Biogeosciences Discuss., 12, C2119–C2122, 2015
www.biogeosciences-discuss.net/12/C2119/2015/

Biogeosciences
Discussions
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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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Received and published: 12 May 2015

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 for to find the relevant passages in the pdf, we included the lines in brackets.

Please consider adding a few lines to the introduction why the phytic acid/phosphate pathway is of wide relevance, and why it is necessary to understand the associated isotope effects.

Authors: we have added a few lines to the introduction clarifying the relevance of the

hydrolysis of phytic acid by phytases in soils and why it is important to know the associated isotope effects.

In the same fashion, revise the conclusion and comment on the implications that the finding of rather uniform and temperature-independent isotope effects during soil phosphate hydrolysis has for future research. Is there a relevance for our understanding of soil P cycling beyond isotope mechanisms? Future interpretation of d18Op signatures?

Authors: we have revised the conclusion, mentioning the implication of the temperature-independent isotope effect for future interpretation of δ18O values of phosphate, as well as the importance of the substrate dependency on the oxygen isotope effects in future studies.

Page 5056 Line 4, and Page 5057 Line 1 - Explain the abbreviation "IP6"

Authors: we have added an explanation for the abbreviation "IP6" [ll.40-41 and ll. 61]

Page 5057 Line 4 - Any quantitative information how "dominant" phytic acid can be in the soil organic P pool? A reference would be handy. [ll. 66-67]

Authors: we have added the sentence: "In soils, IP6 can comprise 25-50% of organic phosphorus (Dalal, 1977; Anderson, 1988),"

Page 5059 Line 3ff - Is this total phosphate yield relevant for the isotope mass balance of the assays? Explain how it is referenced. You pick this up in the Results section 3.2., but it would be good to have the information that it corresponds to the IP6->IP2 pathway before.

Authors: we have added a sentence giving the information that a turnover of 65% indicates that IP6 is hydrolyzed to IP2 and not further. [ll. 130-133]

Page 5060 Line 22f - Analytical precision or accuracy?

Authors: we changed the sentence into "Analytical error (precision) calculated on replicate analysis of standards was better than $\pm$ 0.06‰. [ll. 177-178]

Page 5064 Line 12ff - Please explain the reaction mechanism more detailed. Is it always all the way from IP6 to IP2? Figure 1 only explains the IP6 -> IP5 step. Would it then matter stochastically if different Pi groups were isotopically distinct, also in light of a potential back reaction that may have equilibrated IP6 isotopically in a natural system (t->∞)?

Authors: we have included a more detailed explanation of the reaction steps at section 4.2 [ll. 258 and following]. Varying δ18O values of the hydrolyzed phosphate moieties would not influence the determination of the δ18O value of the substrate, because their δ18O value is averaged. However, a varying δ18O value of the remnant substrate IP2 compared to IP6 does influence the determination of the δ18O value of the substrate, because IP2 is not hydrolyzed and therefore not a substrate per se. The effect of a potential backreaction in a natural system with t -> ∞ can be ruled out as well, because the amount of released phosphate molecules from IP6 would always exceed the amount of reformed inositol phosphate molecules to the same extent as during 72 hours. The slope of 0.25 is a strong indicator, that the backreaction does not occur at all.

Page 5066 Line 7 - should read "result“

Authors: we changed “results” into “result” [ll. 302]

Page 5066 Line 17 - correct to something like "... for the observed positive isotopic fractionation“

Authors: we changed the sentence into “. . .there is another reason for the observed positive isotopic fractionation.”[ll. 311-313]

Page 5066 Line 18 - I am not sure if the concept of a hidden equilibrium is clear to readers here. You mention a potential back-reaction earlier, but at this point, this concept needs definitely better explanation.

Authors: The same issue has been raised by reviewer#1: There is no study which

shows that the backreaction occurs under the assay conditions. The authors only wanted mention that the finding of this study gives further evidence that the backreaction does not occur. However, in order to avoid confusion we therefore think it is better to omit the sentence. [ll. 237-242]

Page 5067 Line 7ff - Is the amino acid pattern (or structure?) of the active sites strictly relevant to their function (which is the reaction mechanism)?

Authors: we have extended the explanation of why the amino acid sequence motif at the active sites of the enzymes are relevant to their function. A more detailed discussion of the reaction mechanisms of phosphatases and their potential effect on isotopic fractionation can be found in von Sperber et al. 2014. [ll. 326-340]

Page 5068 Line 5ff - Though an interesting idea, this paragraph leaves me somewhat baffled. Could you come up with a reason why the C-O-P oxygen should be isotopically lighter than the P-O oxygen?

Authors: we have included a sentence describing the possibility that the synthesis of phosphate esters by kinases might also lead to an isotope fractionation. Though only hypothetical, it is the only explanation we have for our observation so far. [ll. 369 and following]

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/12/C2119/2015/bgd-12-C2119-2015-supplement.pdf

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Interactive comment on Biogeosciences Discuss., 12, 5055, 2015.