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ORGANIC FERTILIZATION IN TRADITIONAL MEDITERRANEAN GRAPEVINE ORCHARDS MEDIATES CHANGES IN SOIL MICROBIAL COMMUNITY STRUCTURE AND ENHANCES SOIL FERTILITY

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ABSTRACT

Soil microbial populations and their functions related to nutrient cycling contribute substantially to the regulation of soil fertility and the sustainability of agroecosystems. A field experiment was performed to assess the medium-term effect of a mineral fertilizer and two organic fertilization systems with different nitrogen sources on the soil microbial community biomass, structure, and composition (phospholipid fatty acids, pattern, and abundance), microbial activity (basal respiration, dehydrogenase, protease, urease, β-glucosidase, and total amount of phosphomonoesterase activities), and physical (aggregate stability) and chemical (total organic C, total N, available P and water-soluble carbohydrates) properties in a vineyard under semiarid Mediterranean conditions after a period of 10 years. The three fertilization systems assayed were as follows: inorganic fertilization, addition of grapevine pruning with sheep manure (OPM), and addition of grapevine pruning with a legume cover crop (OPL). Both treatments, OPM and OPL, produced higher contents of total organic carbon, total N, available P, water-soluble carbohydrates, and stable aggregates. The organic fertilization systems increased microbial biomass, shifted the structure and composition of the soil microbial community, and stimulated microbial activity, when compared with inorganic fertilization. The abundances of fungi and G+ bacteria were increased by treatments OPM and OPL, without significant differences between them. Organic and inorganic fertilization produced similar grapevine yields. The ability of the organic fertilization systems for promoting the sustainability and soil biological and chemical fertility of an agroecosystem under semiarid conditions was dependent of the organic N source. Copyright © 2016 John Wiley & Sons, Ltd.

KEY WORDS: enzyme activity; organic fertilization; phospholipid fatty acids; semiarid Mediterranean agroecosystem; microbial community structure

INTRODUCTION

The exclusive use of mineral fertilizers, along with soil tillage, usually leads to a loss of soil organic matter (SOM) and reduction of soil water holding capacity and soil structural stability as well as groundwater contamination, resulting in a decline of soil quality and environmental degradation (Melero *et al*., 2011). This situation is aggravated in semiarid agroecosystems, which are highly vulnerable to erosion and degradation processes (García-Orenes *et al*., 2012). Intensive arable agriculture and semiarid climatological conditions cause a progressive decline in soil organic matter levels and associated soil fertility (Caravaca *et al*., 2002). Thus, organic fertilization systems have been amply implemented to increase plant productivity, reduce inputs, and increase the sustainability of such agroecosystems (García-Orenes *et al*., 2013; Macci *et al*., 2013). Organic fertilization includes the use of organic nutrient sources for crop production because of their importance in enhancing soil organic matter, thus potentially improving soil biology fertility and physical fertility indicators like soil aggregation, porosity, and water retention. Organic fertilizers should release their nutrients without subsequent microbial immobilization (Geisseler *et al*., 2010), the input of available plant nutrients resulting in enhanced crop growth. However, organic fertilizers such as pruning residues may involve an environmental risk because such residues may act as a reservoir for fungal pathogens, insects, and termites (Jackson, 2008). Thus, it is recommended to avoid using pruning residues as a soil amendment when infection is suspected. Another conservation practice currently performed in semiarid agroecosystems is cover crops that help prevent erosion by protecting soil aggregates against the impact of rain drops (García-Orenes *et al*., 2012), increase soil nitrogen and carbon inputs, and when properly managed, may conserve soil moisture, and reduce the need for herbicides and pesticides (Hartwig & Ammon, 2002; Mazzoncini *et al*., 2011).

The soil biota plays a decisive role in ecosystem functionality, by mediating the processes of organic matter turnover, nutrient capture and cycling (Van der Heijden *et al*., 2008; Murray *et al*., 2009), and formation and stabilization of soil aggregates (Chenu & Cosentino, 2011). Thus, maintaining a taxonomically and functionally diverse microbial community may be a key to ensuring sustainable agricultural management. Because of their relationship with soil functionality,

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the soil microbial population and activity have been proposed as useful indicators of both soil improvement and degradation in soil quality monitoring tasks (Schloter *et al*., 2003). In particular, biochemical properties such as soil enzyme activities have been widely used as indicators of soil quality because they contribute to cycling of soil essential nutrients, are easily measured, and respond sensitively and promptly to changes in soil by anthropogenic perturbations (Kandeler *et al*., 1999). There are several studies on the effects of different land uses and agricultural management systems on biological characteristics of semiarid Mediterranean and temperate soils (Kandeler *et al*., 1999; Riffaldi *et al*., 2002; García-Orenes *et al*., 2010). Management of soil fertility based on the use of organic fertilizers can stimulate microbial processes and increase crop yields, compared with inorganic fertilizers (IFs). In contrast, it has been shown that conventional agricultural practices reduce the soil microbial biomass (Esperschütz *et al*., 2007; Ngosong *et al*., 2010) and various enzyme activities, leading to a decrease in functional diversity in the soil ecosystem (Caravaca *et al*., 2002; Ros *et al*., 2006) and changes in the microbial community structure (Lazcano *et al*., 2013). However, changes in the soil microbial community structure following land use changes have been observed without changes in the microbial community function because of high levels of functional redundancy (Farrell *et al*., 2010). Among the approaches available to assess the soil microbial community structure, phospholipid fatty acid (PLFA) analysis has been successfully used to study changes as a consequence of different perturbations or management practices (DeGrood *et al*., 2005; Van der Wal *et al*., 2006; Zornoza *et al*., 2009; García-Orenes *et al*., 2013). Nevertheless, scarce information is available on the shifts in the structure and function of the soil microbial community in response to medium-term and long-term applications of conservation management practices.

We hypothesized that shifting from inorganic to organic fertilization could alter the structure and function of the soil microbial community on a medium-term basis, promoting more efficient nutrient cycling in agroecosystems, and that this change could be dependent on the type of organic fertilizer. In order to verify this hypothesis, we compared the medium-term (10 years) impacts of a mineral fertilizer and two types of organic fertilization systems with different nitrogen sources on soil microbial community structure and composition, microbial activity, and physical–chemical properties in a vineyard under semiarid Mediterranean conditions. The soil microbial community structure was assessed by measuring PLFAs. The information obtained will permit the identification of suitable fertilizer management practices that maintain and enhance soil quality and crop yield.

MATERIALS AND METHODS

Study Site

This research was conducted at "Pago Casa Gran" vineyard located in Mogente municipality (coordinates 38°49′24″N, 0°48′17″W), in Valencia Province (southeast Spain). The experimental area is mainly cultivated with grapevine (*Vitis vinifera* L.) under rainfed conditions. The climate is typically Mediterranean with a mean annual precipitation of 413 mm and a mean annual temperature of 16·3 °C (http://www.carm.es/econet/sicrem/PU7/sec29.html). The soil is a Typic Xerofluvent (Soil Survey Staff, 2014). The analytical characteristics of the soil at the beginning of the experiment are indicated in Table I.

Experimental Design

The field assay was conducted as a factorial randomly arranged in a split-plot design with six replicates, each replication plot measuring 0·5 ha for a total experimental surface of 9 ha. Three different agricultural managements were investigated: (1) application of pruning of grapevine and addition of manure from sheep (0·63% N, 0·27% P_2O_5, and 0·81% K_2O), at a rate of 20 Mg ha^{-1}, with subsequent shallow ploughing (OPM, organic pruning + manure); (2) application of pruning of grapevine, shallow ploughing, and sowing of a native leguminous crop vetch (*Vicia villosa*) (OPL, organic pruning + legume cover crop); and (3) fertilization with NPK 8/4/12 at a rate of 250 kg ha^{-1} y^{-1}, application of herbicide glyphosate five times per year and ploughing (IF). IF is the prevalent local management system. The grapevines were regularly inspected during the growing season to check the appearance and development of diseases. If evidence of disease was detected, the diseased grapevines were marked for future removal. The pruning of grapevines and its addition to soil was annually performed in the months of January. Pruning of grapevine consisted of chipped pruned branches and weeds from a previous harvest, which C/N ratio was about 80. Annual leguminous crops were sown in the month of October at a rate of 50 kg ha^{-1} y^{-1}.

Soil Sampling

Ten years after applying the treatments (in March 2014), soil samples (one per replication plot) were collected at 0–15 cm depth from individual grapevine for each agricultural management system. Field-moist soil samples were dried at room temperature and divided into two subsamples: One subsample was sieved at 2 mm for physical–chemical, chemical, and biochemical analyses, and the other subsample was sieved between 0·25 and 4 mm for aggregate stability. An aliquot of field-moist soil samples was frozen at −20 °C and stored until PLFA analysis.

Table I. Characterization of the soil at the beginning of the experiment

pH (H_2O)	8·48 ± 0·03[a]
EC (1:5, μs cm^{-1})	145 ± 2
Texture	Loam
$CaCO_3$ (%)	35·6 ± 0·8
Total organic C (g kg^{-1})	9·3 ± 0·3
Dehydrogenase (μg INTF g^{-1})	30·1 ± 2
Water-soluble carbohydrates (μg g^{-1})	28 ± 2
Total N (g kg^{-1})	0·35 ± 0·02
Available P (μg g^{-1})	30 ± 3
Extractable K (μg g^{-1})	322 ± 4

[a]Mean ± standard error (n = 6).
EC, electrical conductivity; INTF, iodo-nitrotetrazolium formazan.

Soil Physical–Chemical, Microbiological, and Biochemical Analyses

In a 1:5 (w/v) soil aqueous extract, soil pH and electrical conductivity were measured. In this extract, water-soluble carbohydrates were determined by the method of Brink *et al.* (1960) using anthrone and measuring spectrophotometrically at 630 nm. Total soil organic carbon was determined using potassium dichromate oxidation method (Nelson & Sommers, 1982). The basal respiration of soil was measured in a multiple sensor respirometer (Micro-Oxymax, Columbus, OH, USA). Aggregate stability was measured according to Roldán *et al.* (1994), based on Benito *et al.* (1986). This method examines the proportion of aggregates that remain stable after a soil sample is subjected to an artificial rainfall of know energy ($279\,J\,min^{-1}\,m^{-2}$). Available phosphorus was determined by the Burriel–Hernando method (Díez, 1982).

Urease activity (EC 3·5.1·5) was measured according to the method of Tabatabai (1994), using urea as substrate. Alkaline phosphomonoesterase (EC 3·1.3·1) and β-glucosidase (EC 3·2.1·21) activities were determined using *p*-nitrophenyl phosphate disodium and *p*-nitrophenyl-β-D-glucopyranoside as substrates, respectively. The assay is based on the release and detection of *p*-nitrophenol (PNP) according to Tabatabai (1994). Dehydrogenase activity was determined according to García *et al.* (1997).

Phospholipid fatty acids from soil samples were extracted and fractionated following the procedures described by Bossio *et al.* (1998). Methylated fatty acids were analysed by gas chromatography (Hewlett Packard 6890 Gas Chromatograph, Palo Alto, CA, USA). The internal standard nonadecanoic acid (19:0) was used in order to quantify the fatty acids. The peaks were identified on the basis of their retention times in comparison with a standard mixture using an identification software (Microbial ID, Inc., Newark, DE, USA). We identified 48 fatty acids. Microbial groups were assigned using the PLFAs: i14:0, i15:0, a15:0, i16:0, 16:1 ω7, 16:1 ω9, 17:0, i17:0, a17:0, cy17:0, cy19:0 for bacteria; 18:2 ω6·9 for fungi; cy17:0, cy19:0, 16:1 ω7, 16:1 ω9 for Gram-negative (G−) bacteria; i14:0, i15:0, a15:0, i16:0, i17:0, a17:0 for Gram-positive (G+) bacteria; and 16:0 10-meth, 17:0 10-meth, and 18:0 10-meth for actinobacteria following Federle (1986), Olsson *et al.* (1995), and Zelles (1999). The fungal/bacterial PLFAs ratio was calculated by dividing the fungal PLFAs, estimated by the PLFA 18:2 ω6·9, by the sum of bacterial specific PLFAs. The total biomass was estimated as the sum of all the extracted PLFAs (total PLFAs).

Statistical Analyses

Before performing analysis of variance (ANOVA), the homogeneity of variances was tested with the Levene's test, and normality of data was checked with the Kolmogorov–Smirnov normality test at $p < 0{\cdot}05$. Measured variables were submitted to a one-way ANOVA, and comparisons among means were made using the Tukey's honest significant difference test calculated at $p < 0{\cdot}05$.

The concentrations of individual fatty acids and soil characteristics were analyzed by principal component analysis (PCA) with Varimax normalized rotation in order to determine the influence of the types of agricultural managements on soil microbial community structure. PCA was performed on PLFA data transformed to mol% of the total fatty acid concentration. The abundance of distinct microbial groups such as bacteria, actinobacteria, G+ and G− bacteria, and fungi was calculated. The scores of the first two principal components of the PLFA data were subjected to a correlation analysis with soil parameters measured using Pearson's correlation coefficients. For statistical analyses, SPSS program (Statistical Program for the Social Sciences 23·0; IBM, Armonk, NY, USA) was used.

RESULTS AND DISCUSSION

Physical–Chemical Parameters

After 10 years of treatments, the highest concentrations of total organic carbon, total N, and available P were recorded in the treatments with organic fertilization (OPM and OPL), without significant differences between them (88%, 200%, and 50% higher, respectively, than with inorganic fertilization), as shown in Table II. The labile fraction of carbohydrates also responded effectively to changes in management practices, the highest values being obtained with the treatment OPM followed by the treatment OPL. There is much evidence that residue quality affects short-term N and SOM dynamics (Gentile *et al.*, 2011). Palm *et al.* (2001) recommended that residues with an elevated C/N ratio like grapevine pruning (C/N ratio 80) should be applied in combination with an N source, to accelerate their decomposition and increase soil nutrients. In this study, both sheep manure (a residue rich in N) and the legume crop in combination with grapevine pruning were more effective as a source of N, P, and C inputs to improve soil fertility than N/P/K fertilizer application. The high values of total N obtained with the treatment OPL could be related to the potential for biological N_2 fixation of leguminous species, due to their associated rhizobial symbioses (Sainju *et al.*, 2002). The fact that the highest values of available P were found in the organic fertilization systems suggests that P, mainly present in organic forms, was released gradually, enriching the soil and supplying P to the crop. In the period of time of our study, enhancement of SOM and fertility was observed (Table II), which is consistent with other long-term organic fertilizer experiments (Flieβbach & Mäder, 2000; Gentile *et al.*, 2011; Ge *et al.*, 2013). However, at the end of the experiment, the grapevine yields following implementation of the conservation practices were similar to those obtained with IF, being the average yield of Merlot grapevines of $6500\,kg\,ha^{-1}$.

Additionally, soil pH was significantly lower in the organic treatments relative to the inorganic treatment (Table II). In contrast, soil electrical conductivity reached higher values in the organic treatments, especially in the treatment OPM, possibly because of the elevated soluble salts content of the manure,

Table II. Soil physical–chemical, biological and biochemical properties of the different treatments [Organic pruning + manure (OPM), organic pruning + legume cover crop (OPL) and inorganic fertilizer (IF)]

	OPM	OPL	IF
pH (H_2O)	8·24 ± 0·03 b	8·31 ± 0·03 b	8·51 ± 0·01 a
EC (1:5, $\mu s\,cm^{-1}$)	200 ± 9 a	167 ± 8 b	120 ± 2 c
AS (%)	43·4 ± 2·4 a	44·7 ± 1·2 a	35·7 ± 3·1 b
TOC ($g\,kg^{-1}$)	15·8 ± 0·4 a	16·5 ± 0·7 a	8·6 ± 0·2 b
TN ($g\,kg^{-1}$)	1·39 ± 0·08 a	1·28 ± 0·08 a	0·44 ± 0·02 b
Available P ($\mu g\,g^{-1}$)	33 ± 3 a	30 ± 3 a	21 ± 1 b
Water-soluble CH ($\mu g\,g^{-1}$)	74 ± 3 a	41 ± 2 b	29 ± 1 c
BR ($\mu g\ C–CO_2\,g^{-1}\,h^{-1}$)	3·8 ± 0·4 a	2·9 ± 0·4 a	1·0 ± 0·1 b
BR/TOC mineralization quotient	0·24 ± 0·02 a	0·18 ± 0·03 b	0·12 ± 0·01 c
Dehydrogenase ($\mu g\ INTF\,g^{-1}$)	87·9 ± 2·7 a	54·8 ± 3·3 b	24·1 ± 1·9 c
Protease ($\mu mol\ NH_4^+\,g^{-1}\,h^{-1}$)	1·5 ± 0·1 a	1·1 ± 0·1 b	0·5 ± 0·0 c
Urease ($\mu mol\ NH_4^+\,g^{-1}\,h^{-1}$)	2·6 ± 0·2 a	1·8 ± 0·1 b	1·3 ± 0·1 b
β-glucosidase ($\mu mol\ PNP\,g^{-1}\,h^{-1}$)	1·8 ± 0·2 a	1·5 ± 0·1 a	0·7 ± 0·1 b
Phosphomonoesterase ($\mu mol\ PNP\,g^{-1}\,h^{-1}$)	1·8 ± 0·1 a	1·9 ± 0·2 a	0·8 ± 0·1 b

Values are mean ± standard error ($n = 6$).
EC, electrical conductivity; AS, aggregate stability; TOC, total organic carbon; TN, total nitrogen; CH, carbohydrates; BR, basal respiration; INTF, iodo-nitrotetrazolium formazan; PNP, *p*-nitrophenol.
Values in rows sharing the same letter do not differ significantly ($p < 0·05$) as determined by the Tukey's honest significant difference test.

although the value reached in this soil (200 $\mu s\,cm^{-1}$) was not enough to limit crop growth. The percentage of stable aggregates was significantly higher in the treatments OPM and OPL, corresponding with increases of aggregating agents – like water-soluble carbohydrates and total soil organic carbon – recorded in these treatments.

Biochemical and Microbiological Parameters

Medium-term application of organic fertilization resulted in significant changes in the dehydrogenase, protease, urease, β-glucosidase, and phosphomonoesterase activities and in soil basal respiration (Table II). The greatest differences between the organic and inorganic fertilization systems were found in protease and dehydrogenase activities and basal respiration, particularly in the treatment with grapevine pruning and sheep manure (265%, 200%, and 280% higher, respectively, with respect to the inorganic fertilization). The β-glucosidase and phosphomonoesterase activities involved in C and P cycling were also higher in the organic treatments, although no significant differences were observed between the two organic systems. In contrast, both organic treatments significantly differed in the levels of protease and urease activities, which are involved in N cycling.

The input of specific organic compounds following organic fertilization may have promoted the activity of microflora involved in the cycling processes of such substrates. Among the organic compounds related to microbial activity are the soluble carbohydrates, which can be used as carbon and energy sources by soil microflora and may also have a structural function (Roldán *et al*., 1994). We detected a higher content of water-soluble carbohydrates with the treatment OPM, which can be related to high carbohydrates contents contained in the manures (Moral *et al*., 2005). This trend was also observed in the dehydrogenase activity, which is considered as an index of the global metabolic activity of the soil microbial community (Alguacil *et al*., 2005). This suggests a close relationship between the availability of labile and easily mineralizable organic matter and the activity of microbial population, as shown in previous studies (Caravaca *et al*., 2002). Likewise, the mineralization quotient (Ojeda *et al*., 2013), defined as soil basal respiration in relation to the total organic C, evidenced that the organic fertilization systems, especially the OPM treatment, caused an increase in the efficiency of the soil microbial populations regarding the decomposition of soil organic matter.

The total PLFA content of the soil, considered as indicative of the viable microbial biomass, was significantly affected by the type of agricultural management (Table III). Increases in

Table III. Total fatty acid content and abundance of signature phospholipids fatty acids in the soil with the different treatments [Organic pruning + manure (OPM), organic pruning + legume cover crop (OPL) and inorganic fertilizer (IF)]

	OPM	OPL	IF
Total PLFA ($nmol\,g^{-1}$)	20·9 ± 2·2 a	22·6 ± 1·3 a	12·1 ± 1·8 b
Gram+ PLFA ($nmol\,g^{-1}$)	3·6 ± 0·6 a	3·5 ± 0·2 a	1·9 ± 0·2 b
Gram− PLFA ($nmol\,g^{-1}$)	3·8 ± 0·4 a	3·9 ± 0·2 a	3·2 ± 0·5 a
Fungi PLFA ($nmol\,g^{-1}$)	2·8 ± 0·4 a	2·6 ± 0·3 a	1·2 ± 0·3 b
Actinobacteria PLFA ($nmol\,g^{-1}$)	0·5 ± 0·2 a	0·5 ± 0·1 a	0·3 ± 0·2 a
Fungi/bacteria	0·4 ± 0·1 a	0·3 ± 0·0 a	0·2 ± 0·0 b

Values are mean ± standard error ($n = 6$).
PLFA, phospholipid fatty acid.
Values in rows sharing the same letter do not differ significantly ($p < 0·05$) as determined by the Tukey's honest significant difference test.

soil microbial biomass were also observed for a maize crop following the imposition of organic cropping systems in a Mediterranean agroecosystem (Kong *et al*., 2011). Microbial biomass C responded in a similar way to the water-soluble carbohydrates fraction, increasing with the adoption of organic fertilization. Decomposition of organic compounds added to soil or derived from the cover crop releases essential nutrients, such as N, P, and S, required for both plant and microbial growth (Roldán *et al*., 2003), leading to enhanced microbial biomass.

Microbial Community Structure and Composition

The major proportion of the identified PLFAs were bacterial biomarkers, followed by fungal PLFAs biomarkers (Table III). The dominance of bacterial over fungal PLFAs in the three systems might have resulted from the high levels of N (Moore *et al*., 2005). Among the microbial groups studied, only the abundances of fungi and G+ bacteria were increased by the organic treatments. In particular, the G+ bacterial and fungal PLFAs were 87% and 125%, respectively, more abundant in the treatments OPM and OPL than with inorganic fertilization. However, previous studies using PLFAs analyses have shown an increase of G− bacteria: after short-term agronomic application of manure in an integrated treatment (Lazcano *et al*., 2013) or following medium-term application of a cellulosic residue-like oat straw in a Mediterranean environment (García-Orenes *et al*., 2013). In our study, the bacterial population, which developed in the soils receiving organic fertilization, was dominated by G+ bacteria with characteristic methyl-branched fatty acids. It is plausible that this bacterial group was faster growing and more competitive for the available organic substrates supplied than the populations of G− bacteria with characteristic mono-unsaturated (16:1ω7) and cyclic (cy17:0 and cy19:0) fatty acids. In fact, it is known that many genera of G+ bacteria are able to degrade complex substrates such as lignin and cellulose, grapevine pruning being rich in such substances, whereas growth rates of G− bacteria are related to the presence of available C (Paul & Clark, 1996).

The increased fungal abundance in the soil microbial community of the organic fertilization systems OPM and OPL, as compared with the inorganic fertilization system, may have facilitated the formation and stabilization of soil aggregates through their extraradical fungal hyphae and extracellular polysaccharides (Roldán *et al*., 1996), which was evidenced in the present study as the higher percentages of stable aggregates in these systems. An increase in fungal biomass following the addition of oat straw in a Mediterranean agroecosystem was also recorded by García-Orenes *et al*. (2013). Likewise, Zhang *et al*. (2005) found that agricultural deintensification stimulated the proliferation of fungi in the soil microbial community. Meanwhile, the abundances of G− and actinobacteria were similar in all three agricultural management systems. The fact that the organic systems had no effect on the total actinobacterial community is consistent with the work of Zhang *et al*. (2005).

Principal components analysis was performed to evaluate the influence of the type of management on the soil physical, chemical, and biochemical properties (Figure 1). The first two components explained 82·4% of the total variation with eigenvalues >1·0; the first principal component (PC1) explained 72·9% of the variation and separated the samples OPM from those IF and OPL (Figure 1(a) and Table IV). Thus, the organic fertilization treatments differed with each other along PC1.

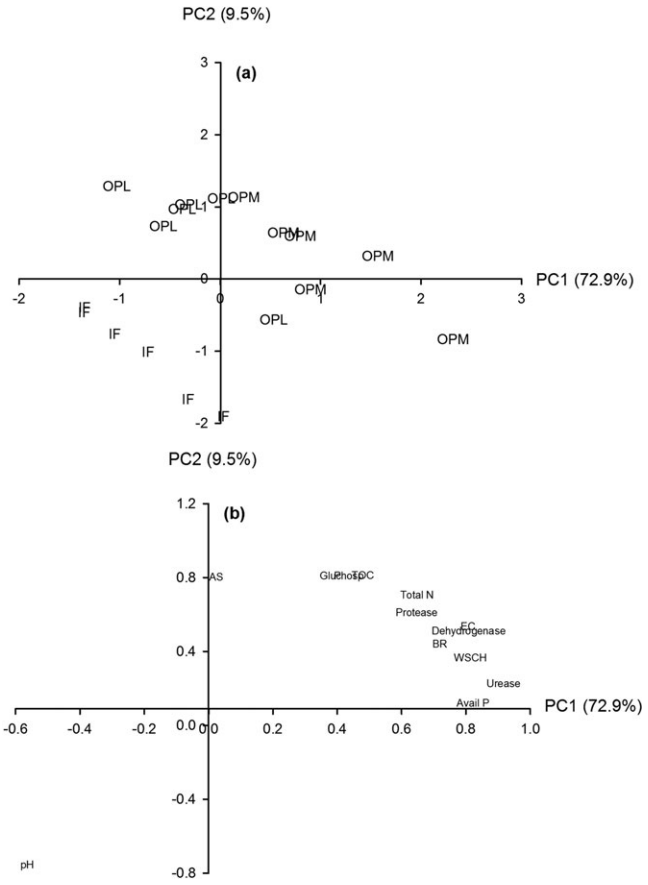

Figure 1. Scores (a) and loadings (b) plot from principal component analysis performed on different parameters studied in the soil samples. TOC, total organic carbon; EC, electrical conductivity, WSCH, water-soluble carbohydrates; AS, aggregate stability; Gluc, β-glucosidase; Phosp, phosphomonoesterase.

Table IV. ANOVA of the loading values of soil properties and the individual PLFAs for the first and second principal components (PC1 and PC2)

	Soil properties		PLFAs	
Treatment	PC1	PC2	PC1	PC2
OPM	a	a	a	a
OPL	b	a	b	a
IF	b	b	c	a

OPM, organic pruning + manure; OPL, organic pruning + legume cover crop; IF, inorganic fertilizer.
For each factor and each plant species, treatment followed by the same letter are not significantly different according to the Tukey's honest significant difference test ($p < 0{\cdot}05$).

Factor loadings indicated that electrical conductivity, available P, soil basal respiration, dehydrogenase and urease activities, and water-soluble carbohydrates were highly weighted on PC1. These data indicate that soil nutrient contents, soil global microbiological activity, and urease activity involved in cycling of N were responsible for the separation between the two organic fertilizers assayed differing in N source. Likewise, PC2 is positively correlated with aggregate stability, total organic carbon, and β-glucosidase and phosphomonoesterase activities. PC2 (which accounted for 9·5% of the total variance) showed a strong separation of the samples OPM and OPL from those IF (Table IV), which seem to be related to increased structural stability and chemical and biological fertility promoted by the organic fertilizers.

Changes in the soil microbial PLFA pattern following the different types of agricultural practices were also analysed by means of PCA (Figure 2). The PC1 explained 46·2% and the second (PC2) 13·3% of the total variance in the PLFAs with eigenvalues >2. The ANOVA of PC1 separated the treatments into three distinct clusters, indicating that they differed in their lipid composition ($p < 0{\cdot}05$, Table IV). An increase in the PLFA related to G− bacteria, 16:1 ω9, was detected as a consequence of inorganic fertilization. According to the eigenvector loading values, the FAs most responsible for the separation of the treatments along the PC1 axis were PLFAs associated mainly with bacteria, like 14:0, 15:0, 16:0, a15:0, 17:0, i17:0, and 16:1 ω9. The second component PC2 exhibited higher loadings of the unsaturated PLFAs 18:1 ω9 and 18:3 ω3·6,9 – although there were no statistically significant differences among treatments along this axis (Table IV). The changes of the microbial community along the PC1 axis were correlated with all enzymatic activities and soil basal respiration to a very significant degree ($p < 0{\cdot}01$), which confirms a close relationship between the soil microbial community composition and function following the replacement of inorganic fertilization by organic fertilization. Likewise, soil nutrient concentrations (available P and total N) were important in determining the composition of the microbial community under the organic fertilization systems, showing significant and positive correlations with PC1 ($r = 0{\cdot}644$** and $r = 0{\cdot}775$**, respectively). It is possible that with the organic fertilization systems, specific microbial communities and more active functional groups involved in the cycling of N, P, and C proliferated. Meanwhile, the first component appeared to be related to total organic C and water-soluble carbohydrates ($r = 0{\cdot}714$** and $r = 0{\cdot}850$**, respectively), which suggests that the dominant microbial community was strongly driven by the organic substrates and nutrients provided to the soil by the organic systems assayed.

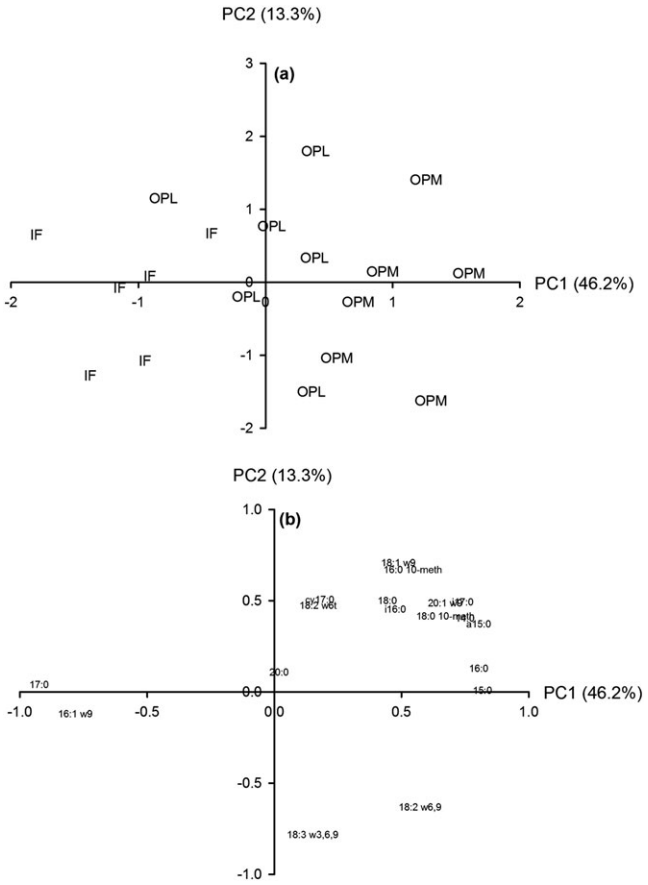

Figure 2. Scores (a) and loadings (b) plot from principal component analysis performed on the phospholipid fatty acids (PLFAs) of soil samples.

In conclusion, the total replacement of inorganic by organic fertilization in a semiarid agroecosystem had a significant medium-term effect on the biomass, composition, and function of microbial community, which may be attributed to the organic carbonaceous substrates and nutrients introduced into the soil. In particular, the addition of grapevine pruning combined with sheep manure or a legume cover crop promoted the proliferation of fungal and G+ bacterial populations in the soil microbial community. The shifts in soil microbial populations and microbial processes related to nutrient cycling promoted by the use of the organic fertilizers improved soil fertility, maintaining crop yields at levels similar to those of the inorganic fertilization system. The effectiveness of the organic fertilization systems for promoting the sustainability and soil biological and chemical fertility of an agroecosystem under semiarid conditions was dependent of the organic N source.

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