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Application of residue, inorganic fertilizer and lime affect phosphorus solubilizing microorganisms and microbial biomass under different tillage and cropping systems in a Ferralsol

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ABSTRACT

Phosphorus is a critical nutrient for plant growth. Several agronomic practices have been promoted to improve soil fertility and crop productivity in Western Kenya. Yet, despite their benefits, a dearth of knowledge exists on their long-term effects on soil microbial biomass and phosphorus solubilizing microbial species (PSMs) abundance especially in this region. In a long-term (15 years) agronomic field trial, we selected 8 treatments that allowed for evaluation of 1) Effect of tillage in a maize-soybean rotation with fertilization and residue retention; 2) Effect of residue retention in a maize-soybean rotation with fertilization under conservation tillage; 3) Effect of N and P fertilization in a maize-soybean rotation with conventional tillage; 4) Effect of liming in a maize-soybean intercropping with conventional tillage; and 5) Effect of maize-soybean rotation versus intercropping under conservation tillage on microbial biomass and PSMs abundance. The study was conducted in a long-term conservation agriculture experiment (30 seasons) in a Ferralsol in Western Kenya in 2016 and 2017. Reduced tillage significantly ($P < 0.05$) increased microbial biomass phosphorus (MBP) and abundance of different PSMs relative to conventional tillage, though the results were not consistent for some species. Residue addition significantly increased MBP and abundance of different PSMs compared to systems without residue addition. Liming significantly reduced PSMs abundance in 2016, though this was inconsistent for 2017. In 2017, no effect of liming on soil pH was found. Fertiliser addition significantly increased PSMs abundance in 2016, but this was also inconsistent for 2017. Some PSMs strains were significantly more abundant in maize and soybean intercropping system compared to rotation, and vice versa. Our study demonstrated that not only the agronomic inputs applied but also tillage and cropping systems employed can variably affect the soil microbial populations.

1. Introduction

Phosphorus (P), the second most limiting plant nutrient in tropical soils (Fageria et al., 2013; Anand et al., 2016), mostly occurs in insoluble forms, thus restricting its availability for plant utilization (Sharma et al., 2013). In soils, acidity, poor solubility and metal-cation complexes (Khan et al., 2009) limit P availability for plants. On average, approximately 1 Mg P ha^{-1} is present in the upper (15 cm) soil depths (Walpola and Yoon, 2012), but characteristic low solubility of P, immobilization and high fixation rates limit its availability to plants (Mahdi et al., 2012; Al-Rohily et al., 2013). Application of inorganic P fertilisers may be a temporary measure to overcome the phosphorus problem (Khan et al., 2009), as part of the applied P may get fixed (Mahdi et al., 2012; Walpola and Yoon, 2012; Al-Rohily et al., 2013), besides hindering biological P cycling by disrupting soil microbial structure, diversity and activities (Sharma et al., 2013).

Phosphorus Solubilizing Microbes (PSMs) represent a pool of heterotrophic beneficial microbial group with the ability to hydrolyze both

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organic P compounds and insoluble inorganic P sources, thereby making P available for plant nutrition and soil enrichment (Khan et al., 2009; Liu et al., 2016; Mahadevamurthy et al., 2016; Kalayu, 2019). Biologically, PSMs can achieve this through solubilisation and mineralization of inorganic and organic P sources, respectively (Oliveira et al., 2009) through mechanisms involving production of metabolites such as organic acids, chelation of cations and hydrolysis of organic P sources (Pradhan and Sukla, 2006; Khan et al., 2009; Kalayu, 2019), as well as production of phosphatase enzymes (like phytases and phosphomonoesterases) that hydrolyse organic P forms to inorganic P (Nannipieri et al., 2011; Jarosch et al., 2019).

Bacteria, fungi, actinomycetes and algae exhibit P solubilization (Alori et al., 2017), with bacteria and fungi being the most predominant P solubilizers (Chen et al., 2006). The most powerful bacterial P solubilizers include *Pseudomonas* spp., *Bacilli* spp., *Rhizobium* spp., *Agrobacterium* spp. (Babalola and Glick, 2012). Other bacterial PSMs include *Enterobacter* spp., *Azotobacter* spp., and *Burkholderia* spp. (Istina et al., 2015; Borham et al., 2017). Fungal PSMs include *Penicillium* spp., *Aspergillus* spp., *Glomus* spp., (the first three being the most dominant fungal PSMs (Igual et al., 2001, Kalayu, 2019)), *Pantoea* spp., *Kebsiella* spp., *Arthrobotrys* spp., *Rhizoctonia* spp., *Rhizopus* spp., *Trichoderma* spp., and *Yarrowia* spp. among others (Chung et al., 2005; Son et al., 2006; Srinivasan et al., 2012; Tandon et al., 2020). Actinomycetes strains in genera *Actinomyces* spp., *Micromonospora* spp., and *Streptomyces* spp. and algal strains like cyanobacteria also have P solubilizing abilities (Sharma et al., 2013; Alori et al., 2017).

Abundance and activities of PSMs are limited by agronomic management practices and soil physico-chemical characteristics (Bengtsson et al., 2005; Das and Dkhar, 2011). Such physico-chemical parameters include soil pH, low soil organic carbon (SOC) (when the microbes are heterotrophic), soil structure and fluctuations in soil nutrient, moisture and temperature (Zhou et al., 2002; Bargaz et al., 2012; Choi et al., 2017; Szoboszlay et al., 2017). Agronomic practices that augment soil pH, promote build-up of SOC, moisture retention, creation of microclimates and reduction of soil disturbances often influence PSMs abundance, diversity and activities (Böhme et al., 2005; Bünemann et al., 2006; Li et al., 2017; van der Bom et al., 2018). Contrary to conventional tillage, reduced tillage minimizes soil disturbance that also favours soil nutrient enrichment, SOC accumulation, protection of fungal hyphae, soil food webs, specialised microbial niches and microsites, thus promoting soil microbial proliferation and activities (Kihara et al., 2012; Mathew et al., 2012; Mårtensson and Olsson, 2012; Choi et al., 2017).

Besides application of inorganic fertilizers, agronomic practices involving residue addition, liming, intercropping and crop rotation under different tillage systems have been widely promoted to bolster soil nutrient status, sustain and improve crop productivity in sub-Saharan Africa (Kihara et al., 2012; Mucheru-Muna et al., 2014; Nziguheba et al., 2016) and the rest of the world (Latati et al., 2016; Ghimire et al., 2017). However, little is known on long-term effects of the above agronomic practices on PSMs abundance (Margenot et al., 2017) and soil biochemical properties, especially in tropical soils. The objectives of this study were to determine how soil biochemical properties, microbial biomass and PSMs are affected by soil management practices involving reduced tillage, residue application, cropping systems, liming and N and P fertilizer use.

2. Materials and methods

2.1. Study site

The study was conducted during the short rains season (August-November 2016) and long rains season (April-July 2017), characterized by different rainfall amounts and levels of crop productivity. The research was carried out in a long-term agronomic trial named CT1 in Siaya County, western Kenya. The trial was established in the year 2003 by International Center for Tropical Agriculture (CIAT) and lies at latitudes 0° 07′ N and longitude 34° 24′ E; under a sub-humid climate with biannual rainfall (1200–1600 mm) and average temperature of 23.2 ± 1.5 °C (Kihara, 2009). Soils in the trial are Ferralsols (Paul et al., 2013), characterized by low pH (5.1 ± 0.3); with sand:silt:clay ratio of 15:21:64 and extractable inorganic phosphorus (Olsen) content of 2.99 ± 2.09 mg kg^{-1} (Kihara, 2009). Crop production in the region is mainly for subsistence, rainfed and mostly practiced under conventional tillage in smallholder farms (of mostly less than 1 ha), with maize being the dominant staple food crop.

2.2. Experimental design

The CT1 long-term experimental site in Nyabeda was set up in a randomized block design, with 12 treatments replicated four times under two tillage systems (reduced and conventional tillage), three cropping systems (maize-soybean rotation, maize-soybean intercropping and continuous maize), two residue application rates (with or without 2 Mg ha^{-1} residue application), four rates of N fertilization (0, 30, 60 and 90 kg N ha^{-1}) as urea, two rates (0 and 60 kg P ha^{-1}) of P application as triple super phosphate, and blanket application of K as muriate of potash at 60 kg ha^{-1}), in plots measuring 4.5 m by 7 m. Maize and soybean were either planted in rotation or intercropped. Maize was planted at a spacing of 25 cm by 75 cm, with 2 seeds placed per hill and later thinned to one plant per hill. Soybean was planted at a spacing of 5 cm by 75 cm. Urea was applied in two splits; a third during planting and two-thirds during topdressing when the plants were knee-high (4 weeks after planting). Potassium was applied as muriate of potash during planting. Hand ploughing and weeding using hoes were restricted to 15 cm depth in the conventional tillage systems. In the reduced tillage systems, weeding was restricted to surface scratching.

2.3. Selection of treatments

Within this long-term field trial, the following management systems (or combinations thereof) were selected for our study (Table 1). The effects of management factors on soil biochemical properties, microbial biomass and PSMs were evaluated as follows: i) Tillage factor was inferred by comparison between treatment codes RTFr versus CTFr treatments; ii) residue application factor by RTFr versus RTF; iii) N and P fertilizer application factor by CTFr versus CTF; iv) cropping systems factor by RT.int versus RT.rot; and v) lime application factor by CTint + L versus CTint-L (Table 1).

2.4. Soil sampling and analysis

Soil samples were collected at 0–20 cm depths in August 2016 during the short rains season and at 0–10 cm depths in May 2017 during long rains season, with maize as the main crop during sampling. Samples were taken from five spots per plot within each treatment following a "W" shaped pattern, using an auger. However, before taking soil samples at every subsequent plot, the auger and buckets were repeatedly cleaned and sterilized to avoid contamination of samples. The samples were pooled in a bucket, thoroughly mixed to make a composite sample for each treatment in each plot. Representative samples were taken, stored in cool box with frozen ice-packs and transported to the laboratory on the same day. In the laboratory, the fresh field-moist soil samples for biological assessments were sieved through 2 mm sieves and stored frozen (-20 °C) until extraction while the soil samples for chemical analyses were first air-dried and ground before sieving.

Soil organic carbon was determined by Carbon Nitrogen (CN) Elemental Analyser. Permanganate oxidizable carbon (POxC) was assessed from 2.5 g of soil following the procedure of Weil et al. (2003) as previously used by Culman et al. (2012). Briefly, 2.5 g of air-dried soil were weighed into centrifuge tubes, 18 mL distilled water (DI) and 2 mL (0.2 M) K_2MnO_4 added, shaken (at 240 revolutions per minute) for 2 min, tubes removed and allowed to settle for 10 min, thereafter 0.5 mL

Table 1
Description of the treatments selected for the study in CT1-Nyabeda site, western Kenya.

Treatment code	Tillage	Cropping system	Residue (Mg ha^{-1})	N (Kg ha^{-1})	P (Kg ha^{-1})	K (Kg ha^{-1})	Lime (Mg ha^{-1})
RTFr ¶§	RT	M−S Rot'n	2	60	60	60	0
CTFr ¶₩	CT	M−S Rot'n	2	60	60	60	0
RTF §	RT	M−S Rot'n	0	60	60	60	0
CTr ₩	CT	M−S Rot'n	2	0	0	60	0
RT.rot ‡	RT	M−S Rot'n	2	0	60	60	0
RT.int ‡	RT	M + S Intcr'p	2	0	60	60	0
CTint + L⊕	CT	M + S Intcr'p	2	0	60	60	2
CTint-L⊕	CT	M + S Intcr'p	2	0	60	60	0

CT = conventional tillage; RT = reduced tillage, M−S Rot'n = Maize and soybean rotation system, M + S Intcr'p = maize and soybean intercropping system. The effects of management factors were evaluated based on the combination of treatment codes (and symbols in superscripts) as follows; ¶= tillage, §=residue application, ₩= fertilizer application, ‡=cropping systems, ⊕=lime application (lime was applied once in 2015 only).

of the supernatant was taken and mixed with 49.5 mL of DI. From each sample, 200 μl aliquots were extracted and their concentrations read with spectrophotometer set at wavelength of 550 nm. Total N was assessed using the Kjeldahl method (Kjeldahl, 1883). Soil pH was determined in water (soil: water; 1:2), while extracts for Mehlich 3 P (Mehlich, 1984), phosphorus sorption index (PSI; Bache and Williams, 1971), Mehlich 3 Mg and Al (Mehlich, 1984) were measured using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

2.5. Soil sample extraction and measurement for microbial biomass

Before extraction begun, the stored field-moist soil samples were removed from the freezer (where they were kept at −20 °C until extraction) and transferred to a cold room (4 °C) for 20 min to ease weighing. The samples were used for both the assessment of microbial biomass C, N and P, as well as extraction of DNA. Microbial biomass C, N and P were determined using chloroform fumigation direct extraction method (Vance et al., 1987). Twenty-five (25) grams of the sieved field-moist soil samples were weighed in triplicates. One set was fumigated for 24 h (in sealed desiccators lined with filter papers to maintain humidity) at 25 °C using ethanol-free chloroform, the other set was non-fumigated while the third set was used for soil moisture determination. The fumigated and non-fumigated soil samples were extracted by addition of 100 mL of 0.5 M potassium sulphate (K_2SO_4) solution, shaken (at 150 revolutions per minute) for 1 h and filtered. Total organic C in the extracts were determined by digestion with potassium dichromate oxidising reagent and colorimetry using spectrophotometer (600 nm) as previously proposed by Bartlett and Ross (1988). Total organic N in the extracts was colorimetrically analysed by determining the nitrate in the digested samples using salicylic acid method. Chloroform fumigation-extraction method for determination of microbial biomass phosphorus involved the extraction of chloroform-fumigated and non-fumigated soil samples with 0.5 M sodium bicarbonate. The extracts were centrifuged, filtered and digested by adding ammonium persulfate, 5 M NaOH and 1.2 M H_2SO_4 sequentially. Total P was determined colorimetrically using a spectrophotometer at 880 nm wavelength after addition of 4.0 mL of ascorbic acid and 3.0 mL of molybdate reagent solutions. Microbial biomass C, N and P were obtained by calculating the difference between the fumigated and non-fumigated samples.

2.6. Deoxyribonucleic acid (DNA) extraction

DNA extraction was done after one week of soil sampling for each period. Soil DNA extraction from soil samples taken in 2016 was done using the procedure of Porteous et al. (1997), as previously used by Kihara et al. (2012). In 2017, Mo-Bio's PowerSoil DNA Isolation Kit was used to extract DNA samples following the manufacturer's protocol. To extract total DNA from the field-moist soil samples taken in 2016 season, 0.5 g of the sample was weighed into 1.5 mL Eppendorf tubes, mixed with 0.25 g glass beads and lysis buffer and homogenized for 2 min at 2500 revolutions per minute (rpm) using a minibead cell disruptor (Biospec products Inc.), before incubating (65 °C) and re-homogenizing for 2 min. This was followed by centrifuging (13,000 rpm) for 15 min at 4 °C, addition of 75 μl of 5 M potassium acetate, 250 μl of 40% polyethylene glycol (PEG), incubation for 1 h at −20 °C, re-centrifugation (13,000 rpm). The pellets were removed, re-suspended in 600 μl of 2% CTAB, re-incubated (68 °C) for 15 min and cleaned with 600 μl of chloroform. DNA was precipitated overnight (−20 °C) by addition of 600 μl of ice cold isopropanol. Pellets were cleaned with 70% ethanol, re-suspended in sterile double distilled water, stored at −20 °C and shipped to MR DNA (www.mrdnalab.com, Shallowater, TX, USA) for sequencing. The DNA yield concentrations ranged between 8.5 ng/μL and 44 ng/μL.

2.6.1. Soil DNA sequencing

At MR DNA, Illumina Sequencing technology was used for bacterial and fungal meta-barcoding. Illumina Miseq, an integrated instrument that performs clonal amplification and sequencing, was used to target the V4 hyper-variable region of the 16S rRNA gene for bacteria while for fungal meta-barcoding, internal transcribed spacer (ITS 1-2) region was targeted. For most fungal phyla, ITS 1-2 spacer regions are the most reliable targets for phylogenetic analysis. However, for most bacterial phyla, V4-V6 variable regions of 16S rRNA are the most reliable for representing the full-length 16S rRNA sequences in the phylogenetic analysis (Yang et al., 2016a), alongside being the optimal sub-regions for the design of universal primers with superior phylogenetic resolution for bacterial phyla. From each DNA sample, 1 μl of each was used for DNA sequencing. Polymerase chain reaction (PCR) primers 515/806 with barcode on the forward primer were used in a 28 cycle PCR (5 cycle used on PCR products) using the HotStarTaq Plus Master Mix Kit (Qiagen, USA). This was performed under the following conditions: 94 °C for 3 min, followed by 28 cycles of 94 °C for 30 s, 53 °C for 40 s and 72 °C for 1 min. This was followed by a final elongation step at 72 °C for 5 min. After amplification, the quality of the PCR products were checked in 2% agarose gel before using them to prepare Illumina DNA library. Sequencing was performed on Illumina MiSeq following the manufacturer's guidelines before processing using MR DNA analysis pipeline. In summary, sequences were joined, depleted of barcodes and sequences less than 150 base pairs and sequences with ambiguous base calls removed, respectively. Sequences were denoised, operational taxonomic units (OTUs) generated and chimeras removed. Clusters of similar 16S/18s rRNA sequences, commonly known as Operational taxonomic units (OTUs), were defined by clustering at 3% divergence (97% similarity). Final OTUs were taxonomically classified using BLASTn against a curated database derived from RDPII and NCBI (www.ncbi.nlm.nih.gov, http://rdp.cme.msu.edu). OTUS are the most widely used basic diversity units for large-scale characterisation of microbial communities (Schmidt et al., 2014).

2.7. Statistical data analysis

Before any statistical analysis was done the data were explored using

scatter plots to identify (and correct) outliers as observations that were either below or above ±2.24 standard deviation units away from the mean (Kutner et al., 2005; Martin and Roberts, 2010; Aguinis et al., 2013). The uncorrected outliers were less than 1% and were removed from the dataset. The microbial species data were square root transformed. Data were analyzed using R project (R Development Core Team, 2016). Fligner-Killeen test was used to check for and confirm homogeneity of variances. Orthogonal contrast analysis was used to compare significant differences in means of different treatments and mean separation done at ($p \leq 0.05$). The treatments were compared pair wise to infer effects of certain management factors embedded in certain agronomic systems. Canonical correspondence analysis (CCA) was used to assess the relationship between the soil microbial community richness and soil chemical parameters. The CCA analysis was performed using *Anacor* library and *cca* function in Vegan library in R, overall significance was tested using *anova* function, and *step* function used to determine significant variables with permutation test at 999-maximum permutations.

3. Results

3.1. Overall microbial species abundance at genus level

Overall, 839 bacterial phylotypes were identified at genus level in 2016, out of which, 107 phylotypes had relative abundance greater than 0.1% (Fig. 1). In 2017, 1350 bacterial phylotypes at genus level were identified, out of which, 137 phylotypes had relative abundance greater than 0.1% (Fig. 2). In both years, *Gemmattimonas* spp. were the most abundant bacterial phylotypes identified at genus level.

3.2. Categories of PSMs studied

Out of the microbial phylotypes identified in 2016 and 2017, 30 PSMs strains at genus level, falling under two broad categories of bacteria and fungi kingdoms, were identified (Table 2). Specifically, 23 bacterial PSMs strains at genus level were observed. The bacterial PSMs strains observed in 2016 comprised of *Burkholderia* spp., *Streptomyces* spp., *Enterobacter* spp., *Actinomyces* spp., *Pseudomonas* spp., *Micromonospora* spp., *Rhizobium* spp., *Bacillus* spp., *Arthrobacter* spp., *Mesorhizobium* spp., *Agrobacterium* spp., *Bradyrhizobium* spp., *Azospirillum* spp., *Gemmatimonas* spp., *Massilia* spp., *Paenibacillus* spp., *Ralstonia* spp., *Rhodococcus* spp., *Sinomonas* spp., *Sphingomonas* spp. and *Thiobacillus* spp. while in 2017, *Erwinia* spp., was recorded in addition to the other species reported in 2016. Fungi were studied in 2017 only, and fungal PSMs strains observed and studied at genus level were six. These fungal PSMs strains comprised of *Penicillium* spp., *Paraglomus* spp., *Aspergillus* spp., *Trichoderma* spp., *Glomus* spp., and *Rhizoctonia* spp. (Table 2).

3.3. Effects of soil management factors on chemical and microbial variables

3.3.1. Reduced tillage versus conventional tillage

In 2017, soil pH was significantly higher in CTFr than RTFr while phosphorus sorption index was significantly higher in RTFr than CTFr (Table 3). In 2016, the population (mean species counts) of *Pseudomonas* spp., *Arthrobacter* spp. and *Thiobacillus* spp. were significantly higher in the reduced tillage system with residue and inorganic fertilizer application (RTFr) compared to the conventional tillage (CTFr) of the same treatment (Table 4). On the other hand, in the same year, CTFr significantly increased the population of *Micromonospora* spp., *Mesorhizobium* spp., *Agrobacterium* spp., *Bradyrhizobium* spp., *Ralstonia* spp., and *Sphingomonas* spp., relative to RTFr (Table 4). In 2017, the populations of *Penicillium* spp., *Glomus* spp., *Aspergillus* spp., *Trichoderma* spp., and *Actinomyces* spp. were also significantly higher in RTFr compared to CTFr system. *Streptomyces* spp., *Enterobacter* spp., *Arthrobacter* spp., *Bradyrhizobium* spp., *Paenibacillus* spp., *Ralstonia* spp. and *Rhodococcus* spp. were significantly higher in CTFr than RTFr systems in 2017 (Table 5).

3.3.2. Residue application versus no residue addition

PSI was significantly ($p < 0.05$) higher in in the reduced tillage system with residue and inorganic fertilizer application (RTFr) than similar system without residue application (RTF) in 2017 (Table 3). MBP significantly increased in RTFr compared RTF in 2016 (Table 4). Interestingly, RTFr significantly increased the population counts of *Burkholderia* spp., *Streptomyces* spp., *Actinomyces* spp., *Pseudomonas* spp., *Micromonospora* spp., *Rhizobium* spp., *Bacillus* spp., *Arthrobacter* spp., *Mesorhizobium* spp., *Agrobacterium* spp., *Bradyrhizobium* spp., *Azospirillum* spp., *Gemmatimonas* spp., *Massilia* spp., *Paenibacillus* spp., *Ralstonia* spp., *Rhodococcus* spp., *Sinomonas* spp., *Sphingomonas* spp. and *Thiobacillus* spp. compared to RTF in 2016. In 2017, RTFr significantly increased soil phosphorus sorption index and populations of *Aspergillus* spp., *Trichoderma* spp., *Glomus* spp., and *Thiobacillus* spp., compared to RTF. However, the populations of *Enterobacter* spp. and *Streptomyces* spp. were significantly higher in RTF compared to RTFr in 2017 (Table 5).

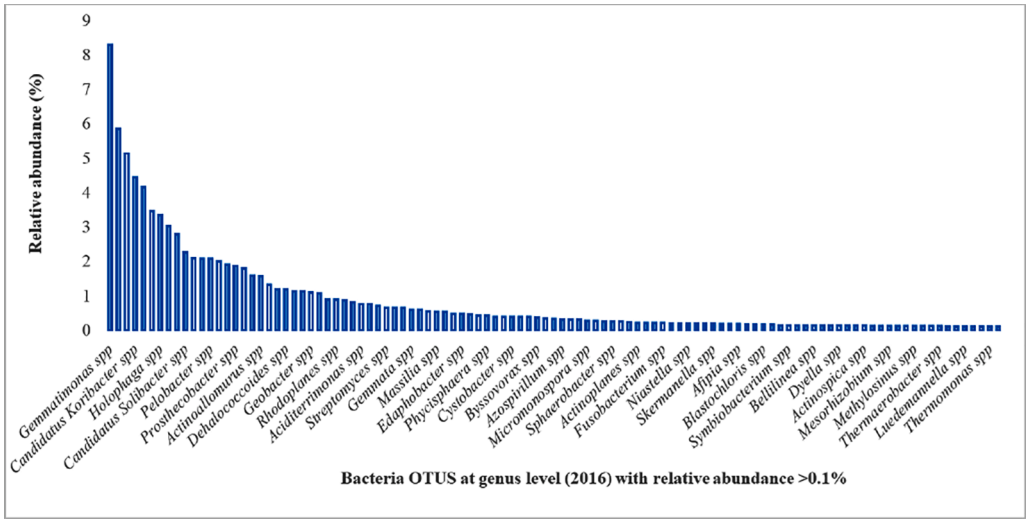

Fig. 1. Relative abundance (>0.1%) of bacterial OTUS identified in the short rains season of 2016 in CT1 site.

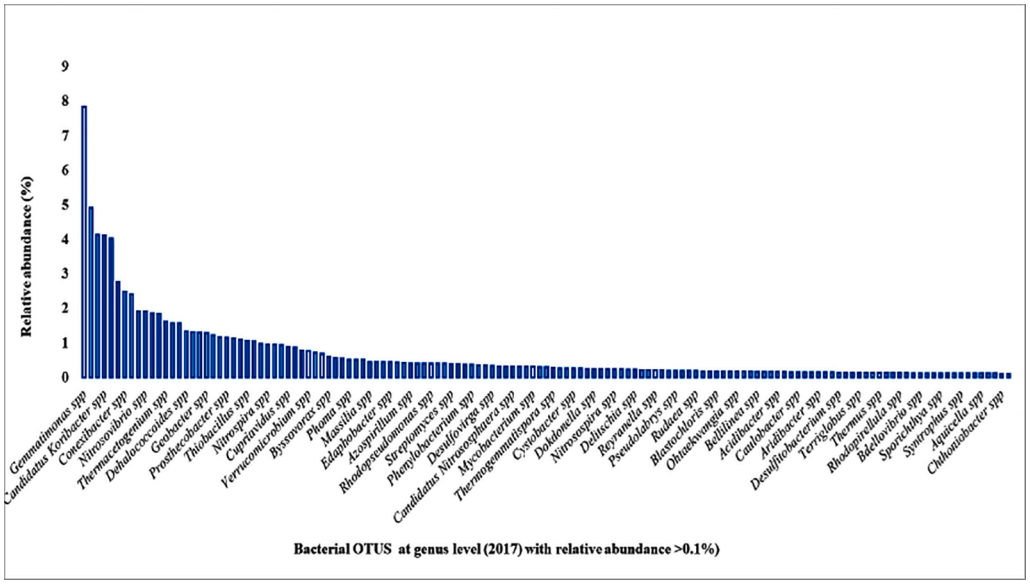

Fig. 2. Relative abundance (>0.1%) of bacterial OTUS identified in the long rains season of 2017 in CT1 site.

Table 2
Taxonomic information of the bacterial and fungal PSMs identified in the study site.

Kingdom	Division	Class	Order	Family	Genus
Bacteria	Actinobacteria	Actinobacteria	Actinobacteridae	Micrococcaceae	*Micrococcus*
			Actinomycetales	Actinomycetaceae	*Actinomyces*
				Micrococcaceae	*Arthrobacter*
					Sinomonas
				Micromonosporaceae	*Micromonospora*
		Actinomycetes		Nocardiaceae	*Rhodococcus*
				Streptomycetaceae	*Streptomyces*
	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*
				Paenibacillaceae	*Paenibacillus*
	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonales	Gemmatimonaceae	*Gemmatimonas*
	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*
				Phyllobacteriaceae	*Mesorhizobium*
				Rhizobiaceae	*Agrobacterium*
					Rhizobium
			Rhodospirillales	Rhodospirillaceae	*Azospirillum*
			Sphingomonadales	Sphingomonadaceae	*Sphingomonas*
		Betaproteobacteria	Burkholderiales	Burkholderiaceae	*Burkholderia*
				Oxalobacteraceae	*Massilia*
				Ralstoniaceae	*Ralstonia*
			Nitrosomonadales	Thiobacillaceae	*Thiobacillus*
		Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	*Enterobacter*
				Erwiniaceae	*Erwinia*
			Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*
					Penicillium
	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*
	Basidiomycota	Agaricomycetes	Cantharellales	Ceratobasidiaceae	*Rhizoctonia*
	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Glomus*
			Paraglomerales	Paraglomeraceae	*Paraglomus*

3.3.3. Maize-soybean intercropping versus maize-soybean rotation

In 2017, POxC was significantly higher in the reduced tillage with intercropping (RT.int) than with maize and soybean rotation (RT.rot) systems (Table 3). The populations of *Arthrobacter* spp., *Mesorhizobium* spp., *Agrobacterium* spp. and *Azospirillum* spp., were significantly higher in the RT.rot than than RT.int whereas *Pseudomonas* spp., were higher in RT.int compared RT.rot in 2016 (Table 4). In 2017, maize and soybean intercropping system (RT.int) significantly increased *Penicillium* spp., *Streptomyces* spp., *Mesorhizobium* spp., *Rhizobium* spp., *Bradyrhizobium* spp. and *Paenibacillus* spp. populations whereas *Erwinia* spp., *Enterobacter* spp., *Actinomyces* spp., *Arthrobacter* spp., and *Gemmatimonas* spp., populations significantly increased in RT.rot than RT.int.

3.3.4. Fertiliser addition versus no fertiliser application

In 2017, POxC and P were significantly higher ($p < 0.05$) while Mehlich 3 Mg and PSI significantly decreased in conventional tillage treatment with residue and fertiliser application (CTFr) than in the same system without NP fertiliser application (CTr; Table 3). MBP was significantly higher ($p < 0.05$) in CTr than CTFr in 2016 (Table 4). With the exception of *Thiobacillus* spp., the populations of *Burkholderia* spp., *Streptomyces* spp., *Actinomyces* spp., *Pseudomonas* spp., *Micromonospora* spp., *Rhizobium* spp., *Bacillus* spp., *Arthrobacter* spp., *Mesorhizobium* spp., *Agrobacterium* spp., *Bradyrhizobium* spp., *Azospirillum* spp., *Gemmatimonas* spp., *Massilia* spp., *Paenibacillus* spp., *Ralstonia* spp., *Rhodococcus* spp., *Sinomonas* spp., *Sphingomonas* spp. and *Thiobacillus* spp.

Table 3
Chemical variables for the different systems studied in CT1 study site in 2017.

Chemical Variables	Tillage factor		Residue factor			Fertilizer factor			Cropping systems factor			Liming factor			
	CT	RT		+	−		+	−		Int	Rot		+	−	
	CTFr	RTFr	P-value	RTFr	RTF	P-value	CTFr	CTr	P-value	RT. int	RT. rot	P-value	CTint + L	CTint-L	P-value
POxC (mgkg^{-1})	258	257	0.98	257	231	0.42	258	339	**0.02**	369	286	**0.03**	391	325	0.42
Total N (%)	0.18	0.18	>0.99	0.18	0.17	0.44	0.18	0.17	0.25	0.19	0.18	0.25	0.18	0.19	0.44
SOC (%)	1.99	1.96	0.73	1.96	1.89	0.55	1.99	1.90	0.39	2.00	1.88	0.27	1.96	2.01	0.55
C:N	11.00	10.95	0.86	10.95	11.05	0.75	11.00	11.04	0.91	10.77	10.79	0.96	10.59	10.79	0.75
Soil pH	4.95	4.67	**0.02**	4.67	4.79	0.29	4.95	5.15	0.11	5.18	5.04	0.23	5.45	5.13	0.29
P* (mgkg^{-1})	36.57	30.69	0.71	30.69	25.58	0.74	36.57	0.43	**0.03**	27.07	18.76	0.59	22	19.28	0.74
Mg* (mgkg^{-1})	99.70	78.83	0.25	78.83	96.7	0.32	99.7	137	**0.05**	141	140	0.98	119	1356	0.32
Al* (mgkg^{-1})	1180	1183	0.95	1183	1103	0.16	1180	1097	0.15	1073	1080	0.90	998	1100	0.16
PSI (mgkg^{-1})	188	234	**<0.01**	235	199	**0.02**	188	223	**0.02**	199	198	0.94	191	195	**0.02**

Values are means of different variables assessed in the year 2017. POxC = Permanganate oxidizable carbon, PSI = phosphorus sorption index, CT = conventional tillage, RT = reduced tillage, Int = maize-soybean intercrop, Rot = maize-soybean rotation, + = with, − = without, CTFr = conventional tillage with 60 kg N, 60 kg P ha^{-1} and 2 Mg ha^{-1} stover, RTFr = reduced tillage with 60 kg N, 60 kg P ha^{-1} and 2 Mg ha^{-1} stover, RTF = reduced tillage with 60 kg N, 60 kg P ha^{-1} minus residue, CTr = conventional tillage with 2 Mg ha^{-1} stover only, RT.int = maize-soybean intercropping under reduced tillage with 60 kg P ha^{-1} and 2 Mg ha^{-1} stover, RT.rot = maize-soybean rotation under reduced tillage with 60 kg P ha^{-1} and 2 Mg ha^{-1} stover targeting soybean phase, CTint + L = conventional tillage intercrop with 0 kg N, 60 kg P ha^{-1}, 2 Mg ha^{-1} stover and 2 Mg ha^{-1} lime, CTint-L = conventional tillage intercrop with 0 kg N, 60 kg P ha^{-1}, 2 Mg ha^{-1} stover and no lime; * Mehlich III extracted.

Table 4
Microbial biomass and PSMs operational taxonomic units (OTUS) across various systems in 2016 in CT1 study site.

Biological Variables	Tillage factor		Residue factor			Fertilizer factor			Cropping systems factor			Liming factor			
	CT	RT		+	−		+	−		Int	Rot		+	−	
	CTFr	RTFr	P-value	RTFr	RTF	P-value	CTFr	CTr	P-value	RT. int	RT. rot	P-value	CTint + L	CTint-L	P-value
MBC (mg kg^{-1})	44.15	57.48	0.60	57.48	87.95	0.23	44.15	88.22	0.23	83	58.9	0.34	75.33	61.88	0.59
MBN (mg kg^{-1})	8.14	6.39	0.45	6.39	6.92	0.80	8.14	11.55	0.80	8.22	5.59	0.21	8.19	8.87	0.74
MBP (mg kg^{-1})	0.41	1.4	**0.05**	1.4	0.25	**0.03**	0.41	1.46	**0.03**	0.76	0.51	0.56	0.92	1.41	0.27
Actinomyces spp.	242	395	0.09	395	56	**<0.01**	242	50	**0.04**	3	21	0.23	62	124	0.41
Agrobacterium spp.	41	18	**0.01**	18	3	**0.05**	41	2	**<0.01**	134	334	**0.05**	4	33	**<0.01**
Arthrobacter spp.	6658	9984	**0.01**	9984	948	**<0.01**	6658	650	**<0.01**	1526	1901	0.47	1036	8524	**<0.01**
Azospirillum spp.	1540	1283	0.26	1283	263	**<0.01**	1540	103	**<0.01**	1608	1377	0.65	199	624	**0.05**
Bacillus spp.	1652	2558	0.13	2558	319	**<0.01**	1652	289	**0.02**	1618	2336	0.39	1954	2767	0.17
Bradyrhizobium spp.	3765	2622	**0.05**	2622	87	**<0.01**	3765	291	**<0.01**	6	26	**0.01**	203	1469	**0.03**
Burkholderia spp.	9310	9774	0.85	9774	118	**<0.01**	9310	1617	**0.01**	5105	8039	0.22	2058	7219	**0.05**
Enterobacter spp.	8	5	0.87	5	2	0.80	8	3	0.72	2439	2636	0.73	2	31	0.06
Gemmatimonas spp.	50,020	40,217	0.33	40,217	2235	**<0.01**	50,020	6236	**<0.01**	449	1145	**0.01**	9798	25,953	0.12
Massilia spp.	2856	2384	0.21	2384	61	**<0.01**	2856	310	**<0.01**	1591	1286	0.40	516	2636	**<0.01**
Mesorhizobium spp.	610	385	**0.03**	385	6	**<0.01**	610	46	**<0.01**	3915	6886	**<0.01**	155	287	0.17
Micromonospora spp.	912	1436	**0.02**	1436	62	**<0.01**	912	282	**<0.01**	4155	2536	**<0.01**	948	796	0.41
Paenibacillus spp.	78	282	0.08	282	4	**0.02**	78	5	**0.04**	85	53	0.76	113	248	0.22
Pseudomonas spp.	1542	3862	**<0.01**	3862	206	**<0.01**	1542	431	**<0.01**	166	267	0.23	685	2001	**<0.01**
Ralstonia spp.	1430	558	**<0.01**	558	9	**<0.01**	1430	113	**<0.01**	694	567	0.42	158	730	**<0.01**
Rhizobium spp.	4560	3308	0.15	3308	168	**<0.01**	4560	267	**<0.01**	1809	1581	0.23	472	1989	**0.05**
Rhodococcus spp.	64	122	0.11	122	10	**<0.01**	64	13	0.15	159	162	0.95	5	141	**<0.01**
Sinomonas spp.	691	722	0.85	722	36	**<0.01**	691	119	**<0.01**	545	378	0.36	77	393	**0.05**
Sphingomonas spp.	11,070	3950	**<0.01**	3950	84	**0.03**	11,070	566	**<0.01**	1508	2273	0.66	751	3325	0.16
Streptomyces spp.	2275	3552	0.07	3552	618	**<0.01**	2275	334	**0.01**	6	3	0.54	818	1530	0.28
Thiobacillus spp.	1314	1928	**<0.01**	1928	794	**0.04**	1314	1516	**<0.01**	590	750	0.35	1853	1851	0.07

Values for MBC, MBN and MBP, respectively are means for Microbial biomass carbon, biomass nitrogen and biomass. Values for PSMs are mean OTUs counts for the year 2016. CT = conventional tillage, RT = reduced tillage, Int = maize-soybean intercrop, Rot = maize-soybean rotation, + = with, − = without, CTFr = conventional tillage (CT) with 60 kg N, 60 kg P ha^{-1} and 2 Mg ha^{-1} stover, RTFr = reduced tillage (RT) with 60 kg N, 60 kg P ha^{-1} and 2 Mg ha^{-1} stover, RTF = RT with 60 kg N, 60 kg P ha^{-1}minus residue, CTr = CT with 2 Mg ha^{-1} stover only, RT.int = maize-soybean intercropping under RT with 60 kg P ha^{-1} and 2 Mg ha^{-1} stover, RT.rot = maize-soybean rotation under RT with 60 kg P ha^{-1} and 2 Mg ha^{-1} stover targeting soybean phase, CTint + L = CT intercrop with 0 kg N, 60 kg P ha^{-1}, 2 Mg ha^{-1} stover and 2 Mg ha^{-1} lime, CTint-L = CT intercrop with 0 kg N, 60 kg P ha^{-1}, 2 Mg ha^{-1} stover and no lime.

significantly increased in the CTFr than CTr treatment in 2016. In 2017, addition of fertilizer significantly increased the populations of *Trichoderma* spp., *Enterobacter* spp., *Bradyrhizobium* spp. and *Paenibacillus* spp (but significantly reduced *Burkholderia* spp., *Glomus* spp., *Mesorhizobium* spp. and *Bacillus* spp) in CTFr compared to CTr.

3.3.5. Addition of lime versus no lime application

Application of lime in the conventional tillage system under maize and soybean intercropping (CTint+L) significantly reduced (there was a minor difference) PSI compared to the same system without lime (CTint-L) (Table 3). However, in 2017, no effect of liming on soil pH was found. In 2016 and 2017, the abundance of PSMs involving *Burkholderia* spp.,

Table 5
PSMs operational taxonomic units (OTUS) across various systems in 2017 in CT1 study site of Western Kenya.

PSMs	Tillage factor			Residue factor			Fertilizer factor			Cropping systems factor			Liming factor		
	CT	RT		+	−		+	−		Int	Rot		+	−	
	CTFr	RTFr	P-value	RTFr	RTF	P-value	CTFr	CTr	P-value	RT.int	RT.rot	P-value	CTint + L	CTint-L	P-value
Actinomyces spp.	245	437	**0.03**	437	312	0.11	245	336	0.24	282	483	**0.02**	279	354	0.28
Agrobacterium spp.	10	2	0.38	2	15	0.15	10	6	0.59	8	10	0.83	19	31	0.19
Arthrobacter spp.	549	263	**<0.01**	263	291	0.65	549	530	0.76	263	393	**0.04**	553	367	**<0.01**
Aspergillus spp.	24	411	**<0.01**	411	94	**<0.01**	24	87	0.39	52	170	0.12	170	113	0.44
Azospirillum spp.	446	468	0.79	468	338	0.18	447	373	0.43	303	328	0.79	530	594	0.53
Bacillus spp.	728	501	0.35	501	716	0.38	728	1524	**<0.01**	1085	1285	0.38	1619	1457	0.47
Bradyrhizobium spp.	2736	1716	**0.01**	1716	2138	0.24	2736	1902	**0.05**	3503	2175	**<0.01**	1673	2576	**0.03**
Burkholderia spp.	1538	1472	0.78	1472	2261	**<0.01**	1538	2474	**<0.01**	2353	2092	0.21	1941	3349	**<0.01**
Enterobacter spp.	24.5	6	**0.05**	6	34	**0.01**	25	5	**0.04**	8	52	**<0.01**	48	11	**<0.01**
Erwinia spp.	11	24	0.38	24	15	0.54	11	6	0.67	7	41	**0.03**	3	36	**0.03**
Gemmatimonas spp.	14,231	12,815	0.58	12,815	13,018	0.94	14,231	16,683	0.35	13,283	17,880	**0.04**	15,710	16,633	0.66
Glomus spp.	82	469	**0.05**	469	68	**0.05**	180	590	**0.04**	136	93	0.74	500	305	0.29
Massilia spp.	124	128	0.96	128	203	0.39	124	200	0.38	280	273	0.92	398	346	0.51
Mesorhizobium spp.	147	161	0.74	161	112	0.26	147	265	**0.02**	356	144	**<0.01**	182	278	**0.04**
Micrococcus spp.	2	3	0.82	2.5	5	0.4	2	5	0.14	4	5	0.45	2	7	**0.02**
Micromonospora spp.	255	249	0.95	249	258	0.93	255	449	0.08	695	597	0.3	508	561	0.57
Paenibacillus spp.	174	68	**0.02**	68	73	0.87	174	48	**<0.01**	275	69	**<0.01**	143	58	**0.05**
Paraglomus spp.	118	198	0.19	198	130	0.22	118	162	0.46	62	23	0.47	32	221	**<0.01**
Penicillium spp.	61	324	**0.04**	324	149	0.13	61	136	0.49	291	18	**0.03**	67	112	0.68
Pseudomonas spp.	285	245	0.71	245	403	0.16	285	407	0.28	734	562	0.17	647	814	0.18
Ralstonia spp.	295	173	**0.05**	173	271	0.12	295	323	0.66	203	223	0.76	165	157	0.89
Rhizobium spp.	696	783	0.55	783	715	0.64	696	723	0.86	1069	691	**0.02**	712	1153	**<0.01**
Rhodococcus spp.	38	20	**0.04**	20	27	0.4	38	40	0.86	35	25	0.19	64	42	**<0.01**
Sinomonas spp.	8	18	0.47	18	45	0.06	8	27	0.17	50	30	0.14	28	16	0.35
Sphingomonas spp.	1370	1580	0.22	1580	1422	0.41	1370	1445	0.66	1364	1240	0.52	1718	1405	0.12
Streptomyces spp.	716	356	**0.01**	356	629	**0.03**	716	463	0.09	1322	566	**<0.01**	683	820	0.24
Thiobacillus spp.	8807	2413	0.21	2413	32	**0.03**	8807	758	0.67	736	1856	0.68	952	3326	>0.99
Trichoderma spp.	586	1508	**<0.01**	1508	8	**<0.01**	586	56	**<0.01**	185	34	0.21	178	1079	**<0.01**

Values for PSMs are mean OTUs counts for the year 2016. CT = conventional tillage, RT = reduced tillage, Int = maize-soybean intercrop, Rot = maize-soybean rotation, + = with, − = without, CTFr = CT with 60 kg N, 60 kg P ha^{-1} and 2 Mg ha^{-1} stover, RTFr = RT with 60 kg N, 60 kg P ha^{-1} and 2 Mg ha^{-1} stover, RTF = RT with 60 kg N, 60 kg P ha^{-1}minus residue, CTr = CT with 2 Mg ha^{-1} stover only, RT.int = maize-soybean intercropping under RT with 60 kg P ha^{-1} and 2 Mg ha^{-1} stover, RT.rot = maize-soybean rotation under RT with 60 kg P ha^{-1} and 2 Mg ha^{-1} stover targeting soybean phase, CTint + L = CT intercrop with 0 kg N, 60 kg P ha^{-1}, 2 Mg ha^{-1} stover and 2 Mg ha^{-1} lime, CTint-L = CT intercrop with 0 kg N, 60 kg P ha^{-1}, 2 Mg ha^{-1} stover and no lime.

Micrococcus spp., *Pseudomonas* spp., *Rhizobium* spp., *Arthrobacter* spp., *Agrobacterium* spp., *Bradyrhizobium* spp., *Azospirillum* spp., *Massilia* spp., *Ralstonia* spp. and *Rhodococcus* spp.; as well as *Paraglomus* spp., *Trichoderma* spp., *Erwinia* spp. and *Mesorhizobium* spp. significantly reduced in the conventional tillage system with addition of lime under maize and soybean intercropping (CTint+L) than similar system without lime (CTint-L) application (Table 4; Table 5). However, *Arthrobacter* spp., *Enterobacter* spp., *Paenibacillus* spp. and *Rhodococcus* spp. abundance significantly increased in CTint+L than CTint-L in 2017.

3.3.6. Relationship between soil chemical parameters and PSMs

Canonical Correspondence Analysis (CCA) results, only in 2017, revealed significant ($p < 0.05$) relationship between soil chemical parameters and abundance of PSMs. Soil pH, total Mg, Al, SOC and P (Mehlich III) significantly correlated ($p < 0.05$) with the PSMs (Fig. 3, Table 6). *Arthrobacter* spp., *Azospirillum* spp., *Bacillus* spp., *Burkholderia* spp., *Gemmatimonas* spp. *Penicillium* spp., *Sphingomonas* spp. and *Trichoderma* spp. were significantly affected by the environmental variables. Available P strongly positively correlated with *Penicillium* spp. and *Trichoderma* spp., but negatively with *Bacillus* spp, *Burkholderia* spp. and *Sphingomonas* spp. Soil pH and total Mg strongly negatively correlated with *Trichoderma* spp., *Penicillium* spp., *Gemmatimonas* spp. and *Burkholderia* spp. but positively correlated with *Bacillus* spp. Most PSMs assumed centroid distribution with their abundance positively correlating with SOC, total nitrogen and POxC (Fig. 3).

Table 6
Bi-plot CCA analysis scores for constraining phosphorus solubilizing microbes and soil chemical properties in CT1-Nyabeda site, western Kenya.

Variables	CCA1	CCA2	R^2	P-value
Soil chemical properties				
Soil pH (soil: water; 1:2)	0.56	−0.83	0.19	**0.03**
Soil P (Mehlich III(mg kg^{-1}))	−0.39	0.92	0.31	**<0.01**
Labile C (mg kg^{-1})	0.19	0.98	0.02	0.74
Total N (%)	−0.37	0.93	0.10	0.16
SOC (%)	−0.14	0.99	0.16	**0.05**
Carbon: Nitrogen ratio	0.67	0.74	0.09	0.18
Total Mg (mg kg^{-1})	0.72	−0.70	0.20	**0.02**
Total Al (mg kg^{-1})	−0.86	0.51	0.31	**<0.01**
Microbial species				
Actinomyces spp.	1.00	−0.01	0.03	0.52
Arthrobacter spp.	−0.46	−0.89	0.18	**0.04**
Aspergillus spp.	−0.66	0.75	0.13	0.08
Azospirillum spp.	−0.78	0.62	0.16	**0.05**
Bacillus spp.	0.40	−0.92	0.21	**0.01**
Bradyrhizobium spp.	−0.92	−0.39	0.14	0.07
Burkholderia spp.	0.96	−0.28	0.23	**0.01**
Gemmatimonas spp.	0.99	0.17	0.71	**<0.01**
Glomus spp.	0.21	−0.98	0.02	0.69
Mesorhizobium spp.	−0.80	−0.61	0.04	0.46
Micromonospora spp.	0.49	−0.87	0.12	0.11
Paraglomus spp.	−0.81	0.58	0.11	0.16
Penicillium spp.	−0.86	0.51	0.24	**0.03**
Pseudomonas spp.	−0.16	−0.99	0.10	0.16
Rhizobium spp.	0.62	−0.78	0.09	0.20
Sphingomonas spp.	−0.97	−0.25	0.16	**0.05**
Streptomyces spp.	−0.84	0.54	0.02	0.75
Thiobacillus spp.	0.61	0.79	0.02	0.72
Trichoderma spp.	−0.67	0.74	0.56	**<0.01**

4. Discussion

4.1. Benefits of reduced tillage for microbial biomass and PSMs species abundance

This study points out the positive aspects of practicing reduced tillage compared to conventional tillage on microbial biomass and abundance of PSMs. In the reduced tillage system, the higher MBP and PSMs abundance may be explained by minimal soil disturbance (Kihara et al., 2012, 2018; Choi et al., 2017) increased accumulation of organic matter and nutrient availability compared to conventional tillage systems (Chu et al., 2007; Sun et al., 2015; Li et al., 2017); but also improved soil environmental conditions favoring microbial growth and activities (Juan et al., 2015; García-Orenes et al., 2013). Increased microbial biomass in the reduced compared to conventional tillage systems observed in this study is consistent with previous studies in the same site (Kihara et al., 2012, 2018), and elsewhere in the United States of America (Mathew et al., 2012) and Asia (Guo et al., 2016), all attributing the higher microbial biomass to either increased quantity of SOC, reduced soil disturbance and/or improved nutrient levels. Soil organic carbon and nutrients are important source of food and energy to the soil microbial population (Logah et al., 2010), and this explains the positive correlation between SOC, total nitrogen and POxC with soil PSMs (Table 3, Fig. 3). Soil chemical characteristics such as soil pH, POxC, SOC and total N are important edaphic parameters that influence the microbial species abundance and distribution, explaining their

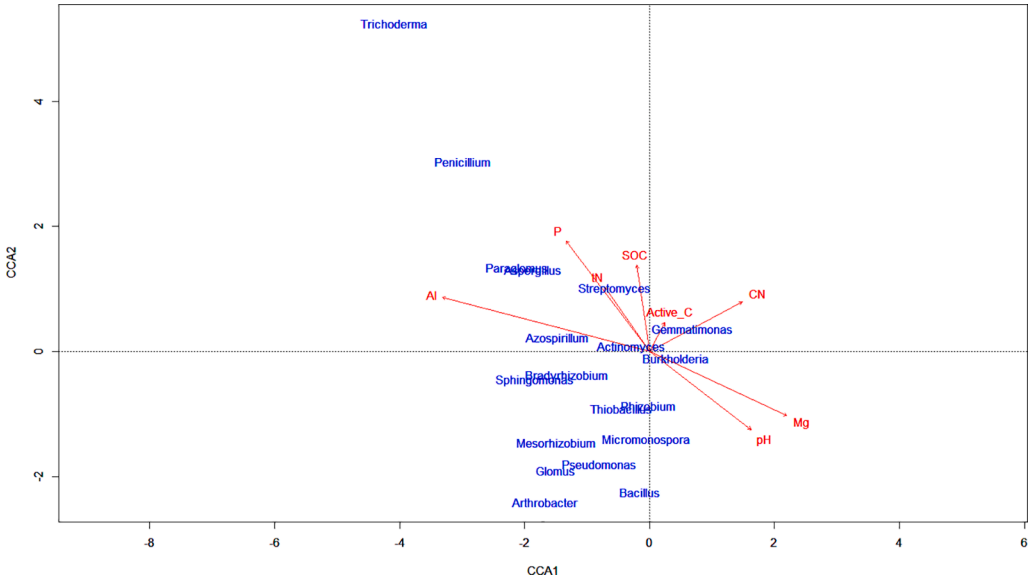

Fig. 3. Canonical Correspondence Analysis (CCA) results showing the relationship between the chemical properties and PSMs species' abundance in CT1 trial in 2017. pH = soil pH, CN = carbon to nitrogen ratio, SOC = soil organic carbon, Active_C = POxC, tN = total nitrogen (%).

significant correlations with PSMs. Generally, the centroid distribution of PSMs in response to soil properties (Fig. 3) indicates these species had similar environmental preferences (Vink et al., 2003).

Tillage associated disturbances in the conventional tillage systems can cause increased soil compaction and reduction of pore volumes, microbial desiccation, mechanical killing, limited substrate availability and disruption of access to food resources, thus rendering soils susceptible to unfavorable conditions for microbial growth and activities (Giller, 1996; Zhou et al., 2002; Bargaz et al., 2012; Choi et al., 2017; Szoboszlay et al., 2017), thus the reduced microbial biomass and abundance of PSMs in the conventional tillage systems. In addition, tillage associated disturbances in the conventional tillage can also lead to microbial communities mostly dominated by aerobic microbes (Mathew et al., 2012) following continuous opening of the soil during cultivation. This explains the increased abundance of PSMs involving *Micromonospora* spp., *Mesorhizobium* spp., *Agrobacterium* spp., *Bradyrhizobium* spp., *Ralstonia* spp. and *Sphingomonas* spp. in 2016 and *Streptomyces* spp., *Enterobacter* spp., *Arthrobacter* spp., *Bradyrhizobium* spp., *Paenibacillus* spp., *Ralstonia* spp. and *Rhodococcus* spp. (in 2017) in the conventional tillage treatment (CTFr) than reduced tillage treatment (RTFr) systems. This is also consistent with previous findings (Muriithi-Muchane, 2013) who worked on related processes and reported higher arbuscular mycorrhiza species (i.e., *Glomus* spp.) diversity in CT than in NT systems in the same locality.

Improved PSMs abundance observed in the current study can be assumed to positively correlate with their activities (Ndungu-Magiroi et al., 2015; Margenot et al., 2017) resulting to increased P availability. However, it remains unclear if the quantities of such P mineralized or solubilized by PSMs, especially in combination with other inputs, would reduce the quantity of inorganic P fertilizer demands, consequently relieving the farmers of some financial burdens incurred in purchasing such inorganic P fertilizers. For instance, PSMs, in combination with either inorganic triple super phosphate (TSP) fertiliser or rock phosphates, respectively, can decrease P fertilizer demands by 25% and 50%, respectively (Sundara et al., 2002).

Moreover, the slightly lower soil pH in the reduced and conventional tillage systems (Table 4) could contribute towards the proliferation of both pH specialist and pH selective species of PSMs (Andrade et al., 2002). The pH specialist and acidophilic microbial PSMs like Thiobacillus spp. were previously reported to be more dominant in relatively acidic environments (Harrison Jr, 1984) and this could also explain their increased abundance in the slightly lower pH in the reduced tillage (RTFr) than conventional tillage system (CTFr).

4.2. Stimulation of microbial biomass and PSMs species abundance with residue addition

The current study recognizes the benefits of residue addition on soil chemical and microbial variables. The consistent increase in some of the PSMs species abundance and MBP (Tables 4; 5) following residue addition (RTFr) relative to residue omission (RTF) indicates increased accumulation, and greater accessibility, of SOC as food by the microbes (Mathew et al., 2012; Mårtensson and Olsson, 2012; Kihara et al., 2012, 2018). This is consistent with the increase in the richness of PSMs observed in *Burkholderia* spp., *Streptomyces* spp., *Actinomyces* spp., *Pseudomonas* spp., *Micromonospora* spp., *Rhizobium* spp., *Bacillus* spp., *Arthrobacter* spp., *Mesorhizobium* spp., *Agrobacterium* spp., *Bradyrhizobium* spp., *Azospirillum* spp., *Gemmatimonas* spp., *Massilia* spp., *Paenibacillus* spp., *Ralstonia* spp., *Rhodococcus* spp., *Sinomonas* spp., *Sphingomonas* spp. and *Thiobacillus* spp. in the reduced tillage system with residue addition compared to the same system with no residue addition. This corroborates previous findings by Muriithi-Muchane (2013) who observed significant increase in P-efficient arbuscular mycorrhizal fungi richness following residue application in the same locality.

Similar observations with other PSMs are documented in other studies (St. John et al., 1983; Albertsen et al., 2006; Gryndler et al., 2006) which are also linked to continuous organic matter inputs that act as important food (carbon) and energy sources for microbial growth and activity (Logah et al., 2010). The added residues could have also improved soil physicochemical conditions, protected microsites, created microclimate, and/or at least, reduced losses of C from soils (Kihara, 2009; Kihara et al., 2012; Sharma-Poudyal et al., 2017; Sommer et al., 2018); directly favouring microbial proliferation and activities (Gougoulias et al., 2014) as observed in the study.

Although there was a significant increase in MBP, addition of residue at 2 Mg ha^{-1} did not increase MBC and MBN, and this indicates that there is probably a threshold for crop residue return into the soil that could effectively and significantly stimulate increases in microbial biomass and SOC. Identification of this threshold for crop residue return may therefore be vital in the management of soil organic carbon and microbial biomass. In a previous study in the same site, Sommer et al. (2017) reported that residue and fertiliser addition only reduced SOC losses but with no effects in soil carbon sequestration. Our observation is consistent with Zhang et al. (2016) who reported that microbial biomass only increased significantly following incorporation of residues at 5 Mg ha^{-1} but not 2.5 Mg ha^{-1}. In our site, residue was applied at 2 Mg ha^{-1}, which is even lower than 2.5 Mg ha^{-1} reported in Zhang et al. (2016). In addition, the faster rates of residue disappearance previously observed in this site (Kihara et al., 2015) could limit SOC and substrate availability thereby suppressing microbial growth and activities and consequently reducing microbial abundance as observed.

The slightly higher SOC content in the residue addition (RTFr) than no residue application (RTF) treatments demonstrates increased microbial activities involving breakdown of residues (Kihara et al., 2015) and mineralization of the added residues following stimulation of microbial abundance by SOC and substrate availability. However, this observation is inconsistent with Paul et al. (2013), perhaps due to difference in the years that the samplings were done. Paul et al. (2013) conducted sampling when the trial was about 5 years old (i.e., 11 cropping seasons) while for our study, sampling was done when the trial was 15 years old (30 cropping seasons). Previous studies found that residue retention combined with mineral fertilizers positively influenced upper SOC (Bationo et al., 2007; Chivenge et al., 2007, 2011; Anyanzwa et al., 2011) and this is consistent with our results.

4.3. Increase in PSMs abundance in the absence of P and lime

Although liming and P fertilizer additions have been promoted to increase P availability in western Kenya (Kisinyo et al., 2014), approximately 80% of soils in the region are still P deficient (Jama and Van Straaten, 2006; Opala et al., 2013); and this affects biological P cycling. Even when P inputs are applied, the high P fixation capacity of the Ferralsols would cause P lock-up in different pools, resulting to less available P and high total soil P (Ayaga et al., 2006), with the reduced P availability likely to stimulate the activities of P-efficient microbial species as previously reported in the same locality (Margenot et al., 2017). This study demonstrated that PSMs abundance was greatly stimulated in the absence of P or lime application compared to application of either lime or fertilizer inputs. In the study, no significant effect of liming on soil pH was found, probably because liming was done only once in 2015. The reduction of PSMs abundance in the lime-applied (CTint + L) and fertilizer-applied (CTFr) treatments compared to no lime (CTint-L) or fertilizer (CTr) additions demonstrates suppressive effects of increased inorganic P availability on PSMs (Gosling et al., 2006). Higher inorganic P can hinder microbial expression of P-associated enzymes, thus reducing their abundance and activities (Nannipieri et al., 2011). Liming often relieves P limitation by modifying soil pH and reducing P fixation (Li et al., 2019), and consequently enhancing P availability. Changes in soil pH following no lime additions could directly affect soil carbon, nutrient mineralization and availability (Kemmitt et al., 2006; Wu et al., 2017), thereby affecting soil microbial

structure, abundance and distribution (Carrero-Colón et al., 2006).

Recently, Olsson et al. (2006) established that high available P levels in a system (and in plants) can lower allocation of carbon to some PSMs species (specifically the arbuscular mycorrhizal fungi reported herein as *Glomus* spp. and *Paraglomus* spp.), and this can compromise their proliferation and activities. Fertilizer addition raises the concentrations of available phosphorus in the soil, but with inhibitory effects on growth and activities of most P-efficient microbial species like PSMs (Gosling et al., 2006; Aislabie et al., 2013; Liu et al., 2016). However, the increase in PSMs in the treatments without lime (CTint-L) nor fertilizer addition (CTr) indicate limited P availability that would otherwise stimulate the proliferation and activities of P-efficient microbial species like PSMs (Olander and Vitousek, 2000; Rosolem et al., 2014). This corroborates the findings from previous studies done on related microbial properties in the same locality. For instance, Margenot et al. (2017) observed that application of poorly soluble P input (i.e., Minjingu phosphate rock; MPR) stimulated P-efficient microbial communities more than application of TSP fertilizers. Similarly, in the same locality, Muriithi-Muchane (2013) reported increased Arbuscular mycorrhizal species (*Glomus* spp.) richness and diversity in unfertilized than fertilized conventional tillage. The reduced P availability following application of poorly soluble MPR (Margenot et al., 2017) or no fertilizer addition (Muriithi-Muchane, 2013) is consistent with the limited P availability following no fertilizer or lime application in this study; with both resulting to the observed increases in PSMs and related activities.

This study also indicates that not all the PSMs species are suppressed by liming and fertilizer addition. Liming and fertilizer addition improves soil nutritional status and crop biomass production favoring greater organic matter return to the soil, and consequently improved food availability for PSMs growth and activities. The increase in some PSMs with liming and fertilizer addition is an indication that increasing P (nutrient) availability can positively influence the proliferation of certain P-efficient but nutrient sensitive microbial species, amongst them being mycorrhizal helper bacterial species (e.g., *Paenibacillus* spp., *Rhodococcus* spp., *Arthrobacter* spp. and *Enterobacter* spp) whose abundance is greatly influenced by soil nutritional status and environmental factors (Zaidi et al., 2009); but play numerous integral roles that promote self-regulating soil microbiome (Frey-Klett and Garbaye, 2005; Frey-Klett et al., 2007; Prasad et al., 2012). This corroborates the findings of a recent study where long-term lime amendments, besides increasing P availability, increased the abundance of PSMs genes with different rhizospheric nutrient cycling properties (Bossolani et al., 2020).

4.4. Benefits of maize-soybean intercropping versus rotation systems on microbial biomass and PSMs species abundance

The current study identifies the positive impacts between maize-soybean intercropping versus rotation systems on microbial biomass and abundance of PSMs. The study demonstrated that the populations of majority of PSMs involving *Pseudomonas* spp. (in 2016), *Penicillium* spp., *Streptomyces* spp., *Mesorhizobium* spp., *Rhizobium* spp., *Bradyrhizobium* spp. and *Paenibacillus* spp. (in 2017) significantly increased in the intercrop system (RT.int) compared to rotation system (RT.rot) and this indicates that these PSMs perhaps had greater ability to effectively utilize root metabolites and decompose the diverse crop residues in the intercrop than rotation systems. Intercropped systems contain a variety of crops compared to rotation systems and this increases residue availability and production of root metabolites, thereby influencing organic carbon and diverse substrate availability (Sun et al., 2015) that could shape the microbial structure, growth and activities (Tan et al., 2019) as observed in the study. In addition, the slightly higher microbial biomass and significantly higher PSMs abundance in the intercropped systems could reflect the possibility of direct contact of the PSMs with the crop roots that likely stimulated the roots to produce more nutrients (Song et al., 2007) and metabolites for microbial growth and activities.

Besides addition of more soil organic matter that could also increase the PSMs abundance in the intercropped than rotation systems, the higher biodiversity in intercropped systems have different rates of P root uptake and can promote stiff competition for nutrients leading to nutrient deficiency (especially P) (Zhang and Li, 2003; Li et al., 2009; Latati et al., 2016; Xue et al., 2016) and this could favor colonization and proliferation of P solubilizers (Liu et al., 2016). The impact of nutrient availability on soil microbial structure, abundance and activities could also influence the proliferation of PSMs as observed. The slightly higher SOC in the intercrop than rotation systems indicate that SOC could contribute in shaping the structure of microbial biomass and community abundance (Lian et al., 2019). Previous reports (Harinikumar et al., 1990; Kihara et al., 2012; Vukicevich et al., 2016; Mandou et al., 2016; Yang et al., 2016b) linked increased microbial abundance in the intercropped systems to accumulation of soil organic carbon and nutrient enrichment. Higher P demand and uptake in maize than soybean probably caused reduction of P availability in the maize-soybean rotation (RT.rot) than RT.int systems (and considering the fact that the study was conducted during maize phase), consequently prompting microbial P release response evidenced through increase in populations of PSMs involving *Arthrobacter* spp., *Mesorhizobium* spp., *Agrobacterium* spp. and *Azospirillum* spp. in RT.rot than RT.int system.

5. Conclusions

In this study, we demonstrated the influence of different agronomic management factors like residue addition, tillage, fertilizer addition, liming and cropping systems have on soil chemical and microbial variables. Residue addition, reduced tillage and maize-soybean intercropping system improved soil chemical characteristics, microbial biomass and PSMs abundance. Fertiliser addition and liming are known to promote self-regulating soil microbiome systems by increasing P availability, yet interestingly, the system can sometimes respond by down-regulating the microbes that provide P under natural conditions (i.e., no fertiliser and no lime). Thus, it would be interesting if further research can focus on; i) estimating the quantity of phosphorus that can be solubilized by the different PSMs species reported in the study, and ii) unravelling the economics of microbial mineralization of nutrients versus the costs of inorganic input application.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Aguinis, H., Gottfredson, R.K., Joo, H., 2013. Best-practice recommendations for defining, identifying, and handling outliers. Org. Res. Methods 16 (2), 270–301.

Albertsen, A., Ravnskov, S., Green, H., Jensen, D., Larsen, J., 2006. Interactions between the external mycelium of the mycorrhizal fungus Glomus intraradices and other soil microorganisms as affected by organic matter. Soil Biol. Biochem. 38 (5), 1008–1014.

Alori, E.T., Glick, B.R., Babalola, O.O., 2017. Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Front. Microbiol. 8, 971.

Al-Rohily, K.M., Ghoneim, A.M., Modaihsh, A.S., Mahjoub, M.O., 2013. Phosphorus availability in calcareous soil amend with chemical phosphorus fertilizer, cattle manure compost and sludge manure. Int. J. Soil Sci. 8 (1), 17–24.

Anand, K., Kumari, B., Mallick, M.A., 2016. Phosphate solubilizing microbes: an effective and alternative approach as biofertilizers. J. Pharm. Sci. 8, 37–40.

Andrade, D.S., Murphy, P.J., Giller, K.E., 2002. The diversity of phaseolus-nodulating rhizobial populations is altered by liming of acid soils planted with Phaseolus vulgaris L. in Brazil. Appl. Environ. Microbiol. 68 (8), 4025–4034.

Anyanzwa, H., Okalebo, J.R., Othieno, C.O., Bationo, A., Waswa, B.S., Kihara, J., 2011. Effects of conservation tillage, crop residue and cropping systems on changes in soil organic matter and maize–legume production: a case study in Teso District. In: Innovations as key to the green revolution in Africa. Springer, Dordrecht, pp. 205–213.

Ayaga, G., Todd, A., Brookes, P.C., 2006. Enhanced biological cycling of phosphorus increases its availability to crops in low-input sub-Saharan farming systems. Soil Biol. Biochem. 38 (1), 81–90.

Babalola, O.O., Glick, B.R., 2012. The use of microbial inoculants in African agriculture: current practice and future prospects. J. Food Agric. Environ. 10 (3&4), 540–549.

Bache, B.W., Williams, E.G., 1971. A phosphate sorption index for soils. J. Soil Sci. 22 (3), 289–301.

Bargaz, A., Faghire, M., Abdi, N., Farissi, M., Sifi, B., Drevon, J.J., Cherkaoui Ikbal, M. & Ghoulam, C., (2012). Low soil phosphorus availability increases acid phosphatases activities and affects P partitioning in nodules, seeds and rhizosphere of Phaseolus vulgaris. Agriculture, 2(2), pp.139-153.

Bartlett, R.J., Ross, D.S., 1988. Colorimetric determination of oxidizable carbon in acid soil solutions. Soil Sci. Soc. Am. J. 52 (4), 1191–1192.

Bationo, A., Kihara, J., Vanlauwe, B., Waswa, B., Kimetu, J., 2007. Soil organic carbon dynamics, functions and management in West African agro-ecosystems. Agric. Syst. 94 (1), 13–25.

Bengtsson, J., Ahnström, J., Weibull, A.C., 2005. The effects of organic agriculture on biodiversity and abundance: a meta-analysis. J. Appl. Ecol. 42 (2), 261–269.

Böhme, L., Langer, U., Böhme, F., 2005. Microbial biomass, enzyme activities and microbial community structure in two European long-term field experiments. Agric. Ecosyst. Environ. 109 (1-2), 141–152.

Borham, A., Belal, E., Metwaly, M., El-gremy, S., 2017. Phosphate solubilization by Enterobacter cloacae and its impact on growth and yield of wheat plants. J. Sustain. Agri. Sci. 43 (2), 89–103.

Bünemann, E.K., Schwenke, G.D., Van Zwieten, L., 2006. Impact of agricultural inputs on soil organisms—a review. Soil Res. 44 (4), 379. https://doi.org/10.1071/SR05125.

Carrero-Colón, M., Nakatsu, C.H., Konopka, A., 2006. Effect of nutrient periodicity on microbial community dynamics. Appl. Environ. Microbiol., 72(5), 3175-3183.

Chivenge, P., Murwira, H., Giller, K., Mapfumo, P., Six, J., 2007. Long-term impact of reduced tillage and residue management on soil carbon stabilization: implications for conservation agriculture on contrasting soils. Soil Tillage Res. 94 (2), 328–337.

Chivenge, P., Vanlauwe, B., Gentile, R., Six, J., 2011. Organic resource quality influences short-term aggregate dynamics and soil organic carbon and nitrogen accumulation. Soil Biol. Biochem. 43 (3), 657–666.

Choi, S., Song, H., Tripathi, B.M., Kerfahi, D., Kim, H., Adams, J.M., 2017. Effect of experimental soil disturbance and recovery on structure and function of soil community: a metagenomic and metagenetic approach. Sci. Rep. 7 (1).

Chu, H., Lin, X., Fujii, T., Morimoto, S., Yagi, K., Hu, J., Zhang, J., 2007. Soil microbial biomass, dehydrogenase activity, bacterial community structure in response to long-term fertilizer management. Soil Biol. Biochem. 39 (11), 2971–2976.

Chung, H., Park, M., Madhaiyan, M., Seshadri, S., Song, J., Cho, H., Sa, T., 2005. Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. Soil Biol. Biochem. 37 (10), 1970–1974.

Culman, S.W., Snapp, S.S., Freeman, M.A., Schipanski, M.E., Beniston, J., Lal, R., Drinkwater, L.E., Franzluebbers, A.J., Glover, J.D., Grandy, A.S., Lee, J., Six, J., Maul, J.E., Mirksy, S.B., Spargo, J.T., Wander, M.M., 2012. Permanganate oxidizable carbon reflects a processed soil fraction that is sensitive to management. Soil Sci. Soc. Am. J. 76 (2), 494–504.

Das, B.B., Dkhar, M.S., 2011. Rhizosphere microbial populations and physico chemical properties as affected by organic and inorganic farming practices. American-Eurasian J. Agri. Environ. Sci. 10, 140–150.

Fageria, N.K., Baligar, V.C., Moreira, A., Moraes, L.A.C., 2013. Soil phosphorous influence on growth and nutrition of tropical legume cover crops in acidic soil. Commun. Soil Sci. Plant Anal. 44 (22), 3340–3364.

Frey-Klett, P., Garbaye, J., 2005. Mycorrhiza helper bacteria: a promising model for the genomic analysis of fungal-bacterial interactions. New Phytologist, 168(1), 4-8.

Frey-Klett, P., Garbaye, J. A., Tarkka, M., 2007. The mycorrhiza helper bacteria revisited. New Phytologist, 176(1), 22-36.

García-Orenes, F., Morugán-Coronado, A., Zornoza, R., Scow, K., 2013. Changes in soil microbial community structure influenced by agricultural management practices in a Mediterranean Agro-Ecosystem. PloS One, 8(11), e80522.

Ghimire, B., Ghimire, R., VanLeeuwen, D., Mesbah, A., 2017. Cover crop residue amount and quality effects on soil organic carbon mineralization. Sustainability, 9(12), 2316.

Giller, P.S., 1996. The diversity of soil communities, the? Poor man's tropical rainforest? Biodivers. Conserv. 5 (2), 135–168.

Gosling, P., Hodge, A., Goodlass, G., Bending, G.D., 2006. Arbuscular mycorrhizal fungi and organic farming. Agric. Ecosyst. Environ. 113 (1-4), 17–35.

Gougoulias, C., Clark, J.M., Shaw, L.J., 2014. The role of soil microbes in the global carbon cycle: tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems: role of soil microbes in global carbon cycle: carbon tracking & agro-cosystem management. J. Sci. Food Agric. 94 (12), 2362–2371.

Gryndler, M., Larsen, J., Hršelová, H., Řezáčová, V., Gryndlerová, H., Kubát, J., 2006. Organic and mineral fertilization, respectively, increase and decrease the development of external mycelium of arbuscular mycorrhizal fungi in a long-term field experiment. Mycorrhiza 16 (3), 159–166.

Guo, L.J., Lin, S., Liu, T.Q., Cao, C.G., Li, C.F., 2016. Effects of conservation tillage on topsoil microbial metabolic characteristics and organic carbon within aggregates under a rice (Oryza sativa L.)–wheat (Triticum aestivum L.) cropping system in central China. PLoS ONE 11 (1), e0146145.

Harinikumar, K.M., Bagyaraj, D.J., Mallesha, B.C., 1990. Effect of intercropping and organic soil amendments on native VA mycorrhizal fungi in an oxisol. Arid Land Res. Manage. 4 (3), 193–197.

Harrison Jr, A.P., 1984. The acidophilic thiobacilli and other acidophilic bacteria that share their habitat. Annu. Rev. Microbiol. 38 (1), 265–292.

Igual, J.M., Valverde, A., Cervantes, E., Velázquez, E., 2001. Phosphate-solubilizing bacteria as inoculants for agriculture: use of updated molecular techniques in their study. Agronomie 21 (6-7), 561–568.

Jama, B., Van Straaten, P., 2006. Potential of East African phosphate rock deposits in integrated nutrient management strategies. An. Acad. Bras. Ciênc. 78 (4), 781–790.

Jarosch, K.A., Kandeler, E., Frossard, E., Bünemann, E.K., 2019. Is the enzymatic hydrolysis of soil organic phosphorus compounds limited by enzyme or substrate availability? Soil Biol. Biochem. 139, 107628.

Juan, L.I., Li, Y.T., Yang, X.D., Zhang, J.J., Lin, Z.A., Zhao, B.Q., 2015. Microbial community structure and functional metabolic diversity are associated with organic carbon availability in an agricultural soil. Journal of Integrative Agriculture, 14(12), 2500-2511.

Kalayu, G., 2019. Phosphate solubilizing microorganisms: promising approach as biofertilizers. Int. J. Agronomy 2019, 1–7.

Kemmitt, S., Wright, D., Goulding, K., Jones, D., 2006. pH regulation of carbon and nitrogen dynamics in two agricultural soils. Soil Biol. Biochem. 38 (5), 898–911.

Khan, A.A., Jilani, G., Akhtar, M.S., Naqvi, S.M.S., Rasheed, M., 2009. Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. J. Agri. Biol. Sci. 1 (1), 48–58.

Kihara, J.M., 2009. Conservation tillage in Kenya: the biophysical processes affecting its effectiveness. Center for Development Research, PHD, p. 141.

Kihara, J., Bolo, P., Ayaga, G., Mukalama, J., Margenot, A.J., Sommer, R., 2018. Agronomic management controls microbial populations in soils of western Kenya. Working Paper. CIAT Publication No. 474. International Center for Tropical Agriculture (CIAT). Nairobi, Kenya. 24 p. Available at: https://hdl.handle.net/10568/97847.

Kihara, J., Martius, C., Bationo, A., 2015. Crop residue disappearance and macrofauna activity in sub-humid western Kenya. Nutr. Cycl. Agroecosyst. 102 (1), 101–111.

Kihara, J., Martius, C., Bationo, A., Thuita, M., Lesueur, D., Herrmann, L., Amelung, W., Vlek, P.L.G., 2012. Soil aggregation and total diversity of bacteria and fungi in various tillage systems of sub-humid and semi-arid Kenya. Appl. Soil Ecol. 58, 12–20.

Kisinyo, P.O., Othieno, C.O., Gudu, S.O., Okalebo, J.R., Opala, P.A., Ng'etich, W.K., Nyambati, R.O., Ouma, E.O., Agalo, J.J., Kebeney, S.J., Too, E.J., Kisinyo, J.A., Opile, W.R., 2014. Immediate and residual effects of lime and phosphorus fertilizer on soil acidity and maize production in western Kenya. Ex. Agric. 50 (1), 128–143.

Kjeldahl, J., 1883. Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern. Fresenius J. Anal. Chem. 22 (1), 366–382.

Kutner, M. H., Nachtsheim, C. J., Neter, J., Li, W. (2005). Applied Linear Statistical Models (Vol. 5). New York: McGraw-Hill Irwin.

Latati, M., Benlahrech, S., Lazali, M., Sihem, T., Kaci, G., Takouachet, R., Alkama, N., Hamdani, F.Z., Hafnaoui, E.A., Belarbi, B., Ounane, G., Ounane, S.M., 2016. Intercropping promotes the ability of legume and cereal to facilitate phosphorus and nitrogen acquisition through root-induced processes. Grain Legum. https://doi.org/10.5772/63438.

Li, F., Chen, L., Zhang, J., Yin, J., Huang, S., 2017. Bacterial community structure after long-term organic and inorganic fertilization reveals important associations between soil nutrients and specific taxa involved in nutrient transformations. Front. Microbiol. 8, 187.

Li, Y.Y., Yu, C., Cheng, X., Li, C.J., Sun, J.H., Zhang, F.S., Lambers, H., Li, L., 2009. Intercropping alleviates the inhibitory effect of N fertilization on nodulation and symbiotic N2 fixationof faba bean. Plant Soil 323, 295–308.

Li, Y., Cui, S., Chang, S.X., Zhang, Q., 2019. Liming effects on soil pH and crop yield depend on lime material type, application method and rate, and crop species: a global meta-analysis. J. Soils Sediments 19 (3), 1393–1406.

Lian, T., Mu, Y., Jin, J., Ma, Q., Cheng, Y., Cai, Z., Nian, H., 2019. Impact of intercropping on the coupling between soil microbial community structure, activity, and nutrient-use efficiencies. PeerJ, 7, e6412.

Liu, W., Zhang, Y., Jiang, S., Deng, Y., Christie, P., Murray, P.J., Li, X., Zhang, J., 2016. Arbuscular mycorrhizal fungi in soil and roots respond differently to phosphorus inputs in an intensively managed calcareous agricultural soil. Sci. Rep. 6 (1).

Logah, V., Safo, E.Y., Quansah, C., Danso, I., 2010. Soil microbial biomass carbon, nitrogen and phosphorus dynamics under different amendments and cropping systems in the semi-deciduous forest zone of Ghana. West African J. Appl. Ecol. 17 (1).

Mahadevamurthy, M., Sidappa, M., Thriveni, M.C., Mythrashree, S.R., Amruthesh, K.N., 2016. Isolation of phosphate solubilizing fungi from rhizosphere soil and its effect on seed growth parameters of different crop plants. J. Appl. Biol. Biotechnol., 4(6), 22-26.

Mahdi, S.S., Talat, M.A., Hussain Dar, M., Hamid, A., Ahmad, L., 2012. Soil phosphorus fixation chemistry and role of phosphate solubilizing bacteria in enhancing its efficiency for sustainable cropping – a review. J. Pure Appl. Microbiol. 6 (4), 1–7.

Mandou, M.S., Adamou, S., Nwaga, D., Etoa, R.-X., 2016. Intercrops influence mycorrhizal symbiosis development, growth and nutrient uptake of banana. Int. J. Curr. Microbiol. App. Sci. 5 (12), 84–94.

Margenot, A.J., Sommer, R., Mukalama, J., Parikh, S.J., 2017. Biological P cycling is influenced by the form of P fertilizer in an Oxisol. Biol. Fertil. Soils 53 (8), 899–909.

Mårtensson, L.-M., Olsson, P.A., 2012. Reductions in microbial biomass along disturbance gradients in a semi-natural grassland. Appl. Soil Ecol. 62, 8–13.

Martin, M.A., Roberts, S., 2010. Jackknife-after-bootstrap regression influence diagnostics. J. Nonparametric Statistics 22 (2), 257–269.

Mathew, R.P., Feng, Y., Githinji, L., Ankumah, R., Balkcom, K.S., 2012. Impact of no-tillage and conventional tillage systems on soil microbial communities. Appl. Environ. Soil Sci. 2012.

Mehlich, A., 1984. Mehlich 3 soil test extractant: a modification of Mehlich 2 extractant. Commun. Soil Sci. Plant Anal. 15 (12), 1409–1416.

Mucheru-Muna, M., Mugendi, D., Pypers, P., Mugwe, J., Kung'u, J., Vanlauwe, B., & Merckx, R. (2014). Enhancing maize productivity and profitability using organic inputs and mineral fertilizer in central Kenya small-hold farms. Exp. Agri., 50(2), 250-269.

Muriithi-Muchane, M. N. (2013). Influences of agricultural management practices on Arbuscular Mycorrhiza Fungal symbioses in Kenyan agro-ecosystems.

Nannipieri, P., Giagnoni, L., Landi, L., Renella, G., 2011. Role of phosphatase enzymes in soil. In: Phosphorus in Action. Springer, Berlin, Heidelberg, pp. 215–243.

Ndungu-Magiroi, K.W., Waswa, B., Bationo, A., Okalebo, J.R., Othieno, C., Herrmann, L., Lesueur, D., 2015. Minjingu phosphate rock applications increase the population of phosphate solubilising microorganisms with a positive impact on crop yields in a Kenyan Ferralsol. Nutr. Cycl. Agroecosyst. 102 (1), 91–99.

Nziguheba, G., Zingore, S., Kihara, J., Merckx, R., Njoroge, S., Otinga, A., Vandamme, E., Vanlauwe, B., 2016. Phosphorus in smallholder farming systems of sub-Saharan Africa: implications for agricultural intensification. Nutr. Cycl. Agroecosyst. 104 (3), 321–340.

Olander, L.P., Vitousek, P.M., 2000. Regulation of soil phosphatase and chitinase activityby N and P availability. Biogeochemistry 49 (2), 175–191.

Oliveira, C.A., Alves, V.M.C., Marriel, I.E., Gomes, E.A., Scotti, M.R., Carneiro, N.P., Guimarães, C.T., Schaffert, R.E., Sá, N.M.H., 2009. Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome. Soil Biol. Biochem. 41 (9), 1782–1787.

Olsson, P.A., Hansson, M.C., Burleigh, S.H., 2006. Effect of P availability on temporal dynamics of carbon allocation and Glomus intraradices high-affinity P transporter gene induction in arbuscular mycorrhiza. Appl. Environ. Microbiol. 72 (6), 4115–4120.

Opala, P.A., Okalebo, J.R., Othieno, C., 2013. Comparison of effects of phosphorus sources on soil acidity, available phosphorus and maize yields at two sites in western Kenya. Arch. Agron. Soil Sci. 59 (3), 327–339.

Paul, B.K., Vanlauwe, B., Ayuke, F., Gassner, A., Hoogmoed, M., Hurisso, T.T., Koala, S., Lelei, D., Ndabamenye, T., Six, J., Pulleman, M.M., 2013. Medium-term impact of tillage and residue management on soil aggregate stability, soil carbon and crop productivity. Agric. Ecosyst. Environ. 164, 14–22.

Porteous, L.A., Seidler, R.J., Watrud, L.S., 1997. An improved method for purifying DNA from soil for polymerase chain reaction amplification and molecular ecology applications. Mol. Ecol. 6 (8), 787–791.

Pradhan, N., Sukla, L.B., 2006. Solubilization of inorganic phosphates by fungi isolated from agriculture soil. Afr. J. Biotechnol. 5 (10).

Prasad, K., Aggarwal, A., Yadav, K., Tanwar, A., 2012. Impact of different levels of superphosphate using arbuscular mycorrhizal fungi and Pseudomonasfluorescens on Chrysanthemum indicum L. J. Soil Sci. Plant Nutr. 12 (3), 451–462.

R Development Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.

Rosolem, C.A., Merlin, A., Bull, J.C.L., 2014. Soil phosphorus dynamics as affected by Congo grass and P fertilizer. Sci. agric. (Piracicaba, Braz.) 71 (4), 309–315.

Schmidt, T.S., Rodrigues, J.F.M., Von Mering, C., 2014. Ecological consistency of SSU rRNA-based operational taxonomic units at a global scale. PLoS Comput. Biol., 10 (4), e1003594.

Sharma, S.B., Sayyed, R.Z., Trivedi, M.H., Gobi, T.A., 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. SpringerPlus 2 (1).

Sharma-Poudyal, D., Schlatter, D., Yin, C., Hulbert, S., Paulitz, T., 2017. Long-term no-till: a major driver of fungal communities in dryland wheat cropping systems. PloS One, 12(9), e0184611.

Sommer, R., Paul, B.K., Mukalama, J., Kihara, J., 2018. Reducing losses but failing to sequester carbon in soils – the case of Conservation Agriculture and Integrated Soil Fertility Management in the humid tropical agro-ecosystem of Western Kenya. Agric. Ecosyst. Environ. 254, 82–91.

Son, H.J., Park, G.T., Cha, M.S., Heo, M.S., 2006. Solubilization of insoluble inorganic phosphates by a novel salt- and pH-tolerant Pantoea agglomerans R-42 isolated from soybean rhizosphere. Bioresour. Technol. 97 (2), 204–210.

Song, Y.N., Marschner, P., Li, L., Bao, X.G., Sun, J.H., Zhang, F.S., 2007. Community composition of ammonia-oxidizing bacteria in the rhizosphere of intercropped wheat (Triticum aestivum L.), maize (Zea mays L.), and faba bean (Vicia faba L.). Biol. Fertil. Soils 44 (2), 307–314.

Srinivasan, R., Yandigeri, M.S., Kashyap, S., Alagawadi, A.R., 2012. Effect of salt on survival and P-solubilization potential of phosphate solubilizing microorganisms from salt affected soils. Saudi J. Biol. Sci. 19 (4), 427–434.

St. John, T. V., Coleman, D. C., Reid, C. P. P. (1983). Association of vesicular-arbuscular mycorrhizal hyphae with soil organic particles. Ecology, 64(4), 957-959.

Sun, R., Zhang, X.-X., Guo, X., Wang, D., Chu, H., 2015. Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. Soil Biol. Biochem. 88, 9–18.

Sundara, B., Natarajan, V., Hari, K., 2002. Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugarcane and sugar yields. Field Crops Res. 77 (1), 43–49.

Szoboszlay, M., Dohrmann, A.B., Poeplau, C., Don, A., Tebbe, C.C., 2017. Impact of land-use change and soil organic carbon quality on microbial diversity in soils across Europe. FEMS Microbiol. Ecol. 93 (12), fix146.

Tan, X., Liao, H., Shu, L., Yao, H., 2019. Effect of different substrates on soil microbial community structure and the mechanisms of reductive soil disinfestation. Front. Microbiol. 10, 2851.

Tandon, A., Fatima, T., Shukla, D., Tripathi, P., Srivastava, S., Singh, P.C., 2020. Phosphate solubilization by Trichoderma koningiopsis (NBRI-PR5) under abiotic stress conditions. J. King Saud Univ.–Sci. 32 (1), 791–798.

van der Bom, F., Nunes, I., Raymond, N.S., Hansen, V., Bonnichsen, L., Magid, J., Nybroe, O., Jensen, L.S., 2018. Long-term fertilisation form, level and duration affect the diversity, structure and functioning of soil microbial communities in the field. Soil Biol. Biochem. 122, 91–103.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19 (6), 703–707.

Vink, A., Baumann, K.-H., Böckel, B., Esper, O., Kinkel, H., Volbers, A., Willems, H., Zonneveld, K.A.F., 2003. In: The South Atlantic in the Late Quaternary. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 101–120.

Vukicevich, E., Lowery, T., Bowen, P., Úrbez-Torres, J.R., Hart, M., 2016. Cover crops to increase soil microbial diversity and mitigate decline in perennial agriculture. A review. Agron. Sustain. Dev. 36 (3), 48.

Walpola, B.C., Yoon, M.H., 2012. Prospectus of phosphate solubilizing microorganisms and phosphorus availability in agricultural soils: a review. African J. Microbiol. Res. 6 (37), 6600–6605.

Weil, R.R., Islam, K.R., Stine, M.A., Gruver, J.B., Samson-Liebig, S.E., 2003. Estimating active carbon for soil quality assessment: a simplified method for laboratory and field use. Am. J. Altern. Agric. 18 (1), 3–17.

Wu, Y., Zeng, J., Zhu, Q., Zhang, Z., Lin, X., 2017. pH is the primary determinant of the bacterial community structure in agricultural soils impacted by polycyclic aromatic hydrocarbon pollution. Sci. Rep. 7 (1), 40093.

Xue, Y., Xia, H., Christie, P., Zhang, Z., Li, L., Tang, C., 2016. Crop acquisition of phosphorus, iron and zinc from soil in cereal/legume intercropping systems: a critical review. Ann. Bot. 117 (3), 363–377.

Yang, B.O., Wang, Y., Qian, P.-Y., 2016a. Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. BMC Bioinf. 17 (1), 135.

Yang, Z., Yang, W., Li, S., Hao, J., Su, Z., Sun, M., Gao, Z., Zhang, C., 2016b. Variation of bacterial community diversity in rhizosphere soil of sole-cropped versus intercropped wheat field after harvest. PloS One 11 (3), e0150618.

Zaidi, A., Khan, M., Ahemad, M., Oves, M., 2009. Plant growth promotion by phosphate solubilizing bacteria. Acta Microbiol. Immunol. Hung. 56 (3), 263–284.

Zhang, B., Gao, Q., Xu, S., Ma, L., Tian, C., 2016. Long-term effect of residue return and fertilization on microbial biomass and community composition of a clay loam soil. J. Agric. Sci. 154 (6), 1051–1061.

Zhang, F., Li, L., 2003. Using competitive and facilitative interactions in intercropping systems enhances crop productivity and nutrient-use efficiency. Plant Soil 248, 305–312.

Zhou, J., Xia, B., Treves, D.S., Wu, L.Y., Marsh, T.L., O'Neill, R.V., Palumbo, A.V., Tiedje, J.M., 2002. Spatial and resource factors influencing high microbial diversity in soil. Appl. Environ. Microbiol., 68(1), 326-334.