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Changes in Soil Phosphatase Activities across a Liming Gradient Under Diverse Long-Term Managements in Subhumid Kenya

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Abstract

Changes in biological drivers of soil P cycling following lime application could contribute to improvements in P availability commonly observed in weathered soils after liming. The effect of liming on soil P cycling was evaluated for soils (Typic Kandiudox) in western Kenya under three long-term managements: no fertilization (UNF), mineral N + P (MIN), and manure (ORG). Ca(OH)₂ was applied at six rates (equivalent to 0 – 7.5 t CaCO₃ ha⁻¹) in soil mesocosms to establish a pH gradient from 4.7 to 6.4. After 27 days, labile inorganic P (Pᵢ) fractions increased by up to 1.2 µg g⁻¹ in response to lime application. Labile organic P (Pₒ) was weakly and inconsistently affected by liming. In MIN, microbial biomass P (Pₘᵦ) decreased at ≥ 6.0 t ha⁻¹ (-24%). Despite similar phosphatase activities in unlimed soils, liming changed
activities depending on management as well as phosphatase type, though changes in activities
did not necessarily reflect commonly proposed pH optima of phosphatases. In UNF and MIN,
acid phosphomonoesterase activity decreased linearly with pH by up to -37% and -29%,
respectively, and activities of alkaline phosphomonoesterase and phosphodiesterase showed
minor or no changes. In contrast, liming in ORG altered activities by up to +16% for acid
phosphomonoesterase, -16% for alkaline phosphomonoesterase, and +36% for
phosphodiesterase. In some cases, similar trends were observed for activities normalized to $P_{\text{mic}}$,
including intensified decreases for acid phosphomonoesterase in UNF (-54%) and increases for
phosphodiesterase in ORG (+68%) with liming. In contrast to phosphatase activities expressed
on a soil basis, when normalized to $P_{\text{mic}}$ the activity of acid phosphomonoesterase in ORG was
unaffected by lime additions and the activity of phosphodiesterase exhibited a marked decreased
in UNF by up to -36%. Across all managements the ratio of acid phosphomonoesterase to
phosphodiesterase activity peaked at pH 5.0 (1.5 t lime ha$^{-1}$) and decreased thereafter. Despite
strong management-induced differences in soil P availability, consistent changes in the ratios of
phosphatase activities indicate a short-term impact of lime on the enzymatic component of P
cycling independent of management, which if persistent could translate to changes in $P_{\text{o}}$
mineralization and available P in the longer-term.

1. Introduction

Lime is commonly applied to increase soil pH and, thus, the availability of native and added
phosphorus (P). The increase in available inorganic P ($P_i$) following liming has been attributed to
abiotic processes driven by pH elevation, such as desorption of mineral-bound P and lowered P
sorption potential (Haynes, 1982, Sánchez and Salinas, 1981). However, it is not clear how
liming impacts soil P availability via biological P cycling. The sudden pH increase following a
liming event could exert short-term effects on P cycling because microbial mineralization of soil
organic matter is sensitive to changes in soil pH (Kemmitt, et al., 2006, Robson and Abbot, 2012,
Rousk, et al., 2010, Rousk, et al., 2009) and because the activities of P-cycling enzymes in soils
(i.e., phosphatases) are pH-sensitive (Nannipieri, et al., 2011, Turner, 2010). Though decreases
in total organic P (P_o) following liming have been proposed to reflect mineralization of labile P_o
(e.g., Condron and Goh, 1990, Condron, et al., 1993, Halstead, et al., 1963), the biochemical
drivers (phosphatases) and biological sinks (microbial biomass) of P_o mineralization have yet to
be examined in conjunction. Given that soil phosphatases catalyze mineralization of P_o and that
their activity is pH-sensitive, coupling measures of phosphatase activities and P_mic offers a
comprehensive evaluation of biological P cycling response to liming events, because changes in
pH-sensitive phosphatase activities can influence the amount of P_o mineralized to P_i that is
available for immobilization in P_mic.

Microbial biomass plays a key role in P-fixing weathered soils because it is both a pool
and driver of P cycling (Oberson, et al., 2006, Richardson and Simpson, 2011). In such soils,
microbial biomass is able to rapidly respond to changes in P availability, such as P_i pulses
(potentially <7 h) microbial immobilization of soil solution P_i into microbial biomass P (P_mic)
(Achat, et al., 2009) avoids its fixation (Oehl, et al., 2001), and turnover of microbial biomass
enables this P to become transiently available for plant uptake, or re-uptake by microbes (Achat,
et al., 2010, Oberson and Joner, 2005). Liming could foment greater P_mic by increasing the
amount of P available for microbial uptake (Gachengo, et al., 1998), and by increasing soil pH to
values favorable for greater microbial activity (Kemmitt, et al., 2006, Robson and Abbot, 2012,
Rousk, et al., 2010, Rousk, et al., 2009). This could explain elevated pulses of soil respiration following liming (Haynes and Swift, 1988) and increases in microbial biomass carbon across lime-induced pH gradients in multi-year field experiments (Acosta-Martínez and Tabatabai, 2000, Ekenler and Tabatabai, 2003), though the response of $P_{mic}$ in such studies was not measured.

Independent of its effect on the soil microbial community, it is conceivable that liming alters phosphatase activity directly by increasing soil pH (Turner and Blackwell, 2013). Since different phosphatases have distinct pH optima, liming effects may be specific to the type of phosphatase. For example, the acidic pH optimum (pH 5 – 6) of acid phosphomonoesterase and alkaline optimum of phosphodiesterase (pH 8) (Eivazi and Tabatabai, 1977, Hui, et al., 2013) means that liming will likely entail decreases in acid phosphomonoesterase activity while increasing alkaline phosphomonoesterase and phosphodiesterase activity. Given that phosphodiesterase is the first and likely rate-limiting step in $P_{o}$ mineralization (Turner and Haygarth, 2005), shifts in the relative activities of different phosphatases (i.e., activity ratios) from liming could impact $P_{o}$ mineralization (Dick, et al., 2000).

Changes in soil pH following a liming event are relatively rapid compared to the multiseason time scales at which field studies have identified changes in soil phosphatases (Acosta-Martínez and Tabatabai, 2000, Ekenler and Tabatabai, 2003). Given the pH-sensitivity of soil phosphatases, it is conceivable that enzyme activities respond rapidly in the post-liming window. While acid phosphomonoesterase activity has been found to respond within several days of liming (e.g., Haynes and Swift, 1988), the response of other phosphatases with alkaline pH optima (alkaline phosphomonoesterase, phosphodiesterase) is not known.
Soil management is likely to condition soil phosphatase response to liming because practices such as fertilization are known to influence soil enzyme activities (Bending, et al., 2004, Bowles, et al., 2014, Nannipieri, et al., 2012). For example, additions of manure or inorganic P could influence pre-lime phosphatase activities by altering the amount of enzyme substrate (i.e., $P_0$) and/or phosphatase production (Acosta-Martínez and Waldrip, 2014). The inverse relationship of phosphatase activity and P availability observed in weathered soils (Olander and Vitousek, 2000) suggests that in conditions of high available P (e.g., P fertilization) alteration of phosphatase activities by liming may have a relatively lesser impact than changes in abiotic controls (e.g., P-fixation) on P availability. Conversely, under conditions of soil P scarcity, in which a greater proportion of available P is thought to be derived from phosphatase mineralization of $P_0$ (Oberson, et al., 1999, Oberson, et al., 2011), changes to phosphatase activities by liming could have a substantial impact on P availability.

To address these knowledge gaps, we evaluated the short-term (<1 month) post-liming response of enzymatic and microbial components of P cycling. To test potential effects of management, we selected soils from fertilization treatments of zero input, low input (manure), and high input (mineral fertilizer) from a long-term field trial (11 years) in western Kenya. Across liming gradients established in soil mesocosms, we hypothesized (1) improved P availability (decreased P sorption, increased labile $P_{i}$); (2) increased $P_{mic}$ with soil pH elevation; (3) changes in activities of acid phosphomonoesterase, alkaline phosphomonoesterase, phosphodiesterase reflective of phosphatase-specific pH optima; and (4) a significant effect of management history on $P_{mic}$ and phosphatase activity response to lime.

2. Materials and Methods
2.1. Soil management and sampling

Soils from a long-term integrated soil fertility management (ISFM) trial in western Kenya were used to test the hypothesized effect of management history on biological P cycling response to liming. The trial was established in 2003 near Sindindi in Siaya County, Kenya (34°24'13.7"E, 00°08'38.3"N) at an elevation of 1330 m above sea level. The region experiences a mean annual temperature of 22.5 °C and a historical mean annual precipitation of 1780 mm distributed over two rainy seasons: a short rain (September – November) and a long rain (March – June) (Sommer, et al., 2018). The soil is classified as a Typic Kandiudox (USDA) or Haplic Ferralsol (WRB), and expresses a clay texture (555 g clay kg\(^{-1}\), 183 g silt kg\(^{-1}\), 261 g sand kg\(^{-1}\)) at 0-15 cm (Jelinski, unpublished).

Three soil fertility managements were selected to evaluate liming effects on soil P cycling: (1) an unfertilized control (0 kg N, P ha\(^{-1}\) season\(^{-1}\); UNF); (2) mineral N (60 kg ha\(^{-1}\) season\(^{-1}\) as urea) and P (60 kg ha\(^{-1}\) season\(^{-1}\) as triple super phosphate; MIN); (3) and bovine manure (4 t ha\(^{-1}\) season\(^{-1}\)) sourced from surrounding homesteads (ORG). Inputs were applied twice per year, for the short and long rainy season. Manure sampled in 2014 had 0.69% N and 0.29% P, corresponding to inputs of 2.8 kg N and 1.1 kg P ha\(^{-1}\) season\(^{-1}\). Such N and P contents are common for manure produced on smallholder homesteads in western Kenya (Sommer, et al., 2018, Waithaka, et al., 2007), and likely results from local manure harvest and storage practices such as inadvertent mixing of manure with soil scraped from the farmyard surface during collection (Lekasi, et al., 2003) and exposed storage of manure (Tittonell, et al., 2010).

These 3 selected treatments represent fertility management scenarios of zero input (UNF) and low input (ORG) that are prevalent in western Kenya due to resource limitation (Tittonell and Giller, 2013, Tittonell, et al., 2013, Tittonell, et al., 2007) whereas the high input treatment...
(MIN) is based on regionally recommended N and P rates (KARI, 1994, Kihara and Njoroge, 2013). Treatment plots (4.5 × 6 m) randomized in a complete block design (Sommer, et al., 2018) were cropped to maize (*Zea mays*) in the long rains and to tephrosia (*Tephrosia candida*) in the short rains. Tephrosia biomass was incorporated by hand tillage into the soil as a green manure. Tillage and weeding was performed by hand hoe as necessary according to local practices. At the time of sampling, soils (0-15 cm depth) from the three treatments have similar soil pH and exchangeable acidity, and comparable SOC (Supplementary Table 1).

In March 2014 (11 years or 21 cropping seasons), soils were sampled at the end of the dry season by auger at 0-15 cm depth as a plot composite (n = 3) for each of three field replicate plots, for each of the three soil fertility management treatments (UNF, MIN, ORG). Soils were air-dried and gently broken by hand to pass a 2 mm sieve and used to establish liming mesocosms.

2.2. Determination of liming requirement

Exchangeable acidity was determined using the Mehlich buffer method (Mehlich, et al., 1976) modified to replace barium chloride with calcium chloride (Hoskins and Erich, 2008). Briefly, 10 g oven-dry equivalent soil was mixed with 10 mL of distilled water for 2 min using a magnetic stir bar in a 50 mL beaker, then allowed to stand for 1 h. The mixture was re-stirred and 10 mL of modified Mehlich buffer (pH 6.64) was added. The resulting solution was stirred for 2 min, then allowed to stand for 30 min, at which point the pH of the buffer-soil mixture (pH_B) was measured (Eq. 1). Triplicate measurements were performed for each soil sample. Exchangeable acidity was calculated as follows:

**Equation 1**

\[
\text{Exchangeable acidity (m_eq 100 g}^{-1}) = \frac{(6.64-pH_B)}{0.25} \times \text{soil mass}
\]
The liming requirement (LR) was calculated as the calcium carbonate equivalent (CCE) of calcium hydroxide Ca(OH)$_2$ necessary to neutralize exchangeable acidity assuming 135% CCE of Ca(OH)$_2$ (Havlin, et al., 2013).

2.3. Soil mesocosms and lime treatments

Six lime rates were applied to soil mesocosms using Ca(OH)$_2$: 0 - 2.5 × LR at 0.5 LR intervals. Since soils under the three management histories had highly similar pH and exchangeable acidity, this corresponded to similar rates of 0, 20.3, 40.6, 60.9, 81.2, and 101.5 mg Ca(OH)$_2$ g$^{-1}$ soil for managements. Based on a mean bulk density of 1.15 g cm$^{-3}$ at 0-15 cm for sampled plots and a depth of incorporation of 15 cm using hand hoe (Paul, et al., 2013), this corresponds to an application rate of 0, 1.5, 3.0, 4.5, 6.0, and 7.5 t CaCO$_3$ ha$^{-1}$.

Triplicate soil mesocosms were used for each lime rate, for each of the three management histories. Soil mesocosms were constructed by placing 30 g (oven-dry basis) of < 2 mm sieved soil into an acid-washed 473 mL glass Mason jar. Soils were pre-incubated at 70% of water-filled pore space (WFPS) for 5 days before applying lime treatments. Ca(OH)$_2$ was added as a dry powder (< 200 µm) and thoroughly incorporated with moist soil by mixing with an acid-washed glass stir rod for 1 min. Soil in the unlimed controls (no Ca(OH)$_2$) was similarly ‘mixed’.

Mesocosms were incubated at 22.5 °C for 27 days post-liming, and harvested at the end of day 27. All further analyses were performed on freshly harvested soils.

2.4. Soil pH and labile P fractions

Soil pH was measured in triplicate in deionized water (1:5) following 30 min of equilibration by horizontal shaking (120 rpm). Labile P$_i$ and P$_o$ fractions were measured using a modified sequential extraction based on Hedley, et al. (1982). Soil from each mesocosm (lime treatment replicate) was analyzed in duplicate. Soils were first extracted by carbonate-loaded
anion-exchange membrane (AEM; 1 × 4 cm, VWR International, West Chester, PA) in deionized water by shaking for 18 h (Dieter, et al., 2010). P$_i$ was desorbed from the membranes by shaking for 1 h in 0.25 mol L$^{-1}$ H$_2$SO$_4$ and analyzed by molybdate colorimetry (Murphy and Riley, 1962). Soils were then extracted with 0.5 mol L$^{-1}$ NaHCO$_3$ (pH 8.5) by shaking for 18 h. Extractions were centrifuged (8000 RCF, 15 min), and an aliquot was analyzed by molybdate colorimetry for P$_i$ and for total P (P$_t$) following acid-persulfate digestion (80 °C, 16 h) (Rowland and Haygarth, 1997). P$_o$ was estimated as the difference between P$_t$ and P$_i$. The AEM-extractable P$_i$ and NaHCO$_3$-extractable P$_i$ were considered labile P$_i$ fractions, and the NaHCO$_3$-extractable P$_o$ was considered a labile P$_o$ fraction (Cross and Schlesinger, 1995, Negassa and Leinweber, 2009).

2.5. P sorption and P$_{mic}$

Sequential fumigation-extraction with chloroform gas according to Brookes, et al. (1982) was used to determine microbial biomass P (P$_{mic}$) using fresh soils 27-days post-liming. For each soil mesocosm, three types of subsamples were processed, in duplicate: fumigated, non-fumigated and P-spiked. Fumigated samples (2 g) were treated with chloroform gas for 18 h followed by extraction with 40 mL of 0.5 mol L$^{-1}$ NaHCO$_3$ (pH 8.5, 1 h). Centrifugation (8,000 × g, 15 min) was used to obtain a clear supernatant, an aliquot of which was used to determine P$_i$ by molybdate colorimetry (Brookes, et al., 1982, Murphy and Riley, 1962). Non-fumigated and P-spiked subsamples were processed in the same way as fumigated subsamples, but without chloroform fumigation. To avoid under-estimation of P$_{mic}$, a P spike (75 µg P g$^{-1}$ soil) was used to estimate P recovery in fumigated samples (Brookes, et al., 1982, Joergensen, et al., 1995, Morel, et al., 1996, Oberson, et al., 1997). P$_{mic}$ was calculated as the difference between fumigated and non-fumigated extractable P [Eq. 2] (Brookes, et al., 1982).

**Equation 2**  
Microbial biomass P ($P_{mic}$) = \( \frac{\text{fumigated P} - \text{nonfumigated P}}{\text{P spike recovery}} \)
The recovery of the $P_i$ spike was used as an indicator of $P$ sorption (i.e., greater % recovery = lower $P$ sorption potential) (Sims, 2000). Also interpretable as a single-point sorption, this method has been used to estimate $P$-fixation potential in weathered soils (Fox and Kamprath, 1970, Henry and Smith, 2003, Sims, 2000).

2.6. Phosphatase activities

Activities of acid phosphomonoesterase (Enzyme Commission 3.1.3.2), alkaline phosphomonoesterase (EC 3.1.3.1), and phosphodiesterase (EC 3.1.4.1) were assayed as described by Tabatabai (1994). Assays were performed in duplicate using 1 g of air-dried soil incubated for 1 h (37 °C) in 5 mL of modified universal buffer (MUB) at pH 6.5 for acid phosphomonoesterase and pH 11.0 for alkaline phosphomonoesterase, and in 5 mL of 0.05 mol L$^{-1}$ Tris (2-amino-2-(hydroxymethyl)-1,3-propanediol) buffer at pH 8.0 for phosphodiesterase. Assays used a final substrate concentration of 0.01 mol L$^{-1}$ per g soil of para-nitrophenyl phosphate (acid and alkaline phosphomonoesterase) or bis-para-nitrophenyl phosphate (phosphodiesterase). Assays were halted by the addition of 4 mL of 0.5 mol L$^{-1}$ NaOH to acid phosphomonoesterase and alkaline phosphomonoesterase assays or 4 mL of 0.1 mol L$^{-1}$ Tris (pH 12.0) to phosphodiesterase assays, and 1 mL of 0.5 mol L$^{-1}$ CaCl$_2$. Centrifugation (2,113 × g, 5 min) was used to remove sediment and para-nitrophenol ($p$NP) in the clear supernatant was quantified colorimetrically (410 nm). Mean absorbance of triplicate negative controls (no soil + substrate) was subtracted from the absorbance of soil assays. Phosphatase activities were expressed in three ways:

1. Activities of individual phosphatases (i.e., $\mu$mol $p$NP g$^{-1}$ soil h$^{-1}$).

2. Activity ratios of phosphatases, in order to evaluate relative changes in phosphatases involved in different steps of $P_o$ mineralization (e.g., mineralization of phosphodiesters vs...
monoesters) (Turner and Haygarth, 2005). This approach has been used to investigate potential changes in soil P cycling because phosphodiesterase is the first and potentially rate-limiting step of mineralization of $P_o$ (i.e., phosphodiester P forms) (Dick, et al., 2000, Turner and Haygarth, 2005, Waldrip and Acosta-Martínez, 2014). Three phosphatase activity ratios were calculated: acid phosphomonoesterase:alkaline phosphomonoesterase, acid phosphomonoesterase:phosphodiesterase, and alkaline phosphomonoesterase:phosphodiesterase.

(3) Phosphate activities normalized to microbial biomass P (i.e., $\mu$mol $p$NP $\mu$g $P_{mic}$ h$^{-1}$), in order to account for the potential influence of microbial biomass changes on measured response of phosphatase activities (Waldrop, 2000; Turner and Haygarth, 2005; Liu, 2017).

2.8. Statistical analyses

The effect of lime treatments on soil P variables was evaluated using analysis of variance (ANOVA) with Proc GLM in SAS v9.4 (Cary Institute, NC) and Tukey’s studentized difference ($p < 0.05$) to test significant mean differences. The F-statistic was used to compare the relative magnitude of lime effects on soil response variables by management history. Relationships between labile P fractions and phosphatase activities were evaluated separately for each management by calculating correlation coefficients (Pearson’s R) with Proc CORR.

3. Results

3.1. Liming effects on soil pH and recovery of $P_i$ spike

Soil pH increased linearly with lime rate in soils across management histories ($R^2 = 0.998$), furnishing a stepwise pH gradient from 4.7 to 6.4 (Table 1). Recovery of a $P_i$ spike (75 $\mu$g P g$^{-1}$) was greater for limed soils but did not necessarily increase linearly across the lime-induced pH gradient (Supplementary Fig. 1). In UNF and ORG, recovery of the $P_i$ spike

...
increased stepwise with pH, from 51% to 62% and from 56% to 66%, respectively. In contrast, recovery in MIN peaked at 76% at intermediate lime rate (3 t ha\(^{-1}\), pH 5.4) and was lowest (63%) at the zero and highest lime rate (pH 4.7 vs 6.4).

3.2. Labile P fractions

The relative change in labile P\(_i\) increased with lime rate for soils with low labile P\(_i\) (UNF) and was least for soils with high labile P\(_i\) (MIN) (Fig. 1A, B). Minor but significant increases in labile P\(_i\) occurred for UNF, with an increase in AEM-P\(_i\) of up to 79% (0.4 to 0.7 µg g\(^{-1}\)) and in NaHCO\(_3\)-P\(_i\) by 44% (0.9 to 1.2 µg g\(^{-1}\)). Soils managed with P inputs showed weak (ORG) or no (MIN) changes in AEM-P\(_i\) and NaHCO\(_3\)-P\(_i\). Irrespective of liming rate, labile P\(_i\) was greatest in MIN by 1-2 orders of magnitude compared to UNF and ORG.

Labile P\(_o\) response to lime depended on rate and management history (Fig. 1C). NaHCO\(_3\)-P\(_o\) was greatest in MIN (24.2 µg g\(^{-1}\) at 0 t ha\(^{-1}\)) and was unaffected by liming. In UNF, which had the least NaHCO\(_3\)-P\(_o\) (15.5 µg g\(^{-1}\)) among managements, labile P\(_o\) decreased by a mean of 10.4% at low lime rates (1.5 – 3 t ha\(^{-1}\)), but did not significantly affect labile P\(_o\) at higher rates compared to no lime. In ORG, NaHCO\(_3\)-P\(_o\) increased by up to 37% from 17.7 to 24.3 µg g\(^{-1}\) at 4.5 t ha\(^{-1}\) (pH 5.8), but at higher rates did not differ from the unlimed control.

3.3. \(P_{mic}\)

\(P_{mic}\) varied by an order of magnitude across managements (2.1 – 24.5 µg g\(^{-1}\) at 0 t ha\(^{-1}\)) but for a given management was similar across lime rates (Fig. 2). \(P_{mic}\) was unaffected by liming in UNF (mean 2.5 µg g\(^{-1}\)) and ORG (mean 5.6 µg g\(^{-1}\)). In MIN, \(P_{mic}\) did not significantly differ between unlimited and limed soils, but was elevated by 24.1% at lower lime rates (1.5 – 4.5 t ha\(^{-1}\)) relative to higher rates (6.0 – 7.5 t ha\(^{-1}\)).

3.4. Phosphatase activities
Changes in activities of individual phosphatases with lime were management- and enzyme-specific, but activity ratios of phosphatases showed similar changes to lime additions regardless of management history. The individual activities of acid phosphomonoesterase were most sensitive to lime in UNF and MIN, and decreased across the lime-induced pH gradient, whereas in ORG the activity of phosphodiesterase was most sensitive to liming and increased across the pH gradient (Fig. 3A,C).

Across management histories, alkaline phosphomonoesterase activity was least responsive to liming (Fig. 3B). Activity of acid phosphomonoesterase in unlimed soils was similar for UNF and MIN (Fig. 3C) despite AEM-P$_i$ differing by two orders of magnitude (Fig. 1A). Across the pH gradient of 4.7 to 6.4, acid phosphomonoesterase activity decreased continuously by up to 37% in UNF, and by up to 29% in MIN. The activity of acid phosphomonoesterase in ORG was elevated by 16% at lower lime rates (1.5 – 3 t ha$^{-1}$) relative to higher rates (6.0 – 7.5 t ha$^{-1}$) but did not differ relative to no lime. Only under ORG did alkaline phosphomonoesterase activity change with liming (Fig. 3B), decreasing transiently at 4.5 t ha$^{-1}$ (pH 5.8) by 16%. The magnitude and direction of change in phosphodiesterase activity following liming were also unique to management history (Fig. 3C). Phosphodiesterase activity was most strongly affected by lime under ORG, increasing by up to 36% at high rates (6 – 7.5 t ha$^{-1}$). In UNF and MIN, phosphodiesterase activity initially decreased by 14% and 13%, respectively, at the lowest lime rate (1.5 t ha$^{-1}$).

Individual phosphatase activities showed similar or contrasting correlations with labile P$_i$ and P$_o$ depending on phosphatase type and management history. In ORG, increases in phosphodiesterase activity were positively correlated with NaHCO$_3$-P$_i$ ($R = 0.65$, $p < 0.0001$) but not AEM-P$_i$ ($R = -0.13$, $p = 0.43$), whereas acid phosphomonoesterase activity was negatively
correlated with labile P in MIN (AEM-P$_i$ R = -0.79, $p < 0.0001$; NaHCO$_3$-P$_i$ R = -0.75, $p < 0.0001$). In UNF, acid phosphomonoesterase as well as phosphodiesterase activity were also negatively correlated with NaHCO$_3$-P$_i$ (R = -0.32, $p = 0.058$ and R = -0.31, $p = 0.066$, respectively), but acid phosphomonoesterase activity was positively correlated with AEM-P$_i$ (R = 0.31, $p = 0.065$). In ORG, labile P$_o$ was negatively correlated with both alkaline phosphomonoesterase activity (R = -0.62, $p < 0.0001$) and phosphodiesterase activity (R = -0.50, $p = 0.002$).

3.4. Ratios of phosphatase activities

Despite management- and enzyme-specific response of individual phosphatase activities to liming, activity ratios (Fig. 4) of acid phosphomonoesterase:alkaline phosphomonoesterase decreased with lime rate for UNF and MIN, and for all managements was lower at 7.5 t ha$^{-1}$ compared to no lime. For all managements, acid phosphomonoesterase:phosphodiesterase increased slightly at low lime rates (1.5 – 3.0 t ha$^{-1}$), and decreased markedly at higher rates. In contrast, there were minor or no changes in alkaline phosphomonoesterase:phosphodiesterase by lime rate across managements (Supplementary Fig. 2).

3.5. Phosphatase activities normalized to microbial biomass P

Activities of phosphatases normalized to P$_{mic}$ exhibited management- and enzyme-specific trends across liming gradients and did not necessarily reflect liming impacts on phosphatase activities on a soil mass basis or on phosphatase activity ratios (Fig. 5). For example, though the activity of acid phosphomonoesterase on a soil basis decreased with lime rate across managements (Fig. 3), acid phosphomonoesterase activity per unit P$_{mic}$ in ORG was similar at 0 and 7.5 t lime ha$^{-1}$ and activity decreases in MIN were limited to high lime rates (4 and 7.5 t ha$^{-1}$), though similar in magnitude (up to -28%) (Fig. 5A). In UNF, the decrease in
activity of acid phosphomonoesterase activity per unit $P_{\text{mic}}$ was greater in magnitude (−54% between 0 and 7.5 t lime ha$^{-1}$) than on a soil basis. Alkaline phosphomonoesterase activity per unit $P_{\text{mic}}$ in ORG increased at high lime rates (7.5 t ha$^{-1}$) compared to no or low lime rates (0 – 3.0 t ha$^{-1}$), in contrast to activity on a soil basis differing between no lime and intermediate (4.5 t ha$^{-1}$) lime (Fig. 5B). Though the activity of alkaline phosphomonoesterase on a soil basis was not influenced by lime in MIN, the activity normalized to $P_{\text{mic}}$ was elevated under high (7.5 t ha$^{-1}$) compared to low (1.5 t ha$^{-1}$) lime rates. Similar to activities on a soil basis, $P_{\text{mic}}$-normalized activity of alkaline phosphomonoesterase in UNF was not influenced by lime. Normalizing phosphodiesterase activity to $P_{\text{mic}}$ revealed a decrease of up to −36% in UNF with liming whereas in ORG the increase in phosphodiesterase activity was greater in magnitude per unit $P_{\text{mic}}$ (+68%) than per unit soil mass (Fig. 5C). The depression of phosphodiesterase activity in MIN at 1.5 t ha$^{-1}$ lime compared to other lime rates also occurred for activity normalized to $P_{\text{mic}}$. Across lime rates, phosphatase activities per unit $P_{\text{mic}}$ were greatest for UNF > MIN > ORG, opposite to phosphatase activities on a soil basis. For a given phosphatase, differences in activities normalized to $P_{\text{mic}}$ among managements were greater than for activities on a soil basis, reflecting differences in $P_{\text{mic}}$ among managements (Fig. 2).

In contrast to phosphatase activities on a soil mass basis, phosphatases activities normalized to $P_{\text{mic}}$ were not correlated with labile $P_i$, either across managements or within a given management. In soils under UNF and MIN, phosphatase activities per unit $P_{\text{mic}}$ were also unrelated to labile $P_o$, which in ORG soils was negatively correlated with activities of phosphodiesterase ($R = -0.71, p = 0.0009$) as well as acid phosphomonoesterase ($R = -0.53, p = 0.024$) and alkaline phosphomonoesterase ($R = -0.57, p = 0.013$). Soil pH in UNF was negatively correlated with $P_{\text{mic}}$-normalized activities of acid phosphomonoesterase ($R = -0.65, p = 0.004$).
and phosphodiesterase ($R = -0.51, p = 0.031$), and for acid phosphomonoesterase were also negatively correlated to soil pH ($R = -0.70, p = 0.0013$) and alkaline phosphomonoesterase ($R = -0.59, p = 0.009$) in MIN. In contrast, soil pH in ORG was not correlated with the $P_{\text{mic}}$-normalized activity of acid phosphomonoesterase and positively correlated with that of alkaline phosphomonoesterase ($R = 0.66, p = 0.0026$).

4. Discussion

4.1. Changes in P availability with liming

Decreased P sorption and increased labile $P_i$ occurred with lime-induced pH elevation, though the favorability of these changes for P availability depended on management history ($\text{UNF} > \text{MIN} > \text{ORG}$). These effects likely reflected differences in P saturation due to varying P inputs (or lack thereof) over 21 cropping seasons of previous managements. Limited decreases in P sorption (i.e., P recovery) and the absence of changes in labile $P_i$ under high P inputs (MIN), despite the same lime rate and pH elevation as for soils under other managements, indicates that soils with already high available P may not necessarily benefit from lime application with respect to enhancing crop-available P. However, liming offers additional soil fertility benefits beyond P, most notably decreasing aluminum (Al) toxicity to roots, increasing available Ca and magnesium (Mg) (depending on lime source), and increasing the availability of micronutrients such as molybdenum (Mo), a common constraint to biological nitrogen fixation in strongly weathered soils (Havlin, et al., 2013).

Though a high background of labile $P_i$ under MIN may have masked lime effects on available P, increases in AEM-$P_i$ for UNF (+0.3 µg g$^{-1}$) and ORG (+0.4 µg g$^{-1}$) were three orders of magnitude lower than AEM-$P_i$ in MIN soils that did not receive lime. Net increases in labile $P_i$
from lime alone appear to offer a limited contribution to P availability in weathered soils in the short-term. This indicates the necessity of P inputs for weathered soils in this region (Margenot, et al., 2016), the efficiency of which can be improved by the use of lime to decrease fixation of added P (Kisinyo, et al., 2015, Kisinyo, et al., 2014) (see also Section 4.4).

4.2. Phosphatase response to liming

This study supports the hypothesized sensitivity of soil phosphatase activity to liming and identifies a strong effect of management history on the direction and magnitude of the response of phosphatase activities on both a soil and P_{mic} basis. A common response of activity ratios of particular phosphatases across diverse managements may indicate a common effect of liming on phosphatase stoichiometry. Liming impacts on P cycling may be similarly mediated by the enzymes that catalyze mineralization of P_{o} despite strong management-induced differences in available and organic P prior to liming.

Contrary to field studies (Acosta-Martínez and Tabatabai, 2000, Ekenler and Tabatabai, 2003), shifts in phosphatase activities with lime-induced pH elevation did not necessarily reflect generally accepted pH optima (e.g., Tabatabai, 2003) depending on management history. For example, strong linear decreases in acid phosphomonoesterase activity and increases in alkaline phosphomonoesterase activity with increasing pH were proposed to reflect enzyme pH optima of 6.5 and 11.0, respectively (Acosta-Martínez and Tabatabai, 2000). At our study site, the extent of acid phosphomonoesterase activity decline across the lime-induced pH 4.6 – 6.4 gradient depended on management history, and alkaline phosphomonoesterase activity did not change (UNF, MIN) or did not consistently increase with pH (ORG). Changes in soil pH alone are therefore insufficient to predict changes in activities of individual phosphatases across the range of managements encompassed by the present study. That in some managements the activities of
phosphatases considered to have acid and alkaline pH optima did not necessarily change or
decreased, respectively, with liming on a soil and/or $P_{mic}$ basis (1) is consistent with evidence
that commonly proposed pH optima may be overgeneralizations (Turner, 2010) and (2) suggests
an effect of management on phosphatase type (e.g., isozymes of differing pH optima).

There are several potential explanations for the strong influence of input history on the
short-term response of soil phosphatase activities to lime. Changes in phosphatase activities
could reflect abiotic changes in activities of enzymes already present in soils expected to occur
with pH alteration, such as mismatch or convergence of soil pH and enzyme pH optima, or
labile P suggests that potential inhibition of phosphatase activity and/or production (Nannipieri,
et al., 2011) were likely minimal, especially given that increases in available P do not necessarily
suppress soil phosphatase activity (Margenot, et al., 2017). Future work should examine
relationships between soil phosphatase activities and phosphatase-encoding gene abundance
and/or expression in order to evaluate how observed response of phosphatase activity may be due
to changes in microbial expression of phosphatases (Fraser, et al., 2015, Lagos, et al., 2016, Luo,
et al., 2017).

Given the same lime rates and matching pH gradients, differences in phosphatase
activities by management history suggests that 11 years of contrasting input quality and quantity
at this site conditioned the response of enzyme activities to liming. For example, though
phosphodiesterase activities in unlimed soils were similar across managements, the increase in
phosphodiesterase unique to ORG indicates a difference in the capacity of phosphatase activities
to respond to lime as the result of input history. This could be mediated by (1) $P_o$ substrate
loading in soils, (2) accumulated differences in the amount or characteristics (e.g., pH optima,
substrate affinity \([K_m]\), velocity \([V_{\text{max}}]\) of phosphatases, and (3) variation in soil properties known to influence soil enzyme activities (e.g., SOC). For example, addition of phosphatase substrates could explain the unique response of phosphatase activities to liming in soils receiving manure \((4 \text{ t ha}^{-1} \text{ season}^{-1})\), because manure is a source of monoester and diester \(P_o\) (He, et al., 2004, Sharpley and Moyer, 2000). Since stabilization of monoester and diester \(P_o\) forms by binding to Fe and Al oxides (Giesler, et al., 2002, Giesler, et al., 2004) can protect these \(P_o\) substrates from mineralization by phosphatases (Giaveno, et al., 2010) and is pH-dependent (maximized at pH < 5) (Condron, et al., 2005), we speculate that elevated soil pH could have led to desorption of mineral-bound \(P_o\) and potentially induced microbial expression of phosphatases.

Despite strong differences in labile \(P_i\) among managements \((10^3)\), potential activities of phosphatases were comparatively similar. This is in contrast to the hypothesized inverse relationship between \(P\) availability and phosphatase activity via negative feedback inhibition of microbial phosphatase production by \(P_i\) (Nannipieri, et al., 2011). Limited studies in forest ecosystems have demonstrated suppression of phosphomonoesterase activity in highly weathered soils under long-term \(P\) application (e.g., triple super phosphate at 100 kg \(P\) ha\(^{-1}\) yr\(^{-1}\)) (Olander and Vitousek, 2000). However, consistent with our findings, \(P\) fertilization in weathered soils in East Africa under agricultural use \((25 – 250 \text{ kg } P \text{ ha}^{-1} \text{ yr}^{-1})\) do not suppress and may even stimulate acid phosphomonoesterase activity (Margenot, et al., 2017, Mukuralinda, et al., 2011, Radersma and Grierson, 2004).

4.3. Lime impacts on biological \(P\) cycling

In the short-term period following liming represented by this study (<4 weeks), the general absence of \(P_{\text{mic}}\) response and management-specific changes in phosphatase activities are in mixed support of the hypothesized stimulation of biological \(P\) cycling by liming. Constant \(P_{\text{mic}}\)
across a lime-induced pH gradient is not necessarily in conflict with the hypothesized
mechanism of increased P availability enabling greater P$_{\text{mic}}$, because labile P$_i$ showed only minor
increases and there were minor or no changes in labile P$_o$ with liming.

Weak or absent changes in P$_{\text{mic}}$ and labile P$_o$ in our short-term study are not inconsistent
with reports of increased P$_{\text{mic}}$ and decreased soil P$_o$ 1-2 years following liming (4 t ha$^{-1}$)
(Condron and Goh, 1989, Condron and Goh, 1990). Though a separate study reported a 2-fold
decrease in P$_{\text{mic}}$ 8 weeks after Ca(OH)$_2$ addition, which increased soil pH from 5.5 to 6.1 – 6.7
(Haynes and Swift, 1988), the lack of correction for P sorption (see Section 2.6) would be
expected to underestimate P$_{\text{mic}}$ in the unlimed control. Additionally, such approaches measure
net changes in an operationally defined P$_o$ fraction rather than directly quantifying P$_o$
mineralization (e.g., Bünemann, 2015). The use of extractions to monitor liming effects on P$_o$
risks artifacts from alteration of P$_o$ solubility. For example, Halstead, et al. (1963) measured high
reductions in NaHCO$_3$-P$_o$ (-44%) and NaOH-P$_o$ (-38%) concomitant with increases in P$_i$
fractions within three days of Ca(OH)$_2$ addition. This could result from formation and
precipitation of P$_o$ – Al complexes following a result of the flush of Al$^{3+}$ from the exchange
complex and the low solubility of Al$^{3+}$ at pH > 5.5 (Condron and Goh, 1990, Condron, et al.,

Changes in phosphatase activities following lime additions support the hypothesized
potential of lime to impact soil P cycling because phosphatase activity assays measure potential
maximum rates of enzymatic mineralization of P$_o$ (Kruse, et al., 2015). In the <4 weeks of the
present study, however, this did not translate to appreciable changes in labile P$_o$, labile P$_i$, or P$_{\text{mic}}$.
That relationships among labile P$_o$ and phosphatase activities were specific to management
history indicates that management can condition the response of biological soil P cycling to
liming events. For example, while the inverse correlation of alkaline phosphomonoesterase and phosphodiesterase activities with labile P₀ in soils receiving manure (ORG) supports the hypothesized mineralization of P₀ due to activity increases for phosphatases with alkaline pH optima, under high input (MIN) and zero input (UNF) managements, labile P₀ concentrations were unrelated to phosphatase activities. Since labile Pᵢ and Pₘic were weakly or not affected by liming, microbial P demand was unlikely to have influenced phosphatase activity (e.g., secretion of phosphatases to scavenge P). The negative correlation of acid phosphomonoesterase activity and labile Pᵢ in MIN and UNF is difficult to ascribe to enzyme inhibition by soluble Pᵢ (Nannipieri, et al., 2011, Olander and Vitousek, 2000) because increased soil pH could also explain loss of acid phosphomonoesterase activity (Acosta-Martínez and Tabatabai, 2000, Nannipieri, et al., 2011). Because phosphatase activities normalized to Pₘic were not correlated to labile Pᵢ but were correlated with soil pH, observed changes in phosphatase activity (1) were unlikely to have resulted from microbial secretion of phosphatases and (2) as hypothesized, can be driven by changes in pH following liming.

Changes in ratios of phosphatase activities across managements indicate potential alteration of P cycling via enzymatic mineralization of P₀ regardless of pre-lime differences in soil P cycling. The relative decrease in acid phosphomonoesterase compared to alkaline phosphomonoesterase and phosphodiesterase suggests that liming could change the relative roles of phosphatases. As phosphodiesterase is considering the first and rate-liming step of P₀ mineralization (Turner and Haygarth, 2005), a decrease in acid phosphomonoesterase relative to phosphodiesterase may not necessarily impact Pᵢ mineralization. On the other hand, given that the magnitude of acid phosphomonoesterase activity was at least twice that of alkaline phosphomonoesterase across soils, decreased acid phosphomonoesterase activity could reduce P₀
mineralization and alter P availability at timescales extending beyond that of the present study.

Elevated phosphatase activity per unit of \( P_{\text{mic}} \) in soils under no P inputs (UNF) relative to soils receiving low to high P inputs would appear to support the hypothesized use of phosphatases by soil microorganisms to scavenge P under conditions of P-limitation (Oberson, 2001; Nannipieri, 2012). However, soils under ORG had the least phosphatase activity per unit \( P_{\text{mic}} \), despite exhibiting an order of magnitude less available P and \( P_{\text{mic}} \) compared to soils under MIN. This indicates that normalizing phosphatase activities to \( P_{\text{mic}} \) may not necessarily provide an indication of P-limitation.

4.4. Implications for P management in acid soils of western Kenya

Our results highlight the limited potential of liming to alleviate constraints on P availability in weathered soils in western Kenya with low or no P inputs: even with liming, available P remained within the range of severe deficiency. Although high lime rates (7.5 t ha\(^{-1}\)) nearly doubled available P in soils under zero-input management, the magnitude of this increase was insufficient to ameliorate severe P deficiency (< 1 µg AEM-P\(_{i}\) g\(^{-1}\)) because AEM-P\(_{i}\) was still below critical levels of AEM-P\(_{i}\) in weathered soils (e.g., 26 – 33 µg P g\(^{-1}\) for maize and soybean) (Schlindwein and Gianello, 2008). On the other hand, high available P under MIN is the result of sustained P inputs at rates (120 kg ha\(^{-1}\) yr\(^{-1}\)) that for many farmers in western Kenya are unaffordable (Nziguheba, et al., 2015), even if recommended (see KARI, 1994, Kihara and Njoroge, 2013). While the use of manure at rates in this study is likely more realistic (accessible and/or affordable) for farmers in this region (Sommer, et al., 2018), the low P content and application rate of manure in ORG entailed low P inputs (1.1 kg ha\(^{-1}\) season\(^{-1}\)). ORG and MIN managements in this study therefore represent P input extremes that bound intermediate rate(s) that are economically affordable and agronomically efficient. Similarly, lime additions in soil
mesocosms corresponded to field applications of 1.5 – 7.5 t ha\(^{-1}\), with pH increasing to the threshold of maximum P availability (pH 6.4) only at the highest rate. This rate is higher than employed in many studies in weathered soils in East Africa, which commonly employ rates ≤ 2 t ha\(^{-1}\) (e.g., Okalebo, et al., 2009), though yield increases can be obtained at this or lower rates in western Kenya (Fund, 2015, Fund, 2016).

5. Conclusion

This study reveals mixed short-term effects of lime on soil P cycling in a weathered soil (Oxisol) and identifies a strong influence of previous soil fertility management on this response. Within 4 weeks of a liming event, soils with P deficiency experienced significant relative increases in available P that were insufficient in magnitude to alleviate deficiency. \(P_{\text{mic}}\) was largely unaffected by liming and was an order of magnitude greater in soils receiving inorganic N and P inputs compared to soils with no inputs or with manure additions at low, albeit regionally realistic, rates (4 t ha\(^{-1}\) yr\(^{-1}\)). Phosphatase activities differed by enzyme type and management history, and there were no clear trends in activities of individual phosphatase activities across the lime-induced pH gradient (pH 4.7 – 6.4). Patterns in P sorption and \(P_{\text{mic}}\) did not match liming response of phosphatase activities, which were strongly influenced by management history. Soils that received manure over the previous 11 years showed a unique phosphatase response to liming compared to soils with zero or high inputs. Since greatest changes in P availability and phosphatase activities occurred at lime rates higher than those usually practiced in western Kenya, current liming practices in this region may not impact short-term soil P cycling. On the other hand, if persistent beyond the time-frame of this study, changes in phosphatase activities could impact soil P availability over longer time frames. Future studies
should examine longer-term response of P cycling to commonly practiced lime rates under field conditions.

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Figure 1: Labile inorganic and organic phosphorus (P) fractions 27 days after lime additions to a Typic Kandiudox under differing fertilization managements (21 cropping seasons) from western Kenya. Managements were no fertilization (UNF), mineral N and P (60 kg ha\(^{-1}\) season\(^{-1}\); MIN), and manure (4 t ha\(^{-1}\) season\(^{-1}\); ORG). Labile fractions include (A) anion-exchange membrane (AEM) extractable P\(_i\); (B) sodium bicarbonate extractable P\(_i\) and (C) sodium bicarbonate extractable P\(_o\).

Figure 2: Microbial biomass phosphorus 27 days after lime additions to a Typic Kandiudox under differing fertilization managements (21 cropping seasons) from western Kenya. Managements were no fertilization (UNF), mineral N and P (60 kg ha\(^{-1}\) season\(^{-1}\); MIN), and manure (4 t ha\(^{-1}\) season\(^{-1}\); ORG).

Figure 3: Activities of P-cycling enzymes (phosphatases) 27 days after lime additions to a Typic Kandiudox under differing fertilization managements (21 cropping seasons) from western Kenya. Assays of phosphatase activities included both phosphomonoesterases, with acid (A) and alkaline (B) pH optima, as well as phosphodiesterase (C). Managements were no fertilization (UNF), mineral N and P (60 kg ha\(^{-1}\) season\(^{-1}\); MIN), and manure (4 t ha\(^{-1}\) season\(^{-1}\); ORG).

Figure 4: Ratios of (A) acid phosphomonoesterase (ACP) to alkaline phosphomonoesterase (ALP) activities and (B) ACP to phosphodiesterase (PDE) activities across a Typic Kandiudox under differing fertilization managements (21 cropping seasons) from western Kenya. Managements were no fertilization (UNF), mineral N and P (60 kg ha\(^{-1}\) season\(^{-1}\); MIN), and manure (4 t ha\(^{-1}\) season\(^{-1}\); ORG).
**Figure 5:** Activities of P-cycling enzymes (phosphatases) 27 days after lime additions to a Typic Kandiudox under differing fertilization managements (21 cropping seasons) from western Kenya. Phosphatase activities are normalized to microbial biomass P (P_{mic}), and include (A) acid phosphomonoesterase, (B) alkaline phosphomonoesterase, and (C) phosphodiesterase.

Managements were no fertilization (UNF), mineral N and P (60 kg ha\(^{-1}\) season\(^{-1}\); MIN), and manure (4 t ha\(^{-1}\) season\(^{-1}\); ORG).

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**Table 1.** Soil pH (1:2 in water) across a liming gradient in a Typic Kandiudox under differing fertilization managements (21 cropping seasons) from western Kenya 27 days after addition of Ca(OH)\(_2\). Lime rates were calculated using the Mehlich buffer liming requirement and bulk densities at the field trial to the estimated depth of incorporation (0-15 cm). Managements were no fertilization (UNF), mineral N and P (60 kg ha\(^{-1}\) season\(^{-1}\); MIN), and manure (4 t ha\(^{-1}\) season\(^{-1}\); ORG). Mean pH values are presented. Standard error was ≤ 0.02 for all mean values.

<table>
<thead>
<tr>
<th>Management</th>
<th>Lime application (t ha(^{-1}))</th>
<th>0</th>
<th>1.5</th>
<th>3.0</th>
<th>4.5</th>
<th>6.0</th>
<th>7.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNF</td>
<td></td>
<td>4.73</td>
<td>5.03</td>
<td>5.37</td>
<td>5.73</td>
<td>6.12</td>
<td>6.44</td>
</tr>
<tr>
<td>MIN</td>
<td></td>
<td>4.69</td>
<td>4.94</td>
<td>5.31</td>
<td>5.64</td>
<td>6.04</td>
<td>6.35</td>
</tr>
<tr>
<td>ORG</td>
<td></td>
<td>4.79</td>
<td>5.08</td>
<td>5.43</td>
<td>5.79</td>
<td>6.18</td>
<td>6.48</td>
</tr>
</tbody>
</table>
Acid phosphomonoesterase: $P_{mic}$

Alkaline phosphomonoesterase: $P_{mic}$

Phosphodiesterase: $P_{mic}$
Changes in soil phosphatase activities across a liming gradient under diverse long-term managements in subhumid Kenya

Supplementary Information

Supplementary Figure 1: Recovery of an inorganic phosphate spike (75 µg g⁻¹) across a liming gradient (0 – 7.5 t ha⁻¹) in a Typic Kandiudox under differing fertilization managements (21 cropping seasons) from western Kenya. Managements were no fertilization (UNF), mineral N and P (60 kg ha⁻¹ season⁻¹, respectively; MIN), and manure (4 t ha⁻¹ season⁻¹; ORG).
Supplementary Figure 2: Ratios of alkaline phosphomonoesterase (ALP) to phosphodiesterase (PDE) activities across a Typic Kandiudox under differing fertilization managements (21 cropping seasons) from western Kenya. Managements were no fertilization (UNF), mineral N and P (60 kg ha\(^{-1}\) season\(^{-1}\); MIN), and manure (4 t ha\(^{-1}\) season\(^{-1}\); ORG).
**Supplementary Table 1**

General soil properties of a Typic Kandiudox under differing fertilization managements (21 cropping seasons) from western Kenya used to assess soil P response to liming using 27-day mesocosms. Significant differences among soil variables among experimental plots (n=3 per treatment) are indicated by different letters (Tukey's HSD test, \( p < 0.05 \))

<table>
<thead>
<tr>
<th>Management</th>
<th>Inputs</th>
<th>pH (1:2 water)</th>
<th>Ex. acidity (m(_{eq} 100 \text{ g}^{-1}))</th>
<th>SOC (mg \text{ g}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean ( \pm ) se</td>
<td>mean ( \pm ) se</td>
<td>mean ( \pm ) se</td>
</tr>
<tr>
<td>UNF</td>
<td>none</td>
<td>4.76 ( \pm ) 0.02 a</td>
<td>3.24 ( \pm ) 0.13 a</td>
<td>15.8 ( \pm ) 0.3 b</td>
</tr>
<tr>
<td>MIN</td>
<td>60 kg N, 60 kg P ha(^{-1}) season(^{-1})*</td>
<td>4.74 ( \pm ) 0.02 a</td>
<td>3.86 ( \pm ) 0.26 a</td>
<td>19.2 ( \pm ) 0.4 a</td>
</tr>
<tr>
<td>ORG</td>
<td>4 t manure ha(^{-1}) season(^{-1})**</td>
<td>4.77 ( \pm ) 0.04 a</td>
<td>3.50 ( \pm ) 0.42 a</td>
<td>18.4 ( \pm ) 0.1 a</td>
</tr>
</tbody>
</table>

*As urea and triple super phosphate

**Corresponds to 2.8 kg N and 1.1 kg P ha\(^{-1}\) season\(^{-1}\)