

Biogeosciences Discuss., author comment AC2
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Reply on RC2

Melanie S. Verlinden et al.

Author comment on "Phosphorus stress strongly reduced plant physiological activity, but only temporarily, in a mesocosm experiment with *Zea mays* colonized by arbuscular mycorrhizal fungi" by Melanie S. Verlinden et al., Biogeosciences Discuss., <https://doi.org/10.5194/bg-2021-168-AC2>, 2021

Below are itemized replies to the referee comments and the suggestions made in the manuscript.

1) This is a well written and interesting paper that reports on the effects of P deficiency on photosynthesis in mesocosm experiment in 2016, and includes an impressive range of measured parameters. It argues that P deficiency has wide range effects on leaf scale photosynthetic parameters, and that mycorrhiza (AMF) can alleviate the low P effects in comparison with fertilized plants, presumably by efficient mobilization of soil P. However, I think there are some issues with the paper, which may require significant changes before publication in BG.

REPLY: We thank the reviewer for these positive notes and appreciate the useful and constructive review.

2) Perhaps the main difficulty I had was due to the fact that this seems to be at least the 5th in a series of papers on the same experiment(s) that cover various aspects of the same story. Notably, the main point of the paper on the AMF compensation for low soil P is already made in the other paper, repeatedly.

REPLY: We believe there was some confusion here. The other papers mentioned by the reviewer do not all deal with the same experiment. There were two different experiments in consecutive years (2016 and 2017), having different treatments and targeting different research questions. In 2016, the experiment included N and P addition treatments, while in 2017 the experiment consisted of a P gradient. So far, one article focussed on the 2016 experiment (Verlinden et al., 2018), while the other three (Ven et al., 2019, 2020a, 2020b) report results from the 2017 experiment.

The manuscript submitted to Biogeosciences contains unique data and analyses that were not included in any of these other papers (and measurements that were done only in 2016, not in 2017).

Ven, A., Verbruggen, E., Verlinden, M. S., Olsson, P. A., Wallander, H. and Vicca, S.: Mesh

bags underestimated arbuscular mycorrhizal abundance but captured fertilization effects in a mesocosm experiment. *Plant Soil*, 446, 563–575, doi: 10.1007/s11104-019-04368-4, 2020a.

Ven, A., Verlinden, M. S., Fransen, E., Olsson, P. A., Verbruggen, E., Wallander, H. and Vicca, S.: Phosphorus addition increased carbon partitioning to autotrophic respiration but not to biomass production in an experiment with *Zea mays*. *Plant Cell Environ.*, 43, 2054–2065, doi: 10.1111/pce.13785, 2020b.

Ven, A., Verlinden, M. S., Verbruggen, E., and Vicca, S.: Experimental evidence that phosphorus fertilization and arbuscular mycorrhizal symbiosis can reduce the carbon cost of phosphorus uptake. *Funct. Ecol.*, 33, 2215–2225, doi:10.1111/1365-2435.13452, 2019.

Verlinden, M. S., Ven, A., Verbruggen, E., Janssens, I. A., Wallander, H. and Vicca, S.: Favorable effect of mycorrhizae on biomass production efficiency exceeds their carbon cost in a fertilization experiment. *Ecology*, 99, 2525–2534, doi: 10.1002/ecy.2502, 2018.

3) Further, the other papers seem to contain complementary information that would be hard work to extract to fully understand the current results in the proper context. For example, it seems that the main point is the 'recovery' from 'P stress' in most leaf parameters in the 2nd campaign (on a quick look this is what Fig. 1 generally show: The P stress is essentially gone in C2). But starting with very low rates of photosynthesis (Amax J, etc.), one must assume the control were small plants in C2. But there is no info on total leaf area and biomass to account for this. There is no information on the root system to support the conclusion that it's only the AMF that extended the P uptake, and not changes in root/shoot or other forms of expanding root system.

REPLY: The paper by Verlinden et al. (2018), which is referred to the most, handles with the same experiment and shows the results on plant above- and belowground biomass and carbon partitioning. It shows indeed that both the partitioning to roots and to AMF is larger in the non-P-fertilized mesocosms as compared to the P-fertilized mesocosms. We will incorporate this in the revised manuscript. In response to comment 8 of reviewer 2, we will also include more explicit links to the ecosystem-scale responses.

4) As the authors indicate, P nutrition is linked to ADP/ATP balance, as well as other P-dependent processes, which can influence the plant functioning. And so, in a detail physiological paper, some of these potential effects could be discussed/mentioned. There are also some textbook type issues but already mentioned in the Discussion so will not repeat, but clearly need to be checked.

REPLY: We agree with the reviewer and will add the following to the discussion:

A decrease in stromal inorganic phosphate (Pi) concentration could diminish the rate of photophosphorylation and thereby reduce the rate of photosynthesis through increased energization of the thylakoid membrane, decreased electron flow (Rychter et al., 2005). In this regard, the Pi translocator provides an indirect shuttle system for transferring ATP and NADPH to the cytoplasm involving exchange of triose-P and PGA (Flugge 1999). The reduced concentration of Pi leads to a reduction in ATP/ADP, which could restrict the activity of Rubisco activase and therefore Rubisco carbamylation (Portis 1992).

Pi also plays a role in carbohydrate metabolism. It is involved in starch synthesis through affecting the key regulatory enzyme for starch synthesis (ADP-glucose pyrophosphorylase)

(Preiss 1994). Pi is involved in the mechanisms that control the enzyme activity of the SPS protein including allosteric control by G6P (activator) and Pi (inhibitor) and (ii) protein phosphorylation (Winter et al., 2000)

Flugge, U. I.: Phosphate translocation in the regulation of photosynthesis. *J. Exp. Bot.*, 46, 1317–1323, doi: 10.1093/jxb/46.special_issue.1317, 1995.

Portis, A. R.: Regulation of ribulose 1,5-bisphosphate carboxylase oxygenase activity. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, 43, 415–437, doi: 10.1146/annurev.pp.43.060192.002215, 1992.

Preiss, J.: Regulation of the C3 reductive cycle and carbohydrate synthesis, in: *Regulation of Atmospheric CO₂ and O₂ by Photosynthetic Carbon Metabolism*, edited by: Tolbert, N. E. and Preiss, J., Oxford University Press, New York, USA, 93–102, 1994.

Rychter, A. M. and Rao, I. M.: Role of phosphorus in photosynthetic carbon metabolism, in: *Handbook of photosynthesis*, edited by: Pessaraki, M., Taylor and Francis, London, UK, 123-148, 2005.

Winter, H. and Huber, S. C.: Regulation of sucrose metabolism in higher plants: localization and regulation of activity of key enzymes. *Crit. Rev. Biochem. Mol. Biol.*, 35, 253–289, doi: 10.1080/10409230008984165, 2000.

5) The lower leaf P in the C2 in all cases (while N increased) is not clearly consistent with the C2 recovery being an all P recovery, which seems to be the main argument. The link to SLA (a parameter not well defined) is hard to make as SLA also decreased. It seems that P per leaf weight and not area could have helped here. And that the leaf recovery in general was not necessarily (or only) due to P buildup in the leaves

REPLY: We are very thankful to the reviewer for this comment. It seems that in the process of editing, the values of leaf P in the table were wrongly copied (same values of SLA are shown). Below we paste the correct P-values (per leaf are, left table), showing the increase in leaf P in C2 for all treatments. We expressed the P concentration not per leaf mass but per leaf area since the CO₂ assimilation rate is also expressed per leaf area. The table on the right shows the P concentration per dry leaf mass.

leaf P

leaf P

g m⁻²

mg (g dry mass)⁻¹

mean

mean

0.018^a 0.90

0.018^a 0.94

0.056^b 1.98

0.066^{bc} 1.99

0.103^d 2.70

0.102^d 2.61

0.092^{cd} 1.87

6) In Fig. 2, panel A can be linked to the Methods, but not panel B, considering the AMF was measured in bags without roots?

REPLY: The reviewer is correct. The methodology on AMF (section 2.2.4 'Mycorrhizal fungi' in the Material and Methods) concerns the hyphal length density determination in mesh bags, of which results are shown in panel A of Fig. 2.

Mycorrhizal colonization (shown in panel B) on the other hand was examined in C1 and C2 by sampling roots from two plants per mesocosm. Per plant, 20 cm of one lateral root containing root hair, was excavated, cut, and stored. Mycorrhizal colonization was quantified by counting arbuscules, vesicles, and hyphae applying the gridline intersection method (Vierheilig et al., 2005). The methodology on root colonization determination is described more elaborately in Verlinden et al. (2018). We will add this information to the revised manuscript.

Vierheilig, H., Schweiger, P. and Brundrett, M.: An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots. *Physiol. Plantarum*, 125, 393-404, doi: 10.1111/j.1399-3054.2005.00564.x, 2005.

7) Table 1 contains a lot of information but, for example, it is difficult to understand how from C1 to C2 N goes up and P goes down but the N:P decreases?

REPLY: We apologize for the mistake we made in the table. See also 2 comments before. The P-values in the manuscript were not the correct ones. Leaf P indeed increased in C2.

8) Finally, another important example of the problematic spread across many papers, is that in some of the other papers (which I just eyed briefly) it seems "ecosystem-scale" GPP (and NPP) was estimated, but there is no discussion on agreement or not with the leaf scale photosynthesis. I think this is a particularly significant point here when what seems to be a purely physiological paper is submitted to a Biogeochemistry journal, but no attempt whatsoever is made to link the P story to biogeochemistry.

REPLY: We thank the reviewer for this suggestion. We will further improve the discussion by including an explicit link between ecosystem-scale GPP and leaf-level measurements. The ecosystem-scale GPP measurements corresponded very well to the leaf-scale

measurements, as both were in the first weeks (very) low in the absence of P addition, but showed a sudden increase about 6 weeks after planting.