J. ISSAAS Vol. 27, No. 1: 87-105 (2021)

RELATIONSHIP BETWEEN MICROBIAL SOIL QUALITY INDICATORS AND SOIL PRODUCTIVITY UNDER CASSAVA-BASED FARMING SYSTEMS IN VARIOUS SITES IN THE PHILIPPINES

Rosario G. Monsalud[1*], Eldrin DLR Arguelles[1], Marilyn B. Brown[1], Mannix S. Pedro[1], Robert A. Nepomuceno[1], Shaun Adrian D. Villadiego[1] and Florentino C. Monsalud[2]
[1]National Institute of Molecular Biology and Biotechnology (BIOTECH),
[2]Agricultural Systems Institute (ASI),
University of the Philippines Los Baňos, College, Laguna, 4031, Philippines
*Corresponding author: rgmonsalud@up.edu.ph

(Received: January 27, 2021; Accepted: May 6, 2021)

ABSTRACT

Soil quality indicators are needed in monitoring the soil property transformations to determine sustainable farming systems. This study sought to determine the relationship between microbial soil quality indicators and soil productivity under cassava-based farming systems in the three major islands of the Philippines. The cassava-based farming systems of productive and marginal farms in each site were documented. Total microbial activity, bacterial diversity, abundance of arbuscular mycorrhizal fungi (AMF) spores, texture, and soil chemical properties were determined. The study showed a positive relationship between total microbial activity and soil productivity in one of the three study sites. The effectiveness of total microbial activity as a soil quality indicator is influenced by soil physical properties affecting drainage. The study also provided evidence on the consistent association of more diverse bacterial and higher AMF populations in productive soils under cassava-based farming systems in all three sites.

Key words: microbial activity; bacterial diversity, arbuscular mycorrhizal fungi

INTRODUCTION

The traditional performance indicator of a crop-based farming system is generally based on crop yield. In most cases, farmers modify their crop production technology in response to the trends in crop yields, oftentimes changing chemical-based nutrients and pest management inputs. The levels of the production technology inputs cause changes in the soil biological quality which could affect sustainability. Soil quality can be defined as the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation (Karlen et al. 1997). In agricultural systems, this definition may be used focusing on the capacity of the soil to perform its production function (Mueller et al. 2010).

Soil quality monitoring is essential to determine the sustainability of farming systems. In doing so, soil quality indicators such physical, chemical, and biological characteristics or processes are needed to examine transformations in the soil (Brady and Weil 2008) and its capacity to sustain crop growth (Arshad and Martin 2002). However, in the Philippines where the current assessment of soil quality is generally based on soil chemical and physical properties, the inclusion of biological soil quality indicators is necessary. It is valuable to incorporate microbial indicators as these react rapidly to fluctuations in environmental conditions than physical and chemical indicators (Zhang and Li 2020). Various potential microbial indicators of soil quality that may be used for agricultural soils in the

Philippines include total microbial activity, bacterial diversity, and abundance of arbuscular, mycorrhizal fungi (AMF).

The estimation of total microbial activity as a biological/biochemical indicator of soil quality with the use of fluorescein diacetate (FDA) hydrolysis was proposed by Green et al. (2006). This was reported to have a good correlation with the more precise measurements of microbial biomass, i.e., amount of adenosine triphosphate (ATP) and cell concentration (Stubberfield and Shaw 1990). The said assay was also found in the studies of Gajda et al. (2017) to exhibit similar patterns as dehydrogenase activity. The FDA hydrolysis assay had been described as a simple, sensitive, and rapid technique (Green et al. 2006; Dutta et al. 2010; Patle et al. 2018). Some of the recent studies that utilized the assay of hydrolysis of FDA include the effects of different farming systems (Gajda et al. 2016) and different tillage systems (Gajda et al. 2018) on microbial activity.

The microbial community structure in soils, primarily of the rhizosphere soil, is of immense value in assessing soil quality in order to attain high yields of crops (Rincon-Florez et al. 2013). It is commonly considered that agricultural soils with medium to high diversity are of good quality (Winding 2004). Variations in soil populations and the loss of soil biodiversity endanger ecosystem processes and sustainability (Wagg et al. 2014). The breakdown of organic matter is affected by the changes in microbial diversity (Juarez et al. 2013). In addition, microbial community structure affect significantly geochemical cycles (Zhang et al. 2020). One of the techniques widely used in studying soil microbial communities in the last decade is the denaturing gradient gel electrophoresis (DGGE). It gives a straightforward image of the diversity of dominant microbes of a given sample, including the unculturables, and allows for their identification through the generation of sequences from cloned excised bands (Garbeva et al. 2004).

The abundance of arbuscular mycorrhizal fungi (AMF) in the soil is another good indicator of soil biological quality. The quantity of AMF spores can be used to estimate soil fertility (Syib'li et al. 2013). The study of Verzeaux et al. (2017) on the influence of soil management techniques indicated a positive correlation between the density of AMF spores in the soil and dehydrogenase enzyme activity. Another study yielded a positive correlation between soil structure and abundance of AMF spores in three soil systems (Sanchez et al. 2015). Mycorrhizal fungi inoculants to enhance the growth of agricultural crops and forest species are widely available in the market in many countries. In the Philippines, mycorrhiza inoculant brands include: VAMRI, MYKOVAM, MYCOGROE, BROWN MAGIC (Brown et al. 2016), among others.

These microbial indicators of soil quality may be adopted in the Philippines as these are based on robustness and simplicity of the methods, and the facility's capability to perform laboratory analysis. The study, therefore, sought to determine the relationship between microbial soil quality indicators and productivity of soils under selected cassava-based farming systems in the Philippines.

MATERIALS AND METHODS

Study site. The study was conducted in three cassava-growing provinces in the three main islands of the country: Pampanga in Luzon, Bohol in the Visayas and South Cotabato in Mindanao. The selection of the study sites was done through a series of key informant interviews (KII) at the provincial, municipal, and village levels. The enquiries for the Offices of the Provincial and Municipal Agriculturists were mainly focused on identifying cassava farms with two levels of productivity. The specific study sites were the following: 1) Porac in Pampanga province (15°4'N; 120°32'E); 2) Carmen (9°49'N; 124°12'E) and Clarin (9°58N; 124°1'E) in Bohol province; and 3) Surallah (6°23'N; 124°45'E) and Tampakan (6°28'N; 125°3'E) in South Cotabato province. Data on average annual rainfall and temperature were taken from the Philippine Atmospheric, Geophysical and Astronomical Services Administration (PAGASA). In Pampanga, the average annual rainfall was 2391 mm and the average annual temperature was 27°C. The average annual rainfall in Bohol was 1916 mm while the average annual temperature was 29°C. In South Cotabato, the average annual rainfall and temperature were 2271 mm and 28°C, respectively. The soils in the study sites were determined from the soil survey reports of the provinces; for Pampanga (Yniguez et al. 1956), Bohol (Mojica et al. 1952), and South Cotabato (Mojica et al. 1963). The taxonomic classification of the soils was taken from the guidebooks on the keys to soil series: for Pampanga (PhilRice 2010) and Bohol (Collado et al. 2017), while the morphological descriptions of the soils in South Cotabato were adapted from the report on the physical land resources of the province (BSWM 1989) (Table 1).

Table 1. Soil types in the study sites.

Particulars	Productive	Marginal
Pampanga		
Soil Name:	Angeles series	La Paz series
Surface texture:	Loamy sand	Loamy sand
Taxonomic classification:	Coarse loamy, isohyperthermic Typic Untipsamment	Sandy, mixed, isohyperthermic, Typic Psammaquent
Drainage:	Well drained	Drainage: Poor
Bohol		
Soil Name:	Ubay series	Batuan series
Surfce texture:	Clay loam	Clay loam
Taxonomic classification:	Fine, mixed, isohyperthermic, Typic Eutrudept	Fine, mixed, isohyperthermic, Aquic Calciudoll
Drainage:	Good	Drainage: Moderate
South Cotabato		
Soil Name:	Dadiangas series	Tupi series
Surface texture:	Sandy loam	Sandy loam
Taxonomic classification:	Not available	Not available
Drainage:	Moderate to rapid	Rapid
General profile description:	The soil has a black sandy loam surface horizon with few gravels down to 25 cm depth. The B horizon has dark brown loamy coarse sand with common gravels down to 40 cm depth.	The surface horizon has black fine sandy loam down to 35 cm depth. The B horizon has dark brown to dark grayish brown sandy loam to loamy sand with few gravels and many highly weathered pumice-like volcanic materials down to 65 cm depth.

Farm productivity categorization and farming systems documentation. The target study sites were initially categorized as village with high or marginal cassava productivity through the KII at the Office of the Municipal Agriculturist. In Pampanga, both farm categories were located in Porac. In Bohol, the village with high cassava productivity was located in Carmen, while the village with marginal cassava productivity was in Clarin. In South Cotabato, the village with high cassava productivity was located in Surallah, while the village with marginal cassava productivity was in Tampakan. Validation of the cassava productivity category was conducted through interview with select farmers and agricultural technicians from the respective municipalities. The farmers' recall method was used in the collection of cassava yield data per farm (Fermont and Benson 2011) using the average production per hectare for the last three cropping periods and the average cassava yields were used to finalize the productivity category at the farm level. The categorization was based on the Food and Agriculture Organization (FAO) land evaluation suitability classes, S (suitable) and N (not suitable), of a land unit for a given land utilization type, with S1/S2 referred to in this study as productive farms, and S3/N1 as marginal farms (FAO 1976). In the FAO framework, the attainable yield of S1 corresponds to 85-100% of the optimum, 60-85% for S2, 40-60% for S3, and 25-40% for N1. The 60% average yield, which is the lower limit of S2 and upper limit of S3, delineated the productive and marginal farms (Sys et al. 1993).

Soil sampling and analytical methods. Soil samples were randomly collected from the productive and marginal farms with standing cassava crop at 0-20 cm soil layer. These were placed in plastic bags and immediately transferred to the laboratory for subsequent analysis.

Physico-chemical properties. The following soil properties were analyzed at the Analytical Service Laboratory of the Agricultural Systems Institute, University of the Philippines Los Banos (ASI, UPLB): texture (soil particle size analysis, hydrometer method); soil pH (1:1 soil-water suspension glass electrode potentiometer); organic matter (OM), Walkley-Black method; total nitrogen (N), Macrokjeldahl method; available phosphorus (P), Bray P2 or Olsen method; cation exchange capacity (CEC), distillation method; exchangeable potassium (K), flame photometry; exchangeable calcium (Ca) and magnesium (Mg), EDTA titration method.

Biological properties

Microbial activity. Total microbial activity was determined by quantifying the rate of fluorescein diacetate (FDA) hydrolysis using the method of Adam and Duncan (2001). Briefly, from the mid-section of the bag, three soil samples of 2.0 g were weighed and placed in each of three 50-mL falcon tubes (two replications and a control). To each falcon tube, 20 mL of 60 mM phosphate buffer (pH 7.6) was added, vortexed, then 100 uL of 2000 ug/mL fluorescein diacetate (FDA) was also added (except for the control) and swirled for a few seconds to blend the components. All tubes were incubated for 45 minutes in 30°C water bath. Immediately after, 20 mL of chloroform/methanol (2:1, v/v) was added to each sample to stop the reaction. The liquid portion was decanted and filtered through Whatman® No. 1, and its absorbance at 490 nm was measured with a spectrophotometer. The concentration of fluorescein produced by the soil samples (expressed g^{-1} oven-dried soil) was determined with reference to the standard curve prepared from freshly prepared fluorescein standards.

Microbial diversity. The microbial community profile of the soil was assessed by Denaturing Gradient Gel Electrophoresis (DGGE) (Sambrook et al. 1989).

DNA isolation. Total genomic DNA of the soil samples was extracted using Invitrogen™ plant/bacteria DNA isolation kit. The purity of the extracted DNA samples was checked using gel electrophoresis. Samples were kept at -80°C until further use.

Denaturing Gradient Gel Electrophoresis (DGGE) analysis. Near full-length 16S rDNA (approximately 1.5 Kbp in length) were amplified through PCR using bacteria-specific forward primer 8F (5'-AGA GTT TGA TCC TGG CTC AG-3') and universal reverse primer, 1512R (5'-ACG GCT ACC TTG TTA CGA CT-3'), DNA amplification was carried-out using Invitrogen™ amplification kit. PCR amplification was performed using the Bio-Rad T100 ™ Thermal Cycler using the conditions as follows: initial denaturation at 95°C, 10 min; 30 cycles consisting of denaturation (93 °C, 1 min); annealing (65 °C, 30 sec); extension (72 °C, 2 min);and final extension (72 °C, 5 min). The PCR-amplified DNA products were evaluated in agarose gel (0.8% (w/v) using Mupid® - 2 Plus submarine electrophoresis system at 100V. After which, stained gels with ethidium bromide were viewed under Gel Doc ™ XR^{+} (Bio-Rad). The V3 region of the 16S rDNA was amplified with the primer set 357F-GC (5'-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3') and 517R (5'-ATT ACC GCG GCT GCT GG-3'). The resulting PCR products were determined electrophoretically and analyzed in agarose gel (2%, w/v) using Mupid® - 2 Plus submarine electrophoresis system at 100V. After ethidium bromide staining, the detection of bands was done using Gel Doc ^{TM} XR^{+} (Bio-Rad). Bands along the 0.15kbp – 0.21kbp position confirm the amplification of the desired product. DGGE analysis was achieved using the Bio-Rad DCode^{TM} Mutation Detection System using acrylamide - bisacrylamide gels (6%-12%) with a 20% - 60% denaturing gradient. A 7M urea and 40% formamide matches a 100% denaturant. Electrophoresis was performed at 200V for five h at a temperature of 60°C in Tris Acetate-EDTA buffer (0.5 times). The polyacrylamide gels soaked for 20 min in SYBR® Green solution (Molecular Probes, Inc.) and viewed with Gel Doc ^{TM} XR^{+} Bio-Rad. This was followed by DNA extraction of the prominent DGGE bands using standard protocols. DNA isolated from the excised bands was re-amplified and loaded to DGGE to verify the relative position of the band in the gel. Single DGGE band is considered to represent a corresponding bacterial species and was subjected to DNA sequencing and analysis.

DNA sequencing. PCR products for all samples were submitted to AITBiotech Pte Ltd in Singapore for DNA sequencing. Sequences obtained were analyzed through BioEdit Sequence Alignment Editor v 5.0.9. Species closest identification was done with the Basic Local Alignment Search Tool (BLAST) from available databases such as the National Center for Biotechnology Information (NCBI).

Arbuscular Mycorrhizal Fungi (AMF). Spores of AMF were extracted from the soil samples following the wet sieving and decanting technique of Gerdemann and Nicolson (1963). Approximately 1 kg of soil was suspended and mixed thoroughly in water, and the slurry passed through a graded series of sieves from 50, 100, 300, 750, and 1,000 μM. Heavy clay suspensions were further processed by the centrifugation technique of Tommerup and Kidby (1979). The materials collected from the 1,000-mesh sieve were scraped-off with a stream of water from a wash bottle and centrifuged for 4 to 5 min at 1,750 rpm. The supernatant liquid was decanted, and the pellet resuspended in sucrose solution (454 g white sugar/L). This was again centrifuged for 0.5 to 1 min and the supernatant rinsed with water onto a 325-mesh sieve to remove the sugar. The materials that were retained on the sieves were washed, decanted into Petri dishes, and observed using a dissecting microscope (10X magnification). Further examination for distinct characteristics was done using a compound microscope with higher magnification.

Statistical analysis. Comparison of means of microbial activity between productive and marginal farms per province was done using pooled t-test procedure. Correlation analysis of the data on microbial activity and soil chemical properties such as pH, OM, total N, available P, exchangeable bases (Ca, Mg, K), and CEC obtained from productive and marginal farms was conducted using Pearson's correlation with the help of the R software.

RESULTS AND DISCUSSION

Description of the cassava-based farming systems. The cassava cropping system in the study sites was reported as generally monocropping in a rotation scheme with corn with no regular cropping cycles. Land preparation was done by one to two plowings and about the same number of harrowings using hired tractor. Some farms did not practice harrowing; other farms did more than two harrowings. The use of animal-drawn plow and harrow was also noted in some farms. These varied tillage practices in land preparation which appeared a common practice in both productive and marginal farms may have influenced the soil quality status of both farm types. It has been reported that soil under reduced tillage had higher soil microbial activity as measured with dehydrogenases and FDA hydrolysis and greater diversity of microbial communities than the same soil under conventional tillage (Gajda et al. 2018).

Cassava planting in Pampanga and South Cotabato was done in the month of March, while in Bohol, planting was done in December. The varieties being planted in both farm types per site were generally the same. In Pampanga, Golden Yellow and Lakan varieties were planted in both farm types. In Bohol and South Cotabato, the common variety planted in both farm types was Golden Yellow. It was noted that, generally, the planting distance in the Pampanga and South Cotabato sites, for both farm types, was 100 cm x 100 cm. In Bohol, the planting distance of 50 cm x 50 cm was common in both farm types. Manual weeding was reported to be a common practice for the control of weeds in both productive and marginal farms. The use of herbicides was also practiced in some farms of both farm types. Use of chemicals for insect pest control was not a regular practice in both farm types in all sites. However, there were some reports of insecticide applications in marginal farms in Bohol and productive farms in South Cotabato.

Some differences in the amounts of applied fertilizer nutrients between the productive and marginal farms in each site were documented (Table 2). In Pampanga, the amounts of N, P_2O_5, and K_2O generally applied to the productive farms were comparable with those supplied to the marginal farms. It was noted, however, that some farms in both farm types applied only nitrogen fertilizer. In Bohol, application of fertilizers was generally practiced in productive farms, while some farms in the marginal farm type were not applying fertilizers. However, in some marginal farms, the amounts of N, P_2O_5, and K_2O applied per hectare were higher than in the productive farm type. In South Cotabato, the amounts of N, P_2O_5, and K_2O fertilizer nutrients generally applied per hectare to the productive farms were comparable with those in the marginal farms. The use of animal manure and compost was also noted in some farms. In Pampanga, animal manure and compost were also applied in some farms in both farm types. In Bohol, the use of compost in productive farms was mentioned but not in marginal farms. In South Cotabato, application of organic fertilizers in both farm types was also reported.

Table 2. Amounts of fertilizer nutrient applied to cassava farms in the study sites.

Study Site	**Productive**			**Marginal**		
	N	**P_2O_5**	**K_2O**	**N**	**P_2O_5**	**K_2O**
			kg ha^{-1}			
Pampanga	48-134	0-42	0-42	60-138	0-35	0-35
Bohol	14-74	14-28	14-28	0-86	0-63	0-63
South Cotabato	24-45	44	44	38	44	44

Soil microbial activity, physico-chemical properties and productivity. The results of the comparison of means of the total MA levels between the two farm types showed a significantly higher total MA in the soil of productive farms than in marginal farms in South Cotabato site (Fig. 1). However, in Pampanga and Bohol sites, the differences were not significant although the MA in the productive farms were also higher than in the marginal farms. The soil chemical properties in the three study sites are presented in Table 3. In South Cotabato, the results of the correlation analysis showed a significant positive correlation between total MA and soil pH (Table 4). No significant correlations of the total MA with soil OM content, total N, available P, exchangeable bases (K, Ca, Mg), and CEC were observed in this site.

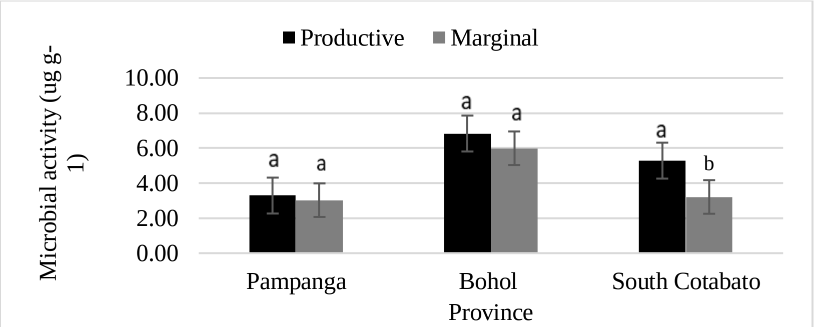

Fig. 1. Comparison of soil microbial activities in productive and marginal cassava farms.

The correlation analysis in South Cotabato indicate that an increase in soil pH by about one unit level improved significantly total MA in the productive farms. Varied observations about the relationship of soil pH with bacterial counts and FDA hydrolytic activity, which is a measure of total MA, indicate the response of microbial populations to soil pH levels may vary with crops and soil types (Higashida and Takao 1986; Tokuda and Hayatsu 2011). The lack of significant correlations of MA with OM, total N, exchangeable bases (K, Ca, Mg), and CEC could be attributed to the comparable levels of these chemical properties between the soils in the productive and marginal farms (Table 3). This observation differs from the reported significant correlations of FDA hydrolytic activity with microbial biomass C and N and other soil chemical properties (Gajda et al. 2016; Bueis et al. 2018).

Table 3. Chemical properties of the soils in the study sites. SD 2 decimal places.

Soil Chemical Properties	Study Sites					
	Pampanga		Bohol		South Cotabato	
	Productive	Marginal	Productive	Marginal	Productive	Marginal
Ph	5.2±0.44	4.1±0.49	5.0±0.20	4.9±0.05	5.4±0.44	4.5±0.15
OM (%)	0.94±0.30	0.95±0.12	2.83±0.63	2.34±0.42	3.53±1.75	3.60±2.69
Total N (%)	0.06±0.02	0.06±0.01	0 .16±0.006	0.17±0.02	0.17±0.08	0.16±0.12
Avail. P (ppm)	69±22.50	38±5.57	3±0.00	2±0.06	61±47.86	50±18.25
Exch. K (cmol$_c$ kg^{-1})	0.18±0.06	0.25±0.14	0.57±0.46	0.35±0.12	0.40±0.32	0.29±0.18
Exch. Ca (cmol$_c$ kg^{-1})	2.15±0.64	2.60±0.38	6.82±4.23	6.14±1.469	3.07±0.68	2.68±1.26
Exch. Mg (cmol$_c$ kg^{-1})	0.73±0.39	0.65±0.46	4.26±0.21	2.64±1.92	1.34±0.54	1.15±0.38
CEC (cmol$_c$ kg^{-1})	6.78±1.46	5.77±0.92	19.82±2.888	22.21±5.37	10.46±4.44	12.59±1.71

The absence of significant correlation between soil OM content and total MA is not in agreement with our initial assumption, based on studies on microbial distribution and farming systems (Monsalud et al. 2009; Monsalud et al. 2016), that the farms which produce more biomass accumulate higher soil OM and consequently favors greater microbial populations and activity than the less productive one. This suggests that the amounts of net biomass being produced and recycled in each farm type had no effect on the accumulation of soil OM. This implies that farm types and the associated farming history and current management practices had no influence on the status of the soil chemical properties with the exception of soil pH, and thus, not related to the level of MA. It is, however, unclear whether the soil pH of the two farm types was the effect of farming history and management practices or due to the inherent property of the soils.

The soil chemical properties from each farm type had no influence on the levels of total MA in Pampanga and Bohol. Except for available P in Pampanga, the levels of soil chemical properties in the productive and marginal farms were comparable (Table 3). As in the case of South Cotabato, it appears that farming history and the current management practices did not affect the measured chemical properties of the soils. Moreover, the differences in the levels of available P in Pampanga, which were both very high in productive (69 ppm) and marginal (38 ppm) farms, apparently had no influence on the levels of the total MA in the soil.

The different findings on the relationship between soil productivity and total MA observed in the three sites, which were generally not related to the soil chemical properties, could be attributed to the soil physical properties. One soil physical property that possibly influenced the productivity of the farms under study was soil drainage. The differences in the drainage conditions between the productive and marginal farms were noted in Pampanga and Bohol but not in South Cotabato (Table 1). The soil in Pampanga was well-drained in the productive farms, but poorly drained in the marginal farms. The soil in the marginal farms, a Typic Psammaquent, is saturated with water for repeated periods of time

(PhilRice 2010). In Bohol, the soil drainage conditions in the productive and marginal farms were good and moderate, respectively. The soil in the marginal farms, an Aquic Calciudoll, is saturated with water for repeated periods of time (Collado 2017). This drainage limitation, which is associated with aquic moisture regime, was not present in the soils in the productive farms, Typic Untipsamment in Pampanga, and Typic Eutrudept in Bohol. Since poor drainage condition is a yield-limiting factor for upland crops like cassava, it can be deduced that the farm productivity levels in Pampanga and Bohol were controlled by the soil physical quality (Sys et al. 1993).

The case of South Cotabato site demonstrated the positive relationship of MA and the soil's productive capacity and the significant correlation between MA and soil pH. Soil microbial populations can significantly contribute to agricultural soil quality due to their participation in soil processes related to nutrient cycling, detoxification, and stabilization of soil aggregates, in addition to other functions (Lovaisa et al. 2017). A number of reports indicated that microbial processes release bound nutrients and substances such as hormones and enzymes that stimulate plant growth (Utobo and Tiwari 2014; Micuti et al. 2017). The study of Wang et al. 2018 on unfertilized arable land showed that the composition and abundance of microbial communities perform important role in determining soil productivity. On the other hand, the case of Pampanga and Bohol sites demonstrated the limitation of MA as a soil quality indicator when applied to cassava farms where soil productivity is influenced more by physical properties affecting soil drainage.

This preliminary study on MA as soil quality indicator conducted in small farms planted to cassava revealed that total MA, measured by FDA hydrolytic activity, as a soil biological quality indicator has a limitation in its application. The findings in this study indicated that MA can be a valuable tool in monitoring the effects of the levels of farming technology and soil management practices on soil acidification which is potentially causing harm to soil biological quality. Future studies should be conducted to establish the response of MA to the technology levels of a given crop-based farming system and specific soil type. Studies on the determination of the threshold values of total MA for various farming systems and soil types are needed to define the appropriate limit of technology levels that will enhance the capacity of the soil to perform sustainably its production function.

Soil bacterial diversity and productivity. The DGGE profile of some of the cassava farm soils in the test sites at different stages of growth of the crop showed marginal farms (with asterisks) have fewer bands detected than the productive soils (Fig. 2). 16S rDNA sequencing of the prominent bands showed that farms with high productivity have higher species richness compared to farms with marginal productivity (Fig. 3).

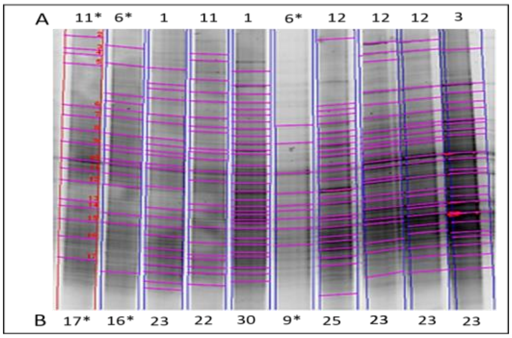

Fig. 2. DGGE microbial profile of some of the cassava soils from the study sites.
(A). The numbers denote the age of the standing crops, in months.
(B). The number of bands detected by automated band detector (BIORAD software) at 20 sensitivity.
(*). Samples from marginally productive soils.

Table 4. Correlation analysis between MA and chemical properties of the soils in South Cotabato.

	Microbial Activity	**pH**	**CEC**	**Avail. P**	**OM**	**Exch. Mg**	**Exch. Ca**	**Total N**	**K**
Microbial Activity	1	0.8628*	−0.5886^{ns}	0.3580^{ns}	−0.2133^{ns}	−0.2039^{ns}	−0.1998^{ns}	−0.1370^{ns}	0.0005^{ns}
pH	0.8628*	1	−0.7082^{ns}	0.0676^{ns}	−0.3725^{ns}	0.0981^{ns}	0.2341^{ns}	−0.2825^{ns}	0.2704^{ns}
CEC	−0.5886^{ns}	−0.7082^{ns}	1	−0.3767^{ns}	0.7390^{ns}	0.4446^{ns}	0.1458^{ns}	0.7367^{ns}	0.2092^{ns}
Avail. P	0.3580^{ns}	0.0676^{ns}	−0.3767^{ns}	1	−0.2198^{ns}	−0.6109^{ns}	−0.6309^{ns}	−0.2575^{ns}	−0.6446^{ns}
OM	−0.2133^{ns}	−0.3725^{ns}	0.7390^{ns}	−0.2198^{ns}	1	0.2136^{ns}	0.2183^{ns}	0.9907*	−0.1641^{ns}
Exch. Mg	−0.2039^{ns}	0.0981^{ns}	0.4446^{ns}	−0.6109^{ns}	0.2136^{ns}	1	0.8195*	0.3034^{ns}	0.8519*
Exch. Ca	−0.1998^{ns}	0.2341^{ns}	0.1458^{ns}	−0.6309^{ns}	0.2183^{ns}	0.8195*	1	0.2719^{ns}	0.5672^{ns}
Total N	−0.1370^{ns}	−0.2825^{ns}	0.7367^{ns}	−0.2575^{ns}	0.9907*	0.3034^{ns}	0.2719^{ns}	1	−0.0555^{ns}
K	0.0005^{ns}	0.2704^{ns}	0.2092^{ns}	−0.6446^{ns}	−0.1641^{ns}	0.8519*	0.5672^{ns}	−0.0555^{ns}	1

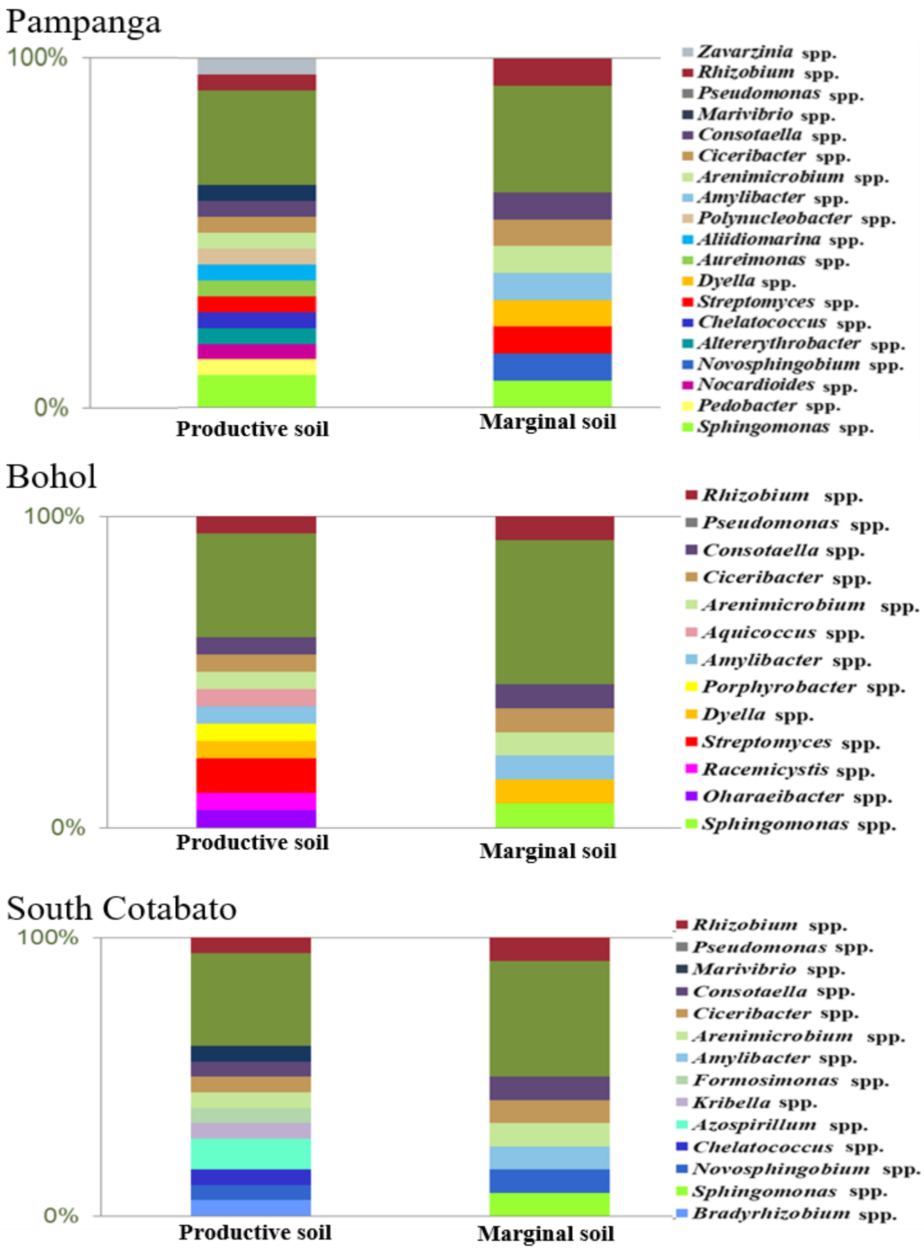

Fig. 3. Dominant bacterial species identified in soils of selected cassava farms in the study sites.

More dominant species were recovered from productive farms (16 in Pampanga, 12 in Bohol, and also 12 in South Cotabato) compared to the marginal farms (10 in Pampanga, and eight each in Bohol and South Cotabato). Similar species were found in productive farms and marginal farms across all provinces/islands. There were more reported beneficial bacterial genera identified in the productive farms of Pampanga and South Cotabato compared to the unproductive farms, while the same number was identified in Bohol farms (Table 5). However, the relative abundance (band intensity) of each genus varies in different farms. The *Pseudomonas* spp. were much more abundant in all farms, whether classified as productive or marginal.

Table 5. List of identified bacteria (reported as beneficial for agriculture) in the soils of the productive (P) and marginal (M) cassava farms.

Beneficial Microbes	Pampanga		Bohol		South Cotabato		Function	References
	P	M	P	M	P	M		
Rhizobium sp.	+	+	+	+	+	+	Nitrogen fixation	Santos et al. 2019
Pseudomonas sp.	+	+	+	+	+	+	Root development	Zamioudis et al. 2013
Consotaella sp.	+	+	+	+	+	+	Nitrogen fixation	Díaz-Cárdenas et al. 2017
Ciceribacter sp.	+	+	+	+	+	+	Nitrogen fixation	Siddiqi et al. 2018
Streptomyces sp.	+	+	+	-	-	-	Biocontrol and plant growth promotion	Olanrewaju and Babalola. 2019
Sphingomonas sp.	+	+	-	+	+	-	Stimulates growth of lateral roots and root hairs	Luo et al. 2019
Chelatococcus sp.	+	-	-	-	+	-	Organic matter decomposition	Zhang et. al. 2017
Nocardioides sp.	+	-	-	-	-	-	Plant growth promoting endophyte	Han et al. 2013
Pedobacter sp.	+	-	-	-	-	-	Accelerates crop maturation and fruit quality	Morais et al. 2019
Novoshingobium sp.	-	+	-	-	+	+	Plant growth promoting endophyte	Zhang et al. 2016
Azospirillum sp.	-	-	-	-	+	-	Nitrogen fixation	Alexandre. 2017
Bradyrhizobium sp.	-	-	-	-	+	-	Nitrogen fixation	Hara et al.2019
Total no. of beneficial microbes	9	7	5	5	9	5		

Moreover, correlation analysis based on species occurrence relative to the productive or marginally productive areas revealed that the higher the occurrence of a particular species in productive cassava farms, the higher its correlation would be (Fig. 4). Generally, 65% of all the identified species have a positive correlation to productive cassava farming areas, with correlation ranging from 26 to 50% ($p<0.05$). This is consistent with the results of Wagg (2014), wherein it was shown that soil biodiversity is associated with higher crop productivity, higher rates of nitrogen turnover, and reduced leaching of essential minerals. Among the positively correlated species are *Pseudomonas azotoformans*, *Pseudomonas parafulva*, *Aliidiomarina soli*, and *Nocordioides phosphati*. In addition, a proportion of 9.76% was identified to be equally prevalent in productive and marginally productive areas. These species are *Amylibacter lutimaris, Ciceribacter thiooxidans, Consotaella salsifontis,* and *Arenimicrobium luteum*. These species can be classified as highly ubiquitous, abundant with vast global distribution, and belongs to Class Alphaproteobacteria (Brenner et al. 2005). Only three microbial species were identified to exhibit a very high correlation (>76%, $p<0.05$) to productive cassava areas, namely *Chelatococcus composti*, *Marivibrio halodurans,* and *Pseudomonas gessardii*. *C. composti* was first isolated from fungal spent substrate and pig manure compost (Zhang et al. 2017). It is a potentially beneficial microorganism that promotes the rapid breakdown of highly cellulosic substrates. High organic matter in the soil inhibits the absorption of essential micronutrients.

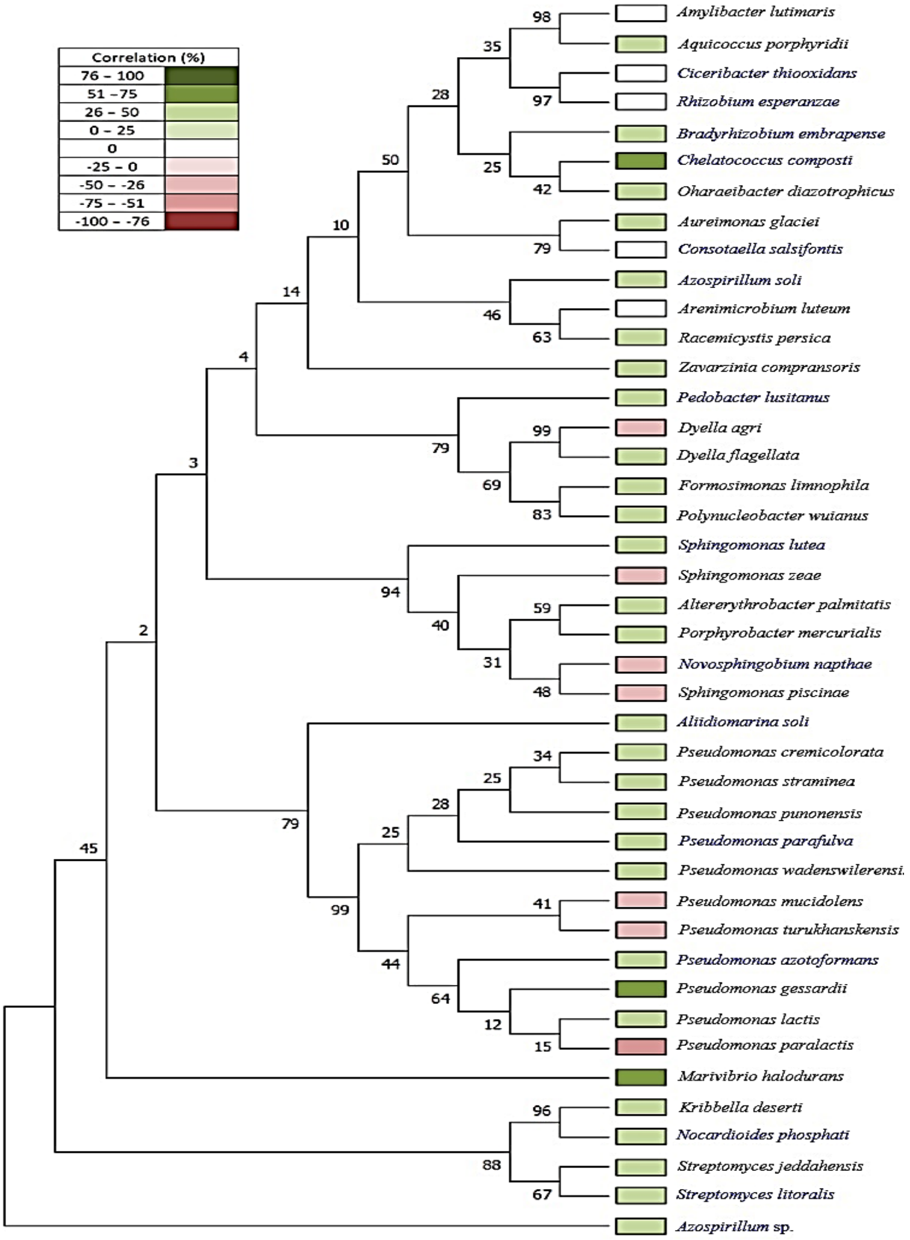

Fig. 4. Phylogenetic tree of the partial 16S rDNA sequences and correlation analysis of the soil microorganisms identified in the study sites (values at the nodes are bootstrap values at 1000 replications).

Microorganisms have been used to facilitate nutrient uptake in various crops such as cassava (Otaiku et al. 2019), corn (Viruel et al. 2014) and rice (Nepomuceno et al. 2020). Thus, the high correlation rate of *C. composti* can be attributed to its enhancement of micronutrient uptake through the rapid breakdown of organic matter. Another highly correlated microorganism to productive cassava farms is *Marivibrio haloduran,* which can withstand a highly alkaline environment (Chen et al. 2017) and could potentially increase the tolerance of cassava under an alkaline environment. Strains of *P. gessardii* can be beneficial in the bioremediation of polycyclic aromatic hydrocarbons (PAHs) and heavy metals (Huanga et al. 2016) and can potentially increase the tolerance of cassava against contaminants. Six species, however, were determined to be negatively correlated to productive cassava farming areas, namely *Sphingomonas zeae, Sphingomonas piscinae, Novosphingobium napthtae, Pseudomonas paralactis, Pseudomonas turukhanskensis, Pseudomonas mucidolens* and *Dyella agri*.

It is inferred that the higher the diversity of microorganisms in the soil, the healthier the soil. The diverse microbial functions brought about by various microorganisms are important and necessary for an ecosystem to be sustainable (Marcel et al. 2008). Diversity had significant effect on the mineralization of soil organic matter (Juarez et al. 2013), soil health, disease suppression and nutrient cycling (Chourasiya et al. 2017), and fertility and biocontrol functions (Zhu et al. 2018). Soil bacterial diversity was used in estimating potato productivity with emphasis on species balance index while correlation to land use and soil physico-chemical properties was attained with high prediction accuracy to soil quality (Jeanne et al. 2019; Hermans et al. 2020).

A major limitation of the technique is the underestimation of bacterial diversity population. In general, dominant species as reflected by band intensity is considered as the major species but a single major band does not always represent a single species (De Mandal et al. 2015). However, DGGE is a much better technique to study microbial diversity than culture-dependent methods. A more recent technique is a high throughput DNA sequencing technology called next generation sequencing or NGS. It is a powerful technique which revolutionized genomic research especially with ecology of microorganisms that can complete millions of sequences directly from a given environmental sample in a single experiment (De Mandal et al. 2015). However, at the time this study was started, NGS was not possible to do as there was no available service provider even in the Southeast Asia region; hence the cost was very limiting.

Soil arbuscular mycorrhizal fungi abundance and productivity. The abundance of three genera of AMF *(Acaulospora* spp, *Gigaspora* spp. and *Glomus* spp.) was determined in the soils of the study farms (Fig. 5). Consistently higher AMF populations were obtained in the productive farms than in the marginal farms. Among the three species, *Glomus* spp. were the most abundant in all farms in the study sites in both farm types. However, it should be noted that in all the marginal farms, lower *Glomus* spp. density were observed and *Gigaspora* spp. were absent.

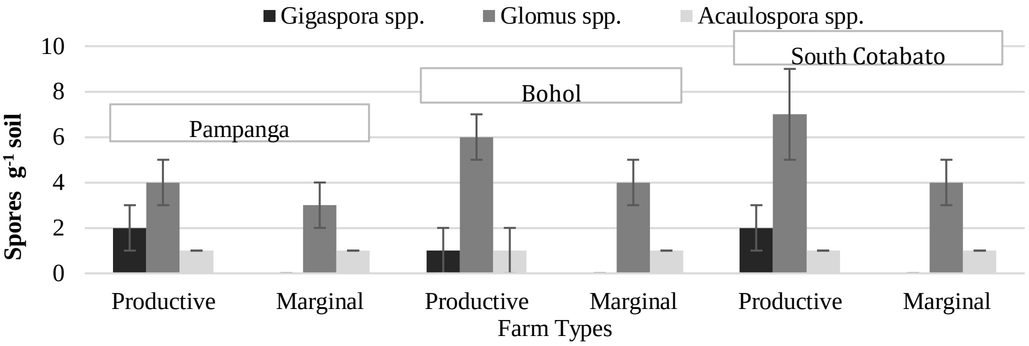

Fig. 5. AMF spore counts in the soils of cassava farms in the study sites.

The results of the study suggest that AMF is a good indicator of cassava farm productivity. Increases in yield of cassava linked to mycorrhiza have been documented. Mycorrhizal fungi help provide nutrients to plants by absorbing these from farther areas in the soil through the fungal mycelia which serve as root extensions. The fungi also solubilize bound phosphorus making this a prominent contribution to the nutrition of the plant. Mycorrhiza is also a biological control agent. Further, antagonistic effect of AMF against a number of bacterial and plant pathogens had been observed by many investigators (Tahat et al. 2010). *Glomus* spp. was the dominant indigenous mycorrhizal spores from the Alfisol soil mycorrhiza source which showed significant improvement in the growth of the indigenous Kirik cassava cultivar in Indonesia (Astuti el al. 2020). *Acaulospora colombiana* significantly improved the growth and enhanced the tolerance of cassava to water stress in Cote d'Ivoire (Sery et al. 2016). *Glomus mosseae*, *G. clarum* and *G. fasciculatus* AMF species yielded 20.7 – 39.3 % root yield increase in Nigeria (Oyetunji and Osonubi 2007). The genera *Glomus*, *Gigaspora* and *Acaulospora*, identified in symbiotic association with cassava, are being considered for mycorrhizal inoculation in Cameroon (Begoude et al. 2016).

CONCLUSION

A positive relationship between total microbial activity and soil productivity was observed in only one of the three study sites indicating the limitation of its application as soil quality indicator particularly in areas where soil productivity is influenced by soil physical properties affecting drainage. The findings also demonstrate the consistent association of more diverse bacterial populations and higher AMF populations in productive soils under cassava-based farming systems in Pampanga, Bohol and South Cotabato sites. It is recommended that future studies include the establishment of the response of microbial activity and diversity to the levels of farming technology for each crop-based farming system and specific soil type.

ACKNOWLEDGMENT

The authors are grateful to the Department of Agriculture-Bureau of Agricultural Research (DA-BAR) of the Philippines for the research funds. We also highly appreciate the assistance provided by the provincial and municipal agricultural offices, as well as the farmer respondents in the project sites. We likewise thank the following staff of BIOTECH for their invaluable contribution in the conduct of this research: Adora M. de Castro, Rose May Anne J. Capanzana, Francisca P. Lagman, and Jovenal C. Lanceras.

REFERENCES CITED

Adam, G. and H. Duncan. 2001. Development of a sensitive and rapid method for the measurement of total microbial activity by fluorescein diacetate (FDA) in a range of soils. Soil Biol. Biochem. 33:943-951.

Alexandre, G. 2017. *Azospirillum brasilense*, a beneficial soil bacterium: isolation and cultivation. Curr. Prot. Microbiol., 47(1):3E-1.1-3E.1.10. DOI:10.1002/cpmc.40.

Arshad, M.A. and S. Martin. 2002. Identifying critical limits for soil quality indicators in agro-ecosystems. Agric. Ecosyst. Environ. 88:153-160.

Astuti, A., M. Haryono and A. E Putri. 2020. Compatibility and effectivity of various mycorrhizal sources with cassava varieties in Gunungkidul. Second International Conference on Sustainable Agriculture IOP Conf. Series: Earth and Environmental Science. 458:1-8. DOI:10.1088/1755-1315/458/1/012005.

Begoude, D.A. B., P.S. Sarr, T.L.Y. Mpon, D.A. Owona , M.N Kapeua and S. Araki. 2016. Composition of arbuscular mycorrhizal fungi associated with cassava (*Manihot esculenta* Crantz) cultivars as influenced by chemical fertilization and tillage in Cameroon. J. Appl. Biosci. 98:9270-9283.

Brady, N.C. and R. Weil. 2008. The Nature and Properties of Soils. 14th Ed. Pearson Prentice Hall. New Jersey. 975 pp.

Brenner, D.J., N.R. Krieg and J.T. Staley. (eds.). 2005. The Proteobacteria. Vol 2C. In. Garrity, G.M. (chief ed.). Bergey's Manual of Systematic Bacteriology. New York. Springer. 1388 pp.

Brown, M.B., C.M.B. Brown and R.A. Nepomuceno. 2016. Regulatory requirements and registration of biopesticides in the Philippines, In: HB Singh, BK Sarma and C Keswani (Eds.) Agriculturally Important Microorganisms Commercialization and Regulatory Requirements in Asia, p. 183-195, Springer-Nature. Singapore. DOI:10.1007/978-981-10-2576-1.

BSWM [Bureau of Soils and Water Management]. 1989. Physical Land Resources: Province of South Cotabato. DA-BSWM, Quezon City, Philippines. 283 pp.

Bueis, T., M.B. Turrión, F. Bravo, V. Pando and A. Muscolo. 2018. Factors determining enzyme activities in soils under *Pinus halepensis* and *Pinus sylvestris* plantations in Spain: a basis for establishing sustainable forest management strategies. Ann. Forest Sci. 75(34):1-13. DOI:/10.1007/s13595-018-0720-z.

Chen S, Y. Xu, C. Zheng and L.X. Ke. 2017. *Marivibrio halodurans* gen. nov., sp. nov., a marine bacterium in the family Rhodospirillaceae isolated from underground rock salt. Intl. J. of Syst. and Evol. Microbiol. 67(10):4266-4271. DOI: 0.1099/ijsem.0.002309.

Chourasiya D., M.P. Sharma, H.S. Maheshwari, A. Ramesh, S.K. Sharma and T.K. Adhya. 2017. Microbial diversity and soil health in tropical agroecosystems. In: Adhya T., Mishra B., Annapurna K., Verma D., Kumar U. (eds) Advances in Soil Microbiology: Recent Trends and Future Prospects. Microorganisms for Sustainability, vol 4. Springer, Singapore. pp. 19-35. DOI:/10.1007/978-981-10-7380-9_2.

Collado, W.B., R.T. Dollentas, J.L. De Dios, J.D. Bibar, R.V. Bermudez, Jr., S.D. Canete. 2017. Simplified Keys to Soil Series: Bohol. Philippine Rice Research Institute, Nueva Ecija, Philippines. 59 pp.

De Mandal, S., A.K. Panda, S.S. Bisht and N.S. Kumar. 2015. Microbial ecology in the era of next generation sequencing. Next Generat. Sequenc. and Applic. S1:1-6. DOI:10.4172/2469-9853.S1-001.

Díaz-Cárdenas, C., L.F. Bernal, A. Caro-Quintero, G. López, J. D. Alzate, L. N. Gonzalez, S. Restrepo, N. Shapiro, T. Woyke, N.C. Kyripedes and S. Baena. 2017. Draft genome and description of *Consotaella salsifontis* gen. nov. sp. nov., a halophilic, free-living, nitrogen-fixing alphaproteobacterium isolated from an ancient terrestrial saline spring. Int. J. Syst. Evol. Microbiol., 67(10):3744-3751.

Dutta, M., D. Sardar, R. Pal and R.K. Kole. 2010. Effect of chlorpyrifos on microbial biomass and activities in tropical clay loam soil. Environ. Monitoring Assessment, 160:385-391.

FAO. 1976. A framework for land evaluation. Soils Bulletin 32.FAO, Rome. 72 p.

Fermont, A. and T. Benson. 2011. Estimating yield of food crops grown by smallholder farmers: A review in the Uganda context. International Food Policy Research Institute (IFPRI) Publication, Washington D.C. 57 p.

Gajda, A.M., E.A. Czyz and A.R. Dexter. 2016. Effects of long-term use of different farming systems on some physical, chemical and microbiological parameters of soil quality. Int. Agrophys. 30: 165-172.

Gajda A.M., E.A Czyż., J. Stanek-Tarkowska, A.R. Dexter, K.M. Furtak and J. Grządziel. 2017. Effects of long-term tillage practices on the quality of soil under winter wheat. Plant Soil Environ. 63: 236-242.

Gajda, A.M., E.A. Czyż, A.R. Dexter, K.M. Furtak, J. Grządziel and J. Stanek-Tarkowska. 2018. Effects of different soil management practices on soil properties and microbial diversity. Int. Agrophys. 32:81-91. DOI: 10.1515/intag-2016-008.

Garbeva, P., J.A. van Veen and J.D. van Elsas. 2004. Microbial diversity in soil: Selection of microbial populations by plant and soil type and implications for disease suppressiveness. Annu. Rev. Phytopathol. 42:243–70. DOI:10.1146/annurev.phyto.42.012604.135455.

Gerdemann, J.W. and T.H. Nicholson. 1963. Spores of mycorrhizal *Endogone* species extracted by wet sieving and decanting. Trans. Brit. Mycol. Soc. 46:235.

Green, V.S., D.E. Stott and M. Diack. 2006. Assay for fluorescein diacetate hydrolytic activity: optimization for soil samples. Soil Biol. Biochem. 38:693-701.

Han, J. H., T. S. Kim, Y Joung, M.N. Kim, K. S. Shin, T. Bae and S. B. Kim. 2013. *Noc endophyticus* sp. nov. and *Nocardioides conyzicola* sp. nov., isolated from herbaceous plant roots. Int. J. Syst. Evol. Microbiol., 63(12):4730-4734.

Hara S., T. Morikawa, S. Wasai, Y. Kasahara, T. Koshiba, K. Yamazaki, T. Fujiwara, T. Tokunaga and K. Minamisawa. 2019. Identification of nitrogen-fixing *Bradyrhizobium* associated with roots of field-grown sorghum by metagenome and proteome analyses. Front. Microbiol. 10(407):1-15. DOI:10.3389/fmicb.2019.00407.

Hermans, S.M., H.L. Buckley, B.S. Case, F. Curran-Cournane, M. Taylor and G. Lear. 2020. Using soil bacterial communities to predict physico-chemical variables and soil quality. Microbiome 8(79):1-13. DOI:10.1186/s40168-020-00858-1.

Higashida, S. and K. Takao. 1986. Relations between soil microbial activity and soil properties in grassland. Soil Sci. Plt. Nutri, 32(4):587-597. DOI:10.1080/00380768.1986.10557540.

Huanga, H., K. Wua and A. Khana. 2016. A novel *Pseudomonas gessardii* strain LZ-E simultaneously degrades naphthalene and reduces hexavalent chromium. Bioresource Tech. 207:370-378.

Jeanne, T., Parent S-E´, and R. Hogue. 2019. Using a soil bacterial species balance index to estimate potato crop productivity. PLOS ONE. 14(3):1-15. DOI:10.1371/journal.pone.0214089.

Juarez, S., N. Nunan, A.C. Duday, V. Pouteau and C. Chenu. 2013. Soil carbon mineralization responses to alterations of microbial diversity and soil structure. Biol. Fertil. Soils. 49(7): 939-948.

Karlen, D.L., M.J. Mausbach, J.W. Doran, R.G. Cline, R.F. Harris and G.E. Schuman. 1997. Soil quality: a concept, definition, and framework for evaluation. Soil Sci. Soc. Amer. J. 61: 4-10.

Lovaisa, N.C., M.F. Guerrero-Molina, P.G. Delaporte-Quintana, M.D. Alderete, A.L.Ragout, S.M. Salazar and R.O. Pedraza. 2017. Strawberry monocropping: Impacts on fruit yield and soil microorganisms. J. Soil Sci. Plant Nutr. 17(4):868-883 DOI:10.4067/S0718-95162017000400003.

Luo, Y., F. Wang, Y. Huang, M. Zhou, J. Gao, T. Yan, H. Sheng and L. An. 2019. *Sphingomonas* sp. Cra20 increases plant growth rate and alters rhizosphere microbial community structure of *Arabidopsis thaliana* under drought stress. Front. Microbiol. 10(1221):1-19. DOI: 10.3389/fmicb.2019.01221

Marcel, G.A., R.D. van der Heijden and B.N.M. van Straalen. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11:296-310.

Micuti, M.M., L. Badulescu and F. Israel-Roming. 2017. A review on the enzymatic indicators for monitoring soil quality. Sci. Bull. Series F. Biotech. 21:223-228.

Mojica, A.E., I.J. Aristorenas and J.A. Tingzon. 1952. Soil Survey Report of Bohol province, Philippines. Soil Report 15. Bureau of Printing, Manila, Philippines. 90 pp.

Mojica, A.E., J.A. Mariano and F.G. Salazar. 1963. Soil Survey Report of Cotabato province, Philippines. Soil Report 28. Bureau of Printing, Manila, Philippines. 145 pp.

Monsalud, F.C., R.G. Monsalud, M.B. Brown and R.D. Badayos. 2009. Distribution of soil microorganisms in selected farming systems in Laguna, Philippines. Phil. J. Crop Sci. 34(3): 90-101.

Monsalud, R.G., M.B. Brown, A.R. Creencia and F.C. Monsalud. 2016. Spatial distribution of microbial populations in soils under various farming systems in Batangas Province, Philippines. Asia Life Sci. 25(1):221-243.

Morais, M. C., Â. Mucha, H. Ferreira, B. Gonçalves, E. Bacelar and G. Marques. 2019. Comparative study of plant growth-promoting bacteria on the physiology, growth and fruit quality of strawberry. J. Sci. Food Agric. 99(12):5341-5349. DOI: 10.1002/jsfa.9773

Mueller, L., U. Schindler, W. Mirschel, T.G. Shepherd, B.C. Ball, K. Helming, J. Rogasik, F. Eulenstein and H. Wiggering. 2010. Assessing the productivity function of soils: A review. Agronomy for Sustainable Development 30:601-614.

Nepomuceno, R.A., C.M.B. Brown, A.M.P. Gargarino, M.S. Pedro and M.B. Brown. 2020. Growth enhancement of rice (*Oryza sativa* L.) by zinc-solubilizing bacteria isolated from vesicular-arbuscular mycorrhizal root inoculant (VAMRI). Phil. J. Crop Sci. 45(1):34-40.

Olanrewaju, O. S., and O.O. Babalola. 2019. *Streptomyces*: implications and interactions in plant growth promotion. Appl. Microbiol. Biotechnol. 103(3):1179-1188.

Otaiku, A.A, P.C Mmom and A.O.Ano. 2019. Biofertilizer impacts on cassava *Manihot esculenta* Crantz) cultivation: Improved soil health and quality, Igbariam, Nigeria. World J. Agric. Soil Sci. 4:1-14. DOI:10.33552/WJASS.2019.04.000578.

Oyetunji, O.J. and O. Osonubi. 2007. Assessment of influence of alley cropping system and arbuscular mycorrhizal (AM) fungi on cassava productivity in derived savanna zone of Nigeria. World J. Agric. Sci. 3(4):489-495.

Patle, P.N., N.P. Navnage and P.K. Barange. 2018. Fluorescein diacetate (FDA): Measure of total microbial activity and as indicator of soil quality. Int. J. Curr. Microbiol. App. Sci. 7(6): 2103-2107.

PhilRice. 2010. Soil Series: Improving Agricultural Productivity in Pampanga. Philippine Rice Research Institute, Nueva Ecija, Philippines. 56 pp.

Rincon-Florez, V.A., L.C. Carvalhais and P.M. Schenk. 2013. Culture-independent molecular tools for soil and rhizosphere microbiology. Diversity 5:581-612. DOI:10.3390/d5030581.

Sambrook, J., E.F. Fritschi and T. Maniatis 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York. 1.659pp.

Sanchez, J.D.L., I. Armbrecht and J.M. Lerma. 2015. Arbuscular mycorrhiza and their effect on the soil structure in farms with agroecological and intensive management. Acta Agronómica. 64 (4): 274-281. DOI:10.15446/acag.v64n4.46045.

Santos, M.S., M.A. Nogueira and M. Hungria. 2019. Microbial inoculants: reviewing the past, discussing the present and previewing an outstanding future for the use of beneficial bacteria in agriculture. AMB Expr 9(205):1-22. DOI:10.1186/s13568-019-0932-0.

Séry, D.-M., Z.G.C. Kouadjo, B.R.R. Voko and A. Zézé. 2016. Selecting native arbuscular mycorrhizal fungi to promote cassava growth and increase yield under field conditions. Front. Microbiol. 7:2063. doi: 10.3389/fmicb.2016.02063.

Siddiqi, M. Z., G. M. Choi and W.T. Im. 2018. *Ciceribacter azotifigens* sp. nov., a nitrogen fixing bacterium isolated from activated sludge. Int. J. Syst. Evol. Microbiol. 68(2):482-486.

Stubberfield, L.C.F. and P.J.A. Shaw. 1990. A comparison of tetrazolium reduction and FDA hydrolysis with other measurements of microbial activity. J. Microbiol. Methods. 12:151-162.

Syib'li, M.A., A. Muhibuddin and S. Djauhari. 2013. Arbuscular mycorrhiza fungi as an indicator of soil fertility. Agrivita. 35(1):44-53.

Sys, C., E. V. Ranst, J. Debaveye and F. Beernaert. 1993. Land Evaluation Part III: Crop Requirements. Agricultural Publications No. 7. General Administration for Development Cooperation. Belgium. 199 p.

Tahat, M.M., S. Kamaruzaman and R. Othman, 2010. Mycorrhizal fungi as a biocontrol agent (Review article). Plant Pathol. J. 9:198-207. DOI: 10.3923/ppj.2010.198.207.

Tokuda, S. and M. Hayatsu. 2011. Soil microbial biomass and fluorescein diacetate hydrolytic activity in Japanese acidic tea field soils. Soil Sci. Plt. Nutri. 48(6):865-869. DOI: 10.1080/00380768.2002.10408713.

Tommerup, I.D. and D.K. Kidby. 1979. Preservation of spores of vesicular-arbuscular endophytes by L-drying. Appl. Environ. Microbiol. 17:831-835.

Utobo, E.B. and L. Tewari. 2014. Soil enzymes as bioindicators of soil ecosystem status. Appl. Ecol. Environ. Res. 13(1):147-169.

Verzeaux, J., E. Nivelle, D. Roger, B. Hirel, F. Dubois and T. Tetu. 2017. Spore density of arbuscular mycorrhizal fungi is fostered by six years of a no-till system and is correlated with environmental parameters in a silty loam soil. Agronomy. 38(7):1-9.

Viruel, E., L.E. Erazzu, L. Martínez-Calsina, M.A. Ferrero, M.E. Lucca and F. Siñeriz. 2014. Inoculation of maize with phosphate solubilizing bacteria: effect on plant growth and yield. J. Soil Sci. Plant Nutr. 14(4):819-831.

Wagg, C., S.F. Bender, F. Widmer and M.G.A. van der Heijden. 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proc. Natl. Acad. Sci. USA.111:5266–5270.

Wang, B., Y. Adachi and S. Sugiyama. 2018. Soil productivity and structure of bacterial and fungal communities in unfertilized arable soil. PLOS ONE 13(9):1-17. DOI:10.1371/journal.pone.0204085.

Winding, A. 2004. Indicators of soil bacterial diversity. In: Agricultural impacts on soil erosion and soil biodiversity: developing indicators for policy analysis. Proceedings of an OECD expert meeting on soil erosion and soil biodiversity indicators. 25–28 March 2003. Rome, Italy. pp. 495–504.

Yniguez, T.M., I.M. Jose and J.P. Dimaano. 1956. Soil Survey Report of Pampanga Province, Philippines. Ad Bureau, Manila, Philippines. 76 pp.

Zamioudis, C., P. Mastranesti, P. Dhonukshe, I. Blilou and C. M.J. Pieterse. 2013. Unraveling root developmental programs initiated by beneficial *Pseudomonas* spp. bacteria. Plant Physiol. 162(1):304–318. DOI:10.1104/pp.112.212597.

Zhang, H.Y., Z. Gao, M. Shi and S. Fang. 2020. Soil bacterial diversity and its relationship with soil CO_2 and mineral composition: A case study of the Laiwu experimental site. Int. J. Environ. Res. Public Health. 17(5699):1-20.

Zhang, J. and P. Li. 2020. Using soil microorganism to construct a new index of soil quality evaluation. Earth Environ. Sci. 440(052023):1-4. DOI:10.1088/1755-1315/440/5/052023.

Zhang, L., J.S. Gao, S.G. Kim, C.W. Zhang, J.Q. Jiang, X.T. Ma, J. Zhang and X.X. Zhang. 2016. *Novosphingobium oryzae* sp. nov., a potential plant-promoting endophytic bacterium isolated from rice roots. Int. J. Syst. Evol. Microbiol. 66(1):302-307.

Zhang, Z., J. Zhao, C. Yu, S. Dong, H. Duan, R. Yu, Y. Liu and C. Wang. 2017. *Chelatococcus composti* sp. nov., isolated from penicillin fermentation fungi residue with pig manure co-compost. Int. J. of Syst. and Evol. Microbiol. 67(3):565-569. DOI:10.1099/ijsem.0.001651.

Zhu, S., Y. Wang, X. Xu, T. Liu, D. Wu, X. Zheng, S. Tang and Q. Dai. 2018. Potential use of high-throughput sequencing of soil microbial communities for estimating the adverse effects of continuous cropping on ramie (*Boehmeria nivea* L. Gaud). PLOS ONE 13(5):1-16. DOI:10.1371/journal.pone.0197095.