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Changes in soil microbial community structure following the abandonment of agricultural terraces in mountainous areas of Eastern Spain

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Abstract

In Eastern Spain, almond trees have been cultivated in terraced orchards for centuries, forming an integral part of the Mediterranean forest scene. In the last decades, orchards have been abandoned due to changes in society. This study investigates effects of changes in land use from forest to agricultural land and the posterior land abandonment on soil microbial community, and the influence of soil physico-chemical properties on the microbial community composition (assessed as abundances of phospholipids fatty acids, PLFA). For this purpose, three land uses (forest, agricultural and abandoned agricultural) at four locations in SE Spain were selected. Multivariate analysis showed a substantial level of differentiation in microbial community structure according to land use. The microbial communities of forest soils were highly associated with soil organic matter content. However, we have not found any physical or chemical soil property capable of explaining the differences between agricultural and abandoned agricultural soils. Thus, it was suggested that the cessation of the perturbation caused by agriculture and shifts in vegetation may have led to changes in the microbial community structure. PLFAs indicative of fungi and ratio of fungal to bacterial PLFAs were higher in abandoned agricultural soils, whereas the relative abundance of bacteria was higher in agricultural soils. Actinomycetes were generally lower in abandoned agricultural soils, while the proportions of vesicular-arbuscular mycorrhyzal fungi were, as a general trend, higher in agricultural and abandoned agricultural soils than in forests. Total microbial biomass and richness increased as agricultural < abandoned agricultural < forest soils.

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Keywords

Mediterranean soils; land use changes; phospholipids fatty acids, microbial community structure; biodiversity

1. Introduction

Understanding the biology and ecology of soils has been considered as a critical factor for the restoration and sustainable management of terrestrial ecosystems in the last years. Soil microorganisms play important roles in organic matter decomposition and nutrients cycles, and consequently provide an integrated vision of soil quality and health. Furthermore, microbiological properties are the most sensitive and rapid indicators of perturbations and land use changes. In this sense, the quantitative description of microbial community structure and diversity has aroused great interest in soil quality evaluation (Zelles, 1999).

In the mountainous region of the Mediterranean Basin of Spain, almond trees have been cultivated in terraced orchards for centuries. These crops are immersed in the Mediterranean forest scenery, part of a landscape mosaic where orchards are integrated into the forest areas. Cultivation of soils represent a type of land use with important effects on soil characteristics and microbiology. Continuous soil tillage leads to a great decrease in soil organic carbon, total nitrogen, cation exchange capacity, aggregate stability, water holding capacity and microbial biomass and activity in topsoils (Caldwell et al., 1999; Gardi et al., 2002). In addition, the incorporation of organic residues or fertilization can provoke alterations in the soil microbial community structure (Lundsquist et al., 1999; Calderón et al., 2000). In the last decades, almond orchards in Eastern Spain are being abandoned due to the low productivity of the crops, industrialization, or the development of tourism (Bonet, 2004). This phenomenon has led to an increase in vegetation cover (González-Bernáldez, 1991; Bonet, 2004), since abandoned fields are naturally colonized by the surrounding natural vegetation. In addition, after abandonment, soil properties such as organic matter content, soil structure and infiltration rate tend to improve, resulting in more effective protection again erosion (Kosmas et al., 2000). So, an improvement in soil quality is expected in these areas, as long as no erosive processes appear. However, there is little information about the impact of the abandonment of former arable soils on soil microbial community structure in the Mediterranean Basin, despite it being a common process in the entire region. Bound to the succession in vegetation, a succession in soil microbial communities should also be initialised. Thus, it is worth focusing on soil microbial community composition after land abandonment, since the functions of soil microbes are crucial for supporting plant growth, vegetation success and the reactivation of biogeochemical cycles, and, therefore, restoration studies should include below-ground community characterization. Furthermore, the understanding of the processes implied in the secondary forest succession initialised after crop abandonment is crucial to avoid degrading processes.

We used phospholipid fatty acid (PLFA) analysis to measure microbial community composition. PLFA analysis uses the lipids of the microbial membranes as biomarkers for specific groups of microorganisms, besides creating a profile or finger print of the community structure. As a consequence, rapid changes in soil microbial community structure can be detected by changes in the PLFAs pattern (Zelles, 1999). In addition, total concentration of PLFAs can be used as a measure of viable microbial biomass, since phospholipids are rapidly degraded after cell death (Zelles, 1997). The use of PLFA analysis has been used to study changes in soil microbial community structure across ecological succession as a consequence of different perturbations or management practices (Hedlund, 2002; Allison et al., 2005; DeGrood et al., 2005; Van der Wal et al., 2006).

An alternative and fast method to measure and monitor soil characteristics is the use of near infrared reflectance (NIR) spectroscopy. In the near infrared region, the radiation is absorbed by the different chemical bonds, such as C-H, N-H, S-H, C=O and O-H of the compounds present in the sample. Moreover, the radiation is absorbed in accordance with the concentration of these compounds. Thus, NIR spectra contain information about the compositional qualities of a soil sample. As a consequence, many authors have observed that NIR spectra contain information about physical, chemical and biological properties of soils, including variables related to the microbial community composition of the soil (Cozzolino and Moron, 2003; Chang and Laird, 2002; Rinnan and Rinnan, 2007; Zornoza et al., 2008).

In this paper, we selected four locations, with similar climate, vegetation composition, and land use history. All sites exhibit similar vegetation mosaic pattern, constituting forest, almond orchards and abandoned lands. Our study aims at investigating: (i) the effects of changes in land use from forest to arable and posterior land abandonment on soil microbial community, and (ii) the influence of soil physico-chemical properties on the microbial community structure.

2. Materials and Methods

2.1. Study sites and soil sampling

Samples were collected from four locations in the province of Alicante (SE Spain): Sierra de Crevillent (C), Sierra del Maigmó (M), Puig Campana (Pu) and Camara (Cam). An undisturbed forest soil was selected in each location. We selected native soils under natural and autochthonous vegetation, as close as possible to potential vegetation, which had not been recently disturbed by human action (>40 years). We also selected an agricultural soil dedicated to almonds, and a former almond orchard abandoned 10-15 years previous to sampling. Hence, for this study, three land uses were chosen: undisturbed forest (f), agricultural (a) and abandoned agricultural (ab). In all locations agricultural and abandoned agricultural soils were situated in the middle of wide forest zones, representing a landscape mosaic, characteristic of the mountainous areas of Eastern Spain. All forest soils have in common the presence of a tree stratum occupied by *Pinus halepensis* Miller (with *Quercus rotundifolia* Lam. in Pu). The understory vegetation is dominated by some shrub species and *Brachipodium retusum* (Pers.) Beauv. as the main herbaceous species. Abandoned almond orchards are characterized by various shrub species (dominated by *Rosmarinus officinalis* L., *Rhamnus lycioides* L., *Helichrisum stoechas* (L.) Moench, or *Cistus albidus* L.), belonging to the first stages of the succession of the vegetation series. All soils are situated on calcaric bedrock. An overview of the sites and soils characteristics is given in Table 1.

A single sampling was carried out in July 2005. A plot of 200 m^2 was defined for each land use at each location, where 5 soil samples (0-10 cm depth) were taken randomly from the mineral A horizon. Prior to soil analysis the samples were air-dried for a week. Afterwards, they were passed through a 2 mm mesh sieve, except for soil aggregate stability determination (4-0.25 mm). For all assays, the average value of two replicates per sample was used, and data have been expressed on an oven dry weight basis.

2.2. Analytical Methods

The following physical and chemical soil properties were assayed: pH and electrical conductivity (EC) were measured in deionised water (1:2.5 and 1:5 w/v, respectively); soil organic carbon (C_{org}) was determined by potassium dichromate oxidation (Nelson and Sommers, 1982); total nitrogen (N) was determined by the Kjeldahl method (Bremmer and Mulvaney, 1982); the soluble carbon (C_{sol}) was extracted with 0.5 M K_2SO_4 and measured at 590 nm (Sims and Haby, 1971); cation exchange capacity (CEC) was measured by the

method described by Roig et al. (1980); available phosphorus (P) was determined by the Burriel-Hernando method (Díez, 1982); water holding capacity (WHC) was assayed by the method exposed by Forster (1995); aggregate stability percentage (AS) was quantified using the method described by Roldán et al. (1994); available Ca, Mg, K and Na were extracted with 1N ammonium acetate (Knudsen et al., 1982) and measured by atomic absorption and emission spectrophotometry.

The following biochemical properties were assayed: microbial biomass carbon (MBC) was determined using the fumigation-extraction procedure (Vance et al., 1987); basal soil respiration (BSR) was monitored for 4 days at 55% WHC and 25°C with a multiple sensor respirometer (Micro-Oxymax, Columbus, OH, USA); urease activity was measured according to the method of Nannipieri et al. (1980); acid phosphatase activity was assayed by the method of Tabatabai and Bremmer (1969); the activity of β-glucosidase was determined according to Tabatabai (1982).

Phospholipid fatty acid (PLFA) analysis was carried out as described in Bossio et al. (1998). Briefly, fatty acids were extracted from 8g soil samples using chloroform:methanol:phosphate buffer. PLFAs were separated from neutral and glycolipid fatty acids on a solid phase extraction column (0.58 Si; Supelco Inc., Bellafonte, PA). After mild alkaline methanolysis, samples were analysed using a Hewlett Packard 6890 Gas Chromatograph with 25 m Ultra 2 (5% phenyl)- methylpolysiloxane column (J & W Scientific, Folsom, CA). Fatty acids were quantified by comparison of the peak areas with those of an internal standard 19:0 peak. The peaks were named using bacterial standards and identification software from the Microbial Identification System (Microbial ID, Inc., Newark, DE). Fatty acid nomenclature used was that described by Frostegard et al. (1993). The fatty acids i15:0, 15:0, a15:0, i16:0, 16:1ω7, i17:0, a17:0, cy17:0, 17:0, 18:1ω7 and cy19:0 were chosen to represent bacteria (Frostegard et al., 1993). The unsaturated PLFA 18:2ω6 was used as indicator of fungal biomass (Federle, 1986). PLFAs cy17:0, 18:1ω7c, cy19:0, 17:1ω9c, 16:1ω9c, 18:1ω9c and 15:1ω4c were chosen to represent Gram-negative [G−] bacteria (Zelles et al., 1994). The branched, saturated i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0 were chosen to represent Gram-positive [G+] bacteria (Zelles et al., 1994). The PLFAs 10Me16:0, 10Me17:0 and 10Me18:0 were selected as indicators of actinomycetes biomass (Zelles et al., 1994). The PLFA 16:1ω5 was used as representative of vesicular-arbuscular mycorrhizal [VAM] fungi (Olsson et al., 1995). Two ratios were also calculated, fungal to bacterial PLFAs (fungi:bacteria) and Gram-positive:Gram negative bacteria (G−:G + bacteria). The total biomass was estimated as the sum of all the extracted PLFAs [total PLFAs].

For NIR measurements we used a Fourier-Transform near infrared (FT-NIR) spectrophotometer (MPA, Bruker Optik GmbH, Germany), equipped with quartz beamsplitter and PbS detector. It is also equipped with an integrating macrosample sphere and rotating sample cup, allowing the scanning of large areas of the samples. NIR spectra of soil samples were obtained with the following procedure: aliquots of around 50 g of soil were placed in glass Petri-dishes, and scanned on reflectance mode from 12000 to 3800 cm^{-1}. In each of the reflectance measurement, 64 scans were averaged. Samples were measured in duplicate. After this, they were averaged again. The time employed for the spectral measurement was approximately 1 min per sample. The resolution used for spectral analysis was 8 cm^{-1}. Background corrections were made before each sample scan. The x-scale of each NIR spectrum was transformed from wavenumber to wavelengh, obtaining a spectrum with 1000 data of absorbance comprising from 830 to 2630 nm.

2.3. PLFA diversity indices

The richness (R) of the fatty acids was expressed as the total number of PLFAs present in each sample. The diversity of the fatty acids was calculated with the Shannon-index H:

$$H = -\sum_{i=1}^{R} pi \ln pi, \quad (1)$$

where pi is the relative abundance of each fatty acid in the total sum, and R is the number of detected fatty acids. The equitability of the fatty acids was calculated with the Shannon's evenness E (Shannon, 1948):

$$E = H/\ln(R) \quad (2)$$

2.4. Statistical Analyses

PLFA data were converted to mol% of the total fatty acids concentration. Canonical Correspondence Analysis (CCA) was used to examine the relationship between the microbial community composition and soil characteristics. Samples with similar PLFA profiles have similar scores and thus will group closer together when plotted. Soil physical and chemical properties were tested for significant contribution to the explanation of the variation in the PLFA data with the Monte Carlo permutation test ($P<0.05$). Only soil properties that were significantly correlated with factor in the CCA were included in the plots. Soil properties are represented by vectors. Vectors of greater magnitude and forming smaller angles with an axis are more strongly correlated with that axis. CCA can be influenced by rare fatty acids. Fatty acids that only appear in a few samples are usually unreliably represented because they have values near the detection limit. Hence, fatty acids that were present in less than 25% of the samples were omitted.

We carried out a one-way ANOVA for each location separately to investigate the differences between land uses regarding microbial groups and ratios based on PLFA biomarkers and diversity indices. The separation of means was made according to Tukey's honestly significant difference at $P<0.05$. We also developed Pearson correlations between the physical and chemical properties and the different PLFA microbial groups.

A discriminant analysis (DA) was independently performed for each set of soil variables: (1) soil physical, chemical and biochemical properties, (2) NIR data, and (3) PLFAs. The main purpose was to confirm that changes in land management had significantly changed soil microbial community structure, as well as to compare the sensitivity of PLFAs against other soil properties and soil spectral characteristics, so that soil samples can be correctly classified by land uses. The discriminant functions produced probability of membership of a given sample in each respectively land use class. A given sample was assigned to the land use class with the highest probability. With the aim of reducing the number of variables with NIR data, a previous principal component analysis (PCA) was applied to the NIR spectral matrix (60 samples ×1000 wavelengths), extracting the first 35 principal components (PCs), that explained the 99.99% of the variability. The factor scores of the first 35 PCs were used, reducing the size of the matrix (from 60 × 1000 to 60 × 35). Thus, the DA was performed on the 60 samples which represented 35 variables (the factor scores of these 35 PCs) in 3 groups or categories.

CCA was performed using CANOCO for Windows, Version 4.5, while ANOVA, correlations and DA were performed with the software SPSS for Windows, Version 13.0.

3. Results

3.1. Multivariate analysis

The CCA performed with the PLFA data showed that a 78.3% of total variation could be explained by the first three axes, and soil samples clearly clustered by land use (Fig. 1). Axis 1 separated forest soils from the other land uses, and explained 56.0% of the variation. Axis 2 explained 11.9% of the variation, and separated, only for agricultural and abandoned agricultural soils, C and Cam locations from M and Pu locations. Axis 3, which explained 10.4% of the variation, separated agricultural from abandoned agricultural soils. All soil characteristics, with the exception of Na and K, were significant ($P<0.05$) in explaining the variation in PLFA data. The variables C_{org}, N, C/N ratio, C_{sol}, WHC, CEC, AS, EC, Mg and Ca showed a positive association with axis 1, and thus with forest soils, while pH was negatively associated with this land use. Thus, soil organic matter content largely accounts for variations in microbial community composition of forest soils, distributed in a positive direction along axis 1. Changes in microbial community composition along axis 2 were associated with both relatively higher values of P and lower values of Mg and pH. No soil property was related to the gradient along axis 3, differentiating between agricultural and abandoned agricultural soils.

Certain PLFAs were strongly associated with the different land uses. Forest soils were characterised by high concentrations of the saturated PLFAs 12:0, 13:0 and 3OH12:0, the monounsaturated i18:1, and the sum of the PLFAs 19:1ω11c, 19:1 and an unknown fatty acid. Agricultural soils (with positive values along axis 3) were associated with higher concentrations of the branched fatty acids a15:0 and i17:1, and the unsaturated fatty acids 2OH16:1 and 18:3ω6*c*. Abandoned agricultural soils (with negative values along axis 3) were characterised by high concentrations of the fungal PLFA 18:2ω6, and the branched saturated fatty acids 10Me19:0 and a19:0. The most positive loading score for axis 2 was found for the fatty acid 30H16:0, associated with samples from Pu and M locations.

3.2. PLFA biomarkers

Microbial biomass, assessed as total PLFAs, showed the following trend in all locations: agricultural < abandoned agricultural < forest soil (Table 2). Specific PLFAs as biomarkers were quantified (Fig. 2). The proportion of the total PLFAs attributable to fungi was significantly higher ($P<0.05$, Fig. 2a) in abandoned agricultural soils, with a general decrease with abandoned agricultural soils > forest soils > agricultural soils. The ratio of fungal to bacterial PLFAs showed the same trend detected with the PLFA indicative of fungi (Fig. 2b). The relative abundance of bacteria was higher ($P<0.05$, Fig. 2c) in agricultural soils, whereas G− bacteria abundance was lower in forest soils, with no differences between agricultural and agricultural soils (Fig. 2d). We observed a high variability in the relative abundance of G+ bacteria in terms of location, although in all cases, there was no significant difference between forest and abandoned agricultural soils ($P>0.05$, Fig. 2e). The ratio G−:G + bacteria (Fig. 2f) showed the same trend observed with G− bacteria. The specific PLFAs indicative of actinomycetes were generally lower in abandoned agricultural soils (Fig. 2g), while VAM fungi proportions were as a general trend higher in agricultural and abandoned agricultural soils (Fig. 2h).

Total PLFAs were strongly correlated with most variables, the strongest correlation established with MBC ($r=0.91$; $P<0.001$) (Table 3). The relative abundance of bacteria was negatively correlated with C_{sol} and Mg ($P<0.001$). The proportion of G− bacteria were negatively correlated with C_{org}, WHC, AS, CEC and MBC, but positively correlated with pH ($P<0.001$). The ratio G−:G+ bacteria was also positively correlated with pH.

3.3. Separation of land uses by means of Discriminant Analysis

The DA performed on the set of physical, chemical and biochemical variables resulted in a good classification of samples in terms of the three land uses, and more than 80% of the samples were correctly classified. Using the DA with NIR data and PLFAs resulted in an excellent classification of land uses, and 100% of the samples were correctly classified (Fig. 3).

3.4. PLFAs diversity indices

As a general pattern, richness increased significantly as follows: agricultural soil < abandoned agricultural soil < forest soil, ranging from 36 in the agricultural soil from Sierra del Maigmó to 49 in the forest soil from Puig Campana (Table 2). The Shannon-index *H* showed no significant differences among land uses, except for the agricultural soil in Puig Campana, which was significantly the highest (3.27). The Shannon evenness *E* revealed an increasing trend with forest soil < abandoned agricultural soil < agricultural soil, with values ranging from 0.79 in the forest soil from Camara to 0.90 in the agricultural soil from Puig Campana.

4. Discussion

4.1. Soil microbial community structure regarding land uses

Soil organic carbon content increased with agricultural soil < abandoned agricultural soil < forest soil, owing to the highest inputs as a result of the highest vegetation cover. As a consequence, microbial biomass, which is strongly correlated with organic carbon, followed the same trend. The total concentration of PLFAs is strongly correlated with microbial biomass carbon determined by the fumigation-extraction method, which verifies the reliability of PLFAs as indicative of microbial biomass. Strong correlations between these two methods have been often reported. Nonetheless, the correlation coefficient achieved in this research (0.91) is high as compared with other studies, where *r* values ranged between 0.60 and 0.75 (Feng et al., 2003; Bünemann et al., 2004; Allison et al., 2005; Hackl et al., 2005).

Differences found among zones with regard to soil microbial communities are explained by the fact that the size, structure and activity of microbial communities depend on climatic, edaphic and topographic characteristics, as well as vegetation and management history, specific to each zone. However, our results suggest a substantial level of differentiation in microbial community structure according to land use, irrespective of location. In all locations, microbial biomass increased from agricultural soil to forest soil, accompanied by increases in richness and similar variations in microbial community structure. Multivariate analysis showed that the microbial community structure of forest soils was highly associated with soil organic matter content. Probably, microorganisms responded to an increment in organic matter levels in soil, involving in increases in carbon and nutrients availability and increments in the capacity for water retention. On the contrary, the differences in microbial community composition found between agricultural and abandoned agricultural soils were not explained by any physical or chemical soil property. Thus, the abandonment of the perturbation caused by agricultural activity per se, and the associated changes in vegetation, rather than direct changes in soil properties, may have led to shifts in soil microbial community structure. It has often been reported that plant species composition influences microbial communities by the release of radical exudates (Grayston et al., 1998; Yang and Crowley, 2000).

Abandoned agricultural soils had the highest relative fungi abundance. Fungi communities play a dominant role in fresh organic matter decomposition (Dilly et al., 2001). In

abandoned agricultural soils, vegetation cover is higher than in agricultural soils. Thus, there is an increased incorporation of litter into the soil, which facilitates the development of fungi populations. As other authors have demonstrated previously, the abandonment of agricultural practices leads to increments in fungi dominance (Hedlund, 2002; Allison et al., 2005). Relative bacteria abundance was highest in agricultural soils. Bacteria tend to dominate decomposition and nutrient cycles in soils that are tilled and fertilized (Lovell et al., 1995; Allison et al., 2005).

The ratio of fungal to bacterial PLFAs increased after agriculture abandonment. When the mature ecosystem is reached, represented by forest, relative fungi abundance and the ratio fungi:bacteria descend. We think that increments in fungi abundances after land abandonment are related to the cessation of perturbations (tillage and fertilization), as often reported in other studies (Allison et al., 2005; van der Wall et al., 2006). Tillage provokes the break of hyphae. Hence, the interruption of this activity permits the development of fungi, stimulated by greater contributions of organic matter. However, succession towards a mature community may have caused a descent and stabilization in fungi abundance, probably as a consequence of the increment in species richness, and so, increases in competence. Allison et al. (2005) also observed that although in the first years after field abandonment fungi increased, in later successional stages (a tallgrass prairie), they started decreasing. The authors explained these findings by the fact that C inputs in the grassland mainly come from root exudates rapidly metabolised, which limit the development of fungi as this group use more recalcitrant sources of C. Nonetheless, this argument is not valid for explaining our results, since the incorporation of recalcitrant organic matter is high in the topsoil of conifer forests.

Although fungi are considered the major contributors to microbial biomass in forest soils as well as other soils with high organic matter content (Bailey et al., 2002), the fungal to bacteria ratio in this study is low, also reported in other studies that developed PLFA analysis (Bååth and Anderson, 2003; Certini et al., 2004; Allison et al., 2005; van der Wall et al., 2006), as compared with direct microscopic methods or selective respiratory inhibition (Alphei et al., 1995; Ananyeva et al., 2006; Busse et al., 2009). However, to be able to calculate actual biomass values from the ratio of fungal to bacterial PLFAs one needs reliable conversion factors. Such conversion factors will be different for fungi and bacteria, but also different for the PLFA and other techniques such as the selective inhibition technique. Since there are currently no reliable conversion factors, it is meaningless to compare the actual values of the fungal to bacterial ratios estimated with different methods (Bååth and Anderson, 2003). It is also probable that more basic research on the concentration of specific indicators PLFAs in soil bacteria and fungi is necessary to obtain the full quantitative information from this method. In fact, a major disadvantage of PLFA analysis is the fact that none of the indicator is fully specific for a certain microbial group (Joergensen and Wichern, 2008). However, changes in this ratio due to changes in land use should be reliable since the same analytical procedure was used and the same extraction efficiency was guaranteed.

The highest proportions of G− bacteria in agricultural and abandoned agricultural soils may be the result of higher pH values in these soils in comparison with forest soils (significant correlations have been found between G− bacteria and pH, Table 3). High values of pH favour the growth of bacteria populations (Nodar et al., 1992). Furthermore, Frostegard et al. (1993) observed that increments in soil pH lead to shifts in the bacterial community, with increments in G− bacteria.

The highest relative abundances in VAM fungi were found in agricultural soils, and in some cases in abandoned agricultural soils. In agricultural soils there is a higher proportion of

herbaceous species, which generally form this type of mycorrhizal association (Rillig, 2004). Although no correlation was found between VAM fungi and aggregate stability with all soils, a significant positive correlation was observed in abandoned agricultural soils (r=0.72; P<0.001). This correlation seems to partly corroborate other researches which highlight the importance of VAM fungi hyphae in the stabilization of soil aggregates (Miller and Jastrow, 1990; Rillig, 2004; Allison et al., 2005), mainly in the first stages of succession after land abandonment. Mycorrhizal fungi directly favour the aggregation of particles by the extension of their mycelium or indirectly by the release of extracellular exudates (Miller and Jastrow, 1990). This relationship has only been observed in abandoned agricultural soils probably because in agricultural soils the continuous tillage provokes the breakage of aggregates. In forest soils, aggregates are mainly stabilised by the higher amount of organic matter.

The results of the DA carried out with the PLFAs supported the presence of different community composition among the three land uses, since all samples were correctly discriminated according to land use. Soil samples classification using PLFA data was better than that obtained with the set of physical, chemical and biochemical properties. Thus, these results confirm the sensitivity of parameters relating to soil microbial community composition in evaluating soil quality, perturbations and effects of management practices, as microbial communities respond very quickly to changes in land use, and give a result earlier than measurements of other variables of a different nature, including total microbial biomass or metabolic activity. Also excellent results were achieved with the NIR spectra. In fact, distances of each sample to the centroid of its group (forest, agricultural or abandoned agricultural) were lower when using NIR data than with PLFAs, mainly for forest soils (see Fig. 3), supporting the high sensitivity of NIR spectra to land use changes. In this sense, NIR spectra offer an integrated vision of soil conditions, since synthesize information regarding mineralogy, soil chemistry, soil biology, organic matter and physical attributes (Cohen et al., 2005). NIR spectrum could be considered as an integrated vision of soil quality, and as consequence offers an integrated vision of perturbations. As a consequence, NIR spectra have been satisfactorily used for land use classification (Velasquez et al., 2005; Elliot et al., 2007). In addition, Johnson et al. (2003) observed that the grouping of soil samples based on their soil reflectance properties was similar to the grouping based on DNA fingerprinting. Thus, since microbial community composition is related to reflectance properties, these techniques could be promising at providing insight into microbial distributions at the landscape scale.

4.2. PLFA diversity indices

Increases in microbial biomass were associated with increases in richness, with significant positive correlations among both parameters (r=0.76; P<0.001). DeGrood et al. (2005) also found increases in richness bound to increases in microbial biomass after the restoration of degraded serpentine soils. It has been reported that increments in biomass must be accompanied by increments in diversity in order to facilitate sustainable restoration on disturbed sites (Bradshaw, 1984). Furthermore, the increment of richness has been proposed as a basic objective in the management of degraded ecosystems (Bonet, 2004). Nevertheless, no correlation has been found between microbial biomass and the Shannon index. Thus, although forest soils showed the highest variety of PLFAs, and so a higher variety of groups of microorganisms, this did not entail highest biodiversity in terms of the relative abundance of each group. In agricultural and abandoned agricultural soils, even though having lower richness, microbial groups were better balanced, and there were no differences in the Shannon index between forest and agricultural soils. Furthermore, regarding the Shannon evenness, we observed that in the zones with lower richness and biomass (agricultural soils), the microbial groups were represented more equitably.

A possible explanation for these findings is that, in agricultural soils, microorganisms are mainly “r” strategists, fast colonisers with high growth rates, as they are characteristic of altered environments. Under these conditions, all microbial groups can colonise the environment with proportional increments in their abundance, according to the availability of substrates. On the contrary, with progressing succession, the detritus food webs would become more complex. Then, slower growing specialists, the “K” strategists, occupy specific niches. This may explain why the Shannon evenness was lower.

5. Conclusions

Different land uses support different microbial community structures. Soil organic matter content seems to be the main agent responsible for the different microbial community structure in forest soils. Agricultural soils have the highest relative abundance of bacteria, probably due to tillage and fertilisation. The abandonment of agriculture has led to increments in microbial biomass, richness and shifts in the microbial community structure, probably due to the cessation of tillage and the associated changes in vegetation, rather than direct changes in soil properties. The principal effect has been the increment in the ratio of fungal to bacterial PLFAs. The fact that increments in microbial biomass are accompanied by increments in richness contributes to reinforce the hypothesis of ecosystem recovery. Future studies should evaluate if the vegetation and microbial community composition of former agricultural soils with higher periods of abandonment move towards advanced successional stages, which would really guarantee the recovery of ecosystems after the cessation of agriculture in the mountainous areas of Eastern Spain.

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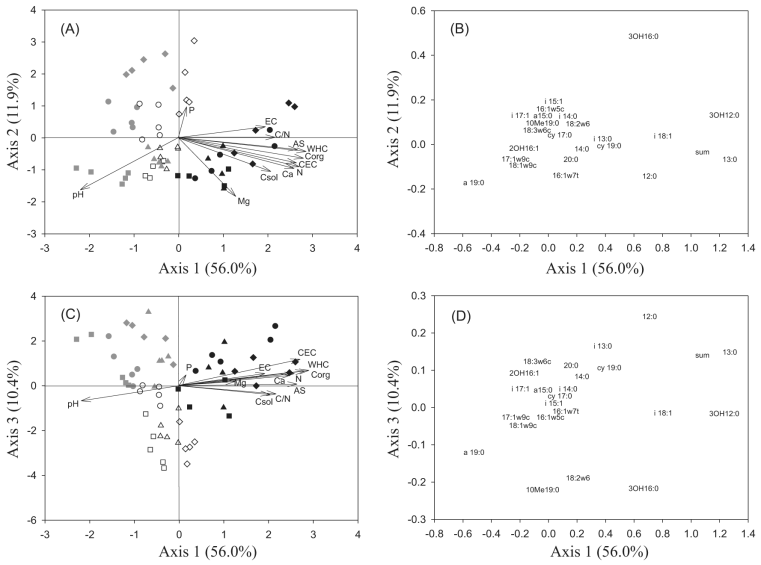

Figure 1.

Samples and soil characteristics biplots (left) and loadings plots (right) from CCA performed on the relative concentration of PLFAs in all land uses: agricultural (grey symbols), abandoned agricultural (open symbols), and forest (black symbols). Locations: Sierra de Crevillent (triangles), Sierra del Maigmó (circles), Puig Campana (diamonds) and Camara (squares).

C_{org}: soil organic carbon; N: total nitrogen; C_{sol}: soluble carbon; EC: electrical conductivity; WHC: water holding capacity; AS: aggregate stability; CEC: cation exchange capacity; sum: sum of 19:1ω11c, 19:1 and an unknown fatty acid.

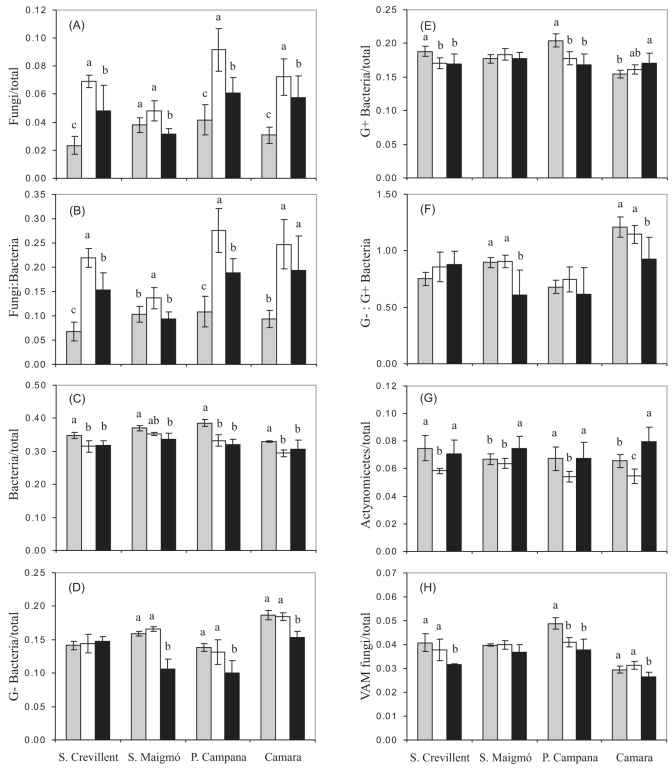

Figure 2.

Values of various PLFA biomarkers (mean ± standard deviation) for all land uses in each location (■ agricultural; □ abandoned agricultural; ■ forest). A one-way ANOVA and Tukey tests (P<0.05) were used to compare differences in land use for each location separately. Letters above the bars indicate significant differences between land uses in each location. Bars with no letter within the same location are not significantly different (P>0.05). G−: Gram-negative; G+: Gram-positive; VAM: vesicular-arbuscular mycorrhyzal.

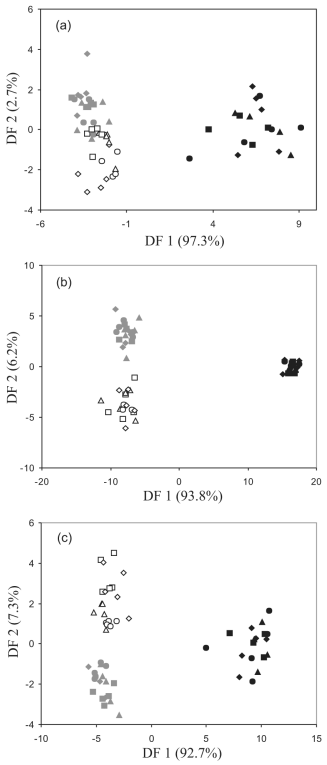

Figure 3.
Discriminant analysis with soil physical, chemical and biochemical properties (a), factor scores from NIR absorbance data (b), and PLFAs (c) of samples taken under three land uses: agricultural (grey symbols), abandoned agricultural (open symbols), and forest (black symbols). Locations: Sierra de Crevillent (triangles), Sierra del Maigmó (circles), Puig Campana (diamonds) and Camara (squares).

Table 1

Sites and soils characteristics.

Site[a]	Land use[b]	Tm[c]	Pm[c]	Vegetation cover	Soil type	Texture[d]	pH	OM[e]	CO_3^{2-}
		°C	mm	%	SSS, 2006	(% sand, silt, clay)		%	%
C	f			95	Calcixeroll	Loam (44, 43, 13)	7.9	14.4	30
C	a	15.8	355	5	Xerorthent	Clay (37, 34, 29)	8.1	3.1	51
C	ab			30	Xerorthent	Clay (37, 34, 29)	8.1	3.8	52
M	f			70	Xerorthent	Clay loam (56, 23, 21)	7.9	11.0	59
M	a	18.2	302	5	Xerorthent	Clay (51, 30, 29)	8.2	1.5	66
M	ab			50	Xerorthent	Clay loam (51, 32, 17)	8.2	2.1	75
Pu	f			80	Calcixeroll	Silty loam (46, 45, 9)	7.6	17.9	25
Pu	a	15.6	424	5	Xerorthent	Silty clay loam (17, 66, 17)	7.7	1.9	59
Pu	ab			70	Xerorthent	Silty loam (21, 70, 9)	7.8	4.1	51
Cam	f			70	Xerorthent	Clay loam (42, 35, 23)	7.9	10.0	32
Cam	a	14.0	308	5	Xerorthent	Clay (37, 36, 27)	8.4	1.1	59
Cam	ab			50	Xerorthent	Silty clay loam (27, 48, 25)	8.3	1.9	54

[a] C: Sierra de Crevillent; M: Sierra del Maigmó; Cam: Camara; Pu: Puig Campana.

[b] Land uses: f (forest); a (agricultural); ab (abandoned agricultural).

[c] Mean annual temperature (Tm) and mean annual precipitation (Pm).

[d] Sand: 2-0.02 mm; silt: 0.02-0.002 mm; clay < 0.002 mm.

[e] OM: organic matter.

Table 2

Microbial biomass and PLFA diversity indices (means and standard deviation) for each land use and location

Soil properties	Sierra de Crevillent[a]			Sierra del Maigmó			Puig Campana			Camara		
	f	a	ab	f	a	ab	f	a	ab	f	a	ab
Total PLFA (nmol g^{-1})	210 (57)a	67 (24)b	77 (16)b	152 (53)a	33 (8)c	52 (9)b	178 (82)a	36 (16)c	93 (15)b	125 (38)a	36 (13)c	70 (14)b
Richness	45 (3)a	41 (3)b	40 (1)b	43 (3)a	36 (2)c	39 (2)b	49 (3)a	38 (2)b	45 (2)a	44 (3)a	37 (3)c	39 (1)b
Shannon index *H*	3.20 (0.03)	3.20 (0.05)	3.12 (0.03)	3.14 (0.05)	3.18 (0.02)	3.19 (0.03)	3.16 (0.02)b	3.27 (0.06)a	3.12 (0.04)b	3.16 (0.05)	3.18 (0.03)	3.12 (0.04)
Shannon Evenness *E*	0.84 (0.02)b	0.86 (0.02)a	0.85 (0.01)a	0.83 (0.03)c	0.88 (0.01)a	0.87 (0.02)b	0.81 (0.02)b	0.90 (0.03)a	0.82 (0.02)b	0.79 (0.07)c	0.88 (0.02)a	0.85 (0.01)b

[a]Land uses: f (forest), a (agricultural) and ab (abandoned agricultural).

Separately for each location, values with different letters indicate significant differences ($P<0.05$) among land uses after one-way ANOVA. Values with no letter are not significantly different ($P>0.05$).

Table 3

Correlation coefficients (*r* values) for relationships between physical, chemical and biochemical properties, and the PLFA biomarkers.

	Total PLFA	Fungi	Fungi:bacteria	Bacteria	G−	G+	G− : G+	Actinomycetes	VAM fungi
C_{org}	0.89*	0.08	0.12	−0.35	−0.48*	−0.20	−0.32	0.25	−0.14
N	0.90*	0.04	0.09	−0.36	−0.44	−0.18	−0.31	0.31	−0.19
C/N	0.58*	0.25	0.26	−0.22	−0.38	−0.12	−0.30	0.03	0.01
C_{sol}	0.61*	0.22	0.27	−0.50*	−0.24	−0.19	−0.14	0.37	−0.40
pH	−0.53*	−0.24	−0.18	−0.11	0.54*	−0.30	0.56*	−0.14	−0.37
EC	0.54*	0.14	0.13	0.08	−0.38	0.11	−0.35	0.35	−0.07
WHC	0.86*	0.11	0.15	−0.30	−0.50*	−0.14	−0.39	0.26	−0.09
AS	0.70*	0.10	0.11	−0.21	−0.49*	0.06	−0.37	0.31	−0.01
CEC	0.87*	−0.02	0.02	−0.28	−0.49*	−0.13	−0.36	0.31	−0.11
P	0.06	−0.03	−0.10	0.29	−0.06	0.22	−0.12	0.08	0.17
Ca	0.84*	0.05	0.10	−0.39	−0.34	−0.19	−0.31	0.28	−0.16
Mg	0.59*	0.02	0.11	−0.50*	−0.03	−0.27	0.07	0.51*	−0.61*
Na	0.14	0.17	0.16	−0.07	−0.24	0.08	−0.24	0.20	−0.15
K	0.50*	0.07	0.10	−0.27	−0.43	−0.06	−0.34	0.23	−0.11
MBC	0.91*	0.25	0.29	−0.40	−0.48*	−0.21	−0.31	0.16	−0.11

C_{org}: soil organic carbon; N: total nitrogen; C_{sol}: soluble carbon; EC: electrical conductivity; WHC: water holding capacity; AS: aggregate stability; CEC: cation exchange capacity; MBC: microbial biomass carbon; G−: Gram-negative bacteria; G+: Gram-positive bacteria; VAM: vesicular-arbuscular mycorrhyzal.

*Significant correlation at $P<0.001$.