

## ***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.***

### **Anonymous Referee #1**

Received and published: 8 April 2015

This is an interesting and topical manuscript on the consequences of enzymatic hydrolysis of inositol hexakisphosphate for the oxygen-18 signature of the released orthophosphate. I thought it was a well-conducted study and a well-written manuscript. Although I'm not sure that Biogeosciences is the most appropriate journal for this work, I recommend acceptance with minor revisions. I have a few minor comments.

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

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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).

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?

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.

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?

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.

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.

Line 17 page 5066: remove 'the' near the end of the sentence (or re-word if I have

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misunderstood the meaning).

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.

Citations: Shears, S.B., Turner, B.L., 2007. Nomenclature and terminology of inositol phosphates: clarification and a glossary of terms. pp. 1–6. In: Inositol Phosphates: Linking Agriculture and the Environment, CAB International, Wallingford, UK. Turner BL (2007) Inositol phosphates in soil: amounts, forms and significance of the phosphorylated inositol stereoisomers. Pp. 186-207. In: Inositol Phosphates: Linking Agriculture and the Environment. CAB International, Wallingford, UK.

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**BGD**

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