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Fungal diversity within organic and conventional farming systems in Central Highlands of Kenya

Edward Nderitu Karanja[1,2], Andreas Fliessbach[3], Noah Adamtey[3], Anne Kelly Kambura[4*], Martha Musyoka[2], Komi Fiaboe[2,5] and Romano Mwirichia[1]

[1]Department of Biological Sciences, School of Pure and Applied Sciences, University of Embu, P. O. Box 6-60100, Embu, Kenya.
[2]International Centre for Insect Physiology and Ecology (*icipe*). P. O. Box 30772-00100, Nairobi, Kenya.
[3]Research Institute of Organic Agriculture, Ackerstrasse 113, 5070 Frick, Switzerland.
[4]School of Agriculture, Earth and Environmental Sciences, Taita Taveta University, P. O. Box 635-80300, Voi, Kenya.
[5]International Institute of Tropical Agriculture, Cameroon, BP. 2008 (Messa); Yaoundé, Cameroon.

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Fungal diversity in agro-ecosystems is influenced by various factors related to soil and crop management practices. However, due to the complexity in fungal cultivation, only a limited number has been extensively studied. In this study, amplicon sequencing of the Internal Transcribed Spacer (ITS) region was used to explore their diversity and composition within long-term farming system comparison trials at Chuka and Thika in Kenya. Sequences were grouped into operational taxonomic units (OTUs) at 97% similarity and taxonomy assigned via BLASTn against UNITE ITS database and a curated database derived from GreenGenes, RDPII and NCBI. Statistical analyses were done using Vegan package in R. A total of 1,002,188 high quality sequences were obtained and assigned to 1,128 OTUs; they were further classified into eight phyla including *Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycota, Calcarisporiellomycota, Kickxellomycota*, *Mortierellomycota* and unassigned fungal phyla. *Ascomycota* was abundant in conventional systems at Chuka site while *Basidiomycota* and *Chytridiomycota* were dominant in conventional systems in both sites. *Kickxellomycota* and *Calcarisporiellomycota* phyla were present in all organic systems in both sites. Conventional farming systems showed a higher species abundance and diversity compared to organic farming systems due to integration of organic and inorganic inputs.

Key words: Long-term farming systems, fungi, internal transcribed spacer (ITS), diversity, Illumina sequencing.

INTRODUCTION

Fungal communities are an essential constituent of soil microbial biomass that is involved, and/or linked to processes of carbon and nitrogen cycles, organic matter decomposition, as well as nitrogen mineralization and immobilization (Bloem et al., 1995; Bååth and Anderson, 2003; Wall et al., 2012; Berthrong et al., 2013; Milner, 2014; Fierer, 2017). On the other hand, fungi which constitute one of the largest groups of eukaryotes, play a

*Corresponding author. E-mail: annnderitu@gmail.com. Tel: +254721235147.

key role in nutrient and carbon cycling as mutualists, symbionts, pathogens and free-living saprotrophs (Barea et al., 2005; Gadd, 2007; Lindahl et al., 2007; McLaughlin and Spatafora, 2014). Fungi are also involved in formation of soil aggregates, elevated water holding capacity, plant growth promotion and suppression of phytopathogens (Sommermann et al., 2018). Mutualistic root endophytic fungi induce systemic resistance in host plants thereby increasing crops tolerance levels to biotic and abiotic stress factors (Lahlali et al., 2014). Therefore, they are a key component of sustainable soil-plant systems that govern major plant nutrient cycles hence sustaining the vegetation cover and ecosystem services (Schreiner and Bethlenfalvay, 1997; Dighton, 2003; Johansson et al., 2004).

Soil fungal community composition is influenced by soil physicochemical properties, plant populations and geo-climatic conditions (Tkacz et al., 2015). However, in agro-ecosystems, they are exposed to added influencing factors associated with soil and crop agronomic management practices. To date, only few studies have been keen to determine the impact of farming systems on microbial diversity. Little information is available concerning the effect of cultivation systems on fungal diversity and the level of fungal diversity between different crops in the same farm (Lentendu et al., 2014; Lopes et al., 2014; Kazeeroni and Al-Sadi, 2016). The fungal diversity ecosystem is still undefined; though, Wang et al. (2008) reported that about 5-13% of the total estimated global fungal species have been described. Since many fungi are unculturable and rarely produce visible sexual structures, molecular techniques have become widely used for taxonomic detection of species to understand shifts in their richness and composition along environmental gradients (Persˇoh, 2015; Balint et al., 2016; Tedersoo and Nilsson, 2016; Tedersoo et al., 2018).

It is still not understood how fungal communities respond to different inputs within organic and conventional farming systems (Hartmann et al., 2015; Wang et al., 2017). In this study, conventional farming systems received inorganic and organic inputs whilst organic systems received organic inputs only. High throughput sequencing of the ITS gene amplicons was used to explore the population diversity and composition of fungal communities within conventional and organic farming systems in system comparison trials within central highlands of Kenya. The study sites were initiated to compare the performance of organic and conventional farming systems in the tropics on farm productivity, profitability and sustainability.

MATERIALS AND METHODS

Study sites characteristics

Samples were collected from the on-going long-term farming systems comparison (SysCom; www.system-comparison.fibl.org) trials in Kenya (Adamtey et al., 2016). The trials were established in 2007 at two locations: Chuka (Tharaka Nithi County) and Thika (Murang'a County) in the Central Highlands of Kenya. These sites are 125 km apart and they have a bimodal rainfall pattern with long rains occurring between March and June and short rains occurring between October and December. The site at Chuka is located at 1458 m above sea level (Longitude 037° 38.792' and Latitude 00° 20.864'), with an annual mean temperature of 20°C and mean annual rainfall of 1500 to 2400 mm. This site is situated in the upper midland 2 agro ecological zone, also referred to as the coffee zone (Jaetzold et al., 2006a). The site at Thika is located at 1500 m above sea level (Longitude 037° 04.747' and Latitude 01° 00.231'), with an annual mean temperature of about 20°C and mean annual rainfall of 900 - 1100 mm. This site is situated in the upper midland agro ecological zone 3, also referred to as the sunflower maize zone (Jaetzold et al., 2006b). The soils at Chuka site are classified as Humic Nitisols and those at Thika as Rhodic Nitisols (Adamtey et al., 2016) in the Food and Agricultural Organization (FAO) World reference base for soil resources (IUSS Working Group WRB. 2006).

Farming systems

Food and Agricultural Organization (FAO) defines farming systems as a set of population of individual farm systems that have broadly similar resource bases, enterprise patterns, household livelihoods and constraints and for which similar development strategies and interventions would be appropriate (Dixon et al., 2001). This was adopted in this study and at each site, conventional (Conv) and organic (Org) systems were compared at low input levels (Conv-Low and Org-Low), where the N and P application rates and management practices mimicked small-scale farmers' practices in the region (Muriuki and Qureshi, 2001). Conventional (Conv) and organic (Org) systems at high input levels (Conv-High and Org-High) represented recommended N and P application rates and other management practices embraced by market-oriented and large-scale production systems farmers (Musyoka et al., 2017). The high input systems received supplementary irrigation during the dry period and management of pests and diseases was guided by scouting reports (Adamtey et al*.,* 2016). The four farming systems in each site were arranged in a randomized complete block design with plot sizes of 8 m × 8 m replicated 4 times. The type of inputs and their application rates in each farming system are indicated in Supplementary Table 1.

Soil sampling

Soil sampling was done before land preparation in March 2015. Surface organic materials were removed and a homogenized composite soil sample collected from 12 single cores within top soil (0-20 cm depth) which is the root zone of majority crops grown in the trial sites. Two batches of sixteen (16) composite samples from each site were packed in sterile 500 g containers. One batch of the soil samples for molecular analysis was preserved on dry ice and transported to the laboratory at International Centre for Insect Physiology and Ecology for preservation at -80°C. The other batch of soil samples was used for soil physicochemical analysis (using methods summarized in Table 1) at Crop Nutrition Laboratory Services, Nairobi in Kenya.

Fungal community analysis

DNA was extracted from 0.25 g of the soil samples in triplicates as described by Sambrook et al. (1989). The DNA from triplicate samples was pooled at precipitation stage, pellets were air dried

Table 1. Soil physicochemical parameters analyzed and their respective methods.

Parameter	Method
pH and Electrical conductivity (EC)	Potentiometric (Okalebo et al., 2002)
Cation exchange capacity (CEC), Potassium (K), Calcium (Ca), Magnesium (Mg), Sulphur (S), Sodium (Na), Copper (Cu), Boron (B), Zinc (Zn) and Iron (Fe)	Mehlich 3 (Mehlich, 1984)
Exchangeable Aluminium (Exch. Al)	Spectrophotometry (Kennedy and Powell, 1986)
Organic Carbon (OC)	Wet oxidation (Anderson and Ingram, 1993)
Total Nitrogen (N)	Kjeldahl acid digestion (Gupta, 1999)
Total Phosphorous (P),	Olsen (Okalebo et al*.,* 2002)
Soil moisture and Temperature	Soil Moisture Meter (IMKO GmbH – Germany)
Aggregate size separation (Small macro-aggregates and micro-aggregates)	Wet sieving (Six et al*.,* 1998)

and sent to Molecular Research DNA Laboratory (www.mrdnalab.com, Shallowater, TX, USA) for amplicon library preparation and sequencing.

Amplicon DNA library preparation and sequencing

Polymerase Chain Reaction (PCR) amplification of ITS region was done using ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) barcode primers (White et al., 1990; Ihrmark et al*.,* 2012). Amplification proceeded in a 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) with initial heating at 94°C for 3 min, followed by 28 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 40 s and extension at 72°C for 1 min, after which a final elongation step at 72°C for 5 min was performed. PCR products were visualized on 2% agarose gel to determine the success of amplification and the relative intensity of bands. Multiple samples were pooled together (e.g., 100 samples) in equal proportions based on their molecular weight and DNA concentrations during sequencing. The pooled samples were purified using calibrated Ampure XP beads (Agencourt Bioscience Corporation, MA, USA) and used to prepare DNA library by following Illumina sequencing protocol (Yu and Zhang, 2012). Sequencing was performed at Molecular Research DNA (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq 2x300 bp Version 3 platform following the manufacturer's guidelines.

Sequence analysis

Sequences were analyzed using QIIME2 pipeline (Bolyen et al., 2018) whereby the input file was created using "convert_fastaqual_fastq.py" script on Qiime v1.9 (Caporaso et al., 2010). Sequences were demultiplexed using barcode information; and quality control and construction of feature tables was done using dada2 software in QIIME2 (Callahan et al., 2016). The pipeline denoises sequences, removes chimeras, creates OTU table, picks representative sequences and calculates denoising statistics. Sequences which were < 200 base pairs after phred20-base quality trimming, with ambiguous base calls, and those with homopolymer runs exceeding 6bp were removed (Callahan et al., 2016). Taxonomic classification of representative sequences obtained from the OTU clustering was done using QIIME feature-classifier classify-sklearn based on UNITE ITS Reference Database (Kojalg et al., 2005; Koljalg et al., 2013) and a curated database derived from GreenGenes, RDPII and NCBI (www.ncbi.nlm.nih.gov; http://rdp.cme.msu.edu) at 97% level of similarity using default settings as implemented in QIIME2 (Bolyen et al., 2018). The sequence reads have been deposited at NCBI Sequence Read Archive with SRA accession: PRJNA532741 (https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA532741).

Diversity analysis was carried out using Vegan Community Ecology Package version 2.5.2 (Oksanen et al., 2012). In order to support OTU-based analysis, taxonomic groups were derived from the number of reads assigned to each taxon at all ranks from domain to species output from QIIME2. Alpha diversity indices (Richness - S and Shannon - H') were used to test statistically significant differences between high and low input farming systems. Rarefaction curves were generated, plotted and customized using Vegan Community Ecology Package. Community and environmental distances were compared using analysis of similarity (ANOSIM) while significance was determined at 95% confidence interval ($P<0.05$). Calculation of Bray-Curtis dissimilarities between datasets and hierarchical clustering were carried out using Vegan package in R (Oksanen et al., 2012). To estimate diversity between samples (β Diversity), Principal Component Analysis of soil physicochemical characteristics and prokaryotic taxa was done using R programming language (R Development Core Team., 2012). Besides Principal Component Analysis, Non-metric multidimensional scaling (NMDS) and hierarchal clustering were also performed for beta diversity. In order to understand the influence of farming systems on soil physicochemical characteristics, analysis of variance was performed at $P<0.05$, 0.01 and 0.001 using a linear mixed-effect model with *lmer* function from lme4 package in R software (Bates et al., 2013) with system and site as fixed effects; while replication was used as random effect. In order to delineate the farming systems within sites, computation of least mean squares was done using *lsmeans* package in R software. Means were separated with Tukey's method implemented using *cld* function from *multicomp* package as developed by Piepho (2004) in R software version Ri386 3.1.1 (R Development Core Team, 2014).

RESULTS

Soil physicochemical properties of the long-term system comparison trials

In this study we assessed the fungal community composition in 32 soil samples collected from long-term farming system comparison trials at Chuka and Thika in Kenya. The physicochemical characteristics of soils are as shown in Table 2. Tukey's separation of means

Table 2. Soil physicochemical characteristics of the long-term system comparison trial sites at Chuka and Thika.

Soil property	Farming systems				System x Site								Source of variation	
					Chuka				Thika				System	System x Site
	Conv-High	Org-High	Conv-Low	Org-Low	Conv-High	Org-High	Conv-Low	Org-Low	Conv-High	Org-High	Conv-Low	Org-Low		
pH	5.68^{a}	6.61^{ab}	5.43^{a}	5.87^{a}	5.64^{ab}	6.50^{bc}	5.58^{ab}	5.75^{ab}	5.72^{ab}	6.71^{c}	5.23^{a}	5.98^{abc}	***	ns
EC.S (uS/cm)	85.75^{a}	113.75^{a}	60.13^{a}	75.50^{a}	48.50^{a}	74.00^{ab}	46.50^{a}	48.50^{a}	123.00^{bc}	153.50^{c}	73.75^{ab}	102.50^{abc}	ns	ns
OC (%)	2.29^{a}	2.52^{a}	2.29^{a}	2.34^{a}	2.60^{cd}	2.89^{d}	2.78^{d}	2.51^{bcd}	1.97^{ab}	2.16^{abc}	1.79^{a}	2.16^{abc}	ns	ns
N (%)	0.19^{a}	0.205^{a}	0.185^{a}	0.196^{a}	0.208^{cde}	0.223^{e}	0.203^{bcde}	0.215^{de}	0.173^{ab}	0.188^{abcd}	0.168^{a}	0.178^{abc}	ns	ns
S (mg/kg)	16.37^{a}	8.00^{a}	15.59^{a}	14.04^{a}	10.09^{ab}	1.22^{a}	9.80^{ab}	8.10^{ab}	22.65^{b}	14.78^{ab}	21.39^{b}	19.97^{b}	ns	ns
P (mg/kg)	30.80^{ab}	42.31^{b}	16.97^{a}	20.18^{a}	35.75^{a}	39.08^{a}	14.55^{a}	19.23^{a}	25.86^{a}	45.55^{a}	19.38^{a}	21.14^{a}	**	ns
K (mg/kg)	472.63^{a}	1077.25^{b}	453.13^{a}	541.63^{a}	339.00^{a}	994.25^{bc}	334.75^{a}	366.00^{a}	606.25^{ab}	1160.25^{c}	571.50^{a}	717.25^{ab}	***	ns
Ca (mg/kg)	1462^{a}	2086^{b}	1438^{a}	1539^{a}	1765^{ab}	2315^{b}	1598^{ab}	1695^{ab}	1159^{a}	1858^{ab}	1279^{a}	1384^{a}	**	ns
Mg (mg/kg)	248^{a}	342^{b}	260^{a}	245^{a}	250^{ab}	344^{c}	237^{a}	235^{a}	246^{a}	340^{bc}	283^{abc}	256^{abc}	***	ns
Na (mg/kg)	21.63^{a}	32.73^{a}	18.03^{a}	18.34^{a}	7.17^{ab}	9.29^{ab}	4.48^{a}	5.70^{ab}	36.10^{bc}	56.18^{c}	31.58^{abc}	30.98^{abc}	ns	ns
Exch. Al (meq/ 100g)	0.07^{a}	0.04^{a}	0.19^{a}	0.11^{a}	0.78^{ab}	0.12^{a}	0.53^{ab}	0.04^{a}	0.06^{ab}	0.07^{ab}	0.33^{b}	0.17^{ab}	ns	ns
B (mg/kg)	0.58^{a}	0.96^{b}	0.55^{a}	0.68^{a}	0.54^{a}	0.93^{ab}	0.53^{a}	0.58^{a}	0.63^{ab}	0.99^{b}	0.58^{a}	0.78^{ab}	***	ns
Mn (mg/kg)	434^{a}	443^{a}	446^{a}	429^{a}	567.50^{b}	533.50^{b}	575.75^{b}	553.75^{b}	300.50^{a}	353.25^{a}	315.25^{a}	303.75^{a}	ns	*
Fe (mg/kg)	89.25^{b}	70.19^{a}	83.70^{b}	77.33^{ab}	97.93^{c}	72.76^{ab}	89.63^{bc}	83.78^{abc}	80.58^{ab}	67.60^{a}	77.75^{ab}	70.88^{a}	**	ns
Zn (mg/kg)	8.89^{a}	10.51^{a}	7.19^{a}	8.06^{a}	12.23^{de}	12.80^{e}	9.55^{cd}	10.80^{cde}	5.49^{ab}	8.23^{bc}	4.82^{a}	5.32^{ab}	ns	ns
Small Macro-aggregate (g)	48.11^{ab}	52.15^{b}	42.17^{a}	42.28^{a}	46.09^{b}	48.56^{bc}	36.53^{a}	36.76^{a}	50.15^{bc}	55.75^{c}	47.82^{bc}	47.80^{bc}	**	ns
Micro-aggregate (g)	21.15^{ab}	17.43^{a}	28.66^{b}	27.13^{b}	25.58b^{c}	22.29^{b}	34.22^{c}	33.81^{c}	16.72^{ab}	12.58^{a}	23.10^{b}	20.46^{ab}	*	ns

Letters designate significant differences at P ≤ 0.05. Means followed by the same letter are not significantly different; ns= not significant; *P ≤ 0.05; ** P ≤ 0.01 and *** P ≤ 0.001.

revealed a trend in the means of soil pH, P, K, Ca, Mg, B and small macro-aggregates that were found to be significantly high (P<0.05) in organic farming systems. Fe and micro-aggregates were high in conventional farming systems (Table 2). Soils from Chuka contained 59.4% primary clay minerals and 40.6% secondary clay minerals, while soils from Thika were characterized by high primary minerals (78.3%) and low secondary clay minerals (21.7%) (Adamtey et al., unpublished results). Congruently, the rate of formation and stabilization of small macro aggregates was found to be higher at Thika than Chuka site.

Fungal sequence coverage analysis within farming systems

After denoising and demultiplexing, a total of 556,135 and 466,053 high quality sequences were obtained from Chuka and Thika sites respectively. Rarefaction analysis of the extent of diversity captured in each farming system and the level of sequence coverage visualized using rarefaction curves showed a steep slope that plateaued to the right in some of the replications within farming systems (Figure 1a and b). This indicated that a good proportion of the fungal diversity had been captured within the represented farming systems and an increase in the number of sequences extracted would only marginally increase the number of OTUs obtained. However, rarefaction curves of some replications within farming systems displayed a steep slope, denoting that more intensive sampling within the replicate plots was likely to

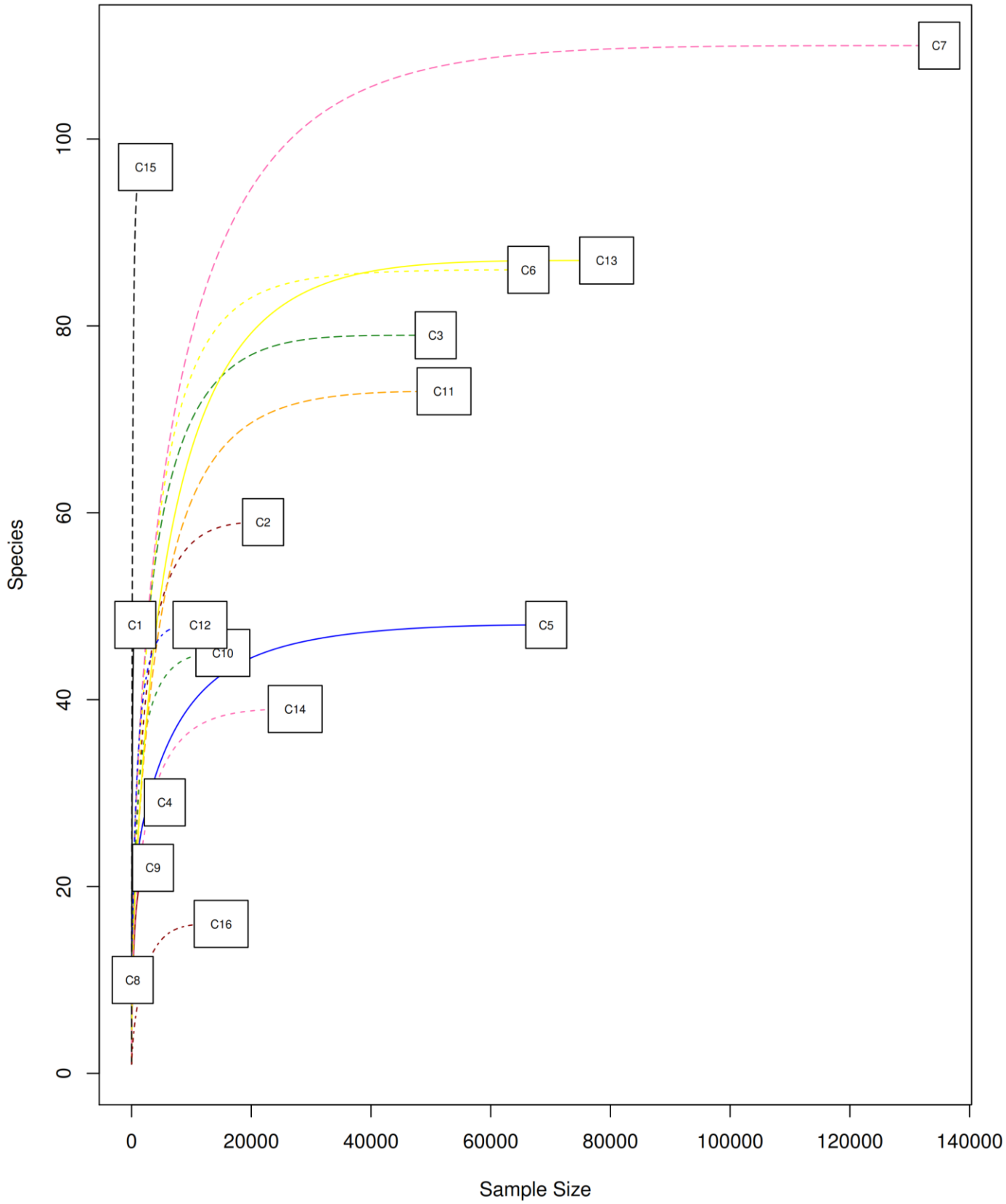

Figure 1a. Rarefaction curves of each farming system replication indicating the level of fungal ITS sequence coverage at Chuka site. C3, C6, C12 and C14 represents Conv-High; C2, C7, C11 and C16 represents Conv-Low; C4, C8, C9 and C15 represents Org-High; C1, C5, C10 and C13 represents Org-Low.

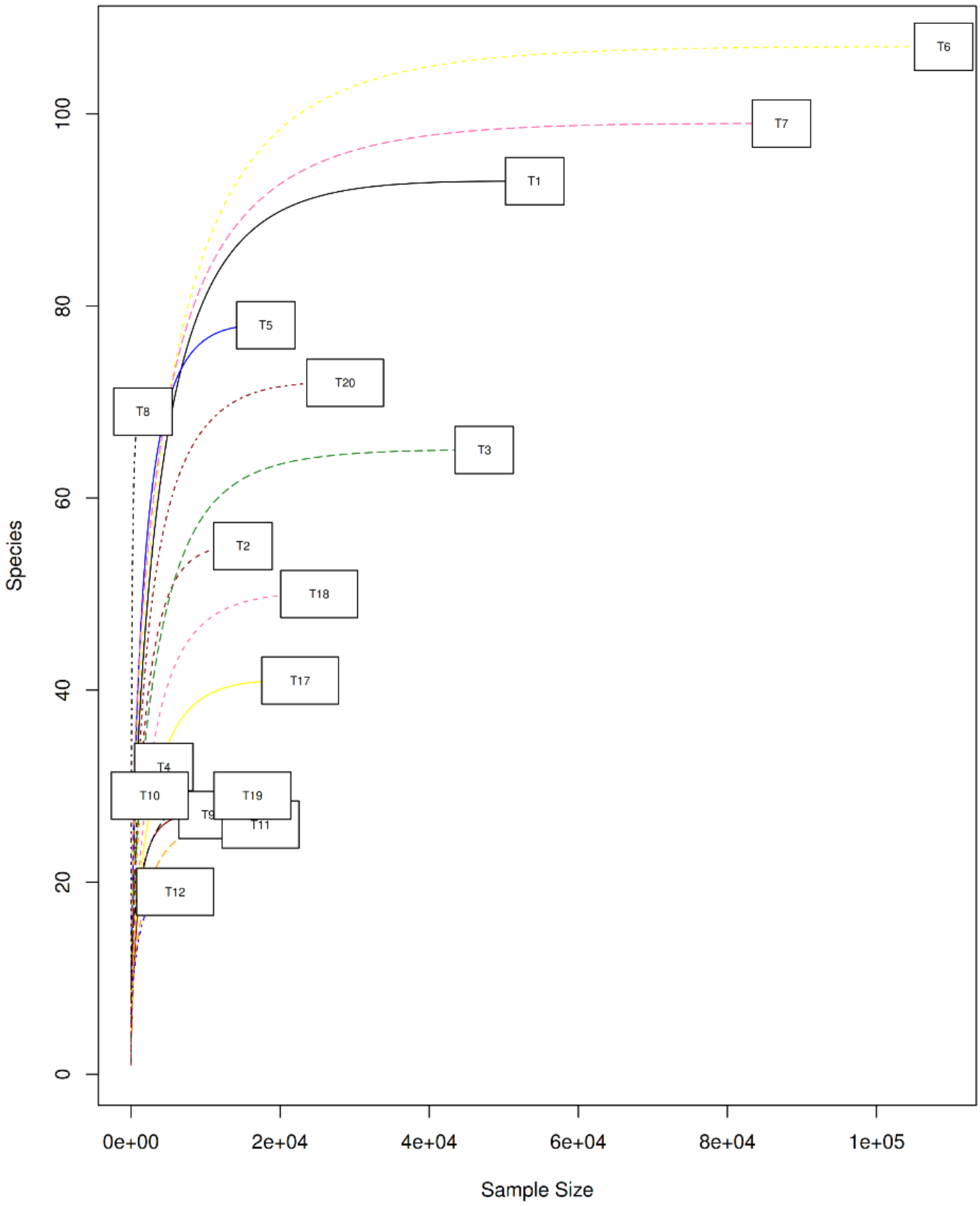

Figure 1b. Rarefaction curves of each farming system replication indicating the level of fungal ITS sequence coverage at Thika site. T2, T7, T9 and T20 represents Conv-High; T1, T6, T12 and T18 represents Conv-Low; T3, T8, T11 and T17 represents Org-High; C1, C5, C10 and C13 represents Org-Low.

Table 3. Distribution of high-quality sequences, Operational Taxonomic Units (OTUs), diversity indices and fungal taxa at Chuka and Thika sites (The farming systems have been sorted as per total number of OTUs in each site).

Site	System	High quality sequences	OTUs	Unique OTUs	Richness (S)	Shannon index (H')	Number of phyla	Number of classes	Number of orders	Number of families	Number of genera	Number of species
Chuka	Conv-Low	224,073	161	82	64.5	1.15	8	18	21	103	134	204
	Org-Low	164,528	155	76	57.0	1.53	8	19	21	103	131	201
	Conv-High	155,879	143	65	63.0	1.53	8	18	21	96	129	196
	Org-High	11,655	113	35	39.5	2.05	8	16	19	92	124	185
Thika	Conv-Low	194,317	168	98	67.3	1.43	8	19	24	101	147	224
	Conv-High	141,355	144	72	63.3	1.44	8	21	24	101	141	213
	Org-High	89,075	128	56	50.3	2.00	8	20	24	101	134	200
	Org-Low	41,306	116	46	42.0	1.49	8	17	20	94	124	189

yield more fungal communities for further classification (Figure 1a and b).

Effect of the farming systems on operational taxonomic units (OTUs)

The high-quality sequences obtained were assigned to 1,128 OTUs at 97% genetic distance. Conventional systems were found to harbor more (both total and unique) OTUs as compared to organic farming systems (Table 3). Taxonomic classification of final OTUs based on UNITE ITS Reference Database and a curated database derived from GreenGenes, RDPII and NCBI grouped the OTUs into a total of eight phyla. Farming systems were dominated by *unassigned fungal* phyla with low input farming systems in both sites scoring the highest relative abundance. Notably, known fungal taxa revealed included *Ascomycota*, *Basidiomycota*, *Chytridiomycota, Glomeromycota*, *Calcarisporiellomycota*, *Kickxellomycota* and *Mortierellomycota*. *Ascomycota* was most abundant in organic systems in both sites while *Chytridiomycota* was dominant in conventional systems in both sites. *Basidiomycota* was dominant in conventional systems at Chuka site whilst *Kickxellomycota* and *Calcarisporiellomycota* phyla were present in all organic systems in both sites but relative abundances were too low to allow their view in Figure 2.

High input systems: unknown fungi, *Basidiomycota* and *Chytridiomycota* were the fungal groups that showed the greatest relative abundance in conventional systems, whereas in the organic systems *Ascomycota* and *Glomeromycota* were the prevalent groups, in both sites. The *Kickxellomycota* phyla occurred more strongly in the Org-High system in Thika site; the same occurred for *Mortierellomycota* phyla in Org-High system in Chuka site (Figure 2).

Low input systems: Unknown fungi and *Chytridiomycota* were more abundant in conventional systems in both sites. In organic systems, unknown fungi, *Basidiomycota* and *Ascomycota* were the most abundant groups, in both sites. In addition, *Chytridiomycota, Glomeromycota* and *Calcarisporiellomycota* phyla were abundant in Org-Low system at Thika site (Figure 2).

Taxonomy assignment at genus level revealed the most abundant genera within farming systems to include; at Chuka site, *Gnomonia*, *Sporobolomyces*, *Saccharomyces* and *Exophiala* in Conv-Low; *Minimedusa*, *Pluteus*, *Macrophomina*, *Leucoagaricus* in Org-Low; *Penicillium*, *Malassezia*, *Aspergillus* and *Marasmius* in Conv-High; and *Alternaria*, *Marasmius*, *Harknessia* and *Laetisaria* in Org-High farming systems. At Thika site, the most abundant genera within farming systems included *Alternaria*, *Spizellomyces*, *Rhizophlyctis* and *Conocybe* in Conv-Low, *Leucoagaricus*, *Marasmius*, *Rhizophagus* and *Mortierella* in Org-Low; *Lepiota*, *Penicillium*, *Phialemonium* and *Conocybe* in Conv-High; and *Racocetra*, *Tomentella*, *Spizellomyces* and *Ramicandelaber* in Org-High farming systems (Figure 3). The

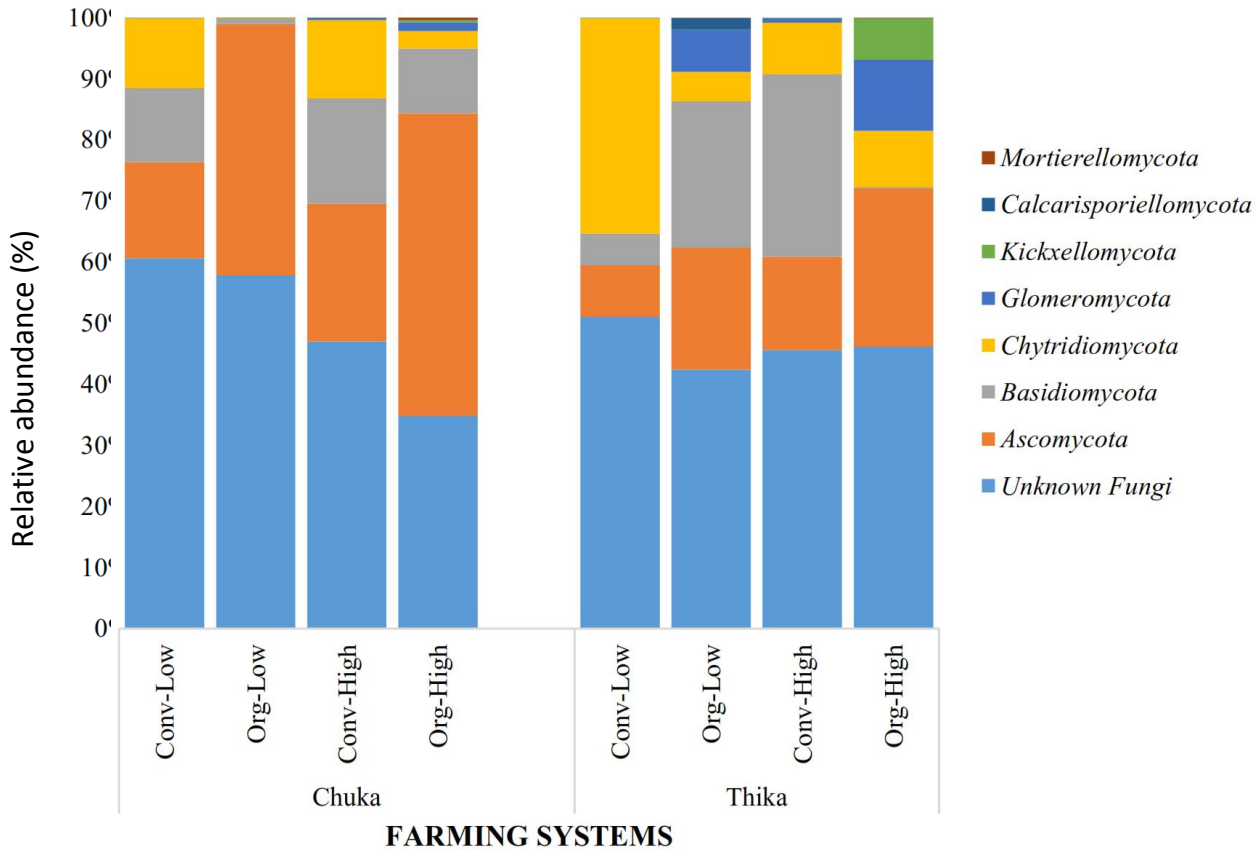

Figure 2. Relative abundance of fungal taxa at phylum level as revealed in the long-term comparison trials at Chuka and Thika sites.

distribution of various fungal OTUs and taxonomic groups within farming systems in both sites are as shown in Table 3.

Diversity indices of soil fungal communities as influenced by farming systems

Alpha diversity was applied to analyze species' diversity in each farming system through Richness (S) and Shannon index (H'). In both sites, there was a higher species richness in conventional farming systems. For instance, at Chuka site, species richness was: Conv-High = 63, Org-High = 39.5, Conv-Low = 64.5 and Org-Low = 57; while at Thika site, the species richness was Conv-High = 63.3, Org-High = 50.3 Conv-Low = 67.3 and Org-Low = 42. However, fungal communities within organic farming systems were more diverse (H) as compared to conventional farming systems (Table 3). At Chuka site, Analysis of Similarity (ANOSIM) of fungal diversity within farming systems indicated significant differences between fungal community OTUs within high and low input farming systems at 95 % level of confidence (P value = 0.05 and R = 0.115). However, there were no significant differences observed at Thika site (P value = 0.17 and R=0.066).

Effect of farming systems on beta diversity of fungal communities

Beta diversity analysis was used to evaluate differences in species complexity among the farming systems. Beta diversities were based on non-metric multidimensional scaling and hierarchical clustering. β-diversity analyzed by community comparison of the Non-metric multidimensional scaling plot indicated the four different ellipses formed by each farming system. There was an overlap of ellipses between farming systems indicating that some fungal taxa were shared across farming systems; while numerous taxa appeared outside the ellipses, signifying that the fungal taxa revealed were highly diverse (Figure 4). At Chuka site, diversity was higher in Org-High system while at Thika, Org-Low system revealed the highest diversity of fungal communities as shown by Shannon index (H') (Table 3).

Hierarchical clustering analysis was done to compare the similarity and dissimilarity of most abundant fungal

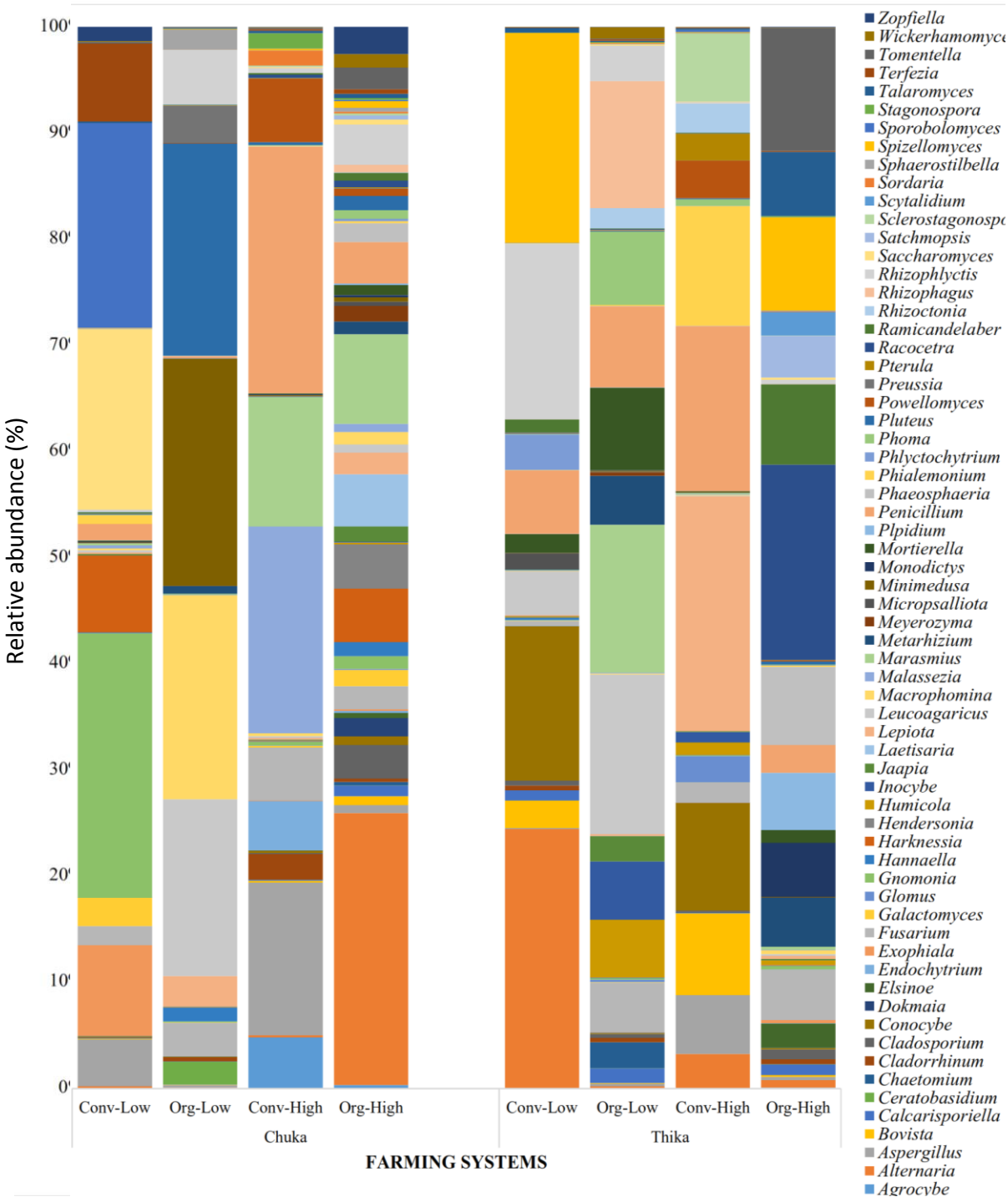

Figure 3. Relative abundance of the most predominant fungal taxa at genus level as revealed in the long-term comparison trials at Chuka and Thika sites.

taxa at family level as well as clustering of the four farming systems in each site. The hierarchical heatmap of fungal community was generated based on bray–curtis distance indices, displaying the relative abundances of fungal communities across farming systems. The dendrogram revealed two main groups within farming systems; the first group consisting of Org-High systems in both sites. Within the second group, Conv-Low systems in both sites and; Chuka Conv-High and Thika Org-Low systems were shown to cluster together. Thika

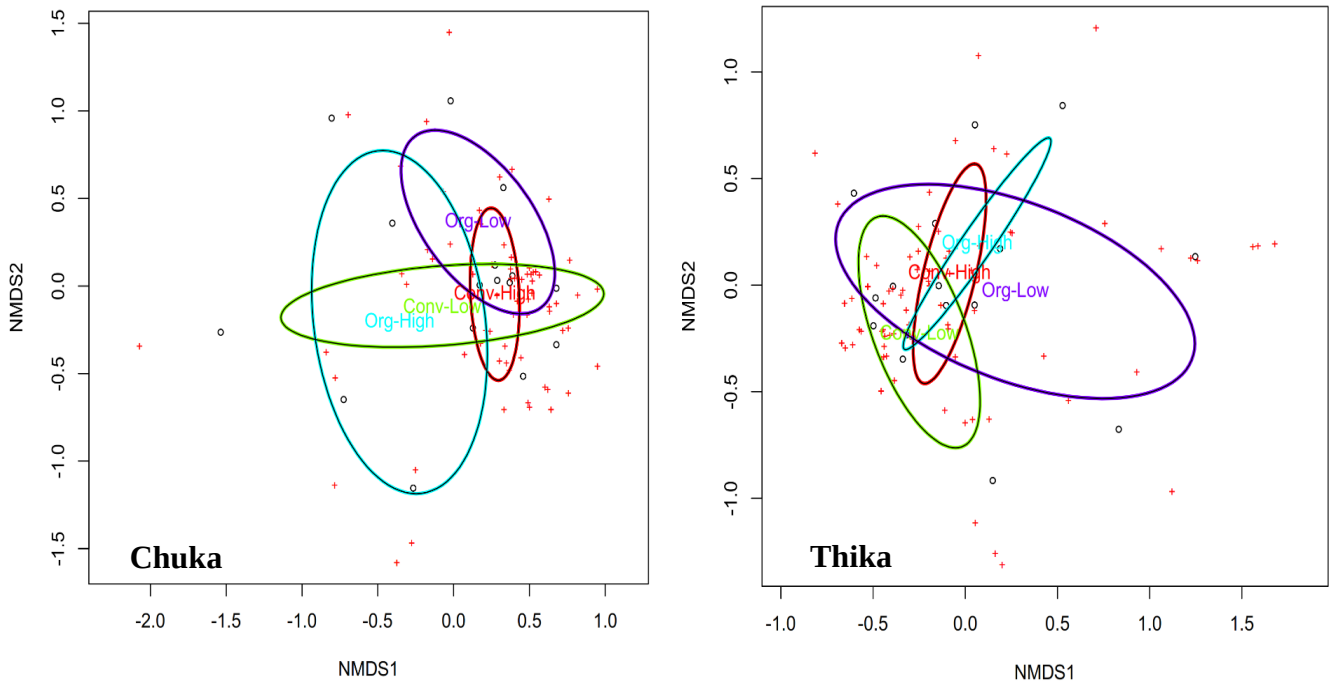

Figure 4. Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities between fungal taxa at species level grouped according to farming systems.

Conv-High and Chuka Org-Low systems were outliers within the second group on the dendrogram as shown in Figure 5. Although some farming systems were shown to cluster together, they harbored different fungal taxa, an indication that soil ecosystem supports a diverse group of microorganisms.

Key environmental drivers of fungal community diversity and structure

In order to assess how environmental variables shaped soil fungal community composition, Principal Component Analysis (PCA) was performed on soil physicochemical characteristics within farming systems and fungal taxa at species level. Each characteristic was assessed on its ability to positively or negatively influence diversity within sites and farming systems. At Chuka, pH, C, N, Zn, Fe and Al were designated as major drivers of fungal diversity within farming systems while at Thika, key properties displayed were pH, EC, C, N, K, Fe, Zn, B and micro-aggregate (MA) as shown in Figure 6. Aluminum (Al) was shown to have a negative influence on fungal diversity at Chuka site.

DISCUSSION

This study combined high-resolution power of Illumina sequencing technology and analysis of fungal ITS amplicon sequences to assess the effects of organic and conventional farming systems on the diversity and composition of fungi and generate a taxonomic profile within long-term experiment trial sites in the central highlands of Kenya. The number of OTUs and alpha diversity analysis gives a glimpse of the resident fungal diversity. Eight fungal phyla (*Ascomycota*, *Basidiomycota*, *Chytridiomycota*, *Glomeromycota*, *Calcarisporiellomycota*, *Kickxellomycota*, *Mortierellomycota* and *unknown fungal phyla*) were identified at Thika and Chuka sites. Taxonomic composition analysis indicated *unknown fungal phyla, Ascomycota*, *Basidiomycota*, *Chytridiomycota* and *Glomeromycota* as the most predominant phyla within both sites and farming systems. *Ascomycota* and *Basidiomycota* are important decomposers in carbon cycle. They break down organic substances such as cellulose, lignocellulose, and lignin within plant residues into micro-molecules hence, promoting the carbon cycle in soil (Purahong et al., 2016). At family level, unique families to Chuka site included; *Unknown Pleosporales*, *Lentitheciaceae, Unknown Eurotiales* and *Unknown Cystobasidiomycetes* while at Thika site, unique families included *Didymellaceae*, *Periconiaceae*, *Phaeosphaeriaceae*, *Thyridariaceae*, *Chaetosphaeriaceae*, *Plectosphaerellaceae*, *Clavicipitaceae*, *Ophiocordycipitaceae*, *Unknown Sordariomycetes*, *Unknown Xylariales*, *Lentinaceae*,

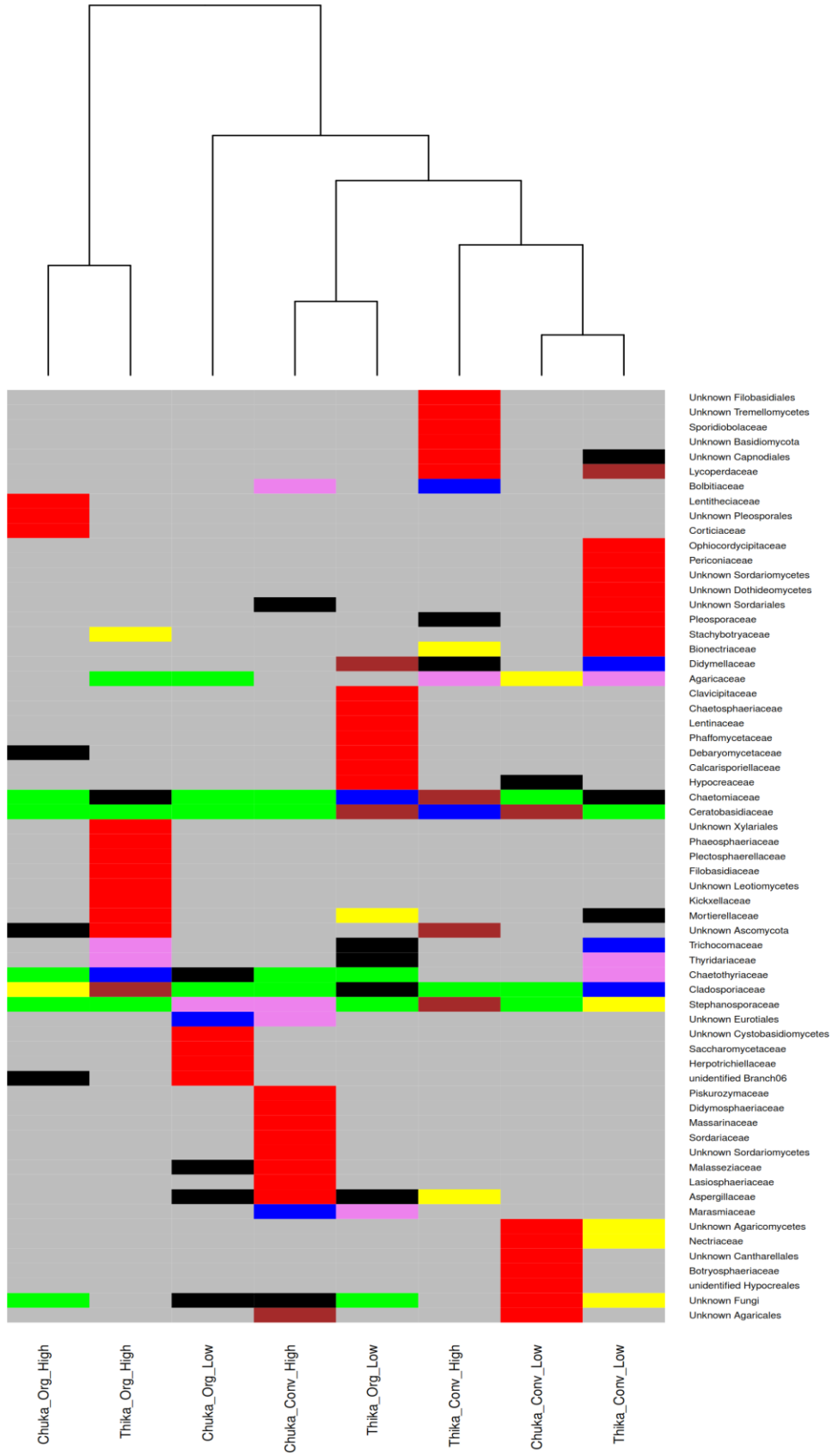

Figure 5. Hierarchical clustering of most predominant fungal taxa at family level in both sites. X-axis indicates the farming systems at Chuka and Thika.

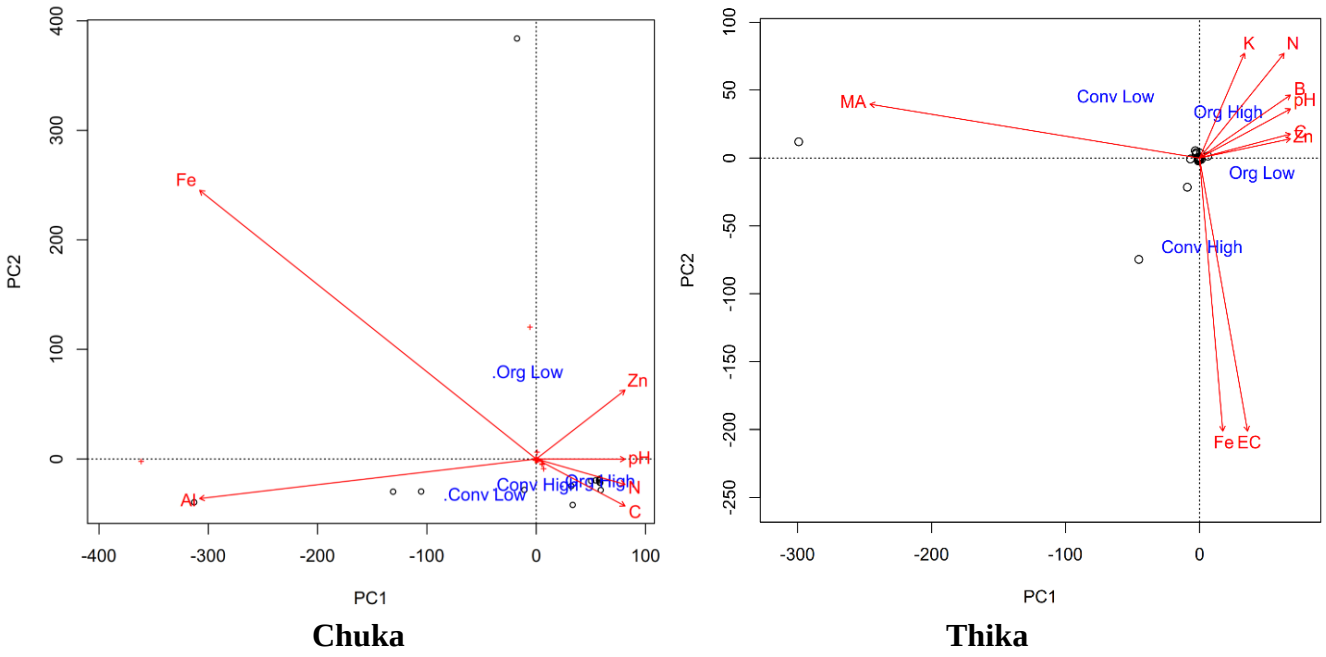

Figure 6. Principal component analysis of soil physicochemical characteristics that drive diversity within farming systems.

Filobasidiaceae, *Unknown Filobasidiales*, *Unknown Tremellomycetes* and *Mortierellaceae*. At genus level, potentially phytopathogenic genera (Sharma-Poudyal et al., 2017; Fraç et al., 2018) were revealed and they included *Alternaria* (scored up to 92% relative abundance at Chuka Org-High and 87% at Thika Conv-Low), *Epicoccum (*1.4% relative abundance at Chuka Org-High and 0.1% at Thika Org-Low), *Fusarium* (17% relative abundance at Chuka Conv-High and 17% at Thika Org-High and Org-Low), *Olpidium* (0.4% relative abundance at Chuka Org-High and 20% at Thika Org-High), *Phoma* (2.3% relative abundance at Chuka Org-High and 26.3% at Thika Org-Low), *Rhizoctonia* (0.2% relative abundance at Chuka Org-High and 10.7% at Thika Conv-High), and *Stagonospora* (5.4% relative abundance at Chuka Conv-High and 0.4% at Thika Org-High). Other major putative plant pathogenic groups revealed included members of *Nectriaceae*, *Ceratobasidiaceae, Bionectriaceae, Phaeosphaeriaceae* and *Mycosphaerellaceae* families.

Some potentially plant beneficial fungal genera (Madi et al., 1997; Harman et al., 2004; Fraç et al., 2018) were revealed within farming systems. They included; *Glomus* (scored up to 0.2% relative abundance at Chuka Org-High and 9.7% at Thika Conv-High), *Trichoderma* (0.5% relative abundance at Chuka Org-High and 0.3% at Thika Org-Low) and *Talaromyces* (1.5% relative abundance at Chuka Org-High and 22.1% at Thika Org-High). *Glomus species* have plant endosymbiotic properties especially arbuscular mycorrhizal fungi which form symbiotic relationships with plant roots (Harman et al., 2004). The species within *Glomus* genus consisted of *Glomus cerebriform*, *Rhizophagus intraradices*, *Rhizophagus diaphanum* and unknown *Glomus species*. *Trichoderma* and *Talaromyces* are prominent biocontrol agents with antagonistic potential and mycoparasitic life-style (Harman et al., 2004). *Trichoderma* genus included *Hypocrea lixii*, *Hypocrea koningii*; while *Talaromyces* genus included *Talaromyces islandicum*, *Talaromyces rotundus* and unknown *Talaromyces* species. Plant inoculation with *Epicoccum nigrum* and *Trichoderma atroviride* has been reported to protect potato against *Rhizoctonia solani* (Lahlali and Hijri, 2010). In this study, *Epicoccum nigrum* and *Epicoccum sorghi* were among the fungal species found within farming systems. The presence of potential phytopathogens, recognized plant beneficial fungi, biocontrol agents, mycoparasites and plant endosymbiont fungal groups within farming systems was similar to a previous study carried out to analyze the fungal community profiles in agricultural soils of a long-term field trial under different tillage, fertilization and crop rotation conditions (Sommermann et al., 2018). The study revealed eight potentially phytopathogenic genera, namely *Alternaria*, *Bionectria*, *Epicoccum*, *Fusarium*, *Olpidium*, *Phoma, Rhizoctonia, Stagonospora, Ophiosphaerella* and *Verticillium*. Among the biocontrol agents identified were *Trichoderma sp*., *Coniothyrium minitans* and *Talaromyces* some of which have designated efficacy against phytopathogens (Sommermann et al., 2018).

A few groups of fast-growing soil-inhabiting saprophytic fungi and root colonizers such as *Humicola* (Family *Chaetomiaceae*), *Mortierella* (Family *Mortierellaceae*) and

Exophiala (Family *Herpotrichiellaceae*) were revealed. Some species within these genera are potential pathogens while others are considered potential biocontrol agents and may benefit plant health (Sommermann et al., 2018). Also common within the farming systems were *Penicillium* and *Aspergillus* (Family *Trichocomaceae*), common cellulolytic colonizers of soil and plant residues (Sharma-Poudyal et al., 2017).

Fungal diversity in all farming systems was majorly dependent on the flow of nutrients within the soil. Composition and diversity assessment of fungal communities within sites and farming systems displayed Thika site to harbor more OTUs as compared to Chuka site. This could be attributed to the presence of high small macro-aggregates that provided unique environmental habitats for soil fungi. Macro-aggregates have been considered as massively concurrent incubators that allow enclosed microbial communities to pursue their own independent progression (Rillig et al., 2017), hence creating more unique habitats for microbial colonization within these farming systems. Chuka soils contained high primary and secondary clay minerals, while Thika soils were characterized by high primary minerals and low secondary clay minerals. Clay minerals and oxides of Fe and Al have been exhibited to play important roles in adsorbing dissolved organic carbon (Singh et al., 2016, 2017b). Since Thika soils contained high Fe levels coupled with high primary clay minerals, this may have created a stable atmosphere for fungal groups to thrive. At Chuka site, fungal diversity was also negatively influenced by high Al levels, hence low OTU numbers obtained. However, in both sites, Conv-Low had the highest number of OTUs (161 and 168 OTUs at Chuka and Thika respectively) compared to other farming systems. This could be attributed to the application of undecomposed farmyard manure as input component in the system during planting. The fungal diversity within farming systems is influenced by complex interactions between a wide range of soil properties and agronomic inputs, thus signifying that fungi within the soils are exceptionally diverse. These inputs change soil properties and microbial diversity, and the microbial community in turn manipulates nutrient cycling processes altering soil fertility, plant productivity and environmental sustainability.

Conclusion

This study revealed that farming systems have a profound impact on soil fungal communities. Conventional farming systems were shown to support diverse fungal communities compared to organic farming systems. This was possibly due to the integration of organic and inorganic inputs into conventional farming systems which enhanced nutrient availability for fungal proliferation, thus increasing their diversity. The results of this study provide a foundation for further studies on the regulation of quality and quantity of farming inputs and could provide guidance for selecting the best farming system model to protect soil ecology.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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ABBREVIATIONS

ITS, Internal Transcribed Spacer; **OTUs,** Operational Taxonomic Units; **DNA,** Deoxyribonucleic Acid; **QIIME,** Quantitative Insights into Microbial Ecology.

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Table S1. SysCom trials soil fertility managment plan; crop rotation cycle, inputs types used, amount of nitrogen and phosphorous contained in the inputs and amounts applied per hectare.

Year	Season		CONV LOW		CONV HIGH		ORG LOW		ORG HIGH	
1	Long	**Crop**	**Maize**		**Maize**		**Maize**		**Maize**	
		Starter	5 t fresh FYM 50 kg DAP	22 kg N; 6 kg P; 9 kg N; 12 kg P;	Approx. 5 t rotten FYM (start with 7.5 t) 200 kg DAP	34kg N; 9 kg P; 36kg N; 46 kg P;	Rotten FYM (started with 5 t fresh FYM) 1.36 t Tithonia mulch (FW) 100 kg Phosphate rock	22 kg N; 6 kg P; 9 kg N; 1 kg P; 11 kg P;	Compost (start with 7.5 t fresh FYM) 5.4 t Tithonia mulch (FW) 364 kg Phosphate rock	34 kg N; 9 kg P; 36 kg N; 3 kg P; 40 kg P;
		Top dressing	No		100 kg CAN	26 kg N;	No	No	3.9 t Tithonia (FW) as mulch or liquid manure	26 kg N; 2 kg P;
		Lime	No		Based on pH monitoring		No		Based on pH monitoring	
		Micro-nutrients	No		Based on soil analysis and crop requirements		No		Based on soil analysis and crop requirements	
		Green manures	No		No		No		Mucuna	
1	Short	**Crop**	**Kales&Swiss chard ("spinach")**		**Cabbage**		**Kales&Swiss chard ("spinach")**		**Cabbage**	
		Starter	1 t fresh FYM 50 kg TSP	4 kg N; 1 kg P; 12 kg P;	Approx. 10 t rotten FYM (start with 15 t) 200 kg TSP	67 kg N; 18 kg P; 46 kg P;	Rotten FYM (started with 1 t fresh FYM) 1.2 t Tithonia mulch (FW) 90 kg Phosphate rock	4 kg N; 1 kg P; 8 kg N; 1 kg P; 10 kg P;	Compost (start with 15 t fresh FYM) 6 t Tithonia as mulch 400 kg Phosphate rock	67 kg N; 18 kg P; 40 kg N; 4 kg P; 44 kg P;
		Top dressing	60 kg CAN	16 kg N	300 kg CAN	78 kg N	1.2 t Tithonia FW as liquid manure	8 kg N; 1 kg P	6 t Tithonia FW as liquid manure	40 kg N; 4 kg P;
		Lime	No		Based on pH monitoring		No		Based on pH monitoring	
		Micro-nutrients	No		Based on soil analysis and crop requirements		No		Based on soil analysis and crop requirements	
		Green manures	No		No		No		Mucuna	
2	Long	**Crop**	**Maize**		**Baby corn**		**Maize**		**Baby corn**	
		Starter	5 t fresh FYM 50 kg DAP	22 kg N; 6 kg P; 9 kg N; 12 kg P;	Approx. 7.5 t rotten FYM (start with 11.3 t) 200 kg DAP	51 kg N; 14 kg P; 36 kg N; 46 kg P;	Rotten FYM (started with. 5 t fresh FYM) 1.36 t Tithonia mulch (FW) 100 kg Phosphate rock	22 kg N; 6 kg P; 9 kg N; 1 kg P; 11 kg P;	Compost (start with 11.3 t fresh FYM) 5.4 t Tithonia mulch (FW) 364 kg Phosphate rock	51 kg N; 14 kg P; 36 kg N; 3 kg P; 40 kg P;
		Top dressing	No		100 kg CAN	26 kg N;	No	No	3.9 t Tithonia (FW) as mulch or liquid manure	26 kg N; 2 kg P;
		Lime	No		Based on pH monitoring		No		Based on pH monitoring	
		Micro-nutrients	No		Based on soil analysis and crop requirements		No		Based on soil analysis and crop requirements	
		Green manures	No		No		No		Mucuna	
2	Short	**Crop**	**Grain legumes & …**		**French beans**		**Grain legumes & …**		**French beans**	
		Starter	No		Approx. 7.5 t rotten FYM (start with 11.3 t) 200 kg DAP	51 kg N; 14 kg P; 36 kg N; 46 kg P;	No		Compost (start with 11.3 t fresh FYM) 5.4 t Tithonia mulch (FW) 364 kg Phosphate rock	51 kg N; 14 kg P; 36 kg N; 3 kg P; 40 kg P;
		Top dressing	No		100 kg CAN;	26 kg N;	No		3.9t Tithonia (FW) as liquid manure	26 kg N; 2 kg P;
		Lime	No		Based on pH monitoring		No		Based on pH monitoring	
		Micro-nutrients	No		Based on soil analysis and crop requirements		No		Based on soil analysis and crop requirements	
		Green manures	No		No		No		Mucuna	
3	Long	**Crop**	**Maize**		**Baby corn**		**Maize**		**Baby corn**	
		Starter	5 t fresh FYM 50 kg DAP	22 kg N; 6 kg P; 9 kg N; 12 kg P;	Approx. 7.5 t rotten FYM (start with 11.3 t) 200 kg DAP	51 kg N; 14 kg P; 36 kg N; 46 kg P;	Rotten FYM (started with 5t fresh FYM) 1.36 t Tithonia mulch (FW) 100 kg Phosphate rock	22 kg N; 6 kg P; 9 kg N; 1 kg P; 11 kg P;	Compost (start with 11.3 t fresh FYM) 5.4 t Tithonia mulch (FW) 364 kg Phosphate rock	51 kg N; 14 kg P; 36 kg N; 3 kg P; 40 kg P;

Table S1. Contd.

		Top dressing	No		100 kg CAN	26 kg N;	No		3.9 t Tithonia (FW) as mulch or liquid manure	26 kg N; 2 kg P;
		Lime	No		Based on pH monitoring		No		Based on pH monitoring	
		Micro-nutrients	No		Based on soil analysis and crop requirements		No		Based on soil analysis and crop requirements	
		Green manures	No		No		No		Mucuna	
		Crop	**Irish potatoes&local vegetables**		**Irish potatoes**		**Irish potatoes&local vegetables**		**Irish potatoes**	
3	**Short**	**Starter**	2 t fresh FYM 100 kg DAP	9 kg N; 2 kg P; 18 kg N; 23 kg P;	Approx. 7.5 t rotten FYM (start with 11.3 t) 300 kg TSP 200 kg CAN	51 kg N; 14 kg P; 69 kg P; 52 kg N;	Rotten FYM (started with 2 t fresh FYM) 2.72 t Tithonia (FW) 200 kg Phosphate rock	9 kg N; 2 kg P; 18 kg N; 2 kg P; 22 kg P;	Compost (start with 11.3 t fresh FYM) 8.2 t Tithonia mulch (FW) 581 kg Phosphate Rock	51 kg N; 14 kg P; 54 kg N; 5 kg P; 64 kg P;
		Top dressing	Nothing		Nothing		Nothing		Nothing	
		Lime	No		Based on pH monitoring		No		Based on pH monitoring	
		Micro-nutrients	No		Based on soil analysis and crop requirements		No		Based on soil analysis and crop requirements	
		Green manures	No		No		No		Mucuna	
		TOTAL	18 t FYM 250 kg DAP 50 kg TSP 60 kg CAN	79 kg N; 21 kg P 45 kg N; 59 kg P; 12 kg P; 16 kg N	Approx. 45 t rotten FYM (start with 68 t) 800 kg DAP 500 kg TSP 900 kg CAN	305 kg N; 83 kg P; 144 kg N; 184 kg P; 115 kg P; 234 kg N;	Rotten FYM (started with 18 t fresh FYM) 9.2 t Tithonia 590 kg Phosphate rock	79 kg N; 21 kg P; 61 kg N; 6 kg P; 65 kg P;	Compost (start with 68 t FYM FW) 65 t Tithonia 2392 kg Phosphate Rock	305 kg N; 83 kg P; 382 kg N; 33 kg P; 268 kg P;
				140 kg N; 92 kg P		**683 kg N; 382 kg P**		**140 kg N; 92 kg P**		**683 kg N; 382 kg P**

Nutrient contents: FYM/compost (DW): 1.12% total N and 0.3% P (Lekasi et al., 2003); DM of FYM is assumed to be 40%; Tithonia diversifolia (DW): 3.3% N; 0.31% P; 3.1% K (Nziguheba et al. 2004); DM of Tithonia = 20%; Phosphate rock from West Africa (Finck): 11 - 13% P; DAP: 18% N; 23% P; TSP: 23% P; CAN: 26% N.