

Chapter 4

Carbon Sequestration in Soils by Hydrophobic Protection and In Situ Catalyzed Photo-Polymerization of Soil Organic Matter (SOM): Chemical and Physical–Chemical Aspects of SOM in Field Plots

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Abstract The application of innovative practices of soil organic matter (SOM) management, based on either soil amendment with hydrophobic mature compost or with a water-soluble biomimetic catalyst, has shown to enhance organic carbon sequestration in agricultural soils. In three different agricultural sites in Italy, field plots under maize were treated with traditional deep tillage, minimum tillage, green manuring and mature compost, whereas field plots under wheat were added with the iron–porphyrin catalyst. In terms of soil physical quality, mature compost additions improved soil aggregate stability by favoring the increase of water-stable macroaggregates. After 3 years of field experiments, both minimum tillage and green manuring treatments confirmed short-term and even negative effects on organic carbon (OC) accumulation, as compared to traditional tillage. Conversely, the hydrophobic protection exerted by compost amendments on SOM successfully fixed OC in soil from 3 to 22 ton ha⁻¹ more than for traditional tillage, depending on the experimental site. Solid-state CPMAS-NMR spectra of humic substances (HS) extracted from treated soils allowed the molecular characterization of the stable organic matter pool. NMR data combined with chemometric methods revealed that compost-added soils progressively incorporated aliphatic and aromatic hydrophobic components into soil humic fractions over the experimental period. Though more variable among experimental sites, the treatment with biomimetic catalyst positively affected both total soil organic carbon (SOC) content and molecular characteristics of humic extracts. An increased aromaticity and hydrophobicity was shown in the spectra of HS from soils treated with the biomimetic catalyst, thus suggesting an effective photo-polymerization of soil aromatic components and their progressive inclusion into the humic pool. In the first and second year of treatments with the water-soluble iron–porphyrin catalyst, the in situ

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catalyzed photo-polymerization of SOM effectively occurred since soils were found to have sequestered from 4 to 24 ton ha⁻¹ more than the control.

4.1 Objectives

Within the MESCOAGR project, two innovative management practices were studied in field experiments (see Chap. 3) to increase OC sequestration in agricultural soils:

1. Soil addition with recycled biomasses, such as mature humified compost, characterized by large hydrophobicity and biochemical stability. This practice is expected to sequester biolabile carbon by hydrophobic protection.
2. Soil treatment with a water-soluble iron–porphyrin biomimetic catalyst (see Chap. 1) to promote an in situ photo-oxidative polymerization of soil organic molecules. The polymerized soil organic matter (SOM) is expected to be less readily mineralized by soil microbes and be fixed permanently in soil.

Our aim was to evaluate the quantitative and qualitative changes brought about in soil organic carbon (SOC) by these two novel techniques, as compared to conventional tillage and other common SOM management practices, such as minimum tillage and green manuring.

The effect of soil management practices on SOC stabilization was evaluated through the following parameters:

- Distribution of water-stable aggregates, and soil structural stability
- OC content in bulk soils and water-stable aggregates
- Physical–chemical characterization of humic substances (HS) extracted from soil

4.2 Soils Characteristics and Experimental Setup

Three different experimental sites along a North–South climate gradient were chosen to set up soil treatments. Soil classification textural composition and organic carbon content of the selected soils are shown in Table 4.1.

The effect of mature compost addition on soil carbon sequestration was evaluated by comparing the following field management during a 3-year experiment (2006–2008), using a monoculture of mais (*Zea mais*) as annual crop (see Chap. 3):

Table 4.1 Textural composition (%), bulk density, and TOC (g kg⁻¹) content of soils from field experiments

Field sites	Soil type	Sand	Silt	Clay	Bulk density	TOC
Torino	Typic Ustifluent	36.9	56.2	6.9	1.50	11.5
Piacenza	Udifluventic Haplustept	17.9	47.1	35.0	1.30	17.2
Napoli	Vertic Haploxeralf	47.0	20.1	32.9	1.40	10.5

- Traditional (TRA): plowing at 35 cm depth, followed by surface harrowing with addition of mineral fertilizers.
- Minimum tillage (MIN): no plowing, with addition of mineral fertilizers.
- Green manure (GMAN): plowing at 30 cm depth, followed by surface harrowing. Leguminous crops were interlaced between two main annual cycles and used as green manure to totally or partially replace nitrogen fertilizer.
- Compost first rate (COM-1): same as the TRA plots but with the addition of an amount of mature compost corresponding to 2.7 ton ha⁻¹ of OC.
- Compost second rate (COM-2): same as the TRA plots but with addition of an amount of mature compost corresponding to 5.4 ton ha⁻¹ of OC.

Each management treatment was established on a 4 m × 4 m plot with four replicates, in a randomized block experiment.

The experiment based on the addition of the biomimetic catalyst was performed on 1 m × 1 m field plots ($n = 4$) cultivated with wheat (*Triticum Durum*) for 3 years, with the following treatments:

- CAT: plowing at 35 cm depth, followed by surface harrowing with the addition of mineral fertilizer and 10 kg ha⁻¹ of a biomimetic catalyst, the water-soluble iron–porphyrin (FeP). FeP was synthesized in the laboratory as *meso*-tetra (2,6-dichloro-3-sulfonatophenyl)porphyrinate of iron(III) chloride, Fe-(TDCPPS) Cl (Piccolo et al. 2005a).
- No-CAT: plowing at 35 cm depth followed by surface harrowing with the addition of mineral fertilizers.

4.3 Water-Stable Soil Aggregates and Soil Stability

The dynamics of SOM is closely related to soil physical properties, such as particle sizes distribution and soil aggregation (Tisdall and Oades 1982). Organic matter components are involved in the different steps of soil aggregate hierarchy, from the formation of organo-mineral complexes up to stabilization of aggregate-size classes (Oades and Waters 1991; Six et al. 2004). Since the role of OM in soil physical aggregation determines the processes of OC decomposition and accumulation (Plante and McGill 2002a), an evaluation of aggregate distribution in soil and their stability is useful to follow SOC dynamics (Kasper et al. 2009).

The classical procedure described by Kemper and Rosenau (1986) was used to separate water-stable aggregates. Briefly, 20 g of <4.75 mm air-dried soil samples was put on the topmost of a nest of three sieves with 1.00, 0.50, and 0.25 mm mesh size and pre-soaked in distilled water for 30 min. Then, the nest of sieves was oscillated vertically in water 20 times, using a 4 cm amplitude at the rate of one oscillation per second. Care was taken to ensure that soil particles on the topmost sieve were always below the water surface during each oscillation.

After wet-sieving, the water-stable soil materials left on each sieve and the unstable (<0.25 mm) aggregates were quantitatively transferred into beakers,

dried in the oven at 50°C for 48 h, weighed and stored for analysis. The percentage ratio of aggregates in each sieve represents the water-stable aggregates for size classes: 4.75–1.00, 1.00–0.50, 0.50–0.25, and <0.25 mm. Mean-weight diameter (MWD) of water-stable aggregates was calculated by the following equation:

$$\text{MWD} = \sum_{i=1}^n X_i W_i$$

where X_i is the mean diameter of the i th sieve, and W_i is the amount of total aggregates in the i th fraction.

4.3.1 Control Soils

The distribution of water-stable aggregates obtained from fractionating the initial soils before the start of field experiments revealed a marked influence of textural composition on soil structural properties in the three field sites (Table 4.2). The lowest aggregate stability was found for the silty-loamy soil of Torino that was characterized by both the lowest clay content (Table 4.1) and the largest yield of unstable microaggregates (<0.25 mm). Conversely, the significantly larger stability index (Table 4.2) shown, in the order, by the sandy-clay loam Napoli soil and the silty-clay loam Piacenza soil, was associated with a large amount of clay particles (Table 4.1). No direct relationship could be drawn by relating aggregate stability to bulk OC content (Tables 4.1 and 4.2).

The finest soil mineral components, such as fillosilicates and Fe and Al hydroxides, exert a strong influence on soil structural properties. The large surface area exposed by clay-size particles, in fact, allows a close interaction among inorganic and organic colloidal constituents, and promote formation and stabilization of soil aggregates (Oades and Waters 1991; Attou et al. 1998).

The modifications induced by field treatments on soil aggregate distribution and structural stability during the three experimental years are reported in Tables 4.3–4.5 for the soils of Torino, Piacenza and Napoli, respectively.

Table 4.2 Percent (%) distribution of water-stable aggregates (mm) and mean-weight diameter index (MWD, mm) of initial control soils

Field sites	Aggregate size				MWD
	4.75–1.00	1.00–0.50	0.50–0.25	<0.25	
Torino	9.7	21.1	27.5	41.8	0.59
Piacenza	52.8	26.2	9.1	11.9	1.76
Napoli	39.2	30.8	14.3	15.7	1.43
LSD 0.05	8.5	NS	4.0	3.9	0.18

LSD least significant difference ($n = 4$), NS not significant

4.3.2 Torino Experimental Site

After the first year, the majority of treatments on maize plots of Torino showed a significant increase of aggregate stability with respect to the initial soil (Table 4.3). Only the addition of green manure (GMAN) and the lower rate of compost (COM-1)

Table 4.3 Torino experimental site, percent distribution (%) of water-stable aggregate sizes (mm) and mean-weight diameter index (MWD) (mm) under different treatments for 3 years of experimentation

Treatments	Aggregate size				MWD
	4.75–1.00	1.00–0.50	0.50–0.25	<0.25	
Control soil	9.7	21.1	27.5	41.8	0.59
Maize					
<i>First year</i>					
TRA	15.7	27.1	26.9	30.4	0.79
MIN	13.2	30.9	27.6	28.3	0.75
GMAN	10.8	26.5	31.1	31.6	0.67
COM-1	9.0	27.9	32.0	31.1	0.65
COM-2	14.7	29.3	27.2	28.9	0.78
LSD	1.7	NS	3.2	1.2	0.04
<i>Second year</i>					
TRA	8.8	25.5	39.8	25.9	0.63
MIN	9.6	23.4	38.7	28.2	0.63
GMAN	10.3	24.1	39.7	25.9	0.66
COM-1	9.6	24.1	38.5	27.8	0.64
COM-2	9.7	26.6	39.9	23.8	0.66
LSD	NS	NS	NS	NS	NS
<i>Third year</i>					
TRA	11.4	25.0	36.2	27.5	0.68
MIN	9.8	21.2	38.0	30.9	0.62
GMAN	9.4	19.4	38.3	33.0	0.60
COM-1	15.3	25.2	34.5	25.1	0.79
COM-2	20.3	27.7	29.8	22.2	0.93
LSD	2.5	2.2	2.1	2.2	0.06
Wheat					
<i>First year</i>					
CAT	19.4 (4.2)a	25.0 (4.4)	25.8 (3.8)b	29.8 (8.3)	0.88 (0.11)
No-CAT	12.9 (2.2)b	28.5 (2.5)	32.3 (1.1)a	26.2 (0.9)	0.74 (0.05)
<i>Second year</i>					
CAT	9.5 (1.2)b	23.4 (1.2)	38.2 (3.8)a	28.9 (8.7)	0.64 (0.03)
No-CAT	11.3 (1.7)a	22.5 (1.3)	33.0 (1.7)b	33.1 (2.8)	0.66 (0.04)
<i>Third year</i>					
CAT	10.3 (1.5)	25.0 (1.9)	36.5 (1.4)	28.2 (2.7)	0.66 (0.05)
No-CAT	10.6 (1.4)	24.3 (5.2)	33.9 (3.3)	31.1 (5.4)	0.65 (0.05)

LSD least significant difference ($n = 4$), NS not significant. Different small letters in columns indicate significant difference at 0.05 probability level ($n = 4$). Numbers in brackets for wheat plots represent standard deviation ($n = 4$)

produced smaller MWD increases. All field treatments revealed a positive effect in the distribution of water-stable aggregates, with an overall decrease in the yield of microaggregates (<0.25 mm), which were steadily incorporated in upper size-classes. The widespread increase of soil aggregation may be explained with the physical action of plant roots and fungal hyphae, as well as root debris and microbial bio-products, which promote association of small soil fractions into meso- and macroaggregates (Tisdall and Oades 1982; Chan and Heenan 1999; Six et al. 2004). For the GMAN and COM-1 treatments, the effect was limited to the intermediate particles size fraction (1–0.25 mm), while for traditional (TRA), minimum tillage (MIN), and greater compost rate (COM-2), there was a significant particle incorporation also in the large macroaggregate class, with a consequent increase of MWD.

As compared to both initial (control) and untreated plots (No-CAT), an increase of MWD index (Table 4.3) was observed for the soil added with biomimetic catalyst (CAT), indicating that this treatment induced a redistribution of macroaggregates into larger sized fractions. However, this effect may have also been partially favored by the action of plant roots and microbial biomass.

After the second year, an overall stabilization of structural properties was noted with an even distribution of water-stable aggregates and a similarity of MWD index for all soil treatments. With respect to previous year, a MWD decrease was found for TRA, MIN, and COM-2, whose values approached those for GMAN and COM-1, which instead maintained a steady effect on soil aggregates. The aggregate distribution for the second year (Table 4.3) showed a further decrease of microaggregate sizes for all treatments, with their prevalent incorporation into the next larger size-fraction (0.50–0.25 mm). Concomitantly, the trend of decreasing aggregate stability for TRA, MIN, and COM-2 was mainly due to decrease of large macroaggregates, whose values remained unvaried only for GMAN and COM-1 treatments.

In the second year, soils treated with the biomimetic catalyst (CAT) showed a breakdown of larger aggregates, with soil redistribution in smaller macroaggregates and MWD index returning to No-CAT values.

Soil samples under traditional soil management did not show a significant variation in structural stability after 3 years of experimentation, except for only a slight increase in microaggregate yield (Table 4.3). Conversely, both COM-1 and COM-2 revealed a positive effect on structural properties. In fact, their stability index increased, due to decrease of both microaggregates and small macroaggregates (0.50–0.25 mm), which apparently aggregated into larger sized fractions. This improvement in soil stability was consistent with the amount of added compost, since the larger compost addition (COM-2) provided a greater response. In fact, the yield of large macroaggregates for COM-2 was twice as large as that for TRA, MIN, and GMAN. No difference in the distribution of water-stable aggregates and MWD index was found between the CAT treatment and its control for the third year.

4.3.3 Piacenza Experimental Site

The soil treatments from the Piacenza site showed, throughout the experimental period, a similar trend of soil aggregation as for Torino. An overall improvement of soil aggregation was shown for all treatments after the first year (Table 4.4). This was mainly due to greater yield of large aggregate size fractions, as compared to the initial distribution at the onset of experimentation (initial soil). The major effects were achieved for the least disruptive MIN, COM-2, and CAT treatments. Both soil aggregation and stability index were significantly larger than the respective TRA and No-CAT control treatments.

Table 4.4 Piacenza experimental site, percent distribution (%) of water-stable aggregate sizes (mm) and mean-weight diameter index (MWD) (mm) under different treatments for 3 years of experimentation

Treatments	Aggregate size				MWD
	4.75–1.00	1.00–0.50	0.50–0.25	<0.25	
Control soil	52.8	26.2	9.1	11.9	1.76
Maize					
<i>First year</i>					
TRA	55.6	21.5	11.0	11.9	1.82
MIN	64.3	17.7	9.1	9.0	2.03
COM-2	63.6	18.3	9.3	8.7	2.01
LSD	5.5	NS	NS	1.7	0.10
<i>Second year</i>					
TRA	36.1	33.4	15.6	14.9	1.37
MIN	45.0	27.1	14.7	13.2	1.57
COM-2	45.4	29.5	14.6	10.6	1.59
LSD	3.2	2.4	NS	1.6	0.08
<i>Third year</i>					
TRA	53.7	25.9	10.8	9.6	1.79
MIN	50.4	28.1	11.7	9.9	1.72
COM-2	58.8	24.1	9.3	7.8	1.92
LSD	NS	NS	NS	NS	NS
Wheat					
<i>First year</i>					
CAT	55.1 (0.9)a	23.8 (1.1)	11.2 (0.3)	9.9 (0.3)b	1.82 (0.02)a
No-CAT	49.0 (1.9)b	25.7 (2.7)	10.5 (0.4)	12.5 (1.6)a	1.67 (0.03)b
<i>Second year</i>					
CAT	38.4 (0.1)a	29.9 (0.4)b	16.2 (0.5)b	15.5 (0.8)	1.41 (0.03)a
No-CAT	34.6 (1.5)b	30.8 (0.4)a	17.9 (0.8)a	16.7 (1.0)	1.31 (0.04)b
<i>Third year</i>					
CAT	47.7 (2.9)	28.2 (1.3)	12.5 (0.6)	11.6 (1.4)	1.65 (0.1)
No-CAT	52.5 (5.5)	25.9 (3.0)	11.8 (1.4)	9.8 (1.1)	1.76 (0.1)

LSD least significant difference ($n = 4$), NS not significant. Different small letters in columns indicate significant difference at 0.05 probability level ($n = 4$). Numbers in brackets for wheat plots represent standard deviation ($n = 4$)

A sharp decrease of soil aggregation and stability index occurred in Piacenza for soil treatments under maize after 2 years of field experiments (Table 4.4). All soils showed a breakdown of large size aggregates, and redistribution of soil particles towards smaller size fractions, including microaggregates, though the loss of structural properties was different for various treatments. A persistent effect on soil stability was shown by MIN and COM-2, both characterized by a preservation of water-stable macroaggregates ($\cong 45\%$), and a limited reduction in the associated MWD index. Moreover, aggregate fractionation of COM-2 provided the lowest yield of microaggregates (10.6%), whose amount was even smaller than for the initial control soil (11.9%).

For the wheat plots, despite the overall loss of structural properties in the second year, both size-aggregate distribution and MWD index (Table 4.4) indicated that the treatment with biomimetic catalyst preserved soil structural quality more than for the No-CAT treatment.

The positive effect on aggregate stability for the maize field plots under compost was also confirmed at the end of the third experimental year (Table 4.4). With respect to the results of the previous years, the aggregate distribution of TRA, MIN, CAT, and No-CAT indicated an occurred recovery of the original structural stability. The values of aggregate yields and stability index for these treatments reached those found at the onset of experiments, thereby suggesting a progressive lower efficacy of both MIN and CAT on soil structural quality. Conversely, COM-2 plots showed a continuous decrease of both microaggregates (-2.8%) and intermediate macroaggregate fractions, which became firmly incorporated into larger sized aggregates ($+13.4\%$), thus improving the overall soil structural stability index (1.92).

4.3.4 *Napoli Experimental Site*

Differently from previous experimental sites, the first year of cultivation produced a deterioration of soil structural properties in the field plots of Napoli (Table 4.5). With respect to the initial control soil, nearly all treatments showed lower yields of large size aggregates, with consequent decrease in soil stability index. The loss of structural stability in maize cultivated plots may be due to specific properties of the sandy-clay loam soil of this site. An exception was the soil treated with the low compost rate (COM-1). This showed a MWD increase, due to an effective association of soil particles into larger aggregate fractions, which included more than 50% of total fraction mass. The addition of the low compost rate (COM-1) may have promoted a microbially induced priming effect with a temporary improvement of macroaggregate formation (Chan and Heenan 1999).

In the case of wheat fields, both CAT and No-CAT treatments revealed an improvement of soil structural properties as compared to maize fields, thus suggesting a crop effect on soil physical parameters (Table 4.5).

Table 4.5 Napoli experimental site, percent distribution (%) of water-stable aggregate sizes (mm), mean-weight diameter index (MWD) (mm), under different treatments for three experimental years

Treatments	Aggregate size				MWD
	4.75–1.00	1.00–0.50	0.50–0.25	<0.25	
Control soil	39.2	30.8	14.3	15.7	1.43
Maize					
<i>First year</i>					
TRA	33.3	32.4	16.6	17.6	1.29
MIN	31.9	35.4	18.4	14.2	1.27
GMAN	28.7	39.8	16.8	14.7	1.20
COM-1	53.6	26.9	9.5	10.0	1.79
COM-2	37.3	31.4	12.7	18.6	1.38
LSD	3.3	1.1	3.7	4.7	0.19
<i>Second year</i>					
TRA	26.4	40.1	20.1	13.4	1.15
MIN	33.1	41.6	15.8	9.5	1.33
GMAN	32.4	38.4	17.3	11.9	1.30
COM-1	37.6	37.2	14.9	10.4	1.42
COM-2	32.6	36.5	18.1	12.8	1.30
LSD	5.3	3.7	2.1	1.2	0.10
<i>Third year</i>					
TRA	38.7	33.7	16.1	11.5	1.44
MIN	40.8	31.9	16.1	11.2	1.49
GMAN	46.1	31.4	13.2	9.3	1.62
COM-1	43.1	29.9	15.5	11.5	1.54
COM-2	43.2	32.6	14.7	9.5	1.55
LSD	2.2	NS	NS	2.4	0.06
Wheat					
<i>First year</i>					
CAT	51.3 (2.4)	25.9 (1.9)	11.2 (2.3)	11.6 (1.7)	1.73 (0.06)
No-CAT	47.5 (7.3)	28.2 (4.3)	13.3 (2.0)	10.9 (2.7)	1.64 (0.18)
<i>Second year</i>					
CAT	31.5(2.7)	40.0(0.4)	17.1(1.7)	11.4(1.5)	1.28(0.1)
No-CAT	36.7(6.0)	40.2(3.0)	13.2(1.9)	9.9(1.1)	1.42(0.1)
<i>Third year</i>					
CAT	41.6(1.9)	32.7(0.3)	15.2(0.6)	10.4(0.9)	1.51 (0.05)
No-CAT	39.7(2.2)	35.0(1.4)	15.2(0.5)	10.1(0.4)	1.47 (0.05)

LSD least significant difference ($n = 4$), NS not significant. Different small letters indicate significant difference at 0.05 probability level ($n = 4$). Numbers in brackets for wheat plots represent standard deviation ($n = 4$)

A slight different distribution of water-stable aggregates was observed in the maize plots after two experimentation years. For all treatments, soil aggregate fractionation indicated a decrease in microaggregate yield and a corresponding, though uneven, increase in macroaggregates, this trend being more effective for COM-1 (Table 4.5). For maize fields, soil aggregate distribution decreased the

MWD index, with respect to the first year, for all treatments except for MIN and GMAN. The MWD stability index for both CAT and No-CAT treatments was also reduced in the second year to the levels similar to those of maize fields.

Similar to other experimental sites, the Napoli field site presented a structural improvement in soils of all treatments after three experimental years (Table 4.4). Either maize or wheat plots produced an increased amount of large macroaggregates fraction, with a consequent improvement of MWD values, which were larger than for the initial undisturbed control soil.

The best structural quality was found for treatments with organic amendments, including green manure, and for CAT, although for the latter the difference from No-CAT was not statistically significant (Table 4.5).

4.3.5 Concluding Notes on Soil Physical Quality

As mentioned earlier, the yields of aggregate fractionation and values of soil stability index suggested a predominant influence of specific texture and clay content on the physical and structural properties of soils. However, all treatments in each site, despite the specific soil structural properties, showed a comparable macroaggregate dynamics throughout the experimental period, consisting first in a decrease and then in a recovery of soil stability. In fact, our results revealed a similar succession of aggregate yields and stability index, whose values varied annually around the average level of the respective undisturbed initial soils.

The effectiveness of clay particle sizes, as binding agent, is strongly related to soil physical–chemical conditions and imposed mechanical stress. Soil pH, ionic strength, and salt composition of soil solution, water holding capacity, freezing/thawing and wetting/drying cycles, raindrop impact, and intensity and frequency of soil tillage, may temporarily overcome the glueing effect exerted by mineral colloids on soil aggregates. In cultivated soils with low SOM content, the lower resilience of clay particles may result in a slow response to the aggregation capacity of soil management (Spaccini et al. 2004), and a significant loss of structural stability for heavy textured soils. In fact, the susceptibility of cultivated soils to loose structural stability is a function of initial aggregation, that is greater for stable clayey than for fragile sandy soils (Spaccini et al. 2001).

Moreover, large soil aggregates, placed in the upper level of aggregate hierarchy, are usually characterized by large porosity, wide planes of weakness, and low tensile strength (Oades and Waters 1991). Therefore, macroaggregates are mostly affected by land use and soil disturbance, and undergo rapid turnover cycles and fast aggregation/disaggregation dynamics, especially for agricultural soils with low OC content (Plante and McGill 2002b). Since turnover time of macroaggregates ranges from 10 to 90 days (Plante et al. 2002; De Gryze et al. 2006), with significant fluctuation during growing seasons, more frequent samplings should better show the effect of management practices on soil aggregation (Daraghmeh et al. 2009). Thus, since the present experiment was mainly focused on the cumulative effects of

soil management on SOC, the unique sampling date at the end of the growing season may have partly masked the differences among soil treatments.

Nevertheless, though results indicate a small effect of soil management as compared to intrinsic soil physical properties, significant differences on aggregate-size distribution and MWD were found among various treatments in the three sites. A similar aggregation process was revealed by fractionating TRA, MIN, and GMAN soils at any experimental site. This indicates that either a reduced soil disturbance by minimum tillage, or green manuring by soil incorporation of residues from leguminous crops, did not significantly modify the aggregate dynamics, neither in the sandy-loam soil of Torino, nor in the heavier textured soils of Piacenza and Napoli. On the contrary, a slight but significant improvement of soil aggregation and structural stability were found for both compost treatments (COM-1 and COM-2), in comparison to either TRA, MIN, or GMAN.

Contrasting results have been reported on the relation between soil aggregate stability and green manuring, when in combination with either reduced tillage or conventional tillage (Biederbeck et al. 1998; Podwojewski and Germain 2005). The main purpose of green manuring relies in the supply to soil of a slow-release source of organic nitrogen, in order to reduce or even replace mineral fertilization. The organic residues added with green manuring are easily decomposable and low in lignified tissues and hydrophobicity (Carvalho et al. 2009). These biolabile and hydrophilic characteristics are recognized to provide at best only a transient effect on soil stability (Piccolo and Mbagwu 1999).

Current findings on soil management methods based on no-tillage practices (NT) indicate an overall improvement of soil physical properties and soil stability, as compared to conventional tillage (CT) (Six et al. 2004). On the other hand, the main effects of reduced tillage methods on soil aggregation are limited to surface horizons, while small differences from conventional tillage are usually found below 10 cm with increasing plow depth (Liebig et al. 2004; Kasper et al. 2009). This emphasizes the importance of investigating the whole soil profile when studying the suitability of NT versus CT for aggregate stability and SOC sequestration (Plaza-Bonilla et al. 2010).

A large literature indicates that soil amendments with different compost materials provide an effective improvement in aggregate stability and related physical properties (porosity, infiltration rate, surface erosion, etc.), regardless of soil type and crop rotation (Weber et al. 2003, 2007; Sodhi et al. 2009). Although soil structure improvement is closely associated with an increase of organic carbon content, the quality and molecular composition of added organic matter play a basic role in the long-term stabilization of soil aggregates (Piccolo and Mbagwu 1999). Soil amendments with compost may promote two possible effects on soil physical quality (1) a transient or temporary soil aggregation through the stimulation of microbial activity and consequent production of stabilizing exopolysaccharides, and, (2) a long-term stabilization due to addition to soil of an effective amount of hydrophobic humified materials (Albiach et al. 2001; Romàn et al. 2003; Bipfubusa et al. 2008). In fact, depending on the type and quality of compost, its amendment to

soils may produce either short term, poor, or even negative effects on soil aggregate stability (de León-González et al. 2000; Kohler et al. 2008).

As reminded above, the transient effect observed on soil aggregates of COM-1 field plots in the Napoli site in the first year (Table 4.5) may be related to an increased amount of microbial bio-products due to a priming effect on SOC. This explanation may be supported by the low OC amount found in bulk soil and large soil aggregates (Table 4.8). However, the results on aggregate-size distribution and stability index reported for COM-1 and COM-2 for all field sites after 3 years (Tables 4.3–4.5) indicate that the progressive incorporation of humified and hydrophobic organic matter from compost allowed a slow but persistent improvement of soil aggregation. The largest effect was found for Torino, where the intrinsically low initial soil structural stability was more easily improved by stable compost (Piccolo et al. 2004), and favored a significant incorporation of fine aggregate sizes into larger stable aggregates.

4.4 Organic Carbon in Bulk Soil and Water-Stable Aggregate-Sizes

Assessing the content of total SOC by an elemental analyzer (Interscience EA1108) is the simplest and most direct measurement to evaluate the effect of soil management practices and cropping systems on SOC accumulation or decomposition. However, an estimate of the total amount of organic carbon (TOC) in the bulk soil does not inform on the mechanism of SOM incorporation, but it is only an indirect evaluation of both SOM dynamics and stabilization process.

A large number of chemical or physical fractionation methods have been applied to SOM studies in order to quantify organic matter pools with different OC turnover time and dynamics. The separation of various SOC fractions, based on both SOM physical protection and biochemical recalcitrance, was generally achieved by fractionation of soil particle sizes or aggregates (Gregorich et al. 1989; Angers et al. 1995; Puget et al. 2000; Six et al. 2004), density flotation of aggregates and organic mineral complexes (Christensen 1992; Gregorich et al. 1997; Janzen et al. 1992), chemical separation (Spaccini et al. 2000; Derenne and Largeau 2001; Helfrich et al. 2007), and a combination of physical and chemical methods (Spaccini et al. 2001; Leifeld and Kögel-Knabner 2005; Quénéa et al. 2006). Although each technique may provide information to clarify SOM accumulation processes, most of the applied methodologies show different specificities according to soil and procedure. Thus, no common protocol has been yet established for univocal or unambiguous determination of SOM pools and fractions (Smith et al. 2002; von Lützow et al. 2007).

In the field experiments of the MESCOSAGR project, we focused on the determination of OC content in both bulk soils and water-stable aggregate sizes. Moreover, we appraised the molecular composition of humic substances which

were annually extracted from soil after each crop cycle during the three experimental years (see Sect. 4.5).

The TOC content and its distribution among soil aggregates showed the short- and medium-term effects of various field treatments on OM incorporation in soil and its interactions with soil particles. On the other hand, the structural characterization of humic substances extracted from soil indicated the effect of field experiments on the molecular composition of the most stable soil organic components, and the potential of different management for a long-term stabilization of SOM.

4.4.1 *Torino Experimental Site*

After 1 year of maize cultivation, TOC values in bulk soils (Table 4.6) did not reveal significant differences among treatments, except for MIN, that showed the lowest OC content with a decrease of about 1 g OC kg^{-1} with respect to TRA. The addition of exogenous organic matter (GMAN, COM-1, and COM-2) did not produce any increase with respect to TRA. This implies that the fresh organic matter stimulated a microbial mineralization that may have counteracted the OC addition to soil.

With the exception of COM-2, the OC distribution in soil aggregate sizes generally showed a progressive larger OC absolute content with increasing aggregate size, while the relative (%) OC amount was mainly related to the aggregate mass distribution. The OC increase in larger soil aggregates is in line with the theoretical organization of aggregate hierarchy, by which macroaggregates become richer in OC due to the progressive mutual association of fine particles in larger aggregates (Puget et al. 1995; Six et al. 2004). The large OC found in the 0.50–0.25 mm aggregate size in COM-2 may be explained with a compost-induced greater microbial activity, whose bio-products are preferentially adsorbed on the large surface areas of fine aggregate sizes (Zech and Guggenberger 1996). This finding is in line with those for GMAN and COM-1, which also showed, with respect to TRA and MIN, the lowest relative (%) OC in large aggregates (4.75–1 mm), and greater absolute and relative OC content in small aggregate sizes (0.50–0.25 and <0.25 mm).

For wheat fields (Table 4.6), a greater OC content in bulk soil was found for plots treated with the water-soluble iron–porphyrin catalyst (13.2 g kg^{-1}), as compared to control plots (12.4 g kg^{-1}). However, aggregate-size fractionation provided a low OC recovery for both CAT and No-CAT treatments, with 91 and 93% of TOC recovered for bulk soil, respectively (Table 4.6). This result suggests a loss of soluble organic matter due to a weaker interaction between organic components and surface of mineral particles. Since these values were lower than those obtained for the same soil under maize, the reason cannot be attributed to differences in soil physical properties, but possibly to a different OC incorporation depending on both treatment and crop. The OC distribution in water-stable aggregates for CAT

Table 4.6 Torino experimental site, amount (g kg^{-1}) and relative distribution (%) of organic carbon in bulk samples and water-stable aggregate sizes (mm) in soil under different treatments for 3 years of experimentation

Treatments	Bulk g kg^{-1}	Aggregate sizes						Sum of fractions				
		4.75–1.00		1.00–0.50		0.50–0.25		<0.25				
		g kg^{-1}	%	g kg^{-1}	%	g kg^{-1}	%	g kg^{-1}	%			
Control soil	11.5	12.1	11.0	10.0	19.9	11.1	28.8	10.3	40.4	10.6	92.2	
Maize												
<i>First year</i>												
TRA	11.6	13.6	19.2	11.9	29.1	10.4	25.3	9.6	26.4	11.0	94.8	
MIN	10.7	10.9	14.3	10.2	31.0	10.0	27.2	9.8	27.5	10.1	94.4	
GMAN	11.8	11.6	11.2	11.9	28.0	11.5	31.9	10.3	29.0	11.3	95.8	
COM-1	11.8	13.1	10.2	12.2	27.7	11.8	32.9	10.2	29.4	11.5	97.5	
COM-2	11.4	9.2	12.2	11.5	30.1	12.2	29.6	11.0	28.1	11.2	98.2	
LSD	0.45	1.0	0.6	1.1	2.4	0.6	1.9	0.2	NS	1.0		
<i>Second year</i>												
TRA	11.5	13.4	10.9	10.9	26.1	10.4	38.9	9.9	24.1	10.7	93.0	
MIN	11.5	17.2	14.2	14.4	28.8	10.0	33.0	10.0	24.2	11.3	98.2	
GMAN	10.8	11.8	11.3	10.9	24.3	11.4	42.2	9.3	22.3	10.7	99.0	
COM-1	12.1	11.4	9.0	10.2	20.3	13.8	43.7	11.7	26.9	12.1	100	
COM-2	12.4	14.9	11.5	13.9	26.2	13.1	41.0	9.4	21.3	12.4	100	
LSD	0.50	1.5	0.6	1.7	1.6	0.7	1.9	0.8	1.2	0.8		
<i>Third year</i>												
TRA	11.6	12.0	11.7	11.9	25.8	11.7	36.7	10.8	25.8	11.5	99.1	
MIN	11.3	11.5	9.9	11.5	21.3	11.4	38.2	11.2	30.5	11.4	100	
GMAN	12.0	12.2	10.1	11.5	19.8	11.4	38.7	10.7	31.3	11.3	94.2	
COM-1	12.7	14.6	17.7	13.4	27.0	11.5	31.6	12.0	23.9	12.6	99.2	
COM-2	13.2	15.0	23.1	15.1	31.8	12.0	27.1	10.7	18.0	13.2	100	
LSD	0.40	0.9	0.7	0.9	1.3	0.4	1.4	0.7	1.0	0.7		

Wheat											
<i>First year</i>											
CAT	13.2a (0.11)	11.9 (1.1)	18.8a (2.0)	11.5 (1.0)	23.6 (2.0)	13.4a (1.1)	28.6 (3.0)	12.6 (0.5)	29.1 (3.0)	11.0 (1.0)	83.3
No-CAT	12.4b (0.40)	11.7 (0.9)	13.0b (1.9)	10.9 (0.6)	27.5 (3.0)	10.9b (0.6)	31.4 (5.0)	12.6 (0.7)	28.2 (4.8)	10.5 (0.9)	84.7
<i>Second year</i>											
CAT	13.0a (0.4)	15.5a (0.9)	12.5 (0.5)	12.8 (0.6)	25.3a (0.2)	12.5 (0.1)	40.0a (1.0)	8.9b (0.1)	22.3b (1.2)	11.8a (0.2)	90.8
No-CAT	11.8b (0.3)	12.4b (0.2)	12.4 (1.0)	11.8 (0.5)	23.6b (0.1)	11.7 (1.0)	34.3b (1.2)	10.1a (0.5)	29.7a (1.7)	11.2b (0.3)	94.9
<i>Third year</i>											
CAT	13.8 (0.5)	14.6 (0.4)	13.0 (0.1)	11.8b (1.0)	26.9a (0.6)	14.0a (0.1)	36.5a (0.9)	14.9a (1.1)	23.6b (0.6)	12.9 (0.4)	93.5
No-CAT	13.7 (0.3)	15.9 (1.2)	11.9 (1.1)	14.7a (0.7)	23.2b (0.1)	11.1b (0.5)	31.9b (0.8)	10.0b (0.7)	33.0a (0.2)	12.7 (0.2)	92.7

LSD least significant difference for p 0.05 ($n = 4$), NS not significant. Numbers in brackets for wheat plots represent standard deviation ($n = 4$). Different small letters in columns indicate significant difference at 0.05 probability level ($n = 4$)

revealed a generally larger OC content in greater aggregate size fractions than for No-CAT, though a significant difference from No-CAT was found only for the 0.50–0.25 mm aggregate size. This microaggregate size-fraction is composed of fine particles (silt and clay) (Six et al. 2004) mutually associated in organo-mineral complexes due to interactions exerted by adsorbed aliphatic and aromatic components (Baldock and Skjemstad 2000; Spaccini et al. 2002). Thus, it is likely that the catalyst added in the CAT treatment has favored the covalent coupling of aromatic and phenolic SOM components, thus further stabilizing the organic constituents adsorbed on this fine soil fraction.

After two experimentation years, both COM treatments determined an increase of TOC in bulk soils, whereas a decrease was observed for GMAN (Table 4.6). Although GMAN may represent an important supply for plant N requirements, the fresh organic matter added to soil with this treatment may not produce a stable incorporation of organic material (Scholes et al. 1997; Puget and Drinkwater 2001; Spaccini et al. 2004). Conversely, with respect TRA, about 0.6 and 1.0 g kg⁻¹ of OC were additionally incorporated in COM-1 and COM-2 bulk soils, respectively. Based on plowed soil depth (0.35 m) and average soil bulk density of 1.4 (Table 4.1), the OC fixed corresponded to about 0.6 and 1.1 g kg⁻¹ for COM-1 and COM-2, respectively. Therefore, at the end of the second year, the COM plots retained around 100 and 90% of OC added with compost. This means that, by comparing the OC content in MIN and COM-1 between first and second experimentation year (Table 4.6), the SOC fraction in COM-1 must have inherited an aliquot of organic matter incorporated in the first year. The MIN treatment was instead able to significantly fix OC during the second crop cycle, thus recovering the difference from TRA observed in the first year.

The OC distribution in water-stable aggregates after the second year showed that the relative OC content was still related to the physical fractionation yield (Table 4.6). An increase of OC content in large aggregate sizes was shown by TRA, MIN, and COM-2, whereas a greater OC concentration in smaller aggregate sizes was found in GMAN (0.50–0.25 mm) and in COM-1 (0.50–0.25 and <0.25 mm), thus suggesting an accumulation of microbially derived organic components in the latter treatments. The low OC recovery after aggregate fractionation of TRA (92.8% of OC in bulk soil) may imply a contribution of coarse debris from crop residues to TOC of bulk soil. The incorporation of fresh plant-derived organic material was suggested by the large OC concentration (17.4 g kg⁻¹) found in MIN macroaggregates (4.75–1.00 mm), thus indicating that little soil disturbance in MIN may have favored the inclusion of particulate organic matter in the building up soil aggregates (Angers et al 1995; Puget et al. 1995; Angers and Giroux 1996; Six et al. 2000).

Despite the low OC recovery in the aggregate fractionation of wheat soils after two experimentation years (Table 4.6), an OC fixation was evident in CAT due to amendment with biomimetic catalyst. Both absolute and relative OC contents in CAT water-stable aggregates showed a preferential OC incorporation in larger size-fractions.

After three experimentation years, the TOC content showed that both COM-1 and COM-2 were able to increase OC with respect to other maize treatments. In comparisons to the first year, when no difference was observed among different treatments, COM-1 and COM-2 revealed, at final experimental time, an additional OC content of about 0.9 and 1.8 g kg⁻¹, respectively, corresponding to the 87% of the total averaged organic carbon added with compost in the second and third year of soil treatment.

Also GMAN showed a 1.2 g kg⁻¹ increase of TOC content in the third year, as compared to the second year. For an average OC content of about 55% in leguminous plants, this additional amount in soil corresponded to about 13 ton ha⁻¹ of green manure material, and implied that the totality of leguminous plants added to soil as GMAN should have been retained. However, leguminous crops are reckoned to rapidly mineralize, once incorporated in soil (Fernandes et al. 1997; Spaccini et al. 2004), and hardly contribute to stable SOM (Scholes et al. 1997; Puget and Drinkwater 2001). Hence, the larger TOC found in GMAN should be mainly accounted to maize crop residues left on soil.

The OC content in soil aggregates of COM plots after 3 years indicates the capacity of this treatment to increase SOM (Table 4.6). All field treatments under maize revealed an increased OC in large aggregate sizes. However, the greatest absolute and relative OC content was found in the >0.50 mm aggregate class for both COM treatments. This confirms the effectiveness of hydrophobic humified matter in improving soil aggregation and stability, as already suggested by mass yield of soil fractionation (Table 4.2). Conversely, the lower OC recovery in soil aggregate size fractions for GMAN further indicates the poor OC stabilization conferred by this treatment that prevalently provides easily biolabile organic matter in soil.

No significant difference was found between CAT and No-CAT after 3 years for TOC content in bulk soils (Table 4.6). However, the OC distribution in separated aggregate sizes clearly indicates that SOM stabilization was induced by the biometric catalyst treatment. In fact, the 0.50–0.25 and <0.25 mm aggregate size fractions revealed a significant OC increase due to the catalyst, reaching 14.0 and 14.9 g kg⁻¹ for CAT, and only 11.1 and 10.0 g kg⁻¹ for No-CAT, respectively. This further suggests that the catalyzed photo-polymerization of organic constituents of SOM had occurred in situ and its products were associated with the finest soil particle fractions.

4.4.2 Piacenza Experimental Site

After 1 year of maize cultivation, all field treatments showed an overall TOC depletion in bulk soils (Table 4.7). Similar to Torino, the largest decrease was found for MIN, while a lesser OC reduction was found for TRA and COM-2 (Table 4.7). Also for Piacenza, the failure of compost amendment to enhance SOM content in the first year may be attributed to an extensive priming effect induced by compost organic matter.

Table 4.7 Piacenza experimental site, amount (g kg^{-1}) and relative distribution (%) of organic carbon in bulk samples and water-stable aggregate sizes (mm) in soil under different treatments for 3 years of experimentation

Treatments	Bulk g kg^{-1}	Aggregate sizes				Sum of fractions				
		4.75–1.00 g kg^{-1}	1.00–0.50 g kg^{-1}	0.50–0.25 g kg^{-1}	<0.25 g kg^{-1}	g kg^{-1}	%	g kg^{-1}	%	
Control soil	17.2	15.8	15.7	14.4	14.0	10.7	15.4	89.5		
Maize										
<i>First year</i>										
TRA	16.7	12.7	11.1	23.4	20.2	16.5	14.4	86.2		
MIN	14.5	14.3	12.7	14.3	14.7	9.4	14.0	96.6		
COM-2	15.1	12.2	9.0	35.5	26.0	15.2	15.0	99.3		
LSD	1.27	1.0	1.6	3.5	2.5	0.7	NS			
<i>Second year</i>										
TRA	16.6	17.6	10.4	15.5	15.7	16.0	14.6	87.9		
MIN	16.3	16.5	17.4	14.5	13.0	10.7	16.0	98.1		
COM-2	17.0	14.8	15.0	20.6	24.3	15.4	16.7	98.2		
LSD	0.70	1.0	0.7	2.6	1.7	0.8	0.4			
<i>Third year</i>										
TRA	18.7	16.7	16.2	27.9	16.8	8.8	17.6	94.1		
MIN	22.4	22.3	21.4	20.7	19.8	8.9	21.5	96.0		
COM-2	23.5	23.3	20.1	25.3	22.8	7.8	22.7	96.6		
LSD	1.30	2.8	1.8	5.2	5.5	0.2	0.7			
Wheat										
<i>First year</i>										
CAT	16.7 (0.4)	16.2a (0.1)	16.0 (0.4)	23.2b (0.2)	18.1 (0.1)	12.4 (0.1)	16.7a (0.1)	10.0b (0.1)	16.4 (0.4)	98.2
No-CAT	16.3 (0.5)	15.3b (0.4)	15.6 (1.1)	26.2a (0.4)	18.1 (0.9)	12.4 (0.3)	15.1b (0.5)	12.3a (0.4)	15.3 (0.1)	93.8
<i>Second year</i>										
CAT	13.4a (0.1)	11.4b (0.2)	12.2a (0.1)	27.8b (0.3)	19.0a (0.5)	23.3a (0.3)	13.2a (0.3)	15.5 (0.1)	13.2a (0.5)	98.5
No-CAT	12.2b (0.3)	13.2a (0.3)	11.7b (0.2)	29.3a (0.1)	12.3b (0.5)	18.0b (0.1)	11.3b (0.3)	15.4 (0.1)	12.2b (0.4)	100
<i>Third year</i>										
CAT	19.4a (0.5)	17.3 (0.8)	18.0 (0.8)	26.7a (0.1)	26.1a (0.8)	17.2a (0.3)	20.9a (2.1)	12.6a (0.1)	19.0a (0.2)	97.9
No-CAT	17.0b (0.9)	16.5 (0.6)	16.4 (1.2)	25.6b (0.1)	17.5b (1.6)	12.4b (0.1)	16.7b (1.6)	9.9b (0.1)	16.5b (0.4)	97.0

LSD least significant difference for p 0.05 ($n = 4$), NS not significant. Numbers in brackets for wheat plots represent standard deviation ($n = 4$). Different small letters in columns indicate significant difference at 0.05 probability level ($n = 4$)

However, the experiment's progression in the following 2 years indicates that an increasing trend of OC content in bulk soils was shown by both MIN and COM-2, as compared to TRA. In fact, both MIN and COM-2 had a significant larger OC values than TRA in the third year.

Contrary to Torino, a larger OC content was generally found, throughout the experimentation, for all maize treatments in the smaller 0.50–0.25 and <0.25 mm aggregate fractions (Table 4.7). This result is attributed to the influence of physical properties in the soil aggregate hierarchy and, particularly, of the role played by soil texture. Therefore, the large mass found in macroaggregates for the heavy-textured control soil of Piacenza (Tables 4.1 and 4.4) was somewhat reduced over the years in the deeply plowed TRA and COM-2 treatments, with the latter showing the greatest OC content in the fine size fractions (Table 4.7). On the other hand, the least disturbed MIN treatment kept a OC distribution in size fractions similar to the control Piacenza soil. These findings were further supported by data on the relative (%) amount of OC in soil aggregate sizes, which were strongly correlated to mass distribution of the same size fractions (Table 4.4). Moreover, the difference in OC stabilization among treatments was also revealed by the low OC recovered from fractionation of TRA soil (86%), thus indicating an unstable incorporation of free coarse organic debris in the water-stable aggregates of this soil.

As for wheat plots, while no effect of biomimetic catalyst was observed in bulk soils in the first year as compared to control (No-CAT), OC increased significantly in CAT bulk soils in both the second and third year (Table 4.7). Moreover, a significant increase in OC was found in the sum of size fractions of CAT, as compared to No-CAT, for both the second and third year. This was the consequence of the significantly larger OC content found in the smallest <0.25 mm size fraction of CAT, as compared to No-CAT. Since the association of very fine soil particles promotes the formation of new microaggregates also in already existing macroaggregates (Oades 1984; Six et al. 2004), this result may be explained with the catalyst effect on soil organic components and their association in recently formed microaggregates.

4.4.3 Napoli Experimental Site

After the first experimentation year, the OC content in Napoli bulk soil decreased in TRA, MIN, and COM-1 to 8.9, 9.8, and 9.5 g kg⁻¹ values, respectively, as compared to the 10.5 g kg⁻¹ level of control soil (Table 4.8). A maintenance of the initial OC content was shown by GMAN (10.5 g OC kg⁻¹), while additional organic carbon (11.3 g OC kg⁻¹) was incorporated in the soil by the COM-2 treatment. This result seems to indicate that also in the Napoli site a priming effect induced by COM-1 promoted the mineralization of previously incorporated OM.

The findings observed for bulk soils were confirmed by the OC content found in water-stable aggregates (Table 4.8). The soil fractionation of TRA, MIN, and GMAN followed the typical hierarchical distribution of aggregates with decreasing

Table 4.8 Napoli experimental site, amount (g kg^{-1}) and relative distribution (%) of organic carbon in bulk samples and water-stable aggregate sizes (mm) in soil under different treatments for 3 years of experimentation

Treatments	Bulk					Aggregate sizes					Sum of fractions	
	g kg^{-1}	$\frac{\text{g kg}^{-1}}{\%}$	$\frac{\text{g kg}^{-1}}{\%}$	$\frac{\text{g kg}^{-1}}{\%}$	$\frac{\text{g kg}^{-1}}{\%}$	4.75–1.00	1.00–0.50	0.50–0.25	<0.25	g kg^{-1}	%	
Control soil	10.5	11.6	43.3	10.5	30.7	9.3	12.7	8.9	13.3	10.5	100	
Maize												
<i>First year</i>												
TRA	8.9	9.4	37.5	7.8	30.7	8.2	16.5	7.2	15.2	8.2	92.0	
MIN	9.8	10.4	34.1	9.9	36.0	9.0	17.0	8.8	12.8	9.8	100	
GMAN	10.5	11.4	32.2	10.3	41.0	8.9	14.8	8.2	12.0	10.1	96.2	
COM-1	9.5	8.4	49.8	9.6	27.6	11.4	11.3	11.1	11.3	9.4	98.9	
COM-2	11.2	11.4	37.3	11.3	31.0	9.3	10.2	13.3	21.4	11.4	100	
LSD	0.5	1.5	1.7	0.5	1.9	1.4	1.8	1.9	2.0	0.40		
<i>Second year</i>												
TRA	9.1	9.5	27.7	8.6	38.6	9.8	22.0	7.8	11.7	8.9	97.8	
MIN	10.3	11.6	36.8	10.5	42.0	9.2	13.9	7.8	7.2	10.3	100	
GMAN	10.0	10.1	35.3	9.4	39.2	8.2	15.4	7.7	10.0	9.2	92.0	
COM-1	10.0	10.7	41.3	9.2	34.9	9.6	14.7	8.6	9.2	9.8	98.0	
COM-2	11.3	12.6	36.9	11.3	37.1	9.7	15.8	8.9	10.2	11.1	98.3	
LSD	0.5	0.6	1.1	0.5	1.0	0.6	1.8	0.8	1.3	0.60		
<i>Third year</i>												
TRA	9.5	10.0	44.4	7.8	30.4	8.5	15.6	7.2	11.1	8.7	91.6	
MIN	10.1	10.1	44.0	9.2	31.3	9.2	15.8	7.5	10.8	9.4	93.1	
GMAN	10.0	9.4	44.9	7.9	25.8	12.0	16.6	13.0	10.2	9.6	96.0	
COM-1	10.5	10.1	41.9	11.8	33.9	10.9	16.6	7.2	11.1	10.2	97.1	
COM-2	12.5	12.2	42.5	11.8	31.2	13.1	15.6	13.9	12.2	12.4	99.2	
LSD	0.5	0.5	1.7	0.9	1.5	0.4	NS	1.2	0.5	0.60		
Wheat												
<i>First year</i>												
CAT	10.4 (0.1)	10.9 (0.2)	54.6a (1.1)	9.6b (0.3)	24.3b (0.2)	10.3 (0.7)	11.1b (0.8)	9.7 (0.3)	9.9 (0.3)	10.2 (0.3)	98.1	

No-CAT	11.0 (0.5)	10.9 (0.4)	49.8b (1.5)	10.5a (0.4)	27.7a (0.4)	10.3 (0.5)	12.9a (0.5)	8.7 (0.8)	9.7 (0.2)	10.6 (0.5)	96.4
<i>Second year</i>											
CAT	11.1 (0.8)	11.6 (0.9)	33.0b (0.4)	10.8a (0.4)	39.0 (0.2)	10.6a (0.4)	16.3a (0.2)	11.2a (1.0)	11.5a (0.1)	11.0a (0.2)	99.1
No-CAT	10.0 (0.2)	10.7 (0.7)	39.4a (0.3)	10.0b (0.1)	40.3 (0.9)	9.0b (0.3)	11.9b (1.0)	8.4b (0.8)	8.3b (0.1)	9.9b (0.1)	99.0
<i>Third year</i>											
CAT	11.6 (0.2)	12.6a (0.5)	47.4a (1.0)	10.2b (0.1)	30.3b (0.1)	10.1a (0.6)	14.0 (1.2)	9.0 (0.4)	8.3 (0.9)	11.0a (0.3)	94.8
No-CAT	11.4 (0.3)	10.8b (0.3)	41.6b (0.2)	10.6a (0.2)	36.1a (0.5)	9.1b (0.1)	13.5 (0.2)	8.7 (0.1)	8.8 (0.1)	10.3b (0.3)	90.2

LSD least significant difference for $p < 0.05$ ($n = 4$), *NS* not significant. Numbers in brackets for wheat plots represent standard deviation ($n = 4$). Different small letters in columns indicate significant difference

OC towards smaller aggregates. Conversely, a significantly larger OC content was found in the 0.50–0.25 mm size fractions for COM-1 and in microaggregates (<0.25 mm) for both COM-1 and COM-2. As already reminded, this result may be attributed to adsorption of microbially processed organic components on fine soil particles and their association in microaggregates. While COM-2 appeared to better incorporate organic carbon in water-stable aggregates, the low OC recovery from fractionation of the TRA soil (92%) suggests that its OC content was due mainly to hardly transformed and, thus, coarse incorporated plant residues.

For soils under wheat, no consistent differences in OC content were found between CAT and No-CAT for either bulk soils or water-stable aggregates (Table 4.8), thus showing that the biomimetic catalyst did not stabilize SOM components in the first year.

Some differences in SOM dynamics among treatments were shown in Napoli after the second experimental year (Table 4.8). An increase of about 0.5 g kg^{-1} in bulk soil OC, as compared to the first year, was noted for MIN and COM-1. The same OC level found in TRA (9.1 g kg^{-1}) and COM-2 (11.3 g kg^{-1}) indicates that both OC deposition by crop in subsoil and OM added with compost were able to maintain the SOC content of the first year. Conversely, the plant residues mixed in soil with the GMAN treatment were not similarly effective and TOC content in this bulk soil decreased in this treatment.

A stable OM incorporation in aggregate size fractions was suggested after the first year in MIN, COM-1, and COM-2 by the large OC recovery in sum of fractions of these treatments. The OC stabilization was particularly evident for both COM rates which showed a significantly greater OC content in <0.25 mm microaggregates (Table 4.8). On the other hand, the low OC recovery in GMAN sum of fractions (92.4%), with respect to the OC of bulk soil, further suggests a weaker interaction between soil aggregates and organic matter added by green manuring.

The treatment with the biomimetic catalyst resulted more effective in the second year of wheat cultivation (Table 4.8). TOC content in CAT bulk soil increased about 1.0 g kg^{-1} with respect to the first year, while that of No-CAT was reduced by 1.5 g kg^{-1} . The effectiveness of SOM stabilization promoted by catalyst addition was further indicated by the OC distribution in water-stable aggregates. An increased absolute OC content was found in <1.0 mm aggregate sizes in CAT soil. However, the relative % OC distribution in the 4.75–1.00 mm size fraction was also significantly larger than for No-CAT. These results are in line with those found for wheat plots in Torino and Piacenza, for which OM was preferentially associated with the finest soil particles due to the catalyzed coupling of humus aromatic compounds.

The superior OM fixation in soils treated with compost and biomimetic catalyst, with respect to conventional management practices, was revealed by the OC content in bulk soils and aggregate size fractions of the third year (Table 4.8). Bulk soils under COM-1 and COM-2 showed an OC content larger than that of second year by 0.5 and 1.2 g kg^{-1} , respectively, and even 1.0 and 2.5 g kg^{-1} greater than for TRA of the same third year. As for COM-1, the final OC value in bulk soil (10.5 g kg^{-1}) was the same as that of the initial undisturbed soil. However,

although the priming effect noted in all soils with this treatment caused SOM losses, the OC incorporated in the Napoli site by COM-1 over control corresponded to $0.5 \text{ g kg}^{-1} \text{ year}^{-1}$. Based on an average 1.4 soil bulk density and 0.35 m plow depth, this amount represents about 80% of the total OC added with COM-1 in the 2 years of experimentation. This consideration, along with the observed large OC fixed by COM-2, confirms that mature compost amendment in the conditions of the Napoli site appears as a reliable method for carbon sequestration in soil.

The same can be argued for the CAT treatment in the third year that was further capable of significantly enhancing OC content in the largest macroaggregate size fraction and firmly sequester carbon, as shown by the OC fractionation losses which were lower than for No-CAT. Finally, the stable OC content for MIN and GMAN soils throughout the experimentation period and its significantly larger values than for TRA indicate that these soil management practices do also effectively sequestered carbon, though to a significantly lesser extent than COM and CAT treatments.

4.5 The Humified SOM Fraction and Its Characterization by Nuclear Magnetic Resonance Spectroscopy

The humified organic matter in soil, operationally separated in humic acid (HA), fulvic acid (FA), and humin (HU), is considered the most microbially stable reservoir of SOC, and the most important component for the maintenance of the soil physical–chemical and biological quality (Piccolo 1996). While the operationally non-extractable HU is the most passive fraction of humified matter (Hayes et al. 1989; Simpson et al. 2007), the extractable humic and fulvic fractions exert the essential role of intermediary compartments between the more labile organic compounds and the stable SOM pool. Humic matter results from the progressive accumulation in soil of hydrophobic organic molecules (Deport et al. 2006; Spaccini and Piccolo 2009) surviving the microbial degradation of plant residues. During humus formation, biolabile compounds present in the soil solution are progressively incorporated in the humic hydrophobic superstructures (Spaccini et al. 2000; Piccolo 2002). Moreover, humic molecules are also stabilized by the formation of complexes with different metals and adsorption on surfaces of soil minerals (Nebbioso and Piccolo 2009; Cornejo and Hermosin 1996).

The operational extraction of HS from soil consists in the alkaline separation of organic matter from soil mineral components and isolation of fulvic and humic acids based on their solubility at different pH values (Swift 1996). However, it is yet to be proven that the humic superstructures observed in extracts maintain the same intermolecular arrangement as in soil before extraction (Piccolo 1996; Spaccini et al. 2000; Kelleher and Simpson 2006; Nebbioso and Piccolo 2011). Nevertheless, due to the HS essential role in conferring soil quality and controlling all SOM transformation processes, their characterization is regarded as a valuable tool to appraise the effects of different management practices on long-term sequestration of organic carbon in cultivated soils (Swift 2001). Moreover, extractable humic

matter (HA and FA) was found to be in short-time dynamic interactions with other soil components (Spaccini et al. 2000). Thus, in this work, HS (jointly HA and FA) were alkaline extracted from soils subjected to the MESCOSAGR project treatments. The extracts were neutralized and purified as customary for humic acids (Spaccini et al. 2009), before being characterized by solid-state NMR spectroscopy.

The non-destructive solid-state ^{13}C cross-polarization-magic-angle-spinning (CPMAS) NMR technique provides the molecular distribution of organic carbons in solid matrices without extensive sample pre-treatment. This solid-state NMR technique is widely used to characterize the composition of litter, SOM, and humic substances, as well as the transformation of plant tissues in soil (Kögel-Knabner 2000; Hatcher et al. 2001; Conte et al. 2004; Zhou et al. 2010). Although CPMAS spectra are not strictly quantitative, a reproducible quantitation of molecular distribution may be achieved from spectra of soil humic fractions when acquisition parameters are correctly adopted (Kinchesh et al. 1995; Piccolo et al. 2005b; Spaccini et al. 2006).

A detailed comparison among NMR spectra may become excessively tedious and time consuming for the large number of CPMAS spectra obtained for HS extracts within the MESCOSAGR project. This burden can be reduced by applying chemometric methods or multivariate analyses. Among these, principal component analysis (PCA) is widely used to simplify interpretation of chemical and spectroscopic data for complex systems (Einax et al. 1997). The main purpose of PCA is to reduce the original data set, represented by an ' n ' dimensional space (where n is the number of variables or experimental results), into a few principal components (PC), which concomitantly retain the maximum percentage of original information contained in the data set. The principal components are derived as a linear combination of the original variables, such as NMR spectral areas. The variables are multiplied by loadings, which are vectors of constants generated during PCA. The numerical values of loadings reflect the importance of original variables in the direction of each PC. The resulting PC can be used to project the originally multidimensional data into only a two- or three-dimensional space, which is called a score plot (Brereton 2003). Even without a specific knowledge of statistical implications, these plots enable a rapid and direct evaluation of similarities, differences, and groupings among the original samples, which were extracted by PC analysis. Principal component analysis performed on ^{13}C NMR data has been successfully applied for statistical differentiation of humic substances and organic matter in agricultural soils (Novotny et al. 2007; Šmejkalova et al. 2008). Previous works have proved that PCA of ^{13}C -CPMAS NMR spectra of humic matter provide the required discrimination among heterogeneous samples and may be useful to evaluate SOM quality.

In this study, HS were extracted for each experimental site from the initial undisturbed soil and from soils at the end of each cropping cycle after harvesting of either maize or wheat. The four soil replicates from field treatments were mixed in order to obtain about 1 kg of composite soil sample. The free-lipid components were first removed from soil with two consecutive extractions with a 1:10 w/v of

dichloromethane–methanol (2:1 v/v) solution. The, humic substances were then extracted by shaking the soil (20 g) overnight with 100 ml of 0.1 M NaOH–Na₄P₂O₇ (1:1 v/v) solution under N₂ atmosphere. After centrifugation, the solid residue was washed with distilled water until pH 7. The supernatant and washings were combined, filtered on a quartz filter (Whatman GF/C), neutralized to pH 6 with 1 M HCl, dialyzed against deionized water, and freeze dried. The extractions were conducted in duplicate.

¹³C-CPMAS-NMR spectra of the compost materials as well as of HS extracted from soils were acquired on a Bruker AV-300, equipped with a 4 mm wide-bore MAS probe. Spectra were obtained with the following parameters: 13,000 Hz of rotor spin rate; 1 s of recycle time; 1 ms of contact time; 20 ms of acquisition time; 4,000 scans. Samples were packed in 4 mm zirconia rotors with Kel-F caps. The pulse sequence was applied with a 1H ramp to account for non-homogeneity of the Hartmann–Hahn condition at high spin rotor rates. For the interpretation of ¹³C-CPMAS-NMR spectra, the overall chemical shift range was divided into the following main resonance regions (Spaccini et al. 2009): alkyl-C (0–45 ppm); methoxyl-C and N-alkyl-C (45–60 ppm); O-alkyl-C (60–110 ppm); unsubstituted and alkyl-substituted aromatic-C (110–145 ppm); oxygen substituted aromatic-C (145–160 ppm); carboxyl- and carbonyl-C (160–200 ppm). The area of each spectral region (R_i^{abs}) was divided by the sum of all spectral areas, in order to obtain a relative percentage ($R_i\%$):

$$R_i\% = \left(\frac{R_i^{\text{abs}}}{\sum_i R_i^{\text{abs}}} \right) \times 100$$

The $R_i\%$ values were used as variables for the multivariate statistical analysis.

In order to summarize the modifications brought about by different soil treatments on the molecular composition of humic extra cts, the following structural indexes were calculated from the relative amount of C distribution over the NMR spectra:

$$\text{HB} = \frac{\Sigma(0 - 45\text{ppm}) + (45 - 60\text{ppm}) + (110 - 160\text{ppm})}{\Sigma(45 - 60 \text{ ppm}) + (60 - 110 \text{ ppm}) + (160 - 190 \text{ ppm})}$$

$$\text{Lignin Ratio} = \frac{(45 - 60\text{ppm})}{(145 - 160 \text{ ppm})} \quad \text{AR} = \frac{(110 - 160\text{ppm})}{(0 - 190\text{ppm})}$$

The hydrophobic index (HB) is the ratio of signal intensity in chemical shift intervals for hydrophobic C components over that in intervals for hydrophilic C components. The Lignin Ratio is the ratio of signal intensity in the 60–45 ppm interval over that in the 160–145 ppm interval. The aromaticity index, Ar, is the relative percentage of aromatic components in the samples. The larger the HB and Ar values of a OM sample, the greater is its hydrophobic character and its content of aromatic molecules, respectively (Zhou et al. 2010). Likewise, the smaller the value

of the Lignin Ratio, the larger is the content of lignin-derived material in the humic extract or the lesser is the amount of biolabile hydrophilic carbon, such as in peptides (C–N in the 60–45 ppm interval) (Spaccini et al. 2009).

4.5.1 *NMR Spectra of Initial Soils and Composts Before Amendments*

The ^{13}C -CPMAS-NMR spectra of compost used for field treatments in the three experimental sites are shown in Fig. 4.1, whereas the relative distribution of signal areas is reported in Table 4.9. Since compost samples had a common origin, a similar C distribution was found in NMR spectra.

The spectra of mature composts were dominated by the alkyl-C (0–45 ppm) and O-alkyl-C (60–110 ppm) signals. The alkyl-C region comprised a prominent peak at around 30 ppm, mostly due to methylene carbon chains $(\text{CH}_2)_n$ and terminal methyl groups in plant lipid compounds, such as waxes and aliphatic biopolyesters.

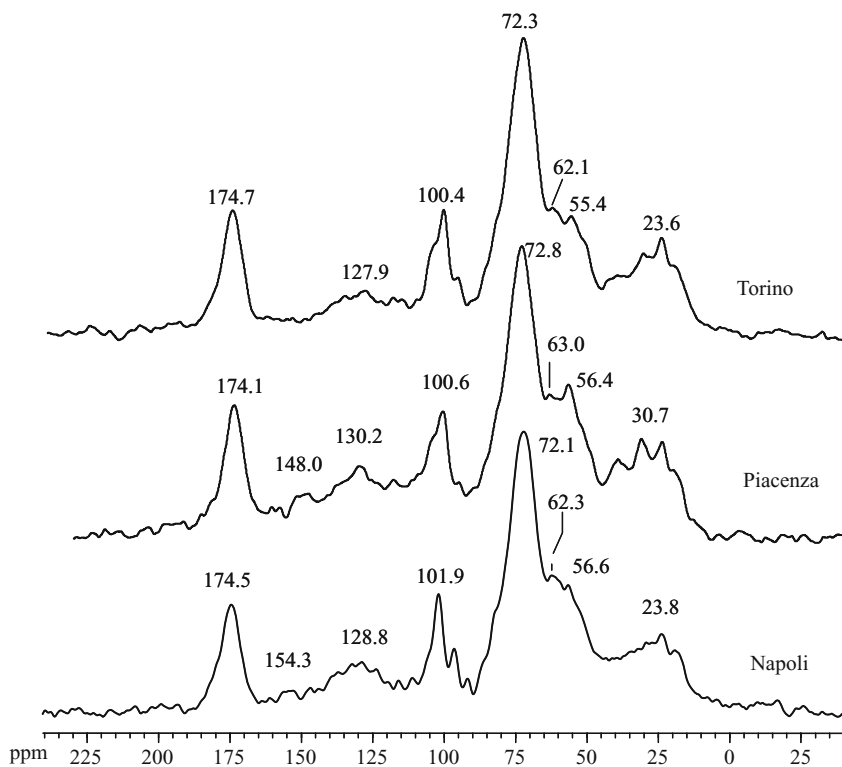


Fig. 4.1 ^{13}C -CPMAS-NMR spectra of the mature compost materials used for soil treatments at Torino, Piacenza, and Napoli

Table 4.9 Relative distribution (%) of signal area over chemical shift regions (ppm) in ^{13}C -CPMAS-NMR spectra of compost samples used for field treatments

Field site	Chemical shift regions						LR ^a	HB ^b
	Carboxylic-C (190–160)	Phenolic-C (160–145)	Aromatic-C (145–110)	O-alkyl-C (110–60)	CH ₃ O/C-N (60–45)	Alkyl-C (45–0)		
Torino	8.2	3.2	12.2	37.1	10.5	28.7	3.3	0.97
Piacenza	7.3	4.2	11.8	39.4	10.6	26.8	2.5	0.93
Napoli	8.7	4.1	11.6	36.9	10.5	28.3	2.6	0.97

^aLignin Ratio (LR) = (45–60 ppm)/(145–160 ppm)

^bHydrophobic index (HB) = $\Sigma[(0-45) + (45-60) + (110-160)]/\Sigma [(45-60) + (60-110) - (160-190)]$

The O-alkyl-C resonances are assigned to monomeric units in oligo and polysaccharidic chains of plant woody tissues (Vane et al. 2001; Wikberg and Maunu 2004). The intense signal around 72 ppm corresponds to the overlapping resonances of C2, C3, and C5 carbons in the pyranoside structure of cellulose and hemicellulose, whereas the signals at 105 ppm (sharp), 64 ppm, and 82–88 ppm (shoulders) are assigned to the anomeric C1, and C6 and C4 carbons, respectively, the latter being split in the presence of both amorphous and crystalline forms of cellulose (Atalla and VanderHart 1999). Plant woody tissues were also indicated by the 56 ppm shoulder of methoxy groups on the aromatic rings of guaiacyl and siringyl units in lignin structures (Liitiä et al. 2002; Zhou et al. 2010). The aromatic region (110–160 ppm) also revealed distinct resonances for O-substituted aromatic carbons in the 147–152 ppm interval, the 128 ppm broad signal for C-substituted or C-unsubstituted aromatic rings of lignin monomers (Albrecht et al. 2008), or cinnamic and ferulic units in suberin biopolymer (Stark et al. 2000; Graça and Santos 2007). Finally the sharp signal at 172 ppm is currently assigned to quaternary carbons of carboxyl groups. The OC composition shown by NMR spectra of compost closely resembles the molecular characteristics found for stable humified organic material in SOM (Spaccini and Piccolo 2007; Caricasole et al. 2011) with a large HB index (Table 4.9) due to a great content of hydrophobic molecules (Spaccini et al. 2008; Canellas et al. 2010).

Conversely, CPMAS-NMR spectra of HS extracted from initial control soils showed a predominance of hydrophilic carbon components and consequent low HB values (Fig. 4.2 and Table 4.10). These extracts revealed that 58–61% of total C was due to carbohydrates (110–60 ppm) and oxidized carboxylic groups (190–160 ppm), whereas less than 15–17% of carbon was in aromatic (160–110 ppm) and alkyl (45–0) structures (Table 4.10).

If humic extracts contained residual lignin carbon, the signal of methoxyl C at around 56 ppm and that of phenolic C in the 160–145 ppm region should bear a close relationship. An intense peak at 56 ppm was shown by NMR spectra of both compost samples and HS from control soils, but the intensity of the phenolic C resonance was different in these two organic materials (Figs. 4.1 and 4.2, respectively). A Lignin Ratio (see above) can be calculated using the relative areas of both 60–45 ppm and 160–145 ppm intervals in NMR spectra (Tables 4.9 and 4.10),

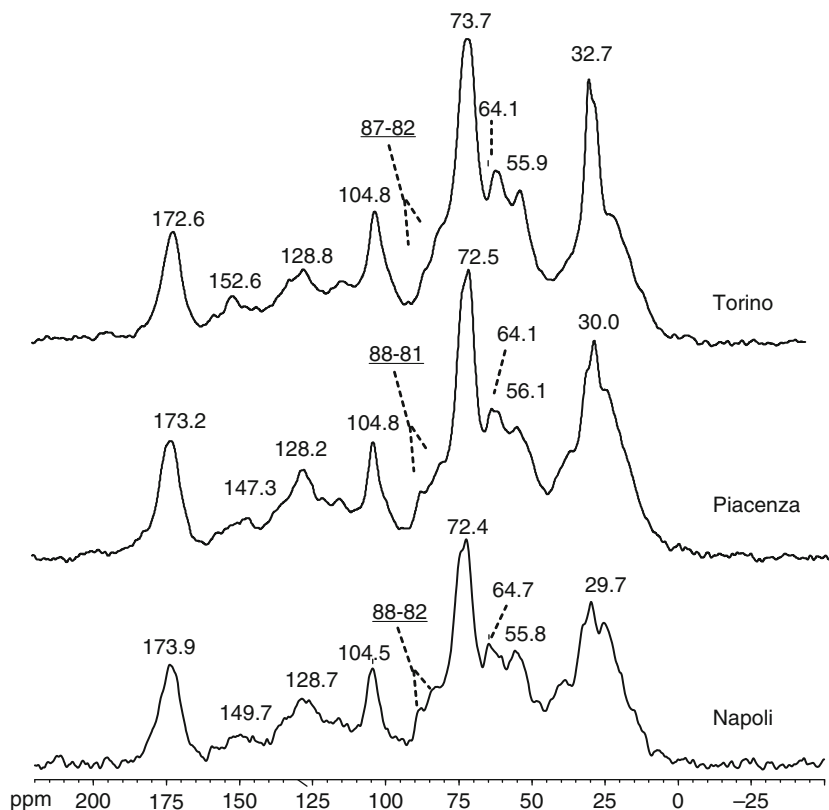


Fig. 4.2 ^{13}C -CPMAS-NMR spectra of humic substances extracted from initial control soils at Torino, Piacenza, and Napoli

Table 4.10 Relative distribution (%) of signal area over chemical shift regions (ppm) in ^{13}C -CPMAS-NMR spectra of humic substances from control soils

Field site	Chemical shift regions						LR ^a	HB ^b
	Carboxylic-C (190–160)	Phenolic-C (160–145)	Aromatic-C (145–110)	O-alkyl-C (110–60)	CH ₃ O/C-N (60–45)	Alkyl-C (45–0)		
Torino	9.9	2.5	12.1	48.3	12.6	14.6	5.0	0.55
Piacenza	11.0	1.7	9.0	51.2	9.6	17.5	5.6	0.49
Napoli	9.2	2.0	10.1	48.9	12.7	17.1	6.3	0.55

^aLignin Ratio (LR) = (45–60 ppm)/(145–160 ppm)

^bHydrophobic index (HB) = $\Sigma[(0-45) + (45-60) + (110-160)]/\Sigma[(45-60) + (60-110) - (160-190)]$

respectively). For compost samples, the Lignin Ratio varied from 2.5 to 3.3, while that for HS from control soils ranged between 5 and 6.3. These values thus indicate a likely content of lignified materials in compost samples (Vane et al. 2003), whereas this appears less probable for HS from control soil. In the latter, the intense

signal at 56 ppm should be better attributed to C nuclei in *alpha* position of amino-acid moieties (Quideau et al. 2001; Hayes et al. 2008) rather than to methoxyl C in Lignin.

In fact, NMR spectra of HS from control soils indicate that long-term cultivation without OM inputs reduced the amount of hydrophobic carbon, with consequent prevalent incorporation of biolabile components such as plant carbohydrates and peptidic derivatives. A small hydrophobicity of humic matter is a common feature in highly exploited and weathered soils (Piccolo et al. 2005b; Spaccini et al. 2006). The simultaneous increase of hydrophilic components with a decrease of alkyl-C in NMR spectra of SOM has been attributed to the presence of highly decomposable materials (Baldock et al. 1997; Webster et al. 2001) with short residence time, fast turnover, and slow accumulation rate.

4.5.2 Principal Component Analysis of NMR Spectra of Humic Substances Extracted from Soil Treatments

In order to compare the effect produced by soil treatments on the molecular composition of HS extracts at different experimental times, the areas of C signals in NMR spectra were integrated and their values elaborated with principal component analysis (PCA). The multivariate analysis for the first and third experimentation year at Torino, Piacenza, and Napoli sites of the MESCOSAGR project is shown in Figs. 4.3–4.5, respectively. The relative values (%) associated with the *x* and *y* axes in PCA diagrams stand for the total variation of NMR data explained by two statistical principal components (PC1 and PC2), while the dotted lines represent the loading vectors associated with the variables (ppm intervals) obtained from NMR spectra. Tables 4.11–4.13 show the variation of HS structural indexes throughout the three experimental years as calculated from NMR spectra for different soil treatments and experimental sites.

4.5.2.1 Torino Experimental Site

For the first and third experimentation years, PCA score plots of HS from TRA were compared with those from MIN and GMAN treatments (Fig. 4.3a). An overall treatment discrimination was given by PC1 between TRA and MIN and between TRA and GMAN. However, no consistent molecular variation was evident in HS composition among treatments for either replicates or experimental years. The loading vectors associated with signals at 110–60 ppm and 45–0 ppm indicate that HS extracted from MIN after the first year were rich in carbohydrates and poor in alkyl components, respectively. At the final year, an opposite distribution of carbohydrates and alkyl-C was revealed by the corresponding loading vectors in the TRA vs. MIN biplots. Moreover, although the principal components, PC1 and PC2,

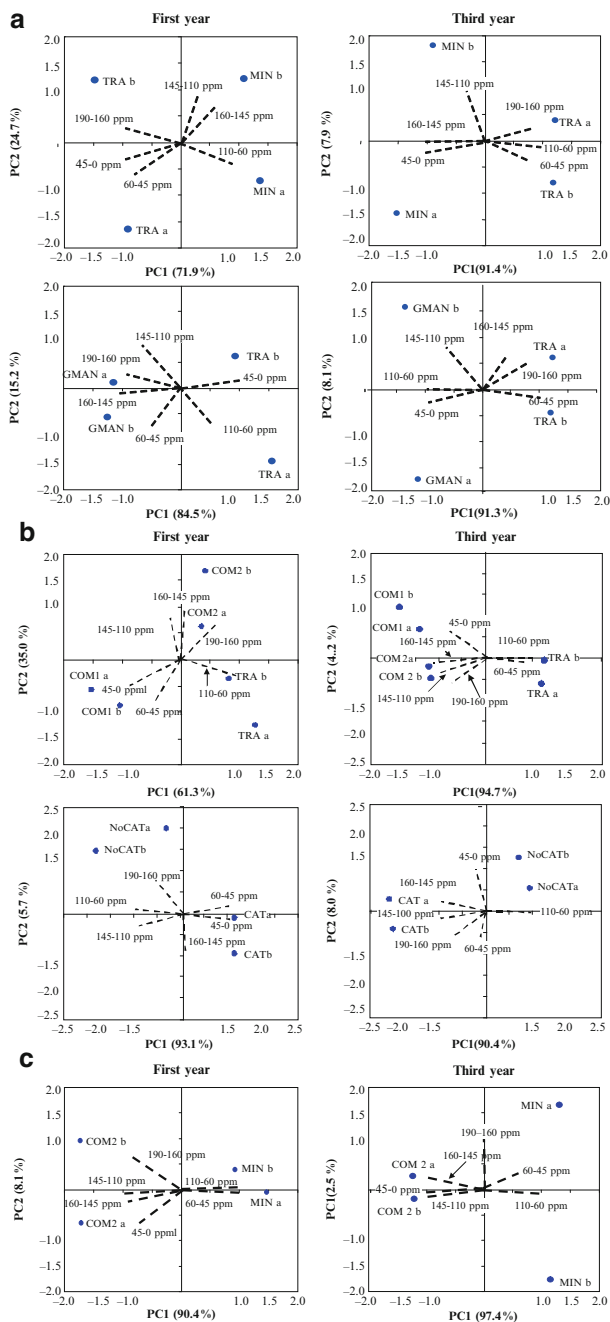


Fig. 4.3 Torino experimental site: biplots generated by principal component analysis of NMR spectra of humic substances extracted from soil treatments (a) TRA vs. MIN and TRA vs. GMAN both at first and third year of field experimentation (*a* and *b* indicate first and second replicates) (b) MIN vs. COM-1 and COM-2 and CAT vs. NoCAT, at first and third year of field experiments (*small letters* indicate replicates) (c) COM-2 vs. MIN at first and third year of field experiments (*small letters* indicate replicates)

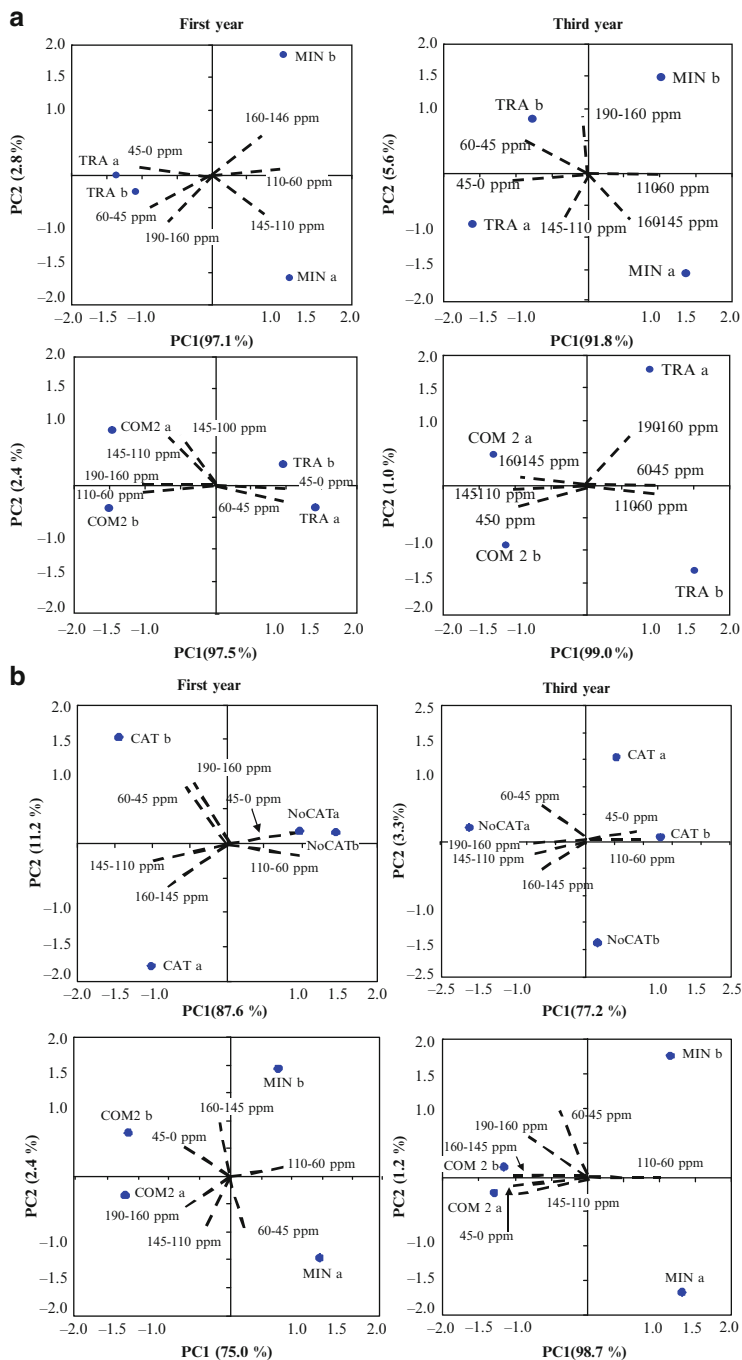


Fig. 4.4 Piacenza experimental site: biplot generated by principal component analysis of NMR spectra of humic substances extracted from soil treatments **(a)** TRA vs. MIN and COM-2 at first year of field experiments (*small letters* indicate replicates) **(b)** CAT vs. NoCAT and COM-2 vs. MIN at first and third year of field experiments (*small letters* indicate replicates)

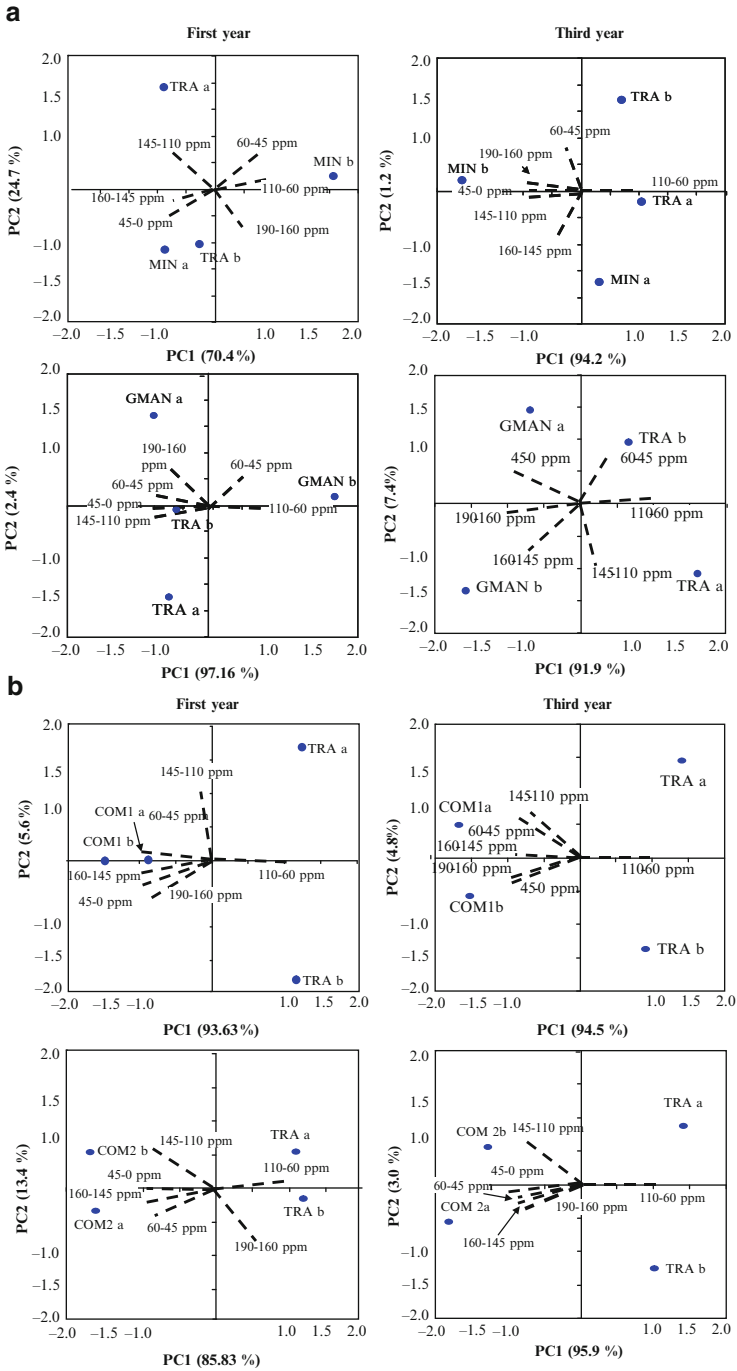


Fig. 4.5 (continued)

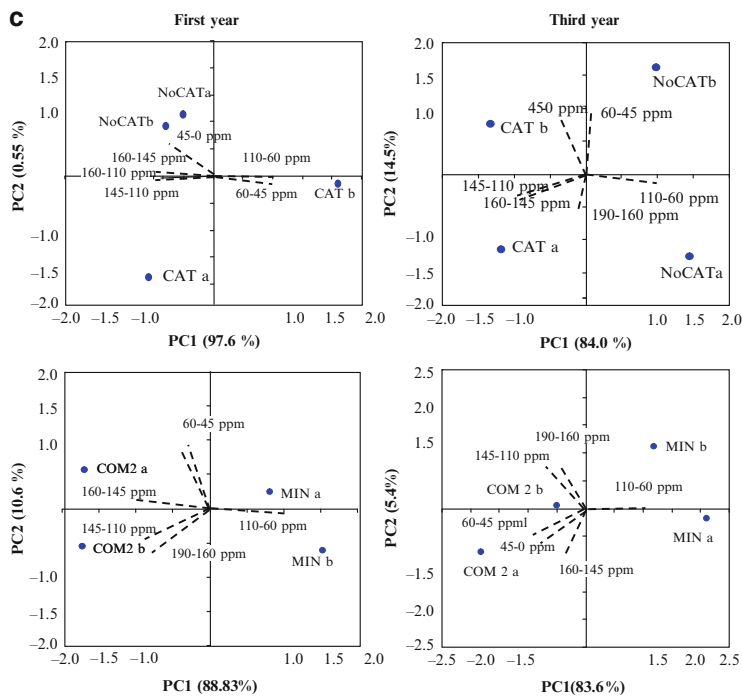


Fig. 4.5 Napoli experimental site: biplot generated by principal component analysis of NMR spectra of humic substances extracted from soil treatments (a) TRA vs. MIN and GMAN at first and third year of field experiments (*small letters* indicate replicates) (b) TRA vs. COM1 and COM2 at first and third year of field experiments (*small letters* indicate replicates) (c) CAT vs. noCAT and MIN vs. COM2 at first and third year of field experiments (*small letters* indicate replicates)

represented from 95 to 99% of total variability, no significant differences were found between HS from MIN and TRA in the loading vectors for molecular components.

A similar PCA was derived by comparing NMR spectra of HS extracted from TRA and GMAN (Fig. 4.3a). After 1 year, the main variation was along the PC1 component (84%) and mainly accountable to distribution of phenolic (160–145 ppm) and alkyl (45–0 ppm) compounds, whose amounts were, respectively, larger and lower in HS from GMAN than from TRA. After 3 years, the alkyl-C became more abundant in HS from GMAN, whereas no longer the distribution of phenolic and aromatic compounds represented a significant difference for HS from TRA. With progression of experimental time, NMR spectra of HS from both MIN and GMAN revealed a larger content of hydrophobic molecules than for HS from TRA (Table 4.11). In fact, both the slight HB increase and the more pronounced decrease of Lignin Ratio suggested a progressive incorporation of alkyl and phenolic compounds in HS from MIN and GMAN as compared to those from TRA.

Table 4.11 Torino experimental site, variation with experimental time of structural indexes HB, Lignin Ratio and Aromaticity (%), calculated from ¹³C-CPMAS-NMR spectra of humic substances extracted from soil treatments for 3 years of experimentation

Treatment	First year		Second year		Third year	
	HB	Lignin Ratio	HB	Lignin Ratio	HB	Lignin Ratio
Maize						
TRA	0.73 (0.02)	4.61 (0.22)	0.76 (0.04)	3.92 (0.35)	0.69 (0.01)	4.19 (0.34)
MIN	0.71 (0.01)	3.95 (0.57)	0.79 (0.01)	3.17 (0.05)	0.83 (0.01)	3.16 (0.19)
GMAN	0.73 (0.01)	3.28 (0.02)	0.75 (0.03)	3.34 (0.44)	0.85 (0.02)	3.40 (0.06)
COM1	0.82 (0.02)	4.70 (0.29)	0.84 (0.03)	2.94 (0.10)	0.91 (0.01)	2.66 (0.39)
COM2	0.82 (0.02)	2.93 (0.03)	1.03 (0.13)	2.66 (0.24)	1.09 (0.01)	2.37 (0.05)
Treatment	First year		Second year		Third year	
	HB	Aromaticity	HB	Aromaticity	HB	Aromaticity
Wheat						
CAT	0.71 (0.01)	12.8 (0.22)	0.76 (0.04)	16.5 (1.01)	0.85 (0.04)	17.5 (0.37)
No-CAT	0.65 (0.01)	13.4 (0.56)	0.76 (0.04)	15.8 (0.49)	0.77 (0.02)	14.6 (0.14)

Number in parentheses indicate standard deviation ($n = 2$)

Hydrophobic index (HB) = $\Sigma[(0-45) + (45-60) + (110-160)]/\Sigma[(45-60) + (60-110) - (160-190)]$

Lignin Ratio = $(45-60)/(145-160)$

Aromaticity = $(110-160)/(0-190)$

Table 4.12 Piacenza experimental site, variation with experimental time of structural indexes HB, Lignin Ratio and Aromaticity (%), calculated from ¹³C-CPMAS-NMR data of humic substances extracted from soil treatments for 3 years of experimentation

Treatment	First year		Second year		Third year	
	HB	Lignin Ratio	HB	Lignin Ratio	HB	Lignin Ratio
Maize						
TRA	0.63 (0.01)	6.46 (0.48)	0.74 (0.01)	3.52 (0.27)	0.73 (0.01)	3.61 (0.27)
MIN	0.60 (0.01)	5.86 (0.30)	0.78 (0.01)	3.45 (0.67)	0.66 (0.09)	3.95 (0.25)
COM2	0.71 (0.02)	5.58 (0.15)	0.98 (0.02)	2.36 (0.18)	0.93 (0.02)	2.01 (0.07)
Treatment	First year		Second year		Third year	
	HB	Aromaticity	HB	Aromaticity	HB	Aromaticity
Wheat						
CAT	0.67 (0.01)	16.2 (0.32)	0.74 (0.02)	14.2 (0.07)	0.70 (0.03)	14.3 (0.25)
No-CAT	0.64 (0.01)	10.9 (1.29)	0.76 (0.07)	15.2 (1.43)	0.73 (0.01)	14.1 (0.95)

Number in parentheses indicate standard deviation ($n = 2$)

Hydrophobic index (HB) = $\Sigma[(0-45) + (45-60) + (110-160)]/\Sigma[(45-60) + (60-110) - (160-190)]$

Lignin Ratio = $(45-60)/(145-160)$

Aromaticity = $(110-160)/(0-190)$

The PCA of NMR data for HS extracted from compost-treated plots revealed, with experimental time, an increased incorporation of exogenous hydrophobic organic carbon. After the first year, a significant discrimination ($PC1 + PC2 > 96\%$), was evident between both compost treatments and TRA (Fig. 4.3b). In fact, the loading

Table 4.13 Napoli experimental site, variation with experimental time of structural indexes HB, Lignin Ratio and Ar, calculated from ¹³C-CPMAS-NMR data of humic substances extracted from soil treatments for 3 years of experimentation

Treatment	First year		Second year		Third year	
	HB	Lignin Ratio	HB	Lignin Ratio	HB	Lignin Ratio
Maize						
TRA	0.80 (0.01)	2.67 (0.12)	0.72 (0.04)	4.07 (0.38)	0.85 (0.01)	3.03 (0.34)
MIN	0.94 (0.13)	2.23 (0.29)	0.85 (0.03)	2.50 (0.02)	0.83 (0.06)	2.85 (0.27)
GMAN	0.92 (0.08)	2.43 (0.32)	0.74 (0.05)	2.67 (0.00)	0.71 (0.05)	3.52 (0.09)
COM1	0.96 (0.06)	2.10 (0.11)	0.89 (0.04)	2.59 (0.15)	0.98 (0.01)	2.28 (0.12)
COM2	1.08 (0.02)	2.31 (0.23)	0.95 (0.03)	2.20 (0.20)	1.06 (0.03)	1.64 (0.13)
Treatment	First year		Second year		Third year	
	HB	Aromaticity	HB	Aromaticity	HB	Aromaticity
Wheat						
CAT	1.07 (0.04)	28.1 (0.16)	0.90 (0.07)	24.8 (1.71)	0.90 (0.00)	25.2 (0.66)
No-CAT	0.91 (0.03)	28.1 (0.84)	0.79 (0.02)	22.9 (0.81)	0.90 (0.04)	25.0 (0.71)

Number in parentheses indicate standard deviation ($n = 2$)

Hydrophobic index (HB) = $\Sigma[(0-45) + (45-60) + (110-160)]/\Sigma[(45-60) + (60-110) - (160-190)]$

Lignin Ratio = $(45-60 \text{ ppm})/(145-160 \text{ ppm})$

Aromaticity = $(110-160)/(0-190 \text{ ppm})$

vectors in the score plot indicated a larger incorporation of alkyl compounds (45–0 ppm) for COM-1 and of both phenolic (160–145 ppm) and aromatic components (145–110 ppm) for COM-2. The NMR spectra of HS from TRA were instead significantly dominated by O-alkyl-C units from carbohydrates and polysaccharides (110–60 ppm). The acquired hydrophobicity of compost-treated soils was also shown by the increased HB index (0.83) for NMR spectra of their HS extracts, as compared to those from TRA, whereas the low values for Lignin Ratio in HS from COM-2 suggested a large incorporation of lignin-like aromatic components (Table 4.11).

The increased hydrophobicity of HS from COM-1 and COM-2 throughout the experimental period shows that compost stabilized material has been progressively incorporated in soil humic fractions (Fig. 4.3b and Table 4.11). After the third year, the PCA of NMR data revealed enhancement of phenolic (160–145 ppm), unsubstituted aromatic (145–110), and alkyl C components (45–0 ppm) in HS from both COM treatments, while the loading vectors associated with NMR signals of hydrophilic C at 160–110 and 60–45 ppm were prevalent in HS from TRA. Furthermore, the large content of C–N bonds from peptidic moieties (60–45 ppm) in HS from TRA was accompanied by the increased Lignin Ratio values as a sign of progressive reduction of hydrophobicity in soil humus from TRA. The contribution of composted matter (mainly alkyl and lignin derivatives) in the molecular composition of stable soil HS was shown by the NMR-derived HB index and Lignin Ratio for COM-1 and COM-2 soil treatments. The enhanced hydrophobicity of HS from COM-2 was also evident in comparison with NMR results obtained for MIN (Fig. 4.3b). The loading vectors associated with alkyl (45–0 ppm) and phenolic

and aromatic (160–110 ppm) C regions, for both the first (PC1 90.7%) and third experimental years (PC1 97%) indicated the occurred incorporation of hydrophobic materials in HS from COM-2.

After the first experimental year, only minor differences were found between HS from soil treated with the biomimetic catalyst (CAT) and those from its control (No-CAT) (Fig. 4.3c). Conversely, after 3 years of treatment, HS from CAT revealed a preferential distribution along the PC1 (90.4%) of signals in the 160–145 and 145–110 ppm regions, thereby showing an increased incorporation of phenolic and aromatic C in the soil humic fraction. Such enhanced content of aromatic components in HS from CAT was consistently accompanied by progressive increase of the NMR aromatic index, as compared to that of No-CAT (Table 4.11).

4.5.2.2 Piacenza Experimental Site

No distinct treatment effects on soil humic composition were revealed by the loading vectors along both PC1 and PC2 in the biplots from NMR of HS extracted from either TRA or MIN (Fig. 4.4a). After the first year, the low values for HB and the large ones for Lignin Ratio for HS from both TRA and MIN indicated the predominance of hydrophilic compounds, such as carbohydrates and peptidic moieties. However, at the experiments end, an incorporation of lignified plant material was revealed by the slight decrease of Lignin Ratio for both soil treatments (Table 4.12).

The low hydrophobic character in HS from TRA and MIN was evident when compared with that resulting from NMR data of HS from COM-2 (Fig. 4.4a, b). In fact, for both sampling times, NMR data provided a positive discrimination along PC1 for loading vectors associated with alkyl (45–0 ppm) and overall aromatic (160–145 and 145–110 ppm) carbons in HS from COM-2. Conversely, the HS from COM-2 were negatively correlated with loading vectors associated with carbohydrates signals (110–60 ppm). Table 4.12 supported the occurred incorporation of hydrophobic molecules from alkyl components and lignin derivatives of compost in HS of COM-2. The molecular distribution, in fact, indicates an increase of HB index and a concomitant progressive decrease of Lignin Ratio throughout the experimental period.

After 1 year of soil treatment with biomimetic catalyst, an increase of aromatic C in HS extracts was suggested by the distribution of phenolic (160–145 ppm) and aromatic (145–100 ppm) C signals along biplot PC1 (87%) (Fig. 4.4b) and by the larger Ar index value (16.2%) with respect to that (10.9%) of control (No CAT) (Table 4.12). However, CAT treatment apparently lost effectiveness later in the experimentation, as revealed by the Ar index of HS from CAT, whose value approached that of control, and by the PCA loading vectors which no longer discriminated between CAT and No-CAT (Fig. 4.4b).

4.5.2.3 Napoli Experimental Site

As in the case of Torino and Piacenza, also for Napoli field experiments, few differences were found by PCA among NMR results for HS from TRA, MIN, and GMAN (Fig. 4.5a). In fact, although from 94 to 99% of total variability for the OC distribution was covered by PCA, at every sampling time, no specific distinction was appreciated for the loading vectors associated with different NMR chemical shift regions in HS from TRA, MIN and GMAN.

However, the latter treatments did induce a significant incorporation of hydrophobic alkyl and lignin compounds in HS, if their values for HB and Lignin Ratio (Table 4.13) were compared to those of the initial soil before the experimentation (Table 4.10). This significant difference may have been due to both the low OC content in the initial undisturbed soil and the large surface area offered by the clay content in this soil. These two conditions may have enhanced the physical–chemical affinity of organic components to soil particles (Staunton and Quiquampoix 1994; van Oss and Giese 1995) and favored the stabilization of the organic molecules that entered the subsoil during cropping seasons (Webster et al. 2001; Piccolo et al. 2004; Winkler et al. 2005).

Hydrophobic molecules from compost were incorporated more in HS from COM treatments than for TRA, as suggested by the PCA of NMR results (Fig. 4.5b). After the first year, this incorporation was already shown by the loading vectors of both aliphatic (45–0 ppm) and phenolic (160–145 ppm) components in HS from both COM-1 and COM-2, though only that associated to alkyl carbon had >95% of statistical significance. Conversely, the loading vector for hydrophilic carbohydrate carbon (110–60 ppm) characterized HS from TRA. After 3 years, the distribution of loading vectors associated to phenolic (160–145 ppm), aromatic (145–110 ppm), and alkyl (45–0 ppm) carbons confirmed the larger hydrophobicity of HS from compost-treated soils, while a persistent content of hydrophilic compounds (110–60 ppm) was found for TRA. The incorporation of alkyl hydrophobic molecules in compost-amended soil was again evident by comparing the OC distribution in HS from COM-2 and MIN (Fig. 4.5c). In fact, the PC1 discrimination represented 88 and 83% of total variation for initial and final experimental time, respectively, showing a positive correlation of hydrophilic O-alkyl-C in HS from MIN, and of alkyl components in HS from COM-2.

The progressive decrease of HB index in HS from MIN and GMAN and the simultaneous increase of Lignin Ratio (Table 4.13) revealed that reduced tillage or green manuring promoted the progressive loss of stable hydrophobic OM from soil (Piccolo 1996; Spaccini et al. 2006). Conversely, HB index and Lignin Ratio for COM treatments indicated incorporation of hydrophobic organic molecules from compost into soil humic substances (Table 4.13). Moreover, the Lignin Ratio in HS from COM-1 (2.28) and COM-2 (1.64) was lower than that of the very compost used for soil treatments (2.5 in Table. 4.9). This suggests that there must have been an incorporation and sequestration into SOM of additional lignin components derived from crop residues left on soils (Spaccini et al. 2000).

After 1 year of experimentation, the PCA of NMR molecular distribution in HS from CAT and that from No-CAT was not significantly different (Fig. 4.5c). This supports the Aromaticity index that indicated a similar content of total aromatic components for both treatments (Table 4.13). However, a significant increase in HB index was noted for CAT for both the first and second experimentation years (Table 4.13). After 3 years, a positive correlation was found for HS from CAT with the loading vector of unsubstituted and alkyl-substituted aromatic components (145–110 ppm) along PC1 (84% of total variation), though no differences were detected in HS total aromatic content between CAT and No-CAT (Table 4.13).

4.6 Concluding Notes on Soil Organic Carbon

The field experiments of the MESCOSAGR project have provided sound indications that the innovative soil treatments, such as amendment with mature hydrophobic compost and in situ SOC photo-polymerization through biomimetic catalysis, sequestered carbon and stabilized SOM more than conventional soil management practices. In fact, the variability in SOC content throughout the experimentation period shown by both MIN and GMAN suggests that these treatments were not able to persistently stabilize OC in both bulk samples and soil particle sizes more than TRA. Moreover, NMR evaluation of soil HS extracted from MIN and GMAN excluded any significant and persistent variation in SOM chemical quality with respect to TRA.

Conversely, results obtained for COM-1, COM-2, and CAT plots for all experimental sites during the whole experimentation, suggest an overall positive effect of soil treatments with both humified compost and biomimetic catalyst on SOC accumulation and stabilization.

Notwithstanding the initial decrease of OC content due to a priming effect observed in the first year after compost addition to soils of Napoli and Torino, compost treatments progressively increased stable OC in bulk soils and soil aggregates in subsequent years. Such a persistent incorporation of hydrophobic components in compost-treated soils was also indicated by the molecular characteristics of HS extracted from different sites throughout the experimentation.

Soil treatment with biomimetic catalyst was found to positively affect both total SOC content and HS molecular characteristics. However, its effect was maintained throughout the whole experimental period only for Piacenza, whereas a decreasing effect was noted for Torino and Napoli in the third year. These contrasting results may be related to the different soil textural composition in the three experimental sites. The Torino sandy-loam soil and the still highly sandy Napoli soil may have not sufficiently adsorbed the water-soluble iron-porphyrin catalyst on soil particles, and it was partially lost by leaching. Conversely, an effective adsorption of biomimetic catalyst on mineral surfaces may have occurred in the heavy textured silty-clay soil of Piacenza, thereby allowing the catalyst to exert a prolonged photo-polymerization activity. However, the effectiveness of the catalyst when in close

interaction with fine mineral particles in all three experimental sites was confirmed by the increased OC distribution in small soil aggregate sizes. In fact, as compared to No-CAT, absolute and relative OC content in CAT soils revealed a significant greater OC incorporation in microaggregates and smallest macroaggregates.

In the quest of summarizing the practical results of our field studies, Tables 4.14–4.16 show the ton ha^{-1} of TOC content in bulk soil for the various treatments in the three experimental sites. The values for MIN and GMAN confirm the short-term and even negative effect of both treatments on SOC accumulation.

Table 4.14 Torino experimental site, total organic carbon (ton ha^{-1}) in bulk soil under different treatments for three experimentation years

Treatments	First year		Second year			Third year		
	TOC ^a	Δc^b	TOC	Δc	Δr^b	TOC	Δc	Δr
Maize								
Control	60.4							
TRA	60.9	0.5	60.4	–	–	60.9	–	–
MIN	56.2	–4.2	60.4	0.5	4.7	59.3	–2.1	–1.6
GMAN	62.0	1.6	56.7	–3.1	–4.7	63.0	1.6	5.8
COM-1	62.0	1.6	63.5	3.7	2.1	66.7	5.3	2.6
COM-2	59.9	–0.5	65.1	5.2	5.8	69.3	7.9	3.7
Wheat								
No-CAT	65.1	–	62.0	–	–	71.9	–	–
CAT	69.3	4.2	68.3	9.4	2.1	72.5	–9.4	–8.9

^aTotal organic carbon = $\text{OC (g kg}^{-1}) \times \text{bulk density (1.5) } \times 10,000 \text{ (m}^2) \times \text{plow depth (0.35 m)}$

^b Δ = yearly difference in TOC for each treatment in respect to TRA (or No-CAT) and to either initial or previous year TOC. E.g.: Δc (cumulative) for MIN 2nd year = (MIN 2nd year–TRA 2nd year) – (TRA 2nd year–TRA 1st year); Δr (relative) for MIN 2nd year = (MIN 2nd year–MIN 1st year) – (TRA 2nd year–TRA 1st year)

Table 4.15 Piacenza experimental site, total organic carbon (ton ha^{-1}) in bulk soil under different treatments for three experimentation years

Treatments	First year		Second year			Third year		
	TOC ^a	Δc^b	TOC	Δc	Δr^b	TOC	Δc	Δr
Maize								
Control	78.3							
TRA	76.0	–2.3	75.5			85.1		
MIN	66.0	–12.3	74.2	–0.9	8.6	101.9	16.8	17.7
COM-2	68.7	–9.6	77.4	2.3	9.1	106.9	21.8	19.6
Wheat								
No-CAT	74.2		55.5			77.4		
CAT	76.0	1.8	61.0	24.1	3.6	88.3	–10.9	5.5

^aTotal organic carbon = $\text{OC (g kg}^{-1}) \times \text{bulk density (1.5) } \times 10,000 \text{ (m}^2) \times \text{plow depth (0.35 m)}$

^b Δ = yearly difference in TOC for each treatment in respect to TRA (or No-CAT) (Δc) and to either initial or previous year TOC. E.g.: Δc (cumulative) for MIN 2nd year = (MIN 2nd year–TRA 2nd year) – (TRA 2nd year–TRA 1st year); Δr (relative) for MIN 2nd year = (MIN 2nd year–MIN 1st year) – (TRA 2nd year–TRA 1st year)

Table 4.16 Napoli experimental site, total organic carbon (ton ha^{-1}) in bulk soil under different treatments for three experimentation years

Treatments	First year		Second year			Third year		
	TOC ^a	Δc^b	TOC	Δc	Δr^b	TOC	Δc	Δr
Maize								
Control	51.5							
TRA	43.6	-7.8	44.6	-	-	46.6	-	-
MIN	48.0	-3.4	50.5	4.9	1.5	49.5	1.0	-2.9
GMAN	51.5	0.0	49.0	3.4	-3.4	49.0	0.5	-2.0
COM-1	46.6	-4.9	49.0	3.4	1.5	51.5	2.9	0.5
COM-2	54.9	3.4	55.4	9.8	-0.5	61.3	12.7	3.9
Wheat								
No-CAT	53.9		49.0	-	-	55.9	-	-
CAT	51.0	-2.9	54.4	10.3	8.3	56.8	-5.9	-9.3

^aTotal organic carbon = OC (g kg^{-1}) \times bulk density (1.5) \times $10,000$ (m^2) \times plow depth (0.35 m)

^b Δ = yearly difference in TOC for each treatment in respect to TRA (or No-CAT) and to either initial or previous year TOC. E.g.: Δc (cumulative) for MIN 2nd year = (MIN 2nd year-TRA 2nd year) - (TRA 2nd year-TRA 1st year); Δr (relative) for MIN 2nd year = (MIN 2nd year-MIN 1st year) - (TRA 2nd year-TRA 1st year)

Table 4.17 Net total organic carbon (ton ha^{-1}) in bulk soil under different treatments for three experimentation years, as obtained by subtracting from measured SOC either 2.7 or 5.4 ton ha^{-1} of OC added with the COM-1 and COM-2 treatments, respectively

Treatments	First year	Second year		Third year	
	$n\Delta c^a$	$n\Delta c$	$n\Delta r^b$	$n\Delta c$	$n\Delta r$
Torino					
COM-1	-1.6	0.4	-1.1	3.1	0.4
COM-2	-6.5	-0.7	-0.2	3.0	-1.2
Piacenza					
COM-2	-12.7	-3.6	3.2	16.4	24.2
Napoli					
COM-1	0.2	1.7	-0.25	2.2	-0.2
COM-2	5.9	5.4	-4.9	9.3	0.5

^a $n\Delta c$ (net cumulative variation) = (TOC COM-1-TOC TRA) - 2.7; (TOC COM-2-TOC TRA) - 5.4

^b $n\Delta r$ (net relative variation) = (TOC COM-1_{2nd year}-TOC COM-1_{1st year}) - 2.7; (TOC COM-2_{2nd year}-TOC COM-2_{1st year}) - 5.4

On the contrary, both cumulative (Δc) and relative (Δr) increments of TOC for compost and catalyst treatments indicate their relevant potential in favoring the long term sequestration and stabilization of organic carbon in soil.

After 3 years, the hydrophobic protection mechanism implied in the compost amendments to soils provided an amount of fixed SOC ranging from 3 to 22 ton ha^{-1} more than TRA, depending on experimental site.

The potential of mature compost treatment to sequester OC in soil is further revealed by the net relative increase of TOC when calculated by subtracting from soil carbon the compost OC annually added to soils (Table 4.17). Except for an

initial SOC decrease at Torino and Piacenza, net SOC increments were generally observed. This shows that the hydrophobic protection mechanism exerted by compost progressively protected, against biotic and abiotic oxidation, both the indigenous SOM and the seasonal OC inputs (microbial biomass, plant roots, and crop residues). The net carbon sequestration obtained over the 3 years by hydrophobic protection of compost resulted thus in much greater than 0.5 ton C ha⁻¹ year⁻¹ achievable by reduced or zero tillage methods (Freibauer et al. 2004).

Though more variable, the OC fixed in the first and second years by the mechanism of in situ catalyzed SOM photopolymerization varied from 4 to 24 ton ha⁻¹. Also these values overcome by far the sequestration potential of simple tillage management practices.

Both innovative methods adopted in the MESCOSAGR project appear then liable to become important to turn agricultural soils into OC sinks and contribute to alleviate the greenhouse effect and the global changes.

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