# Chapter 7
# Effects of Methods of Carbon Sequestration in Soil on Biochemical Indicators of Soil Quality

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**Abstract** Here we describe the effects of carbon sequestration managements on soil enzymatic activities and PLFA patterns, as viable parameters to establish soil biochemical quality and its changes. We extensively review the available scientific literature related to experimental results on soil enzymatic activities and PLFA values from different soil treatments. This knowledge was then compared with the experimental results obtained within the MESCOSAGR project. It was found that MESCOSAGR findings are well in agreement with literature, and they show that the use of mature compost or adoption of reduced tillage practices provides an improvement of soil quality, as shown by a general increase in different enzymatic activities. The carbon sequestration method based on the in situ photo-polymerization of soil organic matter catalyzed by a water-soluble iron–porphyrin spread on soil did not show significant difference in soil biochemical quality from control. Changes in microbial communities at taxonomical level have also been identified with PLFA determinations, but these changes were usually site-specific, and mostly related to expression of ecological functions. Our work confirms the importance of linking structural with functional measurements when assessing the response of soil microbial communities to any experimental factor.

## 7.1 Introduction

Soil is a natural resource that is not renewable at the human time scale and often subjected to a range of alteration events due to human or natural activities. One of the most striking features of soil is its biological complexity, still largely unknown. Soil is indeed the most biological diverse environment on Earth (Dance 2008), and

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its main ecological and productive functions are driven and sustained by the presence and activity of microorganisms (Young and Crawford 2004).

Scientists are still largely debating about the estimations of the size of microbial communities in soils. One of the first estimations, published in 1990, pointed to about 4,000 different bacterial genomes per gram of soil (Torsvik et al. 1990). Later studies have first moved the estimated number of species per gram of soil to 830,000 (Gans et al. 2005) and, then, back down to a number between 20,000 and 50,000 species (Roesch et al. 2007). Of course these estimations are affected by a numbers of factors, starting from the type of soil considered and the methods used for the estimation. Amann et al. (1995) reported that more than 99% of the bacterial species in soils are unculturable, and this has been widely confirmed by recent studies (Handelsman 2004; Deutschbauer et al. 2006). Research is now mining at molecular level into this widely unknown unculturable majority, by taking advantage of new technologies which allow a fine-scale resolution of DNA, RNA and proteins in soils. These approaches will most probably give relevant outcomes at both basic and applied science level in the next years, but at the moment the level of complexity is so high that is difficult to translate the amount of information obtained in, for example, indexes of soil quality and/or alterations due to soil treatments.

Soil scientists have been studying the structure and most importantly the functionality of soil microbial communities for decades, by developing and optimizing methods that are now still applied and widely accepted, even at regulatory levels. We have focused on two of these methods: enzymatic activities and phospholipids fatty acid (PLFA) analyses. Methods and literature evidence are firstly discussed in relation to soil management practices aimed at carbon sequestration, namely amendment with compost and other organic residues, and reduced or no tillage managements. In the second part of the chapter, evidence from MESCOSAGR project is discussed in relation to literature review. Original outcomes about possible effects of the third C sequestration strategy considered in MESCOSAGR (organic matter photo-polymerization under a biomimetic catalyst) on soil PLFAs and enzymes are also presented.

## 7.2 Enzymatic Activities and Soil Carbon Sequestration Strategies

The expression “enzymatic activities” is usually preferred to “enzymes,” the reason being that applied methods do not isolate and measure the enzyme itself in soils, but quantify (mainly by absorbance or fluorescence) the rate of transformation of an enzyme substrate added to soil. Already in the 1940s a paper was published reporting the effect of copper nitrogen complex on soil potential nitrification, as assessed by this substrate-induced approach (Lees 1946), and it was followed by a series of pioneering works on the location and activities of enzymes in soils

**Table 7.1** Number of published literature on enzymes and PLFAs in soil as related to compost and tillage treatments

| Search word | <2008 | 2008 | 2009 | 2010 | 2011 | Total |
|---|---|---|---|---|---|---|
| Soil* AND enzym* | 10,388 | 1,188 | 1,290 | 1,337 | 135 | 14,338 |
| Soil* AND enzym* AND compost | 220 | 48 | 55 | 56 | 3 | 382 |
| Soil* AND enzym* AND tillage | 124 | 16 | 27 | 33 | 3 | 203 |
| Soil* AND PLFA* | 502 | 113 | 95 | 109 | 18 | 819 |
| Soil* AND PLFA* AND compost | 19 | 4 | 1 | 5 | 0 | 29 |
| Soil* AND PLFA* AND tillage | 20 | 4 | 3 | 6 | 3 | 36 |

The enquiry was carried out on Scopus database in February 2011 among titles, abstracts and keywords

(McLaren 1954; McLaren et al. 1957). From then on, the assessment of enzymatic activities (or more generally biological activities, as in the case of nitrification, where a series of enzymatic activities is involved) has become widely popular. A search on the Scopus scientific database using "soil*" and "enzym*" as search words among titles, abstracts and keywords gives a total 14,332 published papers, with more than a thousand papers per year (Table 7.1). If the search is restricted adding either "compost" or "tillage," the number of published papers is, respectively, 382 and 203, with a slight increase in the last years.

Numerous reviews about enzymatic activities have been published in the last years (e.g., Dick 1992; Tabatabai et al. 2002; Nannipieri et al. 2003; Caldwell 2005), as well as a number of books (Burns 1978; Dick and Burns 2002). Thus, the aim of this chapter is not to provide a further review on the topic but to analyse the evidence available in literature on the effects of organic fertilization and tillage on soil enzymatic activities and to assess whether some general conclusions can be drawn from this critical bibliographic investigation.

Soil enzymatic activities are related to the majority of ecological processes in soils such as soil organic matter decomposition, cycling of nutrients, and detoxification of undesired compounds, such as pesticides and other organic contaminants. Enzymes play a main role in relation to presence and activities of soil microorganisms, since their catalytic activity toward transformation of organic substrates allows liberation of the necessary energy for their activities (Kiss et al. 1978) and promotes soil fertility by releasing nutrients for plants growth. Soil enzyme activities have been suggested as suitable indicators of soil quality for a number of reasons: (1) they are an index of soil microbial activity and, thus, they are strictly related to nutrient cycles and transformations; (2) they may rapidly respond to changes in soil caused by both natural and anthropogenic factors; (3) they are easy to measure (Calderon et al. 2000; Drijber et al. 2000; Nannipieri et al. 2002). The information given by a single enzymatic activity is of course important but limited. This is why most works usually consider a range of enzymatic activities, eventually condensing all information in numerical indices obtained by different approaches. In a recent review paper, 13 indexes based on soil enzymatic activities were discussed (Bastida et al. 2008).

Here we present the outcomes of an extended bibliographic review carried out in order to assess, identify, and interpret possible trends in the response of the main experimental factors studied in MESCOSAGR project: organic fertilization (Table 7.2) and reduced or no tillage (Table 7.3). Among the papers cited in Table 7.1, only the ones which allowed extrapolation of quantitative data were selected.

Fourteen studies dealing with the effects of organic fertilizers on soil enzymatic activities have been reviewed. Twelve enzymatic activities have been considered, namely arylsulphatase, β-glucosidase, phosphatase, fluorescein diacetate hydrolysis activity (FDA), urease, dehydrogenase, invertase, phenoloxidase, catalase, protease, nitrate reductase and amylase. For each study the type and amount of organic fertilizer applied is reported, together with information (when available) on soil texture and taxonomy. Soil textures ranged from sandy to clay, whereas 11 different soil types were considered (Table 7.2).

Arylsulphatase activity is usually assessed by adding a substrate such as *p*-nitrophenylsulphate in soil, and quantifying the amount of *p*-nitrophenol produced in time. Up to our knowledge, it is the only enzyme of the S cycle whose activity is assessed in soil. However, it is considered quite representative of the mineralization of organic S in soils, since sulfate esters represent a large fraction (25–93%) of the soil total S (Elsgaard et al. 2002). Six papers were considered about the effects of compost amendment on arylsulphatase (Table 7.2). Five papers indicated an increase in arylsulphatase activity as a result of organic additions, whereas only one (Abdelbasset et al. 2011) indicated no relevant effects after application of up to 80 ton $ha^{-1}$ of municipal solid waste (MSW) compost to a clayey-loamy soil cropped with *Triticum durum* (although the use of sewage sludge had instead a positive effect). In two of the works, arylsulphate activity was more than doubled as a result of application of 2–4 ton $ha^{-1}$ of MSW compost (Albiach et al. 2000) or of composted red clover corresponding to 416 kg of total N $ha^{-1}$ (Elfstrand et al. 2007a). Two other reports dealt with a maize field amended for several years with MSW compost and sewage sludge (Puglisi et al. 2006) and with the application of compost rates up to 45 ton $ha^{-1}$ in a greenhouse and in an open field under Mediterranean conditions (Iovieno et al. 2009). Finally, Darby et al. (2006) assessed the effects of compost from dairy manure solids (56 ton $ha^{-1}$) on sweet corn plots in Oregon.

β-Glucosidase is one of the enzymatic activities involved in C cycling in soils. It is usually assessed using *p*-nitrophenyl-b-D-galactoside as a substrate, and it thus gives an indication of the activity of enzymes involved in cellulose degradation, specifically in the hydrolysis of β-1,4 bonds in β-glucopiranosides. Eight papers were considered here (Table 7.2). Five of them dealt with MSW compost, one with municipal food waste (MFW) compost, one with compost from manure mixed with leguminous residues, and another one with an unspecified compost. Concentrations considered ranged from 5 to 80 ton $ha^{-1}$. According to five studies, β-glucosidase activity was increased after amendment with compost from MSW (Garcia-Gil et al. 2000; Crecchio et al. 2004; Hojati and Nourbakhsh 2009; Abdelbasset et al. 2011), MFW (Iovieno et al. 2009) and manure mixed with leguminous residues (Laudicina

**Table 7.2** Analysis of literature info on the effects of organic fertilization on soil enzymatic activities

| Enzyme activity | Organic fertilizer applied | Effect | Soil texture | Soil taxonomy |
|---|---|---|---|---|
| S cycle | | | | |
| Arylsulphatase | MSW compost (24 ton $ha^{-1}$ $year^{-1}$) | ⇑⇑ Albiach et al. (2000) | Sandy-silty loam | Xerorthent |
| | Composted dairy manure (56 ton $ha^{-1}$ $year^{-1}$) | ⇑ Darby et al. (2006) | Silty loam | Chealis |
| | MSW compost (25 ton $ha^{-1}$ $year^{-1}$) | ⇑ Puglisi et al. (2006) | Sandy loam | Alluvial hydromorphic |
| | MFW compost (45 ton $ha^{-1}$ in 3 rates) | ⇑ Iovieno et al. (2009) | – | Calcaric Cambisol |
| | Composted red clover (15 ton $ha^{-1}$) | ⇑⇑ Elfstrand et al. (2007a) | Silty clay loam | – |
| | MSW compost (80 ton $ha^{-1}$) | - Abdelbasset et al. (2011) | Clay loam | Mollisol |
| C cycle | | | | |
| β-Glucosidase | MSW compost (80 ton $ha^{-1}$) | ⇑ Garcia-Gil et al. (2000) | Sandy | Typic Haploxeralf |
| | MSW compost (24 ton $ha^{-1}$ $year^{-1}$) | ⇑ Crecchio et al. (2004) | Clay | Typic Chromoxerert |
| | MSW compost (25 ton $ha^{-1}$ $year^{-1}$) | ⇓ Puglisi et al. (2006) | Sandy loam | Alluvial hydromorphic |
| | Compost (5 ton $ha^{-1}$ $year^{-1}$) | ⇓ Nayak et al. (2007) | Sandy clay loam | Aeric Endoaquept |
| | MSW compost (100 ton $ha^{-1}$) | ⇑ Hojati and Nourbakhsh (2009) | Silty clay loam | Typic Haplargid |
| | MFW compost (45 ton $ha^{-1}$ in 3 rates) | - Iovieno et al. (2009) | – | Calcaric Cambisol |
| | Compost from manure (30 ton $ha^{-1}$ $year^{-1}$) | ⇑ Laudicina et al. (2010) | – | Hortic Cambisol |
| | MSW compost (80 ton $ha^{-1}$) | ⇑ Abdelbasset et al. (2011) | Clay loam | Mollisol |
| Invertase | MSW compost (25 ton $ha^{-1}$ $year^{-1}$) | ⇑ Puglisi et al. (2006) | Sandy loam | Alluvial hydromorphic |
| | Compost (5 ton $ha^{-1}$ $year^{-1}$) | ⇓ Nayak et al. (2007) | Sandy clay loam | Aeric Endoaquept |
| Phenoloxidase | MSW compost (25 ton $ha^{-1}$ $year^{-1}$) | ⇑ Puglisi et al. (2006) | Sandy loam | Alluvial hydromorphic |
| Amylase | MSW compost (15 ton $ha^{-1}$) | ⇑ Pramanik et al. (2010) | Clay | Aqualfs |
| P cycle | | | | |
| Phosphatase | MSW compost (24 ton $ha^{-1}$ $year^{-1}$) | ⇑⇑ Albiach et al. (2000) | Sandy-silty loam | Xerorthent |
| | MSW compost (80 ton $ha^{-1}$) | ⇓ Garcia-Gil et al. (2000) | Sandy | Typic Haploxeralf |
| | MSW compost (24 ton $ha^{-1}$ $year^{-1}$) | ⇑ Crecchio et al. (2004) | Clay | Typic Chromoxerert |
| | MFW compost (27 ton $ha^{-1}$) | ⇑⇑ Lee et al. (2004) | – | – |
| | MSW compost (25 ton $ha^{-1}$ $year^{-1}$) | ⇑⇑ Puglisi et al. (2006) | Sandy loam | Alluvial hydromorphic |
| | Composted red clover (15 ton $ha^{-1}$) | ⇑⇑ Elfstrand et al. (2007a) | Silty clay loam | |

(continued)

**Table 7.2** (continued)

| Enzyme activity | Organic fertilizer applied | Effect | Soil texture | Soil taxonomy |
|---|---|---|---|---|
| | MFW compost (45 ton $ha^{-1}$ in 3 rates) | - Iovieno et al. (2009) | – | Calcaric Cambisol |
| | Compost (30 ton $ha^{-1}$ $year^{-1}$) | ⇑ Laudicina et al. (2010) | – | Hortic Cambisol |
| | MSW compost (15 ton $ha^{-1}$) | ⇑ Pramanik et al. (2010) | Clay | Aqualfs |
| | MSW compost (80 ton $ha^{-1}$) | ⇑ Abdelbasset et al. (2011) | Clay loam | Mollisol |
| N cycle | | | | |
| Urease | MSW compost (24 ton $ha^{-1}$ $year^{-1}$) | ⇑ Albiach et al. (2000) | Sandy-silty loam | Xerorthent |
| | MSW compost (80 ton $ha^{-1}$) | - Garcia-Gil et al. (2000) | Sandy | Typic Haploxeralf |
| | MSW compost (24 ton $ha^{-1}$ $year^{-1}$) | ⇑ Crecchio et al. (2004) | Clay | Typic Chromoxerert |
| | MSW compost (6 ton $ha^{-1}$) | ⇑ Bhattacharyya et al. (2005) | – | Typic fluvaquent |
| | MSW compost (25 ton $ha^{-1}$ $year^{-1}$) | - Puglisi et al. (2006) | Sandy loam | Alluvial hydromorphic |
| | Compost (5 ton $ha^{-1}$ $year^{-1}$) | ⇑ Nayak et al. (2007) | Sandy clay loam | Aeric Endoaquept |
| | Compost (30 ton $ha^{-1}$ $year^{-1}$) | ⇑⇑ Laudicina et al. (2010) | – | Hortic Cambisol |
| | MSW compost (15 ton $ha^{-1}$) | ⇑ Pramanik et al. (2010) | Clay | Aqualfs |
| | MSW compost (80 ton $ha^{-1}$) | ⇑ Abdelbasset et al. (2011) | Clay loamy | Mollisol |
| Protease | MSW compost (80 ton $ha^{-1}$) | - Garcia-Gil et al. (2000) | Sandy | Typic Haploxeralf |
| | MSW compost (24 ton $ha^{-1}$ $year^{-1}$) | - Crecchio et al. (2004) | Clay | Typic Chromoxerert |
| | Composted red clover (15 ton $ha^{-1}$) | ⇑⇑ Elfstrand et al. (2007a) | Silty-clay-loam | – |
| | MSW compost (15 ton $ha^{-1}$) | ⇑ Pramanik et al. (2010) | Clay | Aqualfs |
| Nitrate reductase | MSW compost (24 ton $ha^{-1}$ $year^{-1}$) | ⇑ Crecchio et al. (2004) | Clay | Typic Chromoxerert |
| Total microbial activity | | | | |
| FDA | Composted dairy manure (56 ton $ha^{-1}$ $year^{-1}$) | ⇑ Darby et al. (2006) | Silt loam | Chealis |
| | Compost (5 ton $ha^{-1}$ $year^{-1}$) | - Nayak et al. (2007) | Sandy clay loam | Aeric Endoaquept |
| | MFW compost (45 ton $ha^{-1}$ in 3 rates) | ⇑ Iovieno et al. (2009) | – | Calcaric Cambisol |
| Dehydrogenase | MSW compost (24 ton $ha^{-1}$ $year^{-1}$) | ⇑ Albiach et al. (2000) | Sandy-silty loam | Xerorthent |
| | MSW compost (80 ton $ha^{-1}$) | ⇑⇑ Garcia-Gil et al. (2000) | Sandy | Typic Haploxeralf |
| | MSW compost (24 ton $ha^{-1}$ $year^{-1}$) | ⇑ Crecchio et al. (2004) | Clay | Typic Chromoxerert |
| | MFW compost (27 ton $ha^{-1}$) | ⇑⇑ Lee et al. (2004) | – | – |

(continued)

**Table 7.2** (continued)

| Enzyme activity | Organic fertilizer applied | Effect | Soil texture | Soil taxonomy |
|---|---|---|---|---|
| | MSW compost (25 ton $ha^{-1}$ $year^{-1}$) | ⇑ Puglisi et al. (2006) | Sandy loam | Alluvial hydromorphic |
| | Compost (5 ton $ha^{-1}$ $year^{-1}$) | ⇑ Nayak et al. (2007) | Sandy clay loam | Aeric Endoaquept |
| | Compost (30 ton $ha^{-1}$ $year^{-1}$) | ⇑ Laudicina et al. (2010) | – | Hortic Cambisol |
| | MSW compost (80 ton $ha^{-1}$) | ⇑ Abdelbasset et al. (2011) | Clay loamy | Mollisol |
| Catalase | MSW compost (80 ton $ha^{-1}$) | ⇑ Garcia-Gil et al. (2000) | Sandy | Typic Haploxeralf |
| | MSW compost (80 ton $ha^{-1}$) | ⇑ Abdelbasset et al. (2011) | Clay loamy | Mollisol |

For each enzymatic activity the referred biogeochemical cycle is reported. (⇑, ⇓ Increase or decrease of less than 100%; ⇑⇑, ⇓⇓ increase or decrease of more than 100%; - no effect)

et al. 2010). Differently from what reported above for arylsulphatase, no differences were found in β-glucosidase activity according to Iovieno et al. (2009). Conversely, Puglisi et al. (2006) and Nayak et al. (2007) even found a slight but significant decrease, possibly due to presence of toxic trace elements in MSW compost and to the low amount applied (5 ton $ha^{-1}$), respectively.

Another important enzymatic activity involved in soil C cycling is invertase. As for urease, for which urea is the substrate, invertase is the only other hydrolase assessed using its natural substrate, namely sucrose (Speir et al. 2002). Only two papers dealing with effects of compost on invertase activity were found in literature (Table 7.2). Puglisi et al. (2006) found that in a sandy loam soil amended with 25 ton $ha^{-1}$ of MSW compost, invertase was significantly enhanced, while according to Nayak et al. (2007) invertase activity was instead reduced in a soil of similar texture (sandy clay loam). In the latter, however, only 5 ton $ha^{-1}$ of compost were tested, and the presence of clays might as well played a role in adsorbing the enzyme and thus reducing its activity (Gianfreda et al. 1991).

Another C cycling enzymatic activity considered here was phenoloxidase, involved in organic matter degradation. Only one study (Puglisi et al. 2006) was found that showed a significant increase in phenoloxidase activity after compost amendment. It was also found that another C cycling enzyme (amylase, responsible for starch degradation) was increased by compost addition at 15 ton $ha^{-1}$ (Pramanik et al. 2010).

Phosphatases are key enzymes controlling phosphorus turnover and availability for plants. Assayed after addition of the synthetic substrate *p*-nitrophenylphosphate to soil samples, phosphatases control the transformation of organic P to inorganic P through dephosphorylation processes. These enzymes can be assessed under either alkaline or acidic conditions. Alkaline phosphatases are mostly of microbial origin, while acid phosphatases are more of plant or fungal origin, though this is not a strict difference and conditions may differ from soil to soil. Ten scientific papers were considered here (Table 7.2): seven reports analysed acid phosphatase, two of them

discussed alkaline phosphatase (Albiach et al. 2000; Abdelbasset et al. 2011), while only one (Lee et al. 2004) evaluated both forms. Most papers (eight) analysed the effects of MSW compost (rates ranging from 15 to 80 ton $ha^{-1}$), one paper described the effect of composted red clover (Elfstrand et al. 2007a) and another one reported effects of an unspecified compost (Laudicina et al. 2010). In eight out of ten papers, phosphatase activities were significantly induced by compost addition. A significant reduction was instead found by Garcia-Gil et al. (2000) for a low organic matter sandy soil amended with an MSW compost, though contaminated with significant levels of Zn (1325 mg $kg^{-1}$), Cu (548 mg $kg^{-1}$), Ni (81 mg $kg^{-1}$) and Pb (681 mg $kg^{-1}$). The authors attributed this phosphatase inhibition to trace elements level and to the large content of soluble P in the amended soil, in agreement with evidence showing an inhibitory effect of inorganic P on phosphatases (Spiers and McGill 1979).

Urease activity is at the basis of nitrogen turnover and soil fertility. Being assessed through determination of the ammonium liberated after soil addition with urea, this enzyme plays a central role in organic nitrogen mineralization, as it is the gateway for nitrification. Urease is sensitive to a number of environmental parameters such as oxygen and trace elements, and it is well correlated with soil quality (Badiane et al. 2001; Coppolecchia et al. 2010). Nine studies were considered here to assess general trends on the effects of organic amendments on urease activity (Table 7.2). Most of these studies have been already cited for the enzymatic activities discussed above. Seven out of nine studies used MSW compost as fertilizer, two of them used an unspecified compost. Amendment rates ranged from 5 to 80 ton $ha^{-1}$, and, as for other enzymes, a wide range of soil textural types were considered. In most cases, urease activity was significantly increased by compost, and in one case (Laudicina et al. 2010) more than doubled. In one case (Garcia-Gil et al. 2000; Puglisi et al. 2006), no significant effect was found, while in another work (Garcia-Gil et al. 2000) a significant inhibition of urease from MSW compost was even found. These effects did not seem to be related to compost rates, while Nayak et al. (2007) showed significant increase with a rate of only 5 ton $ha^{-1}$. The inhibition of urease activity can be due to the presence of trace elements as for other enzymes, or, as suggested by Garcia-Gil et al. (2000), to the large content of $NH_4^+$ (a urease inhibitor) produced by the activity of proteases present in the MSW compost-amended soil.

Proteases belong to a large family of soil enzymes, and, depending on the substrate used for determination, different protease activities can be assayed. We considered four papers about the effects of compost on soil protease activities (Table 7.2). The used substrates were Nα-benzoyl-argininamide (Garcia-Gil et al. 2000; Crecchio et al. 2004), Na-caseinate (Pramanik et al. 2010), and caseine (Elfstrand et al. 2007a). It was found that protease activity was induced by 15 ton $ha^{-1}$ of composted red clover (Elfstrand et al. 2007a) and 15 ton $ha^{-1}$ of MSW compost (Pramanik et al. 2010), while according to Crecchio et al. (2004) and Garcia-Gil et al. (2000), the rates of 80 and 24 ton $ha^{-1}$ of MSW compost did not cause any significant change in protease activities, respectively.

The assessment of nitrate reductase activity in soil can give an important indication of denitrification. Also this activity is enhanced by compost amendment, thus confirming that organic matter addition stimulates microorganism involved in very different cycles (Crecchio et al. 2004).

Fluorescein diacetate (FDA) is a substrate that can be hydrolysed by a variety of nonspecific enzymes and the assessment of FDA hydrolysis activity is thus used as an indicator of total microbial activity in soil (Perucci 1992). Three papers on the effects of composts on FDA in soil were considered here (Table 7.2). It was found that 56 ton $ha^{-1}$ of composted dairy manure (Darby et al. 2006) and 45 ton $ha^{-1}$ of MFW compost (Iovieno et al. 2009) significantly increased soil FDA, while a lower dose of 5 ton $ha^{-1}$ of compost induced no significant changes (Nayak et al. 2007).

Dehydrogenases are ubiquitous in all intact, viable microbial cells: the estimation of dehydrogenase activity as determined by the reduction of 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl-tetrazolium chloride (INT) to iodo-nitrophenyl formazan (INTF) is thus widely used as an estimation of soil total microbial activity (Lagomarsino et al. 2009a). In accordance with the results reported above for the other enzymatic activity used to estimate total microbial activity such as FDA, it was found also through the analysis of papers dealing with the dehydrogenase that compost has a general effect of increase of soil microbial activity. Eight papers were considered (Table 7.2), and all of them showed a significant increase (in some cases more than a doubling) of dehydrogenase as the result of the application of doses of compost ranging from 5 to 80 ton $ha^{-1}$, on different textures and soil types.

Catalase is the third enzymatic indicator of total microbial activity considered here, and it consists of an oxido-reductase associated with aerobic microbial activity (Rodriguez-Kabana and Truelove 1982). Two papers dealing with the effects of compost on soil catalase were found (Garcia-Gil et al. 2000; Abdelbasset et al. 2011), and in line with FDA and dehydrogenase results, they confirmed the stimulation of microbial activity by compost.

Similarly to the effects of organic amendments, also the effects of tillage on soil enzymatic activities were searched in the scientific literature (Table 7.3). Most of the enzymatic activities discussed above in detail have been also considered for tillage effects. Eleven papers in total have been reviewed, covering different soil textural types. Though both no tillage and reduced tillage were included in the bibliographic query, the vast majority of studies considered solely no tillage. Only one (Ramos et al. 2011) assessed the effect of reduced tillage by chisel ploughing on arylsulphatase, β-glucosidase, phosphatase and dehydrogenase activities.

The effect of no tillage in inducing enzymatic activities was even greater and clearer than that observed for amendment of organic materials. Among ten enzymatic activities, nine were generally increased by no tillage: arylsulphatase (5/7), β-glucosidase (6/8), phenoloxidase (1/1), catalase (1/1), phosphatase (8/8), urease (3/4), invertase (1/1), dehydrogenase (6/8) and protease (2/2). In all other cases, no significant difference was reported. In the case of FDA, only one study was found (Nsabimana et al. 2004) that showed no significant differences between control and no till plots for a clay Rhodic Ferrisol.

**Table 7.3** Analysis of literature info on the effects of tillage on soil enzymatic activities

| Enzyme activity | Tillage | Effect | Soil texture | Soil taxonomy |
|---|---|---|---|---|
| S cycle | | | | |
| Arylsulphatase | No tillage | ⇑ Nsabimana et al. (2004) | Clay | Rhodic Ferisol |
| | No tillage | - Mijangos et al. (2006) | Clay loam | – |
| | No tillage (direct drilling) | ⇑ Melero et al. (2008) | Clay | Chromic Haploxeret |
| | No tillage | ⇑ Lagomarsino et al. (2009b) | Silt–clay | Calcaric Gleyic Cambisol |
| | No tillage | ⇑ Mikanovà et al. (2009) | Clay loam | Orthic Luvisol |
| | No tillage | ⇑ Zhang et al. (2010) | – | – |
| | Reduced tillage (chisel ploughing) | - Ramos et al. (2011) | Clay loam | Hypercalcic Calcisol |
| C cycle | | | | |
| β-Glucosidase | No tillage | ⇑ Mijangos et al. (2006) | Clay loam | – |
| | No tillage (direct drilling) | ⇑⇑ Melero et al. (2008) | Clay | Chromic Haploxeret |
| | No tillage | - Mina et al. (2008) | Sandy clay loam | – |
| | No tillage | ⇑⇑ Lagomarsino et al. (2009b) | Silt–clay | Calcaric Gleyic Cambisol |
| | No tillage (mouldboard ploughing) | ⇑ Lopez-Garrido et al. (2010) | – | Eutric Leptosol |
| | No tillage | ⇑⇑ Ulrich et al. (2010) | Sandy loam | Stagnic Luvisol |
| | No tillage | - Zhang et al. (2010) | – | – |
| | Reduced tillage (chisel ploughing) | ⇑ Ramos et al. (2011) | Clay loam | Hypercalcic Calcisol |
| Phenoloxidase | No tillage (mouldboard ploughing) | ⇑ Lopez-Garrido et al. (2010) | – | Eutric Leptosol |
| Invertase | No tillage | ⇑ Mikanovà et al. (2009) | Clay loam | Orthic Luvisol |
| P cycle | | | | |
| Phosphatase | No tillage | ⇑ Nsabimana et al. (2004) | Clay | Rhodic Ferisol |
| | No tillage | ⇑ Mijangos et al. (2006) | Clay loam | – |
| | No tillage | ⇑ Roldan et al. (2007) | Clay | Vertisol |
| | No tillage (direct drilling) | ⇑ Melero et al. (2008) | Clay | Chromic Haploxeret |
| | No tillage | ⇑ Mina et al. (2008) | Sandy clay loam | – |
| | No tillage | ⇑⇑ Lagomarsino et al. (2009b) | Silt–clay | Calcaric Gleyic Cambisol |

(continued)

**Table 7.3** (continued)

| Enzyme activity | Tillage | Effect | Soil texture | Soil taxonomy |
|---|---|---|---|---|
| | No tillage | ⇑⇑ Zhang et al. (2010) | – | – |
| | Reduced tillage (chisel ploughing) | ⇑ Ramos et al. (2011) | Clay loam | Hypercalcic Calcisol |
| N cycle | | | | |
| Urease | No tillage | - Mina et al. (2008) | Sandy clay loam | – |
| | No tillage | ⇑ Mikanovà et al. (2009) | Clay loam | Orthic Luvisol |
| | No tillage | ⇑ Zhang et al. (2010) | – | – |
| | No tillage | ⇑ Qin et al. (2010) | Silt loam | Haplic Cambisol |
| Protease | No tillage | ⇑ Mina et al. (2008) | Sandy clay loam | – |
| | No tillage | ⇑⇑ Zhang et al. (2010) | – | – |
| Total microbial activity | | | | |
| FDA | No tillage | - Nsabimana et al. (2004) | Clay | Rhodic Ferisol |
| Dehydrogenase | No tillage | - Nsabimana et al. (2004) | Clay | Rhodic Ferisol |
| | No tillage | ⇑ Mijangos et al. (2006) | Clay loam | – |
| | No tillage | ⇑ Roldan et al. (2007) | Clay | Vertisol |
| | No tillage (direct drilling) | ⇑⇑ Melero et al. (2008) | Clay | Chromic Haploxeret |
| | No tillage | ⇑ Mina et al. (2008) | Sandy clay loam | – |
| | No tillage | - Mikanovà et al. (2009) | Sandy clay loam | – |
| | No tillage (mouldboard ploughing) | ⇑ Lopez-Garrido et al. (2010) | – | Eutric Leptosol |
| | Reduced tillage (chisel ploughing) | ⇑ Ramos et al. (2011) | Clay loam | Hypercalcic Calcisol |
| Catalase | No tillage | ⇑⇑ Ulrich et al. (2010) | Sandy loam | Stagnic Luvisol |

For each enzymatic activity the referred biogeochemical cycle is reported. (⇑, ⇓ Increase or decrease of less than 100%; ⇑⇑, ⇓⇓ increase or decrease of more than 100%; - no effect)

## 7.3 PLFAs and Soil Carbon Sequestration Strategies

The determination of soil phospholipid fatty acids (PLFAs) is a powerful and still widely used method to assess the structure of viable microbial communities in soils. Phospholipids are at the basis of life itself, since they represent the structural skeleton of most living cells. The double layer of phospholipids found in most cells (exception

represented by Archaea, where tetraether lipids substitute phospholipids) allows the separation of living cells from the surrounding environment, and regulates, together with proteins, sterols and glycolipids, the exchanges between cells and their outside. Phospholipids are made up by a hydrophilic head constituted by a negatively charged phosphate group, usually substituted with a choline, and a hydrophobic tail, usually constituted by two fatty acids. These fatty acids belong to different classes, the most common ones being saturated, monounsaturated, polyunsaturated, branched, and cyclopropanic (Fig. 7.1). As explained below, fatty acids composition of cell membranes differs from species to species (and thus can also be used for community structure assessments), and, within a single species, it is sensitive to a number of environmental parameters (e.g., temperature, nutrients, pollutants).

Bligh and Dyer (1959) published the first method for the identification and estimation of individual phospholipids in biological samples. The rationale of the method is based on isolation of the phospholipidic fraction, and removal from

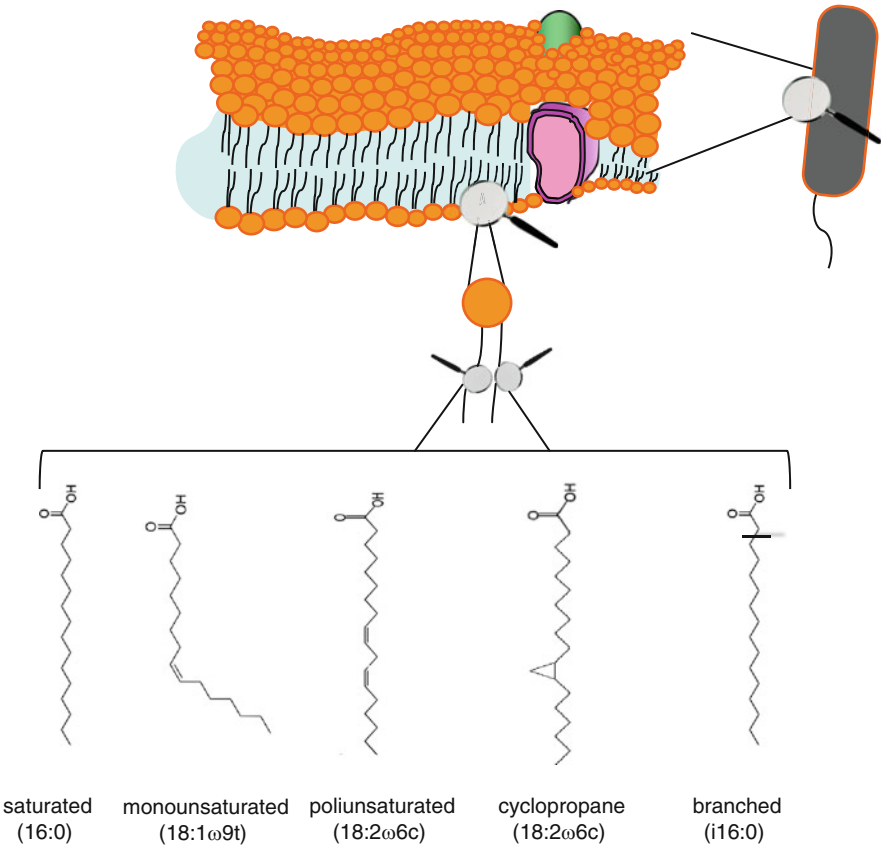


**Fig. 7.1** Schematic representation of a microbial cell, its double layer phospholipidic membrane and examples of the most important classes of phospholipids fatty acids

phospholipids of single fatty acids by an alkaline hydrolysis. Then, a method was devised for the conversion of fatty acids in methyl esters and their easy determination by silica chromatographic analyses (Luddy et al. 1960). Marr and Ingraham (1962) assessed the composition of individual PLFAs in *Escherichia coli* cells grown at 10, 15, 20, 25, 30, 35, 40 and 43°C. They found that the increase of temperature resulted in an increase in hexadecanoic acid (16:0) and a decrease in unsaturated acids such as hexadecenoate (16:1). Furthermore, they found that the composition in fatty acids reflects the composition of the growth medium, but does not correlate with the culture growth stage. This was a milestone paper since it casted the basis of PLFAs use as ecological biomarkers of environmental conditions. Moreover, derived concepts such as the ratio between specific saturated and unsaturated fatty acids as index of specific conditions are still fundamental in the environmental extension of PLFA studies. PLFAs have been also used for the ecological assessment of microbial communities in marine and estuarine sediments (White et al. 1979), and, later, for the more complex soil microbial communities (Tunlid et al. 1985; Nichols et al. 1986; Vestal and White 1989).

Despite the introduction in later years of more advanced methods (especially those based on nucleotides), PLFAs analysis remains a widely used and useful method for the analyses of soil microbial communities. An enquire in SCOPUS using "soil*" and "PLFA*" as a search key among titles, keywords and abstracts gives a total of 819 papers between 1987 and today, with the number of papers per year almost constant in the last years (Table 7.1). Similarly to the case of enzymatic activities, Table 7.1 shows how tillage and organic fertilization represent only a part (around 10%) of the effects studied in the scientific literature.

A number of reasons make PLFAs still a very useful and informative method. First, fatty acids are easily degraded after cells death and, thus, their analyses give a snapshot on the total viable microbial communities. This is a full advantage, since in DNA-based analyses it is often difficult if not impossible to distinguish between living and dead cells. The second feature is related to the fact that specific PLFAs analysis (Table 7.4) can provide reliable information about microbial groups such as Gram-positive and Gram-negative bacteria, actinomycetes, fungi, protozoa, arbuscural mycorrhiza and total fungi (Zelles 1999; Bougnom et al. 2010).

**Table 7.4** PLFA biomarkers and their use for the analysis of specific groups and conditions

| Bacterial group/environmental condition | Biomarker PLFAs |
|---|---|
| Gram-positive bacteria | Sum of i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0 |
| Gram-negative bacteria | Sum of cy17:0, cy19:0, 18:1ω9c, 16:1ω9c, 18:1ω9c, 15:1ω4c and 18:1ω7c |
| Arbuscural mycorrhiza | 16:1ω5c |
| Protozoa | Sum of 20:2ω6,9,c, 20:3ω6,9,12c and 20:4ω6,9,12,15c |
| Actinomycetes | 10Me16:0 and 10Me18:0 |
| Fungal biomass | 18:2ω6c and 18:1ω9 |
| Total microbial biomass | 16:0 |
| Stress conditions | cy19:0/18:1ω7c ratio |

Furthermore, some ratios between specific PLFAs have been proposed as indicators of stress conditions, and a numerical index of soil alteration based on PLFA has also been proposed (Puglisi et al. 2005).

A bibliographic search on PLFA values, as the one described above for enzymatic activities, is reported for the effects of organic amendments to soil (Table 7.5) and for those of soil tillage practices (Table 7.6). As it is indicated in each retrieved paper, aggregated PLFA data have been reported in order to refer to specific groups (i.e., actinobacteria, Gram-positive, Gram-negative, fungi and arbuscural mycorrhiza).

Five papers dealt with the effects of organic fertilizers on PLFAs (Table 7.5). The organic fertilizer additions were: composted red clover at 15 ton $ha^{-1}$ (Elfstrand et al. 2007a), farmyard manure at 4 ton of carbon $ha^{-1}$ (Elfstrand et al. 2007b), wood ash compost mixed with soil at 33% v:v ratio (Bougnom et al. 2010), compost at 10 ton $ha^{-1}$ (Treonis et al. 2010), and composted manure at 373 kg of nitrogen $ha^{-1}$ (Kong et al. 2011). Actinobacteria was the only microbial group that was not affected by organic fertilization, whereas significant changes in the community structure were found for all other groups. Specifically, fungi were always increased (five out of five studies), arbuscural mycorrhiza were increased in four studies and unaffected in one report, while total microorganisms (as estimated by the sum of all PLFAs) were significantly increased in four out of four studies.

Contrasting results about Gram-positive and Gram-negative bacteria were shown in these works. No significant differences for both groups were found in the study of Elfstrand et al. (2007b), while both groups were increased after the organic fertilization conducted by Treonis et al. (2010) and Bougnom et al. (2010). Finally, Kong et al. (2011) reported a decrease in both bacterial groups after addition of composted manure.

Five papers dealing with the effects of soil tillage on PLFAs have also been reviewed (Table 7.6). All these papers compared no tillage versus conventional tillage. No significant reductions on specific microbial groups were experimentally found, although results indicated an increasing trend in some cases. These findings were common for each microbial group, thus suggesting that effects of no tillage on microbial communities structure was quite site-specific, and most probably affected by general variables such as climate, soil type and cultivation. An exception was represented by Gram-positive bacteria, whose numbers were never affected by soil tillage treatments.

## 7.4 The MESCOSAGR Case Study

The MESCOSAGR project aimed at assessing the sustainability of methods for soil carbon sequestration (see Foreword) and their effects on soil physical, chemical, biological parameters as well as crop productivity. Two innovative methods have been compared with traditional and reduced tillage methods (see Chap. 1). The innovative methods were: the soil amendment with mature humified compost to promote a hydrophobic protection against microbial degradation of the more easily

**Table 7.5** Analysis of literature info on the effects of organic fertilizers on soil PLFAs

| Microbial group | Organic fertilizer applied | Effect | Soil texture | Soil taxonomy |
|---|---|---|---|---|
| Total microorganisms | Composted red clover (15 ton $ha^{-1}$) | ⇑ Elfstrand et al. (2007a) | Silty clay loam | – |
| | Farmyard manure (4 ton C $ha^{-1}$) | ⇑ Elfstrand et al. (2007b) | Clay loam | Eutric Cambisol |
| | Wood ash compost (33%) | ⇑⇑ Bougnom et al. (2010) | Clay | – |
| | Compost (10 ton $ha^{-1}$) | ⇑ Treonis et al. (2010) | Loamy sand | – |
| Actinobacteria | Composted manure (373 kg N $ha^{-1}$) | - Kong et al. (2011) | Silt loam | Typic Xerorthent |
| | Compost (10 ton $ha^{-1}$) | - Treonis et al. (2010) | Loamy sand | – |
| Gram-positive | Farmyard manure (4 ton C $ha^{-1}$) | - Elfstrand et al. (2007b) | Clay loam | Eutric Cambisol |
| | Compost (10 ton $ha^{-1}$) | ⇑ Treonis et al. (2010) | Loamy sand | – |
| | Wood ash compost (33%) | ⇑⇑ Bougnom et al. (2010) | Clay | – |
| | Composted manure (373 kg N $ha^{-1}$) | ⇓ Kong et al. (2011) | Silt loam | Typic Xerorthent |
| Gram-negative | Farmyard manure (4 ton C $ha^{-1}$) | - Elfstrand et al. (2007b) | Clay loam | Eutric Cambisol |
| | Compost (10 ton $ha^{-1}$) | ⇑ Treonis et al. (2010) | Loamy sand | – |
| | Wood ash compost (33%) | ⇑⇑ Bougnom et al. (2010) | Clay | – |
| | Composted manure (373 kg N $ha^{-1}$) | ⇓ Kong et al. (2011) | Silt loam | Typic Xerorthent |
| Fungi | Composted manure (373 kg N $ha^{-1}$) | ⇑ Kong et al. (2011) | Silt loam | Typic Xerorthent |
| | Composted red clover (15 ton $ha^{-1}$) | ⇑ Elfstrand et al. (2007a) | Silty clay loam | – |
| | Farmyard manure (4 ton C $ha^{-1}$) | ⇑ Elfstrand et al. (2007b) | Clay loam | Eutric Cambisol |
| | Wood ash compost (33%) | ⇑⇑ Bougnom et al. (2010) | Clay | – |
| | Compost (10 ton $ha^{-1}$) | ⇑⇑ Treonis et al. (2010) | Loamy sand | – |
| Arbuscural mycorrhiza | Composted red clover (15 ton $ha^{-1}$) | - Elfstrand et al. (2007a) | Silty clay loam | – |
| | Farmyard manure (4 ton C $ha^{-1}$) | ⇑ Elfstrand et al. (2007b) | Clay loam | Eutric Cambisol |
| | Wood ash compost (33%) | ⇑⇑ Bougnom et al. (2010) | Clay | – |
| | Compost (10 ton $ha^{-1}$) | ⇑ Treonis et al. (2010) | Loamy sand | – |

⇑, ⇓ Increase or decrease of less than 100%; ⇑⇑, ⇓⇓ increase or decrease of more than 100%; - no effect

**Table 7.6** Analysis of literature info on the effects of tillage on soil PLFAs

| Microbial group | Tillage | Reduced/no tillage effects | Soil texture | Soil taxonomy |
|---|---|---|---|---|
| Total microorganisms | No tillage | ⇑ Chaer et al. (2009) | Loamy | Typic Fragiudults |
| | No tillage | ⇑ Helgason et al. (2010b) | – | – |
| | No tillage | - Treonis et al. (2010) | Loamy sand | – |
| Actinobacterial | No tillage | - Treonis et al. (2010) | Loamy sand | – |
| Gram-positive | No tillage | - Muruganandam et al. (2009) | Sandy clay loam | Typic Kanhapludult |
| | No tillage | - Helgason et al. (2010a) | – | – |
| | No tillage | - Treonis et al. (2010) | Loamy sand | – |
| Gram-negative | No tillage | ⇑ Chaer et al. (2009) | Loamy | Typic Fragiudults |
| | No tillage | - Muruganandam et al. (2009) | Sandy clay loam | Typic Kanhapludult |
| | No tillage | - Helgason et al. (2010a) | – | – |
| | No tillage | - Treonis et al. (2010) | Loamy sand | – |
| Fungi | No tillage | ⇑ Muruganandam et al. (2009) | Sandy clay loam | Typic Kanhapludult |
| | No tillage | ⇑ Helgason et al. (2010b) | – | – |
| | No tillage | - Treonis et al. (2010) | Loamy sand | – |
| Arbuscural mycorrhiza | No tillage | ⇑ Helgason et al. (2010a) | – | – |
| | No tillage | - Treonis et al. (2010) | Loamy sand | – |
| | No tillage | ⇑ van Groenigen et al. (2010) | Sandy loam | Haplic Luvisol |
| Stress biomarker cy19:0/ 18:1ω7c | No tillage | ⇑ Chaer et al. (2009) | Loamy | Typic Fragiudults |
| | No tillage | - Helgason et al. (2010a) | – | – |

⇑, ⇓ Increase or decrease of less than 100%; ⇑⇑, ⇓⇓ Increase or decrease of more than 100%; - No effect

degradable soil organic matter fraction, and an in situ photo-polymerization of soil organic matter catalyzed by a water-soluble iron–porphyrin catalyst spread on soil.

The analytical methods described above (enzymatic activities and PLFA analyses) have been applied in order to assess any possible effects of the tested practices on the biochemical quality of soils as assessed by measures of the activity (enzymatic analyses) and structure (PLFAs) of soil microbial communities.

Experiments were conducted for three consecutive years in the four different Italian locations described in the other chapters: Napoli, Torino, Piacenza and Potenza. The following treatments have been compared at each site:

- MIN: minimum tillage and mineral fertilization with urea (130 kg N $ha^{-1}$)
- COM2: second rate of compost (20 ton $ha^{-1}$, corresponding to 260 kg N $ha^{-1}$)
- TRA: traditional ploughing and mineral fertilization with urea (130 kg N $ha^{-1}$)
- CAT: catalyst treatment (1 g $m^{-2}$ of catalyst porphyrins, traditional ploughing and mineral fertilization with urea at 130 kg N $ha^{-1}$)
- No-CAT: no catalyst treatment

In the Piacenza site, all treatments were under maize cropping. In the Napoli and Torino sites, the MIN, COM2 and TRA treatments were for soil under maize,

whereas CAT and No-CAT treatments were adopted under wheat. Finally, in the Potenza site, a specific comparison between TRA, COM2 and COM1 (equal to COM2 but with half compost rate) treatments were conducted under sorghum. Specific details about soil and climatic conditions in each site are reported in Chap. 3.

At each location, soil samples were collected after harvesting every year for three consecutive years (2006, 2007 and 2008). All samples have been sieved at 2 mm immediately after sampling and stored at 4°C for maximum 2 months until analysed.

Four enzymatic activities were determined. β-Glucosidase (E.C. 3.2.1.21) and phosphatase (E.C. 3.1.2.1) were analysed according to Eivazi and Tabatabai (1990) and Sannino and Gianfreda (2001), using, respectively, *p*-nitrophenyl-β-D-glucoside and *p*-nitrophenylphosphate as substrates; urease (E.C. 3.5.1.5) and invertase (E.C. 3.2.1.26) were determined according to Kandeler and Gerber (1988) and Sannino and Gianfreda (2001), using urea and saccharose as substrates. Enzymatic activities were expressed as μmol (for β-glucosidase, phosphatase and invertase) or μg (for urease) of substrate hydrolyzed per hour and per g of dry soil.

The analysis of PLFAs was conducted according to the original Bligh and Dyer (1959) method, as modified by Ibekwe and Kennedy (1998). The lipidic phase was extracted from soil by a mixture of methanol, dichloromethane and sodium bromide. Phospholipids were separated from glycerolipids and neutralipids on columns, and transesterified by saponification. The obtained fatty acid methyl esters (FAME) were then determined with an Agilent 5973N GC, equipped with a 30 m × 0.25 mm ID cross-linked methyl silicone (0.25 μm film thickness) HP-5-MS capillary column. A splitless injection was employed (injector at 280°C) and the oven was held at 70°C for 2 min after injection. The oven temperature was then ramped to 160°C at 40°C $min^{-1}$, and again to 280°C at 3°C $min^{-1}$, using helium as carrier gas (1 ml $min^{-1}$). The run lasted for 40 min, long enough to allow column elution of fatty acids up to 26 carbons. Each PLFA was identified by comparing both retention time and mass spectra with analytical standards. Concentrations were quantified from peak areas of representative ions injected every five samples, after linear interpolation of standards at concentrations increasing from 0.1 to 15 mg $kg^{-1}$. The concentration of each PLFA was normalized to the 19:0 fatty acid, used as internal standard.

Results for the last year of experimentation are reported here and discussed in order to provide information about the cumulative effects of 3 years of C sequestration strategies. Statistical analyses were carried out by SAS software (1995). A mixed model analysis of variance (ANOVA, PROC MIXED, SAS) was applied. In a mixed model ANOVA, one or more factors are defined as *fixed*, and one or more as *random*. A *fixed* factor has levels that are determined by the operator, while a *random* factor has levels that are chosen randomly from the population of all possible levels (Sit 1995). A main effect to be detected and evaluated is normally a *fixed* factor, while an effect that contributes to the data spreading is a *random* one. In this work, the classification variable TREAT, that is the effect of soil C sequestration strategy (classification levels MIN, TRA, COM1, COM2, CAT and

No-CAT) has been treated as a *fixed* variable, whereas SITE (classification levels Piacenza, Torino, Napoli and Potenza) was assumed as a random one. The interaction TREAT*SITE was *random* as well. Significant effects were confirmed and further investigated for specific differences between class levels by Tukey's test for comparison of means by assessing the effect of treatment separately per each site. The ANOVA results are reported in Table 7.7 for four soil enzymatic activities and for soil microbial groups estimated by PLFAs analysis (total microorganisms, Gram-positive, Gram-negative, protozoa, fungi, actinomycetes).

Both SITE and TREAT*SITE effects were significant for β-glucosidase, whereas TREAT*SITE was the only significant effect for both phosphatase and urease (Table 7.7). Finally, both SITE and TREAT*SITE effects were significant for invertase. The SITE effect was confirmed by the Tukey's test, with data for Piacenza and Potenza being significantly larger than for Napoli and Torino.

**Table 7.7** Application of a mixed model analysis of variance to enzymatic activities and PLFA data from the different sites of MESCOSAGR

| | TREAT | SITE | TREAT*SITE |
|---|---|---|---|
| β-Glucosidase | $0.84^{ns}$ | 0.65** | 7.78*** |
| Phosphatase | $0.14^{ns}$ | $1.85^{ns}$ | 3.41** |
| Urease | $1.29^{ns}$ | $3.76^{ns}$ | 3.72** |
| Invertase | $0.86^{ns}$ | 14.19** | 6.09*** |
| Total microorganisms | $1.31^{ns}$ | $1.33^{ns}$ | $0.76^{ns}$ |
| Gram-positive | 0.66 | $3.61^{ns}$ | 2.37* |
| Gram-negative | $1.42^{ns}$ | $1.29^{ns}$ | $1.90^{ns}$ |
| Protozoa | $1.23^{ns}$ | $0.82^{ns}$ | $0.84^{ns}$ |
| Fungi | $0.19^{ns}$ | $0.19^{ns}$ | $2.75^{*}$ |
| Actinomycetes | $0.77^{ns}$ | 4.95* | $1.93^{ns}$ |

Per each effect $F$ values are reported. TREAT is the effect of soil C sequestration strategy (classification levels MIN, TRA, COM1, COM2, CAT and NO-CAT), SITE represents the location (classification levels Piacenza, Torino, Napoli and Potenza) and TREAT*SITE their interaction

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, $^{ns}$nonsignificant

Specific differences per each enzymatic activity were further analysed by assessing, through the Tukey's test, the effect of treatments separately at each sampling location. For β-glucosidase, MIN values were found significantly larger than all other treatments in Piacenza, while the enzyme's activity of MIN was still greater than TRA in the Napoli site (Fig. 7.2). Again, MIN provided larger phosphatase (Fig. 7.3) and urease values (Fig. 7.4) than other treatments in Piacenza, whereas no significant differences were instead found among treatments for the other sites. Similar findings were also shown for invertase in Piacenza (Fig. 7.5), while both CAT and No-CAT provided larger enzyme values than TRA and COM2 treatments in Torino. However, it should be highlighted that in Torino, differently from Piacenza, the CAT and No-CAT trials were conducted under wheat, while MIN, TRA and COM2 were under maize.

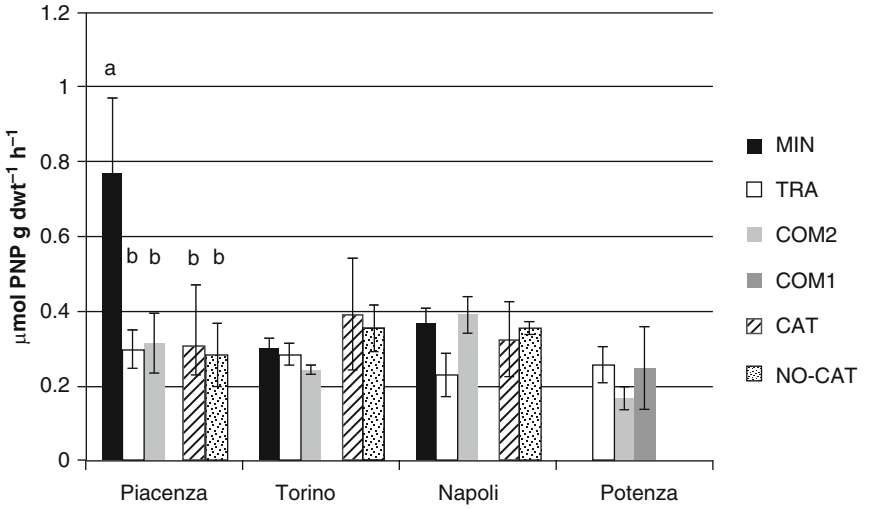


**Fig. 7.2** β-Glucosidase activity in 2008 in the MESCOSAGR sites of Piacenza, Torino, Napoli and Potenza. Statistical differences between treatments were assessed separately within each site by ANOVA. In sites where significant differences were found, specific differences were indicated by Tukey's test for comparison of means

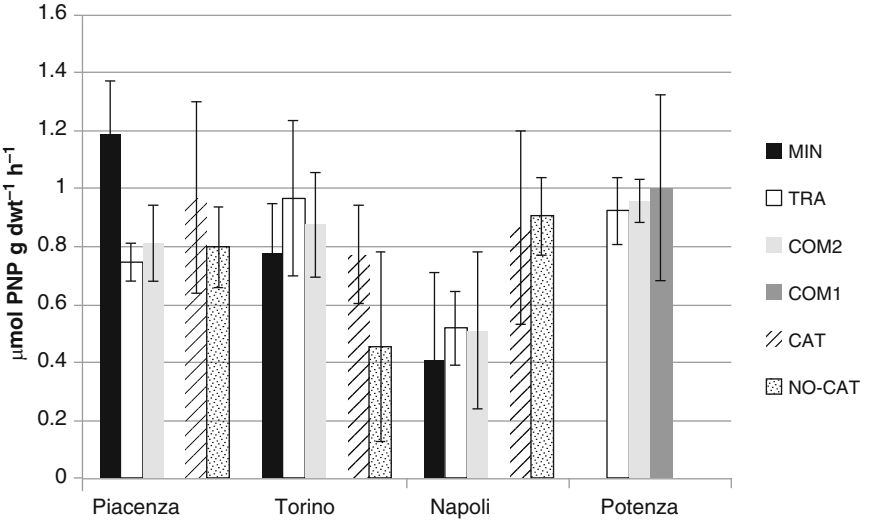


**Fig. 7.3** Phosphatase activity in 2008 in the MESCOSAGR sites of Piacenza, Torino, Napoli and Potenza. Statistical differences between treatments were assessed separately within each site by ANOVA. In sites where significant differences were found, specific differences were indicated by Tukey's test for comparison of means

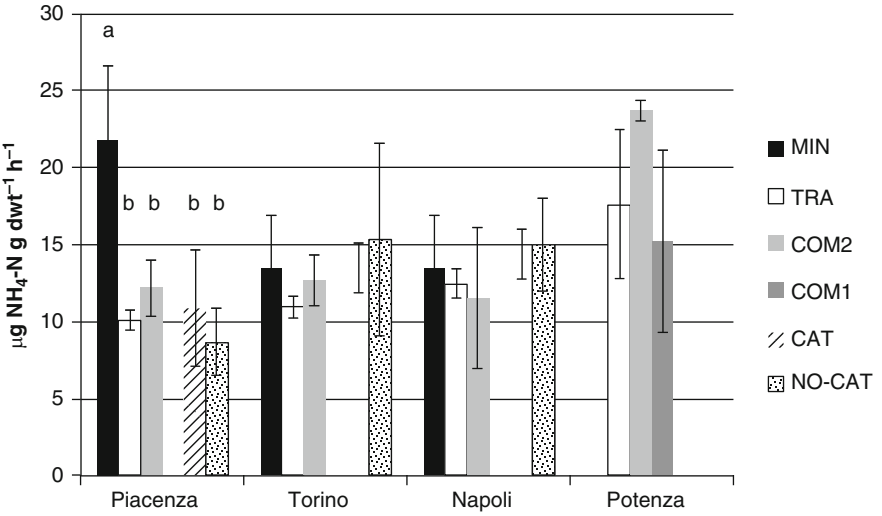


**Fig. 7.4** Urease activity in 2008 in the MESCOSAGR sites of Piacenza, Torino, Napoli and Potenza. Statistical differences between treatments were assessed separately within each site by ANOVA. In sites where significant differences were found, specific differences were indicated by Tukey's test for comparison of means

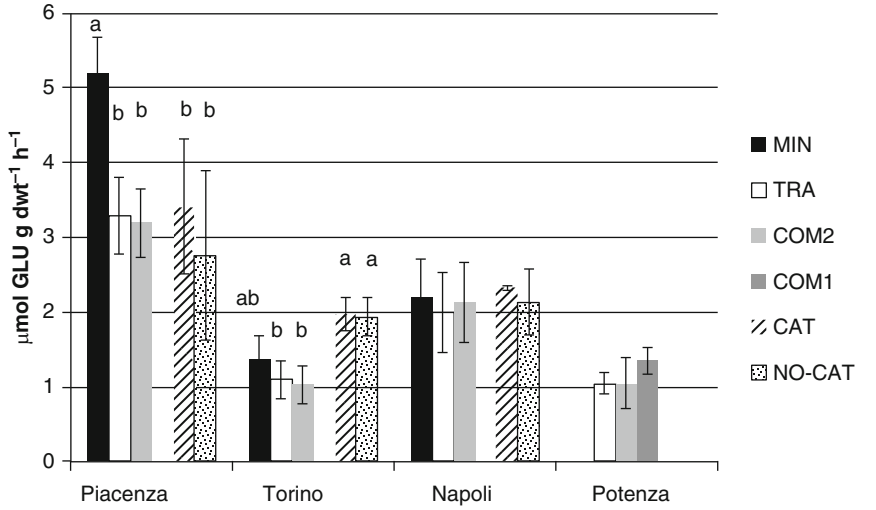


**Fig. 7.5** Invertase activity in 2008 in the MESCOSAGR sites of Piacenza, Torino, Napoli and Potenza. Statistical differences between treatments were assessed separately within each site by ANOVA. In sites where significant differences were found, specific differences were indicated by Tukey's test for comparison of means

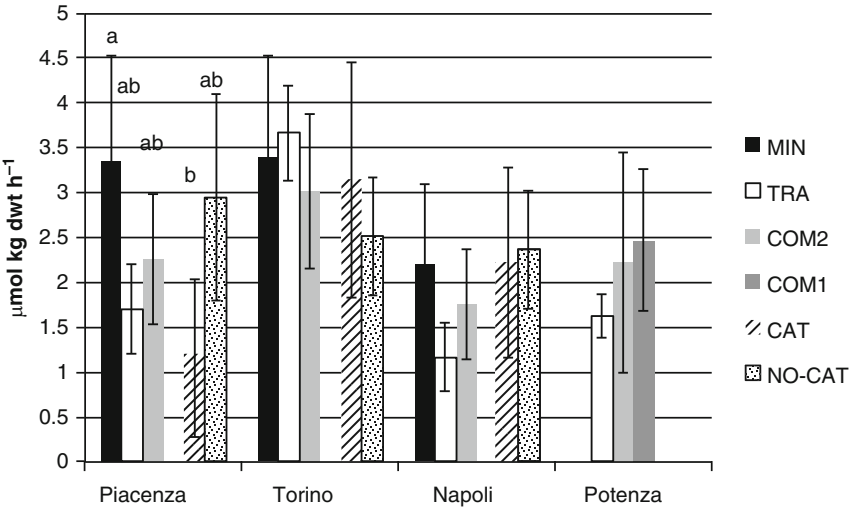


**Fig. 7.6** PLFAs estimation of Gram-positive bacteria in 2008 in the MESCOSAGR sites of Piacenza, Torino, Napoli and Potenza. Statistical differences between treatments were assessed separately within each site by ANOVA. In sites where significant differences were found, specific differences were indicated by Tukey's test for comparison of means

Microbial groups, as estimated by specific PLFAs (identified according to Table 7.4), are also reported in Table 7.7. The statistical elaboration of the PLFA findings indicates that there were no significant effects among treatments in the different sites for total microorganisms, Gram-negative bacteria and protozoa (data not shown). Conversely, it was found that the relation TREAT*SITE was significant for Gram-positive bacteria (Fig. 7.6) and fungi (Fig. 7.7), while for actimomycetes (Fig. 7.8) the SITE variable had a significant effect. Only data for these three groups are presented and discussed here.

According to the Tukey's test for comparison of means that was conducted separately per each site, Gram-positive bacteria (Fig. 7.6) were significantly larger in Piacenza in the MIN treatment as compared to COM2, but no other differences were found among all other treatments and in the other sites. For fungi (Fig. 7.7), the only significant difference was in the Torino site, where the CAT treatment produced a larger level than for MIN, TRA and COM2. However, since no significant difference was found between CAT and No-CAT, the latter finding in Torino is to be attributed more probably to a plant (wheat for CAT and No-CAT and maize for all other treatments) rather than to a treatment effect. Finally, for actinomycetes (Fig. 7.8) the situation was very similar to that for Gram-positive bacteria, with significantly larger values in the Piacenza site for MIN that for COM2, though no other differences were shown among all other treatments and in the other sites.

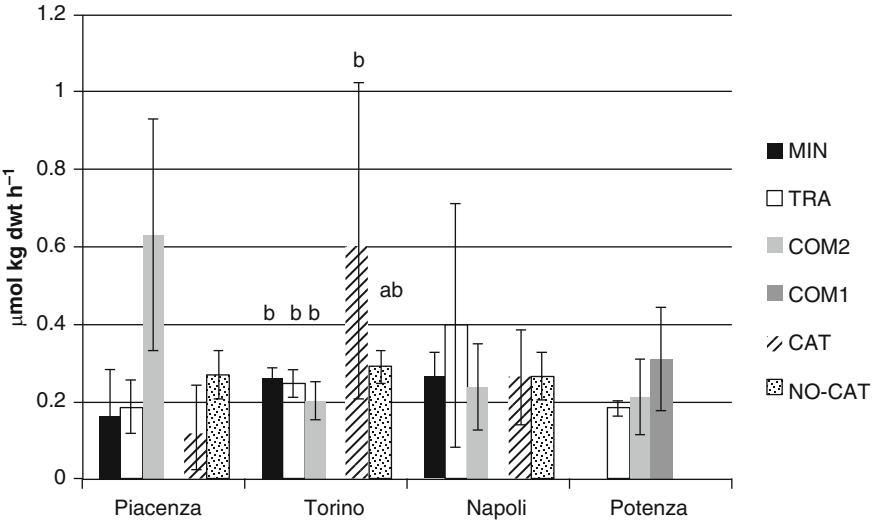


**Fig. 7.7** PLFAs estimation of fungi in 2008 in the MESCOSAGR sites of Piacenza, Torino, Napoli and Potenza. Statistical differences between treatments were assessed separately within each site by ANOVA. In sites where significant differences were found, specific differences were indicated by Tukey's test for comparison of means

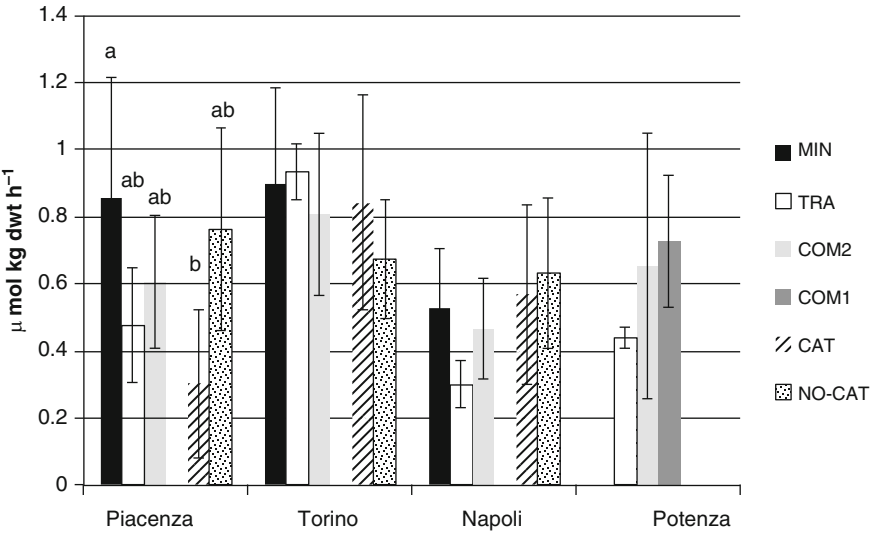


**Fig. 7.8** PLFAs estimation of actinomycetes in 2008 in the MESCOSAGR sites of Piacenza, Torino, Napoli and Potenza. Statistical differences between treatments were assessed separately within each site by ANOVA. In sites where significant differences were found, specific differences were indicated by Tukey's test for comparison of means

## 7.5 Discussion of Results

We have used both bibliographic and original data from the MESCOSAGR project to compare the effects of C sequestration methods on selected biochemical indicators of soil quality, such as enzymatic activities and PLFAs. We found (Tables 7.1–7.6) that much information is available in literature in response to soil treatments such as addition of humified composted matter and different tillage systems, but it has never been summarized and critically evaluated. In the case of the carbon sequestration method based on the catalyst-assisted in situ photo-polymerization of SOM, there are no information yet available on the soil biological responses, and, thus, our results are original, together with those also reported in Chap. 6.

According to literature, some trends were identified for the responses of enzymatic activities to compost addition (Table 7.2), and were even more definite for the responses to tillage (Table 7.3). Based on reports regarding 12 enzymatic activities, these were in most cases induced after addition of different types of compost and manure, at different rates and in different soil types. In some specific cases, no significant differences or inhibitions were found, usually because of presence of contaminants or other compounds, or by the application of too small rates. In response to tillage treatments, the enzymatic activities reported were even clearer in showing a general trend of larger values in no tilled plots (Table 7.3). However, a number of other works indicated that soil organic amendment or reduced tillage had no effects on most enzymatic activities or even resulted in their inhibition. Other soil parameters or management practices (e.g., pest control strategies) were also accounted to play a role on soil biological responses (Omar and Abdel-Sater 2001).

For the MESCOSAGR project, the effects of soil compost amendment and tillage practices were followed on four selected enzymatic activities (β-glucosidase, phosphatase, urease and invertase). The soil treatments were found to hardly have an influence on these enzymes. Specifically, it was found that only the minimum tillage treatment had an effect on urease, β-glucosidase and invertase, and only in the Piacenza site. Neither compost additions nor the biomimetic catalyst spread resulted in significant changes of the four enzymatic activities.

The determination of PLFA values in soil provides indications on the content and changes of different microbial groups (Table 7.3). The soil PLFA patterns were generally altered by compost additions in soil or changes in tillage practices. These alterations lacked, however, a consistent direction, as some microbial groups were found to be either enhanced or reduced, or not affected at all. This variability in literature data was confirmed by results obtained at the third year of experimentation within the MESCOSAGR field trials. These findings showed that some microbial groups (Gram-positive, fungi, actinomycetes) changed according to field site and/or soil treatments, while other showed almost constant levels across sites.

A comparison of the third-year values with those obtained after the first experimentation year (data not shown here) suggests that the content of enzymatic activities and PLFAs in MESCOSAGR soils increased after 3 years of treatments,

but not in all cases. Specifically, it was found an increase in Piacenza for invertase and urease for the MIN treatment, while enzymatic activities were relatively constant in the other sites, if not even progressively reduced in some cases. Analyses of PLFAs data showed some changes in the global pattern with time, though the trends reported in Table 7.7 and in Figs. 7.6–7.8 were quite constant. These outcomes confirm the variability of these parameters in time, and the importance of carrying out experimentation for some consecutive years in order to identify and confirm specific trends.

## 7.6 Conclusions

The results illustrated in this chapter allow drawing some general conclusions. Enzymatic activities are usually correlated with soil quality, and, if not induced by disturbing agents (e.g., organic pollutants stimulating soil microflora activity), their increase is usually positive, as it indicates an efficient microbial recycling of essential plant nutrients.

A more complex issue is the relation between $CO_2$ emission from soils (the main study of the MESCOSAGR project) and the biological parameters dealt with here. An increase in enzymatic activities related to total microbial activity (dehydrogenase, FDA, catalase) will most probably result in larger $CO_2$ emissions, although other factors such as organic carbon quantity and quality should be also considered. On the other hand, an increase in activities of enzymes involved in the biogeochemical cycles of phosphorus, sulfur and nitrogen do not necessary result in an increased $CO_2$ emission. For example, in the case of phosphatase, it was found that this enzyme is more correlated with environmental availability of P than to decomposition rates, and that there is no relationship between $CO_2$ efflux and its activity, at least in litter environments (Johnson et al. 2010). This was an important result and should be confirmed for other enzymatic activities, since it may derive that C sequestration methods can be applied to increase the biochemical quality of soils without affecting, or even reducing, $CO_2$ emission. The relationship with other greenhouse gases should be also considered, especially in the case of N-related enzymatic activities which may affect $N_2O$ fluxes. However, our results within the MESCOSAGR project have shown that in four different Italian locations the adopted field methods for C sequestration did not significantly changed an important component of soil biochemical quality, such as soil enzymatic activities.

Upon a stimulation of soil microbial activities by carbon sequestration practices, the composition of microbial communities should also be affected. This was verified by our PLFA analyses, which also showed that microbial communities were shifted differently than for enzymatic activities. This discrepancy implied that values on enzymatic activities provide soil functional quality, while PLFAs (especially if analysed with a taxonomical approach) account for structural soil composition. Thus, it may be expected that a specific change in a soil function (e.g., an increase in phosphatase activity) can be related to a number of different changes at

taxonomical level. This consideration highlights the importance of including both structural and functional measurements, when verifying the effects of soil treatments on soil microorganisms. Another conclusion of our approach is that changes in microbial communities should not be negatively considered, but they should be evaluated in the wider context of soil ecological functions.

Finally, it must be pointed out that the use of the biomimetic catalyst as a practice to sequester C in soil (the CAT treatment) through photo-polymerization of SOM, failed to bring about any change in the enzymatic activities in four different Italian field site under both maize and wheat cropping. As for PLFAs, the CAT treatment produced only a specific increase in fungal population of the Torino site.

## 7.7 Recommendations for Future Experimentation

Our MESCOSAGR results showed that the applied methods for soil carbon sequestration had no negative effects on the soil biochemical quality. We found no effects in some cases, and even positive effects in many other cases. This was in line with the literature review illustrated above. This work also confirms the importance of linking structural with functional measurements, when assessing the response of soil microbial communities to any experimental factor.

It is recommended that future investigations will continue to apply well-established methods such as enzymatic activities and PLFA analyses for evaluation of soil biochemical quality. However, they will have to be increasingly supported by novel advanced techniques, such as meta-genomics and meta-transcriptomics, in order to reach more valid evidence on the changes induced by soil treatments. Both PLFA and enzymatic methods will be useful as benchmarks for future advanced methods, since they provide easily interpretable information capable to correctly interpret the large amount of data that the new technologies will provide.

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