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REVIEW ARTICLE

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Oxygen isotope ratios of phosphates in the soil-plant system: Limitations and future developments

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Abstract

The oxygen isotope ratio of phosphates is a powerful tool to study phosphorus in the soil-plant system. In the past two decades, the scientific community has made substantial progress in characterizing biogeochemical processes that lead to an alteration of oxygen isotope ratios in phosphates and in evaluating oxygen isotope ratios of phosphates in plants and soils under various environmental conditions around the globe. However, there are still uncertainties with respect to their interpretation in environmental systems. These uncertainties include the comprehensive analysis of isotope endmembers, artifacts during the chemical extractions and the phosphate purification protocols, overlapping isotope values from various processes and sources, gaps of knowledge about isotope effects of metabolic pathways, and the possibly erroneous assumption that insights from experiments under controlled laboratory conditions can be directly translated into the complex soil-plant system. This paper provides a critical discussion of these uncertainties addressing recommendations and needs for future research and gives an outlook on recent technological advances, such as triple oxygen isotope analysis or the use of high-resolution mass spectrometry. We conclude by suggesting that a concerted and systematic effort by scientists from a wide range of disciplines will be necessary to remove the uncertainties in the interpretation of oxygen isotope ratios in phosphates as an environmental tracer.

KEYWORDS

isotope equilibrium, microorganism, oxygen isotopes, phosphorus, plant, soil, water

1 | INTRODUCTION

Phosphorus (P) is a limiting nutrient in many ecosystems around the world. Excess fertilization to overcome the limitation for crops reduces biodiversity and can lead to the transfer of P from agricultural soils to aquatic ecosystems, resulting in eutrophication and toxic algal blooms (Carpenter, 2008; Ceulemans et al., 2014; Schindler et al., 2016). Among the analytical tools used to improve our understanding of the environmental P cycle, the ratio

of oxygen isotopes in phosphate (reported here as $\delta^{18}O_p$ compared to VSMOW in the per mille notation) has provided many new insights during the past two decades.

Under ambient conditions and in the absence of biological activity, $\delta^{18}O_p$ does not change (O'Neil et al., 2003). However, intracellular and extracellular enzymes catalyze the exchange of oxygen between phosphate and water (Blake et al., 2005; Cohn, 1958; Liang & Blake, 2006a, 2009; von Sperber et al., 2014, 2015; Wu et al., 2015). The isotopic imprints caused by these enzymatic reactions have been used in numerous studies for the interpretation of $\delta^{18}O_p$ in the environment, initially in aquatic ecosystems (McLaughlin, Cade-Menun, et al., 2006; McLaughlin, Kendall, et al., 2006; Paytan et al., 2017; Pistocchi et al., 2017; Tonderski et al., 2017) and later in the soil-plant system (Angert et al., 2011; Helfenstein et al., 2018; Pfahler et al., 2013; Tamburini et al., 2012). Even though spatial and temporal patterns in $\delta^{18}O_p$ in environmental systems have been observed (e.g., seasonal variation, Angert et al., 2011), decreasing $\delta^{18}O_p$ values with soil depth, (Bauke et al., 2017; Joshi et al., 2016); increasing $\delta^{18}O_p$ values along riparian buffer strips, (Bauke et al., 2022); and chronosequence studies, (Frkova et al., 2022; Roberts et al., 2015; Tamburini et al., 2012), we are of the opinion that the current interpretation of these patterns is not conclusive (Helfenstein et al., 2018; Pistocchi et al., 2020). Here, we discuss issues that complicate the interpretation of $\delta^{18}O_p$ in the soil-plant system, which include the analysis of different isotopic endmembers, deviations from the oxygen isotope equilibrium between phosphate and water, knowledge gaps on oxygen isotope effects in metabolic pathways, and discrepancies between findings from controlled laboratory conditions and the complex environment of the soil-plant system. In addition, we give an outlook on methodological advances that might help to clarify these issues and promise new insights into the application of this environmental tracer.

Highlights

- Discussion of uncertainties associated with the oxygen isotope ratios of phosphates.
- Isotope endmembers, contamination, overlapping δ-values, isotope effects of metabolic pathways.
- Discussion of new technological advances.
- Triple oxygen isotope analysis, high-resolution mass spectrometry and DUV-Raman.

2 | OXYGEN ISOTOPE RATIOS IN PHOSPHATE FROM DIFFERENT ENDMEMBERS IN THE SOIL-PLANT SYSTEM

In the soil-plant system, P can be broadly categorized into five different pools: the soil inorganic P, the soil organic P, the soil microbial P, the plant biomass P, and the bedrock P. Past studies have analyzed the $\delta^{18}O_p$ in these pools under various environmental settings, providing a range of values found in the environment (Figure 1). However, the methodological approach to determine the $\delta^{18}O_p$ in the different pools in the soil-plant system bears uncertainties, because it relies on extractions, which are subject to controversy (Barrow et al., 2020; Gu et al., 2020; Guppy, 2021). On the one hand, liquid extractions have contributed to our understanding of the environmental P cycle by differentiating soil P pools depending on their solubility in specific chemical extracts (Cross & Schlesinger, 1995; Helfenstein et al., 2020; Syers et al., 2008). On the other hand, the chemical extracts target only operationally defined P pools, which may introduce large biases in the quantification of specific P forms (Condron & Newman, 2011; Klotzbücher et al., 2019). It is important to account for these uncertainties and biases when interpreting $\delta^{18}O_p$ values in the soil-plant system.

2.1 | Uncertainties concerning the oxygen isotope ratio of soil inorganic P

Inorganic P in soils can occur in primary and secondary minerals but also as polyphosphates and orthophosphate (PO_4^{3-}) in solution. The largest amount of inorganic P is not bioavailable but either precipitates with cations (predominantly Ca) or it can be adsorbed on iron (Fe) and aluminum (Al) oxy-hydroxides. Liquid extractions cannot target these specific P forms in soils but rather extract a bulk of all inorganic P forms (Klotzbücher et al., 2019). Some extraction reagents can promote the binding or dissolution of P with elements due to changes in pH and ionic strength. This might lead to an under- or an overestimation of a specific P pool in soils. For example, the alkaline extracts of Hedley sequential fractionation (0.5M $NaHCO_3$ and 0.1M NaOH) can lead to the precipitation of Ca-phosphates through the presence of both dissolved phosphate and exchangeable Ca, causing an underestimation of dissolved P. These newly formed Ca-phosphates present in the alkaline extracts are then filtered out together with the soil particles and subsequently dissolved in the acidic extraction (1M HCl), leading to an overestimation of dissolved P in this extract. Furthermore, the 1M HCl extract does not only dissolve Ca-bound P from crystallized apatite of

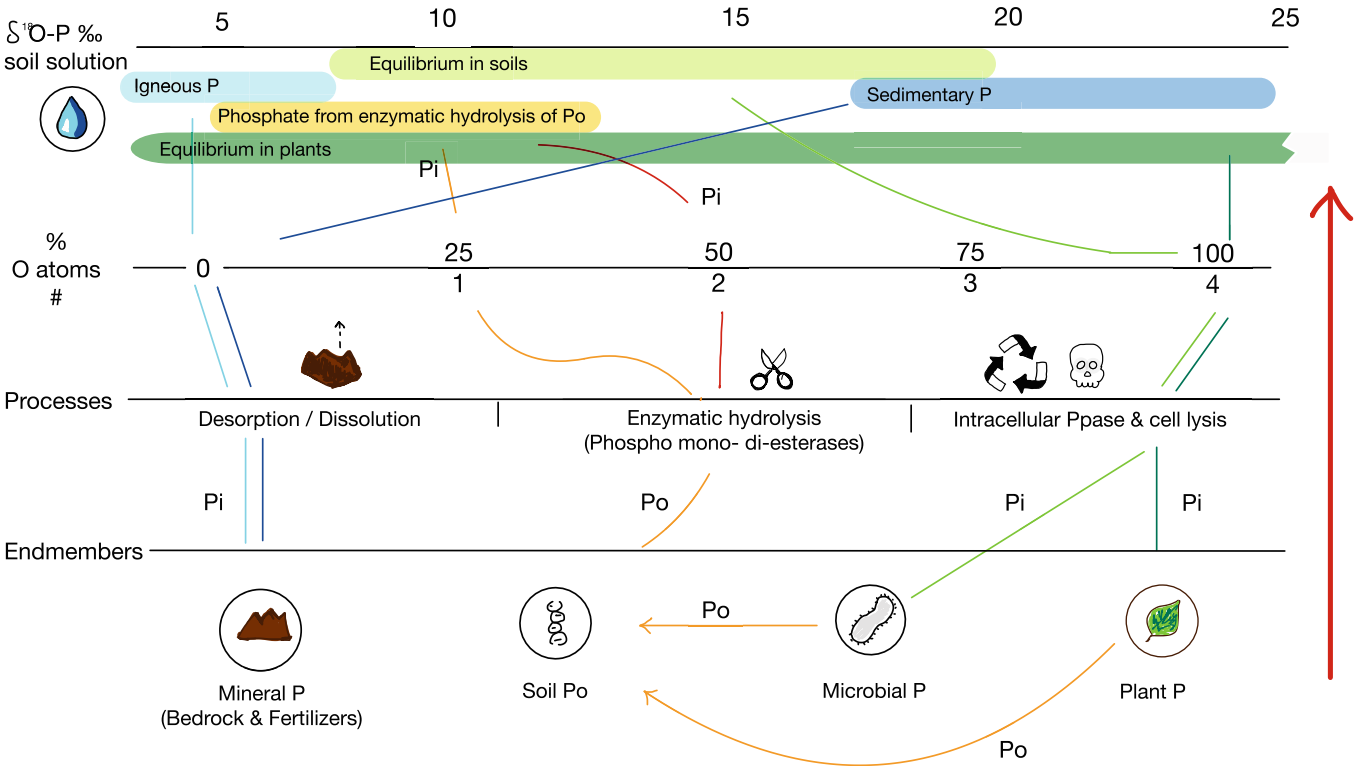

FIGURE 1 Schematic representation (from the bottom) of (1) endmembers, (2) processes, (3) number and percentage of O atoms exchanged, and (4) final $\delta^{18}O_p$ of the inorganic phosphate pools produced. Values for igneous (light blue lines) and sedimentary (dark blue lines) rocks taken from (Ayliffe et al., 1992; Mizota et al., 1992); values of equilibrium in soils (light green line) and in plants (dark green line) determined by using global averages of $\delta^{18}O_p$ in atmospheric precipitation and related temperature (Rozanski et al., 2013) and equation 1 (Chang & Blake, 2015). For plants, we considered the range of temperature and $\delta^{18}Ow$ values of plant leaves and temperature available from the literature (Cernusak et al., 2022). Note the wide range of calculated values (lowest +0.65‰, highest +52‰, out of scale); phosphate from enzymatic hydrolysis of organic P (Po) calculated using $\delta^{18}O_w$ in rain water (reference as above), average $\delta^{18}O_p$ values of organic P in soils (Tamburini et al., 2018), and average epsilon values for mono-esterases (Liang & Blake, 2006a; von Sperber et al., 2014, 2015) (orange lines) and di-esterases (Liang & Blake, 2009) (red lines). The red arrow indicates the direction of processes and fluxes, from the sources to the soil solution.

igneous origin or from secondary hydroxyapatite but also leads to the desorption/dissolution of P bound to iron and aluminum oxides (Benzing & Richardson, 2005; Gu et al., 2020). Another uncertainty arises due to the possible hydrolysis of polyphosphates during the extraction procedure. Polyphosphates represent a biologically mediated pool of P that is often not accounted for but can be abundant in fungi-dominated environments (Bünemann et al., 2008; Ghonsikar & Miller, 1973; Saia et al., 2021). The acidic reactants used during the extraction and/or the $\delta^{18}O_p$ purification procedure can hydrolyse these polyphosphates. This hydrolysis leads to the incorporation of oxygen from the surrounding water molecules into the newly formed orthophosphates, which results in an isotopic artefact. To account for such contamination by oxygen from water, we recommend the use of ^{18}O-labeled and unlabelled acids during the extraction and purification procedures (Tamburini et al., 2010).

2.2 | Uncertainties concerning the oxygen isotope ratio of soil organic P and microbial P

Microbial P can be defined as the fraction contained within the soil microbial biomass and has been typically extracted by fumigation (Kouno et al., 2002). Anion resin membranes are generally used as a sink for inorganic phosphate extracted after cell lysis by hexanol. However, this extraction leads, on the one hand, to an incomplete extraction of the microbial biomass (Ehlers et al., 2008), and, on the other hand, to a lack of differentiation between microbial inorganic and organic P (Brookes et al., 1982; McLaughlin et al., 1986). In addition, with this method only microbial inorganic P is recovered, while microbial organic P and other forms of phosphate, like polyphosphate, which are not adsorbed on the resin membranes remain in solution. These phosphates are lost for analysis or transferred into the NaOH extractable

pool. This means that a method to determine $\delta^{18}O_p$ of microbial organic P is still lacking. One possible approach would be to extract and analyse the microbial P forms remaining in solution after fumigation before the NaOH step through (1) acid hydrolysis to target polyphosphate, (2) UV digestion for organic P forms (Tamburini et al., 2018) and/or (3) the addition of specific phosphatases (Annaheim et al. 2013), whose isotopic fractionation is known. If this separation is not done, the microbial organic P would be diluted too much in the total organic P pool.

Other more complexed forms of organic P can comprise a large part of the total amount of P in soils. Plants and microorganisms exude extracellular enzymes that hydrolyse specific organic P compounds in soils releasing orthophosphate, which can be taken up by organisms or chemically react and bind to the soil mineral phase. Soil organic P can therefore be an important isotopic endmember for the interpretation of $\delta^{18}O_p$ values of other soil pools. To date, there is only one published method to determine the oxygen isotope ratio of soil organic P (Tamburini et al., 2018). This method integrates an anion exchange resin extraction in the presence of a biocide (hexanol) and a 0.25M NaOH-EDTA extraction, to sequentially obtain the microbial and the bulk organic P pools (Cade-Menun & Preston, 1996; Turner et al., 2005). However, most of the organic P extracted by NaOH-EDTA, which is further separated from orthophosphate by gel chromatography, is not hydrolyzed by enzymatic activities (Jarosch et al., 2015; McLaren et al., 2020). This either indicates that the extracted organic P forms are still complexed and/or protected in mineral associations and thus not directly accessible to enzymes (Reusser et al., 2023), or that the high-molecular-weight organic P fraction is recalcitrant and not a viable substrate for enzymes in the soil. In that case, extracted organic P forms are not an isotopic endmember for the interpretation of $\delta^{18}O_p$ values (Jarosch et al., 2015). We suggest that future studies should aim to characterize ecologically meaningful organic P pools by differentiating, as described above, recalcitrant organic P (e.g., extracted by NaOH) from the labile and newly synthesized organic P (e.g., microbial organic P), which constitutes the organic P substrate most likely hydrolysed by enzymes and thus more rapidly turned over in soils.

2.3 | Uncertainties concerning the oxygen isotope ratio of plant biomass P

Similar methodological issues for soil P pools are encountered for plant P pools. For example, labile organic P extracted with trichloroacetic acid might be hydrolysed during the first steps of the extraction process (Pfahler et al., 2013). As with soil acid extracts, this issue can be overcome by using ^{18}O-labeled and unlabeled acids, but the problem of extracting functionally meaningful plant P pools remains. Tracing P from soils to plants using $\delta^{18}O_p$ values is particularly challenging because the metabolism inside plants and, if present, mycorrhizal fungi rapidly causes a partial or complete loss of the isotopic signature of the P taken up (Bauke et al., 2021; Pfahler et al., 2013; Qin et al., 2018). However, without symbiotic association with mycorrhizae, $\delta^{18}O_p$ in roots can retain the signature of the phosphate from the soil solution. Studies that used ^{18}O-enriched irrigation water in the nutrient solution, have been able to trace the ^{18}O-enrichment of P in the xylem sap of *Fagus sylvatica* L. (Hauenstein et al., 2020) and in wheat roots, but not in shoots (Bauke et al., 2021; Qin et al., 2018). These pioneering studies show promise that in the future $\delta^{18}O_p$ can be used as a tracer for P in plants, however, $\delta^{18}O_p$ in plants is still understudied and not routinely characterized, which could lead to erroneous interpretations. As shown in Figure 1, the equilibrium $\delta^{18}O_p$ calculated using the oxygen isotope ratio of water ($\delta^{18}O_w$) in leaves and temperature from different environments (Cernusak et al., 2022) has a wide range, which overlaps with many other possible endmembers. This observation points to the need for a correct and careful characterization of the $\delta^{18}O_p$ of this and all other endmembers to draw meaningful conclusions.

3 | DEVIATIONS FROM THE OXYGEN ISOTOPE EQUILIBRIUM BETWEEN PHOSPHATE AND WATER

If a phosphate molecule is taken up by a living cell, the ubiquitous intracellular enzyme pyrophosphatase catalyzes a complete oxygen exchange between phosphate and cell water, which leads to a temperature-dependent isotope fractionation (Blake et al., 2005; Chang & Blake, 2015; Cohn, 1958). In a carefully designed cell-free enzyme incubation experiment, Chang and Blake (2015) determined the oxygen isotope equilibrium fractionation between phosphate and water in the presence of pyrophosphatase under controlled laboratory conditions which led to the following empirical equation:

$$1000\ln\alpha_{(PO4-H2O)} = 14.43(\pm 0.39) \times 1000/T(K) - 26.54(\pm 1.33) \quad (1)$$

With α being R_{PO4}/R_{H2O} and R being the ratio of ^{18}O:^{16}O in the respective molecule and with T(K) being

the temperature in Kelvin. To date, this study provides the most accurate determination of oxygen isotope equilibrium values caused by pyrophosphatase. Similar observations have been made in the past with experiments on whole organisms (Kolodny et al., 1983; Longinelli & Nuti, 1973; Pucéat et al., 2010). We recommend the use of equation 1 to determine the oxygen isotopic equilibrium of phosphate in environmental systems. The oxygen isotope exchange rate between phosphate and water catalyzed by pyrophosphatase has been determined using Raman spectroscopy (von Sperber et al., 2017) and isotope ratio mass spectrometry (Chang et al., 2021). It ranges from $1.51E^{-05}$ to $3.13E^{-04}$ sec^{-1} and depends on temperature, pyrophosphatase and substrate concentration, and the type and concentration of the co-factor (i.e., Mg). Due to the high reaction rate, the complete oxygen exchange between phosphate and water and the large isotope fractionation (approx. 23‰ at 20°C), the activity of pyrophosphatase is often assumed to dominate the oxygen isotope composition of phosphate in environmental systems (Bauke et al., 2022; Helfenstein et al., 2018; Tamburini et al., 2012). Indeed, multiple studies have found one or more phosphate pools in the environment (soils, waters and plants) that carried the isotope equilibrium value. All these studies concluded that the isotope equilibrium values in environmental phosphates can essentially be assigned to the activity of intracellular pyrophosphatases. However, there are frequently found deviations from oxygen isotope equilibrium, particularly in the soils (Bauke et al., 2022; Helfenstein et al., 2021; Pistocchi et al., 2020; Rodionov et al., 2020; Wang et al., 2021).

The $\delta^{18}O_p$ values below the isotopic equilibrium could be attributed to the contribution of other endmembers or processes in the soil-plant system, for example, the hydrolysis of organic phosphate by extracellular phosphatases, geogenic sources of phosphates (Smith et al., 2021) or fertilizers (Granger et al., 2017; Tamburini et al., 2014). Extracellular phosphatases hydrolyze organic phosphate compounds which causes an exchange of one or more oxygen atoms between water and phosphate. This oxygen exchange leads to an enzyme and substrate-specific isotope fractionation that has been determined for alkaline phosphatases, nucleotidases (Bai et al., 2020; Liang & Blake, 2006a; von Sperber et al., 2014), phosphodiesterases (Liang & Blake, 2009), and acid phosphatases (Bai et al., 2020; Solhtalab et al., 2022; von Sperber et al., 2014; Wu et al., 2015). These specific isotope fractionations leave a distinct isotopic imprint on the released phosphate and usually cause a negative isotope fractionation between oxygen in water and the incorporated oxygen in the released orthophosphate. However, the released phosphate also inherits oxygen atoms from the original organic P compound (e.g., the substrate of the enzymatic reaction). Therefore, the $\delta^{18}O_p$ released by the activity of extracellular phosphatases depends on the oxygen isotope composition of the soil water and the oxygen isotope composition of the organic P compound. This again highlights the importance of the isotopic characterization of soil organic P that is available for enzymatic hydrolysis.

The $\delta^{18}O_p$ values that are above the equilibrium are more difficult to explain. To date the only processes that have been identified to cause a positive isotope fractionation to increase $\delta^{18}O_p$ values above the isotopic equilibrium are (a) the hydrolysis of phytate (von Sperber et al., 2014) and (b) the hydrolysis of RNA (Liang & Blake, 2009), which are both important constituents of soil organic P and the biological uptake of inorganic P (Blake et al., 2005). Future studies should evaluate the $\delta^{18}O_p$ of more organic molecules together with the isotopic fractionation of other relevant environmental enzymes.

4 | OXYGEN ISOTOPIC EFFECTS IN THE METABOLIC PATHWAYS OF MICROORGANISMS AND PLANTS

Microorganisms are central in controlling the P cycle in the soil-plant system (Chen et al., 2019; Kouno et al., 2002; Oberson & Joner, 2005; Spohn & Widdig, 2017). Bacteria and fungi take up available P in the form of orthophosphate from the soil solution. Once taken up, inorganic phosphate enters the microbial metabolism fueling the energy ATP-ADP cycle and contributing to cell growth (DNA synthesis), to genetic information transmission (RNA synthesis), and to cellular function maintenance (phosphorylation of proteins and signaling). Phosphate, or any other phosphate-bearing molecule inside a cell, can be released back into the soil upon cell death and lysis. Despite its importance for cellular functioning and general microbial metabolism, we still lack a full understanding of phosphate homeostasis and cellular P cycling and more specifically, of how microbial metabolism influences $\delta^{18}O_p$ values (Austin & Mayer, 2020). Most biotic processes lead to a modification of the $\delta^{18}O_p$ by breaking the phosphoanhydride bond (P-O) and by promoting a phosphoryl transfer, which is accompanied by an enzyme-specific isotopic effect (Hengge, 2005).

Although cellular metabolism might influence the $\delta^{18}O_p$ in multiple ways, our knowledge is limited and concentrates only on the few specific enzymes as discussed above. The general assumption is that whenever phosphate is taken up by a (micro)organism, intracellular pyrophosphatases will erase any previous $\delta^{18}O_p$ and promote a new isotope equilibrium. However, pyrophosphatases are mostly active during the exponential growth of cells since

it is an intrinsic step of DNA synthase (Kottur & Nair, 2018). Once exponential growth has halted pyrophosphatase activities are likely slowed down, which might then also slow down the isotopic equilibration between phosphate and water. Other enzymes involved in microbial metabolism that have not yet been characterized for their isotopic imprint might change the isotopic composition of microbial P away from the equilibrium. For example, ATPase, which promotes phosphoryl transfer reactions and is generally involved in several steps of the major metabolic cycles (e.g., Krebs cycle), has not yet been studied for its isotopic fractionation. Adenylate kinase is a phosphotransferase enzyme that promotes the reversible transformation of ATP and AMP to two ADP molecules, an essential process for maintaining the energy balance in a cell. Even though this enzymatic process might cause an oxygen isotope equilibrium, it has not been studied yet. The isotope effect of phosphoryl transfer promoted by kinases has never been considered either (Cohn, 1953). Although kinases might not contribute directly to the cytosolic phosphate pool and its $\delta^{18}O_p$, they could influence it by controlling the $\delta^{18}O_p$ of organic substrates.

Biological processes might influence the $\delta^{18}O_p$ not only by phosphoryl transfer but also by discriminating between the lighter or heavier isotopologue (a molecule with a different isotope of one of the elements). As demonstrated in controlled laboratory incubations, cellular uptake of inorganic phosphate can cause such a fractionation (Blake et al., 2005). This fractionation is most likely caused by the activity of high-affinity phosphate transporters (Pst), while low-affinity transporters (Pit) seem not to discriminate between isotopologues (Lis et al., 2019). In plants, $\delta^{18}O_p$ of TCA-extracted phosphate (considered as a proxy for metabolic P) was at the expected equilibrium value during the first stages of plant growth (Pfahler et al., 2013). However, in a subsequent study, it was shown that during senescence and under P limitation, the $\delta^{18}O_p$values in old leaves were lower than equilibrium (Pfahler et al., 2017). By concurrently looking at P fluxes as determined by ^{33}P labeling, the authors concluded that lower $\delta^{18}O_p$ values were most likely the result of enzymatic hydrolysis of intracellular organic compounds happening in old leaves with the released phosphate translocated to new leaves.

To summarize, our knowledge of cell functioning with respect to the oxygen isotope ratio in cytosolic phosphate is limited to the recognition of pyrophosphatase activity and of a few other enzymes. Considering the complexity of the microbial metabolism (Aslan et al., 2017), we suggest that the oxygen isotope ratio in cytosolic phosphate should be determined by the integration and interaction of the different metabolic processes, as already done for other elements (Wijker et al., 2019).

5 | OXYGEN ISOTOPE RATIO OF SOIL WATER, PLANT WATER AND INTRACELLULAR WATER

Arguably the most important endmember controlling the $\delta^{18}O_p$ in the soil-plant system is water, because of the various oxygen exchange mechanisms described above. The oxygen isotope ratio of soil water is highly variable in space and time, and sampling soil water on a single day is not representative of the oxygen isotope composition over longer periods of time, for example, throughout the entire growing season. This has been shown theoretically (Barnes & Allison, 1983; Riley et al., 2002), under controlled laboratory conditions (Rothfuss et al., 2013) and in field studies (Allison et al., 1983; Brinkmann et al., 2018; Hsieh et al., 1998). Therefore, recalcitrant P pools (e.g., 1M HCl extractable P) likely integrate the isotopic signal from the soil water over long periods (years, decades, centuries depending on the soil and the environment (Helfenstein et al., 2020)) compared to more labile pools (e.g., microbial pools), which can turn over in hours or days. Thus, depending on the P pool, using the average $\delta^{18}O_w$ of rainwater during the growing season to estimate the soil water oxygen isotope composition may therefore be a more meaningful determination for this endmember. More generally, the choice of the $\delta^{18}O_w$ range of soil water should take into account the estimated turnover time of the targeted P pool. The $\delta^{18}O_w$ of soil water in a location can be obtained using datasets provided by the Global Network of Isotopes in Precipitation (GNIP). Precipitation varies with season, with generally lower $\delta^{18}O_w$ of rainwater throughout the colder months. However, during the warmer months of the year, evapotranspiration may be a considerable factor influencing the isotopic composition of soil water due to fractionation processes (soil evaporation) (Barnes & Allison, 1983; Gibson et al., 2008; Hacker et al., 2019; Hsieh et al., 1998). In addition, root uptake of bioavailable water can be isotopically different from non-bioavailable water (e.g., water that is held within clay minerals or deep vs. shallow soil water (Ehleringer & Dawson, 1992; von Freyberg et al., 2020). The determination of $\delta^{18}O_w$ in deeper soil layers provides further challenges. In contrast to the topsoil, evaporation does not affect the $\delta^{18}O_w$ in the subsoil and the long residence time of deep soil water as well as the mixing of old and new waters may demand that winter precipitation $\delta^{18}O_w$ values are considered too (Gazis & Feng, 2004).

Like soil water, $\delta^{18}O$ values of plant water can also vary spatially and temporally (Allison et al., 1985; Cernusak et al., 2016; Farquhar & Lloyd, 1993; Gonfiantini, 1965; Pfahler et al., 2013; Seeger & Weiler, 2021). In addition to these variabilities, it is generally assumed that the $\delta^{18}O$ of

intracellular water is indistinguishable from extracellular water, because the diffusion of water through the cell membranes does not fractionate between $H_2{}^{16}O$ and $H_2{}^{18}O$. However, empiric evidence has shown that intracellular water of growing *E. coli* cultures is partly of metabolic origin. Studies in the past found that intracellular $\delta^{18}O_w$ values can be substantially different from $\delta^{18}O_w$ values of the growth medium, with up to 70% of intracellular water derived from metabolic processes (Kreuzer-Martin et al., 2003, 2005, 2006). Another recent study showed that about 30-40% of oxygen in phosphate of intracellular biomolecules is derived from metabolic water (Li et al., 2016). This contribution of intracellular water on the oxygen isotope composition of phosphate may therefore alter our interpretation of $\delta^{18}O_p$ in the environment, in particular from studies that use ^{18}O labeled irrigation water and/or that took place in in arid environments or at high latitudes with relatively high $\delta^{18}O_w$ values of soil water.

6 | CONTROLLED LABORATORY CONDITIONS VERSUS COMPLEX ENVIRONMENTAL SYSTEMS

Considering only specific isotopic fractionations or processes that have been determined under controlled laboratory conditions for the interpretation of $\delta^{18}O_p$ values of environmental samples may be misleading. A petri dish or a cell-free enzymatic assay does not provide an accurate representation of the "dirty reality" of the soil-plant system. In any biological or environmental system, enzymatic processes are seldom isolated and many occur simultaneously in time and space. Their activities and substrate turnover vary depending on the environmental conditions (e.g., temperature, soil water content, soil pH, etc.) and may change with the seasons and/or the land use. Therefore, $\delta^{18}O_p$ values from environmental samples reflect an integrated signal of many processes, biotic and abiotic (see below). For instance, $\delta^{18}O_p$value in soil that is within the range of the isotope equilibrium value could also be derived by mixing of several endmembers with different $\delta^{18}O_p$ values. Cell-free enzymatic assays are generally conducted under optimal conditions to maximize the enzymatic turnover of various substrates. This includes an optimum pH, temperature and co-factor concentrations which are all usually not found under environmental conditions. A similar issue arises with microbial cell cultures since only a few studies have used growing conditions that were suboptimal (Lis et al., 2019). While this approach is very informative to determine microorganism-specific oxygen exchange dynamics (Stout et al., 2014), the conditions of cell cultures generally ensure maximum microbial growth and zero stress on the organisms, which is not a realistic representation of typical soil conditions, where many of the microbes might be inactive or under stress. Therefore, microbial oxygen-exchange dynamics between phosphate and water are likely much slower under environmental conditions compared to controlled laboratory conditions.

Furthermore, a petri dish or an enzymatic assay is a closed system in which one phosphate molecule can potentially be recycled many times. An environmental system, in contrast, is an open system from which phosphate molecules can be sorbed and desorbed from mineral particles or lost via leaching or export. In the case of soil, a continuous exchange of phosphate molecules between the soil solid phase and the soil solution (abiotic process) can replenish phosphate that has been taken up by microorganisms or been lost through leaching (Frossard et al., 2011; Rinderer et al., 2021). This exchange thereby provides a continuous supply of phosphate that does not have the oxygen isotopic imprint of intracellular or extracellular phosphatases. Soils are a particularly complicated environmental matrix because they consist of solid, liquid and gaseous phases. Depending on weather, climate and soil physical properties (e.g., texture, organic carbon content, etc.), the contributions of the liquid and gaseous phase change over time (sometimes as fast as within a day), which is not the case in a petri dish. In order to assign specific enzymatic processes to the measured $\delta^{18}O_p$, it is necessary to acquire additional information. For example, a comparative analysis of similar systems with varying environmental parameters (such as gradient studies) and to closely consider such effects over time. A useful approach to account for temporal changes is the incubation of soil samples with ^{18}O-labelled water to assess the oxygen exchange dynamics over time. Such incubation experiments allow us to manipulate specific environmental or biological conditions while maintaining the complexity of the soil environment and provide unique information on the rate of biological P cycling. However, only a few labelled incubation experiments have been conducted so far, which explore a limited set of conditions and soil types, for example, low and high P availability, drying-rewetting, and so forth. (Hacker et al., 2019; Helfenstein et al., 2021; Pistocchi et al., 2020; Siegenthaler et al., 2020). The mechanistic understanding obtained from such experiments might allow a more conclusive interpretation of *in situ* observations in the environment.

7 | FUTURE METHODOLOGICAL ADVANCES

7.1 | Measurements of oxygen isotope ratios of phosphate

One of the reasons why the analysis of $\delta^{18}O_p$ is not so widespread can be attributed to the cumbersome and

time-consuming analytical procedure prior to the measurement of oxygen isotope ratios by TC/EA-IRMS (Thermal Conversion Elemental Analyser coupled to an Isotopic Ratio Mass Spectrometer). As described above, phosphate has to be extracted and each extract has to be purified, that is, phosphate is concentrated and all other oxygen-bearing species (e.g., nitrates, sulfates, organic compounds) are eliminated. This procedure lasts about one week and depending on the laboratory infrastructure, about 10–15 samples per week can be typically processed if the research uses ^{18}O-labelled and non-labelled extracts. The final product, Ag_3PO_4, is weighed in silver microcapsules, converted to CO in the TC/EA and the oxygen isotope ratio is measured using an IRMS.

In addition, to date no internationally accepted set of standards for oxygen isotope ratios in phosphate is available. This has led to much confusion regarding the $\delta^{18}O_p$ values of the most commonly used reference standards, NBS 120b and NBS 120c (Lécuyer et al., 1996, 2007; Vennemann et al., 2002). This highlights the need for the establishment of a new set of international reference standards by the IAEA (International Atomic Energy Agency). An ideal standard should (Hoefs, 2021):

1: be homogeneous.

2: be available in relatively large amounts.

3: be easy to handle for chemical preparation and measurement.

4: have an isotope ratio close to the natural range of variation.

We believe silver phosphate would be a good candidate because it fulfills these requirements. In addition, silver phosphate is a very stable compound, easy to degas and not hygroscopic, featuring additional benefits to use this material as an international reference standard (Fourel et al., 2011; Lécuyer et al., 2007; Watzinger et al., 2021).

7.2 | Measurement of oxygen isotope ratios of bioavailable soil water

Since the $\delta^{18}O_w$ of the soil water strongly affects $\delta^{18}O_p$, a systematic measurement of the $\delta^{18}O_w$ of the soil water is necessary (Hacker et al., 2019). In the past, soil water was typically sampled and measured for $\delta^{18}O_w$ using cryogenic distillation of the soil sample and isotopic measurement with Cavity-Ring-Down Spectroscopy (CRDS), Off-Axis Integrated Cavity Output Spectroscopy (OA-ICOS) or Isotope Ratio Mass Spectrometry (IRMS). However, recent studies have shown that extraction conditions and physicochemical soil properties affect extracted soil water isotope composition (Orlowski et al., 2018). Also, other approaches to extract liquid soil water like centrifugation or squeezing may be prone to bias.

The development of ICOS, the direct liquid-vapor equilibration method was introduced around 10 years ago. This enabled cheaper, easier isotope analysis with minimum sample handling and less pre-treatment procedures (Herbstritt et al., 2012; Koehler & Wassenaar, 2011; Munksgaard et al., 2011). The equilibration method establishes an isotope equilibrium between the water in the soil sample and CO_2 in the headspace within a closed system. Then, the isotope composition of the equilibrated headspace vapour is analyzed using CRDS or OA-ICOS. However, factors that can affect the accuracy of this method like soil texture, soil saturation, equilibration time and soil-organic matter interaction (Gralher et al., 2018, 2021; Herbstritt et al., 2012) are still unknown and required for the development of a standardized procedure.

Recent developments of *in situ* membrane-based probes for direct measurements of soil water isotopes (Rothfuss et al., 2013; Volkmann & Weiler, 2014) now allow continuous observations of $\delta^{18}O_w$ of soil water. Such a new *in situ* measurement provides an unprecedented combination of high temporal and high spatial resolution data, which help to unravel the partitioning among transpiration, interception and soil evaporation and transport processes, transit times and water ages in distinct compartments of the hydrological cycle (Sprenger et al., 2018).

7.3 | Triple oxygen isotope dynamics and alternatives to TC/EA-IRMS

As discussed above, the overlapping $\delta^{18}O$ values caused by various endmembers and mechanisms that affect the oxygen isotope composition of phosphate often prevent a clear interpretation in environmental systems. The analysis of triple oxygen isotope ratios (^{16}O, ^{17}O, ^{18}O), however, may allow the unequivocal identification of particular enzymatic pathways as well as a distinction between ambient soil, plant and metabolic water pools. Microbial intracellular water can comprise significant fractions of atmospheric oxygen, presenting a distinct mass-independent ^{17}O anomaly, that is passed onto the respective inorganic and organic phosphorus compounds (Herwartz, 2021). Similarly, atmospheric O_2 carries ^{17}O anomalies that have been traced in various geological materials (Pack, 2021) and more recently in the hydrosphere (Sambuichi et al., 2023). This ^{17}O anomaly could also persist in phosphate from ash derived from biomass burning (e.g., wildfires) because phosphate exchanges with air O_2 at elevated temperatures (Bigio & Angert, 2018; Liang & Blake, 2006b). In addition, process-specific slopes in the triple oxygen isotope space can be used to identify individual processes, including the release of inorganic phosphate by

enzymes, which opens new opportunities for understanding the environmental phosphorus cycle.

In the past, most analyses of $\delta^{18}O_p$ have relied on the use of Thermal Conversion Elemental Analysis Isotope Ratio Mass Spectrometry (TC/EA-IRMS). However, recent methodological advances provide alternatives. Raman spectroscopy has been widely used to collect information about the chemical properties of living and non-living samples. In the phosphate-water system, Raman scattering depends on the different vibrational energies of the five phosphate isotopologues ($P^{16}O_4$, $P^{16}O_3{}^{18}O_1$, $P^{16}O_2{}^{18}O_2$, $P^{16}O_1{}^{18}O_3$, $P^{18}O_4$) and can therefore be used to measure the quantity of these isotopologues in solution. This method has been used to monitor the evolution of phosphate isotopologues and determine the kinetics of oxygen isotope exchange between phosphate and water in the presence of pyrophosphatases (Moller et al., 2022; von Sperber et al., 2017). The application of Raman spectroscopy was limited to controlled and purified laboratory conditions. This is because of the fluorescence interference caused by organic matter in environmental samples. Recent methodological advances using Deep Ultraviolet (DUV) Raman spectroscopy now allow for the analysis of different organic and inorganic phosphate compounds in soils (Vogel et al., 2017). Such methodological advances mean that in the future it may be possible to directly monitor oxygen isotope exchange dynamics at a high spatial resolution in environmental systems as well. However, some problems like the photo-hydrolysis of important organic phosphorus compounds upon UV excitation will need to be overcome.

Recently, the use of LC MS/MS (Schryer et al., 2020) and Orbitrap HRMS (high-resolution mass spectrometer (Hilkert et al., 2021; Neubauer et al., 2020)) for the analysis of oxyanions have been proposed for the analysis of $\delta^{18}O_p$. If successful, these two approaches might expedite the analytical procedure and time, since the preparation requirements for the samples could be reduced. They would also overcome the problem of phosphate quantity required for purification by an order of magnitude.

The isotopic shift in ^{31}P NMR spectroscopy caused by the presence of ^{18}O instead of ^{16}O in phosphate has been used to study enzyme-catalyzed reactions (Cohn & Hu, 1978; Pucar et al., 2001). This approach can be powerful in determining the degree of ^{18}O substitution and even the configurational changes of phosphate molecules when working in experimental settings at high levels of ^{18}O enrichment; however, its application to study $\delta^{18}O_p$ at natural abundances needs further development.

8 | CONCLUSION

In this paper, we discussed common problems encountered with the interpretation of oxygen isotope ratios of phosphate and water in the soil-plant system. Some of these limitations can be overcome by (1) carefully considering the pitfalls of the available extraction and purification protocols, (2) by a systematic analysis of relevant endmembers (including phosphates and water) and (3) by identifying and characterizing processes that may contribute to the $\delta^{18}O_p$ in the environment. We think that recent methodological advances such as triple oxygen isotope systematics and Orbitrapp high-resolution mass spectrometry will further eliminate some of these issues and will provide new insights into using oxygen isotope ratios in phosphates to shed new light on the environmental fate of P. Furthermore, we are convinced that future progress in this research field requires a concerted effort across disciplines. Collaborations among soil scientists, geologists, hydrologists, microbiologists and chemists will help to improve our understanding of the processes that determine the oxygen isotope ratios of phosphates in soil-plant systems under environmental conditions.

AUTHOR CONTRIBUTIONS

Christian von Sperber: Conceptualization; writing – review and editing; writing – original draft; visualization. **Chiara Pistocchi:** Conceptualization; writing – review and editing; writing – original draft; visualization. **Markus Weiler:** Conceptualization; writing – original draft; writing – review and editing; visualization. **Federica Tamburini:** Conceptualization; writing – original draft; writing – review and editing; visualization.

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DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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REFERENCES

Allison, G., Gat, J. R., & Leaney, F. W. (1985). The relationship between deuterium and oxygen-18 delta values in leaf water. *Chemical Geology: Isotope Geoscience Section*, *58*, 145–156.

Allison, G. B., Barnes, C. J., & Hughes, M. W. (1983). The distribution of deuterium and ^{18}O in dry soils 2. Experimental. *Journal of Hydrology*, *64*, 377–397.

Annaheim, K. E., Rufener, C. B., Frossard, E., & Bünemann, E. K. (2013). Hydrolysis of organic phosphorus in soil water suspensions after addition of phosphatase enzymes. *Biology and Fertility of Soils*, *49*, 1203–1213.

Angert, A., Weiner, T., Mazeh, S., Tamburini, F., Frossard, E., Bernasconi, S. M., & Sternberg, M. (2011). Seasonal variability of soil phosphate stable oxygen isotopes in rainfall manupulation experiments. *Geochimica et Cosmochimica Acta*, *75*, 4216–4227.

Aslan, S., Noor, E., & Bar-Even, A. (2017). Holistic bioengineering: rewiring central metabolism for enhanced bioproduction. *Biochemical Journal*, *474*, 3935–3950.

Austin, S., & Mayer, A. (2020). Phosphate homeostasis—A vital metabolic equilibrium maintained through the INPHORS signaling pathway. *Frontiers in Microbiology*, *11*, 1367.

Ayliffe, L. K., Veeh, H. H., & Chivas, A. R. (1992). Oxygen isotopes of phosphate and the origin of island apatite deposits. *Earth and Planetary Science Letters*, *108*, 119–129.

Bai, Y., Stout, L., Unal-Tosun, G., Li, J., & Jaisi, D. (2020). Synthesis and degradation of polyphosphate: Isotope effects in enzyme- and bacteria-catalyzed reactions. *ACS Earth and Space Chemistry*, *4*, 2327–2336.

Barnes, C. J., & Allison, G. B. (1983). The distribution of deuterium and ^{18}O in dry soils: 1. Theory. *Journal of Hydrology*, *60*, 141–156.

Barrow, N. J., Sen, A., Roy, N., & Debnath, A. (2020). The soil phosphate fractionation fallacy. *Plant and Soil*, *1*, 1–11.

Bauke, S. L., Schnepf, A., von Sperber, C., Orlowski, N., Lewandowski, H., Selzner, T., Tamburini, F., & Amelung, W. (2021). Tracing uptake and translocation of phosphorus in wheat using oxygen isotopes and mathematical modelling. *New Phytologist*, *230*, 1883–1895.

Bauke, S. L., von Sperber, C., Siebers, N., Tamburini, F., & Amelung, W. (2017). Biopore effects on phosphorus biogeochemistry in subsoils. *Soil Biology and Biochemistry*, *111*, 157–165.

Bauke, S. L., Wang, Y., Saia, S. M., Popp, C., Tamburini, F., Paetzold, S., Amelung, W., & von Sperber, C. (2022). Phosphate oxygen isotope ratios in vegetated riparian buffer strip soils. *Vadose Zone Journal*, *21*, e20193.

Benzing, P., & Richardson, C. (2005). $CaCO_3$ causes underestimation of NaOH extractable phosphorus in sequential fractionations. *Soil Science*, *170*, 802–809.

Bigio, L., & Angert, A. (2018). Isotopic signature of atmospheric phosphate in airborne tree pollen. *Atmospheric Environment*, *194*, 1–6.

Blake, R. E., O'Neil, J. R., & Surkov, A. V. (2005). Biogeochemical cycling of phosphorus: insights from oxygen isotope effects of phosphoenzymes. *American Journal of Sciences*, *305*, 596–620.

Brinkmann, N., Seeger, S., Weiler, M., Buchmann, N., Eugster, W., & Kahmen, A. (2018). Employing stable isotopes to determine the residence times of soil water and the temporal origin of water taken up by *Fagus sylvatica* and *Picea abies* in a temperate forest. *New Phytologist*, *219*, 1300–1313.

Brookes, P. C., Powlson, D. S., & Jenkinson, D. S. (1982). Measurement of microbial biomass phosphorus in soil. *Soil Biology and Biochemistry*, *14*, 319–329.

Bünemann, E., Smernik, R., Marschner, P., & Mcneill, A. (2008). Microbial synthesis of organic and condensed forms of phosphorus in acid and calcareous soils. *Soil Biology and Biochemistry*, *40*, 932–946.

Cade-Menun, B. J., & Preston, C. M. (1996). A comparison of soil extraction procedures for 31P NMR spectroscopy. *Soil Science*, *161*, 770–785.

Carpenter, S. R. (2008). Phosphorus control is critical to mitigating eutrophication. *Proceedings of the National Academy of Sciences*, *105*, 11039–11040.

Cernusak, L. A., Barbeta, A., Bush, R. T., Eichstaedt (Bögelein), R., Ferrio, J. P., Flanagan, L. B., Gessler, A., Martín-Gómez, P., Hirl, R. T., Kahmen, A., Keitel, C., Lai, C., Munksgaard, N. C., Nelson, D. B., Ogée, J., Roden, J. S., Schnyder, H., Voelker, S. L., Wang, L., … Cuntz, M. (2022). Do ^{2}H and ^{18}O in leaf water reflect environmental drivers differently? *New Phytologist*, *235*, 41–51.

Cernusak, L. A., Barbour, M. M., Arndt, S. K., Cheesman, A. W., English, N. B., Feild, T. S., Helliker, B. R., Holloway-Phillips, M. M., Holtum, J. A., Kahmen, A., McInerney, F. A., Munksgaard, N. C., Simonin, K. A., Song, X., Stuart-Williams, H., West, J. B., & Farquhar, G. D. (2016). Stable isotopes in leaf water of terrestrial plants. *Plant, Cell & Environment*, *39*, 1087–1102.

Ceulemans, T., Stevens, C. J., Duchateau, L., Jacquemyn, H., Gowing, D. J. G., Merckx, R., Wallace, H., van Rooijen, N., Goethem, T., Bobbink, R., Dorland, E., Gaudnik, C., Alard, D., Corcket, E., Muller, S., Dise, N. B., Dupré, C., Diekmann, M., & Honnay, O. (2014). Soil phosphorus constrains biodiversity across European grasslands. *Global Change Biology*, *20*, 3814–3822.

Chang, S. J., & Blake, R. E. (2015). Precise calibration of equilibrium oxygen isotope fractionations between dissolved phosphate and water from 3 to 37°C. *Geochimica et Cosmochimica Acta*, *150*, 314–329.

Chang, S. J., Blake, R. E., & Colman, A. S. (2021). Oxygen isotope exchange rates between phosphate and water catalyzed by inorganic pyrophosphatase: Implications for the biogeochemical cycle of phosphorus. *Earth and Planetary Science Letters*, *570*, 117071.

Chen, J., Seven, J., Zilla, T., Dippold, M. A., Blagodatskaya, E., & Kuzyakov, Y. (2019). Microbial C:N:P stoichiometry and turnover depend on nutrients availability in soil: A ^{14}C, ^{15}N and ^{33}P triple labelling study. *Soil Biology & Biochemistry*, *131*, 206–216.

Cohn, M. (1953). A study of oxidative phosphorylation with O-18 labeled inorganic phosphate. *Journal of Biological Chemistry*, *201*, 735–750.

Cohn, M. (1958). Phosphate-water exchange reaction catalyzed by inorganic pyrophosphatase of yeast. *Journal of Biological Chemistry*, *230*, 369–380.

Cohn, M., & Hu, A. (1978). Isotopic (18O) shift in 31P nuclear magnetic resonance applied to a study of enzyme-catalyzed phosphate-phosphate exchange and phosphate (oxygen)-water exchange reactions. *Proceedings of the National Academy of Sciences USA*, *75*, 200–203.

Condron, L. M., & Newman, S. (2011). Revisiting the fundamentals of phosphorus fractionation of sediments and soils. *Journal of Soils and Sediments*, *11*, 830–840.

Cross, A. F., & Schlesinger, W. H. (1995). A literature review and evaluation of the. Hedley fractionation: Applications to the

biogeochemical cycle of soil phosphorus in natural ecosystems. *Geoderma*, *64*, 197–214.

Ehleringer, J. R., & Dawson, T. E. (1992). Water uptake by plants: perspectives from stable isotope composition. *Plant, Cell & Environment*, *15*, 1073–1082.

Ehlers, K., Bünemann, E. K., Oberson, A., Frossard, E., Frostegård, Å., Yuejian, M., & Bakken, L. R. (2008). Extraction of soil bacteria from a Ferralsol. *Soil Biology & Biochemistry*, *40*, 1940–1946.

Farquhar, G. D., & Lloyd, J. (1993). Carbon and oxygen isotope effects in the exchange of carbon dioxide between terrestrial plants and the atmosphere. In *Stable isotopes and plant carbon-water relations* (pp. 47–70). Elsevier.

Fourel, F., Martineau, F., Lécuyer, C., Kupka, H. J., Lange, L., Ojeimi, C., & Seed, M. (2011). 18O/16O ratio measurements of inorganic and organic materials by elemental analysis-pyrolysis-isotope ratio mass spectrometry continuous-flow techniques. *Rapid Communications in Mass Spectrometry*, *25*, 1–6.

Frkova, Z., Pistocchi, C., Vystavna, Y., Capkova, K., Dolezal, J., & Tamburini, F. (2022). Phosphorus dynamics during early soil development in a cold desert: insights from oxygen isotopes in phosphate. *SOIL*, *8*, 1–15.

Frossard, E., Achat, D. L., Bernasconi, S. M., Bünemann, E. K., Fardeau, J.-C., Jansa, J., Morel, C., Rabeharisoa, L., Randriamanantsoa, L., Sinaj, S., Tamburini, F., & Oberson, A. (2011). The use of tracers to investigate phosphate cycling in soil–plant systems. In E. Bünemann, A. Oberson, & E. Frossard (Eds.), *Phosphorus in action* (pp. 59–91). Springer.

Gazis, C., & Feng, X. (2004). A stable isotope study of soil water: Evidence for mixing and preferential flow paths. *Geoderma*, *119*, 97–111.

Ghonsikar, C. P., & Miller, R. H. (1973). Soil inorganic polyphosphates of microbial origin. *Plant and Soil*, *38*, 651–655.

Gibson, J., Birks, S., & Edwards, T. (2008). Global prediction of δA and δ^2H-δ^{18}O evaporation slopes for lakes and soil water accounting for seasonality. *Global biogeochemical cycles*, *22*, 1–12.

Gonfiantini, R. (1965). Some results on oxygen isotope stratigraphy in the deep drilling at King Baudouin Station, Antarctica. *Journal of Geophysical Research*, *70*, 1815–1819.

Gralher, B., Herbstritt, B., & Weiler, M. (2021). Technical note: Unresolved aspects of the direct vapor equilibration method for stable isotope analysis (δ^{18}O, δ^2H) of matrix-bound water: unifying protocols through empirical and mathematical scrutiny. *Hydrology and Earth System Sciences*, *25*, 5219–5235.

Gralher, B., Herbstritt, B., Weiler, M., Wassenaar, L. I., & Stumpp, C. (2018). Correcting for biogenic gas matrix effects on laser-based pore water-vapor stable isotope measurements. *Vadose Zone Journal*, *17*, 170157.

Granger, S. J., Heaton, T. H. E., Pfahler, V., Blackwell, M. S. A., Yuan, H., & Collins, A. L. (2017). The oxygen isotopic composition of phosphate in river water and its potential sources in the Upper River Taw catchment, UK. *Science of the Total Environment*, *574*, 680–690.

Gu, C., Dam, T., Hart, S. C., Turner, B. L., Chadwick, O. A., Berhe, A. A., Hu, Y., & Zhu, M. (2020). Quantifying uncertainties in sequential chemical extraction of soil phosphorus using XANES spectroscopy. *Environmental Science & Technology*, *54*, 2257–2267.

Guppy, C. (2021). Is soil phosphorus fractionation as valuable as we think? *Plant and Soil*, *459*, 19–21.

Hacker, N., Wilcke, W., & Oelmann, Y. (2019). The oxygen isotope composition of bioavailable phosphate in soil reflects the oxygen isotope composition in soil water driven by plant diversity effects on evaporation. *Geochimica et Cosmochimica Acta*, *248*, 387–399.

Hauenstein, S., Nebel, M., & Oelmann, Y. (2020). Impacts of fertilization on biologically cycled P in xylem sap of *Fagus sylvatica L.* revealed by means of the oxygen isotope ratio in phosphate. *Frontiers in Forests and Global Change*, *3*, 542738.

Helfenstein, J., Frossard, E., Pistocchi, C., Chadwick, O., Vitousek, P., & Tamburini, F. (2021). Soil phosphorus exchange as affected by drying-rewetting of three soils from a Hawaiian climatic gradient. *Frontiers in Soil Science*, *1*, 738464.

Helfenstein, J., Pistocchi, C., Oberson, A., Tamburini, F., Goll, D. S., & Frossard, E. (2020). Estimates of mean residence times of phosphorus in commonly considered inorganic soil phosphorus pools. *Biogeosciences*, *17*, 441–454.

Helfenstein, J., Tamburini, F., von Sperber, C., Massey, M. S., Pistocchi, C., Chadwick, O. A., Vitousek, P. M., Kretzschmar, R., & Frossard, E. (2018). Combining spectroscopic and isotopic techniques gives a dynamic view of phosphorus cycling in soil. *Nature communications*, *9*, 3226.

Hengge, A. C. (2005). Mechanistic studies on enzyme-catalyzed phosphoryl transfer. *Advances in Physical Organic Chemistry*, *40*, 49–108.

Herbstritt, B., Gralher, B., & Weiler, M. (2012). Continuous in situ measurements of stable isotopes in liquid water. *Water Resources Research*, *48*, 1–6.

Herwartz, D. (2021). Triple oxygen isotope variations in earth's crust. *Reviews in Mineralogy and Geochemistry*, *86*, 291–322.

Hilkert, A., Böhlke, J. K., Mroczkowski, S. J., Fort, K. L., Aizikov, K., Wang, X. T., Kopf, S. H., & Neubauer, C. (2021). Exploring the potential of electrospray-orbitrap for stable isotope analysis using nitrate as a model. *Analytical Chemistry*, *93*, 9139–9148.

Hoefs, J. (2021). Theoretical and experimental principles. In *Stable isotope geochemistry* (pp. 1–48). Springer International Publishing.

Hsieh, J. C. C., Savin, S. M., Kelly, E. F., & Chadwick, O. A. (1998). Measurement of soil-water δ^{18}O values by direct equilibration with CO_2. *Geoderma*, *82*, 255–268.

Jarosch, K. A., Doolette, A. L., Smernik, R. J., Tamburini, F., Frossard, E., & Bünemann, E. K. (2015). Characterisation of soil organic phosphorus in NaOH-EDTA extracts: A comparison of ^{31}P NMR spectroscopy and enzyme addition assays. *Soil Biology & Biochemistry*, *91*, 298–309.

Joshi, S. R., Li, X., & Jaisi, D. P. (2016). Transformation of phosphorus pools in an agricultural soil: An application of oxygen-18 labeling in phosphate. *Soil Science Society of America Journal*, *80*, 69–78.

Klotzbücher, A., Kaiser, K., Klotzbücher, T., Wolff, M., & Mikutta, R. (2019). Testing mechanisms underlying the Hedley sequential phosphorus extraction of soils. *Journal of Plant Nutrition and Soil Science*, *182*, 570–577.

Koehler, G., & Wassenaar, L. I. (2011). Realtime stable isotope monitoring of natural waters by parallel-flow laser spectroscopy. *Analytical Chemistry*, *83*, 913–919.

Kolodny, Y., Luz, B., & Navon, O. (1983). Oxygen isotope variations in phosphate of biogenic apatites, I. Fish bone apatite—

rechecking the rules of the game. *Earth and Planetary Science Letters*, *64*, 398–404.

Kottur, J., & Nair, D. T. (2018). Pyrophosphate hydrolysis is an intrinsic and critical step of the DNA synthesis reaction. *Nucleic Acids Research*, *46*, 5875–5885.

Kouno, K., Wu, J., & Brookes, P. C. (2002). Turnover of biomass C and P in soil following incorporation of glucose or ryegrass. *Soil Biology and Biochemistry*, *34*, 617–622.

Kreuzer-Martin, H. W., Chesson, L. A., Lott, M. J., & Ehleringer, J. R. (2005). Stable isotope ratios as a tool in microbial forensics—Part 3. Effect of culturing on Agar-containing growth media. *Journal of Forensic Sciences*, *50*, 1–8.

Kreuzer-Martin H. W., Lott M. J., Dorigan J., & Ehleringer J. R. (2003). Microbe forensics: Oxygen and hydrogen stable isotope ratios in Bacillus subtilis cells and spores. *Proceedings of the National Academy of Sciences*, *100*, 815–819.

Kreuzer-Martin, H. W., Lott, M. J., Ehleringer, J. R., & Hegg, E. L. (2006). Metabolic processes account for the majority of the intracellular water in log-phase *Escherichia coli* cells as revealed by hydrogen isotopes. *Biochemistry*, *45*, 13622–13630.

Lécuyer, C., Fourel, F., Martineau, F., Amiot, R., Bernard, A., Daux, V., Escarguel, G., & Morrison, J. (2007). High-precision determination of $^{18}O/^{16}O$ ratios of silver phosphate by EA-pyrolysis-IRMS continuous flow technique. *Journal of Mass Spectrometry*, *42*, 36–41.

Lécuyer, C., Grandjean, P., & Emig, C. (1996). Determination of oxygen isotope fractionation between water and phosphate from living lingulids: potential application to palaeoenvironmental studies. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *126*, 101–108.

Li, H., Yu, C., Wang, F., Chang, S. J., Yao, J., & Blake, R. E. (2016). Probing the metabolic water contribution to intracellular water using oxygen isotope ratios of PO 4. *Proceedings of the National Academy of Sciences*, *113*, 5862–5867.

Liang, Y., & Blake, R. E. (2006a). Oxygen isotope signature of Pi regeneration from organic compounds by phosphomonoesterases and photooxidation. *Geochimica et Cosmochimica Acta*, *70*, 3957–3969.

Liang, Y., & Blake, R. E. (2006b). Oxygen isotope composition of phosphate in organic compounds: Isotope effects of extraction methods. *Organic Geochemistry*, *37*, 1263–1277.

Liang, Y., & Blake, R. E. (2009). Compound- and enzyme-specific phosphodiester hydrolysis mechanisms revealed by $\delta^{18}O$ of dissolved inorganic phosphate: implications for the marine P cycling. *Geochimica et Cosmochimica Acta*, *73*, 3782–3794.

Lis, H., Weiner, T., Pitt, F. D., Keren, N., & Angert, A. (2019). Phosphate uptake by cyanobacteria is associated with kinetic fractionation of phosphate oxygen isotopes. *ACS Publications*, *3*, 233–239.

Longinelli, A., & Nuti, S. (1973). Revised phosphate-water isotopic temperature scale. *Earth and Planetary Science Letters*, *19*, 373–376.

McLaren, T. I., Smernik, R. J., McLaughlin, M. J., Doolette, A. L., Richardson, A. E., & Frossard, E. (2020). In D. L. Sparks (Ed.), *Chapter Two—The chemical nature of soil organic phosphorus: A critical review and global compilation of quantitative data* (pp. 51–124). Academic Press.

McLaughlin, K., Cade-Menun, B. J., & Paytan, A. (2006). The oxygen isotopic composition of phosphate in Elkhorn Slough, California: A tracer for phosphate sources. *Estuarine Coastal and Shelf Science*, *70*, 499–506.

McLaughlin, K., Kendall, C., Silva, S., Young, M., & Paytan, A. (2006). Phosphate oxygen isotope ratios as a tracer for sources and cycling of phosphate in North San Francisco Bay, California. *Journal of Geophysical Research*, *111*, 2156–2202.

McLaughlin, M. J., Alston, A. M., & Martin, J. K. (1986). Measurement of phosphorus in the soil microbial biomass: A modified procedure for field soils. *Soil Biology and Biochemistry*, *18*, 437–443.

Mizota, C., Domon, Y., & Yoshida, N. (1992). Oxygen composition of natural phosphates from volcanic ash soils of the Great Rift-Valley of Africa and East Java, Indonesia. *Geoderma*, *53*, 111–123.

Moller, S. R., Sakhno, Y., Aristilde, L., Blake, R. E., & Jaisi, D. P. (2022). Evolution of oxygen isotopologues in phosphate and pyrophosphate during enzyme-catalyzed isotopic exchange reactions. *ACS Earth and Space Chemistry*, *6*, 1543–1551.

Munksgaard, N. C., Wurster, C. M., & Bird, M. I. (2011). Continuous analysis of $\delta^{18}O$ and δD values of water by diffusion sampling cavity ring-down spectrometry: a novel sampling device for unattended field monitoring of precipitation, ground and surface waters. *Rapid Communications in Mass Spectrometry*, *25*, 3706–3712.

Neubauer, C., Crémière, A., Wang, X. T., Thiagarajan, N., Sessions, A. L., Adkins, J. F., Dalleska, N. F., Turchyn, A. V., Clegg, J. A., Moradian, A., Sweredoski, M. J., Garbis, S. D., & Eiler, J. M. (2020). Stable isotope analysis of intact oxyanions using electrospray quadrupole-orbitrap mass spectrometry. *Analytical Chemistry*, *92*, 3077–3085.

O'Neil, J. R., Vennemann, T. W., & McKenzie, W. F. (2003). Effects of speciation on equilibrium fractionations and rates of oxygen isotope exchange between (PO_4)aq and H_2O. *Geochimica et Cosmochimica Acta*, *67*, 3135–3144.

Oberson, A., & Joner, E. J. (2005). Microbial turnover of phosphorus in soil. In B. L. Turner, E. Frossard, & D. S. Baldwin (Eds.), *Organic phosphorus in the environment* (1st ed., pp. 133–164). CABI Publishing.

Orlowski, N., Breuer, L., Angeli, N., Boeckx, P., Brumbt, C., Cook, C. S., Dubbert, M., Dyckmans, J., Gallagher, B., Gralher, B., Herbstritt, B., Hervé-Fernández, P., Hissler, C., Koeniger, P., Legout, A., Macdonald, C. J., Oyarzún, C., Redelstein, R., Seidler, C., … McDonnell, J. J. (2018). Interlaboratory comparison of cryogenic water extraction systems for stable isotope analysis of soil water. *Hydrology and Earth System Sciences*, *22*, 3619–3637.

Pack, A. (2021). Isotopic tracers of atmospheric O_2 in rocks, minerals, and melts. In *Triple Oxygen Geochemistry* (pp. 217–240). MIneralogical Society of America.

Paytan, A., Roberts, K., Watson, S., Peek, S., Chuang, P.-C., Defforey, D., & Kendall, C. (2017). Internal loading of phosphate in Lake Erie Central Basin. *Science of The Total Environment*, *579*, 1356–1365.

Pfahler, V., Dürr-Auster, T., Tamburini, F., Bernasconi, M. S., & Frossard, E. (2013). ^{18}O enrichment in phosphorus pools extracted from soybean leaves. *New Phytologist*, *197*, 186–193.

Pfahler, V., Tamburini, F., Bernasconi, S. M., & Frossard, E. (2017). A dual isotopic approach using radioactive phosphorus and the isotopic composition of oxygen associated to phosphorus to

understand plant reaction to a change in P nutrition. *Plant Methods*, *13*, 1–12.

Pistocchi, C., Mészáros, É., Frossard, E., Bünemann, E. K., & Tamburini, F. (2020). In or out of equilibrium? How Microbial activity controls the oxygen isotopic composition of phosphate in forest organic horizons with low and high phosphorus availability. *Frontiers in Environmental Science*, *8,* 15. Retrieved from https://www.frontiersin.org/articles/10.3389/fenvs.2020.564778

Pistocchi, C., Tamburini, F., Gruau, G., Ferhi, A., Trevisan, D., & Dorioz, J.-M. (2017). Tracing the sources and cycling of phosphorus in river sediments using oxygen isotopes: Methodological adaptations and first results from a case study in France. *Water Research*, *111*, 346–356.

Pucar, D., Dzeja, P. P., Bast, P., Juranic, N., Macura, S., & Terzic, A. (2001). Cellular energetics in the preconditioned state-Protective role for phosphotransfer reactions captured by O-18-assisted P-31 NMR. *Journal of Biological Chemistry*, *276*, 44812–44819.

Pucéat, E., Joachimski, M. M., Bouilloux, A., Monna, F., Bonin, A., Motreuil, S., Morinière, P., Hénard, S., Mourin, J., & Dera, G. (2010). Revised phosphate–water fractionation equation reassessing paleotemperatures derived from biogenic apatite. *Earth and Planetary Science Letters*, *298*, 135–142.

Qin, Y., Duan, G., Zhao, Z., Tian, H., & Solaiman, Z. M. (2018). ^{18}O-labeled phosphate applied to soil appears in the shoots of maize after uptake by roots but not after uptake by an arbuscular mycorrhizal fungus. *Mycorrhiza*, *28*, 787–793.

Reusser, J. E., Piccolo, A., Vinci, G., Savarese, C., Cangemi, S., Cozzolino, V., Verel, R., Frossard, E., & McLaren, T. I. (2023). Phosphorus species in sequentially extracted soil organic matter fractions. *Geoderma*, *429*, 116227.

Riley, W., Still, C., Torn, M., & Berry, J. (2002). A mechanistic model of $H_2{}^{18}O$ and $C^{18}OO$ fluxes between ecosystems and the atmosphere: Model description and sensitivity analyses. *Global Biogeochemical Cycles*, *16*, 1–14.

Rinderer, M., Krüger, J., Lang, F., Puhlmann, H., & Weiler, M. (2021). Subsurface flow and phosphorus dynamics in beech forest hillslopes during sprinkling experiments: How fast is phosphorus replenished? *Biogeosciences*, *18*, 1009–1027.

Roberts, K., Defforey, D., Turner, B. L., Condron, L. M., Peek, S., Silva, S., Kendall, C., & Paytan, A. (2015). Oxygen isotopes of phosphate and soil phosphorus cycling across a 6500 year chronosequence under lowland temperate rainforest. *Geoderma*, *257–258*, 14–21.

Rodionov, A., Bauke, S. L., von Sperber, C., Hoeschen, C., Kandeler, E., Kruse, J., Lewandowski, H., Marhan, S., Mueller, C. W., Simon, M., Tamburini, F., Uhlig, D., von Blanckenburg, F., Lang, F., & Amelung, W. (2020). Biogeochemical cycling of phosphorus in subsoils of temperate forest ecosystems. *Biogeochemistry*, *150*, 313–328.

Rothfuss, Y., Vereecken, H., & Brüggemann, N. (2013). Monitoring water stable isotopic composition in soils using gas-permeable tubing and infrared laser absorption spectroscopy: Monitoring water stable isotopic composition in soils. *Water Resources Research*, *49*, 3747–3755.

Rozanski, K., Araguás-Araguás, L., & Gonfiantini, R. (2013). Isotopic patterns in modern global precipitation. In P. K. Swart, K. C. Lohmann, J. Mckenzie, & S. Savin (Eds.), *Geophysical monograph series* (pp. 1–36). American Geophysical Union.

Saia, S. M., Carrick, H. J., Buda, A. R., Regan, J. M., & Walter, M. T. (2021). Critical review of polyphosphate and polyphosphate accumulating organisms for agricultural water quality management. *Environmental Science & Technology*, *55*, 2722–2742.

Sambuichi, T., Tsunogai, U., Ito, M., & Nakagawa, F. (2023). First measurements on triple oxygen isotopes of dissolved inorganic phosphate in the hydrosphere. *Environmental Science & Technology*, *57*, 3415–3424.

Schindler, D. W., Carpenter, S. R., Chapra, S. C., Hecky, R. E., & Orihel, D. M. (2016). Reducing phosphorus to curb lake eutrophication is a success. *Environmental Science & Technology*, *50*, 8923–8929.

Schryer, A., Bradshaw, K., Siciliano, S.D. (2020). Methodology and validation of a new tandem mass spectrometer method for the quantification of inorganic and organic 18O-phosphate species. *PloS one 15*, e0229172. https://doi.org/10.1371/journal.pone.0229172

Seeger, S., & Weiler, M. (2021). Temporal dynamics of tree xylem water isotopes: in situ monitoring and modeling. *Biogeosciences*, *18*, 4603–4627.

Siegenthaler, M. B., Tamburini, F., Frossard, E., Chadwick, O., Vitousek, P., Pistocchi, C., Mészáros, É., & Helfenstein, J. (2020). A dual isotopic (^{32}P and ^{18}O) incubation study to disentangle mechanisms controlling phosphorus cycling in soils from a climatic gradient (Kohala, Hawaii). *Soil Biology and Biochemistry*, *149*, 107920.

Smith, A. C., Pfahler, V., Tamburini, F., Blackwell, M. S. A., & Granger, S. J. (2021). A review of phosphate oxygen isotope values in global bedrocks: characterising a critical endmember to the soil phosphorus system. *Journal of Plant Nutrition and Soil Science*, *184*, 25–34.

Solhtalab, M., Moller, S. R., Gu, A. Z., Jaisi, D., & Aristilde, L. (2022). Selectivity in enzymatic phosphorus recycling from biopolymers: Isotope effect, reactivity kinetics, and molecular docking with fungal and plant phosphatases. *Environmental Science & Technology, 56*, 16441–16452.

Spohn, M., & Widdig, M. (2017). Turnover of carbon and phosphorus in the microbial biomass depending on phosphorus availability. *Soil Biology & Biochemistry*, *113*, 53–59.

Sprenger, M., Tetzlaff, D., Buttle, J., Laudon, H., Leistert, H., Mitchell, C. P. J., Snelgrove, J., Weiler, M., & Soulsby, C. (2018). Measuring and modeling stable isotopes of mobile and bulk soil water. *Vadose Zone Journal*, *17*, 1–18.

Stout, L. M., Joshi, S. R., Kana, T. M., & Jaisi, D. P. (2014). Microbial activities and phosphorus cycling: An application of oxygen isotope ratios in phosphate. *Geochimica et Cosmochimica Acta*, *138*, 101–116.

Syers, J. K., Johnston, A. E., & Curtin, D. (2008). *Efficiency of soil and fertilizer phosphorus use. Reconciling changing concepts of soil phosphorus behaviour with agronomic Information*. FAO.

Tamburini, F., Bernasconi, S. M., Angert, A., Weiner, T., & Frossard, E. (2010). A method for the analysis of the δ^{18}O of inorganic phosphate extracted from soils with HCl. *European Journal of Soil Science*, *61*, 1025–1032.

Tamburini, F., Pfahler, V., Bünemann, E. K., Guelland, K., Bernasconi, S. M., & Frossard, E. (2012). Oxygen isotopes unravel the role of microorganisms in phosphate cycling in soils. *Environmental Science and Technollogy*, *46*, 5956–5962.

Tamburini, F., Pfahler, V., von Sperber, C., Frossard, E., & Bernasconi, S. M. (2014). Oxygen isotopes for unraveling phosphorus transformations in the soil–plant system: A review. *Soil Science Society of America Journal*, *78*, 38.

Tamburini, F., Pistocchi, C., Helfenstein, J., & Frossard, E. (2018). A method to analyse the isotopic composition of oxygen associated with organic phosphorus in soil and plant material. *European Journal of Soil Science*, *69*, 816–826.

Tonderski, K., Andersson, L., Lindström, G., St Cyr, R., Schönberg, R., & Taubald, H. (2017). Assessing the use of $\delta^{18}O$ in phosphate as a tracer for catchment phosphorus sources. *Science of The Total Environment*, *607–608*, 1–10.

Turner, B. L., Cade-Menun, B. J., Condron, L. M., & Newman, S. (2005). Extraction of soil organic phosphorus. *Talanta*, *66*, 294–306.

Vennemann, T. W., Fricke, H. C., Blake, R. E., O'Neil, J. R., & Colman, A. S. (2002). Oxygen isotope analysis of phosphates: a comparison of techniques for analysis of Ag3PO4. *Chemical Geology*, *185*, 321–336.

Vogel, C., Ramsteiner, M., Sekine, R., Doolette, A., & Adam, C. (2017). Characterization of phosphorus compounds in soils by deep ultraviolet (DUV) Raman microspectroscopy: Characterization of phosphorus compounds in soils. *Journal of Raman Spectroscopy*, *48*, 867–871.

Volkmann, T. H. M., & Weiler, M. (2014). Continual in situ monitoring of pore water stable isotopes in the subsurface. *Hydrology and Earth System Sciences*, *18*, 1819–1833.

von Freyberg, J., Allen, S. T., Grossiord, C., & Dawson, T. E. (2020). Plant and root-zone water isotopes are difficult to measure, explain, and predict: Some practical recommendations for determining plant water sources. *Methods in Ecology and Evolution*, *11*, 1352–1367.

von Sperber, C., Kries, H., Tamburini, F., Bernasconi, S. M., & Frossard, E. (2014). The effect of phosphomonoesterases on the oxygen isotope composition of phosphate. *Geochimica et Cosmochimica Acta*, *125*, 519–527.

von Sperber, C., Lewandowski, H., Tamburini, F., Bernasconi, S. M., Amelung, W., & Frossard, E. (2017). Kinetics of enzyme-catalysed oxygen isotope exchange between phosphate and water revealed by Raman spectroscopy: Kinetics of enzyme-catalysed oxygen isotope exchange. *Journal of Raman Spectroscopy*, *48*, 368–373.

von Sperber, C., Tamburini, F., Brunner, B., Bernasconi, S. M., & Frossard, E. (2015). The oxygen isotope composition of phosphate released from phytic acid by the activity of wheat and *Aspergillus niger* phytase. *Biogeosciences*, *12*, 4175–4184.

Wang, J., Huang, T., Wu, Q., Bu, C., & Yin, X. (2021). Sources and cycling of phosphorus in the sediment of rivers along a eutrophic lake in China indicated by phosphate oxygen isotopes. *ACS Earth and Space Chemistry*, *5*, 88–94.

Watzinger, A., Schott, K., Hood-Nowotny, R., Tamburini, F., Arppe, L., Cristini, D., Knöller, K., & Skrzypek, G. (2021). New Ag_3PO_4 comparison material for stable oxygen isotope analysis. *Rapid Communications in Mass Spectrometry*, *35*, e9101.

Wijker, R. S., Sessions, A. L., Fuhrer, T., & Phan, M. (2019). 2H/1H variation in microbial lipids is controlled by NADPH metabolism. *Proceedings of the National Academy of Sciences, 116*, 12173–12182.

Wu, J., Paudel, P., Sun, M., Joshi, S. R., Stout, L. M., Greiner, R., & Jaisi, D. P. (2015). Mechanisms and pathways of phytate degradation: Evidence from oxygen isotope ratios of phosphate, HPLC, and phosphorus-31 NMR spectroscopy. *Soil Science Society of America Journal*, *79*, 1615–1628.

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