

Assessment of soil microbial diversity measurements as indicators of soil functioning in organic and conventional horticulture systems

Romina Romaniuk^{a,*}, Lidia Giuffré^a, Alejandro Costantini^{a,b}, Paolo Nannipieri^c

^a Edafología, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, 1417 Buenos Aires, Argentina

^b Instituto de Suelos, INTA, Argentina

^c Department of Soil Science and Plant Nutrition, University of Firenze, Italy

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ABSTRACT

Soil quality assessment is necessary to detect changes on soil properties among different management practices. Some microbial properties could be useful to predict changes in soil providing an integrated and relevant vision of soil functioning. The aim of this work was to evaluate and compare different methods to assess microbial diversity, such as methyl ester fatty acids (PLFA) and catabolic response profiles (CRP) to act as indicators of soil functioning. The study was carried out in an intensive horticulture production system. Undisturbed soil, 5 years plots, and more than 20 years plots under organic and conventional production were studied. Principal component analysis followed by multivariate discriminant analysis showed that D-glucose, D-glucosamine, α -ketobutyric, α -ketoglutaric and uric acids were the substrates with the highest sensitivity to separate situations. The same analysis was performed for PLFA, showing that C18:1w9, C13:0, C16:1w9, C14:0, i15:0 and cy19:0 methyl ester fatty acids were the most sensitive. Multivariate analysis of variance of selected substrates and fatty acids showed that CRP and PLFA techniques were both capable to characterize the studied systems. Saturated/monounsaturated (S/M), iso/anteiso (i/a) and cyclopropyl/precursors (cy/pre) microbial stress indicators were higher in plots under conventional management, presenting also these situations the lowest microbial biomass and fungi/bacteria ratio (F/B), especially in plots under conventional management for more than 20 years. Microbial functional diversity, calculated as evenness (*E*) from CRP was capable to distinguish between all situations and management systems showing the potential of this measurement to act as an integrative indicator of soil functioning.

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1. Introduction

Soil quality assessment is necessary to detect changes on soil properties under different agricultural management practices. Changes in soil physical and chemical properties, and subsequent changes in microbial community composition, will in turn influence soil processes, nutrient cycling, and overall soil quality (Speeding et al., 2004).

Soil quality can be defined as the capacity of a soil to “function” within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health (Doran and Parkin, 1994). Some important soil functions include: water flow and retention, solute transport and retention, physical stability and support, retention and cycling of nutrients, buffering and filtering of potentially toxic materials, resistance and resilience, and maintenance of biodiversity and habitat (Daily et

al., 1997). Soil microorganisms are involved at least in the five last functions mentioned above.

Soil quality cannot be measured directly, but must be inferred by measuring soil attributes or properties that serve as indicators. Change in these indicators can be used to determine whether soil quality is improving, stable, or declining with changes in management (Brejda et al., 2000). The major part of attempts for biological soil quality measuring has been focused principally on indicators such as respiration, microbial biomass, enzyme activities and nematodes. However, there is a substantially uncharted territory as regards soil quality indicators connected with microbial diversity, which is intimately related to soil community and function (Nannipieri et al., 2003; Bastidia et al., 2008). The microbial community is recognized as the essential living component of soil and its diversity has been suggested as a sensitive means of assessing soil quality. Any loss in ability of the microbial biomass to maintain its wide range of functions is seen as a warning sign of decreased soil health (Chapman et al., 2007). Recent research has shown that soil quality can be assessed by some microbial community measurements, in particular phenotypic profiling (phospholipids fatty acids

* Corresponding author. Tel.: +54 11 4524 8059.

E-mail address: romaniuk@agro.uba.ar (R. Romaniuk).

profiles) coupled with functional profiles (substrate induced respiratory responses to different carbon substrates) that yield sufficient data to base management decisions (Ritz et al., 2009). Winding et al. (2005) suggest that only through continued research and application of the different techniques available to characterize the soil microbial community, a better understanding of the diversity and functions of microbial communities will be reached, in order to assess and monitor soil quality.

Several approaches for the measurement of community level physiological profiles have been devised. Since the Biolog method (Garland and Mills, 1991) essentially only targets the small fraction of the microbial community that can grow within the microtitre plate wells, Degens and Harris (1997) developed a multiple carbon-source, substrate induced respiration method (multi-SIR) that measures the response of the whole soil. The more recently described MicroResp™ method (Campbell et al., 2003) technique combines both relevance and convenience, being a 'whole soil' method in a flexible microtitre plate format that is fairly simple to execute. The choice of which of these three methods to use may depend upon the particular hypotheses or questions in mind, as well as on the facilities available within individual laboratories. However, the multi-SIR technique is an accurate methodology that can be performed without the technology requires by the MicroResp™, giving similar results if it is considered that both techniques are based in the same principle. This technique is known to be sensitive to management practices (Sparling et al., 2008), but it has not been really explored under organic and conventional horticulture.

Phospholipids fatty acid (PLFA) composition of microbial community provides a measurement of microbial community diversity at group level (Zelles, 1999). Phospholipids are essential membrane components of all living cells and represent a relatively constant proportion of the biomass of organisms. The analysis of the PLFA is a useful assay for soil microbial community because (i) the concentration of total PLFA is an index of viable microbial biomass since phospholipids are rapidly degraded after cell death, (ii) certain fatty acids may be used as molecular markers for specific taxa and as indicators of microbial stress and (iii) multivariate analysis of the PLFA profiles can be used to detect changes in community composition (Rahman et al., 2008). Differences in the PLFA composition of microbial communities have been found in farming systems receiving different amounts of organic inputs (Bossio and Scow, 1998), land use (Grayston et al., 2001) and management practices (Esperschütz et al., 2007).

There is growing evidence that organic systems exhibit an improved soil quality characterized by higher biological activity than conventional ones. In organic systems, plant production depends almost exclusively on nutrient transformation in soil, since only limited amounts of permitted fertilizers are used. As nutrient transformations are primarily controlled by microbes, an active soil microflora is very important to smooth functioning of organic systems. Organic and conventional management influence on soil properties have been largely studied, however, there are only few studies about the changes in soil microbiological properties with the duration of organic (Monokrousos et al., 2006) and conventional management.

The aim of this work was to compare catabolic response profiles (CRP) and methyl ester fatty acids (PLFA) methods to assess microbial diversity, to be used as indicators of soil functioning in organic and conventional horticulture systems.

2. Materials and methods

2.1. Study area and sampling

The study area is an intensive horticulture system located in La Plata, Buenos Aires province, Argentina. The soil is classified as Ver-

tic Argiudoll. Conventional (C) and organic (O) plots with 5 years (O 5 and C 5) and more than 20 years (O 20 and C 20) of horticulture production plots were evaluated. The size of each plot was approximately 0.5 ha. It is an intensive horticultural production system with 2 crops per year, one in the spring/summer and other in the autumn/winter seasons. The main crops cultivated in both organic and conventional management plots are lettuce, spinach, tomatoes, celery, zucchini, spinach, cabbage, beet, vetch, bean and fennel. The leguminous (vetch and bean) are included in the 2 years rotation, but not necessary all years. Conventional and organic production plots are conventional tilled with moldboard plow.

Organic plots were fertilized with organic amendments and weeds were mechanically controlled. Inputs amendments vary between 2 and 4 Mg ha⁻¹ before planting the crop (1 or 2 times per year). Most of the amendments applied are fresh vegetable residues with high C/N relation, composted vegetable and animal waste, and a minor part of bone meal and chicken manure with low C/N ratio.

Conventional plots were fertilized and weed controlled by agrochemical applications, and there are not vegetable or organic waste amendments. Inorganic fertilizers used were diammonium phosphate and urea, and the application rate varied between 50 and 150 kg ha⁻¹ depending on the requirements of the crop. Insecticides and fungicides are applied in each crop cycle in doses ranging from 1 to 4 kg ha⁻¹ depending on the active ingredient. In the C 5 plot the insecticides applied were endosulfan and methidathion, the fungicides are azoxystrobin, captan and thiram, and the herbicide was glyphosate. For the C 20 plot the most common insecticides applied were chlorpyrifos and cypermethrin, the fungicides used were thiram and carbendazim, and the herbicide was atrazine.

The situation chosen as a control (UN) is an area of approximately 0.5 ha adjacent to the horticultural plots. The soil is completely covered and the vegetation height can range from 30 to 50 cm. The plant species composition has been altered by the invasion of native and exotic species from the production plots. The predominant species are *Paspalum Dilatum*, *Paspalum quadrifarium*, *Bromus unioloides*, *Cynodon dactylon*, *Stipa neesiana*, *Bothriochloa*, *Baccharis sps*. According to information received by producers, this site has not been cultivated, at least for the last 50 years.

The climate can be characterized as temperate with a mean annual rainfall of 1023 mm, and a mean annual temperature of 16.3 °C. The coldest month is July (lowest mean monthly temperature 5.3 °C) and the hottest is January (highest mean monthly temperature 29.5 °C).

Soils were sampled in September 2006, when all production plots were uncultivated. Five single soil samples were randomly collected in each plot from 0 to 10 cm depth. Moist soil samples were sieved (<2 mm), kept at room temperature for 7 days and then stored at 4 °C prior to microbiological analysis.

2.2. Analysis

2.2.1. Catabolic responses profiles

The sieved soil was conditioned for 7 days at 20 °C at field moisture content (18% gravimetric water content) prior analysis. Catabolic responses profiles were measured by short-term respiration responses of soil to the addition of a range of simple organic compounds (Degens and Harris, 1997). The used substrates were two amines (D-glucosamine, L-glutamine), five aminoacids (L-arginine, L-glutamic acid, L-histidine, L-lysine, L-serine), two carbohydrates (D-glucose, D-mannose) and eleven carboxylic acids (L-ascorbic acid, citric acid, tartaric acid, gluconic acid, α-ketobutyric acid, α-etoglutaric acid, D,L-malic acid, malonic acid, pantothenic acid, quinic acid and uric acid). These organic substrates were individually added to 1 g dry soil in McCartney bottles;

the mixture was incubated for 4 h at 25 °C and the respired CO₂ in the headspace of each bottle was determined by gas chromatograph. Functional evenness (*E*) was calculated from the response profile as:

$$E = \frac{1}{\sum p_i^2}$$

where p_i is the percentage of total respiration, obtained by the sum of all respiration rates expressed as $\mu\text{g CO}_2\text{-C g}^{-1}$ soil, due to the respiration rate of each substrate (Magurran, 1988).

2.2.2. Phospholipid fatty acids profiles: methyl ester fatty acids

Methyl ester fatty acids profiles (FAMES) were determined as reported by Schutter and Dick (2000). This method employs a mild alkaline methanolysis, which is thought to extract ester-linked fatty acids but not free fatty acids. Dry soil (3 g) was treated with 15 ml of 0.2 M KOH in methanol. The contents of the tubes were mixed and incubated at 37 °C for 1 h, during which ester-linked fatty acids were released and methylated. Samples were vortexed every 10 min during the incubation period. Then, 3 ml of 1 M acetic acid was added to neutralize the pH. FAMES were partitioned into an organic phase by adding 10 ml of hexane followed by centrifugation at 480 × g for 10 min. The hexane layer was then transferred to a clean glass test tube and the hexane was evaporated under a stream of N₂. Then, FAMES were dissolved in 0.5 ml of 1:1 hexane:methyl-tert butyl ether mixture and transferred to a GC vial for analysis with a chromatograph (Hewlett-Packard 5890 Series II) equipped with HP capillary column Ultra 2 (5% difenil–95% dimetilpolisiloxane, 25 mby 0.2) and compounds were revealed by using a flame ionization detector. The oven temperature was initially set at 150 °C for 1 min, then raised to 210 °C at a rate of 5 °C min⁻¹ and held for 20 min. Identification of peaks was done by comparing retention times of samples to those of known standards (bacterial acid methyl esters standard in mirthyl caproate, “Sigma Aldrich”, cat. N° 47080-U). Fatty acids were named according to the convention: “A; B; and w C”; where “A” is the number of carbon atoms in the chain, “B” is the number of unsaturations, and “w” preceding “C” is the number of carbon atoms between the methyl end of the molecule and the first unsaturation. Prefixes used are: “i” for iso-branched; “a” for anteiso-branched; “cy” for cyclopropyl. The abundance of individual PLFAs was normalized to FAME C16:0 (Drijberg et al., 2000; Speeding et al., 2004) which is often the most abundant FAME in samples and correlate well with total biomass (Zelles et al., 1992). For the calculation of the fungal/bacterial PLFA (F/B) ratio, the saturated FAMES (S) 11:0, 12:0, 13:0, 14:0, i15:0, a15:0, i16:0, 16:0, 17:0, 20:0 and 22:0 and the monounsaturated (M) 16:1w9, cy17:0, cy19:0, 18:1w9 were chosen to represent bacterial biomass, and 18:2w6 was taken as indicator of fungal biomass (Madan et al., 2002). The cy17:0 and 16:1w9 FAMES were considered for calculating the cyclopropyl/precursors (cy/pre) ratio, and i15:0 and a15:0 FAMES for the iso/anteiso (i/a) ratio. Total PLFAs were the sum of all identified GC peaks.

2.3. Statistical analysis

Simultaneous observations of substrates (CRP) and methyl ester fatty acids (FAMES) were taken for each situation. A principal component analysis (PCA) was used to select a subset of variables for CRP and PLFAs important in explaining the variation in the data. The PC receiving high eigen values and variables with high factor loading were assumed to be variables that best represented system attributes. Therefore, the PCs that explained some percentage of the variation in the data were examined. Within each PC, only highly weighted factors variables were retained. Highly weighted factor loadings were defined as having absolute values within 10% of the

highest factor loading. When more than one factor was retained under a single PC, multivariate correlation coefficients were carried out. The variable with the highest correlation sum was considered for further analysis. When highly weighted variables were not correlated (correlation coefficient < 0.7), they were retained. Considering the retained variables, a discriminant analysis (DA) was used to determine whether these variables could be used to discriminate between the sites. DA coefficients were then analyzed to determine how the selected variables contributed to the weighting of each significant DA.

3. Results

3.1. Functional diversity

3.1.1. Catabolic responses profiles, multivariate PCA and discriminant analysis

Only five of the 20 substrates presented high sensitivity to differ among situations: D-glucose, D-glucosamine, α-ketobutyric acid, α-ketoglutaric acid and uric acid. These substrates were capable to discriminate among the studied situations (Fig. 2). The soil samples from the 20 and 5 years plots under organic and conventional production were well separated from each other along axis 1. This axis was largely influenced for the α-ketobutyric acid. Soil samples for undisturbed plot were separated from 20 years production plots along axis 1, and from 5 years production plots along axis 2, mainly represented for D-glucose, uric acid and α-ketobutyric acid respectively.

The α-ketobutyric acid was sensitive to differentiate the production plots for the management system and for the time under production (Fig. 1). The 5 years plots under production have higher values than the 20 years plots; and for the same time of production, the organic systems present higher values than the conventional ones.

3.1.2. Evenness

Functional Evenness (*E*) was calculated from CRP (Fig. 3). The *E* was highly sensitive, differentiating among the study plots both for time under production and management practices. The UN situation presented the highest values. The 5 years production plots (O 5 and C 5) presented highest values than the 20 years plots (O 20 and C 20). The *E* decreased with the increase in time under production for both organic and conventional management systems. However, decrease of the *E* with the time under production was greater for conventional management. For the two periods of time under production considered, the organic systems always presented higher values of *E* than conventional ones.

3.2. Methyl ester fatty acids

3.2.1. Microbial community composition

Microbial communities were compared on the basis of their PLFAs. PCA clearly separated organic and conventional soils along the first principal component (Fig. 4). More than 20 years organic and conventional plots (O 20 and C 20) were separated along the second PC. The UN site was nearly to the O 20 plot along PC 1 and PC 2.

The soil microbial community composition differed among the sites, as measured by FAMES. Monounsaturated PLFAs (markers of Gram negative bacteria) were higher for organic plots than for conventional ones. In contrast, saturated PLFAs, typical markers of Gram positive bacteria, were generally higher for conventional systems. The proportion of iso and anteiso branched saturated PLFA was also lower in the organic sites. Saturated short chain FAMES were higher for UN, while large chain FAMES and fungal markers were higher for organic systems, especially for O 20. Fatty acid i15:0 was

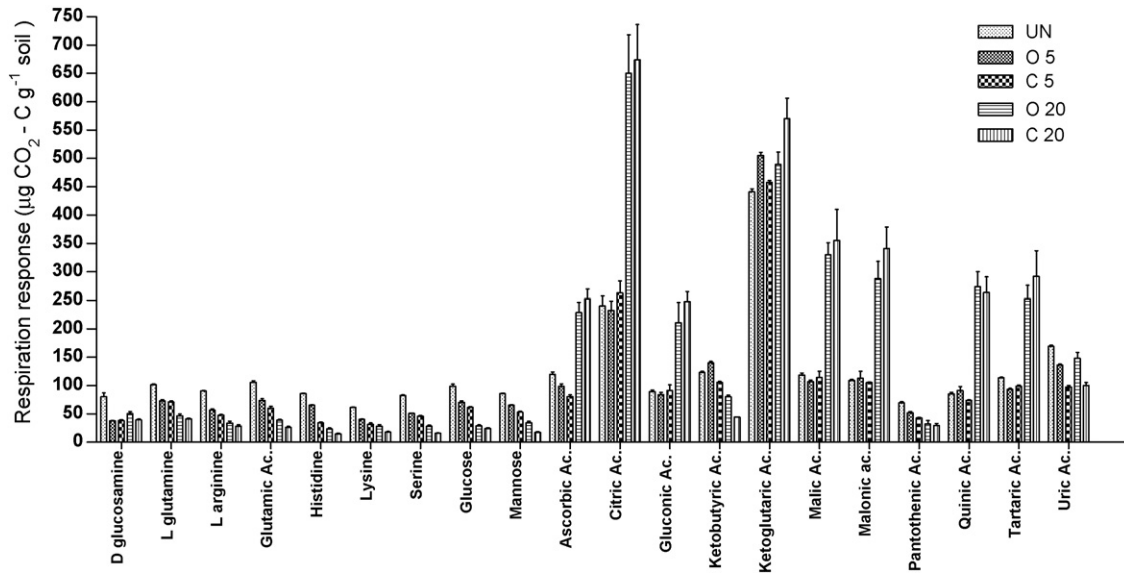


Fig. 1. Catabolic response profiles in undisturbed plot (UN), 5 years organically management plot (O 5), 5 years conventionally management plot (C 5), 20 years organically management plot (O 20) and 20 years conventionally management plot (C 20). Error bars represent standard errors ($n = 3$).

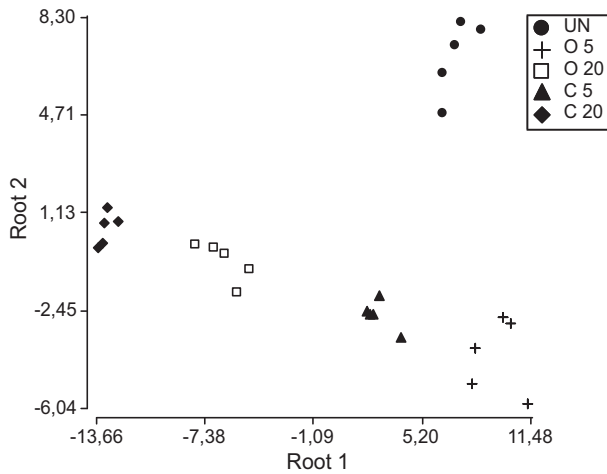


Fig. 2. Discriminant analysis of the five substrates chosen by the PCA (D-glucose, D-glucosamine, α -ketobutyric acid, α -ketoglutaric acid and uric acid) for the undisturbed plot (UN), 5 years organically management plot (O 5), 5 years conventionally management plot (C 5), 20 years organically management plot (O 20) and 20 years conventionally management plot (C 20).

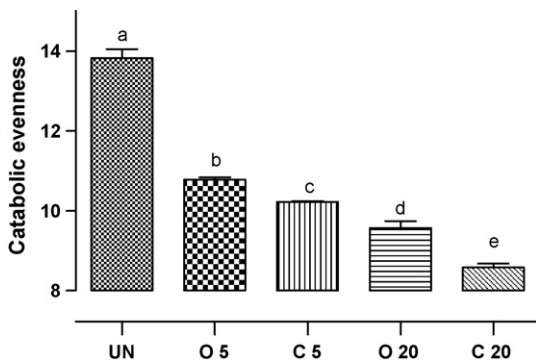


Fig. 3. Response of microbial catabolic evenness profiles in undisturbed plot (UN), 5 years organically management plot (O 5), 5 years conventionally management plot (C 5), 20 years organically management plot (O 20) and 20 years conventionally management plot (C 20). Error bars represent standard errors ($n = 3$). Different letters represent significance differences between treatments ($P < 0.05$).

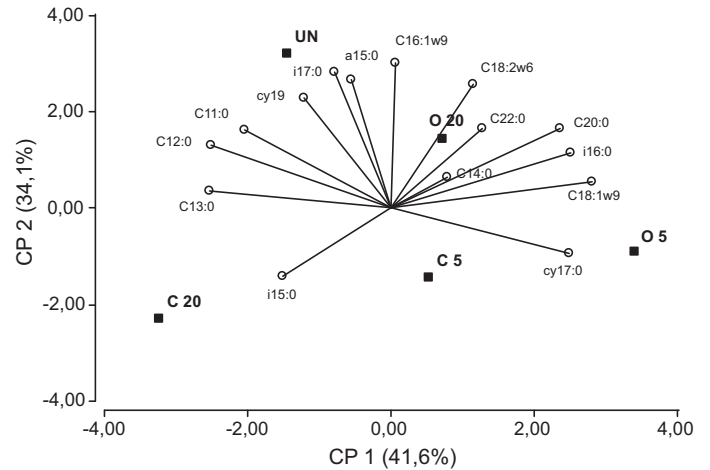


Fig. 4. Principal component analysis of the PLFA data set for the undisturbed plot (UN), 5 years organically management plot (O 5), 5 years conventionally management plot (C 5), 20 years organically management plot (O 20) and 20 years conventionally management plot (C 20).

mainly associated to conventional management plots (C 5 and C 20), while the fatty acid cy17:0 was higher in the 5 years organic plot (O 5).

3.2.2. FAMES selection and discriminant analysis

Six high loading PLFAs from PCA were considered in a discriminant analysis (DA): C18:1w9, C13:0, C16:1w9, C14:0, i15:0 and cy19:0 (Fig. 5). The soil samples from C 20 and C 5 were separated from each other along DA 1. Along this axis, UN and O 20 sites were separated from five years plots under production (O 5 and C 5) and from 20 years conventional plot (C 20). This axis was mainly influenced by fatty acids C16:1w9 and i15:0. The fatty acid C16:1w9 was greater in the UN and in the O 20 compared with the other three sites (Fig. 4). The FAME i15:0, marker of Gram (+) bacteria, was the highest for C 20.

Soil samples for undisturbed (UN) and 20 years plot productions (O 20 and C 20) were separated from 5 years plots (O 5 and C 5) along DA 2, mainly influenced for fatty acid C18:1w9. This FAME was higher for O 5 and O 20 (Fig. 4).

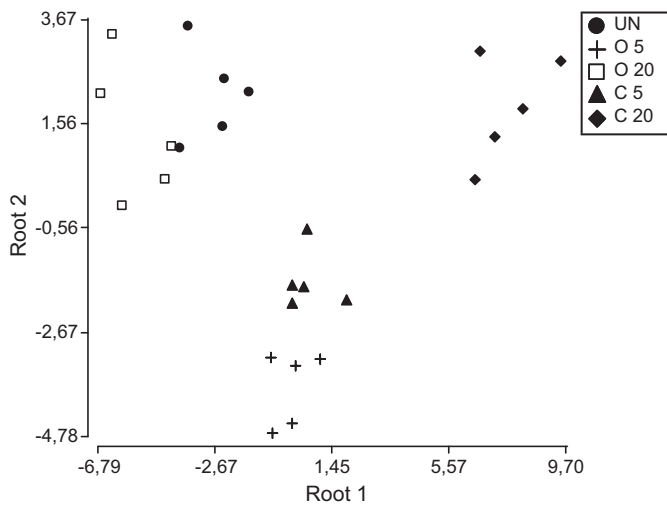


Fig. 5. Discriminant analysis of the six FAMEs chosen by the PCA (C18:1w9, C13:0, C16:1w9, C14:0, i15:0 and cy19:0) for the undisturbed plot (UN), 5 years organically management plot (O 5), 5 years conventionally management plot (C 5), 20 years organically management plot (O 20) and 20 years conventionally management plot (C 20).

3.2.2.1. *TPLFA, F/B and stress indicators.* Total PLFA (Fig. 6) was significantly higher in the UN and organic plots (O 5 and O 20) than in conventional production plots (C 5 and C 20).

Fig. 7 shows the fungal/bacterial ratio (F/B), saturated/monounsaturated (S/M), cyclopropyl/precursors (cy/pre) and iso/anteiso (i/a) ratios considered as stress indicators. The F/B ratio (Fig. 7a) was higher in UN and organic sites than in conventional ones, and was significantly lower for the 20 years plot under conventional management (C 20). The S/M ratio (Fig. 7b) was significantly higher for C 20. The i15:0/a15:0 and cy/pre ratios (Fig. 7c and d) were significantly higher for C 20, followed by the 5 years plots (O 5 and C 5), and the lowest values were for the 20 years organic plot (O 20) and the undisturbed plot (UN).

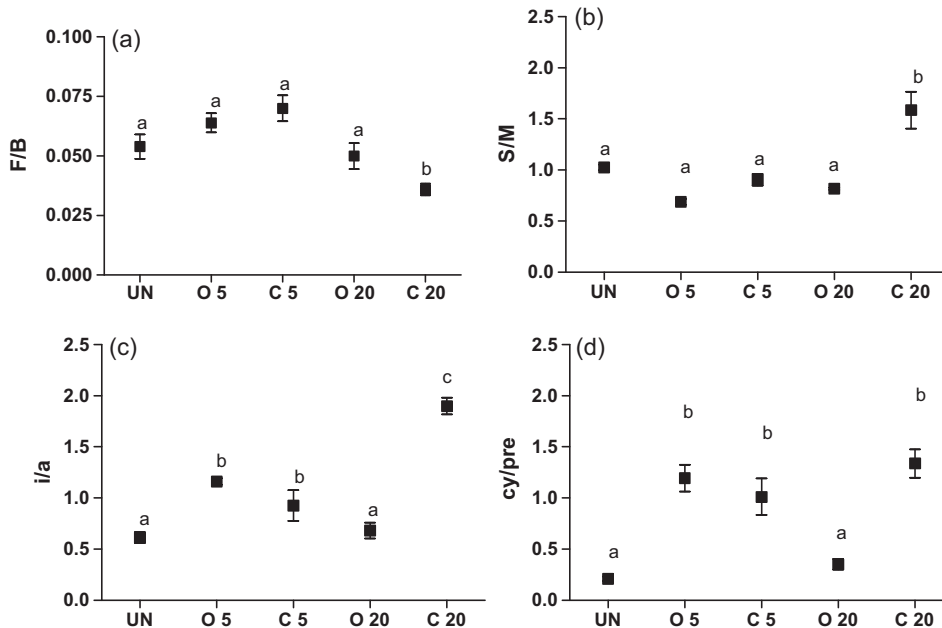


Fig. 7. Ratios of (a) fungi PLFA markers to bacteria PLFA markers, (b) normal saturated to monounsaturated PLFA, (c) iso to anteiso branched PLFA, and (d) cyclopropyl fatty acids to their monoenoic precursors for the undisturbed plot (UN), 5 years organically management plot (O 5), 5 years conventionally management plot (C 5), 20 years organically management plot (O 20) and 20 years conventionally management plot (C 20).

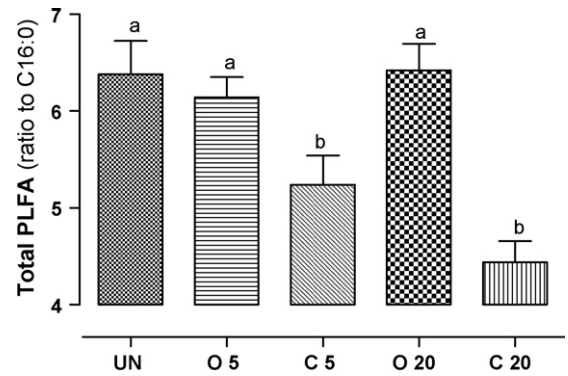


Fig. 6. Total PLFA biomass for the undisturbed plot (UN), 5 years organically management plot (O 5), 5 years conventionally management plot (C 5), 20 years organically management plot (O 20) and 20 years conventionally management plot (C 20). Error bars represent standard errors (n=3). Different letters represent significance differences between treatments at a P < 0.05 level.

There were no statistically significant differences between O 5 and C 5 for the stress indicators evaluated, and the differences between management practices were only evident with long time under production (O 20 and C 20). The UN soil microbial community exhibited the lowest values of indicators of stress or nutritional imbalances.

4. Discussion

4.1. Functional diversity

4.1.1. Catabolic responses profiles, multivariate PCA and discriminant analysis

Only five of the 20 substrates presented high sensitivity to differentiate among situations: D-glucose, D-glucosamine, α -ketobutyric acid, α -ketoglutaric acid and uric acid. Discriminant analysis showed that the most important substrates were α -ketobutyric acid, D-glucose and uric acid. Degens and Harris (1997) reported that the α -ketoglutaric, α -ketovaleric, quinic, fumaric and oxalic

acids were the primary responsible for the differences among management systems. Degens et al. (2001) found that mainly the α -ketobutyric acid, and then the α -ketoglutaric and quinic acids presented high sensitivity to expose the differences in the microbial response due to disturb application to soils under different management systems. Stevenson et al. (2004) studied the catabolic response profiles for soils under different land uses, and only 9 (D-glucose, lysine, glutamine, α -ketobutyric acid, α -ketoglutaric acid, ascorbic acid, tartaric acid, succinic acid and pantothenic acid) were sensitive to differ among pasture, forest native soil and cultivated forest soil.

The α -ketoglutaric acid presented the highest value for C 20 and the lowest for UN (Fig. 1). Soil microorganisms that degrade the α -ketoglutaric acid could be characterized for a high reproductive rate (*r* strategy) associated to disturbed ecosystems. This explanation is in agreement with the higher values presented in the more disturbed plot (C 20). Highest values of catabolic response to glucose in soil samples from UN plot (Fig. 1) could be related to the abundant root density generating great amounts of root exudates (Bardgett and Shine, 1999). Monokrousos et al. (2006) found that only 11 of the 95 substrates used to determine the CRP by *Biolog* were sensitivity to differentiate between organic systems with different time under production, all selected substrates were carbohydrates. Elevated amounts of uric acid are frequently found in urine of animals and in insects. High values for this substrate in the organic plots (Fig. 1) could be a consequence of the organic amendment inputs, which are in part constituted by animal manure. Kandeler and Eder (1993) reported higher urease activity in soils organically fertilized than in reference soils.

4.1.2. Evenness

The UN plot presented the highest values of evenness. Degens et al. (2000) found higher values of evenness under pasture and native vegetation soils than in soils under production. Functional diversity was mainly affected by the period under production. The 20 years under production plots showed lower values of *E* than the 5 years plots. Continuous tillage operation that disturbed the soil microbial habitat and decrease the soil structure could affect the microbial functionality with time. For the same period of time under production the higher values of *E* in the organic systems in comparison with the conventional ones are probably due to lack of organic amendments, and pesticides and insecticides application that could inhibit the microbial community activity (Sall et al., 2006). Increase in microbial diversity under organic management compared with conventional management has been reported by other authors (Mäder et al., 2002; Bending et al., 2002). Esperschütz et al. (2007) found higher size and diversity of microbial communities in organic fertilized systems than in inorganic fertilized ones, probably related to the presence of a more complex microbial community able to degrade the organic compounds present in the amendments.

Increases in the *E* are associated with decreases in the dominance of the catabolic response of the substrates. This could be related with the differences in the soil cover of the plots. The UN situation presents a relevant and diverse vegetable cover, which influences in the abundance and diversity of its microbial community. Soil cropping practices often decrease plant and faunal diversity (Sparling et al., 2000). The vegetable cover of plots under horticulture production is restricted to the cultivated species, and their microbial community is adapted to degrade the residues of these crops. This could be represented by the increments in the substrate induce respiration (SIR) of some substrates and low SIR for those not corresponding to common crops. The differences among the production plots could be consequence of the differences in soil chemical and physical properties that influenced the habitat of the microbial communities and thus the functional evenness.

Sparling et al. (2008) reported that microbial functional evenness was not affected for the period of conventional horticultural management. However, in this study the *E* decreased with the increased time under production for the organic and conventional management systems. Nevertheless, Bossio et al. (1998) and Bending et al. (2002) presented differences in conventional and organic cultivation influenced in higher microbial biomass and diversity in the organic systems at the beginning, while differences in diversity were not related with time.

Sparling et al. (2000) found that the microbial functional evenness showed the same response pattern to cropping pressure as did the other biological and physical properties and may have some potential as an integrated measure of the status of the soil microbial community. Degens et al. (2000) concluded that catabolic evenness shows the potential to be an indicator of microbial diversity that can be applied across different soil types, particularly useful for rapid evaluation.

4.2. Methyl ester fatty acids

4.2.1. Microbial community composition

Microbial communities of the organic and conventional plots were clearly separated on the basis of their PLFAs. The higher proportion of monounsaturated FAMES for organic plots than for conventional ones have been reported by others (Bossio and Scow, 1998; Peacock et al., 2000) that related the increase in monounsaturated fatty acids with augmentation in the availability of organic substrates. The proportion of iso and anteiso branched saturated PLFA was lower in the organic plots, consistent with the presence of higher proportion of Gram (+) bacteria. The fungal markers were higher for organic systems, according to results reported by Monokrousos et al. (2006) that also found higher fungal biomass in organic systems than in conventional ones, probable due to the fungicide application. However, Marschner et al. (2004) did not find differences in the fungal marker (C18:2w6) among organic and conventional management systems. Bossio et al. (1998) identified fungi as one of the major microorganisms responsible for the discrimination between conventional and organic farming system.

In agreement with the results of this research, Lundquist et al. (1999) found high values of the fatty acid cy17:0 in organic management soils. Griffiths et al. (1999) found that the proportion of Gram (–) (indicated by the marker cy17:0) increased with loadings of simulated root exudates, while the proportion of Gram (+) declined. However, McKinley et al. (2005) presented a greater proportion of cy17:0 in agricultural sites than in a virgin prairie soil. The proportion of cy17:0 can be affected by the nutrient status and physiological state of the community and may not always be a reliable indicator of taxonomic differences, remarking the importance of the measures of microbial activity.

Differences in microbial community structure could be associated to management practices. Organic and conventional managements could generate organic matter with different chemical compositions (Quidean et al., 2001) and substrate availability, affecting the differences in the microbial community structure observed in the present study. Although the organic management influenced the microbial structure in the first years, the results showed that the structure could be recovering the original pattern with increase time under organic management, while the conventional management affected markedly the structure of the microbial community for both periods studied in comparison with the UN soil.

Hartman et al. (2006) found that structural diversity was mainly influenced for the fertilization strategy (organic vs. inorganic). Indeed, tillage operations may cause temporary stress that limits the ability of soil microbes to assimilate nutrients and alters the community structure (Calderón et al., 2000).

4.2.2. FAMES selection and discriminant analysis

The fatty acids C16:1w9, i15:0 and C18:1w9 were the most important to discriminate between the study situations. The fatty acid C16:1w9 is indicative of Gram (–) bacteria, which commonly increases in conditions of high labile carbon availability (Peacock et al., 2001). The FAME i15:0, is a marker of Gram (+) bacteria, largely known as low grow organisms (K strategy), frequently found under low resources situations. Most of the FAMES selected as important discriminators are markers for nutritional status (i15:0 and cy 19:0) are typical of certain taxonomic groups (C18:1w9 for fungi, C16:1w9 and cy19:0 for Gram (–), and i15:0 for Gram (+) organisms). Marschner et al. (2004) found a higher concentration of the PLFAs i15:0, a15:0 and 16:1w9 after sewage sludge soil amendment and after mineral fertilization, and the same trend was found for total bacteria and Gram (+) bacterial PLFAs, while treatments did not differ in Gram (–) bacterial or the fungal PLFA 18:2w9. Speeding et al. (2004) reported that FAMES C20:0, C14:0, C18:2w6 and cy19:0 were the most important PLFAs to discriminate among different residue treatments. Peacock et al. (2001) found that the fatty acid 18:1w9 was the most important to discriminate among manure vs. inorganic fertilizer, and to explain the differences in total PLFAs. Esperschütz et al. (2007) found higher amounts of the fungal marker 18:2w6 in organic systems than in conventional ones, however, it was less important than other bacterial fatty acids (bacterial i16:0, i17:0, a15:0, a17:0, cy17:0) to discriminate between sites. These fatty acids presented high factor loadings in the PCA in this experiment too.

4.2.3. TPLFA, F/B and stress indicators

Higher amount of total PLFA in organic production plots could be associated with a greater availability of organic substrates for microbial growth (Hartman et al., 2006). Soil management methods that increase carbon inputs to the soils often enhance microbial biomass, population and activities. Several studies (Scow et al., 1994; Gunapala and Scow, 1998) reported higher stable microbial biomass after several years of increased organic inputs. Peacock et al. (2001) found a significantly increase in total PLFA in manure soils in comparison with inorganic fertilizer soils. They suggested that long term use of manure also supplied large amounts or readily available carbon, resulting in a more diverse and dynamic microbial system than in inorganically fertilized soil. This work showed significant differences in TPLFA between conventional and organic management systems but not between O 5 and O 20.

Differences in soil management could affect the Fungi/bacterial ratio (Klein et al., 1995; Bardgett et al., 1996). The higher F/B ration in UN and organic sites related to conventional ones could be associated with an increase of fungi biomass when organic material is incorporated. Frostegård and Bååth (1996) found a positive correlation between F/B ratio and the organic matter content in soils, although, tillage operations could cause damage in the fungal hyphae. The lowest F/B ratio in C 20 could be due to the high application of pesticides and insecticides and the low amounts of organic inputs. Marschner et al. (2004) and Bittman et al. (2005) reported higher F/B ratios in organic than in conventional systems in relation with the fertilizer strategy. Application of synthetic fertilizers in conventional systems, which is easily used by microorganisms increments bacterial growth instead of fungi population because fungi are mainly responsible of decomposition of more recalcitrant materials (Grayston et al., 2004) that are usually highest in organic and non disturbed situations, where amounts of straw and manure with higher aromaticity are greater than in inorganic fertilized plots. Stahl et al. (1999) found that conventional agriculture practices resulted in a reduction in the amount of fungal biomass compared with uncultivated sites, and thus, could be the result of altered soil environmental conditions resulting from tillage, low annual carbon input, and direct disturbance of fungal mycelial by

tillage. Beare et al. (1993) reported that fungal community structure and hyphal density in soil were both strongly affected by tillage. Fungi often represent the largest component of microbial biomass in arable soils and make critical contributions to soil properties and processes. As a group, fungi are primarily responsible for the decomposition and mineralization of organic residues in soil, make up an important food source for other organisms and contribute to soil aggregate formation. These activities make fungi critically important to soil quality and the sustainability of agroecosystems (Stahl et al., 1999).

Conventional management induces a soil physical degradation because of the tillage operations and the low carbon inputs. Shifts in the iso/anteiso and saturated/unsaturated PLFA ratios have been associated with nutrient stress or physical or chemical disturbance (Pinkart et al., 2002; Fierer et al., 2003). The S/M ratio has been also related with nutrient availability. In pure culture studies this ratio increases in Gram (–) bacteria subjected to starvation conditions (Kieft et al., 1994). Low organic amendments could cause a decrease of the monounsaturated fatty acids, and thus increase the S/M ratio. Similar results have been reported by Bossio et al. (1998). Increases in S/M ratio can be related with higher abundance of saturated fatty acids, which were found to be abundant in Gram (–) bacteria. Monounsaturated FAMES, attributed largely to Gram (–) organisms, have also been found to increase in proportion with increasing amounts and diversity of carbon sources (Peacock et al., 2000).

In bacteria, the ratio of cy/pre have been proposed as an indicator of stress conditions, as it has shown to increase under situations such as acidic conditions, low oxygen, high temperature and low nutrient availability (Kieft et al., 1994). In this research this ratio was significantly higher for the more disturbed situation (C 20). However, Lundquist et al. (1999) did not found increase in the cy/pre ratio in 8 years organic relative to conventional soils.

The lower values of the i15:0/a15:0 and cy/pre ratios for the 20 years organic plot (O 20) could be attributed to the high and continuous inputs of organic amendments, which generate a positive nutrient balance, being this microbial community more resistant to the stress induced by the production practices.

Lack of statistic differences among O 5 and C 5 for the stress indicators evaluated could be associated to the short time under production of these plots, as the differences in management practices are evident only with more time under production (O 20 and C 20). The stress indicators showed that conventional management practices increase the stress of the microbial communities with time (Riffaldi et al., 2002), while the organic management practices maintain the well-being of the soil biota. However, these differences according to the management practices could not be early detected by these stress indicators. The lower values of stress indicators for the UN are probably due to a greater ecosystem stability related to a better soil quality.

5. Conclusions

Both measurements, PCR and PLFA, were sensitive to be used as soil indicators of functioning and adaptation to management. The functional evenness was capable to integrate the catabolic response of all substrates and showed differences among management practices and time under production. The use of PLFA analysis coupled with multivariate statistical analysis, allowed the characterization of changes that occur with different management practices. The results suggested that a relatively small subset of FAMES can be used to discriminate between sites.

Organic and conventional systems resulted in widely diverse microbial communities with different ecological functions. The functional diversity diminished in all the cases under production

in comparison to the undisturbed site, being more affected by the quantity of years under production than by the management practices (organic or conventional). However, for the plots with the same period under production, the organic management presented higher values of functional diversity. The incidence of the period of organic and conventional management practices on the structure of the microbial community was different. Although the organic management affected the microbial structure in the first years with reference to UN soil, the structure showed a tendency to recover the original pattern with the length of the period of organic management. On the contrary, the conventional management affected markedly the microbial structure community with increase time under this management.

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