Dear editor,

We hereby submit a revised version of our manuscript entitled "Phosphorus stress strongly reduced plant physiological activity, but only temporarily, in a mesocosm experiment with Zea mays colonized by arbuscular mycorrhizal fungi". We thank the two reviewers for insightful comments and suggestions, which increased the quality of our manuscript. Below, you can find our point-by-point answers to the comments and questions. Line numbers refer to the revised manuscript with tracked changes.

Sincerely,
Melanie Verlinden, on behalf of all co-authors

REPLIES TO THE EDITOR

The authors provided a detail response to both Reviewers comments in a way that indicates that a significantly improved revised version can be produced. I therefore recommend to invite the authors to go ahead and re-submit a revised paper.

Please note that while there are detail responses to the comments, not in all cases the proposed modifications to the paper are noted. Please insure responses are apparent in the paper itself, rather than remain the response letters.

REPLY: We followed the editor's advice and have included the necessary modifications in the manuscript. These are referred to in the point-by-point answers below.

Also, while there are many detail comments, there are some general ones to note.

For example, Rev 1 questions suitability to BG, while Rev 2 notes the disconnection to ecosystem scale. P limitation is relevant to BG but such relevance should be apparent.

REPLY: We have added a paragraph to the introduction to better clarify the relevance of P limitation for Biogeosciences on L. 32-38 of the revised manuscript:

Phosphorus (P) is a crucial element in natural ecosystems. It is present in the structure of DNA, in cell membranes, in molecules storing and supplying energy and in several enzymes. As a consequence, P plays a crucial role in plant and soil processes, it regulates productivity and ecosystem functions and influences organisms from the individual to the community level. The importance of P for the functioning of the Earth's biogeochemical cycles, especially the carbon cycle, is therefore being increasingly recognized and this is reflected in the recent efforts to include P in terrestrial biosphere models.

We also added extra text on the ecosystem scale results. For example on L. 337-341:
The leaf-scale responses reported here correspond well to the ecosystem-scale GPP measurements reported for the same experiment in Verlinden et al. (2018). In the first weeks, both were (very) low in the absence of P addition, but showed a sudden increase about 6 weeks after planting. Although

ecosystem level GPP remained lower for the non-P fertilized treatments, the photosynthesis system seemed to have fully recovered, as indicated by similar levels of leaf photosynthesis among all treatments during C2.

And another refers to the issues with previous papers and experiments (Rev 2), which the authors claim are due to confusion. Clearly, such confusion should be cleared up by more clearly putting the present paper in the context of the others.

REPLY: We agree with the reviewer and have clarified the similarities and differences between our studies by adding information to materials and methods and linking to the follow-up study in the discussion.

L. 84-86:
For this study, we used the first of two mesocosm fertilization experiments. While the first applied a full-factorial NxP fertilization approach and was first described in Verlinden et al. (2018), the second applied a P gradient. Results for the latter are reported in Ven et al. (2020b).

L. 343-344:
Also our follow-up experiment with a P gradient confirmed the important stimulating role of AMF for plant productivity and photosynthesis (see Ven et al., 2020b).

REPLIES TO REVIEWER 1

We appreciate the very useful and constructive criticisms of the reviewer on our manuscript. Below are itemized replies to the referee comments and the suggestions made in the manuscript. The line numbers (L._ _) in our replies refer to those of the revised manuscript.

1) I was a bit surprised that this manuscript was submitted to Biogeosciences, as I would have thought that a straight physiology or ecophysiology journal would have been more appropriate. However, I will leave that aspect of my feedback to the Editor.

REPLY: We opted for Biogeosciences instead of a plant physiology journal because we envisage a more general audience for our results. Although the measurements presented in the manuscript focus for a large part on leaf-level responses, the main incentive for this study was the importance of nutrient availability and plant-mycorrhiza interactions in determining carbon cycling. We expect that our results will be useful for the land surface modeling community and particularly to those who are aiming to implement carbon-nutrient interactions in these models. In the revised manuscript, we modified the start of the introduction to make this clearer (L.32-38 of the revised manuscript):

Phosphorus (P) is a crucial element in natural ecosystems. It is present in the structure of DNA, in cell membranes, in molecules storing and supplying energy and in several enzymes. As a consequence, P plays a crucial role in plant and soil processes, it regulates productivity and ecosystem functions and influences organisms from the individual to the community level. The importance of P for the functioning of the Earth's biogeochemical cycles, especially the carbon cycle, is therefore being

increasingly recognized and this is reflected in the recent efforts to include P in terrestrial biosphere models.

2) The authors quickly jumped to the conclusion that P effects must all be direct effects on photosynthesis, and appear rather dismissive of the idea that P might effect leaf growth, and the change in sink demand would then affect photosynthetic activity. Even though they cite some of the papers highlighting P effects on sink activity, with effects of photosynthesis being indirect, the message in those papers appears not to have been taken on board. Tools exist to assess feedback inhibition of photosynthesis, but the literature dealing with that aspect wasn't discussed at all. For example:

- Sharkey T D, Stitt M, Heineke D, Gerhardt R, Raschke K and Heldt H W 1986 Limitation of photosynthesis by carbon metabolism: II. O2-insensitive CO2 uptake results from limitation of triose phosphate utilization. Plant Physiol. 81, 1123-1129.
- Sage R F and Sharkey T D 1987 The effect of temperature on the occurrence of O2 and CO2 insensitive photosynthesis in field grown plants. Plant Physiol. 84, 658-664. 10.1104/pp.84.3.658.
- Plaut Z, Mayoral M L and Reinhold L 1987 Effect of altered sink: source ratio on photosynthetic metabolism of source leaves. Plant Physiol. 85, 786-791. 10.1104/pp.85.3.786.

REPLY: We agree with the reviewer that changes in sink demand could have played a role. In our experiment, however, leaves turned yellow at the start, but greened up later, albeit only in the presence of AMF. Plants without AMF died prematurely. These results indicate that sink demand is unlikely to be the main responsible for the observed changes.
In the revised manuscript, we added new text on the direct and indirect effects of P on photosynthesis, including the possibility for changes in sink demand. This is now discussed in the introduction and discussion sections:

L. 63-70:
Pi can also indirectly affect photosynthesis through the changes in stromal pH (Bhagwat 1981), where the consumption of Pi as a substrate of photosynthesis could decrease photosynthesis by a direct effect of low stromal Pi concentration on Rubisco. Moreover, the effect of P on photosynthesis depends on the dynamic interactions between sink and source tissues. Low P can reduce carbon export to sinks, and thus decrease sink strength, and thereby limiting photosynthesis (Pieters et al., 2001). Concomitantly, leaf starch often increases with P stress (Zhang et al., 2014) due to low availability of P for triphosphate translocation (although decreases of leaf starch have also been observed; Halsted and Lynch, 1996). Moreover, low sink strength restricts the recycling of Pi back to the chloroplast, further reducing photosynthesis (Paul and Foyer, 2001).

L. 258-268:
The initial P-limitation present during C1, strongly limited leaf-level photosynthesis as A_{max}, J_{max} and v_{cmax} were three to four times lower in non-P-fertilized than in P-fertilized plants. This inhibitory effect can be attributed to the decrease in the pool size of ribulose-1,5-bisphosphate (RuBP) and its regeneration (Jacob and Lawlor, 1992; Pieters et al., 2001; Calderón-Vázquez et al., 2009), or by feedback inhibition of photosynthesis, but the latter was not specifically tested. Feedback inhibition of

photosynthesis can be induced by elevated soluble sugar levels decreasing the gene expression levels of photosynthetic enzymes (e.g. PEPC, malic enzyme and RuBisCo) (Jeannette et al., 2000; AbdElgawad et al., 2020). This was not likely the case here, since during C1 sugar levels tended to be even lower in the non-P-fertilized than in the P-fertilized treatments. Lower starch and soluble sugar synthesis, like in the non-P-fertilized treatments, might slow Pi regeneration, limit ATP production and eventually the functioning of the Calvin cycle, which is known as short-term feedback regulation of photosynthesis (Griffin and Seemann, 1996).

L. 291-301:

P deprivation has been found to increase the leaf starch concentration in maize (Zhang et al., 2014), although decreases in starch levels under low P conditions have also been reported (Schlüter et al., 2013). In our experiment, P addition had no clear effect on the leaf starch concentration (Table 1), indicating that reduced photosynthetic rates were not due to reduced sink strength. The starch concentration did show a significant campaign effect and more than doubled from C1 to C2. Both sucrose and starch synthesis play important roles in the cellular recycling of phosphate for photosynthesis (Schlüter et al., 2013). A decrease in sugars and starch might lead to lower vitality and productivity of plants, as was previously observed in stressed C_4 leaves (da Silva and Arrabaça, 2004). In our experiment, while there was no effect of P for both sugars and starch, the campaign effect illustrated an increase of sugars and starch from C1 to C2, possibly suggesting that plants in all treatments experienced nutrient stress during C1. Moreover, the increasing sugar and starch levels between C1 and C2 further confirm that the low photosynthetic rates for the low P treatments were not due to reduced sink strength.

3) The authors need to consult a recent textbook to check where different reactions related to carbon metabolism in C4 plants occur, because it is not correct that synthesis of starch and sucrose occur in different cell types. Both require Rubisco, which only occurs in the bundle-sheat cells, and not in mesophyll cells.

REPLY: The interpretation of the cited publication (Friso et al. 2010) was taken from Schlüter et al. 2013, stating 'Starch accumulates almost exclusively in the bundle sheath while sucrose synthesis takes place in the mesophyll'.

We consulted other sources, but found similar information.

"Sucrose was predominantly synthesized in the mesophyll cells and starch in the bundle sheath cells" (Furbank and Kelly, 2021). "In Zea mays L. and Atriplex spongiosa F. Muell., sucrose-phosphate synthase (key enzyme in sucrose biosynthesis) was located almost exclusively in the mesophyll cells" (Lunn and Furbank, 1997).

During revisions, we considered it better to remove this sentence. See modifications on L. 295 of the revised manuscript.

Friso, G., Majeran, W., Huang, M. S., Sun, Q. and van Wijk, K. J.: Reconstruction of metabolic pathways, protein expression, and homeostasis machineries across maize bundle sheath and mesophyll chloroplasts: large-scale quantitative proteomics using the first maize genome assembly. Plant Physiol., 152, 1219‑1250, doi: 10.1104/pp.109.152694, 2010.

Furbank, R. and Kelly, S.: Finding the C_4 sweet spot: cellular compartmentation of carbohydrate metabolism in C_4 photosynthesis. J. Exp. Bot., 72, 6018–6026, doi: 10.1093/jxb/erab290, 2021.

Lunn, J. and Furbank, R.: Localisation of sucrose-phosphate synthase and starch in leaves of C_4 plants. Planta 202, 106–111, doi: 10.1007/s004250050108, 1997.

Schlüter, U., Colmsee, C., Scholz, U., Bräutigam, A., Weber, A. P. M., Zellerhoff, N., Bucher, M., Fahnenstich, H. and Sonnewald, U.: Adaptation of maize source leaf metabolism to stress related disturbances in carbon, nitrogen and phosphorus balance. BMC Genomics 14, 442, doi: 10.1186/1471-2164-14-442, 2013.

4) It is true that mycorrhizas may mobilise organic P or sorbed P, but when it comes to arbuscular mycorrhizas (AM), the cited textbook (Smith & Read) points out that AM are unlikely to do that. Their role is to enhance the volume of soil that can be explored. So, the text needs to be tweaked a bit to acknowledge that.

REPLY: Here we disagree with the reviewer. Many studies have shown that AMF (contrary to ectomycorrhizae) are especially important for plant uptake of P, not only because of the increased soil volume explored, but because they produce exudates that liberate P from the minerals (a.o. Smith et al., 2011; Burghelea et al., 2015; Kobae, 2019; Etesami et al., 2021; Jansa et al., 2021). For example glomalin, a glycoprotein secreted by AMF, aids the uptake of nutrients such as Fe and P that are difficult to dissolve (Miransari, 2010; Emran et al., 2017; Begum et al., 2019). We modified this section of the manuscript, including these additional information and references (L. 326-335 in the revised manuscript).

The hyphal network of mycorrhizae extends over a very large surface area, increasing prominently the absorbing area of roots. Their extraradical hyphae extend beyond the P depletion zone, absorbing P that is otherwise not accessible for the plant (Plenchette et al., 2005; Roy-Bolduc and Hijri, 2011). Besides, mycorrhizal fungi improve phosphate solubility because they produce exudates that liberate P from the minerals (a.o. Smith et al., 2011; Burghelea et al., 2015; Kobae, 2019; Etesami et al., 2021; Jansa et al., 2021). For example glomalin, a glycoprotein secreted by AMF, aids the uptake of nutrients such as Fe and P that are difficult to dissolve (Miransari, 2010; Emran et al., 2017; Begum et al., 2019). Mycorrhizae thus significantly contribute to plant nutrition and to P uptake in particular (Wright et al., 2005), which in turn can positively affect leaf gas exchange rates (Smith and Read, 2008; Augé et al., 2016).

5) SLA is not a simple measure of leaf thickness, but of both leaf thickness and leaf density. Leaf density is affected by carbohydrate concentrations and amount of cell walls.

REPLY: The reviewer made a good point here. With the thinner feel of the leaves in mind, we went too short here. The higher SLA indeed points to lower leaf density and/or leaf thickness. The lower concentration of leaf compounds in the non-P-fertilized mesocosms (as shown in table 1) might suggest lower leaf densities indeed. We corrected this in the manuscript on L 182-183: 'Leaves in the non-P-fertilized mesocosms were thinner and/or had a lower density than in the P-fertilized mesocosms.'

6) 'Content' is generally used when amounts are expressed per plant (part); when amounts are expressed per unit mass or area, 'concentration' is recommended.

REPLY: We agree with the reviewer. We changed 'content' to 'concentration' on L. 60, 277, 291 and 293.

Below we list the additional comments made by Referee 1 in the manuscript.

7) L15: Why start with 'Despite'? I suggest to start with 'Phosphorus' and wrote this as two statements about P. (Despite doesn't make sense here.)

REPLY: We changed this in the revised manuscript (L. 15-16) to ' Phosphorus (P) is an essential macronutrient for plant growth and one of the least available nutrients in soil. P limitation is often a major constraint for plant growth globally.'

8) L17-18: Change '...experiments have been carried out to study the long-term effects on the yield, data on P addition effects to seasonal variation in leaf-level photosynthesis are scarce.' To '...experiments have been carried out to study the long-term effects on yield, data on P addition effects on seasonal variation of leaf-level photosynthesis are scarce.'

REPLY: We modified the text on L. 16-18.

9) L20-22: The primary effect of P is just as likely on growth, rather than photosynthesis, and effects on photosynthesis likely reflect a reduced sink demand on source activity.

REPLY: See reply to comment 2 above.

10) L34: Replace 'participates in the formation' by 'is a component'.

REPLY: We modified as suggested on L. 39.

11) L35-36: I don't know what this means, but do know that P is important in P-containing metabolites that play a role in carbon metabolism. Schulze et al. is an odd reference here. I think this one would be more appropriate: Veneklaas E J, Lambers H, Bragg J, Finnegan P M, Lovelock C E, Plaxton W C, Price C, Scheible W-R, Shane M W, White P J and Raven J A 2012 Opportunities for improving phosphorus-use efficiency in crop plants. New Phytol. 195, 306-320. 10.1111/j.1469-8137.2012.04190.x.

REPLY: We added the suggested reference on L. 41.

12) L38: I can see how plants experience P stress, or, rather plant productivity is limited by P, but lands doesn't really experience P stress.

REPLY: We rephrased this sentence to 'On more than one third of the arable land worldwide, plant productivity is considered to be limited by P. (L. 43-44)'

13) L40: Delete 'the' in '…effect on the yield'.

REPLY: We deleted as suggested on L. 46.

14) L40-41: Reference suggestion:
Rodríguez D, Andrade F H and Goudriaan J 2000 Does assimilate supply limit leaf expansion in wheat grown in the field under low phosphorus availability? Field Crops Res. 67, 227-238. 10.1016/s0378-4290(00)00098-8.

REPLY: We thank the reviewer for this suggestion and added the reference to the manuscript on L. 47.

15) L43-44: Reference suggestions for effects of phosphorus on photosynthesis:

Brooks A 1986 Effects of phosphorus nutrition on ribulose-1,5-bisphosphate carboxylase activation, photosynthetic quantum yield and amounts of some Calvin-cycle metabolites in spinach leaves. Funct Plant Biol 13, 221-237. doi:10.1071/PP9860221.

Brooks A, Woo K C and Wong S C 1988 Effects of phosphorus nutrition on the response of photosynthesis to CO2 and O2, activation of ribulose bisphosphate carboxylase and amounts of ribulose bisphosphate and 3-phosphoglycerate in spinach leaves. Photosyn Res 15, 133-141. 10.1007/bf00035257.

Rodriguez D and Goudriaan J 1995 Effects of phosphorus and drought stresses on dry-matter and phosphorus allocation in wheat. J. Plant Nutr. 18, 2501-2517.

Rodríguez D, Keltjens W G and Goudriaan J 1998 Plant leaf area expansion and assimilate production in wheat (Triticum aestivum L.) growing under low phosphorus conditions. Plant Soil 200, 227-240. 10.1023/a:1004310217694.

REPLY: We thank the reviewer for this suggestions and added these references to the manuscript (L. 50-51).

16) L47 'P is required for adenosine triphosphate (ATP) synthesis': This is true, but ATP is only a minute fraction of the metabolite P pool. See: Veneklaas E J, Lambers H, Bragg J, Finnegan P M, Lovelock C E, Plaxton W C, Price C, Scheible W-R, Shane M W, White P J and Raven J A 2012 Opportunities for improving phosphorus-use efficiency in crop plants. New Phytol. 195, 306-320. 10.1111/j.1469-8137.2012.04190.x.

REPLY: We added the reference to the manuscript on L. 54.

17) L48 'P-deficiency therefore leads to a decrease in RuBP pool size and insufficient ATP, and consequently to a decrease in photosynthetic C assimilation.': Or is that decline due to a decreased sink demand?

REPLY: See reply to comment 2 above.

18) L53: Low sink demand for sugars, and feedback inhibition of photosynthesis: Sharkey T D, Stitt M, Heineke D, Gerhardt R, Raschke K and Heldt H W 1986 Limitation of photosynthesis by carbon metabolism: II. O2-insensitive CO2 uptake results from limitation of triose phosphate utilization. Plant Physiol. 81, 1123-1129.

REPLY: We discussed the low sink demand in the introduction on L. 63-70, see reply to comment 2 above.

19) L61: In science, we never set off to 'verify', but we seek to test. That test may well lead to a verification, but that was never the intention per se.

REPLY: We replaced 'verify' with 'test' on L. 77.

20) L62-64 'At low soil P availability, we expected low leaf-level photosynthetic and respiratory activity, associated with reduced chlorophyll and photosynthetic enzymes.': And how can you discard that these effects are the result of reduced leaf growth and sink demand? See: Pieters A J, Paul M J and Lawlor D W 2001 Low sink demand limits photosynthesis under Pi deficiency. J. Exp. Bot. 52, 1083-1091. 10.1093/jexbot/52.358.1083.

REPLY: See reply to comment 2 above.

21) L92 ‘seasonal evolution’: That's what you would use in French, but not in English.

REPLY: We replaced this with ‘seasonal development’ on L. 110.

22) L96, L101, 105, 109 ‘net assimilation rate’: Do not use that expression here, as it means something different in growth analysis; inserts CO2.

REPLY: We inserted CO_2 as suggested on L. 114, 119, 123 and 127.

23) L108, 111 ‘light response curves’: hyphenate

REPLY: We hyphenated as suggested on L. 123.

24) L115: add ‘to’

REPLY: done on L. 133.

25) L116: delete ‘phoshor-‘

REPLY: done on L. 134.

26) L118: use ‘rate’ instead of ‘measure’

REPLY: done on L. 137.

27) L119: use ‘leaves’ instead of ‘leaf tissue’

REPLY: done on L. 139.

28) L121 : use ‘sugar concentration’ instead of ‘sugars’

REPLY: done on L. 141.

29) L125, 126 ‘(Shimadzu SPD-M10Avp)’: Add city and country

REPLY: We added ‘Kyoto, Japan’, to the instrument name on L. 145, 146.

30) L125: remove ‘different’

REPLY: done on L. 146.

31) L134: use ‘staining’ instead of ‘colouring’

REPLY: done on L. 154.

32) L154: replace ‘as compared to’ with ‘than in’

REPLY: done On L. 181.

33) L157: SLA does not simply reflect thickness, but also density, which is affected by accumulation of carbohydrates. It is very likely that leaf mass density accounts for the difference in SLA, rather than leaf thickness, and Table 1 shows no information on thickness. It would have been easy to include the DW/FW ratio, to get information on density.

REPLY: See reply to comment 5 above.

34) L186 ‘direct rubisco’: what does that mean in this context?

REPLY: The activity of Rubisco was determined directly, without incubation of the extract in the presence of 10 mM HCO^{3-} and 20 mM Mg^{2+} to convert the non-carbamylated Rubisco into the carbamylated form. We clarified this on L. 134-136 of the revised manuscript.

35) L192: replace 'compared to' with 'than during'

REPLY: done on L. 217.

36) L201 'Phosphorus': lowercase p

REPLY: done on L. 227.

37) L202, 217: replace 'statistical' with 'significant'

REPLY: done on L. 228.

38) L210, in table 1: spelling should be 'beta carotene'

REPLY: done

39) L220: soils are never P-limited, but plants are, if grown in low-P soils soils can be P-impoverished, however

REPLY: We changed 'P-limited' to 'P-impoverished' on L. 244.

40) L223: replace 'neither had an' with 'had no'

REPLY: done on L. 246.

41) L228-229: N:P rations are never P limited; what you mean is that these ratios indicate that plant growth is P limited.

REPLY: We modified the sentence (on L. 251-254) to: 'Since growth of plants with leaf N:P ratios higher than 16 up to 20 is considered to be P-limited, the high leaf N:P ratios of about 37 illustrate a clear P-limitation of plant-growth for the non-P-fertilized treatments in C1,… '.

42) L230-231: why optimal (a grossly overused word, when you likely mean favorable)

REPLY: done on L. 255.

43) L232-233 'The initial P-limitation present during C1, strongly limited leaf-level Photosynthesis…': You can't tell that, as the effects on photosynthesis may reflect sink limitation of source activity.

REPLY: See reply to comment 2 above.

44) L234-235 'This inhibitory effect can be attributed to the decrease in the pool size of ribulose-1,5-bisphosphate and its regeneration': Or feedback inhibition of photosynthesis. That could have been tested by assessing O2 sensitivity of CO2 assimilation.

REPLY: We added this information as possible explanation of the inhibitory effect on L. 259-262. We did not assess O2 sensitivity of CO2 assimilation: *'This inhibitory effect can be attributed to the decrease in the pool size of ribulose-1,5-bisphosphate (RuBP) and its regeneration, or by feedback inhibition of photosynthesis, but the latter was not specifically tested.'*

Subsequently we added (L. 262-268): '*Feedback inhibition of photosynthesis can be induced by elevated soluble sugar levels decreasing the gene expression of photosynthetic enzymes (e.g. PEPC, malic enzyme and RuBisCo) (Jeannette et al., 2000; AbdElgawad et al., 2020). This was not likely the case here, since during C1 sugar levels tended to be lower in the non-P-fertilized than in the P-fertilized treatments. Lower starch and soluble sugar synthesis, like in the non-P-fertilized treatments, can slow Pi regeneration, limit ATP production and eventually the functioning of the Calvin cycle, which is known as short-term feedback regulation of photosynthesis (Griffin and Seemann, 1996).'*

45) L239: what does a 'suffering plant' look like? This word must be avoided in academic writing about plants.

REPLY: We changed the sentence (on L. 271-273) to: '*C_4 plants can maintain adequate levels of P in the bundle cells, and their growth is therefore generally less constrained by P limitation as compared to C_3 plants.'*

46) L244, 260: replace 'content' with 'concentration'

REPLY: done on L. 278, 291 and 293.

47) L252: replace 'in case' with 'when'

REPLY: done on L. 286.

48) L258: concentration - use content only when amounts are expressed per plan (part)

REPLY: We replaced content by concentration.

49) L259: was that perhaps 'very low'?

REPLY: The reported decreases in starch levels under low P conditions come from Schlüter et al. They just report 'low P'.

50) L261-262: 'Unlike C3 plants, synthesis of sucrose and starch in C4 leaves takes place in different cell types.': Please check

REPLY: This text was removed from the manuscript. See also reply to comment 3.

51) L262-263 'Whereas starch accumulates in the bundle sheath, sucrose synthesis takes place in the mesophyll (Friso et al., 2010)':This is not correct. The mesophyll cells lack Rubisco, so do not produce triose-P, and therefore neither starch nor sucrose. They have PEP-carboxylase, and export C4 compounds to the bundle-sheath cells.

REPLY: This text was removed from the manuscript. See also reply to comment 3.

52) L263-264 'A shift towards sucrose or starch would thus affect the metabolism of both cell types in different ways.': not really

REPLY: This text was removed from the manuscript. See also reply to comment 3.

53) L267-268 'Due to stress, a larger proportion of starch can possibly be converted to soluble sugars, thereby decreasing the osmotic potential as a form of protection (da Silva and Arrabaça, 2004).': Relevant under water stress, but makes no sense in this context.

REPLY: We removed this sentence from the manuscript.

54) L271: replace ‘and’ with ‘which’

REPLY: We rephrased this sentence (L. 302-303) to:

Foliar respiration rate is suppressed in the light, the abrupt decline in quantum yield of net CO2 assimilation that occurs at very low light, often near the photosynthetic light compensation point, is also known as the ‘Kok effect’ (Kok, 1948).

55) L283: replace ‘was’ by ‘is’

REPLY: done on L. 313.

56) L288 ‘revival’: odd word to use here – change

REPLY: done on L. 318.

57) L293: remove ‘(fungus-root)’

REPLY: done on L. 324-325.

58) L295: correct spelling ‘extends’

REPLY: done on L. 327.

59) L296 ‘Besides, mycorrhizal fungi improve phosphate solubility’: That would be true for ECM, but is not relevant for AM that are the subject here.

REPLY: See reply to comment 4 above.

60) L301-302 ‘The ‘machinery-limited’ photosynthesis system’: You don't know that. It could be limited by demand and feedback inhibition.

REPLY: We removed ‘machinery limited’ on L. 340.

61) L305-306 ‘To conclude, low P availability significantly decreased photosynthetic capacity, associated with reduced concentrations of photosynthetic enzymes and pigments.’: This dismisses any effects of sink, as alluded to above.

REPLY: See reply to comment 2 above.

62) L433 ‘Smith, S. E. and Read, D. J. (Eds.): Mycorrhizal Symbiosis (Third Edition), Academic Press, London, UK, 2008.’: They were the authors of that book, rather than the editors.

REPLY: We removed the ‘(Eds.)’ from the reference on L. 531.

63) L449: replace ‘.’ With ‘,’

REPLY: done on L. 549.

64) L458: replace ‘Victoria’ with ‘Melbourne’

REPLY: done on L. 571.

REPLIES TO REVIEWER 2

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). We clarified this in the revised manuscript by adding information to materials and methods and linking to the follow-up study in the discussion.

L. 84-86:

For this study, we used the first of two mesocosm fertilization experiments. While the first applied a full-factorial NxP fertilization approach and was first described in Verlinden et al. (2018), the second applied a P gradient. Results for the latter are reported in Ven et al. (2020b).

L. 343-344:

Also our follow-up experiment with a P gradient confirmed the important stimulating role of AMF for plant productivity and photosynthesis (see Ven et al., 2020b).

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. Because root harvesting is destructive, root data were only collected at the end of the experiment and root:shoot ratio can only be reported for the end-of-season harvest. Nonetheless, our ecosystem-scale flux data showed that GPP in the non-P-fertilized plots was very low in the first few weeks of the experiment, but increased about 6 weeks after planting. We incorporated this information in the revised manuscript.

L. 319-320:
In the same experiment, we found that the partitioning to roots and to AMF was larger in the non-P-fertilized mesocosms as compared to the P-fertilized mesocosms (Verlinden et al., 2018).

L. 337-344:
The leaf-scale responses reported here correspond well to the ecosystem-scale GPP measurements reported for the same experiment in Verlinden et al. (2018). In the first weeks, both were (very) low in the absence of P addition, but showed a sudden increase about 6 weeks after planting. Although ecosystem level GPP remained lower for the non-P fertilized treatments, the photosynthesis system seemed to have fully recovered, as indicated by similar levels of leaf photosynthesis among all treatments during C2. These results are in line with the study by Řezáčová et al. (2018), who reported photosynthetic upregulation following upon the establishment of mycorrhizal symbiosis. Also our follow-up experiment with a P gradient confirmed the important stimulating role of AMF for plant productivity and photosynthesis (see Ven et al., 2020b).

4) As the authors indicate, P nutrition is linked to ADP/ATP balance, as well as other P-dependent processes, which and 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: In response to this comment, we elaborated on the physiological plant responses in the introduction on L. 53-70:

Among others, plant P limitation typically results in reduced photosynthesis and plant growth, especially aboveground. P is required for adenosine triphosphate (ATP) synthesis (Veneklaas et al., 2012), which is needed to regenerate Ribulose 1,5-bisphosphate (RuBP) in the Calvin cycle of photosynthesis. Inorganic phosphate (Pi) directly affects the activity of Calvin cycle enzymes through the level of activation. For instance, Pi is required for light activation of Rubisco (Parry et al., 2008). It also directly affects maximum rate of CO2-limited carboxylation (vcmax) and triose phosphate utilization (Lewis et al., 1994) and RuBP-regeneration-limited rates of electron transport (Loustau et al., 1999). P-deficiency therefore leads to a decrease in RuBP pool size and insufficient ATP, and consequently to a decrease in photosynthetic C assimilation. The concentration and specific activity of Rubisco, the primary CO2 fixing enzyme in photosynthesis, are generally little affected by P stress (Brooks, 1986; Paul and Stitt, 1993; Pieters et al., 2001, but see Jacob and Lawlor, 1991; Pieters et al., 2001).
Pi can also indirectly affect photosynthesis through the changes in stromal pH (Bhagwat 1981), where the consumption of Pi as a substrate of photosynthesis could decrease photosynthesis by a direct effect of low stromal Pi concentration on Rubisco. Moreover, the effect of P on photosynthesis depends on the dynamic interactions between sink and source tissues. Low P can reduce carbon export to sinks, and

thus decrease sink strength, thereby limiting photosynthesis (Pieters et al., 2001). Concomitantly, leaf starch can increase with P stress (Zhang et al., 2014) due to low availability of P for triphosphate translocation, although decreases of leaf starch have also been observed (Halsted and Lynch, 1996). Moreover, low sink strength restricts the recycling of Pi back to the chloroplast, further reducing photosynthesis (Paul and Foyer, 2001).

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 $g\ m^{-2}$ mean	(SE)
C1	control	0.018^{a}	(0.001)
	+N	0.018^{a}	(0.001)
	+P	0.056^{b}	(0.008)
	+NP	0.066^{bc}	(0.008)
C2	control	0.103^{d}	(0.003)
	+N	0.102^{d}	(0.022)
	+P	0.092^{cd}	(0.005)
	+NP	0.111^{d}	(0.008)

leaf P mg (g dry mass)$^{-1}$ mean	(SE)
0.90	(0.04)
0.94	(0.02)
1.98	(0.24)
1.99	(0.13)
2.70	(0.08)
2.61	(0.62)
1.87	(0.06)
2.49	(0.16)

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, vesicules, 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 added this information to the revised manuscript on L. 161-165

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.

Added to the manuscript on L. 337-344:

The leaf-scale responses reported here correspond well to the ecosystem-scale GPP measurements reported for the same experiment in Verlinden et al. (2018). In the first weeks, both were (very) low in the absence of P addition, but showed a sudden increase about 6 weeks after planting. Although ecosystem level GPP remained lower for the non-P fertilized treatments, the photosynthesis system seemed to have fully recovered, as indicated by similar levels of leaf photosynthesis among all treatments during C2. These results are in line with the study by Řezáčová et al. (2018), who reported photosynthetic upregulation following upon the establishment of mycorrhizal symbiosis. Also our follow-up experiment with a P gradient confirmed the important stimulating role of AMF for plant productivity and photosynthesis (see Ven et al., 2020b).