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ABSTRACT

Aims: Although phytate is the most abundant organic P compound in soil, its sorption makes it poorly bioavailable through enzymatic hydrolysis. The sustainable use of the P resource in agriculture will require improving the supply of P to plants from organic P forms. To this end, further knowledge on dynamics of phytates in the soil is necessary, particularly in alkaline soils. This work aimed to study the factors that control phytate sorption and hydrolysis by phytases in alkaline soils and how this sorption affects other P forms present in soil. *Methods* A phytate sorption experiment was carried out using six soils with basic pH. Subsequently, the hydrolysis by two phytases (from *Aspergillus niger* and *Bacillus subtilis*) of organic P remaining in solution and extracted from the soil was assessed. *Results* Phytate was rapidly sorbed by soils with an estimated maximum sorption of 603 mg kg^{-1} at 360 min. Phytate sorption was positively correlated with carbonates, poorly crystalline Fe oxides, organic C, pH, and electrical conductivity in soils. Phytate adsorption promoted the release of other organic and inorganic P forms with less affinity for sorbent surfaces. For inorganic P, this release was more evident in soils with a low poorly crystalline Fe oxide content, while for organic P, the release increased with carbonates and clay content. Most of the phytate adsorbed was not extracted by NaOH, particularly in soils with high carbonate and poorly crystalline oxide content. This reveals that a significant portion of adsorbed phytate can be bound to carbonates, precipitated as metal phytates, or was not desorbed from Fe oxides. The insights gained from this research contribute to increasing our understanding of organic P cycling in soils and how soil properties influence the dynamics of these P forms.

1. Introduction

As an essential nutrient for living organisms, phosphorus (P) deficiency restricts the productivity of agricultural systems (Delgado and Scalenghe, 2008). Therefore, P fertilization is usually necessary for adequate crop nutrition, particularly on the 30 % of world's agricultural land where agricultural productivity is still severely limited by low P availability in soils (Kvakić et al., 2018). However, P is a non-renewable and strategic resource, whose price is expected to increase in the near future, thus negatively affecting agricultural productivity and food security in many regions of the world (Cordell et al., 2009; Ryan et al., 2012; Cordell and Neset, 2014; Helin and Weikard, 2019). Therefore, it is imperative to consider the recycling of P throughout the entire food chain (van Dijk et al., 2016; Recena et al., 2022). In addition, it is also necessary to evaluate the potential of theoretically non-available P forms in soils, such as some organic compounds, to supply P to plants

(Stutter et al., 2012; Withers et al., 2014; Rowe et al., 2016; Nesme and Withers, 2016; Le Noë et al., 2020). The use of organic fertilizers derived from urban or agro-industrial residues to reduce the dependence on mining resources (Metson et al., 2016; Chojnacka et al., 2020; García-López et al., 2021) will lead to an increased supply of organic P to soils. This supply would increase the organic P reserves accumulated in agricultural soils, which should be mineralized by the action of plant or microbial phosphatases to be used by plants (Liu et al., 2022). Thus, further knowledge of organic P dynamics in soil, particularly its hydrolysis rates, is necessary for the sustainable management of P fertilization.

Organic P usually amounts to 20–80 % of total P in the soil (Saavedra and Delgado, 2005; Recena et al., 2015). However, it is often the missed part of the phosphorus cycle, particularly about nutrient supply to crops (Delgado and Scalenghe, 2008; Recena et al., 2016; 2018). It has been shown that the main organic P compound in the soil is phytate (Turner

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et al., 2002, 2003; Liu et al., 2015; 2022; Recena et al., 2018). Furthermore, phytate is present in organic residues, mainly from livestock and crops (Fuentes et al., 2009; He et al., 2009; Hill and Cade-Menun, 2009; Gerke, 2015; Liu et al., 2022). The main soil properties that control phytate cycle are mineralogy, pH, charge of sorbent surfaces, organic matter, and texture (Celi et al., 2000; Celi and Barberis, 2007; Recena et al., 2018; García-López et al., 2021). Phytate tends to accumulate in soils as it forms insoluble compounds and has a high affinity for sorbent surfaces (George et al., 2005; Tang et al., 2006; Fransson and Jones, 2007). It is strongly adsorbed on hydroxylated surfaces, and this avoids its enzymatic hydrolysis, making it non-available to plants (Giaveno et al., 2008; Menezes-Blackburn et al., 2013; Celi et al., 2020). The sorption of phytate occurs through its phosphate groups, and the number of phosphates bound to mineral surfaces varies depending on the degree of crystallinity, surface porosity, point of zero charge (PZC), and specific surface of these minerals. The negative charge of phytate increases with increasing pH, which enhances its interactions with cations, forming complexes of variable solubility and leading to the accumulation of different metal-phytates in soils (Celi and Barberis, 2007; Crea et al., 2008; Heighton et al., 2012; Celi et al., 2020). These metal-phytates can be solubilized only at acidic pH since they are insoluble at mid-range pH values as Fe4/Al4-phytate (pH = 5–7) or Ca6/Mg6-phytate (pH > 7.5) complexes (Celi and Barberis, 2005; Turner et al., 2005; Liu et al., 2022).

On the other hand, phytate adsorption on oxyhydroxides improves with decreasing pH in soils since the positive charge of these minerals increases (Celi et al., 2001). In this regard, the PZC of each sorbent surface, i.e., the pH at which charge turns from positive (low pH) to negative (high pH) in minerals with pH-dependent surface charging, such as oxyhydroxides, has particular relevance in explaining their contribution to adsorption phenomena (Kosmulski, 2023). Most of the studies have focused on the study of phytate adsorption to Fe and Al oxyhydroxides or to clays (Giaveno et al., 2008; Wang et al., 2017) or in acidic soils (Anderson et al., 1974; Leytem et al., 2002). However, phytate adsorption has received less attention in alkaline soils, where different dominant sorbent surfaces and pH (in some cases above PZC) can lead to different phytate dynamics.

Phytate hydrolysis to inorganic phosphate ions by enzymes (phytases) can occur in the soil under specific conditions and only then can it be a source of P to plants (Mullaney and Ullah, 2003; Martin et al., 2004; Doolette et al., 2010; García-López et al., 2021). Phytases are synthesized by few organisms and most of the time are not present in plant root exudates (Hinsinger et al., 2015). Extracellular phytases can be produced by fungi and bacteria (Vats and Banerjee, 2004; García-López et al., 2021; Liu et al., 2022). These enzymes hydrolyze phytate, releasing lower inositol phosphate esters or orthophosphate ions (Ragon et al., 2008; Recena et al., 2018). Phytases hydrolytic activity is affected by pH: histidine acid phosphatases (HAP) produced by fungi are active at acidic pH, whereas β-propeller phytases (BPP) are active at basic pH (Tang et al., 2006; Hill et al., 2007; Singh et al., 2020). Furthermore, although the adsorption of phytases on soil minerals preserve them from degradation, this can restrict their hydrolytic potential (Giaveno et al., 2010; Celi et al., 2020). Thus, phytate hydrolysis in soils is largely dependent on (i) the release of these hydrolytic enzymes by the soil microbial community, (ii) the amount of active phytases in the soil solution, and (iii) the extent and strength of adsorption of phytate and phytases on soil components (Martin et al., 2004; Berg and Joern, 2006; Tang et al., 2006; Liu et al., 2022).

Faced with the need to ensure sustainable P management in agriculture, it is necessary to increase the use by crops of non-available P forms in soils such as stabilized organic P forms, which are major components of the so-called P legacy. To this end, it will be essential to expand our knowledge of the behavior of organic P as related to soil properties. Therefore, the objective of our work was to study the factors that control the stabilization and mineralization of the major organic P compound, i.e. phytate, in alkaline soils to understand its cycle and relevance for agricultural ecosystems, and how its retention in soil affects other P forms present in soil.

2. Materials and Methods

2.1. Soil sample collection and pretreatment

Samples of six different agricultural soils were collected from the surface horizon (0–20 cm depth) in different locations in Spain. Soils were assigned to the following orders according to the Soil Taxonomy (Soil Survey Staff, 2014): Entisols (Ent. 1 and 2), inceptisols (Incept.), alfisols (Alf. 1 and 2), and vertisols (Vert.) (Table S1). Before soil characterization and batch experiments, soil samples were air-dried at room temperature, homogenized, ground, and sieved through a 2 mm screen.

2.1.1. Soil characterization

For soil characterization, the following properties were analyzed: particle size distribution by the pipette method (Gee and Bauder, 1986), soil organic carbon (SOC) by the oxidation method (Walkley and Black, 1934), total equivalent CaCO3 (CCE) using a calcimeter, soil pH and electrical conductivity (EC) in a 1:2.5 soil: water extract, and cation exchange capacity (CEC) by the NH4OAc method (Sumner and Miller, 1996). The P availability to plants was assessed using Olsen P (Olsen et al., 1954). Total P of the soil was measured after ignition of 2 g of air-dried soil at 550°C for 2 h and subsequent extraction with 0.5 M H_2SO_4 for 16 h (Kuo, 1996). Total inorganic P was determined as the P extracted by 0.5 M H_2SO_4 from an unignited sample. The total organic P in the soil was calculated as the difference between the total P and the total inorganic P. Phosphorus concentration in all extracts was determined using the colorimetric method of Murphy and Riley (1962). Iron bound to oxides was determined by using two different extractions: ammonium oxalate for releasing Fe from poorly crystalline oxides (Fe_{ox}) (Schwertmann, 1965) and citrate-bicarbonate-dithionite for releasing Fe from crystalline and poorly crystalline oxides (Fe_{cbd}) (de Santiago and Delgado, 2006). Both reagents also release organically bound Fe. In these extracts, Fe was determined by atomic absorption spectrometry (Unicam Solaar M instrument from Thermo Fisher Scientific, Madrid, Spain). All analyses of soil properties were performed in triplicate.

2.2. Sorption-Kinetics experiment

To understand how the physical–chemical properties of soils influence the rate of phytate sorption, a sorption kinetics experiment was performed in triplicate using 50-ml polypropylene centrifuge tubes. A 100 mM solution of phytic acid sodium salt hydrate from rice (Sigma, Ref. 68388) was prepared in deionized water, adjusted to pH 7 with 1 M HCl, and sterilized by filtration at 0.45 μm. In the sorption kinetics study, a working solution (0.5 mM) was prepared from the 100 mM phytate solution in deionized water. Then 20 ml of the working solution (equivalent to 9.24 mg of sodium phytate) was added to 3 g of soil. The experiment included two sets of blank samples: (i) samples with sodium phytate solutions in tubes without soil to check the sorption on the tube material and/or degradation of phytate, and (ii) soil samples without added phytate (suspension in water) to assess if soil samples contribute to the phytate concentration in the supernatants. Tubes with suspensions were shaken in an oscillator at 150 rpm and a temperature of 25 °C. Solution samples from these suspensions were collected at 0, 15 min, 30 min, 60 min, 180 min, and 360 min.

At each sampling time, 1.5 ml of soil suspensions were sampled in Eppendorf tubes that were centrifuged at 14,000 g for 15 min. Concentrations of P in the supernatants were determined: inorganic P (Pi) using the malachite green method (Ohno and Zibilske, 1991) at 630 nm, and total P (Pt) using the same colorimetric method after mineralization. Mineralization was carried out in acidified soil extracts with 12 M HCl (1/1 v/v) at 110 °C for 16 h according to Ali et al. (2009). Organic P (Po)

was calculated as the difference between Pt and Pi.

2.2.1. P content in soil after kinetics experiment

Soils subjected to phytate adsorption kinetics were collected at 360 min and air dried. Then, soil extraction with 2 M NaOH at 25 °C for 16 h was performed with a soil-solution ratio of 1:20 and centrifuged at 14,000 g for 15 min. The total P in the NaOH soil extract was determined after digestion with 12 M HCl (v/v) at 110° C for 16 h (Ali et al., 2009). Inorganic P was determined using the malachite green method (Ohno and Zibilske, 1991). Organic P in the extract was estimated by subtracting Pi from Pt.

2.3. Phytase labile P in solid phase and liquid phase

Two recombinant phytases were used, originating either from the fungus *Aspergillus niger* (Phy-A) or from the bacteria *Bacillus subtilis* (Phy-C). Both enzymes (Phy-A and Phy-C) were produced in the yeast *Pichia pastoris* that harbors each gene to overexpress the protein after methanol induction (Laborde et al., 2004; Brunel et al., 2004). The two enzyme extracts have been produced at the 'Halle de Biotechnologie' (UMR IATE, Montpellier, France). The enzyme extracts were of the same batch as described in (Trouillefou et al., 2015) for Phy-A and Dussouy et al. (2021) for Phy-C, releasing 1300 (Phy-A) and 17.5 (Phy-C) μmoles Pi per minute per ml of enzyme extract.

The substrate specificity of both enzymes was determined against Na-phytate and ten additional organic P compounds purchased from Sigma, which were α-D-Glucose 1-phosphate (α-D-Glucose-1-P, ref. G7000), D-Glucose 6-phosphate (α-D-Glucose-6-P ref. G7250), ß-Glycerophosphate (ß-Glycero-P, ref. G9422), D-Fructose 6-phosphate (D-Fructose-6-P, ref. F1502), D-Fructose 1,6-bisphosphate (D-Fructose-1,6-bisP, ref. F6803), Adenosine 5′-monophosphate (AMP, ref. 01930), Pyrophosphate (ref. P8135), Triphosphate (ref. 72061), Adenosine 5′-diphosphate (ADP, ref. A5285), and Adenosine 5′-triphosphate (ATP, ref. A2383). The hydrolysis of these compounds was measured by adding 50 μl of enzyme solution previously diluted 2000 (Phy-A) or 200 (Phy-C) times to 200 μl of a 0.2 M acetate buffer, pH 5.5, containing each substrate at 10 mM. 1 M CaCl₂ was added to all substrate solutions in order to obtain a P to Ca ratio of 1:1. Incubation was performed at 28 °C and the hydrolysis reaction was finished by adding 1 ml of 0.2 M HCl after 0, 10, 15, 20 and 30 min for all substrates except phytate, which was stopped after 0, 4, 7 and 10 min. Substrate hydrolysis was measured by the release of Pi into the solution using the colorimetric malachite green method (Ohno and Zibilske, 1991). The rate of Pi release was calculated from the slope of Pi release as a function of time for each substrate and expressed as the percentage of the phytate hydrolysis rate for both enzymes. The ability of phytases to hydrolyze organic P extracted from soils after the kinetics experiment by 2 M NaOH or in liquid extracts during the kinetics experiment was evaluated. The 2 M NaOH extracts of the soil were first acidified by adding 2 M CH₃COOH (1/1, v/v). Then, in two different batch, 0.1 ml of each acidified soil NaOH extract or water extract collected previously during the kinetics

experiment (liquid extract) was mixed with 0.3 ml of 0.025 M sodium acetate buffer solution pH 5.5 and 0.1 ml of enzyme extract previously diluted 2000 (Phy-A) and 200 (Phy-C) times in ultrapure water. After incubation for 24 h at 30 °C, the reaction was stopped by adding 0.5 ml of 10 % trichloroacetic acid (TCA) (w/v). The Pi concentration in the extract was determined before and after incubation using the malachite green method (Ohno and Zibilske, 1991). Before these enzyme assays, it was necessary to have a calibration curve between Pi concentrations measured after enzyme activity and phytate concentrations in NaOH or water extracts. This was necessary to know the concentration of phytate hydrolyzed by the enzymes. After hydrolysis by enzymes, only the concentration of Pi in the extract was known but not that of hydrolyzed phytate. For this reason, another calibration curve for each phytase with increasing concentrations of phytate under the same conditions as the enzymatic assays with soil extracts. To this end, enzyme reactions were carried out in solutions of Na-phytate (Phy-A) or Ca-phytate (Phy-C) with concentrations ranging from 0.1 to 2 mM. With this, a linear equation that relates the concentration of phytate and Pi was obtained. Thus, by determining the increase in Pi, the actual concentration of the hydrolyzed phytate was found.

2.4. Sorption kinetics, sorbed phytate equations, and ratio of organic phosphorus to added phytate phosphorus during kinetics

The Elovich equation and pseudo-second order equation were used to fit kinetics curves. The Elovich equation is as follows.

$$qt = \left(\frac{1}{\beta}\right)\ln(\alpha\beta) + \left(\frac{1}{\beta}\right)\ln(t) \qquad (1)$$

Where: qt is the phytate amount adsorbed (mg kg^{-1}) at each time (t), α is the constant of initial velocity (mg kg^{-1}min^{-1}), and β is the desorption constant (kg mg^{-1}).

The pseudo-second order equation is:

$$\frac{t}{qt} = \frac{1}{qmax^2 k_2} + \frac{1}{qmax}t \qquad (2)$$

Where k_2 is the rate constant (kg mg^{-1} min^{-1}) and $qmax$ is the maximum adsorption capacity (mg kg^{-1}). Derived parameters from Equation (2) are h, which is the initial sorption rate (kg mg^{-1} min^{-1}), $h = qmax^2 k_2$, and t1/2, which is the half-life time (min), $t1/2 = 1/k_2 qmax$.

Phytate sorbed by soils was calculated using the following equation:

$$Q = \frac{(C_0 - C_i) \times V}{M} \qquad (3)$$

Where Q is the phytate amount sorbed (mg organic P kg^{-1}), C_o is the initial Po concentration in the soil solution (mg organic P L^{-1}), C_i is the Po concentration in the solution at sampling time i after the addition of phytate (mg organic P L^{-1}), V is the solution volume (L), and M is the mass of the soil in the centrifuge tube (kg).

The ratio of organic phosphorus (Po) remaining in the solution after

Table 1
Soil properties.

Soils	Clay	SOC	CCE	Fe$_{ox}$	Fe$_{cbd}$	Olsen P	Total inorganic P	Total organic P	EC	pH	CEC
			g kg^{-1}				mg kg^{-1}		μS cm^{-1}		cmol$_c$ kg^{-1}
Ent. 1	222	9.7	168	0.65	6.65	16.3	1834	184	278	8.1	16.3
Ent. 2	320	18	372	1.54	6.66	19.5	630	181	558	8.1	26
Alf. 1	274	6.4	0	0.74	17.11	13.8	136	100	224	7.9	15.4
Alf. 2	168	5.5	0	0.44	5.42	11.5	54	24	197	7.4	13.1
Incept.	518	11	323	0.85	5.56	17	355	107	492	8.1	47.8
Vert.	700	6.2	41	1.12	11.94	6.6	247	114	173	7.7	35.4
Range	168——700	5.5——18	0 – 372	0.44 – 1.54	5.42 – 19.5	6.6 – 19.5	54–1834	24–184	173–558	7.4 – 8.1	13.1 – 47.8

Ent., Entisols; Incept., Inceptisols; Alf., Alfisols; Vert., Vertisols; Soil organic carbon (SOC); calcium carbonate equivalent (CCE); oxalate extractable Fe (Fe$_{ox}$); citrate-bicarbonate-dithionite extractable Fe (Fe$_{cbd}$); 0.5 M H₂SO₄ extractable inorganic P from intact soil (Total inorganic P); Total P after soil ignition minus total inorganic P (Total organic P); electrical conductivity (EC); cation exchange capacity (CEC).

Table 2
Concentrations of organic P (Po) and inorganic P (Pi), and percentage of phytate remaining in solution (ratio of Po to added phytate phosphorus), in the soil solution during phytate sorption kinetics from 1 to 360 min after phytate application.

Soil	Solution phase after:																	
	1 min			15 min			30 min			60 min			180 min			360 min		
	Po	Pi	% Po to added phytate P[a]	Po	Pi	% Po to added phytate P[a]	Po	Pi	% Po to added phytate P[a]	Po	Pi	% Po to added phytate P[a]	Po	Pi	% Po to added phytate P[a]	Po	Pi	% Po to added phytate P[a]
	mg kg^{-1}		%	mg kg^{-1}		%	mg kg^{-1}		%	mg kg^{-1}		%	mg kg^{-1}		%	mg kg^{-1}		%
Ent. 1	164	5.13	26	19.46	8.02	3.14	17.57	7.37	2.83	13.69	6.92	2.21	9.58	6.29	1.54	6.05	5.52	0.98
Ent. 2	38.30	3.25	6.2	1.89	1.42	0.31	1.42	1.81	0.23	1.10	1.20	0.18	2.00	0.75	0.32	1.40	0.66	0.23
Alf. 1	350	3.97	56	12.77	5.51	2.06	9.48	4.73	1.53	7.00	4.20	1.13	5.53	3.94	0.89	2.41	3.23	0.39
Alf. 2	211	4.93	34	173	5.29	27.90	143.24	5.31	23.10	116	5.28	18.79	84.06	5.44	13.56	52.47	5.44	8.46
Incept.	3.43	3.05	0.55	1.70	2.61	0.27	1.56	2.32	0.25	0.94	2.18	0.15	1.57	1.89	0.25	0.35	1.53	0.06
Vert.	295	2.92	47	2.05	0.65	0.33	1.50	0.56	0.24	0.84	0.50	0.14	1.01	0.39	0.16	0.94	0.33	0.15

Ent, Entisols; Incept., Inceptisols; Alf, Alfisols; Vert., Vertisols.
[a] Na-phytate was added at the rate of 620 mg total P kg^{-1} soil at the starting of the kinetics.

addition of Na-phytate was expressed as a percentage of the initial Na-phytate added in the experiment; this ratio was calculated for all soils and times in the kinetics sorption experiment as:

The ratio of Po to added phytate phosphorus (%) = Organic P in the soil solution (after adding Na-phytate) x 100 / initial Na-phytate added to experiment.

This calculation allows one to compare the change in the Po in the soil solution as a result of the application of Na-phytate.

2.5. Statistical analysis

A one-way ANOVA was performed using Statgraphics Centurion XVI (Technologies, 2013). Previously, normality and homoscedasticity were assessed using the Shapiro-Wilk and Levenne tests, respectively. The mean comparison was made according to the Tukey HSD post hoc test ($P < 0.05$). Correlations and regressions were also performed using the same software. Principal component analysis (PCA) was applied to identify the effects of soil properties and P sorption using the R software version 4.2.1 (R Core Team, 2021).

3. Results

The properties of studied soils varied widely, in particular those affecting P dynamics and availability to plants, such as clay, Fe oxides, and carbonates (Table 1). Soil pH ranged from 7.4 to 8.1. Four soils were calcareous and two noncalcareous. Iron in crystalline oxides (Fe$_{cbd}$) varied between 5.4 and 19.5 g kg^{-1}, meanwhile Fe in poorly crystalline oxides (Fe$_{ox}$) ranged from 0.44 to 1.54 g kg^{-1}. Soil organic carbon (SOC) ranged from 5.5 to 18 and clay content from 168 to 700 g kg^{-1}. Olsen P ranged from 6.6 mg kg^{-1} to 19.5 mg kg^{-1}. The total organic P showed a mean value of 108 mg kg^{-1}; however, the difference between soils was quite wide, ranging from 24 to 184 mg kg^{-1}.

3.1. Phytate sorption kinetics

In the sorption kinetics experiment, Po in soil solution decreased with time (Table 2). The ratio of Po to added phytate phosphorus, ranged from 0.55 % (Incept.) to 56 % (Alf. 1) at 1 min after phytate addition (Table 2). At 360 min, the ratio of Po to added phytate phosphorus was on average 1.71 % and ranged from 0.06 % (Incept.) to 8.46 % (Alf. 2) (Table 2). This reveals a fast sorption process, with most of the phytate adsorbed in less than 1 min, increasing to a maximum of 603 mg kg^{-1} at 360 min (Fig. 1). Alf. 2 showed the slowest sorption although after 360 min the adsorption was similar to the other soils. In all the cases, Pi was higher with phytate addition than without phytate addition (Table 2 and Table S2) and tended to decrease with time in soils Vert., Incept. and Ent. 2 (Table 2). On the other hand, Pi in solution was the highest and tended to increase with time in soils Ent. 1, Alf. 1, and Alf. 2 (Table 2). Both Alfisols were the soils where the difference in Pi in the solution with and without phytate addition was the highest (Table 2 and Table S2).

Overall, fitting phytate sorption kinetics to the pseudo-second order model (average R^2 = 0.85; Table 3) was more accurate than to the Elovich model (average R^2 = 0.76; Table 3). However, in the Alf. 2. soil, kinetics data fitted the Elovich model better than pseudo second order equation. This soil showed the lowest sorption after 360 min and slower sorption kinetics than the other soils. This was reflected in significantly different parameters in the models used to fit phytate sorption kinetics (Table 3). Parameters of both kinetics models were consistent. In this regard, the rate constant (k_2), and the initial sorption rate in the pseudo second order model (h), and the constant of initial velocity in the Elovich model (α) (Table 3) were the highest in the three more calcareous soils (Ent. 1, Ent. 2, and Incept; Table 3; CCE content > 168 g kg^{-1}). This reveals a faster sorption kinetics in these three soils. These soils also had high saturation of sorbent surfaces saturation, which can be assumed from the Olsen P to Fe$_{cbd}$ ratio (Table 1 shows values of these both
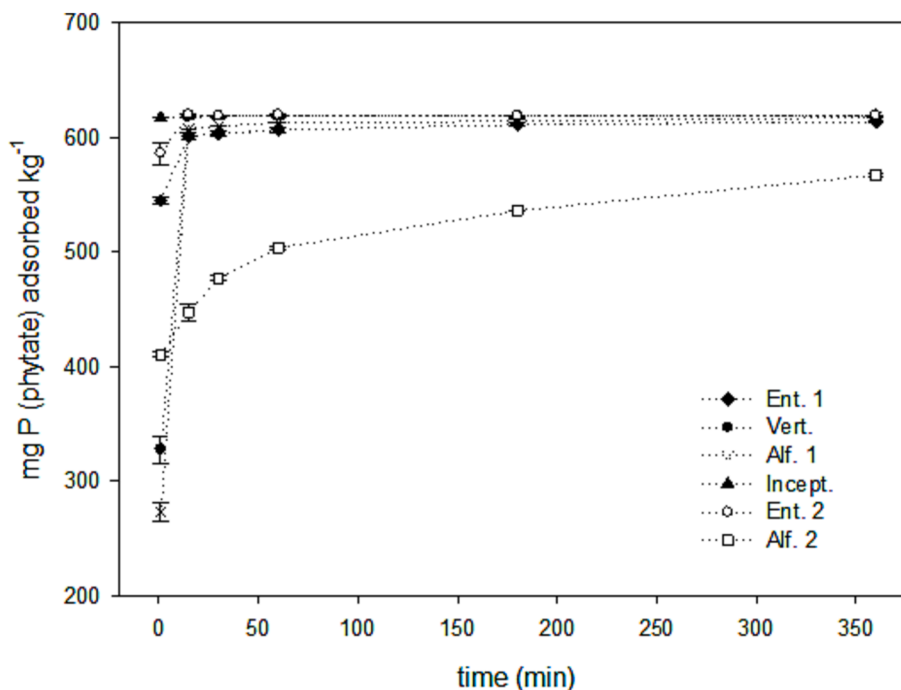
Fig. 1. Sorption kinetics of phytate in the studied soils. The initial amount of phytate-P provided at time zero was 620 mg P kg^{-1} of dry soil. Ent., Entisols; Incept., Inceptisols; Alf., Alfisols; Vert., Vertisols.

Table 3
Elovich and pseudo-second order model for phytate adsorption in different soils.

Parameters	Ent 1	Ent 2	Alf 1	Alf 2	Incept	Vert
Pseudo-second order model						
q_{max} (mg kg^{-1})	609	620.5	630	511	619	632
k_2	0.01	0.028	0.00127	0.007	0.71	0.0018
R^2	0.98	0.99	0.99	0.53	0.62	0.99
h	3708	10,781	504	1828	272,044	718
$t_{1/2}$	0.16	0.06	1.25	0.28	0.00	0.88
Elovich model						
α	$1.8*10^{22}$	$1.14*10^{43}$	31,641	$6.48*10^7$	$1*10^{44}$	221,071
β	0.09	0.16	0.02	0.04	0.17	0.02
R^2	0.8	0.65	0.7	0.93	–	0.7

k_2, Rate constant; h: Initial sorption rate; t1/2, half-life time; α, constant of initial velocity; β, desorption constant.

Table 4
Summary of Pearson correlation coefficient values between different soil properties and P sorbed by soil at 360 min and phytate hydrolyzed by two phytases in liquid phase[a].

	Clay	OC	CaCO$_3$	Fe$_{ox}$	Fe$_{cbd}$	Olsen P	EC	pH	CIC
mg P (phytate) sorbed kg^{-1}	0.21	0.36*	0.41*	0.34*	−0.06	0.29	0.38*	0.45**	0.33
Organic P hydrolyzed by Phy-A (%)	−0.33*	−0.19	−0.43**	−0.08	0.20	−0.26	−0.41*	−0.40*	−0.61***
Organic P hydrolyzed by Phy-C (%)	−0.37*	−0.23	−0.46**	−0.14	0.21	−0.26	−0.45**	−0.41*	−0.64***

Phy-A, phytase of *Aspergillus niger*; Phy-C, phytase of *Bacillus subtilis*.
[a] *, **correlations were significant at the 0.05, and 0.01 levels, respectively.

variables).

The amount of sorbed P after 360 min increased with increased contents of organic C, Ca carbonate, poorly crystalline Fe oxides (Fe$_{ox}$), and with increased values of pH and electrical conductivity, as revealed by the positive correlations between sorbed P and mentioned soil properties (Table 4).

3.2. P hydrolyzed by phytases in the liquid phase during the phytate sorption kinetics at different times

In the experiment to determine the specificity of the substrates of Phy-A and Phy-C, it was found that both enzymes were quite specific for phytate (Table 5). Phy-A was able to hydrolyze seven Po compounds in addition to phytate but at very low rates compared to that of phytate. Phy-C was even more specific to phytate than Phy-A, because it hydrolyzed only two Po compounds in addition to phytate, which were D-Fructose-6-P and ADP. The hydrolytic rate of ADP was, however, one-

Table 5
Substrate specificity of the two recombinant phytases (Phy-A and Phy-C) used in this study. For both enzymes, the maximal rate of free Pi release has been measured with phytate and this value corresponds to 100% of activity.

	Phytase from:	
Substrate	*Aspergillus niger* (Phy-A)	*Bacillus subtilis* (Phy-C)
Phytate	100£	100$
α-D-Glucose-1-P	NM*	NM
D-Glucose-6-P	0.4	NM
ß-Glycero-P	0.2	NM
D-Fructose-6-P	0.04	5.1
D-Fructose-1,6-bisP	6.7	NM
AMP	0.01	NM
Pyrophosphate	0.86	NM
Triphosphate	NM	NM
ADP	1.5	32.5
ATP	NM	NM

£: the substrate is Na-phytate; $: the substrate is Ca-phytate; * NM: not measurable.

third of that of phytate (Table 5). In the liquid phase, during the sorption experiment, the amount of Po hydrolyzable by phytases decreased with time in most soils (Fig. 2a, b). Similar trends were observed for both phytases (Phys. A and C). In the case of Alf. 2, most of the Po in solution was hydrolyzed by phytases, 92 % and 89 % for Phy-A and Phy-C, respectively (Fig. 2a, b). The portion of Po hydrolyzed by both phytases at 360 min negatively correlated with clay, carbonates, EC, pH, and CEC, but was not correlated with Fe_{ox} (Table 4).

The analysis of the principal components of soil parameters suggested substantial differences between soils in the P sorption and organic P hydrolyzed by phytases (Fig. 3). The first principal component (PC1) explained 42.9 % of the variability and the second (PC2) 19.6 % of the total variance. The variable more strongly correlated with sorbed phytate was Fe in poorly crystalline oxides (Fe_{ox}) and, to a lesser extent, EC, pH, carbonates and SOC. On the other hand, variables correlated with the hydrolysis of organic P by phytases in solution were the clay content and total Fe in oxides.

3.3. Quantification and hydrolysis of organic P in the soil after kinetics experiment

The Po extracted with NaOH in the soil after the end of the adsorption experiment ranged from 60 to 320 mg kg^{-1} (Fig. 4a). This accounted for 10 to 52 % of the phytate sorbed at the end of the experiment as estimated using equation (3) (Fig. 1). Phosphorus hydrolyzable by phytase ranged from 22 to 63 % (absolute amounts described in Fig. 4b and 4c) of the organic P released in the NaOH extract (Fig. 4a). The soil Alf. 1 showed the highest ratio of hydrolysed to sorbed P (around 25 %, in the other soils this ratio was less than 17 %) with both phytases. The soil with the second highest ratio of hydrolyzable to sorbed P was Alf. 2, also non-calcareous, and with the lowest poorly crystalline oxides content. On the other hand, the soils Ent. 1 and Vert. showed the lowest ratio of hydrolysable to sorbed P with both phytases (Fig. 4b and c). Ent. 1 soil was calcareous with one of the lowest poorly crystalline Fe oxides, and the Vert. was also calcareous, with the highest clay (mainly montmorillonite; not shown) content and the second highest content of crystalline and poorly crystalline Fe oxides (Table 1).

4. Discussion

4.1. Soil properties affecting phytate sorption in alkaline soils

In our study, soils with a basic pH showed rapid phytate sorption kinetics (Fig. 1) as observed for acidic soils by Anderson et al. (1974) and Leytem et al. (2002). Carbonates and poorly crystalline Fe oxides were the main minerals explaining phytate retention in soils, as revealed by correlations and PCA (Table 4; Fig. 3). The relevance of poorly crystalline Fe oxides as phytate adsorbing surfaces has been largely described in acidic soils where these minerals are the dominant Fe oxides (Turner et al., 2007; Jørgensen et al., 2015), but never in alkaline soils (Table 1). Crystalline Fe oxides appeared to have little influence on phytate sorption as Fe_{cbd} (mostly ascribed to crystalline) was not related to phytate adsorbed at 360 min (Table 4). Thus, despite the dominance of crystalline Fe oxides in the studied soils (Table 1), poorly crystalline oxides seemed to be the main Fe oxides involved in phytate adsorption. This agrees with previous evidence by Recena et al. (2018) where an increase in phytate content is observed with an increase in poorly crystalline Fe oxides in soils with pH ranging from slightly acid to alkaline and Fe oxide mineralogy dominated by crystalline oxides. This is the opposite trend to Pi, which is known to adsorb predominantly to crystalline Fe oxides in this type of soils (Torrent, 1987). The preferential adsorption of phytate on poorly crystalline Fe oxides reveals a high affinity of these P compounds for these sorbent surfaces. This is the consequence of the high concentration of binding sites ascribed to the increased surface area of these oxides compared to crystalline ones (Celi et al., 2020). According to Chen and Arai (2019), there is an inner-surface complexation of phytate on the surface of poorly crystalline Fe oxides such as ferrihydrite. In the formation of these complexes, several phosphate groups of phytate can interact with the surface of oxides. The number of phosphate groups involved in the adsorption depends on the hydroxyl groups distribution on the surface and contributes to explaining the different sorption capacity of oxides and the release of adsorbed phytate and consequently its potential hydrolysis (Celi et al., 2020). With increasing phytate adsorption, the sorption mechanism shifts from bidentate-binuclear surface complexation to unidentified ternary complexation and to amorphous Fe-phytate precipitation on oxides surfaces (Wang et al., 2017). Thus, different phytate adsorption mechanisms are possible, which also affects the release and hydrolysis of phytate. Little differences are expected in the PZC value of the main Fe oxides in soil (Cornell and Schwertmann, 2006). Thus, the pH-dependent surface charge was not likely the main reason for the different contribution of crystalline and poorly crystalline Fe oxides to phytate adsorption. The positive effect of EC on phytate adsorption is explained by a decrease in the thickness of a diffuse double layer in case of a net negative electric surface potential, and therefore reduced repulsion of the negatively charged phytate (Chen and Arai, 2019). A negative surface potential is expectable in Fe oxides above their PZC (Wang et al., 2017). Thus, the effect of EC on phytate sorption indicates that the main sorbent surfaces in the soil were above their PZC.

Although the negative potential of calcite surface increases from pH 6 to 9, phytate adsorption does not decrease in this pH range. This is consistent with a lack of significant change in zeta-potential of phytic acid-reacted calcite, which would mean that the formation of outer-sphere complexes is one of the mechanisms involved in phytate adsorption on calcite (Chen et al., 2022). According to Celi et al. (2000), surface precipitation of Ca-phytate complexes also occur on calcite. Thus, carbonates contribute to phytate adsorption involving several simultaneous mechanisms. Calcium carbonate is also a factor contributing to Ca saturation in soil and buffers the pH at alkaline values. These are factors that affect phytate dynamics in soil since the precipitation of Ca phytate increases with increasing Ca activity and pH in the solution (Crea et al., 2004). The relevance of metal phytate precipitation in the retention of this P compound in soils is evidenced by the increase in adsorption with increasing pH (Recena et al., 2018). This is because, at a higher pH, the negative charge of phytate increases, enhancing cation complexation (Celi and Barberis, 2007). This, however, negatively affects phytate adsorption on Fe oxides due to the increased negative charge on the sorbent surfaces (Johnson et al., 2012; Chen and Arai, 2019). Thus, surface adsorption and precipitation of Ca phytates explain the relevant role of carbonates in phytate dynamics in studied soils.

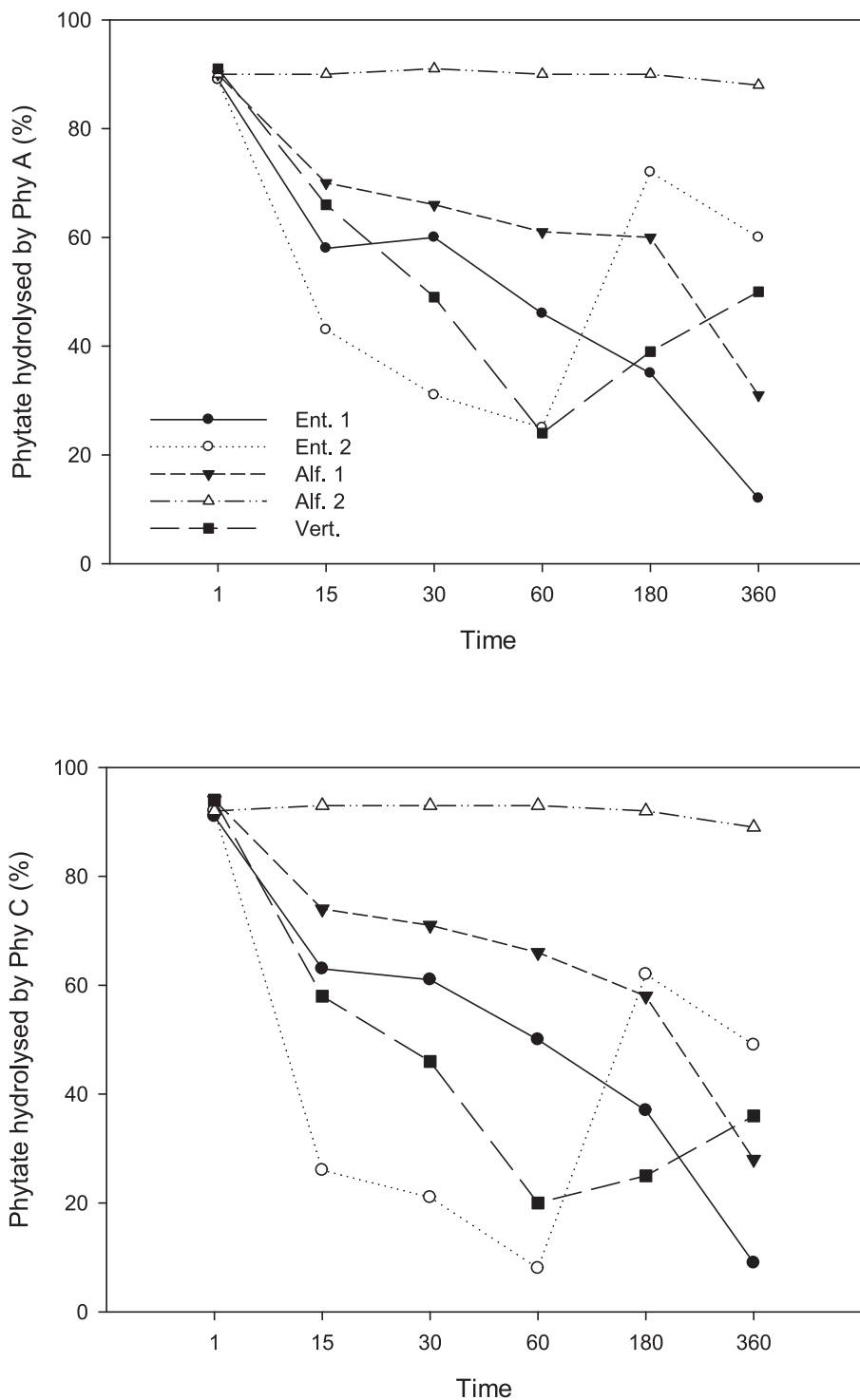Organometallic complexes in organic matter in soils can contribute

Fig. 2. Phytase-hydrolysable P by Phy-A (phytase from *Aspergillus niger*) (a) and Phy-C (phytase from *Bacillus subtilis*) (b) in the solution during the kinetics sorption experiment. Ent., Entisols; Incept., Inceptisols; Alf., Alfisols; Vert., Vertisols. Note: Inceptisol soil is not shown due to a lack of hydrolysis.

to phytate sorption. Phosphate bound to soil organic matter has been mainly observed in the humic fraction, which is typically the dominant fraction of organic matter and is assumed to be mainly bound to the mineral fraction (Adams et al., 2018). Organic matter forms binary complexes with di- and poly-valent cations, such as Ca^{2+}, Mg^{2+}, Al^{3+}, and Fe^{3+}, to which phytate can be bound (Gerke, 2010; Jindo et al., 2023). This sorption mechanism explains the correlation observed between organic C and the amount of phytate adsorbed at 360 min (Table 4). This agrees with previous evidence by Recena et al. (2018)

who concluded that stabilization of phytates was possible through binding to soil organic matter in soils developed under Mediterranean climate.

Overall, the pseudo second order kinetics was the best model to describe phytate retention by soils. This indicates that chemisorption on sorbent surfaces is the rate-determining step in the kinetics (Agbovi and Wilson, 2021). The three more calcareous soils, which also had a high saturation of sorbent surfaces, showed faster sorption kinetics. Thus, it seems that phytate sorption kinetics is much faster when Fe oxides have
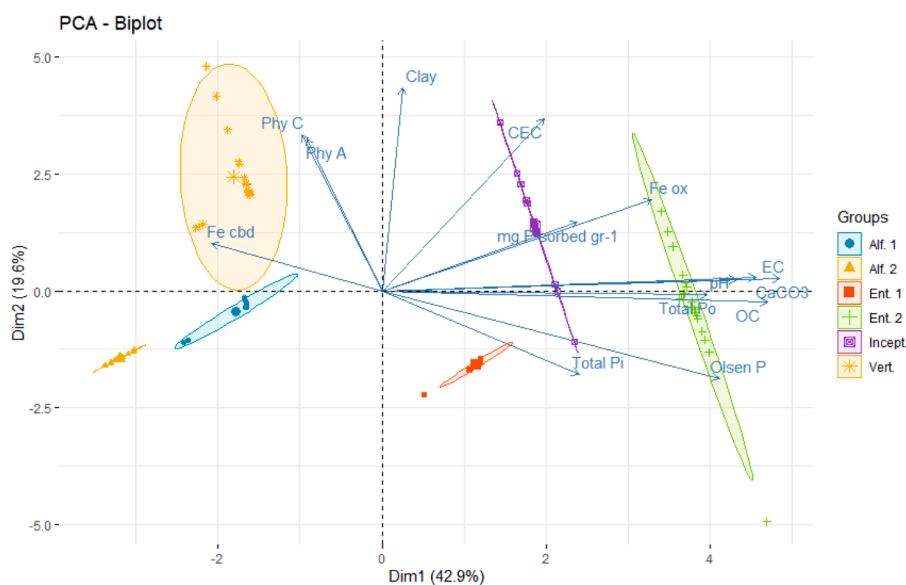
Fig. 3. Biplot of principal component analysis (PCA) of soil properties and parameters studied in sorption kinetics of phytate across soils studied. Each soil is represented by a different color and ellipse. Vectors represent soil properties. Ent., Entisols; Incept., Inceptisols; Alf., Alfisols; Vert., Vertisols; Soil organic carbon (SOC); calcium carbonate equivalent (CCE); oxalate extractable Fe (Fe_{ox}); citrate-bicarbonate-dithionite extractable Fe (Fe_{cbd}); 0.5 M H_2SO_4 extractable inorganic P from intact soil (Total inorganic P); Total P after soil ignition minus total inorganic P (Total organic P); electrical conductivity (EC); cation exchange capacity (CEC); Phy-A (phytase from *Aspergillus niger*), and Phy-C (phytase from *Bacillus subtilis*).

less relevance in phytate sorption and other sorption processes associated with carbonates, which favors the precipitation of metal phytates. In this regard, the slower kinetics in Alf. 2 compared to the other soils may be explained by the absence of carbonates. In addition, since the poorly crystalline Fe oxides content was the lowest in this soil, the availability of sorption sites was lower than in other soils and previous Pi desorption is necessary for the adsorption of phytate. All these may have contributed to the slower phytate sorption kinetics.

4.2. Effect of phytate sorption on the dynamics of other P forms

Phytate sorption was coupled to the release of Pi to the solution, to a different extent depending on soils (Table 2; Table S2). This agrees with Berg and Joern (2006), who showed competition between both P forms, leading to desorption of Pi in the presence of phytate. This is explained by the higher affinity of phytate for Fe oxides relative to Pi (Xu and Arai, 2022). Ent. 1 and both Alfisols (Alf. 1 and Alf. 2) had the lowest poorly crystalline Fe oxides content and showed the highest Pi desorption by competence with phytates. Furthermore, both Alfisols were not calcareous. Thus, lower sorption capacity leading to increased saturation of sorbent surface are conditions leading to increased Pi release with phytate adsorption. On the contrary, the two soils with the highest poorly crystalline Fe oxides content (Ent. 2 and Vert.), which were calcareous, showed the lowest Pi concentration in solution during the kinetics experiment, quite similar to that observed without the addition of phytate (Table S2).

Inorganic P in soil suspension during sorption kinetics can be affected by the activity of soil enzymes that mask the Pi release promoted by phytate and affect the estimated amount of adsorbed phytate. In fact, in the samples without phytate addition, there is a decrease in Po and a slight increase in Pi in solution (Table S2) which may reveal some hydrolysis of Po. However, Pi was always lower without phytate than that with phytate addition (Table 2). This difference could be ascribed to partial hydrolysis of added phytate, but after 15 min there is a general decreasing trend of Pi likely through readsorption which was not observed in samples without phytate. This reveals that there is no evidence of phytate hydrolysis in the solution with time. Thus, it seems that autochthonous Po or added phytate hydrolysis do not explain the

difference in Pi between samples with and without phytate, thus supporting the competitive release of Pi by phytate. On the other hand, the Pi concentrations were minimal compared to the added phytate, supporting that most of the phytate was adsorbed without hydrolysis. This is likely ascribed to the fast sorption of phytate, which decreases its susceptibility to enzyme hydrolysis (Giaveno et al., 2010).

The fraction of organic P corresponding to phytate (hydrolysable by phytases) in the liquid phase decreased with time in the kinetics experiment. Thus, the adsorption of phytate promoted a progressive release of other organic P forms from soil with less affinity for sorbent surfaces. The percentage of organic P hydrolysable by phytases was negatively correlated with carbonates and clay (indirectly with CEC) but not with Fe_{ox}. In fact, the soil with the highest percentage of organic P hydrolysable by phytases was non-calcareous and showed the lowest clay content (Alf. 2). Therefore, it seems that competitive adsorption of phytate with other organic P forms was more relevant in minerals with lower P adsorption capacity due to lower density of hydroxyl groups on the surface than poorly crystalline Fe oxides. In addition, conditions leading to augmented phytate sorption, such as increased EC or pH, also decreased the proportion of phytate in the solution. An increased pH favours the precipitation of Ca phytate as mentioned above. Thus, this precipitation contributes to explaining a decreased ratio of phytate to other organic P forms in solution.

4.3. Hydrolysis of adsorbed phytate

Differences in the organic P extracted with NaOH between soils at the end of the experiment can be attributed to the different initial content of organic P, the amount of sorbed phytate, and the different effectiveness of the extraction method to release organic P depending on the P forms and soil properties. Most of the adsorbed phytate was not extractable by NaOH since the ratio of P hydrolysed by phytases to adsorbed phytate was always less than 25 %. Although part of this hydrolysable P may correspond to phytate previously present in soil, the low recovery with the extractant reveals that phytate was strongly bound to the surfaces of the soil sorbent, not easily accessible to the extractant, and/or in forms such as metal phytates (e.g. Ca phytates) which are not soluble in NaOH. The higher hydrolysis ratios in Alfisols
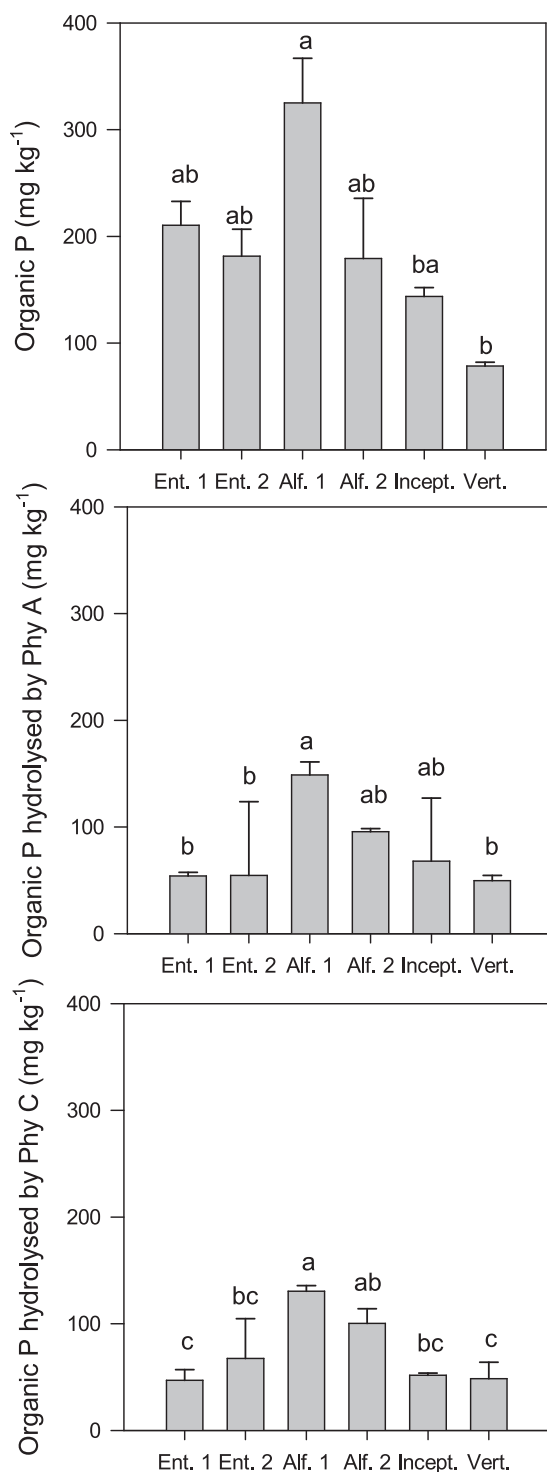
Fig. 4. NaOH-extractable organic P from the studied soils after phytate sorption experiment. Total amount of organic P (a), P hydrolysable by Phy-A (phytase from *Aspergillus niger*) (b) and Phy-C (phytase from *Bacillus subtilis*) (c). Means with different letter were significantly different for soils studied as per Tukey test at $P < 0.05$. Error bars indicate one standard error. Ent., Entisols; Incept., Inceptisols; Alf., Alfisols; Vert., Vertisols.

reveals that the portion of sorbed phytate that was releasable and more accessible to enzymatic hydrolysis increases in non-calcareous soils with low content in poorly crystalline Fe oxides. Thus, conditions prone to Ca phytate precipitation and/or sorption on poorly crystalline Fe oxides

seem to make phytate less easily releasable by NaOH and accessible for enzyme hydrolysis.

5. Conclusions

Phytate sorption was a rapid process which in the studied alkaline soils mainly involved adsorption on poorly crystalline Fe oxides and carbonates and precipitation of metal phosphates. The presence of metal phytates is consistent with the limited efficiency of NaOH extraction of phytates from the solid phase after the experiment. Although poorly crystalline Fe oxides were not the dominant oxides in soils, they play a more relevant role in phytate sorption than crystalline oxides. Phytate competed with other organic P forms and Pi with less affinity for sorbent surfaces. All of this provides new insight into the dynamics of phytate, allowing a better understanding of the properties that affect its bioavailability in alkaline soils.

CRediT authorship contribution statement

Ana Mᵃ García-López: Conceptualization, Investigation, Writing – original drfat, Writing – review. **Antonio Delgado:** Funding acquisition, Writing – review & editing, Project administration, Writing – original draft. **Claude Plassard:** Conceptualization, Investigation, Methodology, Supervision, Validation, Writing – original draft, Resources.

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2024.116800.

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