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Agronomic management controls microbial populations in soils of western Kenya

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Contents

Tables

Figures

Introduction

The provision of food requirements for current and future generations can be guaranteed through agricultural intensification options that safeguard the production resource base. Over the years, the debate on sustainable agricultural intensification has not been matched with due regard to how the intensification options influence the functions and balance of soil organisms and soil biology in general. Soil mesofauna and microorganisms have received very little attention so far. In sub-Saharan Africa (SSA) especially, there is very little knowledge and documentation of soil micro-organismal functioning and how these affect and are affected by the abiotic environment (soil physical and chemical properties, and climatic conditions), as well as agricultural management and intensification. Therefore, there is need to evaluate how measures to restore soil fertility and improve its productivity influence not only crop productivity and soil physical and chemical changes, but also soil biology, i.e. the diversity of macro-, meso- and micro-fauna and flora. In addition, the impact of 'sustainable' intensification on the evolution of greenhouse gas (GHG) emissions and related climate footprint remains to be assessed in a comprehensive manner.

Key intensification options currently under promotion by practitioners include conservation agriculture and various integrated soil fertility management (ISFM) options. Minimum soil disturbance, a minimum soil cover of at least 30% throughout the season and crop rotation/intercropping, all of which are principles of conservation agriculture (CA), have been shown to not only introduce shifts in microbial populations but also improve soil structure (Kihara et al., 2012) and enhance carbon sequestration in the top soil. On the other hand, increased aggregation in CA provides anaerobic microsites suitable for micro-organisms that contribute to nitrogen (N) losses through denitrification, and the release of nitrous oxide (N_2O); a potent GHG. How such losses are influenced by nutrient inputs, such as through application of mineral fertilizer or biological N fixation, remains largely un-assessed in SSA.

The use of industrial fertilizers, one way of increasing crop productivity in SSA, can have variable effects on microbial biomass and activity (Wardle, 1992; Treseder, 2008). For instance, increased amounts of readily available forms of key inorganic mineral nutrients, e.g. N and P, can decrease population and diversity of various microbial functional groups associated with nutrient uptake (e.g. arbuscular mycorrhizae fungi) and nitrogen fixation (e.g. *rhizobium*) (Azcón-Aguilar and Bago, 1994; Geisseler and Scow, 2014; Smith and Read, 1997). On the other hand, fertilizer use can increase plant biomass production which, when returned to the soil via residues as in CA, promotes microbial proliferation and diversity (Álvarez, 2005). The effects of fertilizer use, either alone or in combination of organic resources, on soil micro-organisms need, therefore, to be evaluated in order to guide sound soil management practices.

This study focuses on

1

Determination of microbial community structure for soils of western Kenya

2

Determination of diversity of soil fauna (macrofauna, mesofauna, and microfauna) across a variety of commonly promoted management practices

3

Assessment of effects of these management practices on microbial biomass, soil enzyme activities and denitrification potentials

Photo: Georgina Smith/CIAT

Methods

Study sites

This study was conducted in western Kenya within three ongoing experiments: 1) a long-term (13 yrs) conservation agriculture trial (coded CT1) established in 2003, 2) a long-term (13 yrs) integrated soil fertility management trial (coded INM3) also established in 2003 – both managed by CIAT; and 3) a 6 yr-old conservation agriculture trial in Kakamega (coded Kakamega) managed by Kenya Agricultural and Livestock Research Organization (KALRO). The two long-term trials (CT1 and INM3) are in Nyabeda with only about 2 kilometers between them (complete experimental details are reported in Kihara et al., 2012 and Sommer et al., 2016) while the Kakamega is about 50 km away. Both sites are characterized by two rainy seasons: a long rainy season between March and August and short rainy season between September and February. Maize is the main staple crop grown either as a monocrop or in association with legumes, mainly common beans, groundnuts, and more recently soybean. Although Nyabeda has ferralsols and Kakamega nitisols, both sites are generally P-limited (available P <4 mg kg^{-1}), somewhat low pH and moderate carbon (Table 1). Nyabeda is within Siaya County while Kakamega is within the neighboring Kakamega County. The native vegetation in Nyabeda comprises dominantly *Markhamia lutea*, *Thevetia peruviana, Cassia siamea,* while exotic species include *Grivelia robusta*, *Terminalia mentalis*, *Ecalyptus* sp., *Casuarina equisetifolia* and *Tithonia diversifolia* interspersed with *Lantana camara*. The dominant vegetation around the Kakamega site include *Grivelia robusta, Tithonia diversifolia, Lantana camara, Olea welwitschii* (Elgon teak), *Vitex keniensis* (Meru oak), *Prunus africana* (bitter almond), and *Markhamia lutea*.

The study included 12 selected treatments from the three experiments (Table 2). These represent the common best bet management practices recommended for maize-legume based systems in sub-humid environments of tropical Africa, and a few contrasts for comparison. The management practices under each in general range from tillage practices, cropping systems, amendments use (manure and lime), residue retention (maize stovers in CT1 and Kakamega, and Tephrosia in INM3) and fertilizers.

Table 1 Location, climatic, and soil characteristics of the study sites

PARAMETER	INM3	CT1	Kakamega
Year established	2003	2003	2010
Agro-ecological zone [a]	Lower midland 2	Lower midland 2	Upper midland 1
Latitude	00° 08'38.3'' S	0° 07' 46.96'' N	0° 16.96' N
Longitude	34° 24'13.7'' E	34° 24' 19.15'' E	34° 46.07' E
Altitude (m.a.s.l.)	1420	1420	1534
Total annual rainfall (mm)	1730[b]	1730[b]	1978
Daily temperatures (°C)	23.2 (Min=14; Max=31)	23.2 (Min=14; Max=31)	21 (Min=11; Max=26)
Soil type	Ferralsol	Ferralsol	Nitisol
Sand:silt:clay	26:18:56	15:21:64	13:34:53
pH (water)	4.75	5.37	5.50
Extractable K (cmol+ kg^{-1})	0.27	0.10	0.70
P (mg P kg^{-1})	3.7	3.0	3.40
Total soil organic carbon (SOC) (%)	2.4	2.3	2.4

[a] See Jaetzold and Schmidt (1983) for details, [b] 1997-2013 period.

Table 2 Treatments selected from 3 long-term trials managed by CIAT and KALRO used in the assessment of soil biological indices

TRT	TRIAL	TILLAGE	CROPPING SYSTEM	LIME/MANURE	RESIDUES	FERTILIZERS	TARGET PHASE	YIELD (t/ha)
1	CT1	CT	M-S	None	+R	60N 60P	Maize	5.11 (1.40)
2	CT1	RT	M-St	None	+R	60N 60P	Maize	4.37 (1.60)
3	CT1	RT	M/S	None	+R	0N 60P	N/A	1.82 (1.26)
4	CT1	RT	M-S	-lime	+R	60N 60P	Soybean	1.41 (1.04)
5	CT1	CT	M/S	+lime	+R	0N 60P	N/A	2.58 (0.97)
6	CT1	CT	M/S	-lime	+R	0N 60P	N/A	2.58 (0.97)
7	CT1	CT	M-S	None	+R	0N0P	Maize	1.5 (0.20)
8	INM3	CT	M-T	-manure; +lime	-R	$45P_{PR}$*	Maize	4.99 (1.60)
9	INM3	CT	M/T	+manure; -lime	-R	0N0P	Maize	6.65 (0.55)
10	Kakamega	CT	M/B	None	-R	50N 25P	N/A	4.5 (0.23)
11	Kakamega	RT	M/B	None	+R	50N 25P	N/A	5.2 (0.39)
12	CT1	RT	M-S	None	-R	60N 60P	Maize	5.71 (1.53)

CT = Conventional till, **RT** = reduced tillage, **R** = crop residue, **M-S** = maize soybean rotation, **M/S** =maize soybean intercropping, **M-T** = Maize Tephrosia rotation, **M/B** = maize beans intercrop, **values of yield in bracket** are for rotated or intercropped legume. **+manure** treatment receives 4 t/ha FYM per season (i.e., total of 104 t/ha over the 13 yrs). The manure used during 2014 was characterized by 81.2 ± 3.8 total C, 6.91 ± 0.02 total N, 1.85 ± 0.04 total P and 0.35 ± 0.01 water-extractable inorganic P (all units are g kg^{-1} soil). **+lime treatment** receives 2 t/ha of lime. Both Lime and Minjingu application started in long rains (March) 2015.
* as Minjingu phosphate rock **(PR).**

Soil sampling

The field study was conducted during the September 2016–January 2017 cropping season. Soil samples were taken using an auger from five points within each plot at 0–20 cm depth to adequately represent the plough layer. The samples were thoroughly mixed before taking a composite sample. For microbial studies (DNA extractions), the samples were kept cool in a cooler box and transported to the laboratory the same day where they were sieved over an 2-mm sieve and refrigerated at -20 °C until DNA extraction. The analyses for microbial biomass C, N, and P and enzyme activities were also done with sieved (2 mm) field moist soils that were kept under refrigeration at -20 °C. The procedure followed in DNA extraction at the CIAT laboratory was earlier provided in Kihara et al. (2012). Briefly, total DNA was extracted from 0.25 g of homogenized whole soils as described by Porteous et al. (1997). The soil samples were mixed with lysis buffer and glass beads and homogenized for 2 min at 2500 rpm using a minibead cell disruptor (BioSpec Products, Inc.). Homogenization was repeated for 2 min after incubation at 65 °C for 1 h. After centrifugation (13,000 rpm), potassium acetate (5 M) and polyethylene glycol 40% were added to the supernatants and kept at -20 °C for 1 h. After new centrifugation (13,000 rpm), pellets were resuspended in CTAB 2% and incubated for 15 minutes at 68 °C, cleaned with chloroform, and DNA was precipitated overnight by addition of isopropanol. Pellets were cleaned with 70% ethanol and resuspended in sterile double distilled water.

DNA sequencing

Illumina Miseq, an integrated instrument that performs clonal amplification and sequencing, was used to target the 16S rRNA gene V4 variable region. 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) under the following conditions: 94 °C for 3 minutes, followed by 28 cycles of 94 °C for 30 seconds, 53 °C for 40 seconds and 72 °C for 1 minute, after which a final elongation step at 72 °C for 5 minutes was performed. After amplification, PCR products were checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands before using them to prepare illumina DNA library. Sequencing was performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq following the manufacturer's guidelines before processing using MR DNA analysis pipeline. In summary, sequences were joined, depleted of barcodes then sequences <150 bp removed, and sequences with ambiguous base calls removed. Sequences were denoised, operational taxonomic units (OTUs) generated, and chimeras removed. 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).

Microbial biomass carbon (C), nitrogen (N), and phosphorus (P)

The chloroform fumigation method was used for determining microbial biomass C, N, and P. One set of 25 g of sieved (2 mm) field moist soil sample, previously kept under refrigeration (-20 °C), was fumigated using ethanol-free chloroform for 24 hours in sealed desiccators, after which the fumigated and non-fumigated samples were extracted with 0.5M K_2SO_4 for C and N. These extracted solutions were centrifuged at 5000 rpm for 10 minutes, filtered and concentrations read using a spectrophotometer. For microbial biomass phosphorus, the fumigated and non-fumigated samples were extracted by adding 5M NaOH (until yellow colour was produced) and 1.2 M H_2SO_4 dropwise until yellow colour disappeared, after which 4 ml ascorbic acid and 3 ml of molybdate reagents were added and left for one hour for full colour development before reading at 880 nm using a spectrophotometer. Microbial biomass C, N, and P were obtained by calculating the difference between the fumigated and non-fumigated samples.

Net nitrogen mineralization

Net nitrogen mineralization was determined with the in-situ resin core method. Therefore, cation and anion exchange resin bags were used. Sampling was done at four locations within the sampling plot where resin bags (2 cation exchange resin bags and 2 anion exchange resin bags) were buried in each sampling plot. The bags, at a depth of 0–5 cm, were left in the field for up to 2 months. The first set of bags (2 anion and 2 cation resin bags) were retrieved from each sampling spot after 1 month, and the second set retrieved after 2 months. Extraction was with 2M NaCl (anion resins) and 2M HCl (cation resins), followed by colorimetric determination of ammonium and nitrate ions using spectrophotometer. Net N mineralization was calculated as the change in ammonium-nitrogen (NH_4+-N) plus nitrate-nitrogen (NO_3-N) over the 2 months period. A net nitrification rate was calculated as the final concentration of NO_3-N minus the initial concentration of the NO_3-N. Net ammonification was calculated as the final concentration of NH_4-N minus the initial concentration of NH_4-N.

Denitrification potentials

Five grams of sieved (2 mm) field moist soil was weighed into 50 ml Erlenmeyer flasks. 10 ml of distilled water and 8 ml of Denitrification Enzyme Activity (DEA) solution were added, flasks stoppered and repeatedly evacuated by flushing with helium gas to create anaerobic conditions. A fine-gauge needle was then used to bring each flask to atmospheric pressure before injecting 10 ml of pure acetylene gas to 10% headspace volume and pumping repeatedly to ensure proper mixing. The flasks were then put on a rotary shaker set at 125 rpm and incubated at 25 °C. The gas samples from each flask were first taken after 30 minutes and the second after 90 minutes of incubation. The N_2O gas determination was done at the International Livestock Research Institute (ILRI), Nairobi, using gas chromatography (GC).

Methodology for acid and alkaline phosphatases assay

One gram of fresh soil from the field sieved through 2 mm was weighed into 50 ml Erlenmeyer flasks, except the control sample flasks. For the assay of acid phosphatase, 4 ml of modified universal buffer (MUB) at pH 6.5 was added to the contents in each flask, followed by addition of 0.25 ml of toluene and 1 ml of 115 mM p-Nitrophenyl Phosphate (p-NPP) solution. For alkaline phosphatase, MUB pH 11.0 was used. After thoroughly mixing, they were incubated at a temperature of 37 °C for 1 hour before addition of 1 ml of 0.5M calcium chloride and 4 ml of 0.5M sodium hydroxide solutions. Then 1 ml of p-NPP was pipetted to the control sample flasks. After centrifugation of all samples for 10 minutes at 5000 rpm, the supernatants from each flask were obtained and their absorbance read using a spectrophotometer at a wavelength of 400 nm. But these supernatants were first diluted to 1:100 for acid phosphatases, and 1:10 for alkaline phosphatase using deionised water at room temperature (25 °C).

Macro- and meso-fauna assessments

Using a monolith of size 25 cm x 25 cm x 30 cm, soil samples were taken 8 weeks after planting (in June-July 2016) at the two conservation agriculture experimental trials in western Kenya (i.e. CT1 and Kakamega). Each monolith point was placed over a randomly selected spot and dug with a spade and hoe to the desired level, first for 0–15 and then the 15–30 cm. The animals, sorted out by hand, were separated into major taxonomic groups, recorded and then collected in plastic bottles before subsequent identification and counting at the soil microbiology laboratory of CIAT, *icipe* Duduville Campus, Nairobi, Kenya. For meso-fauna, one sample from each plot was used in the extraction using the behavioural or dynamic method with a locally constructed Berlese-Tullgren. With this apparatus, mesofauna were exposed to a controlled gradient of high to low temperature and light, and low to high humidity from top to bottom, and thus gradually forced downwards and out into the collection jars filled with 75% alcohol. This was followed by sorting and counting of the mesofauna under a light microscope.

Leaching studies

In the Kakamega trial, soil samples were collected at 0–10 cm, 10–30 cm, 30–60 cm, and 60–90 cm from plots treated with 0 t residue + 75 kg N, 2 t residue + 75 kg N 4 t residue + 75 kg N and 8 t residue + 75 kg N ha^{-1}. Control samples were also collected from plots under conventional tillage receiving 75 kg N ha^{-1}. Nitrogen had been top-dresses as calcium ammonium nitrate when the maize was at the 12^{th} leaf stage. The collected soil samples were analysed for ammonium-N and nitrate N.

Data analysis

Various methods have been used for data analysis. First, nonmetric multidimensional scaling of all OTUS was conducted on 16S rRNA sequence reads for each treatment using *metaMDS* function within vegan library in R, and results plotted in two-dimensional space using ggplot2 library also in R. Here, the mean 16S rRNA sequence reads from the three replicates were obtained before determination of Bray–Curtis dissimilarity index commonly used for detecting underlying gradients in the microbial communities. Species richness was determined using *specnum* function in vegan library, and these were used in a gradient of symbol sizes within the multidimensional scaling plots. Also, a hierarchical clustering was implemented using library ggdendro in R to establish which treatments had related microbial communities again utilizing Bray–Curtis dissimilarity index. Using the *adonis* function (from vegan library), an analysis of variance of the resultant distance matrices was undertaken.

For comparison of treatment effects of enzyme activities, soil aggregation, P fractions e.t.c., analysis of variance was undertaken using Genstat 14.1 (2011), and mean separations based on Fischer's least significance difference (LSD).

Photo: CIAT

Results

The microbial communities in soils of western Kenya, based on 16S rRNA gene, constituted 1459 phylotypes of which 96% were bacteria, 1.2% archaea, and 1.4% fungal (the remainder were Metazoa and Eukaryota). Across sites, the number of phylotypes were 468-1115 in CT1, 475-854 in INM3 and 537-991 in Kakamega. Overall, these were represented by 38 phyla and 87 classes of which 15 bacteria classes constituted 95.6% of all rRNA sequence reads. The top five phyla comprised of (relative abundances specified in brackets) *Proteobacteria* (23.2–42.8 %), being the most dominant, followed by *Actinobacteria* (11.1–32.1%), *Acidobacteria* (8.94–20.74%), *Firmicutes* (5.6–26.5%) and *Gemmatimonadetes* (4.41–12.64%). *Cyanobacteria*, one the major groups targeted by this study due to the multiple roles of P mineralization and nitrogen fixation, ranked 13th at the phylum level, with relative abundance ranging from 0.09–0.29%. The other dominant phyla included *Verrucomicrobia* (1.62–5.85%), *Chloroflexi* (2.66–5.8%), *Planctomycetes* (1.1–2.8%), *Bacteroidetes* (0.37–2.27%), *Nitrospirae* (0.41–1.74%) and *Fusobacteria* (0.01–1.01%). Similarly, the first four classes made up 50% of sequence reads and include *Actinobacteria* (10.8–32%), by far the most abundant microbial class, followed by *Alphaproteobacteria* (7.9–16.1%), *Betaproteobacteria* (3.3–14.4%), and *Deltaproteobacteria* (3.7–16.5%; Figure 1). Across sites, an elevation of *Candidatus Nitrosotalea devanaterra* (Archaea Kingdom), an acidophilic ammonia oxidizer was observed in the Kakamega relative to other sites (i.e. 73.9% of this species were in Kakamega while only 11.1% and 14.9% were in INM and CT1, respectively). Overall, while the CT1 and INM3 had *Candidatus Nitrosotalea devanaterra* being only 0–0.12% of the 1459 species observed, it was 0.14 to 0.45% in the Kakamega site.

Microbial communities are influenced by site and residue application (Figure 2). First, microbial communities are more diverse in the long-term trial sites of CT1 and INM (13 yrs) and less diverse in the younger Kakamega trial (6 yrs). Secondly, unique communities emerge from combined application of lime and phosphate rock-based Minjingu fertilizer in one treatment (see x8), no application of fertilizer (x7) and removal of residues under reduced tillage (x12). The change of microbial communities with lime application is also noticeable in CT1 experiment (i.e. the x5 treatment). Reduced tillage without retention of crop residues promotes dissimilar microbial communities relative to other treatments. The two treatments under poor management (i.e, reduced tillage minus residues and the non-fertilized control) also have reduced species richness. The results from clustering using dendrograms are interesting since the treatment with both lime and Minjingu is in the same clade as the two poorly managed treatments, and is of similarly low species richness. Application of FYM as the only source of nutrient input had similar microbial communities as the best-best treatments involving fertilizer application and residues.

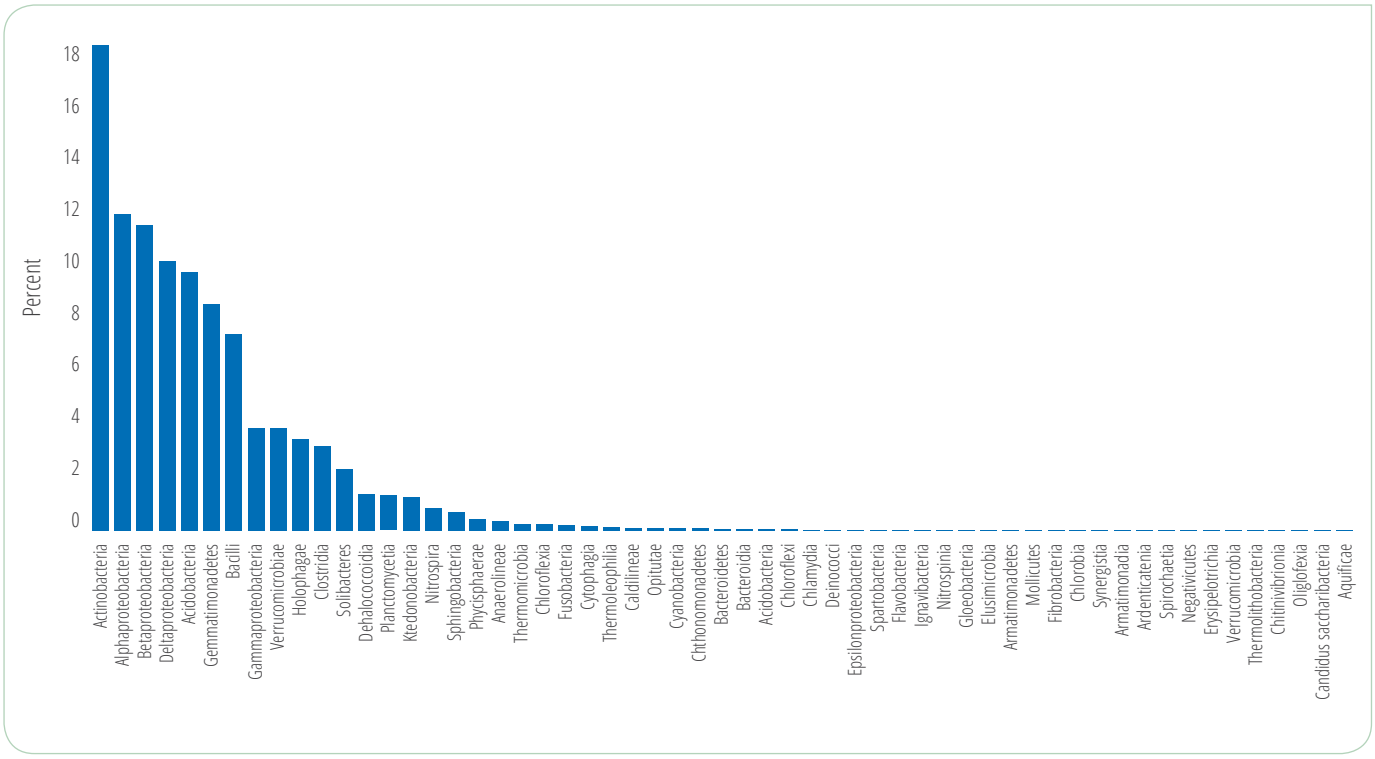

Figure 1 Microbial community composition of arable soils of western Kenya. The relative abundance is represented as a proportion of 16S rRNA gene reads of the total number of reads. Only the most abundant microbial class with relative abundance >0.01 % of total analysed microbial community are plotted.

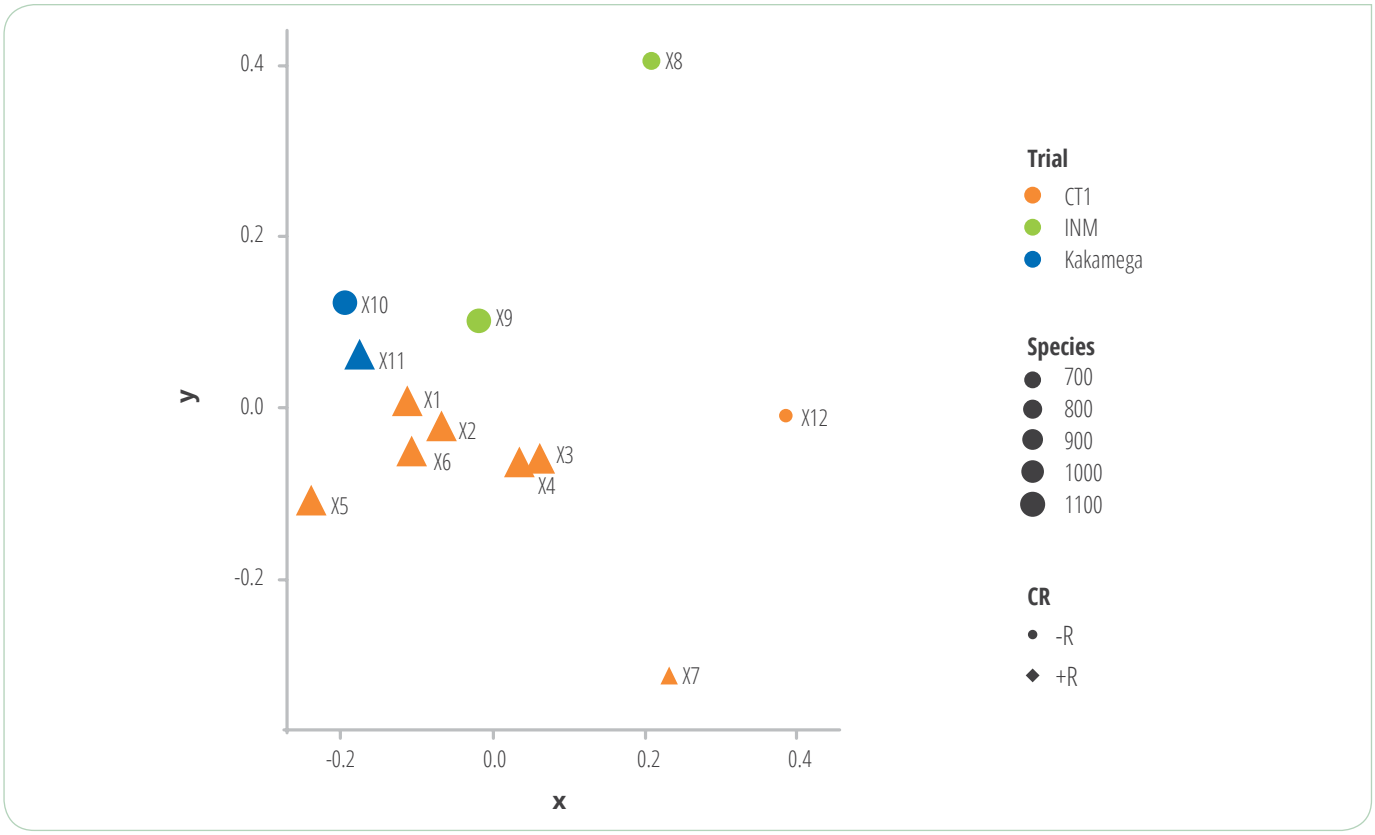

Figure 2a Distribution of microbial communities within the 12 treatments tested, based on 1401 microbial species identified, as multidimensional scaling.

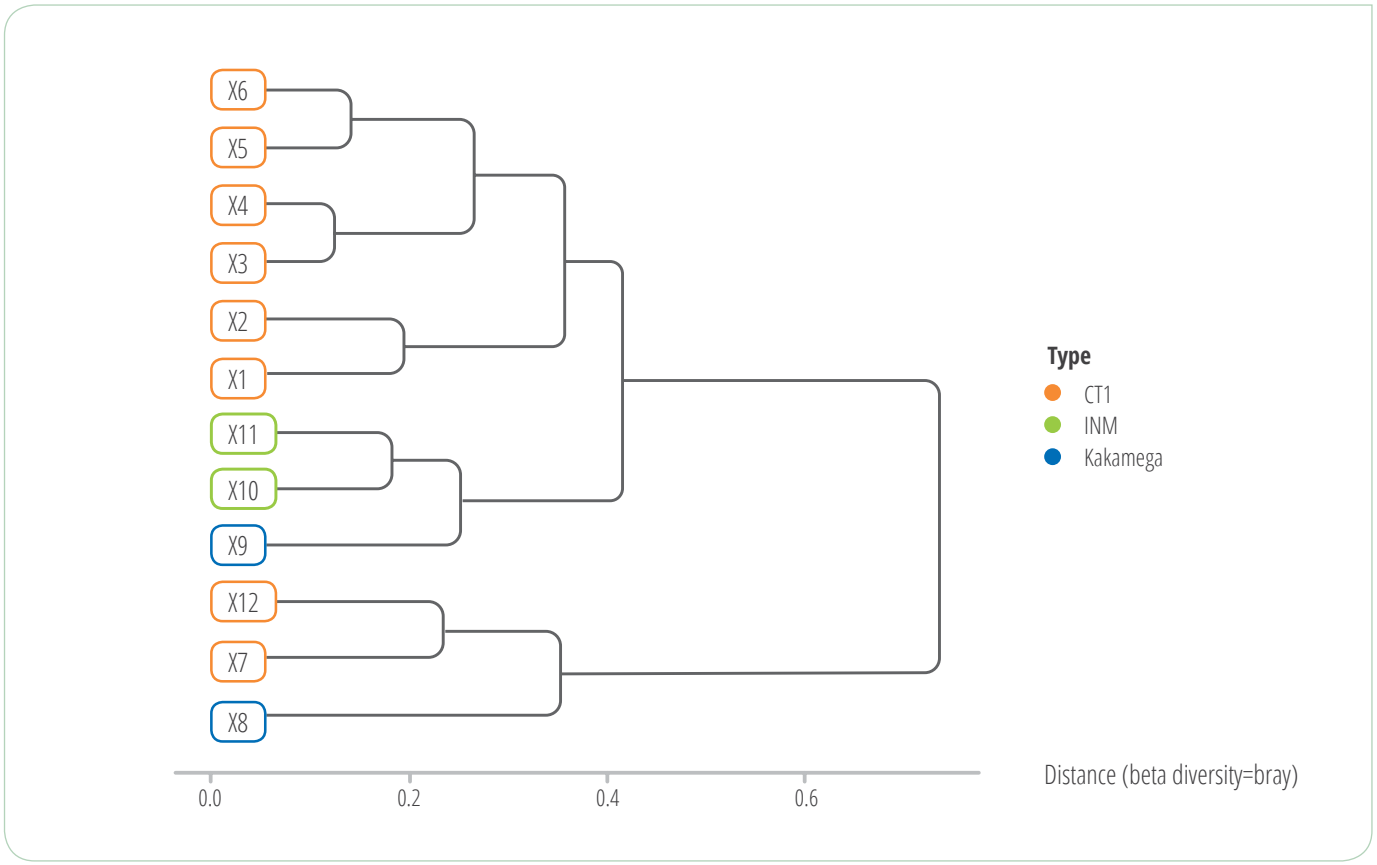

Figure 2b Distribution of microbial communities within the 12 treatments tested, based on 1401 microbial species identified, as dendrogram.

Residue removal under the reduced tillage practice decreased ($p<0.05$) *Cyanobacteria* relative to residue retention (Table 3). Interestingly, treatments with higher *Cyanobacteria* over this treatment were only treatments with N applied in CT1 and the manure only treatment in INM3. *Rhizobia*, i.e. nitrogen-fixing bacteria, fall into the two predominant classes of the *Alpha-* and *Beta-proteobacteria* and most belong to the order *Rhizobiales*. As with *Cyanobacteria, Rhizobiales* (also its genus *Rhizobium*) were depressed under reduced tillage without residue retention and also under no fertilizer application relative to the best bet maize-soybean rotations with fertilizers and residues, even under conventional tillage. Treatments combining nitrogen, fertilizer, and residues had the greatest populations of *Rhizobiales*. *Frankia* and *Kribella*, two nitrogen-fixing genera, were higher under maize/soybean intercropping within reduced tillage than in all other management practices (even though it had no nitrogen application). Besides, residue removal significantly decreased *Frankia* in the long-term CT1 trial. Unlike these two nitrogen-fixing genera, *Actinomyces* were not affected by treatments in our study. In the no-residue system under reduced tillage, *Thermoflavimicrobium* spp. (a thermophilic bacteria), *Gemmatimonas* spp. (adapted to low soil moisture) and *Conexibacter* spp. were the most dominant.

Practicing reduced tillage (with both residues retention and fertilizer application) resulted in higher *Pseudomonas* (except under soybean phase) than the no-fertilizer and the three residual removal treatments under the long-term trials (CT1 and INM3). Lime application together with crop residue retention increased ($p<0.05$) *Cyanobacteria* over all other treatments. *Nitrospira* is involved in ammonium oxidation and, as expected, was significantly reduced when crop residues were removed in reduced tillage and when N and P fertilizers were not applied in conventional tillage system ($p<0.05$). Manure application is associated with higher *Bacillus* than that in the no-fertilizer and the residual removal treatments in CT1 ($p<0.05$).

Table 3 DNA sequence hits for *Cyanobacteria*, *Rhizobiales* and selected microbial general (involved with N-fixing, P-solubilization, nitrification, denitrification and plant growth promotion) under different management practices in western Kenya

#	Treatments	Cyano-bacteria	Frankia	Rhizobiales	Rhizobium	Pseudo-monas	Cyano-bacterium	Nitrospira	Ktedono-bacteria	Kribbella	Bacillus
1	CT1-CT+60N +60P, +R, -L, MS Rotation	18.4a	11.9b	106.a	50.3a	32.3abc	0.80b	54.6a	54.4ab	2.95b	34.4ab
2	CT1-RT+60N +60P, +R, -L, MS Rotation	18.6a	11.9b	110.a	45.9a	50.6a	0.57b	58.7a	70.0a	1.54bc	40.8ab
3	CT1-RT+0N +60P, +R, -L, , MS Intercropping	14.2ab	20.3a	93.5ab	33.3ab	53.2a	1.20b	41.2ab	51.5ab	7.01a	38.9ab
4	CT1-RT+0N +60P, +R, -L, MS Rotation (soybean phase)	15.7ab	10.7bc	88.5abc	35.3ab	39.5ab	0.66b	40.1ab	52.5ab	1.43bc	32.6ab
5	CT1-CT+0N +60P, +R, +L, MS Intercropping	15.7ab	9.81bcd	76.8abc	29.9ab	34.7ab	4.08a	37.5ab	28.2b	2.43bc	34.5ab
6	CT1-CT+0N +60P, +R, -L, MS Intercropping	15.0ab	10.2bcd	84.1abc	36.1ab	36.5ab	0.91b	38.6ab	38.7b	2bc	41.3ab
7	CT1-CT+0N +0P, +R, -L, MS Rotation	6.7ab	6.98bcd	39.0c	16.2b	24.7bc	0b	17.4b	24.9b	0c	16.5b
8	INM3-CT-Manure,-R, +L, +Minjingu (i.e, P and micronutrients), TM Rotation	8.2ab	5.24cd	61.4abc	24.5ab	11.9c	0.33b	19.5b	33.0b	0.66bc	38.4ab
9	INM3-CT+0N +0P, +Manure -R, -L, TMM Rotation	18.0a	10.0bcd	83.6abc	31.4ab	21.5bc	0b	37.3ab	49.8ab	2.30bc	64.4a
10	Kakamega-CT+75N +25P, -R, MB Intercropping	14.6ab	8.54bcd	89.9abc	32.2ab	35.8ab	0.33b	36.4ab	48.5ab	1.47bc	34.1ab
11	Kakamega-RT+75N +25P, +R, MB Intercropping	14.9ab	9.14bcd	101.a	40.9ab	34.2abc	0.66b	37.4ab	50.5ab	2.90b	31.8ab
12	CT1-RT+60N +60P, -R, -L, MS Rotation	3.8b	4.48d	43.6bc	17.2b	21.1bc	0b	22.3b	34.5b	1.57bc	18.7b

Soil microbial biomass

On the Kakamega site, practicing reduced tillage with residue retention increased microbial biomass ($p<0.05$) relative to the conventional tillage practice without residue application (Table 4). None of the treatments in CT1 were significantly different in microbial biomass carbon (MBC) although intercropping increased MBC by 46% over the similarly managed rotation system (i.e., trt 3 vs 4). Application of manure increased MBC by 52% over the N+P+L treatment. That highest microbial biomass P and to some extent N were observed in the no-fertilizer CT1 treatment is startling and could reflect time of sampling, thus calls for further/repeated analysis. The higher C:N ratio of microbial biomass in the no-residue treatment of CT1 is an indication that this system was dominated by fungi.

Soil microbial activities

Soil microbial activity was measured for two soil phosphatases: acid and alkaline phosphatases. Phosphorus application reduced enzyme activities in the INM3 trial. For example, applying lime and P from Minjingu fertilizer (also with a liming effect) significantly decreased the activity of both acid and alkaline phosphatases relative to the manure-only treatment (the accompanying P in the manure was small). In other words, the treatment without significant P application (manure-only) had higher ($p<0.05$) activity of both acid and alkaline phosphatases than the treatment with P application. Similarly, in CT1, the highest phosphatase activities were observed in the treatment with no fertilizer application; the alkaline activity being significantly higher than in 3 of the 7 treatments where P was applied. Neither residue retention in reduced tillage nor implementation of the varied cropping systems nor application of N did affect P-cycling enzyme activities. This is in line with a previous (2012) assessment of phosphatase activity in the same CT1 site, which also found insignificant effects of tillage and residue application (Margenot et al., 2017a). However, a repeat study (Margenot et al., 2018) observed significant effects of tillage and residues and that applying the more soluble forms of P, i.e. TSP, suppressed phosphatase activity relative to phosphate rock, as expected. But unlike the 2016 sampling, the 2013 sampling showed that no P treatment had decreased acid phosphatase activity compared to the treatment with P, even though pH, a key driver of acid phosphatase activity, was still lower and organic P higher without than with P. The three 2012, 2013, and 2016 assessments found quite high acid phosphatase activities compared to the alkaline phosphatase activities, because the soil pH were in the range of optimal activity of acid phosphatase (Hui et al., 2013).

Results obtained from 23 farmer fields (10 Siaya, 8 Kakamega, 5 Bungoma), each with a lime and unlimed plot showed inconsistent results on biological indicators (soil enzymes, earthworms, ants). For example, the activity of 3 P-cycling soil enzymes showed inconsistent responses to lime, with increases (5 farms) or decreases (4 farms) in the case of acid phosphatase. Also, abundance of ants was unaffected while that of earthworms decreased (in 4 farms) with liming. Increases in soil pH due to liming was observed in a majority of fields, as expected, although no change in resin-exchangeable (available) P could as yet be established, perhaps due to an increased P uptake by crops. A striking effect of liming was on water infiltration rates that were greatly increased (more than doubling) in 11 farms, likely due to weakened soil structure. This means that liming may lead to a breakdown of aggregates in the first few years following application (Paradelo et al., 2015). Many farmers also noted the "softer" feeling of limed soil when cultivating it prior to planting (Margenot, personal field observation). Such aggregate breakdown has been noted to stimulate soil C mineralization (Paradelo et al., 2015), and an increase in labile C (POXC) is expected. This was observed in experimental liming mesocosms using INM3 soils, where lime application (0–2.5 t ha^{-1} at 0.5 t ha^{-1} intervals) stimulated respiration and increased POXC in manure and NPK treatments but not in the no-input control (Figure 3). From the long-term trials (as shown in Table 4), lime application at high rates of 2 t/ha (the +L+Minjingu treatment of INM3) decreased soil aggregation indices but no evidence was observed in CT1. The intercropping of maize and soybean, on the other hand, increased soil aggregation over the rotation system in both soil depths tested. Also, reduced tillage resulted in better aggregation indices than conventional tillage treatments especially at the very top soils.

Table 4 Microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP), acid (Acid P) and alkaline (Alkaline P) phosphatase activities and aggregate mean weight diameter (MWD) under different management practices in western Kenya

#	Variables and their treatments	MBC	MBN	MBP	Acid P	Alkaline P	Aggregate MWD (0–5 cm)	Aggregate MWD (5–15 cm)
1	CT1-CT+60N +60P, +R, -L, MS Rotation	44.2b	5.62d	0.28b	124.5ab	21.0ef	1.03f	1.52d
2	CT1-RT+60N +60P, +R, -L, MS Rotation	57.5b	6.39cd	1.04ab	125.6ab	26.4def	1.38cd	1.66bcd
3	CT1-RT+0N +60P, +R, -L, , MS Intercropping	83.0ab	8.22abcd	0.54b	141.3a	27.5cde	1.62a	1.88a
4	CT1-RT+0N +60P, +R, -L, MS Rotation (soybean phase)	58.9b	5.58d	0.51b	124.5ab	26.9cdef	1.52abc	1.84ab
5	CT1-CT+0N +60P, +R, +L, MS Intercropping	75.3ab	8.19abcd	0.92ab	130.2a	25.8def	1.35cd	1.84ab
6	CT1-CT+0N +60P, +R, -L, MS Intercropping	61.9b	8.88abcd	1.05ab	139.2a	27.6cde	1.26de	1.74abc
7	CT1-CT+0N +0P, +R, -L, MS Rotation	88.2ab	11.6a	1.46a	144.7a	33.8abc	1.24de	1.75abc
8	INM3-CT-Manure,-R, +L, +Minjingu (i.e, P and micronutrients), TM Rotation	77.1ab	11.1ab	0.46b	98.5b	20.0f	1.11ef	1.29f
9	INM3-CT+0N +0P, +Manure -R, -L, TMM Rotation	117.6a	11.2ab	0.79ab	138.5a	29.3cd	1.39cd	1.61cd
10	Kakamega-CT+75N +25P, -R, MB Intercropping	50.6b	7.65abcd	0.41b	126.6a	39.2a	1.41bcd	1.67bcd
11	Kakamega-RT+75N +25P, +R, MB Intercropping	117.5a	10.76abc	0.83ab	136.7a	37.4ab	1.60ab	1.72abc
12	CT1-RT+60N +60P, -R, -L, MS Rotation	87.9ab	6.92bcd	0.56b	131.6a	31.7bcd	1.32de	1.52d

MS = maize-soybean, MB = maize-bean, TM = Tephrosia-maize, TMM = Tephrosia-maize-maize.

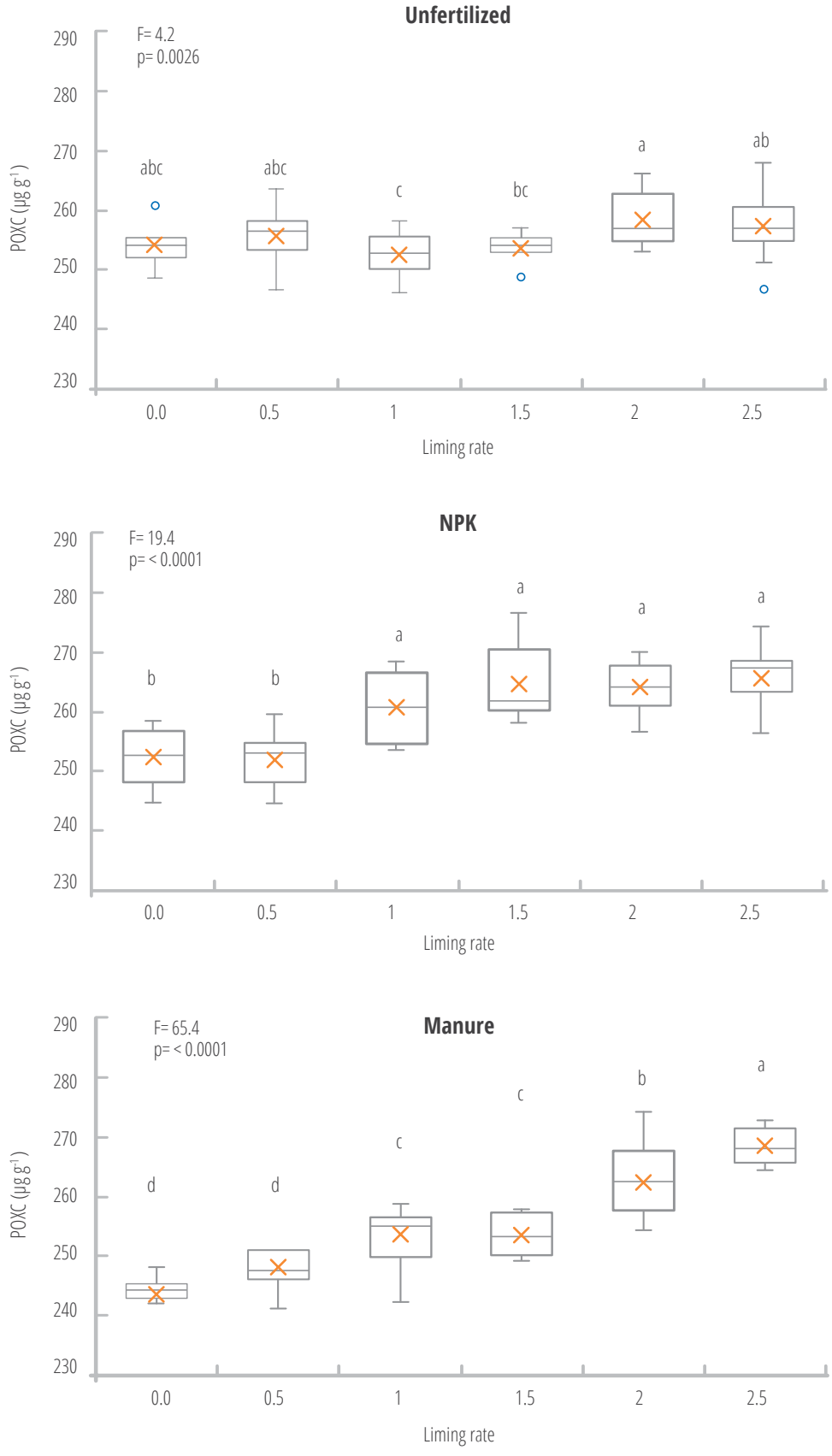

Figure 3 Effect of lime application rates on labile carbon (permanganate oxidizable) for soils under no-input control, NPK and manure treatments in CIAT long-term INM3 trial.

Soil denitrification

Denitrification potentials and rates, assessed in selected treatments of the CT1 experiment, were affected by treatment ($p<0.01$), depth ($p<0.01$), and the interaction between treatment and depth ($p<0.01$). Denitrification potentials reduced sharply with depth. Rates in the 5–15 cm soil depth were at least 6 times lower than in the surface soils (0–5 cm) likely due to higher concentration of residues and microbes on the very top soil (Table 5). Conventional tillage with residue application increased denitrification over a similarly managed reduced tillage treatment at both depths, against our expectation. Application of slow release (polymer-coated) fertilizer had no effect on denitrification rates except in maize-soybean rotation system where it increased the rates. Denitrification increases had been expected since soil sampling was done well after application of fertilizers (3rd month into the cropping season). Also, residue removal in the conventional tillage system more than halved the denitrification rates and potentials. Further, and unlike the elevated potential in the one case comparing CT and RT, using data from four treatments evaluating greenhouse gases mitigation potentials, a slight reduction (though not significant) in CO_2 and N_2O fluxes was observed in conventional tillage relative to reduced tillage (data not shown). These results are, therefore, inconsistent and would need further assessment to confirm.

Table 5 Means for denitrification potentials and denitrification rates at two depths for all the thirteen different treatments studied

TREATMENT CODE	FERTILIZER*	DENITRIFICATION POTENTIALS		DENITRIFICATION RATES	
		0–5 cm	5–15cm	0–5 cm	5–15cm
RT, 60N-60P, -R, -L, ROT	normal	5.36bc	0.58ab	5.89bc	0.64ab
CT, 60N-60P, +R, -L, ROT	normal	6.35a	1.07a	6.98a	1.18a
RT, 60N-60P, +R, -L, ROT	normal	4.16e	0b	4.58e	0b
RT, 60N-60P, +R, -L, ROT	slow	5.09cd	0b	5.6cd	0b
RT, 0N-60P, +R, -L, intercrop	normal	4.18e	0.6ab	4.6e	0.66ab
CT, 60N-60P, -R, CONT. M	normal	4.48de	0.55ab	4.93de	0.61ab
CT, 60N-60P, -R, CONT. M	slow	4.65cde	0.6ab	5.12cde	0.66ab
RT, 60N-60P, +R, CONT. M	normal	6.07ab	0.02b	6.68ab	0.02b
RT, 60N-60P, +R, CONT. M	slow	5.33bc	0.03b	5.87bc	0.04b
CT, 90N-60P, -R, CONT. M	normal	4.1e	0.04b	4.51e	0.05b
CT, 60N-60P, -R, ROT	normal	2.09f	0b	2.3f	0b

* Type of fertilizer applied was either “normal release” or “slow release” urea as indicated. Means followed by same letters in each column are not significantly different from each other.

Soil fauna

Faunal species richness did not vary much between the two trials tested; we observed 32 species in CT1 and 27 in Kakamega trials. For macrofauna groups, termites (i.e., Isoptera) were dominant in Nyabeda (55%) followed by earthworms (i.e., *Oligochaeta*) (21%), ants (i.e., *Hymenoptera*), and beetles (i.e., *Coleoptera*) (6%). In the Kakamega trial, earthworms (64%) were the most dominant of all the macrofauna groups followed by termites (7%), ants (6%), and beetles (5%). Other macrofauna groups were observed in very low numbers, with each group constituting ≤5%. The mesofauna group was dominated by *Acarina* (40–59%) and Collembola (35–46%) with the other groups each constituting ≤ 5%.

At Nyabeda, macrofauna richness of the topsoil was significantly higher in both conventional and reduced tillage practices both under maize-soybean rotation and with crop residues added than in the typical farmer's practice without inputs (Table 6). At 15–30 cm depth, macrofauna richness was significantly higher in conventional tillage+R (rotation) than both the reduced tillage treatments, although it did not differ from the

farmers practice. At the Kakamega trial, no significant differences were noted for macrofauna richness and abundance among the treatments at 0–15 cm and 15–30 cm soil depths. Only mesofauna abundance at the top soil was elevated under reduced tillage (data not shown). As expected, soil fauna richness reduced with depth where these were nearly ≤50% that of top soil for each of the treatments.

Table 6 Macrofauna and mesofauna diversity (richness) across long-term and short-term trials of Embu, Nyabeda, and Kakamega

TREATMENT	MACROFAUNA		MESOFAUNA	
	0–5 cm	5–15cm	0–5 cm	5–15cm
CT1				
Farmer practice	2b	3.7ab	4.3	3
Conventional tillage+R (rotation)	8a	5.3a	5.3	5.7
Reduced tillage+R (rotation)	7a	2.7b	4.3	2.3
Reduced tillage+R (intercropping)	5ab	2.7b	4.7	3.3
Kakamega				
Farmer practice	5.7	5	2	2
Conventional tillage+R (intercropping)	6.7	5.3	3.7	3.7
Reduced tillage+R (intercropping)	11.3	7	5.7	2.3

Leaching

Practicing conventional tillage, as is common among farmers, resulted in increasing amounts of mineral nitrogen at all soil depths tested (Table 7). Although the amount of inorganic N applied is modest (75 kg N ha^{-1}), the resulting average mineral N for this conventional treatment is way beyond the optimal amounts of 6.7 mg N kg^{-1} soil proposed by Peng et al. (2013) for the 0–60 cm depth. At 60–90 cm depth, where nutrient uptake by maize is low, soil nitrate concentrations were almost three times as high in conventional tillage as the conservation agriculture treatments, indicating potential leaching and risk of low nutrient-use efficiency. Averaged over the specific days of measurements and for that 60–90 cm soil depth layer, concentrations translated to at least 12 kg N ha^{-1} more than in CA systems. It is surprising that the no-residue and the residue retention conservation agriculture treatments had similar nitrate levels, and may be a consequence of increased surface losses of applied N without residue and an immobilization of N in the presence of residue. Such immobilization may be responsible for an increased N content of applied residues, such as that of up to 56% in the first 45 days reported within CT1 long-term trial (Kihara et al., 2015).

Table 7 Effect of conservation agriculture on nitrate leaching as observed at Kakamega in western Kenya

TREATMENT	0–10 cm	10–30 cm	30–60 cm	60–90 cm
Conservation0R 75N	18.52	8.87	3.51	2.24
Conservation2R 75N	17.32	9.93	4.24	2.40
Conventional0R 75N	28.57	18.44	8.21	6.17

P fractionation

P application significantly increases all P fractions relative to the control as well as the manure only treatment (except for the NaOH-organic P) in the long-term INM3 trial (Table 8). The observed anion-exchange membrane extractable P of >70 mg /kg of soil was beyond the threshold for crop response to P fertilizer application. Apparently, the >10 years of seasonal P application (60 kg P ha^{-1} twice a year) did supply a high enough amount of labile P to support good crop growth. Following this, two situations should be further studied: one where P application rates are reduced and the other where P-fertilizer application is suspended for a few seasons.

Assuming a bulk density of about 1.05 (Margenot et al., 2017a) for the top 0–15 cm soil in the area, each additional mg P per kg soil translates to 1.58 kg of P per hectare, meaning that considering only the anion-exchange membrane extractable P, the treatment applied with mineral P has 100 kg and 111 kg more plant-available P than the manure only and no-P treatments, respectively. Thus, the significant amount of P fertilizer applied, of which only a fraction is withdrawn by the crop, indeed seems to trigger a slow but steady accumulation of plant-available P.

Analysis of P fractions in the long-term CT1 trial are now published in Margenot et al. (2017a). Here, maize residue application (2 t ha^{-1}) has been observed to have no effect on P fractions. But, reduced tillage increased total and labile P stocks in the top 0–15 cm soil. Because soil pH decreased under reduced tillage, P sorption increased, consistent with greater retention of soil P as Fe- and Al- associated P. In terms of relative distributions, a greater proportion of soil P was present as labile P under reduced tillage compared to conventional tillage. Furthermore, tillage and residue management did not impact the activity of the three soil phosphatases: acid and alkaline phosphomonoesterase, and phosphodiesterase.

There is a greater proportion of P associated with Fe and Al when P-source is the more soluble TSP relative to phosphate rock that is associated with a moderate liming effect (Margenot et al., 2017b). As expected, phosphorus application for 13 cropping seasons, either as phosphate rock or TSP, increased P in different P pools (except the organic P) at both depths relative to no P fertilization. Potential microbial biomass P highly increased by an application of phosphate rock over the no-P application, and was somewhat elevated over TSP treatments, representing up to 3.5% of soil total P. Given evidence that microbial biomass P may be a pool of crop-available P (Ayaga et al., 2006), this suggests MPR can improve traditional measures of available P (e.g., Mehlich, resin-extractable) as well as biological available P. Elevated acid phosphatase activity and labile carbon (POXC) availability under phosphate rock but not TSP application suggest additional benefits for P and C cycling under the use of phosphate rock.

Table 8 Effect of manure and P application, relative to a control, on phosphorus fractions under ferralsols in a long-term trial (INM3) in western Kenya

TREATMENT	AEM_Pi	NaHCO3_Pi	NaHCO3_Po	NaOH_Pi	NaOH_Po	HCl_Pi	HCl_Po
Manure only	11.31a	3.75a	32.55a	86.48a	174.53ab	15.01a	5.63a
P applied	74.52b	31.12b	47.47b	222.12b	207.01a	43.78b	13.39b
Control	3.74a	1.24a	27.96a	55.68a	144.42b	7.44a	3.76a

Residue application and soil pH

We investigated whether application of residues (2 t/ha maize stover) for several seasons influenced soil pH. Within CT1, during the 5th year (10th season) of this trial, pH ranged from 4.84 to 5.36 and there was a tendency of crop residues within the reduced tillage treatments to increase pH of the 0–15 cm soils. A subsequent assessment of the residue application at 11 years (22 seasons) showed no effect on soil pH indicating that the residue application on its own is not a viable strategy to ameliorate soil acidity of the soils tested. This is likely related to the observed high rates of residue disappearance: there were no residues remaining by the end of a season due to high activity of macro- and meso-fauna (Kihara et al., 2015). Despite this, combining reduced tillage with residue application was observed to lead to significantly higher SOC content during some of the periods of long-term assessment, and reduced rates of SOC losses over time relative to other treatments are reported (Sommer et al., 2018). But within the INM3 trial, a significant change in the top soil pH was observed following 22 seasons of manure application

(Figure 4). Interestingly and as in CT1, the manure application did not affect 0–15 cm SOC contents in the INM3 trial but reduced the rates of SOC losses over time relative to other treatments. Thus, residue application did not increase soil pH and P availability but manure application did.

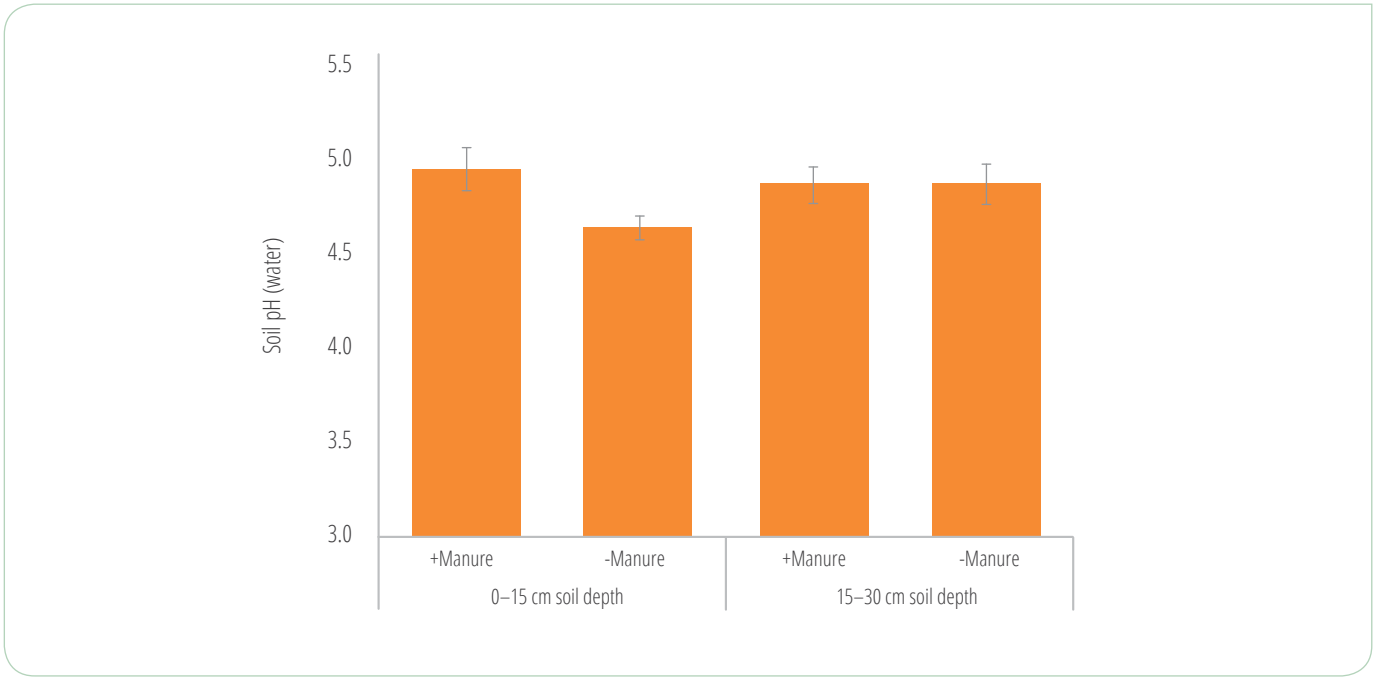

Figure 4 Effect of manure application on soil pH at two depths in the CIAT long-term trial (INM3) in western Kenya.

Total soil carbon and microbial diversity and richness correlated, but no such relationship was observed for variables such as enzyme activity, microbial biomass, or soil aggregation (Figure 5). Active carbon, which is primarily consumed by microbes, is expected to influence microbial abundance/diversity more strongly. Systems that improve/maintain high active carbon have potential to consequently harbour abundance/diversity of microbial species, and this needs to be further investigated. We, therefore, recommend to investigate these relationships with a larger dataset.

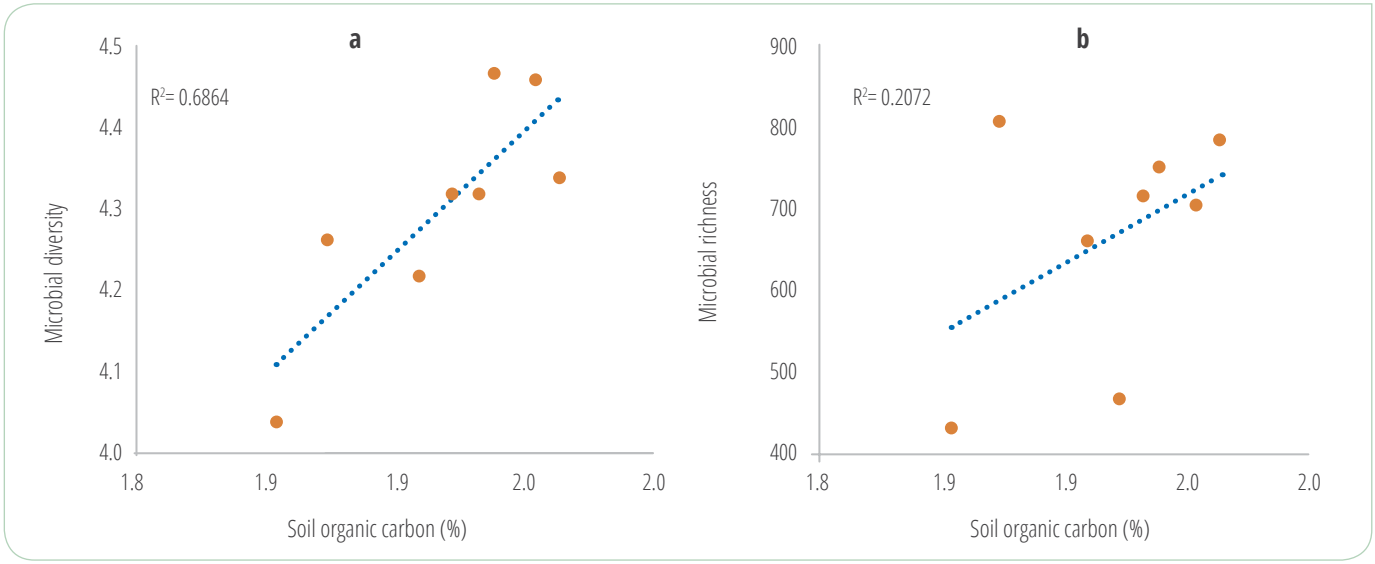

Figure 5 Relationship between soil organic carbon and **a**) microbial diversity and **b**) richness in the long-term conservation agriculture trial (CT1) in western Kenya. SOC data used was taken during the previous year. All plots were applied with P at 60 kg/ha.

Photo: CIAT

Discussions

Soils harbour diverse microbial communities. Up to 39,750 bacterial phylotypes have been observed across the world (Prober et al., 2015). The numbers of bacterial phylotypes reported in our study corresponded to the 1,405 operational taxonomic units (OTUS; 31 phyla, 156 orders, and 481 genera) under acidic soils in China (Lei et al., 2017) and the 1,400 and 1,538 bacterial OTUS under a 78-year old slash pine and eucalyptus forest plantations, respectively (Zhou et al., 2017). Dominance of *Actinobacteria*, *Proteobacteria*, and *Acidobacteria*, compared to other phylotypes is not unique to our soils and is observed elsewhere e.g., under a long-term park grass experiment in the UK (Zhalnina et al., 2015), soils in Antarctic Peninsula (Pessi et al., 2015), and in general worldwide (Prober et al., 2015).

Our study shows that management practices aiming at enhancing soil fertility and productivity do also influence the abundance and diversity of microbes. Shifts in microbial communities and changes in their abundances have been observed following changes in tillage (Ghimire et al., 2014), crop types (Broeckling et al., 2008), residue retention (Govaerts et al., 2007), and manure application (Zhen et al., 2014) mostly under temperate regions and tropical American climates. These influences result from changes in microclimates and provisioning of microbial substrates reflected in soil fertility indices such as C, N, and P (Bergkemper et al., 2016). Similar influence on diversity and abundance of soil micro-organisms occur also under farming conditions and practices of small-holder farmers in SSA as revealed in our study.

Álvarez (2005) has argued that, when returned to the soil, crop residues promote microbial proliferation and diversity. The application of moderate amounts of crop residues of only 2 t/ha of maize stover in our study – a quantity dictated by competing demands for feed and energy in this region – improved microbial diversity and abundance/populations, e.g. *Pseudomonas, Frankia,* and ammonia-oxidizing *Nitrospira*. Also, photosensitive *Cyanobacteria* are affected by removal of residues from a field resulting in decreased populations (Nayak et al., 2001). Residue application results in increased C storage, or at least reduced losses of C from soils (Sommer et al., 2018), but this is also often accompanied by increased feed availability for the microbes. Such organic carbon governs denitrification (Dodla et al., 2008; Stevenson et al., 2011). Despite the benefits and contributions of sequestered carbon to reducing CO_2 emissions into the atmosphere, the expected increases in crop productivity are not realized in all environments.

Use of FYM is a common practice in SSA and its availability varies by region. Long-term application of FYM has resulted in similar or higher productivity than combined N and P applications (see also Krey et al., 2013). The 4 t/ha applied seasonally is quite close to the 3.5 t/ha commonly applied to fields in Babati-Tanzania and where the frequency of application was positively associated with productivity (Kihara et al., 2014). These productivity benefits are associated with similar diversity of soil microbes, as the commonly promoted best-bet integrated soil fertility management (ISFM) practices, and improves soil physical and chemical properties. Under such organic practices, increased populations of microbes e.g. *Cyanobacteria* and *Bacillus* and microbial-

mediated enzyme activities (Krey et al., 2013), are consistent with elevated availability of easily accessible feed (labile carbon) and a resultant microbial biomass carbon. Elsewhere, such effects on microbes have been reported, including *Actinobacteria* and *Firmicutes* in China (Wang et al., 2016), soil microarthropods in tropical USA (Doles et al., 2001), and microbial biomass in Australia (Araújo et al., 2009). The FYM maintains moderate amount of plant-available P, unlike similar P between organic fertilizers (30 t/ha every 3 years) and annual applications of P for 10 years as observed in Germany (Krey et al., 2013), our seasonal P treatments seem to over-supply plant-available P reaching >70 mg P kg^{-1} soil.

Increased availability of N and P in soils can have variable effects on soil micro-organisms. Decreased populations of nitrogen-fixing *Frankia* and *Kribella* but not *Actinomyces* and *Rhizobiales* following nitrogen application could point to decreased functions by these microbes. Although application of mineral N at the modest rate of 60 kg N ha^{-1} in our study did not decrease *Rhizobia*, their functioning may still be affected (Azcón-Aguilar and Bago, 1994; Smith and Read, 1997). In relation to productivity, greater effect of soil micro-organisms on crop yield was reported, e.g. of *Pseudomonas fluorescens* without than with fertilizer applications (organic or inorganic; Krey et al., 2013), and attributed to a stimulated increased colonization by mycorrhizal under P-deficient conditions; not many results relating microbes to crop productivity are available. These results may anyway support proponents of organic agriculture who argue that, unlike organic resources, application of mineral fertilizers is a subversion of microbial roles and result in reduced microbial diversity and activity. On the other hand, some other microbes are favoured by fertilization and, for example, increased ammonium in the soil (through application of N and manure) favours growth of the *Cyanobacteria* (Rückert and Giani, 2004).

Reduced tillage is associated with increased soil microbial biomass contents (Madejón et al., 2007; Álvaro-Fuentes et al., 2013), and higher levels of bacterial and archaea diversity (Dong et al., 2017) compared to conventional tillage systems, as also observed in our study, which is commonly explained by increased carbon storage at the top soil. Effect of tillage on microbial diversity was reported earlier for the same CT1 site also used in our study (Kihara et al., 2012). The pronounced effect of tillage and crop residue application in their study is likely because sampling was restricted to the top 0–10 cm soil where elevated activity is expected under CA following non-inversion and surface residue residence.

An increased presence of acidophilic archaea, i.e., the ammonia-oxidizing *Candidatus Nitrosotalea devanaterra* (Lehtovirta-Morley et al., 2016), in Kakamega relative to other sites probably coincided with increased substrate availability. The growth of this particular archaea greatly coincided with high substrate affinity, being provided by ammonium and ammonia (Lehtovirta-Morley et al., 2016). At lower pH, *Candidatus Nitrosotalea devanaterra* probably survives on high ammonium substrate concentrations, but as the pH increases, this microbe greatly depends on increased ammonia substrate concentrations for growth. This probably explains why despite the Kakamega site having slightly higher pH than INM3 and CT1, increased presence of *Candidatus Nitrosotalea devanaterra* was evident. Despite the 50 km distance and the 200 mm more rainfall of the Kakamega site, no other major differences in microbial indices have been observed relative to INM3 and CT1. Studies of soil microbial diversity across sites have found climate, vegetation type, soil pH, and texture to play a key role in determination of the microbial community structure.

Cropping systems influence microbes through organic inputs and microclimate. Kihara et al. (2012) observed higher diversity under maize-soybean intercropping relative to rotations and maize monocropping systems. Ten years later, an elevated *Frankia*, which is an indication of elevated (free-living) atmospheric nitrogen fixation, is observed under maize-soybean rotation. Combined effects of reduced tillage with residue retention and a continuous presence of a dicot soybean could provide a favourable microclimate responsible for these findings. Consistent with our study, elevated microbial biomass carbon and nitrogen in intercropping have been observed elsewhere, e.g., by Latati et al. (2017) who coincidentally also worked on P-deficient soils. Also, a previous study within the long-term CT1 trial showed increased microbial diversity under the intercropping system relative to maize monocultures and maize-soybean rotation (Kihara et al., 2012). Legume-cereal intercropping favours and sustains high, especially rhizosphere, microbial diversity compared to sole crops (Yang et al., 2016; Vukicevich et al., 2016) and results to increased exudation (amounts and types) of soil extracellular enzymes (Maltais-Landry et al., 2014).

Maize-legume intercropping is associated with higher phosphatase activity compared to monocrops, e.g. in an Orthic Antrosols in China (Wang et al., 2014). Such phosphatase activity, also observed to some extent in our study, results from the increased (concurrent) demand for nutrients (P) by the two crops (Li et al., 2009; Zhang and Li, 2003; Latati et al., 2016a). The competition and resulting microbial activity can contribute to improved nutrient-use efficiency in the intercropping compared to sole cropping systems (Mobasser et al.,

2014; Latati et al., 2016b) and may be responsible for the often observed higher land equivalent ratios of intercropping systems. Legumes normally stimulate and enhance P and N acquisition by the cereal due to the legumes' ability to secrete more P-mobilizing compounds that contribute to increased P availability (Betencourt et al., 2012; Latati et al., 2014; Wang et al., 2015) and have increased phosphatase activities relative to cereals (Maltais-Landry et al., 2014).

Long-term application of lime has been associated with increased microbial biomass (Ekenler and Tabatabai, 2003). In our case, lime was applied for 4 seasons and at lower rates than usually recommended for achieving high pH increase and although a long-term perspective could not be ascertained, yet still the 20% increase in MBC due to liming in CT1 points to a positive change. Also, in the very short-term (27 days of mesocosm experiments using soils from INM3; Margenot et al., 2018), lime application (0–7.5 t ha^{-1} at 1.5 t ha^{-1} intervals) did not significantly alter microbial biomass P, despite an increase in soil pH (0.01 $CaCl_2$) from 4.7 to 6.4. The importance of studying also specific genera is revealed with the increase in Cyanobacterium due to lime application (in presence of crop residues). The *Bacterium* genus has some species such as *Pseudomonas* sp. that produce phytase, commonly associated with plant growth promotion (Singh and Satyanarayana, 2011), while others such as *Pseudomonas putida* are important in soil aggregation through their exopolysaccharides (Sandhya and Ali, 2015).

Photo: CIAT

Conclusions

The following are conclusions derived from this study:

Long-term application of FYM as the only input results in similar microbial communities as treatments integrating organic resources and mineral fertilizers and has a gradual yield increase over time that now stands higher than the stagnated 4–5 t/ha maize grain yield under mineral fertilizer-based treatments.

Long-term application of FYM is a good strategy to promote soil aggregation and microbial activity (phosphatases) and elevate microbial biomass carbon.

Application of mineral P fertilizers, though needed for increased crop yields especially in the absence of FYM, can decrease the activity of micro-organisms involved in P solubilisation and supply to plants e.g. the phosphatase activity, but more research is needed to verify this finding.

Among nitrogen-fixing bacteria, increased interspecific competition between maize and soybean under long-term practice of CA with surface residue retention (and no mineral fertilizer application) increases populations of *Frankia* and Kribella but not rhizobia and actinomyces.

Reduced tillage is clearly a good strategy to promote diversity and abundance of soil micro-organisms. However, soil and residue mixing through tillage promotes greater macrofauna diversity below the very top soil (i.e., in the 5–15 cm soil layer) relative to reduced tillage practices.

Reduced tillage with residues is an opportunity for increased nitrogen-use efficiency through minimization of leaching risk and avoidance of surface losses.

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