Response to Reviewers

Peter Levy

We thank the reviewers for their thorough reading and constructive criticisms.  We hope we have answered their points where possible, and justified where this was not possible.   Editor and reviewers’ comments are shown in *italics*, our responses in normal font.

*Associate Editor’s comments:*

*1) The paper is about the response of a peatbog vegetation community to N addition and the cover responses of more than 60 species have been recorded. Nevertheless, only the responses of 6 species are presented. Please make clear on what this choice was based  and why were not more species taken into account ? I also would like to see more integrative measures of vegetation responses such as diversity indices or dissimilarity indices among treatments.*

 For the univariate analyses, we have now explained why we chose these  six particular species. Much of the focus of the paper is on the multivariate analyses, so all the species were taken into account.  We deliberately do not focus on the simple diversity indices or dissimilarity indices because they are not very robust when the species pool is small, as here (typically 12 species per sample), and are sensitive to observer/classification error.  The multivariate methods we use are much more robust in this respect (den Brink and Braak 1999).  However, we now include the results for Shannon-Weiner diversity and evenness in the Supplementary Information.

*2) The paper is rather descriptive with regards to the vegetation responses. You need to place these responses into a mechanistic context by providing (eco)physiological explanations for the responses of the most important species.*

We now add discussion of this as far as we think useful.  However, we are limited in our understanding of the mechanisms underlying the vegetation change, as is

*3) The description of the (mostly rather common) statistical methods is rather lengthy. Please make this more concise.*

This has been largely removed from the Introduction and Discussion, detailed below.

*4) The writing is in many cases not very clear (some examples are given by referee 2). Please go critically through the text again and try to write in a more concise manner.*

We have re-written several parts - see the responses to referee 2’s points.

*5) The figure legends are not self-explanatory (see the remarks of reviewer 2).*

*6) As reviewer 2 noted, some key references are missing. It is indeed very strange not to see the Limpens et al paper from 2011 or the more recent Robroek et al. 2017 paper being discussed. That is a serious omission !*

Results are now discussed in relation to both of these papers.

*———————-*

*Reviewers’ comments:*

*Reviewer: 1*

*COMMENTS FOR THE AUTHOR*

*This manuscript presents results on changes in the vegetation at a bog peatland in response to the long-term application of nitrogen (in the forms of ammonia, ammonium and nitrate), alone and in combination with potassium and phosphorus. The data are derived from measurements of plant cover, generally in alternate years, and related to the amount of N added and the duration of the study. The conclusions are that nearly plant species are affected by the enhanced N deposition, resulting in a decrease in species coverage, variable among the treatments and a series of statistical analyses were applied to establish the relationships among the drivers of change and the species coverage. Consideration was given to the reasons for differences among treatments and species and caparisons were made to other long-term fertilization studies in bogs and fens. Although ammonia appeared to be the most damaging of treatments, its infrequent significance in N deposition means that it is not likely important, whereas ammonium is likely to have the greatest effect on vegetation cover.*

*The manuscript draws upon a large data base, collected over many years, to reach the results which are generally well presented, though the combination of 7 Tables and 8 Figures may be a bit excessive. I offer the following general, and then specific, comments:*

*The vegetation date base is derived from estimates of cover of > 60 species, of which 6 are presented, a mixture of 3 mosses, 2 vascular plants and a lichen. Why were these species chosen: because of their important coverage (seems that Cladonia was pretty rare), or range of plant functional types? Did any of the other > 50 species show any interesting patterns (were there any ‘winners’, rare species able to take advantage of the demise of others)?*

*Vegetation coverage, as described here, is a fairly simple metric to obtain, but there is the possibility of bias and subjectivity, especially over 14 years, which is admitted in the concluding paragraph (p. 21). It would be more appropriate to bring this caveat on the data earlier in the manuscript, perhaps in the Methods, where this metric is given scant attention (compared to the detail on N treatments, and which may be available in other publications). Point count methods, although tedious, may give a more informative estimate of changes, and are also non-destructive.*

*There are clear differences in the response of the plant species to the treatments, but one wonders whether more consideration could be given to the ecophysiology of the species to explain the pattern. For example, it seems that Eriophorum is the ‘winner’ under some treatments: why is this, does work elsewhere (e.g. Toolik Lake, drained peatlands) give any indication why it responds well to disturbance (apart from large ammonia additions)? Similarly, Pleurozium schreberi increases with PK addition: this moss is generally found below tree canopies in boreal forests, not usually in peatlands except on treed hummocks. Does shading play a role in its success?*

*Comparisons with other similar experiments are made, to put these changes in a larger context. Although I do not dispute that all field experiments of this nature are ‘unreal’, I think that some of the comparisons are not well founded on methodological differences and, being from Mer Bleue, have drawn attention to some of the possible misinterpretations of comparisons with that site.*

*Specific comments by line number:*

*49 and elsewhere – ‘relatively’ is used here and elsewhere, yet ‘close’ (and other terms) are ‘relative’ (here compared to far), so I think relatively is redundant.*

We indeed used the term “*relatively*” nine times, and we have deleted five of these . In the remaining cases, we don’t think it is redundant - we are meaning that the modifier (“close”, “large”, “few”) has to be taken in context, and not in absolute terms.  In this first case - [UK] “peatlands in relatively close proximity to emission sources” - we don’t mean that peatlands in the UK are absolutely proximal to emission sources, but they are close compared to elsewhere (e.g. North America, Russia).

*63 Mer Bleue fertilization is still going on, 18 years in some plots.*

We add “(and are on-going)” for clarity , but note that we are referring  to the results reported in (Bubier et al., 2007, Larmola 64 et al., 2013), in which treatments had been “applied for up to 12 years ” - not 18 years.

*Also, treatments were in 2 mm of water applied at three week intervals seven times during the growing season, starting at the beginning of May and ending generally at the end of August. This compresses the additions into short pulses, with large concentrations of N and P in solutions in contact with leaves and mosses, which one admits is ‘unrealistic’. While not wanting to be picky, it is stated that the sprayer system at Whim was triggered every 15 minutes, while there was sufficient rainwater in the collection tank, temperature was >0oC and wind speed was >5 m/sec, resulting in 120 applications/yr. To what extent does this replicate the rainfall patterns at Whim? What is the interception capacity of the vegetation at Whim (how much ca it retain, before water passes directly through to mosses and then soil water? Perhaps equally important is that at Whim part of the application was made outside the growing season, whereas at Mer Bleue (and Degero Stormyr), it was entirely within the growing season. This further complicates comparison of effects with the same annual N addition.*

We have corrected “five to six applications per year” to  “seven applications per year”.

The distribution of treatment applications over the year are shown in Fig 2 in (Leeson et al. 2017), and show that  the additional nitrogen deposition occurs approximately evenly over the whole year except for the middle of winter.  This follows the even distribution of rainfall at the site across the year, with only a discernible dip in spring.  The treatment applications necessarily follow the rainfall pattern to some extent, as it relies on rainfall being collected (hence a short-term drought means that treatment applications also stop).

We agree that there are differences that complicate comparisons, and now say this in the text, but we still think that the most obvious difference is in using  ~120 annual applications versus 7.

*150 Over a 14 year period, are there significant changes in seasonal or annual temperature and precipitation, which may influence the vegetation changes observed? Similarly, has there been a consistent change in atmospheric N deposition over the 14 years?*

There are slight trends towards a decrease in temperature and in increase in rainfall over the period 2002-2016, but there is no evidence that this variation is driving change in the vegetation - it doesn’t explain the variation statistically, and we left this analysis out of the paper on grounds of space.  We now include this in Supplementary Information.  Similarily, there is no clear change in atmospheric N deposition at the site over the 14 years (although national-scale emissions have decreased over this time).  The background deposition is small compared to the experimental additions.

*270 I think this should be Hill’s.*

Corrected.

*433 Is there any evidence for significant changes in the peat characteristics, such as changes in degree of decomposition and bulk density and depression of the surface? At Mer Bleue, for example, the loss of Sphagnum in the highest fertilization treatments has resulted in a decline of the peat surface, attributed to a combination loss of architectural integrity created by the Sphagnum, replacement by shrub leaves as the major litterfall and faster rates of peat decomposition.*

No, there is no equivalent change at Whim, probably because the shrub layer not not increased nor the *Sphagnum* declined as dramatically.

*500 not quite clear what you mean by 0.44 and 1.4: are these the ranges of NH4-N and NO3-N, or a combination of them?*

This was not clear.  We have now rewritten as “both averaging around 0.5 g N m$^{-2}$ y$^{-1}$ and both with maxima close to 1.5 g N m$^{-2}$ y$^{-1}$”

*520 At Mer Bleue, the loss of Sphagnum could be attributed to the direct effect of increased N deposition, but also the increased coverage of shrub leaves, which shaded out the ground mosses. This latter is covered, in part, in:*

*Chong, M., E. Humphreys & T.R. Moore 2012. Microclimatic response to increasing shrub cover and its effect on Sphagnum CO2 exchange in a bog. Ecoscience 19: 89-97.*

*Thus, shading, as well as different method of application of N may account for differences in the rate of disappearance of Sphagnum. Also, of course, increasing leaf coverage also means less application acting directly on the underlying Sphagnum.*

We now discuss shading as an additional difference between the experiments and refer to the above paper.

*551 As noted above, what was the variability in temperature, precipitation and water table position and to what extent does this help explain the temporal patterns?*

As above, the  variability in does not seem to explain the temporal patterns in the vegetation, but we now include this in the Supplementaty Information for completeness.

*———————-*

*Reviewer: 2*

*L 7 Abstract – it si absolutely necessary to include information on the background Ndeposition rates and its relation to pre-industrial values, already in the abstract, otherwise the reader has no possibility to evaluate the data and its conclusion.*

This has now been added.

*L 14 useless information ”2.2 millilion records” in the abstacts*

This has now been deleted.  We feel it conveys the scale of the sampling effort, so is not quite “useless”, but defer to the reviewer.

*L 17-19 unreadable sentence. “… all six species declined …… The exception was the ….. which dramatically increased. You can not first state that all six species declined and directly after state that there was one exception, E. vaginatum, that dramatically increased. This make no sense at all. I suggest something like “The responses differed among species and nitrogen forms, and/but five out of the six species declined, ….. The exception was ….”*

Corrected as suggested.

*L 21 unclear what you intend to convey here. What is a principal response curve? (I have >30 years experience from multivariate analysis of environmental variables but it is not at all clear to me what this is). Skip the last sentence (l23-24)*

Reworded more clearly as “Multivariate analyses identified similar responses to nitrogen dose across treatments”.

Last sentence deleted.

*L 29-31 hard sentence. Especially last part “….., having dissolved in water on the leaf surface”. I do not understand what you are trying to say.*

This was been reworded more clearly.

*L 38-41 Synthesis: it seems very odd to me to in the synthesis jump to one species “S. capillifolium” that has not at all been mentioned earlier. Make the synthesis paragraph a general conclusion on bog vegetation responses to the experimental treatments.*

We now make it clear that *Sphagnum capillifolium* is chosen as the example because it is the most common species of the keystone genus *Sphagnum*. The Synthesis paragraph is now a bit more general, but its point is to synthesise our measured experimental responses with actual N deposition rates to give our estimate of the magnitude of the real-world responses to the different N forms. The “*general conclusions*” have been stated previously in paras 2-5.

*L 107-135 the large focus on the statistical analyses in the introduction does not add any value to the paper. A few lines on the statistical analysis might be reasonable. This paper is not at all on development of using statistical tools on plant community data. Remove most of this.*

We have removed most of this as suggested.

*L 146 please add data on water table (WT) depths. It is a master variable describing the conditions at the site. The occurrence of Hypnum jutlandicum and Pleurozium schreberi makes me a bit suspicious if the site is disturbed in any sense. I am not used to find any of those species´ in undisturbed mires.*

WT depth is already mentioned (L151) but we add some further description, and include the time series in the Supplementary Information.

We have now added some background to the species chosen, with their status in the UK.   In the UK, occurrence of *Hypnum jutlandicum*and *Pleurozium schreberi*does not particularly indicate disturbance - they are frequent species in the  common mire communities defined by the UK National Vegetation Classification (NVC, Rodwell 1998, Vol 2 Mires & Heaths )

*Pleurozium schreberi* is a constant in M19 (the 3rd most frequent species in plots classed as M19 ) and present in M17 & M18.

*Hypnum jutlandicum* “commonly occurs on a wide range of communities in the UK, although more prevalent on heath” (Rodwell 1998).

*L 149-150 for what time period are the weather/climate data valid?*

We now state that all statistics are for the period 2002-2016

*L 156 what is “unmanaged Calluna vulgaris? To a non UK citizen it is not at all clear exactly what is referred to. Please specify.*

We now clarify this as “The vegetation was dominated by *Calluna vulgaris* which had not been managed by burning or grazing.”

*L 188 Unclear, it says “…. total ….” But continuing reading indicates the given rates are in addition to the background i.e. 0.8 g N m-2  yr -1. Rephrase. Make sure to give true rates of N annually available to the plots i.e. background + additions.*

This has been rephrased to clarify any ambiguity.

*L 195-196 how can four blocks with one treatment level in each result in 44 plots? Please reformulate.*

We now explicitly list them: There were four blocks, with eleven combinations of the treatment levels in each (1 control, 3 NH$\_4^+$ levels without PK, 2 NH$\_4^+$ levels with PK, 3 NO$\_3^-$ levels without PK, 2 NO$\_3^-$ levels with PK), to give a total of 44 plots.

*L 196-200 a total of 120 applications yr-1 worries me. Not because the total amount, that is excellent. However, 120 applications yr-1 means to me that if the system potentially is trigged every 15 minutes it is only at a VERY small proportion of the potentially occasions that nitrogen is actually added. (assuming that air temperature is above 0oC six months per year the potential number of occasions is 6\*30\*24\*4=17280. THIS WOULD GIVE A PROPORTION ). My problem with this very small portion of actual additions relative to the number of potential additions is that it might be VERY high temporal/seasonal bias, i.e. when the containers have enough of rain water. DATA ON THE DISTRIBUTION OF OCCASSIONS FOR N-ADDITIONS ARE NECCESSARRY!*

The distributional data are published in Fig 2 in (Leeson et al. 2017), and show that there is there is very little temporal pattern.  With some random variation, the additional nitrogen deposition occurs over the whole year except for the middle of winter.

*L 227-229 this is not correct. MLR maximizes the explained variance in the response variable (Y). PCA does not relate X and Y at all. What is used is PCR (principal component regression) which is to first maximize explained variance in X and use the derived PC´s in an MLR to explain Y. The latter then maximizing the explained variance in X. PLS maximizes the co-variance matrix of X and Y.*

It is not clear what the referee is saying is not correct - “*PCA does not relate X and Y*” - we don’t say that it does.  “  *PLS maximizes the co-variance matrix of X and Y.* ” - we say this on L231-232.  However, as the general feeling is that the description of the statistical methods is unnecessarily long, we have deleted these sentences anyway.

*L 293 “borderline significance” !!!! what is this? state in M&M what levels you refer to for stat significance and stick to that.*

Because we state the *p* value, it is clear what this is, but we have replaced the word “borderline” with “close to” in one instance.

*L 322-323 fit hyperbole or similar function to data that reaches a plateau after a time period of change. Btu truncating data is also fine.*

We chose truncation as the simpler of the options because it does not introduce extra parameters to estimate.

*L 345-352 Abstract conclusion paragraph!!. Use this paragraph or something very similar as the “conclusion paragraph” in the abstract. This one summarize in a very efficient way the major findings from these years of the experiment.*

Text moved to abstract as suggested.

*L 360-361 did you stop applications after 2011?*

No, the vegetation appears to recover even though the treatment application continued.  We now re-iterate this explicitly here.

*L 423 not a problem. As said before, describe data with another appropriate function e.g. a hyperbole.*

Truncating is simpler as it does not introduce extra parameters, but it does require us to find the extinction point, as we discuss here.

*L 433-435 this is an obvious point to refer to the findings in Robroek et al., 2017 actually counteracting such an interpretation.*

???

*L 436-439 Please stop repeatedly teach basics about the main objectives of using multivariate techniques. Does not fit into a specific journal article like this.*

Text deleted.

*L 439-440 this is also not generally correct. The outcome from general data decomposition methods, e.g. PCA is mathematically very straight forward. PLS to the contrary is much more abstract.*

Yes,  PCA may be mathematically very straightforward, but it is an abstraction of the data which the non-cognizant find hard to interpret. We refer explicity to PRC, not PLS, and this is more easily interpretable in the case of an experimental effect over time, indeed, this is its *raison d’être*  (den Brink and Braak 1999) .

*L 488 Please present a reference to this statement. Why would the occurrence of S. capillifolium be a prerequisite for peat bog systems? Numerous peatlands have hardly any hummocks at all. I assume S. capillifolium occurred in hummocks.*

We made an over-statement that *Sphagnum capillifolium*  is “fundamental”.  We have revised this to make it clear that *Sphagnum capillifolium* was chosen as the example because it is the most common species of the keystone genus *Sphagnum*.

*Figure 1 this is semi-raw data. Move to SI. It is no use to have this in the result section.*

If “it is *no use to have this in the result section* ”, we think better to delete it.???

*Fig 2 - 7 The information in the legends are very much incomplete making its interpretation very hard. You must state in the legends what the different symbols represents, what the lines represents, the grey areas, why is it different amount of data points in the different graphs etc. It is also very hard to connect the different grey areas to a specific line. Please also indicate which of the models of change that are significant.  Also, I would replace the start “Change in cover …” to “ The annual averages/plot cover values of Calluna vulgaris …”. That is what is actually displayed, whether it is a change or not depends on response.  What I also do not understand is what symbols represents the plots without any additions, i.e. just the background levels. Must not that data be the same in all plots or is not data from the control plots included in the graphs?*

*Fig 8 add info on how much variance is explained by PC1. Also add what is the input data to this analysis. Is it all species recognized or species over certain coverage or what?*

*Make sure to clearly explain what the line represents. If you indicate even nonsignificant regressions it has to be clear which ones are significant and which ones are not. If you prefer to include also non-significant regressions I suggests differentiate by having the significant ones as continuous lines and the non-significant ones as hatched lines. Why are the number of lines differing between plant species and treatments? Even if it might be stated in M&M IT HAS TO occur also in the figure legends.*

*Figure 9 according to M&M individual PCA´s are run for each N-form with year and species abundance as inputs. However in this graph you have only one set of loadings across all N-treatments. I do not follow that. Please state that you have chosen to display only control and highest N-dose PRC´s in these displays (if that is the case).*

Separate PRC analyses were run for dry-deposited (NH3) and wet-deposited forms (NH4 and NO3).   Figure 8 shows the former, Figure 9  shows the latter.  We now make this clearer in the M&M text.

*Figure 10 Sorry. This legend is just ridiculous. I have worked with PLS >30 years and I have no help from legend how to interpret what is displayed. Skip the first half of the second sentence. That is some general description of the objective by PLS. Then clearly state what data is in the X- and Y-matrices respectively. I guess the y- and x-axis´ represent score values, right? Then state that in the axis labels If so it should be the score values of the Y-components, right? Now I realize that the chemistry loadings are the same in all three panels. That is confusing. Just display them ones, preferably in a panel by them self as for the species loadings.*

*Figure 11b I do not understand how “dose” and “time” goes in parallel in 11b. this make no sense at all to me. Or is “time” only relating to the control plots and dose to the “treatment plots”?*

We agree this was unclear.  Higher doses tends to move plots to the M17 class but is hard to show this graphically, separate from the effect of cumulative dose over time.  We have removed the “dose” arrow and added text to the caption.???

*Figure 12 ADD TITLES TO THE AXIS´. What does the colours represent? I must not need to guess. It has to be clearly stated in the legend.*

The first sentence in the caption explains what the colours represent - no guessing is required.  These are very obviously maps, so it is clear that the y-axis is North and the x axis is East.  We remove the axis labels in case this causes confusion, and they serve no real purpose here.???

*References*

*Some references that I consider key ones are missing making me quite worried about the quality*

*of the theoretical background. E.g. Limpens et al 2011 Climatic modifiers of the response to*

*nitrogen deposition in peat-forming Sphagnum mosses: a meta-analysis New Phytologist,*

*191:496-507, DOI: 10.1111/j.1469-8137.2011.03680.x. and the recent Nature Communications*

*paper by Robjoerk et al 2017. Especially the missing of Limpens et al 2011 is very hard to*

*understand while it is a synthesis of the effect of N on Sphagnum mosses. (if the authors disagree*

*with the main conclusions in Limpens et al 2011 that is absolutely fine. But than it has to be*

*addressed in the ms. Just leaving that paper out seems very odd.*

*But also the Robroek et al paper is definitely valid according to me. (at least one of the authors of this ms is also co-authoring the*

*Robroek et al paper!!).*

(1) Robroek et al 2017 find that little effect of N deposition (their Supplementary Figure 2)

(2) Personally, I do not find the identification of the two clusters, and therefore most of the analysis, in the Robroek et al 2017 paper convincing.  I can’t speak for all my co-authors.

(3) They focus on functional redundancy of species at different sites,

whereas we look at change in time with experimental addition

# References

den Brink, Paul J. Van, and Cajo J. F. Ter Braak. 1999. “Principal Response Curves: Analysis of Time-Dependent Multivariate Responses of Biological Community to Stress”. *Environmental Toxicology and Chemistry* 18 (2). Wiley: 138–48. doi:10.1002/etc.5620180207.

Leeson, Sarah R., Peter E. Levy, Netty van Dijk, Julia Drewer, Sophie Robinson, Matthew R. Jones, John Kentisbeer, Ian Washbourne, Mark A. Sutton, and Lucy J. Sheppard. 2017. “Nitrous Oxide Emissions from a Peatbog after 13 Years of Experimental Nitrogen Deposition”. *Biogeosciences* 14 (24). Copernicus GmbH: 5753–64. doi:10.5194/bg-14-5753-2017.