

Biogeosciences Discuss., author comment AC1
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Reply on RC1

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-AC1>, 2021

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 original 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.

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 will add extra text on the direct and indirect effects of P on photosynthesis, including the possibility for changes in sink demand:

- 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 (v_{cmax}) and triose phosphate utilization (Lewis et al., 1994) and RuBP-regeneration-limited rates of electron transport (Loustau et al., 1999).
- On the other hand, Pi can 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. The low P level decreases sink strength which imposes the primary limitation on photosynthesis (Pieters et al., 2001). Pi deprivation impacts on photosynthesis can also be explained by diminishing carbon export to sinks (Pieters et al., 2001). Moreover, low sink strength lowers sucrose synthesis and restricts the recycling of Pi back to the chloroplast thus limiting the rate of net photosynthesis (Paul and Foyer, 2001).
- Decreasing cytoplasmic Pi also limits the photosynthesis through end product (feedback) inhibition and this end-product inhibition could be due to high concentrations of triose-P. In this regard The total Pi within the chloroplast is relatively constant, thus high triose-P is automatically coupled with low Pi, which in turn could limit photosynthesis. For example, Pi deficiency drastically decreased RuBP content in the Pi-deficient leaves and hence the rate of photosynthesis. On the other hand, at high Pi supply, triose-P export competes with ribulose 1,5-bisphosphate (RuBP) regeneration and the rate of photosynthesis can be diminished.

Bhagwat, A. S.: Activation of spinach ribulose 1,5- bisphosphate carboxylase by inorganic phosphate. *Plant Sci. Lett.*, 23, 197–206, 1981.

Lewis, J. D., Griffin, K. L., Thomas, R. B. and Strain, B. R.: Phosphorus supply affects the photosynthetic capacity of loblolly pine grown in elevated carbon dioxide. *Tree Physiol.*, 14, 1229-1244, doi: 10.1093/treephys/14.11.1229, 1994.

Loustau, D., Brahim, M. B., Gaudillère, J. P. and Dreyer, E.: Photosynthetic responses to phosphorus nutrition in two-year-old maritime pine seedlings. *Tree Physiol.*, 19, 707-715, doi: 10.1093/treephys/19.11.707, 1999.

Parry, M. A., Keys, A. J., Madgwick, P. J., Carmo-Silva, A. E. and Andralojc, P. J.: Rubisco regulation: a role for inhibitors. *J. Exp. Bot.*, 59, 1569-1580, doi: 10.1093/jxb/ern084, 2008.

Paul, M. J. and Foyer, C. H.: Sink regulation of photosynthesis. *J. Exp. Bot.*, 52, 1383-1400, doi: 10.1093/jexbot/52.360.1383, 2001.

Pieters, A. J., Paul, M. J. and Lawlor, D. W.: Low sink demand limits photosynthesis under

Pi deficiency. *J. Exp. Bot.*, 52, 1083–1091, doi: 10.1093/jexbot/52.358.1083, 2001.

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-sheath 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). We can add these extra references to the manuscript, or if preferred, delete the phrases that are in doubt.

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 C₄ sweet spot: cellular compartmentation of carbohydrate metabolism in C₄ 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 C₄ 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; Burghel 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 will adapt this section of the manuscript, including these additional information and references.

Begum, N., Qin, C., Ahanger, M. A., Raza, S., Khan, M. I., Ashraf, M., Ahmed, N. and

Zhang, L.: Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. *Front. Plant Sci.*, 10, 1068, doi: 10.3389/fpls.2019.01068, 2019.

Burghilea, C., Zaharescu, D. G., Dontsova, K., Maier, R., Huxman, T. and Chorover, J.: Mineral nutrient mobilization by plants from rock: influence of rock type and arbuscular mycorrhiza. *Biogeochemistry*, 124, 187-203, doi: 10.1007/s10533-015-0092-5, 2015.

Emran, M., Rashad, M., Gispert, M., and Pardini, G.: Increasing soil nutrients availability and sustainability by glomalin in alkaline soils. *Agricul. Biosystems Eng.*, 2, 74–84, 2017.

Etesami, H., Jeong, B. R., and Glick, B. R.: Contribution of arbuscular mycorrhizal fungi, phosphate-solubilizing bacteria, and silicon to P uptake by plant. *Front. Plant Sci.*, 12, 1355, doi: 10.3389/fpls.2021.699618, 2021.

Jansa, J., Finlay, R., Wallander, H., Smith, F. A. and Smith, S. E.: Role of mycorrhizal symbioses in phosphorus cycling, in: *Phosphorus in Action. Soil Biology*, vol 26, edited by: Bünemann, E., Oberson, A. and Frossard, E., Springer, Berlin, Heidelberg, Germany, 137-168, doi: 10.1007/978-3-642-15271-9_6, 2011.

Kobae, Y.: Dynamic phosphate uptake in arbuscular mycorrhizal roots under field conditions. *Front. Environ. Sci.*, 6, 159, doi: 10.3389/fenvs.2018.00159, 2019.

Smith, S. E., Jakobsen, I., Grønlund, M. and Smith, F. A.: Roles of arbuscular mycorrhizas in plant phosphorus nutrition: Interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. *Plant Physiol.*, 156, 1050-1057, doi: 10.1104/pp.111.174581, 2011.

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 will adapt this in the manuscript.

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 will change 'content' to 'concentration' where appropriate.

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 will change this in the revised manuscript 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 will make this change as suggested.

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 will adapt as suggested.

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 will add the suggested reference.

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 will rephrase this sentence to 'On more than one third of the arable land worldwide, plant productivity is considered to be limited by P.'

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

REPLY: We will delete as suggested.

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 will add the reference to the manuscript.

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.

Rodríguez 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 will add these references to the manuscript.

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 will add the reference to the manuscript.

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'll add the information and reference to the manuscript.

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'll replace 'verify' with 'test'.

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'll replace with 'seasonal development'.

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'll insert CO₂ as suggested.

23) L108, 111 'light response curves': hyphenate

REPLY: We'll hyphenate as suggested.

24) L115: add 'to'

REPLY: We'll make the addition as suggested.

25) L116: delete 'phoshor-'

REPLY: We'll delete as suggested.

26) L118: use 'rate' instead of 'measure'

REPLY: We'll adapt as suggested.

27) L119: use 'leaves' instead of 'leaf tissue'

REPLY: We'll adapt as suggested.

28) L121 : use 'sugar concentration' instead of 'sugars'

REPLY: We'll adapt as suggested.

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

REPLY: We'll add 'Kyoto, Japan', to the instrument name.

30) L125: remove 'different'

REPLY: We'll remove as suggested.

31) L134: use 'staining' instead of 'colouring'

REPLY: We'll change as suggested.

32) L154: replace 'as compared to' with 'than in'

REPLY: We'll make the replacement.

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.

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

REPLY: We'll make the replacement as suggested.

36) L201 'Phosphorus': lowercase p

REPLY: We'll correct as suggested.

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

REPLY: We'll adapt as suggested.

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

REPLY: We'll correct as suggested.

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

REPLY: We'll change 'P-limited' to 'P-impooverished'.

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

REPLY: We'll adapt as suggested.

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'll adapt the sentence 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: We'll replace the word as suggested.

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'll add this information as possible explanation of the inhibitory effect. We did not assess O₂ sensitivity of CO₂ assimilation.

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

REPLY: We'll change the sentence to: 'C₄ 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 C₃ plants.'

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

REPLY: We'll adapt as suggested.

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

REPLY: We'll change as suggested.

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

REPLY: We will replace 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: See reply to comment 3 above.

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: See reply to comment 3 above.

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

REPLY: See also reply to comment 3 above. If preferred, this sentence can be deleted.

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 will remove this sentence from the manuscript.

54) L271: replace 'and' with 'which'

REPLY: We'll adapt as suggested.

55) L283: replace 'was' by 'is'

REPLY: We'll correct this.

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

REPLY: We'll replace 'revival' by 'recovery'.

57) L293: remove '(fungus-root)'

REPLY: We'll remove as suggested.

58) L295: correct spelling 'extends'

REPLY: We'll correct as suggested.

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'll remove 'machinery limited'.

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'll remove the '(Eds.)' from the reference.

63) L449: replace '.' With ','

REPLY: We'll make this correction.

64) L458: replace 'Victoria' with 'Melbourne'

REPLY: We'll replace as suggested.