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CHANGES OF LABILE FRACTIONS OF SOIL ORGANIC MATTER DURING THE CONVERSION TO ORGANIC FARMING

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Abstract

Purpose: Organic farming can overcome the environmental consequences of intensive conventional farming. The objective of the work was to investigate the changes in labile soil organic matter (SOM) fractions during the conversion from conventional to organic farming in two italian sites, Foggia (FG) and Metaponto (MT), that differed in soil types and initial soil organic carbon (SOC) content.

Methods: The fields were cultivated with lentil and wheat in rotation and treated with either: i) compost or ii) nitrogen or phosphorus (N/P) fertilizers in three field replicates. The SOM was fractionated into light fraction (LF), particulate organic matter (POM) and mobile humic acid (MHA) fraction. Isolated fractions were quantified and analyzed for C and N contents.

Results: Although total SOC responded to the fertilization treatments, the LF and POM fractions were yet more responsive. The MHA represented 15% of SOC at both sites, however, the LF represented only 5–6% of total SOC but was the most responsive fraction to changes in land management. Compost application contributed significantly greater quantities of LF, POM and MHA than did the N/P fertilizers application.

Conclusions: The initial SOC content can play an important role in determining the effects of introducing organic farming practices on SOM fractions. In fact, the site characterized by the lower SOC content (MT) showed a mass increase of all fractions while the FG site showed a mass increment only in the LF.

Keywords

Light fraction, particulate organic matter, mobile humic acids, compost.

1. INTRODUCTION

Conventional farming practices have increased food production to support increasing human demands (Zinati 2002) although, most of the time, they have shown excessive use of energy and agrochemicals, large water consumption and greenhouse gases emissions, loss of soil fertility and productivity (Gomiero et al. 2011). In contrast, organic agriculture is a production system that sustains the health of soils, ecosystems and people (IFOAM 2005), therefore soil of high quality is one cornerstone of sustainable agricultural systems such as the organic ones.

The introduction of organic practices requires a conversion period of at least two years before sowing, or in the case of perennial crops other than grassland, at least three years before the first harvest of organic products (Codex Alimentarius Commission 1999). The transition period is necessary for changes in soil chemical, physical and biological properties that enhance nutrient cycling, plant growth and development of biological pest control within the soil-plant system (Zinati 2002). Various changes occur in soil indeed: Santos et al. (2012) showed that organic farming transitional practices increased the content of the humic fractions and 100 – 300% the soil microbial biomass. Stamou et al. (2011) described the structure of soil networks in agroecosystems constituting a gradient from conventional asparagus cultivation to two and three years of organic cultivation. The newest organic systems were characterized by the engagement of NO_3^- and NH_4^+ to biotic processes since the soil microbial community began to play a significant role in soil processes. A clear shift of N from the mineral pools to the microbial biomass-N was reported by Briar et al. (2011) comparing organic vs. conventional farming, and the increment of the microbial community in organic fields resulted in greater bacterivore nematodes population too. In addition, Sihi et al. (2017) reported that the short-term effects of the introduction of the organic farming resulted in greater (75–90%) soil enzyme activities in organic fields than in the conventional ones after harvest, which was due to the rapid increase of organic substrates useful for the microbial metabolism.

Despite its great importance for soil fertility, the total soil organic carbon (SOC) could not reflect changes of the introduction of organic practices because it often takes years before changes in agricultural management show detectable variations in SOC content (Clark et al. 1998). In contrast, the labile pools of soil organic matter (SOM) are sensitive to short term changes in management and/or environmental conditions (Haynes 2005), especially the light fraction (LF), the particulate organic matter (POM) and the mobile humic acid (MHA; Abdelrahman et al. 2016, 2017; Marriott and Wander 2006). The LF is similar chemically to the original plant material, but a lesser amount of carbohydrates and a greater quantity of sterols in the LF indicates the early stage of its decomposition in soil (Gregorich et al. 1996). The POM consists of partially decomposed plant litter, and it acts as a substrate and center for soil microbial activity, a short-term reservoir of nutrients, a food source for soil fauna and loci for formation of water stable macroaggregates (Haynes 2005). The MHA is a labile fraction of humic substances rich in N, S and H, characterized by phenolic moieties derived from the lignin residues, involved in short-term nutrients cycling (Olk 2006).

Since conventional farming systems range from traditional cultures, low input and environmentally friendly managements (e.g., minimum or zero tillage, integrated pest management, etc.) to intensive industrial monocultures (Gomiero et al. 2011), the introduction of strictly regulated organic practices can influence differently the labile fractions of SOM. Therefore, the objective of this work was to study the evolution of LF, POM and MHA during the conversion period in two sites historically cultivated with wheat followed by a fallow period and managed with the same conventional practices, but different for their soil types and initial SOC content. In particular, the work investigated the effects of the introduction of the lentil-wheat rotation and of the transition i) from the conventional fertilization to the one allowed in organic farming; ii) from the conventional to the minimum tillage; iii) from the removal of aboveground crops residues to their return to soil, on the characteristics of LF, POM and MHA.

2. MATERIALS AND METHODS

2.1. Site Description and Experimental Design

The experimental stations at Foggia (FG; 41°27′35″ N and 15°30′18″ E) and Metaponto (MT; 40° 24′25″ N and 16°48′24″ E) in Italy have been cultivated for decades under conventional farming with wheat followed by a fallow period (July–September). The conventional tillage included moldboard plowing (35 cm deep) in late August, and disk harrowing (15 cm deep) in November to prepare a proper seedbed. The crop was historically rainfed and fertilized with mineral fertilizers, typically about 200 kg diammonium phosphate (18 N, 46 P_2O_5) ha^{-1} before sowing, followed by about 100 kg urea (46 N) ha^{-1} at jointing stage. During the conventional farming, the wheat straw was removed from the plots and placed on the market for livestock.

These sites were converted to organic farming in response to the movement toward organic production in Italy and Europe and to study the changes in SOM under organic farming management. Main characteristics of the soils at the experimental sites at the beginning of the trial are reported in Table 1. The climate at both sites was similar, with mean annual precipitation of 560 and 500 mm at FG and MT, respectively, mainly concentrated in autumn and winter. The mean annual temperature was 15.5 °C (annual temperature range: 44/-10 °C) and 15.8 °C (annual temperature range: 35/0 °C) at FG and MT, respectively.

The experiments started in 2009 and included a 2-year rotation of lentil (*Lens esculenta* Moench, cv Eston) with either durum wheat (*Triticum durum* Desf., cv Svevo) at FG or Emmer wheat (*Triticum dicoccum*) at MT, and all crops were rainfed. Each experimental site was divided into 10 x 10 m plots distributed in a completely randomized block design with three replications for each treatment. Treatments were: i) compost; ii) commercial fertilizer (N/P fertilizer); iii) compost applied at a rate of 13.3 Mg ha^{-1} (compost-A), respecting the N load limit (170 kg ha^{-1} yr^{-1}) defined by the EU nitrate Directive (676/1991); and iv) an unfertilized control. Only the first two treatments were imposed at FG, and their application rate at both sites was calculated to meet crop requirement of 100 kg ha^{-1} N for a subsequent wheat crop (7.82 and 0.8 Mg ha^{-1} of compost and N fertilizer, respectively) or 30 kg ha^{-1} P_2O_5 for a subsequent lentil crop (3.55 and 0.2 Mg ha^{-1} of compost and P fertilizer, respectively). The compost applied annually to both wheat and lentil crops was prepared using olive pomace, olive pruning residues and cattle manure enriched in straw bedding material. For N/P fertilizer treatment, each new wheat crop received only N fertilizer and each new lentil crop received only P fertilizer. These applications were made annually in the autumn, between October and November depending on the weather conditions. All amendments/fertilizers were permitted in organic farming (Annex 1, EC no. 889/2008). Soils were left bare after harvest (July) until September – October then the disc harrowing (15 cm depth) was applied for incorporating the crop residues and for preparing the soil for the subsequent crop.

2.2. Soil Sampling and SOM Characterization

Soils were sampled at the start of the conversion period (September 2009) to represent the initial soil conditions (T0), and after the harvest of each crop in 2010 (T1) and 2011 (T2). Soil samples were collected from the 0–30 cm depth from each treatment, air-dried, passed through a 2-mm sieve and stored at room temperature in dark for subsequent analyses. At the end of each crop cycle, residues of wheat straw and lentil were collected from nine spots of 1 m^2 in each plot and weighed in order to estimate the quantity of crop residues left on each plot and treatment

The total C and N content of soil and SOM fractions were determined through automated combustion analysis (Fisons NA 1500 NC Series 2). Inorganic carbon was determined by the modified pressure-calcimeter (Sherrod et al., 2002) then the organic C was determined by the difference between total C and inorganic C.

Soils were sequentially extracted for the LF, the POM and the MHA using a modified procedure by Cao et al. (2011). Each field replicate was extracted separately for these three SOM fractions. The LF of the whole soil was extracted by floating the soil in a 1.6 g cm^{-3} Na polytungstate (PT) solution. The bottles were shaken for 10 min on a reciprocal shaker at 200 rpm, transferred to 500-mL beakers and allowed to settle overnight. Afterward, the LF was removed from each beaker by vacuum suction and collected on a 20-µm-nylon filter, and then transferred by PT washes into a 50-mL beaker and allowed to stand for 3 h. After 3 h, the floating material was removed from both the 500- and 50-mL beakers by vacuum suction and collected onto the 20-µm-nylon filter, then washed by deionized water and transferred into preweighed drying tins. This material was dried overnight in a forced-air oven at 58 °C.

After extracting the LF, each soil sample replicate was dispersed by Na metaphosphate and shaking. Then each bottle was poured through stacked 53 µm sieves so that clay and silt material was collected in an underlying shallow Pyrex pan. The > 53 µm POM fraction were refloated on 2.0 g cm^{-3} PT solution to obtain its light fractions, which was used for all further analyses.

Following POM extraction, the dried silt plus clay material from each soil was evenly divided into two or three 500-mL centrifuge bottles. The contents of each bottle were extracted by 0.25 mol L–1 NaOH at a 1:10 (w:v) ratio. Specifically, the contents of each bottle were placed under an N_2 atmosphere by bubbling N_2 gas into each bottle for 5 minutes. Bottles were capped and shaken at about 200 rpm on a reciprocal shaker for 30 min every 2 h for a total of 20 h. The bottles were then centrifuged and the supernatants decanted and acidified (2 mol L^{-1} HCl) to pH 1.95 – 2.0 to precipitate the MHA. The soil was shaken overnight in 0.25 mol L^{-1} NaOH two more times and the resulting MHA was combined from all three washes. The silt plus clay was decalcified by 0.2 mol L^{-1} HCl washes with shaking for 10 min at about 200 rpm and centrifuging until the supernatant pH decreased to <1.0. Excess HCl was then removed by one to two deionized water washings until the supernatant pH rose above 2.0, preferably between 2.5 to 3.0. The MHA fractions were then cleansed of soil contaminants by resolubilization in a KOH–KCl solution and reprecipitation with 2 mol L^{-1} HCl (Swift, 1996), followed by a 24-h extraction with 0.2% HCl–0.2% HF, and by 3 d of dialysis in successively weaker HCl solutions and at the end against water. Finally, the fractions were frozen and lyophilized.

2.3. Statistical Analysis

Each treatment was performed in three replicates. Experimental data were tested against the normal distribution using the homogeneity test, then data were analyzed using the General Linear Model procedure (SPSS 17.0, SPSS Inc) with multivariable (fertilization treatment, crop, time and their interactions) on the measured parameters for the LF, POM and MHA.

3. RESULTS

3.1. Crop Residue Masses

Crop residues are one of the main inputs of organic matter into soil. As the LF and POM are plant-like or partially decomposed materials (Gregorich et al. 2006), the input rate of crop residues into soil is an important consideration for understanding their cycling rates. The amounts of aboveground crop residues remaining after crop harvest did not differ among the treatments at either site; they were on average 7.05 Mg ha^{-1} and 10.65 Mg ha^{-1} after lentil and wheat, respectively. The belowground residues were not measured, however they were estimated in about 0.8 Mg ha^{-1} and 3.7 Mg ha^{-1} after lentil and wheat, respectively, according to the root-to-shoot ratio (Arcand et al. 2013).

3.2. Soil C and N contents

Total soil organic C increased by 2–14% and by 1–16% over the 2-year course in the FG and MT sites, respectively. At the FG site, SOC increased numerically with either compost or N/P fertilizer treatment compared to T0. The greater increase in SOC was reported at T2 after compost and wheat, however, the effect of the previous crop is visible in SOC values for T1 (Fig. 1a). Although, at the FG site, compost contributed to numerically greater increases in soil C and N than did the N/P fertilizer, neither crop (P = 0.446) nor fertilization treatment (P = 0.486) significantly affected SOC changes (Table 2). At the MT site, similar increases in SOC occurred after the fertilization treatments, and were more evident after the compost-A treatment with either crop (Fig. 1a). Also, SOC at the MT site was not significantly (Table 2) affected by fertilization treatment (P= 0.074) or crop (P= 0.276).

Soil N increased after both crop cycles and with all treatments at both sites, over the 2-year course. At the FG site, larger numerical increases in soil N were reported after compost and fertilizer and after the lentil wheat crop cycle at the end of the trial (T2). The latter result can be attributed to the lentil effect from T1 (Fig. 1b), even if the changes in soil N in FG were neither significantly affected (Table 3) by crop (P = 0.267) nor by fertilization treatment (P = 0.302).

At the MT site, soil N increased after lentil at T1 with the four treatments, however, no noticeable changes occurred at T2. Interestingly, crop, fertilization and time affected significantly soil N at the MT site (Table 3). The unamended control treatment at MT had minor numeric accruals of total soil organic C and N.

3.3. Mass Distribution of SOM fractions

The introduction of the organic farming management influenced the mass of the SOM fractions at both sites already at the end of the first crop cycle. At the FG site, mean LF mass with compost application was about 3 g kg^{-1} soil for either crop at T1 and T2. In general, the LF increased significantly (P=0.013) by more than 80% with either compost or N/P fertilizer treatment compared to the initial conditions (about 1.2 g kg^{-1} soil).

After lentil, the POM masses of all treatments were similar to the initial value (about 2 g kg^{-1} soil), while POM masses increased numerically (about 2.3 g kg^{-1} soil) after wheat and in the compost amended plots. The compost treatment and the rotation wheat-lentil showed MHA masses similar to the initial value but numerically greater than the N/P fertilizer treatment. In addition, greater MHA masses were associated with lentil, for both compost and N/P fertilizer, than with durum wheat.

At the MT site, compost-A treatment contributed to the largest LF masses after either lentil or wheat (about 2.3 and 1.8 g kg^{-1} soil, respectively) at T1 and T2. In general, the compost treatments contributed to greater LF mass with either crop with respect to the other treatments. Within the control treatment, LF mass was greater after wheat than after lentil (about 1 and 0.8 g kg^{-1} soil, respectively), reflecting the crop effect on the LF (P=0.009). At the end of each crop cycle, the POM was more abundant after the compost treatments with either crop than with the N/P fertilizer, whereas the unfertilized control showed greater (P_{trt} = 0.081) POM mass with respect to the N/P fertilizer treatment. The MHA masses were numerically greater (P_{crop} = 0.103) after lentil than after wheat (about 2.3 and 1.8 g kg^{-1} soil after lentil and wheat, respectively) for all fertilization treatments. However, the unfertilized control showed MHA masses fairly similar to the initial soil endowment (about 1.65 g kg^{-1} soil).

3.4. Carbon content in SOM fractions

The fractional C content of a SOM fraction is the product of the fractional mass multiplied by its C concentration and it represents the contribution of a certain fraction to total SOC. Fractional C concentration, averaged across crops and treatments, differed from LF (351 g C kg^{-1} LF) to POM (314 g C kg^{-1} POM) to MHA (411 g C kg^{-1} MHA) but did not differ greatly within the same fraction by fertilization treatment or crop making the fractional C a function of its mass.

The LF fractional C (LF-C) varied slightly among the fertilization treatments, primarily due to the fractional mass and it represented on average about 5% of total SOC at both sites. At the FG site (Fig. 2a), wheat clearly influenced the LF–C, apparently in a consequence to LF mass as the LF-C increased after both compost and N/P fertilizer treatments clearly after wheat in T2. Similarly, wheat effect on the LF was visible at T1 in the wheat–lentil crop cycle. Although wheat affected the LF clearly, statistical effect of crop on the LF-C was insignificant (P = 0.194). However, the fertilization treatment had significant effects (P = 0.007) on the LF-C as it increased after both compost and N/P fertilizer treatments and reached about 1 g C kg^{-1} soil.

At the MT site (Fig. 2b), the greatest LF-C content was recorded at T1 for compost-A after the lentil–wheat cycle. The compost treatments contributed to greater LF-C with respect to the N/P fertilizer or the control treatment with either crop cycle. Crop (P = <0.001) and fertilizer treatment (P = 0.022) significantly affected the LF-C at both sites at the MT site.

The C concentration of the POM ranged 300–325 g C kg^{-1} POM, and its C/N ratio ranged 12–14 for both sites. At the FG site, both compost and N/P fertilizer treatments increased numerically the POM fractional C (POM-C), reaching the highest value after the wheat-lentil rotation (Fig. 2a). The POM-C increased during the 2-year course reaching the greatest value after compost and with the lentil-wheat rotation (Fig. 2a). Neither crop (P = 0.331) nor the fertilization treatment (P = 0.451) affected significantly the POM-C in FG.

At the MT site (Fig. 2b), the POM-C was significantly influenced by the fertilization (P<0.001) and the time (P=0.001), reaching the highest value (about 0.92 g C kg^{-1} soil) already in T1 with the compost-A treatment after wheat. As per the LF-C, compost treatments showed higher values of POM-C with respect to the other treatments, even if the compost treatment induced a substantial increase in POM-C only at the end of the trial. Except the reported increases in the POM-C, all the treatments did not considerably influence the POM-C; POM-C averaged 0.5 g C kg^{-1} soil for most of the cases at the MT site (Fig. 2b).

At T2, the POM-C represented 4% and 6% of SOC in the FG and MT sites, respectively, showing an increase over time, since POM-C accounted in 2009 for only 2.6% and 4.5% of SOC at the FG and MT sites, respectively.

The MHA fraction contained an average of 411 g C kg^{-1} fraction and had C/N ratios of 12 and 9 at the FG and MT sites, respectively. The MHA contributed the most to SOC among the separated fractions: the MHA-C content (MHA-C) accounted for on average 13.4% and 7.3% of total SOC in T2 at the FG and MT sites, respectively.

At the FG site (Fig. 2a), the MHA-C increased considerably during the experimental course after compost and lentil on one side, and after N/P fertilizer and wheat on the other side, reaching about 2.3 g C kg^{-1} soil, which was more than 15% of total SOC. Despite the reported increases in the MHA-C, neither crop (P = 0.167) nor fertilization treatment (P = 0.498) had significantly affected the MHA-C (Table 2), however, the interaction (Fertilization × Crop × Time) significantly (P = 0.044) influenced the POM-C.

At the MT site, MHA-C increased after all fertilization treatments including the unfertilized control with more evident increases in the MHA-C in 2011. The largest increase in the MHA-C was reported after the compost treatment and lentil; MHA-C reached 0.97 g C kg^{-1} soil (Fig. 2b).

The crop effect was very clear on the MHA, at the MT site, as there were substantial increases in the MHA-C after lentil in 2011 (Fig. 2b). The MHA-C changes during the experimental course were significantly influenced by the crop ($P < 0.001$), the fertilization treatment ($P = 0.001$) and the time ($P < 0.001$).

4. DISCUSSION

The recorded SOC content changes agree to the findings of Herencia et al. (2008) who reported numerical improvement of SOC in plots receiving organic treatments at the end of the transition period, and the SOC increase became significant only after 4–5 crop cycles. In contrast, Gopinath et al. (2009) showed a significant SOC increment already at the end of a two-year transition period of a bell pepper crop.

The results of the present study confirm that the synergy of leaving crop residues on soil surface, introducing the crop rotation, the minimum tillage and the organic fertilization increased soil C and N. In fact, the minimum tillage, introduced to the study sites at T0, slowed the mineralization processes of the C deriving from organic fertilizers and crop residues and built SOM even to the 30-cm depth (Varvel and Wilhelm, 2011). In addition, the studied soils, and especially at MT, are depleted in SOM, which consequently heightens the effectiveness of any fertility enrichment, as reported by Herencia et al. (2008) too.

The introduction of organic farming practices at the FG site influenced mainly the LF mass across all crops and fertilization treatments. At MT, the compost treatments increased the mass of all SOM fractions, including the MHA that increased significantly at the end of the trial after lentil. These increases at the MT site are possibly due to the lower initial SOC despite its greater soil clay content (Abdelrahman et al. 2016), suggesting the larger response to management than at the FG site was due to the soil degradation status at MT. Therefore, the compost based fertilization, the crop residues recycling and the minimum tillage could have influenced more dramatically the masses of the extracted fractions at the MT site. In contrast, the C contribution of the N/P fertilizer treatments was relatively low as their composition was more homogeneous (collagen) than was the compost amendment, and the doses applied were lower than the compost treatment, making the crop residues the main input of organic C. The greater masses of the MHA recorded at the MT site after lentil might have been also due to greater activities of soil microbial communities in response to balanced C/N ratio of the lentil residues compared to the wheat residues (Gan et al., 2011); such high-quality residues might lead to more microbial residues that can be stabilized in SOC (Cotrufo et al. 2013).

Finally, the LF-C increased over time after either compost or N/P fertilizer at both sites. The same trend was observed for the POM-C at the MT site, while the POM-C at the FG site was influenced only by time (Table 2). The MHA-C was dramatically affected by time, crop and treatments only at MT site. Even the changes recorded in the C content of the SOM fractions can be explained by the different level of soil degradation occurring at the two sites (Abdelrahman et al. 2016), with the MT site taking more advantages by the introduction of the organic soil fertility management in comparison to the FG site.

5. CONCLUSIONS

The results of the present study showed that a 2-year conversion period, characterized by the application of compost and organic fertilizers, minimum tillage**,** the management of crop residues and crop rotation, sufficed for demonstrating a starting buildup of physical and chemical fractions of SOC. The initial SOC content can play an important role in determining the effects of the introduction of organic farming practices on SOM fractions. The site characterized by the lower SOC content (MT) showed a mass increase of all fractions, as compared to the FG site that indicated a mass increment only in the LF.

In this study, although SOC responded to the fertilization treatments, the labile fractions were yet more responsive as reported elsewhere (Trigalet et al. 2014) and shown by the significant increases in fractional contents for all three fractions and especially the larger proportional increases in LF and POM (Fig. 2). The fractionation scheme used here successfully integrated uncomplexed physical fractions with humified chemical fractions to depict short-term C cycling in field conditions, which has not been commonly done (Olk and Gregorich 2006). The clear site and crop effects on these fractions illustrate that the relative response of these fractions can vary by soil type and local land use.

Conflict of interest: The authors declare that they have no conflict of interest.

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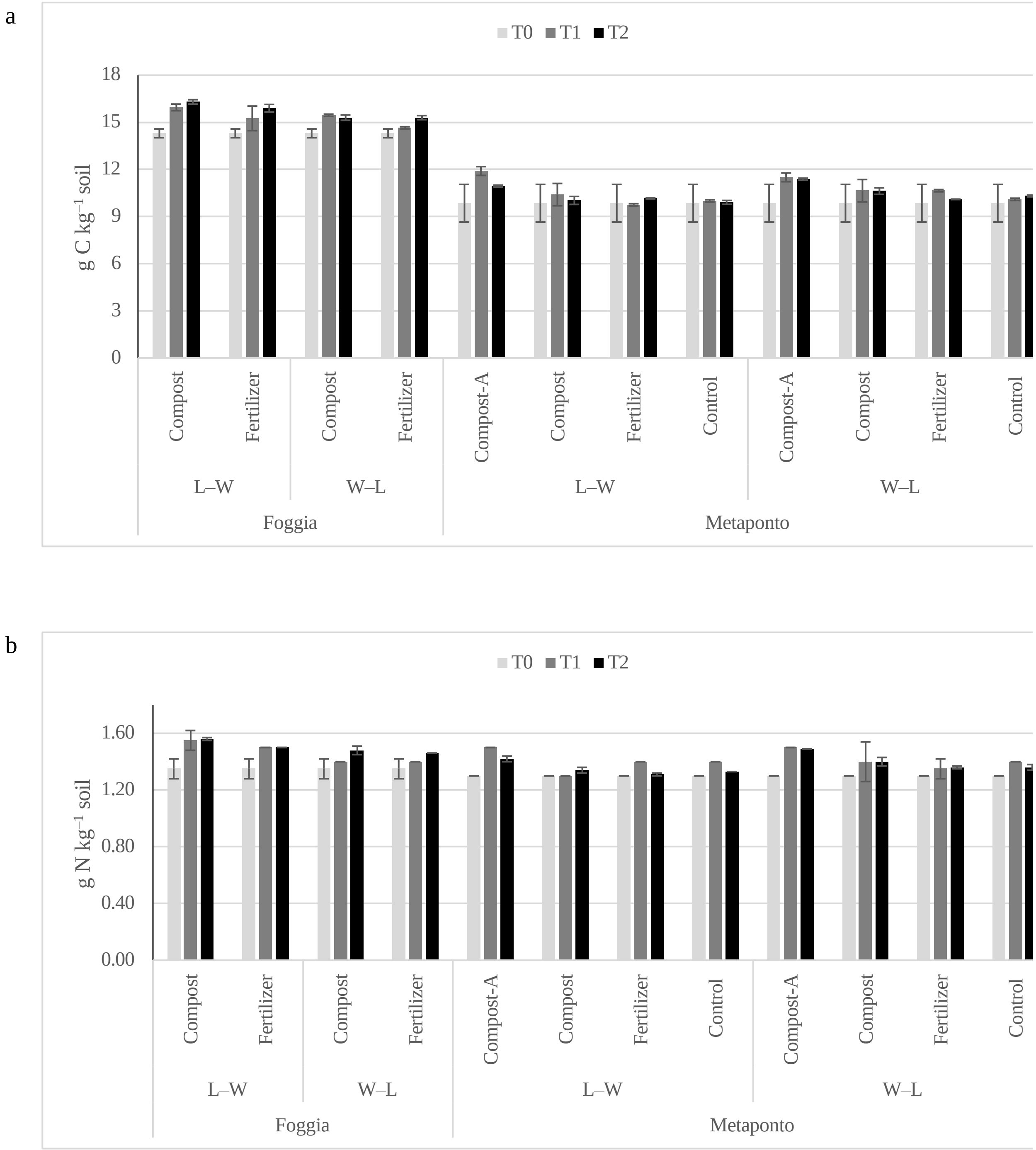
Figure Caption

Figure 1. Total soil organic C (a) and Total soil N (b) at the start of the transition period in 2009 (T0) and after different fertilization treatments and rotations of lentil (L) and wheat (W) in 2010 (T1) and 2011 (T2) at the Foggia and Metaponto sites.

Figure 2. Fractional C (fraction mass × fraction C concentration) in the light fraction (LF), the 500–53 μm particulate organic matter (POM) and the mobile humic acid (MHA) at the start of the transition period in 2009 (T0) and after different fertilization treatments and rotations of lentil (L) and wheat (W) in 2010 (T1) and 2011 (T2) at the Foggia (a) and Metaponto (b) sites.



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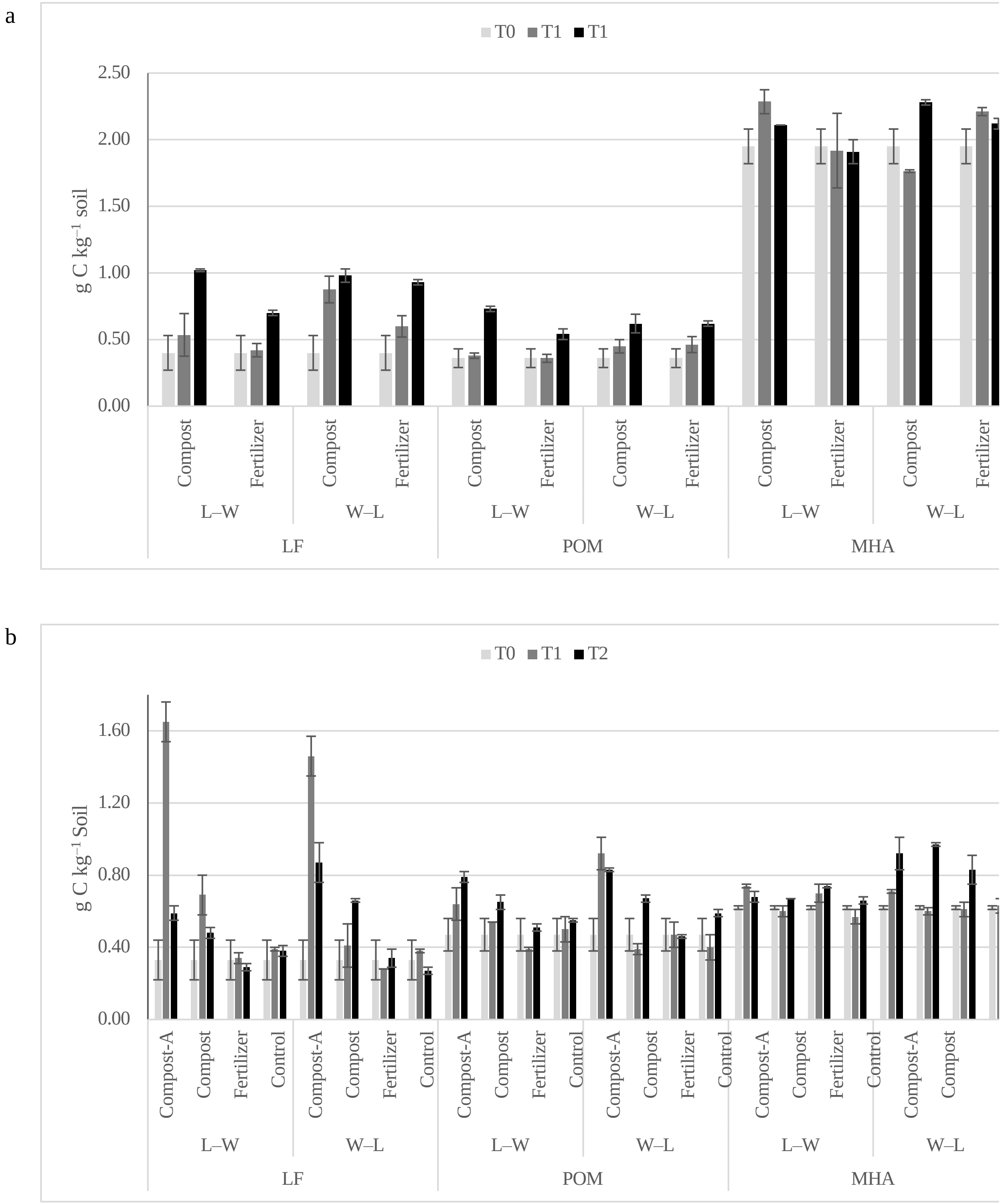

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Table 1. Selected soil characteristics at the start of the transition period in 2009.

Site	pH	Electrical Conductivity (dS m^{-1})	Clay	CaCO$_3$	Organic C	Total N
			g kg^{-1}soil			
Foggia (FG)	7.4	0.17	455	49.9	14.3	1.35
Metaponto (MT)	7.2	0.18	560	143	9.90	1.30

Table 2. Levels of significance (*P* values*) for the effect of crop, fertilization treatment, time and their interactions on the soil C and N fractions during 2009–2011 at the Foggia (FG) and Metaponto (MT) sites.

Variable	FG		MT	
	C	N	C	N
Fertilization (F)	0.486	0.302	0.074	<0.001
Crop (C)	0.446	0.267	0.276	<0.001
Time (T)	0.002	<0.001	0.043	<0.001
F × C	0.365	0.899	0.885	0.309
F × T	0.871	0.728	0.686	0.000
C × T	0.161	0.007	0.736	0.000
F × C × T	0.695	0.674	0.999	0.922

* actual P levels results of the multivariate general linear model, $p \leq 0.05$ is significant

Table 3. Levels of significance (*P* values*) for the effect of crop, fertilization treatment, time and their interactions the SOM fractional C content during 2009–2011 at the Foggia (FG) and Metaponto (MT) sites.

Variable	FG			MT		
	LF–C	POM-C	MHA-C	LF–C	POM-C	MHA-C
Fertilization (F)	0.007	0.451	0.498	<0.001	<0.001	0.001
Crop (C)	0.194	0.331	0.167	0.022	0.735	<0.001
Time (T)	<0.001	<0.001	0.223	<0.001	0.001	<0.001
F × C	0.098	0.544	0.085	0.078	0.536	0.187
F × T	0.112	0.088	0.395	<0.001	0.002	0.005
C × T	0.009	0.592	0.532	0.240	0.971	<0.001
F × C × T	0.420	0.049	0.044	0.146	0.773	0.016

* actual P levels results of the multivariate general linear model, $p \leq 0.05$ is significant