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ORIGINAL ARTICLE

Minjingu phosphate rock applications increase the population of phosphate solubilising microorganisms with a positive impact on crop yields in a Kenyan Ferralsol

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Abstract Soil microbes such as plant growth promoting rhizobacteria play significant roles in the solubilisation of inorganic phosphorus (P), mineralization of organic P and in improving plant P uptake. It is known that phosphate solubilising microorganisms (PSM) populations largely vary depending on the ecosystems, the cropping systems or the soil management. The capacity of Minjingu phosphate rock (PR) to enhance the populations of native PSM under three cereal–legume rotation systems was assessed in the third season of rotation. Triple super phosphate (TSP) was used as a positive control. In comparison to the negative control, application of Minjingu PR increased the total fungal diversity and phosphate solubilising bacteria (PSB) population by 67–90 % while high rates of TSP significantly ($p < 0.05$) reduced bacterial diversity and populations of PSB by 46–69 %. Minjingu PR also resulted in both crop and legume yields increase (41–104 % compared to the control), which were similar to those obtained with TSP application. Cropping systems incorporating sparingly soluble P sources such as Minjingu PR into soils can stimulate the populations of native PSB and agronomic productivity. They may represent a promising way of minimizing the utilization of mineral P fertilizers.

Keywords Minjingu phosphate rock · Phosphate solubilising bacteria · Fungal and bacterial diversity · PCR–DGGE

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Introduction

One of the major challenges Africa has been facing is to produce enough food for its growing population. Good quality and optimal production of crops by African smallholder farmers is strongly influenced by ability of soils to supply nutrients, especially phosphorus (P). For example in Tanzania, over 50 % of cultivated soils are estimated to be deficient in P, and the situation is similar in Kenya (Okalebo et al. 2007). The use of chemical fertilizers is often considered to be an immediate solution to correct nutrient deficiencies in soils. However, water-soluble fertilizers are too expensive for poor smallholder farmers. Farmers could benefit more by using phosphate rock fertilizers that are less expensive (Okalebo et al. 2007). Minjingu phosphate rock (Minjingu PR) has high P concentration (25–30 % P_2O_5) and is highly reactive in soils with pH < 5.5 (Okalebo et al. 2007). According to Kalala and Semoka (2010), in 2008–2009, Tanzania provided about 24,000 tons of its local source of phosphate rock fertilizer, Minjingu PR compared to only 2,000 tons of water-soluble phosphatic fertilizers such as triple super phosphate (TSP). Positive agronomic results have been obtained in Tanzania, Kenya and Rwanda showing optimal crop yields from Minjingu PR applications (Msolla et al. 2005; Nabahungu et al. 2007; Onwonga et al. 2008; Kalala and Semoka 2010; Phiri et al. 2010; Onwonga et al. 2013; Nyambati and Opala 2014) with the direct consequence for Minjingu PR to be approved for wide scale use in Eastern Africa. However so far, the impact of Minjingu PR on soil microbial diversity is not well known.

Soil management practices such as tillage and organic and mineral applications are acknowledged as of major importance to soil fertility management. However, the cropping system has to be also considered as several studies reported their various effects on microbial diversity (Kamaa et al. 2011; Kihara et al. 2012). Cropping systems including legumes in rotation with cereals have been shown to induce shifts in the microbial populations which have been attributed to the release of different types and quantities of exudates by plants, and the influence of bacteria associated with the previous crops on the subsequent ones (Alvey et al. 2003; Suman et al. 2006). These modifications of the micro-flora have resulted in a greater bacterial diversity (Alvey et al. 2003; Govaerts et al. 2007; Acosta-Martínez et al. 2008) and improved soil health. On the other hand, Herrmann et al. (2014) didn't note any impact of the crop management including maize–soybean rotation, maize stem applications and nitrogen (N) fertilization on the genetic diversity of native rhizobial strains.

Soil pH is pivotal in determining the availability of inorganic forms of P and it can influence the fate of organic P as well (Hinsinger 2001). Plant roots can increase or decrease the pH of their rhizosphere by up to 2 or 3 pH units but rhizosphere pH changes also occur as a result of microbial activities (Richardson and Simpson 2011). The production of organic acids that are capable of dissolution of insoluble phosphates is a trait found in many microorganisms amongst the so-called P solubilising microorganisms (PSM). The PSM are generally screened in vitro by culture on selective media containing insoluble forms of inorganic P (Pi) and as reported by Khan et al. (2007). Although potential exists for developing inoculants with PSM, their widespread application remains limited by inconsistent performance over a range of environments.

In this study, we examined the impact of crop rotation regimes and application of a reactive Minjingu PR and TSP fertilizers in an acidic soil after three consecutive seasons on crop yields, populations of native PSM and structure of both soil bacterial and fungal communities.

Materials and methods

Site description and soil sampling

The trial was established in 2007 long rains. This on-farm trial was under the management of the African Network for Soil Biology and Fertility program (AfNet) in Sidada location, Siaya County of Western Kenya. The farm is situated at an altitude of 1,310 m above sea level at 0°08′38.9″ North and 34° 25′27.5″ East. The region receives an average rainfall of about 1,400 mm per annum in two rainy seasons, long rains (March to July) and short rains (September–January). Temperatures ranged from 14 to 34 °C (Information given by the Republic of Kenya, 1997). During the trial period, the area received 1488 mm, and temperatures ranging from 15 to 35 °C, which was within the normal range. Before setting up the trial, a composite soil sample was taken (depth 0–30 cm), air-dried and

soil characterization done as described by Okalebo et al. (2002). Soil characteristics were as follows: pH 5.04, Organic C 3.5 %, Total N 0.30 %, Olsen P 3.67 mg P kg^{-1}, Exchangeable Ca 20 cmol kg^{-1}, Exchangeable Mg 9.8 cmol kg^{-1} and Exchangeable K 0.95 cmol kg^{-1}, clay 49 %, silt 20 % and sand 31 %. The soils in the area are predominantly Rhodic Ferralsol (FAO, 2007) with low soil fertility.

Experimental design, soil sampling and crop harvesting

The treatments consisted of two P sources: TSP (Triple Super Phosphate) and Minjingu PR applied at different levels (TSP—0, 12.5, 25 and 50 kg P ha^{-1}; Minjingu PR—0, 25 and 50 kg P ha^{-1}) and three crops: maize-Mz (*Zea mays)*, common bean-CB (*Phaseolus vulgaris*) and soybean-SB (*Glycine max*). The crops were established during the long rainy season 2007. The treatments were as follows: T1—Control; T2—60 kg N + 60 kg K ha^{-1}; T3—12.5 kg P ha^{-1} TSP; T4—12.5 kg P ha^{-1} TSP + 50 kg P ha^{-1} Minjingu PR; T5—25 kg P ha^{-1} TSP + 25 kg P ha^{-1} Minjingu PR; T6—50 kg P ha^{-1} TSP and T7—50 kg P ha^{-1} Minjingu PR. These fertilizer combinations were applied to three rotation regimes: CB–SB–Mz, Mz–CB and SB–Mz in a randomized complete block design replicated four times in plots of 6 by 6 m. To minimize N and K deficiency, urea and muriate of potash were applied at 60 kg ha^{-1} in all treatments except in the negative control (T1). Phosphorus was applied by banding while N was spot-placed around the plant during top dressing. Minjingu PR (powder) was broadcasted in the plots to increase efficiency of the fertilizer. All the fertilizers were applied every season at planting except urea that was split-applied at planting and at top dressing. Maize (DH04), common bean (Rosecoco GLP92) and soybean-SB 20 (TGx 1448-2E) were sowed at plant densities of 53,333, 100,000 and 266,666 plants ha^{-1} respectively. The crops were hand weeded twice within each season. At 6 weeks after emergence, bull dock was applied on the maize kernels to prevent stem borer attack.

Soil sampling was done on each plot at 6 weeks after planting (WAP) in the third season of rotation in all the plots (4 replicates). At this period, (6 WAP), nutrient demand is quite high and plant–microbe interaction is likely to occur to improve nutrient uptake (Fengping et al. 2008). After mixing thoroughly, a subsample was taken for microbial analysis. The sample was put in sterile plastic bags, packed into an icebox and immediately transported to the laboratory.

During harvest, an effective area of 25 m^2 was considered for plant measurements. Maize cobs were harvested and weights recorded. Maize stover was manually cut at soil level and weighed. Common bean and soybean was uprooted, shaken to remove the soil and weighed to determine the total fresh weight of the biomass. The pods were then plucked and weighed. A subsample of the maize cobs, stover and soybean/common bean haulms was taken for threshing and calculation of grain and total dry matter yields, while the rest of the biomass was incorporated into the soil at land preparation. The subsamples were then oven dried at 70 °C for 48 h.

Determination of the structure of the total microbial communities using PCR–DGGE finger printing technique

Total DNA was extracted as described by Porteus et al. (1997). DNA amplification was performed by PCR using primers 338f and 518r that target a fragment of the V3 region of 16S rDNA of the bacteria (Øvreås et al. 1997) while primers 403f and 662r were used for amplification of the 28S rDNA of the fungal communities (Sigler et al. 2001). These primer pairs have been confirmed to anneal to majority of bacterial and fungal sequences respectively (Heuer and Smalla 1997; van Elsas et al. 1998). PCR amplifications were in a final volume of 25 µl which contained 2 µl of DNA template, 200 µM of each dNTP, 10 pmol of each primer and 1 freeze-dried bead (Ready-To-Go PCR beads, Pharmacia Biotech) containing 1.5 U of Taq polymerase, 10 mMTris–HCL (pH 9 at room temperature), 50 mM KCL and 1.5 mM $MgCl_2$. The PCR cycles were performed in an iCycler Biorad thermocycler. The fungal DNA was subjected to an initial denaturation step of 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 1 min at 53.1 °C and 3 min at 72 °C. A final elongation step of 10 min at 72 °C finished the reaction. Amplification of the total bacteria DNA was done as follows: initial denaturation at 94 °C for 2 min, 20 cycles of 94 °C for 30 s, 65–55 °C for 1 min (regular decrement of 0.5 °C every cycle), 72 °C for 1 min, 10 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, and final elongation at 72 °C for 15 min. PCR products were loaded into a 8 %

acrylamide/bis gel (acrylamide/bisacrylamide solution 37.5:1) containing a linear denaturating gradient (40–70 %). A 100 % denaturating solution contains 7 M of urea and 40 % formamide.

Since the Ingeny apparatus (Leiden, The Netherlands) used for band separation accommodates a maximum of 24 samples, three replicates of each of the eight treatments sampled from the field were considered for each gel. Electrophoresis was conducted at a constant voltage of 120 V for 16 h in 1× Tris–Acetate–EDTA (TAE) buffer bath (Tris Base 4.84 g l^{-1}; Acetic acid glacial 0.02 M, EDTA pH = 8.1 mM) maintained at 60 °C.The gels were then stained with 0.265 µg ml^{-1} of ethidium bromide for 30 min and subsequently distained with distilled water for 10 min. The stained gels were scanned with Gel Doc™ XR (Bio-Rad Laboratories), images were captured using Quantity One software version 46.0 (Bio-Rad Laboratories) and band intensities were calculated using Total Lab software (version 120L). Shannon index of diversity (*H*) was calculated as follows:

$$H = -\sum(\mathrm{pi})(\log_e \mathrm{pi})$$

where pi is the proportion of an individual band area relative to the sum of all band areas detected in a sample.

Enumeration of cultivable phosphate solubilising microorganisms

To determine the impact of treatments on the population of PSM, four treatments (T1, T2, T6 and T7) were considered, based on their positive yield responses. Phosphate solubilising microorganisms were isolated from the rhizosphere soils and enumerated as described by Ndung'u-Magiroi et al. (2012) using the National Botanical Research Institute's NBRIP Phosphate media with 5 g l^{-1} of $Ca_3(PO_4)_2$ as source of P. Serial diluted plates were incubated for seven days at 30 °C and only colonies with clear halos were considered for enumeration (Fankem et al. 2006).

Statistical analysis

An analysis of variance of grain yields, Shannon indices (*H*) was performed using the Proc Mixed procedure of SAS program (SAS 2006). The means were separated and compared using Fisher's Protected LSD Test.

Results and discussion

The effect of crop rotation and fertilizer applications on soybean, common bean and maize grain yields is illustrated in Fig. 1. All the treatments with fertilizer applications significantly improved the crop grain yields (legumes and maize) regardless of the crop rotation and season in comparison to either negative control or application of N and K alone. On the other hand, although a statistical effect ($p < 0.05$) of the crop rotation was observed on soybean (Fig. 1a) and maize (Fig. 1c) grain yields (whatever the treatments and the seasons), this was not the case for common bean grain yields. The highest soybean grain yields were noted in the Mz–SB rotation with an increase of 30 % above the CB–SB–Mz regime. Generally, the highest grain yields were observed from treatments where Minjingu PR was applied alone or in combination with TSP (25 kg P ha^{-1} each). These were similar to those obtained with TSP application of 50 kg P ha^{-1}. Although common bean yields were not significantly ($p < 0.05$) enhanced by crop rotation regime, application of Minjingu PR at 50 kg P ha^{-1} alone or in combination with TSP gave significantly higher yields, with increases of 100–120 % and from 210 to 300 % above the negative control in the CB–SB–Mz and Mz–CB regimes respectively (Fig. 1c). The highest maize grain yields were noted after rotation with common bean and soybean consecutively (3.8 t ha^{-1}) and lowest in the SB–Mz rotation (2.8 t ha^{-1}). For comparison, in Tanzania (Sokoine University), Kisethu and Mtakimwa (2013) showed that exclusive application of Minjingu PR to maize resulted in yields of about 2.94 t ha^{-1}. Our results are much higher after rotation with common bean and soybean consecutively and tend to confirm the good performances of rotation cropping systems. Previous reports have reported agronomic benefits from the use of Minjingu PR in East Africa (Msolla et al. 2005; Nabahungu et al. 2007; Kalala and Semoka 2010; Opala et al. 2010; Phiri et al. 2010; Onwonga et al. 2013; Nyambati and Opala 2014). Our study demonstrated that over the seasons, increment in crop yields due to application of Minjingu PR was similar to TSP added at the same rate. It was also shown that Minjingu PR has a longer residual effect in soils (Kalala and Semoka 2010)

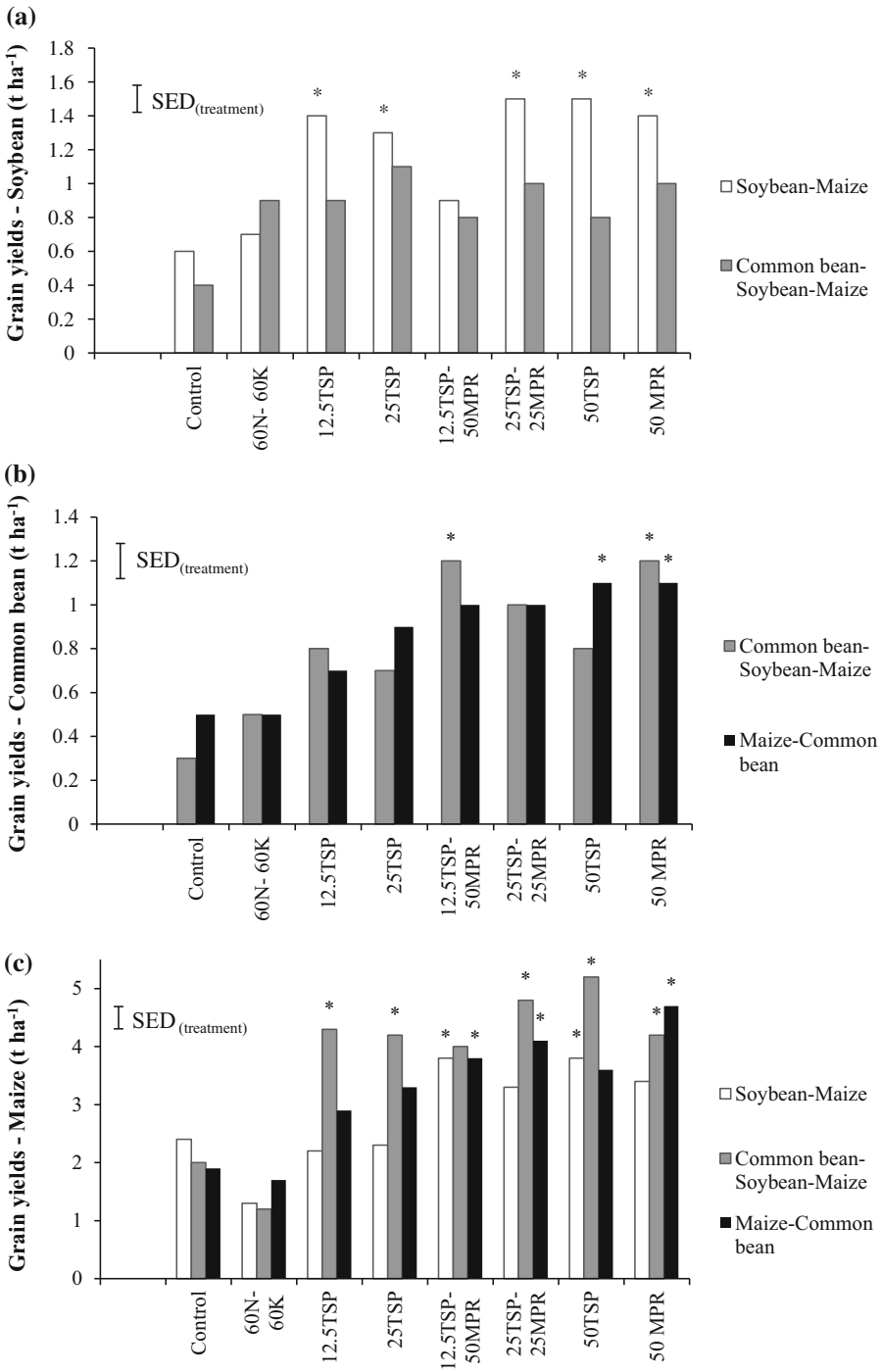

Fig. 1 Effect of fertilizer applications and crop rotation regimes on soybean (**a**), common bean (**b**) and maize yields (**c**) in Siaya County, Western Kenya. The fertilizer rates were added each season as kg P ha^{-1}: *MPR* Minjingu PR, *TSP* triple super phosphate, N—added as Urea (60 kg N ha^{-1}) and K as Muriate of Potash (60 kg K ha^{-1}) in all plots except the negative control. *: significantly higher yields ($p < 0.05$) than the NK control within each rotation regime

and that it was suitable for direct application in the P deficient acidic soil of Western Kenya (Jama and van Straaten 2006). These authors estimated that the cost might be 24–45 % less than P from TSP. Since many small-scale farmers may not afford TSP fertilizers, Minjingu PR could be used as an alternative to expensive and polluting TSP fertilizers in acidic soils without compromising crop yield.

Regression analysis conducted over the three seasons revealed that for every 1 kg increase in crop yield from the recommended fertilizers (TSP) there was a concomitant increase in yield from the use of Minjingu PR of 0.4 kg in maize, 0.9 kg in soybean and 0.7 kg in common bean, implying a higher response of Minjingu PR application in legumes than maize (results not shown). This may be explained by the fact that legumes are more sensitive to acidity and aluminium toxicity than cereals. The double effect of liming and provision of P from the use of Minjingu PR in our acidic soil may probably have improved more significantly yields in legumes compared to maize. In addition, P is very important in the process of the Biological N Fixation since the symbiotic legume-rhizobia association is very much sensitive to any P-deprivations (Olivera et al. 2007; Marschner 2012;). This implies that increase in P availability has a significant positive impact on the legume growth as demonstrated by Rosas et al. (2007), Wasule et al. (2007) and Zaidi et al. (2010).

Intercropping system can play also an important role in increasing solubility of Minjingu PR. Lelie and Onwonga (2014) noted that soil available P, plant P, maize grain and dry matter yields were higher in maize-lupine intercropping treatment with application of Minjingu PR. Minja et al. (2014) confirmed such results and showed that including cabbage in the cropping system sequence increased effectiveness of Minjingu PR for subsequent crops.

The structure of the total bacterial communities was not significantly affected by the crop rotation and the diversity index H reached 1.45 for the 3 rotation regimes (Table 1). On the other hand, for the total fungal communities, H values were significantly higher in the soybean–maize rotation ($H = 1.13$) compared to the other rotation regimes ($H = 1.01$ and 1.09 respectively). The impact of the crop rotation on the structure of the total bacterial communities was quite small and insignificant ($p = 0.145$). However, although the fertilizer treatment effects within the rotation regime were not significant, addition of both N and K significantly improved the diversity index values of bacterial communities for common bean–soybean–maize ($H = 1.49$) and maize–common bean ($H = 1.53$) compared to the other fertilizer treatments. The same trend was observed in the H values of the fungal

Table 1 Effect of crop rotation and mineral fertilization on populations of phosphate solubilizing microorganisms (PSM) and phosphate solubilizing bacteria (PSB) and on Shannon index values (H) of bacterial and fungal communities from a Kenya Ferralsol (Siaya)

Rotation regime	Treatment	CFU × 10^6		Diversity Index (H)	
		PSM	PSB	Bacteria	Fungal
Common bean–Soybean–Maize	50 kg P ha^{-1} Minjingu PR	1.38b	1.20b	1.47a	1.18a
	50 kg P ha^{-1} Triple Super Phosphate	0.45a	0.31a	1.40a	0.99a
	60 kg N ha^{-1} + 60 kg K ha^{-1}	1.74c	1.53c	1.49b	1.15a
	Control	1.47b	1.26b	1.40a	1.05a
	Mean	1.26B	1.08B	1.44A	1.09B
Maize–Common bean	50 kg P ha^{-1} Minjingu PR	1.07b	0.86b	1.50c	1.44d
	50 kg P ha^{-1} Triple Super Phosphate	0.55a	0.35a	1.47b	0.45a
	60 kg N ha^{-1} + 60 kg K ha^{-1}	1.53c	1.26c	1.53c	1.30c
	Control	0.64a	0.45a	1.31a	0.84b
	Mean	0.95A	0.73A	1.45A	1.01A
Soybean–Maize	50 kg P ha^{-1} Minjingu PR	1.76c	1.51c	1.46a	1.28a
	50 kg P ha^{-1}Triple Super Phosphate	1.29b	1.15b	1.46a	0.95a
	60 kg N ha^{-1} + 60 kg K ha^{-1}	0.72a	0.59a	1.41a	1.13a
	Control	1.40c	1.35c	1.44a	1.15a
	Mean	1.29B	1.15B	1.44A	1.13B

The fertilizer rates were added each season as kg P ha^{-1}. N was added as Urea (60 kg N^{-1}) and K as Muriate of Potash (60 kg K ha^{-1}) in all plots except the negative control. Mean values followed by similar letters in the same column and rotation regime are not significantly different from each other at $p < 0.05$ according to Fishers protected LSD test. Letters in small case compare significance within each cropping regime while the letters in upper case compare significance among the rotation regimes

Table 2 Pearson correlation coefficients (r) showing the relationship between microbial diversity and crop yields in different rotation regimes

Pearson correlation coefficients						
Prob > |r| under H0: Rho = 0						
Rotation	Parameter	Fungal diversity	Bacteria diversity	Grain yield	PSM	PSB
Mz-CB	Fungal diversity	1				
	Bacteria diversity	0.41*	1			
	Grain yield	0.38*	0.42**	1		
	PSM	0.58*	0.2	−0.04	1	
	PSB	0.60*	0.2	−0.01	0.97***	1
CB-SB-Mz	Fungal diversity	1				
	Bacteria diversity	−0.06	1			
	Grain yield	0.09	0.15	1		
	PSM	0.16	0.17	−0.07	1	
	PSB	0.16	0.19	−0.05	0.99***	1
SB-Mz	Fungal diversity	1				
	Bacteria diversity	0.14	1			
	Grain yield	−0.45*	0.33	1		
	PSM	0.28	0.48	−0.13	1	
	PSB	0.24	0.49	−0.12	0.95***	1

PSB phosphate solubilizing bacteria, *PSM* phosphate solubilizing microorganisms, *Mz-CB* maize–bean rotation, *CB–SB–Mz* common bean–soybean–maize rotation, *SB–Mz* soybean–maize rotation

*, **, *** indicate significance at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively

communities. This could be attributed to indirect effects of NK fertilizers on soil microorganisms through improved plant growth with concomitant increases in root exudates and microbial populations. Similar results were obtained by Murphy et al. (2007) who reported that optimization of K fertilizer gave a stimulatory effect on microbial activity through increases in root exudation. For both rotation regimes soybean–maize and maize–common bean, the application of TSP decreased the H value of the fungal communities ($H = 0.95$ and 0.45 respectively). In a long-term trial conducted in Kabete, Kenya (>20 years), Kibunja et al. (2010) and Kamaa et al. (2011) reported changes in bacterial populations due to application of NP fertilizers but no clear changes in fungal communities. All these results suggest that changes in fungal communities might be suitable indicators for short-term soil disturbance while bacterial diversity may indicate longer-term effects of agricultural practices.

The populations of PSM and PSB were influenced by both the rotation regime and fertilizers applied with a significant interaction effect between the two factors ($p = 0.005$). In comparison to the negative control (6.4 and 4.5×10^5 cfu g^{-1} for maize–common bean rotation and 1.40 and 1.35×10^6 cfu g^{-1} for soybean–maize rotation), application of Minjingu PR increased PSM and PSB populations in maize–common bean (1.07×10^6 and 8.6×10^5 cfu g^{-1} respectively) and soybean–maize (1.76 and 1.51×10^6 cfu g^{-1} respectively) rotation. However, sole addition of high rates of TSP resulted in a significant reduction of PSM and PSB populations (4.5 and 3.1×10^5 cfu g^{-1} for common bean–soybean–maize and 1.29 and 1.15×10^6 cfu g^{-1} for soybean–maize respectively). Minjingu PR is a sparingly soluble P source, which when applied in soil contributes to both the labile (readily available) and non-labile P pools that are less available. Only a fraction of applied Minjingu PR is immediately available for plant uptake, but P availability is increased with time. The availability of insoluble phosphates may have enhanced the proliferation of P solubilisers. TSP on the other hand is readily available after application and may have suppressed the population of PSM. Our results concur with those obtained by Chabot et al. (1998) who demonstrated a repression in phosphate solubilisation activity of rhizobial strains after addition of 0.5 % soluble P.

There was significant ($p = 0.05$) but weak correlation ($r = 0.389$) between maize yields and fungal

diversity, but no relationship was noted between crop yields and either PSB or PSM populations (Table 2). Although Minjingu PR addition increased PSB and PSM populations, the microorganisms may not have contributed significantly to P nutrition of the test crops. Since the fertilizers were added every season and no residual effects were tested, the rates added sufficiently contributed to plant nutrition of the crops, and the contribution of the microorganisms was negligible (Ndung'u-Magiroi et al. 2012). In the present study, it was observed that whereas TSP applied treatments gave high crop yields, microbial diversity was lower than the negative control, while NK treatments had the lowest crop yields but the highest microbial diversity. Despite this, Minjingu PR had high crop yields and higher microbial diversity and PSM/PSB populations compared to the negative control. This infers that in the short term, systems incorporating sparingly soluble P source (e.g. Minjingu PR) may have positive impacts to both agronomic and microbial yields. However, other indicators of soil health and ecological sustainability must be monitored to assess longer-term effects of different P sources.

Conclusions and recommendations

Microbial communities composition and diversity were influenced by the source of inorganic P and rotation system. Minjingu PR application stimulated the populations of native PSB and enhanced agronomic productivity especially in the maize–common bean and soybean–maize rotation systems. On the other hand, fungal diversity and populations of PSM/PSB were lower when TSP was applied alone. We have demonstrated that where organic inputs are scarce, the use of sparingly soluble P sources such as Minjingu PR could contribute to an increase in indigenous microbial communities; while continuous use of readily soluble P sources (e.g. TSP) encourage the suppression of PSM and total microbial communities. An understanding of microbial interactions in the rhizosphere is required in order to optimize plant–microbial interactions and plant growth promotion. Minjingu PR and crop rotation including legumes represent alternative management practices to mineral fertilizers, and may have a role to play in the restoration and the maintenance of soil fertility in highly P depleted soils in Africa.

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