Soil microbial dynamics and genetic diversity in soil under monoculture wheat grown in different long-term management systems

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Abstract

Organic matter incorporation into soil can increase nutrient availability to plants but it can affect soil microbial communities. These in turn influence soil fertility and plant growth. Soil biochemical and microbiological properties are indicators of soil quality, but there is still no consensus as to how these should be used. Recent developments in molecular biology have provided new tools to obtain a view of the whole microbial community. The long-term impact of crop residue management on the microbial biomass, and on the activity and community structure of soil bacteria was evaluated in a clay soil of Southern Italy, where a monoculture of durum wheat (\textit{Triticum durum} Desf.) was grown in semi-arid conditions, and burning or incorporation of post harvest plant residues were typical practices. The role of N-mineral fertilization, simultaneously with the ploughing in of crop residues and during the plant growth cycle was also investigated. Total bacterial counts of viable cells, biomass C, ATP content of soil microorganisms, genetic fingerprinting of the total eubacterial community and of ammonia oxidizers were evaluated. Burning and incorporation did not affect microbial biomass C, ATP content, and total bacterial counts of viable cells although statistically relevant changes were detected among rhizosphere and bulk soil samples regardless of the crop residue management used. Molecular fingerprinting confirmed that: no significant change in the composition and diversity of total bacteria, as well as of ammonia oxidizers was induced by the crop residue managements; that soil bacteria were more sensitive to N fertilizer application during the plant growth cycle; and that rhizosphere soil samples were significantly different from those of the bulk soil. As microbiological and genetic factors related to soil fertility were not affected significantly, the long-term incorporation of crop residues, under the field conditions investigated, is a sustainable practice to manage post-harvest residues.

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1. Introduction

Traditionally, soil quality has been directly related to its productivity, whereas recently account is taken of the capacity of a soil to sustain biological productivity, environmental quality, and to promote plant and animal health within ecosystem boundaries (Karlen et al., 1997; Doran, 2002).

Soil organic matter (SOM) promotes biological activity, enhances physical and chemical properties, and is a nutrient sink and source (Doran and Parkin, 1994). The mineralization of SOM as a consequence of arable farming is considered to be the major factor in agriculturally induced soil degradation (Paustian et al., 1997) because it affects the soil physical, chemical and biological properties. Thus any system of sustainable agriculture focuses on a goal to maintain and to improve the SOM content (Gregorich et al., 1994).

Organic and inorganic fertiliser and amendments are used to increase nutrient availability to plants. Organic matter can be added to soil by incorporating plant material, animal residues, manure, sewage sludge or municipal waste. On the other hand, agricultural management practices can also affect soil microbial communities (MCs). Changes in microbial activities, and the composition of soil MCs can in turn influence soil fertility and plant growth by increasing nutrient availability and turnover,
disease incidence or disease suppression (Pankhurst et al., 2005). Understanding the response of soil microbiota to agricultural management over time helps to evaluate whether the practices maintain and improve soil quality. Unfortunately, the quantitative and qualitative description of MCs is one of the most difficult tasks facing microbial ecologists. Several microbiological methods have been used to study microbial diversity in agricultural soils (Turco et al., 1994), although most of our knowledge of MCs is based on the estimated 1–10% of total community, which has to date been cultured (Torsvik et al., 1998). Recent developments in molecular biology and biochemical assays have provided new tools to obtain a view of the whole MC. Of the various genetic approaches, the amplification by polymerase chain reaction (PCR) of conserved and variable regions of the 16S ribosomal RNA gene in combination with thermal/denaturing gradient or non-denaturing gel electrophoresis, is undoubtedly the most widely used to estimate the structure and the diversity of MCs including the vast portion that is not culturable (Amann and Ludwig, 2000; Ogram, 2000; Morris et al., 2002; Devereux and Wilkinson, 2004; van Elsas and Boersma, 2004; Muyzer et al., 2000).

The effects of soil improvement treatments on the MC have been extensively investigated by microbiological, biochemical, and molecular approaches. Temperature gradient gel electrophoresis analysis of 16S rRNA gene fragments, amplified with primers targeting eubacteria and ammonia-oxidizing bacteria, was used to analyse changes in bacterial community profiles of an upland pasture following short-term addition of sewage sludge and or lime (Gray et al., 2003). The residual effects on humic acids, enzymatic activities, basal respiration and microbial biomass, 9 and 36 months after sewage sludge amendments, were investigated by Garcia-Gil et al. (2004). The long-term effects of continued applications of sewage sludge on the microbial biomass carbon (C) and nitrogen (N), basal respiration, metabolic quotient, enzymatic activity and MC structure and activity were studied by Marschner et al. (2003) and Fernandes et al. (2005). Physical and chemical properties (texture, pH, organic C, and total N), enzymatic activities and 16S fingerprinting were evaluated in a 6-year trial with amendments of municipal solid waste compost (Crecchio et al., 2004). Autotrophic ammonia-oxidizing bacteria, which carry out the first and rate limiting step of nitrification, namely, the oxidation of ammonia to nitrate, have been investigated by microbiological and molecular approaches in long-term cultivated plots where conventional tillage, treatment with herbicides and insecticides, and fertilization with ammonium nitrate was used (Phillips et al., 2000). Tiquia et al. (2002) determined the effects of mulching and fertilization on soil nutrients, microbial activity and rhizosphere bacterial community structure.

The increasing adoption of conservation tillage, which allows crop residues to remain on the soil surface minimizing soil disturbance, compared with intensive conventional tillage has been widely investigated by community-level physiological profiling (Feng et al., 2003; Bucher and Lanyon, 2005), by soil organic C and N contents and crop yields (Al-Kaisi et al., 2005), and by soil enzyme activities (Ekenler and Tabatabai, 2003; Roldán et al., 2005). In many grain-producing areas, post-harvest residues have been historically burned despite concerns over higher C emissions and accelerated losses of SOM (Biederbeck et al., 1980). Incorporation of residues is another common practice, despite the fact that potentially it could create problems for cultivation equipment (Dickey et al., 1994) and promote the proliferation of residues-borne crop diseases (Cook and Haghlu, 1991; Jenkinson et al., 1995). Recent agricultural policies of the European Community recommend that farmers adopt residues management practices to enhance sustainable agriculture. The effects of crop residue management (burning, bailing and removing, and incorporating residues) on soil chemical, biochemical, and microbiological properties have been recently investigated (Cookson et al., 1998; Bending et al., 2002; Pankhurst et al., 2002; Spedding et al., 2004; Graham and Haynes, 2005). Unfortunately there is a lack of “consensus” about the indicators that should be selected and employed for the evaluation of the structure and activity of MCs and how these indicators should be applied (Gil-Sores et al., 2005). They have suggested that the international scientific community should seek to improve and standardize the analytical methods and to compile databases of biochemical properties. Also the investigations by Cavigelli et al. (2005), suggest significant naturally occurring landscape level variations in microbial activity, size, and community structure. Thus it is clear that this topic needs further investigation using soil with different cropping and management systems.

The objective of this study was therefore to investigate how differences in crop residue management (burning and incorporation of durum wheat crop residues) and fertiliser application (NH4+-N) in a long-term trial, have affected microbial biomass, activity, and the community structure of soil bacteria. Different approaches (total bacterial counting, microbial biomass, ATP content, and genetic fingerprinting) were used in this study.

2. Materials and methods

2.1. Site description and experimental design

The study was carried out on a clay soil (Typic Chromoxerert, USDA, Soil Taxonomy; pH 8.3; clay, 49%; silt, 31%; sand, 20%) at the experimental farm of the Istituto Sperimentale Agronomico (CRA-MiPAF) located near Foggia, in Southern Italy.

According to the UNESCO-FAO classification, the weather is thermo Mediterranean to semi-arid with an average annual rainfall of 625 mm. During the 25-yr trial, the annual rainfall was often 80 to 230 mm below average, and the temperatures were 1 to 18 °C above average.
A field trial was initiated in 1978 to investigate the management of durum wheat (Triticum durum Desf.) crop residues. Randomised blocks (28 m²) with three replicates were subjected to: Incorporation (I), in which all post-harvest residues were mulched and incorporated by ploughing; incorporation and mineral N fertilization (I+N), e.g., 100 kg urea ha⁻¹ applied at the time of incorporation; Incorporation with no N fertilization (I–N), e.g., incorporation of crop residues without the application of mineral fertilizers at the time of incorporation nor during the plant growth cycle; and Burning (B) in which post-harvest residues were burned followed by ploughing. I, I+N, B plots were fertilized with 100 kg NH₄NO₃ ha⁻¹, according to conventional practices, during the plant growth cycle, specifically at the 5th–6th leaf stage. No irrigation was applied to mimic the field conditions of the area. A summary of the residue management and of N fertilization is reported in Table 1.

Bulk soil samples (4 × 7 cm dia. cores per plot, mixed on site) were randomly collected from the surface layer (0–20 cm) in May 2003, 25-yr after the beginning of the trial. Rhizosphere soil samples were obtained by sonication of 2–3 roots for 30 s in sterile phosphate buffer saline (PBS), consisting of 3.56 g Na₂HPO₄ l⁻¹, 1.13 g NaH₂PO₄ l⁻¹, and 7.91 g NaCl l⁻¹ at pH 7.3. Soil pellets were obtained by centrifugation for 10 min at 5000 g, 4 °C; each root sample was sonicated three times and soil pellets were combined. Bulk and rhizospheric samples were collected 4 weeks before harvesting (i.e., about 10 months after crop residues management was applied) and analysed soon afterwards, or stored at 4 °C and analysed within 1 month. Water content of collected soil samples was 40% of water holding capacity, a value that remained constant throughout the storage period.

### 2.2. Total bacterial counts

Soil microorganisms were extracted by shaking 2 g of soil in 20 ml of one-quarter strength Ringer solution (Oxoid). After 10-fold serial dilutions in sterile Ringer solution, suspensions (100 μl) were plated out on Tryptic Soy Broth (TSB) containing 1.5% agar supplemented with cycloheximide (100 μg ml⁻¹) (SIGMA). The plates were incubated at 28 °C in the dark and colonies were counted after 2 days. Data from triplicate readings were expressed as colony forming units (CFU) g⁻¹ dry soil.

<table>
<thead>
<tr>
<th>Plots</th>
<th>Residue management</th>
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<td>B</td>
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<td>I</td>
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<td>I–N</td>
<td>Incorporation</td>
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*No irrigation during ploughing in of crop residues.

100 kg urea ha⁻¹ during incorporation of crop residues.

100 kg NH₄NO₃ ha⁻¹, at the 5th-6th leaf stage of plant growth cycle.

### 2.3. Microbial adenosine triphosphate (ATP)

Microbial ATP was extracted from each soil sample according to Webster et al. (1984). Determinations of ATP contents were measured with the Quantitative ATP Monitoring Kit for free ATP 6410400 (Thermo Labsystems) and with the luciferin-luciferase assay system (Bio-orbit 1253 Luminometer Bio orbitO y, Turku, Finland). An internal standard procedure, as suggested by Ciardi and Nannipieri (1990), was used in order to express the results as ng ATP g⁻¹ dry soil.

### 2.4. Microbial biomass C

Biomass C was measured by the fumigation-extraction method (Vance et al., 1987). Moist soil (50 g dry weight) was split into two half samples. The unfumigated control was immediately extracted with 100 ml 0.5 M K₂SO₄ (1:4 v:w) for 30 min; the second half of each sample, equivalent to 25 g of oven-dry soil, was fumigated in the dark for 24 h at 25 °C with ethanol-free chloroform. After fumigation, CHCl₃ was removed and the soil samples were than extracted with 0.5 M K₂SO₄ as mentioned for the unfumigated sample. Organic C in the soil extract was measured by the K₂Cr₂O₇ acid oxidation-titration method.

### 2.5. Extraction and purification of nucleic acids

A bead beater system consisting of a FastPrep Instrument working with FastDNA SPIN Kit and FastRNA Pro Soil-Direct kit (BIO 101 Systems, Q-BIO gene) was used as a direct method for the extraction from soil of DNA and RNA, respectively. Samples of 500 mg of soil were processed according to the manufacturer’s instructions. Aliquots of DNA and RNA were analysed on 0.7% agarose gel containing 0.5 μg ml⁻¹ of ethidium bromide and quantified spectrophotometrically.

### 2.6. Reverse transcription of total RNA

Extracted RNA was retro-transcribed by Strata Script Two-Tube RT-PCR System (Stratagene). A 30-min incubation for the first-strand cDNA synthesis at 42 °C followed the denaturation of the RNA template at 65 °C for 5 min. The reaction mixture contained: 1 μl of RNA (corresponding to 1/100 of total RNA extracted from 500 mg soil, as described above), 1 × Strata Script RT buffer, 60 ng random primer, 4 mM dNTPs and 20 U Strata Script Reverse Transcriptase added after the denaturation at 65 °C, in a final volume of 10 μl. Then cDNA was further amplified according to the protocol described below.

### 2.7. Polymerase chain reaction

Soil extracted nucleic acids were amplified in a PCR Sprint thermocycler (Hybaid, Ashford, UK) with two
different set of primers purchased by MWG-Biotech (Edersberg, Germany). 968F-1401R eubacterial universal primers were used to amplify a 500 bp region of the 16S rDNA (Heuer and Smalla, 1997). A GC-clamp was added to the forward primer to improve electrophoretic separation of amplicons (Muyzer et al., 1993). PCR amplicons were analysed by denaturing gradient gel electrophoresis (DGGE). DNA fragments of about 1.2 kb relative to the ammonia oxidizer 16S rDNA were amplified by 141F-1314R primers (Smit et al., 1997), digested by Rsal or Taq I and analysed by amplified ribosomal DNA restriction analysis (ARDRA). Each PCR mixture contained 100 ng of DNA template or 1/10 of the retro transcript and analysed by amplified ribosomal DNA restriction analysis (ARDRA). Each PCR mixture contained 100 ng of DNA template or 1/10 of the retro transcript described above, 1 x reaction buffer implemented with 2.5 mM MgCl2, 50 pmol of each primer, 0.2 mM of each dNTP, 3 Units Taq-polimerase (Euroclone) in a final volume of 50 µl. Bovine serum albumin (BSA) (4 µg) was added to minimize any inhibition of amplification by organic compounds co-extracted from soil. The PCR protocol for the 16S rDNA region targeting total bacteria consisted of 3 min at 95 °C followed by 40 cycles each one consisting of a denaturing step (10 s at 95 °C), primer annealing (20 s at 54 °C) and an extension step (40 s at 72 °C); a final extension step (10 min at 72 °C) was finally carried out. Amplification products together with a Low Range ladder (1000–80 bp—MBI Fermentas) were checked by electrophoresis on ethidium bromide stained 1.5% agarose gel run at 10 V cm−1 in 0.5 x TBE buffer. The PCR protocol of DNA fragments relative to the ammonia oxidizers consisted of 3 min at 95 °C; 10 “touch-down” cycles consisting of 95 °C for 10 s, 64–54 °C for 30 s decreasing the temperature by 1 °C every cycle, 72 °C for 2 min; 30 cycles consisting of 95 °C for 10 s, 54 °C for 30 s, 72 °C for 2 min; and a final extension step of 10 min at 72 °C. Amplification products were checked with a Mix Range ladder (10,000–80 bp—MBI Fermentas) by electrophoresis on ethidium bromide stained 1% agarose gel run at 10 V cm−1 in 0.5 x TBE buffer.

2.8. Denaturing gradient gel electrophoresis (DGGE)

DGGE was carried out using the Bio-Rad Dcode system. PCR products (30 µl) were loaded into 6% polyacrylamide gel (acrylamide: bisacrylamide, 37:1) with a parallel gradient of 40% urea formamide on the top and 60% at the bottom of the gel (100% urea-formamide corresponded to 7 M urea and 40% v/v formamide) and run for 16 h at 5 V cm−1 at 60 °C in 1 x TAE buffer. SYBER green (Invitrogen) stained gels were recorded with a Bio-Rad Gel Doc 2000 system.

2.9. Amplified ribosomal DNA restriction analysis (ARDRA)

Aliquots (15 µl) of the amplified rDNA products were digested at 37 °C for 4 h with 5 Units of restriction enzymes (Rsa I, Taq I). Restricted DNA was analysed by horizontal electrophoresis with 16 x 16-cm 2% agarose gels run for 4 h at 150 V in 0.5 x TBE buffer. Ethidium bromide stained gels were recorded with a Bio-Rad Gel Doc 2000 system.

2.10. Statistical analysis

Two-way ANOVA with replicates was used to determine significant differences in ATP, biomass and CFU values among replicates and within the experimental design. The variations among samples extracted from rhizosphere and from bulk soil, and among rhizosphere or bulk samples extracted from plots under different residue managements were evaluated.

Genetic fingerprints were analysed by using the Quantity One software of the Bio-Rad Gel Doc image analyser system. The similarity of the electrophoretic profiles was evaluated by determining the Dice coefficients of similarity and by obtaining the unweighted pair group method with average linkage (UPGMA) relative clusters.

3. Results

3.1. Effects of crop residues management and N fertilization strategies

As previously reported (Maiorana et al., 1992; Maiorana et al., 2004) the average grain yields, over the long-term period, were comparable when residues were burned (B) or incorporated (I); a slight decrease in yield was observed when N fertilization was simultaneously incorporated with crop residues (I+N); and the worst performance, in terms of grain yields as well as of protein content, was observed when no N fertilization was carried out (I−N). Despite the annual variability of chemical determinations, likely due to environmental conditions (rainfall and temperature, as reported in Section 2.1), the long-term trial increased the N content, significantly when inorganic N was added during the incorporation of crop residues and only slightly when residues where burned. On the other hand, total organic C was not affected by the incorporation procedure or by time. It was, however, decreased significantly by the long-term burning of the crop residues.

3.2. Effects of crop residues management on culturable bacteria

Higher numbers of culturable bacteria, as indicated by the CFU values, were isolated from rhizosphere soil samples than from bulk soil plots (Fig. 1). This was particularly relevant for two treatments (B and I) and only slightly detectable when incorporation occurred with a concomitant mineral N fertilization. The same results occurred when no N was added during crop residue incorporation or added at the 5th–6th leaf stage of plant growth. At least for rhizospheric bacteria, higher numbers were observed in B, I and I+N plots, all characterized by
N fertilization during the plant growth cycle, than in unfertilized plots (I/C0). Two-way ANOVA analysis of CFU data, reported in Table 2, clearly indicated that the differences among rhizosphere and bulk soil samples were statistically relevant (P < 0.01) while the different management, within the rhizosphere or the bulk soil microhabitat, was less relevant (P < 0.05). In particular (Fig. 1), CFU data were almost identical for three of the four treatments and the differences observed were not for the same management systems. A decrease of CFU was detected in I/C0 of the rhizosphere soil plots and an increase for I+N in the bulk soil plots.

3.3. Effects of crop residues management on microbial ATP

Average values of microbial ATP (Fig. 2), and the relative statistical analysis of data (Table 2) clearly indicate that the ATP content of bulk soil samples did not depend on crop residue management or on N fertilization and was only slightly but not significantly higher in I+N than B, I, and I–N rhizosphere soil samples. As for CFU, ATP content was increased by root exudates and plant growth, since a significant difference (P < 0.01) was detected among rhizosphere and bulk soil samples.

3.4. Effects of crop residues management on microbial biomass

As indicated in Fig. 3, data for which were statistically analyzed (Table 2), the microbial biomass was not affected by burning or by the incorporation of crop residues. No effect was also seen by N fertilization in rhizosphere as well as in bulk soil habitats. Once again, the only significant difference (P < 0.001) was the higher biomass values detected in rhizosphere soils compared with the bulk soil samples, although the values for I–N treatment was somewhat less than for the other three.

3.5. Effects of crop residues management on the eubacterial community and ammonia oxidizers

In addition to quantitative determinations of soil bacteria, genetic fingerprinting was also carried out to analyse the structure of the total eubacterial community and of ammonia oxidizers, a taxonomic group very likely to be involved in microbial transformations of NH4+-N fertilization applied to some soil plots.

The cluster analysis of DGGE profiles of PCR-amplified soil DNA targeting eubacterial 16S gene is shown in Fig. 4. Dice coefficients of similarity clearly indicate that the eubacterial community of rhizosphere samples clusters separately from bulk soil sample community (Dice coefficient 0.52). Very high values of coefficients (0.83 to 0.89) indicate that the bulk soil eubacterial structure is not affected by burning or incorporation (with or without N fertilization) while bacteria inhabiting the soil–root interface seem to be affected by the complete absence of N fertilization (I–N) (Dice coefficient 0.62), but are not affected by crop residue management (coefficients of similarity, 0.81–0.88).

This fact was even more clear when metabolically active bacteria were investigated by extracting RNA from soil. In fact, cluster analysis of 16S PCR-DGGE fingerprints, and relative coefficients of similarity (Fig. 5), indicate that rhizosphere soil samples of B, I and I+N plots, as well as

![Fig. 1. Effects of crop residue management and mineral N fertilization on total bacterial counts of rhizosphere and bulk soil plots. Values are the means (n = 3) ± standard errors of the mean (SEM). See Section 2.1 and Table 1 for details on residue management practices.](image-url)
Fig. 2. Effects of crop residue management and mineral N fertilization on microbial ATP content of rhizosphere and bulk soil plots. Values are the means ($n = 3$) ± standard errors of the mean (SEM). See Section 2.1 and Table 1 for details on residue management practices.

Fig. 3. Effects of crop residue management and mineral N fertilization on microbial biomass C content of rhizosphere and bulk soil plots. Values are the means ($n = 3$) ± standard errors of the mean (SEM). See Section 2.1 and Table 1 for details on residue management practices.

Fig. 4. Cluster analysis (Unweighted Pair Group Method Average linkage, Dice coefficients of similarity) of eubacterial 16S rRNA Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis profiles. See Section 2.1 and Table 1 for details on residue management practices.
corresponding bulk soil samples clustered together (coefficients of similarity 0.77–0.83 and 0.58–0.67, respectively); while I−N samples from the rhizosphere were more similar to I−N samples from bulk soil (Dice coefficient 0.61) than to B, I, and I+ N from rhizosphere soil; similarly, I−N from bulk soil was less related to B, I, and I+ N from bulk soil. In general, with the slight exception of I−N samples, the electrophoretic profiles relative to rhizosphere soil samples segregated separately from those relative to bulk soil (Fig. 5).

The cluster analysis of ammonia oxidizers bacteria by ARDRA with two restriction enzymes (Fig. 6) confirms the differences among electrophoretic profiles from rhizosphere and from bulk soil samples, although the differences were not particularly relevant (i.e., Fig. 6(a), Dice coefficient 0.81). As for the whole eubacteria, this trophic group did not seem to be affected by crop residues management, as was clearly indicated by the high coefficients of similarity. In this case, there was not any shift in the microbial structure due to N fertilization; in fact, as reported in Fig. 6(a), for example, ammonia oxidizers of non-fertilized plots (I−N) clustered together with samples subjected to N-fertilization (B, I, I+ N) (coefficients of similarity 0.85 and 0.90 for rhizosphere and for bulk soil samples, respectively).

4. Discussion

4.1. Effects of crop residue management on microbial biomass and activity

Our results indicated that, on the basis of data from total bacteria counts, biomass C, and ATP (Figs. 1–3, Table 2) the burning and incorporation of straw residues did not affect microbial biomass and activity. Cookson et al. (1998) detected no significant effect on the population of viable bacteria from cereal crop residue burning, removal or incorporation. Maxima were reached between 60 and 90 d after the treatment, and thereafter declined. As in the present study the soil samples were collected 8–9 months after the last crop residue was burned or incorporated. Thus it is very likely that the numbers of viable bacteria had become stable after that time since these are affected only immediately after the treatment process. Other research has shown that residue management practices can influence size, composition, and the activity of the soil microbial biomass. Gupta et al. (1994) and Ladd et al. (1994) demonstrated that microbial biomass was higher in soils where cereal crop residues were incorporated or direct drilled than where the residues were removed. It has to be stressed that, in our experimental set up (Table 1), the comparison was between burning and incorporation procedures because these are practices currently run by the farmers in the region under investigation. No plot was managed for just the removal of crop residues. Thus the trial did not focus on an untreated control, and that might well give results different from those for the managed plots. That treatment, however, is considered to be very ineffective for the support of a long-term monoculture.

Cookson et al. (1998) reported a residue-specific response to management probably related to differences in the initial chemical composition of plot residues (in
particular, the initial lignin: N ratio). This is an important factor regulating rates of residue decomposition (Swift et al., 1979). Microclimatic conditions are another of the key factors that influence the composition and activity of the decomposer communities that ultimately determine rates of plant residue decomposition and nutrient release. Our investigation was carried in a typical Mediterranean region characterized by limited water resources and low rainfall that was quite often below the average during the trial period. An irrigation plan was not carried out because the trial was designed to mimic the field conditions, and to keep the costs of the trial comparable to those sustained by the farmers of the area. Irrigation would have enhanced microbial activity and plant residue decomposition.

4.2. Effects of crop residue management on molecular fingerprinting of soil nucleic acids

Molecular fingerprinting of soil nucleic acids was also performed in order to investigate in depth the composition of the soil bacterial community and to determine whether traditional approaches (ATP, biomass, and bacterial counts) underestimated the effects of crop residue management. The results reported in Figs. 4–6 confirm that, in the experimental conditions under investigation, there was no significant change in the structure and composition of total bacteria, as well as of ammonia oxidizing communities as a consequence of burning and incorporation of plant residues. Soil bacteria seem to be more sensitive to fertilizer application during the plant growth cycle, according to

Fig. 6. Cluster analysis (Unweighted Pair Group Method Average linkage, Dice coefficients of similarity) of ammonia oxidizers 16S rDNA Polymerase Chain Reaction-Amplified rDNA Restriction Analysis profiles. (a) Taq I restriction enzyme; (b) Rsa I restriction enzyme. See Section 2.1 and Table 1 for details on residue management practices.
Spedding et al. (2004), who demonstrated that the 4th leaf stage fertilization of maize caused broad scale seasonal shifts in MC structure. In our case, these differences, as evidenced by the separate clustering of 1–N samples from the fertilized plots B, I, I+N, were more evident for the eubacterial community studied than for the group of ammonia oxidizers. It seems that N fertilization enhanced a whole group of ammonia oxidizer bacteria within the total bacterial community rather than selecting single species within the same group. Furthermore, the very high values of similarity in Fig. 6 indicate that species within a specific group of microorganisms are more genetically related than bacteria belonging to different groups and confirms that crop residue management does not affect soil bacteria.

The only significant changes detected by the molecular approaches were among rhizosphere and bulk soil samples. That further emphasizes the key role of the rhizodeposition of nutrients for soil microorganisms that occurs, via crop roots, during the plant growing season.

A high degree of similarity between the predominant bacterial populations under different soil improvement treatments is not infrequent (Gray et al., 2003). They detected that most of the differences between populations in soil samples were accounted for by spatial and temporal variations. The relevant effects of organic mulches on the compositions of culturable heterotrophic rhizospheric bacteria and of pseudomonades, detected by terminal restriction fragment length polymorphism of PCR amplified 16S rDNA, were related to bare soil control (Tiquia et al., 2002). On the other hand, despite significant variation in measured nitrification rates among different agricultural treatments, there were no differences in the DGGE banding profiles of ammonia oxidizer populations (Phillips et al., 2000).

5. Conclusions

Multi-approach data sets, such as agronomic indications (crop yields, protein content of wheat grains), soil fertility parameters (C and N content), microbiological determinations (total bacterial counts, biomass, ATP content), molecular characterization (genetic fingerprinting) of eubacteria and of ammonia oxidizers, and world-wide environmental implications (CO₂ emissions by crop residue burning) lead us to conclude that the incorporation of plant residues is a sustainable practice as it does not affect the composition and activity of microbial biomass, while it minimizes the loss of soil fertility as the result of intensive agriculture. In our opinion, that viewpoint is even more relevant as the results reported in the present study refer to a long-term trial under difficult climatic conditions (low rainfall and high temperature) and agronomic strategies (monoculture, and no irrigation during the incorporation of crop residues).

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