UNIVERSITÀ DEGLI STUDI DI SASSARI
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SOIL ORGANIC MATTER QUALITY UNDER DIFFERENT LEVELS OF CROPPING SYSTEMS INTENSIFICATION

Dott.ssa Chiara Cappai

Direttore della Scuola	Prof.ssa Alba Pusino
Referente di Indirizzo	Prof.ssa Rosella Motzo
Docente Guida	Dott.ssa Giovanna Seddaiu

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Summary

The study on the impact of different land uses on soil organic matter (SOM) fractions have important implications for the identification of sustainable land and agricultural management practices and the possibility to develop actions finalized to the soil carbon sequestration and stabilization and, thus, to mitigate climate change processes.

The studies were carried out in Sardinia (Italy) in three different cropping systems under Mediterranean condition: a wooded grassland in an agro-silvo-pastoral area; an intensive forage system in a Nitrate Vulnerable Zone and an artichocke organic farming system.

The overall aim of this research was to evaluate the effects of different land uses and management practices such as presence of trees, type of fertilizer, crop residues, cover crop and rotation, on soil organic matter quality and stability through the determination of well known SOm quality indicators (DOM, WEOM, POMfree, POMoccluded and M-OM).

The results demonstrated (i) the fundamental role of trees for the sustainability of the studied agroforestry systems and their positive effects on soil fertility, (ii) the direct and indirect influence of the type of fertilizer (slurry, manure and mineral) on the C balance and accumulation, mainly due to the different contribution to total C input that the soil receives and (iii) the key role of crop residues management and the cultivation of cover crops on C cycling and storage in organic artichocke systems.

GENERAL INTRODUCTION

General introduction

The concept of soil organic matter quality

Soil quality has been defined as ‘‘the capacity of a soil to function within ecosystem and land-use boundaries, to sustain biological productivity, maintain environmental quality and promote plant and animal health’’ (Doran and Parkin, 1994). Apart from the inherent soil quality, which is based upon the parent geological material and is rather static, the dynamic nature of the soil is strictly related to anthropogenic use and management (Carter et al., 1997). Soil organic matter (SOM) is considered the most important attribute of soil quality since it influences plant growth indirectly and directly. Indirectly, SOM enhances the physical conditions by stabilizing soil aggregates (e.g. Six et al., 2000), and, hence, improving aeration and water retention (e.g., Senesi and Loffredo, 1999). Directly, SOM is a source of energy for microorganisms, it supplies a slow but continuous stream of nutrients for plant growth, it forms compounds with elements such as Fe, making them more available for plant growth and increases the buffer properties of soils (e.g., Stevenson, 1994).

SOM consists of various pools that are stabilized by specific mechanisms and have certain turnover rates. Studies on SOM quality are referred to the analysis of the changes of these heterogeneous SOM pools/fractions. Most applied SOM fractionation methods can be divided into (a) physical procedures and (b) chemical procedures.

SOM physical fractionation methods

Physical fractionation involves the application of various degrees of disaggregating treatments (dry and wet sieving, slaking), dispersion (ultrasonic vibration in water), density separation and sedimentation.

Aggregate fractionation is based on the separation of free SOM and protected SOM that is occluded in secondary organo–mineral assemblages of different sizes. There is some evidence that the degree of decomposition increases with decreasing aggregate size, since chemical compound classes representing plant components decrease from macro- (>250 μm) to microaggregates (<250 μm) (McLauchlan and Hobbie, 2004; Monreal et al., 1997). Angers et al. (1997) demonstrated thatC occlusion in microaggregates only happens after occlusion at the macroaggregate scale. Consequently, microaggregate OM is usually older and in a more decomposed state than macroaggregate OM on average. In fact, turnover times as revealed by 13C natural abundance studies were found to be about 15–50 years for SOM stored in macroaggregates and 100–300 years for SOM in microaggregates (Puget et al., 2000; Six et al., 2002; Yamashita et al., 2006).

Particle size fractionation is based on the concept that SOM associated with particles of different size and therefore also of different mineralogical composition differ in structure and function. While quartz particles that dominate the sand fraction exhibit only weak bonding affinities to SOM, the clay-sized particles provide a larger surface area and numerous binding sites for SOM. Despite , SOM within the sand fraction is traditionally allocated to the active pool and SOM in silt and clay fractions to the intermediate and passive pools, there is still uncertainty on turnover rates of the different particle size fractions. In fact, the generally higher allocation of SOM in smaller particles was not always consistent with longer turnover times (von Lutzow et al., 2007 and references therein).

Density fractionation is applied to isolate SOM that is not firmly associated with soil minerals (light fraction) from organo–mineral complexes (heavy fraction). Associations of SOM to mineral surfaces like e.g. phyllosilicates are most often characterized by a density greater than 1.6–2 g cm^{-3}. Lighter fractions with a density of <1.6–2 g cm^{-3} consist mostly of pieces of plant residues, i.e. as particulate SOM (POM), either free (fPOM) or occluded in aggregates (oPOM) (Christensen, 1992). Generally, the stability of SOM increases from fPOM to oPOM and further to mineral-associated SOM (Golchin et al., 1995; Baisden et al., 2002; John et al., 2005). However, in the literature are reported quite wide turnover times for each SOM density fractions, due to differences in fractionation methods and turnover measurements methodologies (13C, 14C) (von Lutzow et al., 2007 and references therein).

SOM chemical fractionation methods

Chemical fractionation procedures are based on the extraction of SOM in aqueous solutions with and without electrolytes, in organic solvents, on the hydrolysability of SOM with water or acids, and the resistance of SOM to oxidation. Other chemical fractionation procedures are based on destroying the different mineral phases.

Through the extraction of SOM in water the so called dissolved organic matter (DOM) is obtained. In general, the term ‘‘DOM’’ is used unspecifically to indicate organic material truly dissolved in situ (Zsolnay, 1996), whereas WEOM (water extractable organic matter) is the corrected acronym when it refers the organic matter extracted by gently agitating soil samples with aqueous solutions and it includes DOM present in the macropres and some DOM located in smaller pores (Chantigny, 2003; Corvasce et al., 2006). Therefore, WEOM is the fraction of DOM conceptually consisting of the mobile and available portion of the total DOM pool. Water extractable organic matter (WEOM) consists of a heterogeneous mixture of hydrosoluble structures either freely circulating in soil or physically trapped within or loosely adsorbed onto soil minerals (Zsolnay, 2003). Compared to total

SOM the concentrations of WEOM are very small (Embacher et al., 2007). Nevertheless, it plays a significant role in the regulation of nutrients, metals, and microbial activity in soil (Hassouna et al., 2010).

The solubilization of SOM in alkali and acid is used to obtain three SOM fractions: fulvic acid, humic acid and humin fractions. The fulvic acid fraction is soluble in alkali (e.g. 0.1M NaOH+0.1M $Na_4P_2O_7$) and soluble in acid (e.g. HCl). The humic acid fraction is soluble in alkali and insoluble in acid. The humin fraction is insoluble in alkali (Stevenson, 1994). Extraction of humic substances by NaOH and $Na_4P_2O_7$ have been very popular because they generally extract large quantities of humic material in most soils (Stevenson, 1994) and these amounts were found to be very sensitive to soil type (Olk, 2006). Recently, since the article by Kleber and Johnson (2010) an increasing criticism toward the concept oh humus substances in the soil is arising. These authors suggested that extreme caution is needed when studies with classically defined humic substances are intended to explore the physical and chemical nature of SOM. They highlighted that no experimental publications are available showing that materials extracted with alkali, i.e. humic substances, do indeed occur as such in natural soils. On the contrary, these substances were not found when using solid-state NMR and synchrotron spectroscopy that allow to analyse SOM *in situ* in whole soils (Kelleher and Simpson, 2006; Lehmann et al., 2008).

Why studying the quality of soil organic matter in agricultural systems?

Alterations in the different fractions of SOM are more effective in indicating changes in soil use and management than total soil organic matter content.

Soil organic matter labile fractions have efficiently been used instead of total SOM as sensitive indicators of changes in soil quality (Bayer et al., 2002; Haynes, 2005), due to the many important interactions of these components in the soil system (Guimaraes et al., 2013).

Soil organic matter (SOM) levels were found to vary within years or even more, whilst active SOM-fractions like macro- and light fraction-organic matter, soil microbial biomass and microbial functions changed within shorter periods of time (Fließbach et al., 2007).

Tinoco et al. (2010) found particulate free organic matter and humic acids were particularly good biogeochemical proxies of anthropic impact in Mediterranean soils, confirming that the identification of descriptors sensitive to the effect of soil use and management "is not a trivial matter in the case of soils subjected to recent anthropogenic perturbations" (pag. 320).

For the improvement of cropping systems simulation models it would be useful to relate measurable SOM fractions defined with functional SOM pools used to parameterize SOM turnover models.

The strong relationships between SOC pools used in RothC and fractions separated through a fractionation procedure for a range of agricultural sites (arable land, grassland and alpine pasture) highlighted that relatively minor adaptations to classic SOM fractionation procedures were adequate to identify measurable SOC fractions, which could be used to initialize and evaluate RothC (Zimmerman et al., 2007). In particular, a combination of physical and chemical methods resulted in two sensitive (particulate organic matter and dissolved organic carbon), two slow (carbon associated to clay and silt or stabilized in aggregates) and one passive (oxidation-resistant carbon) SOM fractions.

In their review, van Lutzow et al. (2007) highlighted that only few operational methods are useful for characterizing functional SOM pools of SOM turnover models such as microbial biomass or the light fraction. Despite numerous approaches to improve and combine fractionation methods, a major remaining problem is that most procedures are not specific enough with regard to stabilization mechanisms. Especially the conceptual passive pool, which is stabilized by various mechanisms, is still difficult to characterize. All efforts to isolate this pool so far have yielded SOM fractions that are still heterogeneous in terms of turnover times and reveal no causal relationships to stabilization mechanisms. However, there are a range of promising new methods, but data on turnover times in different soil horizons and soil types remain scarce, so that validation of more mechanistic models based on functional SOM fractions is still very complicated.

The assessment of changes of soil quality indicators in the short and medium term is worth for contributing to provide a value of sustainable management practices such as organic farming for which farmers can receive financial support.

Since many soil and crop treatments are also beneficial to the environment without being directly productive, organic but also integrated farmers receive financial support for their environmental services in many countries, mainly within agro-environmental measures of the Rural Development Plans. Apart from the analysis of the economic performances (Stolze et al., 2000), efficiency calculations, nutrient balances, also the assessment of SOM quality indicators was found to be a suitable approach for the quantitative evaluation of the effectiveness of agro-environmental measures (Fließbach et al., 2007).

Moreover, despite many studies on the effect of organic management on SOM fractions are already available for temperate environments (e.g., Kawasaki et al., 2009; Slepetiene et al., 2010), very limited information is available so far for semi-arid Mediterranean climate conditions. For instance, Aranda et al. (2011) observed smaller differences than expected in SOM quality between conventional and organic management of olive groves in a Spanish semi-arid region after 16 years of organic practices adoption. Anyway, further studies are needed to confirm these findings and to identify suitable management practices oriented to C accumulation and stabilization for these types of environments.

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CHAPTER I

The role of cork oak trees on soil organic matter content and quality in Mediterranean silvo-pastoral systems

The role of cork oak trees on soil organic matter content and quality in Mediterranean silvo-pastoral systems

Abstract

The aim of this study was to assess the role of trees (*Quercus suber* L.) on soil proprieties and organic carbon content and pools in an agro-silvo-pastoral system under Mediterranean semi-arid conditions (North-Eastern Sardinia, Italy). Six isolated cork oak trees in a wooded pasture were randomly selected and for each tree were identified two transects in opposite orientations (NE-SW). Along the transects, the measurements were made at five sampling positions following a gradient from underneath the tree canopy to beyond the tree canopy projection. Analyses include the determination of water extractable organic matter (WEOM) and density fractionation of soil organic matter to identify the following pools: free particulate organic matter (POMf), occluded aggregates particulate organic matter (POMo) and mineral-associated organic matter (M-OM). Higher amounts of floor litter dry matter were found in the positions under the tree canopy projection than in the positions beyond the tree canopy. The C contents of bulk soil and all the soil organic matter fractions significantly differ among positions within transect orientation with decreasing values from the closest positions to the trunk to the positions beyond the canopy projection, apart from POMo. These results indicate an overall positive effect of the tree on soil fertility through direct and indirect factors that play a relevant role such as tree litter input and changes in the microclimate beneath the trees. These findings highlight the capacity of these silvo-pastoral systems to store and to stabilize more C in the soil than treeless grassland systems.

Keywords: water extractable organic carbon, particulate organic carbon, mineral-associated organic carbon, wooded pastures.

Introduction

Agroforestry systems provide a range of ecosystem services *sensu* Millennium Ecosystem Assessment (2005) of great importance in the economic and agro-ecological domains, including forage production, livestock and forestry products, maintenance of high levels of biological diversity and protection of soil and water resources. The Association for Temperate Agroforestry, AFTA (www.aftaweb.org) defines an agroforestry system as “an intensive land management system that optimizes the benefits from the biological interactions created when trees and/or shrubs are deliberately combined with crops and/or livestock.”.

Agroforestry systems are believed to have a higher potential to sequester C because of their perceived ability for greater capture and utilization of growth resources (light, nutrients, and water) than in single-species crop or pasture systems (Nair, 2011).

In recent years, an increasing body of knowledge showed that the introduction of trees into temperate croplands and pasturelands such as occurs with the implementation of agroforestry practices, improves C sequestration both in above ground biomass and in the soil (Haile et al 2010). Nair et al. (2010) showed that tree-based agricultural systems, compared to treeless systems, stored more C in deeper soil layers near the tree than away from the tree, and higher soil organic C content was associated with higher species richness and tree density. In addition trees have also other added values, providing shade and shelter for workers and livestock, and combat wind and water erosion processes (Eichorn et al., 2006). Therefore, it is important to quantify the strength and longevity of the C sink in tree-based pasture systems to understand the mechanisms and processes associated with C transformation and storage. SOM has a very complex and heterogeneous composition, and is associated with mineral soil constituents to form soil aggregates (Haile et al 2010). The nature and extent of turnover of soil organic carbon (SOC) is intimately linked to organic matter size fractions as well as to soil structure and extent of aggregation (Martens, 2000). Different pools of SOC have different residence times, ranging from labile to stable forms, different effects on soil quality and respond differently to management practices (Chan, 2001)

In Europe, the most extended agroforestry system is the dehesa, with about 3 million hectares in south-western Spain and Portugal (Eichhorn et al., 2006), but it is also present in other areas such as Sardinia (Caballero et al., 2009; Eichorn et al., 2006). A dehesa is a multipurpose system of widely spaced, scattered oak trees (Holm-oak, *Quercus ilex*; cork oak, *Quercus suber*; a deciduous oak, *Quercus pyrenaica*), which forms an open permanent upperstorey, mixed with pastures or intercropped with cereal and/or fodder. Livestock is the main product of dehesas, but other products such as cereal, cork, firewood and game have been common since at least the Middle Ages (Gòmez-Gutierrez and Pérez-Fernàndez, 1996).

In addition to their direct function as 'fodder trees' (acorn and leaves), oaks are also retained in the belief that they improve soil chemical fertility (Gallardo, 2003), physical fertility (Joffre and Rambal, 1988) and microclimate conditions (Moreno et al., 2005).

The rate of SOC sequestration, and the magnitude and quality of soil C stock depend on the complex interactions between climate, soil, tree species and management, and by the amount and quality of litter input (Yamashita et al 2006, Lagomarsino et al 2011).

Few research has been conducted on the relationship between accumulation and soil organic matter quality and management practices of agroforestry systems in semi-arid environments such as in the Mediterranean agro-silvo-pastoral systems (Moreno et al., 2007; Howlett et al., 2011).

It is therefore important to understand the role of trees in these agro-silvo-pastoral systems and how the tree species might influence the soil fertility *sensu lato*.

The aim of this study was to evaluate the impact on soil organic matter quality and stability of management practices in an agro-silvo-pastoral system under Mediterranean semi-arid conditions. The hypothesis of this study was that the presence of trees in a wooded pasture affects directly and indirectly the soil features mostly limiting the light under the canopy and turns out to be important in the distribution of organic C and principal nutrients along micro-scale gradients. The tree at issue is *Quercus suber* and we evaluated its role by determining: i) the differences in soil properties and carbon contents and pools, namely water-extractable organic matter (WEOM), particulate organic matter (POM-free and POM-occluded) and mineral associated organic matter (MOM).at increasing distances from individual cork oak trees in a wooded grassland.

Materials and methods

Study area

The study was carried out in an area representative of Mediterranean agro-forestry systems, located in the North-Eastern Sardinia (Italy) at Berchidda (40° 47’ 0” N 09° 10’ 0” E) at 320 m above sea level. The bioclimate is pluviseasonal oceanic low meso-Mediterranean low sub-humid (Rivas-Martínez and Rivas y Sáenz, 2007), with a mean annual precipitation of 630 mm (70% from October to March) and a mean annual air temperature of 14.2°C. The soil of the area developed from a granite, a parent material largely diffused in Sardinia (Aru et al., 1990), and was classified as Typic Dystroxerept (Soil Survey Staff, 2010). The potential vegetation of the area is mainly represented by cork oak woods (*Quercus suber* L.) referable to the *Violo dehnhardtii-Quercetum suberis* association (Bagella & Caria, 2011). The study area has been managed according to a flexible rotational scheme consisting of a fallow pasture which is cropped every two to five years, depending on the dynamics of the thorny vegetation, with an annual hay crop mixture (Seddaiu et al 2013). The grazing (Sarda dairy sheep) occurs during the whole year with an average stocking rate of 3 ewes ha^{-1}. This land use is stable since the last two decades as assessed through aerial photos and farmers interviews and at the time of samplings (May and November 2011), the study area was a five-years old fallow pasture where scattered cork oak trees were present at an average density of 30 trees ha^{-1}.

Experimental layout and soil sampling

Six isolated cork oak trees in the wooded pasture were randomly selected in March 2011. The trees had a crown diameter of 11.8 ± 0.9 m and were 10.1 ± 0.3 m in height and 35.3 ± 1.2 cm in diameter at breast height. For each tree, following the Fernandez-Moya (2010) experimental scheme two transects with opposite orientations (NE- SW) were identified. For each transect, measurements were made at five sampling positions (Fig. 1). The five sampling positions were identified taking into account the horizontal projection of the crown onto the ground: two positions were fully underneath the tree canopy (positions 1 and 2), one position was in the edge of the canopy (position 3) and two positions were beyond the tree canopy (positions 4 and 5). Distances from the trunk were 1.2±0.1 m, 3.5±0.3 m, 5.9±0.5 m, 8.2±0.7 m and 10.7±0.9 m respectively in the position 1 to 5.

All the studied variables were measured at each sampling data in all the positions along the two orientations for a total of 60 sampling units.

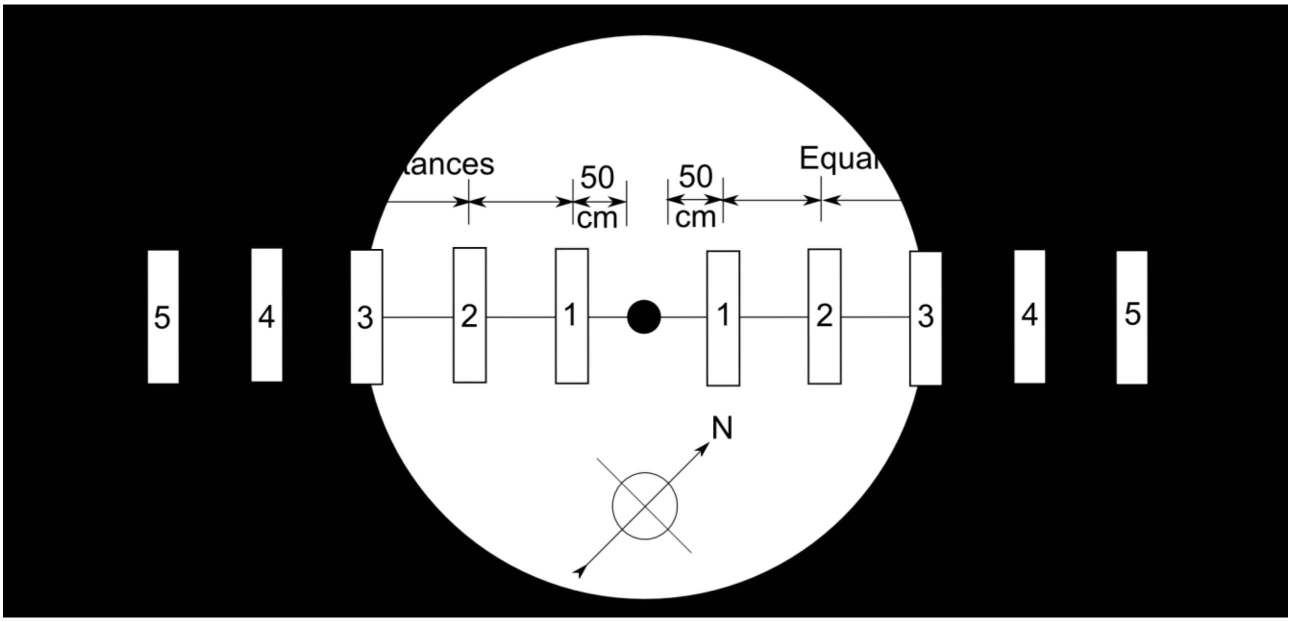

Fig.1. Experimental layout

Data collection

For evaluating the impact of the cork oak trees on the soil traits along the two transects and in relation to the distance from the tree trunk within transect, four kind of tree-related variables were assessed in two sampling dates (May and November 2011): the photosyntetically active radiation (PAR), the soil water content, the soil temperature and the floor litter.

Photosyntetically active radiation

PAR was measured on a clear day at noon by placing Sun Scan Canopy Analysis System SS1-UM-1.05 (Delta-T Devices) at right angles to the transect, parallel to the ground and above the grassland canopy at each sampling positions.

Soil water content and temperature

Soil temperature (T, °C) was measured at 3 cm depth using a digital thermometer HD2101.2 (Delta Ohm, IT). Soil water content (SWC, % on weight) was determined gravimetrically by taking soil cores in the 0-20 cm soil layer and over-drying samples at 105 °C for 24 h.

Floor litter

Floor litter samples were collected at each sampling position within a 25 x 25 cm2 quadrat and all mineral soil was separated from the litter using a brush and a spoon (Hoosbeek and Scarascia-Mugnozza, 2009). Samples were then oven-dried at 60°C until constant weight and floor litter was expressed as the dry mass per unit ground area (kg ha^{-1}).

General soil characterization

In May 2011 soil samples were collected (0-20 cm soil depth) at each sampling position (6 trees x 2 transects x 5 distances from the trunk) for a total of 60 samples. The soil samples were oven-dried at 40°C and sieved at 2 mm to remove skeleton, large roots and organic debris. All the analyses were run on the < 2 mm soil fraction, the fine earth.

The particle-size distribution was determined by the pipette method (Day, 1965) after treating a soil aliquot with 3 M H_2O_2 solution to destroy organic cements. The sand fraction was subdivided into coarse, medium and fine sand by wet sieving at 500, 250 and 20 µm, respectively. The silt was separated from the clay by sedimentation after the samples were dispersed in a 0.08 M sodium hexametaphosphate solution. The pH was measured potentiometrically in the supernatant of a suspension with 1:2.5 soil:liquid ratio, using both distilled water and 1 M KCl solution. Soil available P was determined by the Olsen method (Olsen et al. 1954). Total organic C (TOC) and total N were determined using an elemental analyser (LECO CHN 628).

Water-soluble organic carbon

The water soluble organic matter (WEOM) was determined according to the method described by Burford and Bremner (1975). Fifteen grams of air dried soil (< 2 mm) were placed in a centrifugation tube with 30 ml of distillated water and were shaken for 15 minutes at 250 rpm. The soil suspension was then centrifuged for 10 min at 4000 x g and was filtered (0.45 µm) using vacuum. The C and N contents of extracts were determined using an elemental analyser (LECO CHN 628).

Density fractionation of soil organic matter

Density fractions of soil were then obtained using the procedure described by John et al. (2005). Ten grams of air dried soil (< 2 mm) were placed in a centrifugation tube with 40 ml of sodium polytungstate solution (SPT ACROS) of a density of 1.6 g cm-3. The tube was inverted gently by hand five times, the solution was allowed to settle for 30 minutes and it was centrifuged at 5100 x g for 1 h. The supernatant with floating particles was filtered (0.45 µm) using vacuum and washed with distilled water to gain the free particulate organic matter <1.6 g cm-3 (POMf). The sediment was dispersed with 40 ml of sodium polytungstate solution with a density of 2.0 g cm-3. To break up the aggregates 10 glass beads with a diameter of 5mm were used and the solution was shaken for 16 h with a frequency of 60 movements per minute (Balesdent et al., 1991). The soil suspension was centrifuged for 1 h at 5100 x g and the supernatant with floating particles (occluded POM with a density of 1.6 to 2.0 g cm-3, POMo) was filtered under vacuum and washed. To remove the salt, the pellet containing the mineral fraction (>2.0 g cm^{-3}, MOM) was washed three times with distilled water. Finally, the sample was centrifuged and the supernatant was discarded. All fractions were dried at 40 °C, the POM fractions were ground with a mortar. The C and N contents of the soil organic matter fractions were determined using a LECO CHN 628 elemental analyser.

Statistical analysis

Analysis of variance was carried out for all variables according to a balanced hierarchical design with distances from the trunk nested in transect orientation. Homogeneity of variances was verified using the Cochran C-test, and data were appropriately transformed when necessary (Gomez and Gomez, 1984). Mean comparisons were carried out using the Student–Newman–Keuls test (Winer et al. 1991).

For evaluating the variation of TOC, TN and soil organic matter fractions along the transects removing the effect of the six trees, the significance of regressions between these variables and the normalized distance of the sampling positions from the tree trunk was analysed. The effect of the six trees was removed for each variables analyzing the residuals, which was added the average of the six trees. The normalized distance was calculate from relationship between the distance of sampling position by the tree trunk and the total distance to the tree trunk for each orientation.

In order to assess the degree of association among all the variables, the Pearson's correlation coefficients were calculated.

To group the positions along the transects according to the soil features and to the tree-related variables, a Principal Component Analysis (PCA) and hierarchical clusters of the accessions (Pearson's correlations from PCA coordinates on the first two axes) were performed according to Johnson and Wichern (2002), on a data set comprising all the studied variables averaged among the six trees for the ten sampling positions.

Analysis of variance, the significance of the no linear regressions and principal component analysis were performed with the SAS System (SAS Institute, 1999).

Results

In May 2011 (spring), PAR transmitted below the tree canopy was significantly influenced by the distance from the trunk with the positions 1 and 2 characterized by -94% of PAR with respect to the positions 4 and 5 for both orientations (Fig. 2). At the edge of the tree canopy (position 3), PAR values were -72% and -20% lower than those in the positions beyond the tree canopy for the orientation NE and SW, respectively.

In November 2011 (autumn), PAR values were generally lower than in May and were significantly different among the sampling positions. In the SW orientation, the positions 5,4 and 3 showed higher PAR values than those in the positions 1 and 2, whileI in the NE transect, the position 3 had similar PAR values to the positions 1 and 2 and the highest PAR was observed in the position 5.

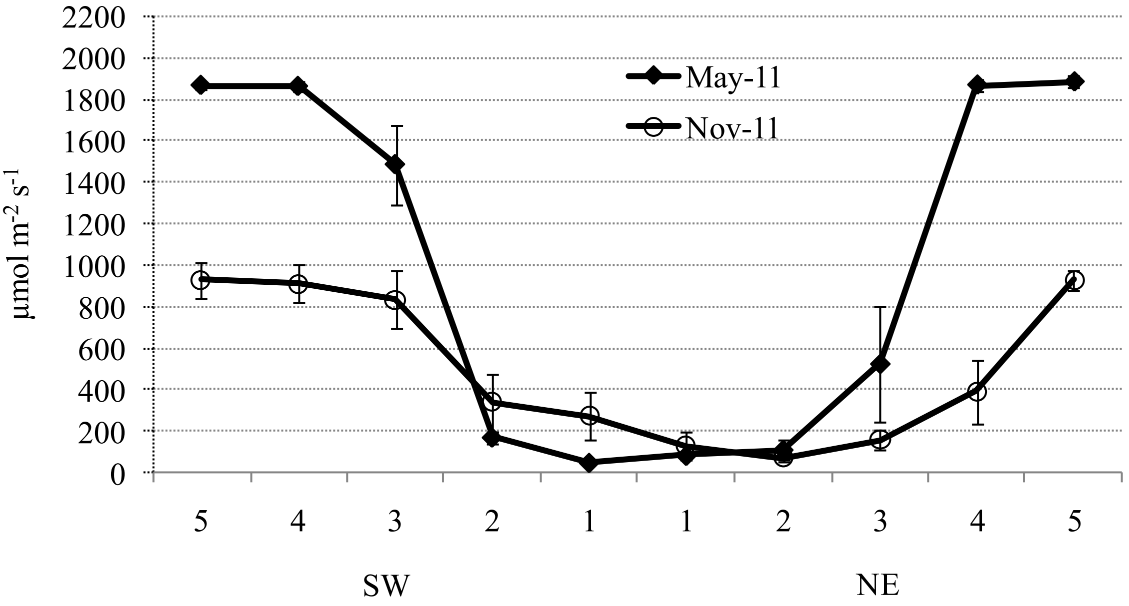

Fig. 2. Photosyntetically active radiation (PAR, μmol m^{-2} s^{-1}) along the two transects SW and NE and in relation to the distances from the trunk within transect measured in May 2011 and November 2011.

Soil T was significantly influenced by the presence of the oak tree in May 2011 when the positions beneath the canopy were cooler (about -8°C) than the positions beyond the canopy projection in both transect orientations (Table 1). In November 2011, the mean soil T of the SW transect was 1.5°C higher than in the NE transect, and the outer position 5 within NE was slightly higher than in the positions beneath the tree.

Regarding SWC (Table 1), no significant differences among positions within orientations were observed apart from the orientation SW in May 2011, when values in the position 1 were significantly higher (+5°C) than the positions 2, and this latter position was +3.1°C warmer than positions 3 to 5.

Table 1. Means and results of the analysis of variance for soil temperature at 3 cm soil depth (T, °C) and soil water content (SWC, % weight) in May and November 2011 in relation to the orientation and distance from the tree trunk.

		May		November	
Orientation	Position	T	SWC	T	SWC
North-East	1	16.5 b	12.2 a	17.1 ab	9.1 a
	2	16.7 b	10.5 a	16.5 b	9.5 a
	3	19.3 b	10.5 a	17.7 ab	10.6 a
	4	23.2 a	10.0 a	18.3 ab	11.5 a
	5	23.9 a	9.1 a	19.4 a	10.8 a
	Mean	19.9 A	10.5 A	17.8 B	10.3 A
South-West	1	16.1 b	16.7 a	18.9 a	9.8 a
	2	16.4 b	11.7 b	19.3 a	11.0 a
	3	22.5 a	8.0 c	19.3 a	10.9 a
	4	25.7 a	9.1 c	20.2 a	11.0 a
	5	24.5 a	8.7 c	19.5 a	10.8 a
	Mean	21.03 A	10.8 A	19.4 A	10.7 A
Source of variation	*d.f.*	*P-value*	*P-value*	*P-value*	*P-value*
Orientation	1	0.674	0.833	0.018	0.428
Distance (orientation)	8	<0.0001	<0.0001	0.307	0.167
CV (%)		*13.2*	*18.9*	*10.2*	*14.6*

Mean values among distances from trunk within orientation with different lower-case letters significantly differ for $P \leq 0.05$.

Mean values between orientations with different capital letters significantly differ for $P \leq 0.05$.

The amount of floor litter did not significantly differ between the two orientation in both sampling dates (spring and fall), while significant differences were observed among the distance from the trunk within the orientations. In May 2011 (Fig. 3) in the SW orientation floor litter dry matter was fivefold higher in the positions 1 and 2 than in the other positions that did not differ among each other, while in the NE orientation no significant difference was found between 1 and 3 position and the position 1 showing less than half floor litter with respect to the position 2. In November 2011 (Fig. 4) in the SW orientation the amount of floor litter showed a decreasing trend from the position 1 to the position 5 with no significant differences between positions 1 and 2 and among the positions 3 to 5. In the NE orientation, the positions 1 and 2 showed twice higher floor litter biomass than the other positions.

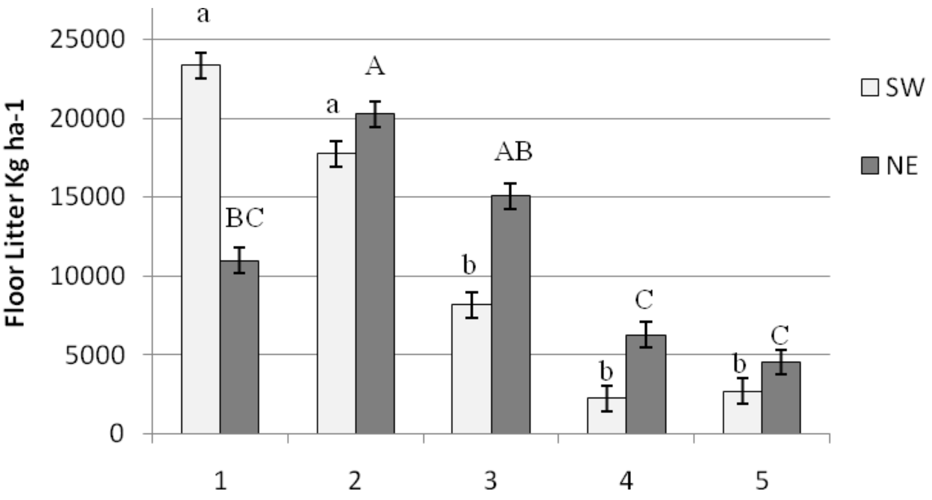

Fig. 3. Floor litter dry matter (kg ha^{-1}) at the spring sampling (May 2011) in the North-East (NE) and South-West (SW) orientations of the transects. Mean values among different positions within NE orientation with different lower-case letters significantly differ for P ≤ 0.05. Mean values among distances from trunk within SW orientation with different capital letters significantly differ for P ≤ 0.05.

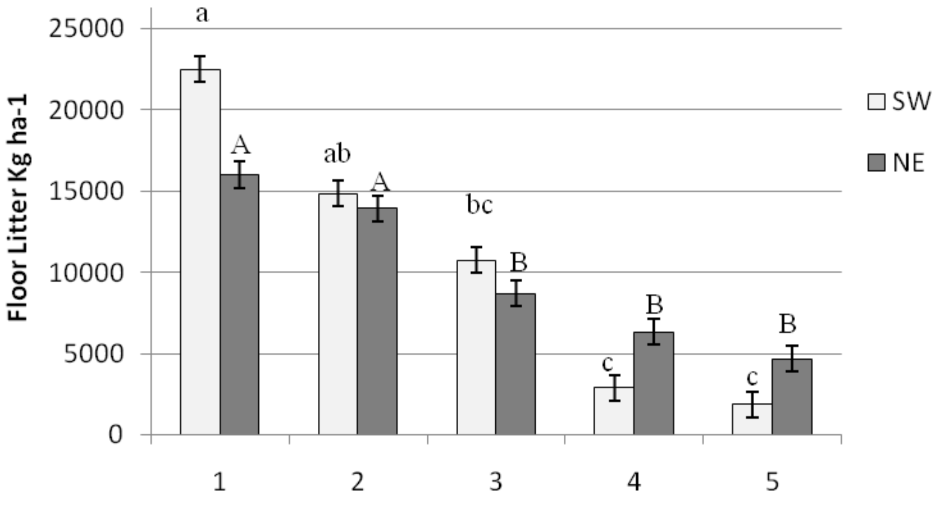

Fig. 4. Floor litter dry matter (kg ha^{-1}) at the autumn sampling (November 2011) in the North-East (NE) and South-West (SW) orientations of the transects. Mean values among distances from trunk within NE orientation with different lower-case letters significantly differ for P ≤ 0.05. Mean values among distances from trunk within SW orientation with different capital letters significantly differ for P ≤ 0.05.

The two orientations did not significantly differ for pH, CEC and available P (Table 2). Regarding pH values, the lowest values were observed in the position 1 for both transects with no or small differences among the other positions. CEC showed the lowest values beyond the tree canopy projection , while the available P did not significantly differ among the distance from the tree trunk in the NE orientation and in the SW orientation it showed higher values under the canopy than beyond the canopy (Table 2).

Table 2. Means and results of the analysis of variance for pH in water, cation exchange capacity (CEC) and available P (mg kg^{-1}) in the top soil layer (0-20 cm) in relation to the orientation and distance from the tree trunk.

Orientation	Position	pH	CEC	Available P
North-East	1	5.5 b	15.5 a	24.9 a
	2	5.8 a	14.7 a	27.4 a
	3	5.9 a	13.7 b	18.7 a
	4	5.9 a	12.3 c	18.7 a
	5	5.9 a	12.3 c	18.7 a
	Mean	5.8 A	13.7 A	21.7 A
South-West	1	5.6 a	16.6 a	23.7 a
	2	5.8 b	15.0 b	19.9 ab
	3	6.0 bc	13.9 bc	16.2 abc
	4	5.9 c	12.6 c	8.7 c
	5	6.0 c	13.1 c	12.5 bc
	Mean	5.8 A	14.3 A	16.2 A
Source of variation	*d.f.*	*P-value*	*P-value*	*P-value*
Orientation	1	0.622	0.552	0.129
Distance (orientation)	8	<0.0001	<0.0001	0.027
CV (%)		*3*	*6.9*	*42.4*

Mean values among distances from trunk within orientation with different lower-case letters significantly differ for P ≤ 0.05.

Mean values between orientations with different capital letters significantly differ for P ≤ 0.05.

The soil C and N contents sharply decreased in the vicinity of the tree trunk between the positions 1 and 2 in both orientations, while no significant differences among the position at the edge of the canopy and the two outer positions were found in the NE transect (Table 3). The C/N ratio was not significantly influenced by orientation and position within orientation (Table 3).

Table 3. Means and results of the analysis of variance for contents of organic C (TOC, g kg^{-1}) and total N (TN, g kg^{-1}), and C/N ratio in the top soil layer (0-20 cm) in relation to the orientation and distance from the tree trunk.

Orientation	Position	TOC	TN	C/N
North-East	1	36.9 a	2.5 a	12.1 a
	2	29.7 b	2.0 b	14.9 a
	3	26.9 bc	1.8 bc	14.6 a
	4	24.5 cd	1.6 c	15.4 a
	5	22.3 d	1.6 c	14.1 a
	Mean	28.0 A	1.9 A	14.2 A
South-West	1	42.8 a	2.9 a	15.0 a
	2	34.8 b	2.4 b	11.9 a
	3	29.0 c	1.9 c	14.9 a
	4	23.0 d	1.6 c	14.9 a
	5	23.5 d	1.6 c	14.9 a
	Mean	30.6 A	2.1 A	14.3 A
Source of variation	*d.f.*	*P-value*	*P-value*	*P-value*
Orientation	1	0.584	0.593	0.531
Distance (orientation)	8	<0.0001	<0.0001	0.732
CV (%)		12.2	15.1	6.8

Mean values among distances from trunk within orientation with different lower-case letters significantly differ for $P \leq 0.05$.

Mean values between orientations with different capital letters significantly differ for $P \leq 0.05$.

The C contents of all the soil organic matter fractions were not significantly influenced by the transects orientation, whereas significant differences were observed among positions within the orientation with decreasing values from the closest position to the trunk to the positions beyond the canopy projection (Table 4). Only the C content as POMo had similar values along both transects ranging from 3.2 to 4.2 g kg^{-1}. No significant differences were found between the two orientations and among the positions within orientation for the N contents of all the fractions apart from the N content as POMf that showed almost three times higher values in the position 1 than in the other positions in the SW orientation (Table 5); in the NE orientation less marked differences among distances from the trunk were observed. The C/N ratio in the soil organic matter fractions showed significant differences between the orientations only for POM free (Table 6). In the POMo fraction the lowest C/N ration was observed in the postion 1 for both orientations and in the MOM fraction significant differences were found among positions only for the SW transect with lower values beyond the tree canopy than beneath the tree (Table 6).

The major part of the TOC content in the top 20 cm of the soil was found in the MOM (about 67%), with no significant differences between orientations and distances from the trunk within orientation (Table 7). Distances from the trunk significantly influenced the contribution of C as WEOM and POMf to TOC, with higher values in the vicinity of the trunk and generally smaller values in the outer positions. On average, C contents as POMf and POMo contributed to TOC for 13%, while C as WEOM for less than 1%.

The relationships between TOC, TN and C contents of all the organic matter fractions, expressed as removed-tree effect values, *vs.* the normalized distances from the trunk were all fitted by a fourth-order polynomial equation and were significant for all the variables apart from POM occluded and MOM (Fig. 5-10). The significant relationships showed higher values near the tree trunk and decreasing values from the positions at the edge the canopy to the positions beyond the canopy projection (Fig. 5-8).

TOC, TN and the C contents of WEOM and MOM were significantly correlated with spring PAR values, spring and autumn floor litter amount and soil T in spring (Table 8). CEC and pH were significantly correlated with TOC, TN and the C contents of most of the soil organic matter fractions, while available P was not correlated with these variables but with litter and soil T of both sampling dates. The C content as POMo was the only variable that did not show any significant correlation with the others (Table 8). Principal component analysis showed that the first component (Prin1) explained about 75% of the variance and the second component about 12% (Fig. 11). The correlation matrix (Table 9) showed that the first component was significantly correlated with almost all the variables ($P<0.01$). Only the C content as POMo was correlated with the second component (Table 9). Along the transects were identified four clusters of positions (Fig. 11). The cluster I encloses the position 1 of both orientations where the highest C contents of bulk soil and organic matter fractions (except C-POMo), the highest litter dry matter and the lowest pH were found. The cluster II includes the position 2 with high soil C contents, high litter production and low pH. The cluster III comprises the position 3 of the both transects characterized by the lowest C content of the POMo and intermediate values for the other variables. The cluster IV includes the positions 4 and 5 of both transect orientations and the position 3 of the NE transect.

and the third cluster the position 3 of both orientations, in the second the variables values were high but in the third were intermediate values in fact the cluster IV include the positions 4 and 5 of both orientations characterized by the lowest values of the majority of variables.

Table 4. Means and results of the analysis of variance for contents of C as WEOM (C-WEOM, g kg^{-1}), POM free (C-POMf, g kg^{-1}), POM occluded (C-POMo, g kg^{-1}) and MOM (C-MOM, g kg^{-1}) in the top soil layer (0-20 cm) in relation to the orientation and distance from the tree trunk.

Orientation	Position	C-WEOM	C-POMf	C-POMo	C-MOM
North-East	1	3.0 a	4.5 a	4.0 a	22.8 a
	2	2.0 b	3.2 ab	3.5 a	19.3 ab
	3	1.6 bc	1.4 b	2.7 a	20.5 ab
	4	1.2 cd	1.9 b	3.5 a	16.6 b
	5	0.9 d	2.6 ab	3.1 a	16.3 b
	Mean	1.7 A	2.7 A	3.4 A	19.1 A
South-West	1	3.5 a	6.8 a	3.7 a	25.4 a
	2	2.3 b	4.6 ab	3.4 a	23.0 ab
	3	1.9 b	2.4 b	2.7 a	22.6 ab
	4	1.1 c	2.0 b	3.4 a	15.8 b
	5	1.1 c	1.9 b	3.6 a	15.2 b
	Mean	2.0 A	3.6 A	3.3 A	20.4 A
Source of variation	*d.f.*	*P-value*	*P-value*	*P-value*	*P-value*
Orientation	1	0.695	0.46	0.884	0.596
Distance (orientation)	8	<0.0001	<0.0001	0.36	0.001
CV (%)		23.1	57.5	30.5	22.7

Mean values among distances from trunk within orientation with different lower-case letters significantly differ for $P \leq 0.05$.

Mean values between orientations with different capital letters significantly differ for $P \leq 0.05$.

Table 5. Means and results of the analysis of variance for contents of N as WEOM (N-WEOM, g kg^{-1}), POM free (N-POMf, g kg^{-1}), POM occluded (N-POMo, g kg^{-1}) and MOM (N-MOM, g kg^{-1}) in the top soil layer (0-20 cm) in relation to the orientation and distance from the tree trunk.

Orientation	Position	N-WEOM	N-POMf	N-POMo	N-MOM
North-East	1	0.6 a	0.3 a	0.2 a	1.8 a
	2	0.5 a	0.2 ab	0.2 a	1.5 ab
	3	0.5 a	0.1 b	0.1 a	1.6 ab
	4	0.5 a	0.1 b	0.2 a	1.4 b
	5	0.5 a	0.1 ab	0.2 a	1.4 b
	Mean	0.5 A	0.2 A	0.2 A	1.5 A
South-West	1	0.6 a	0.4 a	0.2 a	1.9 a
	2	0.6 a	0.3 ab	0.2 a	1.8 a
	3	0.5 ab	0.1 b	0.2 a	1.7 a
	4	0.5 b	0.1 b	0.2 a	1.4 a
	5	0.5 ab	0.1 b	0.2 a	1.4 a
	Mean	0.5 A	0.2 A	0.2 A	1.6 A
Source of variation	*d.f.*	*P-value*	*P-value*	*P-value*	*P-value*
Orientation	1	0.87	0.419	0.821	0.556
Distance (orientation)	8	0.039	<0.0001	0.094	0.015
CV (%)		15.8	59.5	31.7	19.5

Mean values among distances from trunk within orientation with different lower-case letters significantly differ for $P \leq 0.05$.

Mean values between orientations with different capital letters significantly differ for $P \leq 0.05$.

Table 6. Means and results of the analysis of variance for ratio of C/N as POM free (C/N-POMf, g kg-1), POM occluded (C/N-POMo, g kg-1) and MOM (C/N-MOM, g kg-1) in the top soil layer (0-20 cm) in relation to the orientation and distance from the tree trunk.

Orientation	Position	C/N POMf	C/N POMo	C/N MOM
North-East	1	17.6 a	16.7 b	12.5 a
	2	18.2 a	18.1 a	12.5 a
	3	18.3 a	19.0 a	12.7 a
	4	18.2 a	18.3 a	12.0 a
	5	17.4 a	18.4 a	11.6 a
	Mean	17.9 B	18.1 A	12.3 A
South-West	1	17.0 a	15.9 c	13.3 a
	2	16.9 a	16.5 bc	13.1 a
	3	16.9 a	17.5 ab	12.9 a
	4	17.2 a	18.5 a	11.4 b
	5	17.5 a	18.3 a	11.2 b
	Mean	17.1 A	17.3 A	12.4 A
Source of variation	*d.f.*	*P-value*	*P-value*	*P-value*
Orientation	1	0.005	0.261	0.825
Distance (orientation)	8	0.919	<0.0001	<0.0001
CV (%)		7.4	5.3	6.5

Mean values among distances from trunk within orientation with different lower-case letters significantly differ for $P \leq 0.05$.

Mean values between orientations with different capital letters significantly differ for $P \leq 0.05$.

Table 7. Means and results of the analysis of variance for the contribution to TOC of C as WEOM (C-WEOM/TOC, %), as POM free (C-POMf/TOC, %), as POM occluded (C-POMo/TOC, %) and as MOM (C-MOM/TOC, %) in the top soil layer (0-20 cm) in relation to the orientation and distance from the tree trunk.

Orientation	Position	C-WEOM/TOC	C-POMf/TOC	C-POMo/TOC	C-MOM/TOC
North-East	1	8.2 a	12.3 a	11.1 a	61.6 a
	2	6.5 b	10.6 a	12.0 a	64.8 a
	3	5.9 b	5.5 a	10.2 a	75.8 a
	4	4.9 bc	7.8 a	14.1 a	68.1 a
	5	4.1 c	11.7 a	13.8 a	72.7 a
	Mean	5.9 A	9.6 A	12.2 A	68.6 A
South-West	1	8.4 a	16.3 a	8.7 b	58.7 a
	2	6.7 b	13.3 a	10.0 b	65.5 a
	3	6.5 b	8.6 a	9.5 b	77.3 a
	4	4.6 c	8.8 a	15.0 a	68.5 a
	5	4.5 c	8.0 a	15.0 a	64.9 a
	Mean	6.1 A	11.0 A	11.6 A	66.7 A
Source of variation	*d.f.*	*P-value*	*P-value*	*P-value*	*P-value*
Orientation	1	0.848	0.512	0.713	0.691
Distance (orientation)	8	<0.0001	0.017	0.011	0.04
CV (%)		19.6	47.5	30.2	15.1

Mean values among distances from trunk within orientation with different lower-case letters significantly differ for P ≤ 0.05.

Mean values between orientations with different capital letters significantly differ for P ≤ 0.05.

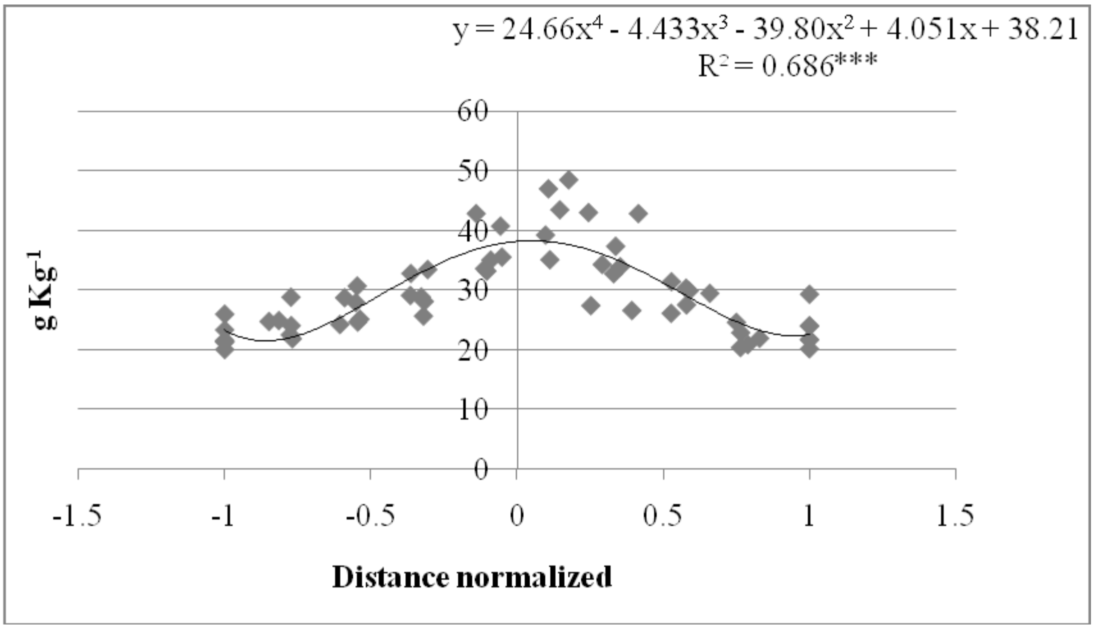

Fig. 5. Relationship between total organic carbon in the top soil layer (0-20 cm) expressed as removed-tree effect and the normalized distance from the trunk.
*** = significant for *P*<0.001.

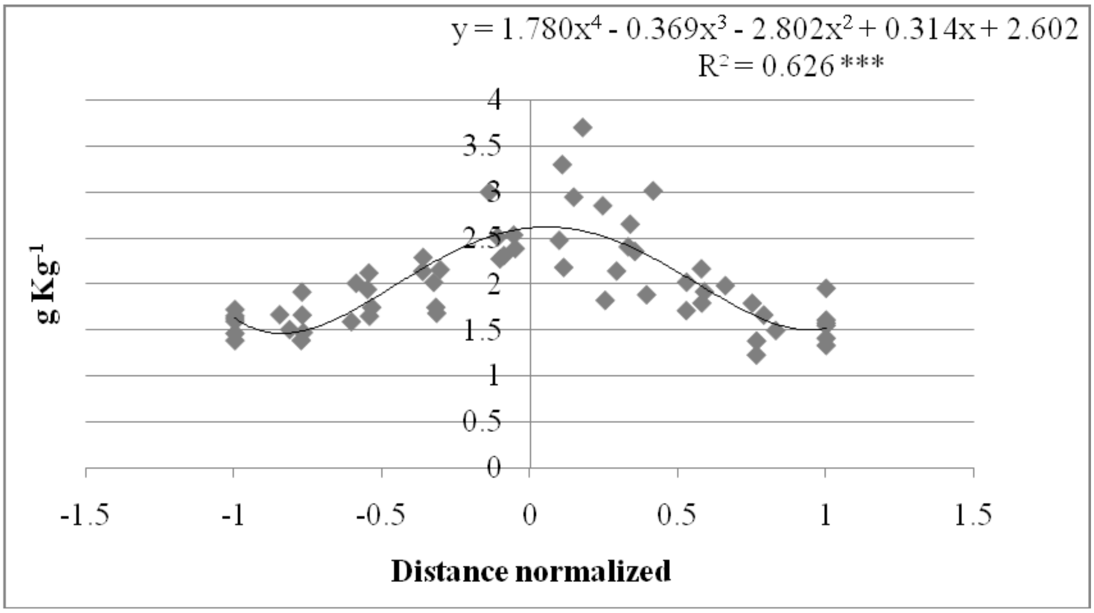

Fig. 6. Relationship between total nitrogen in the top soil layer (0-20 cm) expressed as removed-tree effect and the normalized distance from the trunk.
*** = significant for *P*<0.001.

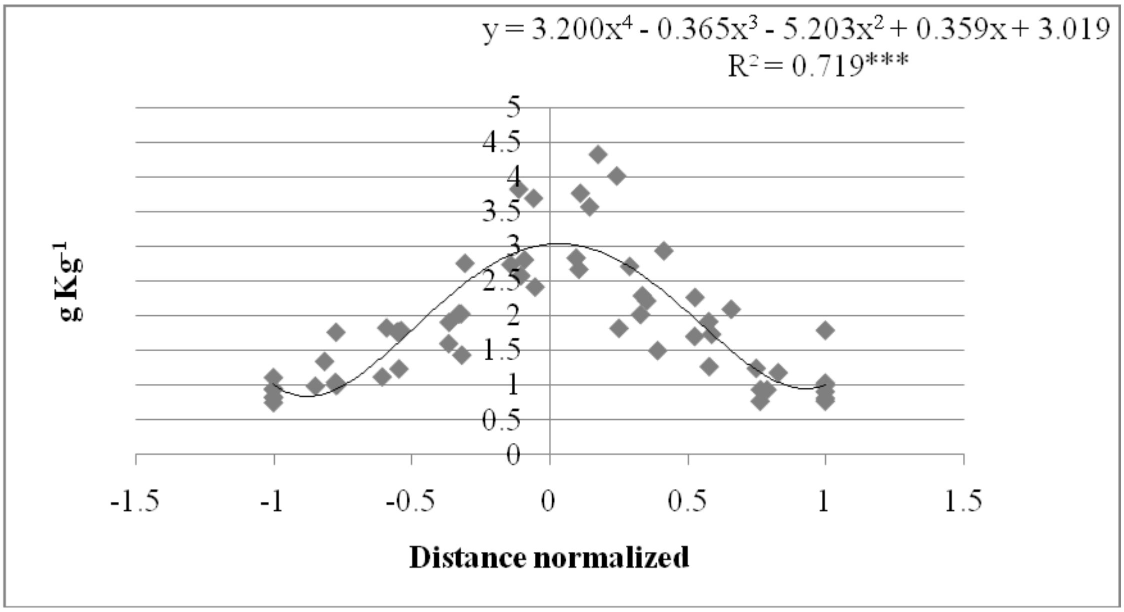

Fig. 7. Relationship between C content as WEOM in the top soil layer (0-20 cm) expressed as removed-tree effect and the normalized distance from the trunk.
*** = significant for *P*<0.001.

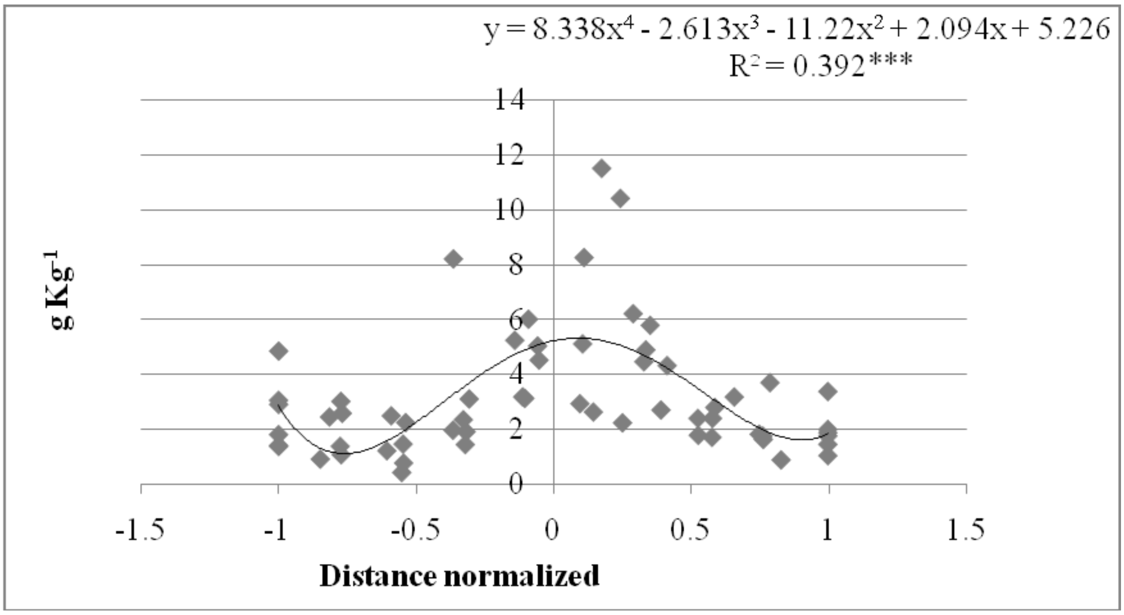

Fig. 8. Relationship between C content as POM free in the top soil layer (0-20 cm) expressed as removed-tree effect and the normalized distance from the trunk.
*** = significant for *P*<0.001.

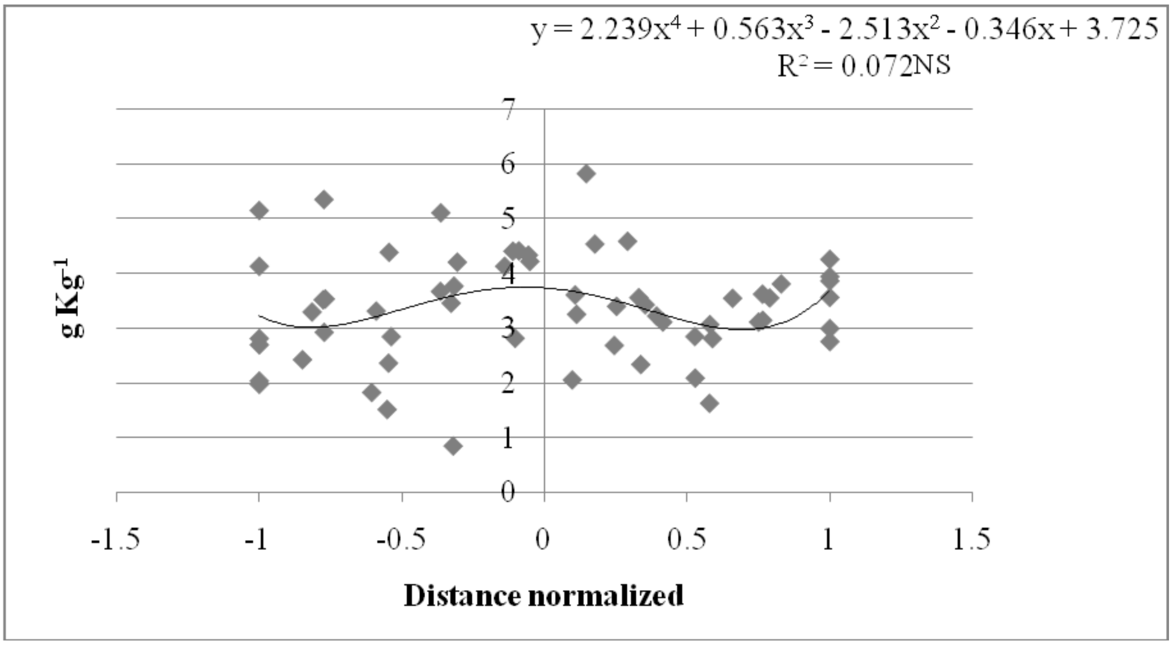

Fig. 9. Relationship between C content as POM occluded in the top soil layer (0-20 cm) expressed as removed-tree effect and the normalized distance from the trunk.
NS = no significant

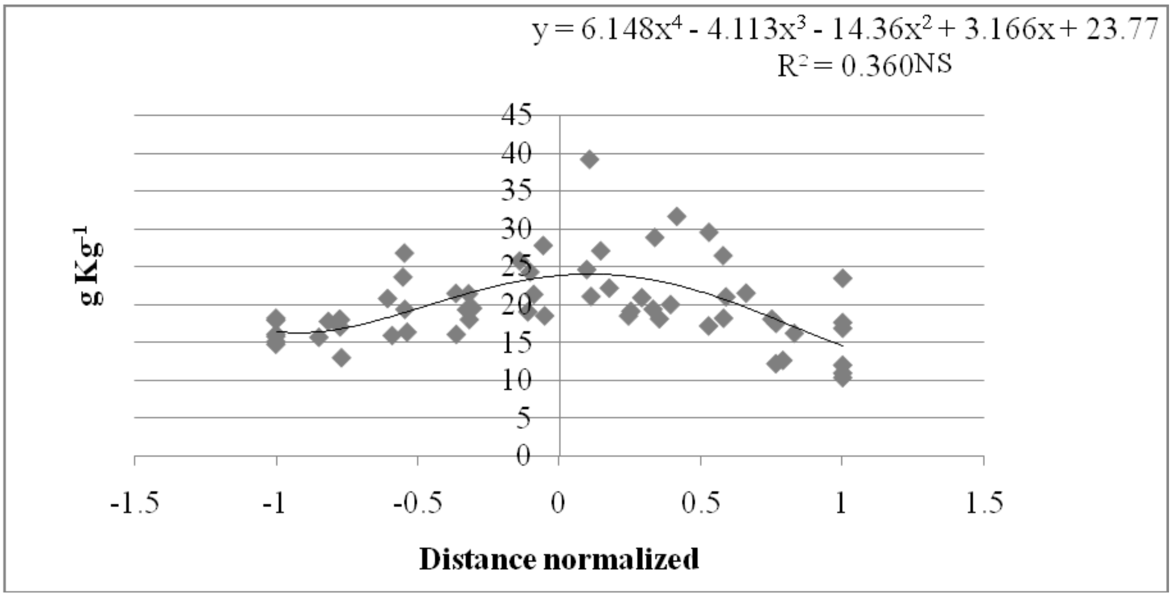

Fig. 10. Relationship between C content as MOM in the top soil layer (0-20 cm) expressed as removed-tree effect and the normalized distance from the trunk.
NS = no significant

Table 8. Pearson’s correlation coefficients among the studied variables measured from ten positions with respect of the tree trunk. Correlation coefficients in bold characters are significant at P < 0.01 (n = 10).

	pH	*CEC*	*PAR May*	*PAR Nov*	*Available P*	*Litter Nov*	*Litter May*	*T May*	*T Nov*	*SWC May*	*SWC Nov*	*TOC*	*TN*	*N-WEOM*	*C-WEOC*	*C-POMf*	*N-POMf*	*C-POMo*	*N-POMo*	*C-MOM*	*N-MOM*
pH	1.00																				
CEC	**-0.77**	1.00																			
PAR May	0.69	**-0.89**	1.00																		
PAR Nov	0.61	-0.63	**0.85**	1.00																	
Available P	-0.67	0.66	**-0.78**	**-0.82**	1.00																
Litter Nov	**-0.77**	**0.95**	**-0.87**	-0.70	**0.78**	1.00															
Litter May	-0.52	**0.83**	**-0.90**	**-0.79**	**0.77**	**0.88**	1.00														
T May	0.72	**-0.89**	**0.98**	**0.87**	**-0.85**	**-0.91**	**-0.92**	1.00													
T Nov	0.45	-0.36	0.62	**0.84**	**-0.81**	-0.42	-0.51	0.63	1.00												
SWC May	**-0.81**	**0.82**	-0.70	-0.61	0.58	**0.84**	**0.76**	-0.74	-0.24	1.00											
SWC Nov	**0.78**	-0.72	0.73	0.60	-0.72	-0.64	-0.55	0.70	0.69	-0.54	1.00										
TOC	**-0.83**	**0.97**	**-0.83**	-0.62	0.65	**0.97**	**0.79**	**-0.86**	-0.30	**0.88**	-0.62	1.00									
TN	**-0.83**	**0.97**	**-0.84**	-0.61	0.65	**0.96**	**0.80**	**-0.87**	-0.28	**0.88**	-0.63	**0.99**	1.00								
N-WEOM	-0.74	0.62	-0.57	-0.51	0.58	0.70	0.48	-0.67	-0.21	0.67	-0.28	0.75	0.75	1.00							
C-WEOM	**-0.86**	**0.97**	**-0.83**	-0.64	0.67	**0.96**	**0.77**	**-0.86**	-0.36	**0.85**	-0.69	**0.99**	**0.99**	0.71	1.00						
C-POMf	**-0.80**	**0.88**	-0.68	-0.43	0.58	**0.89**	0.70	-0.74	-0.10	**0.89**	-0.55	**0.93**	**0.94**	0.74	**0.90**	1.00					
N-POMf	**-0.78**	**0.88**	-0.67	-0.41	0.56	**0.88**	0.70	-0.73	-0.08	**0.89**	-0.53	**0.93**	**0.94**	0.74	**0.90**	**0.99**	1.00				
C-POMo	-0.69	0.39	-0.28	-0.30	0.35	0.32	0.12	-0.33	-0.26	0.49	-0.52	0.42	0.40	0.47	0.43	0.53	0.51	1.00			
N-POMo	**-0.83**	0.66	-0.50	-0.41	0.49	0.61	0.37	-0.55	-0.25	0.70	-0.61	0.71	0.69	0.66	0.71	**0.79**	**0.77**	**0.93**	1.00		
C-MOM	-0.64	**0.89**	**-0.80**	-0.57	0.59	**0.92**	**0.77**	**-0.81**	-0.26	0.70	-0.48	**0.92**	**0.92**	0.66	**0.91**	**0.77**	**0.77**	0.06	0.39	1.00	
N-MOM	-0.69	**0.90**	**-0.79**	-0.54	0.55	**0.90**	0.71	**-0.79**	-0.24	0.68	-0.51	**0.92**	**0.93**	0.69	**0.93**	**0.78**	**0.78**	0.13	0.45	**0.99**	1.00

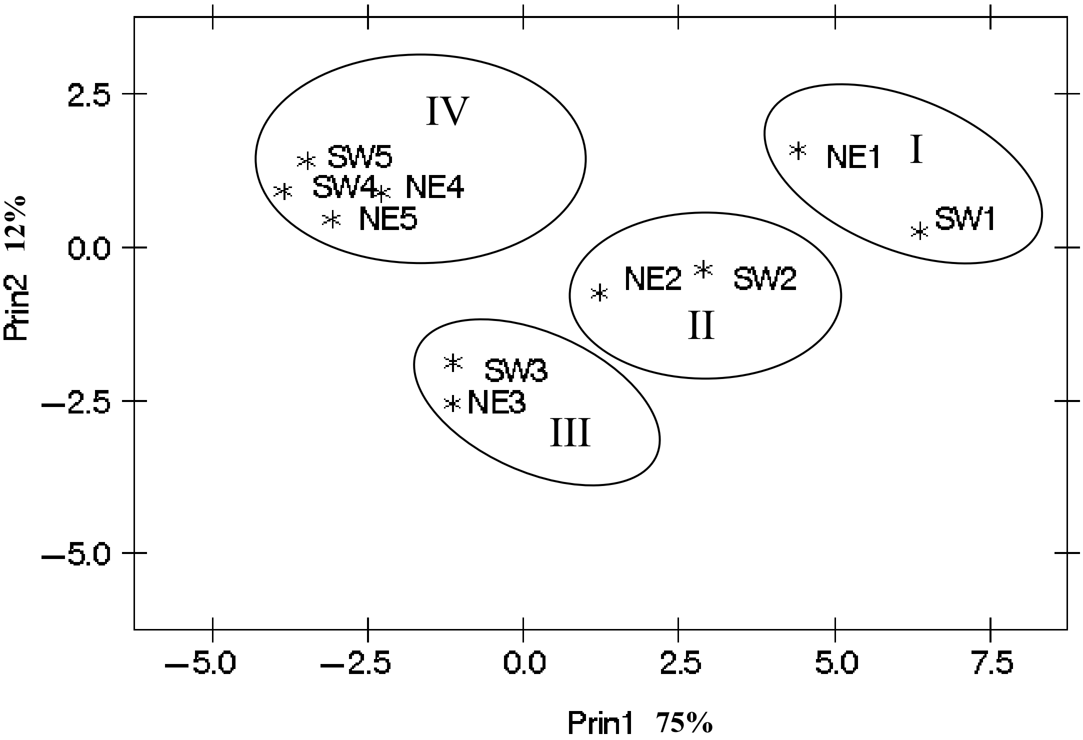

Fig. 11. Plot of the values of the 10 positions (two transects, NE and SW, combined with 5 positions within each transect) for the first two principal components based on 17 variables (pH, CEC, Available P, Litter Nov, Litter May, PAR May, PAR Nov, TOC, TN, C and N contents of WEOM (C-WEOM; N-WEOM), of POMf (C-POMf; N-POMf), of POMo (C-POMo; N-POMo) and of MOM (C-MOM; N-MOM). The ellipses enclose the populations of clusters.

Table 9. Correlation matrix between the first two principal components (Prin) and 17 variables (pH, CEC, Available P, Litter Nov, Litter May, PAR May, PAR Nov, TOC, TN, C and N contents of WEOM (C-WEOM; N-WEOM), of POMf (C-POMf; N-POMf), of POMo (C-POMo; N-POMo) and of MOM (C-MOM; N-MOM).

Variables	Prin1	Prin2
pH	-0.863	-0.339
CEC	0.960	-0.093
Available P	-0.881	0.271
Litter fall	-0.708	0.234
Litter spring	0.756	-0.126
PAR May	0.975	-0.163
PAR Nov	0.828	-0.401
TOC	0.984	-0.015
TN	0.985	-0.026
C-WEOM	0.981	-0.016
N-WEOM	0.775	0.210
C-POMf	0.919	0.202
N-POMf	0.913	0.187
C-POMo	0.472	0.829
N-POMo	0.733	0.664
C-MOM	0.885	-0.356
N-MOM	0.888	-0.268

Discussion

The majority of studied variables were strongly influenced by the presence of cork oak trees confirming that trees play a fundamental role for the sustainability of these agroforestry systems (Nair et al., 2010). We found a positive effect of the tree on soil fertility, also been reported in another studies (Gallardo, 2003; Moreno et al., 2007; Howlett et al., 2011) under Mediterranean climate. In fact, most of the variables influencing the soil fertility such as TOC, essential nutrients like TN and available P, and CEC, showed a similar pattern of distribution around the tree with higher values beneath the tree canopy and a more or less sharp decreasing trend from the trunk to the outer positions. Consistently to the findings of Moreno et al. (2007), TOC and TN in the vicinity of the tree trunk were around twice as high as it was in the sampling points fully beyond the tree canopy projection (40 g C kg^{-1} *vs.* 23 g C kg^{-1}; 2.7 g N kg^{-1} *vs.* 1.7 g N kg^{-1}). TOC decreased exponentially with distance from the tree trunk (for example -20% of TOC from around 1 m to 3.5 m from the trunk) and the positive influence of the trees only a few meters beyond the canopy projection disappeared. However, TOC values were on average similar to what measured (21.8 g C kg^{-1}) by Moreno et al. (2007) for a dehesa system in Central-western Spain dominated by holm oak trees, and they were quite high if compared to those found (11.1 g C kg^{-1} in the open grassland and 15.8 g C kg^{-1} beneath the tree canopy) by Gomez-Rey et al. (2012) for a cork oak woodland in Southern Portugal. These differences may be attributed to differences in the total C input to the soil that, in turn, are associated to above and belowground productivity (Moreno et al. 2007), to stocking rate through sheep dung amount (Gomez-Rey et al., 2012) and to complex interactions between trees and herbaceous communities (Moreno et al., 2005a, 2005b) that could control organic matter stabilization and accumulation.

In our study, we used the floor litter collected in spring and autumn along the transects as proxies of the amount of C input directly influenced by the presence of cork oak trees. On average, floor litter dry matter in the vicinity of the trunk was fivefold to twice higher than beyond the tree canopy projection depending on the season and on the transect orientation. These results could help to explain the patterns along the transects of TOC, TN and the other soil properties.

Overall, we found TOC values in the uppermost soil layer always above 1.0%, which is the lower limit for identifying the status of pre-desertification (CEC, 2002). This result indicates that the traditional soil management practiced in the studied wooded grassland is contributing to maintain a satisfactory long term soil fertility, as already found by Seddaiu et al. (2013) in the same environment.

According to Gallardo (2003) the similar spatial distribution of TOC and TN along the transects indicates that the soil total N content is basically controlled by the soil organic matter mineralization processes. Similarly, lower pH values under the tree than beyond the tree and the strong negative association ($r = -0.83$) between pH and TOC suggest that pH pattern is controlled by the accumulation of SOM.

A previous study (Lagomarsino et al., 2011) carried out in the same area of our study demonstrated that the low rates of basal respiration in grassland soils together with the increase of soil organic C, seem to be the consequence of increased C input which were not completely decomposed by soil microbial communities. This interpretation is consistent to our findings on floor litter as stated above.

In addition to the positive effect on soil fertility is also reported a positive effect of tree on microclimate. Moreno et al. (2005) found that soil and air temperature were significantly lower under the canopy than over the canopy projection on warm days and that the opposite pattern occurred on cold days. Similarly, in our study the soil temperature measured in spring showed the lowest values beneath the canopy and the highest beyond the canopy (16.8° C vs. 23.8° C).

Regarding the C contents in the organic matter fractions, different spatial distribution along the transects were observed. C contents as WEOM and POMf showed a significant trend with higher values near the tree trunk and decreasing values toward the positions beyond the canopy projection. These results were interpreted considering that these fractions are those among SOM fractions much more influenced by fresh C inputs, as evidenced by the strong correlation with floor litter amounts. In fact, WEOM is extracted by gentle stirring of soil samples placed in an aqueous solution, and consists of the mobile and available portion of the total dissolved organic matter (DOM) pool (Zsolnay, 1996), and POM free represents a labile SOM pool with a rapid turnover rate, constituted mainly of relatively fresh plant materials (Yamashita et al., 2006). Other C input sources beyond litterfall could be important, such as animal excreta, roots exudates, mycorrhizas (e.g., Moreno et al., 2005b), but no data are available on these variables for the study site. Moreover we found a strong correlation among TOC and C as WEOM and POMf as also reported by Haynes (2000). On the contrary, the C content as POMo was not significantly correlated with TOC showing a similar distribution along the transects. The C content as MOM represented the major part of TOC and was in fact highly correlated with TOC. MOM is considered among SOM pools as the most stable and with longer turn over

The degree of degradation and humification of the organic matter is indicated by the decreasing C/N ratios as POMf>POMo>MOM (John et al., 2005). In our study the C/N ratio of POMf and POMo was similar but in the MOM fraction was lower than in the other fractions in accordance to other studies (Golchin et al., 1994; John et al., 2005; Yamashita et al., 2006).

The fractions of SOM are affected differently by changes in land cover/land use, soil management or litter input (Yamashita et al., 2006). Labile fractions (WEOM and POMf) being composed of fresh materials are more sensible at the land use or litter input thus is easier to observe changes in the quantity of these fractions in a short term. While the POMo and MOM fractions represent the more stable pools and the changes are visible in a long term. Thus we explain the different spatial distribution of organic matter fractions (WEOM, POMf, POMo and MOM) considering their different composition and stability.

Moreover according to Lagomarsino et al., 2011 it is possible that a more complex chemistry of C inputs to soil combined to their incorporation in more stable soil aggregates could have promoted soil C conservation.

Conclusions

Our results confirm that the presence of trees in Mediterranean wooded pastures is well correlated with a whole range of positive effects. The main impact is the capacity to store and stabilize more C in the soil, thus in the long term these system can play a fundamental role in the C sequestration processes.

Data of SOM fractions indicate that the trees influence the quality and stability of SOM and the spatial distribution of these pools. In particular, the labile fractions (WEOM and POMfree) have proved to be sensitive indicators of short term changes in C status of soils.

Further studies are needed to evaluate the influence of trees in Mediterranean silvo-pastoral system on SOM pools for deeper soil layers and throughout longer time scales.

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CHAPTER II

Soil organic matter content and quality as influenced by the type of fertilizer in Mediterranean intensive forage systems

Soil organic matter content and quality as influenced by the type of fertilizer in Mediterranean intensive forage systems

Abstract

Under the Nitrate Directive, the efficient management of the animal effluents as fertilizers has became crucial in designated Nitrate Vulnerable Zones (NVZs), also for their impact on overall soil fertility. This study aimed to evaluate the dynamics of soluble SOM fractions as influenced by different type of fertilizers and the SOM variation after three years of application of organic *vs.* inorganic fertilizers The field experiment was carried out in the NVZ of the dairy district of Arborea (central-western Sardinia, Italy), with a double cropping silage maize – Italian ryegrass rotation. We compared four types of fertilizers: i) farmyard manure; ii) cattle slurry; iii) mineral fertilizer; iv) slurry + mineral fertilizer. The water extractable organic matter (WEOM) from the top (0-20 cm) soil samples, the dissolved organic matter (DOM) of the soil solution collected with ceramic porous cups installed at varying soil depths, and the C content in the soil at the beginning and at the end of the experiment were determined.

The highest C-WEOM values were observed with the slurry treatment, followed by the manure and slurry+mineral, while lowest values were measured in the mineral. and the unfertilized treatments. The type of fertilizer influenced directly and indirectly the C inputs to the soil, and hence SOM and N contents. Among the organic fertilizers, manure seemed to have a higher C and N accumulation in the soil, even in the deeper soil horizons.

These results confirm that long term organic fertilization and crop residues management are key agricultural practices for maintaining the environmental sustainability of intensive cropping systems.

Keywords: cattle slurry, farmyard manure, water extractable organic carbon, dissolved organic matter, porous cups

Introduction

Nitrogen (N) is considered the most important nutrient limiting crop growth and yield in the world (Huber and Thompson, 2007). Crop responses to N fertilization involve increases in CO_2 fixation and, as a consequence, in aboveground and root biomass production (Tognetti et al., 2005). Consequently, more crop residues return to soil (Studdert and Echeverrıa, 2000; Wilts et al., 2004) which, in temperate agroecosystems, is considered the main factor controlling SOM dynamics (Stevenson and Cole, 1999). Therefore, the rates and quantity of SOM change, as influenced by N rates, crop rotation, soil type, are worth to be evaluated under different environmental and cropping systems conditions (Álvarez, 2005).

Currently to maintain or restore soil nutrients and increase crop yields, the main agricultural practice is the application of mineral N fertilizers. The N used in commercial fertilizers is particularly soluble for easy uptake and assimilation by plants. Therefore N can easily be applied when plants need it most. The mineral fertilizers commonly applied are anhydrous ammonia, urea, ammonium sulfate and ammonium nitrate. Both urea and ammonia are converted to nitrate at different rates depending on the nature of the soil and of the climatic conditions, thus leading to various loss mechanisms either by volatilization for ammonia or runoff for nitrate or urea after heavy rainfall and leaching into groundwater (Vitosh et al., 1995; Jarvis et al 2011).

Nowadays, organic fertilizers are the second nutrient inputs to agricultural systems after mineral fertilizers. However, the contribution of animal effluents remains crucial when there is densely populated livestock nearby. Livestock manure has been used to improve soil fertility for centuries (Kapkiyai et al., 1999), by increasing SOM content (Kaur et al., 2008) and improving soil physical, chemical and biological properties (Fraser et al., 1988; Liebig and Doran, 1999; Hao et al., 2003).

The nutrient content of manure is variable because it depends on the type of farming, grazing systems and nutrient content of different feeds and forages for livestock (Hirel et al., 2011).

The application of manure with different levels of humification, (*i.e* composted),was frequently associated to the increase of soil fertility (Glaser et al., 2002) and to the stimulation of soil microbial activity through the improvement of soil structure (Watson et al., 2002).

Many studies have reported relative increases in OC content of manured fields compared to soils which received only mineral fertilizer (Christensen, 1992; Gerzabeck et al., 2001).

However, despite the many benefits of manure, application at rates higher than crop requirements can lead to excessive nutrient accumulation in the soil and hence to a potential pollution of groundwater (Chang and Entz, 1996; Whalen and Chang, 2001; Evanylo et al., 2008).

Our study was carried out in the Nitrate Vulnerable Zone (NVZ) under Mediterranean condition which have to follow the prescription for N fertilization. The NVZ are implemented very differently in different regions of Europe, and at the European scale it is impossible to generalise about its effects on land use (Freibauer et al., 2004). Limiting the addition of N fertilizer and the type of fertilizer may play a fundamental role of C contents in the soil. Thus it’s important to study as preserve the soil organic matter in terms of quality and stability, and evaluate how management practices and type of fertilizer affect the SOM in these system.

Long-term field experiments have shown that soil organic matter (SOM) is highly influenced by the addition of mineral fertilizers and manures, not only quantitatively but also qualitatively, i.e. in terms of SOM composition (Kaur et al., 2008). For instance, the regular addition of manure enhanced water-soluble fraction of carbon acting as an important source of bioenergy as compared to inorganic fertilizers. The differences in the rate of C mineralized are indicative of variable amounts of labile organic C accumulated in response to crop rotations (Campbell et al. 1992).

It is well known that changes in total organic C in the short- and medium-term are difficult to be detected, while soil labile organic C fractions (i.e., microbial biomass C, dissolved organic C, particulate organic C, and easily oxidizable C), that turn over quickly, can respond more rapidly to soil management than total organic C (Blair et al., 1995; Haynes, 2005). Hence, many studies have suggested these fractions as early sensitive indicators of soil management practices on soil quality (Blair et al., 1995; Yang et al., 2005).

Moreover the dissolved and/or water extractable fraction in soil (DOM, WEOM) has a strong influence on many ecologically relevant processes. Besides its function as substrate for microorganisms (Zsolnay, 1996; Marschner and Kalbitz, 2003), it is well known that solubility and transport of organic contaminants as well as heavy metals through soils are linked to DOM/WEOM properties (Knorr et al., 2005; Tan and Lal, 2005).

The interest in DOM research drastically increased in the last two decades because the ecological significance of DOM/WEOM is not only restricted to the “soil” compartment. It is also environmentally relevant for groundwater quality, desertification and climatic change (Embacher et al., 2007).

The main objectives of this study were to evaluate: (i) the dynamics of WEOM and DOM as influenced by different types of fertilizers, organic vs. and inorganic fertilizers; (ii) SOM changes after three years of application of organic and inorganic fertilizers.

Materials and methods

Experimental design and management

The field experiment was carried out in a private farm located in a Nitrate Vulnerable Zone in the dairy district of Arborea, Italy (39°47’ N 8°33’ E, 3 m a.s.l.) on the west-coast of Sardinia, Italy. The climate of the area is a typical Mediterranean climate, with long, hot, dry summers and short, mild, rainy winters. The mean annual temperature and precipitation are approximately 17°C and 600 mm, respectively. The soils were classified as *Psammentic Palexeralfs* (USDA, 2006). In the Ap horizon (0-45 cm), the soil of the experimental field had a sandy texture (97% sand) with 27 g kg^{-1} of organic C and 15 g kg^{-1} of total N, while in the lower soil horizons both organic C and total N contents decreased sharply (Table 1).

Table 1. Physical and chemical characteristics of the soil at the beginning of the experiment in a typical soil profile (June 2009).

	horizon			
Trait	**Ap**	**C**	**2Btg1**	**2Btg2**
Depth, cm	45	77	99	124
Clay, g kg^{-1}	16	27	64	86
Sand, g kg^{-1}	970	960	932	899
Silt, g kg^{-1}	14	13	4	15
Bulk density, g cm^{-3}	1.59	1.39	1.55	1.80
Organic Matter, g kg^{-1}	26.8	2.0	1.3	1.3
Organic Carbon, g kg^{-1}	15.5	1.2	0.8	0.8
Total N, g kg^{-1}	1.4	0.3	0.3	0.3

The experiment was run from June 2009 until May 2012 with a double cropping silage maize – Italian ryegrass rotation, that is representative of the forage cropping systems for dairy cattle farming in the study area. Four fertilizer sources were compared at the same level of N target rate (316 kg ha^{-1} for silage maize and 130 kg ha^{-1} for the autumn-spring hay crop), set on the basis of the N fertilization prescriptions for Nitrate Vulnerable Zones and on the crop N requirements. The treatments were: i) manure (mature cattle manure applied before sowing with a conventional spreader and followed by rotary tillage); ii) slurry (cattle slurry applied before sowing with a conventional spreader and followed by rotary tillage); iii) mineral (mineral fertilizer (ENTEC 26®) applied before sowing for maize and at the end of tillering for ryegrass); iv) control (slurry+mineral, i.e. slurry as above but at a target rate of 170 kg ha^{-1} N and mineral fertilizer (ENTEC 26®) at a rate of 60 kg ha^{-1} N applied before sowing for maize and at the end of tillering for ryegrass). The experimental design was a randomized complete block with four replicates (16 plots = 4 fertilizer sources x 4 replicates), with a plot size of 12.5 x 60 m^2.

Cropping system

Apart from the type and rate of fertilizers, all cropping operations followed the business as usual practices and were decided by the farm's owner (Table 2). Seed bed preparation consisted of subsoiling (30 cm depth), rotary tillage (30 cm depth) and harrowing (20 cm depth) for both maize and Italian ryegrass. Silage maize (FAO class 700) was sown at distances of 75 cm between rows and 20 cm along rows. Italian ryegrass was sown in rows with a commercial mixture composed by 80% of Italian ryegrass (*Lolium multiflorum* Lam.) and 20% of oat.

Table 2. Agronomic management practices applied to the maize-Italian ryegrass cropping systems in the three experimental years (2009-2012).

	maize			Italian ryegrass		
Management practice	2009	2010	2011	2009-10	2010-11	2011-12
Organic fertilization	8/6/09	9/6/10	26/5/11	7/10/09	20/10/10	5/10/11
Seeding	9/6/09	10/6/10	4/6/11	8/10/09	21/10/10	13/10/11
Mineral fertilization	8/6/09	9/6/10	26/5/11	26/2/10	8/3/11	9/2/12
Sprinkler irrigation*	17; 459	18; 441	23; 485	2; 32	5; 81	7; 113
Harvesting	21/9/09	21/9/10	14/9/11	25/5/10	18/5/11	17/5/12

* number of irrigations and water volumes (mm) are reported.

Cropping system carbon inputs

The C inputs to the soil were estimated from the C left in the soil by the previous crops assuming a mean C content of maize dry matter residuals of 0.44% (Bertora *et al.*, 2009), 0.40% for Italian ryegrass (Bayer et al., 2000) and on the basis of the measured crops yields (*data not shown*), plus the C inputs deriving from the organic fertilizers. The crop residuals were estimated to be 18% and 12% of the harvested biomass for maize and Italian ryegrass, respectively. The Italian ryegrass hay production was determined by weighing the hay round bales obtained at each plot. The C content of organic fertilizers was determined by the Springer-Kleen method (Mipaaf, 2006).

Soil sampling and analyses

Soil samples were collected on seven dates throughout the experiment. In June 2009 and May 2012, i.e. at the beginning and at the end of the experiment, one soil profile for each plot was opened. The profiles were dug down to about 1 m of depth and sampled according to the horizons. The soil samples were oven-dried at 40°C and sieved at 2 mm to remove skeleton, large roots and organic debris. The analyses were run on the < 2 mm soil fraction, the fine earth. C and N contents were determined using an elemental analyser (Thermo Soil NC - Flash EA1112). Soil C and N contents were here expressed as g kg^{-1} in four soil depths (0-20, 20-40, 40-60 and 60-80 cm), rather than keeping for homogeneous soil depths in order to facilitate the comparison among treatments,

In the other five sampling dates, from September 2011 to May 2012 that corresponded to a whole cropping cycle of Italian ryegrass, soil cores were collected at 0-20 cm soil depth from each plot in order to analyze the dynamics of C and N contents as WEOM. The water soluble organic matter was determined according to the method described by Burford and Bremner (1975). Fifteen grams of air dried soil (< 2 mm) were placed in a centrifugation tube with 30 ml of distillated water and were shaken for 15 minutes at 250 rpm. The soil suspension was then centrifuged for 10 min at 4000 x g and was filtered (0.45 µm) using vacuum. The C and N contents of extracts were determined using an elemental analyzer (Thermo Soil NC - Flash EA1112).

Percolation water sampling and analyses

In June 2009, two to three ceramic porous cups (Prenart Soil Disc Samplers, Prenart Equipment, Frederiksberg, Denmark) were installed at varying soil depths from 50 to 90 cm in correspondence of the soil horizons of each profile. A total of thirtysix porous cups were placed horizontally to the soil surface and special care was taken in repacking the soil around the cups by putting the soil from different soil horizons in the original position. Soil solution samples were obtained by applying a suction of 70 kPa for about 45 minutes using a hand pump. Collected samples were stored at 4°C until analysis. The ceramic cups were usually sampled at monthly intervals. In this study, samples collected on six sampling dates (from March to September 2011) were analysed to determine C and N contents of dissolved organic matter. For this purpose, the soil solutions were filtered (0.45 µm) using vacuum and the C and N contents were determined using an elemental analyser (Thermo Soil NC - Flash EA1112).

Statistical analysis

Analysis of variance was carried out for all variables according to a randomized complete block design with repeated measures and four replicates. Types of fertilizer were considered as main plot and sampling dates as subplots (Gomez and Gomez, 1984). Data expressed as concentration were log10 transformed to normalize their distributions before analysis. Means were separated according to the least significant difference using Fisher's protected test (Gomez and Gomez, 1984) at ˛ = 0.05.

Statistical analyses were accomplished using the SAS software (SAS Institute, 1999).

Results

In each cropping cycle, the organic fertilizers provided a higher C input than the preceding crop residues both for the manure and slurry treatments, while in the plots with the slurry+mineral treatment crop residues and organic fertilizer contributed equally to the total C input (Table 3). In relation to the different fertilizers rates that maize and Italian ryegrass received, the C input derived from fertilizers for Italian ryegrass was lower than for maize. On the contrary, maize received at the beginning of each crop cycle a lower C input from the preceding crop, i.e. Italian ryegrass, due to the lower dry matter production of this crop with respect to maize.

No significant relationship was found between the total C input (from fertilizer and preceding crop residues) calculated as the sum of the three cropping cycles (2009-12) and the amount of soil C stock measured at the end of the experiment in May 2012 (Fig 1).

The C content as WEOM (C-WEOM) during the Italian ryegrass cycle 2011-12 was characterized by relatively small variations among sampling dates and significant differences were found only between the values measured in November 2011, i.e. about one month later the crop sowing, and the values in the other dates (Fig. 2 and Table 4). On average, the slurry treatment showed the highest C-WEOM, followed by the manure treatment that did not differ from the slurry+mineral treatment, while the mineral and the unfertilized treatments had the lowest values. Differences among types of fertilizer were particularly marked in March and April 2012: C-WEOM in the top soil of the slurry+mineral plots was significantly higher than that of the mineral and unfertilized plots only in March 2012, whereas in April 2012 the slurry+mineral treatment did not differ from the mineral and unfertilized treatments.

No significant relationship was found between the C content as WEOM averaged among values from November 2011 to May 2012 and the total C input (from fertilizer and preceding crop residues) received by the Italian ryegrass in 2011-12 (Fig. 3).

Regarding the N content as WEOM, no significant differences among treatments during the Italian ryegrass cycle 2011-12 were observed (Fig. 4 and Table 5). Overall, the highest N-WEOM values were measured in November 2011 while from March to May 2012 no significant differences were found.

The C content of DOM in the soil solution from March to September 2011 showed a significant interaction between type of fertilizers and sampling dates (Table 6). Significant differences among the fertilizers were observed only in March 2011 when the mineral fertilizer showed lower DOC than the others, and in September 2011 when lower values were measured for slurry (Fig. 5).

Also for the N content of DOM (DN), a significant interaction between type of fertilization and dates was found (Table 7), in fact only in July and September 2011, the manure treatment showed lower DN values than the other fertilizers (Fig. 6

The soil organic carbon content at the beginning of the experiment showed clearly a gradient along the soil profile with the highest values in the 0-20 and 20-40 soil depths and the lowest in the 60-80 cm. After three experimental years, SOC in the 0-20 cm soil depth did not significantly differ among treatments, whereas in the layers 20-40 cm and 60-80 cm the slurry+mineral treatment shower lower SOC than the mineral treatment. In the 40-60 cm soil depth, higher SOC values were observed for the manure treatment (Table 8). The total N content in all soil layers was significantly influenced by the type of fertilizer apart from the top 0-20 cm layer where no differences were observed among treatments. In the other soil depths, the soil TN of the slurry+mineral plots was significantly lower than the value of the mineral treatment (Table 9).

Table 3. C inputs in the maize-Italian ryegrass cropping systems derived from the type of fertilization (kg ha^{-1}) and preceding crop residues (kg ha^{-1}) during the experiment in 2009-2012.

	C input received by maize		C input received by Italian ryegrass		Total C input	% of total C input from fertilizer	% of total C input from preceding crop residues
	From fertilizer	From preceding crop residues	From fertilizer	From preceding crop residues			
	1st cropping cycle (2009-10)						
Manure	4600	n.a.	1898	1767	-	-	-
Slurry	2978	n.a.	661	1817	-	-	-
Slurry+Mineral	1414	n.a.	99	1834	-	-	-
Mineral	0	n.a.	0	1911	-	-	-
	2nd cropping cycle (2010-11)						
Manure	3704	222	2876	1647	8449	78	22
Slurry	4153	198	1654	1323	7328	79	21
Slurry+Mineral	1314	381	887	1862	4444	50	50
Mineral	0	344	0	1852	2196	0	100
	3rd cropping cycle (2011-12)						
Manure	5298	214	1381	1320	8213	81	19
Slurry	4361	115	697	1733	6906	73	27
Slurry+Mineral	1649	243	374	1695	3961	51	49
Mineral	0	209	0	1543	1752	0	100

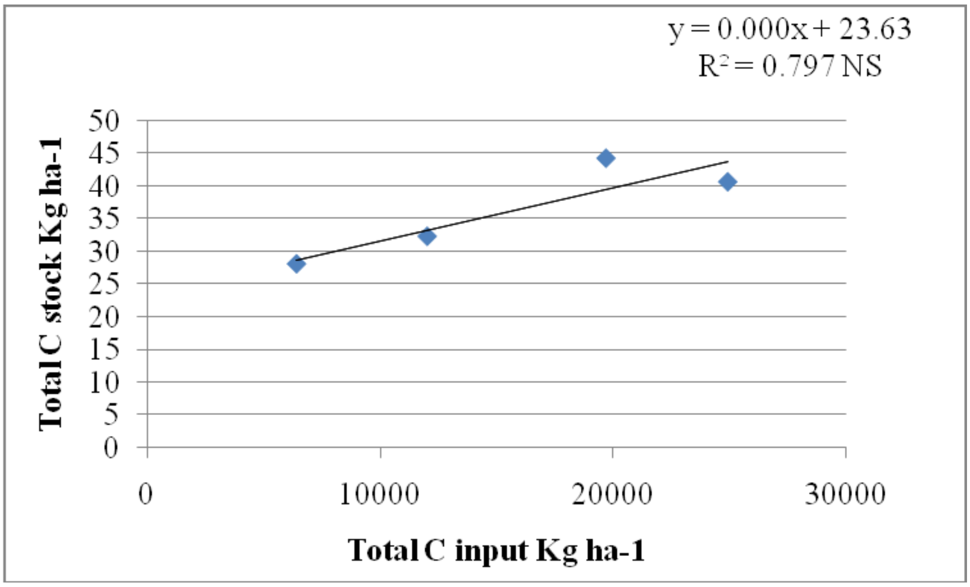

Fig. 1. Relationship between total C input (kg ha^{-1}) in the three cropping cycle maize-Italian ryegrass 2009-12 and total C stock (kg ha^{-1}) measured at the end of experiment in May 2012.

NS= no significant

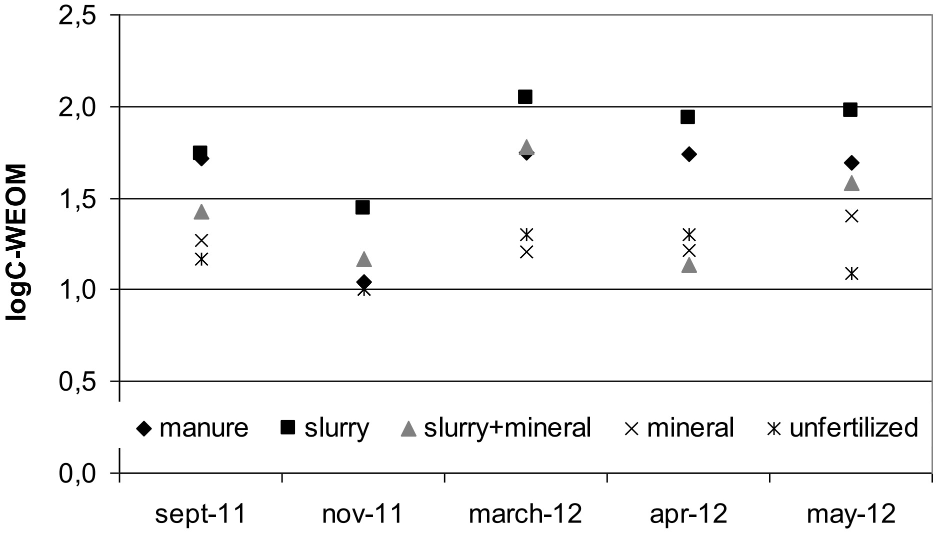

Fig. 2. Changes on C content as WEOM (log C-WEOM) in the top soil layer (0-20 cm) in relation to the type of fertilization during the Italian ryegrass cycle 2011-12.

Table 4. Results of the analysis of variance for the logC content as WEOM.

Source of variation	*d.f.*	*Mean square error*	*P-value*
Block	3	0.121	0.342
Type of fertilization (F)	4	1.359	0.015
Error I	12	0.167	
Date (D)	4	0.686	<0.001
F x D	15	0.090	0.623
Error II	55	0.106	
CV (%)			*22.2*
$LSD_{0.05}$ among F within D			0.50
$LSD_{0.05}$ among D within F			0.46

d.f.= degrees of freedom

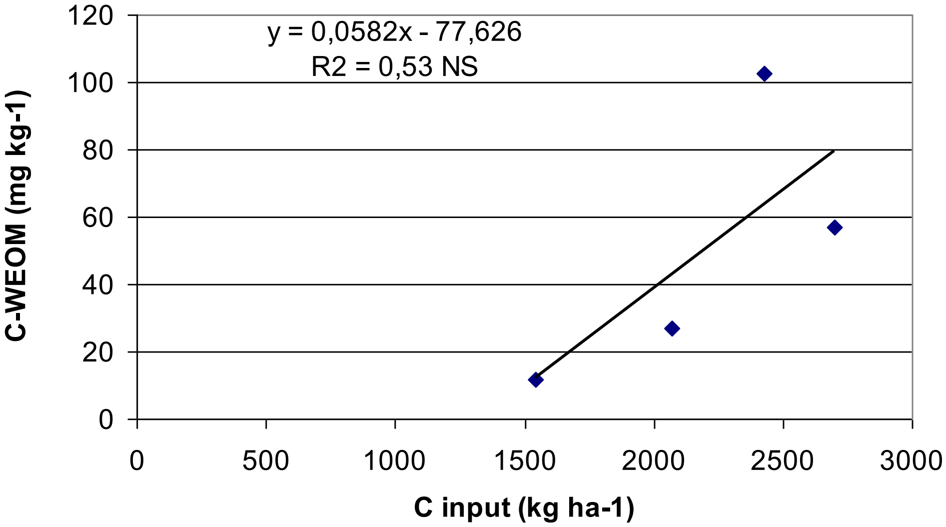

Fig. 3. Relationship between total C input (kg ha^{-1}) received by the Italian ryegrass in 2011-12 and the C content as WEOM (C-WEOM, mg kg^{-1}) calculated as the mean of values from November 2011 to May 2012. NS= not significant.

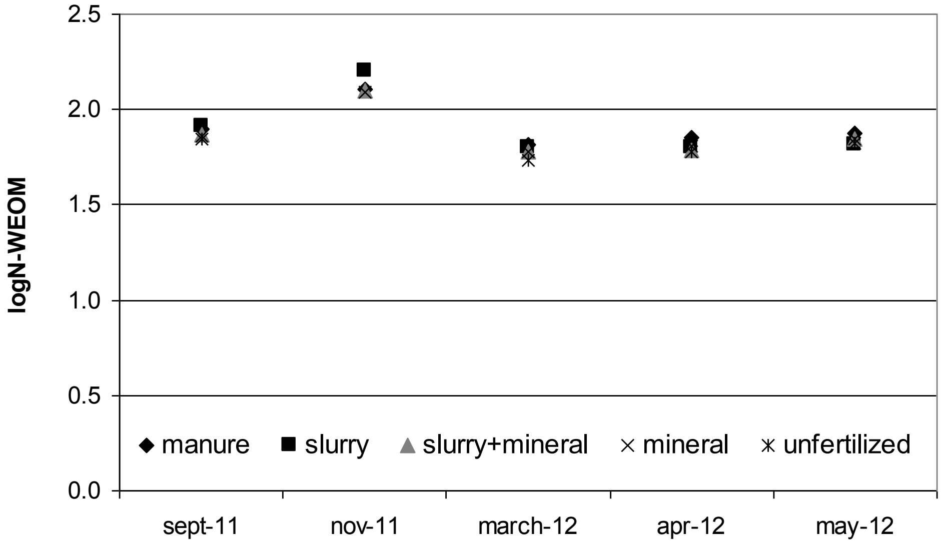

Fig. 4. Changes on N content as WEOM (Log N-WEOM, mg kg^{-1}) in the top soil layer (0-20 cm) in relation to the type of fertilization during the Italian ryegrass cycle 2011-12.

Table 5. Results of the analysis of variance for the logN content as WEOM.

Source of variation	*d.f.*	*Mean square error*	*P-value*
Block	3	0.007	0.103
Type of fertilization (F)	4	0.009	0.174
Error I	12	0.004	
Date (D)	4	0.293	<0.001
F x D	15	0.003	0.524
Error II	55	0.003	
CV (%)			*3.1*
$LSD_{0.05}$ among F within D			0.09
$LSD_{0.05}$ among D within F			0.08

d.f.= degrees of freedom

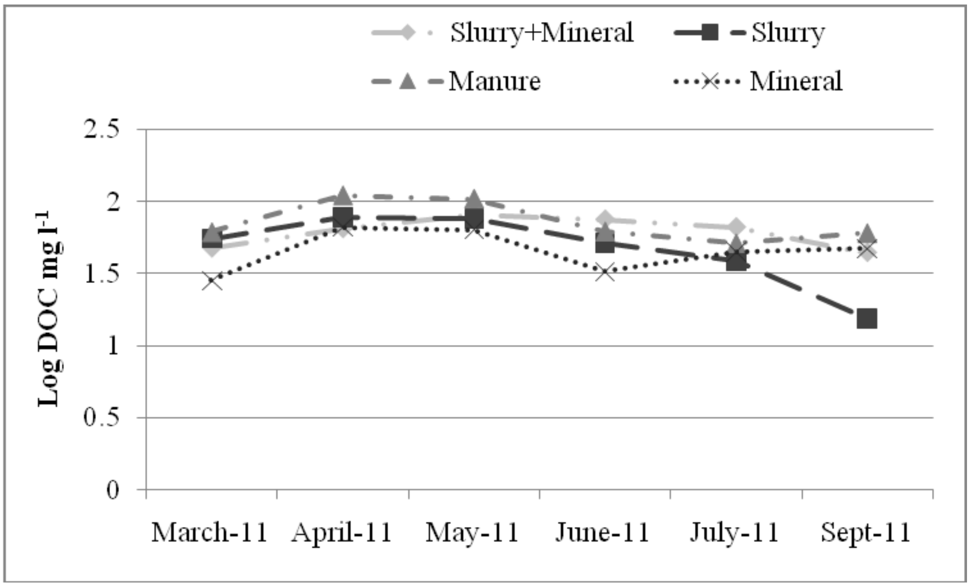

Fig. 5. Changes on DOC (Log DOC, mg l^{-1}) in the soil solution collected by porous cups in relation to the type of fertilization at the end of Italian ryegrass cycle 2010-11 and during the maize cycle 2011.

Table 6. Results of the analysis of variance for the Log DOC.

Source of variation	*d.f.*	*Mean square error*	*P-value*
Block	3	0.110	0.710
Type of fertilization (F)	3	1.579	0.349
Error I	7	1.220	
Date (D)	5	1.486	<0.001
F x D	15	0.311	0.052
Error II	78	0.238	
CV (%)			*12.2*
$LSD_{0.05}$ among F within D			0.98
$LSD_{0.05}$ among D within F			0.69

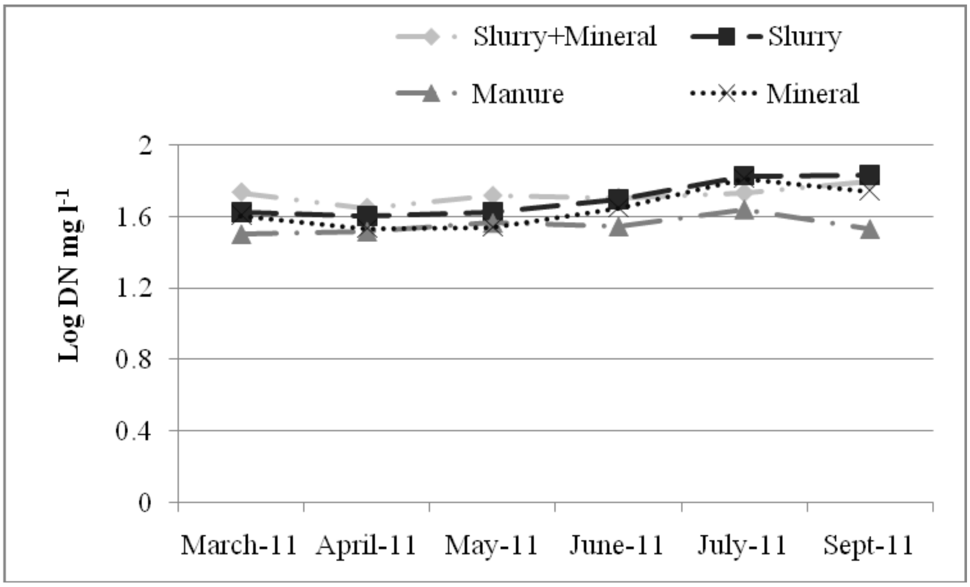

Fig. 6. Changes on N content of DOM (Log DN, mg l^{-1}) in the soil solution collected by porous cups in relation to the type of fertilization at the end of Italian ryegrass cycle 2010-11 and during the maize cycle 2011.

Table 7. Results of the analysis of variance for the Log DN.

Source of variation	*d.f.*	*Mean square error*	*P-value*
Block	3	0.082	0.325
Type of fertilization (F)	3	0.609	0.591
Error I	7	0.896	
Date (D)	5	0.398	<0.001
F x D	15	0.144	0.021
Error II	78	0.070	
CV (%)			*6.9*
$LSD_{0.05}$ among F within D			0.73
$LSD_{0.05}$ among D within F			0.37

Table 8. Soil organic carbon content (g kg^{-1}) at the beginning (June 2009) and at the end (May 2012) of the experiment in relation to the type of fertilization and at different soil depths.

		May-12			
Soil depth	June-09	Slurry+Mineral	Slurry	Manure	Mineral
0-20	12.08 (0.48)	13.6°a	12.4 a	14.1 a	13.1 a
20-40	11.86 (0.49)	10.2°b	11.9 ab	11.7 ab	12.5 a
40-60	6.07 (0.70)	5.0 b	5.8 ab	9.2 a	6.9 ab
60-80	1.86 (0.47)	1.2 b	1.9 b	5.9 a	2.8 b

Table 9. Total nitrogen content (g kg^{-1}) at the beginning (June 2009) and at the end (May 2012) of the experiment in relation to the type of fertilization and at different soil depths.

		May-12			
Soil depth	June-09	Slurry+Mineral	Slurry	Manure	Mineral
0-20	1.29 (0.04)	1.28°a	1.17 a	1.25 a	1.19 a
20-40	1.26 (0.04)	0.97°b	1.16 a	1.12 ab	1.11 ab
40-60	0.72 (0.06)	0.50°b	0.58 ab	0.97 a	0.63 ab
60-80	0.34 (0.05)	0.18°b	0.20 b	0.64a	0.27a

Discussion

The C input from fertilizer increased in the order *mineral<slurry+mineral<slurry<manure,* consistently to what found in a previous study in the same environment by Lai et al. (2012). The major C input received by the Italian ryegrass from the preceding maize residues can be explained by higher maize dry matter yield than Italian ryegrass (data not shown). On average, maize received a major C input by Italian ryegrass residues for the slurry+mineral and mineral treatments due to higher biomass production of Italian ryegrass with these two type of fertilization.

The high variability in the C content as WEOM is to be associated to the possible low uniform spatial distribution of organic fertilizers as well as to their variable composition during the year. Regarding to the dynamics of C as WEOM, similarly to what reported by Angers et al. (2006), we observed a slight increasing trend from March to May, corresponding to the ryegrass growing period from the end of tillering to harvest. Higher C-WEOM values were also observed in September 2011, after the harvest of maize, underlining that senescence and decomposition of plant materials could have promoted an increase of C as WEOM (Angers et al., 2006; Franchini et al., 2001). We observed that after fertilization (November 2011) the levels of WEOC decreased, as also reported by Chantigny et al. (1999) and Giacometti et al. (2013), suggesting that fertilization could have stimulated WEOC degradation processes by providing additional N to soil microbes.

The dynamics of N as WEOM was clearly associated to the distribution of organic fertilizer, in fact higher N-WEOM values were observed in November 2011, about one month later the fertilezers distribution. In this date, however, the mineral fertilizer was not applied, thus, the increase of N-WEOM for this treatment could be explained by an enhanced microbial activity, and in turn, increased organic matter mineralization, as a consequence of adequate availability of organic materials (from maize crop residues) suitable temperatures for soil microbial processes.

After three years of experiment the manure treatment showed a greater capacity of soil C accumulation in the whole soil profile. According to Bertora et al., (2009) the presence of more recalcitrant C compounds in manure than in slurry, means that the soil that received this fertilizer could act as C sink in a long run period. Other studies (Triberti et al., 2008; Fliessbach et al., 2007) have confirmed higher contents of stabilised organic compounds in the manure than in the liquid slurry.

The mineral treatment had an indirect effect on C sequestration, because of the increased plant biomass production, and thus of the increased amount of crop residues returned to the soil and to further soil biological activity (Paustian et al., 1997;Izaurralde et al., 2000).

Conclusions

In our study has emerged that the type of fertilizer (slurry, manure and mineral) influenced directly and indirectly the proportion of C input that the soil receives, increasing the soil organic C and N pools. Among the organic fertilizers, manure seemed to have a higher C and N accumulation in the soil, even in the deeper soil horizons.

The crop residues played a key role in increasing organic materials and C stored in the soil as evidenced by quite high soil C accumulation of the mineral fertilizer due to its positive influence on dry matter production and hence on amount of crop residues that arrived to the soil.

Finally, despite the strong sandy nature of the studied soils, the SOC content was high in the top soil, confirming that long term organic fertilization is a key agricultural practice for maintaining the environmental sustainability of intensive cropping systems.

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CHAPTER III

Water extractable and density fractions of soil organic matter in artichoke under contrasting organic farming practices

Water extractable and density fractions of soil organic matter in artichoke under contrasting organic farming practices

Abstract

The aim of this study was to evaluate the impacts of different management practices in a globe artichocke (*Cynara cardunculus* L. subsp. *scolymus* (L.) Hegi) organic farming system as compared to the conventional cropping system on the soil organic matter (SOM) quality and stability by determining the following SOM pools: water extractable organic matter (WEOM), free and occluded particulate organic matter (POMfree-occluded) and mineral-associated organic matter (M-OM). Our results demonstrated that the organic farming practices increase the SOM in the top soil, in particular when cover crops and incorporation of crop residues are used. The conventional treatment was found to be quite conservative in terms of soil C accumulation, due to the high amount of crop residues left and incorporated into the soil and the high productivity, also of residues, that is associated to the high N use efficiency of the mineral fertilization of artichocke.

Keywords: Organic farming, water extractable organic matter, density fractions, cover crop, artichoke, crop residue.

Introduction

The Organic agriculture was defined by IFOAM (International Federation of Organic Agriculture Movements) as a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved.

In the past decade, there has been increased scientific interest in the organic farming systems, particularly in comparison with conventional agriculture, as one alternative to prevent or to mitigate negative environmental impacts of intensive agriculture (Mäder et al., 2002). Many recent studies compared conventional and organic systems for soil properties, in different regions of the world (e.g., Marinari et al., 2006).

In organic horticultural systems, the crop rotations, the use of organic amendments, the use of intercropping, cover crops and tillage have significant effects on soil quality, on carbon sequestration, on the nitrogen dynamics and fertility management (Gaskell et al., 2000). Several studies show that the use of compost and cover crops in organic horticulture may allow to obtain very similar production in quantity and quality than conventional systems (Delate et al., 2003).

Cover crops were found to offer many benefits to soil fertility and crop production (Dabney et al., 2001), as they can: (1) increase organic matter content by producing a huge amount of biomass that is incorporated into the soil; (2) increase the N and P availability by stimulating the nutrients cycling or by atmospheric N2 symbiotic fixation (legume cover crops); (3) decrease nutrient losses (e.g. by the uptake of exceeding nitrates); (4) reduce soil erosion and organic matter mineralization during otherwise fallow periods (Sapkota et al., 2012). Legume cover crops can enrich soil with N and may therefore reduce the amount of N fertilizers needed for the succeeding crop. This effect, however, may change with different tillage practices due to their different influences on residue decomposition, leading to different rates of soil nutrient mineralization (Cambardella and Elliott, 1993).

The magnitude of soil organic matter (SOM) increase is dependent on soil type, species and biomass input of cover crops, and regional climate (Ding et al., 2006; Santos et al., 2011).

Biologically active SOM fractions that are important for nutrient cycling and supply are likely to be controlled by management to a much greater extent than total SOM. By isolating and quantifying sensitive or responsive SOM fractions one can view the effects of management on SOM and better provide information about the soil fertility status.

Some physical fractions of SOM are more sensitive to soil management and can be good indicators of soil management changes over the short time period (Xavier et al., 2006; Dou et al., 2008).

The quantity and quality of labile SOM pools are important factors to be considered when managing soil fertility in organic farming systems. Labile SOM quantity provides information about the amount of labile C substrate available to support microbial activity and promote aggregation as well as the size of the labile N reservoir in soils, while SOM quality is related to SOM dynamics and nutrient (C or N) supply for plant growth (Bending et al., 1998, Bosatta and Agren, 1999).

However, the impact of this practice on rapidly evolving organic pools such as WEOM is controversial (Gregorich et al., 2000; Hagedorn et al., 2004; Fontaine et al., 2007). In fact the incorporation of fresh crop residues into soil may have significantly different consequences on WEOM dynamics depending on crop type, soil texture, and climatic variations (Embacher et al. 2007).

Many studies applied the particulate organic matter (POM) as an index of labile SOM status and this soil trait proved to be sensitive to management-based differences in SOM quantity regardless of the fractionation strategy used (Cambardella and Elliott, 1992; Christensen, 1992; Golchin et al., 1994; Carter, 2002).

Constituted by partially decomposed plant and animal residues, POM is thought to be an energy source for microorganisms (Janzen et al., 1992; Christensen, 2001) and has been connected to other indices of N supply and nutrient cycling. Both POM-C and -N, when combined with information about recently incorporated crop residues, were found to be good predictors of N mineralization potential in systems using conventional, legume-based organic, and manure-based organic fertility management (Willson et al., 2001).

In addition to accumulation of labile SOM fractions, organically managed soils can accumulate SOM stocks relative to their conventional counter parts (Armstrong Brown et al., 1995; Pulleman et al., 2000; Stockdale et al., 2001).

The artichoke (*Cynara cardunculus* L. subsp. *scolymus* (L.) Hegi) is a typically Mediterranean horticultural species, and in particular; ‘Spinoso sardo’ is the most widely grown and economically important cultivar in Sardinia (Italy). The traditional management of this crop is a conventional monoculture system. To our knowledge, no studies on the relationships between management practices and soil organic matter quality in artichocke are available. The aim of this study was to evaluate the effects of different organic and conventional farming practices on the main indicators of SOM quality in an artichoke cropping system. In particular, we studied the influence of some agricultural practices such as crop rotation, intercropping and cover crops on some SOM fractions (WEOM, POMfree, POMoccluded and MOM).

Materials and methods

Study area

The study was carried out at the experimental farm of the Agriculture Faculty of the University of Sassari, located at Ottava (Sassari) in the North-Western Sardinia (Italy) at 225 m above sea level (40° 43’ 50,16’’ N 8° 33’ 6,84’’ E). The climate of the area is typical Mediterranean, with precipitation about 550 mm, mainly distributed in the autumn and spring. The mean temperature is approximately 16°C. The soil was a calcareous clay-loam (USDA, 2006) with organic C content of 1.11%, N content of 0.89%, C/N ratio 14 and pH 7.73. Before the beginning of the research, the experimental field was cultivated with winter wheat for several years.

The field trial was established in July 2006 with the aim to compare three different cultivation techniques of globe artichoke “Spinoso Sardo”:

1- conventional (CONV)
2- intensive organic (BIO)

3- biannual organic in rotation with cauliflower and with the following different management options for the cover crop (*Medicago polymorpha*) cultivation:

- no cover crop (BBONOC-CBBONOC)
- cover crop with annual sowing (BBOCAS-CBBOCAS)
- cover crop with annual self-reseeding (BBOCA-CBBOCA)

The field size was approximately 4000 m^2, divided into 4 plots of about 1000 m^2 each. For each treatment 4 rows of artichoke were planted, the 2 central rows were used for the observations while the 2 external rows were used as borders. The plants were placed at 0.7 m distance on the row and 1.5 m between rows.

Moreover 2 rows of artichoke were planted between the conventional and organic plots in order to provide a better separation among those treatments.

Here, we reported the results of a whole artichocke growing cycle from May 2011 to May 2012.

Conventional cultivation system (CONV)

The conventional system was based on the adoption of the common cultivation techniques used in Sardinia for the artichoke cropping system based on monoculture of the crop and the incorporation the crop residues (artichoke and weeds) at the beginning of each cropping cycle.

In July 2011 mineral fertilizer was applied (200 Kg/ha 46% triple superphosphate) after milling operation. Furthermore another fertilization with urea (46% of N) was applied. The artichoke was planted in late July using the lower part of dessicated off-shoots called "ovoli".

In August 2011 a chemical weeding was carried out.

Intensive organic cultivation system

The intensive organic cultivation system was based on the adoption of organic farming techniques. Through the early explant of artichoke the cultivation of a short cycle crop, such as French bean, on the same area was possible.

In May 2011, at the end of artichoke production, the crop residues was chop and incorporated in the soil, following the French bean (variety Bronco) was sowed and the first irrigation was carried out.

Beans was harvest in July and the residues was incorporated in the soil by milling, then the artichoke was implanted again and the plant's vegetative activity was forced to start by irrigation. In August was carried out a mechanical weeding and the artichoke was harvest in May 2012, the residues was chop and incorporated in the soil again.

Biennial organic cultivation in rotation with cauliflower

This system provides the artichoke cultivation on biennial rotation with another horticultural crop: the cauliflower. As consequence, this treatment occupied an area double compared to the other treatments. Half of the surface was cultivated with artichoke while the remaining surface was cultivated with cauliflower. Yearly the crops were alternated on the two plots of this treatment. Moreover, these plots were divided in subplots based on the presence or absence of cover crop (*Medicago polymorpha*)

Such as for the "intensive organic cultivation" the rtichoke residues were chopped and incorporated in the soil in May 2011 both in the subplots with cover crop and without cover crop. In July the artichoke was implanted and the vegetative activity started by irrigation. In August a mechanical weeding was carried out and in October the cauliflower (variety Nautilus, crop cycle of 75 days) was transplanted. In the sub-plots with cover crop yearly sowed, the *Medicago polymorpha was sowed.* In February following the damage caused by the wind it was necessary to sow again the Medicago. The residues of artichoke and cauliflower were chopped and incorporated in the soil in May 2012.

Soil sampling

Soil samples were collected in five particular times:

- Pre- chopping cover crop + artichoke (17 may 2011)
- Post- chopping artichoke (7 june 2011)
- Implant artichoke (3 august 2011)
- Post- Implant artichoke (28 november 2011)
- Cycle closing artichoke (9 may 2012).

The total 140 samples (5 date x 28 experimental units at 0-20 cm) were oven-dried at 40°C and sieved at 2 mm for the analyses. For all samples the water soluble organic carbon (WEOC) was determined, and by the first and last sampling the contents of organic C and total N were determined. For the last sampling was carried out the density fractionation in order to determine the SOM pulls (POM free-occluded; M-OM). Total carbonates (g kg−1) were measured through determining CO_2 released after HCl treatment. The C and N contents in the soil samples were determined using an elemental analyser (LECO CHN 628). Prior to TOC measurements, carbonates were systematically removed by HCl acid treatment.

Water-soluble organic carbon

The WEOC was determined according to the method described by Burford and Bremner (1975). Fifteen grams of air dried soil (< 2 mm) were placed in a centrifugation tube with 30 ml of distillated water and were shaken for 15 minutes at 250 rpm. The soil suspension was then centrifuged for 10 min at 4000 x g and was filtered (0.45 µm) using vacuum. The C and N contents of extracts were determined using an elemental analyser (LECO CHN 628).

Density fractionation of soil

Density fractions of soil were then obtained using the procedure described by John et al. (2005). Ten grams of air dried soil (< 2 mm) were placed in a centrifugation tube with 40 ml of sodium polytungstate solution (SPT ACROS) of a density of 1.6 g cm^{-3}. The tube was inverted gently by hand five times, the solution was allowed to settle for 30 minutes and it was centrifuged at 5100 x g for 1 h. The supernatant with floating particles was filtered (0.45 µm) using vacuum and washed with distilled water to gain the free particulate organic matter <1.6 g cm^{-3} (POMf). The sediment was dispersed with 40 ml of sodium polytungstate solution with a density of 2.0 g cm^{-3}. To break up the aggregates 10 glass beads with a diameter of 5mm were used and the solution was shaken for 16 h with a frequency of 60 movements per minute (Balesdent et al., 1991). The soil suspension was centrifuged for 1 h at 5100 x g and the supernatant with floating particles (occluded POM with a density of 1.6 to 2.0 g cm^{-3}, POMo) was filtered under vacuum and washed. To remove the salt, the pellet containing the mineral fraction (>2.0 g cm^{-3}, MOM) was washed three times with distilled water. Finally, the sample was centrifuged and the supernatant was discarded. All fractions were dried at 40 °C, the POM fractions were ground with a mortar. The C and N contents of the soil organic matter fractions were determined using a LECO CHN 628 elemental analyser.

Statistical analysis

Analysis of variance was carried out for all variables separately for each sampling date according to a randomized complete block design with 8 different cultivation techniques. Data expressed as concentration were log10 transformed to normalize their distributions before analysis. Means were separated according to the least significant difference using Fisher’s protected test (Gomez and Gomez, 1984) at ˛ = 0.05.

Statistical analyses were accomplished using the SAS software (SAS Institute, 1999).

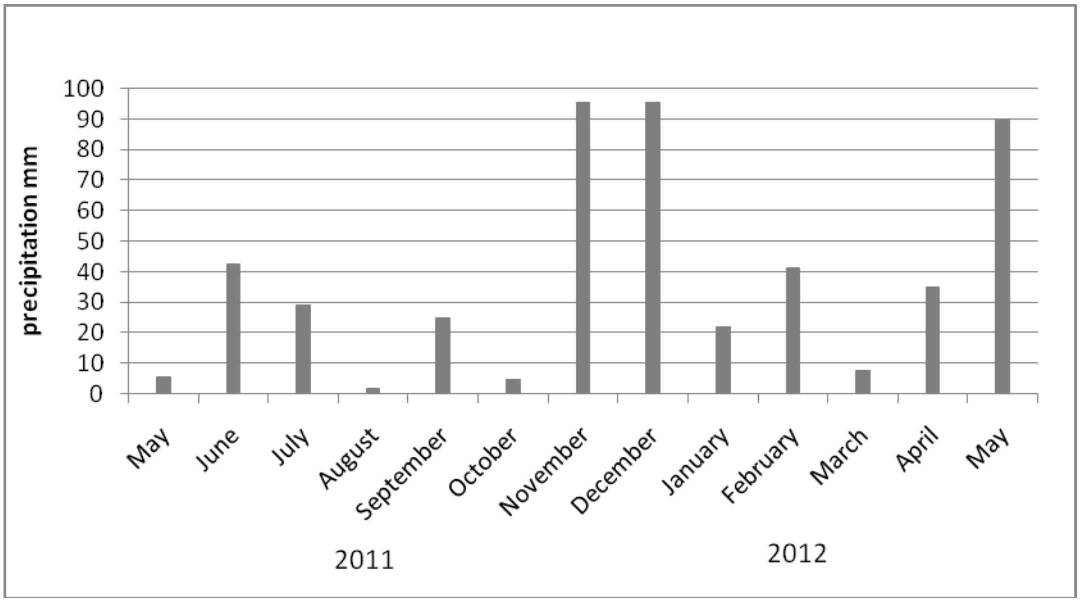

Fig. 1 Dynamics of precipitation (mm) during the artichoke cycle 2011-12 measured in the weather station located at Ottava (SS).

Results

The C contents in the bulk soil (Table 1) in a artichoke cycle, after 1 year didn't significantly differ between them (14.52 *vs* 15.44 g kg^{-1}) that instead were observed in the N contents (Table 2) with significant higher value measured in May 2011 than in May 2012 (1.64 *vs* 1.45 g kg^{-1}).

The dynamic of C contents as WEOM (Table 3) during the cycle showed significant difference only in August with the highest value in the conventional treatment followed by BBOCA and BIO, the other cultivation techniques didn't significantly differ between them. In May-11 and June (Table 3) the values were similar in all treatment but in November and May-12 were observed the lowest values than in the other dates. Instead the dynamic of N contents as WEOM didn't showed significant difference between treatments and respect to the C content were observed higher values in all sampling dates.

Only C content as POMo (Table 5) didn't showed significant difference between the treatments, but higher values were observed in the conventional, CBBOCA and BBOCA cultivation techniques. Instead the C content as POMf (Table 5) significantly differ among techniques, in fact the highest values were measured in the BBOCA and conventional (0.80-0.77 g kg^{-1}) and the lowest 0.25-0.29 g kg^{-1} for CBBONOC and BBONOC respectively. The C content in a mineral associated organic matter (Table 5) showed a similar pattern of C-POMf, apart for BBONOC with the highest value for this fraction and the lowest was measured in CBBOCAS.

Regarding N contents in the fractions of SOM, only N as POMf (Table 6) showed a significant difference among treatments. In the conventional and BBOCA were measured values significant higher than in the other cultivation techniques which didn't significantly differ between them. For the N as POMo (Table 6) were observed similar values among treatments, the N content as MOM was higher in the CBBONOC and lower in conventional treatment (Table 6).

The C/N ratio (Table 7) in the soil organic matter fractions showed significant differences between cultivation techniques only in the mineral associated OM with the lowest ratio (6.66 g kg^{-1}) and the highest (9.46 g kg^{-1}) in the CBBOCAS and CONV respectively. The average of C/N ratio (Table 7) was more higher in POMo fraction (16.27 g kg^{-1}) than the other and the lower in MOM fraction (7.89 g kg^{-1}).

The major contribution to TOC (Table 8) was received from mineral associated OM (about 69%) that didn't significantly differ among treatments. The cultivation techniques significantly influenced the contribution of C as POMf to TOC, with higher values in the BBOCA and CONV and lower values in the no cover treatments (Table 8). On average, C contents as POMf and POMo contributed to TOC about for 4%, while C as WEOM was very smaller (less than 0.5%).

Principal component analysis showed that the first component (PrinC1) explained about 57% of the variance and the second component about 20% (Fig. 2). The correlation matrix (Table 9) showed that the first component was significantly correlated with almost all the variables ($P<0.01$). Instead the N content as WEOM and total nitrogen were correlated with the second component (Table 9). Only the C content as MOM didn't significantly correlated with of both the principal components. Among the treatments were identified four clusters (Fig. 2). The first cluster I encloses the conventional and BBOCA which were measured the higher values in C content in the all fractions of SOM and in the N content as POMf.

The second cluster II includes BIO and the treatments with cauliflower and cover crop . The cluster III comprises no cover and BBOCAS treatments.

Table. 1 Soil organic carbon content (g kg^{-1}) in the top soil layer (0-20 cm) at the beginning of artichoke cycle (May 2011) and at the end of cycle after 1 year (May 2012) in relation to the cultivation techniques.

Cultivation techniques	TOC May 2011	TOC May 2012
BBOCA	14.76	16.05
BBOCAS	14.72	14.32
BBONOC	12.64	14.89
BIO	14.57	16.05
CBBOCA	13.49	15.97
CBBOCAS	14.06	15.23
CBBONOC	14.12	14.35
CONV	17.78	16.71
Mean	14.52	15.44

Table. 2 Total nitrogen content (g kg^{-1}) in the top soil layer (0-20 cm) at the beginning of artichoke cycle (May 2011) and at the end of cycle after 1 year (May 2012) in relation to the cultivation techniques.

Cultivation techniques	TN May 2011	TN May 2012
BBOCA	1.64	1.57
BBOCAS	1.69	1.44
BBONOC	1.61	1.36
BIO	1.59	1.37
CBBOCA	1.62	1.55
CBBOCAS	1.73	1.48
CBBONOC	1.66	1.41
CONV	1.58	1.43
Mean	1.64	1.45

Table. 3 Changes on Log C content as WEOM (log C-WEOM) in the top soil layer (0-20 cm) in relation to the cultivation techniques during the artichoke cycle 2011-12.

		Log C-WEOM				
Cultivation techniques		May-11	June-11	August-11	November-11	May-12
BBOCA		1.67a	1.75a	1.60b	0.30a	1.07a
BBOCAS		1.81a	1.66a	1.54b	0.14a	1.16a
BBONOC		1.73a	1.64a	1.44c	0.48a	0.48a
BIO		1.81a	1.62a	1.56b	0.45a	0.64a
CBBOCA		1.78a	1.74a	1.44c	0.58a	1.11a
CBBOCAS		1.79a	1.64a	1.49c	0.00a	0.25a
CBBONOC		1.92a	1.60a	1.46c	0.00a	0.00a
CONV		1.81a	1.84a	1.84a	1.09a	1.19a
Source of variation	*d.f.*	*p value*	*p value*	*p value*	*p value*	*p value*
Error	20					
Treatment	7	0.8088	0.3112	0.0006	0.0909	0.2187
CV (%)		8	7	5	17	21

Table. 4 Changes on N content as WEOM (log N-WEOM) in the top soil layer (0-20 cm) in relation to the cultivation techniques during the artichoke cycle 2011-12.

		Log N-WEOM				
Cultivation techniques		May-11	June-11	August-11	November-11	May-12
BBOCA		1.76a	1.73a	1.73a	1.97a	1.70a
BBOCAS		1.74a	1.83a	1.74a	1.75a	1.66a
BBONOC		1.68a	1.64a	1.78a	1.89a	1.71a
BIO		1.65a	1.72a	1.77a	1.82a	1.76a
CBBOCA		1.74a	1.77a	1.70a	1.91a	1.68a
CBBOCAS		1.70a	1.83a	1.73a	1.83a	1.63a
CBBONOC		1.72a	1.66a	1.76a	1.91a	1.62a
CONV		1.73a	1.82a	1.56a	1.82a	1.74a
Source of variation	*d.f.*	*p value*	*p value*	*p value*	*p value*	*p value*
Error						
Treatment		0.7714	0.0786	0.4791	0.2208	0.8576
CV (%)		5	4	6	5	6

Table 5. Means and results of the analysis of variance for contents of C as POMfree (C-POMf g kg^{-1}) POMoccluded (C-POMo g kg^{-1}) and MOM (C-MOM g kg^{-1}) in the top soil layer (0-20 cm) in relation to the cultivation techniques.

Cultivation techniques		C-POMf	C-POMo	C-MOM
BBOCA		0.80a	0.71a	12.33a
BBOCAS		0.47b	0.49a	9.96b
BBONOC		0.29c	0.51a	12.46a
BIO		0.39bc	0.60a	10.36b
CBBOCA.		0.49b	0.60a	10.11b
CBBOCAS.		0.53b	0.74a	9.23c
CBBONOC.		0.25c	0.55a	10.78b
CONV.		0.77a	0.79a	11.65a
Source of variation	*d.f.*	*P value*	*P value*	*P value*
Error	20			
Treatment	7	0.013	0.533	0.005
CV (%)		36	35	10

Mean values among treatments with different lower-case letters significantly differ for P ≤ 0.05.

Table 6. Means and results of the analysis of variance for contents of N as POMfree (N-POMf g kg-1) POMoccluded (N-POMo g kg-1) and MOM (N-MOM g kg-1) in the top soil layer (0-20 cm) in relation to the cultivation techniques.

Cultivation techniques		N-POMf	N-POMo	N-MOM
BBOCA		0.07a	0.04a	1.37a
BBOCAS		0.03b	0.03a	1.44a
BBONOC		0.02b	0.03a	1.42a
BIO		0.03b	0.04a	1.31a
CBBOCA.		0.04b	0.04a	1.41a
CBBOCAS.		0.04b	0.04a	1.39a
CBBONOC.		0.02b	0.03a	1.50a
CONV.		0.05a	0.04a	1.23a
Source of variation	*d.f.*	*P value*	*P value*	*P value*
Error	20			
Treatment	7	0.035	0.786	0.074
CV (%)		47	39	7

Mean values among treatments with different lower-case letters significantly differ for P ≤ 0.05.

Table 7. Means and results of the analysis of variance for ratio of C/N as POMfree (C/N-POMf g kg^{-1}) POMoccluded (C/N-POMo g kg^{-1}) and MOM (C/N-MOM g kg^{-1}) in the top soil layer (0-20 cm) in relation to the cultivation techniques.

Cultivation techniques		C/N-POMf	C/N-POMo	C/N-MOM
BBOCA		12.67a	16.61a	9.01a
BBOCAS		15.11a	17.20a	6.93c
BBONOC		14.76a	16.11a	8.74a
BIO		15.09a	13.95a	8.02b
CBBOCA.		13.10a	15.05a	7.17c
CBBOCAS.		14.42a	17.57a	6.66c
CBBONOC.		12.40a	15.75a	7.21b
CONV.		14.85a	17.62a	9.46a
Source of variation	*d.f.*	*P value*	*P value*	*P value*
Error	20			
Treatment	7	0.66	0.116	0.0005
CV (%)		17	11	10

Mean values among treatments with different lower-case letters significantly differ for $P \leq 0.05$.

Table 8. Means and results of the analysis of variance for the contribution to TOC of C as WEOM (C-WEOM/TOC, %), as POM free (C-POMf/TOC, %), as POM occluded (C-POMo/TOC, %) and as MOM (C-MOM/TOC, %) in the top soil layer (0-20 cm) in relation to the to the cultivation techniques.

Cultivation techniques		C-WEOM/TOC	C-POMf/TOC	C-POMo/TOC	C-MOM/TOC
BBOCA		0.17a	4.90a	4.41a	77.10a
BBOCAS		0.12a	3.32b	3.42a	70.07a
BBONOC		0.02a	1.98c	3.40a	83.76a
BIO		0.06a	2.44b	3.77a	64.62a
CBBOCA.		0.15a	3.10b	3.74a	63.28a
CBBOCAS.		0.01a	3.46b	4.91a	60.89a
CBBONOC.		0.00a	1.73c	3.83a	75.12a
CONV.		0.12a	4.67a	4.73a	71.00a
Source of variation	*d.f.*	*P value*	*P value*	*P value*	*P value*
Error	20				
Treatment	7	0.244	0.027	0.721	0.075
CV (%)		107	35	34	13

Mean values among treatments with different lower-case letters significantly differ for P ≤ 0.05.

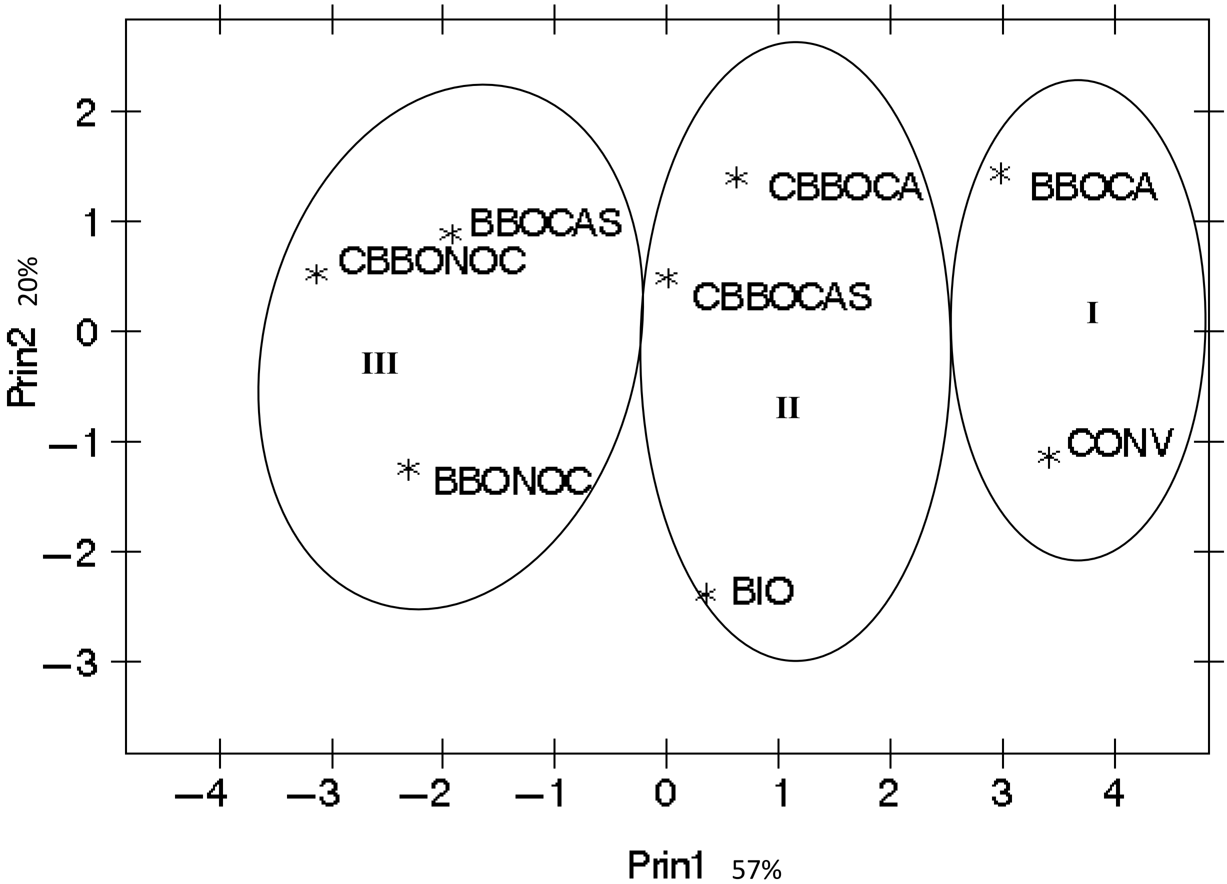

Fig. 2. Plot of the values of the 8 cultivation techniques for the first two principal components based on 10 variables (TOC, TN, C and N contents of WEOM (C-WEOM; N-WEOM), of POMf (C-POMf; N-POMf), of POMo (C-POMo; N-POMo) and of MOM (C-MOM; N-MOM). The ellipses enclose the populations of clusters.

Table 9. Correlation matrix between the first two principal components (Prin) and 10 variables (TOC, TN, C and N contents of WEOM (C-WEOM; N-WEOM), of POMf (C-POMf; N-POMf), of POMo (C-POMo; N-POMo) and of MOM (C-MOM; N-MOM).

Variables	Prin 1	Prin2
TOC	0.921	-0.260
TN	0.521	0.819
N-WEOM	0.554	-0.743
C-WEOM	0.745	0.359
C-POMf	0.923	0.245
N-POMf	0.877	0.396
C-POMo	0.839	-0.016
N-POMo	0.808	-0.150
C-MOM	0.240	-0.196
N-MOM	-0.823	0.528

Discussion

The agronomic management practices in an organic system affects the soil fertility and in particular soil organic matter quality as well as reported by other studies (Marriott and Wander, 2006; Nascente et al., 2013; Conceição et al., 2013).

The absence of significant difference between C contents in the bulk soil at the beginning of cropping cycle (May 2011) and at the end after 1 year (May 2012), it's comprehensible because was a short time to observe difference in SOM, but not for the labile organic matter. The C content as WEOM showed a dynamic with higher values in May, June and August 2011 in all treatments, that can be explained with the incorporate of crops residues in the soil and the high temperature of this period that stimulated the microbial activity. The lower C contents in November can be due at the precipitation (95.2 mm), more higher in this period. Moreover the values more lower in May 2012 than in May 2011, can be explained with the higher precipitation in this month (89.4-5.6 mm respectively). Instead the N contents as WEOM showed similar values in all sampling, this can be attributed at the presence of cover crop (*Medicago polymorpha*), mineral fertilization in the conventional treatment and at the incorporation in the soil of the crops residuals. In this case the precipitation didn't affect the dynamic of N as WEOM.

Regarding the fractions of SOM the data showed a influence from the different cultivation techniques, in particular for the free fraction (POMf) which were measured significant difference among treatments both the content of C that N. The higher values in the conventional can be attributed to a higher plant biomass production due to fertilization (Witt et al., 2000) and at the crop residues both the artichoke that weed.. Instead in the biennial rotation cover auto-sowing for the presence of Medicago and the practice of incorporation of crops residues. As reported to Bayer et al., (2006) and Loss et al., (2009) cover crops have been reported to significantly increase C in free fractions in comparison with treatments that did not include a cover crop. In effect we measured the lowest values in the treatment which did not includes the cover crop (BBNOC; CAVBBNOC).

In accordance to other studies of density fractionation (Golchin et al., 1994 and John et al., 2005) in our data the C/N ratio was lower in the MOM fraction than in the other, but the C/N ratio was higher in the POMo than in the POMf and didn't respect the order proposed POMf>POMo>MOM.

The greater storage of organic C in the mineral-associated pool (about 69%) is confirm from other authors as Six et al., (2000) and Conceição et al., (2013).

Conclusions

Our results demonstrated that the organic farming practices increase the SOM in the top soil, in particular when cover crops and incorporation of crop residues are used. The conventional treatment was found to be quite conservative in terms of soil C accumulation, due to the high amount of crop residues left and incorporated into the soil and the high productivity, also of residues, that is associated to the high N use efficiency of the mineral fertilization of artichocke.

Considering the two studied labile SOM fractions (WEOM and POMf), the POMf fraction seemed to be more sensitive to organic management changes both in the terms of C and N contents.

Further studies are needed to evaluate the role of several other organic management practices on the soil organic matter quality and on the environmental sustainability of these cropping systems that represent an important high income horticultural crop in Sardinia.

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