Communication

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Small Farm Holder Cropping Systems Influence Microbial Profiles in an Equatorial Rainforest Agroecosystem

Christine Matindu [1], Nimalka M. Weerasuriya [2], Francis Muyekho [1], Irena F. Creed [2,3,*], R. Greg Thorn [2] and Anthony W. Sifuna [1]

[1] Masinde Muliro University of Science and Technology, Kakamega, Kenya
[2] Department of Biology, Western University, London, Canada
[3] Department of Physical and Environmental Sciences, University of Toronto, Toronto, Canada
* Correspondence: irena.creed@utoronto.ca

Abstract: Metabarcoding of prokaryotic and fungal ribosomal DNA was used to describe the microbial communities in soils of remnant equatorial rainforest, maize-bean intercrop, and sugarcane in western Kenya. Cropping systems influenced the microbial community composition and functional traits (energy source and nutrient cycling) of bulk soil in each crop. Microbial richness and diversity tended to increase with cultivation intensity. Soil of maize-bean intercrop had lower percentages and sugarcane had higher percentages of unique amplicon sequence variants of both bacteria and fungi compared to the remnant forest. Functional traits were altered by cultivation intensity. Compared to remnant forest soils, maize-bean intercrop soil had lower percentages of aerobic chemoheterotrophic bacteria and higher percentages of N-cycling bacteria, while sugarcane had higher percentages of aerobic chemoheterotrophic bacteria and lower percentages of N-cycling bacteria. In the face of increasing forest loss and pressures for agricultural productivity, this landscape provides a rich site for studying the impacts of cropping systems on soil health.

Keywords: cropping systems; maize-bean; microbial profiles; sugarcane; soil properties; equatorial rainforest

1. Introduction

In the equatorial rainforest region, permanent and continuous cultivation of extractive crops has the potential to fundamentally alter soil properties (e.g., reduction in soil organic matter content, changes in soil structure, losses in nutrient retention and erosion resistance) and soil microbial communities [1], which in turn may lower crop yield [2,3]. Shortened fallow periods and year-round cultivation practices are implemented in smallholder farms [4] for the production of food crops (e.g., sole maize or maize-bean intercropping) [5] or cash crops (e.g., sugarcane) [6]. Soil microbial community health is linked to crop productivity and sustainability [7], so it is invaluable to understand how continuous cropping practices in low-input systems change the structure of these microbial communities.

Within the equatorial rainforest region of the River Isiukhu watershed of Kakamega County in western Kenya, we compared soil properties and soil microbial communities in two cropping systems commonly used by farmers in this region—maize-bean intercrops and pure sugarcane fields—and in pristine remnant forest soils.

2. Materials and Methods

The River Isiukhu watershed is a humid forest agroecological zone with poorly drained clay ferralsols in the south and well-drained sandy clay acrisols in the north [8]. We sampled (1) undisturbed soils in the Kakamega Forest Reserve near Maghaka (Ma) in Kenya (F, $n = 3$), and (2) soils in croplands in Bukhaywa (Bu; sugarcane only), Ikolomani (Ik), and Township (Tw) where maize-bean intercrop (MB, $n = 9$) or sugarcane crops (Sc, $n = 9$) had been planted continuously for at least five years.

Five replicates of approximately 400 g from the top 20 cm of soil were collected and then pooled from each location (Ma, Bu, Ik, Tw) for each cropping system (F, MB, Sc) and analyzed for: soil texture (%Sand, %Silt, %Clay) [9], bulk density (BD; g cm−3) [10], pH [11], % organic carbon (%OC) [12], and % nitrogen (%N) [10]. Total N (TN) and soil organic carbon (SOC) in Mg ha−1 were estimated from % dry weight [13].

A subset of 50 g of soil was pooled for each cropping system and analyzed for microbial community profiles. Each sample underwent DNA extraction using 0.25 g of soil followed by PCR amplification using (1) 16S rDNA V4 region prokaryote primers: U518F/806R [14,15] and (2) 28S rDNA Ascomycota (fungi) primers: LSU200A- F/LSU476A-R [16]. Libraries were sequenced using an Illumina MiSeq at the London Regional Genomics Centre (Robarts Research Institute, London, Canada). Amplicon sequence variants (ASVs; clusters of 100% identical sequence reads) were created and classified using DADA2 v1.10.1 [17] and the SILVA 132 [18] reference dataset.

Kruskal-Wallis one-way analysis of variance on ranks and Dunn's pairwise ($p < 0.05$) comparisons were used to compare soil properties among locations (Ma, Bu, Ik, Tw) and among cropping systems (F, MB, Sc). Microbial data were analyzed and visualized using microeco v0.5.2 [19], with functions assigned to taxa identified using FAPROTAX [20] and FUNGuild [21] in R v4.1.1.

3. Results

3.1. Soil Properties

The remnant forest system occurred in a relatively restricted range of soil properties—sandier, more acidic, and lower organic carbon, both OC (%) and SOC (Mg/ha)—compared to the two cropping systems. In contrast, the two cropping systems occurred in a broad range of soil conditions—sandy to clay/silt rich, low to high acidity, low to high organic carbon and nitrogen (both N (%) and TN (Mg/ha)) (Table 1).

Table 1. A one-way ANOVA on ranks (Kruskal-Wallis) by cropping system and location. Dunn's pairwise ($p < 0.05$) comparisons were adjusted for multiple comparisons using the Holm adjustment, where * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$, ns = not significant ($p \geq 0.05$). Sampling locations include: Ma—Magakha, Bu—Bukhaywa, Ik—Ikolomani, and Tw—Township. Cropping systems include: F—forest, MB—maize-bean, and Sc—sugarcane.

Soil Chemistry		**pH**	**%N**	**TN**	**%OC**	**SOC**	**BD**	**%Sand**	**%Silt**	**%Clay**
Cropping System										
Kruskal Wallis *p*-value		0.016	0.293	0.324	0.034	0.033	0.282	0.03	0.088	0.081
Dunn's Pairwise Comparisons	F-MB	*			ns	ns		*		
	F-Sc	*			ns	ns		*		
	MB-Sc	ns			*	ns		ns		
Location										
Kruskal Wallis *p*-value		0.002	0.125	0.233	0.024	0.038	0.059	<0.001	<0.001	<0.001
Dunn's Pairwise Comparisons	Ma-Bu	**			ns	ns		ns	ns	ns
	Ma-Ik	ns			ns	ns		***	**	**
	Ma-Tw	*			ns	ns		ns	ns	ns
	Bu-Ik	ns			*	ns		****	****	***
	Bu-Tw	ns			ns	ns		ns	ns	ns
	Ik-Tw	ns			ns	ns		**	*	*

3.2. Microbial Diversity

The relative abundance of prokaryotes (bacterial and archaeal classes) and Ascomycota differed among the intact forest and the two cropping systems. Among prokaryotes, the largest differences were among classes Bacilli and Gammaproteobacteria (highest in F), Blastocatellia, Alphaproteobacteria (all Eubacteria), and Nitrososphaeria (Archaea) (highest in MB), and the Eubacteria Actinobacteria, Chitinophagia, and Verrucomicrobia (highest in Sc) (Figure S1A). Among orders of Ascomycota, Hypocreales had the highest relative abundance in all three systems (greatest in F, moderate in MB, and lowest in Sc), followed by Pleosporales, Capnodiales, Onygenales, Sordariales, and Helotiales (Figure S1B). Soils of Sc had by far the highest microbial richness and diversity, followed MB and F (Table 2), and had the largest number of unique prokaryote and ascomycotan ASVs, followed by F and MB (Figure 1). Of the top 40 fungal and prokaryote ASVs, 29 fungi and 20 bacteria were confidently identified to genus (Figure S2).

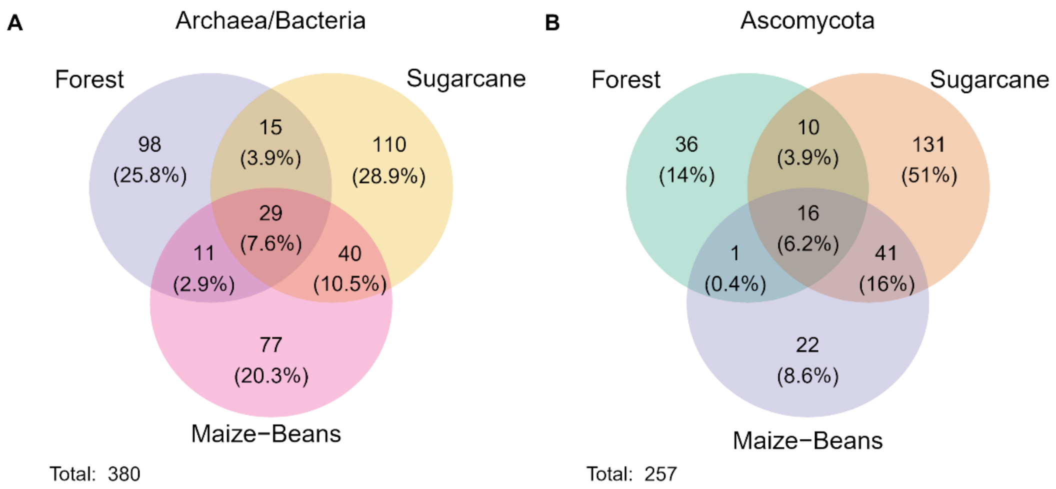

Figure 1. Venn diagram of shared and unique amplified sequence variants (clustered at 100% identity) between (A) prokaryote and (B) ascomycota primers for different cropping regimes. Cropping system include: F—forest, MB—maize-bean, Sc—sugarcane.

Table 2. Observed microbial richness (Richness) and diversity (Fisher's alpha, Shannon, Inverse Simpson) indices under forest (F), maize-bean (MB), and sugarcane (Sc) soils.

		Richness (*S*)	**Fisher's α (*S*)**	**Shannon (*H′*)**	**InvSimpson (λ^{-1})**
Archaea/ Bacteria	**F**	153	17.1	4.7	73.0
	MB	157	17.7	4.7	64.7
	Sc	194	33.0	4.8	75.3
Fungi (Ascomycota)	**F**	63	6.9	2.9	8.1
	MB	80	9.8	3.3	9.5
	Sc	132	15.5	4.1	28.2

3.3. Microbial Functional Traits

Forest soils and the two cropping systems, particularly Sc, all had prokaryotes with energy source functional traits dominated by aerobic chemoheterotrophy. More prokaryotes capable of fermentation (C-cycling) were found in F soil, followed by Sc and then MB with the least. Forest soils had among the highest diversity of microbes representing the largest diversity of N-cycling functional traits (nitrification, nitrate reduction, ammonia oxidation, and nitrate oxidation). In comparison to F, MB soils had similar percentages of N-cycling microbes but these were concentrated in a smaller number of N-cycling functional traits (nitrogen fixation, nitrification, and ammonia oxidation), and Sc soils had fewer still, concentrated in the smallest number of N-cycling functional traits (nitrification and ammonia oxidation) (Figure 2A). All soils had primarily saprotrophic ascomycotan fungi, with a smaller proportion of symbiotrophs and pathotrophs. Again, F soils had the greatest diversity of fungal functional groups, followed by Sc and MB (Figure 2B), a pattern that was not seen in taxonomic diversity, and MB and Sc fungal guilds and trophic modes were proportionally similar in comparison to F.

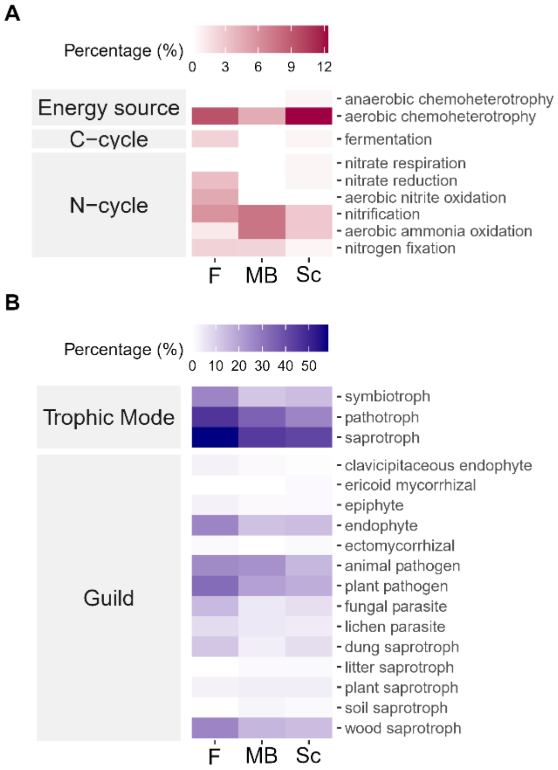

Figure 2. Select functional traits of (A) prokaryotes (FAPROTAX), and (B) ascomycotan fungi (FUNGuild) as a percent of unique amplified sequence variants in each cropping system: F—forest (prokaryotes $n = 25$; fungi $n = 19$), MB—maize-bean (prokaryotes $n = 20$; fungi $n = 26$), Sc—sugarcane (prokaryotes $n = 34$; fungi $n = 43$).

4. Conclusions

This communication presents relatively rare data on the soil properties and microbial communities in arable lands with more than five years of continuous maize-bean or sugarcane cultivation in the River Isiukhu watershed of western Kenya. We found that cropping systems influence the composition and functions of microbial communities relative to that of the remnant original forest. Intercrop maize-bean cultivation had higher SOC and %OC than sugarcane and undisturbed forest soils, with a larger proportion of N-cycling bacteria. However, sugarcane had higher bacterial diversity and richness than maize-bean and forest soils. As this was a descriptive study based on a limited number of samples, future work will benefit from increased replication and the inclusion of other cropping systems common in the region. Extractive cropping practices have the potential to fundamentally alter soil chemistry and microbial communities, and their energy and nutrient cycling processes in complex ways.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figure S1: Relative abundance of amplified sequence variants grouped by (A) prokaryote class (16S RNA) and (B) Ascomycota order (28S RNA) for each cropping system; Figure S2: Log relative abundance heatmap of the top 40 ASVs within (A) prokaryote and (B) Ascomycota samples.

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Data Availability Statement: Raw Illumina MiSeq data was uploaded to ENA under project accession PRJEB44367 and is openly available at https://www.ebi.ac.uk/ena/browser/view/PRJEB44367. Other metadata, R code, and intermediate files are openly available in FigShare at doi:10.6084/m9.figshare.25152740.

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Conflicts of Interest: The authors declare no conflicts of interest.

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