Chapter 6
Impact of Innovative Agricultural Practices of Carbon Sequestration on Soil Microbial Community

Valeria Ventorino, Anna De Marco, Olimpia Pepe, Amalia Virzo De Santo, and Giancarlo Moschetti

Abstract This chapter deals with the impact on soil microbiology of innovative management techniques for enhancing carbon sequestration. Within the MESCOSAGR project, the effect of different field treatments was investigated at three experimental sites differing in pedo-climatic characteristics. Several microbiological parameters were evaluated to describe the composition of soil microbial communities involved in the carbon cycle, as well as to assess microbial biomass and activity. Results indicated that both compost and catalyst amendments to field soils under maize or wheat affected microbial dynamics and activities, though without being harmful to microbial communities.

6.1 Microorganisms in Soil

A huge number of microorganisms reside in soil and exert a variety of functions which contribute to ecosystem-level processes and maintenance of primary productivity in terrestrial ecosystems. Growth and metabolism of soil microbes can alter the solubility of soil mineral components and modify soil structure. Moreover, microbes are able to degrade organic compounds and release nutrients, thus regulating nutrients cycling and availability to plants. Microbial activity is responsible for most of soil respiration, thus including oxygen consumption and CO_2 emission, and for immobilization of nutrients in soil microbial biomass. Soil microbes contribute to processes

V. Ventorino • O. Pepe
Dipartimento di Scienza degli Alimenti, sez. Microbiologia Agraria, Università di Napoli Federico II, Naples, Italy

A. De Marco • A. Virzo De Santo
Dipartimento di Biologia Strutturale e Funzionale, Università di Napoli Federico II, Naples, Italy

G. Moschetti (✉)
Dipartimento DEMETRA, Università di Palermo, Palermo, Italy
e-mail: moschett@unipa.it

of carbon sequestration in the soil humic fraction since they transform dead organic matter in such a recalcitrant pool. Furthermore, microbial activity is responsible for other essential biological processes, among which is nitrogen fixation. In the absence of soil microbial life, all biochemical transformation cease and the ecosystem sustainability is endangered (Wani and Lee 1995).

Although microbial C in natural soil does not exceed 1–2% of the total soil C (Paul and Clark 1989), it is constituted by a huge variety of organisms whose taxonomy and diversity are poorly known in comparison to aboveground organisms (Barot et al. 2007). Soil microbial communities are extraordinary complex and have been estimated to contain more than 4,000 different genomic equivalent in a single gram of soil (Torsvik et al. 1990). However, microbial species in soil are poorly abundant, most likely because conditions for their survival and growth are limited to a few sites where specific environmental factors, physical–chemical characteristics, and nutrient availability occur. Soil is a very heterogeneous environment encompassing solid, gaseous, and liquid phases. Microbial processes take place at the scale of soil aggregate, which is essentially a porous structure that varies both spatially and temporally. Because soil organic matter located within soil aggregates is physically protected from biodegradation, aggregates enhance carbon sequestration and soil structural stability (Six et al. 2000). Microbial dynamics is influenced by soil structure and the pore-size distribution within soil aggregates. Bacteria are restricted to grow and feed on the exposed surfaces of organic matter and/or inorganic particles. Fungi penetrate large pieces of organic matter and can thus extend their hyphae for centimeters and even meters in soil. The location of bacteria and fungi influences their activity as well as their survival to predation. The larger size of fungi may make them more vulnerable to predation, whilst small pores provide refuge for bacteria against predators (Six et al. 2006).

Plants are responsible for a large input of organic carbon into soil, thus becoming the main determinant of microbial life in soil through the complex food web of debris. It has been found that the type of aboveground plant community influences the composition of belowground soil microbial community in natural ecosystems (Reynolds et al. 1997; Côté et al. 2000; Smolander and Kitunen 2002; Rutigliano et al. 2004), as well as in semi-natural grasslands (Singh et al. 2009) and in agro-ecosystems (Marschner et al. 2001; Hedlund 2002). Moreover, arbuscular mycorrhizal fungi require a plant host to survive.

Consequently, plants influence the spatial distribution of bacteria and fungi in soil (Kirk et al. 2004). The site of greatest soil activity is the root–soil interface, or rhizosphere. Roots affect soil structure, aeration, and biological activity and deeply impact soil microbial communities in their immediate vicinity, greatly increasing population densities of bacteria and fungi (Buyer et al. 2002; Marschner et al. 2002). As plants may allocate up to 40% of the assimilated carbon belowground, roots are the major source of organic matter into the surrounding soil through both root debris and exudates. Exudates are made up of sugars (50–70%), carboxylic acids (20–30%), and amino acids (10–20%), i.e., carbon-rich substrates that are able to regulate decomposition of recalcitrant soil organic carbon by controlling the activity and relative abundance of fungi and bacteria (Cheng et al. 2003; de Graaf et al. 2010).

Fig. 6.1 Living fungal hyphae observed by fluorescence microscopy after treatment with the viability stain fluorescein diacetate (FDA)
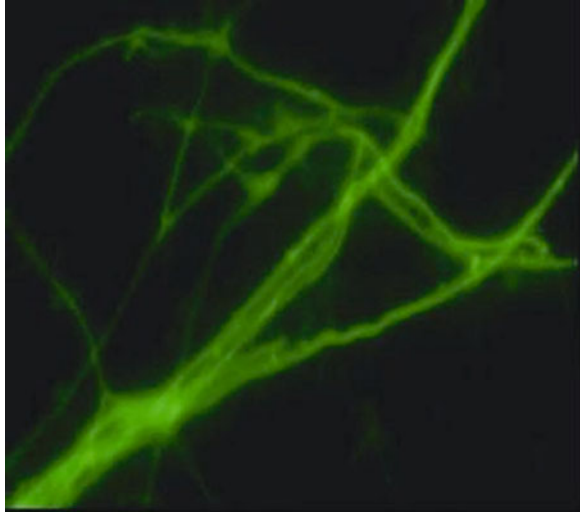
Among soil organisms, actinomycetes, fungi (Fig. 6.1), and bacteria are the most abundant and most metabolically active. Bacteria and fungi generally comprise >90% of the total soil microbial biomass and are responsible for most of soil organic matter decomposition (Six et al. 2006). Fungi incorporate more soil C in their biomass than bacteria, and fungal cell walls are more recalcitrant than bacterial cell walls. Therefore, carbon sequestration may be larger in soils dominated by fungal communities than in those whose communities are dominated by bacteria (Six et al. 2006). Moreover, actinomycetes, fungi, and bacteria include organisms (such as aerobic and anaerobic cellulolytic bacteria), which are able to degrade cellulose and lignin (McCarthy and Williams 1992; Wellington and Toth 1994; Berg and McClaugherty 2008). In fact, degradation of plant biopolymers is the fundamental step in the carbon cycle and this process is important in soil systems. Since plants are the most relevant carbon providers in soil and cellulose and lignin are the most abundant constituents of plant tissues, they consequently represent the largest source of carbon in soil. Microorganisms transform plant polymers into simpler compounds, which are then made available to other microbial populations, and/or are stabilized in humic substances. The mineralization process during metabolic consumption of polymer by-products ultimately produces carbon dioxide that is emitted to the atmosphere. Moreover, actinomycetes regulate the microbial equilibrium in soil through production of antibiotics and probiotics that stimulate microflora and plant growth.

Fungi play a central role in many soil microbiological processes thus influencing the structure and functioning of plant communities and soil ecosystems. Fungi are immensely diversified, both structurally and functionally, and adopt different trophic strategies, since they occur as saprotrophs, symbionts, and pathogens. Individual fungi can often simultaneously colonize different substrates, such as living or dead plant tissues, woody debris, soil animals, and mineral substrates, thus

allowing the transfer of substances. Filamentous fungi are responsible for decomposition of organic matter (e.g., lignin degradation) and nutrient cycling (Parkinson 1994; Van Elsas et al. 2007) and their activity is critical in regulating the availability of nutrients for plant growth. Moreover, fungi are food for nematodes, mites, and other larger soil organisms, which are also predators or parasites of other soil organisms.

6.2 Impact of Agricultural Management on Soil Microbial Communities

Agricultural management produces a disturbance of both abiotic and biotic components of soils. The most negative impact is the loss of soil organic matter (SOM) (Balesdent et al. 1999), with consequent increase in soil erosion and decrease in soil structure stability (Bronick and Lai 2005) and fertility. In agro-ecosystems, soils degradation is the outcome of unsustainable techniques aimed to increase production in the short term without paying attention to the conservation of soil resources. Agricultural land management, such as cropping systems (Kuske et al. 2002) and tillage systems (Peixoto et al. 2006) may affect soil characteristics, including physical, chemical, and biological properties and processes. It has been observed that tillage reduces soil microbial populations (Ibekwe et al. 2002) and different enzymatic activities (Carpenter-Boggs et al. 2003). Tillage has a catastrophic effect on fungi as it physically breaks the hyphae and severely damages the mycelium, thus consequently hampering the stability of soil aggregates whose particles are transiently bound together by fungal hyphae. Six et al. (2006) showed that no-tillage enhances fungal biomass with a consequent quantitative and qualitative SOM improvement that is attributed to the positive influence of fungi on aggregate stabilization.

Alternative agricultural techniques, such as minimum tillage, have been developed to improve soil quality by progressively recovering soil organic matter (Lu et al. 2000). In long-term experiments on tillage comparison along two climatic gradients, Frey et al. (1999) observed that in response to reduced tillage both fungal biomass and fungal/bacterial biomass increased at all sites. Thus, less intensively managed agro-ecosystems, such as those managed with no-tillage practices, more closely resemble natural ecosystems, which are dominated by fungi (Bayley et al. 2002). On the other hand, intensive cultivation leads to progressive SOM depletion with a consequent microbial biomass reduction, loss of microbial diversity and reduction of microbial activities (Bastida et al. 2006). Buckley and Schmidt (2001) performed a large-scale experiment with replicated plots under distinct management regimes ranging from conventionally tilled annual cropping systems to abandoned fields. The effects of tillage, fertilization, and plant community composition on the structure of microbial community were evaluated. They found that microbial communities differed significantly between fields that had never been cultivated and those with a long-term history of cultivation. However, microbial community structure was very similar in plots that shared a long-term history of

cultivation, despite differences in plant community composition, chemical inputs, tillage, and productivity. They argued that microbial communities respond to soil characteristics which require long time periods to recover from disturbance. Indeed, the organic pools of carbon and nitrogen can be depleted by long-term agricultural practices and may require decades or even centuries to recover pre-agricultural levels. In a study dealing with soil quality as related to different land uses in Southern Italy, Marzaioli et al. (2010) report that soil quality, evaluated by a set of parameters including microbial indexes, was strongly and negatively affected by permanent crop management. Moderate grazing activity, as well as crop management comprising mulch cover on soil, had a lower negative impact. Moreover, these authors found that the abandonment of cultivated lands, with consequent development of shrublands, produced an improvement of soil quality, thus suggesting a good recovery capacity.

Microbes are also affected by fertilization (Marschner et al. 2003), both directly and indirectly. Zhong and Cai (2007) showed that the long-term application of P and N indirectly affected microbial parameters in soil by increasing crop yields and promoting SOM accumulation. Fertilizers used in agricultural production systems include mineral (urea, ammonium nitrate, sulfates, and phosphates) and organic (animal manures, biosolids, and composts) fertilizers. Composted materials vary widely in their characteristics such as dry and organic matter content, pH, carbon and nitrogen content, plant residues, and microbial community composition. Application of compost to soil is used to improve soil fertility and structure since it increases the carbon, nitrogen, and phosphorus content in soil (Hartz et al. 2000; Filcheva and Tsadilas 2002; Adediran et al. 2003) and contributes to the stabilization of soil aggregates (Bresson et al. 2001; Barzegar et al. 2002). Although compost amendments differ in origin of material and application rates, organic amendments to soil generally result in an increase of microbial proliferation in soil (Bünemann et al. 2006). In fact, organic-matter-rich amendments are also used to stimulate soil microflora in degraded and arid environments (Ouedraogo et al. 2001; Ros et al. 2003). However, compost amendment can also cause negative effects by altering the microbial biomass, size, function, and diversity, if contaminant residues are present at toxic levels (Gomez 1998; Zheljazkov and Warman 2003). Nevertheless, soil microbial response is generally transient (Calbrix et al. 2007) and microbial characteristics can return to their baseline within a few years (Speir et al. 2003; Garcia Gil et al. 2004) depending on nature of organic amendments and level of compost application (Albiach et al. 2000; Garcia-Gil et al. 2000).

6.3 Microbial Parameters as Indexes of Soil Quality

Because of the fundamental role in mediating soil processes and the responsiveness to soil managements, microbial abundance, diversity, and activity are among the most important soil quality parameters (Andrews and Carroll 2001; Karlen et al. 2001, 2003; Andrews et al. 2003; Anderson and Domsch 2010). In fact, the effect of

agricultural management on microbial community is directly related to changes in soil quality (Schloter et al. 2003), that encompass the size and diversity of specific functional microbial groups (Helgason et al. 1998; Chang et al. 2001).

A great number of methods have been developed to determine the presence and activities of microbial communities in soil. Some of them are internationally standardized (Winding et al. 2005), such as measures of population size for either a single organism type, a functional group, or a whole community. The effect of agricultural managements on soil microorganisms can be measured with changes in both community size (cell number) or microbial biomass, and biological activity, such as soil respiration. However, although addition to soil of good quality compost may increase global microbial biomass and enhance enzyme activity (Albiach et al. 2000; Perucci et al. 2000; Debosz et al. 2002), the specific responses of various bacterial groups to changing environment in agricultural soils are poorly known (Buckley and Schmidt 2001; Kiikkilä et al. 2001; Chander and Joergensen 2002). Moreover, several studies showed that, in order to assess fertilizers' effects, microbial enumeration methods by plate counts (Sarathchandra et al. 1993) and nematode counts (Parfitt et al. 2005) are possibly more sensitive than measurements of microbial biomass.

Fungal and microbial biomass is thought to be a sensitive indicator of soil quality and an early predictor of changes in SOM dynamics. In fact, the rate of microbial fraction turnover is relatively fast (2–6 years) as compared to more than 20 years of SOM turnover (Jenkinson 1990). Thus, fungal and microbial biomass are SOM living components (Jenkinson and Ladd 1981) representing an active soil carbon that is more sensitive to soil management than total organic carbon (Frey et al. 1999; Bayley et al. 2002; Weil and Magdoff 2004; Six et al. 2006). However, microbial biomass C generally reflects the amount of total organic matter content. Both SOM and microbial biomass decline under agricultural or land disturbance, indicating exploitation of organic resources and impact of differing tillage systems, fertilizers, and crop rotations (Luizao et al. 1992; Sparling 1997; Frey et al. 1999; Vineela et al. 2008). Soil respiration is the best indicator of the whole metabolic activity of soil microorganisms, since it allows comparison of different soils and soil management effects (Machulla 2003; Solaiman 2007). Soil respiration, as referred to SOM content to give a coefficient of organic matter mineralization (CEM), may express the potential capacity of soil to accumulate or mineralize carbon (Diaz-Raviñaa et al. 1988).

6.4 Impact of Different Agricultural Practices on Soil Microbial Communities: The Mescosagr Case Study

Within the National project MESCOSAGR, we investigated the impact on soil microorganisms of two innovative technologies applied to sequester carbon in agricultural soils. The hypothesis was that the structure and activity of microbial

communities would be influenced by (1) addition of compost, a humified and hydrophobic material that protects the easily degradable organic fraction, and (2) in situ photo-oxidative polymerization of native SOM under the action of a biomimetic catalyst (CAT) (iron–porphyrin). The effect of these two technologies on soil microorganisms was compared with that exerted by traditional deep tillage and minimum tillage. The latter is an agronomic practice commonly used to reduce SOM depletion and limit CO_2 emission from soil into the atmosphere (see Chap. 3).

Two types of approaches were pursued to estimate soil microbiological parameters (1) one aimed to characterize the composition of soil microbial communities involved into carbon cycle, (2) another one based on a holistic view that considers the microbial biomass as a whole, without distinguishing its individual components. The first approach is based on the Surface Spread Plate Count Method and Pour Plate Method, which use selective media to identify the major microbial groups involved in the different steps of organic matter decomposition, i.e., total aerobic heterotrophic bacteria, cellulolytic bacteria, fungi, and actinomycetes. This approach allows determining the size and composition of microbial communities and has been used to assess changes in the soil biota in response to land management, thereby providing an indicator of soil biological status (Harris and Birch 1992). The second approach is based on the determination of microbial community characteristics which include abundance and activity (1) abundance of fungal cells is measured by fluorescence microscopy; (2) soil microbial biomass is determined as microbial C by the SIR (Substrate Induced Respiration) method; (3) microbial respiration per unit of organic C becomes a coefficient of endogenous SOM mineralization. The combination of these two experimental approaches is expected to provide most information regarding the effects of soil managements on microbial communities.

6.5   Soil Sampling and Microbiological Analyses

The study comprised three different sites (Napoli, Torino, and Piacenza) under different soil and climate conditions. All microbial parameters were assessed in both the bulk soil and the rhizo soil. Soil samples were collected from the experimental plots under either maize or wheat (see Chaps. 3 and 7 for details on the field trials) during three consecutive years (2006–2007–2008). Bulk-soil samples were collected from the 0–15 cm soil layer after maize harvest (September) and before wheat sowing (November). Rhizo-soil samples were collected during stem elongation (April–May for wheat rhizosphere, and July for maize rhizosphere). Bulk-soil samples were a mix of three subsamples collected in three different locations for each treatment plot. Rhizosphere samples consisted of soil adhering to total roots of three crop plants collected from each treatment plot. The roots were shaken vigorously to separate the rhizo soil. All samples were collected in triplicate, brought to laboratories, stored in polyethylene bags at 4°C for no more than 24–48 h before soil microbiological analyses were conducted.

6.5.1 Microbial Counts

Microbial counts were performed according to Italian official methods (Picci and Nannipieri 2003). Briefly, soil samples (10 g) were shaken for 30 min in 90 ml of physiological solution containing 0.162 g of tetrasodium pyrophosphate to detach the bacteria from soil particles. After soil particles were allowed to settle for 15 min, the solution was diluted tenfold in a series. Selected populations of soil microbial community were detected at 28°C by using the Surface Spread Plate Count Method (aerobic bacteria) and the Pour Method (anaerobic bacteria). Three plates were used per each dilution. Total heterotrophic aerobic bacteria were counted in Plate Count Agar (Oxoid Ltd., Oxford, UK). The plates were incubated for 3 days.

Mould and yeast were cultivated on Malt Agar (Oxoid Ltd., Oxford, UK) supplied with chloramphenicol (100 mg L^{-1}) for 3 days (Allievi and Quaroni 2003).

For the isolation of actinomycetes, Starch-Casein Agar (10 g soluble starch, 0.30 g casein, 2 g KNO_3, 2 g NaCl, 2 g K_2HPO_4, 0.01 g $FeSO_4$, 0.05 g $MgSO_4$, 0.02 g $CaCO_3$, 1,000 ml distilled water, 17 g bacteriological agar, pH 7.0) was used (Kuster and Williams 1964). The medium also contained cycloheximide at 100 μg ml^{-1} to minimize fungal contamination. The plates were incubated for 14 days.

The medium used for aerobic and anaerobic cellulolytic bacteria was composed by 5 g L^{-1} carboximethylcellulose (CMC) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), 1 g L^{-1} (NH_4)NO_3, 1 g L^{-1} yeast extract, 50 ml L^{-1} standard salt solution, 1 ml L^{-1} trace elements solution, 15 g L^{-1} bacteriological agar, at pH 7.0. The plates, incubated in aerobic or anaerobic (Oxoid's Anaerogen™ System) (Allievi and Möller 1992) conditions for 7 days, were stained with Congo red (0.1%) for 20 min and bleached with NaCl (5 M) for 20 min to put in evidence cellulolytic activities by developing clear haloes around the colonies (Kluepfel 1988). All microbial counts were carried out in triplicate and microbiological data were expressed as CFU g^{-1} of dry soil.

6.5.2 Active Fungal Mycelium

Metabolically active hyphae were estimated by fluorescence microscopy. Soil samples were sieved through a 2-mm mesh, suspended in a solution (1 g of fresh soil in 100 ml) of phosphate buffer (60 mM, pH 7.5), and homogenized at 6,000 rpm for 2 min. 0.5 ml of suspension were collected and filtered under vacuum on nitrocellulose filter with a pore size of 0.45 μm. The sample was treated with fluorescein diacetate (FDA) (Söderström 1977, 1979). This stain penetrates rapidly in cells and is hydrolyzed to fluorescein by different enzymes such as protease, lipase, and esterase. After clearing by immersion oil, the preparations for active

mycelia were observed at a magnification of $400\times$ and 20 microscopic fields were counted. Active mycelia were estimated by the intersection method (Olson 1950) and their mass calculated on the basis of an average hyphae cross section of 9.3×10^{-6} mm^2, a density of 1.1 g ml^{-1} and a dry mass of 15% of wet mass (Berg and Söderström 1979). The fungal biomass was expressed as mg of fungal biomass per gram of soil dry weight.

6.5.3   Microbial Biomass

Microbial biomass (C_{mic}) was determined by the SIR method (Anderson and Domsch 1978) that is based on the measurement of CO_2 evolution from soil in response to addition of glucose, an easily mineralizable substrate. The magnitude of the respiratory response, as measured after incubation under controlled temperature and humidity conditions, is related to the amount of active biomass in the soil sample, and can be converted to mg of microbial biomass carbon using a conversion factor introduced by Sparling (1995):

$$C_{mic}(\text{mg C g}^{-1}\text{d.w.}) = 50.4 \times \text{respiration rate (ml CO}_2\,\text{g}^{-1}\text{d.w.h}^{-1})$$

Microbial biomass C was measured by mixing in 30 ml vials 1 g of each soil sample (sieved through a 2-mm mesh) with 2 ml of 75 mM D-glucose (27.3 mg g^{-1} soil d.w.). The vials were then sealed tightly and incubated for 4 h in the dark at $25°C$. The evolution of CO_2 was measured by gas chromatography (Fisons GC 8000 series). The CO_2 values were corrected for the CO_2 measured in a blanc vial containing only the soil sample and 2 ml of water, and were reported as mg of microbial carbon.

6.5.4   Microbial Activity

Soil microbial activity can be estimated by measuring CO_2 respired from soil, as a well-established parameter to monitor SOM decomposition (Anderson 1982). Soil respiration is highly variable and its natural fluctuation depends on substrate availability, moisture, and temperature (Alvarez et al. 1995; Brookes 1995). For valid comparisons among soils, respiration measurements must be conducted under controlled laboratory conditions (Anderson 1982). Here, the basal respiration of soil samples was estimated by gas chromatography (Fisons GC 8000 series) as CO_2 evolution in standard conditions (4 h of incubation at $25°C$, at dark), after adding 2 ml distilled water to 1 g of soil (Degens et al. 2000). The basal respiration was expressed in µg CO_2 evolved per gram soil per unit of time. Therefore, the rate of OM mineralization and, hence, the potential capacity of soil to accumulate or

dissipate carbon, is comprised in the coefficient of endogenous mineralization (CEM) that was calculated from soil respiration and SOM content, whereby CEM represents the CO_2 evolved from soil per unit of organic C.

6.5.5 Statistical Analyses

To assess the differences among the project treatments as well as among years in each experimental site, data for cultivable microbial populations were analyzed by using XLSTAT-6.1 and applying standard analyses of variance (one-way and two-way ANOVA) at $p \leq 0.05$ level.

The three-way ANOVA followed by Holm–Sidak post hoc test for pair-wise comparison of means (at $p \leq 0.05$ level) was used to elaborate data of active fungal mycelium (AFM), microbial biomass and microbial respiration and to assess the differences among treatments and experimental sites, as well as those between bulk soil and rhizo soil. A two-way ANOVA was performed for CEM since only bulk-soil values for organic carbon were available due to missing measurements of SOM in the rhizosphere. Statistical analyses were performed by using Sigma-Stat-3.1 for Windows software package.

6.6 Effects of Compost Amendments

Soil managements, such as traditional and minimum tillage, induce reduction of SOM content and decrease of soil structural stability and, thus, have a great impact on functional processes of soil microbial communities. Application of materials rich in organic matter, such as compost, may be used to recover and/or improve soil structure and fertility. Amendments with compost can also strongly influence and modify the size, biodiversity, and activity of the microbial communities in soil (Albiach et al. 2000). Since compost is a source of nutrients which can be used by microorganisms, compost addition usually increases soil microbial biomass and global activity (Bailey and Lazarovits 2003).

6.6.1 Microbial Counts

The effect of compost amendment (COM-2) on the biomass of cultivable communities, as compared to traditional (TRA) and minimum (MIN) tillage, was studied in three different experimental sites (Napoli, Torino, and Piacenza) of the MESCOSAGR project during 3 years (2006, 2007, and 2008).

Microbial populations were significantly affected by agronomic practices. In fact, in all three sites the microbial populations were drastically reduced after

3 years of experimentation in both bulk soil and rhizo soil. This trend was most marked in field plots at Napoli, in which all enumerated microbial populations in COM-2 soils were 1 Log CFU g^{-1} smaller than those found in TRA and MIN soils. In particular, the COM-2 bulk soil showed a negative cumulated effect on total heterotrophic aerobic bacteria, fungi, actinomycetes, and aerobic and anaerobic cellulolytic bacteria due to repeated compost applications to soil. In fact, the
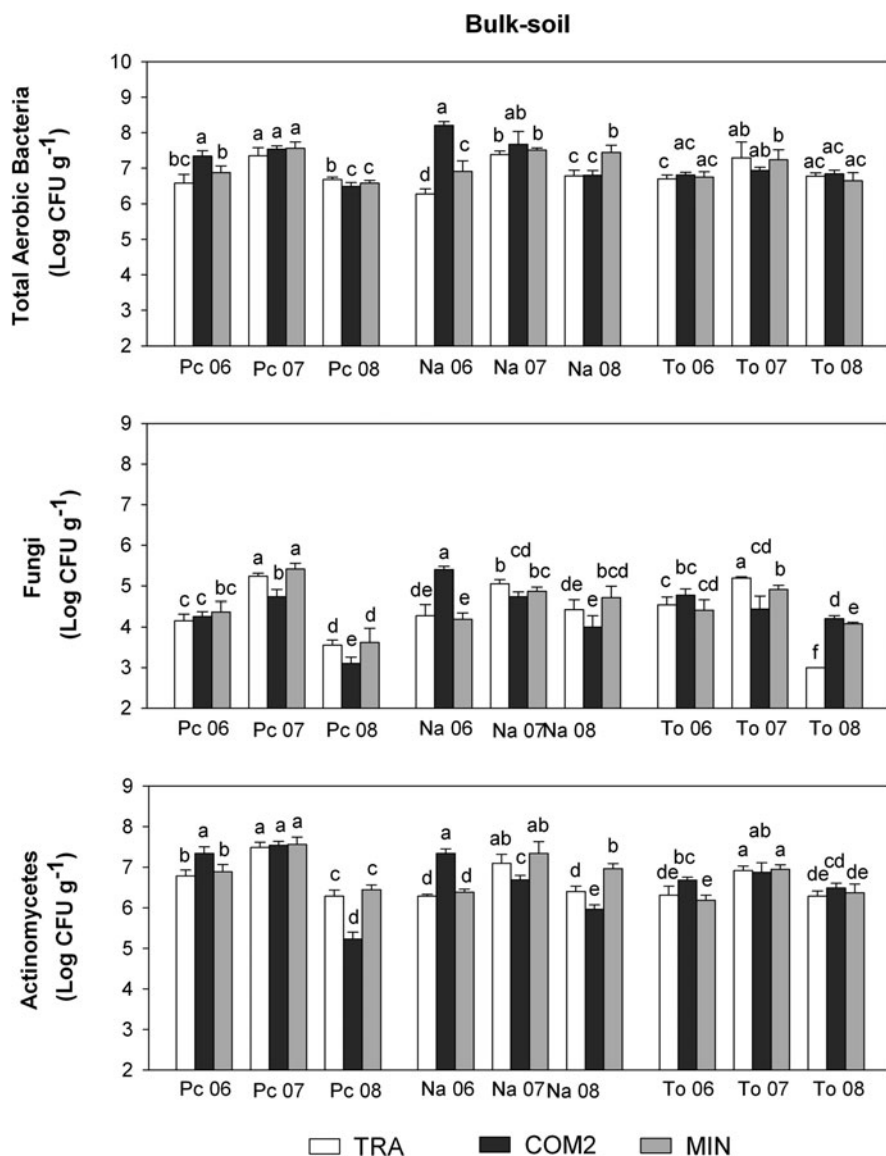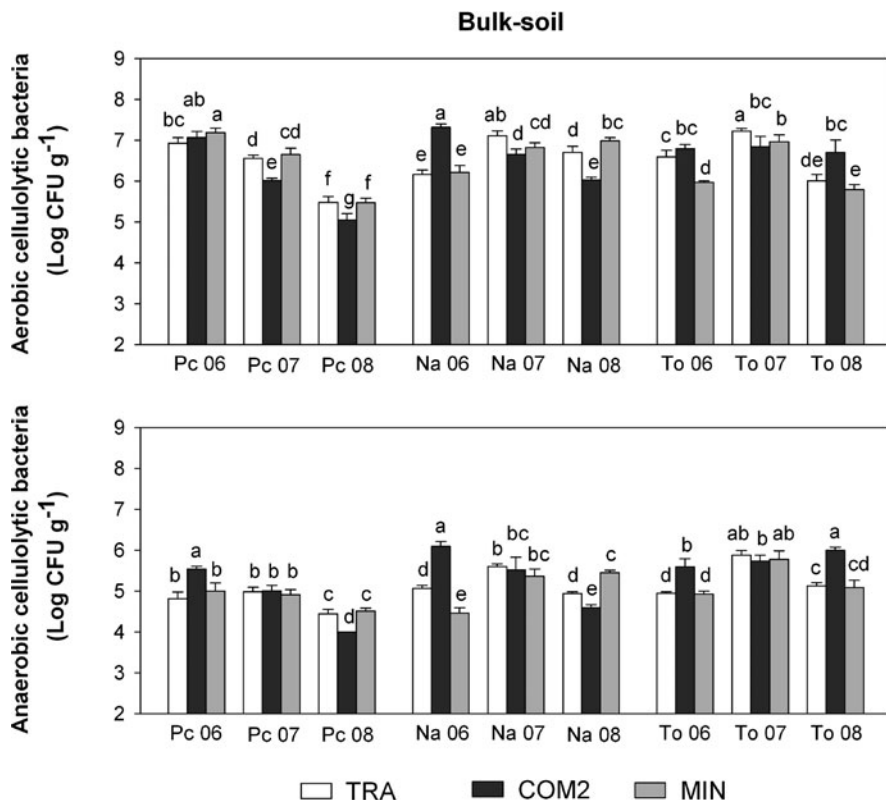

Fig. 6.2 (continued)

Fig. 6.2 Effect of management practices (*TRA* traditional amendment, *MIN* minimum tillage, *COM-2* compost amendment) on (**a**) total aerobic bacteria, fungi, and actinomycetes (mean of Log CFU g^{-1} of soil $\pm$ SE) in bulk soils of Piacenza (Pc), Napoli (Na), and Torino (To). *Different letters* indicate significant difference among treatments and years (ANOVA–Tukey test; $p < 0.05$) within site (**b**) aerobic and anaerobic cellulolytic bacteria (mean of Log CFU g^{-1} of soil $\pm$ SE) in bulk soils of Piacenza (Pc), Napoli (Na), and Torino (To). *Different letters* indicate significant difference among treatments and years (ANOVA–Tukey test; $p < 0.05$) within site

amount of microbial populations after the first compost application (2006) increased significantly more in COM-2 than in TRA and MIN (Fig. 6.2a, b), before declining in the following 2 years (2007–2008). A similar trend was observed in maize rhizo soil at Napoli (Fig. 6.3a, b), whereby a significant abrupt reduction of aerobic (from 8.07 ± 0.08 to 6.21 ± 0.16 Log CFU g^{-1}) and anaerobic (from 6.72 ± 0.12 to 4.93 ± 0.05 Log CFU g^{-1}) cellulolytic bacteria was observed in the third year of compost amendments (Fig. 6.3b).

At the Piacenza site, COM-2 plot was characterized by the lowest microbial values mainly in 2008 year (Fig. 6.2a, b), even though all treatments negatively influenced microbial populations in bulk soil for all 3 years of experimentation. The cumulative negative effect was clearly detectable in aerobic and anaerobic cellulolytic bacteria, which showed a decrease of 1–2 Log cycles in the third treatment
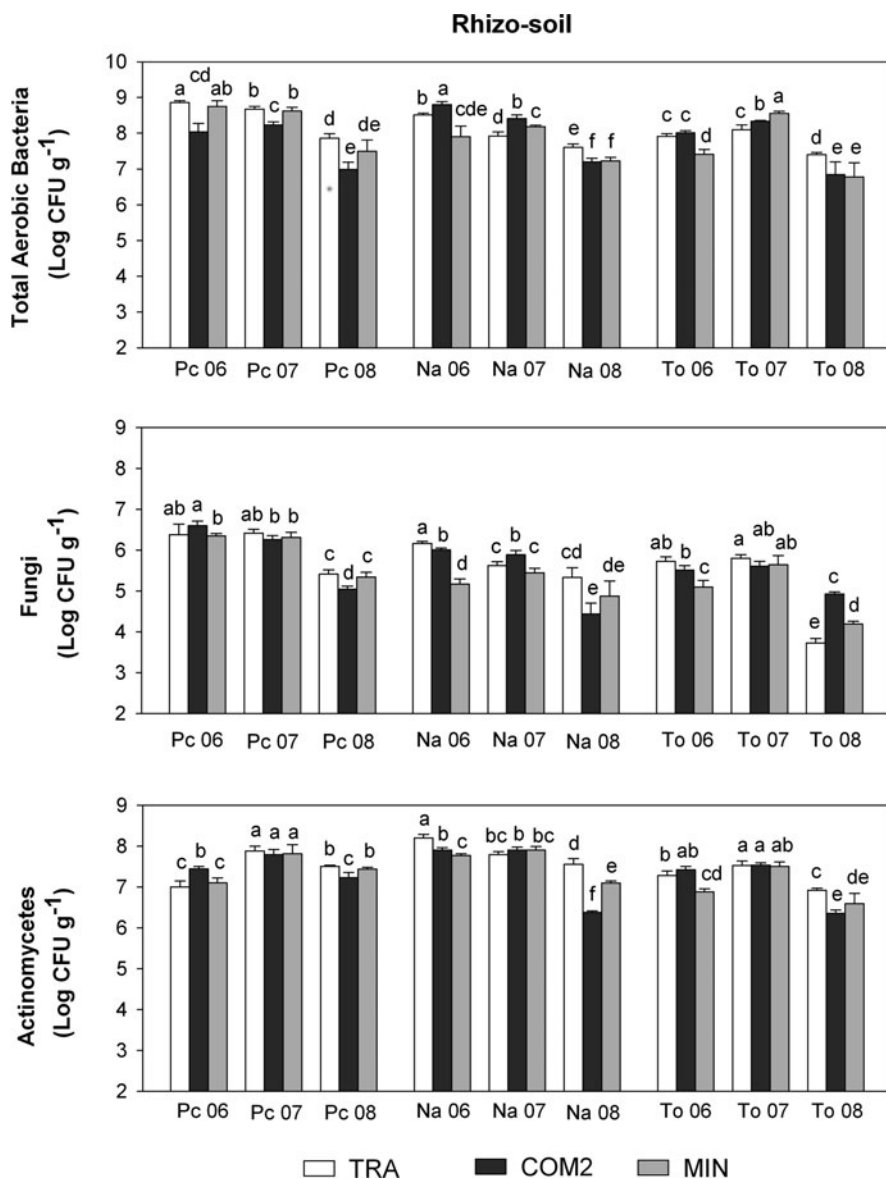
Fig. 6.3 (continued)

year (Fig. 6.2b). The reduction of microbial populations, and particularly of cellu-lolytic bacteria, is interesting since the cellulose degrading enzymes of these populations are directly involved in key OM decomposition steps. In fact, the reduction of functional group of cellulolytic bacteria may result in an increase of organic matter stabilization due to compost addition. Even if this negative behavior
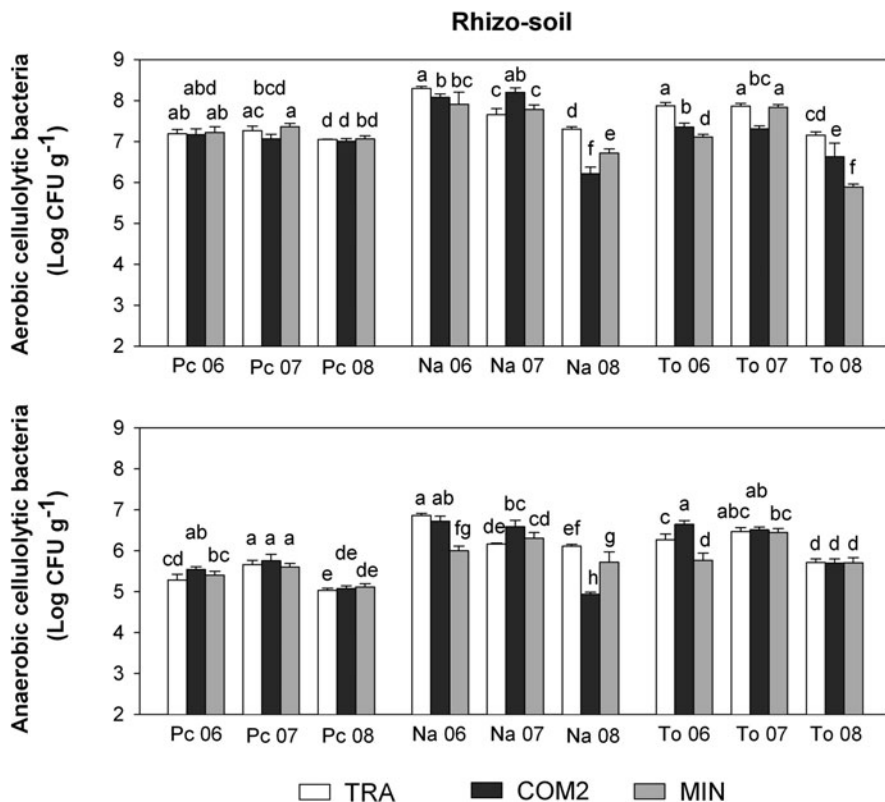
Rhizo-soil

Fig. 6.3 Effect of management practices (*TRA* traditional amendment, *MIN* minimum tillage, *COM-2* compost amendment) on (**a**) total aerobic bacteria, fungi, and actinomycetes (mean of Log CFU g^{-1} of soil $\pm$ SE) in rhizo soils of Piacenza (Pc), Napoli (Na), and Torino (To). *Different letters* indicate significant difference among treatments and years (ANOVA–Tukey test; $p < 0.05$) within site (**b**) aerobic and anaerobic cellulolytic bacteria (mean of Log CFU g^{-1} of soil $\pm$ SE) in rhizo soils of Piacenza (Pc), Napoli (Na), and Torino (To). *Different letters* indicate significant differences among treatments and years (ANOVA–Tukey test; $p < 0.05$) within site

was detected also in the rhizo soil of Napoli, no significant differences among soil management treatments were observed in Piacenza for aerobic and anaerobic cellulolytic bacteria in maize rhizo soil all through the 3 years (Fig. 6.3b). Therefore, the detrimental effect of compost may have been reduced by root exudation in the rhizosphere of maize cropping, as an additional organic carbon source stimulating the microbial growth. The size and composition of rhizosphere microflora is mostly plant-dependent, a phenomenon known as the "rhizosphere effect" (Burr and Caesar 1984) and is attributed to emission of root exudates. Composition of exudates was shown to depend on plant species (Wieland et al. 2001; Singh et al. 2007), as well as on the plant development stage (Jaeger et al. 1999; Yang and Crowley 2000; Feng et al 2003), environmental conditions, and management practices (Paterson and Sim 1999, 2000).

Results different from those of Napoli and Piacenza were obtained from Torino, where compost application did not show the same cumulative effect on soil microbial communities in either bulk or rhizo soil. In fact, bulk soil from COM-2 did not reveal significant difference among the three experimental years in microbial densities of heterotrophic aerobic bacteria, actinomycetes, and aerobic cellulolytic bacteria. By contrast, in 2008, anaerobic cellulolytic bacteria and fungi slightly increased or decreased, respectively (Fig. 6.2a, b). However, the medium used in this study for fungal population mainly selects for a physiological type of fungi characterized by rapid germination of spores and high rate of mycelial growth. Such fungi, which are pioneer colonizers, are able to use ephemeral substrates readily. Their rapid growth results in a sudden spike of activity followed by a rapid decline, since they are unable to degrade abundant substrates such as the resistant ligno-cellulosic structures present in the green compost of this study.

Overall, microbial populations detected in Torino rhizo soil at the third year of experimentation (2008) showed a significant decrease of 1–2 Log CFU g^{-1} for all soil treatments.

The different effect of compost amendment on cultivable microbial biomass at the three field sites should be ascribed to different soil texture and climatic conditions, which are the main determinants of structure and activity of microbial communities. Moreover, the compost used in the experiments was a green waste compost (for chemical composition of the compost see Chaps. 3 and 4). Green waste compost contains both readily decomposable (cellulose) and more recalcitrant (lignin) fractions from plant litter (Standing and Killham 2007). In a short-term experiment, Pérez-Piqueres et al. (2006) evaluated the impact of organic amendments on soil microbial characteristics by using green waste and spent mushroom composts. They found that the microflora in two different soils was influenced by the type of compost. Green waste compost did not modify the densities of cultivable bacteria and fungi in either soil, while the spent mushroom compost significantly increased bacterial and fungal densities in both the clayey and sandy-silty-clay soil, respectively.

Therefore, the cumulated negative effect recorded in Napoli and Piacenza sites (silty-clay-loam soils) may be due to an interaction of compost with the abundant clay particles, which might protect organic matter physically and/or chemically. The mechanism by which organic matter is adsorbed on clay determines its bioaccessibility and the ability of microorganisms to use OM as substrate and to produce extracellular enzymes. Moreover, the presence of chaotropic and antichaotropic ions can influence the nutritional status of microhabitats (Stotzky 1997). By contrast, in Torino bulk soil with a low content of clay (sandy-loam soil), compost amendment led to a significant microflora stimulation, as compared to traditional and minimum tillage.

The largest number of cultivable microorganisms found only in the first experimental year in the bulk soil of COM-2 at all experimental sites should be attributed to the introduction of new community members with the compost rather than to a stimulation of the indigenous community. In fact, both in Napoli and Piacenza, the negative effects on microorganisms were generally observed at the end of the third experimental year.

6.6.2 AFM, Microbial Biomass and Activity

The results of microbial counts were confirmed by evaluating active fungal biomass (AFM), microbial carbon (C_{mic}), and soil respiration. The soils of Torino and Napoli showed statistically significant differences for microbial and fungal biomass and CEM in 2008 and for all investigated parameters in 2007 (Table 6.1). In the third year (2008) of experimentation (Table 6.1), active fungal biomass (AFM) and microbial carbon (C_{mic}) were significantly affected by compost amendment (COM-2). Moreover, when considering the variability within groups, a significant effect was observed for *soil* (bulk/rhizo) and *site* (Napoli/Torino), as well as for

Table 6.1 Levels of significance (*p* values from ANOVA) for effects of compost amendments on microbial biomass and activity in bulk soil and rhizo soil at Napoli and Torino sites, and differences between years

	AFM		C_{mic}	Respiration		CEM	
	dF	p				dF	p
2007 (Three-way)					*2007 (Two-way)*		
Treatments (TRA-COM)	1	0.201	0.524	0.624	Treatments (TRA-COM)	1	0.239
Soil (Bulk–Rhizo)	1	0.164	**<0.001**	**0.039**	Site (Napoli–Torino)	1	**<0.001**
Site (Napoli–Torino)	1	**0.002**	**<0.001**	**0.001**	Treatments × site	1	0.594
Treatments × soil	1	0.469	0.550	0.735			
Treatments × site	1	0.149	0.723	0.850			
Soil × site	1	0.625	**<0.001**	**0.022**			
Treatments × soil × site	1	0.611	0.374	0.891			
2008 (Three-way)					*2008 (Two-way)*		
Treatments (TRA-COM-MIN)	2	**0.013**	**0.019**	0.068	Treatments (TRA-COM-MIN)	2	**0.001**
Soil (Bulk–Rhizo)	1	**<0.001**	**<0.001**	**0.002**	Site (Napoli–Torino)	1	**<0.001**
Site (Napoli–Torino)	1	**<0.001**	**<0.001**	0.147	Treatments × site	2	**0.035**
Treatments × soil	2	**0.007**	0.104	0.405			
Treatments × site	2	0.687	**0.012**	0.079			
Soil × site	1	**<0.001**	**<0.001**	**<0.001**			
Treatments × soil × site	2	**0.019**	0.089	0.091			
2007–2008 (Three-way)					*2007–2008 (Two-way)*		
Years (2007–2008)	1	**<0.001**	**<0.001**	**<0.001**	Years (2007–2008)	1	0.734
Site (Napoli–Torino)	1	**0.004**	0.084	**<0.001**	Site (Napoli–Torino)	1	**<0.001**
Soil (Bulk–Rhizo)	1	**<0.001**	**<0.001**	**0.001**	Years × site	1	**<0.001**
Years × site	1	**<0.001**	**<0.001**	**<0.001**			
Years × soil	1	**<0.001**	**<0.001**	0.095			
Site × soil	1	**<0.001**	**<0.001**	**<0.001**			
Years × site × soil	1	**0.001**	**0.028**	**0.028**			

dF degree of freedom, *AFM* active fungal mycelium, *C_{mic}* Microbial carbon, *CEM* coefficient of endogenous mineralization, *TRA* conventional tillage, *COM* compost amendment, *MIN* minimum tillage. Values in bold are statistically significant

rhizosphere × *site* interaction (Table 6.1). The interaction *treatment* × *soil* was significant only for AFM in 2008, while the interaction *treatment* × *site* was significant only for C_{mic} COM-2 significantly reduced AFM in the Torino bulk soil (Fig. 6.4), while no significant effect was detected in the bulk soil of Napoli (Fig. 6.4). In the maize rhizo soil of both Napoli and Torino, a significant reduction
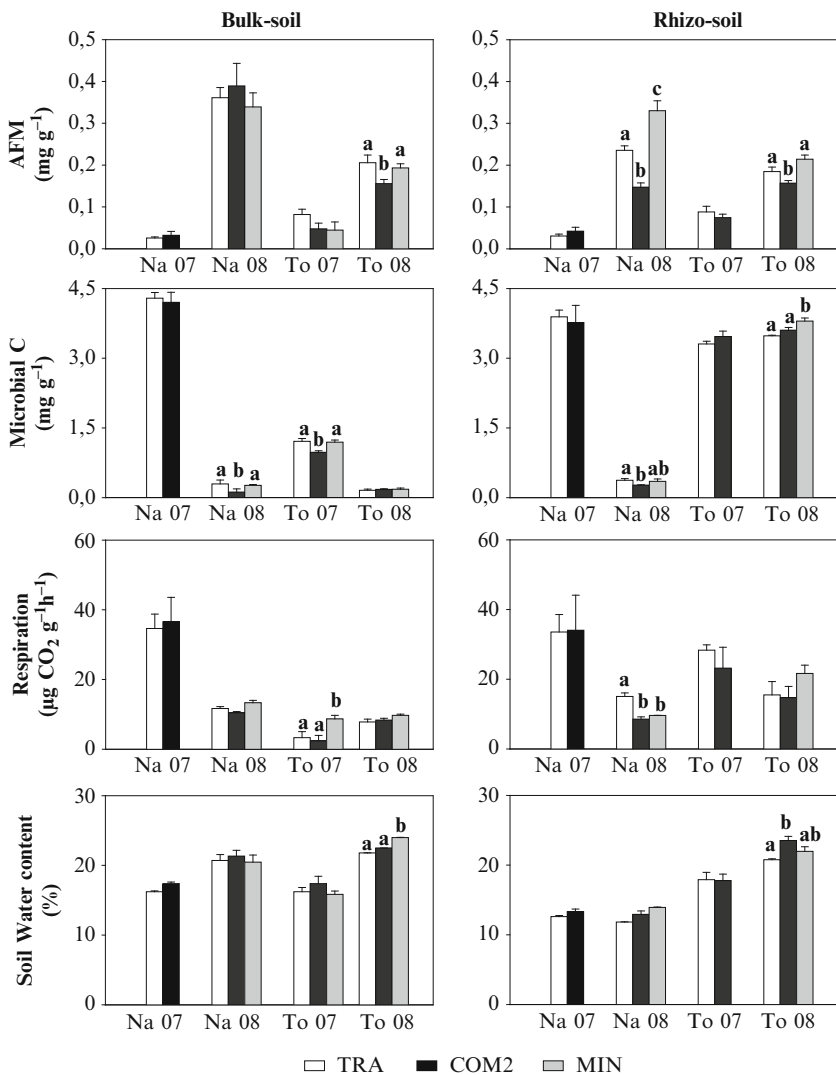
Fig. 6.4 Active fungal mycelium, microbial C, microbial respiration, and water content (mean ± SE) of soil sampled from Napoli (Na) and Torino (To) experimental sites. *TRA* conventional tillage, *COM-2* compost amendment, *MIN* minimum tillage. *Left-hand-side* figures report values for bulk-soil; *Right-hand-side* figures report values for rhizo-soil. *Different letters* indicate significant differences between treatments (ANOVA–Holm–Sidak test; $p < 0.05$) within site and year

in the amount of active fungal mycelium was detected in 2008, as compared to TRA and MIN (Fig. 6.4). Moreover, in both 2007 and 2008, COM-2 reduced microbial biomass of bulk soil and rhizo-soil in Napoli, though differences were statistically significant only in 2008 (Fig. 6.4). In Torino, the microbial biomass of COM-2 bulk soil in 2007 was significantly lower than in both TRA and MIN, while in 2008 the rhizo-soil microbial biomass of COM-2 was significantly lower than in MIN (Fig. 6.4). For both Napoli and Torino, the bulk-soil microbial biomass was significantly larger in 2007 than in 2008, though the difference for rhizo-soil microbial biomass between the 2 years was significant only for Napoli (Fig. 6.4).

Soil respiration was significantly affected by *soil* (bulk/rhizo) or *site* as well as by *soil–site* interaction (Table 6.1). A soil respiration significantly lower than MIN and TRA was found for COM-2 in Torino bulk soil in 2007 and in Napoli rhizo soil in 2008, respectively (Fig. 6.4). In 2008, the coefficient of endogenous mineralization (CEM) was significantly affected by both *treatment* and *site* (Fig. 6.5), and by
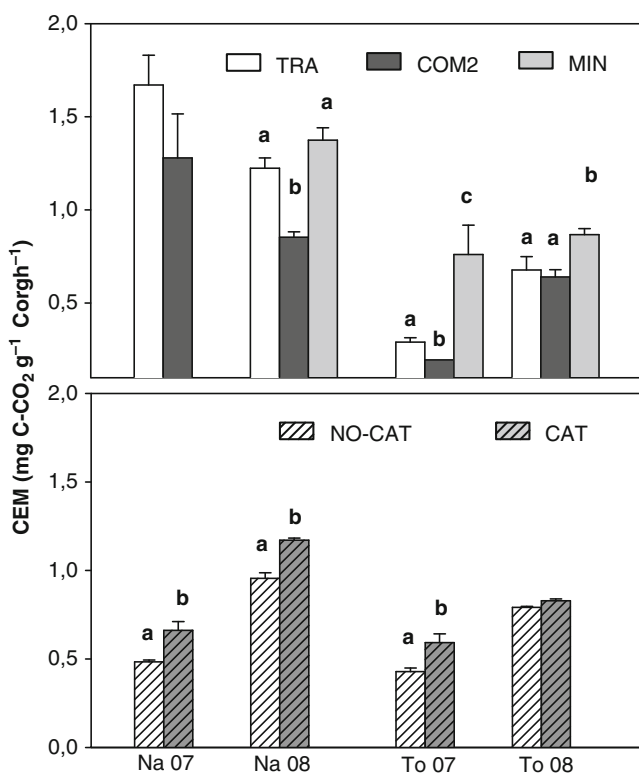
Fig. 6.5 Coefficient of endogenous mineralization (CEM) of soils sampled from the Napoli (Na) and Torino (To) experimental sites. The values are mean ± SE. *TRA* conventional tillage, *COM-2* compost amendment, *MIN* minimum tillage. CAT: conventional tillage with addition of biomimetic catalyst, No-CAT: conventional tillage without catalyst. *Different letters* indicate significant differences among treatments (ANOVA–Holm–Sidak test; $p < 0.05$) within site and year

their interaction. CEM was always lower in COM-2 than in TRA and MIN for both Napoli and Torino field sites.

The general reduction of AFM, microbial biomass and respiration, for both bulk soil and rhizo soil subjected to COM-2 may be explained by the protection that the humified mature compost exerts on bio labile components of soil. This makes them less bio accessible and thus more resistant to microbial degradation (Spaccini et al. 2002; Piccolo et al. 2004).

Another effect of COM-2 was the increase in soil moisture, compared to TRA and MIN. According to Carter (2007), compost amendment improves soil porosity and consequently favors an increase in soil moisture. For both Napoli and Torino sites, water content in bulk soil for the 2008 experimental year was generally larger than for 2007 (Fig. 6.4), whereas the rhizo soil at Napoli had lower water content than the rhizo soil at Torino. More specifically, soil water content tended to be the highest in compost-amended plots at both sites and in both years.

In addition, no difference was found in microbial biomass in soils subjected to TRA and MIN, with the exception of Torino rhizo soil. This result may indicate that the 3-year treatment period is too short to produce a significant improvement in soil biological quality. In fact, various studies (Joergensen and Castillo 2001; Balota et al. 2003; Franchini et al. 2005; Wright et al. 2008; Helgason et al. 2009) indicate that the effects of different agricultural management on soil microbial communities become evident after longer periods (at least 10 years). Nevertheless, AFM and microbial biomass of rhizo soil in MIN were larger in 2008 than in TRA for the Napoli and Torino sites, respectively.

6.7   Effects of the Biomimetic Catalyst

Humic substances comprise the major part of stable organic matter in environmental compartments and their formation and decomposition processes regulate global carbon cycling. An increase in the conformational stability of humus may be achieved by increasing the intermolecular covalent bonds among heterogeneous humic molecules through a photo-oxidative coupling mediated by a biomimetic (enzyme-like) catalyst, such as synthetic water-soluble metal–porphyrins (Piccolo et al. 2005). It was found that soil amendments with the biomimetic catalyst affected the molecular structure of SOM and decreased its biotic degradation, thereby significantly decreasing CO_2 emission from soil (Gelsomino et al. 2010; Piccolo et al. 2011). However, since new molecules added to soil, though apparently harmless and eco-compatible, may deeply alter the behavior of microbial populations through complex and unexpected interaction (biotic and/or abiotic), we studied the impact of the biomimetic catalyst on the dynamics of microbial soil populations in the different experimental fields of the MESCOSAGR project.

6.7.1 Microbial Counts

The CAT treatment showed hardly any long-term effects on cultivable microbial populations, as evaluated in both bulk soil and rhizo soil. In fact, no significant difference was found between CAT plots and their control (No-CAT) in any of the experimental sites for the first 2 years of treatment (Figs. 6.6a, b and 6.7a, b).
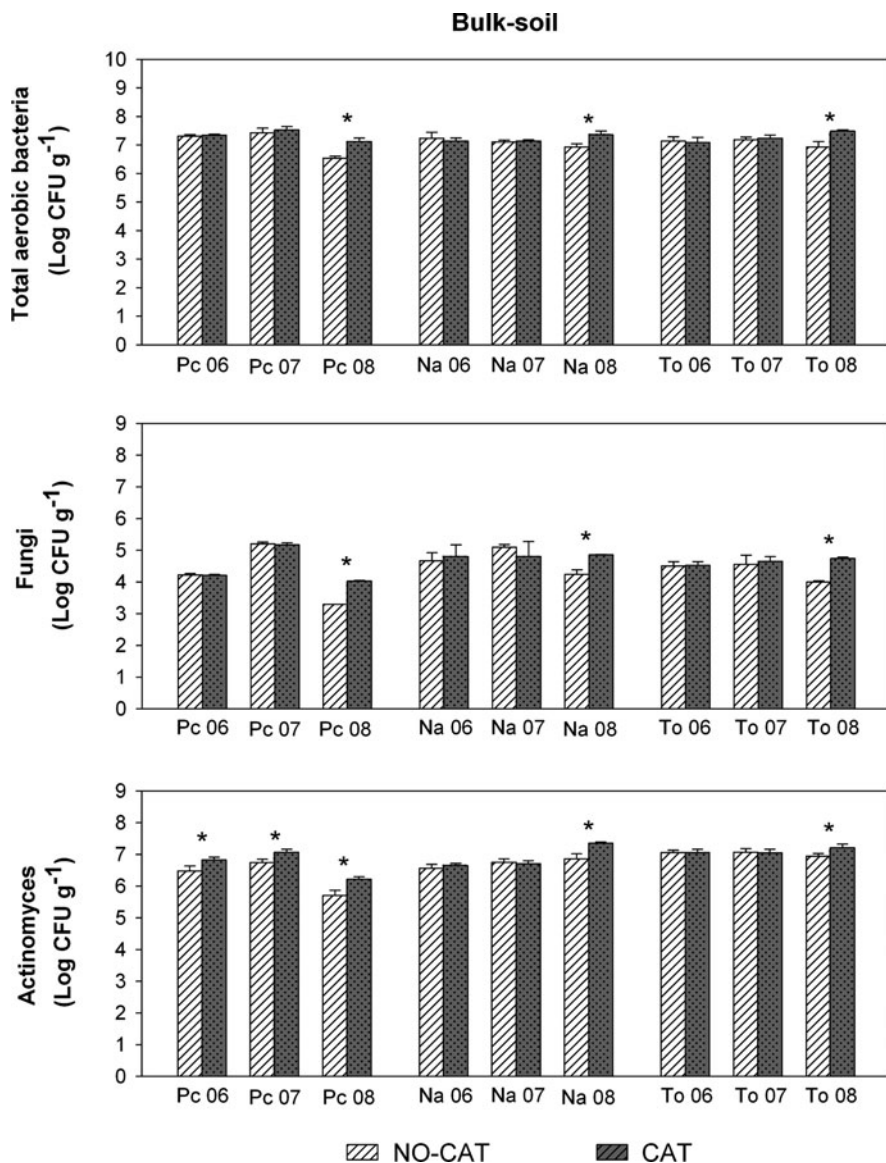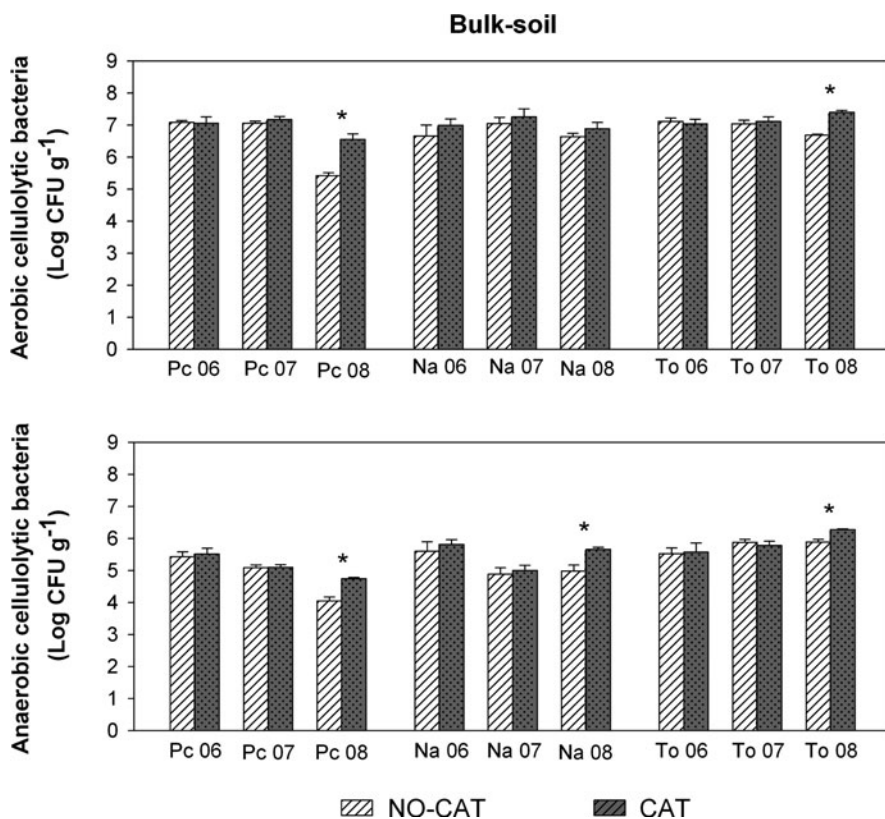

Fig. 6.6 (continued)

Fig. 6.6 Effect of synthetic metal–porphyrins addition on (a) total aerobic bacteria, fungi, and actinomycetes (mean of Log CFU g^{-1} of soil $\pm$ SE) in bulk-soils of Piacenza (Pc), Napoli (Na), and Torino (To). *Asterisk* indicates significant at $p < 0.05$ within site and years. NO-CAT: control, CAT: soil treated with biomimetic catalyst (**b**) aerobic and anaerobic cellulolytic bacteria (mean of Log CFU g^{-1} of soil $\pm$ SE) in bulk-soils of Piacenza (Pc), Napoli (Na), and Torino (To). *Asterisk* indicates significant at $p < 0.05$ within site and years. NO-CAT: control, CAT: biomimetic catalyst

The only exception was in the Piacenza bulk soil that showed an increase in actinomycete populations during the whole experimental period (Fig. 6.6a). This long-term effect was probably due to the firm adsorption of the added metal–porphyrin on the large amount of clay particles present in this soil, thereby resulting in greater catalytic activity upon soil biotic and/or abiotic components.

In the last experimental year (2008), the CAT effect on the bulk-soil was the same in the three different field sites, since it significantly affected the microbial groups directly involved in OM mineralization. In fact, the number of total heterotrophic aerobic bacteria, fungi, actinomycetes, aerobic, and anaerobic cellulolytic bacteria was significantly larger in CAT than in No-CAT by an extent of about 1 Log CFU g^{-1} cycle (Fig. 6.6a, b).

Conversely, it appears that CAT negatively affected the OM mineralization communities in rhizo soils, although the effect on maize rhizo soil (Piacenza) was different from that on wheat rhizo soil (Napoli and Torino). In particular, CAT did not influence the cellulolytic bacteria in the wheat rhizo soils of both Napoli and Torino with respect to No-CAT for all experimental years (Fig. 6.7a, b). By contrast, CAT significantly affected microbial communities in maize rhizo soil.
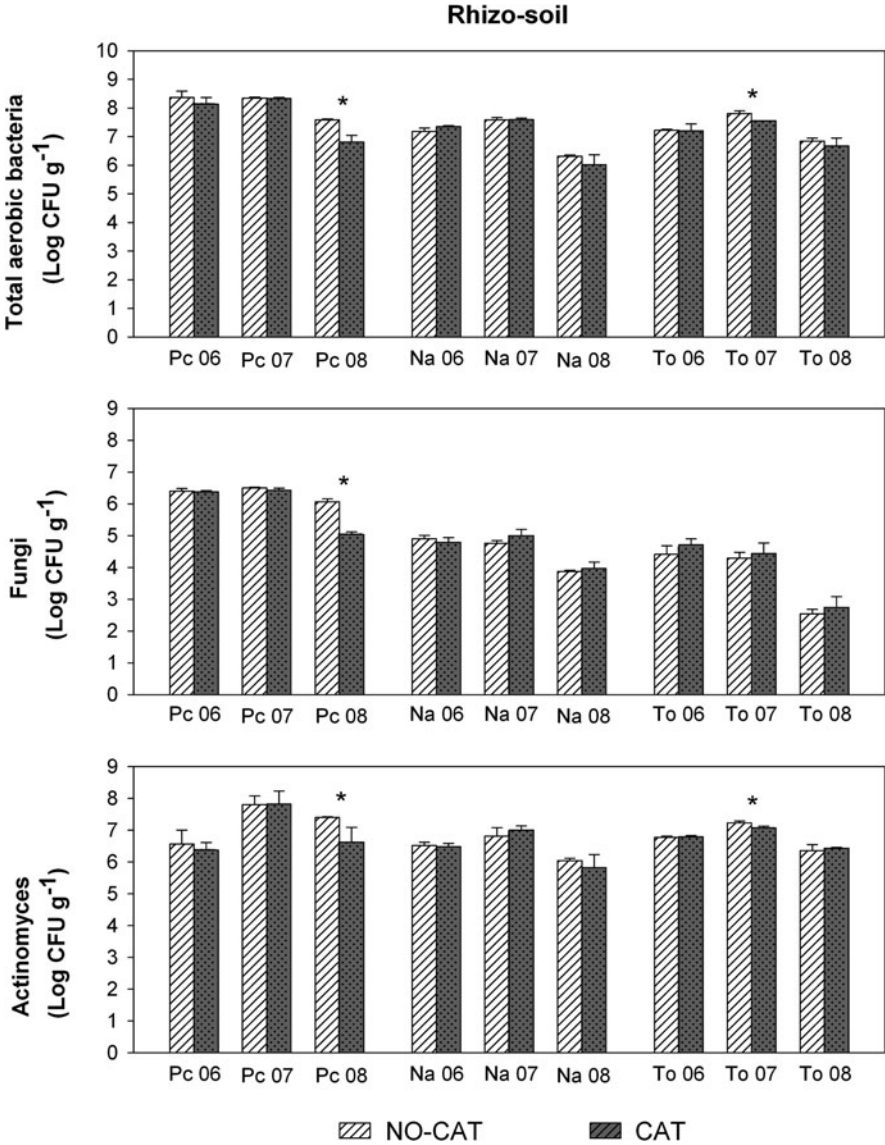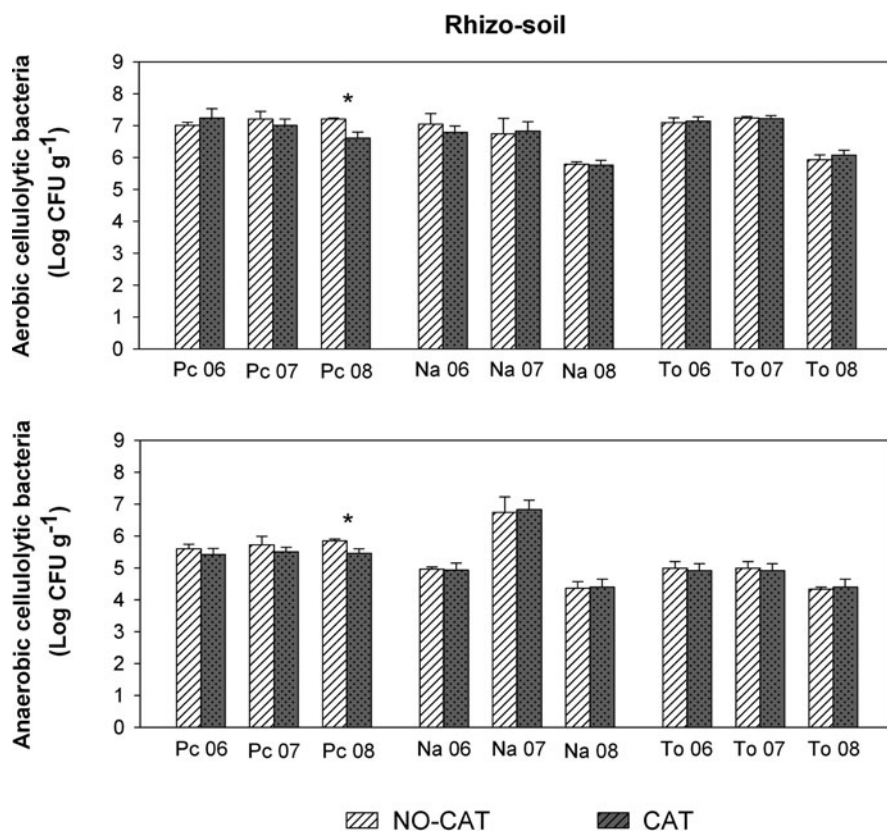

Fig. 6.7 (continued)

Fig. 6.7 Effect of synthetic metal–porphyrins addition on (**a**) total aerobic bacteria, fungi, and actinomycetes (mean of Log CFU g^{-1} of soil $\pm$ SE) in maize rhizosphere of Piacenza agronomic station (Pc), and in wheat rhizosphere of Napoli (Na) and Torino (To) agronomic stations. *Asterisk* indicates significant at $p < 0.05$ within site and years. NO-CAT: control, CAT: biomimetic catalyst (**b**) aerobic and anaerobic cellulolytic bacteria (mean of Log CFU g^{-1} of soil $\pm$ SE) in maize rhizosphere of Piacenza agronomic station (Pc), and in wheat rhizosphere of Napoli (Na) and Torino (To) agronomic stations. *Asterisk* indicates significant at $p < 0.05$ within site and years. NO-CAT: control, CAT: biomimetic catalyst

In fact, a decrease in the number of all cultivable microorganisms in Piacenza was found after three experimentation years (Fig. 6.7a, b). However, this effect was more extensive in fungi populations which decreased from 6.06 ± 0.09 for No-CAT to 5.03 ± 0.08 Log CFU g^{-1} for CAT (Fig. 6.7a).

The different effect of CAT on microbial communities in rhizospheres of maize and wheat may be due to different root systems and root activities. Maize plants have more expanded root systems than wheat, and, thus, explore a greater volume of soil and possibly induce a larger root exudation in the rhizosphere, to promote microbial growth. It is known that when root exudates serve as sole source of C and energy for soil microbes, root exudation is 2–2.6 times greater than in the case of

aseptically grown plants (Vancura et al. 1977; Prikryl and Vancura 1980). Thus, the lower content of soil microbes in CAT suggests that a reduction in root exudate stimulation has occurred, possibly because another source of C and energy was made available to microbes by the CAT treatment. In fact, the enhanced growth of microbial cells following root exudate stimulation is attributed to carbohydrates in exudates (van Overbeek and van Elsas 1995). Therefore in the rhizosphere, the activity of the catalyst on SOM may lead to release of novel organic molecules. The consumption of such molecules by microbes may have depressed the increase of the root exudates/microbial growth cycle observed in No-CAT rhizo soils. Gelsomino et al. (2010) added the biomimetic catalyst on a microcosm soil with and without maize plants and measured CO_2 respiration (see also Chap. 10). They found that while respiration was reduced in catalyst-treated bare soils, there was an enhanced respiration when maize plants were present. Although these results are intriguing, they are difficult to compare since our findings refer to maize rhizo-soil microbial communities and were obtained from field experiments rather than in microcosms.

Nevertheless, the significant decrease observed for maize rhizo soil under CAT treatment suggests that maize plants appear as more suitable indicators than wheat plants in highlighting the effect of the biomimetic catalyst. Furthermore, our findings indicate that the biomimetic catalysts added to soil did not appear to be harmful to cultivable microbial communities, since no lethal effect was recorded.

6.7.2 AFM, Microbial Biomass and Activity

CAT significantly affected soil respiration and CEM (Table 6.2). Moreover, significant differences between bulk soil and rhizo soil were found for all microbial parameters (Table 6.2). The differences between sites were significant for C_{mic} in 2007 and 2008, as well as for respiration and CEM in 2008. Moreover, a significant interaction *soil* × *site* was observed for AFM and C_{mic} (Table 6.2).

As for bulk soils, AFM in Napoli was larger in CAT than in No-CAT, though the differences were not significant (Fig. 6.8), while in Torino AFM was first significantly lower in CAT than in No-CAT in 2007 and, then, significantly larger in 2008 (Fig. 6.8). In the case of rhizo soils AFM found in CAT treatments was always lower than in No-CAT, but the difference was significant only for Torino in 2007 (Fig. 6.8).

Microbial biomass was not found significantly different between CAT and No-CAT in either bulk or rhizo soils throughout the experimental period for either Napoli or Torino (Fig. 6.8).

Respiration showed a similar increasing trend from No-CAT to CAT treatments in both Napoli and Torino and for either bulk soil or rhizo soil. However, the increase was significant only for Napoli bulk soil in 2007 and Napoli rhizo soil in 2008 (Fig. 6.8).

Table 6.2 Levels of significance (p values from ANOVA) for effects of a biomimetic catalyst (iron-porphyrin) addition to soil on microbial biomass and activity in bulk soil and rhizo soil at Napoli and Torino sites, and differences between years

	AFM	C_{mic}	Respiration		CEM	
	dF p				dF p	
2007 (Three-way)				*2007 (Two-way)*		
Treatments (CAT/NO-CAT)	1 0.073	0.510	**0.040**	Treatments (CAT/NO-CAT)	1	**0.010**
Soil (Bulk–Rhizo)	1 **0.019**	**0.004**	**0.005**	Site (Napoli–Torino)	1	0.165
Site (Napoli–Torino)	1 0.422	**0.015**	0.893	Treatments × site	1	0.850
Treatments × soil	1 0.166	0.125	0.138			
Treatments × site	1 0.159	0.857	0.710			
Soil × site	1 0.931	**<0.001**	0.365			
Treatments × soil × site	1 0.571	0.133	0.522			
2008 (Three-way)				*2008 (Two-way)*		
Treatments (CAT/NO-CAT)	1 0.275	0.874	**0.016**	Treatments (CAT/NO-CAT)	1	**0.002**
Soil (Bulk–Rhizo)	1 **<0.001**	**<0.001**	**<0.001**	Site (Napoli–Torino)	1	**<0.001**
Site (Napoli–Torino)	1 0.154	**0.003**	**<0.001**	Treatments × Site	1	**0.008**
Treatments × soil	1 0.140	0.668	0.854			
Treatments × site	1 0.816	0.487	**0.049**			
Soil × site	1 **0.002**	**<0.001**	0.988			
Treatments × soil × site	1 0.864	0.575	0.575			
2007–2008 (Three-way)				*2007–2008 (Two-way)*		
Years (2007–2008)	1 **<0.001**	**<0.001**	**<0.001**	Years (2007–2008)	1	**<0.001**
Site (Napoli–Torino)	1 0.280	**0.030**	**0.033**	Site (Napoli–Torino)	1	**0.008**
Soil (Bulk–Rhizo)	1 **<0.001**	**<0.001**	**<0.001**	Years × site	1	0.082
Years × site	1 0.099	**<0.001**	**0.050**			
Years × soil	1 **<0.001**	**0.003**	0.680			
Site × soil	1 **<0.001**	**0.025**	0.505			
Years × site × soil	1 **<0.001**	**<0.001**	0.512			

dF degree of freedom, *AFM* active fungal mycelium, *C_{mic}* microbial carbon, *CEM* coefficient of endogenous mineralization, *CAT* conventional tillage with addition of biomimetic catalyst, *NO-CAT* conventional tillage without catalyst. Values in bold are statistically significant

The coefficient of endogenous mineralization (CEM) in Napoli was significantly larger in CAT than in No-CAT for both 2007 and 2008 years, while this was true for Torino only in 2007 (Fig. 6.5). Regardless of treatment, CEM for Napoli soils was greater than for Torino (Fig. 6.5).

When comparing wheat soil and maize soil (No-CAT/TRA), C_{mic} and respiration showed significantly lower values in wheat soil ($p = 0.045$ and $p = 0.029$, respectively). Water content in bulk soils was very similar for Napoli and Torino in both years (Fig. 6.8). Rhizo-soil water content was lower in Napoli than in Torino.
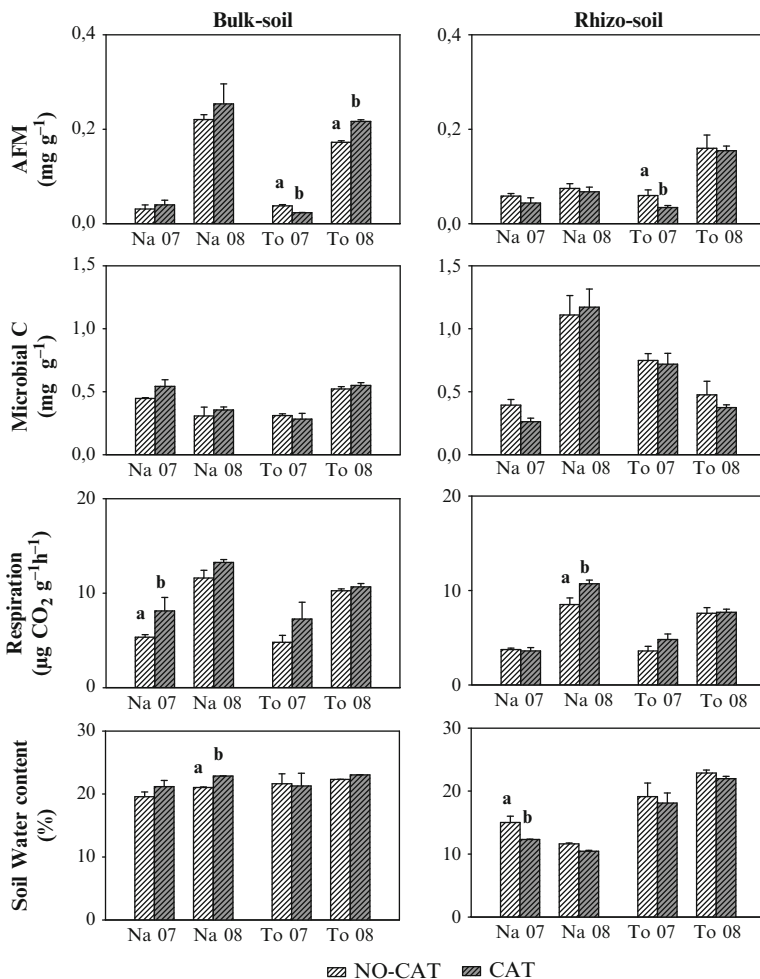
Fig. 6.8 Active fungal mycelium, microbial C, microbial respiration and water content (mean ± SE) of soil sampled from Napoli (Na) and Torino (To) experimental sites. CAT: biomimetic catalyst, NO-CAT: control. Figures on the *left* report values for bulk-soil; figures on the *right* report values for rhizo soil. *Different letters* indicate significant differences between treatments (ANOVA–Holm–Sidak test; $p < 0.05$) within site and years

CAT addition increased water content in bulk soil with respect to No-CAT, whereas it had an opposite effect in rhizo soil (Fig. 6.8).

CAT increased AFM, and microbial biomass in bulk soils, but had an opposite effect in rhizo soil, well in agreement with plate-count results for total aerobic bacteria, cellulolytic bacteria, fungi, and actinomycetes. CAT increased respiration in both bulk and rhizo soils, thus suggesting, in line with CEM values, that the in situ photo-polymerization of SOM unexpectedly favors instead of limiting CO_2

emissions. This result is consistent with the larger CO_2 fluxes measured in the field from CAT soils compared to No-CAT, although the emissions include root respiration (see Chap. 9). Moreover, our findings are in line with the cited microcosm experiment (Gelsomino et al. 2010) that revealed that the addition of iron–porphyrin significantly reduced CO_2 efflux from the unplanted soil, whereas CO_2 emission was stimulated when maize plants were present. Gelsomino et al. (2010) hypothesized that the coarser root system induced by iron–porphyrin favored enhanced destruction of soil macroaggregates, thus exposing physically protected SOM to microbial decomposition. However, they were not able to quantify the contribution to CO_2 emission from soil of autotrophic respiration (maize roots) and heterotrophic respiration (rhizosphere microorganisms).

Our data refer to the effect of CAT treatment on soils under wheat and they do not take into account root respiration. Moreover, we found that respiration increased in both the rhizo and bulk soils. Therefore, at least for bulk soils, the explanation proposed by Gelsomino et al. (2010) should be definitely excluded. However, there were contrasting responses of microbial communities to CAT for either bulk or rhizo soils, and it is likely that root systems inhibit growth of the microbial community. Despite the observed evidence of CO_2 being released as much or more in CAT than in No-CAT, the catalyst-assisted in situ photo-polymerization of SOM has been shown to sequester organic C throughout the experimentation period in all sites (see Chap. 4).

These contrasting results cannot be yet totally explained since the mechanism underlying the interactions among the catalyst, microbial community, and root systems is complex. However, a possible reason for such an opposite behavior may be the fact that substrates for oxidative photo-polymerization are the phenolic or oxidized aromatic moieties of SOM, which produce the free radicals, whose coupling increases covalent bonds among humic molecules. These aromatic photo-polymerized components of SOM certainly become more biologically stable in soil, thus possibly explaining the reduction of AFM in some cases. Consequently, the carbon-chain alkyl compounds of SOM may result more easily accessible to microbial degradation due to alteration of humic conformations following separation of the photo-polymerized aromatic moieties.

It is also interesting to note that water content in rhizo soils is lower in CAT that in No-CAT, while the opposite is true for bulk soils. This may be due to the fact that the interaction of root systems with the catalyst induces an alteration of the surrounding soil structure, thus limiting the water retention capacity. Such alteration may also influence the size of the microbial community.

When comparing results for wheat and maize soils, it is evident that microbial biomass and activity are larger under maize. Given that wheat and maize grow in different seasons, climatic conditions could at least in part explain such differences (Mahmood et al. 2005). However, it is important to recall that different plant species produce different rhizosphere effects (Vancura et al. 1977; Cheng et al. 2003). There was a weak rhizosphere effect on fungal communities at both sites under either maize or wheat. In contrast a positive rhizosphere effect on C_{mic} was observed under maize at Torino, where, an increase of microbial biomass was accompanied by an increase in respiration.

6.8   Conclusion and Future Recommendations

First of all, our results highlight the importance of combining different approaches to obtain complementary information on the microbiological status of agricultural soils.

Amendment with compost appears to have a promising environmental application, although its use depends on soil texture and clay content, as shown by our studied sites. In fact, compost was found to decrease cultivable microorganisms, microbial carbon, and coefficient of SOM mineralization in clayey soils, possibly due to an increased physical and chemical protection of organic matter from microbial attack. On the other hand, such an effect was not equally evident in soil with lower clay content. Van Elsas et al. (2007) denied direct correlation between abundance of microbial populations and their activities (e.g., N-fixation and cellulosolytic activities). The activities are sometimes enhanced by an improved nutrient availability caused by lower competition among microbial cells and by a large concentration of "microbivores" (microbial-feeding microfauna such as mites and nematodes), which keep bacterial abundance at a minimum. Thus, a poliphasic approach including microfauna analyses is necessary to fully understand the complex interactions within the soil food web.

The use of the biomimetic catalyst to fix and/or stabilize soil carbon by photopolymerization caused contrasting responses of soil microbial community. It became evident from concomitant results of other MESCOSAGR groups that the different effects of the catalyst depend on whether the soil is either planted or bare, but also on plant species (maize or wheat), regardless of soil texture and climatic conditions. Such results are consistent with the results obtained by the biotechnological group of MESCOSAGR project (see Chap. 8).

It is thus hoped that further investigations will be conducted, to include analysis of microfauna–microflora interactions, in order to reach a deeper understanding of the long-term effects of compost and metal–porphyrin catalyst on carbon sequestration in soils cultivated with different plant species.

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