4-2-2019

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Article

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Received: 6 March 2019; Accepted: 28 March 2019; Published: 2 April 2019

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Abstract: Intensive agriculture practices often results in decomposition of organic matter, thus causing soil CO_2 emissions. Agro-ecological service crop could be profitably cultivated to improve soil characteristics and reduce CO_2 emissions under Mediterranean environment. Two-year field trials were conducted in central Italy. The treatments were three agro-ecological service crops (hairy vetch, oat, and oilseed rape) and a no-service cover. Plant development, soil characteristics, and CO_2 emissions were measured. Oat and oilseed rape showed a rapid growth, while hairy vetch started to grow rapidly only after the cold period. Soil CO_2 emissions trend was similar among the agro-ecological service crops and tended to decrease during the cold period, then gradually increased until April when warm temperatures were observed. The high soil CO_2 emissions and respiration index observed in hairy vetch probably stimulated mineral nutrients, especially nitrogen, to become more available in the soil compared to oat and oilseed rape throughout the decomposition of soil organic matter. These results confirmed that the cultivation of agro-ecological service crops, especially hairy vetch, could represent a suitable strategy for enhancing carbon sequestration and lead to a mitigation of CO_2 emissions during the fallow period and could thus contribute to the climate change mitigation.

Keywords: cover crop; crop rotation; carbon sequestration; soil respiration index; biomass production

1. Introduction

Agriculture is considered one of the human activities that results in high production of CO_2 [1]. In particular, conventional agricultural practices contribute about 25% of the total anthropogenic CO_2 emissions and cause about 50% of soil organic carbon (SOC) loss [2]. Conventional land management is based on intensive agriculture practices, such as deep tillage, high inorganic nitrogen fertilizer addition, and irrigation, and often results in the rapid decomposition of organic matter through microbial activities, thus causing high soil CO_2 emissions and losses of SOC [2]. However, soil microbes play a number of vital functions in the soil and the microbial breakdown of organic matter of the soil not only affects CO_2 emission, but also contributes to the release of nutrients into the soil, such as nitrogen, phosphorous, and others, that are essential for plant establishment and development [3]. Thus, CO_2 emissions due to soil respiration are also an important indicator to assess nutrient cycling.

For these reasons, sustainable soil management is needed in order to enhance SOC in cropland, based on the chemical, physical, and biological properties of the soils [3–5]. The reduction of CO_2 emissions by soil carbon sequestration is of primary importance as agricultural activities could remove atmospheric carbon by sequestration and thus mitigate climate change by maintaining and enhancing the amount of carbon stored in the soil and plant material. The challenge of sustainable agriculture is to develop crop rotation schemes and increase soil carbon (C) stocks by the adoption of management strategies that could include adding manure or other organic amendments [6], leaving crop residues on the field [7], reducing soil disturbance [8], and including cover crops [5].

Cover crops are commonly identified as agro-ecological service crops because they may bring multiple benefits to agro-ecosystem [9], including suppression of weeds and pests [10], improvement of soil and water quality, and stimulation of nutrient cycles [11]. Among the several benefit of agro-ecological service crops, the enhancement of SOC and nitrogen sequestration through the production of fresh organic matter has the potential to mitigate climate changes [12–15]. In fact, the aboveground and belowground biomass from agro-ecological service crops is considered as net primary production of C and represent a net flux of CO_2-C from the atmosphere into the biomass tissues [16]. Therefore, replacing bare soil with agro-ecological service crops has been widely accepted as a sustainable agricultural practice, even if the species of agro-ecological service crop to cultivate in crop rotation should be based on the biological, environmental, social, cultural, and economic factors where farmers operate [17]. Under Mediterranean climate conditions, agro-ecological service crops are commonly cultivated from early autumn to spring, when they achieve the highest level of biomass production and are then suppressed mechanically or chemically before the cultivation of the main summer vegetable or grain crops [18,19]. The most extensively used agro-ecological service crops are legumes and grasses that are able to accumulate nitrogen and produce a large amount of biomass. However, there is an increasing interest in crucifers which may act as biofumigants and suppress soil pests, especially root pathogens and parasitic nematodes [11]. Grass used as agro-ecological service crop include the annual cereal that are very useful for scavenging nutrients, mainly nitrogen, due to its developed root systems and produce high amount of biomass that can help to add organic matter to the soil [3]. Although several studies evaluated the impact of agro-ecological service crops on pest management and nutrient cycling, little is known about their effect on soil CO_2 emissions and C balance during their cultivation period. This current study hypothesizes that in Mediterranean environment the replacement of bare soil with the adoption of adequate agro-ecological service crop can be profitably used for sustainable agriculture to improve carbon accumulation and soil characteristics also during their growing season. The evaluation of this assumption is of particular interest to improve the knowledge on biomass production, soil characteristics, and soil carbon emissions in the Mediterranean environment. Therefore, the main objectives of this study were to evaluate the effects of agro-ecological service crops at their termination on: (i) Soil CO_2 emissions trend and short-term carbon balance, (ii) biomass production and characteristics, and (iii) soil properties.

2. Materials and Methods

2.1. Description of the Study Area and Experimental Design

Field trials were conducted in the 2009/2010 and 2010/2011 growing seasons of the agro-ecological service crops at the experimental farm of the University of Tuscia located in Central Italy (45°25′ N, 12°04′ E and 310 m above sea level). The soil was classified as Typic Xerofluvent of volcanic origin with the following average characteristics in the 0–30 cm layer: 10.9% clay, 14.3% silt, 74.8% sand; pH 6.5 (water, 1:2.5 *w:v*); organic matter 1.2%; total N 0.89 g kg^{-1}; available P 35 mg kg^{-1}; and exchangeable K 569 mg kg^{-1}.

The climate of the study area is moderate thermo-Mediterranean with average temperature of 14.3 °C. The lowest mean temperature (on average, 0 °C) was observed in winter period, especially in January and February, while the highest mean temperature (on average, 35 °C) was observed in summer period, especially in July and August. Annual rainfall was 752 mm, on average, based on the 30-year period and was mainly concentrated from September to May.

The field experiments were carried out in two adjacent fields of the same site previously cropped with durum wheat (*Triticum durum* Desf.). Each experimental field was 4800 m^2 (60 m × 80 m), which made it possible to perform all farming operations with agricultural machinery. The experimental treatments consisted in: Three agro-ecological service crops (hairy vetch (*Vicia villosa* Roth. var. Capello), oat (*Avena sativa* L. var. Donata), and oilseed rape (*Brassica napus* L. var. Licapo)) and a bare soil with no cover. The experimental treatments were arranged in a randomized block design with three blocks for a total of 12 plots.

2.2. Field Set-Up and Crop Management

In September, at the beginning of each growing season of the agro-ecological service crops, the soil was ploughed at a depth of 30 cm and fertilized with triple super phosphate at the rate of 100 kg P_2O_5 ha^{-1}. The day after, the soil was harrowed to a depth of 10 cm in order to prepare the seedbed. About one week from the seedbed preparation on 24 September 2009, and 13 September 2010, the seeds of hairy vetch, oat and oilseed rape were hand broadcast at the rates of 60, 100, and 15 kg ha^{-1}, respectively, then the seeds were buried by harrowing to a depth of 2 cm. The bare soil plots were treated similarly to the other plots and it was kept bare throughout the agro-ecological service crop growing season by hand weeding when the weeds start to emerge. The growing season of all agro-ecological service crops was mechanically terminated in 20 May 2010, and 4 May 2011, when hairy vetch was at the flowering stage and oat and oilseed rape were in the milk stage.

2.3. Sampling and Measurements

The photosynthetic photon flux density (PPFD, μmol m^{-2} s^{-1}) transmitted by the agro-ecological services crops was measured at ground level. The measurements were performed every seven days from the emergence to mechanical suppression of the agro-ecological services crops by using a linear ceptometer (SS1-UM-2.0, DELTA-T devices LDT, Cambridge, England). The ceptometer was placed horizontally at ground level five times in the central part of each plot. All measurements were carried out under full sunlight on clear days between 12:00 p.m. and 2:00 p.m. The fraction of PPFD intercepted (FiPPFD) was calculated using the following formula:

$$\text{FiPPFD} = [1 - (I_o/I_t)] \quad (1)$$

where the I_o is the average of five measured PPFD on the surface of the ground and I_t is the radiant flux density on the top of the agro-ecological service crop canopy. The FiPPFD equal to 1 or 0 indicates all or no PPFD intercepted, respectively.

Just before the suppression of the agro-ecological service crops, the biomass samples were collected. The aboveground biomass was hand-clipped at the soil surface and collected by placing a quadrat 50 cm × 50 cm (0.25 m^2) randomly four times over the central part. The entire aboveground biomass samples were separated into leaf and stem fractions. The stem fraction included stems and inflorescences. Each fraction was dried, after which they were weighed to determine leaf/stem ratios. The belowground biomass was determined collecting roots from each plot at 0–30 cm depth with hand-operated soil core sampler (8 cm diameter and 15 cm height). The roots were removed from soil sample by pouring slurry of distillated water through a 50 μm screen and the retained roots were rinsed under water. Subsamples of boot shoot and root biomass were dried to calculate the shoot/root ratio, and were then homogenized with a mill for carbon and nitrogen content determination using an elementary analyzer (Thermo Soil NC - Flash EA1112, Lakewood, NJ, USA). The total N or C accumulation were calculated by multiplying the biomass of agro-ecological service crop with the corresponding N or C concentration value, respectively.

At the suppression of the agro-ecological service crops, the sampling of soil was performed. Five soil cores (0–30 cm depth) were taken in the middle part of each plot and then pooled together for the physic-chemical characterization analysis. The collected soil samples were air dried and sieved (<2 mm). The total organic carbon (TOC) and the total nitrogen (TN) contents of the soil samples were determined using an elementary analyzer (Thermo Soil NC - Flash EA1112, Lakewood, NJ, USA). Moreover, soil NO_3-N [20] and soil NH_4-N [21] concentrations were determined after soil sampling in an aliquot of sieved fresh soil in order to assess the soil mineral nitrogen.

In both growing cycles, at the emergence of agro-ecological service crops, soil CO_2 emissions were measured in each plot on a daily basis until their mechanical suppression using a portable dynamic closed-chamber infrared gas analyzer system [22]. All soil CO_2 emission measurements were carried out at the same time of day in order to reduce diurnal variation and performed by placing the closed-chamber of a sensitive infrared gas analyzer instrument (EGM-4, PP Systems, Stotfold, UK) in a permanent PVC collar in the central plot of each agro-ecological service crop. Further information regarding the operation methods adopted for the CO_2 measurements are reported by Mancinelli et al. [5]. The measured CO_2 emission suggested the net carbon mineralization value. In both growing cycles, soil CO_2 emission was estimated as the amount of C-CO_2 accumulated [14,23] throughout the study period. The calculations were made by means of the linear interpolation of the two neighboring measured emissions and the numerical integration over time (trapezoid rule) as reported in the following equation:

$$CO_2 - C = \sum_{i}^{n} [(x_i + x_{i+1}) \times N \div 2] + \ldots + [(x_{n-1} + x_n) \times N \div 2] \quad (2)$$

where i = date of first measurement of CO_2 rate taken, n = date of last measurement of CO_2 rate taken; x = CO_2 rate (kg ha^{-1} day^{-1}), and N = number of days between the two consecutive CO_2 rate measurements.

The soil respiration index was estimated as soil C stock/soil CO_2 emission rate (t ha^{-1}/t ha^{-1}). The soil C content was calculated by applying the formula suggested by Batjes [24]. Carbon input/output ratio was calculated as the ratio between the C added to the system by cover crop biomass at their termination (C input) and the total C content in the CO_2 emissions measured throughout the growing season of the service crops [5]. Soil temperature close to the chamber was measured simultaneously to the measurement of CO_2 emissions at a depth of 5 cm by using the STP-1 Soil Temperature Probe connected to an EGM-4 instrument. Similarly, soil volume water content was measured near the chamber at the same time of CO_2 measurement at a depth of 30 cm using the TDR 300 Soil Moisture Meter (Spectrum Technologies, Inc., Plainfield, IL, USA).

2.4. Statistical Analysis

The data on agro-ecological service crops and soil were analyzed with analysis of variance (ANOVA) using JMP statistical software package, version 4.0 [25]. The data analysis was carried out applying a randomized block design with three blocks and two years of experimentation. Data were analyzed using the ANOVA model with the treatment as fixed factor, the three blocks were included as a random factor and the year (growing cycle) was considered as random effect to account for the repeated measure across time [26]. Percentage data were *arcsin* transformed before analysis in order to homogenize the variance [27]. The data reported in the tables were back transformed for meaningful data interpretation. Fisher's protected least significant difference (LSD) at 0.05 probability level ($p < 0.05$) was used for comparing the treatments. Data on the FiPPFD, CO_2 emissions, soil temperature and soil volume water content are presented as the means value ± standard error (SE).

3. Results

3.1. Weather Conditions

The growing period of agro-ecological service crops lasted for 239 days in 2009/2010 and 233 days in 2010/2011 growing cycles. Generally, the average air temperature was slightly higher in the first year than in the second (on average 10.5 vs. 10.2 °C, respectively), particularly from December to February, when the temperature dropped below 0 °C several times. Furthermore, the first year was drier compared to the second (864 vs. 694 mm, precipitation in 2009/2010 and 2010/2011, respectively), while the evapotranspiration was 420 mm in the first year and 441 mm in second.

3.2. The Fraction of PPFD Intercepted (FiPPFD) during the Agro-Ecological Service Crop Growing Cycle

The fraction of photosynthetic photon flux density intercepted (FiPPFD) from the emergence to mechanical suppression of the agro-ecological service crop in 2009/2010 and 2010/2011 is reported in Figure 1. In both growing cycles, the values of FiPPDF in the agro-ecological service crops increased gradually in time, however, this trend was different among the treatments. Indeed, the first stages of the agro-ecological service crops immediately after their germination until to January, when it was observed the lowest soil temperature, the values of FiPPDF were generally similar and higher in oat and oilseed rape, while hairy vetch grew more slowly showing lower values of FiPPDF compared to oat and oilseed rape (Figure 1). Subsequently, the FiPPDF values in oilseed rape tended to decrease notably, while in hairy vetch they increased considerably and reached similar values observed in oat (Figure 1).

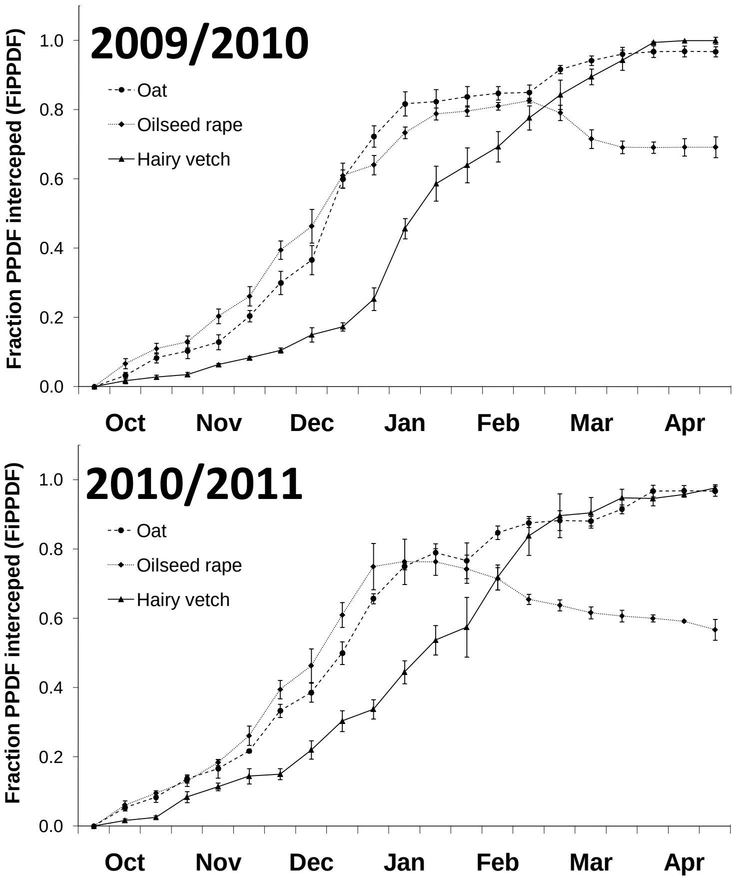

Figure 1. Fraction of photosynthetic photon flux density intercepted at ground level (FiPPFD) during the 2009/2010 and 2010/2011 growing cycles of the agro-ecological service crops. Error bars represent mean ± standard error (n = 3).

3.3. Soil CO_2 Emissions during the Agro-Ecological Service Crop Growing Cycle

The trends of soil CO_2 emissions dynamics, throughout growing period of the agro-ecological service crops are shown in Figure 2. Generally, during the growing period the four adopted treatments affected soil CO_2 emissions with a similar trend in both years. In fact, the CO_2 flux tended to increase immediately after agro-ecological service crop emergence until December, when it was observed low soil temperature (Figure 2). After the cold days in each year, the soil CO_2 flux tended to increase gradually in all treatments reaching a high value in April, just before the agro-ecological service crop suppression. In both years, few differences among the treatments until February were observed, while in the following months the trend of soil CO_2 fluxes among the agro-ecological service crop treatments were largely different. In particular, the main differences were observed between hairy vetch and the other treatments in the 2009/2010 growing cycle, and between hairy vetch and oat compared to oilseed rape and bare soil in the 2010/2011 growing cycle.

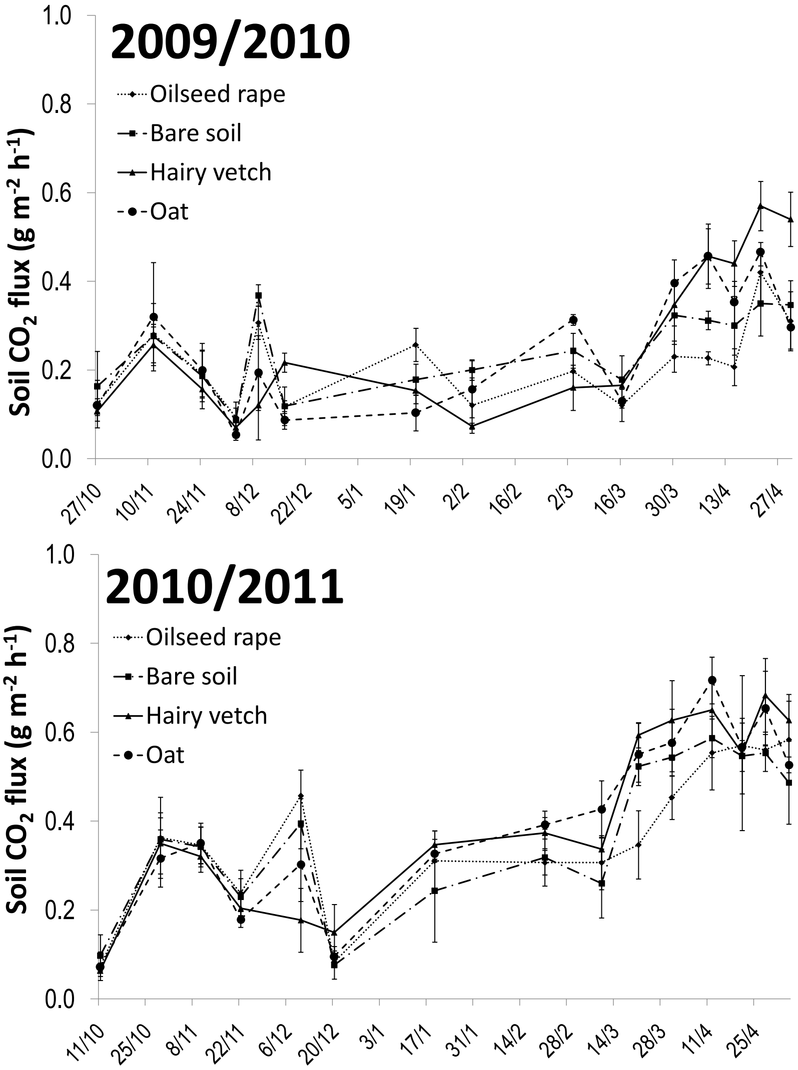

Figure 2. Soil CO_2 emission among the agro-ecological service crops treatments, during the 2009/2010 and 2010/2011 growing season of the agro-ecological service crops. Error bars represent ± standard error from mean (n = 3).

3.4. Soil Temperature during the Agro-Ecological Service Crop Growing Cycle

As expected, the soil temperature and climatic data observed during the cover crop growing period showed similar trends. From March to April in 2010 and from February to April in 2011, significant differences between the agro-ecological service crop treatments were observed. In particular, on 27 April 2010 (22.7 °C), and on 28 March 2011 (27.6 °C), in the oilseed rape treatment high soil temperature values were reached, while on 2 February 2010 (3.4 °C), and on 20 December 2011 (3.5 °C), in the bare soil treatment low values were observed (Figure 3).

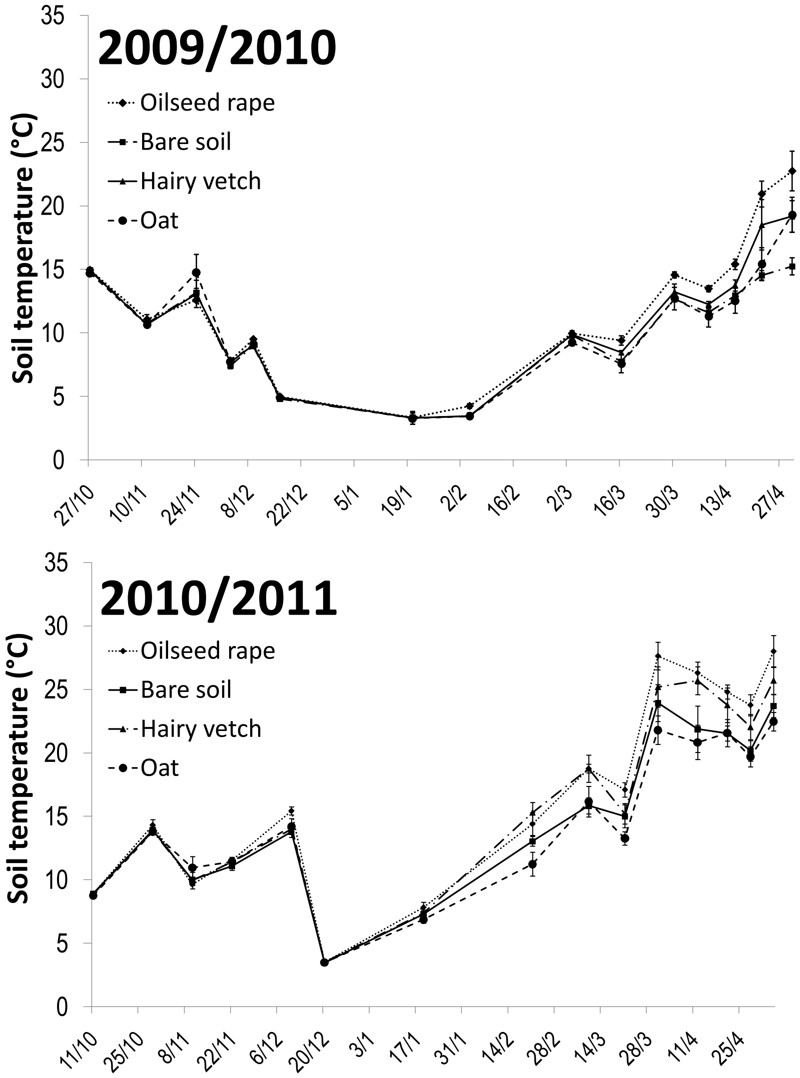

Figure 3. Soil temperature during the 2009/2010 and 2010/2011 growing cycles of the agro-ecological service crops. Error bars represent mean ± standard error (*n* = 3).

3.5. Soil Volumetric Water during the Agro-Ecological Service Crop Growing Cycle

In both years, the soil volume water content was generally influenced by weather conditions and mainly rainfall (Figure 4). Generally, from December onward, the agro-ecological service crop treatments showed higher water volume content than the bare soil. In 2000/2010, oilseed rape treatment showed high values of soil volumetric water content until March, in correspondence of high rainfall period and when all treatments showed similar value of soil volume water content. In the 2010/2011 growing period, a high value of soil volume water content in the hairy vetch treatment was observed, even if only in April, a high difference among the treatments was observed.

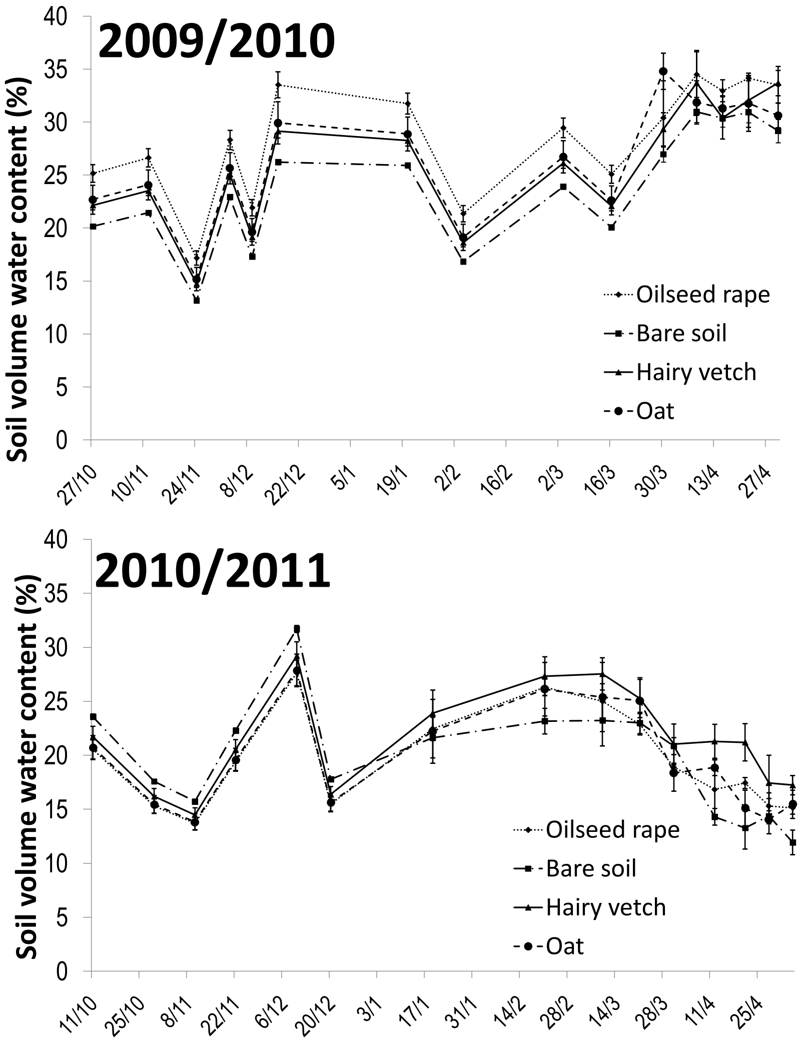

Figure 4. Soil volumetric water content among the agro-ecological service crops treatments, during the 2009/2010 and 2010/2011 growing cycles of the agro-ecological service crops. Error bars represent ± standard error from mean (*n* = 3).

3.6. Agro-Ecological Service Crop Biomass and Soil Characteristics

The total biomass, leaf/stem ratio (L/S), shoot/root ratio (S/R), N, and C accumulated in the total biomass of the agro-ecological service crop measured at their suppression are reported in Table 1. Total agro-ecological service crop biomass was significantly higher in the first year than the second year (averaged 919.7 vs. 695.5 g m^{-2} of dry matter (DM) in 2009/2010 and 2010/2011, respectively). The hairy vetch showed highest biomass production (on average of 1140.2 g m^{-2} DM), the oat resulted in intermediate value (on average of 763.8 g m^{-2} DM), and the amount of total biomass measured in oilseed rape was the lowest (on average of 518.8 g m^{-2} DM). Similarly, the leaf/stem ratio (L/S) was higher in hairy vetch and oat than oilseed rape (on average of 1.8 vs. 0.9, respectively), while no differences were observed between the growing cycles. Conversely, oilseed rape showed the highest value of shoot/root ratio (3.4). The nitrogen accumulated in the total biomass of the agro-ecological service crops at the time of their suppression was higher in hairy vetch than in oilseed rape and oat (on average of 33.1 vs. 7.1 g m^{-2}, respectively, Table 1). At the same time, the C accumulated in the aboveground biomass was highest in hairy vetch, intermediate in oat and low in oilseed rape (476.6, 336.1, and 218.9 g m^{-2}, respectively). The C/N ratio of the agro-ecological service crops was highest in oat, followed by oilseed rape and hairy vetch (data not shown).

Table 1. The main effects of growing cycle and agro-ecological service crop on the total biomass, shoot/root ratio (S/R), leaf/stem ratio (L/S), N and C accumulated in the biomass. Values belonging to the same variable followed by the same letter and are not statistically different according to LSD (0.05).

Treatments	Total Biomass (g m^{-2} of DM)	S/R	L/S	N (g of N m^{-2})	C (g of C m^{-2})
2009/2010	919.7 a	2.8 a	1.6 a	16.5 a	369.2 a
2010/2011	695.5 b	2.6 b	1.4 a	15.0 b	318.6 b
Hairy vetch	1140.2 a	2.1 b	1.9 a	33.1 a	476.6 a
Oat	763.8 b	2.6 b	1.6 a	6.9 b	336.1 b
Oilseed rape	518.8 c	3.4 a	0.9 b	7.3 b	218.9 c
A	*	*	ns	*	*
B	*	*	**	*	**
A × B	ns	ns	ns	ns	ns

LSD = Least Significant Difference, DM = Dry Matter; A = Agro-Ecological Service Crop; B = Growing Cycle.
*, ** or ns: Significance at $p \leq 0.05$, $p \leq 0.01$ or $p > 0.05$, respectively.

The soil total organic C (TOC) and soil mineral N (NO_3 + NH_4), soil CO_2 emission, and soil respiration index of agro-ecological service crop measured at their suppression are reported in Table 2. As expected, the TOC was similar among growing cycles and the agro-ecological service crops (on average 1.24%). The mineral nitrogen in the soil was higher in 2009/2010 than in 2010/2011, while among the agro-ecological service crops, it was higher in hairy vetch than oilseed rape, bare soil, and oat (35.6, 22.0, 19.6 and 17.4 mg kg^{-1}, respectively).

Table 2. The main effects of growing cycles and agro-ecological service crops on the soil total organic C (TOC), soil total N (TN), total mineral N (NO_3 + NH_4), C by CO_2 emission from agro-ecological service crops, soil respiration index and carbon input/output ratio. Values belonging to the same variable followed by the same letter and are not statistically different according to LSD (0.05).

Treatments	TOC (%)	Total mineral N (mg kg^{-1} dry soil)	C-CO_2 Emissions (g C m^{-2})	Soil Respiration Index	Carbon Input/Output Ratio
2009/2010	1.268 a	24.8 a	372 a	0.087 a	0.8 a
2010/2011	1.218 a	22.5 b	320 b	0.075 b	0.6 b
Hairy vetch	1.254 a	35.6 a	408 a	0.094 a	1.2 a
Oat	1.298 a	17.4 c	355 b	0.079 b	0.9 b
Oilseed rape	1.231 a	22.0 b	318 c	0.075 b	0.7 b
Bare soil	1.169 a	19.6 bc	305 c	0.076 b	0.0 c
A	ns	*	*	*	**
B	ns	*	**	**	*
A × B	ns	ns	ns	ns	ns

A = Agro-Ecological Service Crop; B = Growing Cycle. *, ** or ns: Significance at $p \leq 0.05$, $p \leq 0.01$ or $p > 0.05$, respectively.

The soil C-CO_2 emissions during the agro-ecological service crops were significantly higher in the first cycle than in the second (averagely 372 vs. 320 g C m^{-2} in 2009/2010 and 2010/2011, respectively), while among the agro-ecological service crops, the values recorded were highest in hairy vetch, intermediate in oat, and low in oilseed rape and bare soil (408, 355, and 312 g C m^{-2}, respectively, Table 2). In 2009/2010, soil respiration index was higher than in 2010/2011. Hairy vetch showed the highest value of soil respiration index compared to the other agro-ecological service crops (0.094 vs. 0.077, respectively, Table 2). The input/output ratio of C was higher in hairy vetch (1.2), intermediate in oat (0.9) and oilseed rape (0.7), and low in bare soil (0.0, Table 2).

4. Discussion

In this study, the effects of the cultivation of different agro-ecological service crops under Mediterranean climate conditions on short-term carbon balance, soil characteristics, and biomass production were evaluated throughout the growing cycle until their mechanical suppression before the preparation of the transplanting bed of the following main summer vegetable crop. To date, few studies have focused on the effect of agro-ecological service crops on CO_2 fluxes in agricultural soils [28], especially during their growing cycle. In fact, as observed by Papp et al. [4], the majority of studies investigate the effects of agro-ecological service crops after their suppression and throughout the cultivation of the following cash crop when their residues where managed in different way, such as green manuring or mulching the soil. As a consequence, this approach evaluates the combined effects of agro-ecological service crop residues in terms of rhizo-depositions release due to species cultivation and of residue mineralization. Conversely, the approach adopted in this study is aimed to provide knowledge on changes due to the cultivation of a specific agro-ecological service crop during its growth period.

The plant species adopted in this study as agro-ecological service crops were representative of cereal (oat), legume (hairy vetch), and cruciferous (oilseed rape) species, and were chosen because they are common and suitable for the cultivation in the temperate climate of the Mediterranean environment. Although the seedling emergence and establishment were uniform and regular among the three agro-ecological service crops, without any evident gaps, their canopy development followed different trends due to their inherent growth patterns and responses to the weather conditions. Indeed, based on the data regarding the FiPPDF, oat and oilseed rape showed a rapid establishment and growth compared to hairy vetch, resulting in high soil cover and, thus, in biomass production, especially during the first steps after their sowing. Conversely, soil coverage in hairy vetch was slow and started to grow rapidly only after the cold period reaching, before mechanical suppression, a complete soil coverage similar to oat (FiPPDF = 1). The results of this study confirmed that hairy vetch and oat could be well-suited plant species for their cultivation as agro-ecological service crops, in the temperate climate of the Mediterranean environment [3,5,18]. In fact, they were frost resistant and produced the highest amount of total biomass (shoots + roots), thus their cultivation could be encouraged in the Mediterranean environment, even if oat, as grass species, should be preferable for reducing nitrogen leaching risk [29] or suppress winter weeds [18], while hairy vetch, as legume species, should be suggested for its ability to fix high amount of atmospheric nitrogen and enhancing its availability after the suppression in a sustainable way throughout mineralization process [19]. Conversely, oilseed rape that grew fast after seeding was partly damaged (data not shown) by the winter frost, as shown by the FiPPDF data, and provided low amount of total biomass at the suppression. Similarly, Mancinelli et al. [5], in a previous study, observed high sensibility of cruciferous species to cold season in a similar environment. Furthermore, the data on leaf/stem ratio showed a higher number of leaves in hairy vetch and oat compared to oilseed rape, meaning a different canopy structure, mainly composed by materials more suitable to convert, after the mechanical suppression, in organic mulches, especially in the case of the adoption of conservation agriculture practices, as confirmed by the results reported by Mancinelli et al. [5] and Radicetti et al. [19] in similar environmental conditions.

In both growing cycles, the flux of CO_2 emissions follows similar trend among the agro-ecological service crops and tended to decrease during the cold period, then gradually increased until April when warm temperatures were observed. Similarly, Lal et al. [16] showed a gradual decrease of soil CO_2 emissions from summer to winter and an increase of soil emissions with the hotter temperature. According to Jacinthe et al. [30], soil CO_2 emissions strongly depend on weather conditions, indeed, the differences among the agro-ecological crop species were low and follow the soil temperature trend. However, the high biomass production in hairy vetch with low shoot/root ratio compared to oat and oilseed rape resulted in high and denser root biomass thus increasing microbial activity near to the soil surface [4]. As a consequence, rhizosphere and root respiration probably contributed significantly to the high soil CO_2 emission values observed in hairy vetch, even if considering the high amount of carbon accumulated on hairy vetch biomass, the input/output ratio resulted more favorable terms of carbon sink. However, the high soil CO_2 emissions and respiration index observed in hairy vetch was probably due to an intensive microbial activity that began to decompose the organic material from soil and plant residues. As a consequence, mineral nutrients, especially nitrogen, became more available in the soil compared to oat and oilseed rape. Although the soil CO_2 emissions and respiration index in oat and oilseed rape were significantly low compared hairy vetch, the carbon accumulated in their biomass determined a low input/output ratio and thus less efficient in term of carbon sink. These results confirmed that the cultivation of agro-ecological service crops, especially hairy vetch, could be adopted as a suitable strategy for enhancing short-term carbon dynamic and, thus, could contribute to the climate change mitigation if integrated in an integrated management strategy [31]. Furthermore, maximizing organic matter inputs, as observed in hairy vetch, could represent a way to add carbon to the soil (carbon sink) that could be a key factor for improving crop yields and reducing the need for synthetic fertilizers in agreement with Aguilera et al. [32], even if the addition of easily mineralizable carbon from agro-ecological crop residues could results in high CO_2 emissions throughout the cultivation of the following main summer crop in agreement with Bodner et al. [33]. However, as stated by Mancinelli et al. [5,14] the soil tillage practice adopted for managing the residues of agro-ecological service crops represents an important factor that could affect the C balance in the agro-ecosystems.

5. Conclusions

In this study, the replacement of bare soil with agro-ecological service crops during the fallow period under Mediterranean climate represents a sustainable practice for improving carbon sequestration and contribute to mitigate CO_2 emissions, even if the responses varied among the cover crops species. In fact, hairy vetch accumulated the largest amount of total biomass and carbon (input), also resulting in a better performance when soil CO_2 emissions (output) were considered. Furthermore, the high amount of nitrogen accumulated in the total biomass of hairy vetch during the growing cycle assured an elevate availability for the following cash crop through mineralization process. Although oat was less efficient in terms of C end N soil accumulation than hairy vetch, the biomass produced and the carbon sequestered encourages the adoption of legumes in Mediterranean environment, even if the adoption of oat should be well evaluated because could sensibly reduce soil nitrogen availability. Conversely, the adoption of oilseed rape seems unable to cover the need to accumulate carbon on its biomass, due to the risk of frost damage especially in the winter when is frequent in the Mediterranean environment. However, further research should be performed for evaluating the adoption of this species for other agro-ecological service.

Author Contributions: R.M. and E.R. planned the experimental design and developed the concept, E.R. collected and analysed samples with S.M., R.M. and E.R. performed statistical analysis with contribution of O.A.O. and A.R.S.L. All authors contributed to writing, discussing and commenting the manuscript.

Funding: This research received no external funding and it was funded by the University of Tuscia.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Almagro, M.; Garcia-Franco, N.; Martínez-Mena, M. The potential of reducing tillage frequency and incorporating plant residues as a strategy for climate change mitigation in semiarid Mediterranean agroecosystems. *Agric. Ecosyst. Environ.* **2017**, *246*, 210–220. [CrossRef]
2. Sainju, U.M.; Jabro, J.D.; Stevens, W.B. Soil carbon dioxide emission and carbon content as affected by irrigation, tillage, cropping system, and nitrogen fertilization. *J. Environ. Qual.* **2008**, *37*, 98–106. [CrossRef] [PubMed]
3. Radicetti, E.; Campiglia, E.; Marucci, A.; Mancinelli, R. How winter cover crops and tillage intensities affect nitrogen availability in eggplant. *Nutr. Cycl. Agroecosyst.* **2017**, *108*, 177–194. [CrossRef]

4. Papp, R.; Marinari, S.; Moscatelli, M.C.; van der Heijden, M.G.A.; Wittwer, R.; Campiglia, E.; Radicetti, E.; Mancinelli, R.; Fradgley, N.; Pearce, B.; et al. Short-term changes in soil biochemical properties as affected by subsidiary crop cultivation in four European pedo-climatic zones. *Soil Till. Res.* **2018**, *180*, 126–136. [CrossRef]
5. Mancinelli, R.; Marinari, S.; Brunetti, P.; Radicetti, E.; Campiglia, E. Organic mulching, irrigation and fertilization affect soil CO_2 emission and C storage in tomato crop in the Mediterranean environment. *Soil Till. Res.* **2015**, *152*, 39–51. [CrossRef]
6. Safahani Langeroodi, A.R.; Campiglia, E.; Mancinelli, R.; Radicetti, E. Can biochar improve pumpkin productivity and its physiological characteristics under reduced irrigation regimes? *Sci. Hortic.* **2019**, *247*, 195–204. [CrossRef]
7. Langeroodi, A.r.S.; Adewale Osipitan, O.; Radicetti, E. Benefits of sustainable management practices on mitigating greenhouse gas emissions in soybean crop (Glycine max). *Sci. Total Environ.* **2019**, *660*, 1593–1601. [CrossRef]
8. Iocola, I.; Bassu, S.; Farina, R.; Antichi, D.; Basso, B.; Bindi, M.; Dalla Marta, A.; Danuso, F.; Doro, L.; Ferrise, R.; et al. Can conservation tillage mitigate climate change impacts in Mediterranean cereal systems? A soil organic carbon assessment using long term experiments. *Eur. J. Agron.* **2017**, *90*, 96–107. [CrossRef]
9. Wittwer, R.A.; Dorn, B.; Jossi, W.; Van Der Heijden, M.G.A. Cover crops support ecological intensification of arable cropping systems. *Sci. Rep.* **2017**, *7*, 41911. [CrossRef]
10. Osipitan, O.A.; Dille, J.A.; Assefa, Y.; Knezevic, S.Z. Cover crop for early season weed suppression in crops: Systematic review and meta-analysis. *Agron. J.* **2018**, *110*, 1–11. [CrossRef]
11. Hartwig, N.L.; Ammon, H.U. Cover crops and living mulches. *Weed Sci.* **2002**, 688–699. [CrossRef]
12. Zuber, S.M. Long term effect of crop rotation and tillage on soil properties. *IDEALS* **2013**, *18*, 233–237.
13. Farina, R.; Marchetti, A.; Francaviglia, R.; Napoli, R.; Di Bene, C. Modeling regional soil C stocks and CO_2 emissions under Mediterranean cropping systems and soil types. *Agric. Ecosyst. Environ.* **2017**, *238*, 128–141. [CrossRef]
14. Mancinelli, R.; Marinari, S.; Di Felice, V.; Savin, M.C.; Campiglia, E. Soil property, CO_2 emission and aridity index as agroecological indicators to assess the mineralization of cover crop green manure in a Mediterranean environment. *Ecol. Indic.* **2013**, *34*, 31–40. [CrossRef]
15. Sainju, U.M.; Whitehead, W.F.; Singh, B.P. Agricultural management practices to sustain crop yields and improve soil and environmental qualities. *Sci. World J.* **2003**, *3*, 768–789. [CrossRef] [PubMed]
16. Lal, R. Soil carbon sequestration to mitigate climate change. *Geoderma* **2004**, *123*, 1–22. [CrossRef]
17. Gabriel, J.L.; Quemada, M. Replacing bare fallow with cover crops in a maize cropping system: Yield, N uptake and fertiliser fate. *Eur. J. Agron.* **2011**, *34*, 133–143. [CrossRef]
18. Radicetti, E.; Mancinelli, R.; Campiglia, E. Influence of winter cover crop residue management on weeds and yield in pepper (*Capsicum annuum* L.) in a Mediterranean environment. *Crop Prot.* **2013**, *52*, 64–71. [CrossRef]
19. Radicetti, E.; Mancinelli, R.; Moscetti, R.; Campiglia, E. Management of winter cover crop residues under different tillage conditions affects nitrogen utilization efficiency and yield of eggplant (*Solanum melanogena* L.) in Mediterranean environment. *Soil Tillage Res.* **2016**, *155*, 329–338. [CrossRef]
20. Cataldo, D.A.; Maroon, M.; Schrader, L.E.; Youngs, V.L. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Commun. Soil Sci. Plant Anal.* **1975**, *6*, 71–80. [CrossRef]
21. Baillie, I.C.; Anderson, J.M.; Ingram, J.S.I. Tropical Soil Biology and Fertility: A Handbook of Methods. *J. Ecol.* **2006**, *157*, 265. [CrossRef]
22. Pumpanen, J. Comparison of different chamber techniques for measuring soil CO2 efflux. *Agric. For. Meteorol.* **2004**, *123*, 159–176. [CrossRef]
23. Mancinelli, R.; Campiglia, E.; Di Tizio, A.; Marinari, S. Soil carbon dioxide emission and carbon content as affected by conventional and organic cropping systems in Mediterranean environment. *Appl. Soil Ecol.* **2010**, *46*, 64–72. [CrossRef]
24. Batjes, N.H.; Sombroek, W.G. Possibilities for carbon sequestration in tropical and subtropical soils. *Glob. Chang. Biol.* **1997**, *3*, 161–173. [CrossRef]
25. Littell, R.C.; Milliken, G.A.; Stroup, W.W.; Wolfinger, R.D. *SAS System for Mixed Models*; SAS Institute Inc.: Cary, NY, USA, 1996; ISBN 1555447791.
26. Cody, R.P.; Smith, J.K. *Applied Statistics and the SAS Programming Language*, 4th ed.; Prentice Hall: Upper Saddle River, NJ, USA, 1991.

27. Gomez, K.A.; Gomez, A.A. *Statistical Procedures For Agricultural Research*; John Wiley & Sons: New York, NY, USA, 1984.
28. Sanz-Cobena, A.; García-Marco, S.; Quemada, M.; Gabriel, J.L.; Almendros, P.; Vallejo, A. Do cover crops enhance N_2O, CO_2 or CH_4 emissions from soil in Mediterranean arable systems? *Sci. Total Environ.* **2014**, *466–467*, 164–174. [CrossRef] [PubMed]
29. Campiglia, E.; Mancinelli, R.; Radicetti, E.; Marinari, S. Legume cover crops and mulches: Effects on nitrate leaching and nitrogen input in a pepper crop (*Capsicum annuum* L.). *Nutr. Cycl. Agroecosyst.* **2011**, *89*, 399–412. [CrossRef]
30. Jacinthe, P.A. Carbon dioxide and methane fluxes in variably-flooded riparian forests. *Geoderma* **2015**, *241*, 41–50. [CrossRef]
31. Farina, R.; Testani, E.; Campanelli, G.; Leteo, F.; Napoli, R.; Canali, S.; Tittarelli, F. Potential carbon sequestration in a Mediterranean organic vegetable cropping system. A model approach for evaluating the effects of compost and Agro-ecological Service Crops (ASCs). *Agric. Syst.* **2018**, *162*, 239–248. [CrossRef]
32. Aguilera, E.; Guzman, G.; Alonso, A. Greenhouse gas emissions from conventional and organic cropping systems in Spain. I. Herbaceous crops. *Agron. Sustain. Dev.* **2015**, *35*, 713–724. [CrossRef]
33. Bodner, G.; Mentler, A.; Klik, A.; Kaul, H.P.; Zechmeister-Boltenstern, S. Do cover crops enhance soil greenhouse gas losses during high emission moments under temperate Central Europe conditions? *Die Bodenkultur* **2017**, *68*, 171–187. [CrossRef]