



A method to isolate soil organic phosphorus from other soil organic matter to determine its carbon isotope ratio

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ABSTRACT

Despite the importance of soil organic phosphorus (OP) for plant nutrition, its dynamics in soil remain elusive due to the lack of multiple stable P isotopes. Here, we developed a method that isolates the soil OP pool from other soil organic matter to measure its carbon isotope signature to elucidate soil OP decomposition. We tested three extractants (H_2SO_4 , HCl, and NaOH in 0.5 M) for their capacity to preferentially extract OP. For isolating OP from the extract, we evaluated different pH adjustments (pH 1.5–10) and iron- or aluminum hydroxide additions. Finally, we determined the carbon isotope ratio ($\delta^{13}\text{C}$) of the isolated OP pool. We found that the H_2SO_4 extracts had the highest OP content and the lowest organic carbon (OC):OP ratio compared to the HCl and NaOH extracts. The pH adjustments of the H_2SO_4 extracts to pH 4–7.5 removed $\geq 93\%$ of the extracted OP from the solution. The OC:OP ratio of the precipitates was the lowest (11–16) at pH 7.5, showing a strong preferential OP precipitation caused by the pH alteration. Metal hydroxide addition (combined with pH treatment) did not improve the preferential OP removal. The $\delta^{13}\text{C}$ of the OP reflected differences in the vegetation (C_3/C_4) that grows at the soil sampling sites. Overall, this method provides a simple and effective approach to isolate the soil OP pool from other soil organic matter and determine its carbon isotope ratio, which opens new avenues to study soil OP dynamics.

1. Introduction

Organic phosphorus (OP) can comprise up to 90 % of the soil total P pool, and it consists of orthophosphate esters, phosphonates, and organic phosphate anhydrides (Condon et al., 2005). Decomposition of OP provides plants with P since this process turns OP into inorganic P (IP), which can be taken up by plants (Vitousek et al., 2010; Terrer et al., 2019). Thus, it is important to understand soil OP dynamics (Condon et al., 2005; Reed et al., 2015; Sun et al., 2017; George et al., 2018; Spohn, 2024). However, the decomposition rate of the soil OP pool remains elusive due to the lack of multiple stable P isotopes.

Mineralization of OP is commonly determined using isotope pool dilution techniques with radioactive P isotopes (Di et al., 2000; Büne-mann, 2015). Although these techniques provide valuable insights into soil P cycling, the results mostly give a momentary impression of OP mineralization under rather artificial conditions due to the short half-life and radioactivity of ^{32}P (14 days) and ^{33}P (25 days). Besides, this technique does not work well for soils with high microbial activity and strong sorption capacity (Bünemann, 2015; Kruse et al., 2015).

In this study, we propose a new approach to investigate soil OP dynamics by determining the carbon isotopic composition ($\delta^{13}\text{C}$) of the soil OP pool that compensates for the lack of multiple stable P isotopes. This approach builds on previous work based on highly ^{14}C -enriched compounds that were added to soils in incubation experiments (Spohn and Kuzyakov, 2013; Heuck et al., 2015; Spohn and Schleuss, 2019) and a compound-specific isotopic method to determine the $\delta^{13}\text{C}$ values of inositol phytate (Sarangi and Spohn, 2025). Here, we develop a new approach suitable for determining the carbon isotope signature of the soil OP pool under both natural and labelled conditions to assess the overall OP dynamics. This concept builds on established methods in soil organic carbon (OC) research, where $\delta^{13}\text{C}$ has been used to trace carbon turnover. For example, shifts in vegetation from C_3 to C_4 plants (or vice versa) result in distinct changes in $\delta^{13}\text{C}$ values, which have been widely used to study soil OC dynamics over decades (Balesdent et al., 1987, 2018; Bahri et al., 2006). Isotopic labelling with ^{13}C -enriched materials has also been applied to track OC dynamics in experimental settings (Bird et al., 2008). By applying these principles to the carbon bound within OP compounds, our approach opens new possibilities for

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investigating the long-term fate of soil OP across diverse environmental and management contexts.

As the first step of a technique to isolate the soil OP pool, soil extraction should maximize the OP recovery while preferably minimize the extraction of non-phosphorylated organic compounds. To extract soil OP, strong acids and bases, e.g., H_2SO_4 , HCl , and NaOH , are widely used (Condron et al., 2005; Turner et al., 2005). The extraction strengths of acids and bases increase with their concentrations (Turner et al., 2005). However, high concentrations of acids and bases can also cause significant OP hydrolysis. Thus, moderate concentrations of strong acids and bases, for instance 0.5 M H_2SO_4 and 0.5 M NaOH , are commonly chosen for soil OP extraction (Anderson, 1967; Newman and Tate, 1980; Turner et al., 2005).

To preferentially remove OP from solution, metal (hydr)oxides are often used owing to their high affinity to adsorb OP (Douglas et al., 2016; Li et al., 2016; Liu et al., 2018; Spohn, 2024). For example, metal (hydr)oxides have been shown to preferentially remove OP from wastewater (Zhang et al., 2009; Pan et al., 2014; Xu et al., 2017; Liu et al., 2018). This aligns with batch adsorption experiments, which investigated the adsorption of microbial extracellular polymeric substance (EPS) to metal minerals (Omoike and Chorover, 2006; Cao et al., 2011; Mikutta et al., 2011; Zhang et al., 2021). These studies showed that phosphorylated EPS had much higher affinities than non-phosphorylated EPS to adsorb to iron and aluminium (hydr)oxides.

The addition of metal (hydr)oxide to an OP solution can remove OP from the solution through adsorption and precipitation, which are affected by the pH. These processes depend on the protonation of the phosphate group. The phosphate group can donate up to three protons and the corresponding acid dissociation constants (pKa) are 2.1, 7.2, and 12.3, meaning that phosphate has neutral charge at very acidic pH, and the extent to which OP is deprotonated increases with increasing pH. The surfaces of metal (hydr)oxides also have pH-variable charge. When their surface is positively charged, phosphate can adsorb to them, dominantly via ligand exchange-surface complexation and secondly via outer-sphere interactions (Goldberg and Sposito, 1985; Ognalaga et al., 1994; Mikutta et al., 2007; Schneider et al., 2010; Chen and Arai, 2019; Wu et al., 2019). At high pH, both the phosphate group and the surface of metal (hydr)oxides carry negative charges, which likely impairs binding of phosphate to the metal (hydr)oxides (Li et al., 2016).

In a soil extract, the pH affects not only the bonds between phosphate groups and metal (hydr)oxides, but also the protonation of other functional groups, and thus their capacity to interact with charged compounds. Therefore, the effects of pH on OP removal from the solution via adsorption and precipitation are complicated.

This study aims to develop a method that isolates soil OP from other soil organic matter to measure the C isotope signature of the soil OP pool. More specifically, we aim to (1) identify an extractant that extracts the largest amount of soil OP, while extracting only little non-phosphorylated organic carbon (OC), (2) identify the conditions under which OP is preferentially removed from the solution (i.e., removal of OP without concomitant removal of non-phosphorylated organic matter) and (3) measure the $\delta^{13}\text{C}$ of the isolated soil OP. For this purpose, we evaluated several extractants that are commonly used for extracting OP from soil, and we tested how changes in pH and addition of metal hydroxides affect OP removal from the solution.

2. Material and methods

2.1. Soils and metal hydroxides

The soil samples (0–10 cm) come from three croplands (Lanna, Friemar, and Tänikon) located in Sweden, Germany, and Switzerland (Table 1). Lanna and Friemar have a C_3 vegetation, whereas Tänikon experienced a transition from C_3 to C_4 vegetation 17 years before sampling (Poeplau and Don, 2014; Spohn et al., 2023). The soils have contrasting characteristics, in terms of soil type and clay content

Table 1

Geographical information of the three sites.

	Lanna	Friemar	Tänikon
Country	Sweden	Germany	Switzerland
Latitude	58°20′49.9″N	50°58′30.63″N	47°28′42.19″N
Longitude	13°07′36.1″E	10°46′22.31″E	8°54′17.02″E
Elevation (m a.s.l.)	75	283	541
MAT (°C)	7.3	7.3	8.4
MAP (mm)	584	550	1185
Soil type	Udertic Haploboroll	Chernozem	Orthic Luvisol

(Tables 1 and 2). All samples were dried at 40 °C and sieved to 2 mm.

We prepared $\text{Fe}(\text{OH})_3$ and $\text{Al}(\text{OH})_3$ for evaluating their effect on the removal of OP from the soil extracts. $\text{Fe}(\text{OH})_3$ and $\text{Al}(\text{OH})_3$ were produced through a precipitation process (Mikutta et al., 2011). For this purpose, we slowly mixed either a 2 M FeCl_3 solution or a 2 M AlCl_3 solution with 10 M NaOH solution. The resulting precipitates (i.e., $\text{Fe}(\text{OH})_3$ and $\text{Al}(\text{OH})_3$) were washed with Milli-Q water three times, freeze-dried, and stored in air-tight glass bottles at room temperature.

2.2. Soil extraction and OP precipitation

We compared several extractants in order to determine which one extracts the largest amount of OP from soil, while concurrently extracting the smallest amount of OC. For this purpose, we extracted each soil in three extractants (0.5 M H_2SO_4 , 0.5 M HCl , and 0.5 M NaOH) in a ratio of 1:40 (mass: volume) on a horizontal shaker at 180 rpm for 16 h at room temperature. Subsequently, soil extracts were centrifuged (2800 G, 15 min) and filtered using acid- and base-resistant filter papers (Whatman™ 542).

After determining the best-performing extractant (0.5 M H_2SO_4), we conducted a series of precipitation experiments to evaluate, first, the conditions under which the largest amount of OP is removed from the filtered 0.5 M H_2SO_4 extracts by precipitation, and second, which precipitates have the lowest molar OC:OP ratio. Precipitation was achieved either by changing the pH or by a combination of pH change and metal hydroxides addition. All precipitation experiments were conducted with three replicates.

There were nineteen pH treatments, i.e., the original pH and every half-pH unit from pH 1.5 to pH 10. We tested these pH treatments in three experimental rounds. We first evaluated pH 3.5 and pH 8 treatments. Then, we expanded the pH treatments from pH 3.5 to pH 1.5 and from pH 8 to pH 10. Finally, we tested the treatments of pH 4–8. In each round, filtered soil extracts were divided into multiple aliquots, i.e., the original pH aliquot and pH-adjusted aliquots. We adjusted the pH by adding dropwise 10 M NaOH or 2 M H_2SO_4 on a magnetic stirrer while continuously monitoring the pH.

Table 2

Chemical properties of the three soils. Abbreviations: total carbon (TC), total nitrogen (TN), total inorganic carbon (TIC), total organic carbon (TOC), total phosphorus (TP), total inorganic phosphorus (TIP), and total organic phosphorus (TOP).

Parameter	Lanna	Friemar	Tänikon
Soil pH (in KCl)	6.6	6.8	6.6
Clay (%)	46	15	29
Silt (%)	46	76	40
Sand (%)	8	9	31
TC (%)	1.61	1.83	4.17
TN (%)	0.14	0.17	0.37
TIC (%)	0.00	0.11	0.43
TOC (%)	1.76	1.72	3.74
$\delta^{13}\text{C}$ of TOC (‰)	−27.41	−26.66	−21.69
TP (mg P kg d.w. ^{−1})	470	618	1036
TIP (mg P kg d.w. ^{−1})	175	199	479
TOP (mg P kg d.w. ^{−1})	295	419	556
Molar TOC:TOP	154	106	174

In addition, there were three metal hydroxide treatments, namely Fe (OH)₃ addition, Al(OH)₃ addition, and a treatment without metal hydroxide addition. In the first two experimental rounds, the metal hydroxide treatments were added to the filtered extracts with original pH and to the filtered extracts whose pH was adjusted to pH 1.5, pH 2.5, pH 3.5, pH 8, pH 9, and pH 10. Since we observed that the addition of metal oxides did not further increase the OP removal or decrease the OC:OP ratio of the precipitates during the first two experiments, we did not include the addition to metal hydroxides in the subsequent experiment, in which we determined very precisely the optimal pH for OP precipitation. For the treatments with metal hydroxides, we first weighed the amount of metal hydroxide in centrifugation vials before adding the pH-adjusted soil extracts. We chose the amount of metal hydroxides to obtain a molar ratio of 1:2 (metal hydroxide: soil OC).

All treated extracts were placed on a horizontal shaker at 80 rpm for 20 h for equilibration (similar as in Mikutta et al., 2011). We ended the reaction by centrifugation at 2800 G for 15 min. The supernatants were transferred to another set of glass vials and stored at −20 °C prior to analysis, while the precipitates remained in the centrifugation vial and were freeze-dried. The supernatants will be called treated extracts in the following.

2.3. Measurements

We determined the extraction and removal efficiency for OC and OP. For this purpose, total P (TP), IP, and OC were measured in the filtered extracts prior to any treatment, and in the treated extracts, i.e., after OP precipitation. TP was measured using an inductively coupled plasma optical emission spectrometer (ICP-OES; Avio 200, PerkinElmer). IP in the 0.5 M H₂SO₄ samples was determined according to the molybdenum blue method (Murphy and Riley, 1962) using a continuous flow auto-analyser (AA500, Seal). For the samples that were extracted in 0.5 M HCl and 0.5 M NaOH, we applied the malachite green method (D'Angelo et al., 2001) using a colorimetric plate reader (SPARK, Tecan). This is because hydrochloric acid can form chloro-complexes when applying the molybdenum blue method and the low pH requirement (pH < 1) of the molybdenum blue method can cause precipitation in the NaOH extracts (Nagul et al., 2015). To eliminate matrix effects, HCl and NaOH samples were diluted 1:5 prior to measurement, resulting in a final matrix concentration of 0.1 M, which does not interfere with color development in the malachite green method. Organic C was measured using a TOC analyser (Shimadzu TOC-L_{CPH}).

Additionally, we determined the $\delta^{13}\text{C}$ of the precipitates using an elemental analyser (Flash EA, 2000; Thermo Fisher Scientific, Bremen, Germany) coupled to a continuous-flow isotope ratio mass spectrometer (DeltaV, Thermo Fisher Scientific, Bremen, Germany) with three internationally certified standards, IAEA-600, IAEA-CH-6, and USGS40.

2.4. Calculations and statistical analysis

We calculated the OP content of the two solutions, the one at original pH (called aliquot 1 in Fig. 4) and the supernatant after pH adjustment and centrifugation (called supernatant in Fig. 4), as follows:

$$\text{OP (mg/kg)} = \text{TP (mg/kg)} - \text{IP (mg/kg)} \quad (1)$$

This was done separately for each of the two solutions. Subsequently, the amount of OP that precipitated due to pH adjustment (with or without metal hydroxide addition) was calculated as:

$$\text{OP}_{\text{precipitate}} (\text{mg/kg}) = \text{OP}_{\text{original pH}} (\text{mg/kg}) - \text{OP}_{\text{pH-adjusted}} (\text{mg/kg}) \quad (2)$$

Where $\text{OP}_{\text{original pH}}$ is the OP content of the untreated soil extract (aliquot 1), and $\text{OP}_{\text{pH-adjusted}}$ is the OP content of the supernatant after pH adjustment and centrifugation. In addition, the percentage of removed OP was then calculated as:

$$\text{OP removal (\%)} = \frac{\text{OP}_{\text{precipitate}} (\text{mg/kg})}{\text{OP}_{\text{original pH}} (\text{mg/kg})} \times 100\% \quad (3)$$

Equations (2) and (3) were also applied to determine the OC content of the precipitate and the percentage of removed OC by replacing OP by OC, respectively.

We applied the Kruskal-Wallis test to evaluate the effects of pH adjustment and metal hydroxide addition. We performed these statistical analyses for each soil separately. Assumptions of the Kruskal-Wallis test, i.e., ordinal or continuous response variable and independence, were met, and similar distributions among groups were evaluated visually. We considered $p < 0.05$ as the threshold for significance. The data analysis was performed using OriginPro 2024.

3. Results and discussion

3.1. Extraction of OP and OC

We found that 0.5 M H₂SO₄ is the best extractant for soil OP extraction compared to 0.5 M HCl and 0.5 M NaOH (Fig. 1) because it extracted the largest amount of OP before precipitation. In addition, the H₂SO₄ extracts had the lowest OC:OP ratio. Specifically, 0.5 M H₂SO₄ extracted 2.3–3.7-fold more OP than HCl and 1.4–5.9-fold more OP than NaOH, respectively, depending on the soil (Fig. 1a). The OC:OP ratio of the extracts was lower in the H₂SO₄ extracts (molar OC:OP ratio of 35–46) than in the 0.5 M HCl extracts (molar OC:OP ratio of 51–91) and 0.5 M NaOH extracts (molar OC:OP ratio of 173–571; Fig. 1b). Considering that the molar TOC:TOP ratio of the soil is 154, 106, and 174 for Lanna, Friemar, and Tännikon, respectively, 0.5 M H₂SO₄ had the

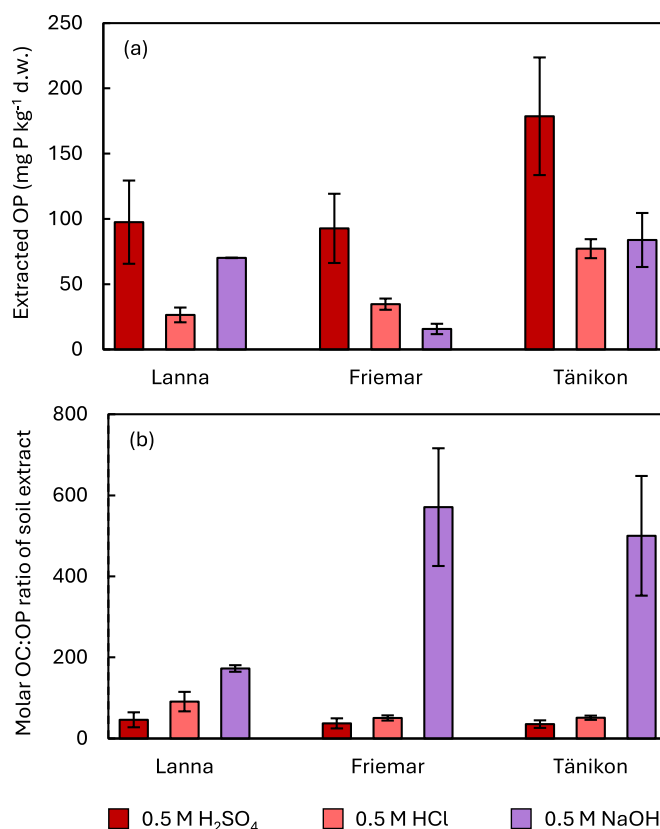


Fig. 1. Extracted soil organic phosphorus (OP; a) and the molar organic carbon (OC):OP ratio of the extracts (b) in three different soils depending on three extractants before precipitation. Bars and error bars represent means \pm standard deviations calculated across 13 replicates for H₂SO₄ and three replicates for HCl and NaOH.

strongest preferential OP extraction whereas 0.5 M HCl showed smaller preferential extraction strength for soil OP, and 0.5 M NaOH did not extract OP preferentially.

The high OP extraction of H₂SO₄ can be attributed to various mechanisms. OP is mainly adsorbed in soils through inner-sphere complexation (ligand exchange surface complexation) and secondly also due to outer-sphere complexation (e.g., electrostatic attraction) (Blume et al., 2016; Spohn, 2024). Sulfate anions can effectively break the inner-sphere complexes and replace phosphate (Liu et al., 2018). Furthermore, H₂SO₄ drastically decreases the pH (pH < 1), which changes the charge of the phosphate group from negative to neutral, thereby diminishing the outer-sphere complexes. Besides, at such low pH, the structural stability of metal oxides is impaired, which further decreases P adsorption (Li et al., 2016).

Both 0.5 M HCl and 0.5 M NaOH were less effective in OP extraction than 0.5 M H₂SO₄ for different reasons. With respect to HCl, the monovalent anion (Cl[−]) is less effective in soil P extraction than multivalent anions, e.g., SO₄^{2−} (Liu et al., 2018; Wu et al., 2019). Regarding NaOH, a high solution pH (pH > 14) may trigger precipitation and/or re-adsorption during extraction, reducing the extraction capacity. To solve this, EDTA is often used together with NaOH as it improves OP extraction by chelating metal cations and eliminating the formation of cationic bridges (Bowman and Moir, 1993; Cade-Menun and Preston, 1996; Dai et al., 1996; Condron et al., 2005). However, C from EDTA would interfere with the subsequent $\delta^{13}\text{C}$ measurement in our application, and thus cannot be applied in this protocol. Overall, 0.5 M H₂SO₄ is the best-tested extractant that extracted the largest amount of OP owing to its strong acidity and high charge density of its anion and the resulting extract had the lowest molar OC:OP ratio.

3.2. Precipitation of OP due to pH adjustment

The pH adjustment had consistent effects on OP removal from the solution across all soils (Fig. 2). The percentage of removed OP was low at pH ≤ 2 and it increased with increasing pH between pH 2.5 and 3.5. For all soils, we observed OP precipitation in the extracts between pH 2.5 and 10. More than 97.4 %, 92.7 %, and 95.2 % of all OP in the extract was removed from the solution by precipitation at pH 4–7.5 for Lanna, Friemar, and Tännikon, respectively, indicating that almost all extracted OP precipitated in this pH range. For the treatments with pH ≥ 8, the percentage of precipitated OP gradually decreased with increasing pH. Notably, this OP removal from the solution was achieved without addition of metal hydroxides.

The main reason for the high OP removal from the solution at pH 4–7.5 is likely that the negatively charged phosphate group binds to positively charged compounds, such as metal cations and metal hydroxides present in the soil extracts, leading to coprecipitation. A recent study showed that soil organic matter strongly co-precipitated with Al, Fe and Si cations released from soil bedrock (Jamoteau et al., 2023). In our study, phosphorylated organic compounds likely precipitated with metal cations and metal (hydr)oxides present in the soil extracts. In this context, it is also important to note that soil OP is largely dominated by phytate, which has six phosphate groups (Darch et al., 2014; Deiss et al., 2018). Thus, one phytate molecule can bind to several metal cations, and multivalent metal cations can bind to two or three phytate molecules or other OP compounds at the same time (Ognalaga et al., 1994; Celi et al., 2000; Chen and Arai, 2019), which likely leads to the formation of big complexes that act as supra-molecules and precipitate (Piccolo, 2002; Tamrat et al., 2019; Jamoteau et al., 2023).

At very low and very high pH, OP removal from the soil extract was low, which is likely because of the charge of the phosphate group and other substances. At very low pH, e.g., pH 1.5 and 2 (Fig. 2), the phosphate group (pK_{a1} = 2.1) is mostly fully protonated, and thus has limited capacities to interact with other extracted substances, such as metal cations (Ren et al., 2020). At very high pH, more functional groups and mineral surfaces are deprotonated and have negative or neutral charge,

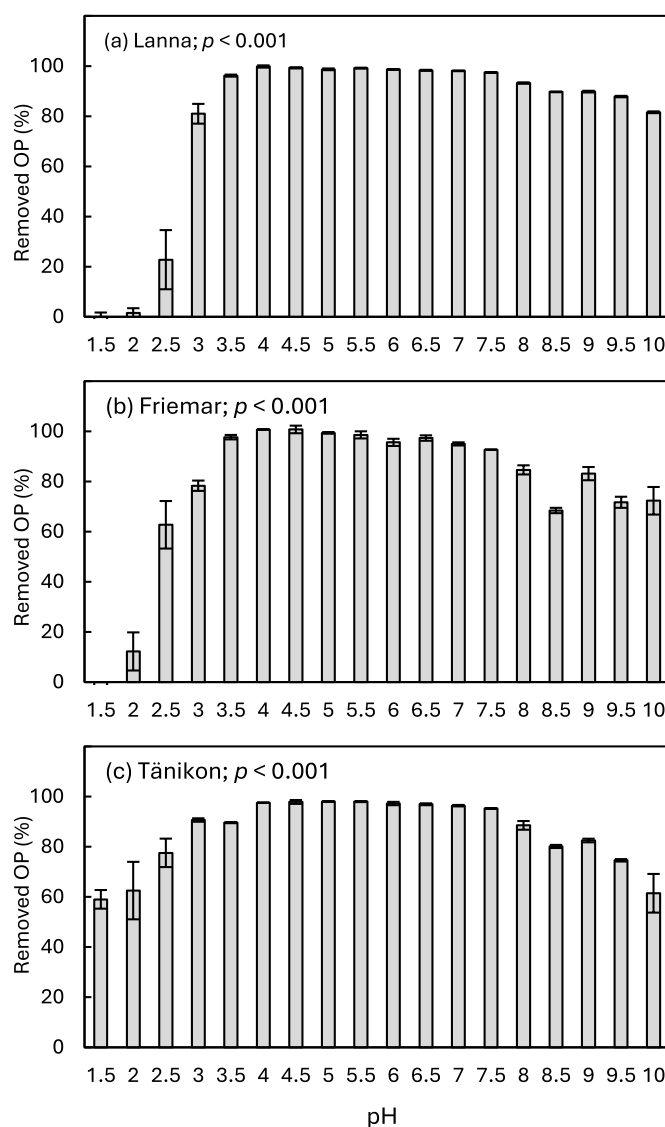


Fig. 2. Effect of pH on the percentage of organic phosphorus (OP) removed from the 0.5 M H₂SO₄ extracts of the three soils ((a) Lanna, (b) Friemar, and (c) Tännikon). Bars and error bars represent means ± standard deviations calculated across three replicates. A p-value of <0.001 indicates significant differences among the pH treatments, tested separately for each soil.

which also limits coprecipitation and adsorption of OP, thereby gradually decreasing the OP removal from the solution (Liu et al., 2018).

3.3. Preferential OP precipitation – OC:OP ratios

The low OC:OP ratio of the precipitates (that is lower than the OC:OP ratio of extracts) indicates that OP precipitated preferentially compared to OC. For all three soils, the molar OC:OP ratios of the precipitates were lower between pH 4 and 7.5 than at pH ≤ 3.5 and pH ≥ 8 (Fig. 3). We observed the lowest molar OC:OP ratio of the precipitates at pH 7.5, which was 16, 11, and 13 for Lanna, Friemar, and Tännikon, respectively.

The low OC:OP ratio of the precipitates (between 11 and 16) suggests that the precipitates are largely composed of OP compounds. The molar TOC:TOP ratio of the soils ranges between 106 and 174 (Table 2), and the molar OC:OP ratio of the 0.5 M H₂SO₄ extracts was 35–46. Thus, OP was preferentially precipitated (compared to non-phosphorylated OC compounds). Considering the molar C:P ratio of common soil OP compounds, for instance: inositol phosphates (1–6), DNA and RNA (9–10), and phospholipids (>25), etc., the low molar OC:OP ratio of the

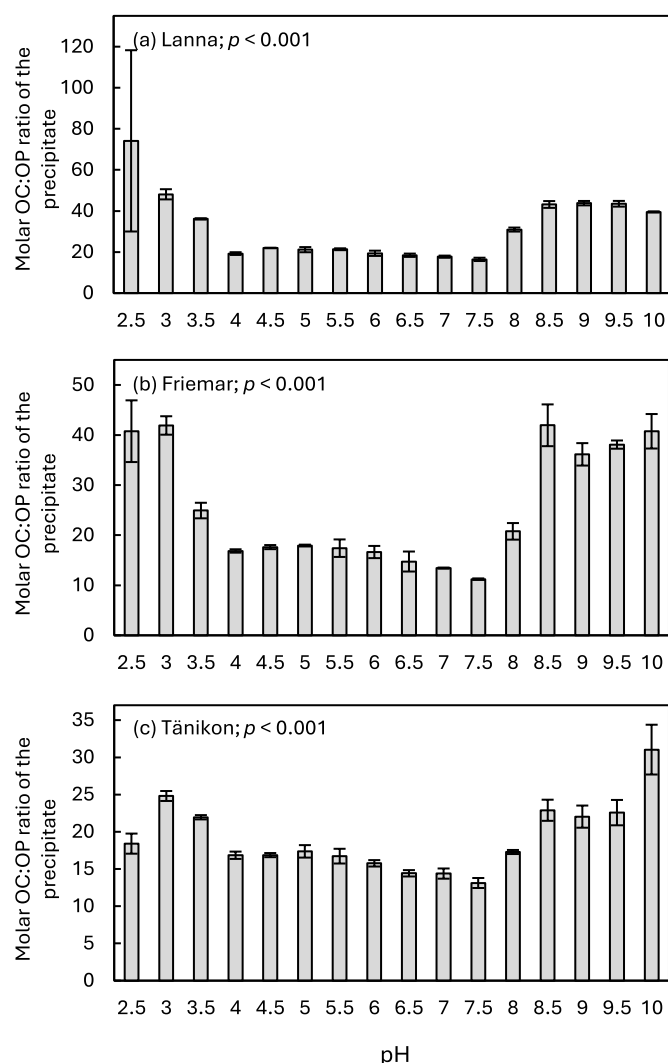


Fig. 3. Effect of pH on the molar organic carbon (OC) to organic phosphorus (OP) ratio of the precipitate from the 0.5 M H₂SO₄ extracts of the three soils (a) Lanna, (b) Friemar, and (c) Tānikon). Bars and error bars represent means \pm standard deviations, calculated across three replicates. A p -value of <0.001 indicates significant differences among the pH treatments, tested separately for each soil.

precipitate (between 11 and 16) indicates that the precipitate is largely composed of OP. This shows that the approach described here allows to isolate soil OP from the soil and from non-phosphorylated soil organic matter.

The preferential OP removal from the solution at pH 7.5 is likely due to several reasons. Phosphorylated organic compounds have a much higher adsorption affinity to various minerals than non-phosphorylated organic matter, in which the carboxyl group is the most abundant function group (Omoike and Chorover, 2006; Scheel et al., 2008; Cao et al., 2011; Mikutta et al., 2011; Swenson et al., 2015; Zhang et al., 2021; for a review see Spohn, 2024). More importantly, the binding form of carboxylates is more pH-dependent than the binding form of phosphate. At low pH, mainly ligand-exchange surface complexation of carboxylates occurs, whereas at neutral and basic pH, non-specific sorption of carboxylates dominates (Boily et al., 2000; Hwang and Lenhart, 2008; Parikh et al., 2011), and a lower proportion of carboxylates is typically adsorbed at neutral pH than at low pH (Gu et al., 1996; Filius et al., 2000; Strahm and Harrison, 2008). Regarding OP compounds, ligand-exchange surface complexation dominates at both low and near neutral pH, implying a consistently high OP precipitation

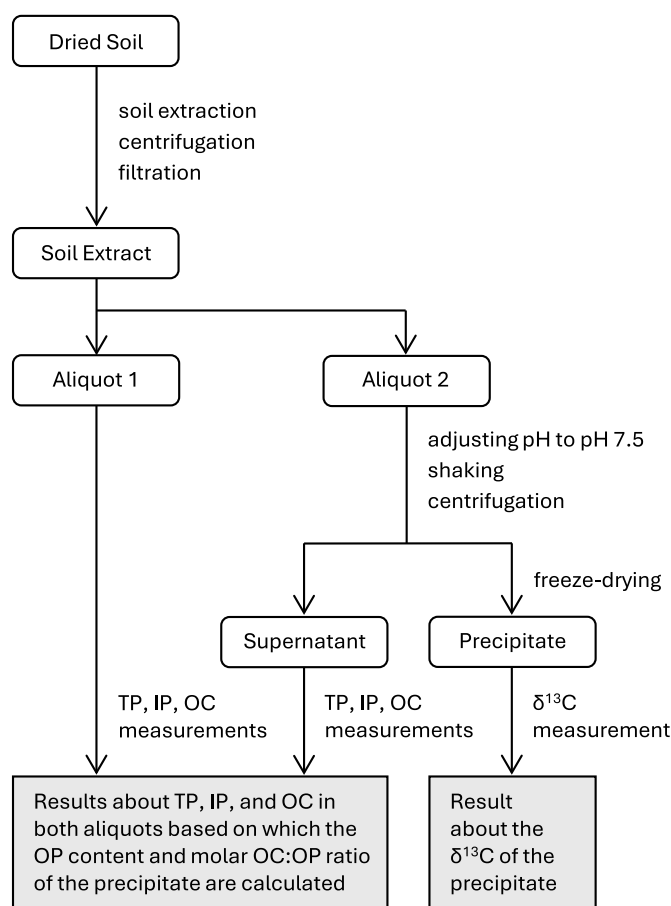


Fig. 4. Workflow of the method to isolate soil organic phosphorus from other soil organic matter to determine its carbon isotope ratio. All steps are performed at room temperature, except for the freeze-drying. Abbreviations: total phosphorus (TP), inorganic phosphorus (IP), and organic carbon (OC).

(Omoike et al., 2004; Cagnasso et al., 2010; Fang et al., 2012; Parikh et al., 2014; Liu et al., 2018; Chen and Arai, 2019). Moreover, the phosphate group mostly carries two negative charges at pH 7.5, allowing the formation of bidentate inner-sphere complexes, which further increases the stability of the bonds (Liu et al., 2018; Wu et al., 2019). Overall, our results show that pH 7.5 is the sweet spot that causes a minimal precipitation of non-phosphorylated organic compound while causing very high OP precipitation.

3.4. Adsorption of OP and OC to metal hydroxides at different pH

Metal hydroxide addition had no significant effect on OP removal from the solution and the molar OC:OP ratio of the precipitate compared to treatments with the same pH adjustment but without metal hydroxide addition (Supplementary Fig. S1 and S2). Especially, when the pH effects were close to their maximum (pH 3.5 and pH 8), metal hydroxide addition did not increase OP removal from the solution compared to the treatments with the same pH adjustment but no metal hydroxide addition. Furthermore, metal hydroxide addition did not cause any consistent effect on the molar OC:OP ratio of the precipitates compared to the treatment without metal hydroxide addition.

Since metal hydroxide addition did not increase OP removal and did not decrease the molar OC:OP ratio of the precipitates, metal hydroxide addition does not have to be included in future applications of this method. Next to the fact that its effects are very limited, adding metal hydroxide is an extra step, implying more efforts, costs, and potentially errors. In addition, metal hydroxides in the precipitate may complicate the $\delta^{13}\text{C}$ measurement.

3.5. $\delta^{13}\text{C}$ of the precipitates

We measured the $\delta^{13}\text{C}$ of the precipitates of the pH treatment that resulted in the highest OP precipitation and the lowest OC:OP ratio (i.e., pH 7.5 treatment). The $\delta^{13}\text{C}$ of the precipitates was $-27.18\text{‰} \pm 0.08\text{‰}$, $-25.96\text{‰} \pm 0.03\text{‰}$, and $-23.23\text{‰} \pm 0.05\text{‰}$ (means \pm standard deviations, calculated across three replicates) for Lanna, Friemar, and Tännikon, respectively. The ^{13}C values correspond to the type of vegetation that grows in these soils. There is a C_3 vegetation at Lanna and Friemar while the vegetation at Tännikon has been changed from C_3 to C_4 vegetation 17 years before the soil samples were taken. The $\delta^{13}\text{C}$ value of C_3 vegetation typically ranges from -24‰ to -30‰ , while the $\delta^{13}\text{C}$ value of C_4 vegetation typically ranges from -10‰ to -14‰ . The $\delta^{13}\text{C}$ of the OP fraction was similar to the $\delta^{13}\text{C}$ of the soil TOC in Lanna, and it was higher or lower, respectively for Friemar and Tännikon (Table 2). Differences in the $\delta^{13}\text{C}$ value between the precipitates and soil total OC pool might suggest different dynamics of the soil OP compared to the non-phosphorylated organic matter that dominates the soil total OC. This should be explored with more detail in future studies.

3.6. Conclusions and outlook

Here, we developed a method to isolate soil organic phosphorus from other soil organic matter to determine its carbon isotope ratio. The procedure can be summarized as follows (Fig. 4 and Supplementary protocol). Briefly, soil is extracted in 0.5 M H_2SO_4 . The filtered soil extract is divided into two aliquots of which one remains untreated while the pH of the second one is adjusted to precipitate the OP. Subsequently, the $\delta^{13}\text{C}$ of the OP precipitate is determined and the TP, IP, and OC concentrations of the treated and untreated extracts are quantified to determine the OP and OC contents as well as the OC:OP ratio of the precipitate. The results show that the tested step of metal hydroxide addition was not necessary to precipitate the OP. This is likely because the soil extract contains sufficient metal cations for the OP to precipitate if the pH allows this.

The method developed here provides a simple and effective approach to determine the carbon isotope ratio of the soil OP pool, which opens new avenues to study soil OP dynamics. For instance, the method can be used in experiments with a change in C_3 to C_4 vegetation to determine the turnover of the soil OP pool, as done similarly for the soil total OC pool and soil organic matter fractions (Balesdent et al., 1987). Furthermore, the method can be used in ^{13}C labeling experiments. In addition, the method can also be used to determine the ^{14}C : ^{12}C ratio using accelerator mass spectrometry. Future studies could explore compound-specific characterization of the precipitated OP, which may provide further insights into the chemical composition and dynamics of soil OP. Thus, given its broad applicability, this method enhances our ability to study P cycling in the soil-plant system. By using the carbon isotope of OP, this method solves the issue of lacking multiple stable P isotope, allowing us to study long-term dynamics of soil OP in both natural and experimental conditions.

CRediT authorship contribution statement

Ye Tian: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Marie Spohn:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2025.109911>.

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