The authors would like to thank the reviewers for the insightful comments which have helped to improve our manuscript. Below we list our detailed responses and the revised manuscript with track changes.

Response to reviewer #1

Line 1 page 5058: the correct terminology here would be "myo-Inositol hexakisphos- phate" (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. Recommenda- tions 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.

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 IP_6 *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. 213-214]

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 com- mercially available sources of phytate. Although I would expect that the phosphomo- noesterase 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 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 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 Chromotography. 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. 232-241]

Line 1-9 page 5065: it's unclear to me whether this apparently minor difference be- tween the gradients is within the bounds of experimental error. The authors should probably assess statistically whether the observed gradients (0.24, 0.23) are signifi- cantly different from 0.25. I think such an analysis ought to be a pre-requisite to spec- ulation 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 and0.23) are significantly different compared to 0.25 (ANOVA, $p < 0.05$). [ll. 232-241]

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. 302]

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

Authors: 'the' was removed [ll. 305-307]

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 under- standing 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. 379-404]

Response to reviewer #2

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 [ll. 65-74].

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 according to the revie [ll.407-425].

Page 5056 Line 4, and Page 5057 Line 1 - Explain the abbreviation "IP6“
Authors: we have added an explanation for the abbreviation "IP6" [ll.38-39 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.

Authors: we have added the sentence: "In soils, IP_6 *can comprise 25-50% of organic phosphorus (Dalal, 1977; Anderson, 1988)," [ll. 63-64]*

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 IP_6 *is hydrolyzed to* IP_2 *and not further. [ll. 123-126]*

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 ± 0.06‰". [ll. 1707-171]

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. 258-261]. Varying $\delta^{18}O$ *values of the hydrolyzed phosphate moieties would not influence the determination of the* $\delta^{18}O$ *value of the substrate, because their* $\delta^{18}O$ *value is averaged. However, a varying* $\delta^{18}O$ *value of the remnant substrate* IP_2 *compared to* IP_6 *does influence the determination of the* $\delta^{18}O$ *value of the substrate, because* IP_2 *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* IP_6 *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. 296]

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. 305-307]

Page 5066 Line 18 - I am not sure if the concept of a hidden equilibrium is clear to read- ers 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. 237-241]

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. 320-334]

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. 359-367]

Title:

The oxygen isotope composition of phosphate released from phytic acid by the activity of wheat and *Aspergillus niger* phytase.

Authors:

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Keywords:

Phytase, phytic acid, oxygen isotope, isotopic fractionation, phosphate, amino acid sequence motif

Abstract

Phosphorus (P) is an essential nutrient for living organisms. Under P-limiting conditions plants and microorganisms can exude extracellular phosphatases that release inorganic phosphate (P_i) from organic phosphorus compounds (P_{org}). Phytic acid (*myo*-Inositol hexakisphosphate, IP_6) is an important form of P_{org} in many soils. The enzymatic hydrolysis of IP_6 by phytase yields available P_i and less phosphorylated Inositol derivates as products. The hydrolysis of organic P-compounds by phosphatases leaves an isotopic imprint on the oxygen isotope composition ($\delta^{18}O$) of released P_i, which might be used to trace P in the environment. This study aims at determining the effect of phytase on the oxygen isotope composition of released P_i. For this purpose, enzymatic assays with histidine acid phytases from wheat and *Aspergillus niger* were prepared using IP_6, adenosine 5'monophosphate (AMP) and glycerophosphate (GPO_4) as substrates. For a comparison to the $\delta^{18}O$ of P_i released by other extracellular enzymes, enzymatic assays with acid phosphatases from potato and wheat germ with IP_6 as substrate were prepared. During the hydrolysis of IP_6 by phytase, four of the six P_i were released, and one oxygen atom from water was incorporated into each P_i. This incorporation of oxygen from water into P_i was subject to an apparent inverse isotopic fractionation ($\varepsilon \sim 6‰$ to $10‰$), which was similar to that imparted by acid phosphatase from potato during the hydrolysis of IP_6 ($\varepsilon \sim 7‰$), where less than three P_i were released. The incorporation of oxygen from water into P_i during the hydrolysis of AMP and GPO_4 by phytase yielded a normal isotopic fractionation ($\varepsilon \sim -12‰$), similar to values reported for acid phosphatases from potato and wheat germ. We attribute this similarity in ε to the same amino acid sequence motif (RHGXRXP) at the active site of these enzymes, which leads to similar reaction mechanisms. We suggest that the striking substrate-dependency of the isotopic fractionation could be attributed to a difference in the $\delta^{18}O$-values of the C-O-P bridging and non-bridging oxygen atoms in organic phosphate compounds.

60 1. **Introduction**

61 *myo*-Inositol hexakisphosphate (phytic acid, IP_6) is synthesized by plants to store phosphorus
62 (P), *myo*-Inositol and nutritionally important minerals (Cosgrove and Irving, 1980, Shears
63 and Turner, 2007). In soils, IP_6 can comprise 25-50% of organic phosphorus (Dalal, 1977;
64 Anderson, 1988), becoming in some instances the dominant form of organic phosphorus
65 (Turner, 2007). It has been shown that under P-limiting conditions, some plants and
66 microorganisms exude phytases, which catalyze the hydrolysis of phosphomonoester bonds
67 in IP_6 leading to the release of inorganic phosphate (P_i) (Hayes et al., 1999; Richardson et al.,
68 2000, 2001; Lung and Lim, 2006; Li et al., 1997 a and b). The exudation of phytases might
69 therefore be an important mechanism of plants and microorganism to utilize a large source of
70 organic phosphorus in many soils of the world. For example, Zimmermann et al. (2003)
71 showed that a transgenic potato expressing a synthetic gene encoding for phytase was able to
72 take up a significant amount of P from IP_6, whereas the potato wild type was not. However,
73 the cycling and bioavailability of IP_6 and the role of phytase in terrestrial ecosystems are still
74 poorly understood (Turner et al., 2002).

75 The oxygen isotopes associated to of phosphorus might be used to trace these
76 enzymatic processes and to shed new light on the cycling and bioavailability of IP_6 in soils. In
77 the terrestrial environment, the oxygen isotope composition ($\delta^{18}O$) of phosphate has been
78 used as a tracer in the terrestrial environment to study the cycling of P in soils (Zohar et al.,
79 2010a and b, Tamburini et al., 2010, 2012; Angert et al., 2011, 2012; Gross and Angert,
80 2015), in plants (Young et al., 2009; Pfahler et al., 2013) and in aerosols (Gross et al., 2013).
81 Under ambient conditions and in absence of biological activity, the $\delta^{18}O$ of phosphate does
82 not change (Kolodny et al., 1983; O'Neil et al., 2003). However, biological uptake of
83 phosphate leads to a substantial alteration of $\delta^{18}O$ values (Paytan et al., 2002; Blake et al.,
84 2005; Stout et al., 2014). This alteration is due to the activity of intracellular

85 pyrophosphatases, which catalyze a complete oxygen exchange between P_i and water leading
86 to an equilibrium isotope fractionation (Cohn, 1958; Longinelli and Nuti, 1973; Blake et al.,
87 2005; Chang and Blake, 2015). Furthermore, the hydrolysis of organic P-compounds by
88 extracellular phosphomonoesterases and phosphodiesterases leads to the incorporation of one
89 or two oxygen atoms from water into released P_i (Cohn, 1949; Liang and Blake, 2006, 2009,
90 von Sperber et al., 2014). This incorporation of oxygen from water is subject to a kinetic
91 isotope fractionation (ε), which has been determined for alkaline phosphatases (Liang and
92 Blake 2006), phosphodiesterases and nucleotidases (Liang and Blake, 2009), and acid
93 phosphatases (von Sperber et al., 2014). To date, the effect of phytases on the $\delta^{18}O$ of the
94 released inorganic phosphate is not known.

95 In the soil/plant system it is important to distinguish between two types of phytases:
96 3-phytase and 6-phytase. The 3-phytases, EC 3.1.3.8, which are typical for microorganisms
97 and most likely the prevalent phytase in the soil environment, first hydrolyzes the ester bond
98 at the 3-position of IP_6 (*myo*-Inositol hexakisphosphate), which leads to the formation of IP_5
99 (*myo*-Inositol 1,2,4,5,6-pentakisphosphate) and free inorganic phosphate. In contrast, 6-
100 phytases, EC 3.1.3.26, which are typical for plants, first hydrolyze the 6-position of IP_6
101 forming IP_5 (*myo*-Inositol 1,2,3,4,5-pentakisphosphate) and free inorganic phosphate
102 (Wodzinski and Ullah, 1996; Dvorakova et al., 1998) (Figure 1). The aim of this study was to
103 investigate the effect of a phytase from wheat, which belongs to the 6-phytases, and a phytase
104 from *Aspergillus niger*, which belongs to the 3-phytases, on the $\delta^{18}O$ values of released P_i.

105

106 **2. Material and Methods**

107 **2.1 *Preparation of enzymatic assays***

108 Enzymatic assays with phytases from two different organisms (phytase from wheat, Sigma
109 Aldrich P1259, and phytase from *Aspergillus niger*, BASF, Natuphos®, Natuphos 5000) were

110 prepared to determine their effect on the oxygen isotope composition of released P_i. Assays
111 consisted of 200 mM acetate buffer (pH 5.5), with either 2 mM of phytic acid (Sigma Aldrich
112 P8810), or 7 mM of glycerophosphate (Sigma Aldrich G6501) or 7 mM adenosine
113 5'monophosphate (Sigma Aldrich A1752) as substrate and with 0.5 UN of phytase (1 UN is
114 defined as activity required to convert 1 µmole of substrate per minute).

115 Assays with acid phosphatases from potato and wheat germ consisted of 200 mM
116 acetate buffer (pH 4.8), 3 mM of phytic acid and 3 UN of enzyme (Sigma Aldrich p3752 and
117 Sigma Aldrich p3627). Proteins were further purified by dialysis with a dilution factor of
118 10000, using a SnakeSkin dialysis tubing 10K MWCO 16mm (Thermo Scientific, SnakeSkin,
119 PI88243). All assay reagents were prepared in ^{18}O-labeled and non-labeled double deionized
120 water (dd-H_2O) and filter-sterilized. Batch assays had a volume of 3 mL and were prepared in
121 15 mL centrifuge tubes. Directly after the addition of the reagents, the tubes were closed and
122 only opened for sampling. The concentration of released P_i in the assays was monitored using
123 the colorimetric malachite green method (Ohno and Zibilske, 1991). After 48 hours P_i yield
124 was usually close to 65% and did not change any more, despite the enzyme being still active,
125 which indicates that the original substrate IP_6 molecule was degraded to *myo*-Inositol
126 biphosphate (IP_2) and 4 P_i molecules ($4 \cdot P_i / 6 \cdot P_i = 66.6\%$). Enzymatic reactions were
127 terminated after 72 hours by adding 2 mL of 7 M ammonia solution. Experiments were
128 carried out in a temperature controlled water bath at 37°C. To test whether temperature had
129 an effect on the isotopic fractionation, enzymatic assays were also prepared at 4°C. The $\delta^{18}O$
130 of water in the assays was measured at the beginning and at the end of each experiment and
131 did not vary over the course of the experiment. Released P_i was purified according to the
132 protocol of Tamburini et al. (2010). In brief, P_i is first precipitated as Magnesium ammonium
133 phosphate (MAP), which can be retrieved by filtration and subsequently re-dissolved,
134 purified and precipitated as silver phosphate (Ag_3PO_4).

135

136 ***2.2 Ultra violet radiation (UVR) digestion***

137 The $\delta^{18}O$ of IP_6 and of the filtrate after the precipitation of MAP were analyzed after UVR
138 digestion. IP_6 and the filtrate were transferred in a solution with 20 mL of ^{18}O labeled and
139 unlabeled dd-H_2O and 3 mL 28% H_2O_2 and left overnight in a 25 mL quartz tube. The next
140 day, the solutions were exposed to UVR (500W mercury lamp) for 4 hours at 27°C. During
141 the photodecomposition of organic P-compounds, only C-O bonds are cleaved whereas O-P
142 bonds remain intact, leading to the release of the original PO_4 moiety from the organic
143 P-compound without any incorporation of oxygen from water (Liang and Blake, 2006).
144 UVR-released P_i was then processed following the protocol of Tamburini et al. (2010). The
145 $\delta^{18}O$ of phosphate from the organic P-compound ($\delta^{18}O_S$) was calculated according to the
146 modified equation from McLaughlin et al. (2006b):

147 $$\delta^{18}O_S = \frac{\left(\delta^{18}O_{P-UVR}{}^{***} \times \delta^{18}O_W\right) - \left(\delta^{18}O_{P-UVR} \times \delta^{18}O_W{}^{***}\right)}{\left(\delta^{18}O_{P-UVR}{}^{***} - \delta^{18}O_{P-UVR}\right) - \left(\delta^{18}O_W{}^{***} - \delta^{18}O_W\right)} \quad (1)$$

148 with $\delta^{18}O_W{}^{***}$ and $\delta^{18}O_W$ being the $\delta^{18}O$ of labeled and unlabeled water and $\delta^{18}O_{P-UVR}$ being
149 the $\delta^{18}O$ of UVR-released phosphate in water which was labeled (***) or nonlabeled. The
150 fraction of oxygen, which exchanged with water during UVR digestion (F_{exch}) can be
151 calculated according to:

152 $$F_{exch} = \frac{\delta^{18}O_{P-UVR}{}^{***} - \delta^{18}O_W{}^{***}}{\delta^{18}O_S - \delta^{18}O_W{}^{***}} \quad (2)$$

153

154 ***2.3 Determination of $\delta^{18}O$ values of phosphate and water***

155 Oxygen isotope analysis of Ag_3PO_4 was carried out with a Vario Pyro Cube (Elementar,
156 Hanau, Germany) connected in continuous-flow to an Isoprime 100 isotope ratio mass
157 spectrometer (Isoprime, Manchester, UK). The pyrolysis of Ag_3PO_4 took place at 1450°C in
158 a carbon-based reactor. A temperature controlled purge and trap chromatography system was

159 used to separate CO from N_2. Results were calibrated against an internal Ag_3PO_4 standard
160 (Acros Organics, Geel, Belgium; $\delta^{18}O$ = 14.2‰ Vienna Standard Mean Ocean Water
161 (VSMOW)) and two benzoic acid standards distributed by the International Atomic Energy
162 Agency (IAEA) (IAEA 601: $\delta^{18}O$ = 23.1‰ and IAEA 602: $\delta^{18}O$ = 71.3‰ VSMOW).
163 Analytical error calculated on replicate analysis of standards was better than ± 0.4‰.
164 For oxygen isotopes analysis of water, a 0.3% CO_2 and He mixture was equilibrated for 18
165 hours at 25°C with the samples in airtight exetainers. Aliquots of the CO_2/He mixture from
166 the headspace were sampled and transferred to a Delta V Plus mass spectrometer (Thermo
167 Fisher Scientific Inc.) using a gas bench (Gas Bench II, Thermo Scientific Inc.). The oxygen
168 isotope composition of water was derived from the isotope analysis of CO_2. The system was
169 calibrated with the international standards VSMOW, Standard Light Antarctic Precipitation
170 (SLAP), and Greenland Ice Sheet Precipitation (GISP), distributed by the IAEA. Analytical
171 error calculated on replicate analysis of standards was better than ± 0.06‰.

172 Oxygen isotope compositions are reported in the conventional delta notation (δ (‰) =
173 (R_x/R_s – 1) x 1000; where R denotes the ratio of the heavy to light isotope and R_x and R_s are
174 the ratios of the sample and standard, respectively) with respect to VSMOW.

175

176 ### *2.4 Statistical Analyses*

177 Standard deviations (SD), linear regressions, ANOVA and Tukey's HSD tests were
178 calculated using the statistical software R. A one-way ANOVA was carried out for isotopic
179 fractionations caused by different phytases and substrates. After rejecting the null hypothesis
180 of the ANOVA, isotopic fractionations were compared with Tukey`s HSD tests.

181

182 ## 3. **Results**

183 ### *3.1 Incorporation of oxygen from water into P_i during hydrolysis of IP_6 by phytases*

184 Purified phytase from wheat and *Aspergillus niger* hydrolyzed approximately 65% of the
185 phosphate molecules bound to IP_6. This indicates that the original substrate IP_6 molecule was
186 degraded to *myo*-Inositol biphosphate (IP_2) and 4 P_i molecules ($4 \cdot P_i/6 \cdot P_i = 66.6\%$).

187 Control experiments with crude protein extract from wheat phytase without any substrate
188 revealed a substantial contamination of approximately 20µmol of P_i/UN protein extract. In
189 order to remove this contamination, crude protein extracts were dialysed. Mean $\delta^{18}O$ values
190 of released P_i ($\delta^{18}O_P$) from assays with both, non-purified and purified proteins, at different
191 $\delta^{18}O$ values of water ($\delta^{18}O_W$) are shown in Figure 2 and Table 2. Mean $\delta^{18}O_P$ values from
192 assays with non-purified wheat phytase ranged from 6.3‰ to 33.9‰ and linear regression of
193 mean $\delta^{18}O_P$ values against mean $\delta^{18}O_W$ values resulted in a slope of 0.17. Mean $\delta^{18}O_P$ values
194 from assays with purified wheat phytase ranged from 6.5‰ to 31.0‰. Mean $\delta^{18}O_P$ values
195 from assays with purified *Aspergillus niger* phytase ranged from 1.4‰ to 37.7‰. Linear
196 regression of mean $\delta^{18}O_P$ values against mean $\delta^{18}O_W$ values from the assays with purified
197 phytases resulted in a slope of 0.23 in the case of wheat phytase and in a slope of 0.24 in the
198 case of *Aspergillus niger* phytase.

199

200 ***3.2 Incorporation of oxygen from water into P_i during UVR digestion***

201 The $\delta^{18}O$ of P_i produced during UVR digestion of IP_6 in water with a $\delta^{18}O$ of -9.8‰ was
202 21.0‰ and 24.4‰ for water with a $\delta^{18}O$ of 51.2‰, corresponding to an incorporation of 6%
203 of oxygen from water into released P_i (Table 1). The filtrate retrieved after precipitation of
204 MAP contains IP_2, which was also analyzed for its $\delta^{18}O$. The $\delta^{18}O$ of P_i produced during
205 UVR digestion of IP_2 in water with a $\delta^{18}O$ of -10.4‰ was 21.7‰ and 22.4‰ for water with a
206 $\delta^{18}O$ of 73.3‰, corresponding to an incorporation of 1% of oxygen from water into the
207 formed P_i (Table 1). These findings confirm that the UVR-induced release of the original PO_4

208 moiety from the organic P-compound proceeded with little incorporation of oxygen from
209 water.
210

211 ***3.3 Oxygen isotope composition of P_i released after hydrolysis of AMP and GPO4 by***
212 ***phytase and after hydrolysis of IP_6 by acid phosphatase.***

213 Phytases can hydrolyze single phosphomonoester substrates and some acid phosphatases can
214 partly hydrolyze IP_6 (Gibson and Ullah, 1988; Oh et al., 2004; Annaheim et al., 2013). For
215 this reason, the effect of wheat phytase on adenosine 5'-monophosphate (AMP) and on
216 glycerophosphate (GPO_4) used in a previous study (von Sperber et al., 2014) was tested.
217 Wheat phytase hydrolyzed approximately 72% AMP and approximately 80% of GPO_4.
218 Experiments with AMP as substrate ($\delta^{18}O_S$ = 15.8‰), which were carried out in assays with
219 a $\delta^{18}O_W$ of -45.5‰, resulted in a mean $\delta^{18}O_P$ of -1.9‰. Experiments with GPO_4 as substrate
220 ($\delta^{18}O_S$ = 16.6‰), which were carried out in assays with a $\delta^{18}O_W$ of -50.4‰, resulted in a
221 mean $\delta^{18}O_P$ of -2.4‰ (Table 3).

222 In addition, two acid phosphatases from potato and wheat germ with IP_6 as substrate were
223 tested. Acid phosphatase from wheat germ hydrolyzed approximately 10% of IP_6 and acid
224 phosphatase of potato hydrolyzed approximately 40% of IP_6. Experiments with acid
225 phosphatase from wheat germ were carried out in assays with a $\delta^{18}O_W$ of -58.5‰ and
226 resulted in a mean $\delta^{18}O_P$ of 3.0‰. Experiments with acid phosphatase from potato were
227 carried out in assays with a $\delta^{18}O_W$ of -9.8‰ and resulted in a mean $\delta^{18}O_P$ of 16.7‰ (Table 3).
228

229 **4. Discussion**

230 ***4.1 Implications of incorporation of oxygen from water into P_i during hydrolysis of IP_6 by***
231 ***phytases***

232 The slopes from assays with purified phytases are close to 0.25, similar to experiments
233 conducted with phosphomonoesterases like alkaline and acid phosphatases (Liang and Blake,
234 2006; von Sperber et al., 2014). However, both slopes (0.23 and 0.24) are significantly
235 different from 0.25 (ANOVA, $p < 0.05$). This indicates that the contamination with P_i from
236 the crude extract, where we observe a strong deviation in the slope, may not have been fully
237 removed by our purification step. The finding of a 0.25 slope implies that one oxygen atom
238 from water is incorporated into each released P_i. From this observation follows that the
239 enzymatic release of P_i from IP_6 proceeds by cleaving the P-O bond of the oxygen connected
240 to *myo*-Inositol via the addition of oxygen from water, a process that is different from the
241 abiotic photodecomposition, where C-O bonds are cleaved and P-O bonds remain intact.
242
243 ***4.2 Oxygen isotope fractionation during the incorporation of oxygen from water into P_i***
244 Assuming that released P_i consists of three oxygen atoms from the original substrate and one
245 oxygen which has been incorporated from water, the following mass balance can be applied
246 to determine the oxygen isotope fractionation (ε) caused by phytases (Liang and Blake,
247 2006):
248 $$\delta^{18}O_P = 0.75 \cdot \delta^{18}O_S + 0.25 \cdot (\delta^{18}O_W + \varepsilon) \quad (3)$$
249 where $\delta^{18}O_P$ is the δ-value of released P_i, $\delta^{18}O_S$ is the δ-value of the substrate (meaning the
250 average value of the 4 phosphate released from IP_6), $\delta^{18}O_W$ is the δ-value of the water and ε is
251 the isotopic fractionation.
252 The analysis of $\delta^{18}O_P$ and $\delta^{18}O_W$ is straightforward, but the determination of $\delta^{18}O_S$ is
253 more complicated. Compared to single phosphomonoesters, such as glycerophosphate or
254 adenosine 5'-monophosphate, phytic acid consists in total of six phosphate molecules, of
255 which all might have different $\delta^{18}O$ values. The direct determination of the $\delta^{18}O$ of each of
256 the phosphate molecules attached to *myo*-Inositol is not possible. However, the bulk isotope

257 composition of the phosphate moieties from IP_6 and IP_2 can be determined, allowing for the
258 calculation of $\delta^{18}O_S$. Our results indicate that the original substrate IP_6 molecule was
259 degraded to IP_2 and 4 P_i molecules ($IP_6 \rightarrow IP_5 + P_i \rightarrow IP_4 + 2P_i \rightarrow IP_3 + 3P_i \rightarrow IP_2 + 4P_i$). In
260 this case, $\delta^{18}O_S$ corresponds to the $\delta^{18}O$ of the 65% of phosphate molecules that were cleaved
261 from IP_6. By using a simple mass balance, $\delta^{18}O_S$ and can be derived indirectly by from $\delta^{18}O$
262 of IP_6 ($\delta^{18}O_{IP6}$) and IP_2 ($\delta^{18}O_{IP2}$) as follows:

263 $$\delta^{18}O_{IP6} = 2/3 \cdot \delta^{18}O_S + 1/3 \cdot \delta^{18}O_{IP2} \quad (4)$$

264 solving for $\delta^{18}O_S$:

265 $$\delta^{18}O_S = 3/2 \cdot \delta^{18}O_{IP6} - 1/2 \cdot \delta^{18}O_{IP2} \quad (5)$$

266 The $\delta^{18}O_{IP2}$ value was determined by oxidation photo-digestion of the filtrate, which consists
267 of IP_2, with UV radiation after the MAP precipitation step. Digestion of the organic P-
268 compounds by UVR led to the release of P_i with a $\delta^{18}O_{IP6}$ value of 22.8‰ (±0.4‰) and a
269 $\delta^{18}O_{IP2}$ value of 22.0‰ (±0.4‰) (Table 1). Using these values in equation (5) we calculate a
270 $\delta^{18}O_S$ value of 23.2‰ (±0.7‰). Solving equation (3) with the obtained $\delta^{18}O_S$ value results in
271 an average ε of 6.4‰ (±2.9‰) in assays with wheat phytase and in an average ε of 6.7‰
272 (±3.4‰) in assays with *Aspergillus niger* phytase (Table 2). The isotopic fractionation is not
273 significantly different between the two types of phytases (ANOVA; p-value > 0.05).

274 We can refine our results by addressing the fact that the linear regression of $\delta^{18}O_P$ vs.
275 $\delta^{18}O_W$ yields a slope of 0.23 in the case of wheat phytase and in a slope of 0.24 in the case of
276 *Aspergillus niger* phytase (Figure 2). These values are slightly below a slope of 0.25,
277 indicating small contaminations with P_i that was not derived from IP_6. These small
278 contaminations are the reason for the linear relationship between $\delta^{18}O_W$ values and ε (Table
279 2). In case of wheat phytase, only 23 percent of oxygen in free inorganic phosphate in
280 solution is derived from water. This means that free inorganic phosphate in solution, which
281 has been released from the organic P substrate by enzymatic activity, only accounts for 92%

282 of total inorganic phosphate in solution (4·23%). Therefore, 8% of free inorganic phosphate
283 in solution is due to contamination. To account for this contamination another term has to be
284 included into the mass balance and equation 3 needs to be rewritten for experiments with
285 wheat phytase as follows:

286 $$\delta^{18}O_P = 0.92\cdot(0.75\cdot\delta^{18}O_S + 0.25\cdot\delta^{18}O_W + 0.25\cdot\varepsilon) + 0.08\cdot\delta^{18}O_{CON} \quad (6)$$

287 and for experiments with *Aspergillus niger* phytase as follows:

288 $$\delta^{18}O_P = 0.96\cdot(0.75\cdot\delta^{18}O_S + 0.25\cdot\delta^{18}O_W + 0.25\cdot\varepsilon) + 0.04\cdot\delta^{18}O_{CON} \quad (7)$$

289 with $\delta^{18}O_{CON}$ being the $\delta^{18}O$ of the contaminant. Analysis of $\delta^{18}O_{CON}$ was not possible,
290 however, $\delta^{18}O_P$ values under environmental conditions usually lie within the range of 15‰
291 (±5)‰ (Tamburini et al. 2014). Assuming a $\delta^{18}O_{CON}$ value of 15‰ results in an average ε of
292 8.2‰ (±0.9‰) in assays with wheat phytase and in an average ε of 7.7‰ (±1.0‰) in assays
293 with *Aspergillus niger* phytase. Taking into account a possible contamination, ε will change
294 depending on the assumed $\delta^{18}O_{CON}$ value. An assumed $\delta^{18}O_{CON}$ value of 20‰ would result in
295 an ε of 6.4‰ (±0.9‰) in case of wheat phytase and in an ε of 6.9‰ (±1.0‰) in case of
296 *Aspergillus niger* phytase, while an assumed $\delta^{18}O_{CON}$ value of 10‰ would result in an ε of
297 9.9‰ (±0.9‰) in case of wheat phytase and in an ε of 8.6‰ (±1.0‰) in case of *Aspergillus*
298 *niger* phytase.

299 These results provide an estimate of 6‰ to 10‰ for the oxygen isotopic fractionation
300 during the release of P_i from IP_6, *i.e.* the oxygen incorporated is enriched in ^{18}O relative to the
301 water it derived from. True inverse kinetic isotope fractionations are rare, and so far have not
302 been observed for oxygen isotope effects in phosphorus cycling. It is unlikely that the
303 apparent inverse isotope effect is caused by the contaminant, as $\delta^{18}O_{CON}$ would have to be
304 +65‰ in experiments with wheat phytase and +117‰ in experiments with *Aspergillus niger*
305 phytase to accommodate for a normal isotope effect (i.e. ε < 0‰). These high δ values are not

306 realistic and we therefore assume that there is another reason for the observed positive
307 isotopic fractionation.
308 An inverse kinetic isotope effect can be caused by a hidden equilibrium isotope
309 fractionation. Unlike kinetic isotope fractionation, equilibrium isotope fractionation is often
310 strongly temperature dependent. The effect of temperature on the isotopic fractionation
311 caused by phytases was tested at 4°C and 37°C. In the case of wheat phytases, ε had a value
312 of 4.9‰ (±1.0‰) and in the case of *Aspergillus niger* phytase, ε had a value of 8.0‰
313 (±0.9‰) at 4°C. The isotopic fractionation was not significantly different between the two
314 temperatures (ANOVA; p-value > 0.05), mirroring the findings with phosphomonoesterases
315 (Liang and Blake, 2006, 2009; von Sperber et al., 2014). This indicates that a hidden
316 equilibrium isotope fractionation may not be the cause of the observed apparent inverse
317 isotope fractionation.
318
319 ***4.3 Comparison of phytase to acid phosphatase oxygen isotope fractionation***
320 Phytases can vary significantly in their catalytic properties and mechanisms. For example,
321 depending on the optimum pH of catalysis, they can either be alkaline, neutral or acid
322 phosphatases (Mullaney and Ullah, 2003). Most of plant and fungal phytases belong to the
323 histidine acid phosphatases, which share the same amino acid sequence motif (RHGXRXP)
324 at their active sites as acid phosphatases and nucleotidases (van Etten et al., 1991; Oh et al.
325 2004; Kostrewa et al., 1997; Kostrewa et al., 1999; Lim et al., 2000). The amino acid
326 sequence motif at the active site of phosphatases drives the reaction mechanisms, which can
327 either lead to the incorporation of an oxygen atom derived from a water molecule into the
328 newly formed phosphate (Lindquist et al., 1994; Knoefel and Straeter, 2001; Ortlund et al.,
329 2004), e.g. acid phosphatases or nucleotidases, or to the incorporation of an oxygen atom
330 derived from a hydroxide ion, e.g. alkaline phosphatases (Kim and Wickoff, 1991; Stec et al.,

331 2000). It has been suggested that these two types of reaction mechanisms are the reason why
332 different phosphomonoesterases cause different isotopic fractionations (von Sperber et al.
333 2014). Based on these findings it can be expected that the isotopic fractionation caused by
334 phytases is similar to that of acid phosphatases and nucleotidases.

335 The action of wheat phytase led to a ε of -12.3‰ (±2.3‰) in the case of AMP and
336 of -12.0‰ (±2.2‰) in the case of GPO4 (calculated according to equations 6 and 7 with a
337 $\delta^{18}O_{CON}$ value of 15‰; Table 3). These fractionations are similar to those reported for acid
338 phosphatases from wheat germ and potato (approximately -10‰ von Sperber et al., 2014).
339 Acid phosphatase from wheat germ hydrolyzed approximately 10% of IP_6, while acid
340 phosphatase of potato hydrolyzed approximately 40% of IP_6. The $\delta^{18}O$ of the *myo*-Inositol
341 phosphate derivate of these reactions were not analyzed. Using a value of 23.2‰ for $\delta^{18}O_{S,}$
342 obtained from the phytase experiment, resulted in an ε of -0.9‰ (±0.6) in the case of acid
343 phosphatase from wheat germ and an ε of 7.2‰ (±2.9) in the case of acid phosphatase from
344 potato (Table 3). The isotopic fractionation caused by potato acid phosphatase is similar to
345 those caused by the two phytases used in this study. The isotopic fractionation caused by
346 wheat germ acid phosphatase differs by approximately 8‰ compared to fractionation caused
347 by phytases. The activity of wheat germ acid phosphatase with IP_6 as substrate was very low,
348 indicating that this enzyme was only able to cleave one phosphate moiety from IP_6. One
349 possibility to explain this observation is that the $\delta^{18}O_S$ value of this single moiety of IP_6 is
350 lower than 23.2‰. The determination of the $\delta^{18}O_S$ values of the single phosphate molecules is
351 a challenge beyond the scope of this study which needs to be addressed in future. The
352 observation of generally strong similarities in the oxygen isotope fractionation between
353 phytases and acid phosphatases supports the hypothesis that the reaction mechanisms
354 catalyzed by these enzymes are similar.
355

356 *4.4 Apparent substrate dependency of oxygen isotope fractionation*

357 The isotopic fractionation caused by phytases and acid phosphatases differ systematically
358 with varying substrates, and encompass a range from inverse (relative enrichment in ^{18}O,
359 positive ε) to normal (relative depletion in ^{18}O, negative ε) isotope effects. At first sight, this
360 striking substrate-dependency of the isotopic fractionation implies a substrate-dependent
361 mode of function of hydrolysis that may involve a multi-step process, with competing inverse
362 and normal isotope effects. While such a scenario is not impossible, there may be a more
363 straightforward explanation for this phenomenon. We hypothesize that there may be a
364 difference in the $\delta^{18}O$ of the bridging oxygen atom (C-O-P) and the three non-bridging
365 oxygen atoms (O-P) in organic phosphate compounds. If the enzymatic hydrolysis of
366 phosphate esters leads to an isotope fractionations, it is by all means possible that the
367 synthesis of phosphate esters by kinases also leads to an isotope fractionation. This is an
368 important aspect which should be addressed in future studies looking at the effect
369 phosphatases on the oxygen isotope composition of phosphate. Therefore, if the C-O-P
370 bridging oxygen atoms are depleted in ^{18}O relative to the non-bridging oxygen atoms, the
371 $\delta^{18}O$ of P_i cleaved from IP_6 and IP_2 by abiotic photodecomposition would be lower than the
372 actual $\delta^{18}O$ of the three oxygen atoms cleaved from IP_6 during enzymatic activity. This would
373 result in an underestimate of $\delta^{18}O_S$ which in turn would lead to a biased calculation of ε, i.e.
374 the true value of ε could be smaller than 0‰ and thus be a normal isotope effect. We consider
375 this issue to be a crucial aspect for the interpretation of the effect of phosphatases on the
376 oxygen isotope composition of phosphate. We have not yet found a way to verify this
377 hypothesis, which would be a highly interesting task for future research.

378

379 ***4.5 Implications to studies of biogeochemical cycling of P in the soil/plant system***

380 It has been shown that phytase activities increase for a wide range of plants grown under P-
381 limited conditions (Li et al., 1997 a and b; Richardson et al. 2001; Lung and Lim, 2006).
382 However, the measurement of these enzymatic activities in soils are usually conducted under
383 pH-buffered and temperature controlled conditions with artificial substrates, e.g. para-
384 nitrophenyl phosphate. In the natural soil environment these conditions can vary substantially
385 and rates of hydrolysis might be much lower. For example, in soils phytic acid might undergo
386 adsorption and/or precipitation reactions, which would prohibit the diffusion of an IP_6
387 molecule into the active site of the enzyme (Anderson, 1980; McKercher and Anderson,
388 1989; Ognalaga et al., 1994). And the use of para-nitrophenyl phosphate as substrate cannot
389 distinguish between extracellular acid phosphatase activity and phytase activity. The isotopic
390 imprint caused by phosphatases might be used to distinguish between different enzymatic
391 processes in situ under natural conditions. In a recent study the effect of
392 phosphomonoesterases and –diesterases on the oxygen isotope composition of phosphate
393 could be traced in alkaline Mediterranean soils. The enzymatic release of phosphate from
394 added organic compounds led, on the one hand to an increase of available P_i concentration
395 and on the other hand to a decrease of $\delta^{18}O$ values of available P_i (Gross and Angert, 2015).
396 This decrease in $\delta^{18}O$ values was attributed to the negative isotopic fractionation caused by
397 alkaline phosphatases (Liang and Blake, 2006). Another recent study comducted on a 6500-
398 year soil coastal dune chronosequence found that $\delta^{18}O$ values of available P_i was in isotopic
399 equilibrium with soil water at younger sites and below isotopic equilibrium at older sites,
400 with higher organic P contents. The low $\delta^{18}O$ values at the older sites indicated higher
401 mineralization rates of labile organic P compounds, in particular DNA, by extracellular
402 phosphatases (Roberts et al., 2015). The findings of our study are therefore of high value in
403 future studies for the interpretation of $\delta^{18}O$ values of available phosphate extracted from soils
404 with high phytic acid contents.

405

406 ## 5. Conclusion

407 The present study indicates that the isotopic fractionation caused by phytases from wheat and
408 from *Aspergillus niger* is similar compared to the fractionation reported for acid phosphatases
409 from wheat germ and potato, and that there is no substantial difference between oxygen
410 isotope fractionation by 6-phytases and 3-phytases. This observation is attributed to the
411 similar reaction mechanisms of phytases and acid phosphatases. Temperature does not have
412 an influence on the observed isotopic fractionations, which alleviates the interpretation of
413 $\delta^{18}O$ values of phosphate extracted from soils under natural conditions with large diurnal and
414 seasonal temperature fluctuations. Furthermore, this study highlights the influence of the
415 substrate on the calculated isotopic fractionation caused by phosphatases. Our results support
416 the hypothesis that $\delta^{18}O$ values of the bridging oxygen atom (C-O-P) and the non-bridging
417 oxygen atoms (O-P) in phosphate molecules of organic P-compounds are different. As the
418 hydrolysis of different organic phosphorus substrates by different phosphatases can lead to
419 very different isotopic signals our findings highlight the potential of oxygen isotopes
420 associated to phosphate as tracer for enzymatic processes in the soil/plant system. Future
421 research should focus on the substrate effect on $\delta^{18}O$ values of phosphate during enzymatic
422 hydrolysis. On the one hand, efforts should be directed to test whether the bridging oxygen
423 atom (C-O-P) has a different $\delta^{18}O$ values compared to the non-bridging oxygen atoms (O-P).
424 On the other hand, it is important to test in the field whether the hydrolysis of different
425 organic phosphate esters leads to different $\delta^{18}O$ values of resin extractable P_i.

426

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433

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600 **Figure Legend:**

601 Figure 1: Phytic acid (IP_6) degradation to IP_5 by 3-phytases and 6-phytases (modified from
602 Dvořáková, 1998). 3-phytases first hydrolyze the ester bond at the 3-position of IP_6
603 (*myo*-Inositol hexakisphosphate), which leads to the formation of IP_5 (*myo*-Inositol 1,2,4,5,6-
604 pentakisphosphate) and free inorganic phosphate. In contrast, 6-phytases, first hydrolyze the
605 6-position, which leads to the formation of IP_5 (*myo*-Inositol 1,2,3,4,5-pentakisphosphate)
606 and free inorganic phosphate. The numbering of the carbon atoms corresponds to the
607 numbering for the D-configuration.

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609 Figure 2: Mean $\delta^{18}O$ values of released P_i ($\delta^{18}O_P$) at different $\delta^{18}O$ values of water ($\delta^{18}O_W$)
610 from (a.) assays with non-purified wheat phytase (dashed line) and purified wheat phytase
611 (solid line) (b.) an assay with purified *Aspergillus niger* phytase.

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625 **Table Legend:**

626 Table 1: Results from UVR digestion of organic P-compounds. The table shows measured
627 δ^{18}O-values of ^{18}O labeled water ($\delta^{18}O_W$***) and non-labelled ($\delta^{18}O_W$) water, as well as δ^{18}O-
628 values of UVR-released phosphate in assays with ^{18}O labeled water ($\delta^{18}O_{IPx}$***) and non-
629 labelled water ($\delta^{18}O_{IPx}$). The $\delta^{18}O_S$ value was calculated according to equation (1).
630 Exchanged F_{exch} is the fraction of oxygen atoms which exchanged with water calculated with
631 equation (2).

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633 Table 2: δ^{18}O-values (‰) of water ($\delta^{18}O_W$), released phosphate ($\delta^{18}O_P$) and phosphate in
634 organic P-compound ($\delta^{18}O_S$) as well as isotopic fractionation (ε), which was calculated
635 according to equations 6 and 7 with an assumed $\delta^{18}O_{CON}$ value of 15‰. Results are from
636 experiments with IP_6 as substrate and with phytases from wheat and *Aspergillus niger*.
637 Table 3: δ^{18}O-values of water ($\delta^{18}O_W$), released phosphate ($\delta^{18}O_P$) and phosphate in organic
638 P-compound ($\delta^{18}O_S$) as well as isotopic fractionation (ε), which was calculated according to
639 equations 6 and 7 with an assumed $\delta^{18}O_{CON}$ value of 15‰. Results are from experiments with
640 IP_6, AMP and GPO4 as substrates and with phytase from wheat and acid phosphatases from
641 wheat germ and potato. * values from von Sperber et al., 2014.

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645 **Table 1:**

Substrate	$\delta^{18}O_W$	$\delta^{18}O_W$***	$\delta^{18}O_{P-UVR}$	$\delta^{18}O_{P-UVR}$***	exch. F	$\delta^{18}O_{IPx}$	SD
IP_6	-9.8	51.2	21.0	24.4	0.06	22.8	0.4
IP_2 (filtrate)	-10.4	73.3	21.7	22.4	0.01	22.0	0.4

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649 **Table 2:**

Enzyme	Substrate	°C	n	$\delta^{18}O_W$	$\delta^{18}O_P$	$\delta^{18}O_S$	ε	mean ε	SD
Phytase wheat (crude)	IP_6	37	6	-67	6.3	23.2	NA	NA	NA
	IP_6	37	3	-10	18	23.2	NA		
	IP_6	37	6	42.3	24.1	23.2	NA		
	IP_6	37	6	94.2	33.9	23.2	NA		
Phytase wheat (dialysed)	IP_6	37	6	-53.7	6.5	23.2	7.0	8.2	0.9
	IP_6	37	5	-10.4	16.7	23.2	8.0		
	IP_6	37	6	29.0	25.9	23.2	8.7		
	IP_6	37	6	51.0	31.0	23.2	9.0		
	IP_6	4	6	-10.6	16.3	23.2	6.5	6.5	1.0
Phytase *A. niger* (dialysed)	IP_6	37	2	-75.4	1.4	23.2	9.2	7.7	1.0
	IP_6	37	3	-10.4	16.5	23.2	7.1		
	IP_6	37	3	48.4	30.6	23.2	6.9		
	IP_6	37	3	77.2	37.7	23.2	7.8		
	IP_6	4	3	-10.6	17.1	23.2	9.5	9.5	0.9

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667 **Table 3:**

Enzyme	Substrate	°C	n	$\delta^{18}O_W$	$\delta^{18}O_P$	$\delta^{18}O_S$	ε	SD
Phytase wheat	IP_6	37	5	-10.4	16.7	23.2	7.7	1.2
	AMP	37	3	-45.5	-1.9	15.8	-12.3	2.3
	GPO4	37	3	-50.4	-2.4	16.6	-12.0	2.2
Apase wheat germ	IP_6	37	3	-58.5	3.0	23.2	-0.9	0.6
	AMP*	37	9	-10.0	7.1	15.8	-8.9	1.7
	GPO4*	37	3	-10.0	7.2	16.6	-11.0	1.3
Apase potato	IP_6	37	2	-9.8	16.7	23.2	7.2	2.9
	AMP*	37	10	-10.0	7.3	15.8	-8.1	1.0
	GPO4*	37	6	-10.0	7.0	16.6	-11.8	1.3

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