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Dr. Roland Bol
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- Submission of revised research paper bg-2015-283 to Biogeosciences

Dear Dr. Bol,

we have now revised the paper by addressing all comments of the reviewers. The main concerns focused on (I) the phrasing of the objectives and expectations raised by our use of “the gradient approach” when describing the design and results of the study, (II) an inadequate presentation of biomass and growth data that makes interpretations difficult to follow, (III) the use of statistics of data presented in Figure 3, and (IV) the approach taken to sample the ^{15}N in biomass that we had applied to the mesocosms. These concerns and criticisms were all valid in our opinion. We think that we could satisfactorily make all the needed changes regarding these points and remove also some unclear writing. Where needed we now better acknowledge the limitations in our approach, such as in the beginning of the Discussion section.

Please find a point by point response with a detailed description of changes made below.

Reviewer 1

The authors show that a legacy of high N deposition reduces N retention in Sphagnum-dominated ombrotrophic peatlands, as well as increases porewater inorganic [N] and transfer of N to shrubs, though Sphagnum N absorption was still high at sites character-ized by high N deposition. Contrary to other studies, mosses preserved their capacity to filter N at deposition levels >1 g N m-2 yr-1. The gradient approach utilized in this study may better inform responses of ecosystem function to increased N deposition, as opposed to fertilization studies that may occur over a short timespan. Overall, the paper falls within the scope of BG and contributes significant new knowledge regarding peatland N dynamics, but could be improved to increase clarity to readers. The following changes/clarifications will help improve this paper.

Perhaps shift the focus of aim 1 to specify which transformations were assessed (ie. NO3- to NH4+) since some were not addressed (ie. organic N).

> We acknowledge the criticism by reviewers 1 and 2 and changed the scope of the objectives. The hypothesis and expected patterns in N retention and mobility are described in the paragraph above the objectives, which were too imprecise and general. We now specify more clearly the focus and kind of information needed to test the hypothesis:
>
> "To address the hypothesis, we specifically examined:
>
> 1. differences in elemental N content in the peat and vegetation before the experiment and dissolved nitrate, ammonium, dissolved organic and total nitrogen concentrations during the experiment," (…)

Regarding aim 2, the N deposition gradient is referred to throughout, which led me to expect regression figures of responses across the gradient.

> We removed the "N deposition gradient" as a phrase from the manuscript because it suggests (as mentioned by both reviewers) that we use a regression approach in analyzing the data. Regression analysis is, however, mostly not a useful approach with only five sites that we could examine with the available resources and replication.
>
> Our new specific objective 2 and 3 instead refer to
>
> 2. differences in the distribution of ^{15}N stemming from experimentally deposited ^{15}N-nitrate between plant functional types, peat, and dissolved nitrate and ammonium , and (…)

3. differences in the 'efficiency' of the vegetation and peat to retain the deposited ^{15}N during the experiment.

I would have liked to see more connection between results and their broader significance, perhaps in the scope of changing N deposition rates. Perhaps bring some of the ideas from the intro back into the end of the paper.

> We carefully examined Introduction and Discussion sections but could not really identify issues where we could provide additional considerations based on the kind of data that we have obtained and presented. This specific paper is primarily concerned with the fate and mobility of deposited nitrate-N between plant functional types, peat and dissolved N, and secondarily with differences in the quantity of solid and dissolved N pools in the plant-peat system. The data are discussed in the context of the differing long-term N deposition received by five sites. The distribution of ^{15}N in the mesocoms and differences between the sites we do discuss in the Discussion section and we also attempt to explain our findings by referring to auxiliary data, such as differences in plant biomass and growth rates of mosses.
>
> We acknowledge that needed information was hidden in the Supplementary Materials, which was the result of our attempt to make the manuscript shorter in the initial revision for BGD. To make the link between the fate of N and background data on the vegetation stronger, we added one more figure with biomass data (now new Figure 1) and also added three more figures to the Supplementary Materials. These figures show growth of vascular plant biomass, moss growth and a gradient analysis of C/N ratios in Sphagnum before, during and after the experiment. We now refer to these data in the Results and also in the Discussion section (see also below).
>
> Some of the significant implications are presented in the conclusions. The main findings are that (I) long-term N deposition leads to higher N mobility in the plant-peat system, and that (II) Sphagnum mosses retains the ability to prosper and take up N in the sites under the chosen experimental conditions. Because *Sphagnum* mosses kept functioning the impact of N deposition on the fate of N seems thus more gradual than sometimes thought. This is what we point out and we think much more cannot be said.

I don't know that you need to address this in the paper, but I'm curious as to whether you think high N deposition increases lateral N movement (perhaps when the water table is raised?) in peatlands and how this could play into N transformations in the field.

> Reviewer 1 and we probably agree that more lateral export should occur if high dissolved nitrate and ammonium concentrations are attained, such as in cores from Frölichshaier Sattelmoor. We also briefly refer to this possibility in the Conclusions section. The possibility for such export drives some of the research on N cycling in peatlands, yet in the context of the kind of analysis presented here a discussion of the magnitude of N export would be speculative.

16928 and Fig. 4 – Retention efficiency of peat and vegetation pools was lowest at intermediate N deposition, but it's unclear to me from the discussion why retention efficiency peaks at this level and why this response is limited to NO3-.

> This is a good point. We cannot explain this finding. The differences were not statistically significant, though, and may just be the result of random variation. We acknowledge this fact now in the Discussion section and clarified the misleading phrases regarding the nitrate (P 16, L 20-25).

16929, 20 – It is suggested that Sphagnum mosses still filter inorganic N under high N deposition perhaps by forming more biomass.
This whole section of the discussion is really interesting and could be stronger if you show data on biomass production.

> We improved this section by adding the new Figure 1 (biomass) and further Figures (plant growth) in the Supplementary Material and refer to these data on P 18, L 1-7.

16931, 25 – Reword. Seems like words are missing (1st sent of 4.2)

> This error was corrected.

16933 - Not sure that I'm following the last paragraph.

Also reviewer 2 had doubts about this paragraph. After looking through it again we decided to delete it. It was not supported by data and only added confusion to the manuscript.

Figure units? ie. Fig. 1 - mg N per g of...?

We changed all Figure units where needed.

Table 2 – Is this porewater N? Could clarify this in the caption.

We added an explanation to Table 2.

Fig. 6 – It may be helpful to the reader if the caption notes that the scale of the X-axes vary across panels to improve clarity. It also appears that font size is smaller for site labels within the bottom panels.

We added caption notes wherever different scales were used.

Fig. 7 – Again, may be useful to note the differences in the scale of X-axes.

See above.

Reviewer 2

I like the idea of the paper, which investigates responses of various N retention pools in peatland ecosystems to different atmospheric N deposition regimes. Also the part of the study relating different N pool sizes to the N deposition regime is valid. The approach taken with the 15N tracer is however problematic and has to be interpreted with care, because the applied methods can not distinguish between 15N assimilation and 15N stuck to the surface of the tissues.

This concern is justified but we took precautions that we omitted to mention in the manuscript before. These are (I) a total of 48 irrigation events with the tracer, in which each one should rinse off some of the previously physically attached ^{15}N tracer. (II) We used distilled water to rinse the tracer off after each irrigation event with the tracer and adjusted the water table at the same time. It is thus unlikely that more than a small portion of the tracer was physically attached to the surfaces when we terminated the experiment and sampled the biomass and peat. We acknowledge, however, that we cannot quantify how much ^{15}N was physically attached to surfaces and now also mention this in the beginning of the Discussion section (P.16, L 6-8). See also comments below.

Further, with the applied methods and
reported results I don't see the main aims of the study (listed as followed) met

1. transformation pathways of N within the peat soil, e.g. from inorganic N to organic
N and from nitrate to ammonium,
2. differences in N transformation in relation to the legacy of atmospheric N deposition,
and
3.whether mosses from "clean" sites would be able to assimilate N more efficiently than
mosses from "polluted" sites

The concern is correct in our view. We thus restated the objectives to better describe how the hypothesis outlined in the paragraph above was examined.

To address the hypothesis, we specifically examined:

1. differences in elemental N content in the peat and vegetation before the experiment and dissolved nitrate, ammonium, dissolved organic and total nitrogen concentrations during the experiment,
2. differences in the distribution of ^{15}N stemming from experimentally deposited ^{15}N-nitrate between plant functional types, peat, and dissolved nitrate and ammonium , and
3. differences in the 'efficiency' of the vegetation and peat to retain the deposited ^{15}N during the experiment.

add. aim 1: Figure 7 and 8 show excess 15N NH4 in pore water. Since NH415NO3 was used as a label any recovered 15N NH4 indicates transformation. However, the results do not show any 15N recovered as organic N, which the authors also acknowledge in the discussion.

We agree with reviewer 2. We corrected and adjusted the objective.

add. aim 2: to answer whether there are responses to the legacy of atmospheric N deposition a different statistical approach needs to be taken. When evaluating a response to a N deposition gradient regression analyses need to be employed. In case of Figure 3 it would be an ANCOVA with N as continuous predictor and dry/wet as categorical. It is certainly not suitable to use t-tests together with an ANOVA. For the regression the 3 cores per site need to be averaged prior to statistical analyses to avoid pseudoreplication. The gradient approach would be in line with how the study is presented in the introduction, the discussion and for most of the description of the results. Hence, either the scope of the paper or the stats need to be adapted.

We mostly agree with reviewer 2 and are grateful for the hint regarding ANCOVA. We (I) conducted the ANCOVA analysis for the data presented in former Figure 3, now Figure 4 and added this Information to the methods section (P 8, L 20f) and to the Results section (P 11, L 5-18). (II) We eliminated the t-test from the manuscript. (III) We added one regression analysis, between long-term N deposition and molar C/N ratio in *Sphagnum* sampled before the experiment, which was significant, to the Supplementary Materials (now Fig. S4). We added the information also in the Results section (P. 11, L 27f).
We did not change the entire data analysis, though. Five sites are in our opinion too few for a useful regression analysis. We rather treat the sites as individuals with variation within and between the sites, as we did before. As proposed by reviewer 2 we consequently eliminated the focus on the “gradient approach” that insinuated a focus on regression analysis in this paper.

add. aim 3: to address assimilation of the applied 15N different methods incorporating a removal step of superficial 15N (that has clearly not been assimilated) need to be used.

As pointed about above we acknowledge this criticism. We maintain, however, that most of the ^{15}N tracer was unlikely to be physically present on surfaces due to the design of tracer application. There were (I) a total of 48 irrigation events with the tracer, in which each one should rinse some of the previously physically attached ^{15}N tracer off. (II) Each time we used distilled water to additionally rinse the tracer off after irrigation with the tracer. Thus there should have been only a small fraction of the total amount of tracer applied physically present on plant surfaces when terminating the experiment. As mentioned above we added a sentence in the Discussion section to draw the reader's attention to this point. Please see also comment on uptake by Sphagnum mosses below.

Please make clear throughout the result section where results from the experiment are reported versus pre- treatment results from the gradient.

We examined the manuscript regarding this comment and added the required information wherever possible.

add chemical analyses: please mention on which material those analyses were conducted

We now mention the materials the analyses were conducted on and also repeat how this material was obtained to better link the information to the previous section.

add nitrogen uptake by plants: it is not surprising that highest retention of 15N was found in Sphagnum species during the dry period. Sphagnum has the highest surface to mass ratio of all investigated plants in the study. When dessicated Sphagnum shows little to no assimilating activity and the applied tracer remains sitting on the surface. Under wet conditions some of the tracer gets diluted.

In the previous version of the paper in BGD we did not report growth of *Sphagnum* mosses during 'dry' and 'wet' periods. We corrected this omission in the new version of the paper. The data show that *Sphagnum* mosses actively grew also during the 'dry' period (see Supplementary Material, Figure S3). We thus do not think that *Sphagnum* was incapable of taking up N during the 'dry' period in the experiment. Also photosynthesis in chamber measurements, which we do not report in this manuscript but can provide on request, remained almost unchanged between dry and wet periods in the experiment. The ‘dry’ conditions thus did not impede biomass growth and N demand. The conditions were ‘dry’ only in that we lowered the water table; however, the cores were irrigated twice a week and mosses and peat stayed moist in the climate chamber, where also an air humidifier had been placed. The situation was thus different from what to expect under drought conditions in the field when *Sphagnum* desiccates.

The applied method does not allow to draw conclusions about 15N assimilation by any of the investigated plants because an unknown proportion of the applied tracer will remain on the surface of any leaf. “The shrubs in cores from Frölichshaier Sattelmoor were particularly efficient in retaining the tracer and accounted for 91 % of the retention in vascular plants (Table 2).” Table 2 shows nicely that the 15N retention is strongly connected to the leaf surface area. Despite the fact that Graminoids show the highest total N content (Figure 1) their 15N retention is compared to the other “pools” minimal. Therefore, vegetation cover estimates need to be reported in the study and ideally the surface area of the respective “pools” should be taken into account.

Regarding the fate of the tracer see our comments above. Again we acknowledge that one could argue this way by looking at the data that were available. Taking into account the plant biomass, however, that is now added in the new Figure 1, the picture looks somewhat different. In the N polluted LV site, for example, shrub biomass was half that of the less polluted WM and CV, yet the same percentage of ^{15}N was recovered (Table 2). Given the biomass and the identical tracer application, shrubs thus retained about twice as much N in the polluted LV site than in the WM and CV sites. In the highly N polluted FS site, ^{15}N recovery from shrubs was more than twice that of the WM and CV sites, yet the shrub biomass was only 30% larger (new Figure 1). These data cannot be explained by physical attachment of the tracer to the biomass assuming that surface area is proportional to biomass. Moreover, the other indicators of N transfer into the peat, where the shrubs would take up the N label, support this pattern. In the shallowest peat in both LV and FS sited more ^{15}N-nitrate was found than in the other sites (now Figure 8). Nitrate concentrations were strongly and ammonium concentrations somewhat elevated compared to less N polluted sites (now Figure 6).

The graminoids to a great extent grew too tall to be sprayed on, yet they were effective in retaining N when standardized to mass (now Figure 5). They were less effective than the shrubs, which may be caused by deeper rooting of these plants that contain aerenchyms and poorer access to the tracer which entered from the surface.

add. Figure 1: what is the unit mg/g what? dry mass? N content from before or after the incubation?

We added the information.

add. Figure 2: what is the unit? Why square meter? How does that work particularly for the shrubs and Graminoids? I would assume the pools have a volume?

We standardized to surface area because this measure takes into account how much biomass/peat mass is present per area. This way the relative importance of N pools can be visualized.

add. Figure 4: “mass” Wet or dry? And, retention pools averaged over wet and dry?

This is retention per dry mass. We added this information to the Figure caption. The retention efficiency is calculated for the entire experiment.

add. Table 2: NH4 and NO3 are measured from pore water?

We added this information to Table 2.

16918/10-20 please use the same units for both the N concentrations in the fertilization slurry during the acclimation period and the 15N concentrations during the treatment period.

The units are the same - mg L^{-1}. As in the acclimation period we added unlabeled ammonium nitrate and in the experimental period ^{15}N labelled ammonium nitrate the units cannot be identical, though, in terms of mass concentration.

16925/26 “Dissolved organic N” (DON) - add abbreviation to all (PN,..) when first introduced in the paragraph

We added this information.

16927/22-23 “transfer of 15N into DON were, for instance, not investigated” – in that case omit it from the aims in the introduction

We acknowledge this comment and followed the suggestion. See above.

16927/25 “While the basic pattern of N distribution appeared to stay intact, ...” – what does that mean?

We agree that the phrase was imprecise. We deleted the sentence and rephrased the section.

16929/21 “most likely by the ongoing formation of new biomass”, where is the growth data? I only see it mentioned in the abstract and the methods.

The information was somewhat hidden in a table in the Supplementary Information. Following the editor’s suggestion we had tried to find a way to show fewer Tables and Figures. In the new version we show the biomass data in new Figure 1.

16931/15 “Shrubs and some Graminoids can experience problems when faced with continuously high water levels since they lack aerenchyma” - what problems? Also the Graminoids (Eriophorum sp.) in this study do posses aerenchyma.

We agree. We rephrased to: “Shrubs can experience oxygen deficiency when faced with continuously high water levels since they lack aerenchyma.”

16931/24 “The fact that the 15N tracer penetrated deeper into the peat and more 15 N recovered” - change to more 15 N was recovered

We changed the whole sentence.

16933/3-8 “at the sites with low N inputs (DS), nitrate was first assimilated by the plant layer and later apparently partly released as ammonium from the vascular plant roots” - where can this time lag be seen in the results?

This time lag cannot be directly seen from the data. We concluded this by inference. At the sites with low N deposition, in particular Degerö Stormyr (DS), nitrate concentrations in the pore water were very small (mostly < 0.5 mg L^{-1} at DS) and it is thus unlikely that the ^{15}N tracer in the ammonium was released by microbial processes. For clarification we rephrased this section:

“At the sites with low N inputs (DS), nitrate was apparently first assimilated by the plant layer and later partly released as ammonium from the vascular plant roots. In these cores a direct microbial transformation from nitrate to ammonium seems implausible, because nitrate concentration levels were very low (Fig. 6). At the sites with elevated N inputs (FS), some nitrate apparently bypassed the living moss layer (Fig. 8c) and was converted to ammonium in the pore water.”

16933/14-15 “From our findings and earlier studies one may infer that N concentration is more important than N deposition rate for the moss filter function and the fate of N.” - how can this be disentangled with the data from this study when high N tissue concentrations result from high N background N deposition?

This whole last paragraph is not quite clear to me. First Pearce and Van der Wal (2008) found that the concentration is more important than the dose. Then Pitcairn and others (2006) concluded that wet deposition is more important than total N deposition, which is slightly different. And then I get lost: “Wu and Blodau (2015) examined if low but frequent doses of N deposition differed in their impact on the distribution of shrubs, peat mosses, and grasses in a model simulation of the long-term N fertilization experiment at the Mer Bleue peatland (Ontario, Canada).” - differed from what? compared to fewer high concentrations? Also model results always depend on the parameters fed into the model.

We agree with reviewer 2 that this whole section wasn't supported by the data and partially confusing. We deleted it.

Best regards,

Christian Blodau