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The effect of sample treatments on the oxygen isotopic composition of phosphate pools in soils

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ABSTRACT

The oxygen isotopic composition of phosphate ($\delta^{18}O_P$) has been increasingly used as an effective tracer for the biogeochemical cycling of phosphorus (P) in soils and other environments. However, diverse pretreatment methods (e.g. storage, preparation and extraction) are being used for soil samples. For the uniformity of methods as well as for the comparison of results, it is important to understand if specific treatment methods can compromise original $\delta^{18}O_P$ values. Here, Ag_3PO_4 and KH_2PO_4 were used to test whether a modified Hedley sequential extraction and purification procedure can alter the $\delta^{18}O_P$ values of phosphate standards. Additionally, to test the effect of sample storage and drying conditions, two types of soils were first processed by using eight different pretreatment methods including sterilizing, storing, drying, and sieving and then the $\delta^{18}O_P$ values of each soil P pool were measured. Results indicate that the extraction and purification procedure, drying temperature (< 0 °C to 80 °C) and sieving mesh (20 to 100) had no significant effect on the $\delta^{18}O_P$ values of P_i (inorganic P) pools, but storage at room temperature (without microbial growth inhibitor-$HgCl_2$ added) can lead to significant changes in $\delta^{18}O_P$ values of almost all P pools. For the two soils studied, the $\delta^{18}O_P$ values of P_i pools decrease from H_2O (H_2O-P_i) to $NaHCO_3$ ($NaHCO_3$-P_i), NaOH (NaOH-P_i) and HCl (HCl-P_i), and organic P was also found in the extraction solution of HCl. Furthermore, the $\delta^{18}O_P$ values calculated from isotope mass balance were different from the measured values suggesting variable extraction of different P pools during single and sequential extraction methods. Collectively these results highlight the need for a unified and standard processing and extraction methods for soil samples to allow meaningful intercomparison of results.

1. Introduction

Phosphorus (P) is an essential nutrient for all life. Low P in soils can limit the growth of plants (e.g. crops), but over fertilization can also lead to P release from soils to aquatic environments and degrade water quality (Sharpley et al., 1994). Therefore, soil P cycling has received widespread attention ((Bunemann, 2015; Hinsinger, 2001; Kruse et al., 2015). Phosphorus in soils can be derived from the weathering of rocks, deposition of aerosols, decomposition of biological debris (e.g. manure and dead body), and chemical fertilizers (Bunemann, 2015; Kruse et al., 2015). Due to the reactivity of P, it exists in different pools in soils and can exchange from one pool to another (Hedley et al., 1982a; Joshi et al., 2016; Walker and Syers, 1976). Therefore, the sources, bioavailability, and interconversion of different P pools are key issues central to soil P research (Bray and Kurtz, 1945; Hinsinger, 2001; Olsen, 1954). The sequential extraction (Hedley et al., 1982a; Tiessen and Moir, 1993), ^{31}P-NMR (Condron et al., 1985) and enzymatic hydrolysis (He et al., 2004) techniques are commonly used to address these questions.

On the other hand, stable isotope ratios have been widely used to trace the biogeochemical cycling of C, N, and S. Phosphorus has only one stable isotope (^{31}P), but occurs in nature mainly as orthophosphate

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(PO_4^{3-}) containing four O atoms. Because the P—O bond in PO_4^{3-} is very strong and in the absence of enzymatic/biological activity, phosphate oxygen does not exchange isotopes with water under normal temperature and pH ranges in earth surface environments (Blake et al., 1997; Kolodny et al., 1983; Longinelli et al., 1976; O'Neil et al., 2003). However, oxygen isotope exchange is rapid in the presence of enzymes/biological activity (Blake et al., 1998; Blake et al., 1997; Blake et al., 2005; Chang and Blake, 2015; Kolodny et al., 1983; Paytan et al., 2002; Stout et al., 2014). Since different soil P pools could derive from different sources or soil biota exert different degrees of influence, they could have different $\delta^{18}O_P$ (Amelung et al., 2015; Roberts et al., 2015; Zohar et al., 2010a; Zohar et al., 2010b). Therefore, $\delta^{18}O_P$ can offer an ideal tracer for the identification of sources, bioavailability and interconversion of different P pools in soils.

In recent years, there has been a surge in publications involving measurements of phosphate oxygen isotopic composition in soils. However, most of these studies have focused on resin-extraction (Gross et al., 2015; Tamburini et al., 2012; Weiner et al., 2011), dilute HCl-extraction (Angert et al., 2012; Tamburini et al., 2010) or both (Angert et al., 2012; Angert et al., 2011; Tamburini et al., 2012). The resin-extracted P is believed to represent the most bioavailable fraction. Single-step extraction methods such as resin extraction are straightforward, relatively easy to perform and save both time and resource, and still they can provide some useful information. In contrast, there are only a handful of studies employing sequential extraction methods to target more specific P pools for $\delta^{18}O_P$ analysis in soils (Joshi et al., 2016; Roberts et al., 2015; Zohar et al., 2010a; Zohar et al., 2010b) or marine/lake sediments (Jaisi and Blake, 2010; Joshi et al., 2015; Markel et al., 1994). One challenge for direct comparisons among these studies is the variability of the sample treatment methods have varied greatly between studies. For example, the published sample storage conditions varied from 4 °C (after drying at 40 °C) (Angert et al., 2012; Angert et al., 2011; Gross et al., 2013; Gross et al., 2015; Weiner et al., 2011) to − 20 °C (Gross et al., 2015; Jaisi and Blake, 2010) and − 80 °C (Joshi et al., 2015). At the same time, the soil drying temperature is quite variable as well including 24 °C (Zohar et al., 2010b), 30 °C (Roberts et al., 2015), 40 °C (Angert et al., 2012; Angert et al., 2011; Tamburini et al., 2010; Weiner et al., 2011), 60 °C (Gross et al., 2015), and freeze-drying (Jaisi and Blake, 2010; Joshi et al., 2015; Joshi et al., 2016). Unfortunately, the effects of these different sample pretreatment and storage methods on soil $\delta^{18}O_P$ values have not been evaluated. We seek here to address these issues by conducting a carefully controlled comparative study including evaluation of the effects of different sample processing and sample storage protocols. We tested the reliability of the extraction-purification procedures by Ag_3PO_4 and KH_2PO_4, and then the effects of sterilizing, storing, drying and sieving methods on $\delta^{18}O_P$ of soil P pools were studied. Finally, a comparison of $\delta^{18}O_P$ values between single-step and sequential extraction method was made. Our results provide important insights into the causes and impacts of treatment methods on $\delta^{18}O_P$ values of soil P pools.

2. Materials and methods

2.1. Materials

The reagents used in this study include: $NaHCO_3$ and KH_2PO_4, both are ultra-pure reagents and produced by Tianjin Guangfu Chemical Co. Ltd.; HCl, $MgCl_2{\cdot}6H_2O$, $HgCl_2$ and Mg $(NO_3)_2{\cdot}6H_2O$, all are analytical grade reagents produced by Xilong Chemical Co. Ltd.; NaOH is an analytical grade reagent produced by Sinopharm Chemical Co. Ltd.; AG50W-× 8 cation exchange resin (H^+ form) and AG1-× 8 anion exchange resin (OH^- form), both are biotechnology grade produced by Bio-Rad Laboratories Inc., USA.

2.2. Collection and processing of soil samples

For the comparison of soil processing and storage protocols for phosphate O-isotope analyses, two types of soils (soil A and B) were collected and analyzed. Soil A was collected from a cropland near Xiamen University (N: 24°3645′, E: 118°3979′) on March 30, 2015. This farm is mainly used for the cultivation of strawberry corn and potato and chemical fertilizer is the main source of P_i. Soil B was collected from a wasteland near the soil A site on March 18, 2015. It has not been farmed and fertilized for many years. From both sites, surface soil (0–3 cm) was collected by using a stainless steel scoop and size separated by a 20 mesh sieve (Φ = 0.84 mm).

To preserve original $\delta^{18}O_P$ values of the soil P pools, a subset of soil sample was treated with $HgCl_2$ (0.4%, w:w) solution to prevent microbial growth and extracted immediately following this treatment. For comparison, another subset of soil sample was processed in parallel (without $HgCl_2$ treatment) to investigate the effect of microbial activity that might alter original isotopic compositions. To investigate the effects of storage time, the third subset of soil sample was stored in a plastic bag (without sealing) at room temperature for 10 and 50 days. All other sub-samples were stored at − 20 °C immediately after collection. In order to investigate the effects of drying temperature, a split of sample was taken from the one stored at − 20 °C and freeze dried or air dried at 40 °C (for 48 h) or 80 °C (for 24 h) until the weight became constant. In order to investigate the effect of soil size, a subset of freeze-dried and air-dried (at 40 °C) samples were further sieved in 100 mesh (Φ = 0.15 mm). Table 1 shows the detailed treatment methods and terminologies used in this communication.

For all treatment methods, soil P extraction was initiated within 3 h after the specific treatment. Soil A was chosen for all treatment methods of Table 1, except 80 °C-drying, and each method included six replicate samples processed in parallel. Because no 80 °C-drying temperature was used in the literature and we believe the actual drying temperature does not need not as high as 80 °C. So 80 °C-drying method was not conducted on soil A. Soil B was limited to four treatment methods namely 0d sto.-$HgCl_2$, 0d sto.-No $HgCl_2$, − 20 °C sto. & 40 °C dry and − 20 °C sto. & 80 °C dry. All treatment included three replicate samples and processed in parallel.

Table 1
Sample treatment methods.

Treatment method	$HgCl_2$	Storage time (days)	Storage temperature	Drying method	Sieving mesh
0d sto.-$HgCl_2$	100 mL, 0.4% (w:w)	No	No	No	20
0d sto.-No $HgCl_2$	No	No	No	No	20
24 °C 10/50d sto.-No $HgCl_2$	No	10/50	24 °C	No	20
− 20 °C sto. & Fre./40/80 °C dry	No	Immediately after collection	− 20 °C	Freeze dried or air dried at 40 °C or 80 °C	20
− 20 °C sto. & Fre./40 °C dry-100 mesh	No	Immediately after collection	− 20 °C	Freeze dried or air dried at 40 °C	100

d = days.
sto. = storage.
Fre. = freeze dried.

2.3. Phosphate extraction and purification methods

2.3.1. Sequential extraction method

To extract different P pools in soil samples for oxygen isotope analysis, we followed Zohar et al. (2010a) and Joshi et al. (2016) methods, which were slightly modified from the widely used (Hedley et al., 1982a) method. Soil samples were sequentially extracted by H_2O, 0.5 M $NaHCO_3$, 0.1 M NaOH and 1 M HCl (Fig. 1). In each case, 15 g (100 mesh) or 30 g (20 mesh) of soil was reacted with 1 L of extractant. We found P_i concentrations extracted by H_2O were almost constant after 4 h (Fig. S1; Table S1). Therefore, to avoid the risk of compromising isotopic values caused potentially by microbial activity in extracted solutions, the duration of H_2O extraction was shortened to 4 h. The additional three P pools were extracted for 16 h on a reciprocal shaker at room temperature. After the supernatant was separated by centrifugation (6000 rpm), the residual soil was rinsed with 1 M $MgCl_2$ to desorb any phosphate extracted by the particular extractant, but resorbed to residual mineral surfaces (Ruttenberg, 1992). Before the extraction of samples with NaOH, residual soil needed to be rinsed with DI water to remove Mg^{2+}, which could react with the extractant to form $Mg(OH)_2$ precipitate. Following extraction with NaOH, residual soils were rinsed again with DI water to remove OH^- ions which could react with $MgCl_2$ in the following step to form $Mg(OH)_2$ precipitate. In place of 1 M $MgCl_2$, some studies have used saturated NaCl (Chang and Jackson, 1957; Markel et al., 1994), 0.5 M $NaHCO_3$ and/or H_2O (Joshi et al., 2016) as a rinse solution to remove the P_i that resorbed to residual mineral surfaces. The extraction and rinse solutions for particular P pool were combined and the mixed solution was processed to further purify and finally convert dissolved PO_4^{3-} to Ag_3PO_4 by using the protocol shown in Fig. 1. The P_i concentration was measured by the method of Tiessen and Moir (1993), which was modified from Murphy and Riley (1962). To measure the total P concentration, acid 5% $K_2S_2O_8$ was added into subsets of extracted solution at a ratio of 1:10 (V:V) and autoclaved at 125 °C for 2 h to convert all organic P into inorganic, then the P_i concentration was measured. Organic P concentration was calculated by the difference between the concentration of total P and P_i.

2.3.2. Single-step extraction method

Subsets of − 20 °C sto. & 40 °C dry-100 mesh soil samples (soil A) were extracted by a single extractant reagent: 0.5 M $NaHCO_3$, 0.1 M NaOH or 1.0 M HCl for 16 h (with six replicate samples extracted in parallel). The extractant solutions were separated from the residual soil by centrifugation and were further processed to purify and convert dissolved PO_4^{3-} to Ag_3PO_4 by the purification protocol described in Fig. 1. The total and P_i concentrations were measured by the same way as sequential extraction.

2.3.3. Purification protocol

Several methods and modifications are currently in use to purify phosphate for isotope analysis (Colman et al., 2005; Joshi et al., 2016; Kolodny et al., 1983; O'Neil et al., 1994). We found the methods of (Blake et al., 2010) and Joshi et al. (2016) to be more effective for complex soil matrix samples and these methods were adopted in the present study (Fig. 1). Magnesium-induced co-precipitation (MagIC) (Karl and Tien, 1992) was used to concentrate PO_4^{3-} and simultaneously remove some interfering ions and dissolved organic matter (DOM) from the extractant (additional DAX-8 resin treatment was needed for DOM-rich samples). Next, PO_4^{3-} was further purified by sequential precipitation of PO_4^{3-} as ammonium phosphomolybdate (APM), followed by dissolution and reprecipitation as magnesium

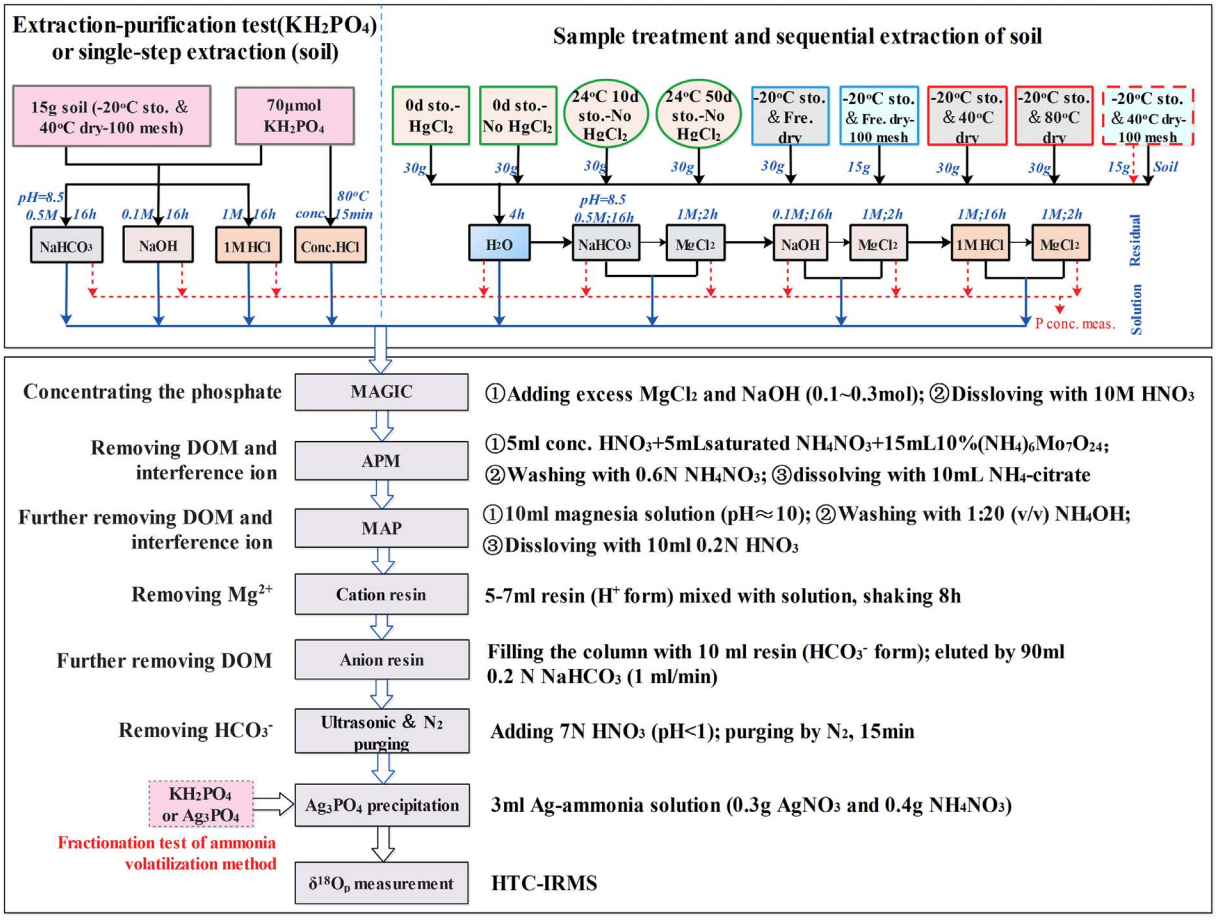

Fig. 1. Sample handling, extraction and purification protocols for $\delta^{18}O_P$ measurement of different P pools in soil (modified after Blake et al., 2010, Joshi et al., 2016).

ammonium phosphate (MAP). Then solutions were further treated with cation resin (AG50W-× 8, BIO-RAD) followed by anion resin (AG1-× 8, BIO-RAD) to remove cations and anions (as well as residual dissolved organic matter), respectively. 0.2 M $NaHCO_3$ was used to elute the P_i from anion resin. Any residual CO_3^{2-} was removed by adding 7 M HNO_3 into the eluate (pH < 4.0) and purging by N_2 gas under ultrasonication. The purified PO_4^{3-} was finally converted to Ag_3PO_4 by the ammonia volatilization method (Firsching, 1961). Lastly, the Ag_3PO_4 crystals were rinsed several times with Milli-Q deionized water and separated by centrifugation. Rinsing and centrifugation were repeated until no Ag^+ was detected in the supernatant. The Ag_3PO_4 was dried at 110 °C and stored in a desiccator in the dark until $\delta^{18}O_P$ measurements in in continuous flow mode HTC-IRMS. The yellow and shiny Ag_3PO_4 crystals were ground and 0.4–0.8 mg were prepared in silver capsules and then pyrolyzed at 1380 °C. During measurement, CO yield of the Ag_3PO_4 was monitored and found to be similar to that of Ag_3PO_4 standard (Fig. S2). This further verified the purity of the Ag_3PO_4 precipitates. The helium carrier gas flow rate was maintained at 80 mL/min, and the GC temperature was chosen to be 85 °C for better separation of peaks based on our past studies (Chen et al., 2015; Yin and Chen, 2014).

2.4. Tests for isotopic fractionation during extraction-purification procedures

To test whether the extraction and purification procedure can alter original $\delta^{18}O_P$ values, the following three experiments were carried out using Ag_3PO_4 working reference and KH_2PO_4 reagent: i) Ag_3PO_4 working reference ($\delta^{18}O_P$ = 8.6‰, determined by direct pyrolysis) was dissolved and reprecipitated as Ag_3PO_4 by the ammonia volatilization method to test the stability of isotopic values during the Ag_3PO_4 precipitation process; ii) KH_2PO_4 reagent was dissolved and directly precipitated as Ag_3PO_4 by the ammonia volatilization method to obtain primary $\delta^{18}O_P$ values of the KH_2PO_4 reagent; iii) aliquots of KH_2PO_4 reagent solutions were treated with a single extractant solution: 0.5 M $NaHCO_3$, 0.1 M NaOH, 1.0 M HCl or concentrated HCl, with six replicate samples extracted in parallel. The extraction time and purification process were the same as that of sequential extractions (Fig.1). In some past studies, concentrated HCl (Conc. HCl) has also been used to extract P from soil samples (Zohar et al., 2010a; Tiessen and Moir, 1993). To validate that this reagent does not compromise isotope values, KH_2PO_4 was also extracted by concentrated HCl (80 °C, 15 min).

3. Results and discussion

3.1. Isotope fractionation tests

The mean $\delta^{18}O_P$ value of the re-precipitated Ag_3PO_4 reference was 8.8 ± 0.1‰ (n = 8), which is similar to the original values (8.6‰) and within the precision of the measurement method (± 0.3‰). This means that the ammonia volatilization method does not compromise $\delta^{18}O_P$ values. The mean $\delta^{18}O_P$ values of Ag_3PO_4 that were precipitated directly from the KH_2PO_4 reagent by the ammonia volatilization method were 13.2 ± 0.2‰ (n = 16) (Dir. Pre. in Fig. 2, Table S2). This value represents the primary isotope composition of the KH_2PO_4 reagent.

For the KH_2PO_4 reagent extracted by a single extractant solution $NaHCO_3$, NaOH, 1 M HCl or concentrated HCl (Conc. HCl), the mean measured $\delta^{18}O_P$ values were 13.2 ± 0.2‰ (n = 6), 13.3 ± 0.2‰ (n = 6), 13.4 ± 0.2‰ (n = 6) and 13.2 ± 0.2‰ (n = 6), respectively (Fig. 2, Table S2). These values are similar to the primary $\delta^{18}O_P$ values of KH_2PO_4 reagent (13.2 ± 0.2‰) but within the error of the measurement method. These results are consistent with the previous studies that the oxygen isotope exchange between phosphate and water is negligible under the abiotic condition (Blake, 1997; Kolodny et al., 1983; Longinelli et al., 1976; O'Neil et al., 2003). One note of caution,

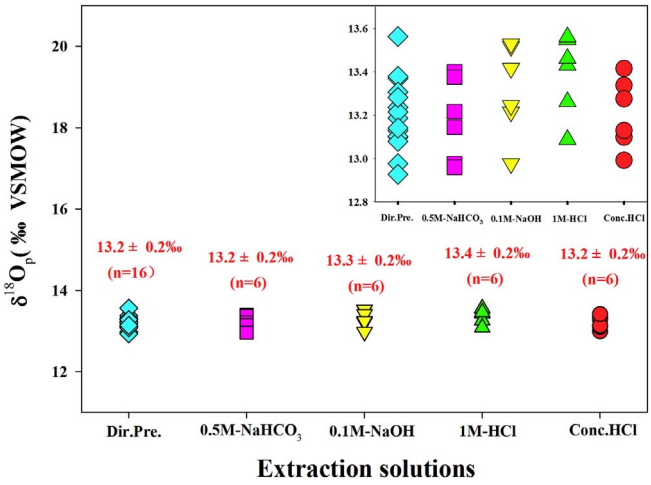

Fig. 2. $\delta^{18}O_P$ values of Ag_3PO_4 precipitated from KH_2PO_4 that was extracted by various reagents and purified as the procedure of Fig. 1. Please note Dir. Pre. represents Ag_3PO_4 being precipitated directly from KH_2PO_4 solution.

however, is the time of extraction because whether longer time could compromise the isotope values especially in extreme pH cases. Therefore, our results reconfirm that the reagents used in sequential extraction methods, time length of treatment, and purification procedures used in this study do not alter oxygen isotopic composition of phosphate.

3.2. Effects of sample treatment methods on the oxygen isotope composition of soil P pools

3.2.1. Effect of microbial activity

We tested the impact of post-sampling microbial activities on measured isotope values. For example, for the 0d sto.-$HgCl_2$ treatment soil samples were extracted by the reagents immediately after collection and microbial activity was suppressed by $HgCl_2$ treatment. Therefore, $\delta^{18}O_P$ values for this sample group should most closely reflect the primary $\delta^{18}O_P$ values of the soil. Please note that the only difference between the 0d sto.-No $HgCl_2$ and 0d sto.-$HgCl_2$ treatment was that the former did not have microbial inhibitor ($HgCl_2$) added. It means comparison between these two treatments can elucidate the effects of microbial activity on the measured $\delta^{18}O_P$ values of soil P pools assuming that the addition of $HgCl_2$ has no direct impact on $\delta^{18}O_P$ values, e.g., release of intracellular P during cell lysis.

Soil A: The difference in average $\delta^{18}O_P$ values, $\Delta\delta^{18}O_P$, of the four P_i pools for 0d sto.-No $HgCl_2$ versus 0d sto.-$HgCl_2$ treatment was 0.6‰, 0.3‰, 0.6‰ and 0.7‰ for H_2O-P_i, $NaHCO_3$-P_i, NaOH-P_i, and HCl-P_i pools, respectively (Fig. 3, Fig. S3). The differences are close to the precision of $\delta^{18}O_P$ measurements. For NaOH-P_i and HCl-P_i pools, the mean $\delta^{18}O_P$ values of the 0d sto.-No $HgCl_2$ treatment are slightly heavier than that of all other treatments except the 24 °C 10/50 ds sto.-No $HgCl_2$ treatments. Although the exact mechanism is still unclear, we believe this should not be caused by the biological activity. Therefore, in the following sections results from all other treatments are compared to the 0d sto.-$HgCl_2$ treatment to evaluate the impact and reliability of particular treatments in preserving original $\delta^{18}O_P$ values of soil P pools.

Soil B: The difference in average $\delta^{18}O_P$ values, $\Delta\delta^{18}O_P$, of the three P_i pools for 0d sto.-No $HgCl_2$ versus 0d sto.-$HgCl_2$ treatment was 0.5‰, − 0.2‰ and 0.2‰ for H_2O-P_i, $NaHCO_3$-P_i and NaOH-P_i pools, respectively (Fig. 3, Fig. S3). The differences are close to the precision of $\delta^{18}O_P$ measurements. This is consistent with the results from Soil A.

3.2.2. Effect of room temperature storage

Compared to samples treated with microbial inhibitor (0d sto.-$HgCl_2$), $\Delta\delta^{18}O_P$ values of the samples stored untreated (without $HgCl_2$)

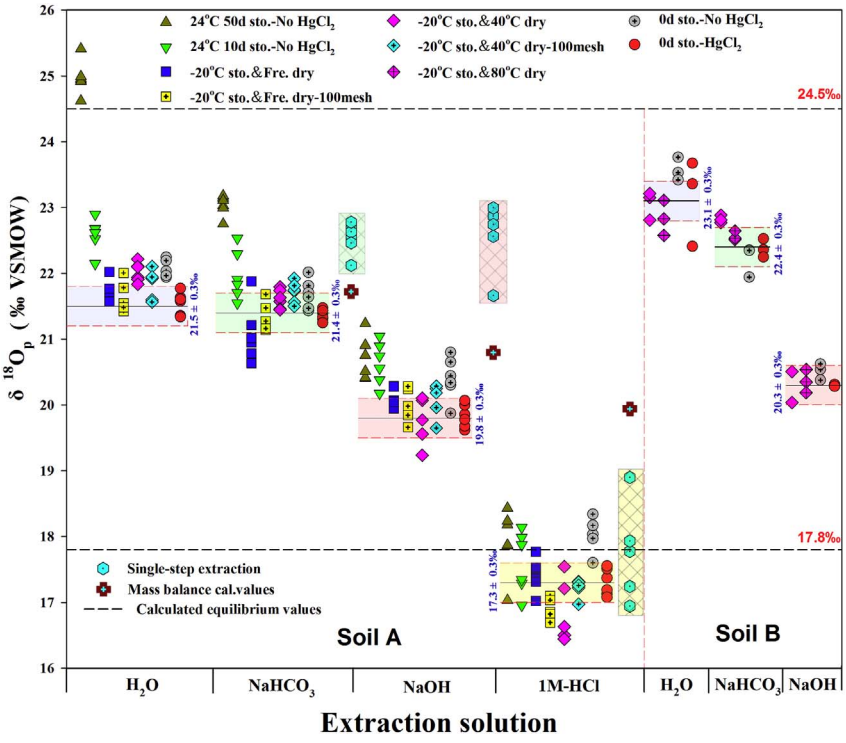

Fig. 3. The $\delta^{18}O_P$ values of soil P pools after different pre-treatment methods. The red numbers are the calculated equilibrium values and the vertical blue numbers are the average $\delta^{18}O_P$ values of the 0d sto.-$HgCl_2$ method. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

at 24 °C for 10 days (24 °C 10ds sto.-No $HgCl_2$) are 1.1‰, 0.6‰, 0.8‰ and 0.3‰ for H_2O-P_i, $NaHCO_3$-P_i, NaOH-P_i, and HCl-P_i, respectively (Fig. 3, Fig. S3). Similarly, $\Delta\delta^{18}O_P$ values of the samples stored untreated at the 24 °C for 50 days (24 °C 50 days sto.-No $HgCl_2$) were 3.5‰, 1.6‰, 0.8‰ and 0.6‰ for the four P pools, respectively (Fig. 3, Fig. S3). These data show that the longer the room temperature storage and more bioavailable the P pool, the larger the change in $\delta^{18}O_P$ values. Two possible mechanisms can generate these results. The first one is the preferential uptake of $P^{16}O_4^{3-}$ by microorganisms in untreated samples as has been previously demonstrated in controlled experiments (Blake et al., 2005) and similar results can be anticipated from soil microorganisms. The second one could be that the soil water $\delta^{18}O_W$ values could gradually be heavier (due to evaporation) for the samples stored untreated. Although we did not measure the $\delta^{18}O_W$ of the soil water in this study, other studies have shown that the $\delta^{18}O_W$ of the soil water varies depending on soil moisture and evaporation conditions (Joshi et al., 2016) and can become heavier with the storage of soil samples due to evaporation (unpublished results). Under constant temperature and with the presence of biological activity, the heavier $\delta^{18}O_W$ values leads to heavier $\delta^{18}O_P$ values due to equilibrium fractionation between them. The actual reason behind excursion of $\delta^{18}O_P$ values may be dominated by one or both of these factors. Additional studies are being undertaken in the laboratory at Xiamen University to develop a quantitative role of these factors under specific conditions.

3.2.3. Effect of soil drying temperature and aggregate size

To investigate the effect of soil drying temperature and aggregate size, results from three drying temperatures (freeze-drying or air drying at 40 °C and 80 °C) and two sieve sizes (20 and 100 mesh) are compared. For all these treatments, when compared to the 0d sto.-$HgCl_2$ treatment, the $\Delta^{18}O_P$ values of all four P_i pools ranged from − 0.4‰ to + 0.5‰ (mean = 0.07, STDEV = 0.26, n = 22) (Fig. 3, Fig. S3). These data show that for all treatments used, the $\delta^{18}O_P$ values of the corresponding P_i pools are similar to that of the 0d sto.-$HgCl_2$ treatment (within the measurement precision). This means that under the experimental conditions used of this study, the drying temperature (freeze-drying to air drying/heating to 80 °C) and sieving mesh (20 and 100) did not have a significant effect on the $\delta^{18}O_P$ values of soil P_i pools. One note of caution is that the different temperatures used to air dry soil could promote alteration of isotope values depending on the length of time used in drying and levels of microbial activity within samples. Most previous studies report sieving soils with 20 mesh, thus freeze-drying and sieving with > 20 mesh is suggested to be a better choice to minimize artifacts.

3.3. Comparison of single-step and multi-step sequential extraction methods

3.3.1. Comparison of P pool size

Extracted P_i. For the sequential extraction method, the average P_i concentration of H_2O-P_i, $NaHCO_3$-P_i, NaOH-P_i and HCl-P_i pools was 3.13 ± 0.07 μmol/g (n = 6), 15.31 ± 0.34 μmol/g (n = 6), 14.38 ± 0.18 μmol/g (n = 6) and 12.68 ± 1.00 μmol/g (n = 6), respectively (Fig. 4). For the single-step extraction method, the average concentrations of $NaHCO_3$-P_i, NaOH-P_i and HCl-P_i were 16.72 ± 0.21 μmol/g (n = 6), 25.31 ± 1.84 μmol/g (n = 6) and 45.59 ± 0.62 μmol/g (n = 6), respectively.

As expected, in single step extractions extractants removed a higher amount of P from soil than that of the corresponding sequential extractants because the single step extracts multiple P_i pools simultaneously. However, for the single-step extraction, the $NaHCO_3$-P_i pool content is about 90.7% (i.e., lower than) of the sum of H_2O-P_i and $NaHCO_3$-P_i pool contents from the sequential extraction, and the NaOH-P_i pool content is about 77.1% of the sum of H_2O-P_i, $NaHCO_3$-P_i and NaOH-P_i pool P_i contents from the sequential extraction. The single-step extraction HCl-P_i pool content, however, is approximately equal to the sum of H_2O-P_i, $NaHCO_3$-P_i, NaOH-P_i and HCl-P_i pool contents from the sequential extraction protocol (Fig. 4). These results indicate that $NaHCO_3$ and NaOH reagents cannot extract 100% of P_i from those pools that precede these extractants in the sequential extraction scheme, in contrast to 1 M HCl which can completely extract (total) P_i from all P_i pools that precede this step in the sequential extraction scheme.

Extracted P_o. Organic P (P_o) was determined as the difference between the total P extracted using the autoclave methods and inorganic P_i pools. For the sequential extraction method, the average

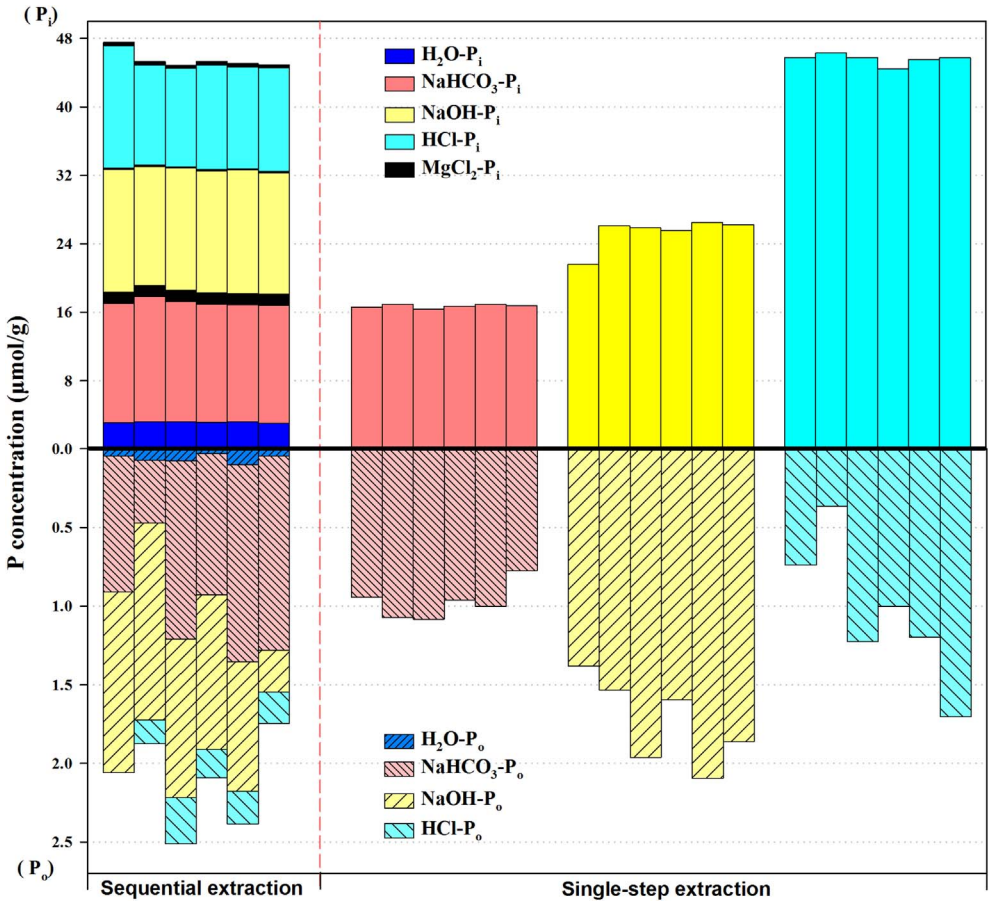

Fig. 4. The inorganic P (P_i) and organic P (P_o) content of soil P pools.

concentrations of H_2O-P_o, $NaHCO_3$-P_o, NaOH-P_o and HCl-P_o were 0.06 ± 0.03 μmol/g (n = 6), 0.96 ± 0.32 μmol/g (n = 6), 0.91 ± 0.35 μmol/g (n = 6) and 0.17 ± 0.10 μmol/g (n = 6), respectively (Fig. 4). It is traditionally assumed that only inorganic P exists in the 1 M HCl fraction (Hedley et al., 1982b; Tiessen and Moir, 1993), while this study and some former studies show that P_o exists in 1 M HCl fraction (He et al., 2006; Joshi et al., 2016). Therefore, P_o measurement of 1 M HCl fraction is suggested to be done routinely for all soil types. For the single-step extracted method, the average concentrations of $NaHCO_3$-P_o, NaOH-P_o and HCl-P_o were 0.97 ± 0.11 μmol/g (n = 6), 1.74 ± 0.28 μmol/g (n = 6) and 1.04 ± 0.46 μmol/g (n = 6) respectively (Fig. 4), showing an increasing trend of P_o pool size. The single-step P_o concentration for a particular extractant is always less than the cumulative sum of potential extractable P_o pools preceding and including the extractant determined by the sequential extraction protocol.

3.3.2. Comparison of phosphate oxygen isotopic compositions

The average $\delta^{18}O_P$ values of the single-step extracted $NaHCO_3$-P_i, NaOH-P_i and HCl-P_i were 22.5 ± 0.2‰ (n = 6), 22.6 ± 0.5‰ (n = 5) and 17.8 ± 0.8‰ (n = 5), respectively (Fig. 3, Table S2). Compared to the $\delta^{18}O_P$ values of the P pools extracted by these extractants in the sequential extraction protocol, the corresponding $\Delta\delta^{18}O$ values were 0.8‰, 2.5‰ and 0.5‰, respectively. It is clear that the $\delta^{18}O_P$ values of single-step extractants are heavier than those of corresponding sequential extractants, with the NaOH-P_i single extractant being heaviest (2.5‰).

Based on the size and $\delta^{18}O_P$ value of each P pool obtained from sequential extraction, we can calculate the anticipated $\delta^{18}O_P$ values of the single-step extractant by using an isotope mass balance model with the assumption that the P content of a single-step extractant is equal to the cumulative sum of preceding sequential extractants. The calculated single-step $\delta^{18}O_P$ values of $NaHCO_3$-P_i, NaOH-P_i and HCl-P_i were 21.7‰, 20.8‰ and 19.9‰ respectively (Fig. 3). Interestingly, the calculated $\delta^{18}O_P$ values are off from measured values: for $NaHCO_3$-P_i and NaOH-P_i pools, they were 0.8‰ and 1.8‰ lower than the measured values, respectively. Similarly, the calculated $\delta^{18}O_P$ values of HCl-P_i were 2.2‰ higher than that of the measured values (Fig. 3).

For $NaHCO_3$-P_i and NaOH-P_i pools, the relatively heavier measured $\delta^{18}O_P$ values of the single-step extractants versus the corresponding calculated cumulative values imply that the unextracted P_i is relatively lighter than the P_i extracted during the single step extraction. There could be several reasons for the observed results. Given that different P pools have quite distinct isotope values (Fig. 3), mixing or partial extraction of specific P pools changes the isotope values. We should point out that we did not rinse the residual solids after centrifugation during the single-step extraction. Therefore, some P_i may be left out of the calculations due to re-adsorption to solid mineral surfaces following extraction. The study of (Jaisi et al., 2010) showed that during the initial stage of absorption, $P^{16}O_4$ was preferentially adsorbed by ferrihydrite, which also led to a relatively high $\delta^{18}O_P$ value in a single-step extraction.

For HCl-P_i pool, with the exception of one treatment, the measured $\delta^{18}O_P$ values of the single-step HCl-P_i pool are lower than the corresponding calculated cumulative values. This implies that although the P_i content is equal, the single-step extracted HCl-P_i is not identical to the sum of the sequentially extracted H_2O-P_i, $NaHCO_3$-P_i, NaOH-P_i and HCl-P_i pools. One possible explanation for this is that similar to $NaHCO_3$ and NaOH, HCl in the single-step extraction did not completely extract the cumulative sum of H_2O-P_i, $NaHCO_3$-P_i, NaOH-P_i and HCl-P_i found for the sequential extraction and/or some of its extracted P_i was adsorbed by the solid residuals, while at the same time, additional P_i could be added due to hydrolysis of organic P compounds. This is possible because 1 M HCl can hydrolyze labile organic P, and P_i

released from hydrolysis could alter $\delta^{18}O_P$ values to be lighter (Colman et al., 2005; Liang and Blake, 2006; Liang and Blake, 2009; McLaughlin et al., 2013).

3.4. Comparison of measured and equilibrium $\delta^{18}O_P$ values

For both soils and under all treatment methods, the $\delta^{18}O_P$ values became successively lighter from H_2O-P_i to $NaHCO_3$-P_i, NaOH-P_i and HCl-P_i pools (Fig. 3). Similar trend has been observed in other soils (Roberts et al., 2015; Zohar et al., 2010a; Zohar et al., 2010b) and this trend is dictated by relative bioavailability, original P sources and equilibrium isotope values at the ambient environment (Joshi et al., 2016).

The original source of P on the earth surface is derived from igneous rocks, which has a relatively lighter $\delta^{18}O_P$ values (2.4–12.2‰) (Holmden et al., 1997; Mizota et al., 1992; Taylor and Epstein, 1962). After release from igneous rocks, P_i could approach equilibrium $\delta^{18}O_P$ values with the surrounding environment (determined by temperature and $\delta^{18}O$ values of water) due to the uptake and metabolism of P by living organisms. Therefore, the $\delta^{18}O_P$ value of an environmental sample is a combined result of the source value, biological activity, temperature and $\delta^{18}O$ of ambient water. In general, the more extensive of the biological cycling, the closer approach to equilibrium $\delta^{18}O_P$ values. Therefore, the $\delta^{18}O_P$ values of H_2O-P_i and $NaHCO_3$-P_i pools (more bio-available) were usually heavier and closer or similar to equilibrium values than the NaOH-P_i and HCl-P_i pools (less bio-available) (Joshi et al., 2016; Roberts et al., 2015; Zohar et al., 2010a; Zohar et al., 2010b).

To calculate the equilibrium $\delta^{18}O_P$ values, we need $\delta^{18}O$ values of ambient environmental water ($\delta^{18}O_W$) and temperature. Unfortunately, we did not measure the $\delta^{18}O_W$ values of these soils from original sampling. However, our later collection of soil from the same site showed that the $\delta^{18}O_W$ values ranged from −4.4‰ to 2.2‰ (mean = −1.9‰; STDEV = 1.8; n = 27) (unpublished results). If we use these $\delta^{18}O_W$ values and the soil temperature of the experiment (24 °C), the corresponding calculated equilibrium $\delta^{18}O_P$ values range from 17.8‰ to 24.5‰ (mean, 20.3‰) based on the equation of (Chang and Blake, 2015). We observe that except for the H_2O-P_i pools of the 24 °C sto. 50d-No $HgCl_2$ treatment (higher) and most HCl-P_i pools (lower), the measured $\delta^{18}O_P$ values are all in the range of the calculated equilibrium values (Fig. 3), which suggests significant effect of biological activity.

The large variation of the calculated equilibrium $\delta^{18}O_P$ values requires a discussion. The calculated equilibrium $\delta^{18}O_P$ values determined by $\delta^{18}O_W$ values and soil temperature. For the variable character of the surface soil temperature and $\delta^{18}O_W$ values, the calculated equilibrium value usually depended on the choice of timescale. At present, the $\delta^{18}O_W$ values of irrigation waters (Zohar et al., 2010b), soil water (Joshi et al., 2016; Roberts et al., 2015), corrected rainwater (Roberts et al., 2015) or calculated rainwater values (Zohar et al., 2010b), and the temperature of soil (Joshi et al., 2016; Roberts et al., 2015) or air (Zohar et al., 2010a; Zohar et al., 2010b) have been used to calculate equilibrium values. While there is no discussion on the choice of particular water and temperature, the range is suggested to be within the timeframe under which isotope values could be impacted (Bear, 2016). This is quite straightforward for river P (Bear, 2016) and for ^{18}O-labeled study (Joshi et al., 2016), but not for other soils. Therefore, studies of the effects of temperature and $\delta^{18}O_W$ on $\delta^{18}O_P$ values of soil P pools at different time scales are needed.

3.5. The implications of $\delta^{18}O_P$ values of HCl-P_i

The HCl-P_i pool corresponds to Ca-P such as apatite-P (Tiessen and Moir, 1993). Although the measured $\delta^{18}O_P$ values of HCl-P_i in the soil studied are relatively lighter, they are much heavier than that of igneous rocks. Some former studies also showed a relatively higher $\delta^{18}O_P$ value of HCl-P_i (8.6‰ to 22‰) (Joshi et al., 2016; Roberts et al., 2015). This implies the HCl-P_i of this and former studies should not completely come from the igneous apatite. One explanation is that HCl also extracts out some P_i from the marine/sedimentary rocks debris and/or other forms of P (Hedley et al., 1982a) in soils. Another explanation is that the phosphate moiety in apatite can exchange oxygen isotope with soil water or new secondary apatite can be formed in the soil. For the 24 °C sto. 50d-No $HgCl_2$ treatment, the $\delta^{18}O_P$ values of the sequentially-extracted HCl-P_i pool are clearly heavier than that of the 0d sto.-$HgCl_2$ treatment. Zohar et al. (2010b) also found that the $\delta^{18}O_P$ of HCl-P_i pool change during 31 days of incubation. These results imply that it is possible to form new secondary apatite in the soil or the phosphate moiety in apatite can exchange oxygen isotope with soil water. But this still need more studies to verify.

4. Conclusions

In this work, we investigated the effects of sample treatment on $\delta^{18}O_P$ values of soil P pools, and compared $\delta^{18}O_P$ values for sequential extraction vs. single-step extraction. The conclusions of this study are:

1) The extraction and purification procedure did not change the original $\delta^{18}O_P$ values.
2) The $\delta^{18}O_P$ values of different P pools in the soils studied were distinctly different. The isotope values became gradually lighter from H_2O-P_i to $NaHCO_3$-P_i, NaOH-P_i and HCl-P_i, which is same as the trend of P bioavailability. It means that the $\delta^{18}O_P$ signature can be used to trace the bioavailability and interconversion of soil P pools.
3) The optimal storage condition of soil samples to avoid compromising isotope values are immediate freezing at low temperature (−20 °C or −80 °C) or freeze-drying.
4) The $\delta^{18}O_P$ values of single-step extractants differ from both the corresponding sequential extractant and mass balance model calculated values suggesting variable extraction of different P pools during single and sequential extraction methods.

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References

Amelung, W., Antar, P., Kleeberg, I., Oelmann, Y., Lücke, A., Alt, F., Lewandowski, H., Pätzold, S., Barej, J.A.M., 2015. The $\delta^{18}O$ signatures of HCl-extractable soil phosphates: methodological challenges and evidence of the cycling of biological P in arable soil. Eur. J. Soil Sci. 66 (6), 965–972.
Angert, A., et al., 2011. Seasonal variability of soil phosphate stable oxygen isotopes in rainfall manipulation experiments. Geochim. Cosmochim. Acta 75 (15), 4216–4227.
Angert, A., Weiner, T., Mazeh, S., Sternberg, M., 2012. Soil phosphate stable oxygen isotopes across rainfall and bedrock gradients. Environ. Sci. Technol. 46 (4), 2156–2162.
Bear, K., 2016. Tracking Sources of Particulate Phosphorus in River Waters: A Case Study in East Creek, a Chesapeake Bay Watershed. University of Delaware, Newark (MS: 95).
Blake, R.E., 1997. Experimental Investigations of Organic and Microbially Mediated Reactions of Aluminosilicates and Phosphates. Ph.D. Thesis. University of Michigan, pp. 119–135.
Blake, R.E., O'neil, J., Garcia, G.A., 1997. Oxygen isotope systematics of biologically mediated reactions of phosphate: I. Microbial degradation of organophosphorus compounds. Geochim. Cosmochim. Acta 61 (20), 4411–4422.
Blake, R., O'Neil, J., Garcia, G., 1998. Enzyme-catalyzed oxygen isotope exchange between inorganic phosphate and water: reaction rates and temperature dependence at 5.7–30 °C. Mineral. Mag. 62, 163–164.
Blake, R.E., O'Neil, J.R., Surkov, A.V., 2005. Biogeochemical cycling of phosphorus: insights from oxygen isotope effects of phosphoenzymes. Am. J. Sci. 305 (6–8), 596–620.

Blake, R.E., Chang, S.J., Lepland, A., 2010. Phosphate oxygen isotopic evidence for a temperate and biologically active Archaean ocean. Nature 464 (7291), 1029–1032.
Bray, R.H., Kurtz, L., 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Sci. 59 (1), 39–46.
Bunemann, E.K., 2015. Assessment of gross and net mineralization rates of soil organic phosphorus – a review. Soil Biol. Biochem. 89, 82–98.
Chang, S.J., Blake, R.E., 2015. Precise calibration of equilibrium oxygen isotope fractionations between dissolved phosphate and water from 3 to 37°C. Geochim. Cosmochim. Acta 150, 314–329.
Chang, S., Jackson, M.L., 1957. Fractionation of soil phosphorus. Soil Sci. 84 (2), 133–144.
Chen, Z.-G., Yin, X.-J., Zhou, Y., 2015. Effects of GC temperature and carrier gas flow rate on on-line oxygen isotope measurement as studied by on-column CO injection. J. Mass Spectrom. 50 (8), 1023–1030.
Colman, A.S., Blake, R.E., Karl, D.M., Fogel, M.L., Turekian, K.K., 2005. Marine phosphate oxygen isotopes and organic matter remineralization in the oceans. Proc. Natl. Acad. Sci. U. S. A. 102 (37), 13023–13028.
Condron, L.M., Goh, K.M., Newman, R.H., 1985. Nature and distribution of soil phosphorus as revealed by a sequential extraction method followed by 31P nuclear magnetic resonance analysis. Eur. J. Soil Sci. 36 (2), 199–207.
Firsching, F.H., 1961. Precipitation of silver phosphate from homogenous solution. Anal. Chem. 33 (7), 873–874.
Gross, A., Nishri, A., Angert, A., 2013. Use of phosphate oxygen isotopes for identifying atmospheric-P sources: a case study at Lake Kinneret. Environ. Sci. Technol. 47 (6), 2721–2727.
Gross, A., Turner, B.L., Wright, S.J., Tanner, E.V.J., Reichstein, M., Weiner, T., Angert, A., 2015. Oxygen isotope ratios of plant available phosphate in lowland tropical forest soils. Soil Biol. Biochem. 88, 354–361.
He, Griffin, Honeycutt, C.W., 2004. Enzymatic hydrolysis of organic phosphorus in swine manure and soil. J. Environ. Qual. 33 (1), 367–372.
He, Z., Fortuna, A.M., Senwo, Z.N., Tazisong, I.A., Honeycutt, C.W., Griffin, T.S., 2006. Hydrochloric fractions in Hedley fractionation may contain inorganic and organic phosphates. Soil Sci. Soc. Am. J. 70 (3), 893–899.
Hedley, M.J., Stewart, J., Chauhan, B., 1982a. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Sci. Soc. Am. J. 46 (5), 970–976.
Hedley, M.J., White, R.E., Nye, P.H., 1982b. Plant-induced changes in the rhizosphere of rape (*Brassica napus* var. Emerad) seedlings: III. Changes in L value, soil phosphate fractions and phosphatase activity. New Phytol. 91, 45–56.
Hinsinger, P., 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant Soil 237 (2), 173–195.
Holmden, C., Papanastassiou, D.A., Wasserburg, G.J., 1997. Negative thermal ion mass spectrometry of oxygen in phosphates. Geochim. Cosmochim. Acta 61 (11), 2253–2263.
Jaisi, D.P., Blake, R.E., 2010. Tracing sources and cycling of phosphorus in Peru Margin sediments using oxygen isotopes in authigenic and detrital phosphates. Geochim. Cosmochim. Acta 74 (11), 3199–3212.
Jaisi, D.P., Blake, R.E., Kukkadapu, R.K., 2010. Fractionation of oxygen isotopes in phosphate during its interactions with iron oxides. Geochim. Cosmochim. Acta 74 (4), 1309–1319.
Joshi, S.R., Kukkadapu, R.K., Burdige, D.J., Bowden, M.E., Sparks, D.L., Jaisi, D.P., 2015. Organic matter remineralization predominates phosphorus cycling in the mid-bay sediments in the Chesapeake Bay. Environ. Sci. Technol. 49 (10), 5887–5896.
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 Sci. Soc. Am. J. 80 (1), 69–78.
Karl, D.M., Tien, G., 1992. MAGIC: a sensitive and precise method for measuring dissolved phosphorus in aquatic environments. Limnol. Oceanogr. 37 (1), 105–116.
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 Planet. Sci. Lett. 64 (3), 398–404.
Kruse, J., Abraham, M., Amelung, W., Baum, C., Bol, R., Kühn, O., Lewandowski, H., Niederberger, J., Oelmann, Y., Rüger, C., Santner, J., Siebers, M., Siebers, N., Spohn, M., Vestergren, J., Vogts, A., Santner, J., 2015. Innovative methods in soil phosphorus research: a review. J. Plant Nutr. Soil Sci. 178 (1), 43–88.
Liang, Y., Blake, R., 2006. Oxygen isotope signature of P i regeneration from organic compounds by phosphomonoesterases and photooxidation. Geochim. Cosmochim. Acta 70 (15), 3957–3969.
Liang, Y., Blake, R.E., 2009. Compound-and enzyme-specific phosphodiester hydrolysis mechanisms revealed by $\delta^{18}O$ of dissolved inorganic phosphate: implications for marine P cycling. Geochim. Cosmochim. Acta 73 (13), 3782–3794.
Longinelli, A., Bartelloni, M., Cortecci, G., 1976. The isotopic cycle of oceanic phosphate, I. Earth Planet. Sci. Lett. 32 (2), 389–392.
Markel, D., Kolodny, Y., Luz, B., Nishri, A., 1994. Phosphorus cycling and phosphorus sources in Lake Kinneret: tracing by oxygen isotopes in phosphate. Isr. J. Earth Sci. 43 (3–4), 165–178.
McLaughlin, K., Sohm, J.A., Cutter, G.A., Lomas, M.W., Paytan, A., 2013. Phosphorus cycling in the Sargasso Sea: investigation using the oxygen isotopic composition of phosphate, enzyme-labeled fluorescence, and turnover times. Glob. Biogeochem. Cycles 27 (2), 375–387.
Mizota, C., Domon, Y., Yoshida, N., 1992. Oxygen isotope composition of natural phosphates from volcanic ash soils of the Great Rift Valley of Africa and east Java, Indonesia. Geoderma 53 (1), 111–123.
Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36.
Olsen, S.R., 1954. Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate. USDA.
O'Neil, J.R., Roe, L.J., Reinhard, E., Blake, R., 1994. A rapid and precise method of oxygen isotope analysis of biogenic phosphate. Isr. J. Earth Sci. 43 (3–4), 203–212.
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. Geochim. Cosmochim. Acta 67 (17), 3135–3144.
Paytan, A., Kolodny, Y., Neori, A., Luz, B., 2002. Rapid biologically mediated oxygen isotope exchange between water and phosphate. Glob. Biogeochem. Cycles 16 (1) (13-1-13-8).
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 6500year chronosequence under lowland temperate rainforest. Geoderma 257, 14–21.
Ruttenberg, K.C., 1992. Development of a sequential extraction method for different forms of phosphorus in marine sediments. Limnol. Oceanogr. 37 (7), 1460–1482.
Sharpley, A.N., Chapra, S.C., Wedepohl, R., Sims, J.T., Daniel, T.C., Reddy, K.R., 1994. Managing agricultural phosphorus for protection of surface waters: issues and options. J. Environ. Qual. 23 (3), 437–451.
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. Geochim. Cosmochim. Acta 138, 101–116.
Tamburini, F., Bernasconi, S., Angert, A., Weiner, T., Frossard, E., 2010. A method for the analysis of the $\delta^{18}O$ of inorganic phosphate extracted from soils with HCl. Eur. J. Soil Sci. 61 (6), 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. Environ. Sci. Technol. 46 (11), 5956–5962.
Taylor, H.P., Epstein, S., 1962. Relationship between O^{18}/O^{16} ratios in coexisting minerals of igneous and metamorphic rocks. Geol. Soc. Am. Bull. 73 (4), 461–480.
Tiessen, H., Moir, J., 1993. Characterization of Available P by Sequential Extraction. In: Carter, M.R., Gregorich, E.G. (Eds.), Soil Sampling and Methods of Analysis, pp. 293–306.
Walker, T., Syers, J.K., 1976. The fate of phosphorus during pedogenesis. Geoderma 15 (1), 1–19.
Weiner, T., Mazeh, S., Tamburini, F., Frossard, E., Bernasconi, S.M., Chiti, T., Angert, A., 2011. A method for analyzing the $\delta^{18}O$ of resin-extractable soil inorganic phosphate. Rapid Commun. Mass Spectrom. 25 (5), 624–628.
Yin, X., Chen, Z., 2014. Measuring oxygen yields of a thermal conversion/elemental analyzer-isotope ratio mass spectrometer for organic and inorganic materials through injection of CO. J. Mass Spectrom. 49 (12), 1298–1305.
Zohar, I., Shaviv, A., Klass, T., Roberts, K., Paytan, A., 2010a. Method for the analysis of oxygen isotopic composition of soil phosphate fractions. Environ. Sci. Technol. 44 (19), 7583–7588.
Zohar, I., Shaviv, A., Young, M., Kendall, C., Silva, S., Paytan, A., 2010b. Phosphorus dynamics in soils irrigated with reclaimed waste water or fresh water—a study using oxygen isotopic composition of phosphate. Geoderma 159 (1), 109–121.