

# ***Interactive comment on “The oxygen isotope composition of phosphate released from phytic acid by the activity of wheat and *Aspergillus niger* phytase” by C. v. Sperber et al.***

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We would like to thank the reviewer for the insightful comments which have helped to improve our manuscript. Below we list our detailed responses and attach the revised manuscript as pdf. In order to make it easier to find the relevant passages in the pdf, we included the lines in brackets.

Line 1 page 5058: the correct terminology here would be “myo-Inositol hexakisphosphate” (i.e. lower case italic ‘myo’ and upper case ‘I’ in Inositol). In addition, the authors might consider using “a subscript ‘6’ in myo-inositol hexakisphosphate. Recommendations for the terminology for the inositol phosphates were made in Shears and Turner

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(2007) and the authors might like to follow that in this manuscript.

Authors: the terminology has been changed in the whole manuscript according to the recommendations by Shears and Turner (2007).

Line 4 page 5058: please provide a citation for the statement that IP6 can accumulate to form the dominant form of organic P in soils – perhaps the review by Turner (2007).

Authors: the review by Turner (2007) has been used as citation for the statement that IP6 can accumulate to form the dominant form of organic P in soils. [ll. 60-63]

Line 14 page 5062: I am not sure Anaheim et al. 2013 is the most appropriate citation here – perhaps cite one of the major reviews on the substrate specificity of the various phytases, or examples where purified phytases have been studied?

Authors: Gibson and Ullah, 1988 and the review by Oh et al. (2004) have been cited for the substrate specificity of phytases. [ll. 218-219]

Line 23 page 5062: I think there is an alternative explanation for phosphate release by the phosphomonoesterases from phytate, which is that the phytate preparation contained some lower-order inositol phosphates. This is quite common for the commercially available sources of phytate. Although I would expect that the phosphomonoesterase preparations can indeed release a small amount of phosphate from phytate of course, this might be via contaminant phytases?), I suspect that hydrolysis of some lower-order esters is also part of the explanation.

Authors: we agree with the reviewer that slight contaminations in the enzymatic assays might have been present, as purification in analytical chemistry is only possible to a certain degree. However, we argue that these contaminations are either too small to have a substantial effect on the results of this study or would be in support our findings: 1.: A substantial contamination of acid phosphatase assays by phytases can be ruled out, because the purification of the same crude acid phosphatase extract in an earlier study did not suggest the presence of phytase (von Sperber et al. 2014). As phytases

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have a much higher molecular weight (70-200 kDa) compared to acid phosphatases (40-58 kDa) (Gellatly et al., 1994; Kawarasaki et al. 1996; Dvorkova, 1998; Nakano et al., 1999), it can be expected that they would elute in another fraction during Fast Protein Liquid Chromatography. This was not observed during the purification process in von Sperber et al. 2014. 2.: The presence of a very small amount of low-order phosphate esters in phytic acid is a possibility. However, in this case the isotope fractionation caused during the hydrolysis of low order phosphate esters would be negative (Liang and Blake, 2006; von Sperber et al. 2014) and therefore reducing the apparent positive fractionation caused during the hydrolysis of phytic acid. In this case, the real isotope fractionation during the hydrolysis of phytic acid would be even more positive.

Line 12 page 5063: it's not clear here why the authors expect 'back-reaction' of enzymatic hydrolysis to re-form IP3 or IP4 esters. Can they provide a citation or two here and some supporting evidence that such a reaction could occur under the conditions of the assay?

Authors: There is no study which shows that the backreaction occurs under the assay conditions. The authors wanted to mention that the finding gives further evidence that the backreaction does not occur. However, this sentence might be confusing to the reader, and we therefore think it might be better to omit it. [ll. 237-242]

Line 1-9 page 5065: it's unclear to me whether this apparently minor difference between the gradients is within the bounds of experimental error. The authors should probably assess statistically whether the observed gradients (0.24, 0.23) are significantly different from 0.25. I think such an analysis ought to be a pre-requisite to speculation on factors that might lead to a slightly smaller gradient than expected – if the slopes are not significantly different from 0.25 then there is not much point in discussing possible explanations for the difference.

Authors: The authors have statistically assessed that the observed gradients (0.24 and 0.23) are significantly different compared to 0.25 (ANOVA,  $p < 0.05$ ). [ll. 237-242]

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Line 12 page 5066: Phosphorus is almost certainly the most misspelled element, but the authors shouldn't contribute to that here. Please correct the spelling of phosphorus.

Authors: the spelling of phosphorus was corrected. [ll. 308]

Line 17 page 5066: remove 'the' near the end of the sentence

Authors: 'the' was removed [ll. 312]

A general comment is that for a publication in Biogeosciences I would expect some broader discussion on the potential importance of the results for our overall understanding of phosphorus biogeochemistry. How do the results help us understand and interpret patterns of phosphorus cycling in nature? At present, the manuscript reads like a very focused biochemical study with limited appeal to the wider biogeochemical community.

Authors: we have included another section in the discussion (4.5) which discusses the implication of our findings for future studies investigating the biogeochemical cycling of phosphorus in the soil plant system. [ll. 385 and following]

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/12/C2109/2015/bgd-12-C2109-2015-supplement.pdf>

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