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 $CaCO_3$ ha^{-1}) in soil mesocosms to establish a pH gradient from 4.7 to 6.4. After 27 days, labile inorganic P (P_i) fractions increased by up to 1.2 μg g^{-1} in response to lime application. Labile organic P (P_o) was weakly and inconsistently affected by liming. In MIN, microbial biomass P (P_{mic}) 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_{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_{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_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 (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 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,

70 Rousk, et al., 2010, Rousk, et al., 2009). This could explain elevated pulses of soil respiration
71 following liming (Haynes and Swift, 1988) and increases in microbial biomass carbon across
72 lime-induced pH gradients in multi-year field experiments (Acosta-Martínez and Tabatabai,
73 2000, Ekenler and Tabatabai, 2003), though the response of P_{mic} in such studies was not
74 measured.

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

85 Changes in soil pH following a liming event are relatively rapid compared to the
86 multiseason time scales at which field studies have identified changes in soil phosphatases
87 (Acosta-Martínez and Tabatabai, 2000, Ekenler and Tabatabai, 2003). Given the pH-sensitivity
88 of soil phosphatases, it is conceivable that enzyme activities respond rapidly in the post-liming
89 window. While acid phosphomonoesterase activity has been found to respond within several
90 days of liming (e.g., Haynes and Swift, 1988), the response of other phosphatases with alkaline
91 pH optima (alkaline phosphomonoesterase, phosphodiesterase) is not known.

92 Soil management is likely to condition soil phosphatase response to liming because
93 practices such as fertilization are known to influence soil enzyme activities (Bending, et al.,
94 2004, Bowles, et al., 2014, Nannipieri, et al., 2012). For example, additions of manure or
95 inorganic P could influence pre-lime phosphatase activities by altering the amount of enzyme
96 substrate (i.e., P_o) and/or phosphatase production (Acosta-Martínez and Waldrip, 2014). The
97 inverse relationship of phosphatase activity and P availability observed in weathered soils
98 (Olander and Vitousek, 2000) suggests that in conditions of high available P (e.g., P fertilization)
99 alteration of phosphatase activities by liming may have a relatively lesser impact than changes in
100 abiotic controls (e.g., P-fixation) on P availability. Conversely, under conditions of soil P
101 scarcity, in which a greater proportion of available P is thought to be derived from phosphatase
102 mineralization of P_o (Oberson, et al., 1999, Oberson, et al., 2011), changes to phosphatase
103 activities by liming could have a substantial impact on P availability.
104 To address these knowledge gaps, we evaluated the short-term (<1 month) post-liming
105 response of enzymatic and microbial components of P cycling. To test potential effects of
106 management, we selected soils from fertilization treatments of zero input, low input (manure),
107 and high input (mineral fertilizer) from a long-term field trial (11 years) in western Kenya.
108 Across liming gradients established in soil mesocosms, we hypothesized (1) improved P
109 availability (decreased P sorption, increased labile P_i,); (2) increased P_{mic} with soil pH elevation;
110 (3) changes in activities of acid phosphomonoesterase, alkaline phosphomonoesterase,
111 phosphodiesterase reflective of phosphatase-specific pH optima; and (4) a significant effect of
112 management history on P_{mic} and phosphatase activity response to lime.
113
114 **2. Materials and Methods**

115 *2.1. Soil management and sampling*

116 Soils from a long-term integrated soil fertility management (ISFM) trial in western Kenya
117 were used to test the hypothesized effect of management history on biological P cycling response
118 to liming. The trial was established in 2003 near Sindindi in Siaya County, Kenya (34°24'13.7"E,
119 00°08'38.3"N) at an elevation of 1330 m above sea level. The region experiences a mean annual
120 temperature of 22.5 °C and a historical mean annual precipitation of 1780 mm distributed over
121 two rainy seasons: a short rain (September – November) and a long rain (March – June)
122 (Sommer, et al., 2018). The soil is classified as a Typic Kandiudox (USDA) or Haplic Ferralsol
123 (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
124 (Jelinski, unpublished).

125 Three soil fertility managements were selected to evaluate liming effects on soil P
126 cycling: (1) an unfertilized control (0 kg N, P ha^{-1} $season^{-1}$; UNF); (2) mineral N (60 kg ha^{-1}
127 $season^{-1}$ as urea) and P (60 kg ha^{-1} $season^{-1}$ as triple super phosphate; MIN); (3) and bovine
128 manure (4 t ha^{-1} $season^{-1}$) sourced from surrounding homesteads (ORG). Inputs were applied
129 twice per year, for the short and long rainy season. Manure sampled in 2014 had 0.69% N and
130 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
131 are common for manure produced on smallholder homesteads in western Kenya (Sommer, et al.,
132 2018, Waithaka, et al., 2007), and likely results from local manure harvest and storage practices
133 such as inadvertent mixing of manure with soil scraped from the farmyard surface during
134 collection (Lekasi, et al., 2003) and exposed storage of manure (Tittonell, et al., 2010).

135 These 3 selected treatments represent fertility management scenarios of zero input (UNF)
136 and low input (ORG) that are prevalent in western Kenya due to resource limitation (Tittonell
137 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_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 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 $$\text{Microbial biomass P } (P_{mic}) = \frac{\text{fumigated P} - \text{nonfumigated P}}{\text{P spike recovery}}$$

206 The recovery of the P_i spike was used as an indicator of P sorption (i.e., greater % recovery =
207 lower P sorption potential) (Sims, 2000). Also interpretable as a single-point sorption, this
208 method has been used to estimate P-fixation potential in weathered soils (Fox and Kamprath,
209 1970, Henry and Smith, 2003, Sims, 2000).

210 *2.6. Phosphatase activities*

211 Activities of acid phosphomonoesterase (Enzyme Commission 3.1.3.2), alkaline
212 phosphomonoesterase (EC 3.1.3.1), and phosphodiesterase (EC 3.1.4.1) were assayed as
213 described by Tabatabai (1994). Assays were performed in duplicate using 1 g of air-dried soil
214 incubated for 1 h (37 °C) in 5 mL of modified universal buffer (MUB) at pH 6.5 for acid
215 phosphomonoesterase and pH 11.0 for alkaline phosphomonoesterase, and in 5 mL of 0.05 mol
216 L^{-1} Tris (2-amino-2-(hydroxymethyl)-1,3-propanediol) buffer at pH 8.0 for phosphodiesterase.
217 Assays used a final substrate concentration of 0.01 mol L^{-1} per g soil of *para*-nitrophenyl
218 phosphate (acid and alkaline phosphomonoesterase) or *bis*-*para*-nitrophenyl phosphate
219 (phosphodiesterase). Assays were halted by the addition of 4 mL of 0.5 mol L^{-1} NaOH to acid
220 phosphomonoesterase and alkaline phosphomonoesterase assays or 4 mL of 0.1 mol L^{-1} Tris (pH
221 12.0) to phosphodiesterase assays, and 1 mL of 0.5 mol L^{-1} $CaCl_2$. Centrifugation (2,113 × *g*, 5
222 min) was used to remove sediment and *para*-nitrophenol (*p*NP) in the clear supernatant was
223 quantified colorimetrically (410 nm). Mean absorbance of triplicate negative controls (no soil +
224 substrate) was subtracted from the absorbance of soil assays. Phosphatase activities were
225 expressed in three ways:

226 (1) Activities of individual phosphatases (i.e., μmol *p*NP g^{-1} soil h^{-1}).

227 (2) Activity ratios of phosphatases, in order to evaluate relative changes in phosphatases
228 involved in different steps of P_o mineralization (e.g., mineralization of phosphodiesters vs

229 monoesters) (Turner and Haygarth, 2005). This approach has been used to investigate potential
230 changes in soil P cycling because phosphodiesterase is the first and potentially rate-limiting step
231 of mineralization of P_o (i.e., phosphodiester P forms) (Dick, et al., 2000, Turner and Haygarth,
232 2005, Waldrip and Acosta-Martínez, 2014). Three phosphatase activity ratios were calculated:
233 acid phosphomonoesterase:alkaline phosphomonoesterase, acid
234 phosphomonoesterase:phosphodiesterase, and alkaline phosphomonoesterase:phosphodiesterase.
235 (3) Phosphate activities normalized to microbial biomass P (i.e., μmol *p*NP $\mu g^{-1} P_{mic} h^{-1}$), in order
236 to account for the potential influence of microbial biomass changes on measured response of
237 phosphatase activities (Waldrop, 2000; Turner and Haygarth, 2005; Liu, 2017).

238 *2.8. Statistical analyses*

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

245

246 **3. Results**

247 *3.1. Liming effects on soil pH and recovery of P_i spike*

248 Soil pH increased linearly with lime rate in soils across management histories (R^2 =
249 0.998), furnishing a stepwise pH gradient from 4.7 to 6.4 (Table 1). Recovery of a P_i spike (75
250 μg P g^{-1}) was greater for limed soils but did not necessarily increase linearly across the lime-
251 induced pH gradient (Supplementary Fig. 1). In UNF and ORG, recovery of the P_i spike

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

255 *3.2. Labile P fractions*

256 The relative change in labile P_i increased with lime rate for soils with low labile P_i (UNF)
257 and was least for soils with high labile P_i (MIN) (Fig. 1A, B). Minor but significant increases in
258 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
259 $NaHCO_3$-P_i by 44% (0.9 to 1.2 μg g^{-1}). Soils managed with P inputs showed weak (ORG) or no
260 (MIN) changes in AEM-P_i and $NaHCO_3$-P_i. Irrespective of liming rate, labile P_i was greatest in
261 MIN by 1-2 orders of magnitude compared to UNF and ORG.

262 Labile P_o response to lime depended on rate and management history (Fig. 1C).
263 $NaHCO_3$-P_o was greatest in MIN (24.2 μg g^{-1} at 0 t ha^{-1}) and was unaffected by liming. In UNF,
264 which had the least $NaHCO_3$-P_o (15.5 μg g^{-1}) among managements, labile P_o decreased by a
265 mean of 10.4% at low lime rates (1.5 – 3 t ha^{-1}), but did not significantly affect labile P_o at higher
266 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}
267 at 4.5 t ha^{-1} (pH 5.8), but at higher rates did not differ from the unlimed control.

268 *3.3. P_{mic}*

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

274 *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

298 correlated with labile P_i in MIN (AEM-P_i R = -0.79, $p < 0.0001$; $NaHCO_3$-P_i R = -0.75, $p <$
299 0.0001). In UNF, acid phosphomonoesterase as well as phosphodiesterase activity were also
300 negatively correlated with $NaHCO_3$-P_i (R = -0.32, $p = 0.058$ and R = -0.31, $p = 0.066$,
301 respectively), but acid phosphomonoesterase activity was positively correlated with AEM-P_i (R
302 = 0.31, $p = 0.065$). In ORG, labile P_o was negatively correlated with both alkaline
303 phosphomonoesterase activity (R = -0.62, $p < 0.0001$) and phosphodiesterase activity (R = -0.50,
304 $p = 0.002$).

305 *3.4. Ratios of phosphatase activities*

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

313 *3.5. Phosphatase activities normalized to microbial biomass P*

314 Activities of phosphatases normalized to P_{mic} exhibited management- and enzyme-
315 specific trends across liming gradients and did not necessarily reflect liming impacts on
316 phosphatase activities on a soil mass basis or on phosphatase activity ratios (Fig. 5). For
317 example, though the activity of acid phosphomonoesterase on a soil basis decreased with lime
318 rate across managements (Fig. 3), acid phosphomonoesterase activity per unit P_{mic} in ORG was
319 similar at 0 and 7.5 t lime ha^{-1} and activity decreases in MIN were limited to high lime rates (4
320 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_{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_{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_{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_{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 P_{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_{mic}. Across lime rates, phosphatase activities per unit 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 for activities on a soil basis, reflecting differences in P_{mic} among managements (Fig. 2).

In contrast to 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 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 in UNF 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), 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_{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 (UNF > MIN > 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

367 from lime alone appear to offer a limited contribution to P availability in weathered soils in the
368 short-term. This indicates the necessity of P inputs for weathered soils in this region (Margenot,
369 et al., 2016), the efficiency of which can be improved by the use of lime to decrease fixation of
370 added P (Kisinyo, et al., 2015, Kisinyo, et al., 2014) (see also Section 4.4).

371 *4.2. Phosphatase response to liming*

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

379 Contrary to field studies (Acosta-Martínez and Tabatabai, 2000, Ekenler and Tabatabai,
380 2003), shifts in phosphatase activities with lime-induced pH elevation did not necessarily reflect
381 generally accepted pH optima (e.g., Tabatabai, 2003) depending on management history. For
382 example, strong linear decreases in acid phosphomonoesterase activity and increases in alkaline
383 phosphomonoesterase activity with increasing pH were proposed to reflect enzyme pH optima of
384 6.5 and 11.0, respectively (Acosta-Martínez and Tabatabai, 2000). At our study site, the extent of
385 acid phosphomonoesterase activity decline across the lime-induced pH 4.6 – 6.4 gradient
386 depended on management history, and alkaline phosphomonoesterase activity did not change
387 (UNF, MIN) or did not consistently increase with pH (ORG). Changes in soil pH alone are
388 therefore insufficient to predict changes in activities of individual phosphatases across the range
389 of managements encompassed by the present study. That in some managements the activities of

390 phosphatases considered to have acid and alkaline pH optima did not necessarily change or
391 decreased, respectively, with liming on a soil and/or P_{mic} basis (1) is consistent with evidence
392 that commonly proposed pH optima may be overgeneralizations (Turner, 2010) and (2) suggests
393 an effect of management on phosphatase type (e.g., isozymes of differing pH optima).

394 There are several potential explanations for the strong influence of input history on the
395 short-term response of soil phosphatase activities to lime. Changes in phosphatase activities
396 could reflect abiotic changes in activities of enzymes already present in soils expected to occur
397 with pH alteration, such as mismatch or convergence of soil pH and enzyme pH optima, or
398 desorption of mineral-bound enzymes (Allison, 2006, McLaren, et al., 1958). Minor changes in
399 labile P_i suggests that potential inhibition of phosphatase activity and/or production (Nannipieri,
400 et al., 2011) were likely minimal, especially given that increases in available P do not necessarily
401 suppress soil phosphatase activity (Margenot, et al., 2017). Future work should examine
402 relationships between soil phosphatase activities and phosphatase-encoding gene abundance
403 and/or expression in order to evaluate how observed response of phosphatase activity may be due
404 to changes in microbial expression of phosphatases (Fraser, et al., 2015, Lagos, et al., 2016, Luo,
405 et al., 2017).

406 Given the same lime rates and matching pH gradients, differences in phosphatase
407 activities by management history suggests that 11 years of contrasting input quality and quantity
408 at this site conditioned the response of enzyme activities to liming. For example, though
409 phosphodiesterase activities in unlimed soils were similar across managements, the increase in
410 phosphodiesterase unique to ORG indicates a difference in the capacity of phosphatase activities
411 to respond to lime as the result of input history. This could be mediated by (1) P_o substrate
412 loading in soils, (2) accumulated differences in the amount or characteristics (e.g., pH optima,

substrate affinity [K_m], velocity [V_{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 t ha^{-1} $season^{-1}$), 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 (10^2), 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 kg P ha^{-1} 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_{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_{mic}

436 across a lime-induced pH gradient is not necessarily in conflict with the hypothesized
437 mechanism of increased P availability enabling greater P_{mic}, because labile P_i showed only minor
438 increases and there were minor or no changes in labile P_o with liming.

439 Weak or absent changes in P_{mic} and labile P_o in our short-term study are not inconsistent
440 with reports of increased P_{mic} and decreased soil P_o 1-2 years following liming (4 t ha^{-1})
441 (Condron and Goh, 1989, Condron and Goh, 1990). Though a separate study reported a 2-fold
442 decrease in P_{mic} 8 weeks after $Ca(OH)_2$ addition, which increased soil pH from 5.5 to 6.1 – 6.7
443 (Haynes and Swift, 1988), the lack of correction for P sorption (see Section 2.6) would be
444 expected to underestimate P_{mic} in the unlimed control. Additionally, such approaches measure
445 net changes in an operationally defined P_o fraction rather than directly quantifying P_o
446 mineralization (e.g., Bünemann, 2015). The use of extractions to monitor liming effects on P_o
447 risks artifacts from alteration of P_o solubility. For example, Halstead, et al. (1963) measured high
448 reductions in $NaHCO_3$-P_o (-44%) and NaOH-P_o (-38%) concomitant with increases in P_i
449 fractions within three days of $Ca(OH)_2$ addition. This could result from formation and
450 precipitation of P_o – Al complexes following a result of the flush of Al^{3+} from the exchange
451 complex and the low solubility of Al^{3+} at pH > 5.5 (Condron and Goh, 1990, Condron, et al.,
452 1993, Haynes, 1984).

453 Changes in phosphatase activities following lime additions support the hypothesized
454 potential of lime to impact soil P cycling because phosphatase activity assays measure potential
455 maximum rates of enzymatic mineralization of P_o (Kruse, et al., 2015). In the <4 weeks of the
456 present study, however, this did not translate to appreciable changes in labile P_o, labile P_i, or P_{mic}.
457 That relationships among labile P_o and phosphatase activities were specific to management
458 history indicates that management can condition the response of biological soil P cycling to

459 liming events. For example, while the inverse correlation of alkaline phosphomonoesterase and
460 phosphodiesterase activities with labile P_o in soils receiving manure (ORG) supports the
461 hypothesized mineralization of P_o due to activity increases for phosphatases with alkaline pH
462 optima, under high input (MIN) and zero input (UNF) managements, labile P_o concentrations
463 were unrelated to phosphatase activities. Since labile P_i and P_{mic} were weakly or not affected by
464 liming, microbial P demand was unlikely to have influenced phosphatase activity (e.g., secretion
465 of phosphatases to scavenge P). The negative correlation of acid phosphomonoesterase activity
466 and labile P_i in MIN and UNF is difficult to ascribe to enzyme inhibition by soluble P_i
467 (Nannipieri, et al., 2011, Olander and Vitousek, 2000) because increased soil pH could also
468 explain loss of acid phosphomonoesterase activity (Acosta-Martínez and Tabatabai, 2000,
469 Nannipieri, et al., 2011). Because phosphatase activities normalized to P_{mic} were not correlated to
470 labile P_i but were correlated with soil pH, observed changes in phosphatase activity (1) were
471 unlikely to have resulted from microbial secretion of phosphatases and (2) as hypothesized, can
472 be driven by changes in pH following liming.

473 Changes in ratios of phosphatase activities across managements indicate potential
474 alteration of P cycling via enzymatic mineralization of P_o regardless of pre-lime differences in
475 soil P cycling. The relative decrease in acid phosphomonoesterase compared to alkaline
476 phosphomonoesterase and phosphodiesterase suggests that liming could change the relative roles
477 of phosphatases. As phosphodiesterase is considering the first and rate-liming step of P_o
478 mineralization (Turner and Haygarth, 2005), a decrease in acid phosphomonoesterase relative to
479 phosphodiesterase may not necessarily impact P_i mineralization. On the other hand, given that
480 the magnitude of acid phosphomonoesterase activity was at least twice that of alkaline
481 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 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_{mic}, despite exhibiting an order of magnitude less available P and P_{mic} compared to soils under MIN. This indicates that normalizing phosphatase activities to P_{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

505 mesocosms corresponded to field applications of 1.5 – 7.5 t ha^{-1}, with pH increasing to the
506 threshold of maximum P availability (pH 6.4) only at the highest rate. This rate is higher than
507 employed in many studies in weathered soils in East Africa, which commonly employ rates ≤ 2 t
508 ha^{-1} (e.g., Okalebo, et al., 2009), though yield increases can be obtained at this or lower rates in
509 western Kenya (Fund, 2015, Fund, 2016).
510

511 **5. Conclusion**

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

528 should examine longer-term response of P cycling to commonly practiced lime rates under field
529 conditions.
530
531 **Acknowledgements**
532 This trial was made possible with the financial support of Africa Students Research
533 Network (AfNET), as well as the CGIAR Research Program on Water, Land and Ecosystems,
534 WLE. A.J.M was supported by a US Borlaug Research Fellowship in Global Food Security and a
535 Henry A. Jastro Award from the Soils and Biogeochemistry Graduate Group at the University of
536 California Davis. We thank CIAT staff, in particular John Mukalama, Evonne Oyugi, Francis
537 Njenga and Margaret Muthoni for providing field and technical support, as well as Prof. Nic
538 Jelinski (University of Minnesota-Twin Cities) for soil profiling.
539
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844 **Figure 1:** Labile inorganic and organic phosphorus (P) fractions 27 days after lime additions to a
845 Typic Kandiudox under differing fertilization managements (21 cropping seasons) from western
846 Kenya. Managements were no fertilization (UNF), mineral N and P (60 kg ha^{-1} $season^{-1}$; MIN),
847 and manure (4 t ha^{-1} $season^{-1}$; ORG). Labile fractions include (A) anion-exchange membrane
848 (AEM) extractable P_i; (B) sodium bicarbonate extractable P_i and (C) sodium bicarbonate
849 extractable P_o.
850
851 **Figure 2:** Microbial biomass phosphorus 27 days after lime additions to a Typic Kandiudox
852 under differing fertilization managements (21 cropping seasons) from western Kenya.
853 Managements were no fertilization (UNF), mineral N and P (60 kg ha^{-1} $season^{-1}$; MIN), and
854 manure (4 t ha^{-1} $season^{-1}$; ORG).
855
856 **Figure 3:** Activities of P-cycling enzymes (phosphatases) 27 days after lime additions to a Typic
857 Kandiudox under differing fertilization managements (21 cropping seasons) from western
858 Kenya. Assays of phosphatase activities included both phosphomonoesterases, with acid (A) and
859 alkaline (B) pH optima, as well as phosphodiesterase (C). Managements were no fertilization
860 (UNF), mineral N and P (60 kg ha^{-1} $season^{-1}$; MIN), and manure (4 t ha^{-1} $season^{-1}$; ORG).
861
862 **Figure 4:** Ratios of (A) acid phosphomonoesterase (ACP) to alkaline phosphomonoesterase
863 (ALP) activities and (B) ACP to phosphodiesterase (PDE) activities across a Typic Kandiudox
864 under differing fertilization managements (21 cropping seasons) from western Kenya.
865 Managements were no fertilization (UNF), mineral N and P (60 kg ha^{-1} $season^{-1}$; MIN), and
866 manure (4 t ha^{-1} $season^{-1}$; ORG).

867

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

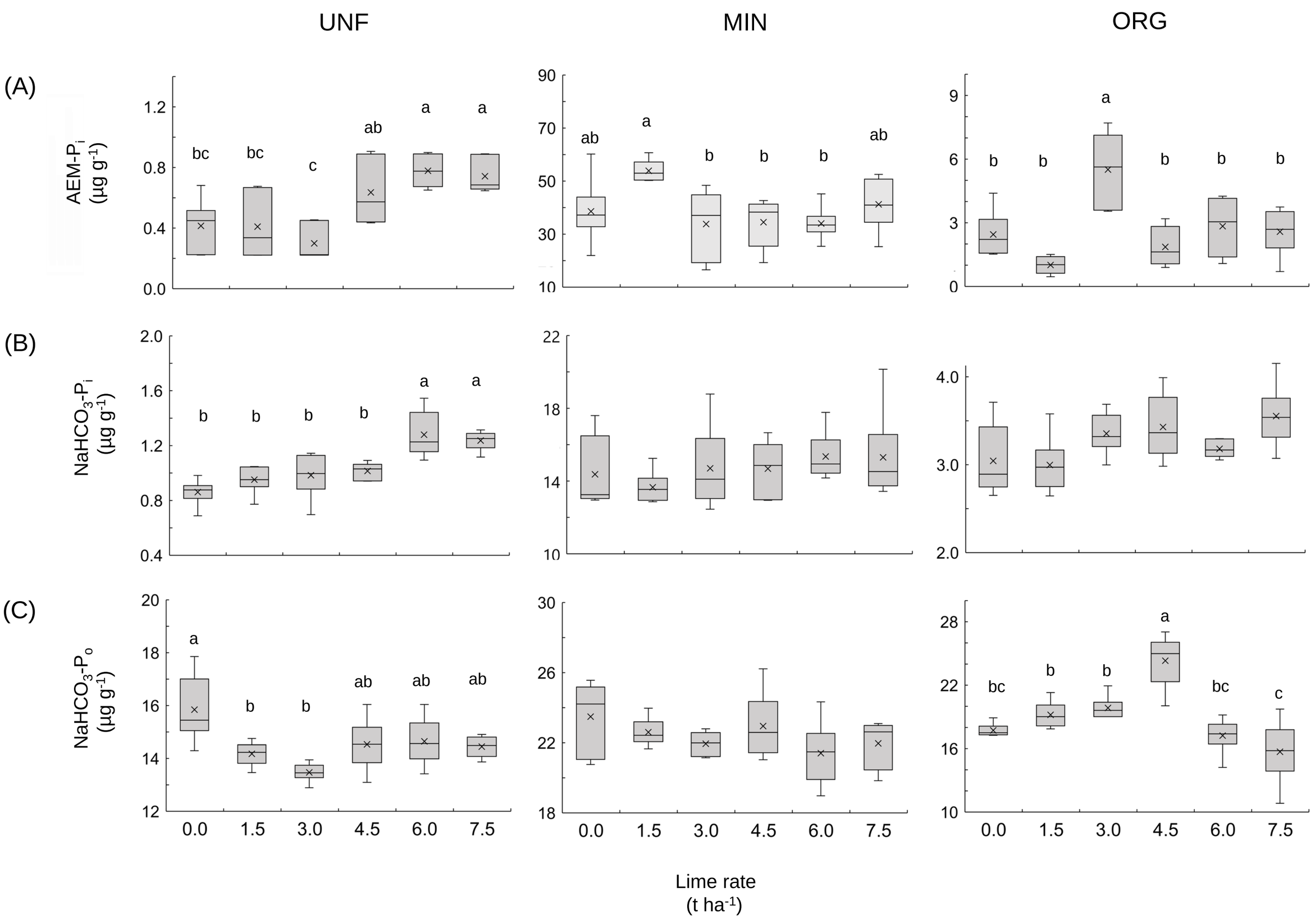
874

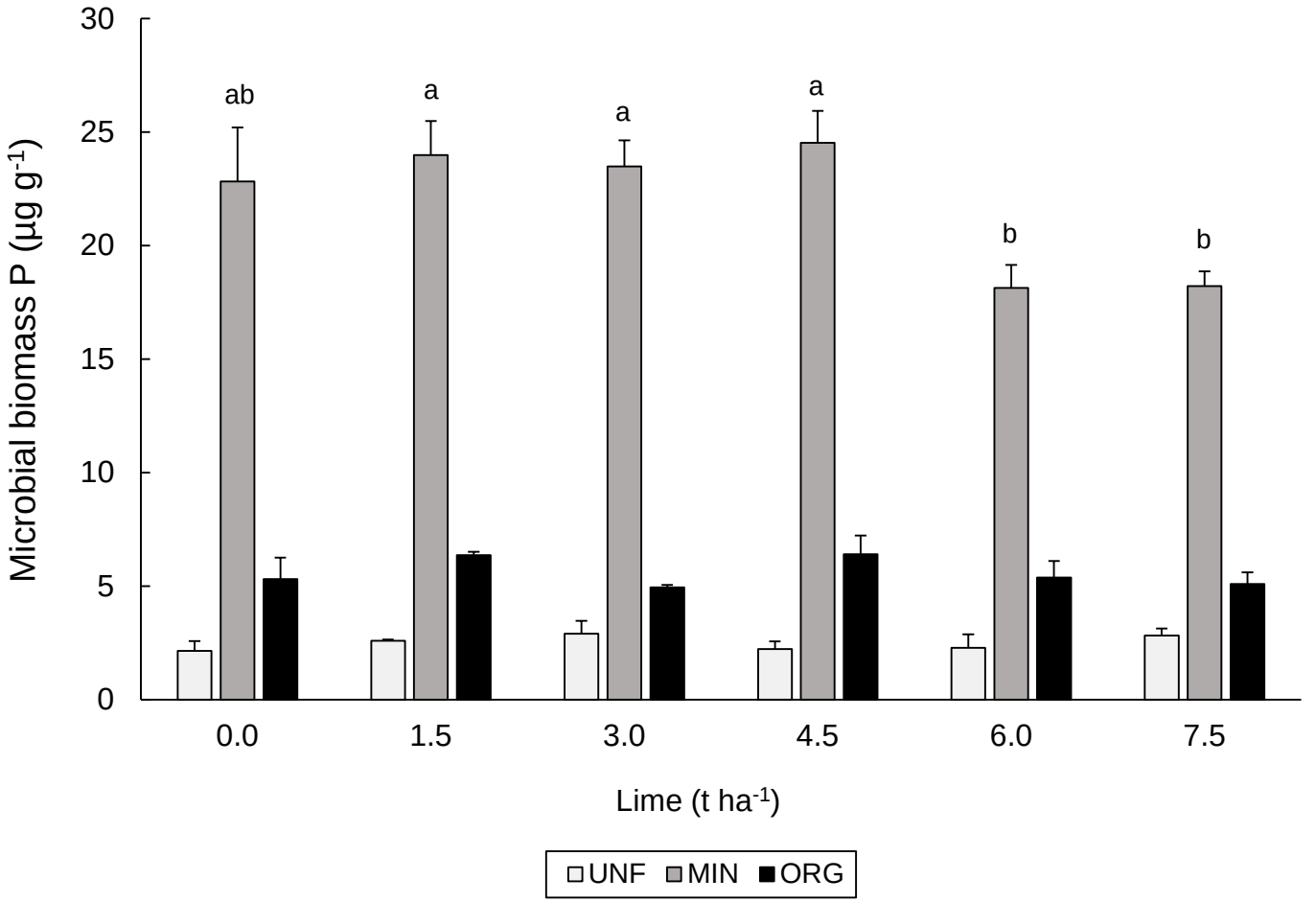
875
876

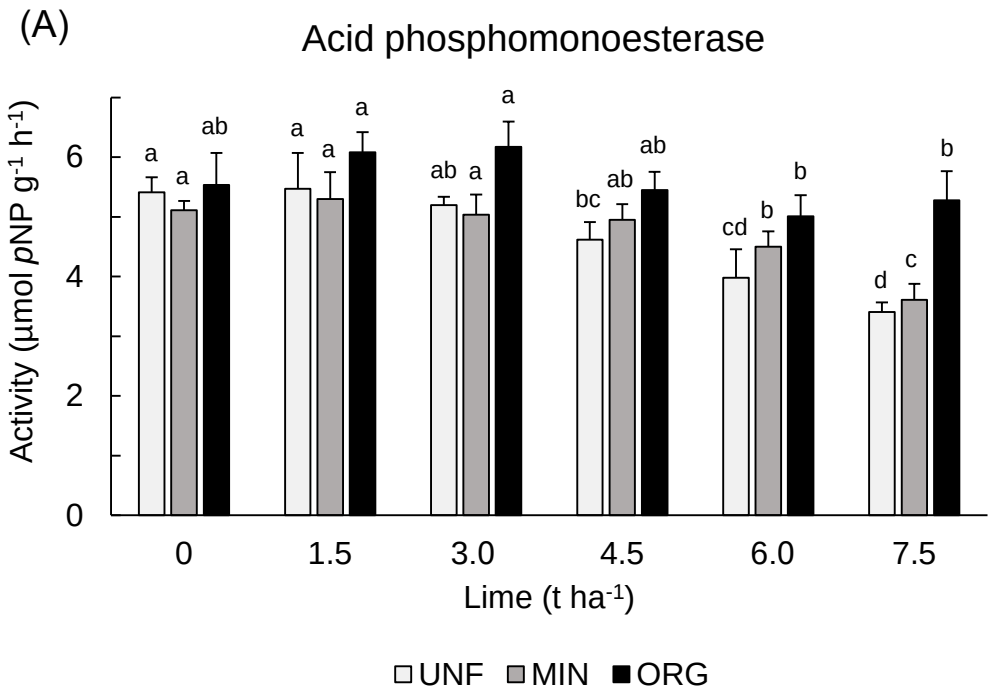
877 **Table 1.** Soil pH (1:2 in water) across a liming gradient in a Typic Kandiudox under
878 differing fertilization managements (21 cropping seasons) from western Kenya 27 days
879 after addition of $Ca(OH)_2$. Lime rates were calculated using the Mehlich buffer liming
880 requirement and bulk densities at the field trial to the estimated depth of incorporation
881 (0-15 cm). Managements were no fertilization (UNF), mineral N and P (60 kg ha^{-1}
882 $season^{-1}$; MIN), and manure (4 t ha^{-1} $season^{-1}$; ORG). Mean pH values are presented.
883 Standard error was ≤ 0.02 for all mean values.

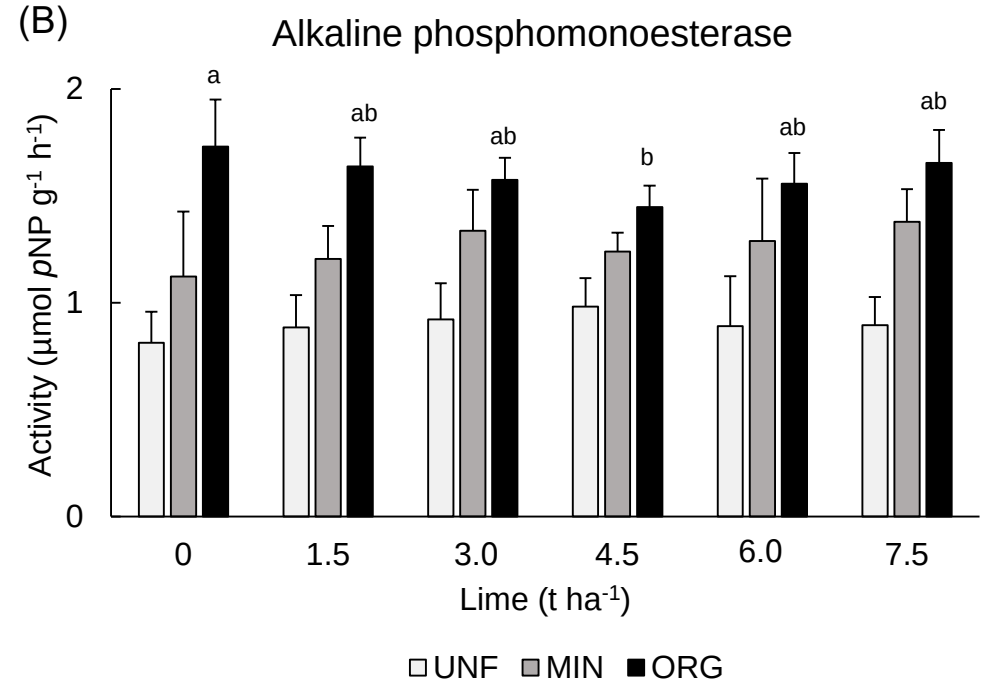
	Lime application (t ha^{-1})					
Management	0	1.5	3.0	4.5	6.0	7.5
UNF	4.73	5.03	5.37	5.73	6.12	6.44
MIN	4.69	4.94	5.31	5.64	6.04	6.35
ORG	4.79	5.08	5.43	5.79	6.18	6.48

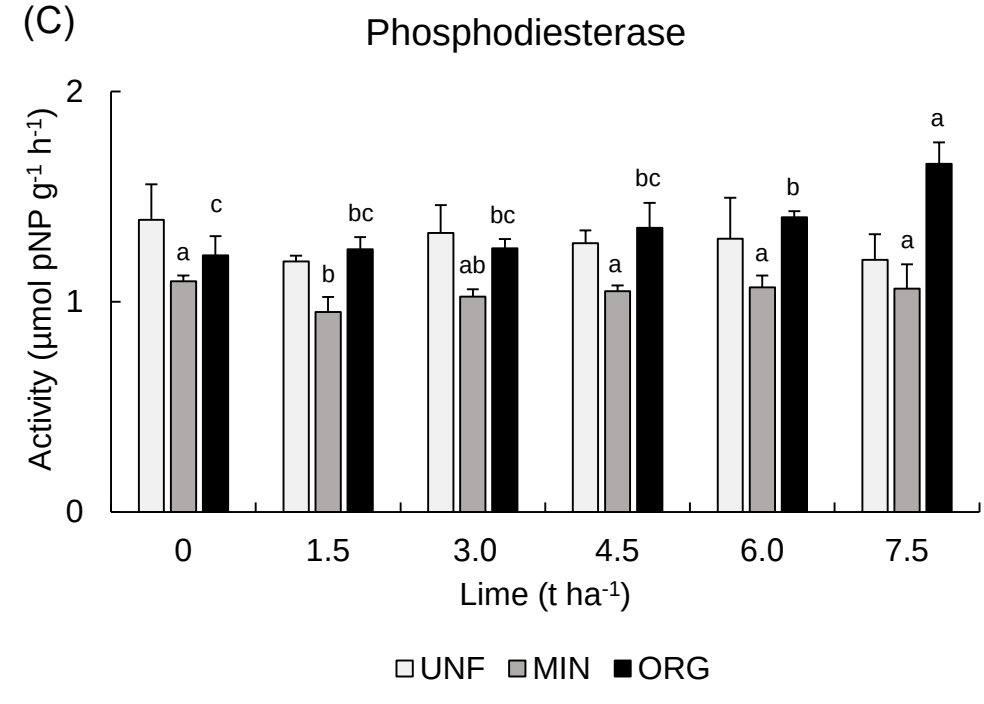
884

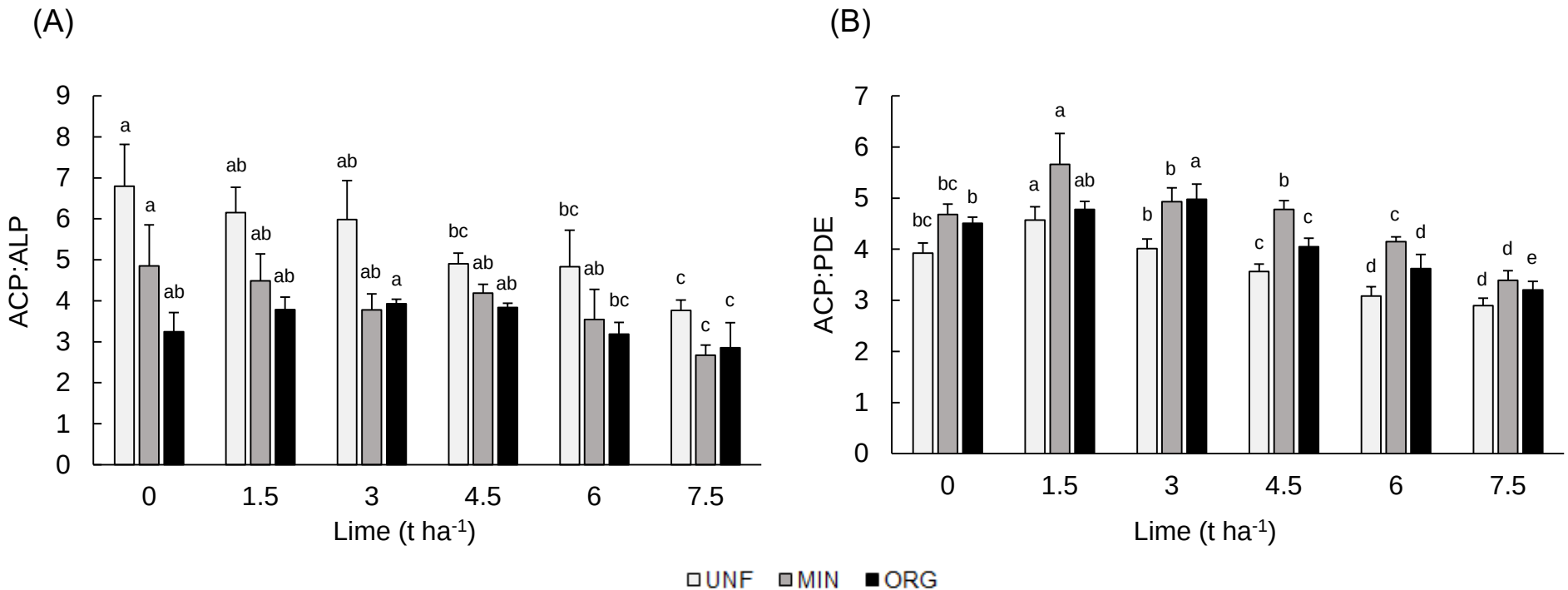

UNF
MIN
ORG
(A)
AEM-Pi (µg g-1)
(B)
NaHCO3-Pi (µg g-1)
(C)
NaHCO3-Po (µg g-1)
Lime rate (t ha-1)
0.0
1.5
3.0
4.5
6.0
7.5

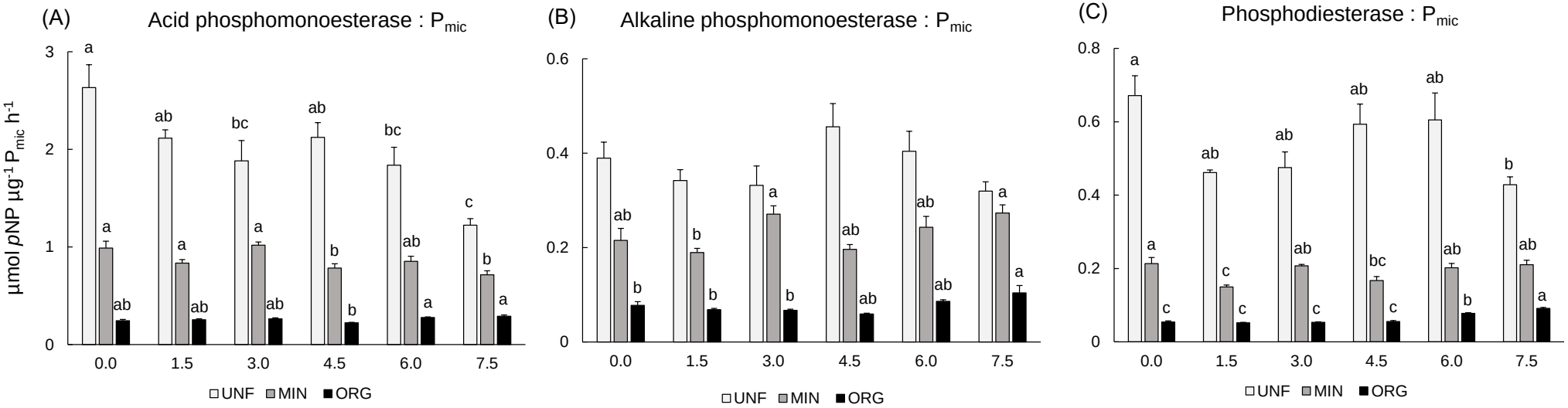

Microbial biomass P (µg g-1)
30
25
20
15
10
5
0
ab
a
a
a
b
b
0.0
1.5
3.0
4.5
6.0
7.5
Lime (t ha-1)
UNF
MIN
ORG


(A)
Acid phosphomonoesterase
Activity (µmol pNP g-1 h-1)
0
2
4
6
a a ab
a a a
ab a a
bc ab ab
cd b b
d c b
0 1.5 3.0 4.5 6.0 7.5
Lime (t ha-1)
UNF MIN ORG


(B)
Alkaline phosphomonoesterase
Activity (µmol pNP g-1 h-1)
0
1
2
a ab ab b ab ab
0 1.5 3.0 4.5 6.0 7.5
Lime (t ha-1)
UNF MIN ORG

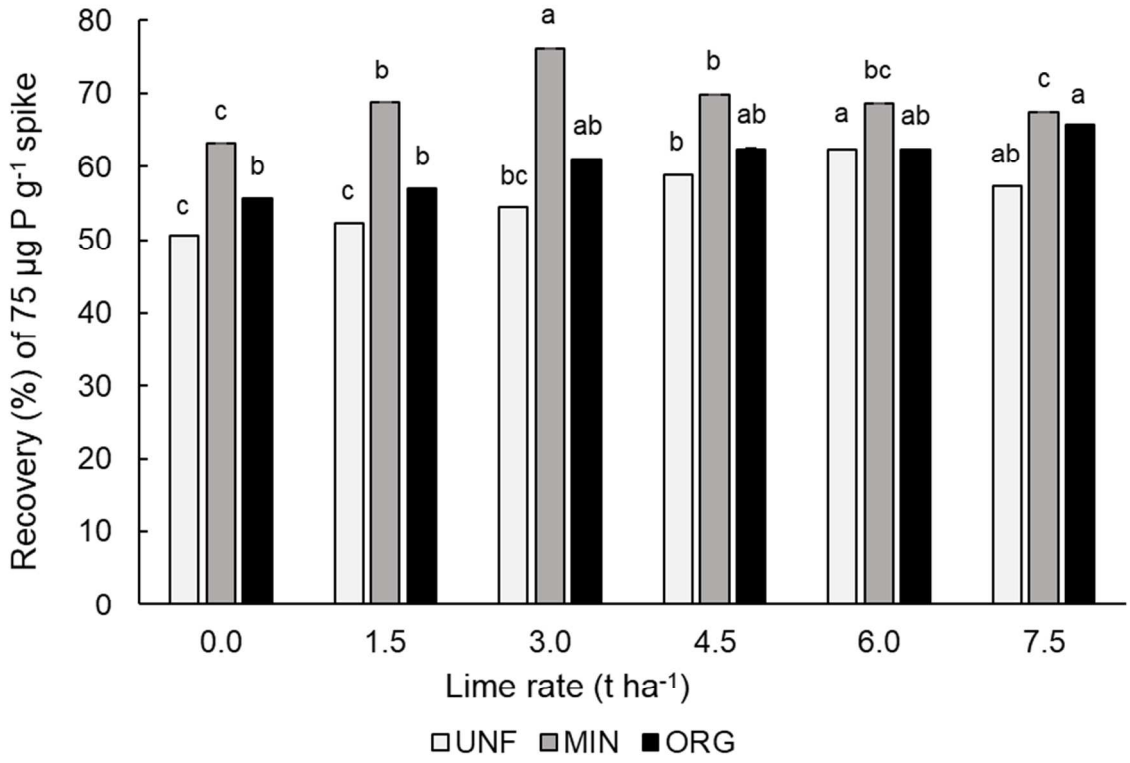

(C)
Phosphodiesterase
Activity (µmol pNP g-1 h-1)
0
1
2
c a bc b bc ab bc a bc a b a a a
0 1.5 3.0 4.5 6.0 7.5
Lime (t ha-1)
UNF MIN ORG


(A)
ACP:ALP
Lime (t ha-1)
(B)
ACP:PDE
Lime (t ha-1)
UNF
MIN
ORG

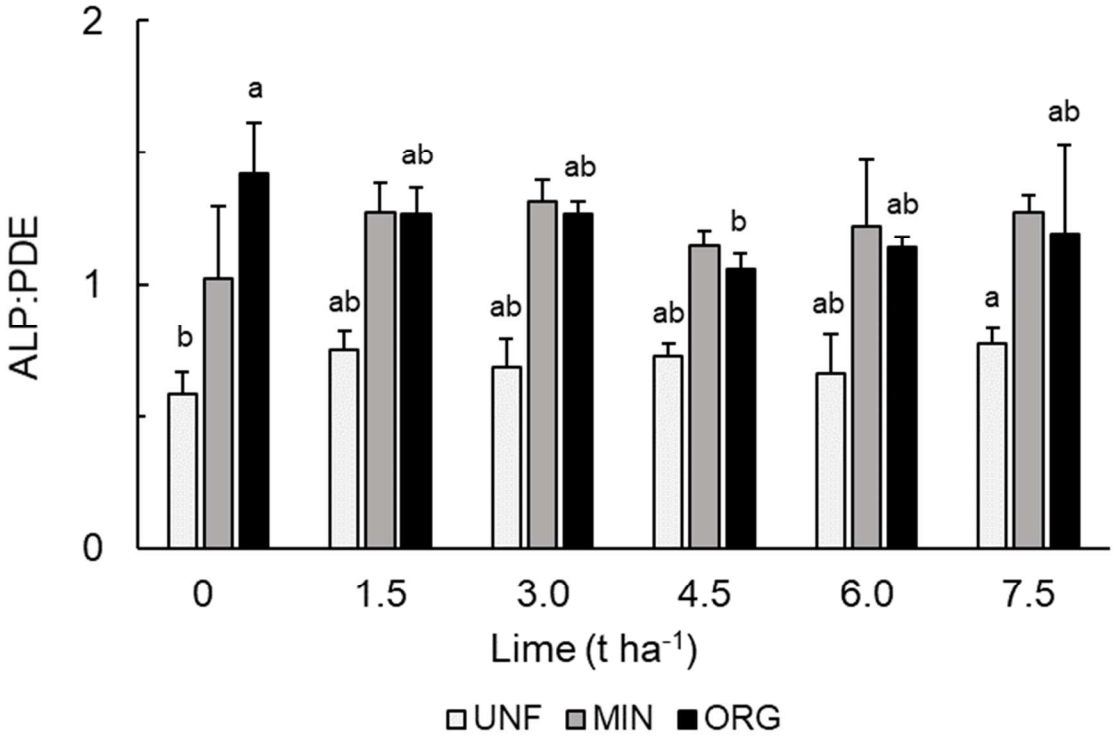

(A)
Acid phosphomonoesterase : P mic
µmol pNP µg-1 Pmic h-1
3
2
1
0
a
a
ab
ab
a
ab
bc
a
ab
ab
b
b
bc
ab
a
c
b
a
0.0
1.5
3.0
4.5
6.0
7.5
UNF MIN ORG
(B)
Alkaline phosphomonoesterase : P mic
0.6
0.4
0.2
0
ab
b
b
b
a
b
ab
ab
ab
a
a
0.0
1.5
3.0
4.5
6.0
7.5
UNF MIN ORG
(C)
Phosphodiesterase : P mic
0.8
0.6
0.4
0.2
0
a
a
c
ab
c
c
ab
ab
c
bc
c
ab
ab
b
b
ab
a
0.0
1.5
3.0
4.5
6.0
7.5
UNF MIN ORG

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, $p < 0.05$)

Management	Inputs	pH (1:2 water)			Ex. acidity (m_{eq} 100 g^{-1})			SOC (mg g^{-1})		
		mean	se		mean	se		mean	se	
UNF	none	4.76	0.02	a	3.24	0.13	a	15.8	0.3	b
MIN	60 kg N, 60 kg P ha^{-1} $season^{-1*}$	4.74	0.02	a	3.86	0.26	a	19.2	0.4	a
ORG	4 t manure ha^{-1} $season^{-1**}$	4.77	0.04	a	3.50	0.42	a	18.4	0.1	a

*As urea and triple super phosphate

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