

Interactive comment on “Symbiosis revisited: phosphorus and acid buffering stimulate N₂ fixation but not *Sphagnum* growth” by Eva van den Elzen et al.

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Received and published: 1 December 2016

We would like to thank the referee for his/her interest and input to the manuscript. We have considered all comments and below you can find our itemized list of responses (b) to the referees comments (a) with changes to the manuscript included (supplement). Pages and lines refer to the revised manuscript with revisions highlighted, which can be found as a supplement file.

General comments:

1a) This paper is of environmental importance as authors have discussed the symbiosis of peat plants and symbiotic microorganisms. They are of recent importance as

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they play vital role in carbon sequestration. It is an interesting paper as the outcomes obtained were not as obvious expected results. However, there are certain flaws in the approaches they have chosen and discussion made. Moreover, it does not have any broader impacts. Though the methodology is very meticulously designed; some pictures or a graphical abstract would make the approach more clear.

1b) We thank the referee for the interest and input with respect to the manuscript, and the statement that our paper is of environmental importance. We do indeed believe that our results have broader impacts, i.e. that the regulation of nitrogen fixation by phosphorus is essential for our understanding of the nitrogen cycle, and how it influences the sequestration of carbon in peatlands. Besides, high additional doses of nitrogen by phosphorus-induced nitrogen fixation to already nitrogen-loaded peatlands can well be expected to lead to serious degradation of these important C storing systems. This is important in the context of ecosystem restoration in high nitrogen areas in which the input of phosphorus is simultaneously abundant and not able to offset nitrogen loads as could be expected.

We also thank the reviewer for the idea of adding a graphic figure of the experimental set up. We added both a picture and a graphical representation as Figure 1 on Page 23. We referred to this picture in the methods section on Page 5, lines 22-23: “A graphic figure of the experimental set up and photo’s can be found in Figure 1.”

Specific comments:

2.1a) Word "symbiosis" in the title of paper is little ambiguous as the paper is only about the relation of P and N fixation and plant growth. Nowhere the microbial community had been addressed.

2.1b) We did indeed not assess the full microbial community, because that was not the purpose of our research. However, we did study the activity of nitrogen fixing microorganisms, and how this affects *Sphagnum* growth. We choose to keep the word symbiosis in the title of the manuscript, because the interaction between the host *Sphagnum*

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and its diazotrophic microbiome is central to this manuscript.

2.2a) Abstract is quite general; more specific results could have been included.

2.2b) In attempt to keep the abstract sufficiently concise, we decided to only include our main results. To be more specific, we have now added the exact rates of nitrogen fixation and the results of the light compared to dark incubations for nitrogen fixation to Page 2, line 7: "at a rate of 40 nmol N gDW⁻¹ h⁻¹" and to Page 2, line 14-15: "In addition, nitrogen fixation was found to strongly depend on light, with rates 10 times higher in light conditions suggesting high reliance on phototrophic organisms for carbon."

2.3a) Actual field conditions should have been studied and mentioned in the paper. Possibly, few revelations could have been seen like for eg. presence of other growth promoting microorganisms in natural environment which could affect the P/N uptake and plant growth.

2.3b) We thank the reviewer for this remark and agree that the context of the field conditions would benefit our manuscript. Therefore, we added a table (Table 1A) with the abiotic conditions of the field site where the mosses were collected to Page 20 and referred to it in the method section on Page 5, line 1-2: "Field conditions of the site where the mosses were collected are shown in Table 1A."

However, additional information on the microbial community of peat soil we did not assess, since this would be out of our scope, very elaborate, and a different study by itself.

2.4a) Time course studies have not been well defined.

2.4b) We thank the reviewer for noticing this. The time course of the experiment was 10 weeks. We added this information to the methods section on Page 5, line 28-29: "Treatment solutions were supplied during ten weeks, after which plant, microbial and abiotic measurements were conducted."

2.5a) Three way ANOVA is the statistical technique used here using three independent

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variable (P, HCO₃ and spp.) which is an appropriate technique. But, three way ANOVA is a technique in which dependent variables should be at continuous level. Here, some dependent variables do not come under this assumption. Moreover; the independent variable should have two or more categorical groups. Authors fail to do so. Authors can read: f Also, post-hoc analysis would make the scenario more clear as it would give precise idea of dependency of each of the independent variable.

2.5b) All dependent variables assessed with three-way ANOVA are at a continuous level, including nitrogen fixation rate, relative growth rate, number of capitula, length increment, pore water nutrients, alkalinity. Since there are only two groups for each variable, post-hoc analyses cannot be applied. All independent variables have two categorical groups: i.e. +/- phosphorus, +/- bicarbonate, *S. palustre* or *S. squarrosum*.

For clarification, we added this information to the method section on Page 7, line 19-21: "...independent variables (fixed factors) with two categorical groups. All dependent variables were quantitative and at a continuous scale, i.e. nitrogen fixation rate, photosynthetic activity, relative growth rate, number of capitula, Sphagnum length increment, and pore water and tissue nutrient concentrations."

Technical comments:

3a) Language used in the paper is pretty precise and clear.

3b) We thank the reviewer for this comment.

3.1a) Number of keywords can be reduced

3.1b) We removed 'ecophysiology' and changed 'nutrients' and 'nitrogen deposition' to 'nitrogen' on Page 2, line 25.

3.2a) Flow of introduction can be changed. Mention all the required introduction first and then mention your assumptions and reason for doing this study at the end.

3.2b) We have now made changes in order to move all hypotheses to the last paragraph

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of the introduction, as suggested by the reviewer.

On Page 4, lines 5-6 we adapted: "It is therefore expected that the addition of P can improve...N deposition areas". The next sentences of this paragraph "In addition...becomes limiting" we moved to Page 4, lines 23-26. In this last paragraph, we also made adjustments to lines 22-23 and 26-27. Leading to a changed last paragraph in lines 22-28: "Our prime research question was whether P availability and alkalinity were key regulators of both diazotrophic and Sphagnum activity, with P increase having a positive effect on both partners, and alkalinity increase a negative effect. In addition, in view of a direct mutualistic relationship between the moss and its diazotrophs, as with *Azolla* spp and its cyanobacteria, we expect that higher N₂ fixation rates provide additional N. Combined with higher P availability, this may increase Sphagnum photosynthesis and growth even further, as long as no other resource or condition becomes limiting. By testing this hypotheses, we are able to explore the nature of the symbiotic interaction, i.e. which benefits or costs the diazotrophic microbial community experience through the close association with their host, and vice versa."

3.3a) If your mentioning anything in your paper for first time mention it clearly. Like page 3, line 25, it was mentioned "our field sites"; as it was being mentioned for the first time it is better to mention the name.

3.3b) We added the specifics of our field site to this line, now on Page 3, line 29.

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/bg-2016-384/bg-2016-384-AC2-supplement.pdf>

Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2016-384, 2016.