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Soil microbial community response to different farm managements in Santa Clara, Cuba

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ABSTRACT
Seven farms (two state, two cooperatives, and three private farms) were selected for assessing effects of farm management on the microbial biomass and the structure of the microbial community, as well as, the responses to seasonality on these two bio-indicators in these three representative farming systems. All farms are located on brown calcareous soil. Soil samples from the 0-20 cm depth were collected from two fields of each farm. Soil microbial community was assessed through two analyses: microbial biomass carbon and phospholipid fatty acid. The technological differences in soil management, among the three farming systems, affected both microbial biomass carbon and the microbial community composition. The differences were most pronounced between the private and the state farms. The statistical analyses demonstrated that the total of phospholipid fatty-acid were significantly higher in cooperative farms. The use of fallow in these farms seems to have positive effects on soil microbial communities. Seasonality has a clear effect on both indicators. Summarizing, both indicators demonstrated sensible responses to disturbances caused by farm management and seasonality in the conditions of Cuban agriculture.

Keywords: farming systems, microbial biomass carbon, phospholipid fatty-acid analysis (PLFA), soil quality

Respuesta de la comunidad microbiana del suelo a manejos agrícolas diferentes en Santa Clara, Cuba

RESUMEN
Siete fincas (dos estatales, dos cooperativas y tres privadas) fueron seleccionadas para evaluar los efectos del manejo agrícola en la biomasa microbiana y en la estructura de la comunidad microbiana, así como, la respuesta a la estacionalidad de estos dos bio-indicadores en los tres sistemas agrícolas. Todas las fincas se encontraban sobre suelos pardos con carbonatos. Las muestras fueron colectadas en dos campos de cada finca a una profundidad de 0-20 cm. La comunidad microbiana fue evaluada a través de dos análisis: carbono de la biomasa microbiana y los fosfolípidos. Las diferencias tecnológicas en el manejo de los suelos, entre los tres sistemas agrícolas, afectaron el carbono de la biomasa microbiana y la estructura de la comunidad microbiana. Las diferencias fueron más pronunciadas entre los sistemas privados y estatales. Los análisis estadísticos demostraron que el total de los fosfolípidos fue significativamente superior en los campos de las cooperativas. El uso del barbecho en estas fincas parece tener efectos positivos en la comunidad microbiana del suelo. La estacionalidad tuvo un claro efecto en los dos indicadores. En resumen, estos bioindicadores demostraron respuestas sensibles a los disturbios causados por el manejo y la estacionalidad en las condiciones de la agricultura cubana.

Palabras clave: análisis de ácidos grasos fosfolípidos (PLFA), calidad del suelo, carbono de la biomasa microbiana, sistemas agrícolas

INTRODUCTION

Soils are complexes, diverse, and heterogeneous and provide several important functions to the ecosystems (García-Orenes *et al*., 2013). Nowadays, improving of soil quality constitutes the base for enhancing food security and ecosystem functioning. From this perspective soil, biology has been addressed as a crucial indicator of soil quality, due to the major role of soil biota in organic matter turnover and because of the quick response of biological soil properties to changes in soil caused by crop management practices (D´Hose *et al.*, 2014).

Microbial habitat is intimately related to soil functioning and its genesis (Gupta *et al.*, 2008). Microorganisms play an important role in nutrient cycling, mineralization of nutrients, soil aggregation; hence, they are important for the maintenance of soil fertility and the sustainability of the ecosystems and any kind of agriculture (Esperschütz *et al.*, 2007).

The composition of SMC may be influenced by environmental conditions, vegetation and soil properties (Hackl *et al.*, 2005), but also cultivation (McKinley and White, 2005; Moore-Kucera and Dick, 2008).

The soil microbial biomass (SMB) represents a significant compartment of the terrestrial biomass and microbial residues in soil are an important source material for humus formation (John, 2003). As active fraction, SMB changes continually and responds much more rapidly to changes in the environment than total organic matter, being reported as an important sensitive indicator to environmental stress and changes in agricultural managements (Campos, 2008).

Moreover, agricultural management influences soil microbial communities, altering their functioning and structure, which in turn may have significant implications for soil quality. Management practices have a direct effect on soil microbiota. For assessing changes in soil microbiota due to agricultural has been used the phospholipid fatty acid (PLFA) analysis. The PLFA method is very useful and efficient when assessing soil microbiota composition; using the lipids of the microbial membranes as biomarkers of specific groups of microorganisms; which permit to analyze the profile of the microbial community structure (Frostegard *et al.*, 2011; García-Orenes *et al.*, 2013).

In Cuba, farming systems can be grouped into three major groups: private, cooperative, and state farms. These three groups differ in land ownership, technological complexity and agricultural intensification. State farms comprise large areas and follow a conventional agriculture model, producing food for the satisfaction of social demands (schools, hospitals, and also, population). These farms have access to improved seeds, fuel, chemical fertilizers, pesticides and mechanized technologies for soil preparation and harvesting (McCune *et al.*, 2011).

The cooperative farms are formed by the aggregation of several private farmers. In these farms, farming is done under similar organizational structures as the large state farms. Their aim is also producing food for distribution on a regional scale. The average size of cooperative farms range from 350 to 550 hectares (ONE, 2010). Private farms are owned and managed by independent farmers producing food for local consumption. These farms are characterized by a small production scale, high crop diversity, the use of alternative technologies for agricultural production, such as animal traction and intensive crop rotation, and the applications of intercropping (Figueroa, 2002; Cruz, 2005).

The impacts of farm management on soil microorganisms and carbon storage potential has been poorly explored (Moore-Kucera and Dick, 2008). In Cuba, studies approaching the influence of farm management on soil biological indicators are scarce in the country. The goal of this research was to assess the effects of different farm managements on SMB and SMC composition, as well as, the responses to seasonality on these two bio-indicators in these three representative farming systems.

MATERIALS AND METHODS

Description of the area and experimental design

This study was carried out in Santa Clara municipality, Villa Clara province, Cuba. Santa Clara has a tropical climate with a humid

summer (Aw). The rainy season starts in May and ends in October. Annual rainfall is 1100 mm on average, and the 80% of the total precipitation falls in the rainy season. Mean temperature is about 24 degrees, the hottest months are June, July and August, and the coolest is January. Mean relative humidity is usually around 81% and can go up to 86% (CITMA, 2008).

All selected farms were located on brown calcareous soil classified as orthi-calcaric cambisol in the FAO-UNESCO system (Hernández, *et al.,* 2005). The cambisols are the most common soil group throughout the country, accounting for 2 355 800 ha that represent 26.99% of all agricultural land in Cuba. Particularly in Villa Clara, this soil type occupies 249 400 ha, representing 33.29% of all productive lands (INS, 1999).

In Santa Clara, there are more private farms than state and cooperative farms. In the soil type selected for the study, private farms are more distributed throughout the municipality; meanwhile the state and cooperative farms are located in the northwest and northeast of Santa Clara city. Due to the uneven distribution of the private farms, three of them were selected for the research, but only two cooperative and two state farms (seven in total).The main characteristics of the technologies used by farming systems are described in table 1.

Table 1. Main characteristics of the technologies used by the selected farming systems.

Technology	Private farms	Cooperative farms	State farms
Agroforestry	Used for field delimitation, and in association with other crops	Trees are associated with crops and border the fields	Permanent fruit trees
Soil conservation measures	Practiced using agroecological techniques: fields with cover crops all year round. The use of crop association, crop rotation, mulching, green manure, and others	Practiced combining conventional and agroecological techniques (crop rotation and fallow)	Practiced using conventional techniques Fields are covered year round
Soil preparation	Animal traction	Tractor and animal traction	Tractor traction
	Low tillage intensity (2 times per year)	Medium tillage intensity (3 times per year)	High tillage intensity (5 times per year)
	Depth: 10 cm	Depth: Tractor (30 cm), animal traction (10-20 cm)	Depth: 30 cm
Soil fertility management	Cow manure and compost as a main source of organic matter (average 7/ha/year) No chemical fertilizers	NPK and urea (same doses as in the state farms) Organic matter (plant residues and manure) in different stages of decomposition: fresh, semi-composted and compost (average 200kg/ha/year)	NPK compound fertilizers (average of 1.45 t ha^{-1}) Urea (=200kg ha^{-1})
Water management	Seasonality	Seasonality	According to the crop needs
	Gravity	Gravity	Electric pumps

Table 1. Continuación.

Residue management	Plant residues are incorporated in the soil or used for animal feeding	Plant residues are not incorporated in the soil. In some cases, residues are used for animal feeding	Plant residues are not incorporated in the soil
Crop rotation	Typical crops: maize (*Zea mays* L.), sweet potato (*Ipomoea batatas* L.) , tomato (*Solanum lycopersicum* L.), cassava (*Manihot esculenta* Mil.), beans (*Phaseolus vulgaris* L.), cabbage (*Brassica oleracea* var. *capitata* L.), butternut squash (*Cucurbita moschata* Duch.), sorghum (*Sorghum halepense* L.), rice (*Oryza sativa* L.), and different fruits and other vegetables	Typical crops: potatoes (*Solanum tuberosum* L.), sweet potatoes (*Ipomoea batatas* L.), tomato (*Solanum lycopersicum* L.), maize (*Zea mays* L.), cassava (*Manihot esculenta* Mil.), beans (*Phaseolus vulgaris* L.) and butternut squash (*Cucurbita moschata* Duch.)	Typical crops: potatoes (*Solanum tuberosum* L.), sweet potatoes (*Ipomoea batatas* L.), cassava (*Manihot esculenta* Mil.), cabbages (*Brassica oleracea* var. *capitata* L), tomato (*Solanum lycopersicum* L.), beans (*Phaseolus vulgaris* L.), maize (*Zea mays* L.) and butternut-squash (*Cucurbita moschata* Duch.)
Cultivation and weeding	Manual weed control	Manual and chemical weed control	Combining intensive herbicide use and mechanized techniques for weed control
Intercropping	Two or more crops are combined	Maximum of two crops are combined	Not used

The main crops, crop rotations and field cover in the moment of the study are described by fields in table 2.

Soil sampling

Soil sampling was done in two consecutive years (2009 and 2010), during the rainy season (September) and the dry season (April). At each farm, two representative fields of 1-2 ha were selected for the research. Three composites samples were collected by field. Each sample was formed by mixing and homogenizing soil from 6 cores, of 20 cm depth each. The analyses were done in laboratories of the Department of Soil Management at the University of Ghent in Belgium. Soil sampling was done 24 h before transporting the samples to Belgium. The samples were kept in the freezer until laboratory analyses.

Microbial Biomass Carbon (MBC)

The MBC was determined using the fumigation-extraction technique (Jenkinson and Powlson, 1976; Vance *et al.*, 1987; Joergensen, 1996). Both fumigated soil and un-fumigated controls (25 g) were extracted in duplicate with 50 ml 0.5 M K_2SO_4. Extracts were stored at -18°C until analysis. Organic carbon (OC) contents of the extracts were determined with a Total Organic Carbon (TOC) analyzer (TOC-VCPN, Shimadzu Corp., Kyoto, Japan). For conversion from OC contents in the extracts to MBC in the soil a kEC value of 0.35 was assumed (Joergensen, 1996).

Phospholipids fatty-acid (PLFA) analysis

The structure of the microbial community was described by the fatty acid composition of the phospholipids in the soil. Determination of PLFA

followed a procedure modified from Balser (2001). The procedure requires avoid light, oxygen, water and heat, which will destroy fatty acids. Four gram freeze-dried soil was weighed in glass centrifuge tubes. Then, 3.6 ml phosphate buffer pH 7.0, 4 ml chloroform and 8 ml methanol were added. The tubes were shaken for 1 h and afterwards centrifuged for 10 min at a centrifugal force of 699 x g. The supernatant was decanted in new glass tubes and 3.6 ml phosphate buffer and 4 ml chloroform were added. Samples were left overnight for phase separation, cover with aluminum foil and away from heat sources. The next day, the lipid layer was transferred to smaller glass tubes. The remaining phase was washed with 3 ml chloroform to remove any remaining lipids. The combined lipid fraction was dried under N_2. Phospholipids were separated from the lipid extracts by solid phase extraction, using silica columns (Chromabond, Macherey-Nagel GmbH, Düren, Germany). After discarding neutral and glycolipids by chloroform and acetone respectively, phospholipids were eluted using methanol. The methanol fraction was dried under N_2. The dried phospholipids were then dissolved in 1 ml methanol:toluene (1:1 vol:vol) and 1 ml 0.2 M methanolic KOH. Samples were incubated at 35°C for 15 min to allow transesterification to methyl esters. After cooling to room temperature, 2 ml hexane:chloroform (4:1 vol:vol), 1 ml 1 M acetic acid and 2 ml water were added to the tubes. After vortexing, the samples were centrifuged for 5 min at a centrifugal force 447 x g. The hexane layer, containing the methylated PLFAs, was transferred to pointed glass tubes. The aqueous phase was washed twice with hexane:chloroform for removing any remaining lipids. The combined hexane phase was dried under N_2. The fatty-acid methyl esters were finally dissolved in 0.3 ml hexane containing methyl nonadecanoate fatty acid (C19:0) as an internal standard. PLFAs were determined by GC-MS on a Thermo Focus GC combined with a Thermo DSQ quadrupole MS (Interscience BVBA, Louvain-la-Neuve, Belgium) in electron ionization mode. Samples were injected on a Varian capillary column CP Sil 88 (100 m x 0.25 mm i.d., 0.2 ìm film thickness; Varian Inc., Palo Alto, USA). The PLFA markers used for calculating the microbial groups are listed in table 3.

Data analysis

For the MBC, results were tested for each season independently using one way ANOVA analysis by Tukey test ($p<0.05$).

The relative composition of the microbial community was calculated as average value of the total PLFA concentration by microbial group in each farming system. The final value used was expressed in nmol g^{-1} of dry soil. Significant differences among the total amounts of PLFA per farming system for each season were tested using the Bonferroni test at significance level of $p<0.05$.The variability was expressed by the Principal Components Analysis (PCA) using biplots and vector graphs. This analysis was performed for each season. All the statistical analyses were performed using SPSS v17 and Statgraphics Centurion, as well as Microsoft Excel 2007.

RESULTS AND DISCUSSION

Microbial Biomass Carbon

Fields on private farms had significantly higher MBC in both seasons than state farms in all field, with the exception of only one field. The MBC tended to be higher also in private farms than in cooperative farms, but this was only significant in 50% of the cases (Figure 1). This result suggests a significant effect of the farm management on this soil quality indicator.

The results demonstrated the farm management has significant influences on MBC and on soil microbial communities in general. Similar results have been reported in the literature (Balota and Martins, 2011; Oyedele *et al.*, 2015; Amaral and Abelho, 2016). Several authors have reported the negative effects of agricultural practices on MBC (Enkenler and Tatabai, 2003; Alvear *et al.*, 2006). In this case, the differences among these three farming systems related to soil management seems to have direct effects on SMB. In the state farms, the intensive cropping, high tillage intensity, application of agricultural chemicals and limited crop rotation among other agricultural practices affect soil organic matter quality and; therefore, soil MBC.

Table 2. Main crops, crops rotation and field cover by fields.

Farming system	Farm	Field	Crop rotation	Season	Field cover
Private farms	Private farm 1	1	M-T-Y-SP	Dry	M
				Rainy	Y
		2	B-M-T-SP	Dry	M
				Rainy	T
	Private farm 2	1	SP-M-Y-T	Dry	M
				Rainy	Y
		2	SP-M-T-Y	Dry	M
				Rainy	T
	Private farm 3	1	M-T-Y- SP	Dry	M
				Rainy	Y
		2	B-M-T-SP	Dry	M
				Rainy	T
State farms	State farm 1	1	P-SP-T-P	Dry	SP
				Rainy	P
		2	P-SP-T-P	Dry	T
				Rainy	P
	State farm 2	1	P-SP-M-P	Dry	P
				Rainy	M
		2	P-SP-M-P	Dry	SP
				Rainy	M
Cooperative farms	Cooperative farm 1	1	B-M-SP-C	Dry	M
				Rainy	SP
		2	P-M-SP-T	Dry	M
				Rainy	SP
	Cooperative farm 2	1	B-M-SP-C	Dry	M
				Rainy	C
		2	P-M-SP-T	Dry	M
				Rainy	C

Legend: M (maize); T (tomato); Y (cassava); SP (sweet potato); P (potato); B (beans); C (cabbage)

Table 3. PLFA markers used for calculating the taxonomic microbial groups.

Taxonomic group	Specific PLFA markers
Total bacteria	Sum of the marker PLFAs for Gram positive and Gram negative bacteria
Gram positive bacteria	Sum of iC15:0, aC15:0, iC16:0, Ac16:00, iC17:0 and aC17:0
Gram negative bacteria	Sum of cyC19 and cyC17:0
Actinomycetes	Sum of 10MeC16:0 and 10MeC18:0
Fungi	C18:2c9, 12
AMF	C16:1c11

Legend: AMF (Arbuscular mycorrhizal fungi)

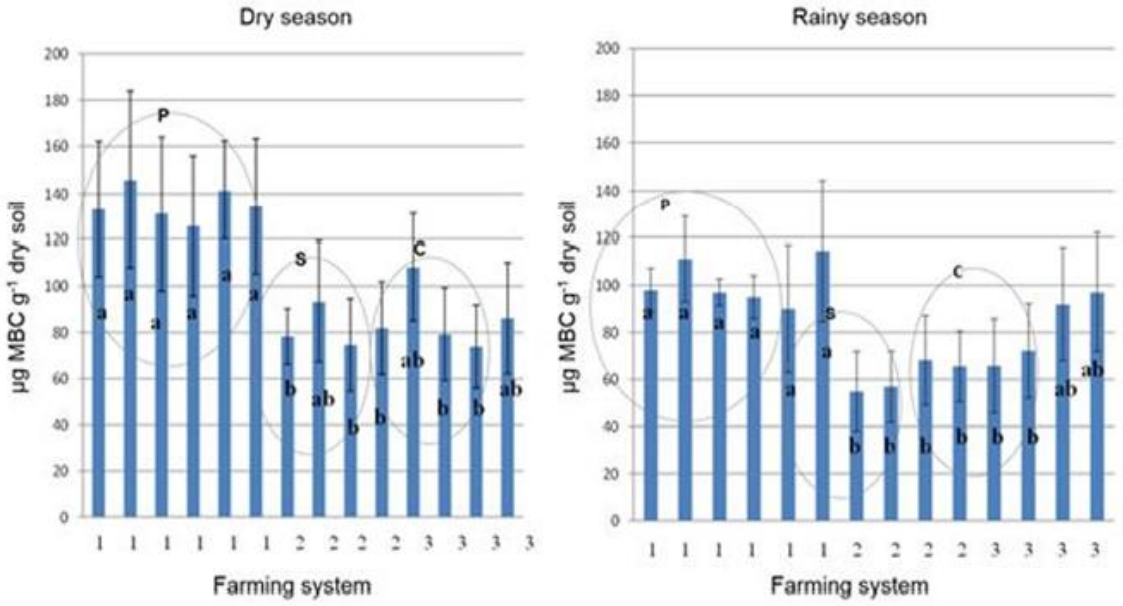

Figure 1. Microbial Biomass Carbon (MBC) by season and farming system. Different letters in the bars indicate the differences among farming systems, according to Tukey test for ($p<0.05$) n=9. The numbers 1, 2, and 3 indicate the private, state, and cooperative farming systems, respectively.

A higher MBC in private farms may indicate greater carbon accumulations in the organic pool, and could represent either a sink or a source of plant-available nutrients, depending on the soil management. Different practices such as intercropping, different cover species and tillage systems have been pointed out due to their significant effects on MBC (Balota and Martins, 2011; Oyedele *et al.*, 2015).

Long-term organic farming, but also, low input farming have been pointed out to be beneficial to MBC and soil biota in general. The regular application of organic fertilizers provides easily available carbon sources, which favor an increment in soil microbial communities (Esperschütz *et al.*, 2007).

PLFAs by farming systems and seasons

Differences in the total amount of PLFA between farming systems were strongly dependent on the season. In the rainy season, there were no differences at all between the farming systems (only a much larger variability in the private farming fields), whereas in the dry season, the three farming systems were significantly different, with the largest total PLFA in the cooperative farming fields (Figure 2). In that sense, the total PLFA concentration did not follow the same trend as MBC, where often private farming fields had the highest values of these indicators.

The PCA analysis based on the PLFAs (Figures 3,4) also showed a very different image between the rainy and the dry season. During the dry season, there was very little discrimination between the PLFA profiles for the different farming systems. The state and cooperative fields are concentrated in a small area of the PCA, and partially overlap, but are completely enveloped by the private fields, which showed a much larger spread. In the rainy season, the farming systems are almost completely separated and are spread mainly along the PC1 axis. Once again, the private farming fields show the highest spread, but much less than in the dry season. The factor loadings showed that no particular fatty acid had a much more pronounced influence than other fatty acids. All contributed equal to the PC's, and pointing all in the same direction along the PC1 axis.

The different trends found of PLFA and MBC is particularly surprising, since normally a good correlation is expected between MBC and PLFA (both are a measure of microbial biomass) (Bailey *et al.*, 2002; Rinklebe and Langer, 2010; Kujur and Patel, 2014).

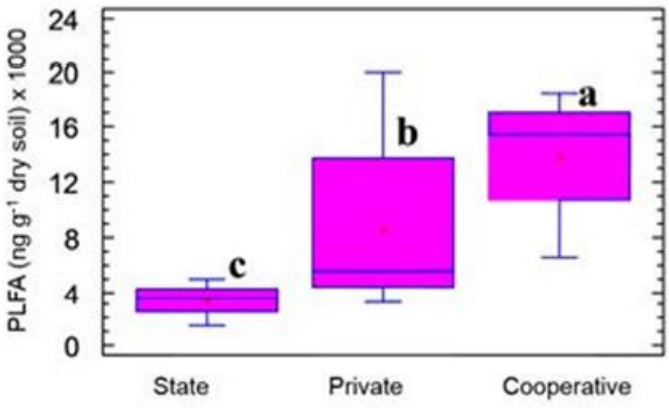

Figure 2. Box plots of total amounts of PLFA per farming system for dry season. Significant differences were tested using the Bonferroni test (at significance level of $p<0.05$).

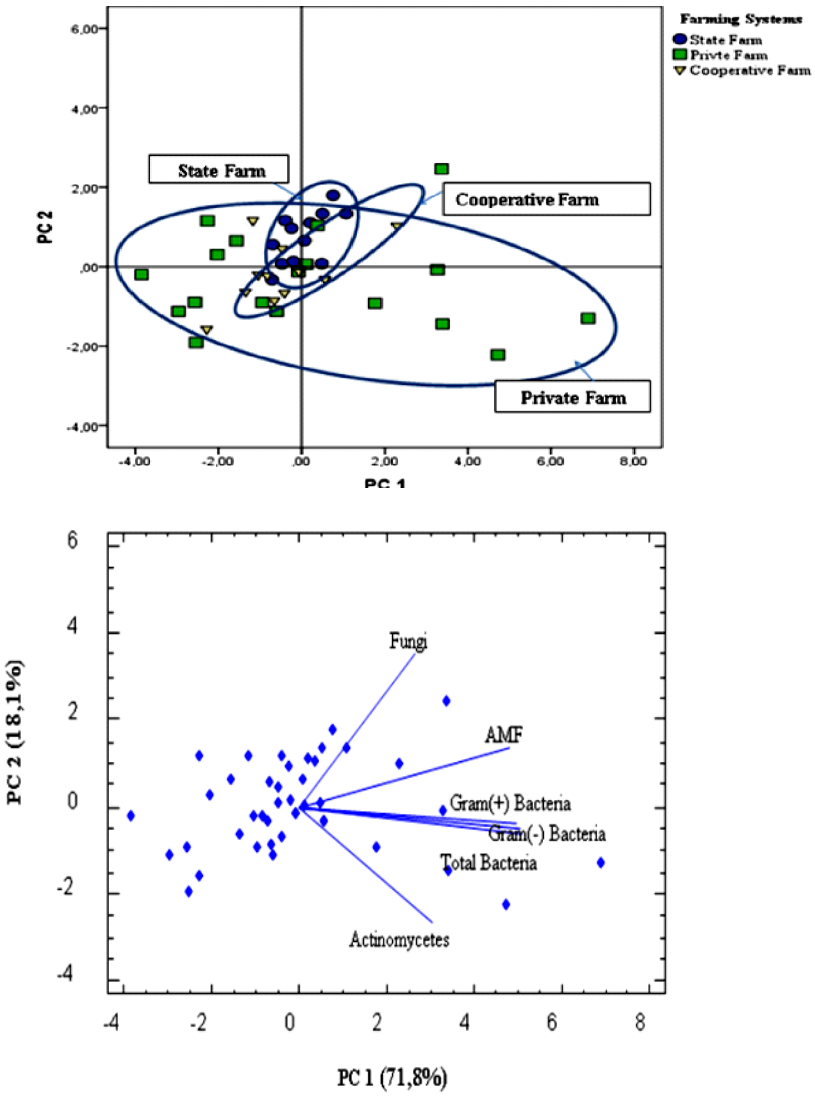

Figure 3. Multivariate analysis (PCA) of PLFAs data by farming systems on dry season.

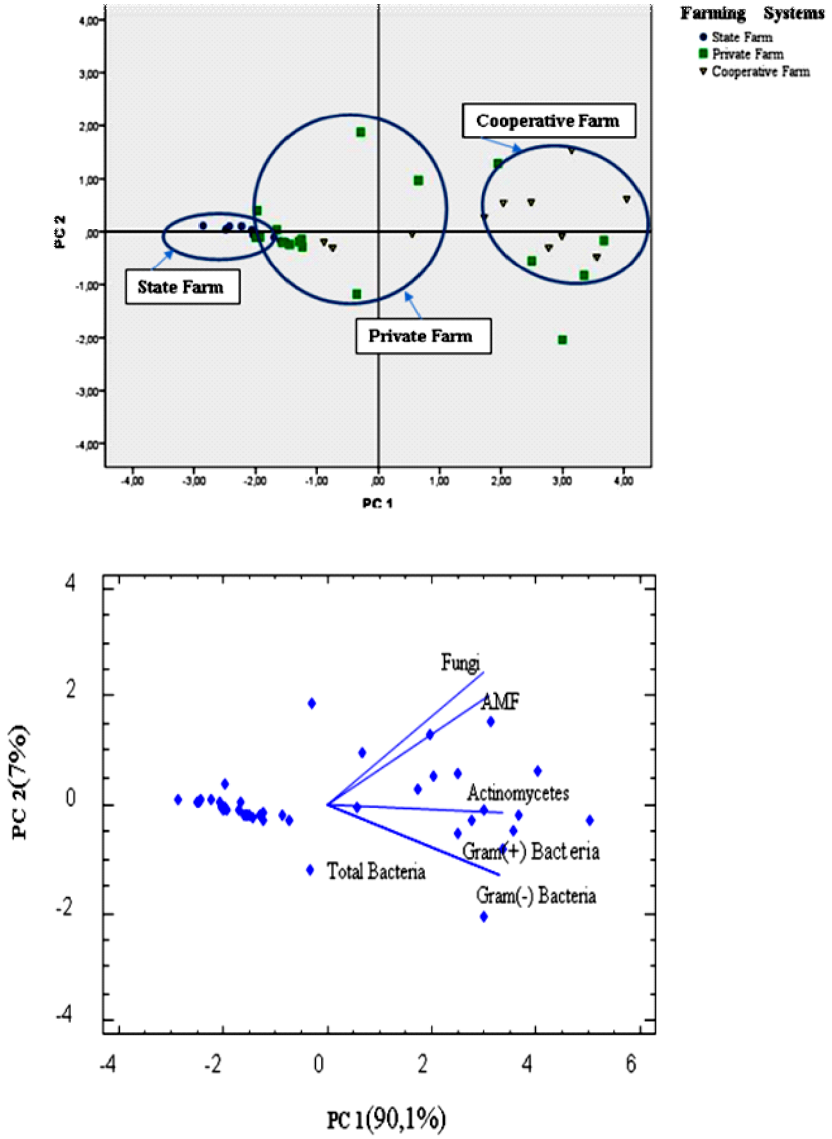

Figure 4. Multivariate analysis (PCA) of PLFAs data by farming systems on rainy season.

It was not found a direct explanation for these discrepancies in soil biological parameters. In the cooperative farms, there is a practice of fallow periods. It has been shown that discontinuation of cultivation can be a factor with positive influence on soil quality (McKinley and White, 2005). However, then it would be found an effect on MBC as well, and not on PLFA only. The fact that differences in total PLFA were only visible in the dry season is not in contradiction with the strongest differences in dry season also in MBC. However, this is also surprising because in the dry season very little microbial activity would be expected at all.

PLFA profiles have been used previously with success to discriminate between farming systems. Moeskops *et al.* (2010) found a very clear discrimination between intensive conventional and organic vegetable farming systems in West Java, Indonesia. In this study, no such strong discriminating power of PLFAs was observed. This may be because these farming systems probably differ less in management and inputs than those very intensive conventional and organic vegetable production systems from the study of Moeskops *et al.* (2010).

CONCLUSIONS

The technological differences in soil management, among the three farming systems, affected both SMB and SMC

composition. The differences in the composition of soil microbial communities were most pronounced between the private and the state farms. The statistical analyses demonstrated that the concentrations of marker fatty acid, that describe the total PLFA of this study, were significantly higher in cooperative farms. The use of fallow in these farms seems to have positive effects on soil microbial communities. Seasonality has a clear effect on microbial communities (MBC, PLFA). The high sensitivity of these biomarkers under Cuban climatic conditions suggests that they can be a useful tool for assessing soil microorganism responses to farm management.

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