UNIVERSITÀ DEGLI STUDI DI SASSARI

CORSO DI DOTTORATO DI RICERCA
Scienze Agrarie

Curriculum: Produttività delle Piante coltivate

Ciclo XXX

SOIL ORGANIC CARBON DYNAMICS AND LAND USE CHANGE ASSESSMENT IN A NO-FOOD MEDITERRANEAN CROPPING SYSTEM

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CHAPTER 1: **USING COVER CROPS AS AN ALTERNATIVE MANAGEMENT OF AGRO-ENERGETIC CROPPING SYSTEMS TO MITIGATE CO_2 EMISSIONS**

1. Introduction

Development of mitigation strategies for tackling global climate change is an important issue influencing management of ecosystems around the world (Lal 2011). Among these strategies soil carbon sequestration can play a relevant role in order to enhance carbon stores in the biosphere (Anthony et al. 2011; Nair et al. 2009) especially in agroecosystems (Lal 2011). Increasing the Soil Organic Carbon (SOC) content has considerable benefits, since it improves physical, chemical and biological soil properties (Lal 2011). Consequently SOC is crucial for sustaining and enhancing crop productivity (Aguileira et al. 2013) and thus for achieving food security (Lal 2016). Specifically in the Mediterranean region characterized by a semi-arid climate conditions (Albadalejo et al. 2013; Muñoz-Rojas et al. 2012) in which SOC decomposition rates increase associated to temperatures rise (Al-Adamat et al. 2007). Furthermore in this context the organic matter inputs are low and mostly rely on crop residues whereas the substantial C losses are due to intensive and no-conservative agricultural practices (Farina et al. 2011). As a consequence of their low SOC contents, these areas are often degraded and vulnerable to environmental variations and climate change is predicted to have a large impact on an additional SOC reduction (Metz et al., 2007).

Different authors have highlighted the potential role of perennial bioenergy crops (e.g. cardoon, mischantus, arundo donax, etc.) in terms of contributing to reduce CO_2 emissions and to foster SOC storage. For instance, Chimento et al. (2014) underlined that the continuous C inputs to the soil and minimal soil disturbance of energy crops systems increased SOC stock following 6 years of cultivation. Furthermore Sartori et al. (2006) pointed out positive effects of perennial crops to reduce climate-altering impacts of anthropogenic CO_2 emissions. Cardoon (*Cynara cardunculus* L. var. *altilis* DC.) is considered by various authors an important species for energy purpose that is able to provide good biomass production level and it shows drought tolerance particularly in semi-arid environments (Christodoulou et al. 2014; Fernández et al. 2006; Vasilakoglou and Dhima, 2014). In addition cardoon is generally cultivated in rainfed conditions adopting an agricultural management based on external inputs use such as different doses of N fertilizers (Deligios et al. 2017; Francaviglia et al. 2016).

However further research is warranted to verify if alternative agricultural managements of energy systems - such as the adoption of biochar, cover crops and biochar plus cover crops used in intercropping systems - might lead a SOC reduction and, as a consequence, mitigate climate change. Austin et al. (2017), Blanco–Canqui et al. (2011), and Fronning et al. (2008) put on evidence the positive effect on C content due to cover crops and biochar adopted in annual cropping systems.

Biochar use can foster the C sequestration enhancement in favors of crop productivity (Lal 2016) because of its highly recalcitrant nature (Woolf et al. 2010).

Cover crops – especially legumes – might have considerable positive effects with respect to: 1) mitigation of greenhouse gases emissions (e.g. CO_2) in comparison to cropping systems characterized by mineral N fertilization; 2) C soil sequestration and 3) low fossil energy inputs requirement for crops cultivation. (Stagnari et al 2017). Specifically cover crops might supply additional residual biomass that in turn might foster soil N and C storage. On the basis of these considerations this research is aimed to assess – with respect to perennial energy cropping system - the soil organic carbon dynamics applying different agricultural managements (i.e. low and high nitrogen fertilizer doses, biochar use and cover crop cultivation both separately and concurrently). The specific objective was to analyze the performance of each agricultural management for cardoon cultivation in order to put on evidence their strengths and weakness with respect to SOC storage and CO_2 emissions from climate change mitigation point of view.

2. Materials and methods

2.1 Site description

The experimental trial was carried out from 2014-15 to 2016-17 growing season at the "Mauro Deidda" experimental station of the University of Sassari, in Ottava (North-West Sardinia, Italy: 41° N; 8° E; 81 m a.s.l.). Prior to the experimental period, the study site was continuously cropped with cardoon (*Cynara cardunculus* var. *altilis* DC. cv. "Bianco Avorio") under mineral fertiliser treatments for 8 years (Deligios et al. 2017; Ledda et al. 2013).

The daily minimum and maximum temperatures and the rainfall during the study period were collected by means of a meteorological station located *in situ*. The climate of the area is Mediterranean warm temperate with dry and hot summers (Kottek et al. 2006). The long-term mean annual rainfall is 550 mm, unevenly distributed in the winter-spring season. The long-term average annual air temperature is 16.2 °C.

Soil chemical and physical analyses were carried at the beginning of the experiment to assess soil initial conditions. The soil of the experimental field was classified as Typic Haploxerepts, sandy-clay loam with 66% sand, 15% silt, and 19% clay (Soil Taxonomy, 2014). The soil surface layer (0–40 cm depth) had an average pH of 8.4 (soil:water=1:2.5), total C content of 49 g kg^{-1} and total N of 1,8 g kg^{-1} (elemental analyzer, Leco CHN628 Series, LECO Corporation, St Joseph, MI, USA), soil organic matter (SOM) of 31 g kg^{-1} (SOM = total organic C × 1.72; Francaviglia et al. 2017), Olsen P of 20 ppm (bicarbonate-extractable; Kuo 1996), exchangeable K of 151 ppm ($BaCl_2$ extraction followed by AAS determination), and total carbonate of 25.9% (volumetric calcimeter method; Loeppert and Suarez 1996).

2.2 Experimental design and treatments details

The trial was established in November 2014 to evaluate the impact of non-conventional energy cropping systems on soil fertility preservation and on crop yield and it is still ongoing. Treatments were arranged in a split-plot scheme with four replicates. Management treatment was assigned to the main plot and genotype to the sub-plot. Experimental field measured 4156 m^2, and main plot and subplot areas were 132 m^2 (22 m × 6 m) and 45 m^2 (7.5 m × 6 m), respectively. All subplots were separated by inter-plot spacing of 1.5 m.

There were five management treatments: i) high rate of N fertilizer application (HI); ii) low rate of N fertilizer application (LW); iii) low rate of N fertilizer application plus cover-crop use (LW-C); iv) low rate of N fertilizer application plus biochar distribution (LW-B); and v) low rate of N fertilizer application plus both cover crop and biochar use (LW-CB).

The first two managements (HI and LW) were considered conventional, whereas the three managements with cover crop and/or biochar use (LW-B, LW-C, LW-BC) were considered as alternative managements.

At the beginning of the experiment, urea (80 kg N ha^{-1}) and ammonium phosphate (100 kg P_2O_5 ha^{-1}) were supplied on the entire experimental field. In addition, ammonium phosphate (65 kg P_2O_5 ha^{-1}) was top-dressed each year to all plots at sprouting (BBCH code 07) to avoid P soil depletion.

The HI treatment plots were managed by top-dressing 100 kg ha^{-1} of mineral N (urea) at stem elongation (BBCH code 51, Archontoulis et al. 2010).

In all treatments with a low rate of N mineral fertilizer application (LW; LW-C; LW-B, and LW-CB), 50 kg N ha^{-1} of urea was supplied at stem elongation stage.

LW-C; LW-B, and LW-CB were differentiated when the plants reached the 3 to 4 leaf stage (BBCH code 13-14).

In LW-C plots, a self-reseeding legume cover crop (*Trifolium subterraneum* L. var. Antas) was introduced in inter-row spaces (Figure 1). In November 2014, subterranean clover was manually sown at a rate of 30 kg ha^{-1} and at 5 cm depth and it was left to reestablish year after year. Cover crop residues were not removed or incorporated into the soil, since the aim was to promote the establishment of a stable litter by providing additional biomass to maintain and sequester SOC.

In the same period, in LW-B treatment, biochar was spread manually on the soil surface at a rate of 10 t ha^{-1}, and it was incorporated into soil to a depth of 10 cm using a rotary hoe (Figure 2). Biochar was produced from rape straw and by a slow pyrolysis procedure at 600 °C (Desogus et al. 2016). No further biochar application was performed throughout the experiment.

LW-CB treatment combined cover crop over-sowing and biochar application. The choice of biochar dose applied in the LW-B and LW-CB plots was decided taking into consideration the findings of Mia et al. (2014).

The Bianco Avorio (BA), Gobbo di Lucca (GL) and Gigante di Romagna (GR) genotypes were selected and used for the field experiment according to results obtained from previous trial that was conducted to characterize and analyze cardoon growth and yield potential in Mediterranean environment (Spissu 2015).

Figure 1: LW-C management

Figure 2: LW-B management

2.3. Crop management

Seedbed preparation consisted in moldboard plowing at a depth of 35 cm followed by two passes of disk harrowing.

Cardoon was manually sown in mid-autumn (November 13, 2014), adopting a plant density of 1.5 plant m^{-2} (1 m × 0.67 m).

No synthetic pesticide application was performed, except during the year of establishment when a weed control was provided (450 g a.i. ha^{-1} Linuron, Sipcam S.p.A., Lodi, Italy).

Every year, total cardoon aboveground biomass was cut off a few centimeters above the soil surface in summer at achenes ripening stage (BBCH code 89, ~ 70-80% DM). The biomass was baled and removed completely from the soil.

2.4 Soil organic carbon stock and soil respiration dynamics

Soil sampling was yearly conducted on each sub-plot in November. Soil cores were collected at the depth of 0-20 and 20-40 cm, each sample was dried at 40° C per 72 h, and crushed and sieved at 0.2 mm mesh-size before analysis. The Wang and Dalal methodology (2006) was used in order to determine SOC stocks (Mg ha^{-1}) for both depths. It is based on the following equation:

$$\text{SOC stock} = \text{SOC content} \times \text{Bd} \times \text{D}$$

SOC content (g kg^{-1}) was determined by dry combustion method with an elemental analyzer (Leco CHN628 Series, LECO Corporation, St Joseph, MI, USA). Bulk density (Bd, g cm^{-3}) was estimated by pedotransfer function (Tsuji et al. 1998), D is soil depth (m). Soil respiration monitoring was conducted at weekly interval, during 2015-2016 and 2016-2017 growing seasons, on each plot (from 9:00 to 13:00 am hours). An infrared gas analyser (EGM-4, PP Systems Hitchin, Hertfordshire, UK) connected to a closed dynamic chamber (SRC, PP Systems Hitchin, Hertfordshire, UK) was used to measure soil CO_2 efflux (g CO_2 m^{-2} $hour^{-1}$) from 30 permanent collars inserted into the soil. In order to monitor only heterotrophic soil respiration, external PVC cylinders (40 cm diameter and depth) according to Alberti et al. (2010), were positioned. At the same time, the soil temperature (°C) and the soil volumetric water content (VWC), close to the collars, were measured by using the "STP-1 Soil Temperature Probe" connected to the EGM-4 instrument, and the "TDR FieldScout 300" (Spectrum Technologies, Inc., Plainfield, IL, USA), respectively.

2.5 Statistical analysis

The normality of the data was confirmed by the Kolmogorov–Smirnov test and the homogeneity of variances by the Levene's test. Soil properties data (organic C, SOC stock, total N and C:N ratio) were subjected to analysis of variance using the MIXED model (SAS v 9.2, 1999). According to the split-plot design, blocks and interaction with blocks were considered as random factors; while the five soil management treatments (factor 1), the three genotypes (factor 2), and the two depths (factor 3) were considered as fixed factors. Means were compared using the SAS least square means with Tukey adjustment at $P \leq 0.05$ to test comparisons across means. The repeated nature of the CO_2 flux, soil temperature and moisture data, with recordings made over time in the same plots, was accounted by including data of measurements as a repeated effect. An autoregressive correlation structure and variance was assumed between data of sampling. Significance was determined using the F statistic and α=0.05. The denominator degrees of freedom in F-test for mean separation were calculated according to Kenward and Roger (1997). All data, when necessary, were log-transformed to obtain homogeneity of variance.

3. Results and discussion

3.1 Soil properties

The SOC content was significantly affected by depth (Table 1) and by two second-order (growing season × genotype and management × genotype) interactions (Tables 2 and 3). The highest SOC content was observed in the surface layer (0-20 cm). Throughout the three growing seasons (from 2014-2015 to 2016-2017) the SOC content showed a reduction with significant differences within BA and GR genotypes (Table 2). Specifically, considering each growing season the decline in soil SOC was in order BA > GL > GR with 39%, 38% and 35% respectively (Table 2). Bianco Avorio genotype (Table 3) showed statistically higher SOC values than other genotypes in LW-C and LW-CB (both 19 g kg^{-1}). Management with the use of cover crop and biochar alone (LW-C and LW-B) or combined with each other (LW-CB) significantly affected SOC content in BA, the same trend was also observed within GR and GL but with a smaller, even if statistically significant, range of variation among managements (Table 3).

SOC stock showed a similar trend within genotypes and among managements (two-way management × genotype interaction; table 3) with LW that showed the lowest SOC stock (Table 3). Because growing season × depth interaction was significant, variations in SOC stock at 0-20 and 20-40 cm depths with growing season were examined (Table 1). The SOC stock decreased throughout the three different growing seasons in both depths (Table 4) ranging from 56.2 Mg ha^{-1} to 39.6 Mg ha^{-1} (0-20 cm) and from 106.2 Mg ha^{-1} to 71.1 Mg ha^{-1} (20-40 cm). The C stock was always lower in the surface layer (0-20 cm) and the increase, in 20-40 cm layer, was equal to +90% and +80% in the first and third growing season, respectively (Table 4).

Soil organic carbon variations, which can be positive or negative, may depend on several factors, such as previous land use, crop management, soil type, and species (Garten and Wullschleger 2000; Brandão and Milà i Canals 2013).

Our findings confirm that a number of factors were involved in affecting SOC content, such as growing seasons, crop management, soil depth, genotypes and their interactions.

Table 1. Mixed model analysis of variance associated with the growing seasons, management and depth studied and for three genotype analyzed.

Factors		Organic C ($g\ kg^{-1}$)	SOC stock ($Mg\ ha^{-1}$)	Total N ($g\ kg^{-1}$)	C:N ratio
Growing season					
2014-2015		21 a	81.7 a	1.8 a	11.3
2015-2016		15 a	63.3 b	1.3 b	11.8
2016-2017		13 b	55.4 c	1.3 b	10.2
Management					
LW		15	60.6 c	1.5	10.4 c
HI		16	64.8 b	1.5	10.9 b
LW-B		13	67.6 a	1.5	11.0 a
LW-C		17	67.7 a	1.4	11.0 a
LW-CB		18	73.2 a	1.5	12.1 a
Depth					
0-20		17 a	47.8 b	1.5 a	11.1
20-40		15 b	58.8 a	1.4 b	11.1
Genotype					
BA		17 a	69.2 a	1.5 a	11.1
GL		16 b	65.4 b	1.5 a	10.9
GR		15 c	65.2 c	1.4 b	11.3
Analysis of variance	Df	P>F	P>F	P>F	P>F
Growing season	2	*	*	**	N.S.
Management	4	N.S.	*	N.S.	*
Depth	1	***	***	***	N.S.
Genotype	2	**	*	*	N.S.
Growing season × depth	2	N.S.	***	**	N.S.
Growing season × genotype	4	**	N.S	N.S.	*
Management × genotype	8	**	**	N.S.	N.S.

LW: low rate of N fertilizer, HI: high rate of N fertilizer, LW-B: low rate of N fertilizer plus biochar, LW-C: low rate of N fertilizer plus cover crop, LW-CB low rate of fertilizer plus cover crop and biochar.
BA: Bianco Avorio, GL: Gobbo di Lucca, GR: Gigante di Romagna genotypes.
Component of variation: *, **, *** significant according to Tukey's test at P levels of P≤0.05, 0.01, 0.001, respectively; N.S. not significant.
For each parameter (growing season, management, depth and genotype), within each column, means followed by different letters are significantly different according to Tukey's test (P ≤ 0.05).

Table 2. Mixed model analysis of variance associated with the growing season × genotype interaction.

Factors	Organic C ($g\ kg^{-1}$)			C:N ratio		
	BA	GL	GR	BA	GL	GR
2014-2015	23 a	21	20 a	11.74	11.12	10.94
2015-2016	16 b	15	16 ab	11.40 B	11.65 AB	12.48 A
2016-2017	14 b	13	13 b	10.05	9.85	10.61

BA: Bianco Avorio, GL: Gobbo di Lucca, GR: Gigante di Romagna genotypes.
Different letters indicate significant differences according to Tukey's test (P ≤ 0.05) among genotypes (upper case, within row) and growing seasons (lower case, within column).

Table 3. Mixed model analysis of variance associated with the management × genotype interaction.

Factors	Organic C (g kg^{-1})			SOC stock (Mg ha^{-1})		
	BA	GL	GR	BA	GL	GR
HI	16 b	16 a	17 a	62.7 b	63.5 ab	68.2 a
LW	16 b	15 ab	14 ab	62.7 b	60.7 b	58.4 b
LW-B	17 ab	17 a	16 a	67.7 b	69.5 a	65.4 ab
LW-C	19 aA	16 aBC	16 aB	75.2 abA	62.4 aB	64.6 abB
LW-CB	19 aA	18 aAB	17 aB	79.5 aA	70.5 aB	69.3 aB

LW: low rate of N fertilizer, HI: high rate of N fertilizer, LW-B: low rate of N fertilizer plus biochar, LW-C: low rate of N fertilizer plus cover crop, LW-CB low rate of fertilizer plus cover crop and biochar.
Different letters indicate significant differences according to Tukey's test ($P \leq 0.05$) among genotypes (upper case, within row) and managements (lower case, within column).

Table 4. Mixed model analysis of variance associated with the growing season × depth interaction.

Factors	SOC stock (Mg ha^{-1})		Total N (g kg^{-1})	
	a	b	a	b
2014-2015	56.2 B	106.2 aA	1.81 aA	1.69 aB
2015-2016	47.5 B	79.0 bA	1.43 bA	1.15 bcB
2016-2017	39.6 B	71.1 bcA	1.36 bA	1.26 bB

a: 0-20 cm depth; b: 20-40 cm depth.
Different letters indicate significant differences according to Tukey's test ($P \leq 0.05$) between depths (upper case, within row) and among growing seasons (lower case, within column).

In 2014, before starting the experiment, biomass residues from previous 8-year cardoon long-term trial (Ledda et al. 2013; Deligios et al. 2017) were incorporated into the soil. Analyzing our results, it rises that in the year of crop establishment (2014-2015) SOC resulted higher than other growing seasons, and this outcome was likely due to soil residues incorporation of the previous cardoon crop. This hypothesis is also supported by Lehtinen et al. (2014) that after reviewing the effects of crop residues incorporation on SOC across Europe, indicated an overall positive impact of crop residues incorporation on SOC. However, the change in SOC (decrease) during the three-year period, although significant for two genotypes, were somewhat negligible and this result is consistent with studies demonstrating that changes in SOC in a few-year period may be small or insignificant, taking a 7-year period or longer for differences to be appreciable (Bonin and Lal 2014).

The management of the short-term experiment described in this paper was characterized by no crop residues (both cardoon and cover crop) and weeds incorporation; consequently, partially decomposed litter was available in the surface layer. This might explain the SOC concentration increase in the surface layer and the slight decline in the lower layers as also found by Ferchaud et al. (2016) after a 5-year study on perennial grasses. Indeed as stated by Conant et al. (2001) and

Fonte et al. (2012) the aboveground plant litter, more easily decomposable than root material, has positive effects in stimulating the soil fauna, which plays a major role in the translocation and/or decomposition of fresh detritus, and therefore it accelerating the C sequestration mechanisms in the first soil cm depth. By contrast, the high below-ground biomass growth of cardoon (Francaviglia et al. 2016; Nocentini and Monti 2017) provided a continuous C input associated to the minimal soil disturbance that increased SOC stock in the deeper soil layer if compared to the surface soil layer (0–20 cm). In addition, the increased of SOC stock at 20–40 cm, can be explained by soil bulk density rise across treatments and years, equaled to 1.32, and 1.55 g cm^{-3} (data not shown) at 0–20, and 20–40 cm, respectively. The higher SOC and SOC stocks at the LW-CB and LW-C managements are likely a result of the use of cover crop alone and combined with biochar, and BA genotype also have had some slight and additive effect. Both biochar and cover crop use are considered as management strategies able to counteract the adverse effect of residues removal on SOC (Blanco-Canqui 2013). The inclusion of a cover crop in a perennial bioenergy system provides additional biomass, mitigating the negative effects of residue removal by adding to soil stable C pools (McDaniel et al. 2014; Kallenbach et al. 2015; Tiemann et al. 2015). Additionally, some authors highlighted the key roles of cover crop rhizodeposition as a source of soil C (Austin et al. 2017), and particularly of legumes species below-ground biomass in increasing soil organic matter and C pool (Crème et al. 2017). Our findings were consistent with Sainju et al. (2003) that reported that crimson clover cover crop treatments can sequester SOC at a higher rate compared with no cover crop ones. Besides additional biomass residues, legume cover crops such as subterranean clover also supply N to the soil by fixing N from the atmosphere, which might have increased the above and belowground cardoon biomass yields, thereby increasing SOC, as also indicated by Gauder et al. (2016) and Sainju et al. (2002) on other species, compared with no cover crop managements. Although biochar is known to affect the soil nutrient balance (Ding et al. 2016), in our study its effect was not equivalent to that of cover crop, as cover crop alone was more effective for storing soil C. Despite the absolute beneficial effects of this compound on soil carbon sequestration and soil fertility, there was evidence of a likely synergistic effect when biochar, cover crop, and fertilizer were applied (LW-CB management). Indeed, root nodulation by rhizobia is generally increased by biochar, presumably because conditions associated with efficient N-fixation, such as access to P, are improved (Biederman and Harpole 2013), and because its an excellent substrate for rhizobia, immobilizing inorganic C in biochar structure particles (Mia et al. 2014). No significant differences in SOC and stock between conventional (LW and HI managements) and alternative managements were found. Therefore, our finds indicate that N mineral fertilization rates in perennial energy cropping systems of cardoon can be limited to 50 kg N ha^{-1} y^{-1} thus reducing the cost of N fertilization without altering soil C storage. These results suggest that the adoption of a legume cover crop and 50 kg N ha^{-1} may be an effective management strategy to promote soil C storage under cardoon. In accordance with Richter et al. (2015) and Mishra et al. (2010) genotype was found to exhibit a statistically significant influence on SOC and SOC stock. The increased C stock in BA and GR compared with GL was probably related to above- and belowground biomass yields. Although belowground biomass was not examined in this study, but total biomass partitioning in cardoon is stable enough (roots contributing 30% of the total biomass; Raccuia and Melilli 2010) to allow affirming that the abundance and depth of the roots of BA and GR are higher than GL and can provide a source of organic material for enhancing the SOC stock as also found by other authors on different perennial energy crops. Angelini et al. (2009) and Spissu (2015) supported our hypothesis by reporting that aboveground biomass of BA and GR genotypes was higher than GR and with no significant differences between the formers.

Total N concentration was significantly lower in the GR genotype (Table 1). The 0-20 cm depth showed significantly higher nitrogen level in comparison to 20-40 cm within each growing season. This last result was likely due to mineral N fertilizer use which fosters the N availability in the soil. However, over the years, a declining trend has been observed for both depths with the highest and statistically different values in the first year compared to other two years (Table 4). Here, we hypothesized that since the amount of N applied as mineral fertilizer was scheduled to meet cardoon demand, the microbial growth, which requires N to build proteins, occurred by depleting soil N pool in the short-term. Indeed, N from organic material source was not readily available due to the fact that root decomposition process and organic N release occur slowly and over a long period of time.

The lowest C and N content observed in LW and HI entailed a decrease of C:N ratio with statistical differences compared to the other three alternative managements (LW-B, LW-C, LW-CB). The lowest C:N ratio detected in LW and HI treatments might be due to higher nitrogen availability for microbial community that can speed up SOC mineralization process (Sinsabaugh et al. 2002). By contrast, the high C:N ratio maintenance in managements with both biochar and cover crop use - also useful for the energy cropping system resilience - might be due to the presence of substantially stabile C resulting from both biochar incorporation and greater below-ground biomass production providing SOM. The C:N ratio, within each growing season and among genotypes, was rather stable and significant differences among genotypes were detected only in the second growing season (growing season × genotype interaction, Tables 1 and 2).

3.2 Soil respiration

Soil respiration (SR) rates were monitored for two growing seasons (2015-2016 and 2016-2017) starting from sprouting to senescence stage (Figures 3-7).

A rise of CO_2 efflux was observed for each genotypes and in all managements in both growing seasons specifically in October after fertilisation. The increase of nutrient content associated with favorable temperature (15 °C) promoted microbial activity speeding up the SOM decomposition and the CO_2 release to the atmosphere. This finding is consistent with previous studies that reported higher soil respiration, after nitrogen fertilization, from the cropland ecosystems, showing also a high correlation between nutrient addition and soil temperature (Zhou et al. 2014; Chen et al. 2017).

CO_2 emissions from soil showed a reduction only throughout the wet season. The decrease of SR rate was probably both due to low winter temperature that caused a reduction of SOM mineralization process, and to high soil water content that limited oxygen availability, as indicated by Kiese and Butterbach-Bahl (2002) and Ding et al. (2007).

SR peaked at early spring when soil water availability decreased and soil temperature increased fostering soil metabolic rate. SR trend showed similar in summer and winter of both growing seasons. Specifically SR showed a steady decrease with a minimum value in summer and then it gradually increased in autumn at the beginning of rainfall events. The seasonal pattern of SR linked to the variation of soil temperature was already highlighted by other studies demonstrating that SR shows twofold values for every 10 °C soil temperatures increase up to a maximum of 35 to 40 °C, beyond which soil temperature is too high, limiting plant growth, microbial activity and SR (Davidson and Janssens 2006; Lellei-Kovács et al. 2011; Laudicina et al. 2014).

Basically the first observed growing season showed higher SR values than the second one for all managements and genotype under consideration. This result might depend on residual effects of tillage practices for seedbed preparation in the first growing season which might have fostered soil aeration and, as a consequence a SR increase. On the other hand, throughout the 2016-2017 growing season the SR rate showed the same trend but more low compared to the previous season always following the thermo-pluviometric variations.

The SR rate showed a variation in each adopted management throughout the considered growing seasons. Specifically the SR followed the seasonal variation in terms of soil temperature and moisture in LW management (Figure 3).

SR followed a clear seasonal pattern even in the case of HI management, namely it showed the highest CO_2 emissions in March-April and the lowest value in winter and summer (Figure 4). This outcome confirms the relevance of soil temperature and moisture with respect to CO_2 flux. The highest CO_2 flux occurred immediately after fertilization in both the considered growing seasons peaking in the first growing season. The measured average CO_2 fluxes were 0.19, 0.52 and 0.25 g CO_2 m^{-2} h^{-1} in winter, spring and summer, in LW management. Very similar values to the latter occurred in HI management (0.19, 0.45 and 0.26 g CO_2 m^{-2} h^{-1} in winter, spring and summer, respectively). In both monitored growing seasons SR in conventional managements (LW and HI) was higher than in the alternative ones. The N fertilization effects on SR remain controversial. Some studies underlined that the increase of N availability for microbial biomass might foster bacteria growth and consequently producing a SR raise (Contosta et al. 2015; Zhang et al. 2014). By contrast, Cardon et al. (2001) and Giardina et al. (2004) showed that N fertilization resulted in a SR rates reduction. In our study SR increased in LW, although the low SOC content did not supply an organic substrate suitable to promote an adequate microbial activity (Bae et al. 2013; Yu et al. 2014), while it decreased in HI management. Similar outcomes were reported by Bae et al. (2013) although with respect to agro-forestry systems.

In LW-B management CO_2 fluxes rates showed a seasonal pattern (Figure 5). Specifically for each growing seasons, the lowest efflux was observed in winter and summer and the highest one in early spring. The SR rates achieved approximatively 0.24, 0.39 and 0.34 g CO_2 m^{-1} h^{-1} in winter, spring and summer, respectively. LW-C management showed the lowest SR effluxes in both monitored growing seasons. The CO_2 emission rates from soil showed a seasonal trend, the highest value was achieved in March-April (0.41 g CO_2 m^{-2} h^{-1}) and the lowest in winter and summer (0.20 g CO_2 m^{-2} h^{-1} and 0.23 g CO_2 m^{-2} h^{-1}, respectively) (Figure 6). LW-CB management showed higher SR fluxes than the other managements in both growing seasons and throughout the year (Figure 7). Specifically the SR values were high at the beginning of the monitoring activity, then it showed a steady reduction achieving the minimum value (0.27 g CO_2 m^{-2} h^{-1}) in November and December, namely corresponding to low temperatures. The higher rates were detected in early spring (0.48 g CO_2 m^{-2} h^{-1} on average). From the end of May SR gradually declined up to 0.33 g CO_2 m^{-2} h^{-1} achieved in August.

Our results evidenced that the seasonal dynamic of SR was affected by growing seasons and managements. In detail, as indicated by Yuste et al. (2007), SR was influenced by soil temperature specifically during autumn, winter and spring, and soil moisture content in summer.

The temporal trends of SR were similar during the two growing seasons.

The SR increase in LW-B might results from the mineralization of the labile C fraction that was able to provide a suitable substrate for microbial activity (Deng et al. 2017). Indeed as underlined by Ventura et al. (2014), the microbial enzymatic activity also showed an increase after the addition

of biochar. This might be able to provide easily exploitable resources for microbial community and strengthening consequently their action.
The LW-C management was the most conservative in terms of CO_2 emissions in both growing seasons under consideration. Temperatures and moisture value partially affected the SR process also in this case. The presence of the cover crop increase SOC content and consequently its stock. However it has also been suggested that belowground inputs (both from main and cover crop) decompose slowly due to the chemically complex components of root biomass (e.g. lipids or waxes such as suberin; Rasse et al. 2005; Mendez-Millan et al. 2010). This condition might cause a lower microbial community development per soil square meter and, as a consequence a SR reduction; indeed, as stated by some authors (Fierer et al. 2009; Kallenbach and Grandy 2011), the quantity and quality of SOM and carbon (C) and nitrogen (N) inputs are the overriding controls on soil microbial biomass and activity. In addition, the N derived from N fixation process was likely less available to soil microbial activity, and was presumably used to meet cover crop and cardoon nutritional demand as found in other studies (Pérez-Álvarez et al. 2015). Moreover, Cellete et al. (2009) reported that a lower soil N availability might be due to a lower soil moisture under cover crops, which would lead to a decline in N mineralization rates.
Our results could suggest that in the LW-C management the SR reduction might be due to the lack of suitable substrate available for microbial activity. This last hypothesis is consistent with results by Graham et al. (2002) that showed a microbial growth reduction when the soil nutritional conditions are inadequate, and an increase of microbial activity after N source replenishment.
In the LW-BC, SR observations are also confirmed by the high SOC content and C stock. Indeed, LW-CB provides considerable C for microbial community activity, but despite a significant C storage in the soil, a relevant amount is lost as CO_2 emissions.

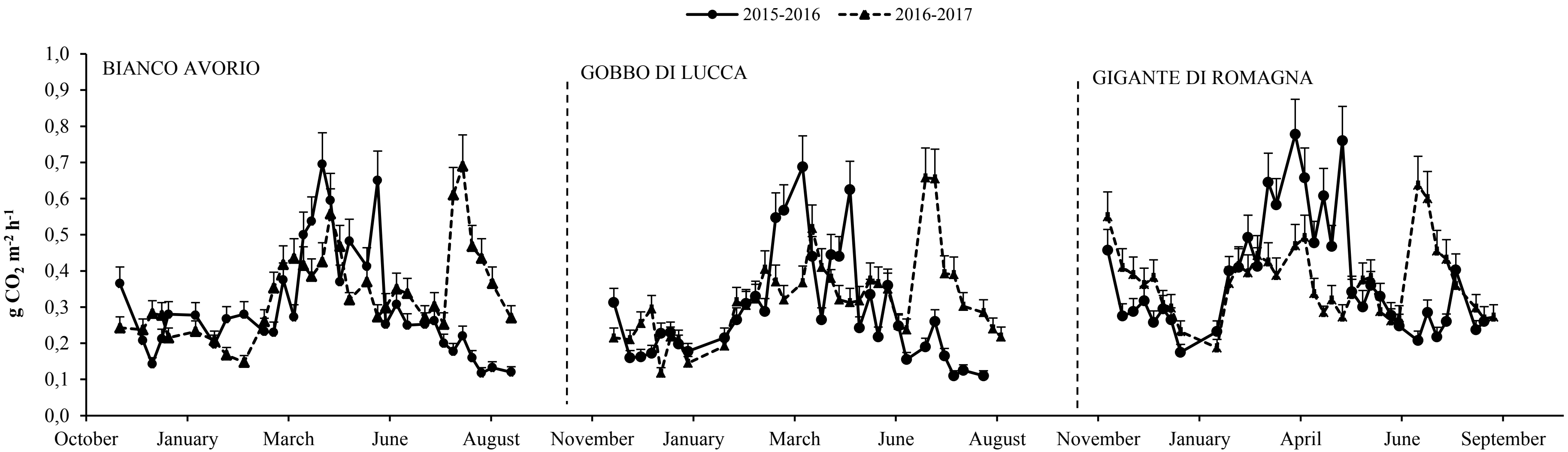

Figure 3: Seasonal dynamics of soil respiration rates ± SE in two growing seasons for the LW (low rate of N fertilization) management.

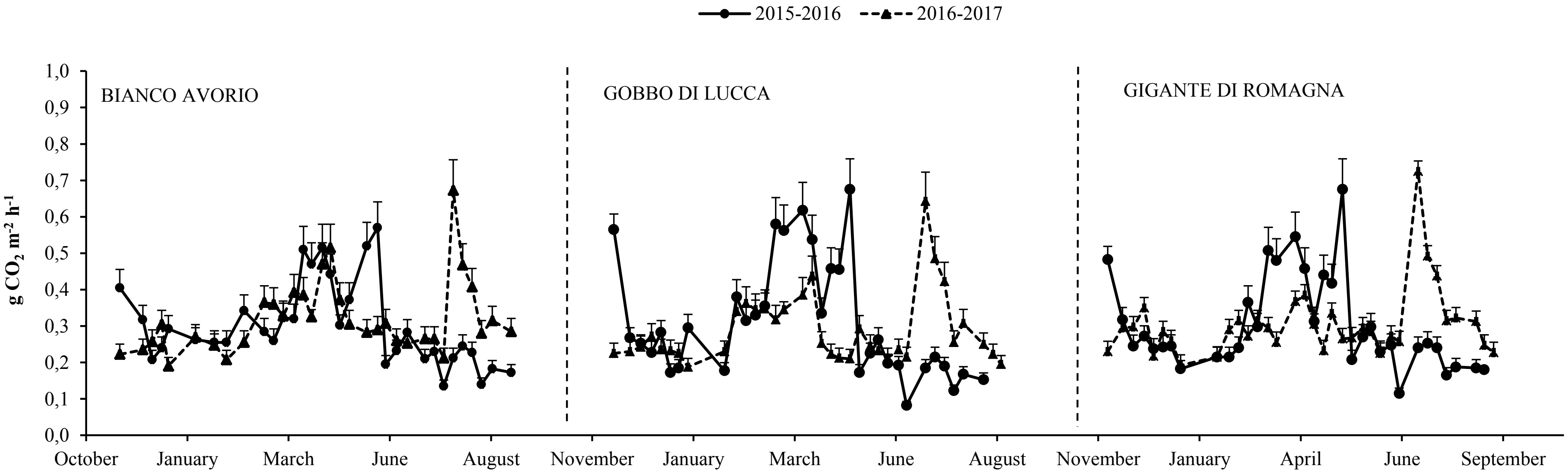

Figure 4: Seasonal dynamics of soil respiration rates ± SE in two growing seasons for the HI (high rate of N fertilizer) management.

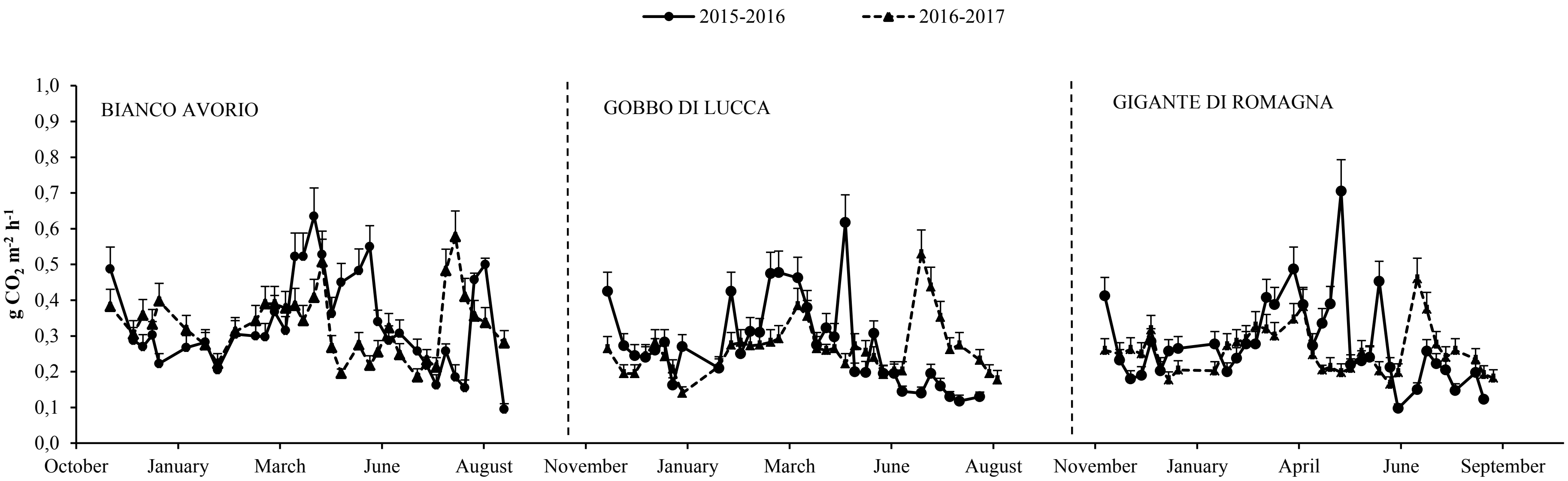

Figure 5: Seasonal dynamics of soil respiration rates ± SE in two growing seasons for the LW-B (low rate of N fertilizer plus biochar) management.

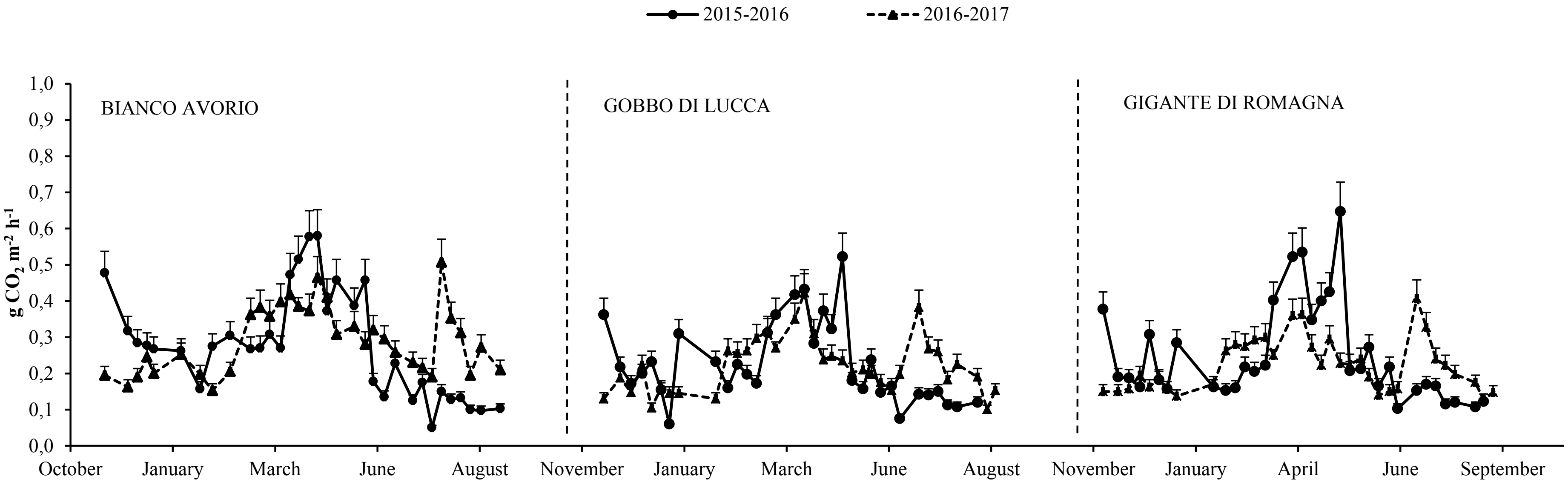

Figure 6: Seasonal dynamics of soil respiration rates ± SE in two growing seasons for the LW-C (low rate of N fertilizer plus cover crop) management.

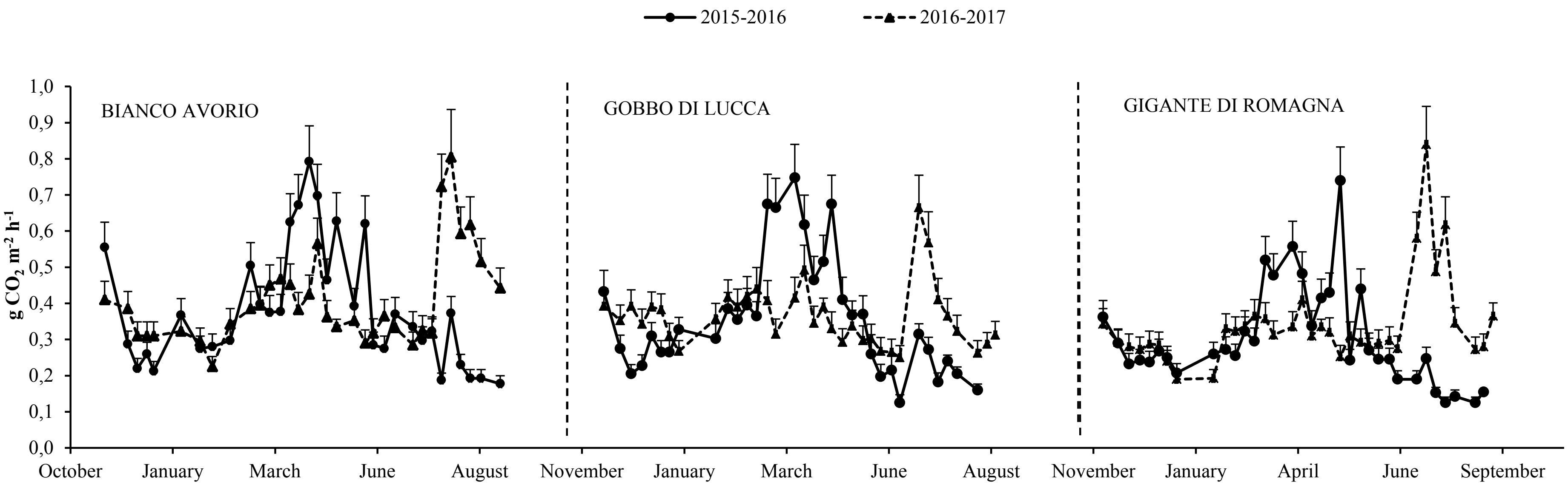

Figure 7: Seasonal dynamics of soil respiration rates ± SE in two growing seasons for the LW-CB (low rate of fertilizer plus cover crop and biochar) management.

4. Conclusions

The understanding of soil carbon dynamics in perennial systems for bioenergy production is crucial since it might influence soil fertility and agro-ecosystem sustainability. The SOC dynamics assessment requires long term measurements since the annual changes are rather small. The research activity addressed the cardoon cultivation monitoring throughout three years after a previous multi-year (8 years) experimental trial based on continuous cardoon crop. The analysis highlighted that benefits in terms of soil C stock, following the previous perennial energy crop, were partly lost as consequence of the establishment of the experimental trial reported in the current paper. Among the three considered cardoon genotypes BA showed the most relevant SOC content. At the same time, within BA genotype, soil heterotrophic respiration showed the lowest rates. Thereby this research basically underlined that a preliminary choice of genotype for energy purpose might be done both in terms of potential productivity and SOC storage capacity. The choice of management might play a key role in order to limit over time SOC decrease and as a consequence the CO_2 losses. The best performance was showed by LW-B and LW-C managements. Furthermore the biochar incorporation within the soil provided an important stable C resource that might foster C sequestration in the long term and thus contributing to climate change mitigation strategies. The inclusion of a N-fixing cover crop (i.e. subterranean clover) in perennial energy cropping systems also might be a successful strategy in terms of soil fertility enhancing and consequently ecosystem services supply. Specifically they might include: 1) C soil sequestration and thus crop productivity improvement; 2) nutrients cycle enhancement to foster a more efficient use by plant and microorganism community and an economic benefit specifically in terms of N fertilizers saving; 3) CO_2 emissions reduction. These findings might provide to farmers useful information regarding different agricultural managements of perennial bioenergy systems, especially in case of continuous crops cultivation. In addition, the considered managements might represent a relevant support for policy makers in order to preserve and improve soil fertility. Finally, the research outcomes might be used for parameterizing C dynamics simulation models in order to better understand strengths and weaknesses resulting from the different agricultural managements application in energy cropping systems in the long term.

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CHAPTER 2: **LONG TERM EFFECTS ASSESSMENT ON SOIL ORGANIC CARBON DYNAMIC AND LAND USE CHANGE FOLLOWING ARABLE LAND CONVERSION TO ENERGY CROPPING SYSTEM IN MEDITERRANEAN ENVIRONMENT**

1. Introduction

The global energy demand from renewable sources is increasing in developed countries. In order to face this need the agricultural land uses might undergo a diversification in terms of production purposes in the coming decades. This shift might cause: a) the conversion of agricultural cropping systems, especially from food/feed to energy cropping systems specifically dedicated to biomass production to be used for energy purposes (Bellido et al. 2014; Fargione et al. 2008); b) the agricultural cropping systems conversion might lead to continuous energy crops cultivation. However the Land Use Change (LUC) resulting from the new cropping systems development is a debated issue worldwide. Some authors put on evidence the question of land completion of food/nonfood crops and possible effects that might cause on food security especially in the developing countries (Smith et al. 2010; Albanito et al. 2016). Other studies underlined that LUC might trigger both a biodiversity reduction if the energy cropping systems cultivation is extended over time (Eggers et al. 2009; Huston and Marland 2003; Robertson et al. 2008) and cause an alteration on soil organic carbon (SOC) dynamics (Anderson-Teixeira et al. 2009).

On the contrary, different researches focused on LUC positive effects that perennial energy cropping systems might provide in terms of ecosystem services. For instance, Peyne (2010) and Ceotto and Di Candilo (2011) highlighted that the switch from food/feed crops to perennial energy cultivations might enhance carbon sequestration within soil, contributing to the mitigation of CO_2 emissions in the atmosphere. Furthermore Blanco-Canqui (2010) underlined that the conservative agricultural management of the perennial energy cropping systems might improve the soil structure, thus preserving its fertility.

The LUC effects might be different depending on: a) fertility and soil capability to store the SOC (Crossman et al. 2011; West et al. 2010); b) site- specific climatic conditions (Trail et al. 2013) and, c) the LUC type and the adopted agronomic management (Post and Kwon, 2000, Guo and Gifford 2002). A crucial issue in order to foster the energy cropping systems development might be their environmental sustainability without jeopardizing soil fertility in the future. To this end it would be necessary to perform an impacts assessment with respect to the SOC dynamics arising from the switch to the energy continuous cropping systems from arable cropping systems. SOC is a priority factor in order to preserve soil fertility since it also affects its physical, chemical and biological properties (Lal et al. 2011). In addition, a SOC content increase can improve crop productivity (Smith and Olesen 2010) and it can also foster a CO_2 emissions reduction (Harris et al. 2013; Lal et al. 2010). Andreson-Teixeira et al. (2009) indicated that the conversion from uncultivated soil to both annual (e.g. maize) and perennial energy cropping systems (e.g. miscanthus and switchgrass) might produce a SOC content decrease that is higher in the conversion to annual energy cropping systems compared to perennial crops. Furthermore, as reported by Mishra et al. (2013) and Qiu et al. (2012) the carbon dynamics evaluation performed by simulation models applied to energy cropping systems (e.g. miscanthus and switchgrass) showed a potential SOC content increase in the long term. Frequently, the LUC effects assessment are focused on SOC dynamics in the surface soil layers neglecting the deepest ones. This is basically due to the fact that the greater soil organic matter (SOM) contribution occurs in the first 20-30 cm of soil. On the contrary Harrison et al. (2011) and Knops and Bradley (2009) underlined the relevance of addressing C dynamics analysis over 30 cm, especially when the cropping systems conversion regards perennial crops characterized by a deep root system. Generally the highest soil C stock was detected in perennial cropping systems in the deep layers (beyond 30 cm) where the C mineralization processes are slower (Fornara and Tilman 2008; Monti and Zatta 2009).

The agricultural managements might play a crucial role in terms of LUC. Indeed soil tillage can reduce the SOC content (20-40%) (Davidson and Ackerman 1993; Murty et al. 2002). On the contrary, conservative management (e.g. no-tillage) - generally carried out for perennial energy cropping systems - might increase soil C accumulation and thus play a role in terms of climate change mitigation strategy (Benacchi et al. 2005; Lal 2004; DeLuca and Zabinski 2011). The time after conversion from food/feed cropping systems to the energy one might also affect the soil capacity in order to achieve its steady-state. The short-term experimental trials aimed to assess LUC impacts arising from the switch from food/feed cropping systems to perennial energy cropping systems might not provide adequate explanations about C dynamics and its sequestration rate in the soil, thus contributing to increase the uncertainty about the actual contribution of land use changes on C dynamics. Therefore the research objective is to analyze the long term effects on SOC dynamic arising from LUC owing to switch from an annual food/feed cropping system to a perennial energy crop cultivation.

2. Materials and methods

2.1 Site characterization and experimental design

The field study was conducted at the "Mauro Deidda" experimental station of the University of Sassari, Sardinia, in Southern Italy (41° N, 8° E, elevation 81 m a.s.l.) from 2014 to 2017. The climate of the site is typically Mediterranean (Köppen classification, Kottek et al. 2006) with mild and humid winters and warm dry summers. The mean air temperature is 16.1 °C and the annual average precipitation of the site is 550 mm, mainly distributed between October and April. The experiment was set up to evaluate the impact of land use change in terms of CO_2 fluxes and soil organic carbon (SOC) stocks following a shift from a barley-field bean 2-year rotation to a perennial cardoon cropping system (Figure 1).

Figure 1: ***a*** *Cynara cardunculus* cropping system for bio-energy purpose, ***b*** and ***c*** traditional biannual rotation of barley (*Hordeum vulgare* L. var. Multie) and field bean (*Vicia faba* L. cv. Prothabat)

The perennial cropping system was already described in the previous chapter. The experimental design was a split-plot with four replicates. For the objective of the current paper, only Bianco Avorio genotype was accounted for, and low rate of N fertilizer (LW), high rate of N fertilizer (HI) and low rate of N fertilizer plus biochar (LW-B) treatments were considered as land use compared to biannual rotation. The cardoon cropping systems management are detailed in figure 2. Before the cardoon implanting a trial on cardoon with different mineral fertilizer doses was conducted for eight years (Deligios et al. 2017; Ledda et al. 2013) in the same field and yet even before barley to field bean rotation were cultivated.

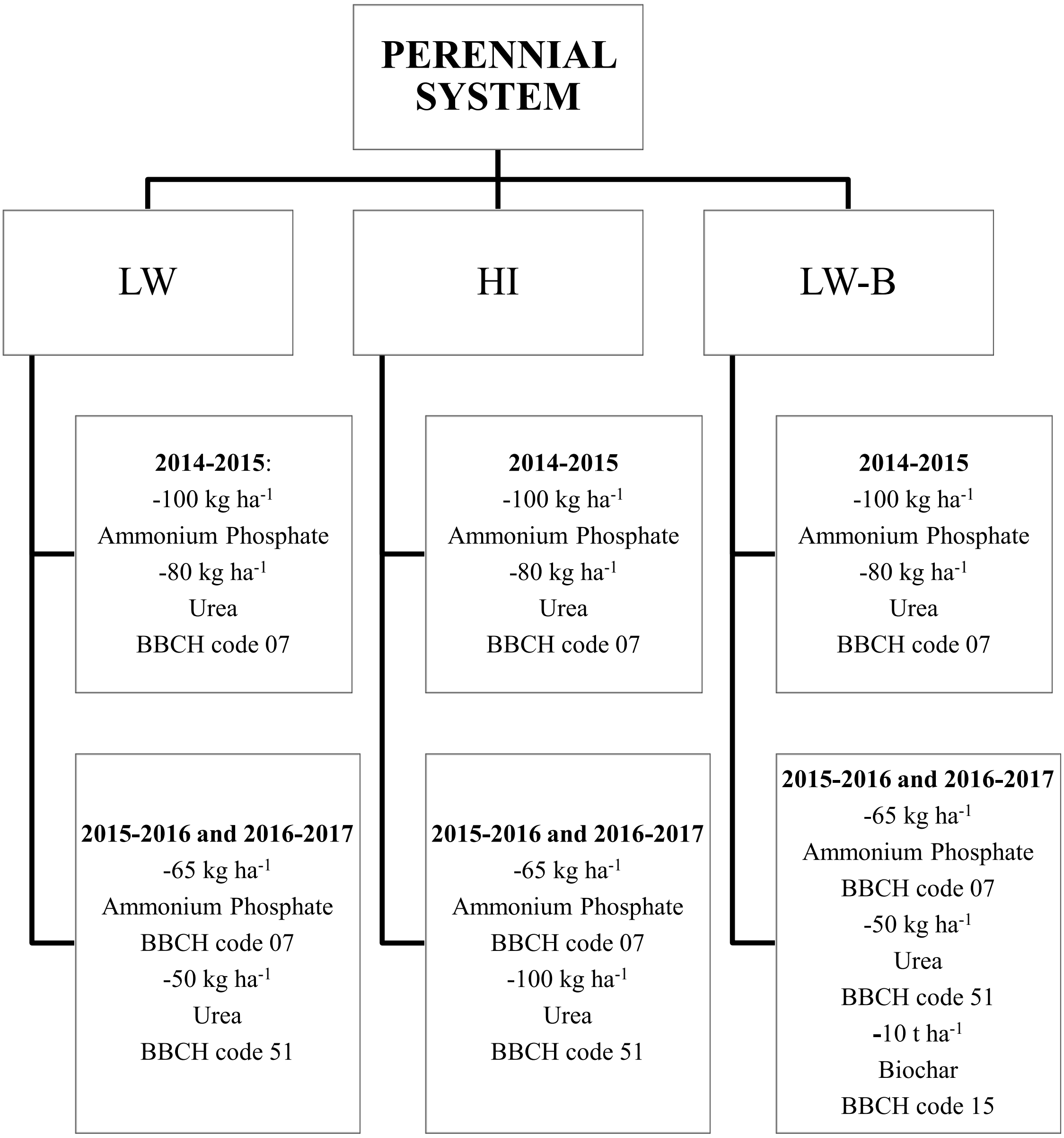

Figure 2: Scheme of perennial cropping systems management for the three growing seasons. LW: low rate of N fertilizer; HI: high rate of N fertilizer and LW-B: low rate of N fertilizer plus biochar. BBCH code (Archontoulis et al. 2010) refers to phenological growth stage during which fertilizer was applied.

All the phases of the rotation were present each year arranged in a completely randomize blocks design with four replicates and placed in a field adjacent to the cardoon one.

Each year, mouldboard ploughing was carried out in September, and seed bed preparation was carried out in early October, just prior to field bean sowing. In both year, barley (cv. Multie, RAGT Italia Srl) and field bean (cv. Prothabat, CGS Sementi Spa, Italy) were sown in January and October at a density of 350 and 30 seeds m^{-2}, respectively. Barley was annually fertilized with 92 kg P_2O_5 ha^{-1} (as diammonium phosphate) and 82 kg N ha^{-1} (as diammonium phosphate and urea) applied pre-sowing. No fertilizer was applied in the field bean plots (Figure 3).

A mixture of tifensulfuron metile and tribenuron metile (Nautius, Belchim Crop Protection SPA, Italy) was applied each year to barley at Zadocks stage 30-32 at a rate of 40 and 15 g a.i. ha^{-1}, respectively. In field bean, broad-leaved weeds were controlled using an annual application of imazamox at a rate of 40 g a.i. ha^{-1} (Imazamox 40, Agrimag srl), carried out in later winter. Herbicides were applied at a spray pressure of 300 kPa with fan-type noozles. Both crops were harvested for grain, field bean in early July, and barley in mid-June. Crop residues were incorporated into the soil.

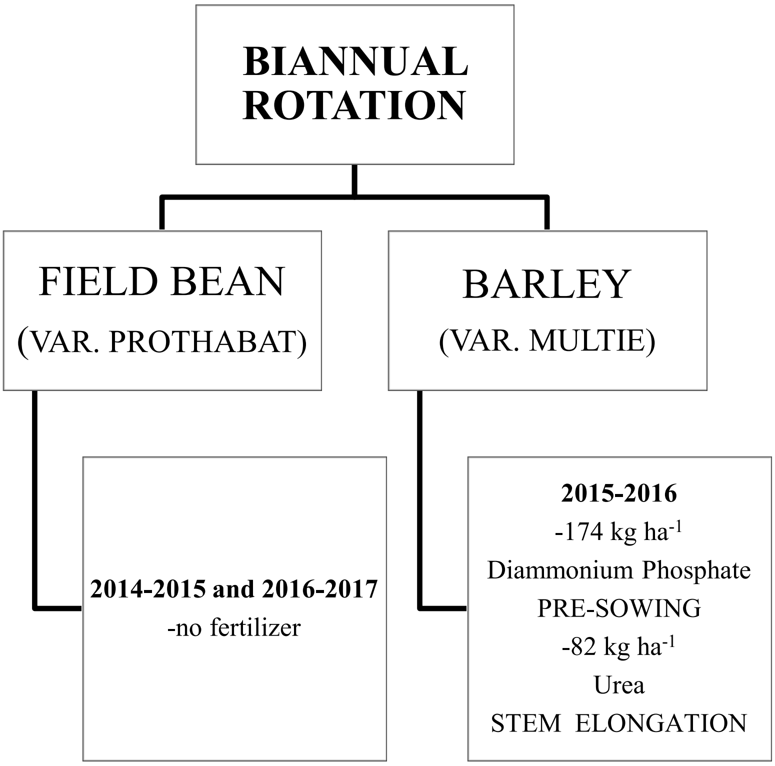

Figure 3: Scheme of biannual rotation management.

In both experimental fields (perennial system and biannual rotation), the soil are sandy clay loam (Typic Haploxerepts, Soil Taxonomy, 2014) overlay on limestone. The main physical and chemical characteristics of the soils are: 65% sandy, 15% silt and 20% clay. The average total nitrogen content is 1.8 g kg^{-1} and 1.2 g kg^{-1} respectively. The total carbon content and organic matter content varies between fields. It is equal to 50 g kg^{-1} and 31 g kg^{-1} in the first experimental field and 40 g kg^{-1} and 25 g kg^{-1} in the second field

2.2 Soil sampling and carbon stock evaluation

In order to evaluate over time the change in SOC stock in both experimental fields soil sampling was carried out at the same point and time during each growing season (November 2014, 2015 and 2016). Soil samples were collected with a hand probe at two depth intervals: 0–20 and 20–40 cm. All soil samples were transported in the laboratory and dried at 40 °C per 72 h. Successively each sample was sieved to 2.0 mm and any possible crop and roots residue was removed. Chemical soil parameters were determined according to standard methods (Supplement issue to G.U. n 248 of 21st October 1999). Specifically soil pH was determined in a 1:2.5 soil/water suspension ratio. Total organic carbon and nitrogen contents were measured by dry combustion method using an elemental analyzer LECO (CHN628 Series, LECO Corporation, St Joseph, MI, USA). Soil sample for soil organic carbon determination, was treated at 550 °C muffle furnace for 2 hours before analysis. Soil organic matter content (g kg^{-1}) was determined according to Francaviglia et al. (2017), multiplying total organic C by 1.72. To assess the SOC stock in both experimental trials we used the procedure of Wang and Dalal (2006) in which:

$$\text{SOC stock} = \text{SOC content} \times \text{Bd} \times \text{D}$$

where SOC stock was expressed in Mg ha^{-1}. SOC content (g kg^{-1}) is soil organic carbon, Bd is the bulk density (g cm^{3}), estimated using a pedotransfer function (Tsuji et al. 1998), and D (m) is soil depth interval.

2.3 Soil respiration measurement

Soil respiration rates (SRr) was measured in order to improve knowledge of the link between SRr and land use change. A total of 18 PVC collars (10 cm diameter and depth, with perforated walls in the first 5 cm) were placed permanently in cardoon cropping systems only in two of the four replicates (in the middle of the field) to minimize border effect. Furthermore, in the soil was inserted an external PVC cylinder (40 cm diameter and depth) to exclude autotrophic respiration (Alberti et al. 2010). In the biannual rotation field, 8 collars (10 cm diameter and depth) were inserted into the soil before the sowing of crops according to the randomize block design. During the measurement the collars were situated at 1 cm from the soil surface to reduce the impact of chamber installation. SRr was measured with a portable non-dispersive infrared gas analyzer (IRGA) EGM-4 (PP-Systems, Hitchin, UK) connected to a SRC-1 chamber fitted to the collar. In both trials SRr measurements were performed weekly for all growing seasons and between 9:00 and 12:00 am. Simultaneously to SRr measurements, soil temperature and soil water content were measured using a probe inserted in the soil to the 10 cm depth and FieldScout TDR 300 (Spectrum Technologies Inc.) respectively.

2.4 Statistical analysis

The analysis was performed using SAS Software (1999, version 9.02. SAS Institute Inc, Cary).
The comparisons between initial (2007) and final soil conditions (2014) of the previous cardoon growing cycle were made by using the Student's t-test. Data on soil organic carbon (g kg^{-1}), SOC stock (Mg ha^{-1}), total N (g kg^{-1}) and C:N ratio were reported as mean and standard error of the mean (±SE). Data of the final soil condition were considered as baseline conditions for the experiment reported in the current paper.
After testing for normality (Kolmogorov–Smirnov test) and homogeneity of variance (Levene test), a three-way ANOVA (main effects and interactions) for soil respiration, moisture and temperature involved the year (2015-2016, and 2016–2017) (factor 1), the management (HI, LW, LW-B, and biannual rotation) (factor 2), and depth (0-20 cm and 20-40 cm) (factor 3). In three-way ANOVA the differences among means were identified by post hoc Tukey tests.
For the Student’s t-test and ANOVA p-values < 0.05 were considered significant.

Results and discussion

3.1 Biannual cropping system conversion to perennial cropping system

The soil chemical parameters at different depth and sampling year are shown in Table 1. The analysis highlighted statistically significant differences in soil organic content (SOC) between 2007 and 2014 sampling years at surface layer, with an increase of 41%, while there was not significant effect of the sampling year on SOC at 20-40 cm.

In both depths and sampling years significant difference from SOC stocks were observed. The SOC stock was always higher in second sampling year (2014) and in the deepest layer. Specifically, the increase of SOC stock was +52.7% at 0-20 cm and +50% at 20-40 cm.

Table 1: Chemical soil properties of two sampling years

Depth (cm)	Sampling year	Soil Organic Carbon (g kg^{-1})	SOC stock (Mg ha^{-1})	Total N (g kg^{-1})	C:N ratio
0-20	2007	12.8±0.11 b	36.6±0.92 b	1.13±0.03 b	11.3±0.39
	2014	18.1±0.29 a	55.0±2.84 a	1.81±0.04 a	11.3±0.51
20-40	2007	9.8±0.82	69.3±0.72 b	1.03±0.03 b	11.3±0.35
	2014	16.3±0.33	104.4±5.51 a	1.69±0.05 a	10.8±0.57

For sampling years (2007; 2014), within each column, means followed by different letters are significantly different according to Student's t-test ($P \leq 0.05$).

The results of Student's t-test applied to SOC content and SOC stock put on evidence that sampling year and consequently the management adopted before soil sampling affected these soil chemical properties. The higher organic carbon and SOC stock observed in the second sampling year may depend on soil management practices and cropping systems adopted. Conversion of biannual system characterized by conventional tillage with residue incorporation to no tillage management (perennial system) increase organic carbon and SOC stock. Our findings are supported by Almagro et al. (2017) that in Mediterranean condition found an increase of SOC sequestration in no-till compared to conventional tillage with residue incorporation.

Furthermore, the increase of SOC stock in the long run (after 8 years) in both depths are in agreement with studies conducted by Ceotto and Di Candilo (2011) and Chimento et al. (2014) that reported that a conversion from an annual feed cropping system to a perennial one provides a substantial SOC sequestration after 7 and 6 years, respectively.

The SOC stocks during the eight years of cardoon cultivation (2007-2014) showed an increasing trend over time. This increase of SOC stock was of +26.7 Mg ha^{-1} (yearly average increase of +3.34 Mg ha^{-1}). Although there is a large potential to sequester SOC following land use change to perennial system, this potential could be attributed to the extensive roots system of perennial plant. This roots system had a high capacity of allocation of C and it contributes to provide organic matter to soil (Jungers et al. 2017). In addition the increase of SOC stock can be due to formation of root exudates (organo-mineral complexes) that promote microbial activity and SOC sequestration in the long run (Bolan et al. 2011; Miltner et al. 2012). Our studies confirmed that the conversion of biannual rotation to perennial bioenergy crops increase SOC sequestration contributing to mitigation of CO_2 emissions. In addition, increase SOC stock can provide further beneficial services such as increase productivity, soil conservation and fertility.

Total N content showed a trend similar to SOC stock within sampling year and depth (Table 1). In the second sampling year significant differences in both depths were observed. Maximum total N content was observed in 2014 sampling year in surface layer, while minimum total N value was found at 20-0 cm in the first sampling year. Specifically, the increase throughout the eight years (2007-2014) were equal to +60.2% at 0-20 cm and +64% at 20-40 cm, respectively. The effects of depth and sampling year on C:N ratio were not significant.

During the eight years of cardoon cultivation N fertilization was supplied (Deligios et al. 2017; Ledda et al. 2013). Analyzing our results, increase of total N content at the end of cardoon cultivation cycle (2014) in the surface layer was likely due to mineral fertilizer applied over the entire cultivation period. Furthermore, the highest total N value at 20-40 cm depth might be explained by cardoon capacity to store N within below-ground biomass (Fike et al. 2006), since N is translocated to roots system that after a decomposition process provide N to the sub-soil. This outcomes is also supported by Mauromicale et al. (2014) who detected the same trend of total N after seven years of cardoon cultivation. By contrast, the continuous conventional tillage, adopted before cardoon planting, might have produced the lowest N values observed at both depth in 2007 fostering mineralization process and as a consequence N release (FAO, 2001).

3.2 Soil properties in second crop cycle of cardoon

Some properties of soil observed during the 2015-2016 and 2016-2017 growing seasons are reported in table 2. The SOC content was significantly affected by growing season, management and depth. The highest SOC content was observed in the 2015-2016 growing season and in the surface layer (0-20 cm), with significant differences compared to 2016-2017 and deep layer respectively. Management significantly affected soil organic C. Specifically, the lowest SOC content was observed in biannual rotation and only in LW-B treatment of perennial system.

SOC stock was also significantly affected by growing season with a reduction of the 13.5% in 2016-2017 compared to the 2015-2016 (Table 2). Furthermore, a significant management x depth interaction was observed (Table 2).

Table 2: Mixed model analysis of variance associated with the growing season, management and depth analyzed.

Factors		Organic C (g kg^{-1})	SOC stock (Mg ha^{-1})	Total N (g kg^{-1})	C:N ratio
Growing season					
2015-2016		15 a	60.2 a	1.3	11.6 a
2016-2017		12 b	52.1 b	1.3	9.7 b
Management					
LW		15 a	60.9 a	1.4 a	11.3
HI		14 ab	57.4 a	1.3 ab	10.8
LW-B		12 b	518 bc	1.3 a	9.9
Biannual rotation		13 b	54.5 b	1.2 b	10.8
Depth					
0-20		14 a	42.8 b	1.4 a	10.6
20-40		13 b	69.5 a	1.2 b	10.7
Analysis of variance	Df	P>F	P>F	P>F	P>F
Growing season	1	***	***	N.S.	**
Management	3	**.	*	*	N.S.
Depth	1	**	***	***	N.S.
Growing season × management	3	N.S.	N.S.	N.S.	N.S.
Growing season × depth	1	N.S.	N.S	N.S.	N.S.
Management × depth	3	N.S.	**	N.S.	N.S.
Growing season × management × depth	3	N.S.	N.S.	N.S.	N.S.

LW: low rate of N fertilizer, HI: high rate of N fertilizer, LW-B: low rate of N fertilizer plus biochar,
Component of variation: *, **, *** significant at P levels of P≤0.05, 0.01, 0.001 (Tukey's Test) respectively; N.S. not significant.
For each parameter (growing season, management, depth), within each column, means followed by different letters are significantly different according to Tukey's test (P ≤ 0.05).

The SOC stocks showed always lowest values in the surface layer (0-20 cm) and an increase in deep layer, with a significant differences within management of the perennial system that showed the highest SOC stock in deep layer. Specifically, LW-B management showed higher values (76.5 Mg ha^{-1}) followed by HI (74.8 Mg ha^{-1}) and LW (66.1 Mg ha^{-1}) managements (Table 3).

Table 3: Mixed model analysis of variance associated with the management x depth interaction

Factors	SOC stock (Mg ha^{-1}) a	b
LW	37.6 bB	66.1 cA
HI	40.1 aB	74.8 bA
LW-B	45.2 aB	76.5 aA
Biannual rotation	48.2 aB	60.8 dA

LW: low rate of N fertilizer, HI: high rate of N fertilizer, LW-B: low rate of N fertilizer plus biochar.
a: 0-20 cm depth, b:20-40 cm depth.
Different letters indicate significant differences according to Tukey's Test ($P \leq 0.05$) among depth (upper case, within row) and management (lower case, within column) .

The relationship that links the variation of organic carbon content and SOC stock to growing season, depth and land use is very clear from the data obtained. However, there is high difference between depths due to crop management and land use. Analyzing our results SOC stock was higher in perennial system than biannual system at deep layer and this outcome can be explained with two hypothesis.

The first is the decrease of microbial oxidation activity on carbon organic in perennial system due to no-tillage compared to biannual system. Therefore no tillage permits a longer physical protection of SOC within aggregates reducing SOC microbial mineralization (Six et al. 1999). Alvaro-Fuentes et al. (2009) supported our hypothesis by showing that the SOC, in no-tillage conditions, is included inside the micro-aggregates (> 250 μn) avoiding microbial decomposition.

The second hypothesis is the great amount of below-ground biomass and the nature of this that returned to the soil in perennial system. This hypothesis is also supported by a review of Don et al. (2012) and Chimento and Amaducci (2015) that underlined the increase of SOC accumulation following the annual cropland conversion to perennial crop, indicating positive effects, on SOC sequester, of roots and rhizomes from these crops in the deep layer (> 30 cm).

By contrast, the higher value of SOC stock observed in biannual system at 0-20 cm (Table 3) is likely a result of positive relationship between C and N mineralization processes (Bendi and Chand 2007). In the biannual system dry residues (straw barley and field bean) were incorporated. These residues are characterized by high C:N ratio and lignin content that limited SOC mineralization. Our results are in accordance with Wang et al. (2015) that reported the mineralization kinetics of crop residues when changing quality. Higher C content reduce SOC mineralization.

The higher SOC content at the LW-B management in deep layer is likely a result of the addition of biochar. The incorporation of biochar in perennial bioenergy system, as substitute of mineral fertilizer, increased SOC sequestration since it provided a recalcitrant substrate that minimized soil microbial activity and SOC mineralization (Mc Cormack et al. 2013).

Total N concentration was significantly influenced by management and depth (Table 2). The higher total N was observed in perennial system with significant differences among managements (LW, HI and LW-B) while the lower total N was observed in biannual system (Table 2). Management with the use of a high rate of nitrogen (100 kg ha^{-1}) and the incorporated biochar showed the lower total N (1.3 g kg^{-1}) compared to LW management (1.4 g kg^{-1}).

The lower value of total N in biannual system was likely due to higher N mineralization following residues incorporation that stimulate microbial activity. This hypothesis is supported by Kumar et al. (2018) that observed a positive correlation between N mineralization and crop residue incorporation in annual system with inserted legumes. On the contrary adopting no tillage in perennial system contributed to reduction of N mineralization process.

The total N decrease along soil depth (Table 2) reached values of 1.2 g kg^{-1} at 20-40 cm. This outcome might be due to the surface application of N and P fertilizers in both cropping systems.

3.3 Soil respiration pattern in perennial and biannual cropping systems

Soil respiration (SR) was monitored for two different growing seasons 2015-16 and 2016-17 in both systems (perennial and biannual). All figures below depict on the left the SR rates of the 2015-2016 growing season and on the right the SR rates of the 2016-2017 growing season. In biannual system the SR observations occurred from crop establishment (February) onwards.

Soil respiration showed similar seasonal and annual patterns in the perennial and biannual system (Figures 4-6) with differences among managements adopted. The fluxes of SR followed seasonal dynamics of soil temperature and moisture (Figures 4a-6a) as it is typical in semiarid and dry Mediterranean area (Almagro et al. 2009). The maximum values occur in spring with increasing temperature while the minimum values were observed in winter and summer season in both systems. At the sprouting stage (BBCH code 07) in the perennial system at all growing season and managements was supplied mineral fertilizer (ammonium phosphate). This fertilization might have promote nutrients availability for microbial community activity and thus rising CO_2 emissions from the soil. This trend is also confirmed by Chen et al. (2018) that reported a positive feedback on heterotrophic respiration following mineral fertilizer application.

A peak of CO_2 efflux was observed for each annual crop, even if in during different periods, in both growing season. In the first growing season the peak occurred in the middle of January, while in 2016-2017 season the peak was observed in October, after ploughing and residues incorporation. The increase of soil respiration due to soil tillage and the creation of favorable soil nutrient conditions was already highlighted by some authors demonstrating that tillage induces change in oxidation status of soil enhancing exposition of crop residues to mineralization (Eriksen and Jensen 2001; Ussiri and Lal 2009).

Figure 4 shows the SR pattern of the perennial system managed with low N rate compared to biannual rotation. CO_2 efflux followed a similar trend in both growing seasons showing gradually increase in spring and reduction at the beginning of summer. Specifically the measured average CO_2 effluxes in the first growing season were 0.44 g CO_2 m^{-2} h^{-1} (spring) and 0.28 g CO_2 m^{-2} h^{-1} (summer) in perennial system and 0.47g CO_2 m^{-2} h^{-1} (spring) and 0.16 g CO_2 m^{-2} h^{-1} (summer) in biannual system. By contrast, in the second growing season perennial system showed lowest average CO_2 emissions during all seasons. Very similar trends of SR rate was observed in HI management of the perennial system (Figure 5). SR was higher in biannual system and followed the seasonal thermo-pluviometric variation (Figure 5a) with highest CO_2 emission in spring in both systems and growing seasons and the lowest value in winter and summer.

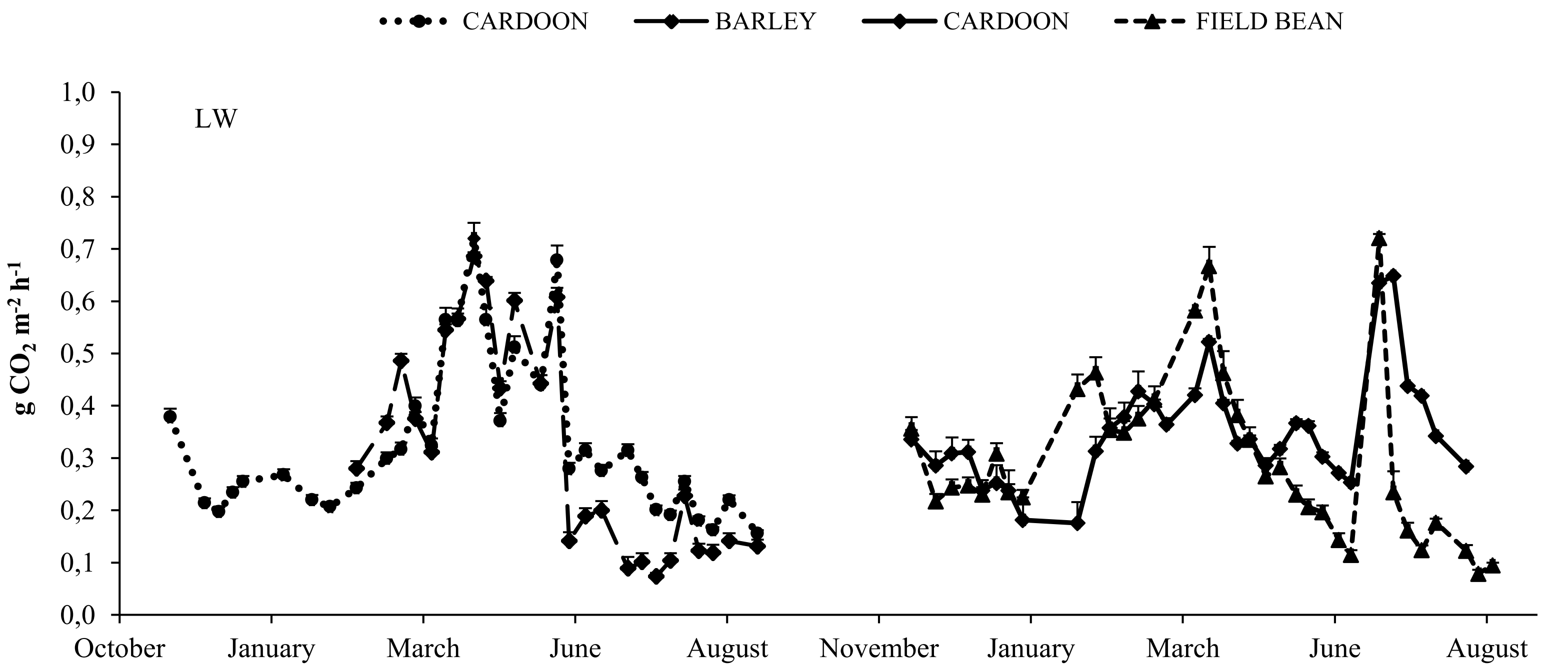

Figure 4: Seasonal variation of soil respiration in perennial system (LW: low rate of N fertilization) and biannual rotation during 2015-2016 and 2016-2017 growing seasons. Bars represent standard errors.

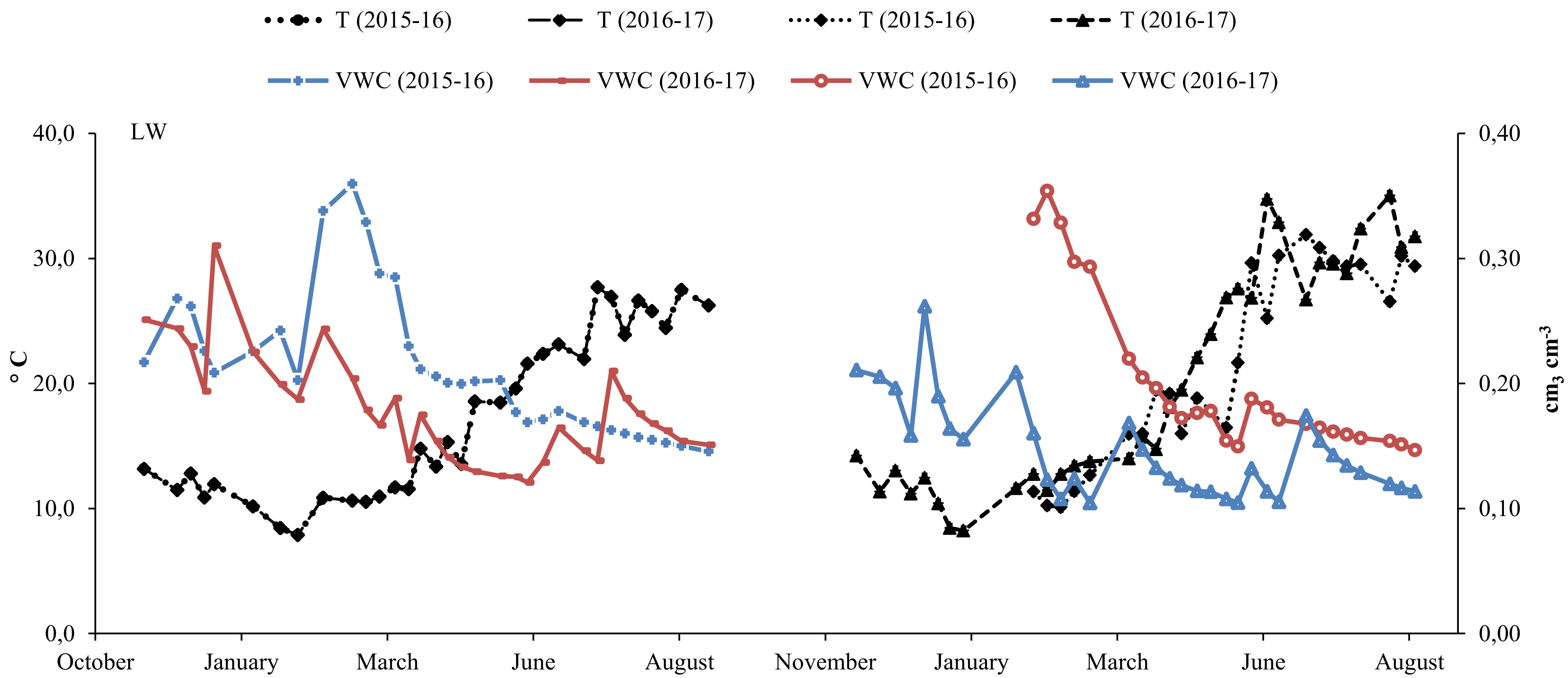

Figure 4a: Seasonal variation of soil temperature and water content over time (from October 2015 to August 2017) for perennial system (left) and biannual system (right). LW: low rate of N fertilizer; T: soil temperature; VWC: volumetric water content.

The average SR in cardoon was 0.22, 0.41 and 0.23 g CO_2 m^{-2} h^{-1} in winter, spring and summer respectively, while the values increased in biannual system by +9.1% and +14.6% in winter and spring, respectively and decreased by −30.4% in summer after barley harvest.

These outcomes confirmed the relevance of soil temperature and moisture with respect to CO_2 efflux (Reichstein et al. 2003; Savage and Davidson 2001) and underlined the positive effects on SR reduction owed to high N rate fertilizer supplied in perennial system. The surplus of N fertilization (+50 kg ha^{-1}) could be likely not responsible of increasing in microbial activity and thus CO_2 emissions. This hypothesis is also supported by Chen et al. (2017) that after mineral N fertilization observed an increase of autotrophic respiration but not heterotrophic one.

By contrast, a greater SR under biannual system indicates a significant contribution from barley and field bean residues to promote microbial activities. Furthermore, SR is positively correlated with crop residues quality incorporated into soil that provide a source of readily available C and N stimulating microbial activity (Aulakh et al. 2001; Huang et al. 2004).

An et al. (2015) have highlighted - through laboratory experiments - that straw addition in soil characterized by low SOC content intensifies the microbial activity compared to the opposite condition (high SOC content). In our trial the biannual rotation soil was C poor compared to cardoon system; thus crops residues incorporation might trigger - in oxidation conditions - an increase of C decomposition activity and SR process.

The LW-B management of the perennial cropping system was the most conservative in terms of CO_2 emissions (Figure 6). In each growing season the lowest SR was observed in cardoon. Specifically the average of SR values were 0.30 g CO_2 m^{-2} h^{-1} and 0.28 g CO_2 m^{-2} h^{-1} in cardoon in the first and second growing season, respectively and 0.33 g CO_2 m^{-2} h^{-1} and 0.28 g CO_2 m^{-2} h^{-1} in biannual system. The CO_2 emissions showed a seasonal pattern of soil temperature and moisture (Figure 6a). Incorporation of biochar into the soil could have promoted reduction of SR rates likely due to depletion of labile C fraction in favor of aromatic compounds hardly decomposed by microbial community (Streubel et al. 2011).

Furthermore, as reported by Alburquerque et al. (2013) the biochar application enhances relevant soil physico-chemical properties (e.g soil porosity, water retention capacity, cation exchange capacity, etc.), soil fertility, habitat for microbial community development, and crop growing. In our experiment a potential soil fertility enhancement might have fostered an increase of cardoon productivity and a greater root system development. As a consequence, more recalcitrant C was released to soil and SR process was positively influenced.

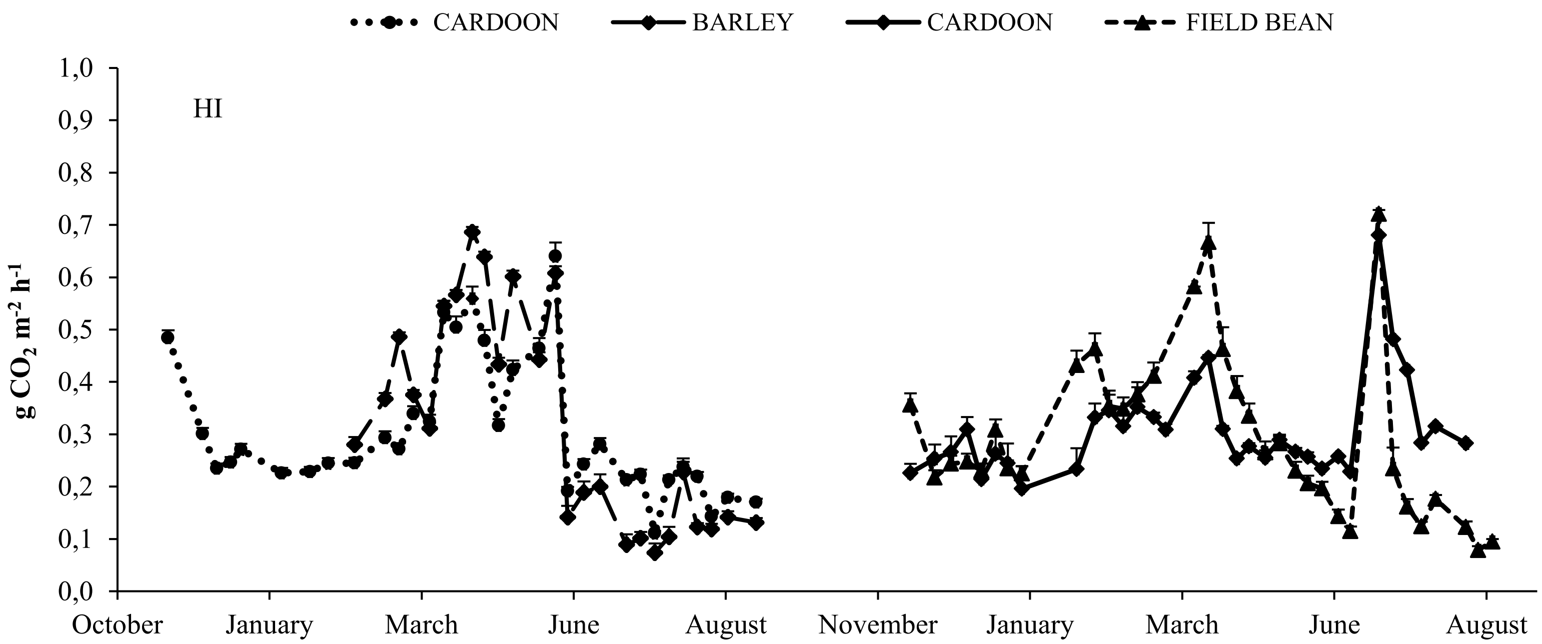

Figure 5: Seasonal variation of soil respiration in perennial system (HI: high rate of N fertilization) and biannual rotation during 2015-2016 and 2016-2017 growing seasons. Bars represent standard errors.

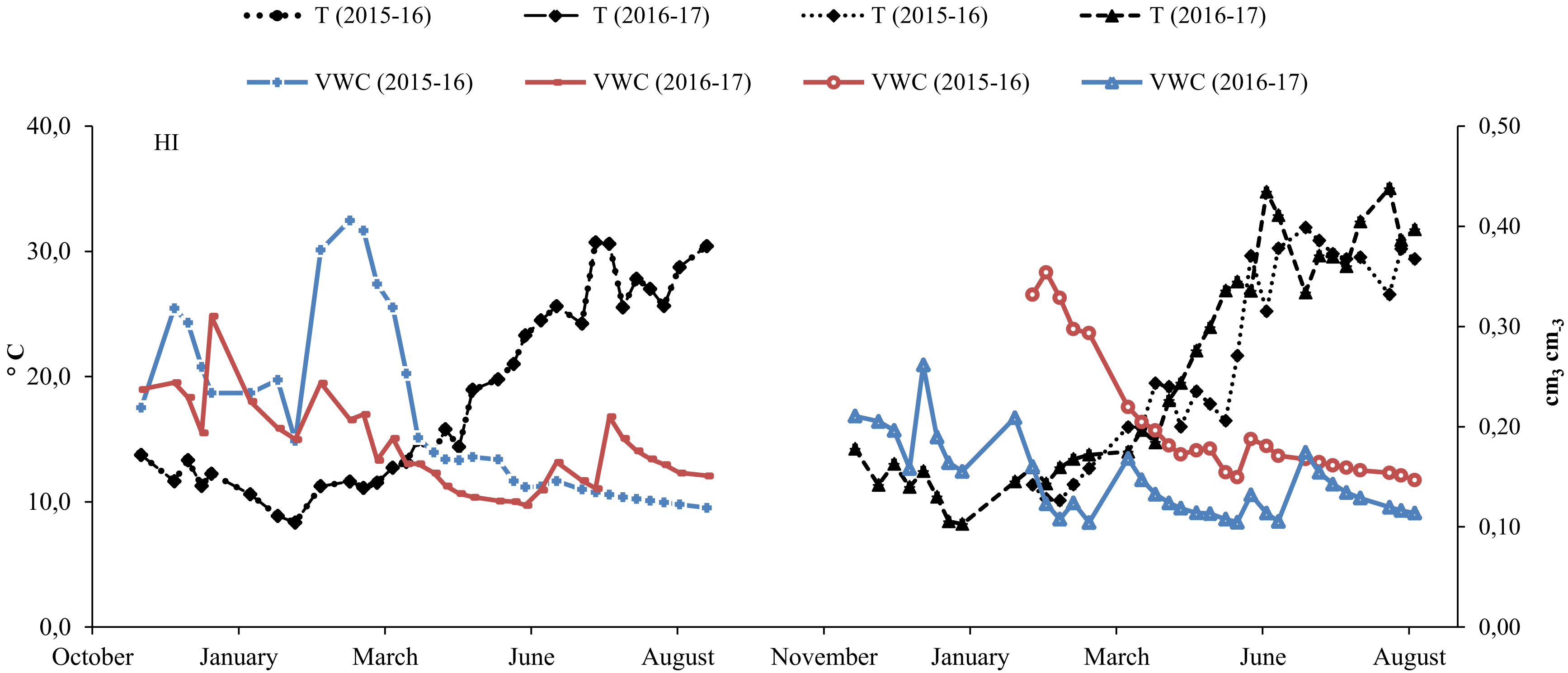

Figure 5a: Seasonal variation of soil temperature and water content over time (from October 2015 to August 2017) for perennial system (left) and biannual system (right). HI: high rate of N fertilizer; T: soil temperature; VWC: volumetric water content.

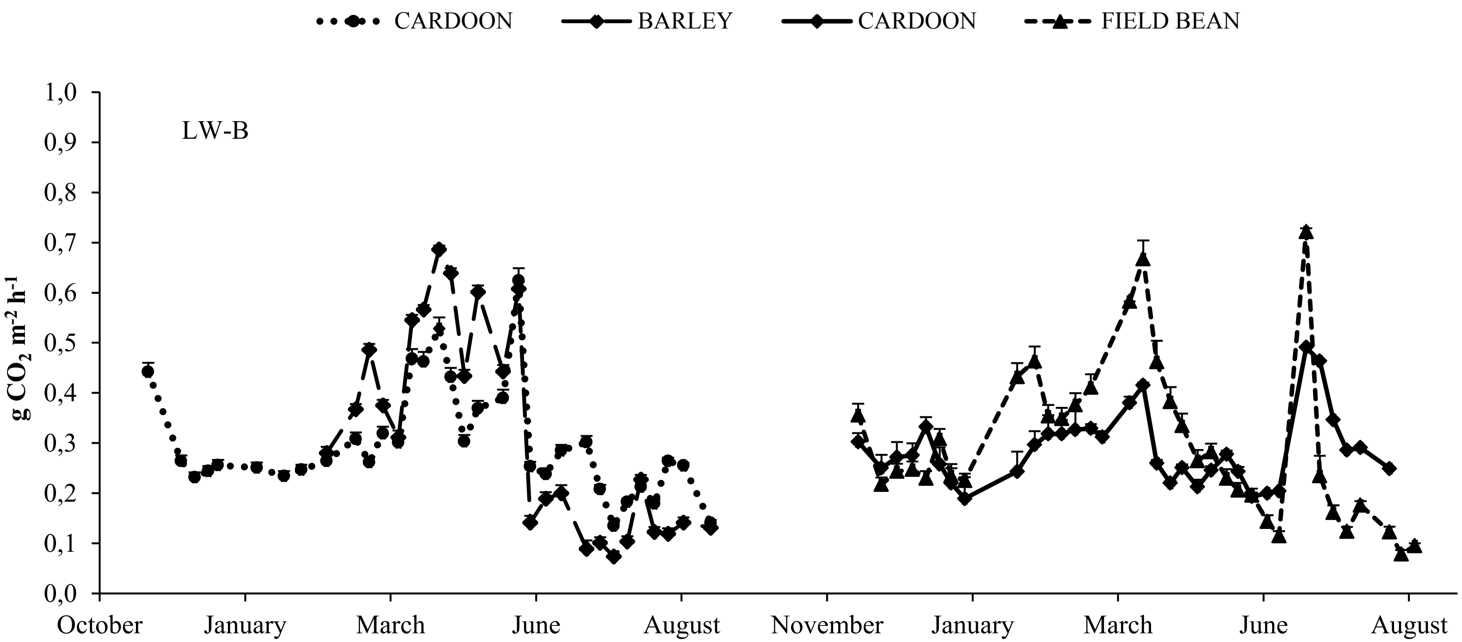

Figure 6: Seasonal variation of soil respiration in perennial system (LW-B: low rate of N fertilization plus biochar) and biannual rotation during 2015-2016 and 2016-2017 growing seasons. Bars represent standard errors.

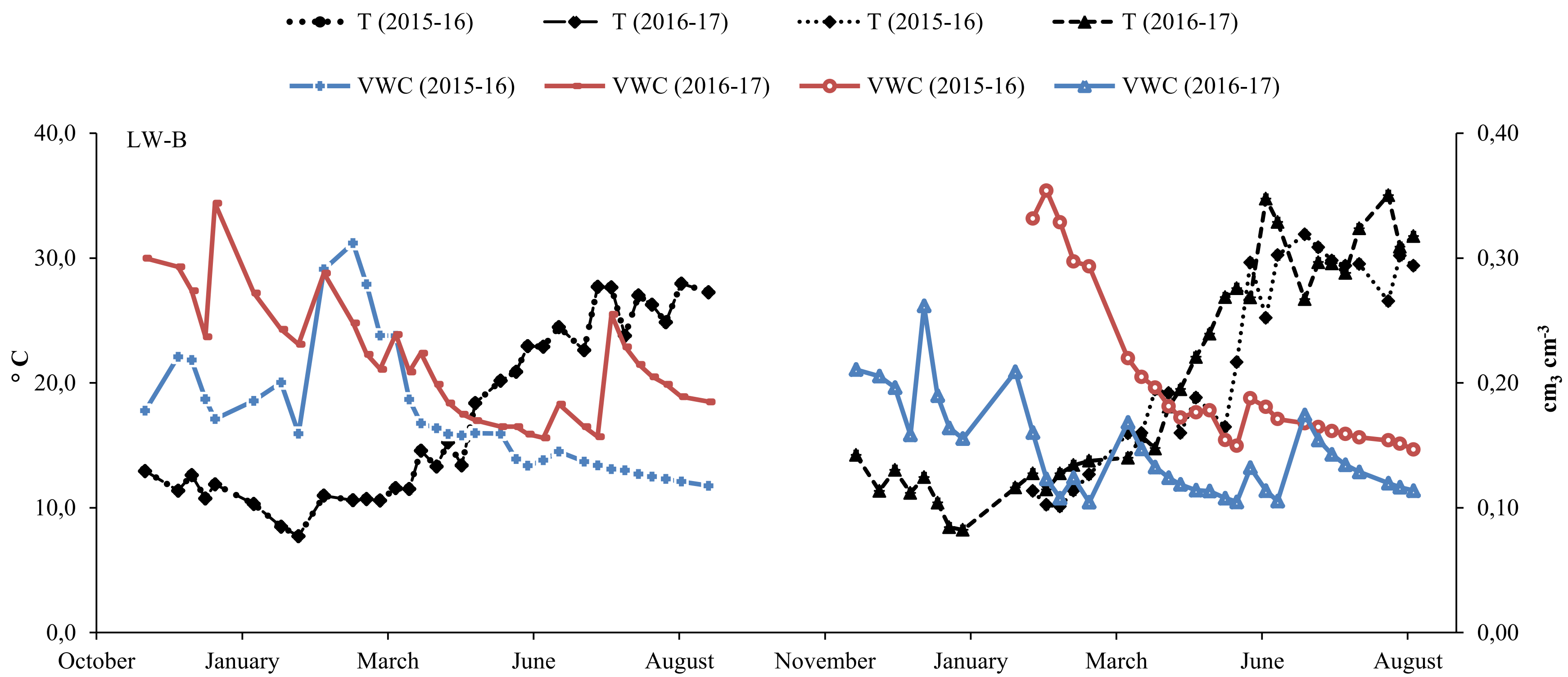

Figure 6a: Seasonal variation of soil temperature and water content over time (from October 2015 to August 2017) for perennial system (left) and biannual system (right). LW-B: low rate of N fertilizer plus biochar; T: soil temperature; VWC: volumetric water content.

Conclusions

The Land Use Change (LUC) arising from the conversion of a food/feed cropping system to energy cropping system - based on cardoon cultivation - have effects on soil carbon dynamics and storage.

In the first cardoon crop cycle (2007) Soil Organic Carbon (SOC) content and SOC stock were lower in deep layer than the second one (2013). The average value of C stock was equal to 53 Mg ha^{-1} at the beginning of the first cardoon crop cycle. The low SOC content observed in the first growing season was mainly due to the adopted agricultural management. Before 2007, a biannual rotation - based on barley-field bean cropping system - was carried out. This agricultural management - characterized by annual ploughing and the crops residues incorporation - caused a fast mineralization process and higher CO_2 losses in the atmosphere in Mediterranean climatic conditions.

Prior to the experimental period, the study site was continuously cropped with cardoon (cv. "Bianco Avorio") under mineral fertiliser treatments for 8 years (Deligios et al. 2017; Ledda et al. 2013). The average of C stock was equal to 80 Mg ha^{-1} at the end of the first cardoon crop cycle. The increase of SOC content and SOC stock at the end of the first cardoon growing season might be due to both: 1) residual effects arising from the previous agricultural management (no tillage) adopted before the cardoon planting and 2) residual root biomass of previous cardoon cultivation. The belowground root biomass - not being undergone any agricultural practices for eight years - was the primary source of soil organic matter in the soil maintaining the dynamic balance between mineralization and humification processes. The LUC produced by the switch from biannual rotation to cardoon cropping system has entailed benefits in terms of SOC content enhancement, nutrients availability, soil fertility and in terms of CO_2 emissions reduction.

In 2014 a second experimental trial was conducted to evaluate the consequence of LUC arise from cropland for food/feed to perennial energy crop and to identify which alternative management of energy system provides the best performance in terms of climate change benefit and improving of soil fertility.

Conversion of biannual system to a perennial cropping system based on cardoon showed significant SOC sequestration with differences among managements. LW-B showed the best performance since it compensated for losses as a result of crop re-implanting. The average of SOC stock was increased +10.4% compared to biannual system evidencing that C dynamics might be considerably affected by LUC and by agricultural management adopted. The research outcomes may have important implications for energy cropping systems carbon monitoring providing useful information to understand carbon dynamics in soil following conversion. In addition these results could be used to evaluate soil emission in the overall life cycle of energy crop as the indicator for assessing the performance of various management.

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