PHILIPP AGRIC SCIENTIST
Vol. 107 No. 2, 133-143
June 2024

ISSN 0031-7454
https://doi.org/10.62550/BQ020022

Short-term Response of Soil Microbial Community and Soil Bio-chemical Properties to Soybean Intercropping in a Cassava-based Cropping System

Nolissa D. Organo[1,*], John Lester V. Pide[1], Michelle Anne M. Calubaquib[1], Elmer E. Enicola[2], Evelyn F. Delfin[2], Vanessa F. Calderon[3], Raymund M. Antiola[4], Hosne A. Dilzahan[5], and Andre F. Cruz[6]

[1] Division of Soil Science, Agricultural Systems Institute, College of Agriculture and Food Sciences, University of the Philippines Los Baños, College, Laguna
[2] Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines Los Baños, College, Laguna, Philippines
[3] Cagayan Valley Research Center (CVRC)
[4] Davao Regional Central Experiment Station (DARCES)
[5] Plant Pathology Division, Bangladesh Rice Research Institute, Bangladesh
[6] Graduate School of Life Sciences, Kyoto Prefectural University, Kyoto, Japan

Author for correspondence; Email: ndorgano@up.edu.ph

Received: February 16, 2022/ Revised: November 17, 2023/ Accepted: February 6, 2024

A cassava-based cropping system study assessed the short-term response of soil chemical and biochemical properties and soil microbial communities to soybean intercropping. Two soybean varieties ('Select Manchuria' and 'Tiwala 12') were tested as intercrops. The treatments did not affect the soil's chemical (pH, OM, N, P, K) and biochemical (basal respiration and dehydrogenase and urease enzyme activities) properties after one cropping period. Amplicon sequencing analysis found that intercropping promoted the abundance of bacterial orders Actinomycetales, Solibacterales, Sphingomonadales, and Rhodospirillales. These groups play active roles in organic matter decomposition and can potentially improve the soil quality in an area by enhancing the soil's organic matter content. In terms of community analysis, principal coordinate analysis (PCoA) and redundancy analysis (RDA) showed separation between mono and intercropping systems, while ANOSIM and PEMANOVA did not detect any significance between varieties and cropping systems for both bacterial and fungal data. These results suggest that, in the short term, introduction of soybean in a cassava-based cropping system affects selected microbial groups, but the overall influence in the microbial community is not distinctly detected.

Keywords: soil metagenome, fungi, bacteria, soybean, intercropping

INTRODUCTION

Intercropping, the growing of two or more crops simultaneously on the same piece of land (Brooker et al. 2015; Zaeem et al. 2019), is increasingly being adopted as a more sustainable practice in modern agricultural production systems throughout the world (Zaeem et al. 2019). Intercropping systems maximize productivity and resource utilization per unit of land (Iqbal et al. 2019).

Cassava (*Manihot esculenta* Crantz) is an important food crop for over 22 800 million people (Leone et al. 2021). In the Philippines, cassava is grown for human food, starch, animal feed, and industrial uses, such as alcohol (Evangelio 2001). The Philippines was one of the leading cassava-producing countries in the Asia Pacific region in 2020, producing approximately 2.61 Mmt (Statista 2022).

Soybean (*Glycine max* L.) is known as one of the "world's wonder crops" due to its nourishment ability and minimal growth requirement (Agcopra and Piadozo 2018, p.78). In the Philippines, local soybean production is low. It is considered one of the major imports along with other agricultural commodities, such as wheat and milk (Agcopra and Piadozo 2018; Galang 2020).

Intercropping cassava with soybean or other leguminous crops is highly possible because of the space available

during the first 3 mo when the growth of cassava is slow, and its nutrient, light, and water requirements are minimal (Amanullah et al. 2007). This practice is widely accepted and practiced in Nigeria (Mbah et al. 2009; Mbah and Ogidi 2012), Congo (Jackson 2018), and Sierra Leone (Mansaray et al. 2021). In the Philippines, cassava is commonly intercropped with maize, peanut, and sweet potato (Aye and Howeler 2012). Intercropping various crops with soybean has been reported to promote soil health by increasing the available phosphorus (P) (Zaeem et al. 2019; Li et al. 2022) and improving the stability of soil aggregates (Zhang et al. 2022) and other soil health indicators (Regehr 2014). Although the cassava-soybean intercropping system has the potential to boost soybean production and improve soil health, Filipino farmers are still hesitant about adopting the practice because it is not well-documented and studied. There are no local and recent studies on the effects of cassava-soybean intercropping on cassava yield and its subsequent effect on soil properties, especially on soil microbial communities.

Microbial communities are essential parts of the soil, and their activity is critical to soil health. Beneficial aspects of microbial populations include their contribution to substantial geochemical cycles, their ability to avoid environmental change through bioremediation, and their ability to provide a wealth of new energy conversion, catalysis, and synthesis of natural products (Deutschbauer et al. 2006; Morong et al. 2021). Understanding the response of microbial communities to changes in agricultural management practices could give insights into the resulting changes in the soil. Recent advances in molecular biology offered a new vision of microbial ecology. They allowed the study of highly complex communities in a short period of time using high-throughput next-generation sequencing (NGS) technology (Escobar-Zepeda et al. 2015). NGS technologies detected changes in the bacterial communities under soybean-corn intercropping (Fu et al. 2019; Li et al. 2022; Liu et al. 2022). While there are a few studies on the microbial community associated with cassava and soybean rhizosphere (Tang et al. 2020; Ha et al. 2021; Liu et al. 2022), there is limited knowledge on the change in the microbial community of the soil in a cassava-soybean intercropping system.

Thus, this research was conducted to determine the short-term response of soil biochemical properties and microbial communities to intercropping cassava with soybean with the view that the incorporation of soybean in a cassava-based cropping system will enhance soil quality and soil health through the enhancement of the soil biochemical properties and beneficial microbial communities.

MATERIALS AND METHODS

Site Description and Field Setup

The field experiment was set up in the Davao Regional Central Experiment Station (DARCES) in Manambulan, Davao City from July 2018 to May 2019. The experimental plot has an area of 1500 m^2 and an elevation of 344 m asl, with the following coordinates: N 7° 05'24.669" and E 125° 26'57.227". The soil in the experimental area belongs to the Tugbok soil series (Typic Hapludults) (Carating et al. 2014). Baseline soil analysis done in preparation for the setup showed a pH of 5.2 and an organic matter (OM) content of 1.83%. It also has low total nitrogen (N) content (0.11%), low available P (2.13 ppm), and moderately high exchangeable Potassium (K) (0.62 centimole positive charge per kg of soil [cmolc/kg soil]) content.

The trial was laid out in a randomized complete block design with five treatments and three replications. The treatments were: (1) cassava alone (MONO), (2) cassava intercropped with 'Select Manchuria' soybean variety (CSIR-'Select Manchuria'), (3) cassava intercropped with 'Tiwala 12' soybean variety (CSIR-'Tiwala 12'), (4) cassava intercropped with 'Select Manchuria' plus *Rhizobium* inoculant, mycorrhizal inoculant, and basal organic fertilizer (chicken manure) at a rate of 10 bags/ha (CSIRplus-'Select Manchuria'), (5) cassava intercropped with 'Tiwala 12' plus *Rhizobium* inoculant, mycorrhizal inoculant, and basal organic fertilizer (chicken manure) at 10 bags/ha (CSIR plus-'Tiwala 12'). The 'Select Manchuria' variety is a registered variety with the National Seed Industry Council (NSIC) while 'Tiwala 12' is a Germplasm and Technology Release and Registration Office (GTRRO)-released variety of UPLB-IPB. Each plot measured 5 m x 6 m consisting of 5 rows of cassava (var. Lakan) spaced 1 m apart. In the MONO treatment, cassava cuttings were planted following a spacing of 75 cm between hills and 100 cm between rows. Soybean was planted in the 1-m space between the cassava rows with intercropping treatment (CSIR and CSIRplus) on the same day. It was harvested in September 2018.

The microbial inoculants were applied based on package instructions. Briefly, seed inoculation was done by thoroughly mixing the moist soybean seeds with the *Rhizobium* (Nitroplus) inoculant until each seed was evenly coated with the inoculant. The mycorrhizal inoculant (Mykovam) was applied by hand under the furrows at 20 g per m before seeding or transplanting. The recommended fertilizer rate (96-60-30) was applied to all the treatments. Cassava was harvested in the last week of April, and sample collection was conducted a week after.

Soil Sampling

Field evaluation and sample collection activities were conducted one wk after the cassava harvest (May 2019). The research team decided that this was the most appropriate timing because this would provide soil analysis values closer to the establishment of the succeeding crop. Five random spot samples were then collected at a depth of 20 cm per plot using a soil auger. The spot samples were homogenized in a clean container and strained using a clean 2-mm sieve. The materials used, such as auger, sieve, and containers, were cleaned and surface sterilized before sample processing to avoid cross-contamination of samples. Samples for chemical analyses (pH, total N, available P, exchangeable K, and OM) were placed in sealed and labeled bags and kept at ambient temperature. On the other hand, samples for microbial biomass, enzyme assays, and molecular analyses were kept in a cooler filled with refrigerants during transport. Upon arrival at the laboratory, samples for chemical analyses were air-dried. In contrast, samples for enzyme assays were immediately stored in the refrigerator. Samples for molecular analyses were frozen at -20°C until DNA extraction.

Soil Chemical Analysis

Soil chemical analyses were determined using established protocols. The soil pH was analyzed following the potentiometric method using a 1:2.5 soil-diluent and distilled water ratio (PCARR 1980). Total N was analyzed using the micro-Kjeldahl method (Bremner and Mulvaney 1982). Available P was determined through the Bray P-2 extraction method using 0.1 N HCl and 0.03 N NH_4F extractant (Bray and Kurtz 1945). The amount of P in the extract was quantified following the method of Murphy and Riley (1962). The exchangeable K was extracted using the ammonium acetate method (PCARR 1980) and quantified using a spectrophotometer. The Walkley and Black (1934) method was used to determine the soil sample's OM content (%).

Measurement of Soil Biochemical Activities

Basal respiration was measured using titration (Schinner et al. 1996). Soil dehydrogenase activity was measured using the colorimetric method (Achuba and Okoh 2014). The urease activity of the soil was determined using the non-buffered colorimetric method (Kandeler and Gerber 1988; Schinner et al. 1996). The chloroform fumigation-incubation method was used to determine the microbial biomass of the soil sample (Jenkinson and Powlson 1976).

Measurement of Microbial Communities

Amplicon sequencing analysis was performed to assess microbial communities. To do this, a subsample of 0.25 g of soil was used for DNA extraction. The genomic DNA of the samples was isolated using DNeasy PowerSoil™ DNA Isolation Kit (Qiagen, United States) according to the manufacturer's protocol. Agarose gel electrophoresis was carried out to confirm the presence of DNA after the extraction. Soil DNA extracts were also quantified using a MultiSkan GO spectrophotometer at 260 nm absorbance. The ratio of the absorbance at 260 nm and 280 nm was used to assess the concentration and quality of genomic DNA.

Amplicon polymerase chain reactions (PCR) and library construction of 16S rRNA and internal transcribed spacer (ITS) genes in each sample were performed for bacteria and fungi, respectively. The following steps were performed from PCR for ITS amplicon sequencing analysis using the Illumina MiSeq 250 bp at Genome Quebec in Montreal, Canada. The ITS gene sequences were analyzed by amplicon PCR using the primer pair ITS1/ITS2 regions for fungal communities with their respective adapters (Table 1), followed by index PCR to construct their library. The amplicon PCR condition and library construction for bacterial metagenome (16S ribosomal RNA [rRNA]) were performed using the V3-V4 primers within the Fluidigm protocol (https://www.standardbio.com/). On the other hand, amplicon PCR for fungal metagenome was performed in a 20 µL mixture containing 10 µL KOD FX buffer, 1 µL genomic DNA, 0.4 µL KOD FX Neo enzyme (https:/www.toyobo-global.com/seihin/xr/lifescience/products/pcr_017.html), 2 µL forward primer, 2 µL reverse primer, 4 µL dNTPs (deoxyribonucleotide triphosphate), and 0.6 µL sterile water. PCR conditions were as follows: 94°C for 2 min, 30 cycles of 94°C for 15 s, 55°C for 30 s, and 68°C for 1 min, held at 4°C.

After amplicon PCR, all resulting PCR products for the ITS samples were cleaned using Promega Wizard SV Gel and PCR Clean-Up System was performed according to the manufacturer's procedure. The cleaned PCR products were subjected to index PCR (library construction) to attach dual indices and Illumina sequencing adapters using the Nextera XT Index Kit (https://jp.illumina.com/techniques/

Table 1. Forward and reverse primers with adapters for 16S rRNA and ITS.

	16S	ITS
Adapters	www.fluidigm.com	https://jp.illumina.com
Forward	Fluidigm adapter – CS1	TCGTCGGCAGCGTCAGATGTG-TATAAGAGACAG
Reverse	Fluidigm adapter – CS2	GTCTCGTGGGCTCGGAGATGTG-TATAAGAGACAG
Primers	Klindworth et al. (2013)	Gardes and Bruns (1993)
Forward	CCTACGGGNGGCWGCAG	TCCGTAGGTGAACCTGCGG
Reverse	GACTACHVGGGTATCTAATCC	GCTGCGTTCTTCATCGATGC

sequencing/ngs-library-prep.html). The DNA libraries were validated, pooled, and applied to amplicon sequencing using the Illumina MiSeq 250bp.

The gene sequences for bioinformatic analysis were processed using the QIIME 2 (Bolyen et al. 2019) pipeline, including the decompressing and reassembling. The taxonomic base analysis for the 16S gene was accessed by the 16S classifier provided on the QIIME 2 website (https://qiime2.org/), while the ITS classifier was downloaded from the UNITE community (https://unite.ut.ee/). QIIME 2 software was used to estimate the taxonomic metrics between communities to determine the relative abundance and operational taxonomic unit (OTUs) of the soil microbes.

Statistical Analysis

The analysis of variance for the chemical and biochemical properties of the soil samples was computed using the Statistical Tool for Agricultural Research (STAR 2.0.1). Normality was tested using the Shapiro-Wilk test. The treatment means were compared using the least significant difference (LSD) test at a 5% significance level. Correlation and Redundant analysis (RDA) between soil chemical properties and microbial community were performed using Statistical Package for the Social Sciences (SPSS 19.0) and R statistical (ggplot and vegan packages) software respectively. Principal coordinates analysis (PCoA) was performed for the microbial community using R statistical software packages, where the PERMANOVA and ANOSIM were used to compare treatments. Other complementary calculations were performed using MS Excel 365.

RESULTS AND DISCUSSION

Soil Chemical and Biochemical Properties

After one cropping period, the treatments were found to have no significant effect on the chemical and biological properties tested (Tables 2 and 3). The values obtained after one cropping period were almost similar to the baseline data, indicating that the first cropping period has minimal effect on soil chemical and biochemical properties. Previous research reported changes in soil properties in experiments set up for at least 2 yr (Fu et al. 2019; Zaeem et al. 2019; Zheng et al. 2022). It appears that the effect of intercropping is not yet manifested in the chemical or biochemical properties of the soil after the 1st cropping period.

Microbial Community Analysis

The number of bacterial sequences per sample ranged from 14 914 to 43 600, with an average of 25 837. The total number of sequences obtained was 439 234. The number of bacterial OTUs

Table 2. Differences in chemical properties of the soil in a cassava-based soybean intercropping system[1].

Treatment[2]	Soil Chemical Properties				
	Soil pH	Total N (%)	Avail P (ppm)	Exch K (cmolc/kg soil)	OM (%)
Baseline data	5.20	0.11	2.13	0.62	1.83
MONO	4.67a	0.13a	3.73a	0.83a	1.67a
CSIR-Select Manchuria	4.60a	0.12a	4.17a	0.83a	1.73a
CSIR-Tiwala 12	4.63a	0.13a	3.80a	0.83a	1.84a
CSIRplus-Select Manchuria	4.60a	0.13a	3.90a	0.95a	1.59a
CSIRplus-Tiwala 12	4.93a	0.11a	3.40a	1.05a	1.58a
CV	3.37%	5.09%	21.58%	15.10%	4.17%
p-value	*0.5257*^{ns}	*0.1667*^{ns}	*0.1957*^{ns}	*0.2580*^{ns}	*0.3160*^{ns}

[1] Means within a column followed by different letters are significantly different at α=0.05, least significant difference (LSD)
[2] MONO (Cassava alone), CSIR-Select Manchuria (Cassava intercropped with 'Select Manchuria' soybean variety), CSIR-Tiwala12 (Cassava intercropped with 'Tiwala 12'), CSIRplus-Select Manchuria (Cassava- intercropped with 'Select Manchuria' plus *Rhizobium* inoculant, mycorrhizal inoculant, and chicken manure), CSIR plus-Tiwala 12 (Cassava- intercropped with 'Tiwala 12' plus *Rhizobium* inoculant, mycorrhizal inoculant, and chicken manure)

Table 3. Differences in the biochemical properties of the soil in a cassava-based soybean cropping system[1].

Treatment[2]	Biochemical Properties				
	Basal Respiration (mg CO_2/g dry soil)	Biodiversity Index	Number of Earthworms	Urease (µg N/g dry soil *2h)	Dehydrogenase (µg TF/g dry soil)
Baseline data	0.18	0.98	5.00	3.10	4.41
MONO	0.16a	1.37a	14.33a	2.72a	4.93a
CSIR-Select Manchuria	0.11a	1.45a	9.67a	3.23a	4.93a
CSIR-Tiwala 12	0.18a	0.97a	17.00a	2.23a	4.61a
CSIRplus-Select Manchuria	0.16a	1.02a	11.67a	3.16a	4.67a
CSIRplus-Tiwala 12	0.15a	1.44a	7.00a	2.94a	4.79a
CV	20.31%	29.81%	116.64%	16.68%	24.07%
p-value	*0.7800*^{ns}	*0.3217*^{ns}	*0.4417*^{ns}	*0.7784*^{ns}	*0.4867*^{ns}

[1] Means within a column followed by different letters are significantly different at α=0.05, least significant difference (LSD).
[2] MONO (Cassava alone), CSIR-Select Manchuria (Cassava intercropped with 'Select Manchuria' soybean variety), CSIR-Tiwala12 (Cassava intercropped with 'Tiwala 12'), CSIRplus-Select Manchuria (Cassava- intercropped with 'Select Manchuria' plus *Rhizobium* inoculant, mycorrhizal inoculant, and chicken manure), CSIR plus-Tiwala 12 (Cassava- intercropped with 'Tiwala 12' plus *Rhizobium* inoculant, mycorrhizal inoculant, and chicken manure).

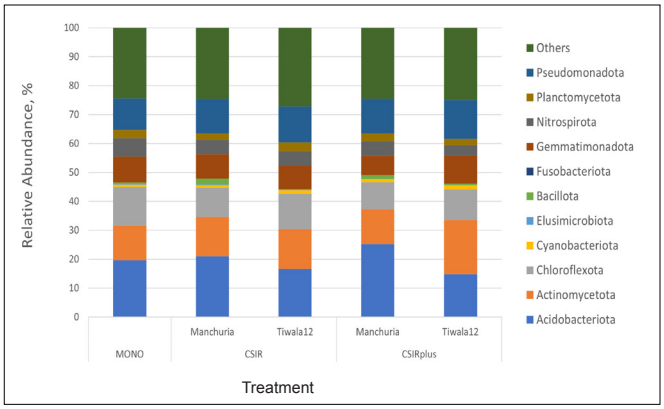

Fig. 1. Relative abundance of dominant bacterial phyla in a cassava-based soybean cropping system.

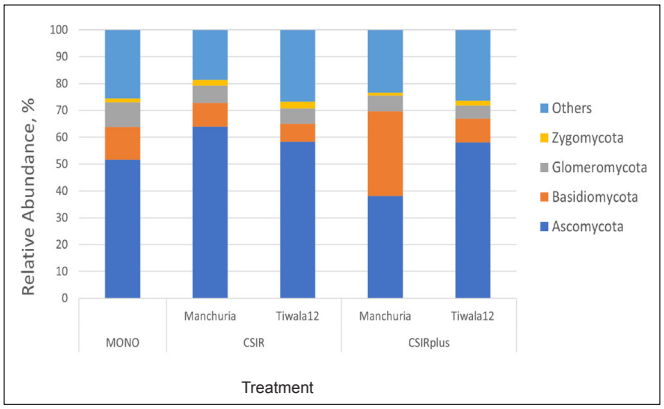

Fig. 3. Relative abundance of dominant fungal phyla in a cassava-soybean intercropping system.

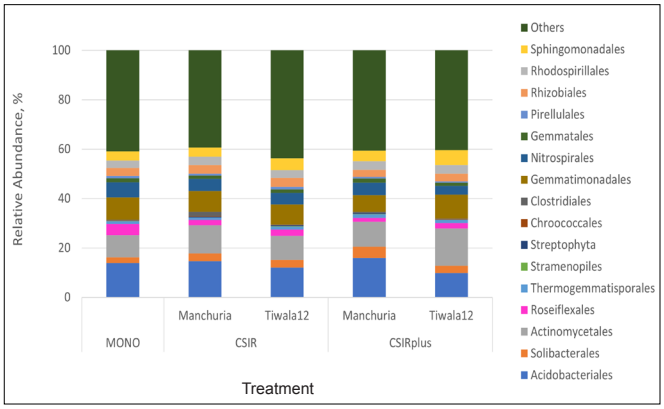

Fig. 2. Relative abundance of dominant bacterial orders in a cassava-based soybean cropping system.

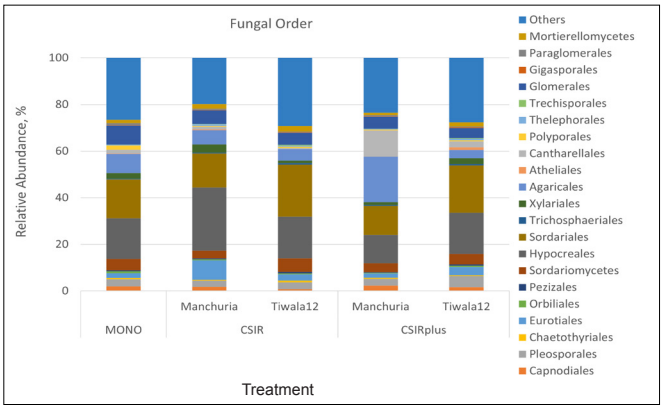

Fig. 4. Relative abundance of dominant fungal orders in a cassava-soybean intercropping system.

ranged from 16 000 to 20 000. The dominant bacterial phyla across samples include Acidobacteriota, Actinomycetota, Pseudomonadota, and Chloroflexota (Fig. 1). Dominant bacterial orders included Acidobacteriales, Actinomycetota, Gemmatimonadota, and Nitrospirota (Fig. 2).

Fungal metagenome analysis resulted in sequence reads that ranged from 19 369 to 60 933. The average number of sequences per sample was 51 377, totaling 873 412 sequences. The number of fungal OTUs ranged from 27 000 to 32 000. Dominant fungal phyla included Ascomycota, Basidiomycota, and Glomeromycota (Fig. 3), while dominant fungal orders included Hypocreales, Sordariales, and Glomerales (Fig. 4).

Shifts in microbial community composition were already detected after only one cropping period. The relative abundance of bacterial orders Actinomycetales, Solibacterales, Sphingomonadales, and Rhodospirillales was higher in intercropping systems (CSIR and CSIRplus) compared with MONO, regardless of soybean variety. Bacterial orders Nitrospirales and Roseiflexales were more abundant in the MONO treatment. The fungal orders Hypocreales and Eurotiales generally increased with soybean incorporation (CSIR and CSIRplus), except in the CSIRplus-Select Manchuria treatment. On the other hand, the fungal order Agaricales decreased with soybean incorporation (CSIR and CSIRplus) except in the CSIRplus-Select Manchuria treatment. The fungal orders Glomerales and Polyporales decreased with intercropping, regardless of soybean variety.

The bacterial and fungal orders that increased in relative abundance in intercropping systems were previously reported to elicit beneficial functions in the soil (Yeager et al. 2017; Wang et al. 2018). Actinomycetales has been reported to have roles in inducing plant disease suppression and plant growth promotion in host plants (Wang et al. 2018). This bacterial order is also regarded as the quintessential degrader of complex polysaccharides in soils and has been associated with secondary metabolite production; some species are also efficient solubilizers of rock phosphate (Yeager et al. 2017). Sphingomonadales was reported to utilize a great diversity of carbon sources, including recalcitrant xenobiotic molecules (Wang et al. 2016). Bacteria belonging to Sphingomonadales are photosynthetic and play a vital role in synthesizing sugars,

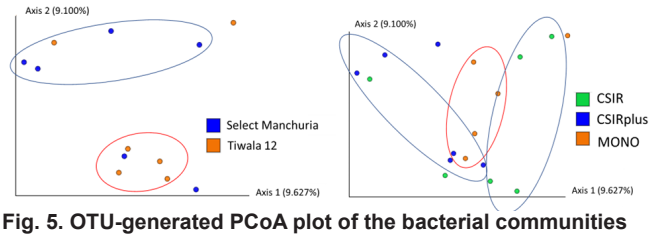

Fig. 5. OTU-generated PCoA plot of the bacterial communities at the family level under different varieties (left) and cropping systems (right).

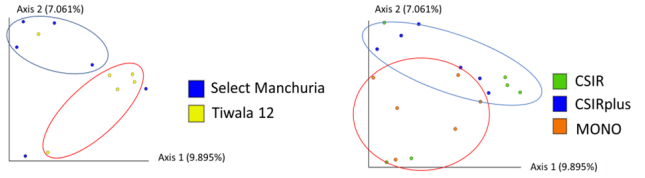

Fig. 6. OTU-generated PCoA plot of the fungal communities at the family level under different varieties (left) and cropping systems (right).

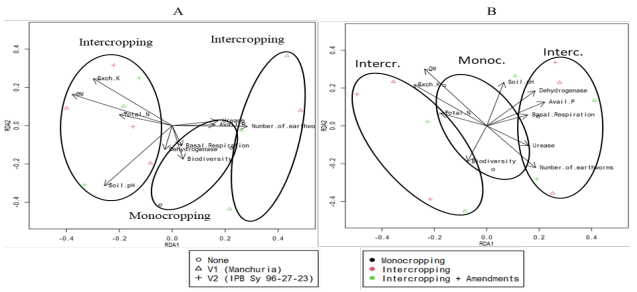

Fig. 7. Redundant analysis (RDA) graph showing the relationship of bacterial communities (left) and fungal communities (right) with environmental data.

amino acids, vitamins, and other bioactive substances (Huang et al. 2017). Rhodospirallales was associated with organic farming (Wang et al. 2018) and positively correlated with corn yield (Ma et al. 2016). The order Solibacterales was reported to have a role in the enhanced cycling of essential micro- and macronutrients, which may partially improve soil fertility and plant growth efficiency (Xu et al. 2013). The fungal order Hypocreales includes the saprophytic fungi such as *Trichoderma*. It contains the largest number of entomopathogenic fungi, and its species have been used extensively when eradicating soil- and plant-borne pests (Lacey et al. 2009). The Eurotiales fungal order contains *Aspergillus* and *Penicillium*, two of the most economically important genera of fungi that can survive low pH environments (Houbraken and Samson 2011). Based on these descriptions from the literature, it can be said that the intercropping treatment promoted the growth of organisms that play active roles in OM decomposition. The increase in these organisms would benefit the area since baseline analysis reports a low OM content of only 1.8%.

The relative abundance of Nitrospirales was reported to increase in the rhizosphere of sugarcane when intercropped with soybean (Malviya et al. 2021), and cassava intercropped with peanut (Tang et al. 2020). In this research, however, an increased abundance of Nitrospirales was observed in the monocropping treatment and not in the intercropping treatments. It appears that any increase in the relative abundance of Nitrospirales during cropping is no longer reflected in the bulk soil for the next cropping season. Soil samples were collected after only one cropping season, and it is also possible that any increase in the relative abundance of Nitrospirales in the rhizosphere was minimal and not yet reflected in the bulk soil.

The effect of plant variety or genotype on the microbial community has been documented previously (Delmo-Organo et al. 2017; Dilla-Ermita et al. 2021), and the current study found evidence for this as well. The effect of soybean variety was observed in the relative abundance of the bacterial orders Acidobacteriales and Clostridiales and fungal order Sordariales. Regardless of the main treatment, the relative abundance of both bacterial groups increased when the 'Select Manchuria' soybean variety was used as an intercrop and decreased when 'Tiwala 12' was used. The relative abundance of Sordariales increased when cassava was intercropped with 'Tiwala 12' and decreased when 'Select Manchuria' was used. Different plant varieties have properties that make them unique, and therefore release varying amounts and types of metabolites in the soil, which would consequently result in a change in bacterial community composition. This was not investigated in the current study and it is strongly recommended that the mechanisms eliciting variable response be investigated in further studies.

PCoA showed that both the cropping system and soybean variety affect the bacterial and fungal community of the soil (Figs. 5 and 6). Relationships between the important ecological parameters and the microbial community were discerned by RDA (Fig. 7). RDA showed that the bacterial and fungal community positively influenced OM, Total N, and exchangeable K. Soil pH was influenced by the bacterial community alone. The RDA graph also shows the separation between monocropping and intercropping systems but does not separate between CSIR and CSIR Plus. Although these graphs visually show the separation between the groups, the general evaluation performed by ANOSIM and PEMANOVA did not detect any significance between varieties and cropping systems for both bacterial and fungal data.

Relationship between Soil Chemical and Biochemical Properties and Microbial Community

Several bacterial families (i.e., Nocardioidaceae, Methylobacteriaceae, Acetobacteraceae) were positively

correlated with soil pH. In contrast, the families Acidobacteriaceae and Ktedonobacteraceae were negatively correlated (Table 4). Aureobasidiaceae, Chaetothyriaceae, Elsinoaceae, Sympoventuriaceae, and Russulaceae (ectomycorrhiza) fungal families were negatively correlated with soil pH while a positive correlation was observed between soil pH and Lasiosphaeriaceae (Table 5).

A negative correlation was observed between OM and the relative abundance of various bacterial families, including Acidobacteriaceae, Thermomonosporaceae, Ktedonobacteraceae, Thermogemmatisporaceae, Xenococcaceae, Bradyrhizobiaceae, Sphingomonadaceae, and Gemmataceae. Fungal families whose relative abundances negatively correlated with OM included Lipomycetaceae and Bionectriaceae. In contrast, Pezizaceae, Cordycipitaceae, Meripilaceae, Trichosporonaceae, and Basidiobolaceae positively correlated with OM.

Table 4. Pearson correlation between soil properties and relative abundance of selected bacterial families.

Family	OM (%)	Number of Earthworms	Soil pH	Total N (%)	Avail P (ppm)	Exch K (cmolc/kg soil)
Acidobacteriaceae	–0.589*	0.404	–0.719**	–0.176	0.125	–0.360
Nocardioidaceae	0.036	–0.135	0.705**	0.088	–0.251	–0.224
Thermomonosporaceae	–0.571*	–0.047	–0.291	–0.065	–0.218	–0.084
Ktedonobacteraceae	–0.524*	–0.181	–0.666**	–0.194	0.203	–0.232
Thermogemmatisporaceae	–0.618*	–0.286	–0.134	0.006	–0.303	–0.052
Xenococcaceae	–0.664**	–0.042	0.244	–0.458	0.224	–0.382
Bradyrhizobiaceae	–0.576*	0.467	–0.332	–0.421	0.47	–0.544*
Methylobacteriaceae	0.013	–0.398	0.557*	–0.100	–0.293	0.375
Acetobacteraceae	–0.147	–0.161	0.592*	–0.186	0.081	–0.266
Sphingomonadaceae	–0.803**	0.261	0.228	–0.438	0.081	–0.649**
Gemmataceae	–0.698**	0.451	–0.205	–0.167	0.035	–0.648**

Note: * and ** indicate significance at 95% and 99%, respectively.

Table 5. Pearson correlation between soil properties and relative abundance of selected fungal families.

Family	OM (%)	Number of Earthworms	Soil pH	Total N (%)	Avail P (ppm)	Exch K (cmolc/kg soil)
Aureobasidiaceae	0.140	0.249	–0.698**	0.050	0.258	–0.099
Elsinoaceae	–0.147	0.493	–0.564*	–0.099	0.116	–0.171
Sympoventuriaceae	–0.261	0.521*	–0.550*	–0.181	0.106	–0.233
Chaetothyriaceae	–0.383	0.474	–0.769**	–0.180	0.225	–0.353
Pezizaceae	0.584*	–0.013	–0.265	0.44	–0.117	0.083
Lipomycetaceae	–0.543*	0.069	–0.089	–0.165	–0.127	–0.173
Bionectriaceae	–0.537*	0.080	0.460	–0.366	–0.068	–0.494
Cordycipitaceae	0.533*	–0.146	–0.050	0.024	0.234	0.032
Ophiocordycipitaceae	–0.144	0.559*	–0.638*	0.069	0.019	–0.210
Lasiosphaeriaceae	0.325	–0.506	0.588*	0.103	–0.261	0.725**
Trichosphaeriaceae	0.463	–0.429	0.092	–0.025	–0.202	0.702**
Meripilaceae	0.720**	0.130	–0.079	0.300	0.035	–0.015
Russulaceae	0.239	0.118	–0.526*	–0.068	0.175	0.525*
Trichosporonaceae	0.595*	–0.294	–0.107	0.028	0.135	0.372
Basidiobolaceae	0.708**	–0.326	0.312	0.129	–0.068	0.524*
Glomeraceae	–0.256	0.593*	–0.260	–0.290	0.241	–0.581*

Note: * and ** indicate significance at 95% and 99%, respectively.

Exchangeable K was negatively correlated with bacterial families Bradyrhizobiaceae, Sphingomonadaceae, and Gemmataceae. A positive correlation was observed between exchangeable K and multiple fungal families (i.e., Lasiosphaeriaceae, Trichosphaeriaceae, Basidiobolaceae, and Glomeraceae).

The number of earthworms positively correlated with the relative abundances of fungal families, Sympoventuriaceae, Ophiocordycipitaceae, and Glomeraceae.

CONCLUSION

The results of this study provided insights into the short-term response of the soil microbial community upon the introduction of soybean as an intercrop in a cassava-based cropping system. It was found that after one cropping period, the chemical and biochemical properties of soil (pH, OM, N, P, K, basal respiration, and enzyme activities) were neither affected by the cropping system nor the soybean variety used. Despite this, a shift in microbial community composition was observed among treatments. Intercropping increased the relative abundance of beneficial microorganisms active in biogeochemical transformations, which could improve the OM content of the soil.

This study also unveiled interesting issues that need to be addressed in follow-up studies. It is highly recommended that the experiment be extended to at least 3 yr to determine if intercropping with soybean will elicit a long-term effect on the soil microbial community and other soil properties. It is also suggested that the succeeding experiments include simultaneous bulk soil and rhizosphere analysis throughout the cropping calendar to elucidate the microbial succession, temporally and spatially. Including a monocropping treatment using soybean alone is also advised. It would be interesting to unravel more information surrounding plant-soil-microbe in cassava-soybean intercropping systems.

ACKNOWLEDGEMENT

This study was supported by the UPLB-DOST-PCAARRD funded project, Enhanced Soil Health and the project, Soybean for Higher Income and Enhanced Soil Health Under Different Cropping Systems under the program, Improvement of Soybean (*Glycine max* (L.) Merr.) for Better Nutrition, Higher Income and Enhanced Soil Health. We would also like to express appreciation to Dr. Naoaki Ono of Nara Institute of Science and Technology, Japan, for his assistance during the bioinformatics analysis.

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