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Rainfed olive farming in south-eastern Spain: Long-term effect of soil management on biological indicators of soil quality

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ABSTRACT

Deteriorating soil quality in arid areas is related primarily to inappropriate farming techniques. The use of environmentally friendly agriculture practices has proven to be effective in restoring or improving soil quality and health in these areas. In this study, four long-term approaches to olive–orchard management, categorized by the presence/absence of two main factors – cover crops and chemical weed control – were evaluated using biological indicators of soil quality. Soil bacterial population size and community structure were estimated by real-time PCR assays targeting 16S rRNA genes and denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA fragments (PCR–DGGE), respectively, and the activity of six enzymes (dehydrogenase, *o*-diphenoloxidase, β-glucosidase, phosphatase, urease and arylsulphatase) representative of the C, P, N, and S cycles were determined. Covered soils exhibited greater bacterial biomass and diversity, as well as higher microbial functional diversity than non-covered soils. The elimination of weeds with herbicides reduced the microbial functional diversity in covered soil but did not affect the other microbiological parameters, revealing that the effect of the cover crop predominated. When non-covered soil was considered, the lowest values of microbial activity and diversity were found when weeds were controlled with chemical methods. The results of this study reveal that covered soils are the best option when olive orchards are managed under rainfed conditions.

1. Introduction

The economic and social importance of olive cultivation in Spain is immense. There are more than 2500 oil mills that produce more than 30% of the world production of olive oil, generating 2,000,000,000 € in Spain. The ecological impact of this crop is also enormous, as the olive orchard constitutes the landscape – or a fundamental part thereof – throughout vast tracts of land in the Mediterranean Basin (Pajaron, 2000). About 80% of the olive crops are concentrated in Andalusia, the biggest olive-growing area on the planet. Within Andalusia, the most important olive oil-producing areas are in the province of Jaen (south-eastern Spain). In these areas, dry climatic conditions determine the viability of any crop. The success of olive farming under rainfed conditions is due mainly to the physiological behaviour of the olive tree (*Olea europaea* L.), a species which has developed a series of physiological mechanisms to tolerate drought stress and grow under adverse climatic conditions (Sofo et al., 2007). Nevertheless, arid regions are particularly susceptible to soil degradation. Deteriorating soil quality in these areas is related primarily to inappropriate farming techniques (Eitzinger et al., 2008). Conventional tillage in olive-production systems typically include primary tillage with a mouldboard or chisel plough in the fall, spring disking or harrowing, and inter-row cultivation for weed control during the crop-growing season. These operations promote soil erosion and rapid depletion of soil organic matter (Fleskens and Stroosnijder, 2007). The use of environmentally friendly agriculture practices has proven to be effective in restoring or improving soil quality and health in these areas by reducing the mechanical disturbance of soil, protecting the soil surface with mulch cover, and adding organic matter to the soil (Balkcom et al., 2007; Castro et al., 2008).

Evaluation of agricultural soil quality is a very complex task, beginning with the difficulty of providing a definition (Bastida et al., 2008). However, it is generally assumed that the first step to estimate soil quality should be to identify critical functions (Karlen et al., 2003). The difficulty is that soil functions are affected not only by soil properties, but also by climate, landscape, and management, and the relations among these variables are complex. In any case, selected parameters such as enzyme activities give helpful information concerning functionality and productivity because of their central role in nutrient cycling and their sensitivity to management (Nannipieri et al., 1990; Benitez

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et al., 2004). Despite that elucidating the link between soil functions and microbial diversity is still a major challenge, it has been established that reductions in the diversity of soil biota reduce the stability of the soil community with possible degeneration in ecosystem functioning (Brussaard et al., 2004). Consequently, changes in soil-microbial diversity, measured in terms of microbial community structure and function, have also been included as feasible biological indicators of soil quality (Puglisi et al., 2006).

The aim of the present study was to assess the impact of four long-term management practices under rainfed conditions, on various biological indicators of soil quality. To do this, soil bacterial population size and community structure were estimated by real-time PCR assays targeting 16S rRNA genes and denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA fragments (PCR–DGGE), respectively. The activity of six enzymes (dehydrogenase, *o*-diphenoloxidase, β-glucosidase, phosphatase, urease and aryl-sulphatase) representative of the C, P, N, and S cycles were also determined.

2. Materials and methods

2.1. Site description, trial and sampling

The study was conducted in Jaen (south-eastern Spain). At this site, an experiment designed to study the impact of different soil-management systems on olive production began in 1976 and continues to date. The main characteristics of the experiment are fully described in Castro et al. (2008). Briefly, the average annual rainfall for the area is 557 mm, the common soil is an Anthropic Regosol (FAO, 1998) and the plantation consisted of unirrigated adult olive trees planted in a pattern of 11 m × 11 m. For our study, a random-block design of four treatments and four plots from within each treatment were selected from the overall trial (Castro et al., 2008). The elementary plot consisted of 16 olive trees: the central four were controlled and the rest constituted guard rows. The treatments tested were:

- Tillage (T): 3–4 annual passes with disk harrow (depth 30 cm) and/or cultivator in the spring, followed by a tine harrow in the summer.
- Non-tillage and no-cover (NC): vegetation was eliminated by applying the pre-emergence herbicides simazine and diuron in the autumn. These have been replaced by oxyfluorfen for five years until now. In the spring, glyphosate was applied locally.
- Cover crop + herbicides (CH): weeds were left to grow each year to be eliminated in March with herbicides. Initially, diquat/paraquat was used before being replaced at a later date by glyphosate.
- Cover crops + mower (CM): weeds were controlled with various passes of the chain mower at the end of spring, usually when the plants had completed or almost completed their vegetative cycle.

Two samples were collected in the center of each plot. We used a modified soil-sample ring kit (Eijkelkamp) which includes a cylinder of 30 cm (depth of sampling) specifically manufactured for this purpose. The bryophyte layer was eliminated (when present) in cover treatments CH and CM, and any weeds were cut to ground level. Samples were bulked to give three replicates per plot. Field-moist soil was stored at 4 °C until enzymes and nucleic-acid analyses were made.

2.2. Soil organic carbon and enzymes activities

Soil organic-carbon content was determined using the Walkley–Black wet dichromate oxidation method (M.A.P.A., 1986).

Dehydrogenase activity was determined by the method of Von Mersi and Schinner (1991) modified by Garcia et al. (1997). One gram of soil was incubated for 20 h at 25 °C with 0.2 ml of 0.4% 2-*p*-iodophenyl-3 *p*-nitrophenyl-5 tetrazolium chloride (INT) as a substrate. Iodonitrotetrazolium formazan (INTF) produced in the reduction of INT was extracted with a mixture of acetone:tetrachloroetene (1.5:1) and measured in a spectrophotometer at 490 nm. Assays without soil and without INT were carried out simultaneously as controls.

For the determination of urease activity, 2 ml of 0.1 M, pH 7.0 phosphate buffer and 0.5 ml 1.066 M urea were added to 1 g of soil (Nannipieri et al., 1980). Shaken incubation was carried out at 37 °C for 1.5 h. The ammonium released in the hydrolytic reaction was measured using an ammonium selective electrode (ORION Research Inc., Beverly, MA, USA) mod. 95–12. Assays without soil and without urea were made at the same time as controls.

For the determination of β-glucosidase, phosphatase and arylsulphatase activity, 0.5 ml of 0.05 M 4-nitrophenyl-β-D-glucanopyranoside, 0.115 M 4-nitrophenyl phosphate and 5 mM 4-nitrophenyl sulphate were used as substrate respectively (Tabatabai and Bremmer, 1969, 1970; Nannipieri et al., 1980). Soil portions (1 g) were incubated at 37 °C for 1.5 h with 2 ml of maleate buffer at pH 6.5. The samples were then kept at 2 °C for 15 min to stop the reaction, and the *p*-nitrophenol produced in the enzymatic reactions was extracted and determined at 398 nm. Assays without soil and without substrates were made at the same time as controls.

For the analyses of *o*-diphenol oxidase, reagent solutions of 0.1 M phosphate (pH 6.5) containing 0.2 M of catechol or 0.2 M of proline were oxygenated for 3 min and incubated for 10 min at 30 °C. Then, 1 g of soil was added to 3 ml of reagent solution (1.5 ml of catechol solution and 1.5 ml of praline solution) and 2 ml of phosphate buffer (0.1 M, pH 6.5). The mixture was incubated for 10 min at 30 °C and the reaction was stopped by cooling in an ice-bath and adding 5 ml of ethanol. The mixture was centrifuged at 5000 *g* at 4 °C for 5 min. The absorbance of the supernatant fraction was measured at 525 nm. Assays without soil and without catechol were made at the same time as controls (Perucci et al., 2000).

2.3. Soil–DNA extraction and PCR–DGGE analysis

The total DNA was extracted from subsamples (2 extractions per each one of the three replicates per plot) of 250 mg of soil by the bead-beating method, following the manufacturer's instructions MoBio UltraClean Soil DNA Isolation kit (MoBio Laboratories Inc., Solana Beach, CA, USA). To remove trace concentrations of PCR inhibitors, the repetition of the second step (Inhibitor Removal Solution) was carried out. The DNA samples were checked for concentration and quality using the NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, Delaware; USA).

PCR was performed with the 16S rDNA universal bacterial denaturing gradient gel electrophoresis (DGGE) primers (TIB®-MOLBIOL, Berlin, Germany) P1, P2, and P3 to amplify the V3 hypervariable region of 16S rDNA genes (Muyzer et al., 1993).

Two successive amplifications were carried out following Muyzer et al. (1993) modified by Moreno et al. (2008). The total reaction mixture of the first PCR consisted of 25 μl with the following ingredients: 1 μl volume (approx.10 ng) of extracted DNA, 1 μM primer P1, 1 μM primer P2, 10 μl Eppendorf® Master Mix (2.5×) and sterile Milli-Q water to a final volume. The second amplification was performed by using 1 μl of the products of the first reaction as template. In this, primers P2 and P3 were used under the same conditions described above.

DGGE analyses were conducted using 20 μl of this latter PCR product loaded into a 40–70% urea-formamide–polyacrylamide

gel. An INGENYphorU System (Ingeny International BV, The Netherlands) was run at 75 V for 17 h at 58 °C to separate the fragments. Gels were silver stained with the Bio-Rad Silver Stain according to the standard DNA-staining protocol and photographed under UV light (λ = 254 nm) using an UVItec Gel Documentation system (UVitec Limited, Cambridge, UK).

2.4. Real-time PCR assay

Real-time PCR was performed to quantify the number of 16S rRNA copy in triplicate soil–DNA extracts. Universal primers specific for V3 hypervariable region of 16S rRNA of eubacteria P1 and P2 (Muyzer et al., 1993) were used. Each 21-μl PCR reaction contained from 2 to 5 ng of the DNA, 10.5 μl 2× iQ SYBR Green Supermix (Bio-Rad, Munich, Germany), and 400 nM each primer. For each extracted DNA, real-time PCR experiments were carried out three times with the threshold cycle (Ct) determined in triplicate. The real-time PCR program consisted of 1.5 min at 50 °C for carryover prevention, 15 min at 95 °C for enzyme activation, followed by 35 cycles of 15 s at 95 °C, 30 s at 55 °C and 30 s at 72 °C, when the fluorescence signal was measured. PCR amplification procedure was checked with a heat dissociation protocol (from 70 °C to 100 °C) after the final cycle of the PCR. Quantification of the DNA copy number was performed on a iQ5 thermocycler using iQ5-Cycler software (Bio-Rad, Munich, Germany).

The standard curve was generated by using a plasmid recombinant containing one copy of 16S rRNA fragment. The curve was drawn by plotting the Ct value as a function of the log of the copy number of 10-fold dilution serial of plasmid DNA. As Ct values may vary slightly between experiments, parallel sets of the three dilution series of pure standard DNA were run in all experiments. The relationship between Ct and the target gene copy number, and the copy numbers of the real-time standard were calculated as described by Quian et al. (2007).

2.5. Data analyses

All biochemical results are the means of 12 replicates (three per plot). Data of the real-time PCR represent the average of three independent soil-extracts replicates. Data were subjected to analysis of variance using the program STATISTICA (StatSoft Inc., Tulsa, Oklahoma, USA) and Duncan's Multiple Range Test was used to separate the means with an overall significance level of 0.05. Sun-ray plots (Dilly and Blume, 1998) were constructed to show graphically the enzyme activities of the different treatments. The star shape for each one allows a comparison of visual and numeric presentations of multivariate data and it has been proposed as an integrated fingerprinting for assessing microbial functional diversity (Nannipieri et al., 2002).

The DGGE band patterns in different lanes were compared with the UVImap Analysis software (UVitec Limited, Cambridge, UK). The lanes were normalized to contain the same amount of total signal after background subtraction and the gel images were straightened and aligned to give a densitometric curve. Band positions were converted to Rf values between 0 and 1, and profile similarity was calculated by determining Nei and Li's similarity coefficients (Nei and Li, 1979) for the total number of lane patterns from the DGGE gel. The similarity coefficients calculated were then used to construct a dendrogram using the unweighted pair-group method with arithmetical averages (UPGMA).

DGGE banding data were used to estimate two diversity indices by treating each band as an individual operational taxonomic unit (OTU). For these analyses, each band was presumed to represent the ability of that bacterial species to be amplified (Ibekwe and Grieve, 2004) and the intensity of the bands was reflected as peak heights in the densitometric curve. The Shannon index of general diversity H' (Shannon and Weaver, 1963) was calculated from the following equation:

$$H' = -\sum(\mathrm{Pi}\log \mathrm{Pi})$$

where Pi = ni/N; ni = height of peak, N = sum of all peak heights in the curve.

To test whether similarities observed within and between samples were stronger or weaker than would be expected by chance, band-matching data was stored as a binary matrix and analysed using Raup and Crick's probability-based index of similarity S_{RC} (Raup and Crick, 1979). The S_{RC} is the probability that the randomized similarity would be greater than or equal to the observed similarity, and S_{RC} values above 0.95 or below 0.05 signify similarity or dissimilarity, respectively, which are not random assortments of the same species (bands or OTUs) (Rowan et al., 2003). The S_{RC} and cluster analyses were calculated using the PAST (Palaeontological statistics, version 1.82b) program (Hammer et al., 2001).

3. Results

3.1. Soil organic carbon and enzymes activities

The treatments T, CH, and CM provided significantly more soil organic carbon concentration than did NT (Table 1).

Fig. 1 shows the sun-ray diagrams plotting the assayed enzyme activities along different radial axes. No differences in the soil dehydrogenase activity were detected between T, CH and CM treatments (5.9, 6.1 and 7.4 μg INTF $g^{-1}h^{-1}$, respectively), whereas NC provoked a 3-fold decrease in the dehydrogenase activity. Meanwhile, *o*-diphenoloxidase was higher in CH and CM, but no difference between the latter and T or NC was detected. The highest β-glucosidase activity was recorded in the CM treatment (1087 μg PNP g^{-1} h^{-1}.) The replacement of the mower by the herbicides in CH significantly decreased ($P \leq 0.05$) the activity to 519 μg PNP g^{-1} h^{-1}. The T and NC treatments decreased the activity at the same magnitude. The rest of hydrolytic enzymes (phosphatase, urease and arylsulphatase) followed the same trend, i.e. highest and similar ($P \leq 0.05$) values when cover crops were used.

3.2. Bacterial population size and community structure

A real-time PCR standard curve was generated for bacterial 16S rRNA quantification. Serially diluted DNA originating from recombinant plasmid including 16S rRNA fragment showed the single expected amplicon of 193 bp. The equation describing the relationship between Ct and the log number of 16S rRNA copies was $Ct = -3329 \times \log(16S\ rRNA) + 48{,}059$, $R^2 = 0{,}997$. Target molecules were linear from 10^4 to 10^9 copies. The soils under cover crops showed the highest median density of bacterial copies per gram (Table 1). Comparison of the T and NC treatments revealed a significant decrease ($P \leq 0.05$) of the number of 16S rRNA copies.

Table 1
Soil organic carbon, bacterial population number and Shannon diversity index values for DGGE profiles for tillage (T), non-tillage and no-cover (NC), cover crop + herbicides (CH), and cover crops + mower (CM) soils. Mean values corresponding to four plots for each treatment ± standard error. For each column, significant differences are indicated by different letters ($P \leq 0.05$).

	Organic carbon (g C × kg $soil^{-1}$)	Bacterial population number (copy numbers × g $soil^{-1}$)	Shannon diversity index values
T	9.94 ± 1.37 a	$2.33 \times 10^{+08} \pm 1.11 \times 10^{+07}$ b	2.999
NC	5.36 ± 0.62 b	$3.22 \times 10^{+07} \pm 1.54 \times 10^{+06}$ c	2.815
CH	8.32 ± 1.78 a	$1.37 \times 10^{+09} \pm 1.30 \times 10^{+08}$ a	3.412
CM	9.91 ± 1.56 a	$9.52 \times 10^{+08} \pm 7.47 \times 10^{+07}$ a	2.933

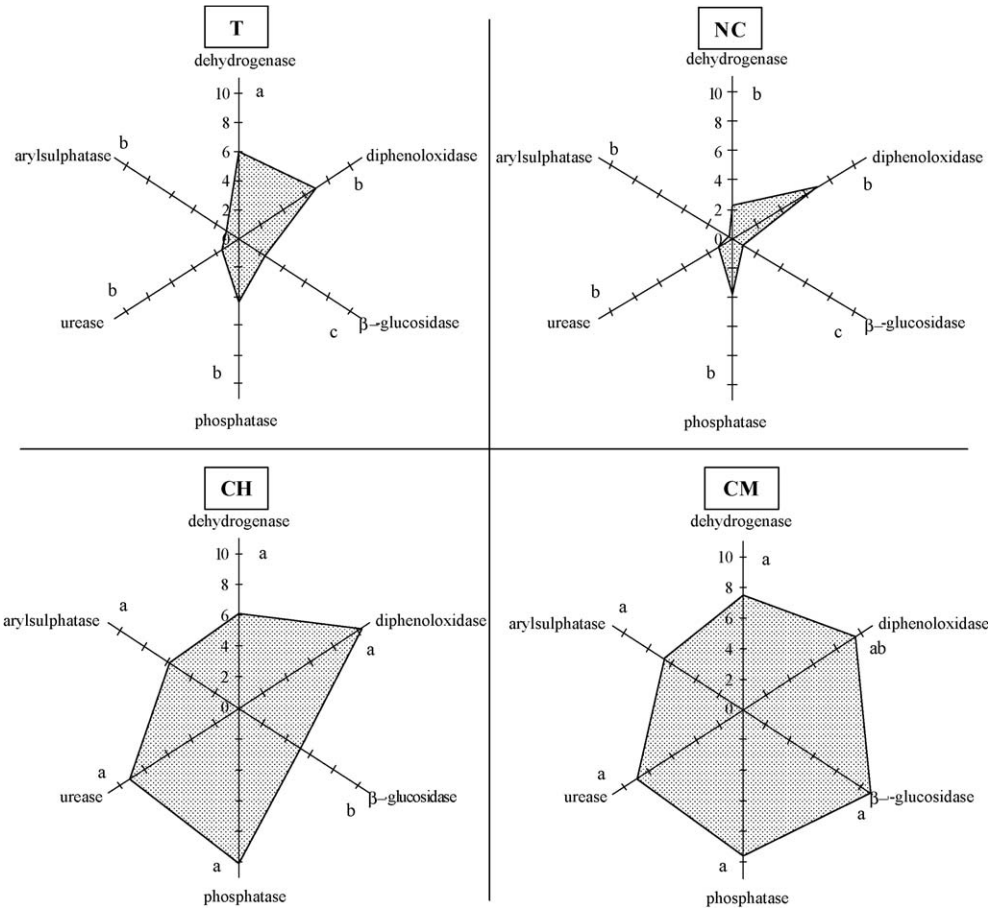


Fig. 1. Enzyme activities for tillage (T), non-tillage and no-cover (NC), cover crop + herbicides (CH), and cover crops + mower (CM) soils. Mean values corresponding to four plots for each treatment are presented for dehydrogenase (μg INTF g^{-1} h^{-1}) × 10, *o*-diphenol oxidase (μmol catechol 10 min^{-1} g^{-1})/1.5, β-glucosidase (μg PNP g^{-1} h^{-1})/100, phosphatase (μg PNP g^{-1} h^{-1})/100, urease (μg NH_4 g^{-1} h^{-1}) and arylsulphatase (μg PNP g^{-1} h^{-1}). For each enzyme activity, significant differences are indicated by different letters ($P \leq 0.05$).

Comparison of the bacterial communities from T, NC, CH, and CM showed markedly different profiles across the DGGE gel (Fig. 2). The generated UPGMA dendrogram evidenced two clear groups of soils (Fig. 3a). The first one was composed by T and NC, which clustered together with a homology coefficient of 50%. The second group (38% homology) included CH and CM treatments. The diversity of the bacterial community was also examined by the Shannon (H') diversity index. NC and CH displayed the lowest and the highest H' from the DGGE banding patterns, respectively, while T and CM showed similar H' values (Table 1).

The S_{RC} values found by comparing the four treatments are summarized in Fig. 3b. Similarities within replicas were significant in all by-products ($S_{RC} = 1$ data not shown). NC was significantly dissimilar to CH and CM ($S_{RC} < 0.05$). For the rest of pair compared DGGE profiles, the similarity was no greater than expected by chance ($0.95A > S_{RC} > 0.05$). Fig. 3b also shows the dendrogram generated by the Raup and Crick cluster analyses.

4. Discussion

4.1. Soil enzymes activities

Dehydrogenase has been considered to be a measure of the total oxidative activity of the soil microflora and therefore of the metabolic activity of soil (Nannipieri et al., 2002). In our experiment, dehydrogenase activity did not differ between permanent arable land and plant-covered soils. Non-tillage following herbicides application lowered the activity of this enzyme in the soil. Evidence of the effect of herbicides on the soil dehydrogenase has been widely reported (Reinecke et al., 2002; Benitez et al., 2006; Garcia-Ruiz et al., 2008), but, since herbicides are not designed to inhibit soil enzymes (Speir and Ross, 2002), direct inhibition seems to be doubtful. Soils with higher levels of plant remains show greater dehydrogenase activity than do uncovered soils (Kremer and Li, 2003), this being related mainly to the additional supply of labile C to soil, which can stimulate biochemical activity (Carpenter-Boggs et al., 2000). In agreement with this, NC did not produce any biomass as the surface was kept vegetation free with herbicides (Castro et al., 2008), and a straight relation was found between SOC levels and dehydrogenase activity.

Organic matter decomposition in soil depends on multi-component enzyme systems, in which a series of inducible enzymes act in cascade (Sinsabaugh et al., 1991). In our study, hydrolytic enzymes involved in biochemical cycles of carbon, nitrogen, phosphorus, and sulphur have been chosen to indirectly determine the impact of cover crops on nutrient release and thus on soil quality.

The β-glucosidase activity was significantly higher in covered soils, but a difference between herbicide treated/non-treated soils was noted, showing that β-glucosidase was the most sensitive enzyme to a long-term herbicide management. Previous studies show the sensitivity of the β-glucosidase enzyme to herbicides, because of a reduction in activity of the stabilized extracellular fraction rather than that associated with viable microbial population (Knight and Dick, 2004). Nevertheless, other authors

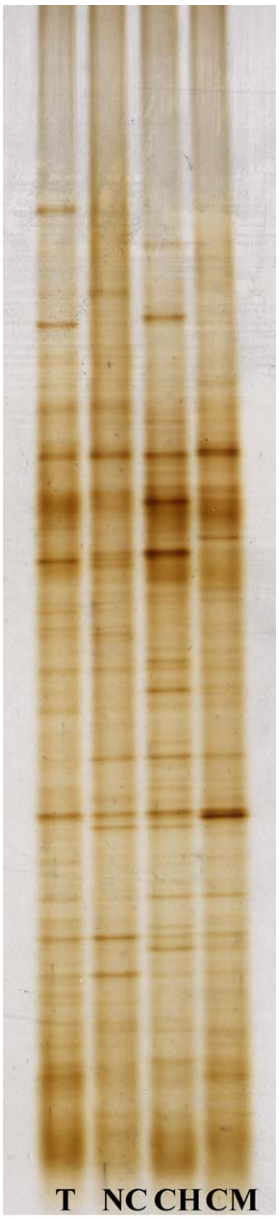
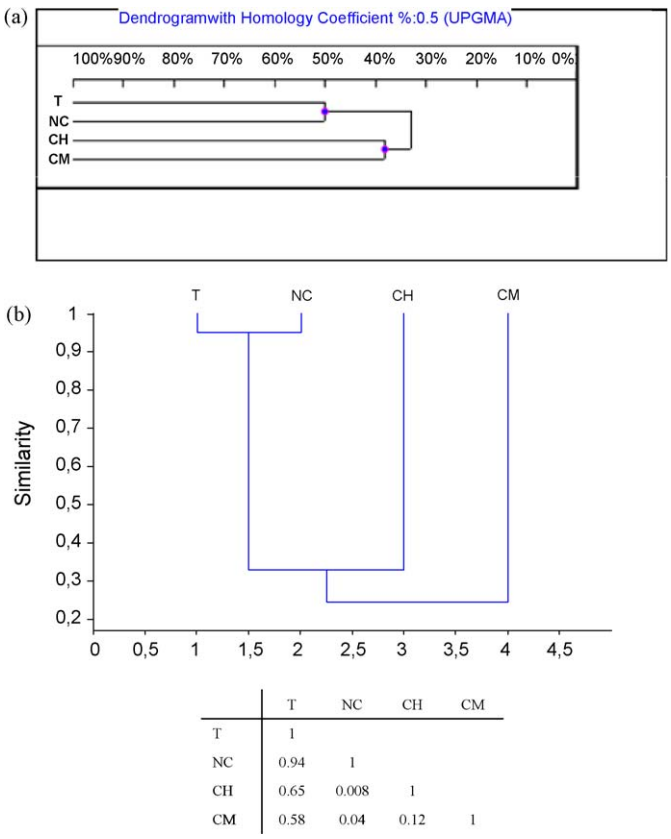


Fig. 2. DGGE profiles for tillage (T), non-tillage and no-cover (NC), cover crop + herbicides (CH), and cover crops + mower (CM) soils.



	T	NC	CH	CM
T	1			
NC	0.94	1		
CH	0.65	0.008	1	
CM	0.58	0.04	0.12	1

Fig. 3. (a) Nei and Li's similarity coefficients (UPGMA dendrogram, tolerance 0.5%) and (b) Raup and Crick probability-based index of similarity cluster analyses, similarity values (S_{RC}) between samples for DGGE profiles for tillage (T), non-tillage and no-cover (NC), cover crop + herbicides (CH), and cover crops + mower (CM) soils.

indicate that general soil microbial properties, including those involving C transformations, are not sensitive to detect the effects of glyphosate on soil microbial activity (Means et al., 2007). The results of our study seem to agree with the first hypothesis, since herbicides decreased the β-glucosidase activity and no changes in the overall microbial activity were detected. This last, estimated as dehydrogenase activity, only decreased when soils where permanently uncovered.

The *o*-diphenoloxidase, phosphatase, urease, and arylsulphatase activities showed similar patterns of behaviour, responding similarly to the management and land-use systems, i.e. significantly higher in covered soils, providing the evidence that cover crops increase the nutrient release of N, P, and S from decomposing plant remains (Rosolem et al., 2002; Ha et al., 2008).

Sun-ray plots graphically show the enzyme activities set for uncovered and covered soils. In these diagrams, different patterns of enzyme activity are indicated by different shapes. The shapes showed different functional diversity, depending on the management. The ranking, from the higher to the lower in terms of functional diversity, was: CM > CH > T > NC. Evidence for the effect of the cover crops and the use of herbicides on soil microbiological parameters was widespread (Tu et al., 2006; Abraham and Chudek, 2008). Cover crop plus mechanical control of weeds characterized the soils with the higher functional microbial diversity, which decreased when weeds were eliminated once a year with glyphosate. The latter finding disagrees with other experimental studies reporting increased soil-microbial activity due to herbicides (Araujo et al., 2003; Moreno et al., 2007). Our study reveals that the effect of the cover crop predominated over soil functional diversity. The absence of cover crops, due to mechanical or chemical weed suppression, decreased the soil functional diversity, the former more strongly than the latter.

4.2. Bacterial population size and community structure

The use of glyphosate once a year did not affect the number of soil bacteria, in agreement with the findings of several authors (Haney et al., 2000; Lupwayi et al., 2009), while soils under cover crops presented the highest bacteria population, particularly under CH management. Nevertheless, uncovered soil management hardly reduced the bacterial biomass. Once more, the weight of cover crop management was higher than the use of herbicides when microbiological parameters were considered.

Bacterial DNA profiles provided by DGGE can be used as a semi-quantitative measure of bacterial diversity (Dilly et al., 2004). Although the real number of genotypes is surely underestimated, the patterns discerned in our study indicate that the structure and diversity of bacterial communities changed significantly with the soil management. The results indicated that presence/absence of cover

crops was the main factor responsible for the differences in the soil-bacterial community structure. In fact, the cluster analysis (UPGMA, Nei and Li coefficient of similarity) of molecular banding patterns generated by PCR–DGGE discriminated the soils into two clear groups; covered and non-covered soils, within a range of similarity of 50%. The DGGE profiles evidenced a small number of strong signals and a large number of weak bands. This would imply that a limited number of dominant, ubiquitous, and ecologically well-adapted bacterial types, along with several equally abundant populations, were the common pattern of each soil (Crecchio et al., 2004).

The Shannon index of general diversity H' indicated that soils where any weed were eliminated with chemicals, permanently or once a year, exhibited the lowest and the highest bacterial diversity, respectively. On the other hand, no differences between T and CM bacterial diversity were observed. The last pattern has also been described by other authors when comparing organic from other land-management systems (conventional, continuous removal of vegetation, undisturbed lands), concluding that soils may exhibit similar bacterial diversity even though they differ in genetic composition (Wu et al., 2008). Therefore, the above assumptions must be considered carefully because most diversity indices do not reflect differences in species richness, species evenness, or simply sampling differences (Gotelli and Colwell, 2001). To test whether the similarities observed within and between samples were stronger or weaker than would be expected by chance, band-matching data was analysed using Raup and Crick's probability-based index of similarity S_{RC}. Values of less than 0.05, which denotes differences among microbial communities and not random assortments of the same OTUs or bands (Rowan et al., 2003; van der Gast et al., 2005), were found between NC and covered soils, but not between the first and T management. Again, the Raup and Crick cluster analyses grouped the non-covered soils, but, in this case, those covered did not cluster together. The results also indicated that soils under cover were phylogenetically distant from the rest.

It is well-known that bacterial community structure and diversity are strongly influenced by plant type (Greyston et al., 2001; Marschner et al., 2001), root zone effects (Yang and Crowley, 2000) and, particularly, by soil type (Girvan et al., 2003). In our study, these effects on community attributes were removed since similar cover crops and soils were investigated, and bulk soil instead of rhizosphere soil was examined.

5. Conclusions

In this study, four long-term approaches to olive–orchard management, categorized by the presence/absence of two main factors – cover crops and chemical weed control – were evaluated using biological indicators of soil quality.

Covered soils exhibited greater bacterial biomass and diversity, as well as higher microbial functional diversity than non-covered soils. The elimination of weeds with herbicides reduced the microbial functional diversity in covered soil but did not affect the other microbiological parameters, revealing that the effect of the cover crop predominated. When non-covered soil was considered, the lowest values of microbial activity and diversity were found when weeds were controlled with chemical methods.

Since these biological indicators are believed to be essential for the sustainability of low-input and organic-farming systems (Biederbeck et al., 2005), the results of this study reveal that covered soils are the best option when olive orchards are managed under rainfed conditions.

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