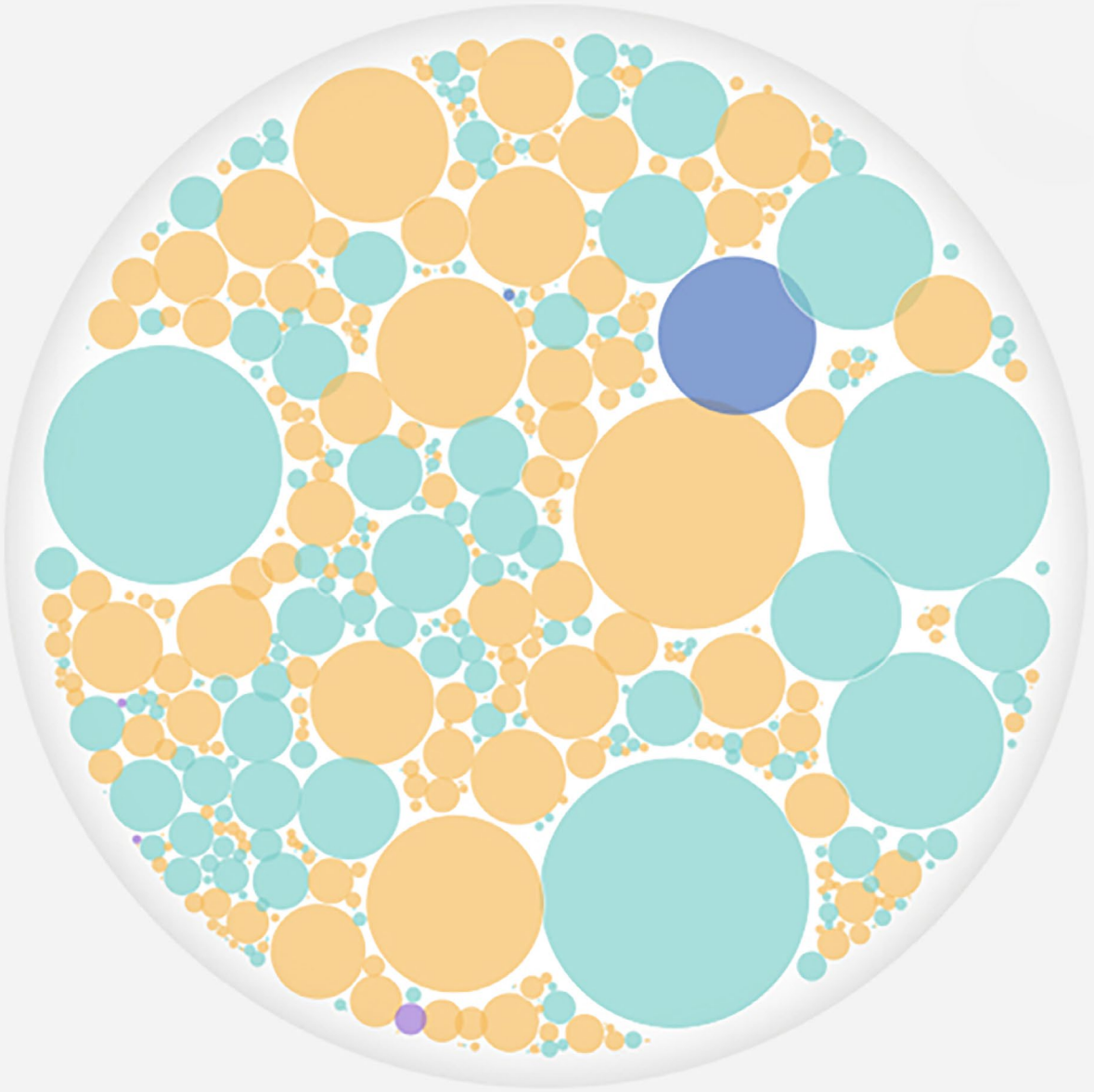

Microbes matter:
unravelling trade-offs between integrated management options and microbial functions

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Several students have benefited from and, in one way or another, supported this project. These students include one PhD student from New Zealand, one MSc student from Kenyatta University and three undergraduate students under attachment at KEFRI, Maseno Station (i.e., one student from Kenyatta University; one from Eldoret University and one from Kabianga University) who received short-term training on different aspects of fieldwork including soil, plant biomass and gas sampling, sample management including drying and sieving, plant biomass and grain handling, drying and grinding, weighting, data recording and entry. In addition, one student from Embu University has benefited by sharpening his skills in DNA extraction from the soil.

Presentation structure

We start with key highlights. We then present the soils as influenced by management practices followed by influences to overall microbes. Effects on specific functional groups including zinc and phosphorus solubilization follows before a presentation of how these relate with the soil properties. This is followed by a section on enzyme activities and another on specific functional genes and fungal to bacterial ratio. We then present data on CO_2 evolution and mineralization of nitrogen and phosphorus. We finish with application of micro-biology through bio-inoculants and then a summary of research gaps.

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Key highlights

Application of farm yard manure (FYM) provides sufficient amounts of phosphorus (P) needed by crops and also alleviates inherent deficiencies of important soil micronutrients. The concentrations of important micronutrients involving zinc, boron and iron were above the critical thresholds in systems with FYM application as opposed to systems without FYM.

Reduced tillage and conventional tillage practices result in different populations/communities of soil fungi that are distinct from the microbial communities occupying soils in undisturbed natural sites.

Application of FYM alone or in combination with fertilizers increased microbial richness and diversity, and microbial structures for zinc solubilization, phosphorus solubilization and plant growth promoting rhizobacteria. Application of FYM alone resulted in higher bacteria richness relative to FYM combined with multinutrients (that include lime).

Application of FYM stimulated CO_2 fluxes compared to either omission or application of nitrogen at higher rates; and this positively coincided with increase in soil moisture. For instance, the CO_2 fluxes in the treatment with application of FYM was 45.2 mg/m²/h, and this was significantly higher than the CO_2 fluxes in the plots without FYM (32.9 mg/m²/h) or plot with the application of 90 N (28.6 mg/m²/h). This is an indication that FYM systems enhance soil microbial activity.

Mineralization of N is increased with reduced tillage relative to conventional tillage, with surface residue application relative to no residues. Application of N at the moderate rates of 60 kg N ha^{-1} seems to depress N mineralization.

Residue addition increases soil microbial pool and activities. Relative to systems without residues, application of residues in the reduced tillage systems significantly increased PhoD gene abundance, pointing to increased P-cycling. In addition, the counts of unique organisms were higher in the agronomic systems with residue addition compared to systems without residue application.

Practicing reduced tillage with nitrogen addition increased diversity of phosphorus solubilizing microorganisms compared to reduced tillage without nitrogen addition and conventional tillage with nitrogen application.

Enzyme activities (Alkaline phosphatase (ALP) and acid phosphatase (ACP)) are increased with combined or sole applications of FYM and residues compared to “fertilizer only” without organic resources, pointing to the inhibitory effects of increased availability of fertilizers on phosphatases and their activities. Also, reduced tillage has more potential for enzymatic P cycling as observed with higher ACP enzyme activity in the reduced relative to conventional tillage systems.

Application of bio-inoculants, in addition to other amendments slightly increase crop and forage yields relative to non-incorporation of bio-inoculants. The use of bio-inoculants in forages increased Napier yields by approximately 2 t ha^{-1}.

Effects of management on soil chemical parameters

Soil chemical analysis across the key treatments of the long-term trials in western Kenya (Table 1) shows that:

- INM3 has a greater range of soil test values for most of the parameters relative to CT1. This relates to the range of integrated soil fertility management practices implemented in INM3 including combinations with FYM, crop residues and agroforestry (Tephrosia).
- In the INM3 site, treatments with manure (alone or in combination with other soil management practices) mostly had elevated pH, Ca, Mg, Zn, SOC, K and CEC. The lowest soil test values were under no input and fertilizer only treatments.
- P application increased available P. Manure also increased P.
- In CT1, the lowest values for most parameters are under reduced tillage with no fertilizer inputs. For B, however, the highly intensified systems (maize-soybean rotations applied with both N and P) had the lowest boron consistently, an indication of year by year nutrient mining. A more balanced soil fertility regime, e.g. including FYM could reverse this decline.
- The 2 long-term sites differ profoundly in Cu (5.9[±0.55] in INM3 vs 15.8[±1.8] in CT1), in Zn (2.2[±1.1] in INM3 vs 10.3[±8.1] in CT1) and in Ca (630[±274] in INM3 vs 914[±210] in CT1).
- In comparison, the undisturbed site has the highest overall pH, SOC, N, Ca, Mg, B, Na, EC and CEC. Here, concentrations of Mn and B were several times higher than in the treatments in INM3. In addition, concentration of Na of 27.9 ppm in the undisturbed site was 70-200% higher than in the treatments of the two long-term trials (INM3 and CT1) while that of SOC was 40-90% higher.
- Available P and exchangeable K in the soil were extremely low (being 1.5 ppm and 91.5 ppm, respectively) in the undisturbed site compared to the experimental site. The low P is because of fixation of P without replenishments in the high P fixing soils of the experimental sites.

The most interesting finding is that sustained application of FYM at the rate of 4 t/ha per season, as done in the long-term trials, is adequate to supply the needed P (at the current level of yields) and alleviate inherent deficiencies of important soil micronutrients such as zinc, boron and iron. Without application of FYM, the concentrations of these micronutrients are below the critical thresholds. Although not as high as for other micronutrients, the application of FYM increased Cu by 10%.

While plots without fertilizer application had average soil available P just at the lower critical level of 15 mg/kg soil, application of FYM alone increased the P to 22 mg/kg of soil while application of P fertilizer and also its combination with FYM increased the soil available P to values way above even the 30 mg/kg of soil, where no P application is needed. Interestingly, reduced tillage without P treatments have very low soil available P, and also the lowest zinc (1.6 to 4 times lower than other treatments) and both nutrients are often lost through erosion. Low biomass production and therefore cover could expose these treatments to erosion through runoffs. The increase in soil pH with application of farmyard manure from 4.84 to 5.25 is consistent with findings of Opala et al. (2012), Mucheru-Muna et al. (2014) and Liu et al. (2017) attributed to liming effects of farmyard manure when humic materials adsorb Al and Fe Oxides with corresponding release of hydroxyl ions (Hue et al., 1986; Neina, 2019).

Among the soil physical properties assessed, only bulk density was influenced consistently by tillage with reduced tillage having 1.19 compared to 1.10 g/cm^{-3} under conventional tillage (data not shown).

Table 1. Effects of soil fertility management practices on the concentrations of selected soil chemical parameters in INM3 and CTI long-term experimental sites. The averages for a recently undisturbed site are also shown.

	pH	SOC (%)	N (%)	OlsenP (mg/kg soil)	K	Ca	Mg	Mn	S	Cu	B	Zn	Fe	Na	EC	CEC
INM3																
No Input	4.84^{de}	1.7^{cd}	0.13^{ef}	18.1^{e}	245^{ef}	382^{e}	38^{d}	394^{ab}	17.1^{d}	5.3^{c}	0.13^{cd}	1.00^{c}	118^{c}	9.5^{bc}	31.7^{b}	6.5^{d}
P + N	4.51^{f}	1.64^{d}	0.12^{f}	79.0^{a}	146^{g}	357^{e}	38^{d}	431^{ab}	22.1^{bc}	5.5^{bc}	0.17^{bcd}	1.30^{c}	128^{bc}	15.0^{ab}	43.6^{ab}	6.8^{d}
Residue Only	4.88^{de}	1.83^{bcd}	0.14^{def}	13.0^{e}	310^{cde}	356^{e}	45^{d}	400^{ab}	19.0^{cd}	5.6^{abc}	0.18^{abcd}	1.23^{c}	117^{c}	13.4^{abc}	32.0^{b}	6.6^{d}
FYM Only	5.16^{abc}	2.05^{a}	0.17^{ab}	23.8^{de}	424^{b}	743^{bc}	122^{ab}	396^{ab}	19.8^{cd}	5.9^{abc}	0.23^{abc}	2.61^{ab}	143^{b}	14.2^{abc}	42.0^{ab}	11.8^{ab}
P + N + Residue	4.66^{ef}	1.68^{cd}	0.13^{f}	71.2^{ab}	209^{fg}	335^{e}	37^{d}	441^{a}	23.3^{ab}	5.7^{abc}	0.12c^{d}	1.28^{c}	133^{bc}	8.0^{c}	44.0^{ab}	6.7^{d}
P + N + FYM + Residue	5.06^{bcd}	1.96^{ab}	0.16^{bcd}	65.6^{ab}	364^{bc}	762^{bc}	139^{a}	397^{ab}	21.1^{bc}	6.3^{ab}	0.19^{abcd}	3.57^{a}	169^{a}	14.6^{abc}	52.2^{a}	12.9^{a}
P + N + FYM	4.95^{cd}	1.86^{abc}	0.15^{cde}	55.9^{bc}	286de	818^{abc}	127^{ab}	366^{b}	23.7^{ab}	6.1^{abc}	0.17^{bcd}	3.29^{a}	174^{a}	16.2^{a}	50.4^{a}	12.6^{a}
Residue + FYM	5.43^{a}	2.07^{a}	0.18^{a}	20.5^{e}	544^{a}	939^{ab}	152^{a}	420^{ab}	21.8^{bc}	6.4^{a}	0.29^{a}	3.46^{a}	126^{bc}	12.1^{abc}	42.5^{ab}	12.1^{ab}
P Only	4.98^{cd}	1.70^{cd}	0.13^{ef}	56.7^{bc}	199^{fg}	695^{cd}	90^{bc}	389^{ab}	24.0^{ab}	5.9^{abc}	0.17^{bcd}	1.84^{bc}	126^{bc}	16.1^{ab}	42.^{7ab}	10.1^{bc}
P + FYM	5.32^{ab}	1.83^{bcd}	0.16^{bc}	59.3^{abc}	334^{cd}	998^{a}	162^{a}	405^{ab}	26.2^{a}	6.2^{ab}	0.27^{ab}	2.58^{ab}	139^{bc}	16.7^{a}	47.8^{a}	13.0^{a}
P + 90N Only	4.54^{f}	1.74^{cd}	0.14^{ef}	42.5^{cd}	162^{g}	463de	56^{cd}	425^{ab}	24.3^{ab}	5.8^{abc}	0.12d	1.61^{bc}	129^{bc}	15.3^{ab}	48.2^{a}	8.6^{cd}
CT1																
RT; 0N+60P+R; M/S Intercrop	5.02^{a}	1.85^{ab}	0.14a^{b}	54.4^{ab}	189^{bc}	1021^{ab}	153^{a}	477^{abc}	23.1^{ab}	15.5^{a}	0.19^{abc}	7.33^{bc}	162^{bc}	9.2^{ab}	42.8^{ab}	14.1^{a}
RT; 0N+60P+R; M-S Rotation	5.09^{a}	1.85^{ab}	0.14^{ab}	71.2^{a}	233^{b}	1047^{a}	147^{ab}	482^{abc}	22.5^{ab}	15.8^{a}	0.17^{bc}	12.15^{ab}	179^{ab}	7.8^{bc}	41.1^{ab}	14.0^{a}
CT; 0N+60P+R; M/S intercrop	5.14^{a}	1.82^{ab}	0.14^{ab}	23.9^{bc}	183^{bc}	864^{abcd}	144^{abc}	546^{a}	19.0^{abc}	14.3^{a}	0.24^{a}	8.41^{abc}	143^{cd}	6.6^{bc}	44.3^{ab}	13.7^{a}
CT; 0N+60P-R; M/S Intercrop	4.98^{a}	1.88^{a}	0.14^{ab}	54.6^{ab}	154^{cd}	912^{abcd}	115c^{d}	535^{a}	23.8^{a}	15.3^{a}	0.19^{abc}	8.63^{abc}	168^{ab}	8.2^{bc}	51.5^{ab}	12.6^{a}
RT; 0N +0P+R; M-S Rotation	5.20^{a}	1.82^{ab}	0.14^{ab}	2.9^{c}	306^{a}	797^{d}	163^{a}	509^{ab}	14.1^{c}	15.2^{a}	0.20^{ab}	5.23^{c}	143^{cd}	4.7^{c}	48.4^{ab}	11.5^{a}
RT; 0N+0P-R; M-S Rotation	4.82^{a}	1.70^{b}	0.13^{b}	2.9^{c}	298^{a}	740^{d}	141^{abc}	407^{c}	17.3^{bc}	16.5^{a}	0.17^{bc}	4.63^{c}	128^{d}	6.2^{bc}	61.6^{a}	13.1^{a}
RT; 0N+60P-R; M/S Intercrop	5.04^{a}	1.83^{ab}	0.14^{ab}	43.2^{ab}	168^{cd}	1015^{abc}	120^{bcd}	493^{ab}	21.9^{ab}	15.9^{a}	0.20^{ab}	8.46^{abc}	186^{a}	9.1^{ab}	41.5^{ab}	13.4^{a}
CT; 60N+60P+R; M-S Rotation	4.93^{a}	1.81^{ab}	0.14^{ab}	40.9^{ab}	150^{cd}	783^{d}	117^{bcd}	546^{a}	22.7^{ab}	16.2^{a}	0.13^{c}	13.30^{a}	182^{ab}	7.3^{bc}	31.0^{b}	11.4^{a}
RT; 60N+60P+R; M-S Rotation	4.85^{a}	1.88^{a}	0.14^{ab}	61.3^{a}	162^{cd}	810^{bcd}	116^{cd}	487^{ab}	23.1^{ab}	16.4^{a}	0.13^{c}	7.96^{abc}	182^{ab}	13.6^{a}	40.9^{b}	12.^{5a}
RT; 60N+60P-R; M-S Rotation	4.88^{a}	1.85^{ab}	0.15^{a}	56.3^{ab}	117^{d}	808^{cd}	102^{d}	456^{bc}	20.8^{abc}	17.2^{a}	0.15^{bc}	9.29^{abc}	181^{ab}	8.5^{bc}	51.0^{ab}	12.8^{a}
Undisturbed natural site	*5.66*	*3.10*	*0.27*	*1.5*	*92*	*1700*	*485*	*423*	*23.2*	*6.9*	*0.61*	*2.49*	*116*	*27.9*	*74.9*	*17.1*

Means with similar letters across each column are not significantly different. P applied at 45 kg/ha (unless specified); N applied at 60 kg/ha (unless specified), residue applied at 2 t/ha; Manure applied at 4 t/ha.

Microbial DNA extractions and analysis

Two sets of microbial sequencing were conducted. The first one was for SR2019 season followed by a second sampling in SR2020 season. In both cases, fresh soils were collected at plot level separately for each 3 replicates of implemented treatments. In the first set, DNA was extracted from 0.2 g fresh soil samples using Phenol-Chloroform Isoamyl (PCI) Alcohol DNA Extraction procedure in Embu University laboratory and analyzed using Illumina sequencing in MR. DNA labs in USA. Briefly, 0.2 g soil were suspended in 200 µl of solution A (containing 100ul Tris-HCl (pH8.0), 100 mM EDTA (Ph8.0); vigorously vortexed, 5 µl of Lysozyme (20mg/ml solution) added and mixture incubated in a water bath (37 °C) for 30 minutes. 400 µl of lysis buffer (containing 400 mM Tris-HCl (pH 8.0), 60 mM EDTA (pH 8.0), 150 mM NaCl and 1 % sodium dodecyl sulfate) was added, incubated at room temperature for 10 minutes. Thereafter, 10 µl of Guanidinium thiocyanate (GITC; for protein digestion) was added and solution incubated (65 °C) in a water bath for 2 hours. After digestion, 615 µl of phenol chloroform isoamyl (i.e., 1:1 volume) was added, solution centrifuged (13200 rpm) for 5 minutes (at 4 °C) and the step repeated again. The supernatant was taken, 150 µl of sodium acetate and 600 µl isopropyl alcohol (2-propanol) added, solution briefly mixed by inversion and left at room temperature until the precipitation settled down. The contents were centrifuged (13200 rpm) for 10 minutes, supernatant discarded and resultant DNA pellets washed in 300 µl of 70% ethanol. The DNA pellets were re-centrifuged (10000 rpm) for 1 minute, supernatant discarded, pellets air-dried and thereafter dissolved in 30 µl of PRC water. DNA quality was checked on 1% agarose gel electrophoresis for further studies. The DNA pellets were lyophilized and shipped to MR DNA (www.mrdnalab.com, Shallowater, TX, USA) for sequencing.

In the second set, fresh soils were sampled and shipped to BeCrop for DNA extraction and sequence analysing. All the samples were of topsoil taken within a depth between 5-15 cm. Each sample from a single plot was made pooling together top soil from three random spots in each plot and extracting the DNA from this composite sample. Soil samples were stored at −80 °C until DNA extraction. DNA extraction was performed using the DNeasy PowerLyzer PowerSoil Kit (Qiagen). Libraries were prepared following the two-step PCR protocol from Illumina and sequenced on an Illumina MiSeq using pair end sequencing (2×300 bp). Libraries were prepared by amplifying the 16s rRNA V4 region and the ITS1 region using Biome Makers® custom primers (Patent WO2017096385). Raw sequences were analyzed using Vsearch using default parameters 54. Briefly, raw paired-end fastq sequences were merged, filtered by expected error 0.25, dereplicated, and sorted by size. We filtered out chimera sequences and clustered the remaining sequences into 97% identity OTUs, considering in further analyses only groups with at least two sequences. Combined sequences were then mapped to the list of OTUs with at least 97% identity, resulting in an OTU table with OTU sequences quantified per biological sample. OTUs were classified with the SILVA 123 database through the SILVA-NGS pipeline.

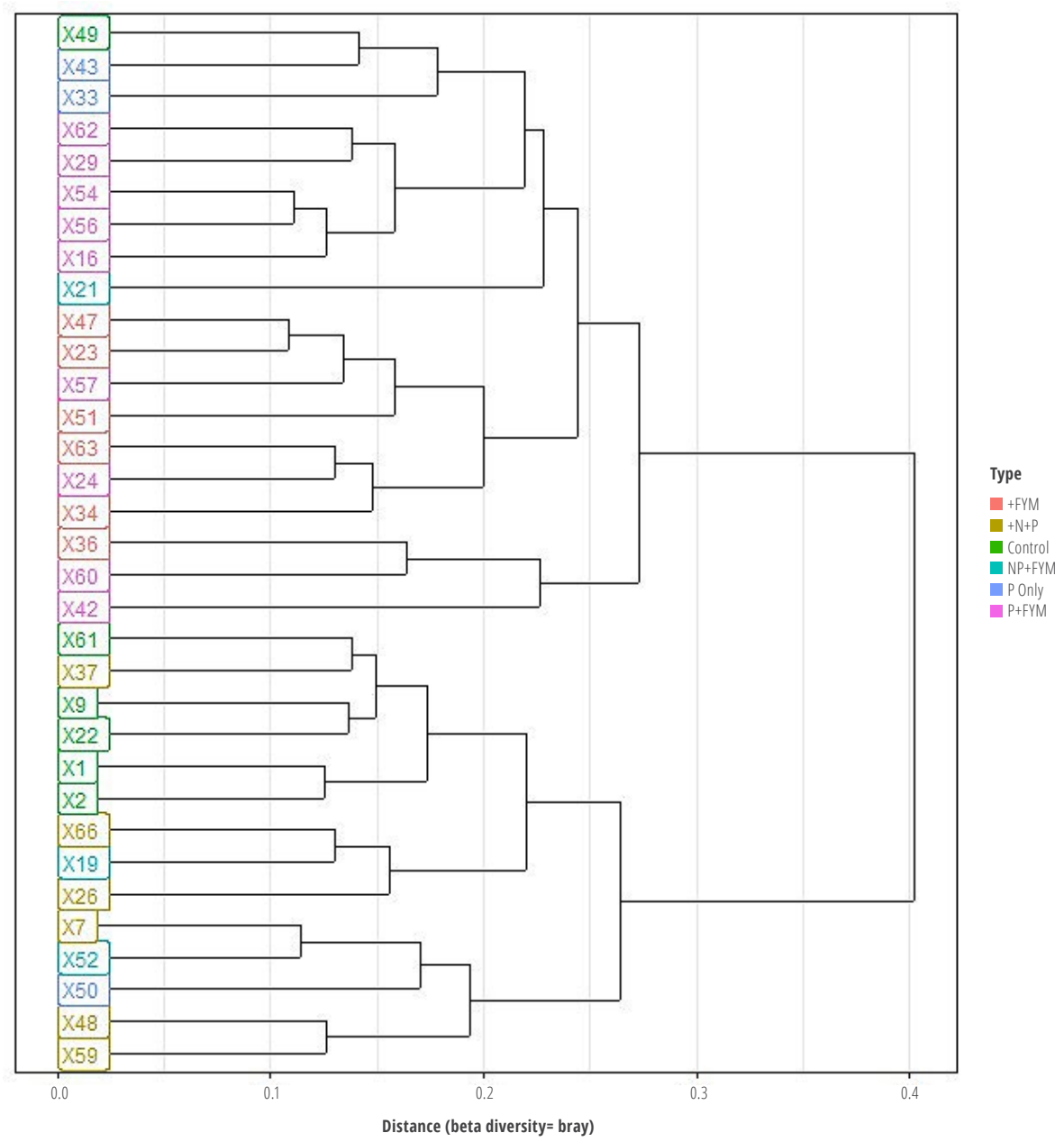
The distribution of soil bacteria and fungi observed, based on the first set of DNA analysis in SR2019, were similar across the two long-term trials (Table 2).

Table 2. Phylotypes and most abundant groups observed for fungi and bacteria in the two long-term trials of western Kenya.

	Bacteria		Fungi	
Long-term trial	CT1	INM3	CT1	INM3
Individual phylotypes (OTUs)	86	85	76	79
Total count	974375	1139440	1012354	1207070
OTUS (class level) abundance > 0.05%	40	35	33	39
Ten most abundant phylotypes at class level	Actinobacteria (17.33%), Bacilli (11.17%), Alphaproteobacteria (10.46%), Gemmatimonadetes (8.83%), Deltaproteobacteria (8.01%), Betaproteobacteria (6.8%), Acidobacteria (6.36%), Verrucomicrobiae (4.4%), Planctomycetia (4.14%), Ktedonobacteria (3.67%).	Actinobacteria (15.51%), Alphaproteobacteria (11.39%), Gemmatimonadetes (9.58%), Bacilli (8.59%), Betaproteobacteria (7.79%), Acidobacteria (6.2%), Ktedonobacteria (5.33%), Clostridia (4.69%), Gammaproteobacteria (2.85%), Verrucomicrobiae (2.52%).	Agaricomycetes (21.21%), Sordariomycetes (19.7%), Dothideomycetes (16.64%), Eurotiomycetes (10.28%), Mortierellomycotina (5.24%), Chytridiomycetes (4.6%), Eukaryota (3.39%), Lecanoromycetes (2.68%), Glomeromycetes (2.64%), Orbiliomycetes (2.21%).	Sordariomycetes (23.26%), Dothideomycetes (16.01%), Agaricomycetes (14.17%), Chytridiomycetes (10.56%), Mortierellomycotina (7.63%), Eukaryota (6.27%), Eurotiomycetes (5.3%), Glomeromycetes (2.42%), Kickxellomycotina (1.57%), Saccharomycetes (1.19%).

The initial analysis of the first set of data showed the key influences of soil microbes are related to addition of manure alone or in combinations with residues or chemical fertilizers in INM3 (Figure 1). The tested treatments are therefore evaluated mostly with regard to these management practices while relating to nutrient application within these management practices. The analysis presented is mostly with the second set of microbial DNA analysis which we consider more robust compared to the first set obtained through a commercial laboratory. Towards the end of the section, analysis relating soil parameters to the first set of microbial analysis is done since these were collected during the same season.

Figure 1. Hierarchical clustering of bacteria (genus level) at the long-term soil fertility management (INM3) trial during SR2019 season.



Microbial abundances as affected by management practices

Microbial populations in undisturbed sites (predominantly under grasses and scattered shrubs) are distinctly different from cropped fields (Figure 2). Practicing reduced tillage with surface residues also results in microbial populations that are distinguished from conventional tillage systems (also with residues). Also, a shift in microbial populations is observed due to application of FYM. The greatest shift in soil microbes is observed with combined application of FYM and micronutrients such as Zn and S (Figure 3). This treatment had been applied with lime, at 2 t/ha, during long rains seasons of 2016 and again in 2017. The profound microbial shifts in the FYM+MN is therefore most likely due to the liming.

Figure 2. Dendrogram (a) and non-metric multidimensional scaling plot of soil total bacteria populations (species level) observed in a conservation agriculture (CT1) long-term trial in Nyabeda, western Kenya in 2020.

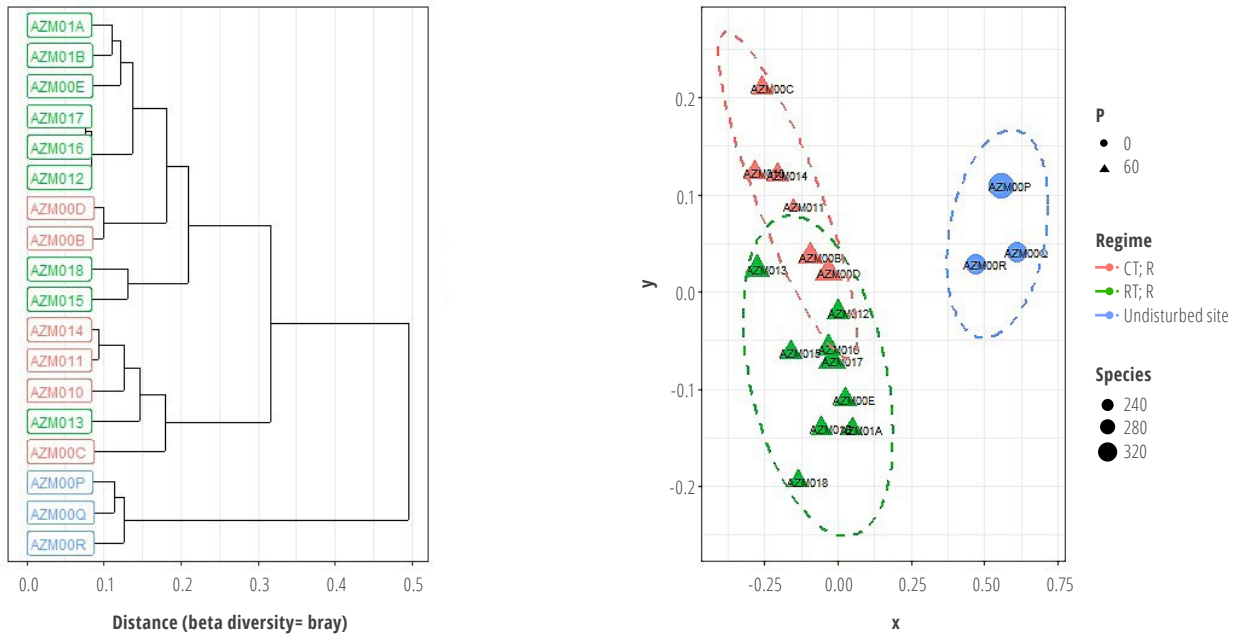
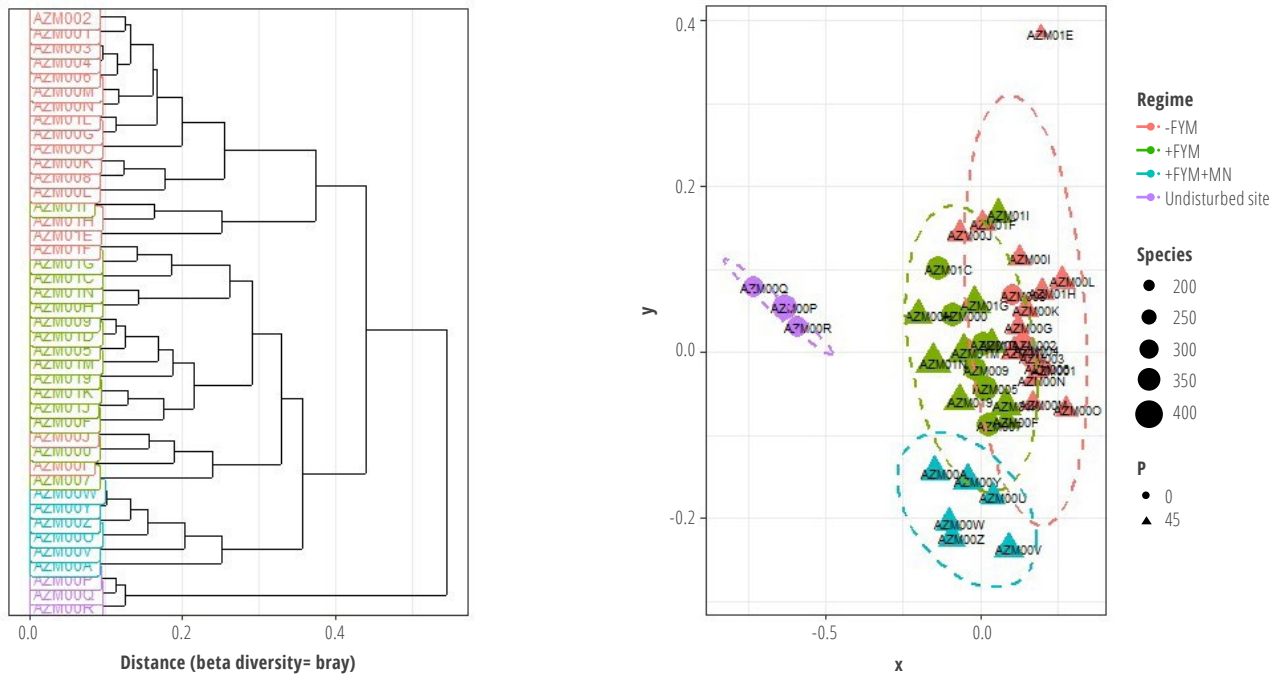
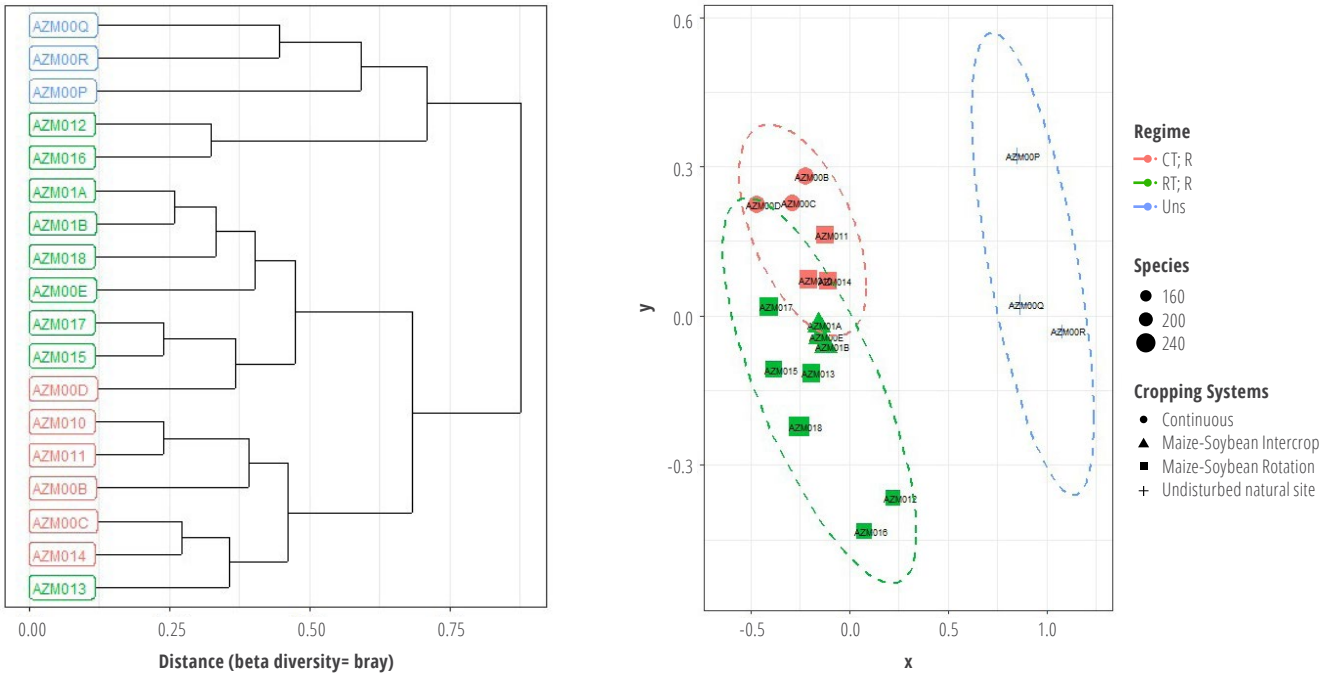


Figure 3. Dendrogram (a) and non-metric multidimensional scaling plot (b) of soil total bacteria populations (species level) observed in an integrated soil fertility management (INM3) long-term trial in Nyabeda, western Kenya in 2020.



Reduced/minimum tillage and conventional tillage practices result in different populations/communities of soil fungi (Figure 4). These are distinct from the microbial communities occupying soils in undisturbed natural sites. Continuous maize monocropping has also a microbial community that is slightly shifted from the maize-soybean rotations and intercrops.

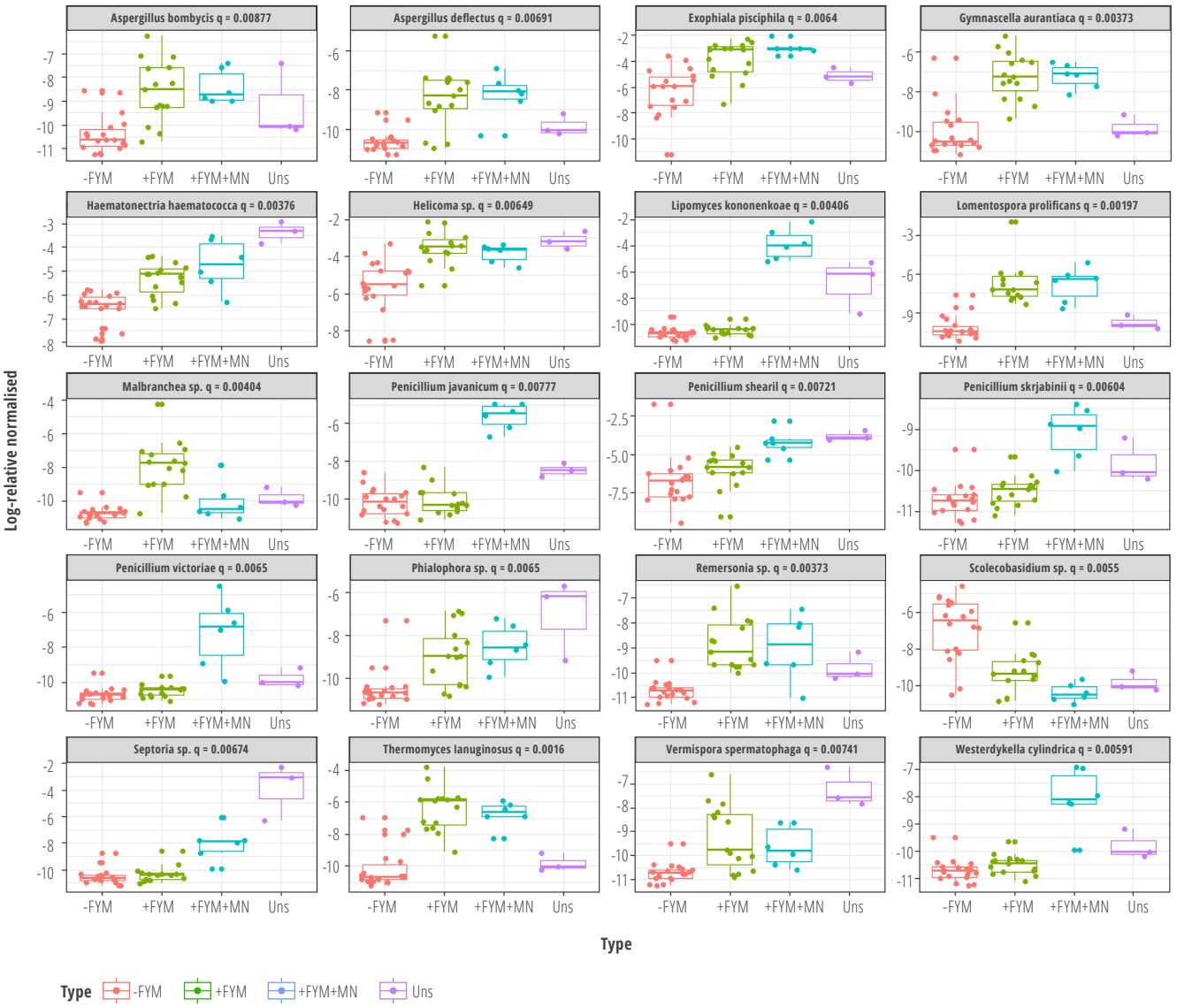
Figure 4. Dendrogram (a) and non-metric multidimensional scaling plot of soil fungi populations (species level) observed in a conservation agriculture (CT1) long-term trial in Nyabeda, western Kenya in 2020.



Out of all the fungi taxa observed (at species level), only 20 were significantly affected by management practices namely, presence or absence of FYM and its combinations with micronutrients analyzed together with an undisturbed natural site (Figure 5). Consistently, with the exception of *Scolecobasidum* sp., management practice without application of FYM had the lowest abundance. With exception of a few cases, both application of only FYM, and its combination with micronutrients, increased abundance of specific fungi taxa in the same way. Combined application of FYM and micronutrients had particularly high effects on *Penicillum victoriae, Penicillum javanicum, Lipomyces kononenkoae* and *Penicillum skrjabinii*. *Penicillium* species are known heterotrophs, depending on different sources of organic carbon, mainly plants and animal matter, for their metabolic nutrition (Krebs et al., 1997). Incorporation of FYM enhances nutrient and organic matter availability that most microbes utilize for growth and development while micronutrients are important for microbial DNA formation and replication. Key highlights on affected species include:

- *Scolecobasidum* sp. are amongst oligotrophic microorganisms (Samerpitak et al., 2015), with the ability to survive in environments with very low nutrients. This explains the increased abundance of *Scolecobasidum* sp. in the agronomic management system without application of FYM.
- *Aspergillus bombycis*, an aflatoxigenic fungi (Peterson et al., 2001), is an aerobic fungus dominating oxygen-rich environments, and prefer growing on carbon rich sources (as contributed by FYM), explaining their increase in systems with application of FYM. This fungal species also doubles up as an oligotroph, characterized by slow growth, low rates of metabolism, and generally low population density.
- *Penicillium* species are known zinc solubilizers (Khande et al., 2017); and their abundance is likely to be positively influenced with zinc availability elevated with micronutrients applications. This partly explains the increased abundance of *Penicillium javanicum*, *Penicillium shearii*, *Penicillium skrjabinii* and *Penicillium victoriae* in the systems with combined application of FYM+micronutrients compared to FYM alone.

Figure 5. Fungi taxa (species level) significantly influenced by management practices in INM3 trial alongside an undisturbed natural site (Uns) as observed in 2020.



Besides, out of all the fungal taxa (at genus level) identified, only 11 were significantly affected by soil management systems (Figure 6). Except for 2 genera (*Septoria* spp. and *Scolecobasidium* spp.) out of the 11, FYM application increased the abundance of the remaining fungi in INM3. *Septoria* spp. are renowned fungi that cause numerous leaf spot diseases on field crops, forages, among others, and the reduction in their abundance following FYM application is beneficial for crop production.

Figure 6. Fungal species (genus) significantly affected by management systems in INM3.

Effects of management practices on fungal and bacteria diversity

As with microbial abundances, application of FYM alone or in combination with multinutrients significantly increase both diversity and richness of bacteria and fungi (Figure 7; Table 3). Interestingly also, application of FYM alone resulted in higher bacteria richness relative to FYM combined with multinutrients. This points to the possibility of reduced tolerance/increased susceptibility of some microbial species (both bacteria and fungi) to the micronutrients applied. On the other hand, application of organic inputs such as FYM are accompanied by microbes that may be absent previous in a system (Ye et al., 2017). Without manure application (-FYM treatment), agroforestry practices slightly improve the Shannon index of the bacteria (green vs red dots). Increased Shannon diversity means more diverse yet more even populations of microbial species present.

Shannon indices for fungi (ITS) was 3.48 in the undisturbed site and this was not different to those of INM3 (3.19 to 3.48; Figure 7). Most bacteria prefer colonizing environments with low C/N ratios (Eiland et al., 2001), thus explaining the increased diversity in systems with application of both FYM and FYM+micronutrients.

Figure 7. Shannon diversity indices of Bacteria (left) and fungi (right) in the INM3 experiment.

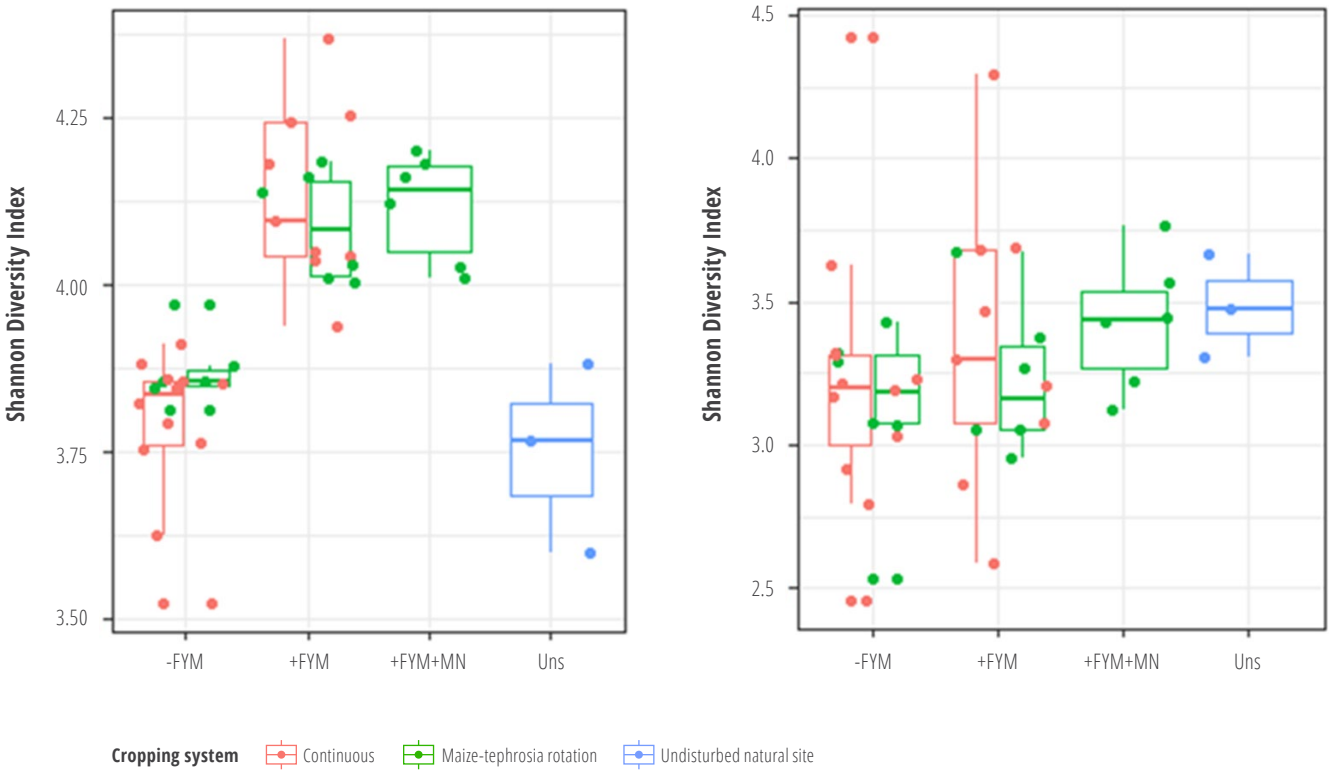


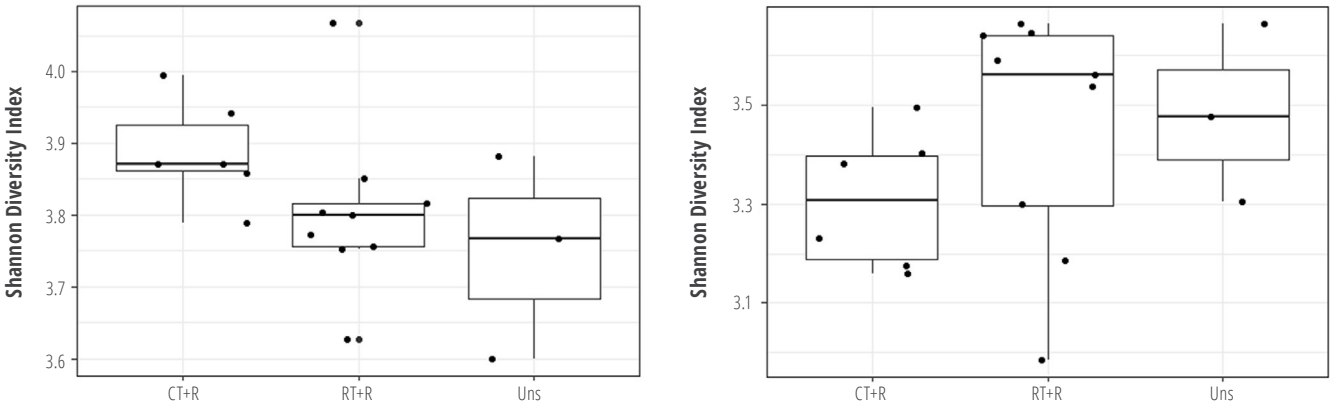
Table 3. Bacteria and fungi richness in different management practices and undisturbed site as observed in western Kenya in 2020.

Richness	Bacteria richness	Fungi richness
Long-term integrated soil fertility trial (INM3)		
+FYM	322[a]	235[a]
+FYM+MN	284[b]	210[ab]
-FYM	241[c]	220[a]
Undisturbed site	302[ab]	133[b]
Long-term conservation agriculture trial (CT1)		
CT+R	249[b]	205[a]
RT+R	252[b]	211[a]
Undisturbed site	302[a]	133[b]

Values with different letters in the same column for a specific long-term trial are significantly different at P<0.05.

Shannon indices for bacteria and richness were not different comparing treatments of CT1 although richness was different (higher under cropped systems) when compared to the undisturbed site (Figure 8). The cropped fields are characterized by increased incorporation of external inputs as well as different plant species (cereals and legumes) while the undisturbed site is mostly on grasses and scattered shrubs thus resulting is different environmental conditions and nutrients availability (Alemu and Bayu, 2005; Bolo et al., 2021; Upchurch et al., 2008). Nevertheless, practicing conventional tillage with residue incorporations increases (not significantly) diversity of soil bacteria but reduces that of fungi relative to conservation agriculture practices. Tillage is detrimental to fungi through occasional breaking of their hyphae. Conservation tillage practices (reduced tillage with surface residues) has similar diversity indices with the undisturbed sites.

Figure 8. Shannon diversity indices of bacteria (left) and fungi (right) in the CT1 experiment. CT+R = Conventional tillage with residue added; RT+R = Reduced tillage with residue added; Uns = Undisturbed natural site.

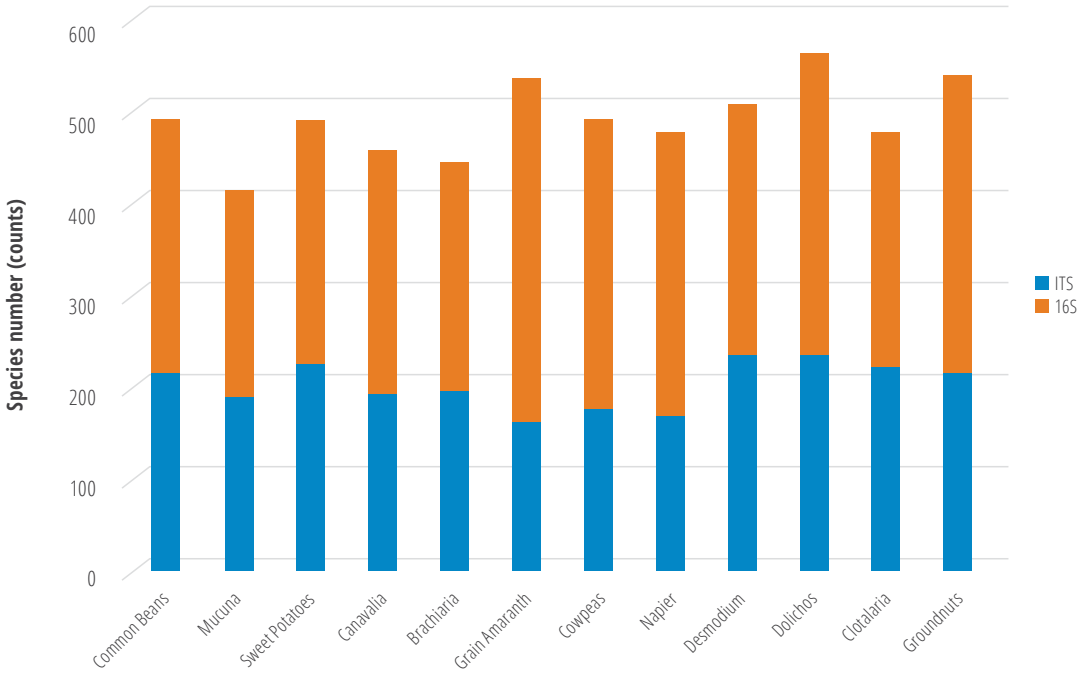


In summary, shifts in microbial populations result from the influence of reduced tillage systems in CT1, sole application of FYM or a combination of FYM and micronutrients in INM3 site, as a result of organic matter and nutrients availability. Soil organic matter provides organic carbon that acts as food source for the microbial populations (Khatoon et al., 2017).

Fungal and bacterial diversity and abundance under selected farmer fields

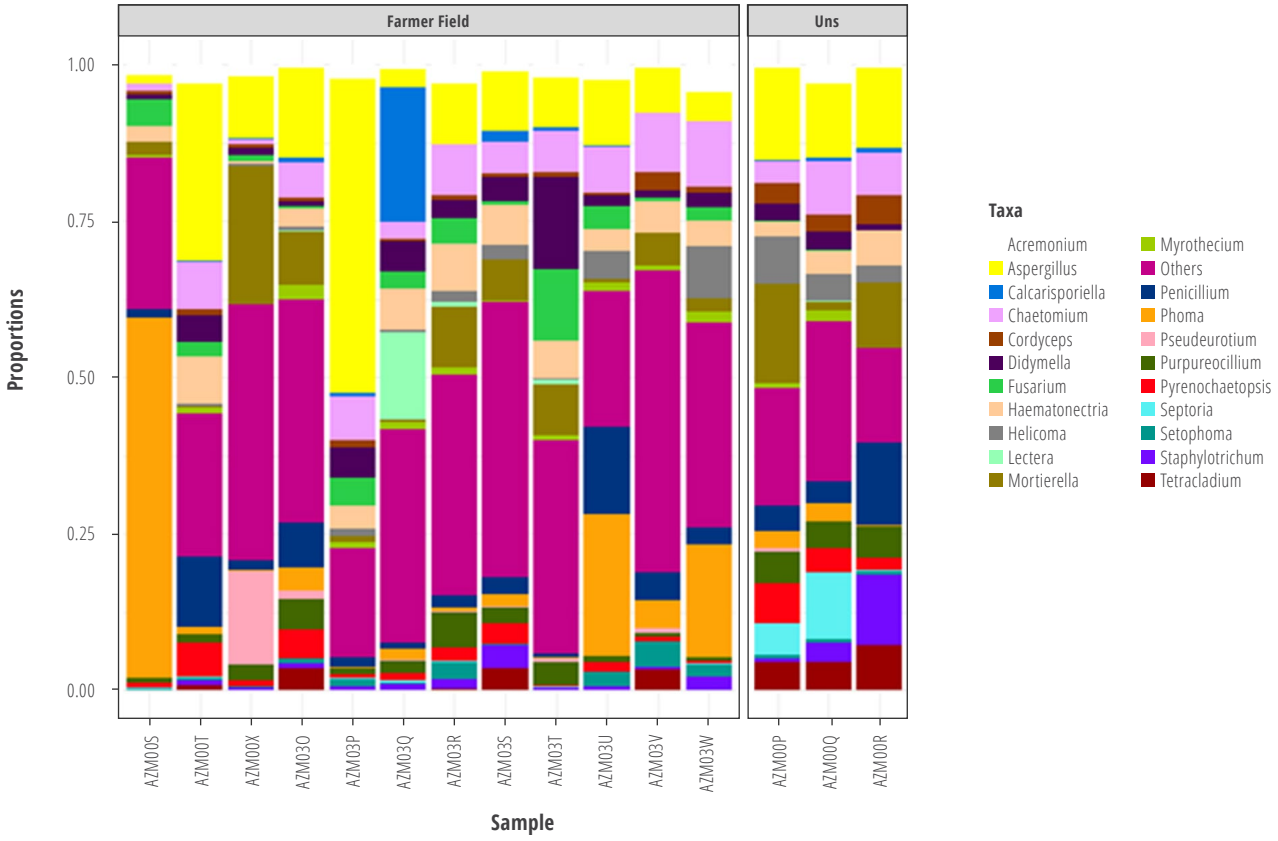
A few farmer fields (12) with various cover crops were assessed for microbial abundances to start understanding microbial conditions outside researcher-controlled fields. Fungi species richness ranged from 163 to 235 while bacteria species richness was 223 to 374 (Figure 9). The field with highest richness was under dolichos and was the only field where lime had been applied.

Figure 9. Fungal (ITS) and bacterial (16S) counts under selected cover crops in selected farmer fields.



The 20 most abundant fungi and bacterial species were identified and those of fungi are the most variable across farmer fields. For these fungal species, *Phoma* spp, a fungal phytopathogen, was absent under the dolichos system (also with lime, see AZM00X in Figure 10) but was increasingly abundant in fields with grain amaranth. All the fields had less *Septoria* spp., also a plant phytopathogen, relative to the undisturbed sites. On the other hand, *Fusarium* spp. was mostly common in the farmer fields but was least common in the undisturbed site. One farmer field with Canavalia had particularly high *Aspergillus* spp., a fungi involved in aflatoxin infestations (Figure 28, AZM03P), but also a phosphorus and zinc solubilizer. In another field (AZMO3Q), *Calcarisporiella* was quite abundant compared to other fields. The high variations in abundances of specific species under farmer fields represent the diversity in management practices employed. However, a detailed assessment of these on-farm management practices, their intensity and how they influence microbial abundances and functions is still needed, including the influences of landscape positions (due to transfers of microbes through soil movements).

Figure 10. Proportions of fungal species under selected farmer field crops and undisturbed natural site. AZM00S= Grain amaranth; AZM00T= Napier; AZMOOX=Dolichos; AZM030=Brachiaria; AZM03P= Canavalia; AZM03Q=Mucuna; AZM03R= Crotalaria; AZM03S=Desmodium; AZM03T=Cowpeas; AZM03U=Beans; AZM03V=Sweet potatoes; AZM03W= Groundnuts.



The next section deals with only the soil microbes involved for known functions such as solubilization of Zn and P. These are often referred to as functional groups and were identified from our overall microbial dataset.

Effect of soil fertility management on soil microbial functional groups

Extensive literature reviews were conducted in this study to identify and map specific genera and species of soil bacteria and fungi (contained in the data obtained from sequencing) to their specific functions including solubilization of phosphorus and zinc. An evaluation of management practices on overall community of plant growth promoters was undertaken followed by specific focus on phosphorus and zinc solubilizes.

The diversity of plant growth promoting rhizobacteria (66 species of bacteria) is reduced with application of multi-nutrients (Figure 11). The low Shannon diversity index with application of multi-nutrients is accompanied by a strong shift in the community structure while presence of manure results to only a small shift in the microbial structure. With the exception of *Paenibacillus turicensis* promoted by presence of multinutrients, and *Clostridium* sp. consistently elevated with both FYM and FYM+MN, 18 other plant growth promoting rhizobacteria were highly depressed in the FYM+MN treatment (Figure 12). The remaining 46 species were either not or only slightly affected (q>0.01) by the management practices.

Figure 11. Diversity (boxplot on left) and ordination plot of community structure (right) of plant growth promoting rhizobacteria (from 66 species of bacteria) as observed in INM3 long-term trial in western Kenya.

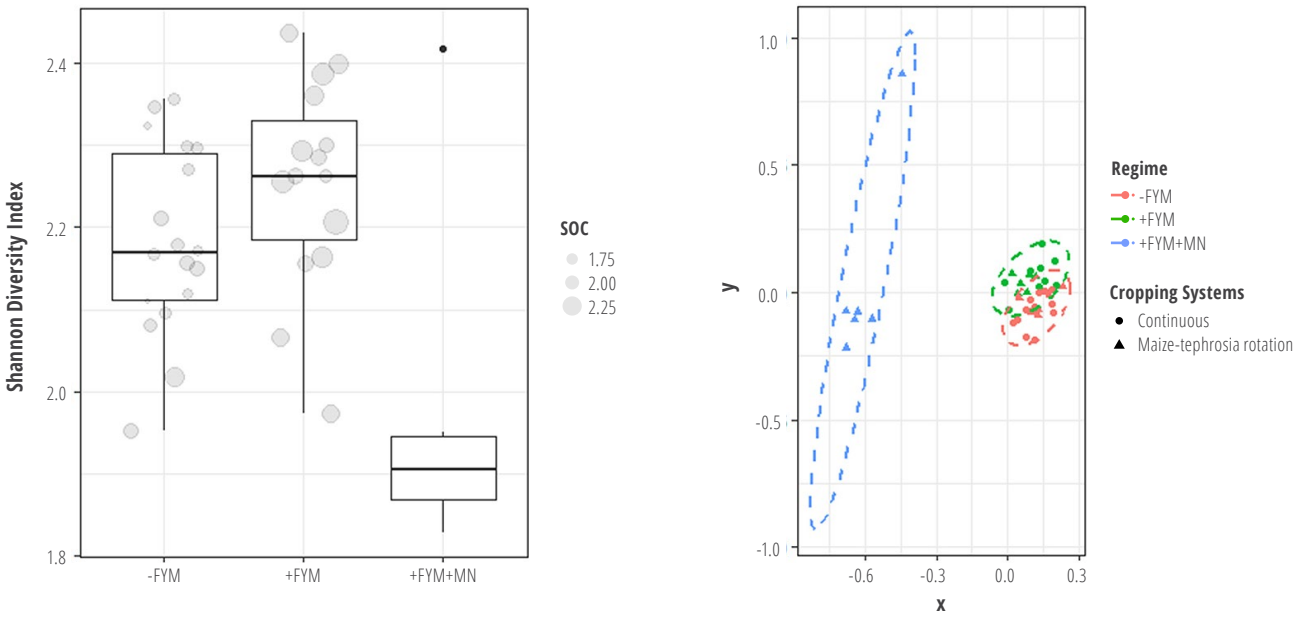
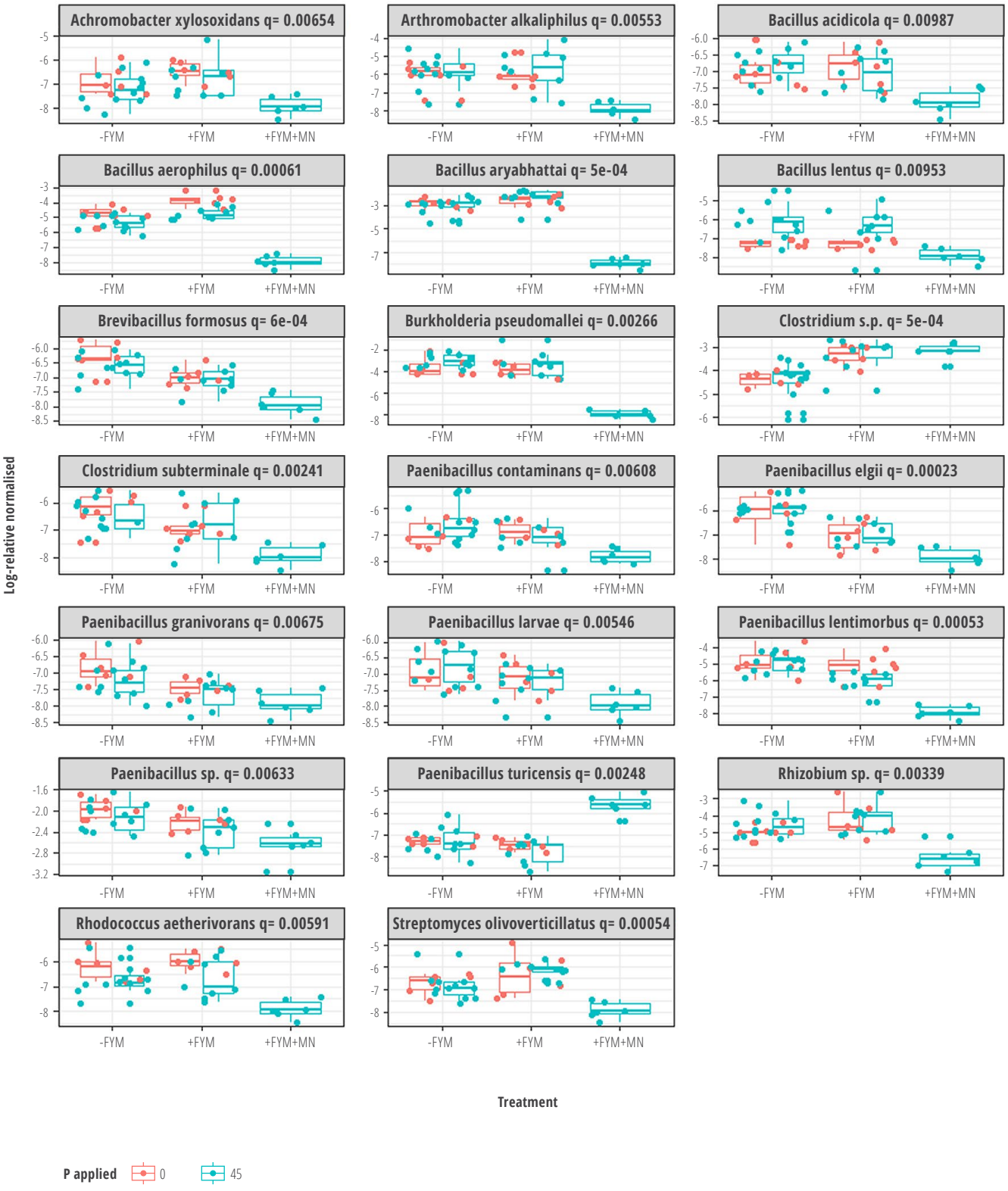
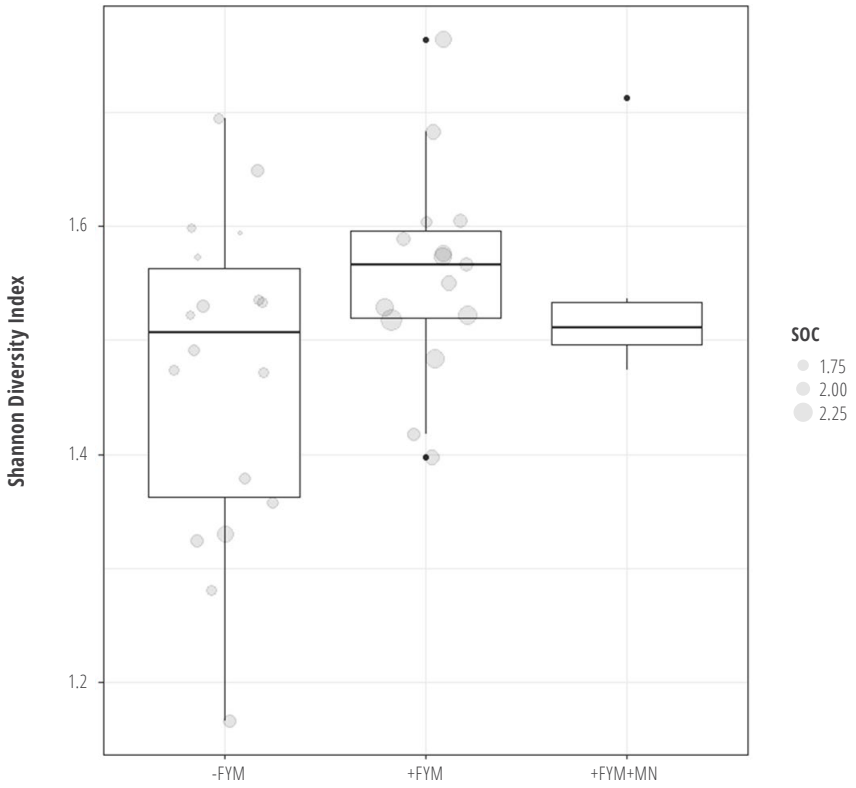


Figure 12. Specific bacteria species of plant growth promoting rhizobacteria significantly affected (q<0.01) by soil fertility management practices (from 66 species of bacteria) in INM3 long-term trial in western Kenya.



The application of FYM improves the diversity of zinc solubilizing soil bacteria in western Kenya (Figure 13). The increase is related to the SOC observed in these systems. Ordination plots show that zinc solubilizing bacteria are shifted under FYM+MN relative to the other management practices. The communities of Zn solubilizing bacteria with and without FYM are essentially the same (data not shown).

Figure 13. Effect of management on diversity of Zinc solubilizing bacteria (18 genus) in INM3. Fertility of the plots (using SOC) is shown with the gray circles. Increasing size denotes increasing SOC. BeCrop data.



Of the 18 bacteria genus involved in zinc solubilization that are identified and analysed with regard to treatment effects, the following results are observed (see Figure 14):

- Application of multi-nutrients highly increased *Bradyrhizobium* spp. but reduced *Rhizobium* spp., *Sphingomonas* spp. and *Sporosarcina* spp. As opposed to Rhizobium spp., *Bradyrhizobium* spp. are slow growing microbial species (Sameshima et al., 2003).
- Application of P without multi-nutrients, greatly increased *Sporosarcina* spp. both with and without FYM.
- Application of FYM highly increased *Lysinibacillus* spp. Just like *Sporosarcina* spp., *Lysinibacillus* spp. are amongst the copiotrophic Firmicutes that respond quickly to changes in labile nutrient availability (Schostag et al., 2019), as potentially contributed by the FYM.
- Nitrogen application increases the Shannon diversity index of the overall community of zinc solubilizing soil bacteria (Figure 15).

Figure 14. Effect of soil management systems on zinc solubilizing genus of bacteria in INM3 site based on Becrop Data.

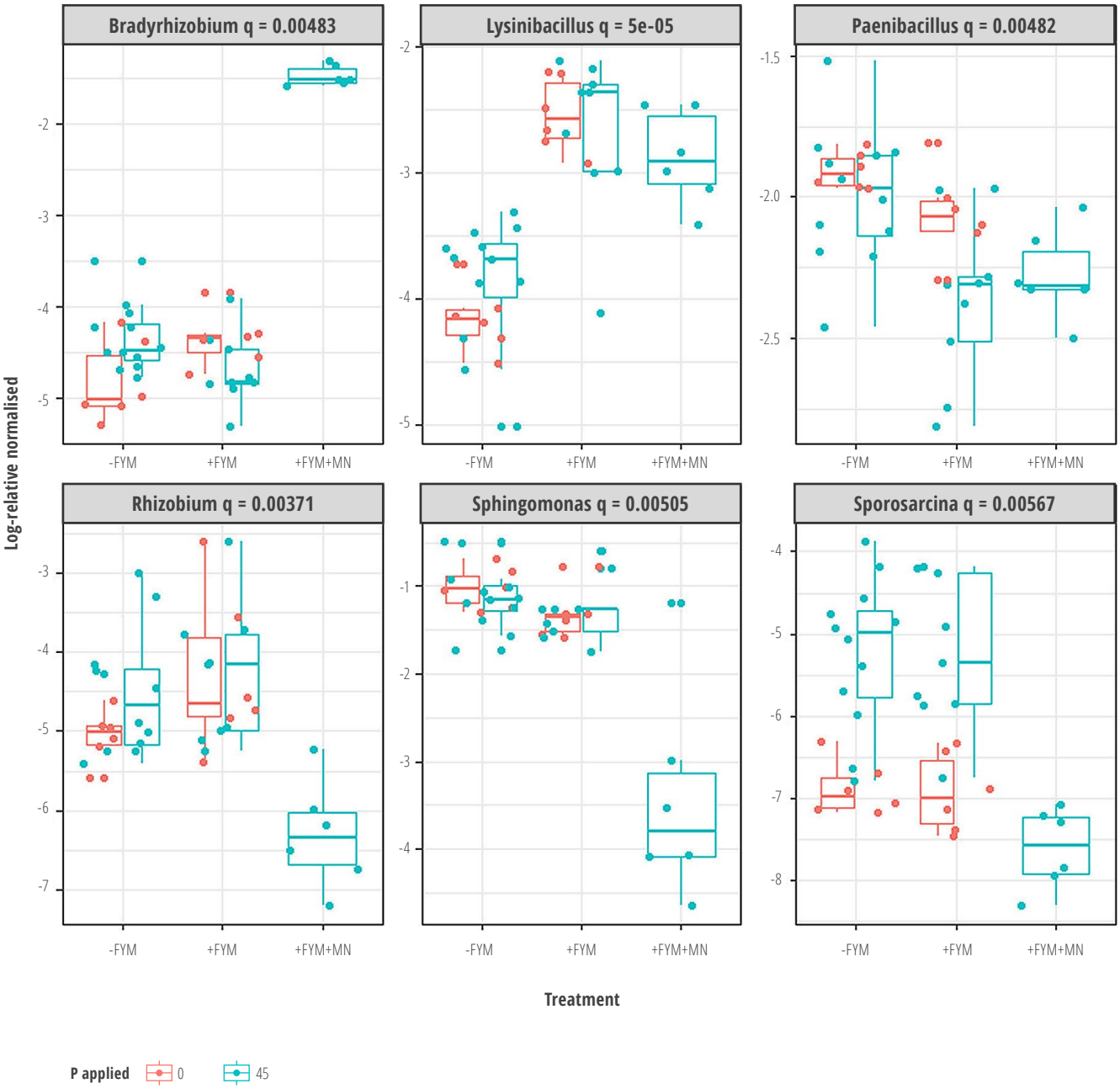
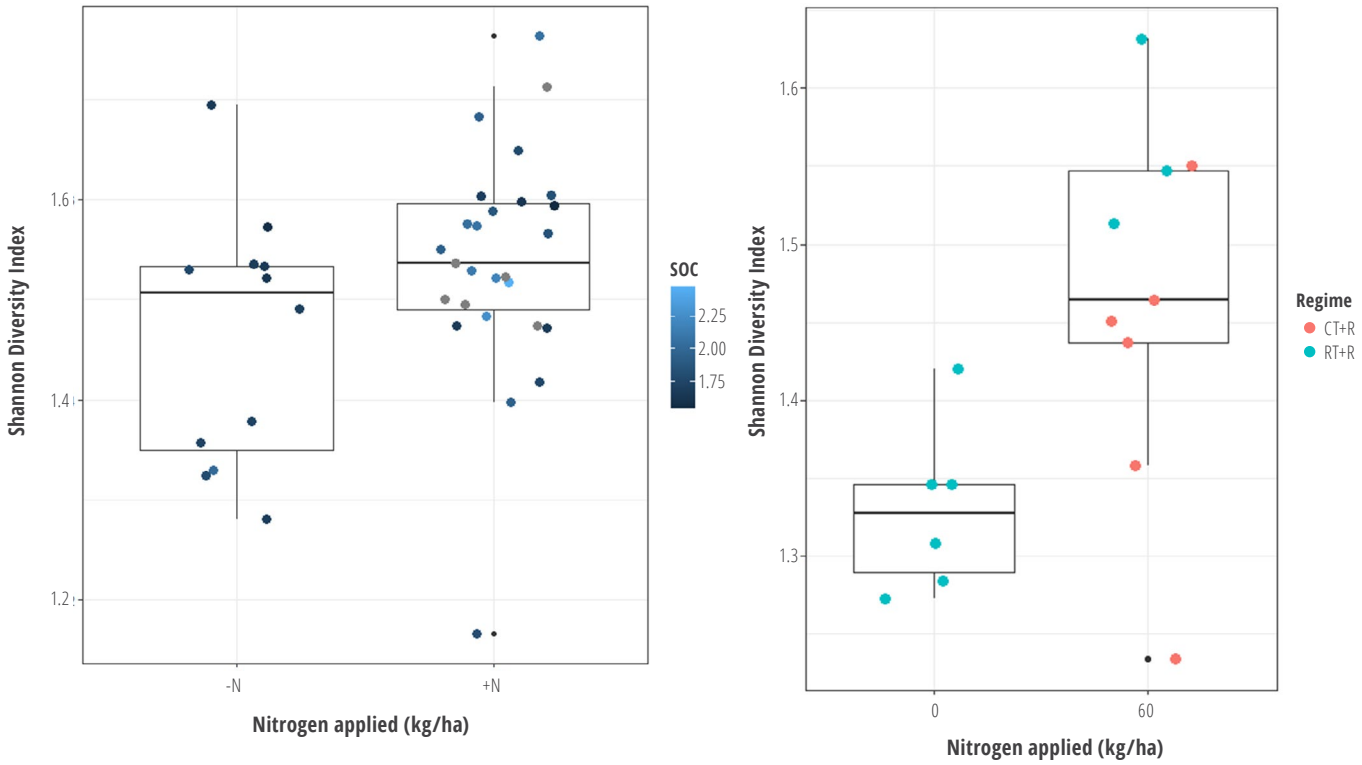


Figure 15. Effect of nitrogen application and tillage on the diversity (Shannon index) of zinc solubilizing soil micro-organisms (at genus level) in the integrated soil fertility management (left) and conservation agriculture trial (right) in western Kenya.



The community of P solubilizing bacteria under FYM+MN was shifted away from the cluster of P solubilizing bacteria without FYM application. The community with only FYM application was only slightly shifted from that with no FYM application (data not shown). Of 22 P solubilizing bacteria genera evaluated, 3 were significantly affected by soil fertility management (Figure 16). Application of multinutrients highly increased *Bradyrhizobium* but decreased Rhizobium, both nitrogen fixers yet essentially different species. Also, application of FYM especially in absence of chemical phosphorus, increased *Flavobacetrium*. Besides *Bradyrhizobium* and the *Sphingomonas* (reported earlier under zinc solubilizers), *Kitasatospora* are clearly distinguished with application of FYM+MN demonstrating the microbial shifts (Figure 17). CCA plots (not shown) confirmed these 3 organisms to clear follow FYM+MN treatment group while all others (except Enterobacter) had a centroid distribution.

Figure 16. Effects of soil fertility management on counts of specific genera of bacteria involved in P solubilization with the Integrated Soil Fertility Management (ISFM) long-term trial (INM3).

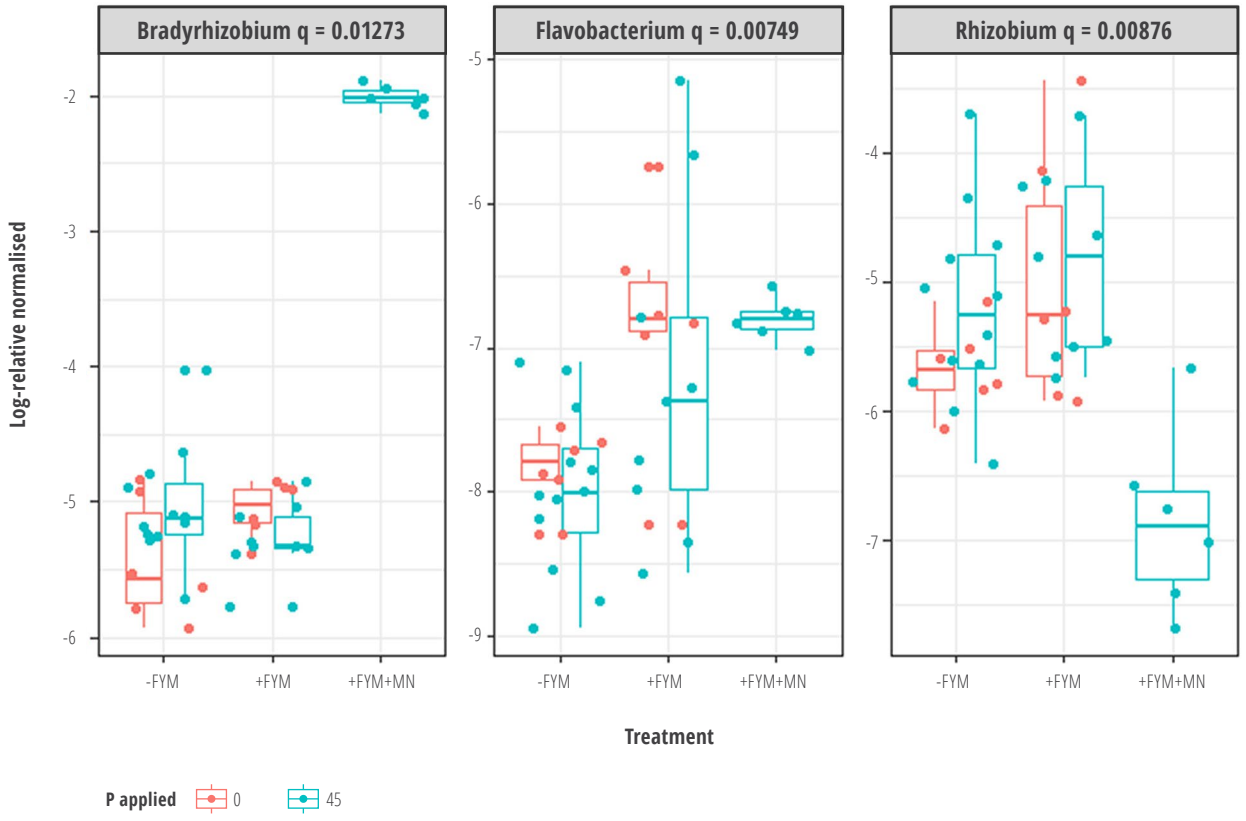
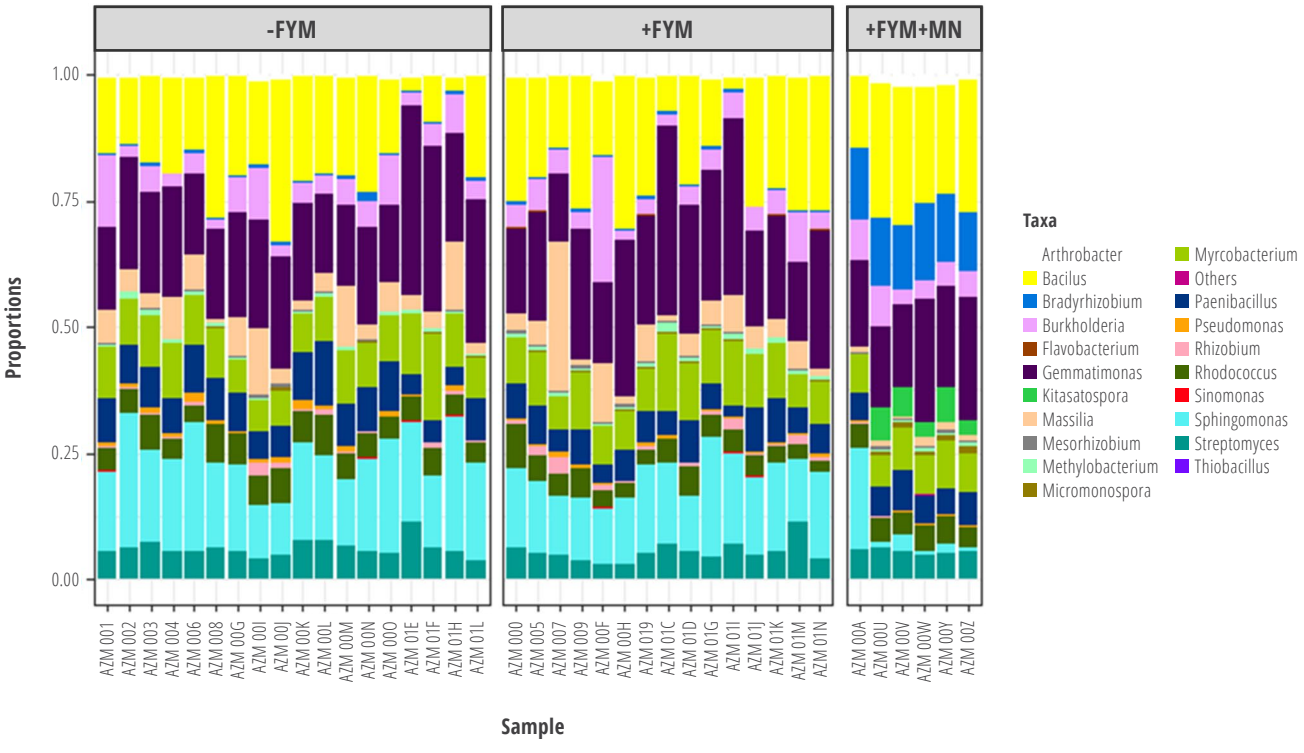


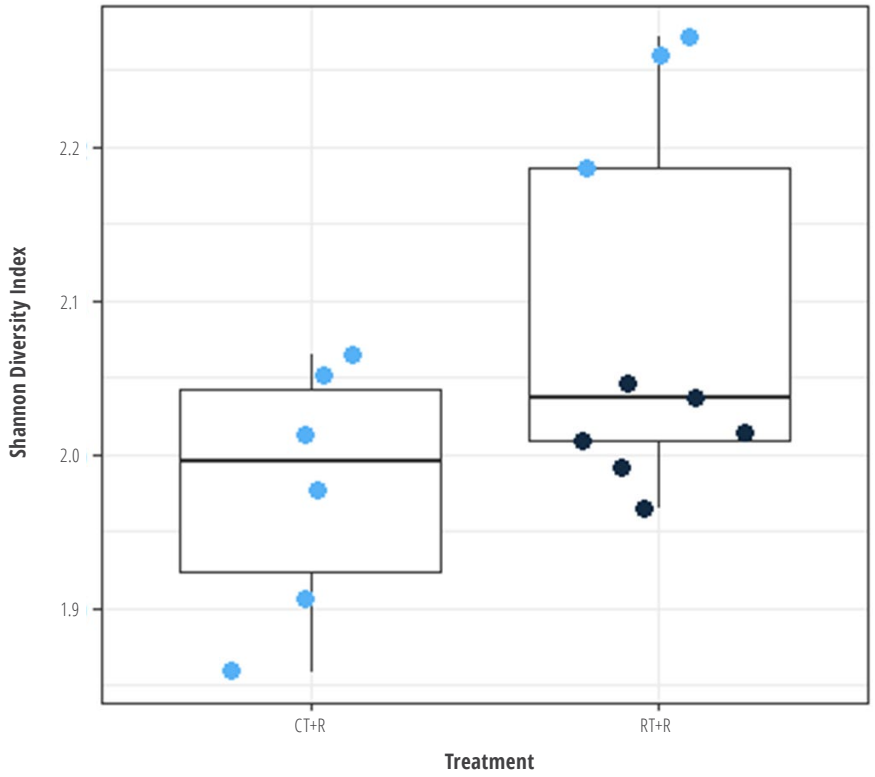
Figure 17. Effects of soil fertility management under long-term trial (INM3) on relative proportions of specific genera of bacteria involved in P solubilization.



Reduced tillage accompanied by nitrogen application increased the diversity of P solubilizing bacteria (Figure 18). Without nitrogen, the diversity of P solubilizing bacteria is similar to that of conventional tillage with nitrogen application. The two genera influenced ($P<0.05$) by the tillage are *Mesorhizobium* spp. (elevated under reduced tillage) and *Gemmatimonas* spp. (decreased under reduced tillage; data not shown).

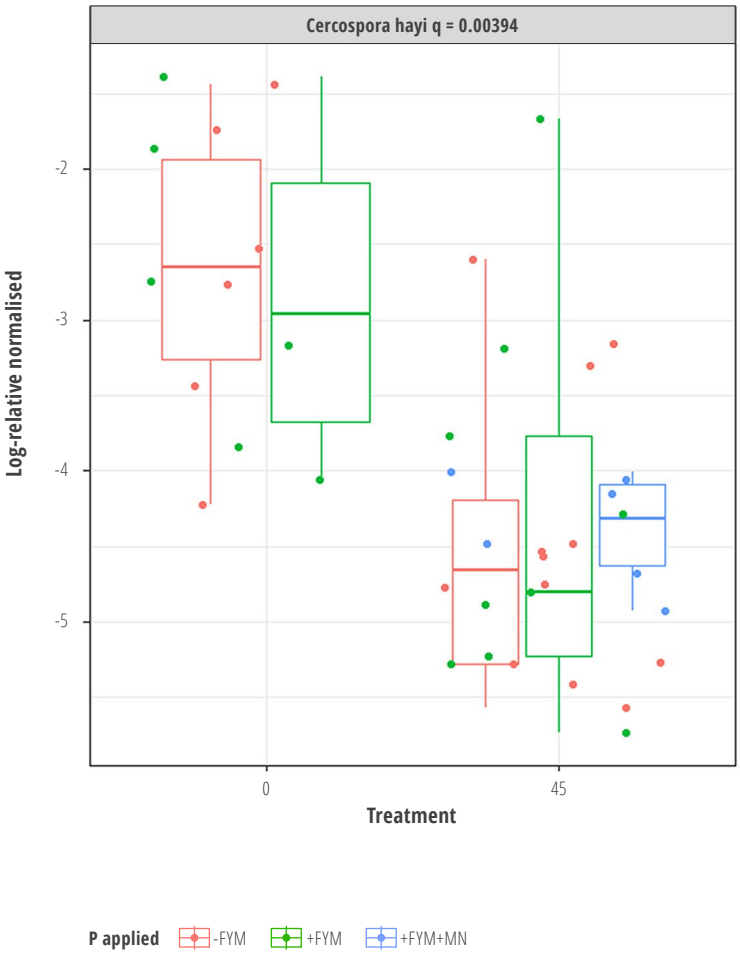
Under CT1 and also INM3, there was no overall effect on diversity for fungi and also none of the fungi genera was affected by treatments tested.

Figure 18. Shannon diversity of P solubilizing bacteria (at genus level) in long-term conservation tillage experiment in Western Kenya. Blue dots=nitrogen applied plots. Black dots=no nitrogen applied plots.



A total of 39 pathogenic fungi identified based on literature were assessed with regard to soil fertility management. Application of FYM or its combination with multinutrients had no significant effect on any of the pathogenic fungi species. Only *Cercospora hayi*, a pathogenic fungi associated with Brown spot disease in banana fruit, was decreased with application of P under systems both with and without FYM (Figure 19). None of the analyzed pathogenic fungi were affected by treatments in CT1 long-term trials.

Figure 19. Effect of phosphorus application (x-axis) on *Cercospora hayi*, a pathogenic fungus, as observed in INM3 long-term trials in western Kenya.



New partnerships were created through this project. One is partnership between the Alliance of Bioversity/CIAT and Field4Ever, a metagenomics group using BeCrop platform which allowed for a fresh set of soils to be collected and analyzed. A second partnership is with University of Guelph in Canada where specific genes, indicators of soil microbial functioning, were analyzed.

The metagenomic data provides insights on the vulnerability of the microbial ecosystem to develop common plant diseases based on pathogens detected. Risk of diseases within the two trial sites and across the management practices is mostly related to *Aspergillus* rot, charcoal rot and red root rot (Figure 20). *Fusarium* rot and *Nigrospora* rot are also potentials. These risks are observed across all treatments. For legumes, Fusarium wilt and Anthracnose are the expected risks observed from the soil metagenomic data (Figure 21).

Figure 20. Risk of developing maize plant diseases detected from soil micro-organisms.

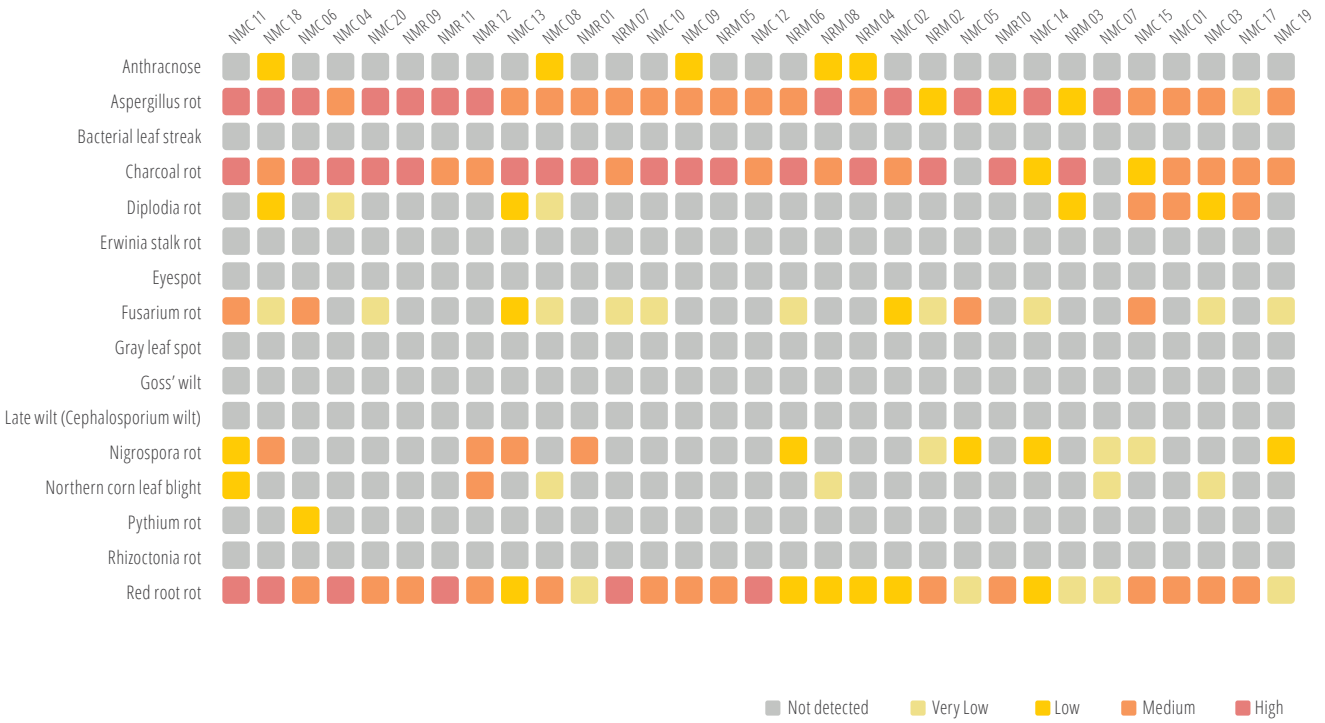
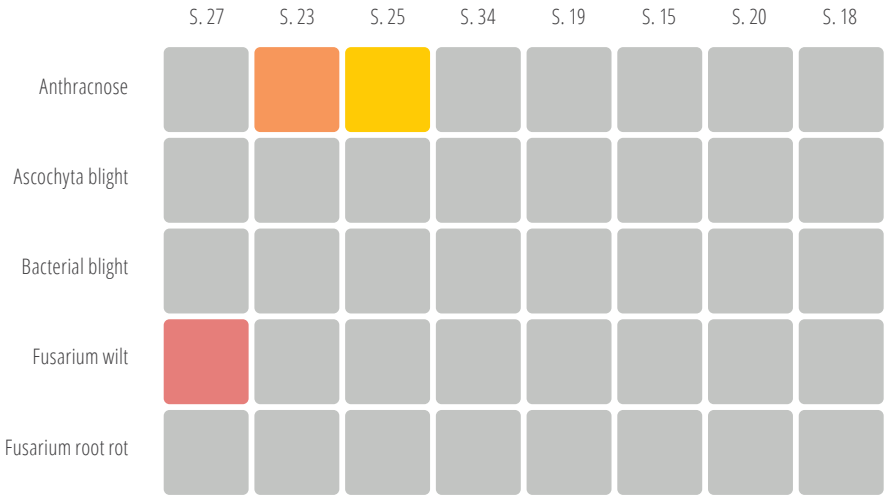


Figure 21. Risks of developing legume diseases across 8 farmer fields within Siaya County. We sampled farmer fields representing various crops and management and included those in metagenomics analysis.

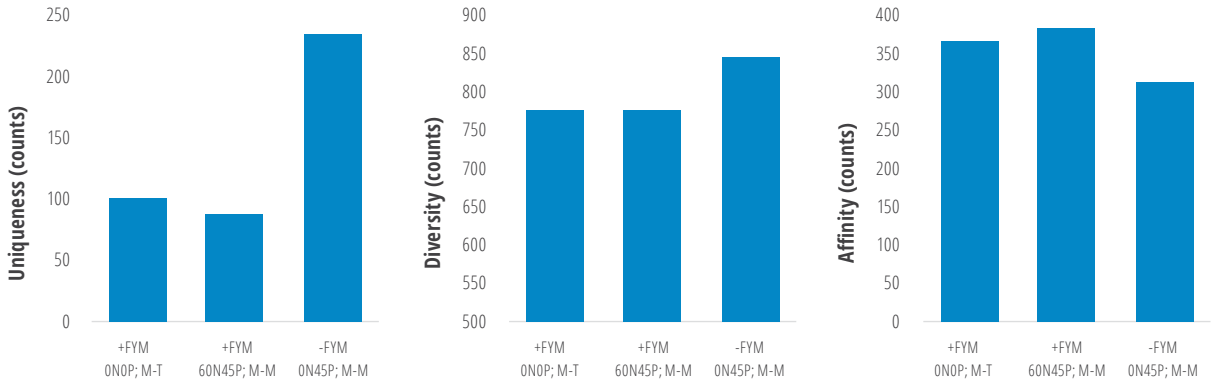


Farmers should conduct agronomic practices cognizant of real risk of Aspergillus rot, charcoal rot and red root rot on cereals and Fusarium wilt and Anthracnose on legumes.

Stability of soil microbes

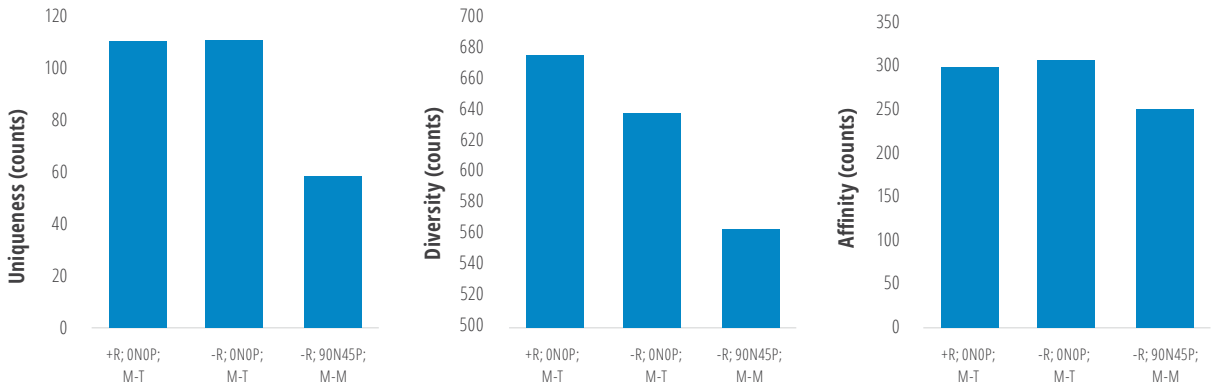
The assessment of stability of soil microbes is performed with limited comparisons that were possible through the BeCrop analysis platform implemented by Field4Ever. Here, only 3 treatments could be compared at a time. From the results, the application of FYM seems to stabilize soil biodiversity (Figure 22). The number of organisms found in some samples of the no FYM treatment and that were not present in any sample of the other treatments (uniqueness; with FYM) were 232, way higher than the <100 considering application of FYM. On the other hand, the common organisms (affinity) to all 3 samples analyzed in each treatment were fewer without than with FYM. The huge variations in microbial diversity from sample to sample within the treatment without FYM may indicate instability. These are only preliminary assessments of microbial stability and further work will be needed with the data already at hand.

Figure 22. Occurrence of soil organisms across three treatments (all with residues) within the INM3 long-term trial based on BiomeMakers analysis. M-T= Maize-tephrosia rotation, M-M=continuous maize cropping.



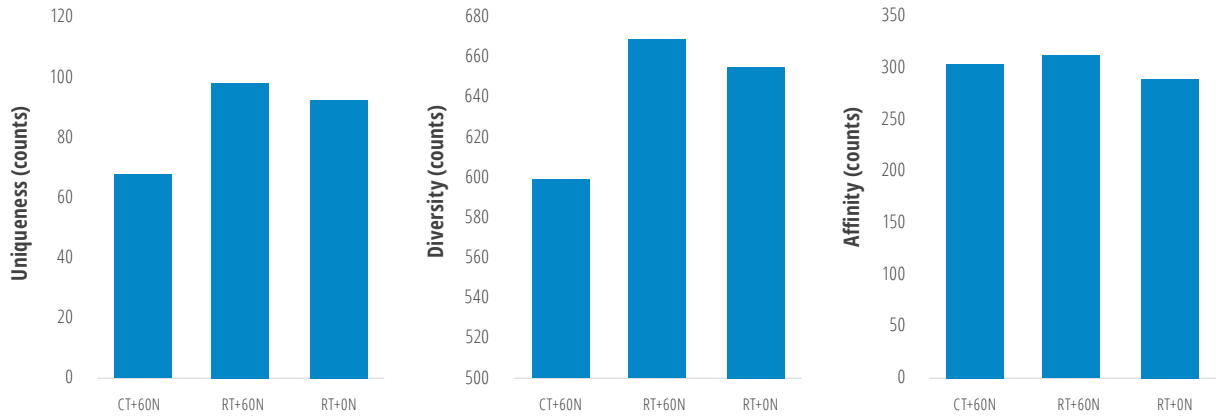
Implementation of agroforestry system of maize in rotation with tephrosia combined with some residue application consistently increased diversity and the number of unique organisms followed by maize-tephrosia rotation with no fertilizers while continuous maize monocropping with addition of inorganic fertilizers (a high amount of N) had the lowest diversity, uniqueness and affinity (Figure 23). The high uniqueness simultaneously with high presence of common organisms within samples of the agroforestry practice is a good indicator of stability.

Figure 23. Occurrence of soil organisms comparing agroforestry (maize-tephrosia rotation; M-T) without fertilizers and continuous maize (M-M) with fertilizers within the INM3 long-term trial based on BiomeMarkers analysis. R= 2 t/ha residue addition, N=nitrogen, P=phosphorus.



Conservation agriculture practices of reduced tillage with and without application of nitrogen fertilizer have elevated diversity of soil micro-organisms and have more unique species relative to conventional tillage system (Figure 24). Thus, although the number of common organisms within all the samples of a treatment are the same, overall diversity is better under reduced tillage.

Figure 24. Occurrence of soil organisms comparing conservation and conventional tillage with different fertilizer N inputs within the CT1 long-term trial based on BiomeMarkers analysis. All treatments are under maize-soybean rotation, had residues applied at 2 t/ha (surface for reduced tillage (RT) and incorporated under conventional tillage (CT)) and all had P application at 60 kg/ha. N=nitrogen.



Key messages:

- Application of FYM is important in stabilizing the microbial populations
- Conservation agriculture practices support increased microbial diversity and unique species relative to conventional tillage
- The practice of agroforestry (maize-tephrosia rotation) with or without additional residues and no fertilizers increases microbial diversity and their uniqueness relative to the growing of continuous maize monocropping with inorganic inputs.

Relationships between soils and soil microbes

Overall analysis of the 2019 microbial data, consistent with the 2020 dataset, shows that application of FYM either alone or in combination with fertilizers and crop residues plays an important role in increasing the abundance of a majority of soil bacteria that perform several roles in nutrient cycling. Compared to no input treatment and fertilizer only treatments, addition of manure alone or in combinations with residues or chemical fertilizers in INM3 significantly increased the abundance of several microbial groups/species (Table 4). However, there were cases, albeit fewer, where combined application of inorganic N and P fertilizers and residue only significantly increased the abundance of microbes e.g., *Nitrosovibrio spp.* and *Clostridium* spp. (also *Aspergillus* spp., *Burkholderia xenovorans*) even over a manure applied treatment.

Table 4. Effects of different ISFM practices on the abundance of different soil micro-organisms as observed in INM3 long-term trial during the 2019/2020 short rains cropping season.

Treatment	Nitrosovibrio spp	Nitrospira japonica	Azospirillum sp	Azospirillum spp	Burkholderia spp	Mesorhizobium spp	Clostridium spp	Bradyrhizobium lupini	Micromonospora sp	Thiobacillus spp	Actinomyces spp	Bradyrhizobium spp
No Input	29.5^{bc}	4.1^{ef}	2.7^{d}	3.6^{b}	14.3^{c}	4.1^{cde}	24.0^{a}	10.9^{d}	1.9^{cd}	7.4^{d}	4.54^{d}	5.9^{bcd}
P+N	38.9^{a}	3.1^{f}	1.8^{d}	4.5^{ab}	17.2^{abc}	4.1^{cde}	23.8^{a}	10.7^{d}	1.4^{d}	8.0^{cd}	2.8^{e}	5.4^{bcd}
+Residue Only	30.4b^{c}	4.9^{def}	3.4^{cd}	3.7^{b}	16.0^{bc}	4.0^{de}	22.5^{a}	11.4^{cd}	2.3^{bcd}	10.0^{bcd}	4.7^{d}	5.8^{bcd}
+FYM Only	20.9^{d}	9.4^{abc}	6.1^{b}	4.7^{ab}	20.9^{a}	6.0^{a}	12.7^{cde}	14.4^{ab}	3.5^{ab}	14.8^{a}	5.0^{bcd}	7.8^{a}
P+N+Residue	34.7^{ab}	3.2^{f}	2.5^{d}	4.3^{ab}	15.7^{bc}	3.5^{ef}	23.2^{a}	13.1^{abcd}	1.9^{cd}	9.5^{bcd}	3.1^{e}	4.4^{d}
P+N+FYM +Residue	24.2^{cd}	8.8^{bcd}	6.1^{b}	5.5^{ab}	19.0^{ab}	5.5^{abc}	15.8^{bcd}	14.6^{a}	2.2^{bcd}	14.0^{a}	4.9^{cd}	6.7^{abc}
P+N+FYM	25.5^{cd}	7.2^{bcde}	5.6^{bc}	5.9^{a}	18.2^{abc}	4.7^{abcde}	18.8^{abc}	13.0^{abcd}	2.3^{bcd}	14.1^{a}	6.6^{a}	6.7^{abc}
R+FYM	22.2^{d}	11^{ab}	8.6^{a}	5.5^{ab}	20.2^{a}	5.7^{ab}	11.7^{de}	12.6^{abcd}	3.1^{abc}	14.5^{a}	5.7^{abcd}	6.8^{ab}
+P only	29.1^{bc}	6.4^{cdef}	5.1^{bc}	4.8^{ab}	17.0^{abc}	3.2^{ef}	18.8^{abc}	11.8^{bcd}	1.9^{cd}	11.4^{abc}	6.3^{ab}	5.4^{bcd}
P+FYM	22.3^{d}	9.8^{abc}	6.9^{ab}	4.7^{ab}	18.2^{ab}	5.1^{abcd}	15.0^{bcd}	12.6^{abcd}	2.5^{abcd}	12.8^{ab}	5.7^{abcd}	6.7^{abc}
P+90N	30.5^{bc}	4.3^{ef}	2.6^{d}	4.2^{ab}	18.4^{ab}	2.2^{f}	20.1^{ab}	12.0^{abcd}	1.3^{d}	9.4^{bcd}	4.7^{d}	5.2^{cd}
Uns	21.1^{d}	12.7^{a}	5.4^{bc}	4.8^{ab}	20.3^{a}	4.4^{bcde}	8.5^{e}	13.8^{abc}	3.9^{a}	9.6^{bcd}	6.3^{abc}	6.4^{abc}

In each site, means followed by similar letters in each column are not significantly different. P applied at 45 kg/ha (unless specified); N applied at 60 kg/ha (unless specified), residue applied at 2 t/ha; Manure applied at 4 t/ha; Uns=uncultivated site at least for the last 6 years. Grey rows represent treatments with FYM.

Canonical correspondence analysis with soil bacteria and fungi at the INM3 site show that the microbial communities with FYM differ from those under fertilizer application (Figure 25 and 26). The influencing soil parameters are pH ($P<0.001$), and somewhat P and S ($P<0.1$) for bacteria and pH and Nitrogen ($P<0.01$), P, S, and K ($P<0.05$) for fungi.

Figure 25. Canonical correspondence analysis plot for all bacteria (691 genera) within the long-term integrated soil fertility experiment (INM3) based on 2019 Illumina dataset.

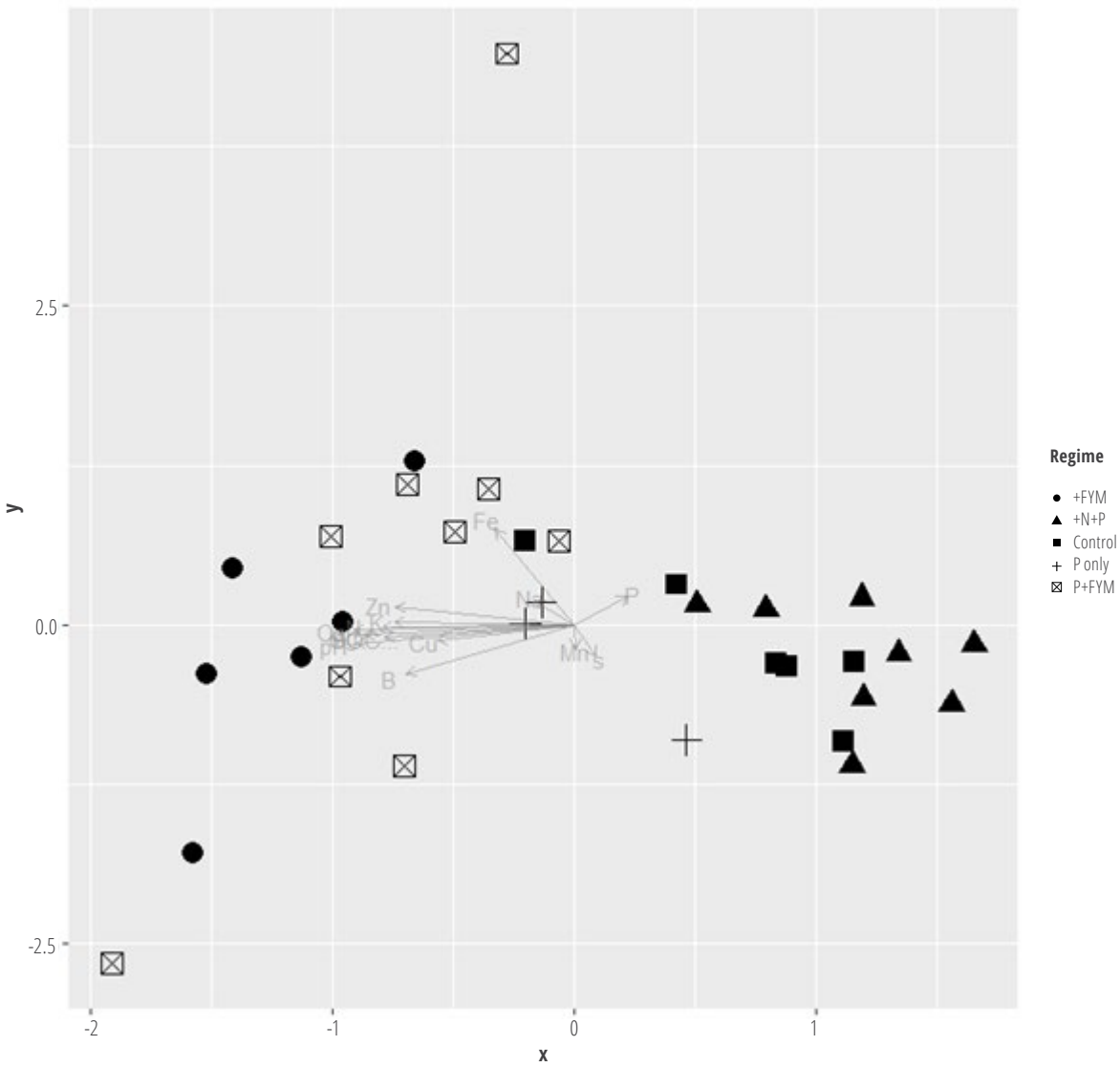
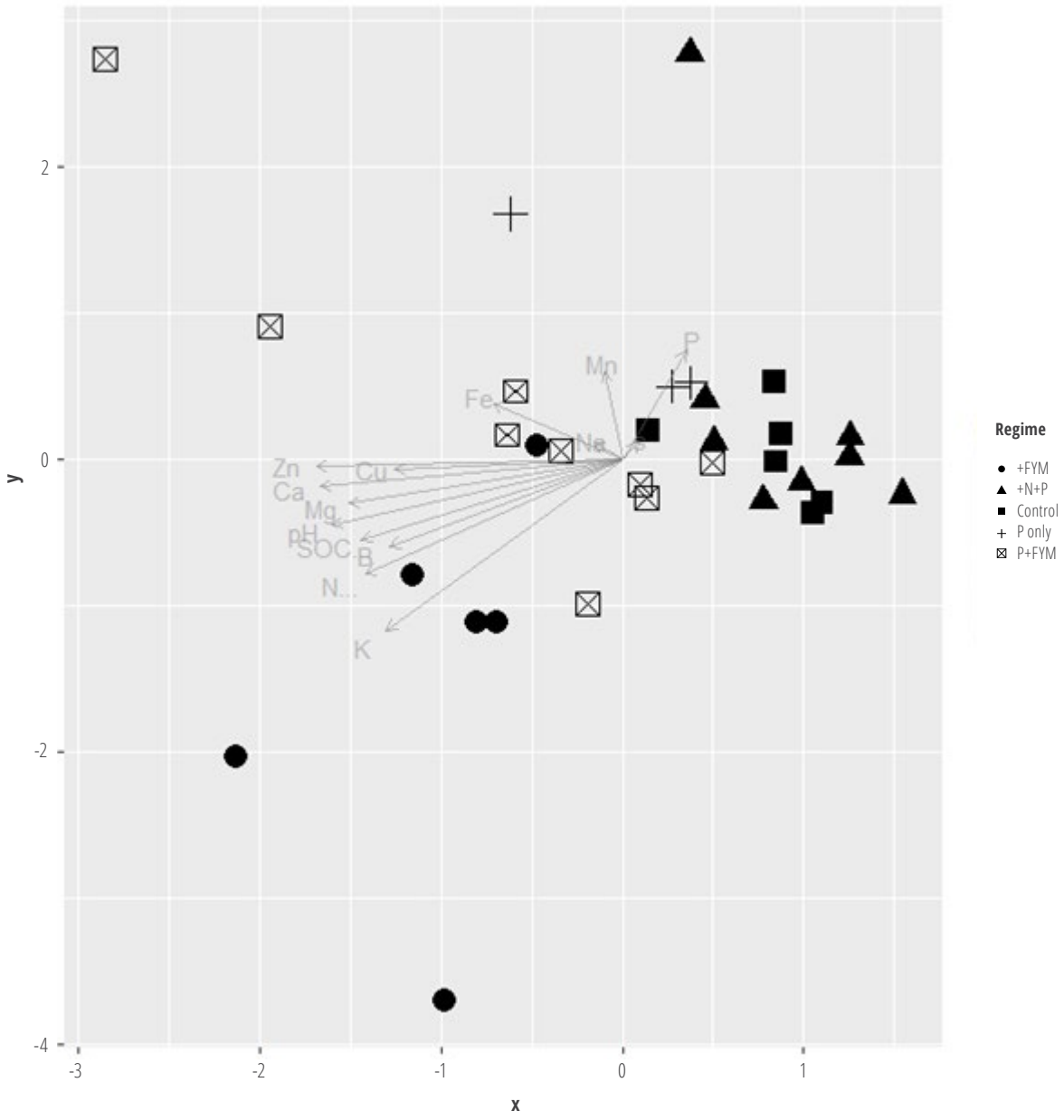
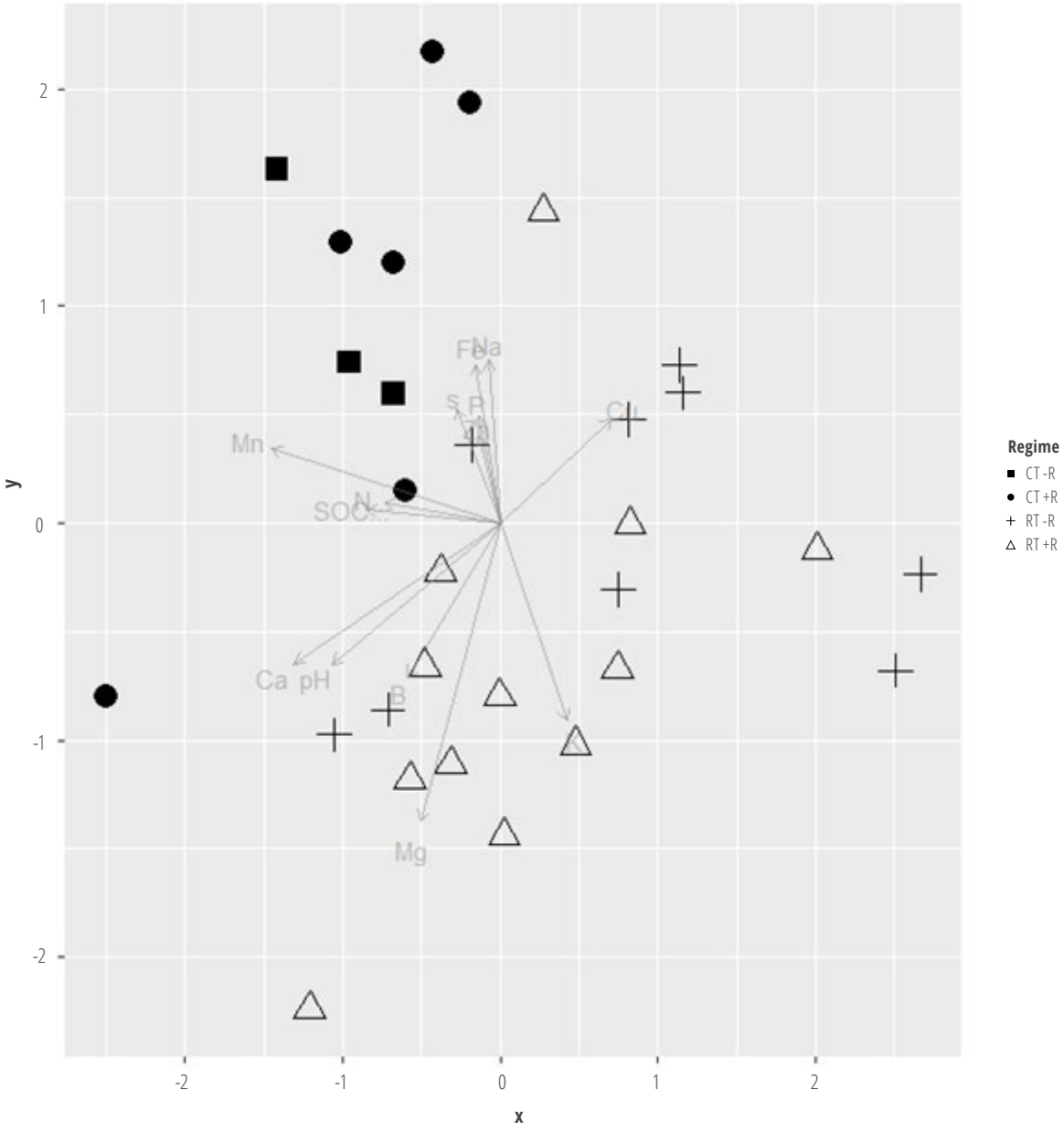


Figure 26. Canonical correspondence analysis plot for all fungi (721 genera) within the long-term integrated soil fertility experiment (INM3) based on 2019 Illumina dataset.



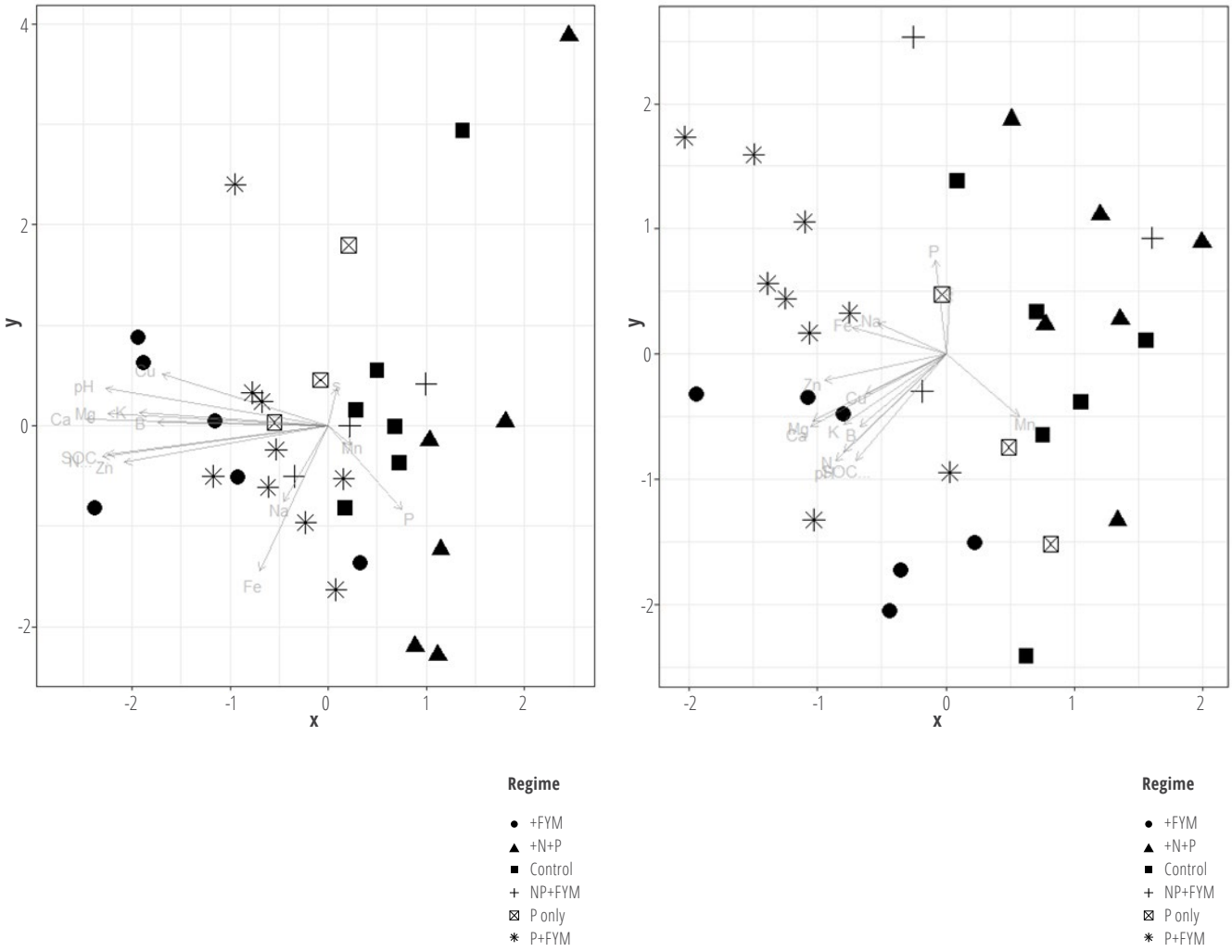
Practicing conservation agriculture also results in a community of soil microbes distinguished from conventional tillage practices (Figure 27). The significant soil factors positively influencing the bacterial communities under long-term reduced tillage are pH, Ca, Mg, K all of which are related to a liming effect of the conservation tillage practice. These factors (except Ca) also significantly and positively influence the fungal community under conservation tillage. On the other hand, the microbial community under conventional tillage (both for fungi and bacteria) are positively related to Mn. Under both reduced and conventional systems, nitrogen (related to SOC) is influencing bacterial community ($P=0.051$) and Boron influence fungi ($P<0.05$).

Figure 27. Canonical correspondence analysis plot for all bacteria (673 genera) within the long-term integrated conservation agriculture (CT1) trial based on 2019 Illumina dataset.



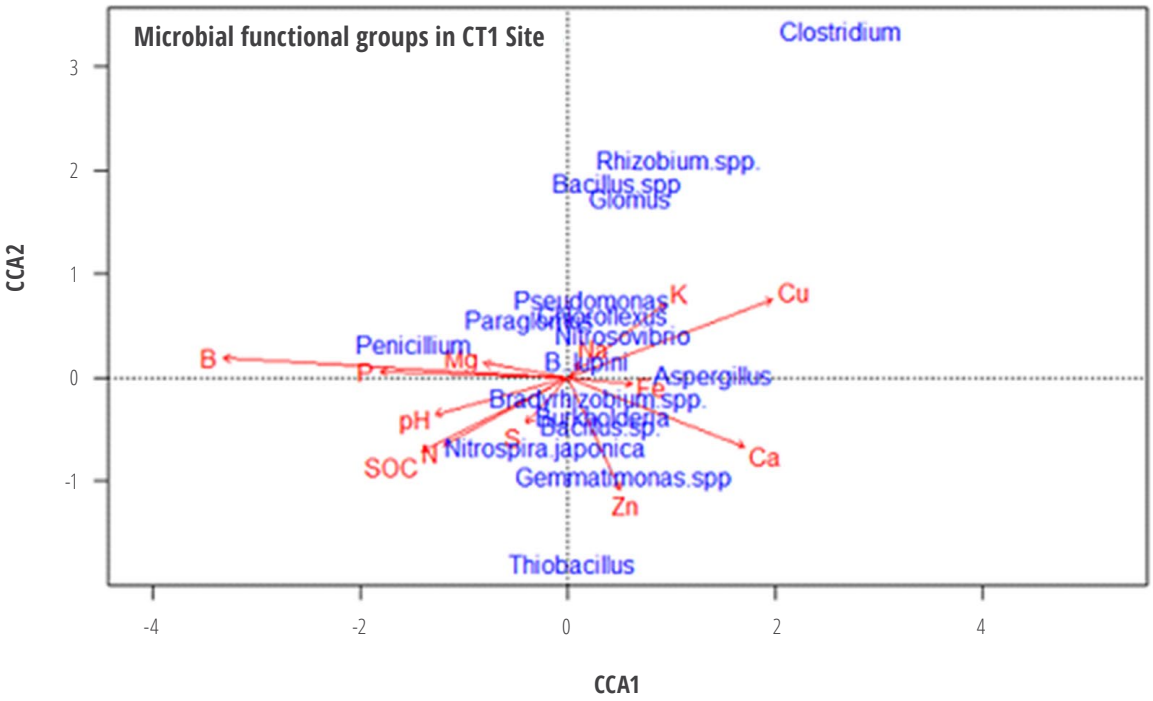
Canonical correspondence analysis shows a distribution of soil microbes (plant growth promoting bacteria) with a continuum ranging from only organics (FYM) to only chemical fertilizers (+N+P plots; Figure 28), consistent with trend for all bacteria and fungi. The significant ($P<0.05$) soil chemical properties influencing the microbes are pH, K, Ca, S, Fe and N. The application of FYM increases concentration of these soil parameters. At the genus level, Zinc solubilizing bacteria followed similar distribution as the plant growth promoting bacteria and were significantly influenced by P and B in addition to the pH, Ca, S and Fe.

Figure 28. Canonical correspondence analysis plot for plant growth promoting bacteria (23 species; left) and zinc solubilizing bacteria (18 genus; right) within the long-term integrated soil fertility experiment (INM3) based on 2019 Illumina dataset.

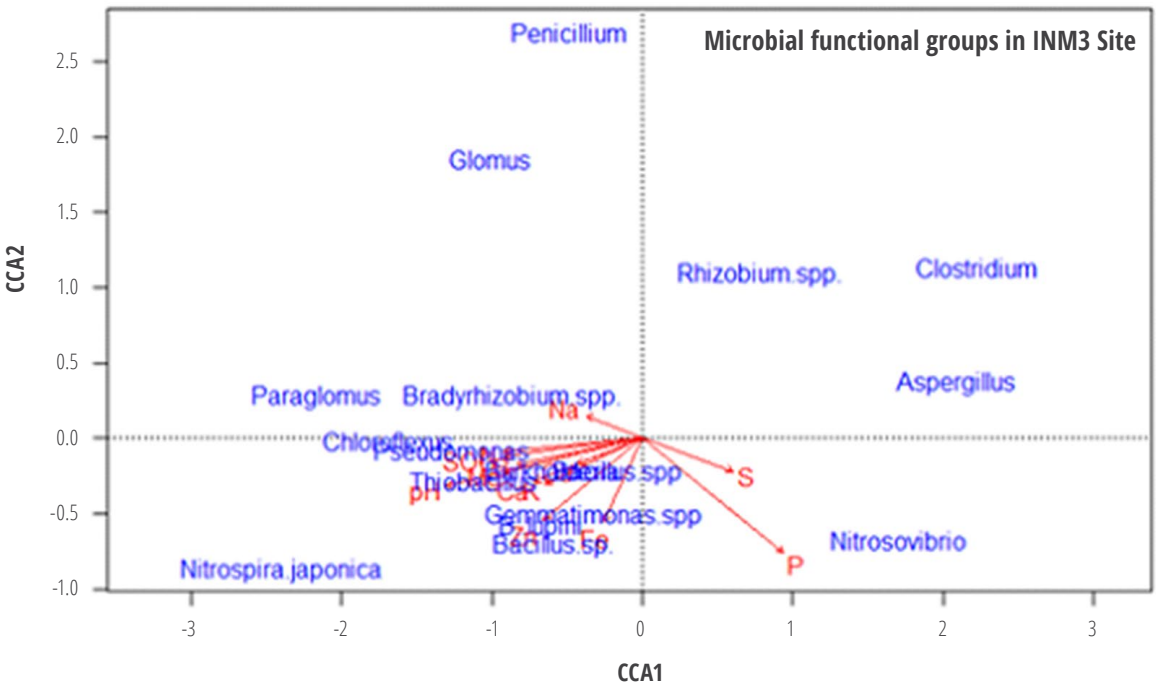


In the subsequent analysis, the bacteria and fungi are combined in analysis. In CT1, nitrogen cycling *Nitrospira japonica*, *Bacillus* spp. and *Bradyrhizobium* spp.; N and P cycling *Gemmatimonas* spp. and sulfur oxidizing *Thiobacillus* spp. are more positively correlated with sulfur, zinc and iron availability than the rest of the species (Figure 29a and b). Nitrogen cycling *Nitrospira* species was also correlated with available nitrogen and soil organic carbon. Phosphorus cycling arbuscular mycorrhizal fungi (*Glomus* spp.) was negatively correlated with soil P and pH. *Clostridium* spp., *Rhizobium* spp., *Bacillus* spp. and *Glomus* spp. were more correlated with potassium and copper availability in CT1.

Figure 29. Canonical correspondence analysis plot for functional microbial groups in CT1 (a) and INM3 (b) sites.



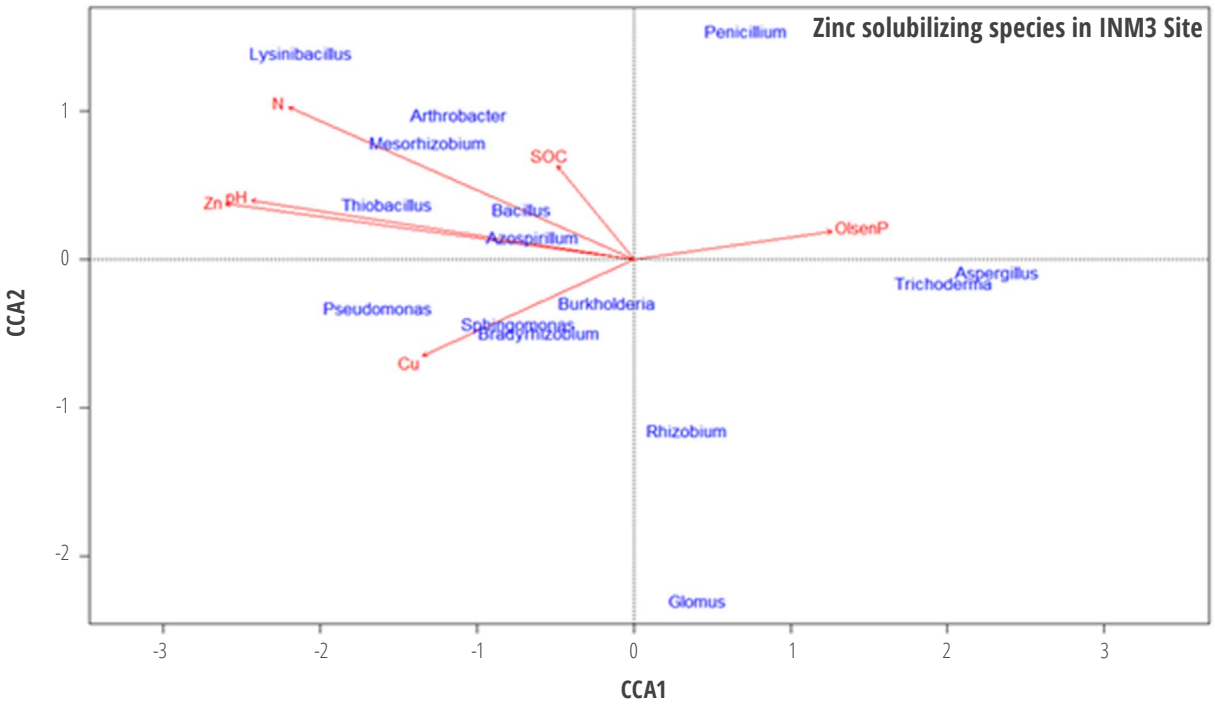
(a)



(b)

Specific microbial species (combining bacteria and fungi) involved in solubilization of zinc are significantly affected by micronutrient (Zn) availability (Figure 30), and these zinc solubilizers are grouped in two major categories, with systems applied with FYM grouped separately from those without FYM application (data not shown). Application of FYM increased the population of key zinc solubilizing microbes including *Burkholderia* spp., *Bacillus* spp., *Bradyrhizobium* spp., *Azospirilium* spp., *Pseudomonas* spp., *Gluconacetobacter* spp., and *Mesorhizobium* spp. Manure application is associated with increased soil fertility necessary for the plant and microbial growth (Malosso et al., 2005). Zinc solubilizing genera belonging to *Pseudomonas* spp., and *Bacillus* spp., *Rhizobium* spp. (Kamran, et al., 2017) are copiotrophs (Fierer et al., 2007), that is, thriving in nutrient-rich environments.

Figure 30. Relationship between soil chemical characteristics and zinc solubilizing microbial species abundance (both fungi and bacteria combined) in INM3 site (This is based on 15 genera with relative abundance >0.5%).



Influences of soil management on potential soil extracellular enzyme activities involved in C, N and P cycling

Extracellular enzymes are secretions by a cell, in this case microbial cells, which function outside of that cell to break down complex macromolecules into smaller units to be taken up by the cell for growth and assimilation. These enzymes are therefore involved in cycling of nutrients. Here, four main enzymes cycling C, N and P in the soil were investigated. These are:

1. Beta-glucosidases (GLU, also known as Cellobiases) are important enzymes that catalyze the hydrolysis of cellobiose to glucose in the soil, thus important for C cycling.
2. Beta-glucosaminidase (NAG, also known as N-Acetyl-β-d-glucosaminidase) is an important enzyme that catalyzes the hydrolysis of chitin, which is converted to amino sugars that are major sources of mineralizable N in soils; thus very important in nitrogen and carbon cycling in soils.
3. Alkaline phosphatase (ALP) and acid phosphatase (ACP) are important enzymes exuded by fungi and bacteria and catalyze the hydrolysis of organic phosphorus compounds to inorganic polyphosphates that occur in soils, important for soils deficient in phosphorus. ACP enzymes are predominant in acid soils while ALP enzymes, specifically originate from soil microbes, predominates in neutral or alkaline soils (Spohn & Kuzyakov, 2013).

Colorimetric methods were used to assess the enzymes following the protocol developed by Tabatabai (1994). Briefly, for ALP and ACP, 1 gram fresh soil was weighed in three replicates (one for moisture, control and enzyme activity assessment), and 4 ml of modified universal buffer (MUB; pH 6.5 for acid phosphatase (ACP); pH 11.0 for alkaline phosphatase (ALP)); 0.25 ml of toluene and 1 ml of 115 mM para-Nitrophenyl Phosphate (p-NPP) solution added (Plate 1). The flasks were stoppered, briefly mixed, incubated (370C; 1 hour) followed by addition of 1 ml of 0.5 M CaCl, 4 ml of 0.5M NaOH; centrifugation (5000 rpm, 10 minutes) and reading the supernatant concentrations at 400 nanometres (nm) using a spectrophotometer. The procedure for assessment of Beta-glucosidase enzyme activity (GLU) was similar to that of ACP except for the difference in substrates (50 mM para-Nitrophenyl –β-D-glucopyranoside (p-NPG)) and use of Tris (hydroxyethyl) amino-methane (THAM) as opposed to NaOH (for ACP) to terminate the reaction. The procedure for assaying N-acetyl-β-glucosaminidase (NAG) commonly known as Beta-glucosaminidase was similar to that of GLU, except for the difference in substrates (10mM para-Nitrophenyl-N-acetyl-β-D-glucosaminide (pNP-NAG)) and buffers (100 mM Acetate buffer; pH 5.5) used. A unit of potential enzyme activity was expressed as the amount of either p-NPP, p-NPG or pNP-NAG released in one hour per gram dry soil.

Plate 1. Colorimetric assay of potential soil extracellular enzyme activities in the laboratory (Photo: Obadiah Mwangi/Alliance).

The results from the extracellular enzyme assays showed that;

i. Application of FYM has more potential for enzymatic nutrient cycling especially when applied in combination with residues; for example, ACP enzyme activity is significantly higher in system with combined FYM+residue addition compared with either sole application of FYM and/or combined application of residue and fertilizers (Table 5).

ii. Application of "fertilizer only" without organic resources reduces ALP activity compared to combined application of fertilizers and organics.

iii. Reduced tillage increases ACP enzyme activity compared to conventional tillage systems. The higher ACP activity under reduced than conventional tillage systems was similarly observed in a recent study

(Margenot et al., 2017; Kihara et al., 2018) in the site; and this is attributed to increased organic matter content, substrate availability and microbial biomass pool in the reduced than conventional tillage systems.

In addition, assessment of microbial resource allocation to nutrient cycling (data not shown) showed that;

i) Overall microbial resource allocation to P acquisition, as a ratio of GLU/ACP, ranged from 0.15 to 0.30; being highest in the system with combined application of P+FYM, implying increased P cycling in the system.

ii) Microbial resource allocation to N acquisition, as a ratio of GLU/NAG, ranged between 2.36 to 4.8, being highest in the system with FYM application, indicating the benefit of FYM in improving N cycling.

The increase in extracellular enzyme activities following addition of FYM, important for improving soil health, is related to soil organic matter, nutrients availability, improved soil chemical and physical characteristics. Previous studies have reported increases in the activities of Alkaline phosphatase (Chen et al., 2019), Acid phosphatase (Liang et al., 2014), B-glucosidase (Liang et al., 2014) and B-Glucosaminidase (Acosta-Martinez et al., 2011; Brennan and Acosta-Martinez, 2019) following application of FYM.

The increase in the enzyme activities under reduced tillage relative to conventional tillage can also be attributed to high organic matter content at the top soil that stimulate the proliferation of the microbes associated with the production of nutrient-cycling enzymes. Within the same site, Margenot et al. (2017) observed 40% increase in Acid phosphatase enzyme activities in the reduced tillage relative to conventional tillage systems. Similarly, reduced tillage has been reported to increase B-glucosidase (Chen et al., 2019) and B-glucosaminidase enzyme activities (Zhang et al., 2014).

Table 5. Soil extracellular Alkaline phosphatase (ALP), Acid phosphatase (ACP), Beta-glucosidase (GLU) and Beta-glucosaminidase (NAG) enzyme activities (µmol P-nitrophenol g-dry soil^{-1} hr^{-1}) in INM3 and CT1 experimental sites.

	ALP	ACP	GLU	NAG
INM3 Site				
No Input	38.3^{ab}	126.2^{cd}	26.6^{b}	7.8^{a}
P+N	22.1^{b}	120.9^{cd}	20.3^{b}	8.1^{a}
Residue Only	31.2^{b}	182.8^{ab}	26.8^{b}	9.5^{a}
FYM Only	27.1^{b}	154.8^{bcd}	37.6^{a}	9.1^{a}
P+N + Residue	29.2^{b}	119.7^{d}	22.1^{b}	11.4^{a}
P+N + FYM + Residue	32.4^{b}	158.3^{abc}	26.4^{b}	8.4^{a}
P+N + FYM	44.3^{ab}	156.1^{abcd}	25.3^{b}	7.2^{a}
Residue and FYM	36.0^{b}	193.8^{a}	37.6^{a}	11.9^{a}
P only	39.6^{ab}	136.5^{cd}	22.0^{b}	9.3^{a}
P + FYM	61.2^{a}	153.9^{bcd}	46.3^{a}	12.5^{a}
P+90N	28.7^{b}	135.7^{cd}	24.9^{b}	7.8^{a}
Natural Site				
Natural Site	119.1	244.4	82.9	33.9
CT1 Site				
RT;0N+60P+R; M/S Intercrop	45.3^{a}	152.1^{ab}	19.4^{ab}	9.0^{abc}
RT;0N+60P+R; M-S Rotation	50.8^{a}	160.6^{a}	20.6^{ab}	10.8^{ab}
CT;0N+60P+R; M/S Intercrop	48.7^{a}	158.4^{a}	23.8^{ab}	9.6^{abc}
CT;0N+60P-R; M/S Intercrop	35.7^{a}	174.2^{a}	19.5^{ab}	9.4^{abc}
RT;0N+0P+R; M-S	39.9^{a}	168.6^{a}	16.0^{b}	7.6^{bc}
RT;0N+0P-R; M-S Rotation	45.8^{a}	144.8^{ab}	20.5^{ab}	11.3^{a}
RT;0N+60P-R; M/S Intercrop	50.6^{a}	162.8^{a}	20.5^{ab}	10.4^{ab}
CT;60N+60P+R; M-S Rotation	39.7^{a}	124.2^{b}	28.7^{a}	6.8^{c}
RT;60N+60P+R; M-S Rotation	49.0^{a}	162.7^{a}	21.0^{ab}	7.6^{bc}
RT;60N+60P-R; M-S Rotation	51.4^{a}	155.1^{ab}	20.1^{ab}	8.3^{abc}

Values are means of the different enzyme activities (µmol P-nitrophenol g-dry soil^{-1} hr^{-1}) in the two sites. Means followed by similar letters in each column in each site are not significantly different. P applied at 45 kg/ha (unless specified); N applied at 60 kg/ha (unless specified), residue applied at 2 t/ha; Manure applied at 4 t/ha.

Relating the enzyme activities with soil parameters and diversity of bacteria also revealed interesting results. GLU and ACP were positively influenced by soil pH, N, SOC, K, Ca, Mg, B, Zn and CEC (Table 6). They also related positively with bacteria richness and diversity. In addition, ALP related positively with pH, Ca, Mg, Cu and CEC. Increasing amount for soil available P decreased ACP and to some extent GLU, as also observed elsewhere (Olander and Vitousek 2000). It has also been suggested that mineral phosphates act as competitive inhibitors depressing phosphatase activities (Lemanowicz et al., 2016). Soil pH influences nutrient availability and thereby microbial biomass, richness and diversity hence the concentration of inhibitors or activators in the soil solution (Dick et al., 2000). The observed simultaneous increase in GLU and ACP point to increased microbial pool involved in production of the two enzymes.

Table 6. Correlation coefficients between soil chemical and biological variables (microbial richness and diversity; and extracellular enzyme activities (Beta-glucosidase (GLU), Beta-glucosaminidase (NAG), Acid Phosphatase (ACP) and Alkaline Phosphatase (ALP)) potential enzyme activities) in INM3 site.

	Beta-glucosidase (GLU)#	Beta-glucosaminidase (NAG)#	Acid Phosphatase (ACP)#	Alkaline Phosphatase (ALP)#
pH	.681**	.238	.547**	.350*
N (%)	.757**	.153	.539**	.171
SOC (%)	.727**	.110	.514**	.223
P	-.312	-.070	-.454**	.065
K	.574**	.218	.635**	.116
Ca	.654**	.159	.418*	.427*
Mg	.707**	.213	.409*	.416*
Mn	-.047	-.085	-.255	.069
s	.040	.140	-.009	.226
Cu	.246	.121	.239	.438*
B	.594**	.215	.404*	.140
Zn	.478**	.012	.532**	.133
Fe	.035	-.245	-.022	.146
Na	-.031	-.116	-.027	.026
EC	.164	.068	-.019	.187
C.E.C	.579**	.065	.355*	.400*
MBC (Max)	.505**	.105	.314	.102
SD_16S_genus	.639**	.076	.485**	.307
Richness_16S_genus	.631**	.137	.545**	.331
SD_ZnSolubilizer_16SGenus	.550**	.043	.393*	.267
SD_PGPR_16S_Species	.347*	.288	.520**	.081

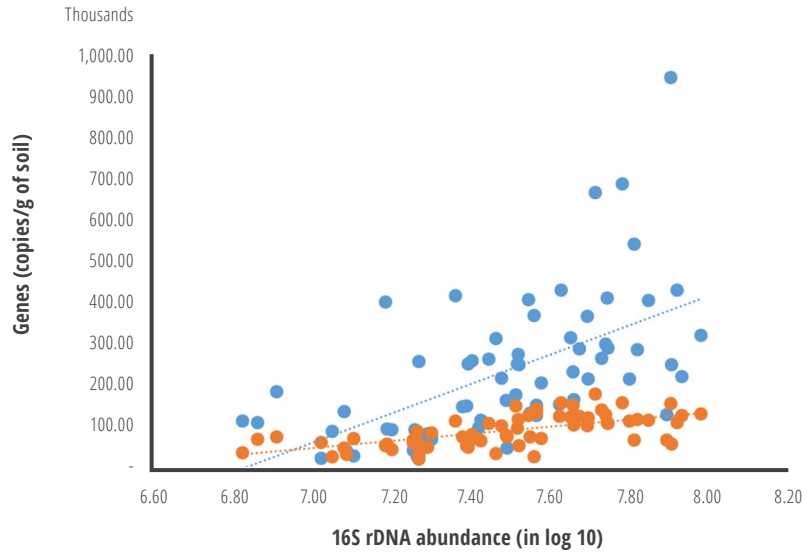
represents μmol P-nitrophenol g-dry soil^{-1} hr^{-1}. MBC (Max) = microbial biomass carbon (potential maximum), SD=Shannon diversity, PGPR= Plant growth promoting rhizobacteria.

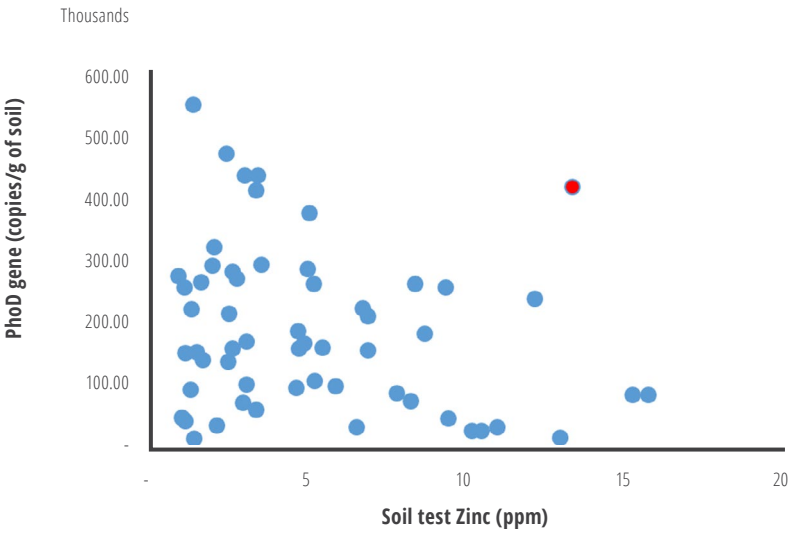
Influences of soil management on specific genes

Within microbes, there are specific genes that encode the specific enzymes involved in nutrient solubilization. These genes were studied to provide insights into microbial activities. Two genes, PhoD and PhoC, are important in P cycling as they encode phosphatase enzymes (acid and alkaline phosphatase) that take part in solubilization of recalcitrant inorganic P compounds. PhoD gene is responsible for encoding alkaline phosphatase while PhoC gene is responsible for encoding acid phosphatase enzymes. The activities of these two enzymes were investigated.

Data obtained through partnership with Guelph University show that bacterial 16S rDNA abundance strongly positively correlated with *Pho*D and *Pho*C gene abundances (P<0.0001; Figure 31a). It is interesting that zinc is also influencing the availability of *Pho*D gene with increasing zinc levels reducing the attainable counts of gene copies (Figure 31b).

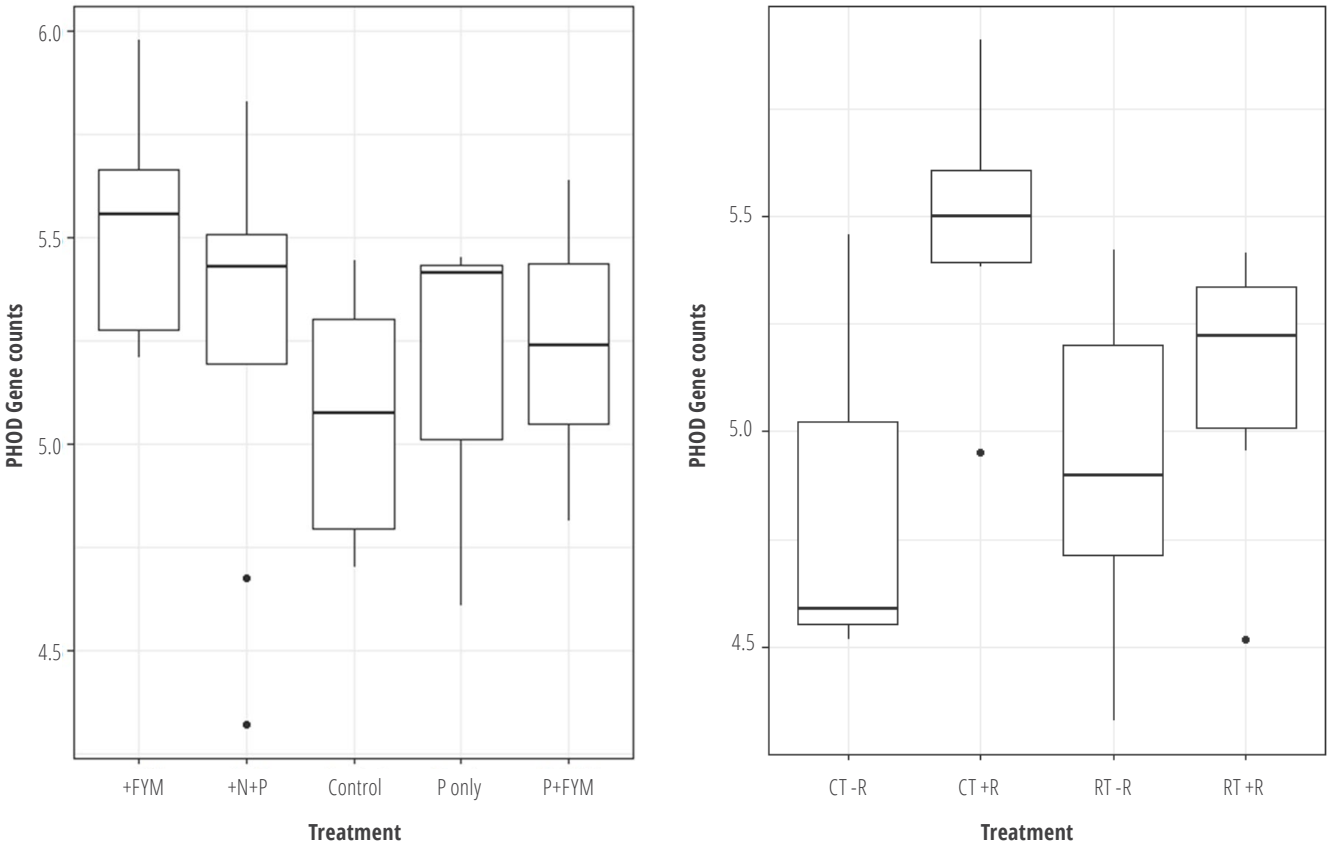
Figure 31. PhoD (blue) and PhoC (orange) functional gene abundance versus bacterial 16S rDNA abundance (a) and relationship between soil test Zinc and PhoD gene abundance (b) within long-term trials (combined INM3 and CT1) in western Kenya.





*Pho*D and *Pho*C abundance were both highest in long-term sole manure application and lowest in no input (control) treatment for *Pho*D and in sole residue application for *Pho*C (see Figure 32 for *Pho*D). Within the conservation tillage treatments (CT1 trial), residues are key in driving the abundances of *Pho*D genes. Significantly higher *Pho*D was also observed with residue relative to no residue application in the reduced tillage maize soybean rotation with fertilizer application treatment (data not shown). Tillage, fertilizer management and cropping systems did not produce any particular pattern of influence on the tested genes.

Figure 32. Distributions of *Pho*D gene across different management practices in Integrated soil fertility management (left) and conservation agriculture (right) long-term trials in western Kenya.



Additional relationships between the enzyme activities and the different genes encoding for phosphorus cycling enzymes (i.e., *Pho*D for ALP and *Pho*C for ACP) revealed that GLU activities was positively correlated with ACP and ALP. In addition, *Pho*C positively correlated with *Pho*D gene abundances (Table 7).

Table 7. Correlation coefficients between Beta-glucosaminidase (NAG), Acid Phosphatase (ACP) and Alkaline Phosphatase (ALP) potential enzyme activities with *Pho*C and *Pho*D gene abundances in INM3 site.

	Beta-glucosaminidase (NAG)#	Acid Phosphatase (ACP)#	Alkaline Phosphatase (ALP)#	PhoD gene abundance (counts g-1 soil)	PhoC gene abundance (counts g-1 soil)
GLU	.314	**.453****	**.455****	.067	-.059
NAG		.199	.128	-.283	-.266
ACP			.155	-.004	-.297
ALP				.245	.017
PhoD					**.393***

represents μmol P-nitrophenol g-dry soil^{-1} hr^{-1}

While manure elevates phosphatase production demonstrated through high *Pho*D and *Pho*C (Luo et al., 2019), these are suppressed by application of fertilizer P. Zhang et al. (2012) observed that phosphatase production is induced at low phosphate levels. Soils with low available P favor the activity of phosphatases and *Pho*C and *Pho*D genes (Nannipieri et al., 2011). When microbes are faced with P scarcity, they upregulate the expression of the specific functional genes (i.e., *Pho*C and *Pho*D) that encode phosphatases (Vershinina and Znamenskaya, 2002). The reduction of *Pho*C and *Pho*D gene abundance with elevated nutrient supplies (e.g. manure application combined with inorganic fertilizers) in our study reflects higher concentrations of plant-available P; and this is consistent with the negative feedback mechanism proposed by Olander and Vitousek (2000).

It is startling that N application did not seem to influence the tested genes. Low *Pho*D and *Pho*D gene copies following inorganic N fertilizer application is observed in different parts of the world and attributed to lowered soil pH i.e., increased soil acidity and overall decreased soil microbial abundance and diversity (Chen et al., 2019; Jorquera et al., 2014). Other studies (Mandal et al., 2007; Marklein and Houlton, 2012; Tan et al., 2013), however, reported increased phosphatase following N application and attributed it to increased demand for P by the increased crop biomass. The inconsistent results could be due to the quantities of N applied, or other environmental factors and management.

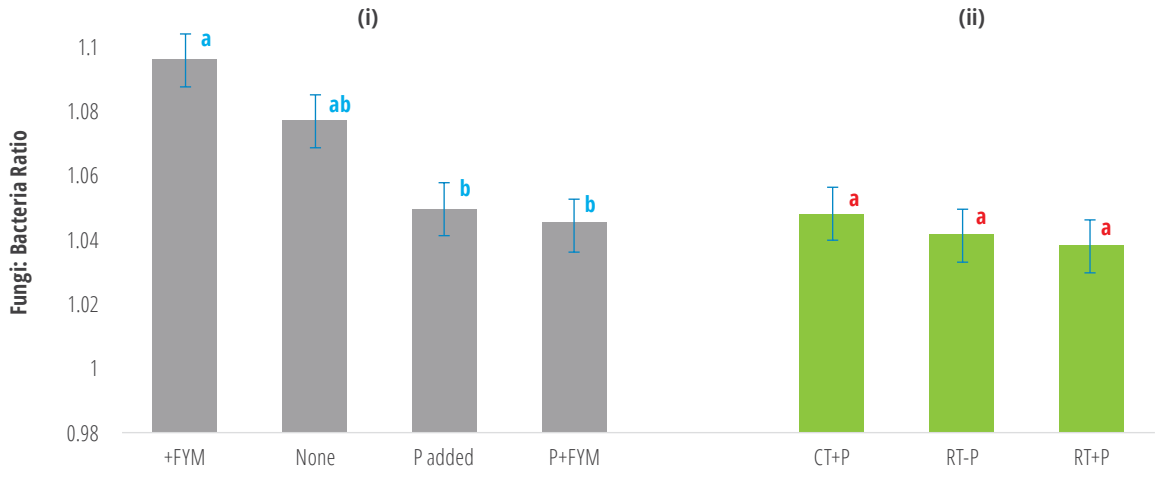
Management influences on soil fungal to bacterial (F:B) ratio

F:B ratio is a good indicator of environmental changes in soil. Application of fertilizer only may promote the proliferation of nutrient dependent microbial species, resulting to increased individual species counts, but poor microbial structure. The F:B ratio (based on sequences analyzed at MR DNA) was higher in the management systems with FYM compared to treatments with either P applied or combined application of P and FYM (Figure 33), implying that sole application of FYM has positive influence on soil microbial structure. Higher F:B ratio implies fungal dominance while lower ratio is bacteria dominance often in the nutrient rich environments (Strickland and Rousk, 2010). When manure and/or plant residues are applied as mulch, fungi often prosper because their hyphae are able to grow into the litter.

Under the long-term conservation tillage (CT1) trial, there was not much variation in the F:B ratio due to treatments applied, only a slight increase due to residue application in both conventional and reduced tillage.

In INM3, F:B ratio were negatively correlated with soil available P (significantly at $P<0.05$; R^2=-0.37*) but not to any other variable even in CT1.

Figure 33. Fungal: Bacteria ratio (based on species counts) under different external inputs in INM3 (i) and tillage systems in CT1 (ii) sites.



Effects of management systems on mineralization of nitrogen and phosphorus

Microbial nutrient mineralization was assessed using in-situ resin core incubation method previously employed by Kihara et al. (2018). Briefly, ion exchange resins were buried, protected within PVC pipes (see Plate 4), in selected agronomic management systems 2 weeks after planting and retrieved on monthly basis (i.e., after 1 and after 2 months), and the concentrations of nitrates, ammonium and phosphates calorimetrically determined. This was followed by determination of the market value of the equivalent quantities of N and P nutrients mineralized, based on the current market prices of the nutrients (i.e., assuming N source as urea and P as Triple superphosphate).

Eight treatments (i.e., 4 management systems in each trial of INM3 and CT1) were selected for the study. The following considerations were made:

- A no-input treatment was required against which the effects of sole P fertilizer input on nutrient mineralization would be determined.
- All the other treatments had P for INM3 and P+residues for CT1. Being common across treatments, these applications were assumed to affect mineralization the same way.
- There was no topdressing in any of these systems to avoid direct interception of fertilizer-based nitrogen by the resins.

We also hypothesized that:

- Nutrient mineralization (and the subsequent monetary benefits) would be higher after 60 days compared to 30 days since by then, more organic matter (i.e., provided by manure, residues, etc.) would have been decomposed and mineralized by the microbes
- More nutrients would be mineralized in the reduced tillage than conventional tillage systems due to more microbial biomass in the reduced than conventional tillage systems.
- Addition of nitrogen at the moderate rate of 60 kg N ha^{-1} as done in the long-term trials would reduce nutrient mineralization as microbes don't have to work for it.
- More nutrient demands under intercropping would result in more nutrients mineralized than under rotation systems.
- Combined application of inorganic fertilizer and organic (FYM) result in nutrient-rich systems causing low nutrient mineralization by microbes.

In general, and although not always, higher N mineralization and monetary value of nutrients are observed after 60 days compared to 30 days of incubation in both sites. Both highest N and P mineralization were observed after 60 days of resin incubation in both the conservation tillage systems in CT1 and the integrated soil fertility management systems in INM3.

Plate 4. PVC installations for protection of ion-exchange resin incubated in the field for mineralization assessment (Photo: Peter Bolo/Alliance).

The following observations are made on N and P mineralization for the periods of 30 and 60 days of resin incubation:

i. Reduced tillage resulted in more N mineralization than conventional tillage systems, as expected, being 6 and 18 kg N ha^{-1} for 30 and 60 days of resin incubation, respectively.

ii. Application of crop residues within reduced tillage increased N mineralization by 7 and 18 kgs for 30 and 60 days of resin incubation.

iii. In the CA system, maize-soybean intercropping conferred highest N mineralization (45.81 Kg N ha^{-1} equivalent to Kshs 5976 ha^{-1}) and highest P mineralization (3.98 Kg P ha^{-1} equivalent to Kshs 1559 ha^{-1}) after 60 days of incubation (Table 8).

iv. In the ISFM long-term trial, no-input agroforestry system (i.e., maize-tephrosia) had the highest N mineralization (20.6 Kg N ha^{-1} equivalent to Kshs 2682 ha^{-1}) after 30 days of incubation, and the highest also at 60 days. This indicates increased microbial activity in this no-input maize-tephrosia system.

v. As expected, addition of N (in CT1 where both systems had P added), decreased N mineralization by 21 and 13 kg N ha^{-1} during 30 and 60 days of resin incubation, respectively.

vi. The control treatment (no-input) in INM3 consistently increased N mineralization and economic benefits after 30 and 60 days of incubation. Nitrogen mineralized was increased by at least 12 and at least 4 kg N ha^{-1} during the 30 and 60 days of resin incubation, respectively.

vii. Phosphorus mineralization varied from 0 to 4.9 Kg P ha^{-1}, with this higher value being equivalent to Kshs 1900.

Table 8. Gross N and P mineralization and monetary value after 30 and 60 days of resin incubation.

	N Mineralization (kg/ha)		P Mineralization (kg/ha)		Mineralized N benefits (Kshs/ha)		Mineralized P benefits (Kshs/ha)	
	30 days	60 days	30 days	60 days	30 days	60 days	30 days	60 days
CT1 Site (2019)								
CA, 0N+60P+R, M/S	20.03^{a}	45.81^{a}	0.18^{a}	3.98^{a}	2613^{a}	5976^{a}	69^{a}	1559^{a}
CA, 0N+60P+R, M-S	32.46^{a}	31.99^{ab}	4.87^{a}	2.12^{ab}	4234^{a}	4172^{ab}	1907^{a}	831^{ab}
CA, 0N+0P+R, M-S	33.8^{a}	5.19^{b}	0.73^{a}	0.02^{b}	4408^{a}	677^{b}	284^{a}	8.07^{b}
CA, 60N+60P+R, M-S	10.93^{a}	18.43^{ab}	1.64^{a}	2.07^{ab}	1425^{a}	2404^{ab}	643^{a}	810^{ab}
INM3 Site (2019)								
0N0P0R0FYM	20.56^{a}	14.69^{a}	0.06^{b}	0.02^{a}	2682^{a}	1916^{a}	25^{b}	8.7^{a}
60N+45P only	8.01^{ab}	10.66^{ab}	0.41^{ab}	0.59^{a}	1045^{ab}	1390^{ab}	160^{ab}	230.2^{a}
60N+45P+FYM	7.59^{ab}	9.44^{ab}	0.84^{a}	1.24^{a}	990^{ab}	1231^{ab}	327^{a}	485^{a}
0N+45P only	2.67^{b}	1.83^{b}	0.35^{ab}	1.24^{a}	348^{b}	239^{b}	138^{ab}	485^{a}
CT1 Site (2016)								
CT, 60N+60P+R, M-S	11.68^{a}	12.41^{b}	-	-	1523^{a}	1619^{b}	-	-
RT, 60N+60P+R, M-S	18.11^{a}	30.15^{a}	-	-	2362^{a}	3932^{a}	-	-
RT, 0N+60P+R, M/S	15.33^{a}	18.27^{b}	-	-	2000^{a}	2383^{b}	-	-
RT, 60N+60P-R, M-S	10.53^{a}	12.67^{b}	-	-	1373^{a}	1653^{b}	-	-

Means followed by similar letters across each column are not significantly different. CA=Conservation Agriculture, CT=conventional tillage; R = crop residue (at 2 t/ha), P=phosphorus, N=nitrogen, M/S= maize and soybean intercropping, M-S = maize and soybean rotation; FYM=Farm yard manure (at 4 t/ha).

CA systems often have higher residue concentrations and higher microbial abundance compared to CT systems (Kihara et al., 2018) and this could contribute to the increased nutrient mineralization, and subsequently the observed higher monetary values in the CA than CT systems. Similarly, intercropping systems often have higher plant and microbial diversity than rotation systems (Kihara et al., 2018). The higher nutrient mineralization (and economic benefits) in the intercropped than rotation systems could also reflect increased substrate availability that is utilized by the soil microbes in the mineralization.

Microbial activity is often limited by P availability in P-deficient soils (Cleveland et al., 2002) and P application stimulates organic N mineralization (White and Reddy, 2000). In a recent study, Mehnaz et al., (2019) reported that addition of P caused higher incorporation of N in microbial biomass, preventing its further mineralization into NH_4^+. The responses also depend on the fertility conditions: after application of N and P the activities of denitrifiers increased by 15–228% in the low fertility soil, but reduced by 18–46% in the high fertility soil (Wang et al., 2019).

The increased mineralization with 60 days of incubation is expected due to extended periods of microbial activity in decomposition. However, Hanselman et al. (2004) provided caution with studies involving extended incubations. They argued that under short-term basis (<45 days), in situ incubation methods may provide reasonable estimates of nitrogen mineralization compared to long-term incubations (>45 days). As time increases, there is consistent depletion of nutrient sources, immobilization, and other external interferences coupled with deterioration of soil conditions.

Role of FYM in microbial activity using proxy of CO_2 evolution

The activity of microbes is implied by the evolution of CO_2. We measured CO_2 comparing sole application of manure, nitrogen and a no nitrogen treatment in 2019 (all treatments were applied with P and K). The measurements were taken across a whole season (12 different dates of measurements; Plate 3). For the data analysis, repeated measures analysis of variance was done in Genstat while boxplots to show the overall distributions of the fluxes were plotted in R statistics. For the analysis of variance and CO_2 released on the 3 different treatments, timing was included as the variate, treatment structure were the treatments while the blocking structure were the four replicates. CO_2 fluxes were significantly ($P<0.01$) influenced by treatment with application of FYM resulting in significantly higher fluxes (45.2 mg/m²/h) than both the omission and the application of 90 N (32.9 and 28.6 mg/m²/h, respectively; Figure 34). The model also showed a significant effect of timing of sampling ($P<0.01$). Following this, we regressed the fluxes with ambient temperatures and soil moisture and observed that the attainable fluxes were correlated with these climatic variables (Figure 35). The fluxes increased with increase in soil moisture. On the other hand, increased soil temperatures beyond 35 °C decrease CO_2 fluxes. As CO_2 is a proxy of microbial activity, these results may imply that practices that promote moisture conservation and regulate soil temperatures from reaching higher extremes favor microbial activity. On the other hand, N_2O fluxes were also significantly influenced by treatments, and were significantly (and equally) higher in both treatments with either application of FYM or nitrogen at 90 kgN/ha compared to no-input control (Figure 36).

Figure 34. Boxplots of CO_2 fluxes observed in different nitrogen (N) and farmyard manure (FYM) treatments in a long-term trial in western Kenya. The notches indicate that the median for treatment with FYM is significantly higher than the median of the other two treatments. The means for boxes with different letters above them are significantly different ($P<0.05$) based on the repeated measures analysis of variance.

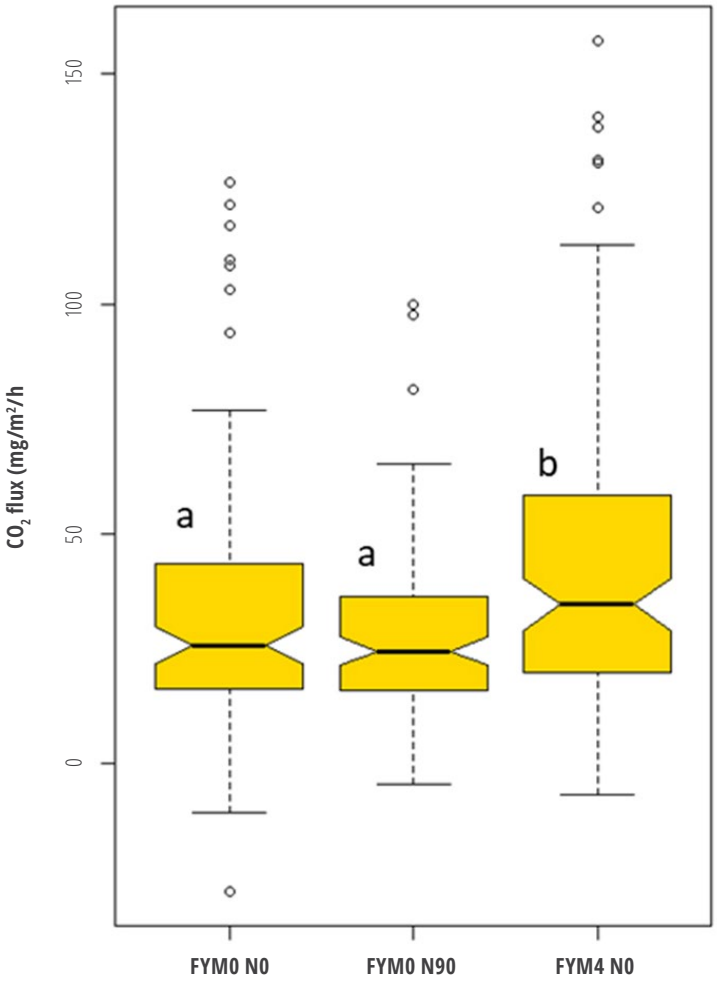


Figure 35. The relationships between CO_2 and both soil temperature and moisture.

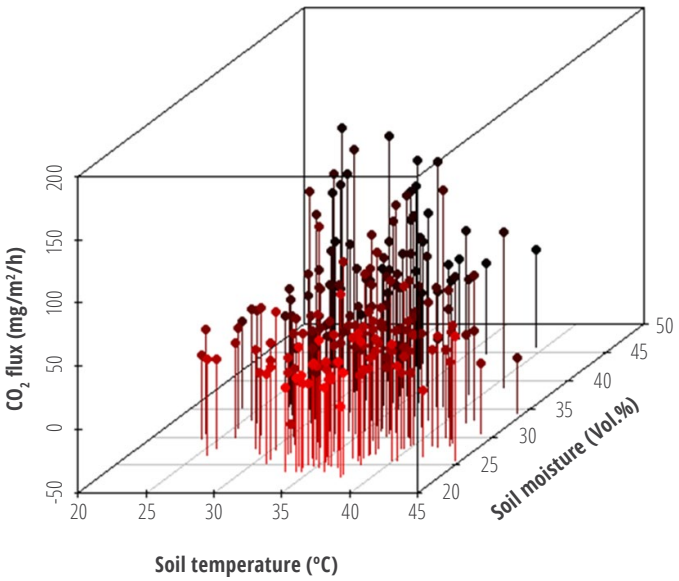


Figure 36. Boxplots of N_2O fluxes observed in different nitrogen (N) and farmyard manure (FYM) treatments in a long-term trial in western Kenya.

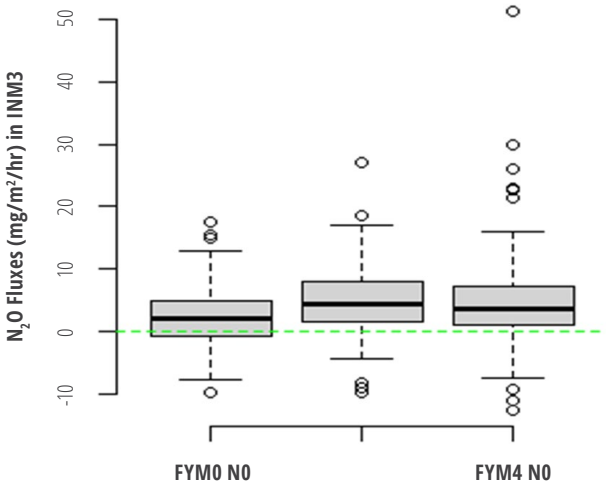


Plate 3. Installation of chamber bases and sampling of greenhouse gases under selected ISFM systems in INM3 (Photo: Peter Bolo/Alliance).

Application of microbiology through bio-inoculants

Forage and food crops in Thika trial

The use of bio-inoculants is important to maximize on the beneficial role of soil microbes such as in nutrient acquisition, and increase crop resistance to pests and diseases and thereby promote crop growth. Used as biofertilizers, microbial inoculants can reduce dependence on fertilizers and support agroecology and regenerative agriculture. We applied inoculants to different food crops and forage crops under CIAT-managed long-term on-station trials in western Kenya and Kenya Agricultural and Livestock Research Organization (KALRO) station, Kandara, Murang'a County. We used combinations of Symbion VAM Plus and Legumefix during the long rains (LR2019) season and Symbion VAM Plus and Biofix during the short rains season (SR2019). Symbion VAM Plus is a carrier-based microbial inoculant consisting of *Bacillus megathrium* and a combination of different species of Vesicular Arbuscular Mycorrhiza (VAM) fungi like Glomus sp. that solubilise and mobilize phosphorus and other plant nutrients. Legumefix and Biofix are Rhizobium inoculants supporting nitrogen fixation. Symbion VAM Plus was used under maize rotation phase while for legumes rotation phase and intercrop treatments, Symbion VAM Plus was combined with either Legumefix (in LR2019) or Biofix (in SR2019). Orthogonal contrasts of inoculants vis a vis no inoculants although insignificant in most cases revealed important effects on yields of crops and forages; yields improved in the inoculated (B) relative to non-inoculated (A) systems during both the LR2019 and SR2019 seasons in KALRO trial (Table 9). The increase in Napier due to inoculants, consistently in the two seasons, was by ~2 t/ha. Inoculation also improved crop physiological characteristics, with inoculated plots having slightly taller plants and deep green leaf color compared to non-inoculated plots (Plate 2).

Table 9. Forage yields (t ha^{-1}) in Thika trial in Inoculated and Non-inoculated treatments during the long rains (LR2019) and short rains (SR2019) cropping seasons.

Treatment	Yield (t ha^{-1}) for LR2019 season			Yield (t ha^{-1}) for SR2019 season		
	Crop	A	B	Crop	A	B
CA+ Napier/ desmodium intercrop	Desmodium	2.45[a]	3.62[a]	Desmodium	3.76[a]	2.63[a]
	Napier	6.96[a]	8.99[a]	Napier	13.27[a]	15.75[a]
CA+ brachiaria/ desmodium intercrop	Brachiaria	1.79[a]	2.19[a]	Brachiaria	4.93[a]	6.19[a]
	Desmodium	1.21[a]	1.23[a]	Desmodium	4.77[a]	2.99[a]
CA + Napier only	Napier	4.61[a]	4.63[a]	Napier	6.85[a]	8.43[a]

Means followed by the same letters in each column are not significantly different. LR2019=Long rains season of 2019; SR2019=Short rains season of 2019; A= yield (t/ha) for plots not inoculated with Symbion VAM and Legumefix (LR2019) or Symbion VAM and Biofix (SR2019). B= yield (t/ha) for plots inoculated with Symbion VAM and Legumefix (LR2019) or Symbion VAM and Biofix (SR2019).

Plate 2. Physiological variations in forages inoculated (B) and non-inoculated (A) with biofertilizers in Thika trial.

Effects of Inoculation on maize and soybean performance in Nyabeda, western Kenya

Gross margins capture overall treatment effects and are particularly useful when multiple crops are involved. Maize total aboveground biomass yield ranged from 3.48 t/ha in reduced tillage no-fertilizer treatment to 11.42 in the conventional tillage full fertilizer plus residue treatment. The yields were similar to those observed in other seasons in the long-term trial. Out of the 10 treatments included, 6 increased gross margins by 50 to 246 US$ in inoculated relative to un-inoculated (Table 10). On the other hand, 4 treatments had reduced gross margins by 37 to 193 US$ with inoculation relative to no-inoculation. Besides, inoculation with biofertilizers had slight effects on maize height (3.30%), circumference at basal diameter (2.13%) and leaf area index (6.04%), maize stover yields (4.21%), maize grain yields (10.47%) and total maize yields (2.52%) relative to non-inoculated plots (data not shown). A similar observation is made with soybean where, at the 50% podding stage, inoculation with biofertilizers slightly increased biomass (11.0%), canopy cover (27.09%), basal diameter (21.13%), nodules counts (1.52%), nodule fresh (8.42%) and dry weights (4.77%) compared to no inoculation. At harvest, inoculation with biofertilizers slightly increased soybean grain yield (4.15%). While these results point to potential for a positive result with bio-inoculants, they are not convincing enough for applications by farmers.

Table 10. Total above-ground biomass maize yield and gross margins for combined maize and soybean in the inoculated (B) and non-inoculated (A) systems in CT1 long-term trial.

Treatments	Total above-ground biomass yield		Gross benefits (USD/ha)		Differences in gross benefits B-A; (USD/ha)
	A	B	A	B	
RT;0N+60P+R;M/S Intercrop	7.22^{bc}	6.38^{bc}	1567.3^{def}	1637.2^{bc}	69.9
RT;0N+60P+R;M-S Rotation	4.53^{d}	4.97^{cd}	1574.2^{def}	1721.5^{bc}	147.3
CT;0N+60P+R;M/S Intercrop	7.14^{bc}	7.61^{abc}	1858.8^{bcd}	1821.5^{b}	-37.3
CT;0N+60P-R;M/S Intercrop	5.31b^{cd}	7.78^{ab}	1568.2^{def}	1702.6^{bc}	134.4
RT;0N+0P+R;M-S	4.91^{cd}	5.66^{bcd}	1284.2^{ef}	1264.7^{cd}	-19.5
RT;0N+0P-R;M-S Rotation	3.95^{d}	3.48^{d}	1026.7^{f}	982.9^{d}	-43.8
RT;0N+60P-R;M/S Intercrop	5.68^{bcd}	4.94^{cd}	1796.1^{cde}	1603.5^{bc}	-192.6
CT;60N+60P+R;M-S Rotation	11.42^{a}	9.19^{a}	2779.3^{a}	2829.3^{a}	50
RT;60N+60P+R;M-S Rotation	7.6^{b}	7.88^{ab}	2401.9^{ab}	2648.3^{a}	246.4
RT;60N+60P-R;M-S Rotation	7.3^{bc}	7.79^{ab}	2246.5^{abc}	2404.8^{a}	158.3

Means followed by similar letters in each column are not significantly different from each other. RT = Reduced tillage; CT= Conventional tillage; +R = residue added (2t/ha); -R = lacking residue; M-S = maize- and soybean rotation; M/S = Maize and soybean intercrop.

The effectiveness of biofertilizers is an issue of ongoing debate. The effects of bioinoculants on crop yields have been inconsistent, some studies either reporting increases or decreases. A previous study within the region (Majengo et al. 2013) reported an increase in soybean yields following inoculation with commercial biofertilizers; whereas Faye et al (2020) reported little effects of different inoculants, either for sole or combined application, on the soybean yield, nutrient uptake and other physiological attributes. It is not clear where to peg the inconsistency in crop yields with biofertilizer applications; whether it is the quality of biofertilizers, suppression effects of the indigenous organisms. At the moment, we are not aware of any policy dimension around biofertilizers nor a system in place to ensure high quality. Thus, more research and policies should be formulated in terms of the quality and effectiveness of the biofertilizers.

In our case, combined application of biofertilizer and inorganic P did not seem to confer more benefits than biofertilizers alone. Also, plant tissue concentrations for various macronutrients and micronutrients were in general not affected by inoculations except for some increases in Zinc for both maize and soybean and Iron for maize (see the means and ranges in Table 10 and 11). Interestingly, the concentrations of important micronutrients are still below the required thresholds for human nutrition. For instance, the critical threshold for zinc in maize grain is between 40-60 mg/kg, but this is higher than the maize grain zinc concentration levels in both the inoculated and non-inoculated plots. This is a pointer that although yield performance of cereals may be high, more management efforts are required to simultaneously address both yield and the micronutrient gaps for proper nutrition.

Table 11. Biomass and grain yield nutrient concentrations in inoculated and non-inoculated plots in CT1. Numbers in brackets are standard deviations from the means.

Plant part	Nutrient	Maize		Soybean	
		Inoculated	Non-inoculated	Inoculated	Non-inoculated
Biomass	N (%)	0.79 (0.23)	0.78 (0.23)	2.75 (0.23)	2.71 (0.26)
	P (%)	0.06 (0.06)	0.06 (0.05)	0.23 (0.06)	0.24 (0.06)
	K (%)	1.53 (0.45)	1.52 (0.47)	2.13 (0.3)	2.13 (0.23)
Grain	N (%)	1.43 (0.12)	1.4 (0.16)	5.04 (0.31)	5.17 (0.26)
	P (%)	0.37 (0.07)	0.35 (0.06)	0.55 (0.11)	0.56 (0.11)
	K (%)	0.51 (0.06)	0.5 (0.06)	1.7 (0.1)	1.71 (0.1)
	Ca (%)	-	-	0.25 (0.02)	0.25 (0.02)
	Mg (%)	0.16 (0.02)	0.15 (0.02)	0.23 (0.01)	0.23 (0.02)
	S (%)	0.11 (0.01)	0.11 (0.01)	0.31 (0.02)	0.31 (0.02)
	Mn (ppm)	9.51 (1.32)	10.07 (2.42)	84.7 (22.31)	86.65 (20.73)
	B (ppm)	2.57 (0.76)	2.48 (0.51)	33.56 (3.76)	33.06 (6.65)
	Zn (ppm)	33.25 (3.66)	29.95 (4.72)	52.23 (7.86)	54.11 (8)
	Fe (ppm)	52.17 (12.86)	45.43 (11.72)	170.8(31.10)	178.2 (40.17)
	Cu (ppm)	2.83 (0.57)	2.89 (0.86)	13.92 (1.91)	14.39 (1.8)
	Na (ppm)	12.57 (5.81)	13.64 (8.02)	12.80 (11.84)	30.66 (23.03)
	Mo (ppm)	-	-	0.22 (0.1)	0.2 (0.1)

Despite that inoculation had no effect on grain nutrient concentrations, three important treatment effects are observed with soybean:

1) reduced tillage with P application resulted in the highest concentrations in soybean grain zinc that was by +6 ppm more compared to same treatments under conventional tillage,

2) reduced tillage with no fertilizer inputs had the lowest concentration of soybean grain zinc being by -15 ppm lower than with fertilizer application (it also had lowest grain K and P) and,

3) growing pure crop of soybean resulted in the highest concentrations of soybean grain Zn and Fe with or without inoculation (Table 12).

The poor soil fertility treatments (with no fertilizers or no residues) had the lowest grain nutrient concentrations relative to other treatments. Improved understanding of soil fertility management influences on quality of produce is important and is one important indicator under regenerative agriculture practices.

Table 12. Effects of soil management practices on concentrations of selected nutrients within soybean grains in CT1 in the inoculated (B) and non-inoculated (A) systems.

Treatments	Zinc (ppm)		K (ppm)		P (ppm)	
	A	B	A	B	A	B
RT;0N+60P+R;M/S Intercrop	57.07^{a}	53.57^{ab}	1.71^{a}	1.72^{ab}	0.56^{b}	0.58^{a}
RT;0N+60P+R;M-S Rotation	62.83^{a}	62.17^{a}	1.76^{a}	1.81^{a}	0.58^{ab}	0.64^{a}
CT;0N+60P+R;M/S Intercrop	53.33^{ab}	49.57^{bc}	1.8^{a}	1.73^{ab}	0.68^{a}	0.62^{a}
CT;0N+60P-R;M/S Intercrop	51.87^{ab}	52.57^{abc}	1.7^{a}	1.68^{b}	0.57^{b}	0.54^{a}
RT;0N+0P+R;M-S	45.43^{b}	43.27^{c}	1.57^{b}	1.56^{c}	0.39^{c}	0.37^{b}

Note: Values in the same column with different letters are significantly different. RT = Reduced tillage; CT= Conventional tillage; +R = residue added (2t/ha); -R = lacking residue; M-S = maize- and soybean rotation; M/S = Maize and soybean intercrop.

Research gaps for future focus

1. Food supply should be matched with appropriate nutrition. Little is known on the effects of soil management on nutritional quality of the produce including supply of sufficient quantities of important proteins and micronutrients. Alleviating health disorders related to micronutrient deficiencies is a health goal.

2. Reduction in crop productivity and yields due to damage by pests and pathogens has continually been met by application of chemical pesticides; that may not only accumulate in edible plant tissues, prompt pathogen-resistance, but may also alter the functional and beneficial soil microbial structure. There is need for research to identify good agricultural practices that control abundance and activity of soil-borne microbial pathogens in environmentally friendly, economical and sustainable ways.

3. There is now overwhelming evidence of soil fertility management effects on microbial diversity, abundances and activity under researcher-controlled fields. Future research should focus on how these are influenced under farmers' own conditions and management practices. This should extend to the range of cover crops and soil protection measures employed by farmers, including tropical forages.

4. Application of manure and of lime clearly have good influences on soil life. It will be good to understand if also antihill soils (and their combinations with manures), and vermicomposts and vermijuices (bio-pesticides from vermicomposting) can achieve similar positive influences as these are easily accessible to farmers

5. The current methods of assessing soil health are complex, expensive and require technical expertise. There is need to develop simple, observation-based, soil health assessment tool(s) that can rapidly, and universally, be used infield by both researchers and small-holder farmers to assess the ecosystem health of their agronomic management practices.

6. The inconsistent responses of different biofertilizers/bio-inoculants to crop productivity should be further explored. We are not aware of a policy dimension around biofertilizers nor a system in place to ensure high quality. Also, the influence of biofertilizer applications on shifts to soil microbial community and functional structures was not given attention in the current study.

7. Development of a better understanding of soil biota under different agro-ecological zones, land use and management, and soil types is necessary for improvement of soil health and realization of self-sustaining ecosystem. Microbial study geared towards identification, quantification and mapping of soil biota and their activities under these different contexts will be important in developing better soil health management approaches.

Acronyms and abbreviations

ACP	Acid phosphatase
ALP	Alkaline phosphatase
C	Carbon
CA	Conservation agriculture
CCA	Canonical correspondence analysis
CT	Contentional tillage
CT1	Conservation tillage long-term experimental site in Nyabeda
DNA	Deoxyribonucleic acid
FYM	Farm yard manure
GLU	Beta-glucosidase
INM3	Integrated nutrient management long-term trial in Madeya
ISFM	Integrated soil fertility management
ITS	Internal transcribed spacer region
KALRO	Kenya Agricultural and Livestock Research Organization
LR2019	Long rains season of 2019
MN	Micronutrient
N	Nitrogen
NAG	Beta-glucosaminidase
OTUS	Operational taxonomic units
P	Phosphorus
R	Residue
RT	Reduced tillage
SOC	Soil organic carbon
SR2019	Short rains season in 2019
UNS	Undisturbed natural site
VAM	Vesicular arbuscular mycorrhiza

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