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Interactive comment on "Symbiosis revisited: phosphorus and acid buffering stimulate N_2 fixation but not *Sphagnum* growth" *by* Eva van den Elzen et al.

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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 comment (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 is an interesting contribution in which the authors demonstrate that nitrogen fixation and plant growth are not affected in the same way by phosphorus or bicar-

bonate. Their expectation was that increased N fixation, especially when coupled with additional phosphorus, would stimulate sphagnum performance. However, while both phosphorus and bicarbonate increased N fixation, they had neutral or negative effects on sphagnum photosynthesis, respectively. Based on this discrepancy between expected result and the actual result, the authors question the concept of a "direct mutualism". I am not sure what a "direct mutualism" is. This should be defined.

1b) We thank the referee for distinguishing this manuscript as an interesting contribution. With a direct mutualism we refer to a mutualism between microbial symbiont and a plant host, in which the host benefits from a direct transfer of nutrients from the symbiont (bacteria) to the host (Sphagnum), as explained by Ho & Bodelier (2015) (see references manuscript). This is in contrast to the uptake of nutrients after die-off of bacteria, which cannot be regarded as a real and direct mutualism, as this is the case for any plant taking up nutrients from decomposed bacterial biomass.

We agree that this term should be explained to readers and defined more thoroughly in the introduction of the manuscript. Therefore we added the following clarification to Page 3, lines 17-18 and lines 21-23: "...a process that we refer to as a direct mutualism, with reference to the direct transfer of chemicals between host and symbiont (Ho and Bodelier, 2015)" and "There may also be a different, indirect type of interaction in which Sphagnum receives a flow of nutrients from dead and lysed microorganisms."..."i.e. a direct mutualism or an indirect interaction,"

2a) I wonder whether the effect of P on plant performance is determinable in the time frame of this study. If, in response to P addition, N fixation increases, if this added N is retained by the microbes, they would have to turn over before the plant could have access to it. Thus, some more time may be necessary to see a P effect on plant performance.

2b) We thank the referee for bringing up this point, as we can indeed improve our manuscript by elaborating on this.

In various studies on peatlands the stimulating effect of phosphorus on Sphagnum growth was shown. During one growing season, phosphorus was shown to increase photosynthesis by 14% (Fritz et al 2001) and the length of photosynthetic material of Sphagnum significantly by 42% (Carfrae et al., 2007), being 6-7 mm. Although our experiment lasted for 10 weeks, which is shorter than a growing season, it would still be sufficient to be able to see the effects of phosphorus addition on Sphagnum growth, being around 3 mm additional growth.

The additional citations were added on Page 4, line 5: "...and lead to an increase in photosynthesis (by 14%) (Fritz et al., 2012) and moss growth (by 42%) (Carfrae et al., 2007)."

Besides, we expected the mutualistic interaction between Sphagnum and its diazotrophs to be of a direct nature (i.e. direct transfer of nutrients between symbiont and host, see previous point) and therefore an increase in NÂň2 fixation rates to result in a direct increase of N availability for Sphagnum, and thus an increase in growth rate. Such a mutualism is for instance known for Azolla spp, were addition of P can in a short time (four weeks or less) greatly increase growth and also N content (Cheng et al., 2010). From our results such a direct symbiotic relationship between Sphagnum and its microbial community seems not to be the case, pointing towards an indirect interaction.

To clarify this point, we added information on Azolla spp to the introduction Page 4, lines 2-3: "...and in Azolla spp, a fern species with symbiotic cyanobacteria within its leaves, P was shown to drastically increase plant growth and N content (Cheng et al., 2010)." And to the discussion we added to Page 11, line 3-6: "In stark contrast with Azolla spp, where P addition is known to directly increase the growth rate and N content of the host plant (Cheng et al., 2010) (direct mutualism), the symbiosis between Sphagnum and its microbial community seems to be based on the indirect transfer of nutrients from microbial die-off (Ho and Bodelier, 2015)."

Specific comments:

3a) The timing of events is not clear. How long were the treatments administered? How much time elapsed between treatment initiation and photosynthesis measurement or nutrient analysis?

3b) We agree that the timing was not stated clearly. The treatments were added during 10 weeks, after which nutrient content, nitrogen fixation and photosynthesis were measured. 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."

4a) The device used to measure photosynthesis is not designed specifically for measuring photosynthesis. One question is whether it is accurate and stable enough to actually detect small but significant differences in photosynthesis between treatments. Also, were the light levels used (not specified) representative of those expected in the field (as opposed to the mesocosm)? If not, the results from this measurement could be irrelevant.

4b) The device used is a small chamber connected in a closed loop to a near infra red spectroscopy (NIRS) gas analyzer with cavity ring down spectroscopy (CRDS), which is at present the most accurate instrument to measure CO2 fluxes (and CH4, N2O fluxes), including decreased CO2 levels as a result of photosynthesis. The set-up is therefore exactly similar to, e.g., those using Infrared Spectroscopy Gas Analyzers (IRGA) to measure photosynthesis (Hunt, 2003). The laser-based NIRS-CRDS devices can, however, measure changes in CO2 concentration much faster and with an extremely high resolution (Crosson, 2008). The light levels used were 150 μmol PAR m-2 s-1, similar to mesocosm and field light levels.

In order to make this more clearly, we added more details on this method from Page 5, line 31 to Page 6, line 4: "...fast greenhouse gas analyzer (NIRS) with cavity ringdown spectroscopy (CRD)" "at similar light conditions as used in the experimental set up (150

μmol m-2 s-1 PAR)" "...in a closed loop with the NIRS-CRDS gas analyzer capable of measuring concentration changes at a very high resolution (Crosson, 2008) and of accurately measure photosynthesis (Hunt, 2003).

5a) The natural conditions of the site where the mosses were collected should be indicated (pH, bicarbonate concentration, phosphate concentration, etc.) in order to place in context the experimental treatments.

5b) We agree that this information would benefit our manuscript. Therefore we added an additional table, table 1A 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."

6a) There appears to be a significant interaction between bicarbonate and P with respect to photosynthesis. That is P did not have a significant effect in the absence of bicarbonate, but it did in the presence of bicarbonate. So, when bicarbonate is present, P may be beneficial.

6b) We are very grateful that the referee noticed this error. Apparently, the wrong figure was added to the manuscript, which we very much regret and apologize for. We changed this figure for the correct one, in which there is no appearance of an interaction effect, in agreement with the statistics, as stated on Page 7, line 23-24: "No interaction effects were found for any of the parameters".

Technical corrections: None

Additional references:

Carfrae, J., Sheppard, L., Raven, J., Leith, I., and Crossley, A.: Potassium and phosphorus additions modify the response of Sphagnum capillifolium growing on a Scottish ombrotrophic bog to enhanced nitrogen deposition, Applied Geochemistry, 22, 1111-1121, 2007.

Cheng, W. G., Sakai, H., Matsushima, M., Yagi, K., and Hasegawa, T.: Response of the

floating aquatic fern Azolla filiculoides to elevated CO2, temperature, and phosphorus levels, Hydrobiologia, 656, 5-14, 2010.

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Fritz, C., Van Dijk, G., Smolders, A. J. P., Pancotto, V. A., Elzenga, T. J. T. M., Roelofs, J. G. M., and Grootjans, A. P.: Nutrient additions in pristine Patagonian Sphagnum bog vegetation: can phosphorus addition alleviate (the effects of) increased nitrogen loads, Plant Biology, 14, 491-499, 2012.

Ho, A., and Bodelier, P. L. E.: Diazotrophic methanotrophs in peatlands: the missing link?, Plant and Soil, 389, 419-423, 2015.

Hunt, S.: Measurements of photosynthesis and respiration in plants, Physiologia Plantarum, 117, 314-325, 2003.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/bg-2016-384/bg-2016-384-AC1-supplement.pdf

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