and complex controlling factors as well. By providing long-term responses of N2O fluxes to mild dry and wet N deposition, the dataset of the manuscript is undoubtedly important and interesting. The manuscript is generally well written and the methodology is fine. However, I doubt whether the manuscript provided enough novelty relative to its companion paper, i.e., Sheppard et al. (2013). The main results of both papers are similar, or the same, i.e., N2O emission was stimulated by ammonia but not by ammonium or nitrate. The previous study covered a period of eight years of N addition, but this manuscript reported the results over another five years. Whether this difference supports a new publication in Biogeosciences needs to be well addressed. Similarly, the three objectives were mostly covered in the previous paper. So they should not be used as the main objectives.

Our paper is a very substantial advance on Sheppard et al. (2013). The focus of that paper was on change in the vegetation cover and the fate of the added N. As regards N2O fluxes, there was only a single bar chart, only two sentences in the Results section, and no appropriate statistical analysis. Those data constitutes only 13 % of the data set analysed in our paper. We present N2O fluxes over the full range of the NH3 transect, whereas Sheppard et al.(2013) had only a single location. We apply a sophisticated mixed-effect statistical model, which accounts for the hierarchical structure of the data (chambers nested within plots within blocks, repeated measurements over time).

Specific comments: Statistical analysis Page 4, Lines 14-16: How did you judge that the four points were outlying measurements?

Visually, these points were clearly out-lying. Any formal test identifies these points as outliers. Out of +700 data points, these do not influence the results much.

Results *Please present only the results or description of data in the result section and exclude any discussion.*

We have moved some of the text as suggested.

Page 5, Lines 16-18: Did you exclude the measurements which were close to the detect limits of the technique? If not, there should be large uncertainty in the data since most of measurements were close to the detect limits.

No, there is no reason to exclude these measurements. The uncertainty is shown explicitly in Fig 3 in the form of the 95 % CIs in each measurement.

Discussion

Page 6, Line 9: The reference should be cited as Sheppard et al. (2013).

This has now been corrected here and elsewhere.

Page 6, Line 14: NH4+NO3 is misused as NH4+NO3. In addition, there are lots of similar misuses, such as NO3- as NO3, especially in the figure titles.

These have now been corrected.

Page 7, Line 2: but both have limited capacities for uptake of what?

Changed to "both have limited capacities for uptake of nitrate".

Page 7, Line 4: Data in (Sheppard et al., 2013, Figure 7)? This is wrong in edit.

This has now been corrected.

Page 7, Line 5: What do you mean by saying belowground vegetation? Roots? I can't see that there is such information in Figure 7 in terms of all the additional N deposited on the wet treatment plots accumulated in the top 10 cm of peat and belowground vegetation. In the method section, N accumulation in vegetation was not presented. In addition, I checked the article (i.e., Sheppard et al., 2013), it seemed that there also was no such information.

What we actually meant was the data that were used in Sheppard et al., 2013 Figure 7, rather than the Figure itself. Summing the two classes which they refer to as "peat" and "vegetation", and comparing with the control, we can estimate how much N has been immobilised. We have now expanded the text to make this clearer.

Page 7, Line 5: If most of the added N was accumulated in the top 10 cm of peat, there should be substantial N2O production. Is there evidence showing that no N2O production in the peat layer?

The N is accumulated in organic form, and therefore not readily available to microbes, hence we say it is immobilised. Beyond the data presented in the paper, it is not clear what other evidence for N2O production the referee refers to.

Figure 3: This figure is about the responses to different forms of N inputs. It is confusing that 1) all the panels showed dry and wet N deposition, 2) when ammonium and nitrate were applied, the rates should be 16, 32 and 64 kg N ha-1 yr-1, 3) Dotted lines show the emission predicted with the IPCC default emission factor, but where is the dotted line?

We feel this figure is appropriate to the structure of the data. 1) all the panels

show dry and wet N deposition because all the plots receive both dry and wet N deposition, albeit that some of this is ambient deposition. 2) total ambient deposition is 8 kg N ha-1 yr-1, made up of approximately 4 kg N ha-1 yr-1 ammonium and 4 kg N ha-1 yr-1 nitrate. If 56 kg N ha-1 yr-1 nitrate is applied, the total nitrate-N deposition is estimated to be 60 kg N ha-1 yr-1. 3) The dotted lines are clearly visible, but may have been mistaken for the x axis. The caption now makews this clear.

Figure 4: The figure should be stand alone, so please provide the necessary explanation.

Some additional text is now added to the caption to make this clearer.

Nitrous oxide emissions from a peatbog after thirteen years of experimental nitrogen deposition

Sarah R. Leeson, Peter E. Levy, Netty van Dijk, Julia Drewer, Sophie Robinson, Matthew R. Jones, John Kentisbeer, Ian Washbourne, Mark A. Sutton, and Lucy J. Sheppard

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Abstract.

Nitrogen deposition was experimentally increased on a Scottish peat bog over a period of thirteen years (2002-2015). Nitrogen was applied in three forms, NH_3 gas, NH_4^+ solution, and NO_3^- solution, at rates ranging from ambient (8) to 64 kg N ha^{-1} y^{-1}, and higher near the NH_3 fumigation source. An automated system was used to apply the nitrogen, such that the deposition was realistic in terms of rates and high frequency of deposition events. We measured the response of nitrous oxide (N_2O) flux to the increased nitrogen input. Prior expectations, based on the IPCC default emission factor, were that 1 % of the added nitrogen would be emitted as N_2O. In the plots treated with NH_4^+ and NO_3^- solution, no response was seen, and there was a tendency for N_2O fluxes to be reduced by additional nitrogen, though this was not significant. Areas subjected to high NH_3 emitted more N_2O than expected, up to 8.5 % of the added nitrogen. Differences in the response are related to the impact of the nitrogen treatments on the vegetation. In the NH_4^+ and NO_3^- treatments, all the additional nitrogen is effectively immobilised in the vegetation and top 10 cm of peat. In the NH_3 treatment, much of the vegetation was killed off by high doses of NH_3, and the nitrogen was presumably more available to denitrifying bacteria. The design of the wet and dry experimental treatments meant that they differed in statistical power, and we are less likely to detect an effect of the the NH_4^+ and NO_3^- treatments, though they avoid issues of pseudo-replication.

1 Introduction

Since the industrial revolution, peatlands have been subject to increased deposition of anthropogenic nitrogen (N), as a result of fossil fuel burning and agricultural use (Fowler et al., 2005). The overall consequences of enhanced N deposition in ombrotrophic peat bogs are poorly understood, but bogs are likely to be sensitive to enhanced N inputs, because they are adapted to conditions of very low N availability (Bobbink et al., 1998). When N deposition exceeds plant demand, the additional N may be used by soil microbes, and can result in the production of the greenhouse gas nitrous oxide (N_2O) via nitrification and denitrification (Regina et al., 1996; Bobbink et al., 1998; Silvan et al., 2005). Of the total N applied to agricultural land and arising from livestock waste which is subsequently deposited on semi-natural land, it it estimated that 1 % is re-emitted as N_2O (De Klein, 2006). This so-called "indirect" emission of N_2O is a large, but uncertain, term in the national inventory of greenhouse gas emissions. There have been relatively few experimental attempts to determine these emissions on peat bogs, and most information comes from Fenno-Scaninavian bogs (Regina et al., 1998; Nykanen et al., 2002; Lund et al., 2009).

N deposition may lead to changes to peat bog ecosystems which influence the emission of N_2O in complex ways, particularly via soil chemistry and vegetation composition (Simek and Cooper, 2002; Juutinen et al., 2010). For example, N deposition may affect soil pH, which affects the composition of the microbial community (Nicol et al., 2008), and affects the relative prevalence

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This paper reports measurements of N_2O emissions, as part of a long-term experiment in which additional N has been deposited on a peatbog in central Scotland, for over thirteen years, in three different forms (as NH_3 gas, as NH_4^+ solution or NO_3^- solution). The automated experiment was designed to provide realistic N deposition, in terms of doses, frequency (>100 spray events y^{-1}) and exposure concentrations, reflecting the pollution climate experienced in the UK. Ambient N inputs at the site are relatively low, so that the responses should be representative of the more pristine northern European peat bogs. Previous results from the experiment have demonstrated that high doses of NH_3 reduces the cover of several plant species, but that the effects of NH_4^+ and NO_3^- on vegetation composition and cover are not large (Sheppard et al., 2008, 2011, 2014). Here, we examine the effects of the dose and form of N deposition on emissions of N_2O. Preliminary data on N_2O fluxes were reported by Sheppard et al. (2013), showing an increase with NH_3, but no effect of NH_4^+ and NO_3^-. Here, we analyse an additional five years of data collected at a wider range of locations, and with further time for any treatment effects to accumulate. The aims were to investigate (i) the extent to which N_2O emissions are stimulated by N deposition, and whether the 1 % emission factor used in IPCC inventories is accurate, (ii)whether the form of N deposition is important, and (iii) whether other changes induced by N deposition (e.g. on soil chemistry or vegetation) have an indirect effect on N_2O emissions.

2 Materials and methods

2.1 Field site

Whim bog in the Scottish Borders (3°16' W, 55°46' N) represents a transition between a lowland raised bog and blanket bog, on 3-6m of deep peat. Mean temperatures of the air and soil (at 10-cm depth) were 8.6 °C and 7.7 °C respectively (2003-2009 means). The annual rainfall was 1092 mm (734-1462 mm range). On average, the water table was 10 cm below the peat surface, i.e. relatively wet for most of the year. The peat was very acidic, with pH 3.4 (3.27-3.91 in water). The vegetation was classified as a *Calluna vulgaris- Eriophorum vaginatum* blanket mire community (M19 in the UK National Vegetation Classification, Rodwell, 1998). Replicate plots were highly variable and dominated by unmanaged *Calluna* of variable age and stature occurring as mosaics containing *Calluna vulgaris* and *Sphagnum capillifolium* hummocks and hollows containing *S. fallax* and *S. papillosum*. Other common species included *Erica tetralix* and the mosses *Hypnum jutlandicum* and *Pleurozium schreberi*.

2.2 Experimental Treatments

Nitrogen was applied to the site using two different treatment systems, for dry deposition of NH_3 gas, and wet deposition of NH_4^+ and NO_3^- in solution. Treatments commenced in June 2002 and continued all year round, except when temperatures were near freezing.

NH_3 deposition was manipulated using a free-air release system (Leith et al., 2004). NH_3 was supplied from a cylinder of pure liquid NH_3, diluted with ambient air and released from a perforated 10-m long pipe, 1 m off the ground. NH_3 was released only when the wind direction was between 180 and 215°, temperatures exceeded freezing and wind speed exceeded 2.5 m s^{-1}.

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This produced a sector downwind wherein NH_3 decreased with distance from the fumigation source. NH_3 concentrations were measured 0.1 m above the vegetation using passive 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). Ammonia deposition was calculated from the concentration measurements, using the method of Cape et al. (2008). The deposition at these locations was interpolated using ordinary kriging, as shown in Figure 1.

Wet deposition of NH_4^+ and NO_3^- was experimentally increased in a number of replicated plots in a randomised block design, using a water sprayer system (Sheppard et al., 2004). Concentrated solutions of either NH_4Cl or $NaNO_3$ were diluted in rainwater, and transferred to each plot via 100-m 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^2 plot. The volume of soulution applied to each plot was monitored using a water meter on each supply line. Three treatment levels were applied, aiming to provide total N deposition rates of 16, 32 and 64 kg N ha^{-1} y^{-1}, in addition to a control treatment which only received ambient N deposition (8 kg N ha^{-1} y^{-1}). The three treatment levels were achieved by applying either NH_4Cl or $NaNO_3$ solution at concentrations of 0.57, 1.71 or 4.0 mM. Wet treatments increased precipitation amounts by ca. 10%. Control plots receive the additional rainwater without any additional nitrogen. There were four blocks, with one treatment level in each, to give a total of 28 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 s^{-1}. This produced a realistic pattern of high frequency, extensive nitrogn deposition, with ca. 120 applications y^{-1}.

Soil water samples were extracted from dipwells in all plots at the same time as gas flux measurements were made. Concentrations of soil water NH_4^+ and NO_3^- were measured by ion chromatography following filtration. The detection limits were 0.014 and 0.062 mg l^{-1} for NH_4^+-N and NO_3^--N respectively. Vegetation species composition was surveyed in all plots every few years, and the percent cover recorded within each chamber location.

2.3 Greenhouse gas exchange

Nitrous oxide fluxes were measured by the static chamber method (Hutchinson and Mosier, 1981). Cylindrical PVC collars (38 cm in diameter and typically 25 cm high) were inserted into the peat at the locations shown by green circles in Figure 1.

On each sampling occasion, a lid was sealed on top, and left in place for 30-40 minutes. Four 20-ml samples were removed by syringe through a 3-way tap or rubber septum, stored in vials or tedlar bags, and analysed on a gas chromatograph (5890 series II, Hewlett Packard), together with replicates of three or four standard gases with known concentrations. For each sequence of gas samples from a chamber, the flux was calculated as:

$$F = \frac{\mathrm{d}C}{\mathrm{d}t_0} \cdot \frac{\rho V}{A} \quad (1)$$

Where F is gas flux from the soil (μmol m^{-2} s^{-1}), $\mathrm{d}C/\mathrm{d}t_0$ is the initial rate of change in concentration with time in μmol mol^{-1} s^{-1}, ρ is the density of air in mol m^{-3}, V is the volume of the chamber in m^3 and A is the ground area enclosed by the chamber in m^2.

The parameter $\mathrm{d}C/\mathrm{d}t_0$ was calculated using linear and non-linear asymptotic regression methods Levy et al. (2011). Using a mixture of goodness-of-fit statistics and visual inspection, the regression method that provided the best fit for the time series of concentration was chosen for each individual measurement. With this method of flux calculation, any non-linearity should

concentrations were measured 0.1 m above the vegetation using passive 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). Ammonia deposition was calculated from the concentration measurements, using the method of Cape et al. (2008). The deposition at these locations was interpolated using ordinary kriging, as shown in Figure 1, assuming the deposition velocity was spatially homogeneous.

Wet deposition of NH_4^+ and NO_3^- was experimentally increased in a number of replicated plots in a randomised block design, using a water sprayer system (Sheppard et al., 2004). Concentrated solutions of either NH_4Cl or $NaNO_3$ were diluted in rainwater, and transferred to each plot via 100-m 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^2 plot. The volume of soulution applied to each plot was monitored using a water meter on each supply line. Three treatment levels were applied, aiming to provide total N deposition rates of 16, 32 and 64 kg N ha^{-1} y^{-1}, in addition to a control treatment which only received ambient N deposition (8 kg N ha^{-1} y^{-1}). The three treatment levels were achieved by applying either NH_4Cl or $NaNO_3$ solution at concentrations of 0.57, 1.71 or 4.0 mM. Wet treatments increased precipitation amounts by ca. 10%. Control plots receive the additional rainwater without any additional nitrogen. There were four blocks, with one treatment level in each, to give a total of 28 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 s^{-1}. This produced a realistic pattern of high frequency, extensive nitrogn deposition, with ca. 120 applications y^{-1}.

Soil water samples were extracted from dipwells in all plots at the same time as gas flux measurements were made. Concentrations of soil water NH_4^+ and NO_3^- were measured by ion chromatography following filtration. The detection limits were 0.014 and 0.062 mg l^{-1} for NH_4^+-N and NO_3^--N respectively. The percent cover of each vegetation species was recorded within each chamber location every few years.

2.3 Greenhouse gas exchange

Nitrous oxide fluxes were measured by the static chamber method (Hutchinson and Mosier, 1981), typically on a monthly basis. Cylindrical PVC collars (38 cm in diameter and typically 25 cm high) were inserted into the peat within each plot.

On each sampling occasion, a lid was sealed on top, and left in place for 30-40 minutes. Four 20-ml samples were removed by syringe through a 3-way tap or rubber septum, stored in vials or tedlar bags, and analysed on a gas chromatograph (5890 series II, Hewlett Packard), together with replicates of three or four standard gases with known concentrations. For each sequence of gas samples from a chamber, the flux was calculated as:

$$F = \frac{\mathrm{d}C}{\mathrm{d}t_0} \cdot \frac{\rho V}{A} \quad (1)$$

Where F is gas flux from the soil (μmol m^{-2} s^{-1}), $\mathrm{d}C/\mathrm{d}t_0$ is the initial rate of change in concentration with time in μmol mol^{-1} s^{-1}, ρ is the density of air in mol m^{-3}, V is the volume of the chamber in m^3 and A is the ground area enclosed by the chamber in m^2.

The parameter $\mathrm{d}C/\mathrm{d}t_0$ was calculated using linear and non-linear asymptotic regression methods Levy et al. (2011). Using a mixture of goodness-of-fit statistics and visual inspection, the regression method that provided the best fit for the time series of concentration was chosen for each individual measurement. With this method of flux calculation, any non-linearity should be accounted for as far as possible. However, the time resolution (approximately 10 minutes) limits the detectable degree of

be accounted for as far as possible. However, the time resolution (approximately 10 minutes) limits the detectable degree of non-linearity in the initial concentration change, so there remains some potential for underestimation of fluxes Cowan et al. (2014).

2.4 Statistical analysis

The data were first analysed using a linear mixed-effects model (Pinheiro and Bates, 2006), after removing four outlying measurements above 10 nmol $m^{-2} s^{-1}$ and two below -2 nmol $m^{-2} s^{-1}$. We fitted fixed-effect terms for soil temperature, T_{soil}, water table height, z_{water}, ammonia-N deposition rate, F_{N-NH_3}, ammonium-N deposition rate, F_{N-NH_4}, and nitrate-N deposition rate, F_{N-NO_3}, and random-effect terms with a design matrix $Z_{i,j}$ to account for the repeated measures on each chamber location, *j*, nested within each experimental block, *i* :

$$F_{N_2O,ij} = \beta_0 + \beta_1 \cdot T_{soil,ij} + \beta_2 \cdot z_{water,ij} + \beta_3 \cdot F_{NH_3,ij} + \beta_4 \cdot F_{NH_4,ij} + \beta_5 \cdot F_{NO_3,ij} + b_i \cdot Z_{i,j} + b_{ij} \cdot Z_{ij} + \epsilon_{ij} \quad (2)$$

$b_i \sim N(0,\sigma_1^2)$ $b_{ij} \sim N(0,\sigma_2^2)$ $\epsilon_{ij} \sim N(0,\sigma_3^2)$.

The data were also analysed using a general additive mixed-effects model (Wood, 2006), with the same fixed- and random effect terms, but allowing for non-linearity in the fixed-effect responses. To analyse the relationship between N_2O flux and vegetation species composition, we used a multivariate approach, partial least squares regression (PLS, Mevik and Wehrens, 2007). The approach is an extension of principal components analysis (PCA), but whereas PCA focuses on the variance in a matrix of variables, $\mathbf{X}$, PLS computes the scores and loadings in such a way to describe the covariance between $\mathbf{X}$ and a response variable or matrix, $\mathbf{Y}$. In this context, we have a matrix consisting of the percent cover of each plant species in each chamber, and the response variable is the N_2O flux. PLS should perform better than PCA in situations where an infrequent species (contributing little to the variance in $\mathbf{X}$) is highly correlated with $\mathbf{Y}$. In PLS, such a component would automatically be present in the first component, but would be a minor component in PCA.

3 Results

Figure 1 shows the spatial distribution of N deposition in the dry and wet deposition treatments. Deposition of NH_3 peaks at around 100 kg N ha^{-1} y^{-1} just downwind of the fumigation source. NH_3 deposition decreases with downwind and cross-wind distance from the fumigation source, and approximates a Gaussian plume pattern expected from micrometeorological theory. The plume is aligned slightly to the east of the boardwalk transect, although there is some uncertainty in the interpolation berween NH_3 samplers. Deposition of NH_4^+ and NO_3^- on the wet deposition plots are shown on the same colour scale. These are known with much greater certainty, as no modelling step is required.

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2.4 Statistical analysis

The data were first analysed using a linear mixed-effects model (Pinheiro and Bates, 2006). There were 729 flux measurements in total, after removing four outlying measurements above 10 nmol $m^{-2} s^{-1}$ and two below -2 nmol $m^{-2} s^{-1}$. We fitted fixed-effect terms for soil temperature, T_{soil}, water table height, z_{water} (negative values indicate depth below the surface), ammonia-N deposition rate, F_{N-NH_3}, ammonium-N deposition rate, F_{N-NH_4}, and nitrate-N deposition rate, F_{N-NO_3}, and random-effect terms with a design matrix $Z_{i,j}$ to account for the repeated measures on each chamber location, *j*, nested within each experimental block, *i* :

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Figure 2 shows the time series of nitrogen deposition in the NH_4^+ and NO_3^- treatments, and on the NH_3 transect at 16 m, where annual deposition was similar to that in the NH_4^+ and NO_3^- treatments. Distribution of deposition events over time is similar in both treatments. Deposition events were spread over most days of the year, with only no deposition in a short period in mid-winter. NH_3 deposition is calculated as a function of stomatal conductance (Cape et al., 2008), so rates are higher in daytime and in the summer.

Whilst there is considerable scatter in the response of N_2O fluxes to mean annual nitrogen deposition, an increase in N_2O

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Whilst there is considerable scatter in the response of N_2O fluxes to mean annual nitrogen deposition, an increase in N_2O flux with NH_3 deposition was apparent (Figure 3). No trend with NH_4^+ or NO_3^- deposition was obvious. In many of the flux measurements, the magnitude of N_2O fluxes was close to the measurement error in the static chamber method. In both the NH_4^+ and NO_3^- treatments, only 9% of fluxes (respectively) had 95 % confidence limits which did not include zero. Detecting a clear response is inevitably difficult when measurement noise contributes substantially to the variability in the data. By contrast, in the NH_3 treatment, 40 % of fluxes had confidence limits which did not include zero.

The output from the linear mixed model analysis is shown in Table 1, with the coefficients representing the response to the fixed factors. As well as showing significant responses to temperature and water table depth, N_2O fluxes responded to NH_3 deposition (Figure 3). This response was greater than the default 1 % IPCC emission factor, and comes close to 8.5 % (with the appropriate unit conversion). The relationship may not be linear (Philibert et al., 2012), and the general additive mixed model (GAMM) was fitted to allow for non-linearity in the fixed effects. However, the exact form of the response to NH_3 deposition was not well constrained by the data, especially at the lower values, and a simple linear fit was justified (Figure 4). The modelled effect of NH_4^+ and NO_3^- deposition was slightly negative on average, although positive and negative slopes are both plausible (Table 1,Figure 4).

NH_4^+ concentrations in the soil water were elevated in the high NH_4^+ deposition treatment, by around 1 mg N dm^{-3} on average (Figure 5). By contrast, the high NO_3^- deposition treatment had no clear effect on NO_3^- concentrations in the soil water. On the NH_3 deposition transect, there was a clear trend in soil water concentrations of both NH_4^+ and NO_3^- with NH_3 deposition (Figure 5), right-hand plots). At the equivalent level of NH_3 deposition, NH_4^+ concentrations in the soil water were elevated by a similar amount to that in the high NH_4^+ deposition treatment. There were weak relationships between N_2O flux and NH_4^+ and NO_3^- concentrations in the dry treatment, but no clear relationship in the wet treatment.

Vegetation composition provided reasonably good explanatory power for N_2O flux, and the PLS regression explained 56 % of the variance in N_2O flux (Figure 6). The first two components explained 27 % of the variance, and gave an interpretable ordination of the chambers (Figure 7). The chambers high on the first axis were dominated by *Eriophorum vaginatum*, often damaged by NH_3, with little or no moss cover, and had high fluxes. The chambers low on the first axis had high cover of *Sphagnum capillifolium*, and had low fluxes. The second axis differentiates hummock and hollow vegetation, and a soil moisture difference. The drier hummocks with *Calluna vulgaris* and *Deschampsia flexuosa* had lower fluxes than the hollows, dominated by *Sphagnum capillifolium*.

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4 Discussion

Our results confirm the early findings of Sheppard et al. (2013), that there was no clear response of N_2O flux to deposition of NH_4^+ or NO_3^-, whereas high doses of NH_3 reduced the cover of *Calluna vulgaris* and *Sphagnum* species, and increased N_2O flux. Other results in the literature show a range of responses of N_2O emission to experimental N addition, from no response to substantial increases. Lund et al. (2009) found no effect of experimental N addition (NH_4NO_3) on N_2O emissions from two

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Some clearer positive responses have been observed where bogs have been drained, or where very high levels of nitrogen have been applied. Regina et al. (1998) found that N_2O emissions were increased by up to 0.8 nmol $m^{-2}\,s^{-1}$ for around nine months after a single experimental N addition of 100 kg N $ha^{-1}\,y^{-1}$ on a drained and forested peatland in Finland. Here, KNO_3, NH_4Cl, and urea gave a similar range of responses. Zhang et al. (2007) observed an increase of 0.3 nmol $m^{-2}\,s^{-1}$ with the application of 240 kg N $ha^{-1}\,y^{-1}$ (NH_4NO_3) to a freshwater marsh in China, bi-weekly over the summer growing season. Clear responses can, however, be very short-lived. For example, Gao et al. (2014, 2015) found a short-term response of N_2O efflux to NH_4NO_3 addition in an *in vivo* study of soil from an alpine peatland in Tibet, but differences from the control lasted less than ten days.

A response of N_2O emissions to nitrogen addition is more often detectable in laboratory incubations, where there are fewer feedbacks and interactions. Field studies commonly show complex interactions with other variables, resulting in no effect in the field, or making interpretation of results complicated. In a Finnish spruce swamp buffer zone, Saari et al. (2013) found that nitrogen addition increased N_2O efflux in laboratory incubations, but *in situ* N_2O effluxes were low and unresponsive. Regina et al. (1996) found that N_2O fluxes were positively correlated with the numbers of nitrite oxidizers, nitrification potential, N, P and Ca and pH of the soil and negatively with the level of water table and K content of the soil. In a study by Silvan et al. (2005) on a restored peatland in Finland, N_2O emissions showed an asymptotic increase with nitrate concentration, and

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We can intrepret our results similarily in terms of resource competition for nitrate. The wet deposition of NO_3^- did not increase concentrations of NO_3^- in the soil water. Previous data suggest that all the additional N deposited on the wet treatment plots accumulated in the top 10 cm of the peat and below-ground vegetation (based on the accumulation of N in the different pools shown in Sheppard et al. (2013), Figure 7, although estimates are rather uncertain because of sensitivity to errors in bulk density, C and N concentrations and sampling error). The deposited nitrogen was thereby immobilised, and not available to soil microbes, and was not denitrified to N_2O. The wet deposition of NH_4^+ did increase concentrations of NH_4^+ in the soil water, but NH_4^+ is less directly related to the microbial production of N_2O. In the dry deposition treatment at high levels of NH_3 deposition, although there was similar accumulation of the deposited N in the peat, much of the vegetation cover was killed, leaving only a sparse cover dominated by *E. vaginatum*. The deposited N was therefore more available to soil microbes

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The multivariate analysis of the vegetation provided the best means for explaining the variation in N_2O fluxes. Shifts in the species composition appeared to be the clearest sign that N deposition was affecting the long-term outcome of resource competition for nitrate, and thereby influencing N_2O flux. A similar result was found in a multivariate analysis of vegetation in relation to methane flux in the UK ecosystems (Levy et al., 2012; Gray et al., 2013). Direct relationships between N_2O flux and nitrate and ammonium were poor, possibly because these are influenced by feedback from the flux itself.

The unique aspect of the experimental design, in providing a very realistic simulation of nitrogen deposition rates, in terms of frequency and exposure concentrations, makes detecting effects more difficult. Unlike measuring N_2O fluxes on agricultural land, where large peak emissions typically follow fertiliser applications, here we are measuring small, diffuse N_2O fluxes spread over the whole year, which is necessarily more difficult.

Experimental designs of the wet and dry deposition experiments were quite different, because of the logistics of applying gases and liquids to ecosystems. This may partly explain why the effect of NH_3 was easier to detect. NH_3 was applied on a larger spatial scale, with very high rates of deposition adjacent to the fumigation outlet. Implicitly, we assume that differences with distance are effects of NH_3, as there was no true replication (i.e. there was only one transect). Given this assumption, we take n to be 60 in the NH_3 experiment. The probability of detecting an effect size of +0.068 nmol m^{-2} s^{-1} (= 1 % of 60 kg N ha^{-1} y^{-1}) is reasonably high ($p = 0.97$), from a standard power analysis (Cohen, 1988), given the typical measurement error in static chamber data (and ignoring between-plot variability). In the case of the wet depostion experiment, there were true replicate plots in a randomised block design, but sample size was small (4 plots per treatment). The probability of detecting the same effect size with $n = 4$ is rather low ($p = 0.14$).

In our experimental results, neither NH_4^+ nor NO_3^- deposition increased fluxes of N_2O (indeed the mean effect of both was negative), and all the deposited N appeared to be retained in the peat and below-ground vegetation. However, we need to be careful before concluding that the true effect was zero (or negative), given that the effect size we were looking for was small. An emission factor of 1 % is not inconsistent with the data, given the between-plot variability, measurement precision and small sample size. If this were the true emission factor, we can estimate the N_2O emission resulting from N deposition over peatlands in the UK. Combined deposition rates of NH_4^+ and NO_3^- are generally less than 10 kg N ha^{-1} y^{-1} on UK peatbogs (Department for Environment, Food and Rural Affairs, 2016). With a 1 % emission factor, this would imply a mean emission

because there was less competition with plants, and could be denitrified to N_2O.

One of the difficulties in interpreting these results is that there are multiple microbial processes occurring (nitrification, denitrification and N_2O consumption), but the chamber flux measurements only give the net exchange of N_2O. The treatments could affect these multiple processes in different ways, e.g. via their effects on pH (and thereby influencing the microbial populations), or more directly by altering the amount of substrate for denitrification. However, without detailed information on the nitrification and denitrification potentials, it is difficult to draw stronger conclusions. Some studies have suggested that gross uptake of N_2O can be substantial, at least in forest soils (Chapuis-Lardy et al., 2007; Frasier et al., 2010). In our study, the negative fluxes are so small compared to the measurement error, that no real pattern can be discerned, and this is a common finding in agricultural systems (Cowan et al., 2014b).

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In our experimental results, neither NH_4^+ nor NO_3^- deposition increased fluxes of N_2O (indeed the mean effect of both was negative), and all the deposited N appeared to be retained in the peat and below-ground vegetation. However, we need to be careful before concluding that the true effect was zero (or negative), given that the effect size we were looking for was small. An emission factor of 1 % is not inconsistent with the data, given the between-plot variability, measurement precision and small sample size. If this were the true emission factor, we can estimate the N_2O emission resulting from N deposition over peatlands in the UK. Combined deposition rates of NH_4^+ and NO_3^- are generally less than 10 kg N ha^{-1} y^{-1} on UK peatbogs (Department for Environment, Food and Rural Affairs, 2016). With a 1 % emission factor, this would imply a mean emission

of 11 pmol m^{-2} s^{-1} or 1.3 g C - CO_2 eq m^{-2} y^{-1}. (To express N_2O flux in units of C - CO_2 equivalents, we convert to a mass of N_2O and multiply by 298 (the global warming potential of N_2O over a 100-year time period including carbon cycle feedbacks, IPCC, 2013), and report only the mass of carbon in the CO_2 equivalents i.e. 12/44.) This compares with contemporary net ecosystem carbon balance measurements in the range of 28-50 g C - CO_2 equivalents in UK peatlands (Helfter et al., 2014; Levy and Gray, 2015).

NH_3 has a different spatial distribution, being deposited much closer to its sources, so with high "hotspots", but with lower overall mean deposition rates (<5 kg N ha^{-1} y^{-1}). In the immediate vicinity of a large agricultural source, high mean emissions would be predicted, in the order of 0.58 nmol m^{-2} s^{-1} or 65 g C - CO_2 eq m^{-2} y^{-1} (i.e. 8.5 % of 60 kg N ha^{-1} y^{-1}), but over relatively small areas. This approaches the large values observed by Repo et al. (2009) in Arctic bare peat circles (averaging 0.86 nmol m^{-2} s^{-1} or 97 g C - CO_2 eq m^{-2} y^{-1}), but upscaling these emissions to national scale is challenging.

Author contributions. LS and MS designed the experiment. SL, MJ and LS maintained the experiment. SL collected the bulk of the flux data, with additional data collected by JD, SR, JK and IW. NvD collected vegetation data and analysed the soil chemistry data. PL performed the statistical analysis and wrote the manuscript.

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11

	DF	F.value	p.value	Coefficient
Intercept	391	23.88	0.000	-0.1655
T_{soil}	391	14.10	0.000	0.0527
z_{water}	391	25.36	0.000	0.0181
$F_{\mathrm{NH_3}}$	40	29.89	0.000	0.0092
$F_{\mathrm{NH_4}}$	40	1.27	0.267	-0.0046
$F_{\mathrm{NO_3}}$	40	1.74	0.194	-0.0041

Table 1. Results of fitting the linear mixed-effects model (Equation 2) to the 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 β coefficients in Equation 2.

	DF	F.value	p.value	Coefficient
Intercept	392	23.90	0.000	-0.1664
T_{soil}	392	16.75	0.000	0.0181
z_{water}	392	22.81	0.000	0.0528
$F_{\mathrm{NH_3}}$	40	29.96	0.000	0.0092
$F_{\mathrm{NH_4}}$	40	1.27	0.267	-0.0046
$F_{\mathrm{NO_3}}$	40	1.74	0.194	-0.0041

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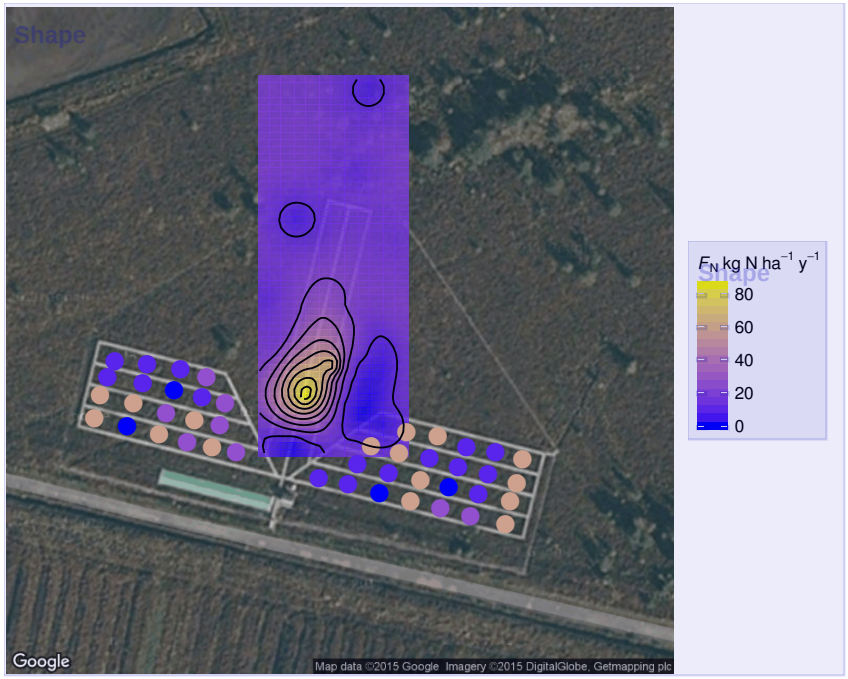
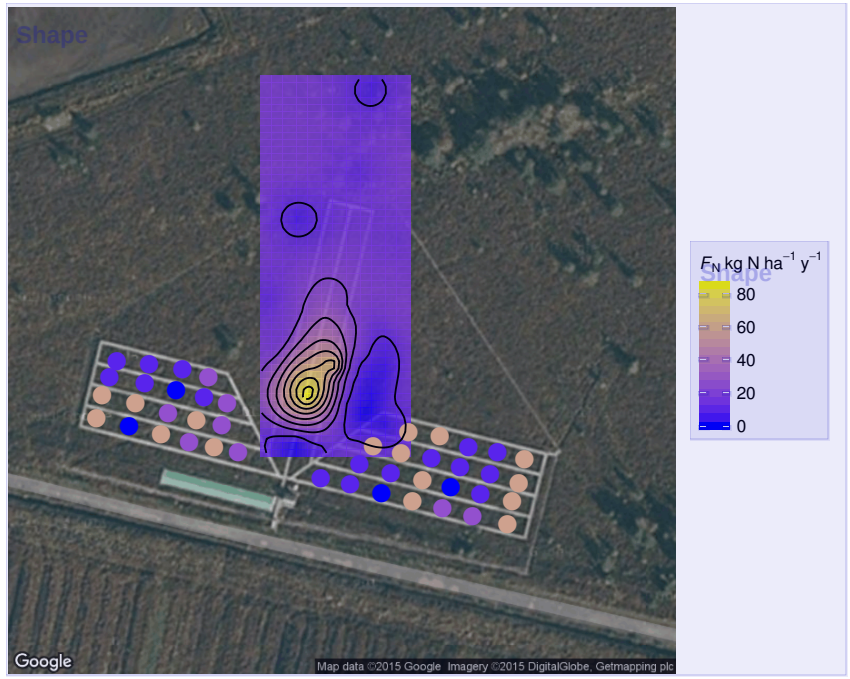


Figure 1. Spatial distribution of N deposition in the Whim experiment. The ammonia release system was located at the centre of the boardwalk structure in the middle of the image. Ammonia deposition was calculated from concentration measurements downwind of the ammonia source using the method of Cape et al. (2008), and interpolated across the central rectangular area of the image using ordinary kriging. In the wet deposition plots (circles in the lower part of the image), the nitrogen solution applied was sprayed evenly across the whole 12.8 m^{2}plot.

12



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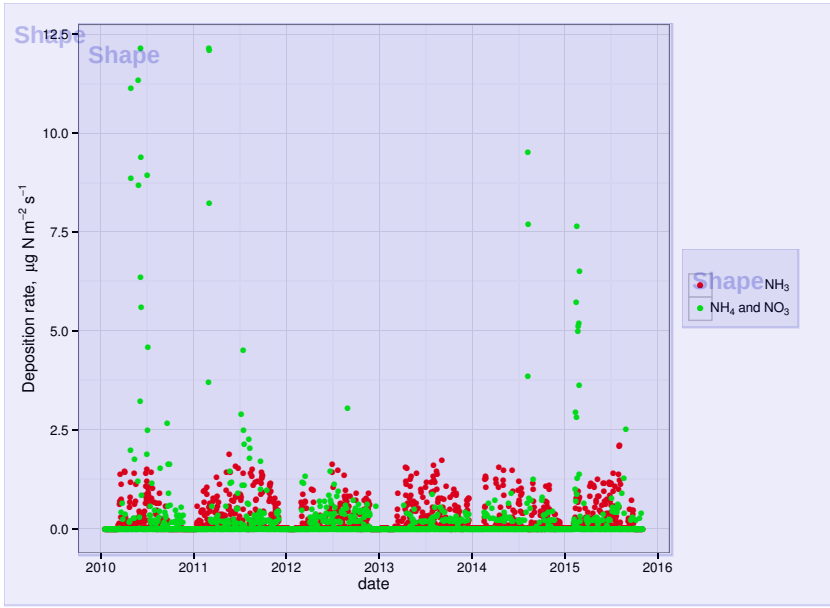
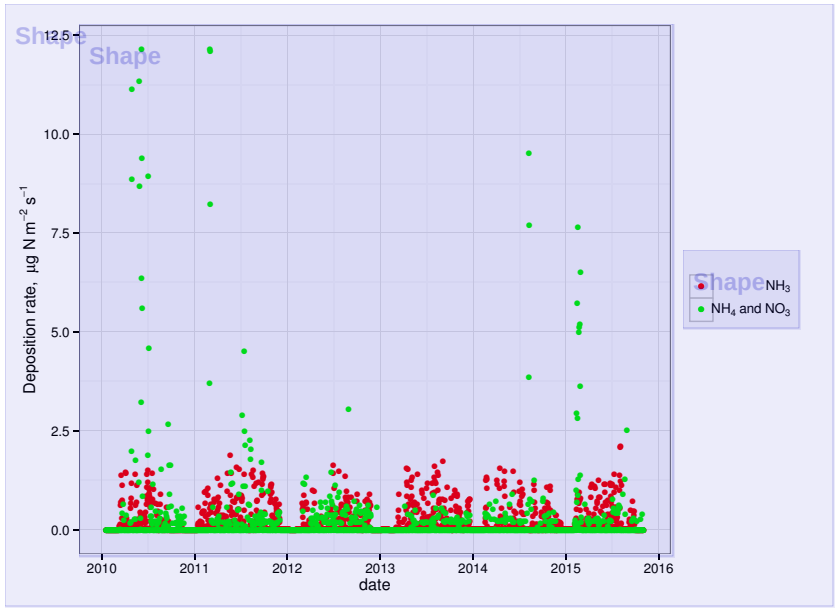


Figure 2. Daily mean nitrogen deposition rates in the NH_4^+ and NO_3^- treatments and at 16 m downwind on the NH_3 transect. NH_4^+ and NO_3^- deposition was calculated from the duration of spraying events each day, multiplied by the NH_4^+ and NO_3^- concentration in the solution, and accounting for the area covered by the spray. NH_3 deposition was calculated from the duration of fumigation events each day, measurements of NH_3 concentration, and meteorological data, using the method of Cape et al. (2008).

13



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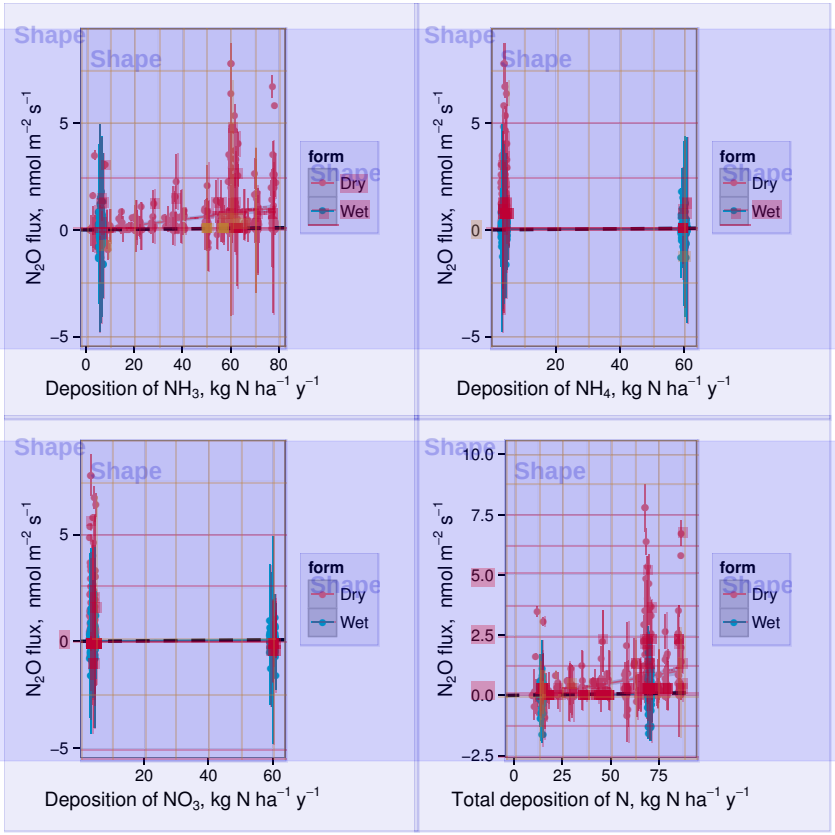
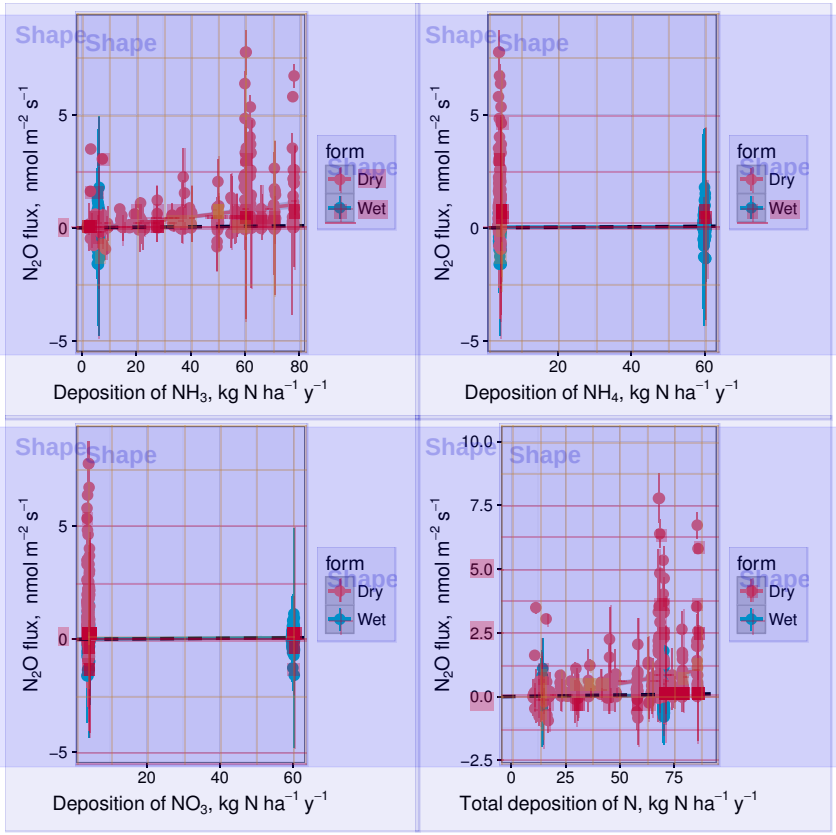


Figure 3. Response of N_2O flux to deposition of NH_3, NH_4, NO_3, and total N deposition. Error bars show the 95 % confidence interval, based on the regression slope for each flux measurement. Dotted lines show the emission predicted with the IPCC default emission factor, as 1 % of the deposited N.



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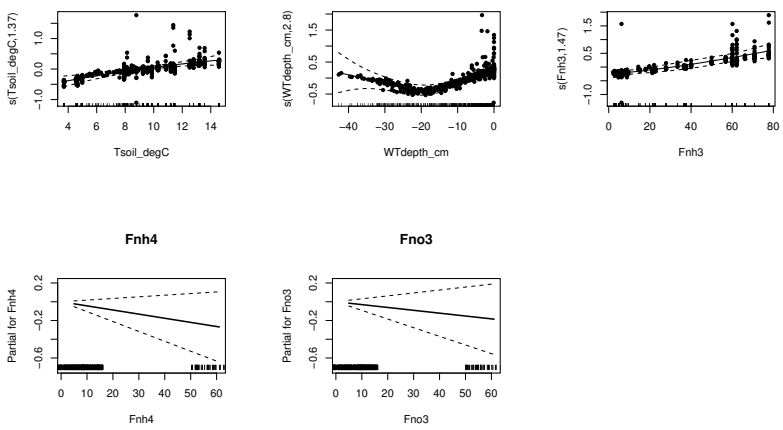
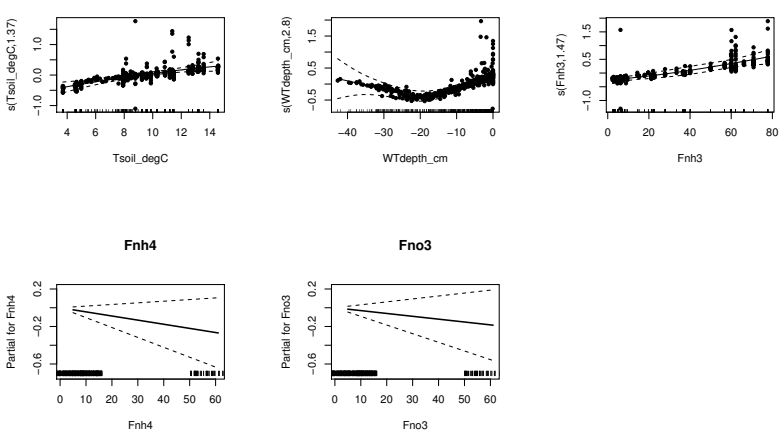


Figure 4. Fitted response of N_2O flux to soil temperature, water table height, and deposition of NH_3, NH_4^+ and NO_3^-, as estimated by the general additive mixed model. Partial residuals are shown for the smooth terms, which are the residuals that would be obtained by dropping the term concerned from the model, while leaving all other estimates fixed. NH_4^+ and NO_3^- were treated as linear terms. Upper and lower lines show 2 standard errors above and below the fitted estimate.



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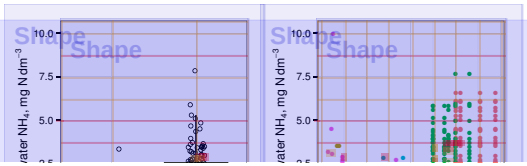
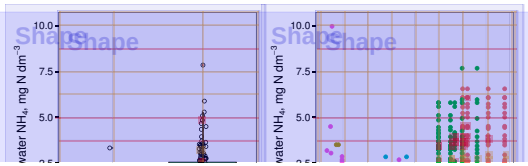




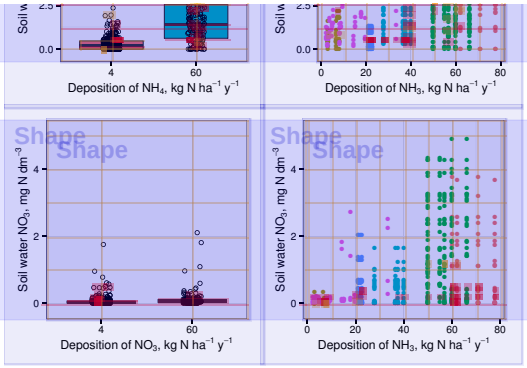
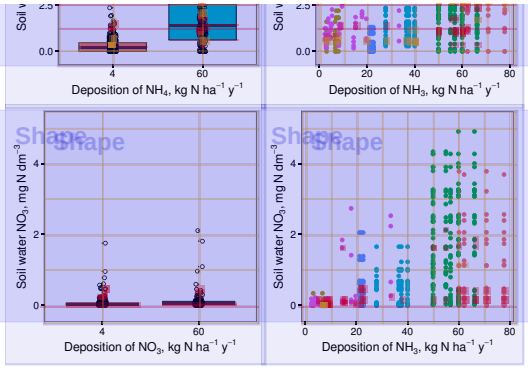


Figure 5. NH_4^+ and NO_3^- concentrations in the soil water in the experimental treatments. In the right-hand plots, colours show measurements grouped by distances downwind of the fumigation source.



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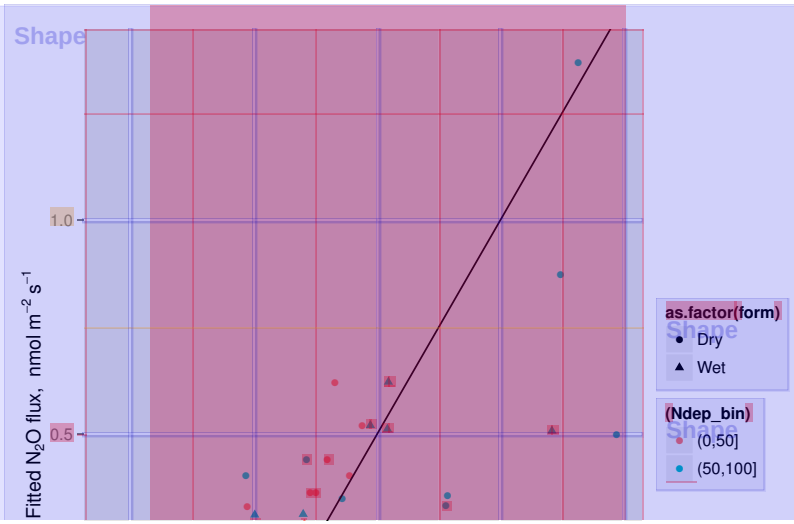
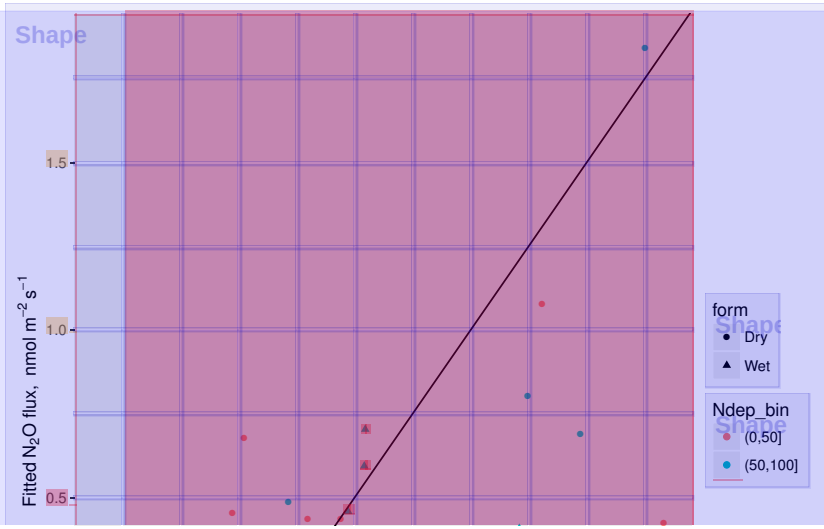




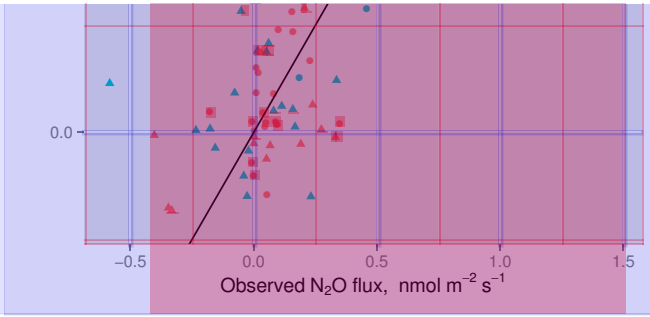
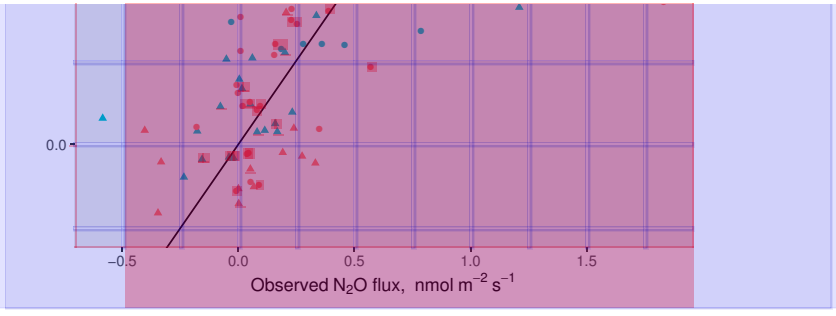


Figure 6. Mean N_2O flux at each chamber location fitted by the PLS model based on vegetation species composition plotted against observed mean fluxes. The data are grouped by nitrogen treatment form (symbol shape) and dose (symbol colour).



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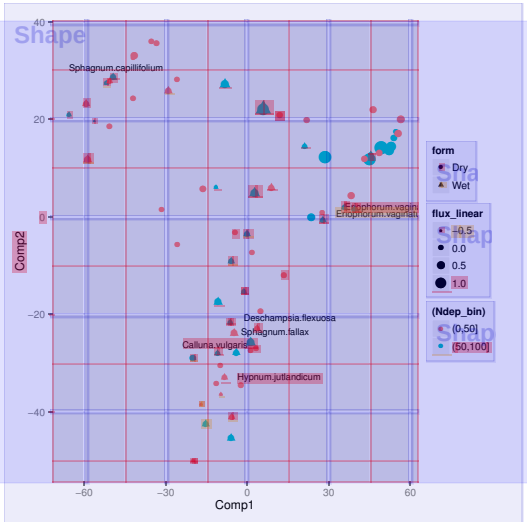
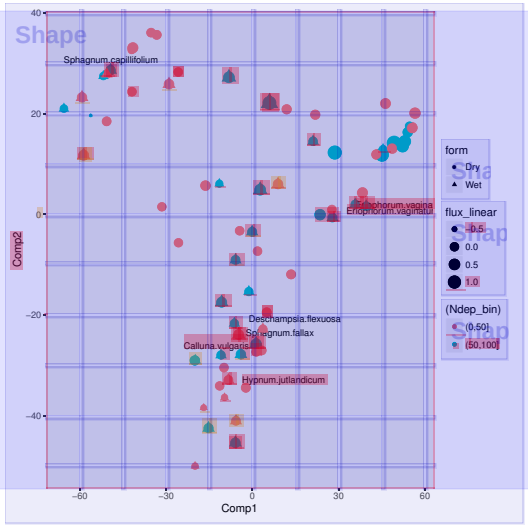


Figure 7. Biplot showing the scores for each chamber location for the first two PLS components. The first two PLS components represent the orthogonal indices of vegetation species composition which are the best linear predictors of mean N_2O flux at each chamber location. The



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