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Soil Phosphatase Activities across a Liming Gradient under Long-Term Managements in Kenya

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Changes in the biological drivers of soil P cycling could contribute to improving P availability in weathered soils after liming. The effect of liming on P cycling was evaluated for a Rhodic Kandiudox in western Kenya under no fertilization (UNF), mineral N + P (MIN), and manure (ORG). $Ca(OH)_2$ was applied at six rates in soil mesocosms to establish a pH gradient (4.7–6.4). Labile inorganic P (P_i) increased by ≤1.2 μg g^{-1} in response to lime and labile organic P (P_o) was weakly affected. In MIN, microbial biomass P (Pmic) decreased at ≥6.0 Mg ha^{-1} (–24%). Liming changed phosphatase activity depending on the management and phosphatase type but did not reflect commonly proposed pH optima of phosphatases. In UNF and MIN, acid phosphomonoesterase activity decreased with pH, and alkaline phosphomonoesterase and phosphodiesterase activity showed minor changes. Liming under ORG altered activity by up to +16% for acid phosphomonoesterase, –16% for alkaline phosphomonoesterase, and +36% for phosphodiesterase. Similar trends were observed for P_{mic}–normalized activity, including decreased acid phosphomonoesterase under UNF and increased phosphodiesterase under ORG. P_{mic}–normalized acid phosphomonoesterase activity under ORG was unaffected by liming whereas P_{mic}–normalized phosphodiesterase activity exhibited a marked decrease under UNF. Across managements, the acid phosphomonoesterase/phosphodiesterase activity ratio peaked at pH 5.0 and decreased thereafter. Despite management-induced differences in soil P availability, consistent changes in phosphatase activity ratios indicate a short-term impact of lime on the enzymatic component of P cycling, which could translate to longer-term changes in P_o mineralization and available P.

Abbreviations: AEM, anion exchange membrane; MIN, mineral N + P fertilization; ORG, fertilization with manure; P_i, inorganic phosphorus; P_{mic}, microbial biomass phosphorus; P_o, organic phosphorus; UNF, no fertilization.

Core Ideas

- **We investigated liming in soil mesocosms from 11-yr managements: no input, mineral N+P, and manure.**
- **There were minor or no changes in labile inorganic P, labile organic P, and microbial biomass P.**
- **Changes in the activity of different phosphatases following liming depended on management.**
- **Liming decreased acid phosphomonoesterase/phosphodiesterase across management systems.**
- **Liming can impact the enzymatic component of soil P cycling in the short term.**

Lime is commonly applied to increase soil pH and thus the availability of native and added 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, 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 P_o following liming have been proposed to reflect the 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

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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 activity can influence the amount of P_o mineralized to P_i that is available for immobilization in P_{mic}.

Microbial biomass is both a pool and a driver of P cycling (Oberson et al., 2006; Richardson and Simpson, 2011). In P-limited weathered soils, microbial biomass is able to respond rapidly to changes in P availability, such as P_i pulses (Ayaga et al., 2006; Bünemann et al., 2012; Oberson and Joner, 2005; Oehl et al., 2001). Rapid (potentially <7 h) microbial immobilization of soil solution P_i into 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 reuptake by microbes (Achat et al., 2010; Oberson and Joner, 2005). Liming could stimulate P_{mic} by increasing the amount of P available for microbial uptake (Gachengo et al., 1998) and by increasing soil pH to values that are favorable for greater microbial activity (Kemmitt et al., 2006; Robson and Abbot, 2012; Rousk et al., 2010, 2009). This could explain the elevated pulses of soil respiration following liming (Haynes and Swift, 1988) and increases in microbial biomass C across lime-induced pH gradients in multiyear field experiments (Acosta-Martínez and Tabatabai, 2000; Ekenler and Tabatabai, 2003), though the response of P_{mic} in these studies was not measured.

Independent of the effect of liming 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 the alkaline optimum of phosphodiesterase (pH 8) (Eivazi and Tabatabai, 1977; Hui et al., 2013) suggest that liming is likely to entail decreases in acid phosphomonoesterase activity while increasing alkaline phosphomonoesterase and phosphodiesterase activity. Given that phosphodiesterase is the first and most likely rate-limiting step in P_o mineralization (Turner and Haygarth, 2005), shifts in the relative activity of different phosphatases (i.e., activity ratios) resulting from liming could impact P_o mineralization (Dick et al., 2000).

Changes in soil pH following a liming event are relatively rapid compared with 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 activity responds rapidly in the post-liming window. Though 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 (i.e., alkaline phosphomonoesterase, phosphodiesterase) is not known.

Soil management is likely to condition the response of soil phosphatase 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 activity by altering the amount of enzyme substrate (i.e., P_o) 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 activity 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_o (Oberson et al., 1999, 2011), changes to phosphatase activity by liming could have a substantial impact on P availability.

To address these knowledge gaps, we evaluated the short-term (<1 mo) post-liming response of the enzymatic and microbial components of P cycling. To test the 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 yr) in western Kenya. Across liming gradients established in soil mesocosms, we hypothesized (i) improved P availability (i.e., decreased P sorption and/or increased labile P_i) with soil pH elevation; (ii) increased P_{mic} with soil pH elevation; (iii) changes in the activities of acid phosphomonoesterase, alkaline phosphomonoesterase, phosphodiesterase reflective of phosphatase-specific pH optima; and (iv) a significant effect of management history on P_{mic} and the response of phosphatase activity to lime.

MATERIALS AND METHODS

Soil Management and Sampling

Soils from a long-term integrated soil fertility management trial in western Kenya were used to test the hypothesized effect of management history on the response of biological P cycling to liming. The trial was established in 2003 near Sindindi in Siaya County, Kenya (00°08′38.3″ N, 34°24′13.7″ E) at an elevation of 1330 m asl. 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 Rhodic Kandiudox (USDA) or Rhodic Ferralsol (World Reference Base for Soil Resources), and has a clay texture (555 g clay kg^{-1}, 183 g silt kg^{-1}, 261 g sand kg^{-1}) at 0- to 15-cm depth (N.A. Jelinski, unpublished data, 2018).

Three soil fertility managements were selected to evaluate the effects of liming on soil P cycling: (i) an unfertilized control (0 kg N and P ha^{-1} per season; UNF), (ii) mineral N (60 kg ha^{-1} per season as urea) and P (60 kg ha^{-1} per season as triple superphosphate; MIN), (iii) and bovine manure (4 Mg dry matter ha^{-1} per season) 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}

per season. These N and P contents are common for manure produced on smallholder homesteads in western Kenya (Sommer et al., 2018; Waithaka et al., 2007) and are likely to result from local manure harvesting 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 three selected treatments represent the fertility management scenarios of zero input (UNF) and low input (ORG) that are prevalent in western Kenya as a result of resource limitation (Tittonell and Giller, 2013; Tittonell et al., 2007, 2013), whereas the high input treatment (MIN) is based on regionally recommended N and P rates (Kenya Agricultural Research Institute, 1994; Kihara and Njoroge, 2013). Treatment plots (4.5 by 6 m) randomized in a complete block design (Sommer et al., 2018) were cropped to maize (*Zea mays* L.) in the long rains and to tephrosia [*Tephrosia candida* (Roxb.) DC.] in the short rains. Tephrosia biomass was incorporated by hand tillage into the soil as a green manure. Tillage and weeding were carried out by hand-hoeing as necessary according to local practices. At the time of sampling, soils (0- to 15-cm depth) from the three treatments had similar soil pH and exchangeable acidity and comparable soil organic C (Supplemental Table S1).

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

Determination of Liming Requirements

Exchangeable acidity was determined via the Mehlich buffer method (Mehlich et al., 1976) modified to replace barium chloride with calcium chloride (Hoskins and Erich, 2008). Briefly, 10 g of oven-dried equivalent soil was mixed with 10 mL of distilled water for 2 min with a magnetic stir bar in a 50-mL beaker and allowed to stand for 1 h. The mixture was restirred 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 (m_{eq} 100 g^{-1}) was calculated as follows:

$$\text{Exchangeable acidity} = \frac{(6.64 - pH_B)}{0.25} \times \text{soil mass} \quad [1]$$

Liming requirement was calculated as the $CaCO_3$ equivalent of calcium hydroxide $Ca(OH)_2$ to neutralize exchangeable acidity, assuming 135% $CaCO_3$ equivalence (Havlin et al., 2013).

Soil Mesocosms and Lime Treatments

Six lime rates were applied to soil mesocosms using $Ca(OH)_2$: 0 to 2.5× the liming requirement at 0.5× liming requirement intervals. Since soils under the three management histories had 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 all three management histories. Based on a mean bulk density of 1.15 g cm^{-3} at 0- to 15-cm depth for sampled plots and a depth of incorporation of 15 cm with a hand hoe (Paul et al., 2013), this corresponds to application rates of 0, 1.5, 3.0, 4.5, 6.0, and 7.5 Mg $CaCO_3$ 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-type jar. Soils were preincubated at 70% of the water-filled pore space for 5 d before applying lime treatments. The $Ca(OH)_2$ was added as a dry powder (<200 μm) and thoroughly incorporated with moist soil by mixing it with an acid-washed glass stirring 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 d after liming and harvested at the end of Day 27. All further analyses were performed on freshly harvested soils.

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 via a modified sequential extraction method based on Hedley et al. (1982). Soil from each mesocosm (lime treatment replicate) was analyzed in duplicate. Soils were first extracted with a 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). Inorganic P was desorbed from the membranes by shaking for 1 h in 0.25 mol L^{-1} H_2SO_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 *g*, 15 min), and an aliquot was analyzed by molybdate colorimetry for P_i and for total P following acid persulfate digestion (80°C, 16 h) (Rowland and Haygarth, 1997). Organic P was estimated as the difference between total P 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).

Phosphorus sorption and P_{mic}

Sequential fumigation–extraction with chloroform gas according to Brookes et al. (1982) was used to determine P_{mic} in fresh soils 27 d after liming. For each soil mesocosm, three types of subsamples were processed in duplicate: fumigated, nonfumigated, 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 (8000 *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). Nonfumigated

and P-spiked subsamples were processed in the same way as fumigated subsamples but without chloroform fumigation. To avoid underestimation 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). Microbial biomass P was calculated as the difference between fumigated and nonfumigated extractable P (Eq. [2]) (Brookes et al., 1982).

$$P_{mic} = \frac{\text{fumigated P} - \text{nonfumigated P}}{\text{P spike recovery}} \quad [2]$$

The recovery of the P_i spike was used as an indicator of P sorption (i.e., a greater percentage of recovery equates to a 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).

Phosphatase Activity

The activity of acid phosphomonoesterase, alkaline phosphomonoesterase, and phosphodiesterase (Enzyme Commission numbers 3.1.3.2, 3.1.3.2, and 3.1.4.1, respectively) 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 at pH 6.5 for acid phosphomonoesterase and pH 11.0 for alkaline phosphomonoesterase and in 5 mL of 0.05 mol L^{-1} 2-amino-2-(hydroxymethyl)-1,3-propanediol buffer at pH 8.0 for phosphodiesterase. The 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). The 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} 2-amino-2-(hydroxymethyl)-1,3-propanediol (pH 12.0) to phosphodiesterase assays and 1 mL of 0.5 mol L^{-1} $CaCl_2$. Centrifugation (2113 *g*, 5 min) was used to remove sediment and *para*-nitrophenol (*p*NP) in the clear supernatant was quantified colorimetrically (410 nm). The mean absorbance of triplicate negative controls (no soil + substrate) was subtracted from the absorbance of the soil assays. Phosphatase activity was expressed in three ways:

1. Activity of each individual phosphatase (μmol *p*NP g^{-1} soil h^{-1}).
2. Activity ratios of phosphatases were used to evaluate relative changes in the activities of phosphatases involved in different steps of P_o mineralization (such as 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 (that is, 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 activity normalized to P_{mic} (μmol *p*NP μg^{-1} P_{mic} h^{-1}) was used to account for the potential influence of microbial biomass changes on the measured response of phosphatase activities (Waldrop et al., 2000; Turner and Haygarth, 2005; Liu et al., 2017).

Statistical Analyses

The effect of lime treatments on soil P variables was evaluated using analysis of variance (ANOVA) with Proc GLM in SAS v.9.4 (SAS Inst., Cary, 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.

RESULTS

Liming on Soil pH and Recovery of P_i Spikes

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 μg P g^{-1}) was greater for limed soils but did not necessarily increase linearly across the lime-induced pH gradient (Supplemental Fig. S1). 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 an intermediate lime rate (3 Mg ha^{-1}, pH 5.4) and was lowest (63%) at the zero and highest lime rate (pH 4.7 vs. 6.4).

Table 1. Soil pH (1:2 in water) across a liming gradient in a Rhodic Kandiudox under different fertilization managements (21 cropping seasons) from western Kenya 27 d after addition of $Ca(OH)_2$. Lime rates were calculated via the Mehlich buffer liming requirement and bulk densities at the field trial to the estimated depth of incorporation (0–15 cm). Management systems were no fertilization (UNF), mineral N and P (60 kg ha^{-1} per season; MIN), and manure (4 Mg ha^{-1} per season; ORG). Mean pH values are presented; SE ≤ 0.02 for all mean values.

	Lime application					
Management	**0**	**1.5**	**3.0**	**4.5**	**6.0**	**7.5**
	Mg ha^{-1}					
UNF	4.73	5.03	5.37	5.73	6.12	6.44
MIN	4.69	4.94	5.31	5.64	6.04	6.35
ORG	4.79	5.08	5.43	5.79	6.18	6.48

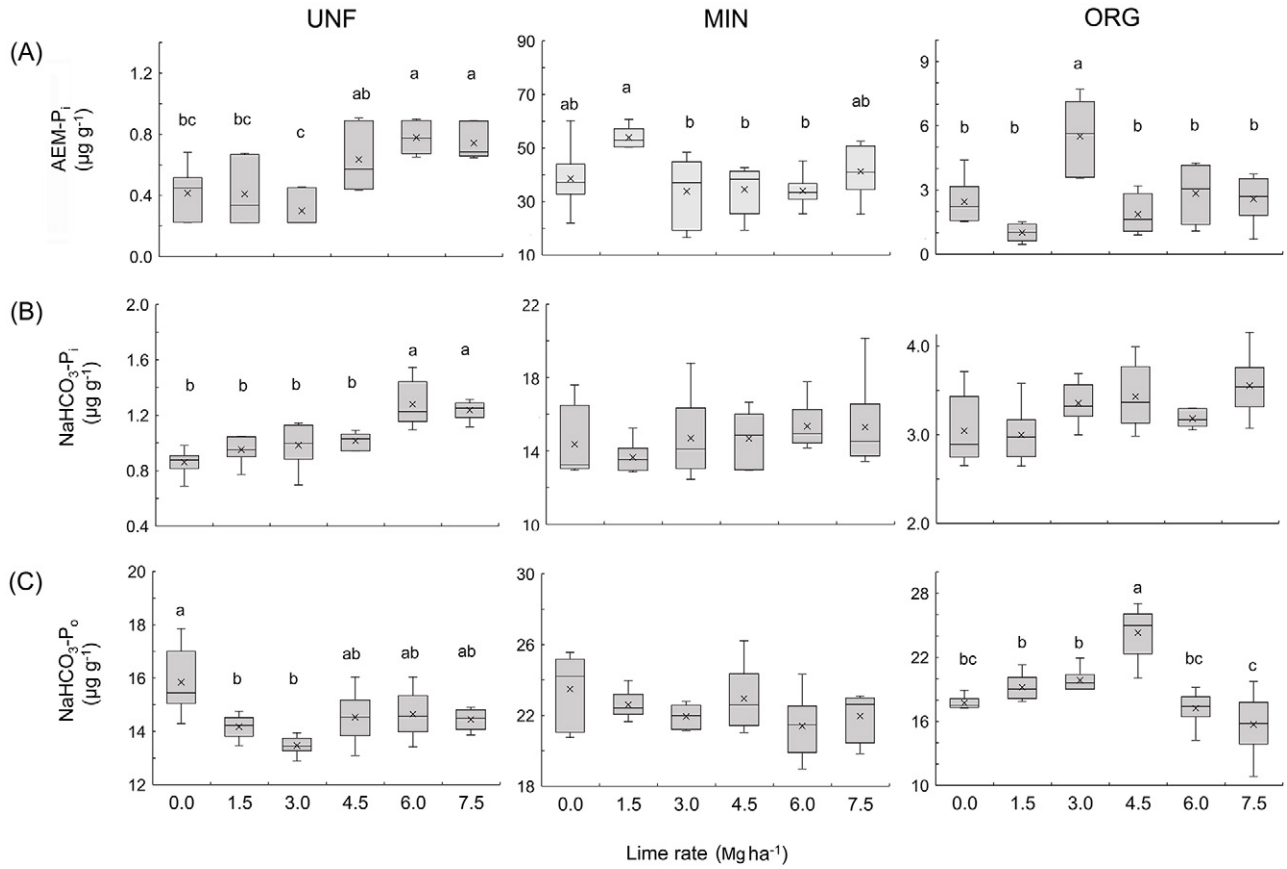

Fig. 1. Labile inorganic and organic P fractions 27 d after lime additions to a Rhodic Kandiudox under different fertilization managements (21 cropping seasons) from western Kenya. Management systems were no fertilization (UNF), mineral N and P (60 kg ha^{-1} per season; MIN), and manure (4 Mg ha^{-1} per season; ORG). Labile fractions include (A) anion exchange membrane (AEM)-extractable inorganic P (P_i); (B) sodium bicarbonate-extractable P_i and (C) sodium bicarbonate-extractable organic P (P_o).

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% (from 0.4 to 0.7 μg g^{-1}) and in $NaHCO_3$–P_i by 44% (from 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 one to two orders of magnitude compared with UNF and ORG.

The response of labile P_o to lime depended on the rate and management history (Fig. 1C). $NaHCO_3$–P_o was greatest in MIN (24.2 μg g^{-1} at 0 Mg 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 (1.5–3.0 Mg ha^{-1}) but did not significantly affect labile P_o at higher rates than 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 Mg ha^{-1} (pH 5.8), but did not differ from the unlimed control at higher rates.

Microbial Biomass P

Microbial biomass P varied by an order of magnitude across managements (2.1–24.5 μg g^{-1} at 0 Mg ha^{-1}) but for a given management was similar across lime application rates (Fig. 2). Microbial biomass P 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 unlimed and limed soils but was elevated by 24.1% at lower lime rates (1.5–4.5 Mg ha^{-1}) relative to higher rates (6.0–7.5 Mg ha^{-1}).

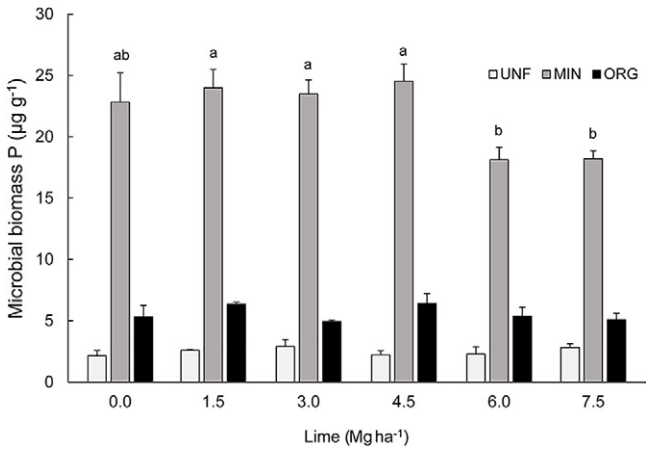

Fig. 2. Microbial biomass P 27 d after lime additions to a Rhodic Kandiudox under different fertilization managements (21 cropping seasons) from western Kenya. Management systems were no fertilization (UNF), mineral N and P (60 kg ha^{-1} per season; MIN), and manure (4 Mg ha^{-1} per season; ORG).

Phosphatase Activities

Changes in the activity of individual phosphatases with lime were management-specific and enzyme-specific, but the 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). The 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 (1.5–3.0 Mg ha^{-1}) relative to higher rates (6.0–7.5 Mg 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 Mg ha^{-1} (pH 5.8) by 16%. The magnitude and direction of the 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 Mg ha^{-1}). In UNF and MIN, phosphodiesterase activity initially decreased by 14 and 13%, respectively, at the lowest lime rate (1.5 Mg ha^{-1}).

Individual phosphatase activities showed similar or contrasting correlations with labile P_i and P_o depending on the 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_i 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 and 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).

Ratios of Phosphatase Activities

Despite the management history-specific and enzyme-specific response of individual phosphatase activities to liming, the acid phosphomonoesterase/alkaline phosphomonoesterase activity ratio (Fig. 4) decreased with lime rate for UNF and MIN and was lower at 7.5 ha^{-1} than no lime for all management histories. For all managements, acid phosphomonoesterase/phosphodiesterase increased slightly at low lime rates (1.5–3.0 ha^{-1}) and

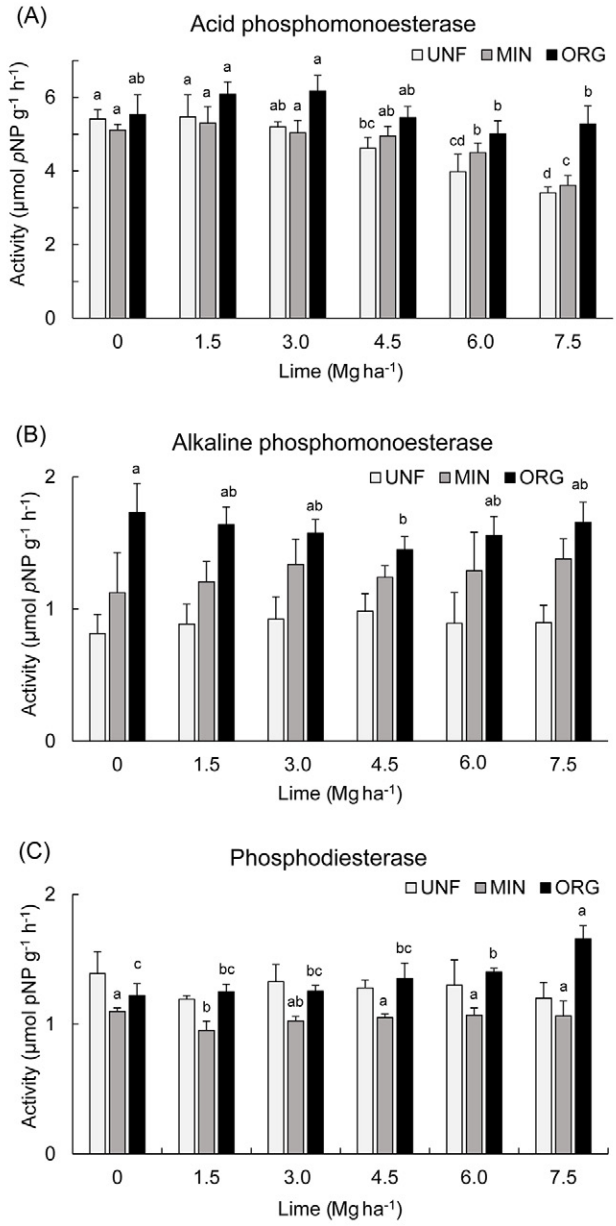

Fig. 3. Activities of P-cycling enzymes (phosphatases) 27 d after lime additions to a Rhodic Kandiudox under different fertilization management systems (21 cropping seasons) from western Kenya. Assays of phosphatase activity included both phosphomonoesterases, with acid (A) and alkaline (B) pH optima, as well as phosphodiesterase (C). Managements were no fertilization (UNF), mineral N and P (60 kg ha^{-1} per season; MIN), and manure (4 Mg ha^{-1} per season; ORG).

decreased markedly at higher rates. In contrast, there were minor or no changes in alkaline phosphomonoesterase/phosphodiesterase by lime rate across managements (Supplemental Fig. S2).

Phosphatase Activities Normalized to P_{mic}

Activities of phosphatases normalized to P_{mic} exhibited management- and enzyme-specific trends across liming gradients and did not necessarily reflect the impacts of liming 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 man-

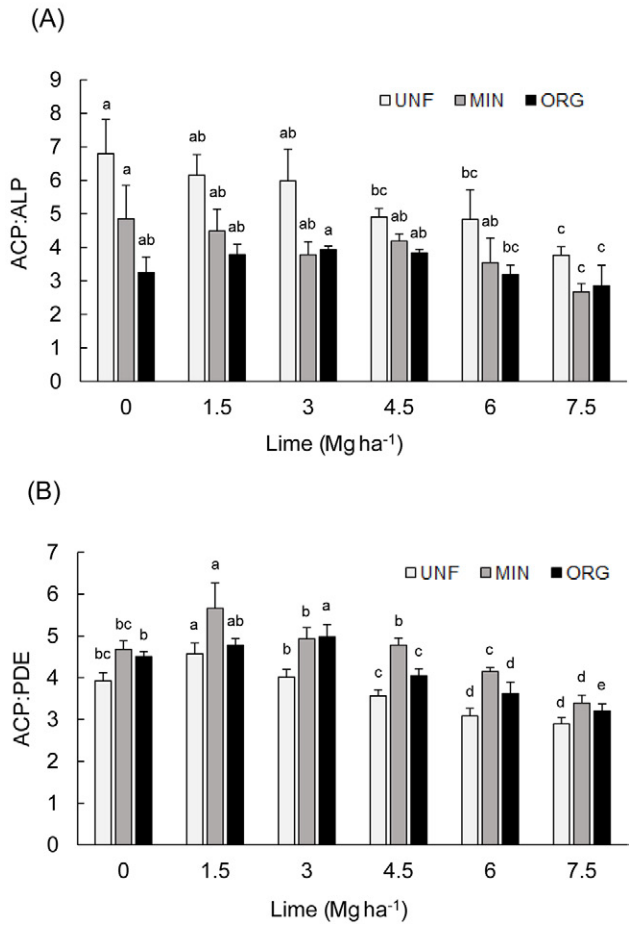

Fig. 4. Ratios of (A) acid phosphomonoesterase (ACP) to alkaline phosphomonoesterase (ALP) activities and (B) ACP to phosphodiesterase (PDE) activities across a Rhodic Kandiudox under different fertilization managements (21 cropping seasons) from western Kenya. Management systems were no fertilization (UNF), mineral N and P (60 kg ha^{-1} per season; MIN), and manure (4 Mg ha^{-1} per season; ORG).

agements (Fig. 3), acid phosphomonoesterase activity per unit of P_{mic} in ORG was similar at 0 and 7.5 Mg lime ha^{-1} and activity decreases in MIN were limited to high lime rates (4 and 7.5 Mg ha^{-1}), though they were similar in magnitude (up to −28%) (Fig. 5A). In UNF, the decrease in acid phosphomonoesterase activity per unit of P_{mic} was greater in magnitude (−54% between 0 and 7.5 Mg lime ha^{-1}) than on a soil basis. Alkaline phosphomonoesterase activity per unit of P_{mic} in ORG increased at high lime rates (7.5 Mg ha^{-1}) compared with no or low lime rates (0–3.0 Mg ha^{-1}), in contrast to activity on a soil basis, which differed between no lime and intermediate lime rates (4.5 Mg ha^{-1}) (Fig. 5B). Though the activity of alkaline phosphomonoesterase on a soil basis was not influenced by lime in MIN, the activity normalized to P_{mic} was elevated under high (7.5 Mg ha^{-1}) compared with low (1.5 Mg ha^{-1}) lime rates. Similar to activities on a soil basis, the P_{mic}–normalized activity of alkaline phosphomonoesterase in UNF was not influenced by lime. Normalizing phosphodiesterase activity to P_{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 of

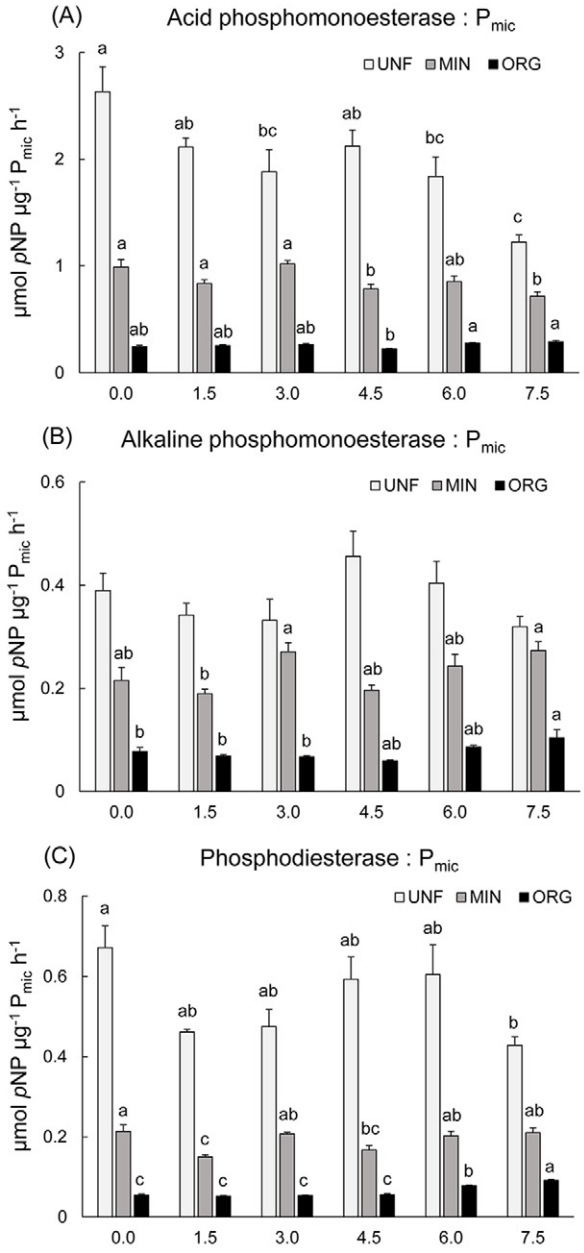

Fig. 5. Activities of P-cycling enzymes (phosphatases) 27 d after lime additions to a Rhodic Kandiudox under different fertilization managements (21 cropping seasons) from western Kenya. Phosphatase activities were normalized to microbial biomass P (P_{mic}), and included (A) acid phosphomonoesterase, (B) alkaline phosphomonoesterase, and (C) phosphodiesterase. Management systems were no fertilization (UNF), mineral N and P (60 kg ha^{-1} per season; MIN), and manure (4 Mg ha^{-1} per season; ORG).

P_{mic} (+68%) than per unit of soil mass (Fig. 5C). The depression of phosphodiesterase activity in MIN at 1.5 Mg ha^{-1} lime compared with other lime rates also occurred for activity normalized to P_{mic}. Across lime rates, phosphatase activities per unit of P_{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_{mic} among managements were greater than those for activities on a soil basis, reflecting differences in P_{mic} among managements (Fig. 2).

In contrast with phosphatase activities on a soil mass basis, phosphatases activities normalized to P_{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 of P_{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 was negatively correlated with P_{mic}–normalized activities of acid phosphomonoesterase ($R = -0.65$, $p = 0.004$) and phosphodiesterase ($R = -0.51$, $p = 0.031$) in UNF and with P_{mic}–normalized activities of acid phosphomonoesterase ($R = -0.70$, $p = 0.0013$) and alkaline phosphomonoesterase ($R = -0.59$, $p = 0.009$) in MIN. In contrast, soil pH in ORG was not correlated with the P_{mic}–normalized activity of acid phosphomonoesterase and was positively correlated with that of alkaline phosphomonoesterase ($R = 0.66$, $p = 0.0026$).

DISCUSSION

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 (UNF > MIN > ORG). These effects probably reflected differences in P saturation caused by varying P inputs (or the lack thereof) over 21 cropping seasons under the previous managements. The 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 soils under other managements, indicate 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 Al toxicity to roots, increasing available Ca and Mg (depending on the lime source), and increasing the availability of micronutrients such as Mo, a possible constraint to biological N fixation in strongly weathered soils (Havlin et al., 2013).

Though a high background of labile P_i under MIN may have masked the effects of lime on available P, increases in AEM-P_i for UNF (+0.3 $\mu g\, g^{-1}$) and ORG (+0.4 $\mu g\, g^{-1}$) 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 the fixation of added P (Kisinyo et al., 2014, 2015) (see also "Implications for P Management in Acid Soils of Western Kenya" below).

Response of Phosphatase 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 the 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 P and P_o 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 4.7 to 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 the activity of individual phosphatases across the range of managements encompassed by the present study. Under some management systems, the activity of phosphatases considered to have acid and alkaline pH optima did not necessarily decrease or increase, respectively, with liming on a soil or P_{mic} basis, which is consistent with evidence that commonly proposed pH optima may be overgeneralizations (Turner, 2010) and suggests an effect of management history 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 the abiotic changes in the activities of enzymes already present in soils expected to occur with pH alteration, such as a mismatch or convergence of soil pH and enzyme pH optima, or desorption of mineral-bound enzymes (Allison, 2006; McLaren et al., 1958). Minor changes in labile P_i suggest 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 the relationships between soil phosphatase activities and phosphatase-encoding gene abundance and/or expression to evaluate how the observed response of phosphatase activity may be caused by changes in the 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 suggest that 11 yr of contrasting input quality and quantity at this site conditioned the response of enzyme activities to liming. For example, though phosphodiesterase activity in unlimed soils was similar across managements, its increased activity under ORG only indicates a difference in the capacity of phosphatase activities to respond to lime as the result of input history. This could

be mediated by (i) P_o substrate loading in soils, (ii) accumulated differences in the amount or characteristics (e.g., pH optima, substrate affinity, and velocity) of phosphatases, and (iii) variation in soil properties known to influence soil enzyme activity (e.g., soil organic C). For example, addition of phosphatase substrates could explain the unique response of phosphatase activity to liming in soils receiving manure (4 Mg ha^{-1} per season), 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, 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 the microbial expression of phosphatases.

Despite strong differences in labile P_i among management (100-fold), 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 superphosphate 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 kg P ha^{-1} yr^{-1}) does not suppress and may even stimulate acid phosphomonoesterase activity (Margenot et al., 2017; Mukuralinda et al., 2011; Radersma and Grierson, 2004).

Impacts of Lime on Biological P Cycling

In the short-term period following liming represented by this study (<4 wk), the general absence of a P_{mic} response and the management-specific changes in phosphatase activities are in mixed support of the hypothesized stimulation of biological P cycling by liming. Constant P_{mic} across a lime-induced pH gradient is not necessarily in conflict with the hypothesized mechanism of increased P availability enabling greater P_{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_{mic} and labile P_o in our short-term study are not inconsistent with reports of increased P_{mic} and decreased soil P_o after 1 to 2 yr following liming (4 Mg ha^{-1}) (Condron and Goh, 1989, 1990). Though a separate study reported a twofold decrease in P_{mic} 8 wk 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 "Phosphorus Sorption and P_{mic}" in the Materials and Methods) would be expected to underestimate P_{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 3 d of $Ca(OH)_2$ addition. This could result from formation and precipitation of P_o–Al complexes as 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., 1993; Haynes, 1984).

Changes in phosphatase activities following lime addition 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 wk of the present study, however, this did not translate to appreciable changes in labile P_o, labile P_i, or P_{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, although the inverse correlation of alkaline phosphomonoesterase and phosphodiesterase activities with labile P_o in soils receiving manure (ORG) supports the hypothesized mineralization of P_o as a result of activity increases for phosphatases with alkaline pH optima, under high input (MIN) and zero input (UNF) managements, labile P_o concentrations were unrelated to phosphatase activities. Since labile P_i and P_{mic} 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 P_i in MIN and UNF is difficult to ascribe to enzyme inhibition by soluble P_i (Nannipieri et al., 2011; Olander and Vitousek, 2000) because increased soil pH could also explain the loss of acid phosphomonoesterase activity (Acosta-Martínez and Tabatabai, 2000; Nannipieri et al., 2011). Because phosphatase activities normalized to P_{mic} were not correlated to labile P_i but were correlated with soil pH, the observed changes in phosphatase activity were unlikely to have resulted from microbial secretion of phosphatases and, as hypothesized, could be driven by changes in pH following liming.

Changes in the ratios of phosphatase activities across managements indicate potential alteration of P cycling via enzymatic mineralization of P_o regardless of pre-lime differences in soil P cycling. The relative decrease in acid phosphomonoesterase compared with alkaline phosphomonoesterase and phosphodiesterase suggests that liming could change the relative roles of phosphatases. As phosphodiesterase is considered to be the first and rate-liming step of P_o mineralization (Turner and Haygarth, 2005), a decrease in acid phosphomonoesterase relative to phosphodiesterase may not necessarily impact P_i 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 P_o mineralization and alter P availability at timescales extending beyond that of the present study. Elevated phosphatase activity per unit of P_{mic} in soils under no P input (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 et al., 2001; Nannipieri et al., 2012). However, soils under ORG had the least phosphatase activity per unit of P_{mic}, despite exhibiting an order of magnitude less available P and P_{mic} than soils under MIN. This indicates that normalizing phosphatase activity to P_{mic} may not necessarily provide an indication of P limitation.

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 Mg 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 (*Glycine max* [L.] Merr.)] (Schlindwein and Gianello, 2008). On the other hand, the high available P under MIN is the result of sustained P inputs at rates (120 kg ha^{-1} yr^{-1}) that are unaffordable for many farmers in western Kenya (Nziguheba et al., 2015), even if they are recommended (see Kenya Agricultural Research Institute, 1994; Kihara and Njoroge, 2013). Although the use of manure at rates in this study is likely to be 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} per season). The 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 to 7.5 Mg 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 that used in many studies in weathered soils in East Africa, which commonly employ rates of ≤2 Mg ha^{-1} (e.g., Okalebo et al., 2009), though yield increases can be obtained at this or lower rates in western Kenya (One Acre Fund, 2015, 2016).

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 four weeks of a liming event, soils with P deficiency experienced significant relative increases in available P that were insufficient in magnitude to alleviate deficiency. Microbial biomass P was largely unaffected by liming and was an order of magnitude greater in soils receiving inorganic N and P inputs than in soils with no inputs or with manure additions at low, albeit regionally realistic, rates (4 Mg 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_{mic} did not match the liming response of phosphatase activities, which were strongly influenced by management history. Soils that received manure over the previous 11 yr showed a distinct phosphatase response to liming compared with soils with zero or high inputs. Since the 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 the longer-term response of P cycling to commonly practiced lime rates under field conditions.

SUPPLEMENTAL MATERIAL

Supplemental Table S1 shows general soil properties. Supplemental Fig. S1 shows recovery of inorganic P spikes across liming gradients. Supplemental Fig. S2 shows the ratio of alkaline phosphomonoesterase (ALP) to phosphodiesterase (PDE) activities across liming gradients.

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