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Research article

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The distribution and kinetics of phosphatase activity in European agricultural soils under long-term tillage reduction practices

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Abstract

Achieving sustainability for agriculture is a significant challenge, particularly with respect to improving nutrient management in the field. This goal requires reducing the use of chemical fertilizers inputs and minimizing nutrient losses whilst maintaining crop yields. Phosphatases, enzymes produced by soil microorganisms and plants, play a key role in phophorus cycling by mobilizing phosphorus (P) from organic sources. This study examined the effects of three long-term tillage practices (no-tillage, reduced tillage, and standard tillage) on acid and alkaline phosphatase activity at two soil depths (0–10 and 10–20 cm) in seven agricultural field trials in Europe over a decade. Despite considerable variation among sampled sites, plots under reduced- and/or no-tillage regimes exhibited higher phosphatase activity in the topsoil compared with those under standard tillage. Both acid and alkaline phosphatases were influenced by the same major soil drivers, such as total nitrogen, total organic carbon, and total organic phosphorus contents. A predictive model incorporating total nitrogen alongside other soil features best explained changes in both phosphatase activities resulting from tillage treatments. Overall, our findings support that reduced-tillage practices enhance phosphatase activity, promote organic P mineralization, and could therefore reduce reliance on inorganic P fertilizers, contributing to more sustainable management of agricultural systems.

Sustainability statement

Implementing reduced and no-tillage practices enhances soil enzymatic activity, particularly phosphatases involved in organophosphorus mineralization, thereby improving nutrient cycling and reducing reliance on fertilizers. This contributes to maintaining crop yields while minimizing environmental impacts, ultimately promoting resilient and sustainable agricultural systems. This aligns with UN SDG 2: Zero Hunger, target 2.4–"ensuring sustainable food production systems and implementing resilient agricultural practices that increase productivity and help maintain ecosystems"

Keywords phosphatases, phosphate availability, no-tillage, reduced tillage, conservation agriculture, cropping systems, soil sustainability

Abbreviations

long-term agricultural experiments:	LTAE
standard tillage:	ST
reduced tillage:	RT
no-tillage:	NT

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p-nitrophenol:	PNP
4-nitrophenyl phosphate:	PNPP
segmented flow analysis:	SFA
maximum reaction rate:	V_{max}
Michaelis-Menten constant:	K_m

Introduction

The imperative to develop more sustainable agricultural systems that meet the growing food demand of the world's population is a pressing concern, crucial for global health. Traditionally, chemical phosphorus (P) fertilizers have been applied to boost agricultural productivity, compensating for the low bioavailability of P in most soils. However, widespread and excessive use of these fertilizers has resulted in severe environmental problems (Zou et al. 2022), including eutrophication of aquatic ecosystems and contamination with hazardous compounds such as heavy metals in fertilizer formulations or released during their production (Bouwman et al. 2017, Mekonnen and Hoekstra 2018, Chen et al. 2020). These environmental concerns, together with the expected shortage of rock phosphate and escalating fertilizer costs, underscore the urgency of improving P management (Cordell and White 2013, Gómez-Gallego et al. 2025). Specifically, strategies to access surplus P accumulated in soils, commonly referred to as legacy P, should be explored (Tóth et al. 2014, Recena et al. 2022, Zou et al. 2022, Turner and Kim 2024, Gómez-Gallego et al. 2025). Understanding how alternative agronomic practices affect P cycling and dynamics could aid policymakers and farmers in developing more sustainable production systems (Ros et al. 2020, Zhang et al. 2023, Gómez-Gallego et al. 2025).

Several studies have reported the benefits of minimizing tillage on physical, chemical, and biological soil properties, including increased water content, improved aggregate stability, enhanced cation exchange capacity, greater organic carbon content, and higher abundance and/or biodiversity of soil microorganisms and earthworms (Singh et al. 2018, Francaviglia et al. 2023, Gómez-Gallego et al. 2025). Reduced tillage has also been associated with increased activity of soil enzymes, including phosphatases (Deng and Tabatabai 1997, Holland 2004, Green et al. 2007, Madejón et al. 2009, Wen et al. 2023, Campdelacreu Rocabruna et al. 2024, Korav et al. 2024). However, the long-term persistence of these effects remains unclear, as some studies report no significant changes (Singh and Kumar 2021, Gerke 2021, Nugroho et al. 2023), while others report strong responses. Therefore, important knowledge gaps remain regarding the long-term effects of tillage on phosphatase activity, particularly in terms of enzyme kinetics.

Phosphomonoesterases, frequently referred to as phosphatases, a term we adopt in this manuscript, are key enzymes involved in mobilizing organic P, which represents one the most abundant P pools in many soils (Eivazi and Tabatabai 1977, Nannipieri et al. 2011). This is particularly relevant in soil enriched with organic inputs, a common management strategy in conservation agriculture (Page et al. 2020, Janes-Bassett et al. 2022). Phosphatases are hydrolytic enzymes that release P from complex organic molecules that would otherwise remain unavailable for plant uptake (Richardson and Simpson 2011, Margalef et al. 2017). Phosphatases are typically secreted into the soil by plant roots and microorganisms, including bacteria, mycorrhizal fungi and saprophytic fungi (Neal et al. 2018, Touhami et al. 2020, Udaondo et al. 2020). They are a complex group of enzymes belonging to different families, displaying substantial evolutionary diversity in substrate specificity, regulatory mechanisms, and responses to nutrient and environmental cues (Rogers et al. 1982, Thaller et al. 1994, Fahs et al. 2016, Neal et al. 2018, Udaondo et al. 2020, Park et al. 2022, Ramos et al. 2022, Recio et al. 2024). One of the simplest and most widely used classification of these enzymes is based on their pH optimum, distinguishing between acid and alkaline phosphatases (Eivazi and Tabatabai 1977). Although several studies have highlighted the importance of phosphatases in P cycling in diverse ecosystems, including agricultural soils (Hui et al. 2013, Azeem et al. 2015, Margalef et al. 2017, Akhtar et al. 2018, Janes-Bassett et al. 2022, Wen et al. 2023), their long-term response to management practices remain inadequately understood.

The kinetics of soil enzymes often follow the Michaelis-Menten equation (Hui et al. 2013). In soils, the calculated kinetic values represent a weighted average of the entire enzyme pool, with individual enzymes typically originating from multiple sources whose relative contributions are unknown (Nannipieri et al. 2011). Thus, kinetic parameters provide an integrated measure of overall enzymatic activity in soil, which reflects both the catalytic activity of the enzymes and broader soil biochemical processes, including nutrient cycling (Nannipieri et al. 2011; Moscatelli et al. 2012; Zhang et al. 2018).

Enzyme activities respond to differences in soil management and soil properties (Gianfreda et al. 2005, Khadem and Raiesi 2019, Wen et al. 2023). Owing to their minimal sample requirements and relatively straightforward analytical protocols, enzymatic activities and associated kinetic parameters have been proposed as bio-indicators of soil fertility (Moscatelli et al. 2012; Leite et al. 2018). This study aimed to investigate the impact of reduced- and no-tillage practices, after at least a decade under these management regimes, on acid and alkaline phosphatase activities in seven agricultural soils across Europe. To better understand how tillage influences phosphatase activity, we further analyzed acid and alkaline phosphatase kinetics at two sites with strongly contrasting soil pH, organic matter content, and climatic conditions. Sampling across diverse soils and climates enabled us to identify the key soil properties predicting phosphatase activity, and to discern general patterns of these soil enzymes across European agro-ecosystems.

Materials and methods

Site description and experimental layout

Seven long-term agricultural experiments (LTAE) were selected in different countries, namely Lithuania, Sweden, Denmark, the Netherlands, France, Switzerland and Spain. These sites span a c. 3 000 km northeast-southwest transect. Each experimental site featured cereal crops cultivated for more than a decade with a consistent set up (see Table S1 in Appendix A), which enabled the study of long-term effects of reduced soil disturbance on various soil properties. The experimental design at each site included three tillage treatments: standard tillage (ST), reduced tillage (RT), and no-tillage (NT), each treatment with two sampling depths (0–10 and 10–20 cm). The full description of the sampled sites, including details of the tillage treatments, is provided in Table S1

(Sánchez-Moreno et al. 2024), while soil characteristics of each LTAE site are presented in Table 1 (an extended version is available in Table S2 and S3).

Soil sampling

Soil sampling was conducted in 2021 in all the LTAEs during the period between harvest and subsequent agricultural operations, which comprised the period between late summer (August 24, 2021) and early autumn (November 18, 2021) depending on the pedoclimatic zone (Table S1). Soil samples were collected at two soil depths (0–10 cm and 10–20 cm) following the protocols described by Fernández-Ugalde et al. (2018). In each plot and at each depth, five sub-samples (ca. 300 g each) were taken from the center of the plot and in its four cardinal points at a distance of 1-2 m from the center depending on the plot size. Sub-samples were then mixed to obtain 24 composite soil samples per LTAE. The sampling strategy resulted in a total of 168 soil samples, namely, 4 experimental blocks × 3 tillage treatments (ST, RT, NT) × 2 soil depths (10 cm, 20 cm) × 7 LTAEs. The samples were placed in insulated containers with ice packs, maintaining a temperature of approximately 4° C, and transport from the field to the laboratory took less than 2 h. Subsamples for microbial biomass were stored at -80 °C until analysis. The remaining soil was sieved to less than 2 mm immediately upon arrival and kept at 4°C to preserve enzymatic activities. Samples exchanged among different laboratories were shipped under refrigeration, and upon arrival stored at 4°C or–80°C, as required for physical, chemical or biological analyses. To avoid inter-laboratory variability, each parameter was analyzed in a single laboratory. Enzymatic assays were performed within 30 days after soil sample reception. Repeated activity measurements conducted on the same soil samples stored at 4 °C confirmed that reliable activities could be maintained for up to 2 months, provided the cold-chain was preserved (Abellán et al. 2011).

Soil physical and chemical analyses

Soil gravimetric water content (GWC) at the time of sampling was calculated from the weight loss of oven-dried soil samples at 105°C for 24 h. Soil texture was determined in 20 g of soil samples by the pipette method as described by Gee and Bauder (1986). The soil pH was determined using a glass electrode in a 1:5 (volume fraction) suspension of air-dried soil in 1 M KCl according to international standard ISO 10390:2005. Total organic carbon (TOC) content was determined in air-dried and ground soil samples using a TruSpec C/N analyzer (Leco Corp., MI, USA) after a 55°C acidic (HCl) treatment.

For the determination of soil total phosphorus (TP), organic phosphorus (Po), and inorganic phosphorus (Pi) content, an aliquot of sample was oven-dried at 40°C for 48 h. Subsequently, TP, Pi and Po were determined according to Kuo (1996). Briefly, 2 g of the dried soil was extracted for 16 h with 50 mL of 0.5 M H_2SO_4 with and without an ignition step for 4 h at 550°C before extraction. Subsequently, the extracts were centrifuged and orthophosphate concentrations measured using the molybdenum blue method (Murphy and Riley 1962) facilitated by segmented flow analysis (SFA; Skalar, SAN^{++}). Soil TP and Pi concentrations were derived from the concentrations measured in ignited and unignited samples, respectively, and Po concentration was then calculated as the difference between these two measurements.

Soil total nitrogen (TN) content was determined after digestion with a mixture of H_2SO_4, salicylic acid, H_2O_2, and selenium (Novozamsky et al. 1983). Briefly, the dried soil samples were finely ground using a ball mill, and 0.3 g soil placed in digestion tubes with 2.5 mL of the digestion mixture. After heating to 100°C, 3 mL H_2O_2 was added and the temperature raised to 330°C for 2 h to complete the digestion. The final volume was adjusted to 50 mL with water and TN quantified using SFA. To estimate the organic nitrogen pool, soil mineral N was determined by extracting 3 g dried soil with 30 mL 0.01 M $CaCl_2$ and, after filtration, total soluble nitrogen was analyzed in the supernatant using SFA (Houba et al. 2000). Organic nitrogen (Norg) was estimated by subtracting mineral nitrogen from TN.

Soil microbial biomass

Soil microbial biomass (MB) was determined on the basis of phospholipid fatty-acid amounts (PLFA; Frostegård et al. 1991). Briefly, PLFAs were extracted according to Kaur et al. (2005) using an adapted Bligh and Dyer solution (1959) (4:8:3 mixture of chloroform: methanol: 0.15 M citrate buffer; pH 4). The lipids were then fractionated into neutral, glyco- and phospholipids methylated and quantified using gas chromatography.

Soil phosphatase activity assays

Enzyme assays were conducted on field-moist samples stored at 4°C. Phosphatase activity was estimated by quantifying the amount of *p*-nitrophenol (PNP) produced from 4-nitrophenyl phosphate (PNPP) (Tabatabai and Bremmer 1969). To minimize buffer effects, we used the HAM polybuffer (40 mM HEPES, 40 mM glacial Acetic acid, 40 mM MES) (Recio et al. 2024) to determine both acid and alkaline phosphatase activity (Table 1). The pH was adjusted to pH 5.5 for acid phosphatase activity as the literature indicates that its activity peaks around this pH (Acosta-Martínez and Tabatabai 2000, Hui et al. 2013, Fraser et al. 2024) and to pH 8.0 for alkaline phosphatase which was the upper limit of the alkaline pH range of soils used in this study (Sinsabaugh et al. 2008).

For the assay, 0.5 g soil was added to 2 mL of HAM buffer at the specified pH. Subsequently, 0.5 mL of 0.115 M PNPP was added, and the samples incubated at 37°C for 30 minutes with agitation at 200 rpm. Phosphatase activity was confirmed to be linear over time under the assay conditions, and the pH was maintained at its set value without significant variations during the incubation. After the incubation period, 0.5 mL of 0.5 M $CaCl_2$ and 2 mL of 0.5 M NaOH were added. The samples were then centrifuged for 5 minutes at 1,200 g, and the supernatants collected. Concentration of PNP was determined at 405 nm using a Tecan Sunrise spectrophotometer (Austria). Soil sample controls were prepared and incubated as above, except that the substrate (PNPP) was added after treatment with the $CaCl_2$-NaOH solution to account for any background of non-enzymatic hydrolysis. The concentration of PNP in the samples was corrected by subtracting those of the corresponding controls. The results were expressed as μmol PNP h^{-1} g^{-1} dry weight (dw) soil.

Table 1 Topsoil properties of the sites under study subjected to the different tillage treatments.

		pH	TP	Pi	Po	TOC	TN
CH	**ST**	5.97 ± 0.37	629.00 ± 61.33	402.75 ± 51.27	225.75 ± 14.51	1.72 ± 0.22	1.48 ± 0.17
	RT	6.05 ± 0.59	807.25 ± 62.24	520.75 ± 58.47	286.75 ± 6.54	2.33 ± 0.11	1.98 ± 0.12
	NT	6.17 ± 0.50	685.75 ± 35.07	433.75 ± 28.07	252.00 ± 9.82	2.09 ± 0.11	1.60 ± 0.09
DK	**ST**	5.69 ± 0.13	1106.50 ± 60.30	531.50 ± 39.09	575.25 ± 34.41	2.44 ± 0.29	1.68 ± 0.14
	RT	5.58 ± 0.00	1162.25 ± 42.83	567.50 ± 27.04	595.00 ± 21.37	2.82 ± 0.31	1.85 ± 0.12
	NT	5.50 ± 0.06	1142.75 ± 56.94	545.50 ± 50.79	597.25 ± 20.64	2.58 ± 0.18	1.78 ± 0.06
ES	**ST**	7.50 ± 0.26	292.25 ± 26.69	193.75 ± 24.10	98.25 ± 4.78	0.74 ± 0.04	0.65 ± 0.05
	RT	7.55 ± 0.17	372.75 ± 35.73	289.50 ± 40.48	83.00 ± 16.48	0.75 ± 0.04	0.78 ± 0.03
	NT	7.27 ± 0.27	376.75 ± 45.79	257.50 ± 54.98	119.25 ± 9.27	1.07 ± 0.07	0.98 ± 0.06
FR	**ST**	5.45 ± 0.14	484.50 ± 12.77	299.50 ± 12.70	185.00 ± 4.71	1.70 ± 0.06	1.20 ± 0.04
	RT	4.99 ± 0.13	525.50 ± 5.61	317.00 ± 8.26	208.50 ± 3.28	2.01 ± 0.06	1.45 ± 0.03
	NT	5.07 ± 0.19	563.75 ± 13.95	341.00 ± 20.52	222.50 ± 11.95	2.34 ± 0.11	1.65 ± 0.06
LT	**ST**	6.20 ± 0.04	410.25 ± 6.24	272.75 ± 11.76	137.75 ± 7.69	1.29 ± 0.05	1.00 ± 0.04
	RT	6.21 ± 0.11	464.75 ± 48.56	271.00 ± 33.05	193.75 ± 48.91	1.33 ± 0.05	1.00 ± 0.04
	NT	6.26 ± 0.10	425.75 ± 27.77	286.00 ± 31.07	139.50 ± 3.77	1.31 ± 0.07	1.05 ± 0.05
NL	**ST**	7.61 ± 0.02	552.75 ± 15.43	489.25 ± 8.60	63.50 ± 15.64	1.18 ± 0.06	0.90 ± 0.00
	RT	7.56 ± 0.02	634.50 ± 29.95	530.50 ± 31.08	103.75 ± 4.48	1.48 ± 0.06	1.00 ± 0.00
	NT	7.54 ± 0.04	669.75 ± 23.16	535.75 ± 19.78	134.00 ± 4.45	1.74 ± 0.37	1.10 ± 0.04
SE	**ST**	5.40 ± 0.06	536.75 ± 18.09	292.25 ± 5.47	244.50 ± 13.47	1.68 ± 0.19	1.70 ± 0.07
	RT	5.39 ± 0.02	533.75 ± 10.69	291.50 ± 6.17	241.75 ± 8.32	1.99 ± 0.22	1.80 ± 0.06
	NT	5.16 ± 0.06	580.50 ± 20.04	302.50 ± 8.85	278.00 ± 13.86	3.13 ± 0.32	2.23 ± 0.08

Notes: Means (± sd) of total phosphorus (TP, mg P/kg), inorganic phosphorus (Pi, mg P/kg), organic phosphorus (Po, mg P/kg), total organic carbon (TOC, %) and total nitrogen (TN, g N/kg) for different treatments (ST, standard tillage; RT, reduced tillage; NT, no-tillage) at 0–10 cm depth. The extended version of this table, including data from both soil depths (0–10 cm; 10–20 cm) is available in Table S2 in Appendix A. Abbreviations: CH, Switzerland; DK, Denmark; ES, Spain; FR, France; LT, Lithuania; NL, Netherlands; SE, Sweden.

Soil enzyme kinetic parameters

For the estimation of soil enzyme kinetic constants, both acid and alkaline phosphatase activity was assayed across a range of PNPP concentrations (0.96, 0.48, 0.24, 0.12, 0.06, 0.03, 0.015, 0.0075, 0.00375 and 0.001875 M) using the assay described above. For each sample, Michaelis–Menten kinetics were confirmed by plotting the rate of PNP production (y-axis) against the substrate concentrations (x-axis), and the data were then analyzed using the Lineweaver-Burk transformation (Hui et al. 2013). A ten-point linear regression, based on the reciprocal of both substrate concentration and PNP rate, was obtained and used to estimate the maximum rate of reaction (Vmax) and the Michaelis-Menten constant (Km). Additionally, the catalytic efficiency factor was calculated as the ratio of Vmax to Km (German et al. 2012; Moscatelli et al. 2012; Zhang et al. 2018, Khadem and Raiesi 2019). All enzyme activity measurements, including kinetics, were performed by the same user, to avoid inter-user variability.

Statistical analyses

Statistical analyses were performed using the R software v. 4.3.2. (R Core Team 2023). We first analyzed the existence of generalizable effects of reducing tillage on soil phosphatase activity across soil depths and pedoclimatic region. To do so, we used a generalized linear mixed model (GLMM) with "tillage" and "depth" and their interaction as the fixed factors and "site" (LTAE) and "block" as the random factors, with "block" being nested into"site" (model 1; Table S4). Alternatively, to assess the effect of "site" on phosphatase activity patterns, a similar model was constructed but including "site" as a fixed factor along with its interactions with "tillage", and "depth", whilst "block" remained as a random factor (model 2; Table S4). Additionally, differences in phosphatase activity were further explored separately for each LTAE by using "tillage" and "depth" and the interaction between them as a fixed factors and "block" as a random factor.

Regarding phosphatase kinetics, due to the large sample number, kinetic analyses were restricted to soil samples collected at 0–10 cm depth, where the highest phosphatase activity was detected, as described in the results section. We initially explored the dynamics of each kinetic variable under standard tillage using a GLMM including "site" as a fixed factor and "block" as a random factor, to obtain an overall view of the enzyme kinetics in the sites. The effect of tillage on phosphatase kinetics was then analyzed via a GLMM, using "tillage" as a fixed factor and "block" as a random factor in the Spanish and Swedish locations. All the GLMMs were analyzed with lmer and Anova (type III) functions of the lmerTest and car R packages, respectively. Post hoc comparisons were conducted using the Tukey's test to differentiate group of means (P value < 0.05) (multcomp and emmeans packages for R; Hothorn et al. 2008; Lenth et al. 2025).

Regarding the analyses of phosphatase predictors, a correlation matrix based on Spearman's correlation test was first used to explore relationships amongst phosphatase activity and differ-

ent soil properties by using the rcorr function of the Hmisc R package (Harrell and Dupont 2019). This correlation matrix was visualized by using corrplot function with the parameters "hclust" and "ward.D" for hierarchical clustering order included in the corrplot package (Wei and Simko 2021). This analysis was used to identify potential phosphatase drivers and to detect highly correlated variables. Then, to elucidate the most important variables that explained the differences in phosphatase activity by the tillage treatments in the LTAEs, various GLMMs were run as in model 1 but including different soil parameters as covariates. The significance of each explanatory variable was tested using the Anova function of the car package as detailed before. Multicollinearity amongst the variables used in each model was checked using the variance inflation factor (VIF) with the function vif included in the car package. Finally, we used the r.squaredGLMM function included in the MuMIn package to test the adjustment of each model (Bartoń 2024). In addition, the best-fitting model was estimated with the model.sel function based on the Akaike information criterion (AIC) in the same package (Bartoń 2024). When necessary for the analyses mentioned above, data were square-root or log transformed prior to analysis to meet assumptions of normality and homogeneity of variances. Additionally, model residuals were assessed by the testUniformity function of the DHARMa package for R (Hartig 2022). Graphical representations were generated using the ggplot2 package (Wickham 2016). Finally, to achieve a global view of the effect of tillage on the main soil characteristics evaluated in this study—including both phosphatase activities—we constructed a heat map and performed clustering of the different soil parameters assessed under the different tillage treatments using the METABOANALYST web-based metabolomic package (https://www.metaboanalyst.ca/).

Results

Reducing tillage intensity increases the phosphatase activity of agricultural soil

Our results revealed significant effects of both depth and tillage on acid and alkaline phosphatase activities, with a significant interaction between tillage and depth for alkaline phosphatase activity (Table S4). Notably, alkaline phosphatase activity was up to six-fold higher than the acid phosphatase activity, and was more pronounced in samples collected from 0–10 cm depth compared to those from 10–20 cm (Fig. 1). In general, long-term tillage reduction increased both acid and alkaline phosphatase activities. Whilst alkaline phosphatase activity increased under both RT and NT compared to ST, the acid phosphatase activity showed significant increases only under RT. For both enzymes, this effect was mainly restricted to the topsoil (Fig. 1 and model 1 in Table S4).

In an alternative analysis (model 2), where "site" was included as a fixed factor, site had a significant effect on both acid and alkaline phosphatase activities. However, only the effect of depth on phosphatase activity depended on site, with no significant interactions observed between tillage and site, or between tillage, depth and site (Table S4). These results support a consistent effect of tillage on phosphatase activity throughout the LTAEs. Although slight differences in phosphatase activity patterns amongst sites were found when the effects of tillage and depth were explored separately (Figs. 2 and 3), a significant increase in at least one phosphatase activity under reduced tillage (RT or NT alone or both practices) was detected in all the LTAEs, except for the Lithuanian site, which did not show a significant response to the different tillage treatments. It should be noted that the lowest acid and alkaline phosphatase activities in topsoil were recorded in the Spanish soil, whilst soils from France and Sweden showed the highest activity (Fig. S1).

Phosphatase kinetics in European agricultural soil

Since changes in phosphatase activity were primarily observed in the topsoil, we carried out the subsequent kinetic analyses using soil samples collected from a depth of 0–10 cm. As an initial step to explore trends in phosphatase kinetic patterns, we determined Km, Vmax and the catalytic efficiency factor (Vmax/Km) under standard tillage conditions for all the locations.

All the kinetic parameters assessed, except for the Km of acid phosphatases, showed significant differences throughout the LTAEs (Fig. 4 and Table S5). The highest enzyme affinity (indicated by the lowest Km) at each site was associated to soil pH. In this study, soil pH under standard tillage ranged from 5.40 to 7.61 among the different LTAEs (Table 1). In alkaline soils (Spain and the Netherlands), the lowest Km were measured for alkaline phosphatases, whilst in acidic soils such as those in Switzerland, France and Sweden, the Km for alkaline phosphatases was the highest (Fig. S2). The Km of acid and alkaline phosphatases did not differ significantly in the Danish and Lithuanian sites (Fig. S2).

Both the Km and Vmax of alkaline phosphatases showed consistent directional changes across all sites (Fig 4B and 4D), with only slight differences in their catalytic efficiencies throughout the LTAEs (Fig 4F). In contrast, the catalytic efficiency of acid phosphatases varied notably between sites (Fig 4E), reflecting the higher variability in Vmax relative to Km, which did not differ significantly among sites (Fig. 4A and 4C). Amongst all the LTAEs, soil from Sweden exhibited the highest catalytic efficiency for acid phosphatase, whilst the Spanish and the Dutch soils displayed the lowest (Fig. 4E).

Effect of tillage on phosphatase kinetic parameters

The Spanish and Swedish locations were selected for full kinetic characterization under all tillage conditions. These sites represent the strongest contrast in pedoclimatic and edaphic conditions within the LTAEs. The Spanish LTAE site is characterized by alkaline soil, an arid climate, and low organic matter content (Table S1); whereas the site in Sweden features acidic soil, cooler climatic conditions, and higher organic matter content (Table 1 and Table S1). These environmental differences are known to influence enzyme stabilization and soil-enzyme interactions, making both sites suitable for comparing enzyme kinetic responses to tillage (German et al. 2012, Margalef et al. 2021, Spinoni et al. 2021, Francaviglia et al. 2023). Kinetic analyses were carried out using soil samples collected at a depth of 0–10 cm, consistent with previous sections, and included all tillage practices to assess their impacts on both acid and alkaline phosphatase kinetics.

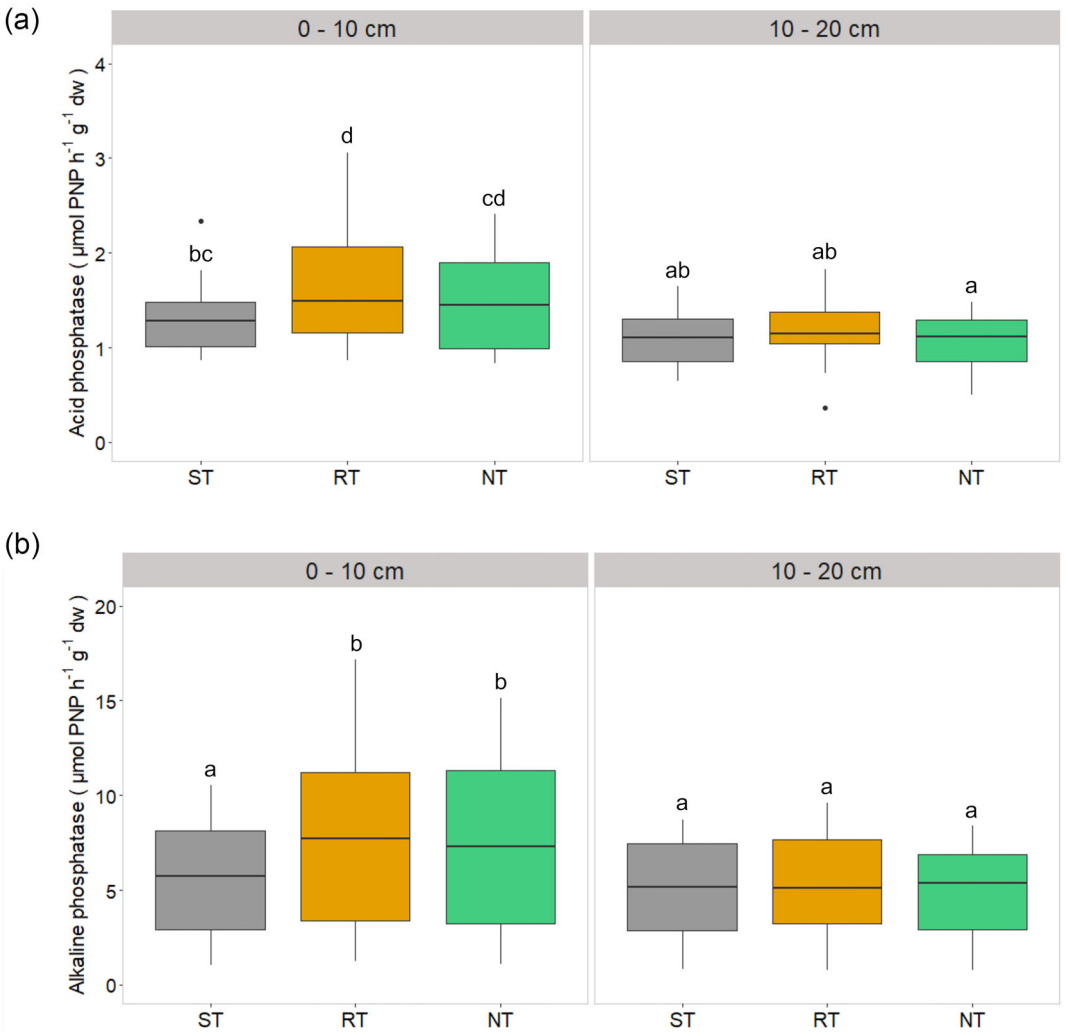


Figure 1 Influence of tillage practices on soil acid (A) and alkaline phosphatase (B) activity throughout various sampling depths. Significant differences between treatments ($P < 0.05$) are denoted by different letters according to the Tukey's test. *P*-values obtained in the GLMM using "tillage" as fixed factor and "site" as a random factor are presented in Table S3. Abbreviations: ST, standard tillage; RT, reduced tillage; and NT, no-tillage.

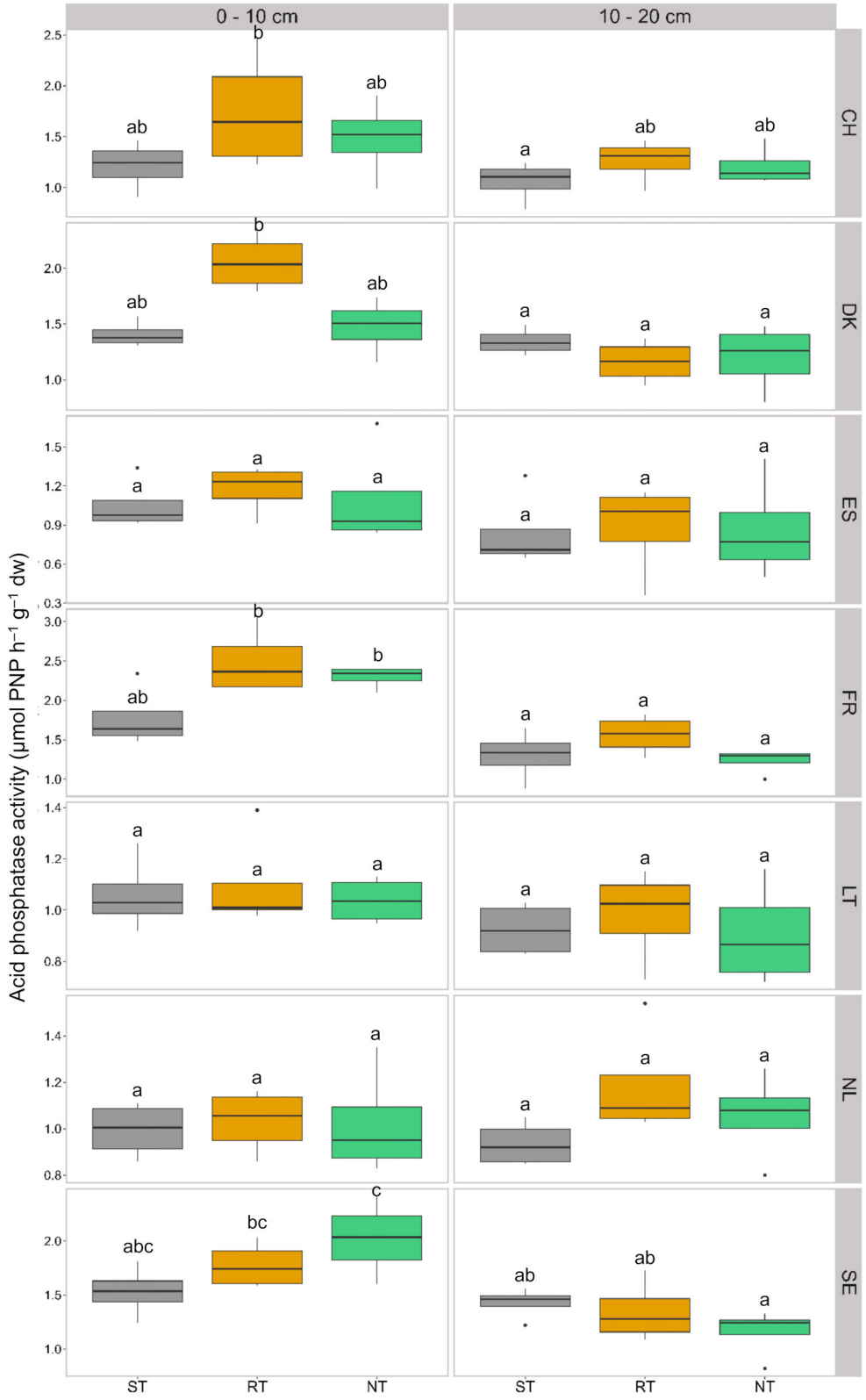
In both locations, NT practices increased catalytic efficiency of phosphatases relative to other tillage treatments, and this response reflected the prevailing soil pH at each site (Table 2). Specifically, in the alkaline soil of the Spanish LTAE, NT enhanced alkaline phosphatase catalytic efficiency, whilst in the acidic soil of the Swedish LTAE, NT enhanced the catalytic efficiency of acid phosphatases. This increase in catalytic efficiency of phosphatases under NT practices was driven primarily by higher Vmax rather than by changes in Km (Table 2; Table S6).

Identifying predictors of phosphatase activity

In order to relate the variability in acid and alkaline phosphatase activities in the different tillage treatments and LTAEs, we examined the influence of key soil parameters, including pH, TP, Po, Pi, TN, Norg, mineral N, TOC, GWC, clay content and microbial biomass (MB). We also analyzed several ratios such as TN/TP, Po/TP, TOC/TP, TN/Po, TOC/Po and TOC/TN, to identify the principal drivers of both enzymatic activities (Table 1 and Table S2).

The Spearman correlation matrix (Fig. 5) revealed a positive correlation between acid and alkaline phosphatase activity ($\rho = 0.80$; $P < 0.001$). Both activities correlated positively with TN, Norg, mineral N, TOC, Po, Po/TP, TOC/TP, MB and GWC, and showed weaker associations ($\rho < 0.50$) with TP, TN/TP, TOC/TN and clay content. Phosphatase activities were negatively correlated with soil pH (Fig. 5 and Table S7), with acid phosphatases displaying a stronger response ($\rho =$ -0.84) than alkaline phosphatases ($\rho =$ -0.67). Neither of the two activities correlated significantly with Pi, while alkaline phosphatase activity was negatively associated with TOC/Po. Soil pH exhibited negative correlations with Po, Po/TP, TOC, TN/TP, TN, MB, GWC and clay content, and positive correlations with Pi, showing trends opposite to those observed for phosphatase activity (Fig. 5 and Table S7).

To elucidate which variables explained differences in phosphatase activity among tillage treatments, we incorporated different soil parameters as covariates in our modeling approach. Strongly correlated variables ($\rho > |0.80|$) were removed to avoid



Switzerland	*P*-value
Tillage	*
Depth	*
Tillage * Depth	ns

Denmark	*P*-value
Tillage	ns
Depth	***
Tillage * Depth	*

Spain	*P*-value
Tillage	ns
Depth	ns
Tillage * Depth	ns

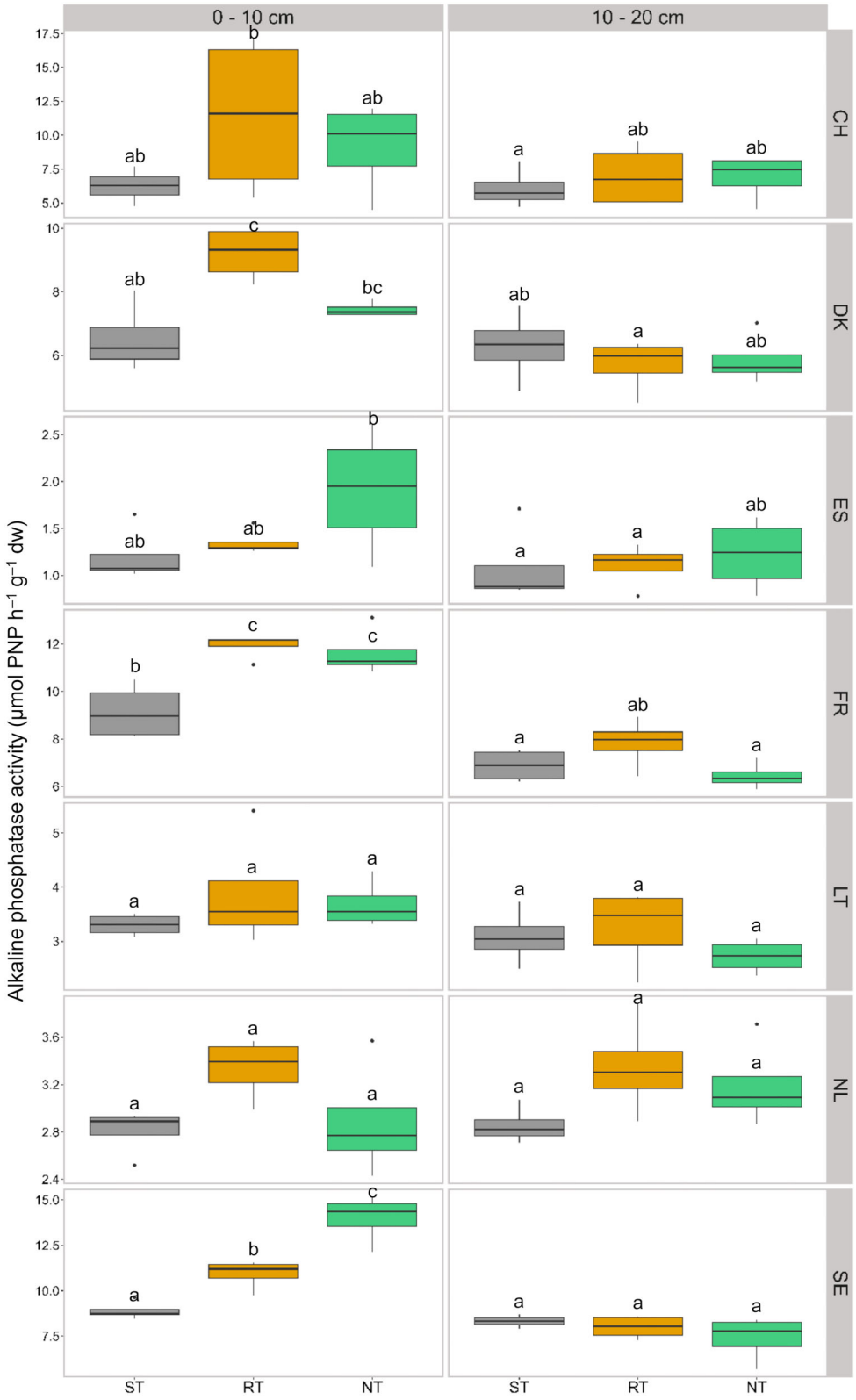
France	*P*-value
Tillage	*
Depth	***
Tillage * Depth	ns

Lithuania	*P*-value
Tillage	ns
Depth	*
Tillage * Depth	ns

Netherlands	*P*-value
Tillage	ns
Depth	ns
Tillage * Depth	ns

Sweden	*P*-value
Tillage	ns
Depth	***
Tillage * Depth	*

Figure 2 Impact of tillage practices on acid phosphatase activity at the different sampling depths within each LTAE. Significant differences between treatments ($P < 0.05$) are denoted with different letters based on the Tukey's test. GLMM analysis using "tillage" and "depth" as fixed factors and "block" as a random was performed. *P*-values obtained for each fixed factor and their interaction are summarized in the table adjacent (right column) to the corresponding LTAE graph, using the following code to denote significance: '.' $P < 0.1$; '*' $P < 0.05$; '**' $P < 0.01$; '***' $P < 0.001$. Abbreviations: ST, standard tillage; RT, reduced tillage; NT, no-tillage; CH, Switzerland; DK, Denmark; ES, Spain; FR, France; LT, Lithuania; NL, Netherlands; SE, Sweden.



Switzerland	*P*-value
Tillage	*
Depth	*
Tillage * Depth	ns

Denmark	*P*-value
Tillage	ns
Depth	***
Tillage * Depth	**

Spain	*P*-value
Tillage	*
Depth	*
Tillage * Depth	ns

France	*P*-value
Tillage	**
Depth	***
Tillage * Depth	*

Lithuania	*P*-value
Tillage	ns
Depth	*
Tillage * Depth	ns

Netherlands	*P*-value
Tillage	*
Depth	ns
Tillage * Depth	ns

Sweden	*P*-value
Tillage	**
Depth	***
Tillage * Depth	***

Figure 3 Impact of tillage practice on alkaline phosphatase activity at the different sampling depths within each LTAE. Significant differences between treatments ($P < 0.05$) are denoted with different letters based on the Tukey's test. GLMM analysis using "tillage" and "depth" as fixed factors and "block" as a random was performed. *P*-values obtained for each fixed factor and their interaction are summarized in the table adjacent (right column) to the corresponding LTAE graph, using the following code to denote significance: '.' $P < 0.1$; '*' $P < 0.05$; '**' $P < 0.01$; '***' $P < 0.001$. Abbreviations: ST, standard tillage; RT, reduced tillage; NT, no-tillage; CH, Switzerland; DK, Denmark; ES, Spain; FR, France; LT, Lithuania; NL, Netherlands; SE, Sweden.

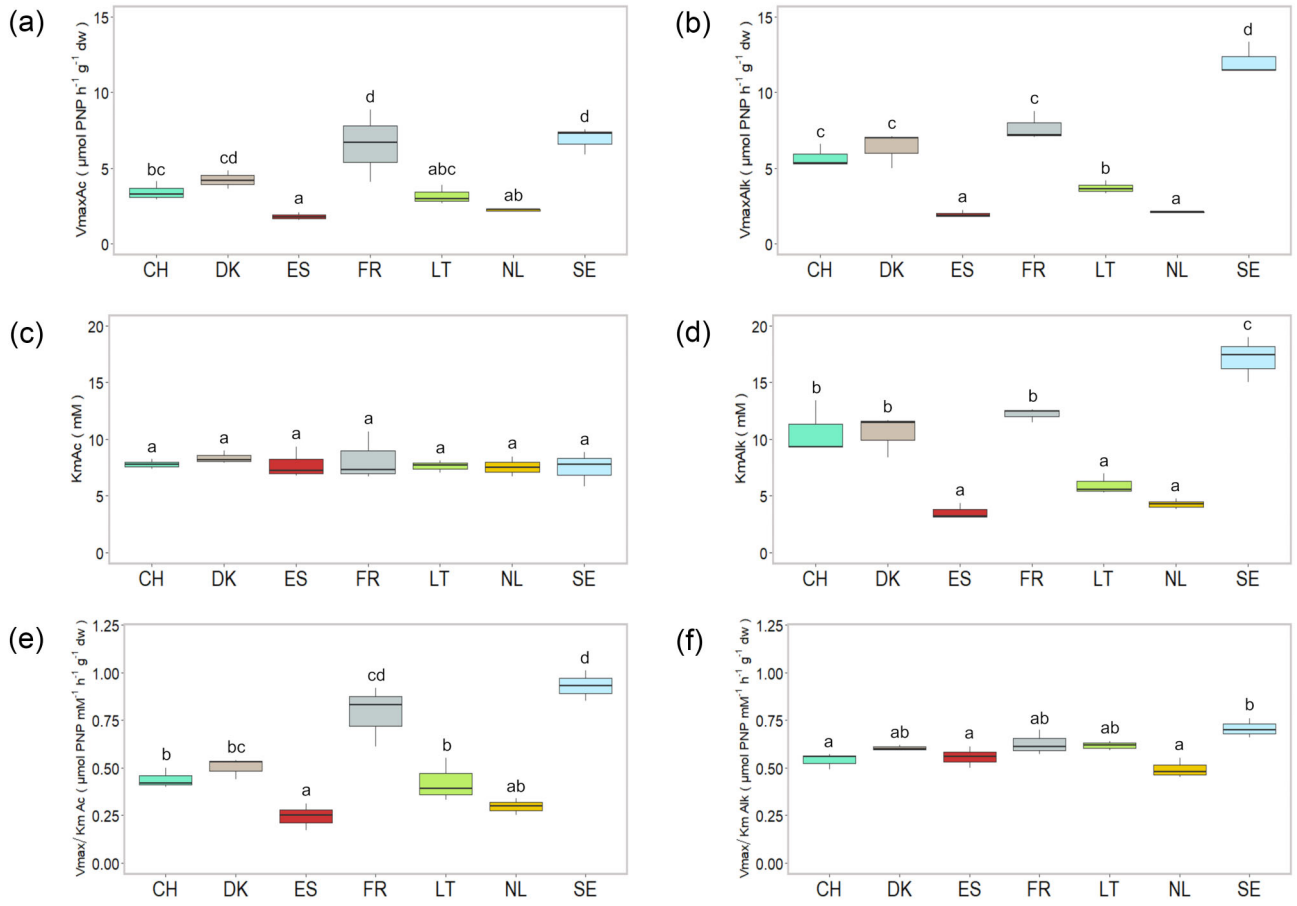


Figure 4 Global trends in soil phosphatase kinetics in agricultural soil. The maximum rate of reaction (Vmax; A-B), the half-saturation Michaelis-Menten constant (Km; C-D) and the catalytic efficiency factor (Vmax/Km; E-F) of soil acid ("Ac") and alkaline ("Alk") phosphatases sampled at a depth of 0–10 cm under standard tillage throughout the LTAEs are presented. Significant differences ($P < 0.05$) between treatments are indicated by different letters based on the Tukey's test. GLMM used "site" as a fixed factor and "block" as a random factor. The results are summarized in Table S4. Abbreviations: CH, Switzerland; DK, Denmark; ES, Spain; FR, France; LT, Lithuania; NL, Netherlands; SE, Sweden.

Table 2 Effect of tillage on phosphatase kinetic parameters.

	V_{max}Ac	V_{max}Alk	K_mAc	K_mAlk	V_{max}/K_mAc	V_{max}/K_mAlk
Spain (ES)						
ST	1.80 ± 0.15 **a**	1.95 ± 0.12 **a**	7.77 ± 0.80 **a**	3.56 ± 0.41 **a**	0.24 ± 0.04 **a**	0.56 ± 0.03 **a**
RT	1.77 ± 0.26 **a**	2.34 ± 0.15 **a**	7.77 ± 0.53 **a**	4.10 ± 0.24 **a**	0.22 ± 0.02 **a**	0.57 ± 0.04 **a**
NT	2.14 ± 0.38 **a**	2.82 ± 0.33 **a**	8.73 ± 0.85 **a**	4.16 ± 0.68 **a**	0.24 ± 0.02 **a**	0.69 ± 0.05**b**
P-value	ns	ns	ns	ns	ns	**
Sweden (SE)						
ST	6.91 ± 0.50 **a**	12.07 ± 0.62 **a**	7.51 ± 0.89 **a**	17.12 ± 1.15 **a**	0.93 ± 0.05 **a**	0.71 ± 0.03 **a**
RT	8.81 ± 0.99 **ab**	13.25 ± 1.03 **a**	9.64 ± 1.85 **a**	14.40 ± 2.52 **a**	0.94 ± 0.07 **a**	0.95 ± 0.10 **a**
NT	9.95 ± 0.89 **b**	14.51 ± 1.43 **a**	8.14 ± 1.14 **a**	13.46 ± 0.85 **a**	1.24 ± 0.07 **b**	1.10 ± 0.17 **a**
P-value	*	ns	ns	ns	*	ns

Notes: Means (± sd) of the maximum reaction rate (Vmax), the half-saturation Michaelis-Menten constant (Km) and the catalytic efficiency factor (Vmax/Km) of soil acid ("Ac") and alkaline ("Alk") phosphatases obtained under different treatments (ST, standard tillage; RT, reduced tillage; NT, no-tillage) in the Spanish (ES) and Swedish (SE) sites. Significant differences between treatments ($P < 0.05$), according to the Tukey's test are denoted by different letters. Additionally, *P*-values obtained in the GLMM using "tillage" as fixed factor and "block" as a random factor for their corresponding LTAE are indicated with the following significance level codes: 'ns' not significant;'*' $P < 0.05$; '**' $P < 0.01$; '***' $P < 0.001$. Detailed statistics are provided in Table S5.

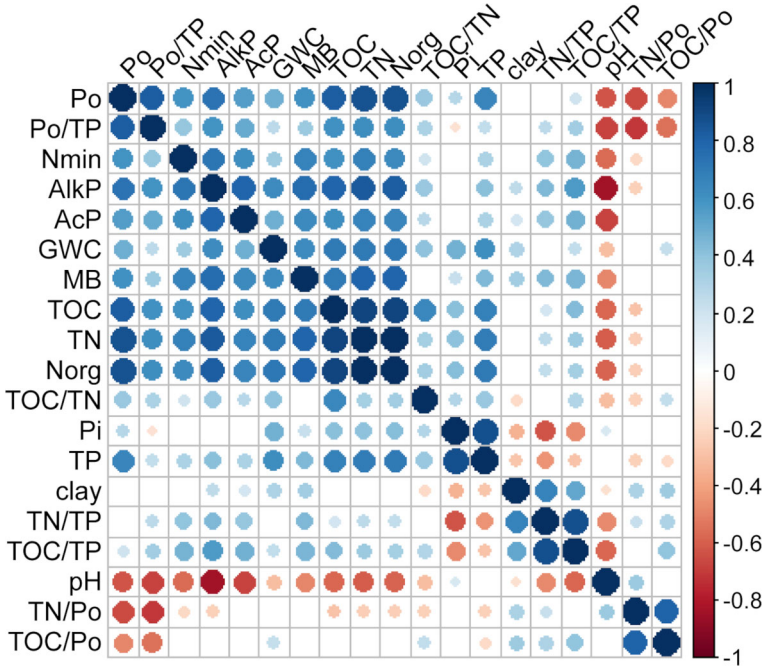


Figure 5 Correlation matrix depicting relationship between soil phosphatase activity and various soil properties for all agricultural fields. Significant correlations ($P < 0.05$) are denoted with circles, with colors indicating positive correlations (blue) and negative correlations (red). Blank spaces indicate non-significant correlations. Circle size and color intensity correspond to the correlation coefficients. Detailed Spearman's correlation coefficients and *P*-values are provided in Table S7. Abbreviations: AcP, acid phosphatase activity; AlkP, alkaline phosphatase activity; GWC, gravimetric water content; TOC, total organic carbon; TN, total nitrogen; Nmin, mineral nitrogen; No, organic nitrogen; TP, total phosphorus; Pi, inorganic phosphorus; Po, organic phosphorus; Clay, clay content; MB, microbial biomass; TN/TP, total nitrogen to total phosphorus ratio; Po/TP, organic phosphorus to total phosphorus ratio; TN/Po, total nitrogen to organic phosphorus ratio; TOC/Po total organic carbon to organic phosphorus ratio; TOC/TN total organic carbon to total nitrogen ratio; TOC/TP total organic carbon to total phosphorus ratio.

multicollinearity (Fig. 5; Table S7), except for TN, Po and TOC, which were retained given their demonstrated relevance for phosphatase activity. These three parameters were independently incorporated in the models, both alone and in combination with other soil parameters. A detailed description of the different model formulations are provided in Table S8. When used as single covariates, TN explained up to 55% of phosphatase activity variance, whilst Po explained up to 27% and TOC up to 34% (marginal R^2 values are given in Table S8). However, single-covariate models showed a high proportion of variance explained by random effects, particularly for alkaline phosphatases. Model explanatory power increased when pH, clay content, GWC and MB were added in combination with TN, Po or TOC (R2 m and R2c values detailed in Table S8). All variables except clay content significantly affected phosphatase activity in at least one model. (Table S8). The best-fitting model, based on the lowest AICc score, was Model 1f, which incorporated TN combined with pH, GWC and MB for both enzyme activities (Table S9).

To gain an overall view of how tillage and depth shaped soil properties, we generated a heat map including the main soil parameters analyzed. In the heatmap (Fig. 6), samples collected at 0–10 cm under NT and RT treatments clustered together, and were clearly separated from the other treatments. The NT and RT samples presented the highest phosphatase activities and the greatest P and N contents, as well as elevated TOC, MB and GWC, but lower pH and clay content. Notably, the highest Po/TP ratio was observed in NT at both 0–10 and 10–20 cm compared to the other treatments (Fig. 6). Despite these predominant soil property patterns, it is important to acknowledge minor deviations in the dataset (Table 1 and Table S2). These deviations do not undermine the analysis but provide useful context for site-specific interpretation. For instance, Swiss soil under RT showed slightly higher TOC and N accumulation at depth than under NT conditions. In Spain and Sweden, only NT resulted in significantly higher TOC compared to ST, with no significant differences between ST and RT (Table S2).

Discussion

Impact of long-term tillage reduction on phosphatase activity

In the LTAEs assays, tillage reduction practices generally increased at least one type of phosphatase activity in the upper 10 cm of soil, with Lithuania as the only exception. This pattern suggests that reducing tillage intensity promotes topsoil phosphatase activity across most European sites, despite large variation in climate and soil properties. The decline in phosphatase activity with soil depth reported here aligns with previous studies (Deng and Tabatabai 1997, Green et al. 2007, Zhang et al. 2014, Akhtar et al. 2018). Likewise, increases in phosphatase activity under no-till (NT) or reduced-till (RT) practices have been documented globally (Deng

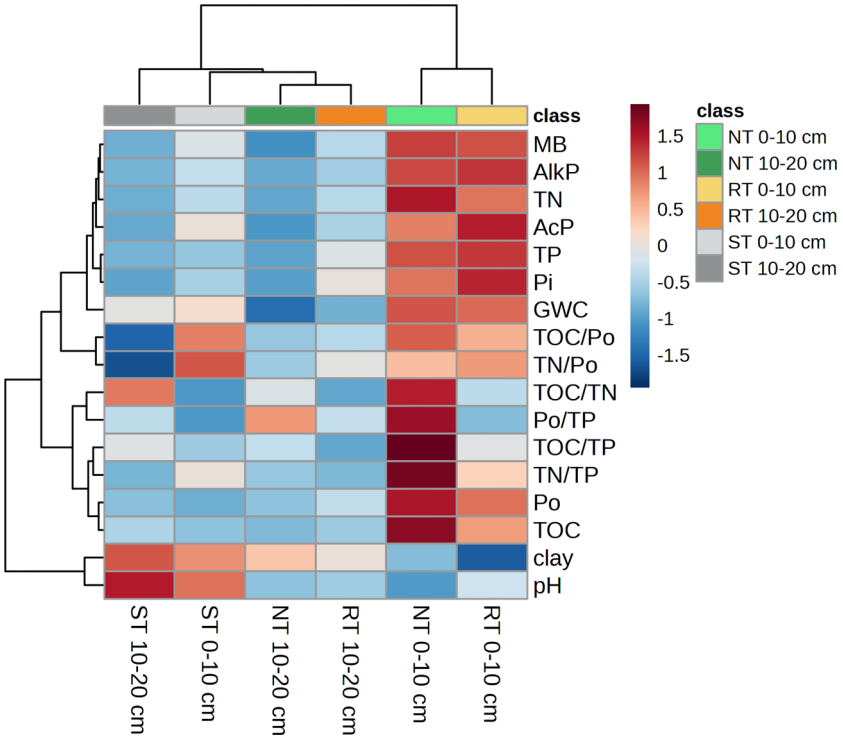


Figure 6 Heat map and clustering analysis revealing predominant soil property patterns in fields subjected to the different tillage treatments at the two sampling depths. Each colored cell of the map is based on the concentration value of the different soil parameters (in rows) in each treatment (in columns). Two distinct groups were identified: soil samples collected under no-tillage (NT) and reduced tillage (RT) at 0–10 cm, whilst other treatments exhibited differential soil property patterns. Abbreviations: ST, standard tillage; RT, reduced tillage; and NT, no-tillage; AcP, acid phosphatase activity; AlkP, alkaline phosphatase activity; GWC, gravimetric soil water content; TOC, total organic carbon; TN, total nitrogen; Pi, inorganic phosphorus, Po, organic phosphorus, TP, total phosphorus; Clay, clay content; MB, microbial biomass; TN/TP, total nitrogen to total phosphorus ratio; Po/TP, organic phosphorus to total phosphorus ratio; TN/Po, total nitrogen to organic phosphorus ratio; TOC/Po total organic carbon to organic phosphorus ratio; TOC/TN total organic carbon to total nitrogen ratio; TOC/TP total organic carbon to total phosphorus ratio.

and Tabatabai 1997, Green et al. 2007, Yang et al. 2016, Akhtar et al. 2018, Wen et al. 2023, Korav et al. 2024). However, contrasting cases, such as the lack of tillage effects under high Pi fertilization, demonstrate that enzyme responses depend not only on tillage but also on nutrient availability (Singh and Kumar 2021, Nughoro et al. 2023). A recent meta-analysis further showed that alkaline and acid phosphatases respond differentially to land management practices (Campdelacreu Rocabruna et al. 2024): acid phosphatase activity increased under organic fertilisation combined with crop rotation or irrigation, whilst alkaline phosphatase activity responded more strongly to organic fertilization coupled with tillage reduction (Campdelacreu Rocabruna et al. 2024). According to these findings, our multi-site results reinforce the importance of assessing both phosphatase types simultaneously in order to capture tillage-driven changes in P cycling.

Patterns of soil phosphatase kinetics in European agricultural soil

In all studied LTAEs, both acid and alkaline phosphatase activities were detected, with unexpectedly high alkaline phosphatase activity even in acidic soils. Although alkaline phosphatases are typically associated with alkaline environments (Nannipieri et al. 2011), recent studies highlight their importance in organic P mineralization even in acidic ecosystems, consistent with the widespread presence of *phoD*–harboring microorganisms (Li et al. 2021b, Bergkemper et al. 2016). Their ecological relevance may have been underestimated, partly because standard alkaline phosphatase assays rely on artificially high pH conditions.

The interplay between soil pH and enzyme activity was further reflected in substrate-affinity patterns. In line with previous studies (Eivazi and Tabatabai 1977, Nannipieri et al. 2011, Margalef et al. 2017), acid phosphatases exhibited the highest substrate affinity in acidic soils, while alkaline phosphatases showed the highest affinity in alkaline soils. These results emphasize the importance of measuring enzymatic activities at pH values close to field conditions; accordingly, we used pH 5.5 for acid phosphatase and pH 8.0 for alkaline phosphatase assays.

Because substrate-affinity reflects both enzyme properties and soil constraints, it also provides insight into microbial strategies under different management conditions (Moscatelli et al. 2012; Marx et al. 2005, Chen and Arai 2023). In addition to intrinsic enzyme affinity, apparent Km values in soils may reflect diffusion constraints, adsorption processes, and substrate accessibility. Lower Km values therefore suggest the prevalence of high-affinity enzyme systems, often associated with k-strategist microbes under nutrient-limited conditions. In our LTAEs, analysis, acid phosphatase Km values showed little variation among sites, while alkaline phosphatase Km differed substantially, and

was lowest (highest affinity) in alkaline soils (Fig. 4). This pattern may reflect microbial adaptation to Pi-limited in alkaline soils.

Interestingly, whilst the catalytic efficiency of alkaline phosphatases was relatively stable across all the LTAEs, the catalytic efficiency of acid phosphatases varied markedly among sites. This variability primarily arises from changes in Vmax rather than Km. These contrasting patterns may reflect differences in isoenzymes composition or soil-enzyme interactions rather than clear long-term evolutionary strategies (Wang et al. 2020).

Response of phosphatase kinetics to long-term tillage reduction

Enzyme kinetic parameters provide system-level indicators of soil biochemical functioning, but they should not be interpreted as intrinsic biochemical constants, as they arise from heterogeneous pools of intra- and extracellular enzymes interacting with soil colloids (Moscatelli et al., 2012; Marx et al. 2005, Nannipieri et al. 2011, Khadem and Raiesi 2019, Paul et al. 2022, Chen and Arai 2023). In this study, we found that the catalytic efficiency (Vmax/Km) of soil phosphatases increased under NT, suggesting that it is a more sensitive indicator of soil management effects than either Km or Vmax alone.

Because Km represents the substrate concentration at half-maximal velocity, it is often used as an ecological proxy for enzyme affinity (German et al. 2012). Under NT conditions, vertical stratification can develop, leading to Pi accumulation in the surface layer with deeper soil layers becaming Pi-limited (Zuo et al. 2018, Nunes et al. 2020, Barker et al. 2025). In contrast, ST practices which periodically redistributes Pi, may favour opportunistic r-strategist microorganisms. Accordingly, contrasting Km responses between NT and ST could be expected, reflecting shifts in the dominance of k- and r-strategists among microbial groups (Srour et al. 2020) However, we observed no consistent tillage effects on Km, likely because, in soils, Km is not a pure measure of intrinsic enzyme affinity and is strongly influenced by enzyme–soil interactions. Our results suggest that the increased catalytic efficiency under NT was mostly driven by changes in Vmax rather than Km. Interpreting Vmax of soil phosphatases is complex, as it integrates contributions from both intra- and extracellular enzymes (Kelleher et al. 2004, Kedi et al. 2013). Thus, higher Vmax values under NT cannot be attributed solely to enhanced microbial enzyme production; they may also result from shifts in phosphatase isoenzymes, changes in sorption dynamics, or increased stabilization of extracellular enzymes. Our dataset cannot distinguish among these possibilities.

We found that NT effects aligned with soil pH: NT increased the catalytic efficiency of alkaline phosphatase in the Spanish alkaline soil, and that of acid phosphatase in the Swedish acidic soil. This supports the hypothesis that soil physicochemical properties, rather than microbial community composition alone, shape enzyme responses. Moreover, intensive tillage practices can disrupt soil structure and soil food-web interactions, with cascading effects on phosphatase production (López-Fando and Bello 1995, Chan 2001, Neal et al. 2017, Wang et al. 2017; Li et al., 2021a; Gao et al. 2022, Madejón et al. 2023). Given the limited functional resolution of PLFA analyses, additional approaches such as metagenomics, *phoD/phoC* qPCR or transcriptomics would be required to attribute the observed kinetic changes to specific microbial guilds (Neal et al. 2017, Zhu et al. 2021, Shi et al. 2023).

Encompassing phosphatase predictors and expected changes under global change

Correlations between acid and alkaline phosphatase activities in LTAEs indicate that shared environmental drivers influence their activities. Negative correlations with soil pH and positive associations with TOC, Po and TN are consistent with the role of organic matter and nutrient availability in stimulating enzyme production (Zhang et al. 2014, Margalef et al. 2017, Luo et al. 2019). N-availability, in particular, has been identified as primary driver of phosphatase activity, as increased N inputs enhance microbial enzyme synthesis (Margalef *et al*., 2017; Olander and Vitousek 2000, Marklein and Houlton 2012, Zhang et al. 2018). Organic matter further promotes phosphatase activity both by supporting microbial growth and by stabilizing extracellular enzymes (Gianfreda et al. 2005, Rosas et al. 2008). In fact, a meta-analysis by Luo et al. (2019) reported that long-term organic matter inputs increased both acid and alkaline phosphatase activities, and concomitantly increased the abundance of *phoC phoD* genes. The widespread global abundance of *phoD* gene, even in acidic soils (Li et al. 2021b, Ragot et al. 2015), highlights the central role of organic matter in sustaining microbial biomass and phosphatase activity (Li et al. 2021b, Hu et al. 2018, Luo et al. 2019, Janes-Bassett et al. 2022).

Although phosphatase gene expression can be repressed by Pi availability (Park et al. 2022), we observed no inhibitory effect, likely due to enzyme stabilization, constitutive expression or rapid turnover (Rodríguez and Fraga 1999). Similarly, inhibition of phosphatase activity is rarely observed in systems with organic fertilization or managed under NT practices (Kremer and Li 2003, Garg and Bahl 2008, Luo et al. 2019, Janes-Bassett et al. 2022, Campdelacreu Rocabruna et al. 2024). Marklein and Houlton (2012) further showed that NP fertilization impacts depend on the relative proportions of N and P added to the soil, with biomes-specific thresholds. Apparent inconsistencies in the literature likely arise from differences in Po *vs* Pi pools, highlighting the importance to consider complete P pools when interpreting phosphatase responses (Romanyà et al. 2017, Hu et al. 2018, Zheng et al. 2021). Altogether, these findings indicate that P cycling in agroecosystems is regulated by interacting C, N, and P pools rather than a single dominant driver.

Additionally, tillage reduction also enhances soil moisture retention, a key factor influencing enzyme activity (Sardans et al. 2008, Margalef et al. 2017, 2021, de-Bashan et al. 2021, Campdelacreu Rocabruna et al. 2024). GWC was positively correlated with both phosphatase activities and was retained in all models assessing tillage effects. This highlights the central role of soil moisture in sustaining P cycling, especially in water-limited regions such as the Mediterranean basin (Sardans et al. 2008, Sun et al. 2020; de-Bashan et al., 2021). Under ongoing global climate change, characterized by rising temperatures, increased evapotranspiration, and a higher risk of more frequent and intense droughts, along with expansion of arid regions (Spinoni et al. 2021) soil moisture is likely to become even more critical. In fact, management practices that enhance water retention,

such as RT and NT, may buffer ecosystems against climate-driven changes.

Finally, the positive correlation between clay content and both phosphatase activities suggests that soil colloids stabilize extracellular enzymes, protecting them from proteolysis and abiotic degradation, and thereby extending their lifetime (Nannipieri et al. 2011, Chen and Arai 2023). However, clay content did not emerge as a significant predictor in mixed models, likely due to interactions among mineralogy, enzymatic adsorption, and substrate availability. These findings illustrate the complex and site-specific controls governing soil phosphatase activities across LTAEs.

Conclusions

Our multi-site analysis demonstrates that long-term tillage reduction consistently enhances phosphatase activity in the upper soil layers across diverse European agrosystems. These increases have important implications for improving organic phosphorous mineralization and, consequently, P-use efficiency. Although shifts in phosphatase activity may reflect changes in microbial functional groups, the absence of direct molecular data prevents precise attribution, highlighting the need for complementary microbial analyses. Nevertheless, the observed enzymatic responses underscore that by enhancing organic matter retention, improving moisture dynamics, and influencing nutrient stoichiometry, minimizing tillage can support more resilient agroecosystems and improved long-term fertilization efficiency. Furthermore, our findings provide robust evidence that reduced tillage creates soil conditions favorable for sustained phosphatase activity and active P turnover. Our results further underscore the importance of evaluating both alkaline and acid phosphatase activities when assessing P cycling in agricultural soils.

Interpretation of kinetic parameters requires caution, because Vmax and Km in soils reflect emergent properties of heterogeneous enzyme pools and soil-enzyme interactions rather than intrinsic biochemical constants. Although catalytic efficiency increased under NT at the two sites examined, the underliyng mechanisms cannot be resolved with the present dataset. Further studies incorporating metagenomics, functional gene quantification, and direct measurements of P losses (i.e. via runoff or leaching) will be required to disentangle microbial contributions, and enzyme stabilization processes. Such efforts will help fine-tune management practices that enhance nutrient-use efficiency, while supporting productive and sustainable agriculture in a changing climate.

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Author contributions

Tamara Gómez-Gallego (Conceptualization [equal], Formal Analysis [equal], Investigation [equal], Methodology [equal], Writing—original draft [equal], Writing—review & editing [equal]), Mart B. H. Ros (Investigation [equal], Methodology [equal], Resources [equal], Writing—review & editing [equal]), Sara Sánchez-Moreno (Investigation [equal], Methodology [equal], Resources [equal], Writing—review & editing [equal]), Dalia Feiziene (Methodology [equal], Resources [equal], Writing—review & editing [equal]), Gerard L. Velthof (Methodology [equal], Resources [equal], Writing—review & editing [equal]), Søren O. Petersen (Methodology [equal], Resources [equal], Writing—review & editing [equal]), Lars Munkholm (Methodology [equal], Resources [equal], Writing—review & editing [equal]), Laurent Philippot (Methodology [equal], Resources [equal], Writing—review & editing [equal]), Luca Bragazza (Methodology [equal], Resources [equal], Writing—review & editing [equal]), Marcel van der Heijden (Methodology [equal], Resources [equal], Writing—review & editing [equal]), Sara Hallin (Investigation [equal], Methodology [equal], Resources [equal], Writing—review & editing [equal]), Marta Goberna Rosa (Conceptualization [equal], Funding acquisition [equal], Investigation [equal], Methodology [equal], Project administration [equal], Supervision [equal], Writing—original draft [equal], Writing—review & editing [equal]), and Juan Luis Ramos (Conceptualization [lead], Formal Analysis [equal], Investigation [equal], Project administration [equal], Resources [equal]), Supervision [equal], Writing—original draft [equal], Writing—review & editing [equal]

Supplementary material

Supplementary material is available at *Sustainable Microbiology Journal* online.

Conflicts of interest

None declared.

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Data availability

The data underlying this article are available in the article and in its online supplementary material.

Appendix A. Supplementary data

Supplementary data to this article can be found online.

Figure S1. Patterns of phosphatase activity in the top layer of agricultural soils;

Figure S2. Comparison of Km of acid and alkaline phosphatases in each LTAE;

Table S1: Metadata corresponding to description of sampled sites and characteristics of each LTAE including detailed treatments (Sánchez-Moreno et al. 2024 in Zenodo repository);

Table S2: Detailed soil properties of the sampled sites subjected to the different tillage treatments at the two sampling depths (extended version of Table 1);

Table S3: Detailed P-values of the GLMMs performed for the different soil parameters, corresponding to data in Table S2.

Table S4: GLMM summary tables corresponding to data shown in Figure 1;

Table S5: GLMM results corresponding to data shown in Figure 4;

Table S6: GLMM summary tables corresponding to data shown in Table 2;

Table S7: Correlation coefficients (rho values) between phosphatase activity and different soil traits used to feed the correlation matrix shown in Figure 5;

Table S8: GLMM summary tables of main treatment effects and interactions on phosphatase activity including different soil parameters as a covariate;

Table S9: Model selection table based on AICc scores of the different models shown in Table S8 used to predict acid (AcP) and alkaline phosphatase activity (AlkP).

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