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Curriculum Produttività delle Piante Coltivate

Ciclo XXIX

CROPPING SYSTEMS FOR BIOMASS PRODUCTION UNDER MEDITERRANEAN CONDITIONS: IMPLANTATION TECHNIQUES AND SOIL CARBON BALANCE

dr. Pasquale Arca

Coordinatore del Corso	Prof. Antonello Cannas
Referente di Curriculum	Prof.ssa Giovanna Attene
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La presente tesi è stata prodotta durante la frequenza del corso di dottorato in Scienze Agrarie dell’Università degli Studi di Sassari, a.a. 2015/2016 - XXIX ciclo, con il sostegno di una borsa di studio cofinanziata con le risorse del P.O.R. SARDEGNA F.S.E. 2007-2013 - Obiettivo competitività regionale e occupazione, Asse IV Capitale umano, Linea di Attività l.3.1 “Finanziamento di corsi di dottorato finalizzati alla formazione di capitale umano altamente specializzato, in particolare per i settori dell’ICT, delle nanotecnologie e delle biotecnologie, dell'energia e dello sviluppo sostenibile, dell'agroalimentare e dei materiali tradizionali”.

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Chapter 1.

Chapter 2.

Chapter 3. **Published article on Catena**

Abstract

The reduction of GHG emission by replacing fossil fuel with biofuel is possible, but the agronomic practices for crop biomass production can have an important effect in the achievement of this objective.

In the rural district of Sulcis, in Southern Sardinia (Italy), we evaluated the effect of different implantation techniques of giant reed (*Arundo donax* L.) on biomass yield and the effect of different N fertilization levels on soil respiration and soil Carbon (C) balance in the early year of cultivation. In the dairy district of Arborea, in the Central-western coast of Sardinia (Italy), we evaluated the biomass production and soil C balance of maize (*Zea mays* L.)-based cropping system in relation to the different management of N fertilization sources.

The results suggested that, under fertile soil conditions, giant reed can be implanted using the cheapest propagation method (stem cutting) in spring or autumn, while under poor soil conditions, the rhizomes proved to be the best propagation method, independently by implantation season.

In the early stages of giant reed growth, compared to the unfertilized treatment, high N fertilization rates determined higher biomass yield, a depressing effect on soil heterotrophic respiration rates and a soil C balance not significantly different from equilibrium.

The fertilization with just organic fertilizer reduced the soil C budget in maize-based irrigated cropping systems, in sandy soils under Mediterranean climate.

Preface

The increment of greenhouse gas (GHG) emissions in the atmosphere is contributing to increase the demand of scientific knowledge on the emission reduction and soil C sequestration strategies (Perez-Lopez et al., 2013). The cropping systems for biomass production can contribute to achieve these objectives.

The cultivation of dedicated biomass crops on arable land is in competition for land availability, which can be used for food production and may contribute to increase the prices of forages and food commodities (Venturi, 2013). However, the cropping systems for biomass production can contribute to produce indirect environmental, agronomic and economic services to society depending on the context where they are developed.

Biomass is an important source of renewable energy that can be used to replace fossil fuels. The reduction of GHG emission by replacing fossil fuel with biofuel is possible, but the agronomic management for crop biomass production can have an important effect in the achievement of this objective (Pawlowski et al., 2017). The soil C sequestration can be enhanced in relation to the Nitrogen (N) fertilization management of the energy crop (Stewart et al., 2016).

The biomass crops can be cultivated also on marginal lands (Ghezehei at al., 2015; Stoof et al., 2015). The marginal and degraded lands are characterized by low productive potential. They are defined as land less suitable or totally unsuitable for food production (Gopalakrishnan et al., 2011). In addition, marginal lands characterized by environmental, social and economic constraints are likely to be abandoned (Heilig, 2002). In this context, biomass crops can represent an opportunity (Nasso e Di Nasso et al., 2012).

The biomass used for biofuel production can derive from dedicated energy crops, crop residues or food crops specifically cultivated for biomass production. The integration of perennial crops in the rotation plans of the current cropping systems can be more difficult than the annual crops. However, perennial crops need lower inputs than the annual crops (Wayman et al., 2014).

The hypothesis of this PhD thesis is that under Mediterranean conditions the agronomic management of annual and perennial crops for biomass production can affect the biomass yield and soil C balance, in the short-term.

The objective is to evaluate the short-term response of two different cropping systems for biomass production, based on annual and perennial crop, in terms of biomass yield and soil C balance in relation to different agronomic management under Mediterranean conditions.

Two experimental studies were carried out on maize (*Zea mays* L.)-based cropping system and giant reed (*Arundo donax* L.) cropping system in two different productive districts.

In the dairy district of Arborea, in the Central-western coast of Sardinia, the main cropping system is a double- crop rotation of silage maize and an autumn-spring hay crop. Such forage crops are used to feed the dairy cattle in a farming system, but the biomass produced from this cropping system can be used to biofuels production. The district is identified as Nitrate Vulnerable Zone (NVZ) according to the European Nitrate Directive (ND) (91/676/EC), which imposed a restriction on the N supplied from organic effluents (slurry and manure). In this context, it is important to evaluate the biomass production and soil C balance in relation to the different management of N fertilization sources.

In the rural district of Sulcis, in Southern Sardinia, there are marginal areas characterized by uncultivated, degraded and contaminated lands. In this context, the cropping systems for biomass production can represent an opportunity to reclaim these areas and to provide of additional income for farmers. Biochemtex agro is a company of the Mossi & Ghisolfi Group, specialized in second generation bioethanol production by transformation of lignocellulosic biomass. This company has interest in developing a new industry for bioethanol production in the disused industrial area of Portovesme, located in South Sardinia. Giant reed (*Arundo donax* L.) was identified a suitable to produce lignocellulosic biomass to feed the processing plant. Pulighe et al. (2016) showed that, among the perennial crops, giant reed is a “best options” for developing an agro-industry system for second generation bioethanol production in the Sulcis area. The cultivation of this bioenergy crop presents high implantation costs that can heavily affect the production costs of biomass feedstock. This phase also affects crop establishment and biomass yield especially in the early years of cultivation. In addition, in the long-term, under Mediterranean environment, the agroecosystem services produced by giant reed cultivation as soil C sequestration in relation to different N fertilization level were observed (Fagnano et al., 2015). In the short-term, there are little information on the impact of giant reed cultivation in terms of biomass yield and soil C balance in relation to different N fertilization management.

This thesis consists of three chapters:

Chapter 1. Effects of implantation techniques on giant reed (*Arundo donax* L.) biomass yield in the early years under Mediterranean conditions. The objective of this chapter was to evaluate the effect of different implantation techniques of giant reed on biomass yield and crop biometric characteristics. This study was carried out in two sites located in Central and Southern Sardinia in the first two years following implantation.

Chapter 2. Short-term effects of nitrogen fertilization on soil respiration and soil C balance in a Mediterranean giant reed (*Arundo donax* L.) cropping system. The objective of this chapter was to evaluate the short-term response of giant reed cultivation in terms of soil C balance in relation to different mineral N fertilization level. Soil respiration, soil C output, soil C input and biomass yield were evaluate during the first growing season of giant reed implanted in an experimental field located in the Sulcis area, South Sardinia.

Chapter 3. Manure fertilization increases soil respiration and creates a negative carbon budget in a Mediterranean maize (*Zea mays* L.)-based cropping system. This chapter was published in January 2017 on Catena journal:

“http://www.sciencedirect.com/science/article/pii/S0341816216305276”

The objective of this chapter was to analyze the effects of different fertilization sources on soil respiration, sensitivity of soil respiration to T and soil C balance in a Mediterranean maize (*Zea mays* L.)-based cropping system. This study was carried out in an experimental field located in Central Sardinia during a year of cultivation.

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CHAPTER I

Effects of implantation techniques on giant reed (*Arundo donax* L.) biomass yield in the early years under Mediterranean conditions

Effects of implantation techniques on giant reed (*Arundo donax* L.) biomass yield in the early years under Mediterranean conditions

1. Introduction

The increased greenhouse gas concentration in the atmosphere and the global warming are contributing to increase the sensitivity of policy makers on the renewable energies and sustainable development systems (Randazzo et al., 2007). Among the renewable energies, biofuels are viable alternative to fossil fuels used in transport and are encouraged by global policies (Tomei and Helliwell, 2016). Biomass for biofuels production can derive from crop residues and dedicated energy cops. The perennial energy crops need lower input than annual crops (Chandel and Singh, 2011) and in Mediterranean environments giant reed (*Arundo donax* L.) is considered particularly promising for second generation biofuels production because it is characterized by high yield potential, good attitude to energy conversion and positive environmental impact (Nassi o Di Nasso et al., 2013a).

This crop does not produce viable seeds and the agamic propagation is the only way for its establishment (Lewandowski et al., 2003) using rhizomes, stem cuttings and micropropagated plants.

The implantation of giant reed is a critical phase of the agronomic technique because it heavily affects the production costs of biomass feedstock. In addition, this phase can affect crop establishment and biomass yield especially in the early years of cultivation (Di Candilo and Ceotto, 2012). To date, rhizomes are considered the best propagation method to implant giant reed. However, the rhizomes present economic and technical limitations, related to the availability of propagation material (Cosentino and Copani, 2012). The rhizome propagation is characterized by high cost of explant, division and transplanting operations; in addition, the availability of this material is limited because there is not adequate nursery activity (Di Candilo and Ceotto, 2012). Stem cutting implantation is characterized by higher availability of propagation material and lower implantation costs than rhizome or micropropagated plant (Cosentino and Copani, 2012), even if the lack of a mechanization system for the material collection is still limiting this technique. Di Candilo and Ceotto (2012) reported that the production performance of implantation with stem cutting or micropropagated plant in the first two years is significantly lower than that obtained from rhizomes, due to the different dry matter content of propagules, which is higher in rhizomes.

Conventionally, in Central Italy rhizomes are implanted in spring (Angelini et al., 2005; Angelini et al., 2009; Nassi o Di Nasso et al., 2010; Nassi o Di Nasso et al., 2011a; Nassi o Di Nasso et al., 2011b; Ragaglini et al., 2014) because at the end of winter the rhizomes have the highest content of nutrients (Cosentino and Copani, 2012), while in Southern Italy Copani et al. (2013) obtained good results also with stem cuttings implanted in autumn. In Mediterranean environments, some authors obtained about 4 Mg ha^{-1} of dry matter yield in the first year by transplanting micropropagated plants in spring (Nassi o Di Nasso et al., 2013b).

Giant reed biomass yield increase from first to third year following implantation, when crop reached the maturity phase and the maximum annual production (Nassi o Di Nasso et al., 2013a).

In this paper we hypothesized that the propagation method and the implantation time could affect the crop establishment, the biometric characteristics and the biomass yield of giant reed in the early years after implantation.

The objectives of this study were: (i) to evaluate the interaction between year, propagation method (rhizomes and stem cuttings) and implantation season (autumn and spring) on biomass yield and culm biometric characteristics of giant reed in the first two years following implantation; (ii) to evaluate the interaction between year and propagation method (rhizomes, stem cuttings and micropropagated plantlets) under spring implantation on biomass yield and culm biometric characteristics of giant reed in the first two years following implantation.

2. Materials and Methods

2.1. Site description and crop management

The study was carried out in two areas located in the Southern and Central Sardinia. The experimental fields were established between October 2013 and March 2014 in Masainas (39°02'N 8°37'E, 57 m a.s.l.) and in Serramanna (39°24'N 8°52'E, 38 m a.s.l.). In Masainas the soil was characterized by sandy loam texture, while in Serramanna the soil texture was sandy clay loam (Table 1).

Two different experiments were conducted in each site: in the experiment (A) we tested two propagation methods, rhizomes and stem cuttings, and two implantation times, spring and autumn. In the experiment (B), three propagation methods, rhizomes, stem cuttings and micropropagated plants in the spring implantation were tested. At harvest in both experiments, biomass yield and biometric characteristics were measured at the end of the first and second growing season.

Part of rhizomes with a couple of buds weighing 250 g and entire stem cuttings used for new implantations were collected from river banks in a local riparian area. Rhizomes and stem cuttings were collected one week before implantation. Micropropagated plantlets derived from ecotypes selected in Tuscany (Central Italy).

The rhizomes and micropropagated plants were planted at a density of 1.1 plants m^{-2} (0.75 x 1.20 m spacing). Cuttings were buried overlapping 2 culms per row with distance between rows of 0.75 m. In both sites soil was tilled in autumn 2013, with medium deep plowing (35 cm) and shallow deep harrowing (15 cm). The autumnal implantation was made in November in both sites, the spring implantation in February and March in Serramanna and Masainas, respectively. In each site 76 kg ha^{-1} P and 65 kg ha^{-1} N were distributed as diammonium phosphate before implantation. In Masainas the top-dressing fertilization was made in June by applying 35 kg of N ha^{-1} as ammonium nitrate in the first growing seasons and 100 kg of N ha^{-1} as ammonium nitrate in the second year. In Serramanna the top dressing N fertilization was not applied in both growing seasons. Drip irrigation was supplied to avoid crop water stress by distributing overall about 600 mm year^{-1} of water in both sites. No weeding or pest controls were necessary.

A completely randomized design with three replications for autumnal transplanting and four replicates for the spring one was adopted for experiment (A). A completely randomized design with four replications was adopted for experiment (B). The plot size was 220 m^2 (36.6 x 6 m).

2.2. Aboveground biomass yield and biometric characteristics

Aboveground biomass yield and biometric characteristics were measured in February at the end of the first and second growing season. In the central rows of each plot the plants were sampled in an area of 12 m^2, while the plants from outer rows were not included in the sampling area. After cutting plants at the ground level, the number of culms was recorded and the harvested fresh biomass was weighted. Sub-samples of harvested biomass were chopped in the field, stored in plastic bags until they were dried at 60 °C in a forced-air oven until constant weight (Burle et al., 1997) to determine the dry matter (DM) content. Fifteen culms among the harvested plants in each sampling area were randomly selected to measure culm height and culm diameter.

2.3. Statistical analysis

Dry biomass yield, number of culms m^{-2}, culm height and culm diameter data were submitted to Shapiro-Wilk normality test and Levene's test (for homogeneity of variance) to verify if the ANOVA assumptions were met. Subsequently, the data were submitted to the analysis of variance using as fixed factors year, propagation method and implantation time for experiment (A) and year and propagation method for experiment (B). The treatment means were compared using a Tukey test at $P < 0.05$. To explain the relationships between biomass dry yield and biometric characteristics the regression analysis was performed using all values recorded in two experiments in both sites.

All statistical analyses were performed using the R software (R Core Team, 2015).

3. Results

3.1. Weather

The Masainas site is about 60 km from Serramanna and the climate in both sites is similar. The study area is characterized by Mediterranean climate with hot dry summers and mild rainy winters (Figure 1). The mean annual temperature is 16.3 °C and the long-term (from 1995 to 2016) average annual rainfall is 447 mm. The long-term average monthly maximum temperature is 32.3 °C in August and 13.5 °C in January. The long-term average monthly minimum temperature is 4.6 °C in February and 18.4 °C in August. The most rainy month is November with an average rainfall of 71 mm, July is the most dry with 2.2 mm of average rain. The average annual aridity index (rainfall/reference evapotranspiration) is 0.51, which corresponds to a semi-arid climate (Lai et al., 2017; Middleton and Thomas, 1992).

The annual rainfall in Serramanna was 425 mm in both growing seasons (2014 and 2015), while in Masainas it was 581 and 658 mm in the first and second year, respectively. In November 2013, during soil tillage and giant reed implantation, 123 mm of rain (52 mm above the long-term average) were observed. Autumn 2014 was particularly dry with 92 and 48 mm of rain below the average (from September to November, corresponding to a -56% and -33%) in Serramanna and Masainas, respectively. The precipitation recorded in Masainas was abundant in February and March 2015, with 360 mm of rain *vs* the long-term average value of 85 mm.

3.2. Aboveground biomass yield and biometric characteristics

Experiment (A)

We observed a significant increment of aboveground biomass yield from the first to the second year in both site (Table 2). In Masainas we recorded a dry biomass yield respectively of 7.2 and 26.9 Mg ha^{-1} DM in the first and in the second year, with a 73% increment. In the unfertilized field of Serramanna we observed lower dry biomass yield than in Masainas, with 3.9 Mg ha^{-1} DM in the first year, 14.8 Mg ha^{-1} DM in the second year and a 74% increment (Table 3).

Biomass DM yield showed a significant year x implantation time interaction in both sites and a significant year x propagation method interaction on biomass yield only in Serramanna (Table 2). In addition, in Serramanna biomass DM yield showed a significant implantation time x propagation method, with higher values observed in spring with rhizome implantation (Table 2).

In Masainas, in the first year we recorded a yield of 10.2 and 4.9 Mg ha^{-1} DM in autumn and spring implantation, respectively; in the second year we observed mean values of 22.8 and 29.9 Mg ha^{-1} DM respectively with autumn and spring implantation (Figure 2a). In Serramanna, autumn and spring implantation yielded respectively 2.4 and 4.9 Mg ha^{-1} DM in the first year and 10.7 and 17.9 Mg ha^{-1} DM in the second year (Figure 2b). With stem cuttings and rhizomes implantation, we observed respectively 3.5 and 4.2 Mg ha^{-1} DM in the first year and 12.2 and 17.5 Mg ha^{-1} DM in the second year (Figure 2c).

Culm density was 16.8 and 18.1 culms m^{-2} in Masainas and Serramanna, respectively. A significant year x implantation time interaction on culm height we observed in Serramanna (Table 2 and Figure 3), where higher values were achieved in the second year with spring implantation. We observed in both sites a significant effect of the year on culm diameter and culm height (Table 2), with higher values in the second year (Table 4). The implantation time influenced the culm height (Table 2), with higher values with spring implantation in Masainas and Serramanna (Table 4).

Experiment (B)

Biomass DM yield showed a significant year x propagation method interaction only in Serramanna (Table 5). In the second year, giant reed planted with rhizomes showed significantly higher yield (21.9 Mg ha^{-1} DM) than stem cuttings and micropropagated plants (13.9 and 12.6 Mg ha^{-1} DM, respectively), but in the first year no significant differences were observed between the three propagation methods, which yielded on average 5.2 Mg ha^{-1} DM (Figure 4). The mean values of aboveground biomass yields in the second year were 16.10 Mg ha^{-1} DM corresponding to a 68% average increment. In Masainas, aboveground biomass yield was influenced only by the year independently by propagation method (Table 5), with 82% average increment from the first to the second year (5.5 and 30.0 Mg ha^{-1} DM, respectively).

Culm density was not significantly influenced by any of the treatments (Table 5), being on average 19.4 culms m^{-2} in both sites. In Masainas, culm diameter was influenced by year (Table 5), with means values of 10.5 and 14.4 mm in the first and second year, respectively. Implantation with stem cutting showed higher culm diameter than that with micropropagated plants (13.9 *vs* 11.4 mm respectively), while rhizomes showed intermediate values (12.4 mm). Culm height was influenced by year (Table 5), with mean values of 199 and 350 cm in the first and second year, respectively. In Serramanna we observed a significant year x propagation method interaction for culm diameter and culm height (Table 5). From the first to the second year, rhizome and micropropagated implantation showed opposite behavior in terms of culm diameter and culm height (Figure 5). In the first year higher values of culm diameter and culm height were observed with micropropagated propagation and lower values with rhizome propagation (10.9 *vs* 10.1 mm and 175 *vs* 155 cm, respectively). In the second year maximum values were observed with rhizome, while with micropropated plants lower values were observed (14.3 *vs* 11.6 mm and 312 *vs* 213 cm, respectively). Intermediate values were observed using stem cuttings in both years.

3.3. Relationship between DM yield and biometric characteristics

The biomass yield increased linearly with culm height and culm diameter (R^2 = 0.79*** and 0.61***, respectively) (Figure 6). Culm height and culm diameter were also positively correlated (R^2 = 0.81***), while no significant relationship was observed between the culm density and the other crop parameters.

4. Discussion

This study confirms that giant reed is one of the most promising candidate bioenergy crops for the cultivations in Mediterranean area related to its suitability and productive potential observed in the early years after implantation, according to a previous study carried out in Southern Sardinia (Pulighe et al., 2016).

The crop showed good establishment and yield performance in the first two growing seasons independently from the propagation method and implantation time, as long as the crop nutritional requirements were met and the implantation operations were accurately managed. In fact in the site of Masainas, where the top dressing fertilization was made in both two years, the biomass yields obtained from different propagation methods were not significantly different (Table 2 and Table 5). Instead, in the experimental fields of Serramanna, where only pre-implantation fertilization was applied in the first year, the implantation with rhizomes in the second year produced higher yield than the other propagation methods (Figure 2c and Figure 4). This result can be explained by the higher nutrient content in rhizomes compared to stem cuttings and micropropagated plants (Di Candilo and Ceotto, 2012), which favored this propagation method under poor soil conditions. In addition, the establishment of the crop planted in autumn in Serramanna was negatively affected by the abundant rainfall that occurred during the soil tillage and implantation operations. Rhizomes and stem cuttings were implanted under critical structural soil conditions and the biomass yield of the autumn implantation was lower than that of the spring implantation. This difference was observed especially in the second year (Figure 2b), when autumn and spring implantations had equal length of the growing season. The results observed under poor soil conditions and without careful management of implantation operations confirmed that spring was the best implantation time and rhizome was the best propagation method, as reported in numerous studies carried out under Mediterranean conditions. However, successful implantation with stem cutting and in the autumn season is possible (Copani et al., 2013).

The yield increment from the first to the second year was observed in all experimental trials (Table 2 and Table 5). This result confirmed that giant reed in the second year had not reached yet the maturity phase, that usually corresponds to the third year after implantation (Nassi o Di Nasso et al., 2013a). The average yield increment of 74% was in agreement with that observed by Di Candilo and Ceotto (2012) in Northern Italy. In contrast with our results, in Central and Northern Italy other authors (Angelini et al. 2005; Angelini et al, 2009; Di Candilo et al., 2005) reported high values of yield (20 - 30 Mg ha^{-1} DM) already in the first year, with increments from the first to the second year of about 50%. In Southern Italy, under irrigation, Copani et al. (2013) observed a biomass production in the second year comparable with the yields observed in our experimental trials, even if the yield increment was lower. However, in Southern Italy under rainfed conditions Fagnano et al. (2015) in the second year of the cultivation reported lower values of biomass yield (about 10 Mg ha^{-1} DM) and yield increment (about 50%). The productive performance of giant reed in the early year could be influenced by several factors as the different implantation density (Di Candilo and Ceotto, 2012). In addition, differences of crop behavior and yield performance could be linked to different crop management, soil characteristics and genetic characteristics of the propagation material (Angelini et al., 2009).

The biomass yield increment from the first to the second year observed in all experiments was due to the crop growth in terms of culm height and culm diameter, as indicated by positive relationships between biomass yield and the both biometric parameters (Figure 6). The culm density did not contribute to the yield increment, differently from what observed by Cosentino et al. (2006). These results were in agreement with Angelini et al. (2009), which showed that biomass yield increased linearly with culm height and culm diameter. In addition, consistently with our results, they reported a positive relationship between culm height and culm diameter. The effect of the different tested factors on culm height and culm diameter was similar to that observed on biomass yield, confirming the relationship between the culm biometric parameters and yield.

5. Conclusion

Our findings indicate that under fertile soil conditions and with an accurate management of the implantation operations giant reed can be implanted using the cheapest propagation method in spring or autumn. Stem cutting is the less expensive propagation method and it showed good establishment and yield performance already in the early years. A careful management of the soil tillage and implantation allowed to obtain same results from giant reed cultivated in autumn or spring, which is an important aspect to consider for the giant reed implantation on large-scale.
However, adverse weather conditions during implantation can prevent the success of the crop establishment if the soil tillage and implantation operations are not accurate. Under poor soil

conditions without fertilization, the rhizomes proved to be the best propagation method, independently by implantation season.

The biomass yield increments from the first to the second year were remarkable; in particular, the growth of the crop was determined by plant development in terms of height and diameter.

Future studies are needed to evaluate the behavior and the productive response of the crop planted with different techniques after plant maturity.

Tables

Table 1 Soil characteristics before the experimental fields establishment in both sites (0-0.60 m; October 2013)

Soil characteristics	Unit	Masainas	Serramanna	International Ufficial Method
Sand (2–0.05 mm)	%	63.4	54.7	ISO 11277
Silt (0.05–0.002 mm)	%	17.4	24.3	ISO 11277
Clay (<0.002 mm)	%	19.3	21.0	ISO 11277
pH		7.5	6.7	ISO 10390
Organic matter	%	1.62	1.49	ISO 10694
Total N	‰	0.83	0.72	ISO 10694
Available P (Olsen)	ppm	25.7	30.2	Italian Ufficial Method n. XV.3
Exchangeable K	ppm	299	138	ISO 11260

Table 2 Experiment (A) - Summary of analysis of variance of the dry aboveground biomass yield, culm density, culm diameter and culm height of giant reed in both sites in the first and second growing season

	Masainas				**Serramanna**			
	CDN ($n. m^{-2}$)	**CDM (mm)**	**CHG (cm)**	**DMY ($Mg ha^{-1}$ DM)**	**CDN ($n m^{-2}$)**	**CDM (mm)**	**CHG (cm)**	**DMY ($Mg ha^{-1}$ DM)**
A	NS	***	***	***	NS	***	***	***
B	NS	NS	NS	NS	NS	NS	NS	*
C	NS	NS	**	NS	NS	NS	***	***
A x B	NS	NS	NS	NS	NS	NS	NS	*
A x C	NS	NS	NS	***	NS	NS	*	*
B x C	NS	NS	NS.	NS	NS	NS	NS	NS
A x B x C	NS	NS	NS	NS	NS	NS	NS	NS

A = first year/second year; B = rhizomes implantation/stem cuttings implantation; C = autumn implantation/spring implantation. *, **, *** and NS significance at $P \leq 0.05$, 0.01 and 0.001 level of probability and non-significant. CDN : culm density, CDM : culm diameter, CHG : culm height, DMY : dry matter biomass yield.

Table 3 Experiment (A) - Mean values of dry aboveground biomass yield (Mg ha-1 DM) and yield increment from the first to the second year in both sites

	Masainas ($Mg ha^{-1}$)	Serramanna ($Mg ha^{-1}$)
Year I	7.2 ±1.04	3.9 ±0.55
Year II	26.9 ±1.30	14.8 ±1.48
Yield increment	+ 73%	+ 74%

± standard error

Table 4 Experiment (A) – Mean values of culm diameter (mm) and culm height (cm) in the first and second year and mean values of culm height (cm) in autumn and spring implantation in both sites.

Site	Culm diameter (mm)		Culm height (cm)			
	Year I	Year II	Year I	Year II	Autumn time	Spring time
Masainas	10.8 ±0.26	14.6 ±0.34	193 ±4.91	346 ±7.02	258 ±23.05	284 ±20.09
Serramanna	10.0 ±0.36	13.1 ±0.37	146 ±7.60	249 ±14.65	164 ±12.20	222 ±18.04

± standard error

Table 5 Experiment (B) - Summary of analysis of variance of the dry aboveground biomass yield, culm density, culm diameter and culm height of giant reed in both sites in the first and second growing season

	Masainas				**Serramanna**			
	CDN ($n. m^{-2}$)	**CDM (mm)**	**CHG (cm)**	**DMY ($t ha^{-1}$ DM)**	**CDN ($n m^{-2}$)**	**CDM (mm)**	**CHG (cm)**	**DMY ($t ha^{-1}$ DM)**
A	NS	***	***	***	NS	***	***	***
B	NS	**	NS	NS	NS	NS	*	*
A x B	NS	NS	NS	NS	NS	*	**	*

A = first year/second year; B = rhizomes implantation/stem cuttings implantation/micropropagated implantation. *, **, *** and NS significance at $P \leq 0.05$, 0.01 and 0.001 level of probability and non-significant. CDN : culms density, CDM : culm diameter, CHG : culm height, DMY : dry biomass yield.

Figures

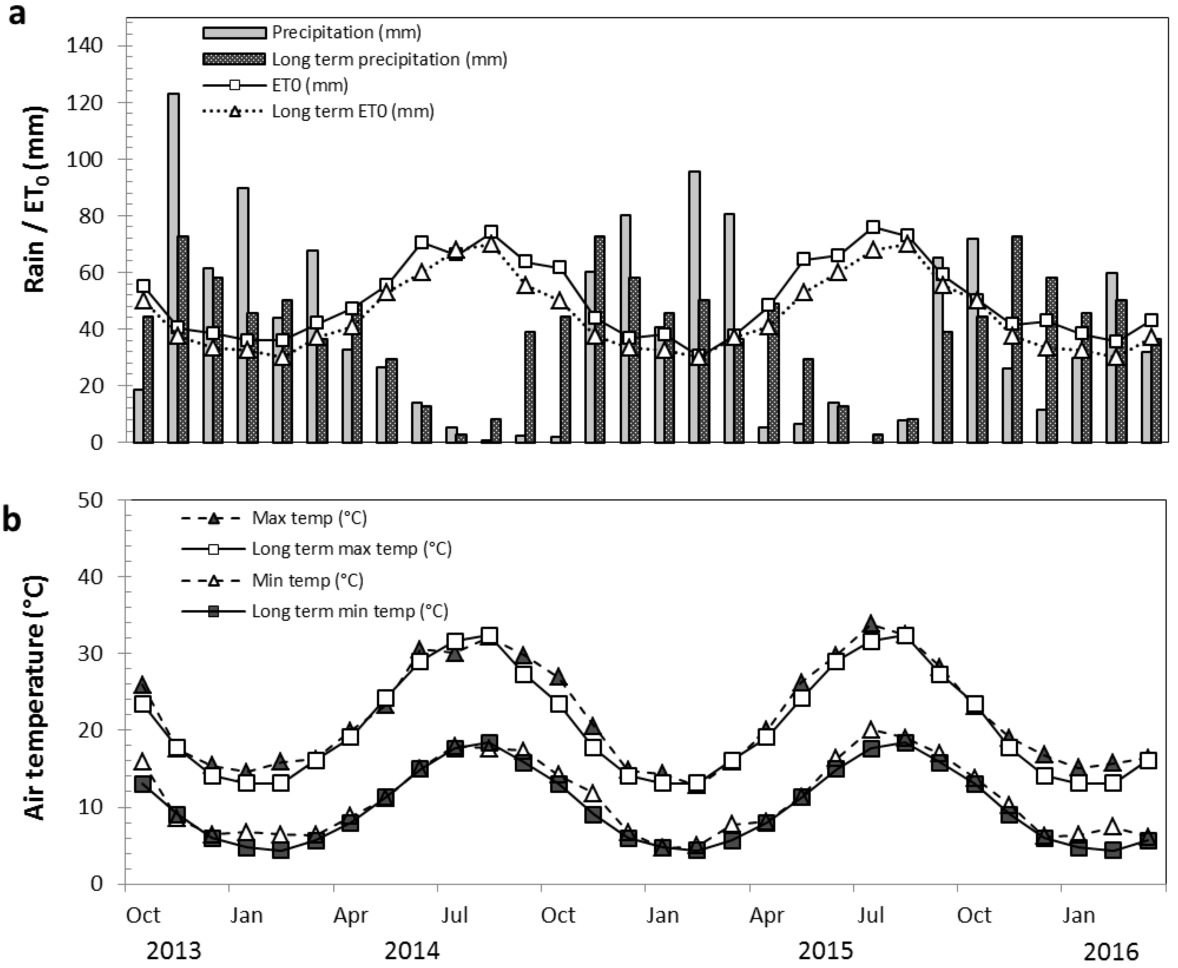

Figure 1 (a) Long-term and monthly precipitation (mm) and long-term and monthly reference evapotranspiration (ET0) (mm), (b) long-term and monthly minimum and maximum air temperature (°C) recorded in Samassi meteorological station between October 2013 and March 2016. This station was located 5 km to Serramanna experimental field and it belongs to Regional Agency for Environmental Protection of Sardinia (ARPAS).

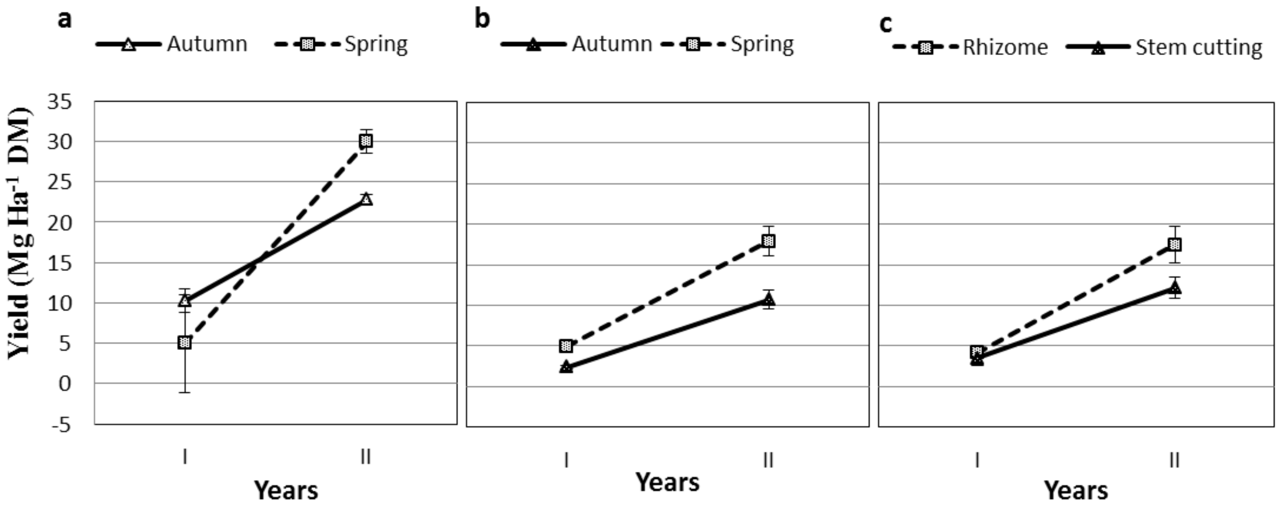

Figure 2 Experiment (A) - Effect of year x implantation time interaction on dry aboveground biomass yield in Masainas (a) and Serramanna (b) and effect of year x propagation method interaction on dry aboveground biomass yield in Serramanna (c). Vertical bars indicate ± standard errors.

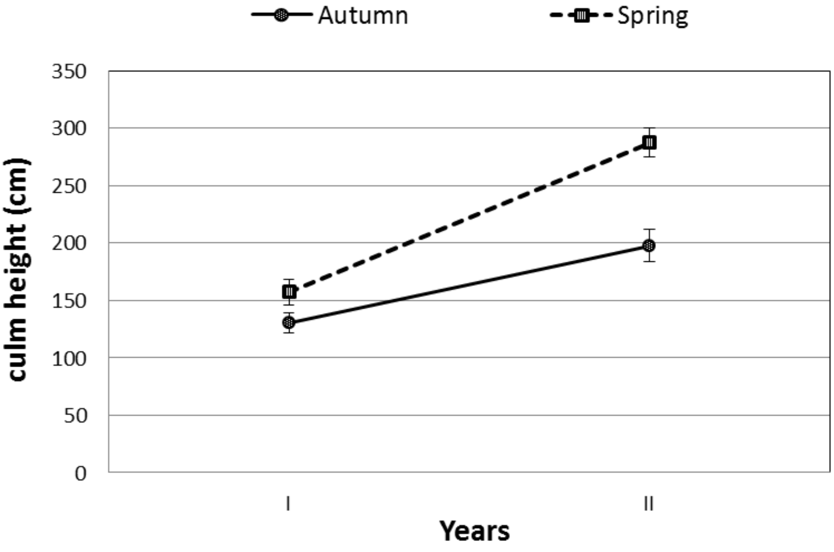

Figure 3 Experiment (A) - Effect of year x implantation time interaction on culm height in Serramanna. Vertical bars indicate ± standard errors.

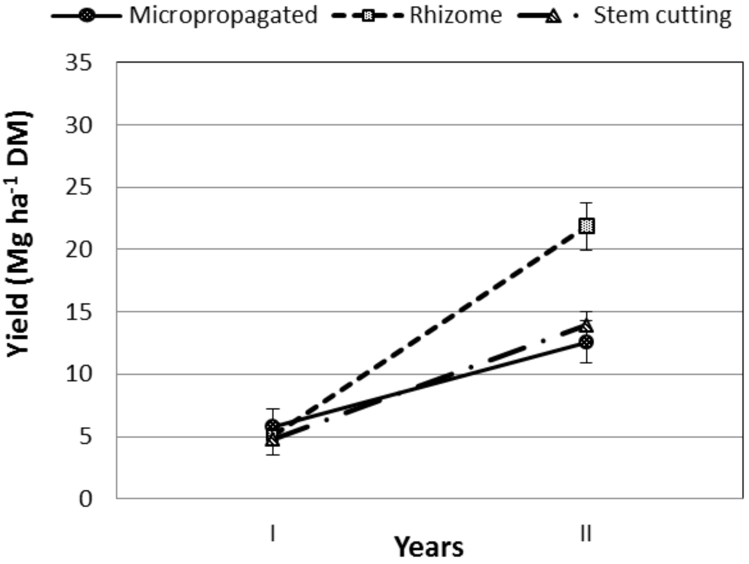

Figure 4 Experiment (B) - Effect of year x propagation method interaction on dry aboveground biomass yield in Serramanna. Vertical bars indicate ± standard errors.

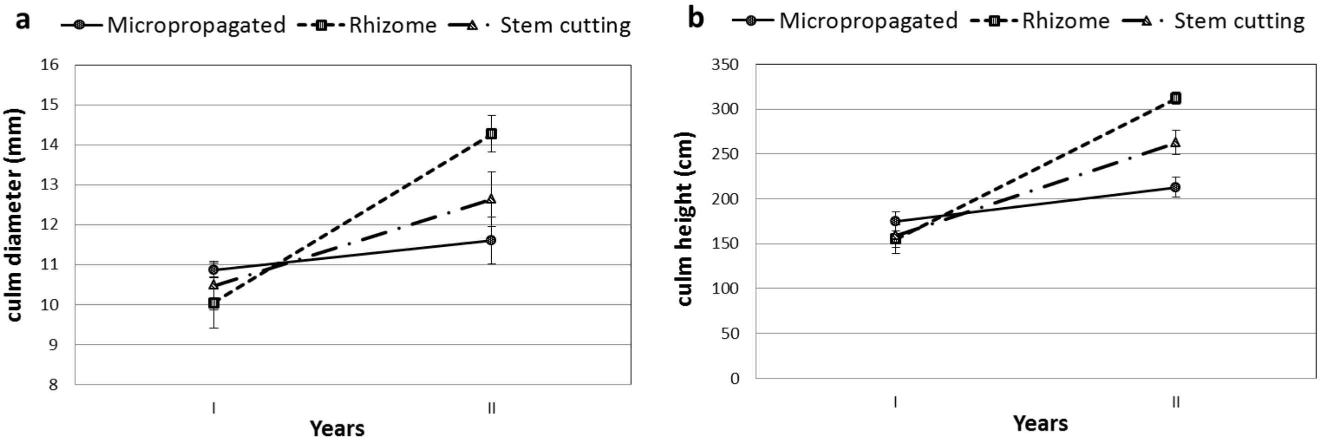

Figure 5 Experiment (B) - Effect of year x propagation method interaction on culm height (a) and culm diameter (b) in Serramanna. Vertical bars indicate ± standard errors.

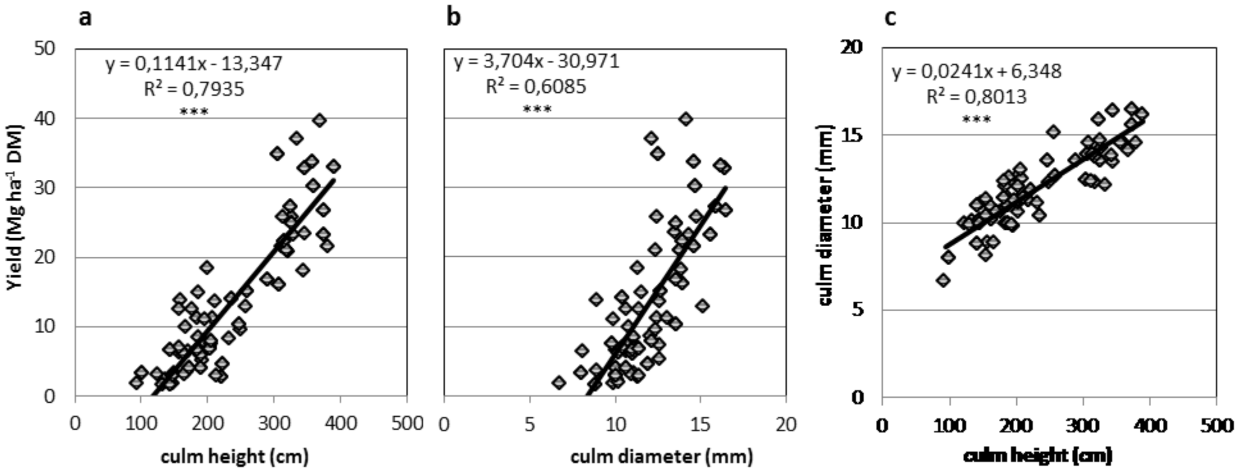

Figure 6 Relationship between (a) culm height (x) and dry yield (y), (b) culm diameter (x) and dry yield (y), (c) culm height (x) and culm diameter (x) using all values recorded in the two experiments in both sites. *, **, *** Significant at the 0.05, 0.01 and 0.001 probability level.

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CHAPTER II

Short-term effects of nitrogen fertilization on soil respiration and soil C balance in a Mediterranean giant reed (*Arundo donax* L.) cropping system

Short-term effects of nitrogen fertilization on soil respiration and soil C balance in a Mediterranean giant reed (*Arundo donax* L.) cropping system

1. Introduction

Perennial bioenergy cropping systems play an important role in the programs of climate change mitigation, through their potential in reducing greenhouse gas (GHG) emissions and improving soil carbon (C) sequestration (Robertson et al., 2016). Among the perennial rhizomatous crops for biomass production, giant reed (*Arundo donax* L.) is a promising energy crop for the Mediterranean environment because of its low input requirements and high biomass productivity (Pulighe et al., 2016; Nassi o Di Nasso et al., 2013). In addition, this crop is adapted to marginal land and can improve the soil fertility (Fagnano et al., 2015). The environmental sustainability and the productive performance of giant reed cropping systems depend on the management practices (Cosentino et al., 2014). Despite giant reed can produce interesting amount of biomass at low Nitrogen (N) fertilization rates, higher yield can be obtained with N addition (Fagnano et al., 2015; Cosentino et al., 2014). Overall, N fertilization is a common field practice used to improve yield and soil fertility (Dietzel et al., 2015; Van Grinsven et al., 2015). However, some authors showed that the N addition can have variable effects on the soil C stock (Halvorson et al., 2002; Liang and Mackenzie, 1992).

Aboveground residues and belowground biomass are the primary sources of soil C stock (Wilson & Al-Kaisi, 2008). In perennial grasses the high amount of belowground and aboveground biomass that returns to the soil as residues can enhance the soil C stock capacity (Agostini et al., 2015). Among these, giant reed has the advantages to develop over years a remarkable root system (Chimento & Amaducci, 2015; Monti & Zatta, 2009) which can be stimulated by N fertilization. Belowground biomass affects C sequestration through rhizomes, fine roots and rhizodeposition biomass (Agostini et al., 2015).

N fertilization can influence soil respiration (SR). SR is composed by two fractions, the heterotrophic respiration (Rh), due to the soil microbial activity, and the autotrophic respiration (Ra), due to the root activity. SR, Rh and Ra are differently influenced by biotic and abiotic factors, including soil mineral N availability (Hanson et al., 2000). The application of mineral N fertilizers has a depressive effect on the microbial activity (Kowalenko et al., 1978), but in the long-term, the increment of biomass crop related to N addition determines high amount of plant residues, which stimulate an increase of Rh (Morell et al., 2010). The root activity is stimulated by increasing levels of N supply (Zhou et al., 2014). The effects of the mineral N on SR emission rates depend by the combined response of Ra and Rh to N availability (Liu et al., 2016; Wilson & Al-Kaisi, 2008). Overall, seasonal variation of SR and Rh is affected by environmental conditions, such as soil temperature and soil moisture level. Under Mediterranean irrigated conditions, the main environmental driver of seasonal variation of SR and Rh is soil temperature (T), whereas soil water content (SWC) is less important (Lai et al., 2017). The response of SR to the variation of soil T can be considered an useful index to evaluate the C balance of agroecosystems (Liu et al., 2016; Jia et al., 2013). Temperature sensitivity of SR can also be influenced by the N fertilization management (Liu et al., 2016).

The soil C balance can be estimated as the difference between soil C input from crop residues and soil C output from accumulated Rh (Lai et al., 2017). The effect of the N fertilization management on soil C balance emerges from the combined response of soil C input and soil C output to different N fertilization rates.

While there is evidence that in the long-term giant reed can improve the soil C stock of degraded soils (Fagnano et al., 2015), there is not enough information about the short term effects in the early stages of the crop following implantation. The establishment year of giant reed cultivation is characterized by the soil tillage before implantation and lower biomass production compared with the maturity phase. Thus, during the establishment year the amount of C sequestration in the soil at harvesting may be lower than the soil C lost by Rh, due to the low biomass accumulation potential of the crop and the higher mineralization rate of soil. The behavior of giant reed in relation to the N fertilization management during the establishment year can provide relevant information to explore the short-term effect of implantation on SR and to identify best options to maximize C sequestration in the early stages.

The hypothesis of this study is that in a Mediterranean irrigated giant reed cropping system, in the first year after implantation, N additions could influence (i) the crop yield and consequently the amount of soil C input from aboveground residues and belowground biomass; (ii) the SR and Rh dynamics and their relationship with T and SWC, as well as the total C-CO_2 losses from soil; (iii) the soil C balance.

The objectives of this study were to evaluate the effects of different N fertilization rates on (i) the temporal dynamics of SR and Rh rates; (ii) the relationships between soil T and SWC with SR or Rh; (iii) the soil C inputs deriving from above- and belowground biomass and soil C output from cumulative Rh; (iv) the soil C balance at the end of the first growing season.

2. Materials and methods

2.1. Study site

This study was carried out in an experimental field in Tratalias (39°06′ N 8°35′ E, 17 m a.s.l.) in the rural district of Sulcis, Southern Sardinia, Italy. The site is characterized by Mediterranean climate with warm, dry summers and mild, wet winters. The long-term (last 70 years) mean annual average temperature is 17.0 °C and the average annual rainfall is 447 mm. The pluriannual average maximum temperature in July and August is 32.8 °C, the pluriannual average minimum temperature in January-February is 5.6 °C. The average annual aridity index (rainfall/reference evapotranspiration) is 0.51, which corresponds to a semi-arid climate (Middleton and Thomas, 1992).

Soil samples were collected in each plot of the experimental trial (see below), at 30 cm depth, in June 2014 and analyzed for texture, bulk density, pH, total calcium carbonate, organic matter, total nitrogen, available phosphorus and exchangeable potassium. Analytical procedures and results of physical and chemical soil characterization are reported in Table 1. Soil type was clay with high organic matter content. The bulk density and the soil hydraulic properties were estimated with the SPAW hydrology model (Saxton & Rawls, 2006). Bulk density was estimated to be 1.38 g cm^{-3} and the SWC at 0, -33 and -1500 kPa was 0.48 m^3 m^{-3}, 0.41 m^3 m^{-3} and 0.24 m^3 m^{-3}, respectively.

2.2. Experimental layout and crop management

The field experiment was conducted during the establishment year of a giant reed plantation treated according to three different levels of N supply. The following N levels were compared following a randomized complete block experimental design with three replications: i) N0, no N fertilizer supply; ii) N50, fertilization with 50 kg of N ha^{-1} supply; iii) N100, fertilization with 100 kg of N ha^{-1} supply. Top dressing fertilization was performed manually on 20th June using ammonium nitrate. The plot size was of 528 m^2 (12 x 44 m) with no spacing between adjacent plots.

In the years, previous to the experiment, the field was used for annual forage crops such as barley and oats. Soil was ploughed in winter 2015 and all farming operations were carried out with conventional agricultural machines. Following tillage at 30 cm depth with mouldboard plough, each plot was fertilized with 185 kg of P_2O_5 ha^{-1} as a triple superphosphate. The seedbed was prepared by harrowing at 20 cm soil depth prior to manually planting two part of rhizomes (with a couple of buds, weighing 250 g) per square meter on 4 March 2015 at 10-20 cm depth with 75 cm row spacing.

During the entire growing season soil water availability was never limiting in all treatments as drip irrigation was supplied in spring and summer (Figure 1). No weeding or pest controls were necessary.

2.3. Soil C balance

Soil C balance was assessed at the end of the first growing season as the difference between soil C inputs from plant residues and soil C-CO_2 losses from heterotrophic soil respiration (Lai et al., 2017). Similarly to Robertson et al. (2016), the C balance was estimated for the soil tilled layer (30 cm depth), the most sensitive to variation of soil organic C (SOC) content. Soil C inputs were assessed by measuring the aboveground and belowground plant residues left in the soil at harvest. C output were estimated from the amount of soil C-CO_2 losses as cumulative heterotrophic soil respiration at the harvest at the end of the establishment year. The C losses from leaching and erosion were negligible and hence not considered in the C balance calculation (Mancinelli et al., 2010).

2.4. Soil C input from plant residues

The soil C inputs were measured at harvest at the end of the establishment year assuming a biomass C content of 45%, as reported by other authors (Bolinder et al., 2007; Agostini et al., 2015). Aboveground and belowground biomass was measured by sampling plants and residues left onto the soil at harvest.

Aboveground biomass

The harvest was conducted manually on March 4, 2016 and the aboveground biomass was partitioned into three fractions: i) cut and removed biomass (harvestable biomass), ii) uncut biomass and iii) residual aboveground biomass. The last two fractions represented the aboveground biomass residues considered in the soil C input calculation.

Harvestable biomass was measured in two sampling areas, each of 18 m^2 (6 x 3 m) in each plot. All culms inside the sampling areas were cut at 20 cm above the ground level and were immediately tied into bundles.

The uncut biomass was represented by basal portion of culms remaining on the ground after cutting and removal of the harvestable biomass fraction. It was measured in the same sampling areas (18 m^2) where yield biomass was measured. Basal portion of culms after cutting at ground level were stored in plastic bags until lab processing.

The other fraction of residual aboveground biomass was represented by crop litter left to the soil after harvesting. Inside one of the two sampling areas of each plot, this fraction was measured in a sub-sampling area of 3 m^2 (1.5 x 3 m). It was mowed with mechanical scissors at ground level and then collected in plastic bags.

The three fractions of aboveground biomass were weighed in the field using an electronic dynamometer with a precision of 20 g. For each fraction, a sub-sample was chopped and dried in a forced-air oven at 60 °C until constant weight (Nassi o Di Nasso et al., 2013) to determine dry matter (DM) content and to calculate dry biomass weight (Mg ha^{-1}).

Belowground biomass

The belowground biomass considered for the soil C input calculation was represented by three different fractions: i) biomass from fine roots, ii) biomass from rhizomes and iii) biomass from rhizodeposition. The first two fractions were measured, while the rhizodeposition was estimated.

The fine root biomass was measured collecting soil samples in two different random points per each plot, along and between the rows. Soil core sampler of known volume (5.2 cm diameter and 30 cm length) was pressed manually into the soil keeping equal row and inter-row spacing, avoiding rhizomes. The two samples collected in the same plot were combined to have one sample per plot at 30 cm soil depth. The soil sample was immediately put in hermetic plastic bags, transported in a refrigerated bag and then stored in the lab at -20 °C before root separation and analysis. The root separation from soil and the fine roots dry biomass weight (FRB, Mg ha^{-1}) was determined using the methodology described by Chimento & Amaducci (2015).

The rhizome biomass considered in the soil C input calculation was represented by the weight increment of rhizomes from implantation to harvest. Rhizome samples were taken where aboveground biomass was sampled, excavating an area of 1.80 m^2 (1.5 x 1.2 m) to a depth of 30 cm per each plot. The collected rhizomes were washed to remove soil particles and weighted to determine total wet rhizome biomass, as reported by Nassi o Di Nasso et al. (2013).

Rhizome sub-samples were stored in plastic bags and refrigerated until they were dried in a forced-air oven at 60 °C until constant weight, to calculate the dry matter content and dry biomass yield (Mg ha^{-1}). The dry weight increment was determined as difference between the dry weight at the implantation and at the harvest (Mg ha^{-1}).

The rhizodeposition biomass was represented by root exudates and it was estimated as 65% of the fine root biomass measured in each plot, following Bolinder et al. (2007). This value is the same applied to the root biomass of perennial forage crops and natural grassland consisting mainly of C3 perennial species as giant reed. Same authors used also this coefficient for switchgrass that develops a root system that ought to have similar C turnovers than that of the giant reed (Chimento & Amaducci, 2015).

2.5. Soil CO_2 efflux, temperature and soil water content

The soil CO_2 effluxes were measured adopting a specific methodology to monitor total soil respiration (SR) and heterotrophic soil respiration (Rh) from implantation to harvest. In the same period, soil temperature (T) and soil water content (SWC) were also measured.

SR emissions

The soil CO_2 efflux due to SR was measured in situ using a portable, closed chamber soil respiration system (EGM-4 with SRC-1, PP-Systems, Hitchin, UK) with a measurement time of 120 s. The measurements were carried out during an entire growing season following implantation, from March 2015 to March 2016. The measurements were performed with a frequency from one to four times a month, depending on crop phenological stage, crop management and weather conditions (Lai et al., 2017). The soil CO_2 efflux was measured by fitting the closed chamber to PVC collars (10-cm inner diameter and 10-cm long, with perforated walls in the first 5 cm) inserted into to soil at 9 cm depth. Two collars per plot were placed in the inter-rows before rhizomes implantation. The average value of the two SR measurements per plot in each sampling date was used for data analysis. SR was monitored on 19 different dates, with 18 measurements for each sampling date used in the data analysis. The measurements were performed between 8:30 and 12:00 am so that the data collected were representative of the daily means (Davidson et al., 1998; Xu & Qi, 2001; Almagro et al., 2009).

Rh emissions

The soil CO_2 efflux from Rh was measured using the same detection system described for SR measurements. Rh was measured by connecting the closed chamber to a PVC collar in each plot inserted into to soil isolated inside a PVC cylinder (40-cm diameter, 40-cm height) open at both ends. This methodology, described by Alberti et al. (2010), allows to measure the heterotrophic component of soil respiration by the exclusion crop roots. The frequency and time of sampling of Rh measurements was the same as that indicated for SR and it was monitored on 17 different dates, with 9 values of Rh for each sampling data.

Soil T and SWC

Soil T and SWC were measured simultaneously with the Rh and SR measurements. The soil T (T_c) was measured near to each collar using a digital thermometer (HD2101.2, Delta Ohm, Padua, Italy). In each sampling date, a measurement for each collar was carried out. The SWC was measured near to each collar using an electrical equipment with reflectometry domain technology (FieldScout TDR 300 Soil Moisture Meter, Spectrum Technologies, East Plainfield, Illinois, USA). At each sampling date three measurements for each collar were carried out. The soil T and SWC were monitored at 10 cm soil depth. The average of two soil T and six SWC measurements per plot monitored near to the collar for SR in each sampling date were used for data analysis. Similarly, one measurement of T and the average of three SWC measurements related to Rh were used for data analysis. Moreover, hourly values of soil T (T_h) and SWC were continuously monitored at 10 cm soil depth during the entire growing season, from the implantation to the harvest, using T (external soil temperature sensor) and SWC (WaterScout SM 100 Soil Moisture Sensor) sensors connected to a datalogger (WatchDog 1000 Series Micro Sations, Spectrum Technologies, Thayer Court Aurora, Illonois, USA).

2.6. Data analysis

Soil T, SWC, Rh and SR data analysis

A generalized least squares (GLS) model was used to evaluate the effect of interaction between N treatment and date on SWC, soil T, SR and Rh temporal dynamics. This model allowed to evaluate different variance and covariance matrices of the dataset were tested, according to Onofri et al. (2016). Least-square means within dates were computed on the best fitted GLS model. The SR and Rh data were transformed into log values prior to submit to the GLS model.

The relationships between SWC and SR or Rh within each treatment were analyzed using linear regression analysis, according to Rey et al. (2002).

The relationships between soil T and SR or Rh within each treatment were analyzed according to the exponential regression model SR or Rh = $a*e^{bT}$ (Davidson et al., 1998), where a and b are equation parameters and T is T_c. An F test was performed to analyze the effect of N treatment on model parameters, then a multiple comparison between them was performed in case the F test was significant ($P < 0.05$), according to Soliani (2005). The SR and Rh data were transformed into log values prior to perform the analysis of variance and to meet the assumption of homogeneity of the error variance (Gomez and Gomez, 1984).

Cumulative Rh, soil C input and soil C balance data analysis

The annual Rh per hectare (Mg C-CO_2 ha^{-1}) was calculated as the cumulative daily Rh from implantation to harvest.

The daily Rh (g C-CO_2 m^{-2} day^{-1}) for each plot was estimated using the average daily T_h, obtained from the monitored hourly values, as independent variable of the model parametrized with soil T (T_c) measured at Rh sampling. The annual cumulative Rh data were analyzed with an ANOVA test for a randomized complete block design with N fertilization as factor. A protected least significant difference test at $P < 0.05$ was performed to compare the means per N level.

An ANOVA test for each fraction of the soil C input and soil C balance was also performed. The treatment means were compared using a protected least significant difference test at $P < 0.05$.The percentage variation of soil C stock was calculated as soil C balance – soil C stock (in the first 30 cm depth) ratio. The Student test was performed to evaluate if the treatment mean values of soil C stock variation were different from zero.

All statistical analyses were performed using R software (R Core Team, 2015).

3. Results

3.1. SWC, soil T, SR and Rh

SWC (Figure 1a) was not significantly influenced by fertilization treatments but it was significantly influenced by the sampling date ($P < 0.001$). During the entire monitored period, SWC was always above the wilting point (WP). The minimum values were observed in spring (on average 0.33 m^3 m^{-3}), while the maximum values were observed in autumn (on average 0.60 m^3 m^{-3}). The irrigation water was supplied from May to September, with a total volume of 342 mm distributed in eight irrigation interventions.

The soil T at 10 cm depth (Figure 1b) significantly varied during the entire monitoring period ($P < 0.001$), while it was not significantly influenced by the treatments. The average annual soil T measured in the sampling date was 19.0 °C, with minimum average values in January (9.7 °C) and maximum average values in July (26.5 °C).

The temporal dynamics of SR and Rh were similar (Figure 2) and varied markedly during the entire growing season ($P < 0.001$). Treatment effect and date x treatment interaction were not significant for SR. The minimum values of SR (on average 1.27 μmol m^{-2} s^{-1}) were observed at the start and at the end of the trial, in March 2015 and March 2016. The maximum values (on average 5.95 μmol m^{-2} s^{-1}) were observed in June and July, one week and one month after the top dressing fertilization, respectively. SR steadily increased from establishment until a maximum in June and July, to decrease steadily until harvest. A peak was observed in April, in correspondence to soil T increase.

Rh was significantly affected by the fertilization treatments ($P < 0.01$), but no significant treatment x date interaction was observed. The minimum values of Rh (on average 1.07 μmol m^{-2} s^{-1}) were observed in March in both years. Rh increased from implantation to top dressing fertilization with a fluctuating trend, reaching a maximum in April 2015. Maximum Rh values were observed in N50 and N100 in June, a week after the N fertilization (3.81 and 4.65 μmol m^{-2} s^{-1}, respectively). Subsequently, a decrease was observed, with a fluctuating trend until the end of October and then a steady trend until harvest. The maximum Rh under N0 was observed in July (4.38 μmol m^{-2} s^{-1}). After the maximum, a steady decrease of Rh until harvest was observed. On 4th July Rh was significantly higher under N0 than N100, while under N50 Rh was not significantly different than the other two treatments.

The relationships between SWC and SR or Rh were not significant (data not shown), while exponential relationships between soil T and SR and (Figure 3a) and between the soil T and Rh (Figure 3b) were significant ($P < 0.001$) for each N level. The N treatments did not affect the relationship between soil T and SR, while the relationship between soil T and Rh was influenced by the fertilization level. In the case of Rh, the regression equation calculated for N50 was similar to those calculated for N0 and N100, while the regression equation calculated for N0 was significantly different from that calculated for N100.

At harvest the C output represented by the annual cumulative Rh was significantly higher under N0 (9.67 Mg C-CO_2 ha^{-1}) than under N50 (8.68 Mg C-CO_2 ha^{-1}) and N100 (7.36 Mg C-CO_2 ha^{-1}) respectively and the cumulative Rh estimated in N50 was higher than that observed in N100 (Figure 4). Differences between the values of cumulative Rh for each treatment emerged some 30 days after the supply of N fertilizer in June.

3.2. Soil C input and soil C balance

The harvestable biomass yield was significantly influenced by the treatment ($P < 0.05$) and it was higher under N50 and N100 than under N0 (7.12, 6.73 and 4.20 Mg of DM ha^{-1} with ±0.66 standard error, respectively), but no significant differences were observed between the two fertilized treatments (data not shown). The harvestable biomass was on average 27% of total crop biomass (above and belowground biomass) and 78% of aboveground biomass (excluding litter residues). The soil C inputs from aboveground and belowground biomass residues were reported in Table 2. The treatments did not affect the distinct fractions of soil C input deriving from different parts of aboveground and belowground biomass. The total soil C input was not influenced by N fertilization and it was on average 7.02 Mg ha^{-1}. The C inputs from belowground biomass were on average 62% of total soil C input in each treatment (Figure 5).

At harvest, the soil C balance was significantly lower in N0 than in N50 and N100, but no differences were observed between N50 and N100 (Table 3). On annual basis the soil C losses were higher than the soil C inputs of 3.1 Mg ha^{-1} in N0, while they were 1.09 and 0.45 Mg of C ha^{-1} under N50 and N100, respectively. The soil C balance calculated in N100 was not significantly different from equilibrium (zero), whereas the net soil C losses observed in N0 and N50 were significantly different from equilibrium ($P < 0.05$).

4. Discussion

The temporal dynamics of SR and Rh rates were similar to those observed for soil T during the monitoring period (Figure1 and Figure 2). In fact we observed a significant exponential relationship between the soil T and soil CO_2 efflux, in agreement with Davidson et al. (1998). Rey at al. (2002) under Mediterranean rainfed conditions, observed that the soil CO_2 effluxes were linked to the soil T only when the SWC was not limiting for the soil respiration. In our experiment, the SWC never fallen below 0.32 m^3 m^{-3} (Figure 1) that is just about -100 kPa, below which the soil CO_2 emission was recognized to be affected by SWC (Lado-Monserrat et al., 2014). Our results were in agreement with these assumptions because we did not observe significant relationships between SWC and SR o Rh but only between soil T and SR or Rh (Figure 3).

The SR rates rapidly increased after top dressing fertilization (Figure 2a), but the N fertilization did not influence the temporal dynamic of SR. A peak observed in April could be explained with an increase of soil T. For Rh instead, the maximum values were reached in June, immediately after N fertilization in N50 and N100, while in N0 the peak was observed in July, almost a month after top dressing fertilization (Figure 2b). N supply was recognized to decrease of Rh CO_2 effluxes in N100 two week after fertilization. This result could be explained by the inhibitory effect of inorganic N additions on soil microbial activity and soil respiration (Wilson & Al-Kaisi, 2008). Kowalenko et al. (1978) reported that the lowering of soil pH by N addition was largely responsible for the decreased microbial activity. Our outcomes were consistent with those reported by Ni et al. (2012) that in a maize field, after N fertilization, observed an increase of autotrophic respiration from root activity, which compensated the lower emissions from Rh, resulting into a no significant difference in SR between fertilized and no fertilized treatments. However, some authors (Dick, 1992; Kuzyakov et al., 2000; Liu et al., 2016) reported that N fertilization in the long-term determined higher yield and higher soil C inputs from the plant residues, which over time resulted into higher microbial activity. Moscatelli et al. (2005) showed that the microbial activity was linked to the soil mineral N and organic C availability and hence the emission rates of Rh were associated to the soil C:N ratio, which varied in relation to N additions.

Similar effects of N fertilization on SR and Rh rates were observed for the sensitivity of SR and Rh to the variation of soil T. SR sensitivity to soil T changes was not affected by N fertilization (Figure 3a), contrarily to Rh where N0 showed higher values than N100 at the different levels of soil T

(Figure 3b). Liu et al. (2016) reported that the decrease of soil C:N ratio after N fertilization determined lower sensitivity of the microbial activity to the soil T changes because it was necessary a lower activation energy to decompose the organic material (Bosatta & Ågren, 1999; Davidson et al., 2006). Same authors showed that the sensitivity of SR rates to the soil T changes was not affected by N treatments because with increase of soil T the root respiration was stimulated by N additions that counterbalanced the behavior of microbial respiration.

Soil C losses, estimated as the cumulative Rh at harvest, reflected the temporal dynamics of Rh related to the N treatments. The soil C losses estimated in the unfertilized treatment were significantly higher than in the two fertilized treatments, due to the depressing effect of N addition on microbial activity in the short-term period (Wilson & Al-Kaisi, 2008). In fact the differences in cumulative Rh between N0 and the two fertilized treatments were more evident following the N fertilizer applications (Figure 4). Liu et al. (2016) showed opposite results in the long-term, with soil C output higher in the fertilized treatments, where they observed over time residues returned to the soil higher than in the unfertilized treatment. However, in the short-term, this behavior was not observed (Wilson & Al-Kaisi, 2008).

The high proportion of soil C inputs deriving from belowground biomass (Figure 5) was mainly characterized by a high amount of root biomass produced (Table 2). The amount of fine roots measured in the first 30 cm was higher than that reported by some authors (Chimento & Amaducci, 2015; Monti & Zatta, 2009) during the maturity phase in Northern Italy under rainfed conditions. In addition, some authors showed that giant reed developed fine roots until about 1 m depth. In our study, it was considered only the first growing season of giant reed, cultivated under drip irrigation conditions, hence it is possible that this crop developed most of the root biomass in the tilled layer. The soil C input from belowground biomass was not significantly affected by N fertilization. Similar results were observed by Strullu et al., (2011) on Miscanthus (*Miscanthus* × *giganteus*). Contrarily to belowground biomass, the effect of N fertilization observed on the biomass yield was in agreement with Angelini et al. (2005), that showed higher aboveground biomass production in the fertilized treatments, in the early years of the giant reed cultivation. This effect was also observed by Monti & Zegada-Lizarazu (2016) in the years following the first growing season.

The final calculation of soil C balance was mostly affected by the high values of cumulative Rh (Table 3). The higher amount of soil C losses observed in the unfertilized treatment determined lower values of soil C balance and a less conservative behavior in terms of soil C stock compared to the fertilized treatments. The fertilization at rates of 100 kg N ha^{-1} determined negligible net soil C losses, whereas in N50 and N0 the soil C balance was negatively deviated from equilibrium, at the end of the first growing season. Monti & Zegada-Lizarazu (2016) reported that long-term high N fertilization rates determined a substantial increment in SOC, especially in surface soil layers. In a long-term conversion simulation from cotton (*Gossypium hirsutum*) to switchgrass (*Panicum virgatum*) Chamberlain et al. (2011) showed that SOC decreased, unchanged and increased with N fertilization rates of 0, 45 and 135 kg ha^{-1}, respectively. In the short-term observation, the low values of soil C balance can be attributed to the first growing season of giant reed that was characterized by soil tillage before implantation with low residues left on the soil. In the long-term, the giant reed crop may be able to compensate this temporary negative soil C balance by absence of tillage practices and by increasing above and belowground biomass and soil C input (Impagliazzo et al., 2017).

5. Conclusion

This study provides some relevant information about the behavior of giant reed in relation to the N fertilization management in the first growing season. In particular, the high N fertilization rates determined higher giant reed biomass yield and soil C balance not significantly different from equilibrium if compared to the unfertilized management. The negative soil C balance in the unfertilized crop was mainly due to higher losses of soil C from heterotrophic respiration, whereas in the fertilized treatments the inhibitory effect of N additions on microbial activity determined lower soil C effluxes and higher values of soil C balance. The N fertilization demonstrated a depressing effect on Rh rates, annual cumulative Rh and Rh sensitivity to the soil T changes, but no effect was observed on SR because the root respiration counterbalanced the behavior of Rh. The total soil C input from residues was not significantly affected by N fertilization, contrarily to the crop yield, which reached higher values when N fertilizer was applied. The soil C input from root biomass represented the most abundant fraction of the total C input in the first year after implantation.

These results showed that in the early stages of growth, the soil C balance of giant reed cropping system may be temporarily negative or near the equilibrium, depending also on N fertilization. However, in the long-term, positive and higher values of soil C balance have been observed by many authors thanks to the absence of soil tillage and abundance of crop residues that every year return to the soil.

Further investigations are needed to evaluate yield biomass and soil C balance related to the N fertilization management of giant reed cultivation in the long-term period.

Tables

Table 1. Physical and chemical soil characteristics of the field site in the tilled layer (0-30 cm).

Soil characteristics	Unit	Values	International Ufficial Method
Sand (2–0.05 mm)	%	33.0	ISO 11277
Silt (0.05–0.002 mm)	%	27.0	ISO 11277
Clay (<0.002 mm)	%	40.0	ISO 11277
pH		7.9	ISO 10390
Total $CaCO_3$	%	2.3	ISO 10693
Organic matter	%	2.8	ISO 10694
Total N	‰	7.9	ISO 10694
Available P (Olsen)	ppm	0.7	Italian Ufficial Method n. XV.3
Exchangeable K	ppm	799.3	ISO 11260

Table 2. Biomass yield and soil C inputs from the different portions of aboveground and belowground biomass at harvest (March 2016). Data are expressed as Mg ha^{-1}.

	Dry matter yield (Mg ha^{-1})	C input from uncut culms (Mg ha^{-1})	C input from litter residues (Mg ha^{-1})	C input from rhizomes (Mg ha^{-1})	C input from fine roots (Mg ha^{-1})	C input from rhizodeposition (Mg ha^{-1})	Total C input (Mg ha^{-1})
N0	4.20 b	0.62	1.81	0.92	1.96	1.28	6.58
N50	7.12 a	0.94	1.67	0.70	2.59	1.68	7.59
N100	6.73 a	0.78	2.15	0.68	2.00	1.30	6.91
Means	6.02	0.78	1.88	0.77	2.18	1.42	7.03
CV	33%	36%	23%	40%	20%	20%	11%

Means followed by different letters indicate significant differences at $P < 0.05$. N0: no fertilization; N50: fertilization with 50 kg of N ha^{-1}; N100: fertilization with 100 kg of N ha^{-1}.

Table 3. Total soil C inputs, soil C output as cumulative Rh and soil C balance at the end of the first growing season. Data are expressed as Mg ha^{-1}.

	Soil C input (Mg ha^{-1})	Soil C output (Mg ha^{-1})	Soil C budget (Mg ha^{-1})
N0	6.58	9.67 a	-3.10 b $^{(*)}$
N50	7.59	8.68 b	-1.09 a $^{(*)}$
N100	6.91	7.36 c	-0.45 a $^{(NS)}$
CV	11 %	12 %	86 %

CV : coefficient of variability. Means followed by different letters indicate significant differences at $P < 0.05$. In brackets significant difference from equilibrium (zero) at level: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; NS: no significant. N0: no fertilization; N50: fertilization with 50 kg of N ha^{-1}; N100: fertilization with 100 kg of N ha^{-1}.

Figures

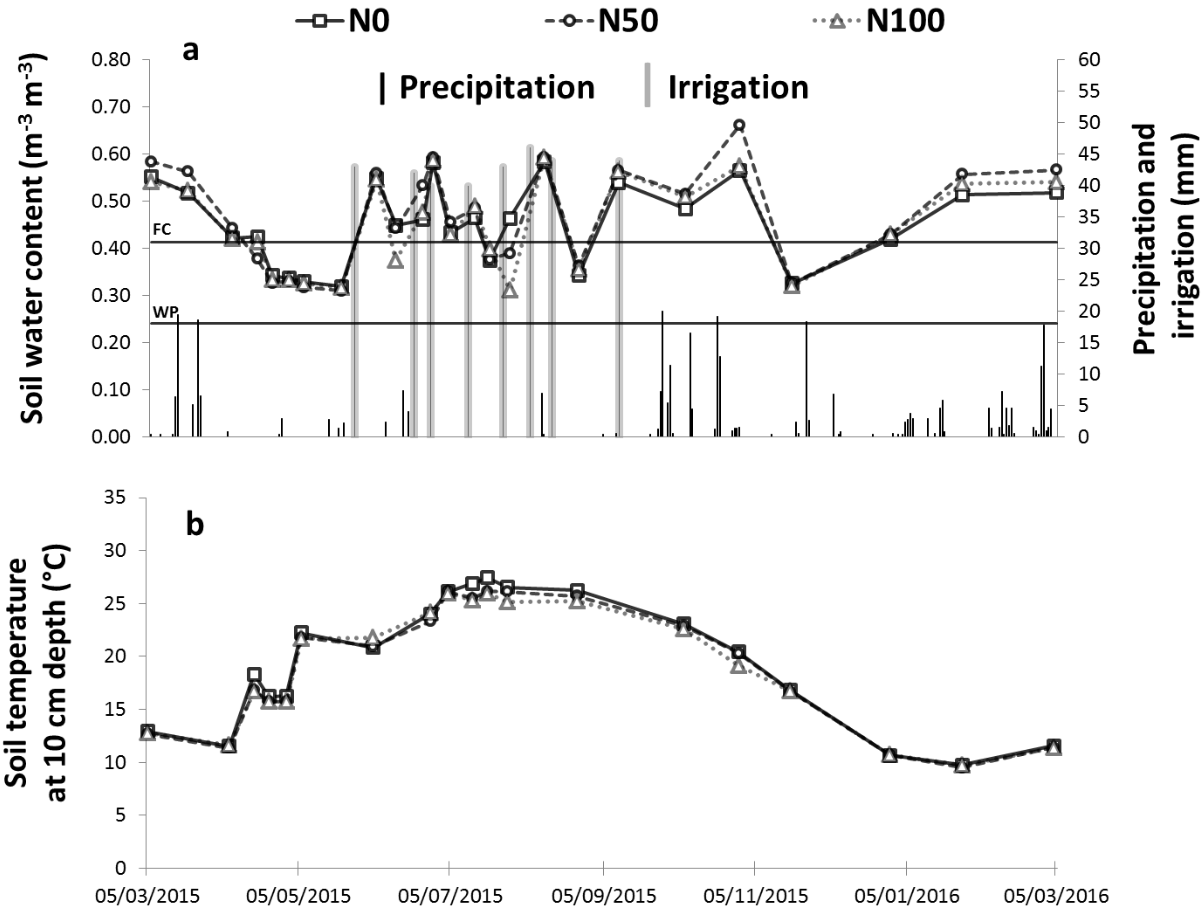

Figure 1. Temporal dynamics of soil water content (a) and soil temperature (b) monitored in the Rh and SR sampling dates at 10 cm soil depth during the entire growing season (March 2015 – March 2016). Vertical bars represent ± standard error of each treatment within sampling date. FC: field capacity; WP: wilting point. N0: no fertilization; N50: fertilization with 50 kg of N ha^{-1}; N100: fertilization with 100 kg of N ha^{-1}.

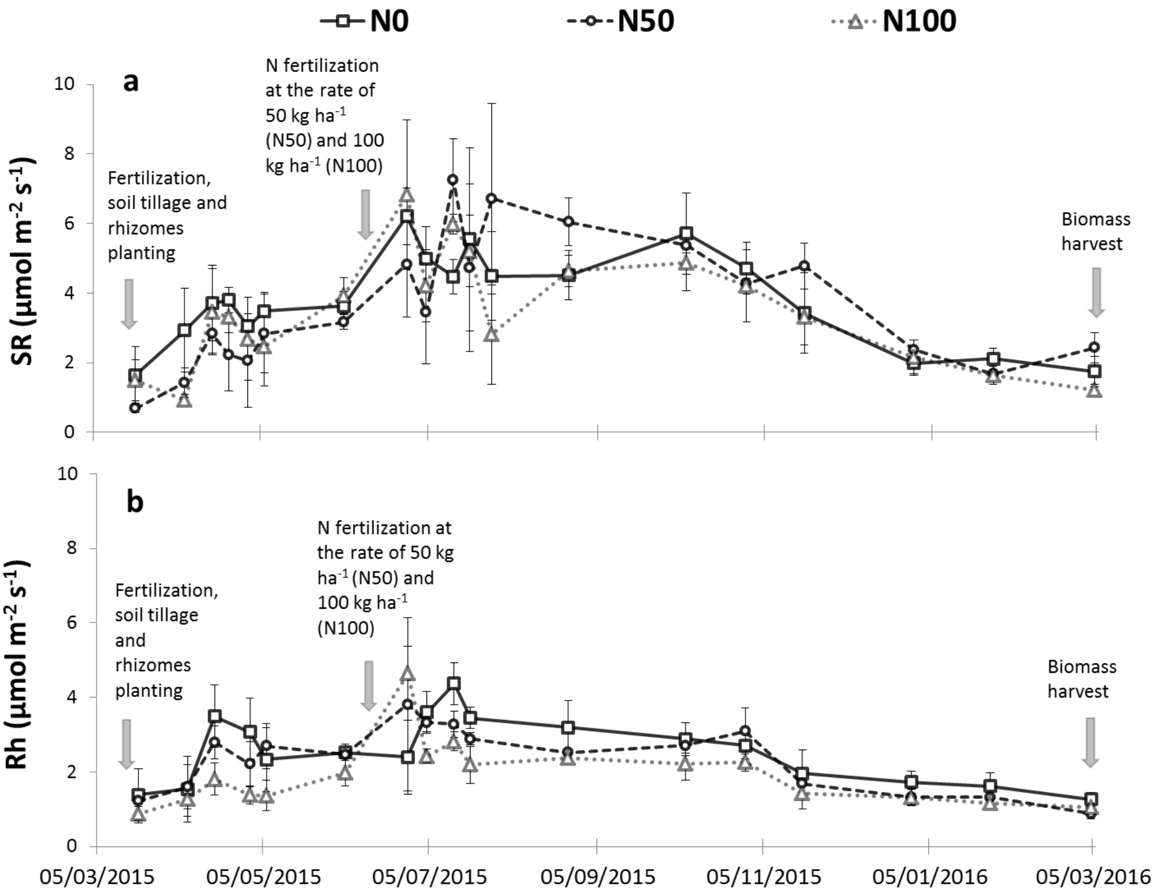

Figure 2. Temporal dynamics of SR, total soil respiration (a), and Rh, heterotrophic soil respiration (b), in the sampling dates during the entire growing season (March 2015 – March 2016). Vertical bars represented ± standard error of each treatment within sampling date. N0: no fertilization; N50: fertilization with 50 kg of N ha^{-1}; N100: fertilization with 100 kg of N ha^{-1}.

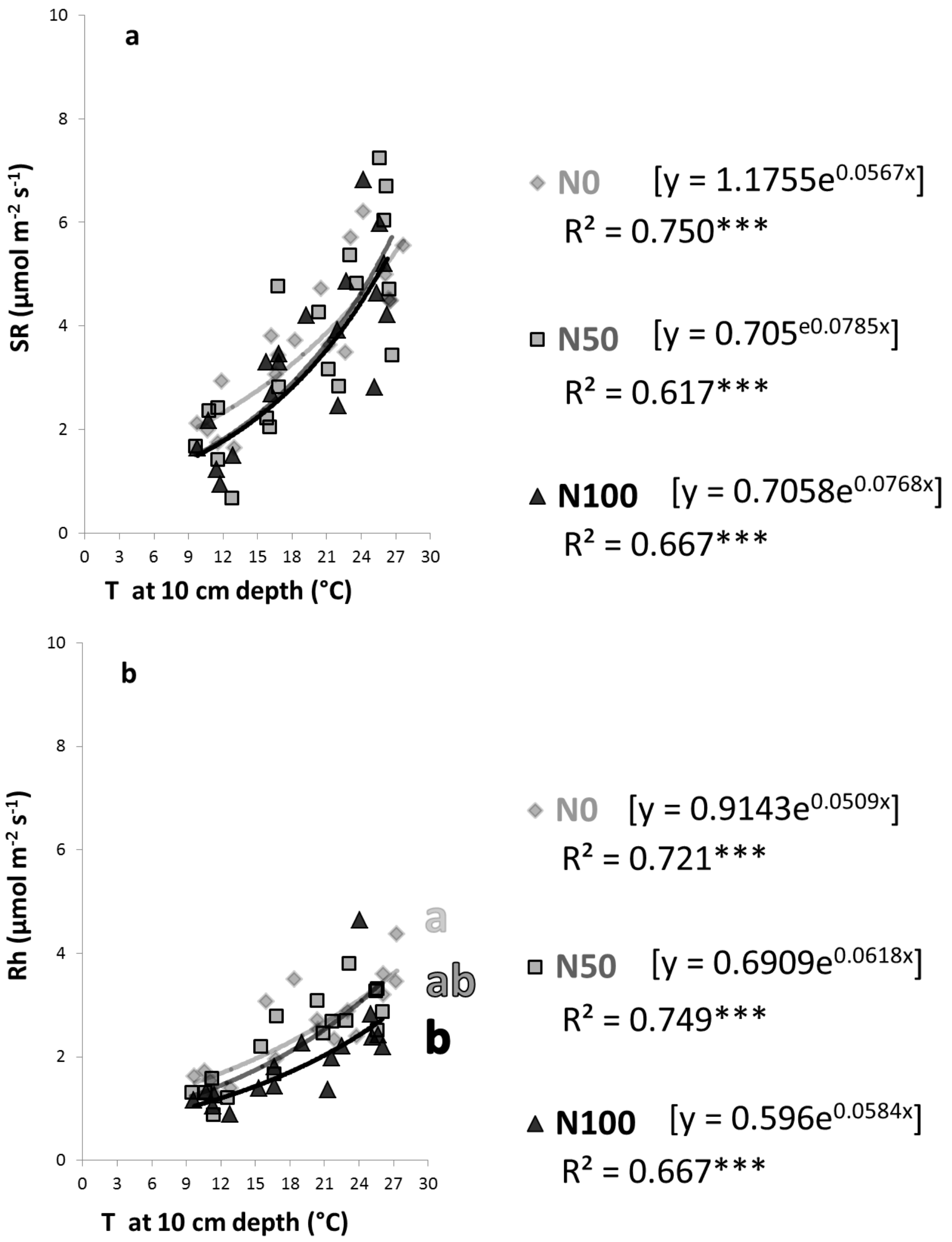

Figure 3. Relationship for each treatment between the soil temperature (T) at 10 cm depth vs (a) the total soil respiration (SR) and (b) the heterotrophic soil respiration (Rh) monitored during the entire growing season (March 2015 – March 2016). Different letters beside regression curves indicate significant differences between regression lines at $P < 0.05$. Asterisks indicate the significance level: $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$. N0: no fertilization; N50: fertilization with 50 kg of N ha^{-1}; N100: fertilization with 100 kg of N ha^{-1}.

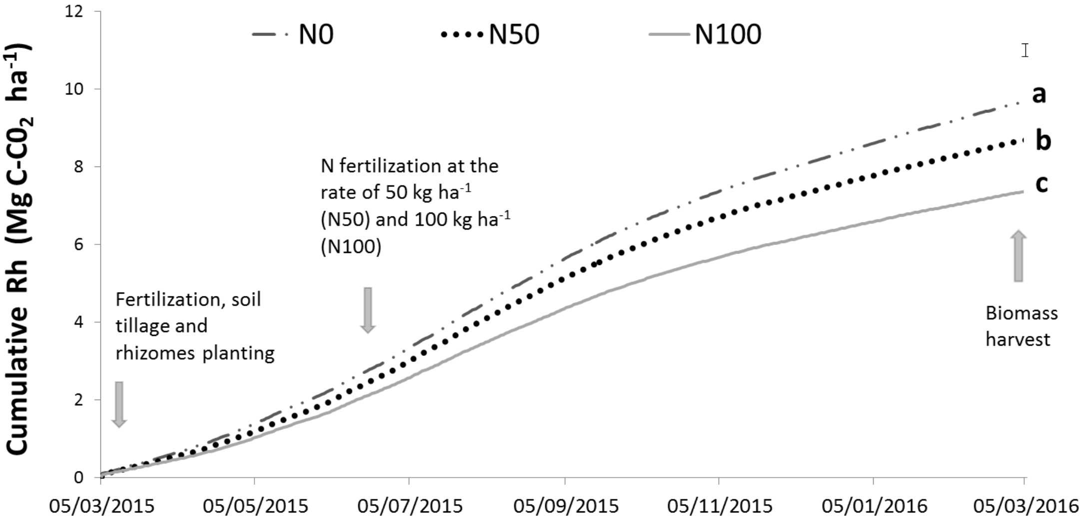

Figure 4. Cumulative heterotrophic soil respiration (Rh) from implantation to harvest (March 2015 – March 2016). Vertical bar represents least significant differences for treatment comparisons at $P < 0.05$. Different letters indicate significant differences at $P < 0.05$. N0: no fertilization; N50: fertilization with 50 kg of N ha^{-1}; N100: fertilization with 100 kg of N ha^{-1}

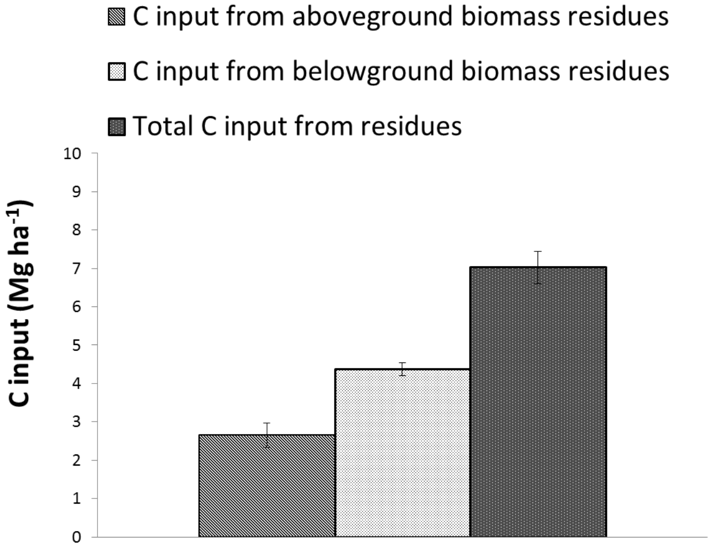

Figure 5. The average values of soil C inputs (Mg ha^{-1}) from aboveground and belowground biomass residues and the total soil C input. Vertical bars represented ± standard error.

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CHAPTER III

Manure fertilization increases soil respiration and creates a negative carbon budget in a Mediterranean maize (*Zea mays* L.)-based cropping system

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Manure fertilization increases soil respiration and creates a negative carbon budget in a Mediterranean maize (*Zea mays* L.)-based cropping system

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Abstract

Agronomic research is important to identify suitable options for improving soil carbon (C) sequestration and reducing soil CO_2 emissions. Therefore, the objectives of this study were i) to analyse the on-farm effects of different nitrogen fertilization sources on soil respiration, ii) to explore the effect of fertilization on soil respiration sensitivity to soil temperature (T) and iii) to assess the effect of the different fertilization regimes on the soil C balance. We hypothesized that i) the soil CO_2 emission dynamics in Mediterranean irrigated cropping systems were mainly affected by fertilization management and T and ii) fertilization affected the soil C budget via different C inputs and CO_2 efflux. Four fertilization systems (farmyard manure, cattle slurry, cattle slurry + mineral, and mineral) were compared in a double-crop rotation based on silage maize (*Zea mays* L.) and a mixture of Italian ryegrass (*Lolium multiflorum* Lam.) and oats (*Avena sativa* L.).

The research was performed in the dairy district of Arborea, in the coastal zone of Sardinia (Italy), from May 2011 to May 2012. The soil was a Psammentic Palexeralfswith a sandy texture (940 g sand kg^{-1}). The soil total respiration (SR), heterotrophic respiration (Rh), T and soil water content (SWC) were simultaneously measured in situ. The soil C balance was computed considering the Rh C losses and the soil C inputs from fertilizer and crop residues. The results showed that the maximum soil CO_2 emission rates soon after the application of organic fertilizer reached values up to 12 μmol m^{-2} s^{-1}. On average, the manure fertilizer showed significantly higher CO_2 emissions, which resulted in a negative annual C balance (-2.9 t ha^{-1}). T also affected the soil respiration temporal dynamics during the summer, consistently with results obtained in other temperate climatic regions that are characterized by wet summers and contrary to results from rainfed

Mediterranean systems where the summer SR and Rh are constrained by the low SWC. The sensitivity of soil respiration to temperature significantly increased with C input from fertilizer. In conclusion, this research supported the hypotheses tested. Furthermore, the results indicated that i) soil CO_2 efflux was significantly affected by fertilization management and T, and ii) fertilization with manure increased the soil respiration and resulted in a significantly negative soil C budget. This latter finding could be primarily explained by a reduction in productivity and, consequently, in crop residue with organic fertilization alone as compared to mineral, by the favourable SWC and T for mineralization, and by the sandy soil texture, which hindered the formation of macroaggregates and hence soil C stabilization, making fertilizer organic inputs highly susceptible to mineralization.

Keywords: Biomass, C turnover, GHG emission, Microbial activity, Soil moisture

1. Introduction

Soil carbon (C) sequestration is considered to be as cost-effective and a win–win option to offset anthropogenic CO_2 emissions to the atmosphere (Lal et al., 2015). Croplands are considered as crucial sinks or sources of C, and the agronomy influences soil C dynamics and balance (Lal et al., 2015). Thus, ‘on-farm’ research of soil management, particularly fertilization, is important to identify suitable options for improving soil C sequestration and reducing soil CO_2 emission (Paustian et al., 2016).

Soil CO_2 efflux or soil respiration (SR) is principally the sum of root metabolic activity and soil heterotrophic respiration (Rh), which is the result of mesofaunal and microbial metabolism and is strongly correlated with total soil C losses (Hanson et al., 2000). Factors influencing soil CO_2 efflux are the soil organic mass concentration and quality (Ferréa et al., 2012), root biomass composition (Ryan et al., 1996), soil chemical and physical properties and site productivity (Subke et al., 2011), soil temperature (T) and soil water content (SWC). T and SWC are the primary environmental factors that regulate the seasonal variation of SR (Davidson et al., 1998). Large seasonal variations in SR are evident in a Mediterranean climate due to the marked dynamics of both soil T and SWC (Rey et al., 2002). Furthermore from winter to early spring, the primary factor that affects SR is T, while during the dry period, SWC plays the most important role in controlling the process (Almagro et al., 2009). Management practices such as irrigation (de Dato et al., 2010), tillage, crop residue management and fertilization regulate the microbial activity, which mediates the processes of organic mass turnover and nutrient cycling and, consequently, the soil C balance (Lal et al., 2015).

Nitrogen (N) fertilization affects SR dynamics in agroecosystems and its relationship with abiotic factors (Ding et al., 2007). Different N fertilization sources affect soil metabolic activity as well as the soil C balance (Mancinelli et al., 2010; López-López et al., 2012; Wang et al., 2015).

Although organic fertilization is considered the most effective approach to create a positive soil C budget in croplands (Lal et al., 2015), recent studies have shown that a negative soil C budget can also occur under this fertilization system (Wang et al., 2015; Mori and Hojito, 2015). Therefore, the effect of fertilization on soil organic C dynamics is still unclear and requires further study. Maize-based cropping systems in the Mediterranean region are irrigated and supplied with high inputs of N fertilization compared to North European countries (e.g. Kayser et al., 2011) or arid climate areas (e.g. Gheysari et al., 2009).

The agricultural practices employed are therefore important drivers of the economic and environmental benefits of these systems (Casa et al., 2011). Such cropping systems are suitable for studying the relationships between SR and its driving factors at a non-limiting SWC and nutrient availability (Shrestha et al., 2013) and for assessing soil C balance dynamics (Grignani et al., 2007). Therefore, the objectives of this study were i) to analyse the effects of different fertilization sources on SR and Rh, ii) to explore the effect of the fertilization regime on the sensitivity of SR and Rh to T and iii) to assess the effect of the different fertilization regimes on the soil C balance.

We hypothesized that i) soil CO_2 emission dynamics in a Mediterranean irrigated cropping system were primarily influenced by fertilization management and T, and ii) fertilization affected the soil C budget via different C inputs and CO_2 efflux.

2. Materials and methods

2.1. Study site

The study was conducted on a private farm located in a Nitrate Vulnerable Zone (NVZ) in the dairy district of Arborea (39°47′ N 8°33′ E, 3 m a.s.l.) in the Central-western coast of Sardinia, Italy. The district was identified as NVZ according to the European Nitrate Directive (ND) (91/676/EC) because the groundwater nitrate concentration was found over the threshold of 50 mg L^{-1}. The ND implementation imposed a restriction on the N supplied from organic effluents (slurry and manure) to a maximum of 170 kg N ha^{-1} $year^{-1}$, a ban on spreading organic fertilizer during the wet and cool season (from15 November to 15 February) and compliance with the coded prescriptions of "good agricultural practices" to prevent nitrate leaching (Nguyen et al., 2014).

The area has a Mediterranean climate with hot dry summers and mild rainy winters. The mean annual temperature is 16.7 °C, and the long-term average annual rainfall is 572 mm (1959-2013), with 73% of it occurring between October and March. The average annual aridity index (rainfall/reference evapotranspiration) is 0.49, which corresponds to a semi-arid climate (Middleton and Thomas, 1992). The 55-year (1958-2013) average monthly maximum temperatures are 31.0 °C in August and 14.1 °C in January. The 55-year average monthly minimum temperature is 18.1 °C in August and 5.5 °C in January and February.

The soil was classified as a Psammentic Palexeralfs (USDA, 2006) with a sandy texture (940 g sand kg^{-1}) and parental material of coastal sediments. At the beginning of the study, the upper soil depth (0- 45 cm) had an average bulk density of 1.5 g cm^{-3}, a soil organic C concentration of 13.6 g kg^{-1} corresponding to a C stock of 76.2±5.2 t ha^{-1}, a total N concentration of 1.3 g kg^{-1} with a C/N ratio of 10, a soil pH of 6.3 and Olsen P of 141 mg kg^{-1}. Further data on the whole soil profile are available in Demurtas et al. (2016). The soil hydraulic properties were estimated with the SPAW hydrology model (Saxton and Rawls, 2006). The estimated SWC at 0, -33 and -1500 kPa was 0.47 m^3 m^{-3}, 0.08 m^3 m^{-3} and 0.04 m^3 m^{-3}, respectively. The drain upper limit measured via a field determination was 0.20 m^3m^{-3}, corresponding to a matric potential of approximately -23 kPa (Lai et al., 2012).

The main cropping system in the dairy district of Arborea is a double- crop rotation of silage maize (*Zea mays* L.) and an autumn-spring hay crop, usually Italian ryegrass (*Lolium multiflorum* Lam). Such forage crops are used to feed the dairy cattle in a farming system consisting of some 28,000 animals in a 5000 ha irrigated plain, which is organized as a cooperative system.

2.2. Study design and management

We compared four fertilization systems at the same target of total N rate, that is, 316 kg ha^{-1} for silage maize and 130 kg ha^{-1} for the autumn- spring hay crop, set on the basis of the crop requirements determined via a N balance as prescribed for NVZs. While for mineral fertilizers the N rate can be set exactly on the basis of the fertilizer nominal rating, the amount of N input from organic fertilizers is set on the basis of mean rating based on literature data or local assessments, but varies in each distribution according to the very variable N concentration in the manure or slurry and hence this may lead to actual N rates that can be very different, in single plots, from the target rate, while at district scale the mean N rates would be compliant with the target set by the norms. For this reason, in our plot scale experiment we measured the actual rates distributed in each plot. The characteristics of the organic fertilizers were described by Demurtas et al. (2016):

i) Manure (MA): Cattle farmyard manure was applied with a conventional spreader before each crop was sown and followed by rotary tillage. Approximately 70% of the total organic fertilizer was spread on the maize at the end of May (50 t $ha^{-1}$with an average C concentration of 1.06 g kg^{-1}) and 30% on the hay crop in October (20 t ha^{-1} with an average C concentration of 0.70 g kg^{-1});

ii) Slurry (SL): A cattle slurry was applied with a conventional spreader before each crop was sown and immediately incorporated into the soil via rotary tillage. Approximately, as done for MA, we applied 70% of the total organic fertilizer on the maize (250 t ha^{-1}, with an average C concentration of 0.18 g kg^{-1}) and 30% on the hay crop (100 t ha^{-1}, with an average C concentration of 0.07 g kg^{-1});

iii) Slurry + mineral (SM): A slurry was applied at a target rate of 100 and 70 kg N ha^{-1} to the maize (80 t ha^{-1}, with an average C concentration of 0.20 g kg^{-1}) and the hay crop (52 t ha^{-1}, with an average C concentration of 0.08 g kg^{-1}), respectively, combined with the mineral fertilizer ENTEC 26®, which contained 0.75 g kg^{-1} nitrate and 1.85 g kg^{-1} ammonium, sulphur dioxide (3.2 g kg^{-1}) and a nitrification inhibitor (3,4-dimethylpyrazole phosphate, DMPP), at a rate corresponding to 216 kg N ha^{-1} applied at sowing to the maize and 60 kg N ha^{-1} applied at the end of tillering to the Italian ryegrass.

iv) Mineral (MI): The mineral fertilizer ENTEC 26® was applied at sowing on the maize at a rate corresponding to 316 kg N ha^{-1} and on Italian ryegrass at the end of tillering (mid-February) at a rate corresponding to 130 kg N ha^{-1}.

Samplings of fertilizers were performed at each distribution event (Demurtas et al., 2016). The C input from fertilizer was determined after analyses by the Springer-Klee method (Mipaaf, 2006) (Table 1). The mineral N/total N ratio in the fertilizer was determined by the official method for fertilizers (Mipaaf, 2006) (Table 1).

The differences between the target and actual N rates from the organic fertilizers were related to the variable N concentration in the effluent as previously described by Demurtas et al. (2016).

The experimental design was a 4 × 4 Latin square with a treatment size of 12 × 60m^2. A buffer area of at least 3 m in width was placed between adjacent treatments. The treatments were applied from June 2009 to May 2012; however, this study occurred during the last year.

Calcio hybrid (FAO class 700) maize was sown in June 2011 following organic fertilization, and rotary tillage (15 cm depth) and harrowing (20 cm depth) were used in each treatment. Mineral fertilization was applied between rotary tillage and harrowing. The sowing spacing was 75 cm between the rows and 20 cm along the rows. After sowing, Lumax® herbicide and Force® insecticide (Sygenta, Zurich, Swiss) were applied. Sprinkler irrigation (4600 m^3 ha^{-1}) was applied until 1 September at rates of 220 m^3 ha^{-1} at intervals between 5 days and 2 days (Fig. 1a), depending on the crop phenological phase and evapotranspiration rate, to keep the SWC above 0.15 m^3 m^{-3}. The silage maize was harvested in mid-September 2011 using combined forage harvester.

Organic fertilization was applied during the first week of October, and the whole field (i.e., each treatment) was tilled using a rotary tillage (15 cm depth) and a harrow (20 cm depth). A mixture of 80% Italian ryegrass and 20% oats (Avena sativa L.) was sown in mid-October and harvested for hay-making in mid-May. Sowing was done with a seed drill with a 12 cm row width. Mineral fertilization was applied to the SM and MI treatments in mid-February 2012. In April and in May, auxiliary sprinkler irrigation was provided when necessary to avoid crop water stress (Fig. 1a). The hay crop was cut using a mower conditioner and harvested using a star wheel rake combined to variable-chamber round baler.

2.3. Measurements

The soil CO_2 efflux was measured in situ using a portable, closed chamber soil respiration system (EGM-4 with SRC-1, PP-Systems, Hitchin, UK) with a measurement time of 120 s. The measurements were carried out from May 2011 to May 2012, at a frequency from once a week to once a month depending on the weather, the crop phenological phase and management. The measurements were performed at least one day after irrigation and at least one day after the end of precipitation.

The soil CO_2 efflux was measured using three PVC collars (10 cm inner diameter and 10 cm long, with perforated walls in the first 5 cm), inserted into the soil to a depth of 9 cm after both the maize and hay crop were sowed. The three collars represented three sub-treatments in each treatment (Lai et al., 2012). Rh was measured in each treatment in a root exclusion sub-treatment in which the soil was isolated with a PVC cylinder (40 cm diameter, 40 cm height) open at both ends, according to Alberti et al. (2010). Therefore, in each treatment, we had two SR sub-treatments and one Rh sub-treatment. The data from two SR measurements per treatment were pooled. Therefore, at each sampling date, 16 values for the SR and 16 values for the Rh, i.e., one per treatment for each, were used in the data analysis. SR was monitored on 24 dates and Rh on 25 dates. The measurements were performed between 8:30 and 12:00 am (standard time) so that the data collected were representative of the daily means (Davidson et al., 1998; Xu and Qi, 2001; Almagro et al., 2009). The soil T and SWC were measured at the same time as the CO_2 efflux measurements were taken. Soil T was measured adjacent to each collar using a digital thermometer (HD2101.2, Delta Ohm, Padua, Italy), and SWC was measured in each treatment using a capacitance probe (Diviner 2000, Sentek, Stepney, Australia). T and SWC were measured at soil depths of 10 cm and over 0-20 cm, respectively, to analyse the effects of T and SWC on SR or Rh (Davidson et al., 1998).

2.4. Soil C balance

The soil C balance was determined at the harvest of each crop and at the end of the crop rotation, as the difference between the soil C inputs and C-CO_2 losses (soil C outputs). The soil C outputs considered in the C balance were estimated as the amount of Rh accumulated at the harvest of each crop and at the end of the crop rotation. For the purpose of this study, C losses from leaching were not accounted for.

2.4.1. Soil C input

The soil C input of each crop was represented by the C input from organic fertilizer (manure or slurry) and from the crop residue, which in turn was derived from the estimated aboveground residue and the estimated belowground biomass, according to Buysse et al. (2013). The soil C input was determined in each treatment for both crops according to the following formula, assuming no treatment effects on plant biomass partitioning and composition:

Soil C input = $C_{fer} + C_{ere} + C_{roo} + C_{rhi}$

where C_{fer} is the C input from organic fertilizer supplied before crop sowing (Table 1), C_{ere} is the C input from aboveground crop residues, C_{roo} is the C input from the root biomass, and C_{rhi} is the C input from rhizodeposition. The sum of C_{ere}, C_{roo} and C_{rhi} represents the crop residues, which were assumed as 11.0% and 44.7% of the harvested dry mass (DM) for the maize and hay crops, respectively, applying indexes described below that were found in the literature.

2.4.1.1. Maize crop residue

The maize C_{ere} (C_{erem}) was determined as follows:

$$Cerem = TEB_m \times iL \times iC$$

where iL is the maize harvest loss index (0.087) according to Perin and Dassie (1982), iC(0.40) is the dry mass conversion index into C (Burle et al., 1997; dos Santos et al., 2011), and TEB_m is the maize total aboveground biomass, which was determined as follows:

$$TEB_m = B_{harm} \times (1-iL)^{-1}$$

where B_{harm} is the maize harvested DM assessed from the crop yield (Demurtas, 2014).

Each treatment was harvested using farm machinery, and the fresh aboveground maize was weighed immediately after harvest using electronic weighing cells positioned in a flat place under the tractor cart. The biomass DM concentration at harvest was assessed by sampling 1 kg of fresh chopped maize, which was immediately cooled in plastic bags and then dried in a forced-air oven at 60 °C to constant weight (Burle et al., 1997).

The maize C_{roo} (C_{room}) was calculated as follows:

$$C_{room} = B_{room} \times iC$$

where B_{room} is the maize root biomass, which was determined as follows:

$$B_{room} = TEB_m \times i(S : R)_m^{-1}$$

where $i(S:R)_m$ (10.0) is the maize shoot/root ratio according to dos Santos et al. (2011).

The maize C_{rhi} (C_{rhim}) was determined as follows:

$C_{rhim} = C_{room} \times iR_z$

where iR_z is the rhizodeposition index (0.65), expressed in relation to the root biomass (Bolinder et al., 2007).

2.4.1.2.Hay crop residue

The hay crop C_{ere} (C_{ereh}) was estimated as follows:

$C_{ereh} = B_{harh} \times (iL_m + iR_{ac}) \times iC$

where B_{harh} is the harvested hay DM (Demurtas, 2014), which was determined using the same method as described for maize, iL_m is the mechanical harvest loss index (0.075) (Borgioli, 1982), iR_{ac} (0.11) is the index of the unmown aboveground residue biomass left after cutting, which was measured in sampling areas of $1m^2$ per treatment by cutting the residue biomass with shears at the harvest, and iC (0.40) is the DM conversion index to C (Burle et al., 1997; dos Santos et al., 2011).

The hay crop C_{roo} (C_{rooh}) was estimated as follows:

$C_{rooh} = B_{rooh} \times iC$

where iC (0.40) is the DM conversion index to C (Burle et al., 1997; dos Santos et al., 2011) and B_{rooh} is the hay crop root biomass, which was determined as follows:

$B_{rooh} = TEB_h \times i(S : R)_h^{-1}$

where $i(S:R)_h$ is the hay crop shoot/root ratio excluding rhizodeposition according dos Santos et al. (2011). The i(S:R)h weighted average of Italian ryegrass (2.0) and oats (2.5) was estimated at 2.1 (Bolinder et al., 2007). TEB_h is the total aboveground biomass of the hay crop, according to the following equation:

$TEB_h = B_{harh} \times (1 + iL_m + iR_{ac})$

The hay crop C_{rhi} (C_{rhih}) was estimated as follows:

$C_{rhih} = C_{rooh} \times iR_z$

where iR_Z (0.65) is the rhizodeposition index (Bolinder et al., 2007) and C_{rooh} is the C input from the hay crop root biomass, which was estimated as above.

2.4.2. Soil C output

The soil C output was assessed assuming that the Rh data collected were representative of the daily means (Davidson et al., 1998; Xu and Qi, 2001; Almagro et al., 2009). The daily output of soil C between two successive measurement dates was estimated via linear interpolation according to Almagro et al. (2009). The estimated daily means of Rh C-CO_2 losses (t ha^{-1}) were accumulated from the beginning of the measurements (i.e., the day after the previous crop harvest) to the maize harvest (119 days), from the day after the maize harvest to the hay crop harvest (246 days) and at the end of the crop rotation (365 days).

2.5. Data analysis

T, SWC, SR and Rh data were submitted to the analysis of variance with repeated measurements over time by using the general linear model in the SAS software (proc GLM, repeated option) (SAS Institute, 1999). The factors were considered to be N fertilization and the measurement dates. The data were transformed into log values prior to the analysis of variance to meet the assumption of homogeneity of the error variance (Gomez and Gomez, 1984). The treatment means were compared using a protected least significant difference test at $P < 0.05$.

The SWC effects on SR and Rh temporal dynamics were assessed by regression analysis (Rey et al., 2002).

The relationships between T and SR or Rh were analysed according to the model: $SR = a\ e^{bT}$ (Davidson et al., 1998), where T is the soil temperature at 10 cm depth and a and b are equation parameters. The parameters of the different regression equations for the four fertilization treatments were compared using the analysis of covariance.

The effects of the fertilizer C input on the average SR and Rh values were tested by linear regression analyses.

The data for the soil C balance were analysed with an ANOVA for a Latin square with the four fertilization systems as treatments using the PROC GLM in SAS (SAS Institute, 1999). The effects of fertilizer or crop residue C inputs on the soil C balance were tested by linear regression analyses.

3. Results

3.1. SWC, soil T, SR and Rh

SWC varied markedly ($P < 0.001$) with the season, consistently with the water provided (Fig. 1a), and was not significantly affected by treatment.

The maximum SWC (approximately 0.30 m^3m^{-3}) was observed in December (Fig. 1a). Over the entire monitoring period, SWC was always higher than 0.085 m^3 m^{-3}, and the lowest SWC was observed in the spring (Fig. 1a). The soil T (10 cm depth) ranged from 8 to 25 °C, with minimum values in January and February and a maximum in July (Fig. 1b). During the maize crop phase, T never fell below 19 °C. During the hay crop phase, T was always lower than 21 °C. No treatment effect on T was observed.

SR and Rh showed similar seasonal dynamics (Fig. 2). Maximum values of SR and Rh were reached soon after organic fertilization was applied, up to 12 µmol m^{-2} s^{-1} in June 2011 in SL and MA (Fig. 2). Another maximum of SR and Rh, but to a much lower extent, was observed soon after the organic fertilization was applied in October, followed by a steady decrease until February 2012 (Fig. 2). An increasing and then fluctuating pattern in SR was observed from early spring to the hay mowing, followed by a sharp decrease in SR at the hay crop harvest (Fig. 2a). Both SR and Rh were significantly affected by treatment ($P < 0.001$). During the maize crop phase, SR and Rh were significantly higher on average in MA than in MI (Fig. 2). SL showed significantly higher Rh values on average than SM (Fig. 2b). In autumn-spring after the hay crop was sowed, SR was significantly lower in the MI than in the other treatments and was significantly higher in the MA than MI in April and May (Fig. 2a). Rh in relation to SR was higher on average in the organic treatments until the maize harvest (Figs. 2, 3a and c). During the crop rotation Rh was on average 59%, 54%, 52% and 47% of SR in MA, SL, SM and MI, respectively.

Significant relationships were observed between the average soil CO_2 efflux rate and the C input from fertilizer in each crop phase; these were stronger for Rh (Fig. 3c and d) and less strong for SR in the autumn-spring hay crop phase (Fig. 3b).

No significant relationship was observed between SWC and SR or Rh (data not shown), while a significant ($P < 0.05$) and distinct exponential relationship was observed between the soil T and SR or Rh in both crop phases (Figs. 4 and 5). The regression equation for the soil T and SR or Rh was similar for MA and SL and differed significantly from that for SM and MI, and between SM and MI (Figs. 4 and 5), with the exception of the regression between T and SR for the autumn-spring hay crop phase (Fig. 5a). The slope of the T *vs* SR or Rh regression line was particularly high for T N 20 °C, corresponding to the maize crop phase (Fig. 4).

3.2. Soil C balance

Significant differences ($P < 0.05$) in terms of C input (crop residues + organic fertilizers) were observed only for the maize crop phase, and the average C input values followed the pattern

MA =SL > SM > MI (Table 2).

The cumulative soil C-CO_2 efflux measured at the harvest of both crops was significantly affected by the fertilization system (Table 2). MA and SL showed higher values than MI. The C losses during the maize crop phase were between 50% (MI) and 67% (MA) of the entire crop rotation losses on average (Table 2).

At the maize harvest, the C balance was significantly negative and lower in MA than SL and SM, while no significant difference was observed between MI and the other treatments (Table 2). A different trend was observed during the hay crop phase, where the C balance was significantly higher in MI than in MA, while no significant differences were observed between SL and both MA and SM (Table 2). Considering the entire crop rotation, the C balance was significantly negative and lower in MA than in the other treatments, while no significant differences were observed between MI, SL and SM (least significant difference = 1.2 t ha^{-1}).

The linear regression analyses showed a significant and negative effect of fertilizer C input (Fig. 6b) and a significant and positive effect of residue C input (Fig. 6d) on hay crop C balance. No significant relationships were observed for the maize crop (Fig. 6a and c).

4. Discussion

4.1. SWC, soil T, SR and Rh

During the observation period, SWC never fell below 0.085m^3m^{-3}, which corresponds to a matric potential of approximately -33 kPa (Saxton and Rawls, 2006). Lado-Monserrat et al. (2014) reported significant effects of the SWC on soil CO_2 efflux below a threshold of -100 kPa. Therefore, the observed SWC is consistent with the assumption that the SWC was never a constraint to the SR or Rh at our research site.

In a Mediterranean climate in a rainfed cropping system, T was shown to display inverse seasonal dynamics with respect to SWC (Rey et al., 2002; Almagro et al., 2009; Lai, 2011). Our results are consistent with those findings only for the winter-early-spring period, when SWC in a rainfed soil is usually not limiting for SR. Under irrigation, the soil CO_2 efflux dynamics were mainly limited by the T and the fertilization system.

We observed maximum SR or Rh values following fertilization and tillage in the spring and autumn. Similar dynamics were reported by Steenwerth et al. (2010) in California, where sharp peaks of SR rates were interpreted as an effect of the autumn rain and tillage. In our study, because SWC was non-limiting with respect to SR or Rh, rain or irrigation events did not significantly affect SR or Rh. Tillage changes the soil structure by breaking soil aggregates and increasing soil aeration (Six et al., 2004). These two effects may trigger microbial activity in the soil (Quincke et al., 2007) by making the soil organic mass previously protected in the aggregates available to the soil microbial community (Six et al., 2004). In our study, the formation of soil aggregates was limited by the sandy soil texture; therefore, we concluded that the maximum SR or Rh rates, which were observed in the spring and soon after autumn ploughing, were a result of the fresh C inputs (Liu et al., 2006) with a non-limiting T and SWC (Almagro et al., 2009). Moreover the first maximum of soil respiration immediately after the application of the organic fertilizers can be interpreted as a positive priming effect, consistent to what reported by Kuzyakov et al. (2000). The differences in the SR rate observed between treatments were interpreted as an effect of the fertilizer source on the organic mass input and the related proportion of mineral to total N (Table 1). These differences were consistent with the strong relationships observed between the fertilizer C input and the average SR or Rh for each crop phase. MA and SL applications affect soil organic matter pools (Kaur et al., 2008) and generate a high concentration of soil dissolved organic C (Abalos et al.,

2013) that are strongly related to soil microbial and enzymatic activity (Kiikkilä et al., 2014). At the same time, rate and time of fertilization affect crop growth, root density (Rasmussen et al., 2015) and the gaps between SR and Rh (Hanson et al., 2000). Consistently, hay yield was higher in MI than MA and SL (Demurtas, 2014).

The response of SR or Rh to T was independent of the type of organic input (i.e., manure or slurry) and, therefore, the amount of C input from fertilizers, as well as the ratio of mineral N/total N(see Table 1), had a negligible role on the Rh sensitivity to T, in agreement with Fang et al. (2005).

The differences in T *vs* Rh regression equations, observed between treatments involving organic fertilization alone and treatments with mineral fertilizer, were associated with a combined effect of a higher fresh organic mass input (Mancinelli et al., 2010) and the inhibitory effect of mineral N on Rh in N-abundant soils (Carreiro et al., 2000; Ding et al., 2007). Consistently, the Rh to SR ratio was higher under organic than mineral fertilization and we observed significant differences between SL, SM and MI in terms of response of Rh to T while no significant differences were observed between SL and MA.

4.2. Soil C balance

In terms of the C input, the differences observed during the maize crop phase were mainly associated with the C input from fertilizers, while the lack of significant differences in the C input during the hay crop phase was associated with an increase in crop productivity (Demurtas, 2014) and, consequently, crop residue, in the treatments with a top dressing of mineral N fertilizer (Lai et al., 2012).

The highest impact in terms of total soil C losses was observed for maize, despite the relatively short duration of the crop cycle and was mainly associated with the high C input provided by organic fertilizer (Liu et al., 2006) and with the favourable T and SWC for mineralization in summer (Davidson et al., 1998). The C balance at the harvest of each crop and for the entire crop rotation showed a lower efficiency in soil C sequestration of the MA as a result of the high C losses. This result was consistent with the findings by Cappai (2014), who showed a not univocal relationship between fertilizer C input and soil organic C concentration, in a study carried out in the same field. On the other hand, our result was contrary to the findings reported in several studies in different soils and weather conditions, in which higher C inputs corresponded to higher soil C stocks (Lal et al., 2015). In Japan, in a humid, continental climate and with 710 g sand kg^{-1} in the top soil, Shimizu et al. (2009) observed a greater annual C budget with manure fertilization than with mineral fertilization, although they showed a higher CO_2 efflux after manure fertilization as an effect of a higher soil C input. In another Japanese field study carried out by Mori and Hojito (2015), a soil organic C balance was observed following fertilization with manure or slurry. In the latter case, after two years of treatment, fertilization with slurry decreased the soil organic C concentration seven times more than manure did. The authors associated this trend with the effect of the different chemical composition of the two organic fertilizers due to the different storage and management methods. In our study, the high soil C losses observed in the MA treatment were interpreted as the combined effect of i) the favourable SWC and T for soil organic mass mineralization (Rey et al., 2002), ii) the sandy soil texture (Zinn et al., 2005) that limited the formation of macroaggregates and consequently soil stabilization of C from fertilizer (Garcia-Franco et al., 2015), iii) the straw concentration in the fertilizer that enhanced Rh as effect of increasing in microbial biomass carbon (Abro et al., 2011), and iv) decrease in crop productivity and, consequently, in the residue C input as result of a reduction of the N use efficiency associated with leaching in autumn-winter (Demurtas, 2014). Moreover, the negative soil C balance observed following manure application alone confirmed the results of a recent study carried out by Wang et al. (2015) in China and could be associated with the progressive achievement of a new C turnover equilibrium after changes in fertilizer management. In addition, in our study, the observed negative correlation between fertilizer C input and soil C balance and the positive relationship between crop residue C input and soil C balance, raise questions on the effectiveness of the supply of just exogenous organic matter in improving the soil C budget in sandy soils under Mediterranean climate.

5. Conclusions

Our findings clearly show that the fertilization with just organic fertilizer did not increase the soil C budget in maize-based irrigated cropping systems, in sandy soils under Mediterranean climate. Organic fertilization increased the soil CO_2 efflux response to T at a non-limiting SWC. MI showed lower sensitivity of SR or Rh to T than MA and SL.

In the autumn-spring hay crop, the top dressing of mineral N fertilizer in late winter improved the soil C balance, also because of an increase in crop productivity. Variations in SR and Rh, induced by different fertilization regimes, were strongly associated with the C input from fertilizers. Fertilization with manure alone provided a higher C input to the soil, but it was the least efficient treatment in terms of CO_2 emission mitigation and overall showed a negative annual C balance. Between treatments, differences in soil C budget were associated with the crop productivity and the limited soil stabilization of the C provided with the organic fertilizer. Concluding, from this work we can recommend the top dressing N fertilization as best practice to improve the soil C budget in sandy soils under Mediterranean conditions when SWC is not limiting.

Further studies are worthwhile to evaluate whether different soil texture can enhance soil organic stabilization when crops are fertilized only with organic fertilizer, in Mediterranean irrigated cropping systems. Given the limitations of the methodology adopted in this study, further studies are also recommended to confirm the different components of the soil C input from crop residues, which we estimated on the basis of indexes taken from the literature, such as the treatment effects on plant biomass partitioning and composition.

In conclusion, this study can be considered a significant step in improving the understanding of soil C dynamic, which generates new research questions, which are worth to be investigated further to verify the long-term effects of different fertilization regimes on the soil C budget of intensive irrigated cropping systems in sandy soils under Mediterranean climate.

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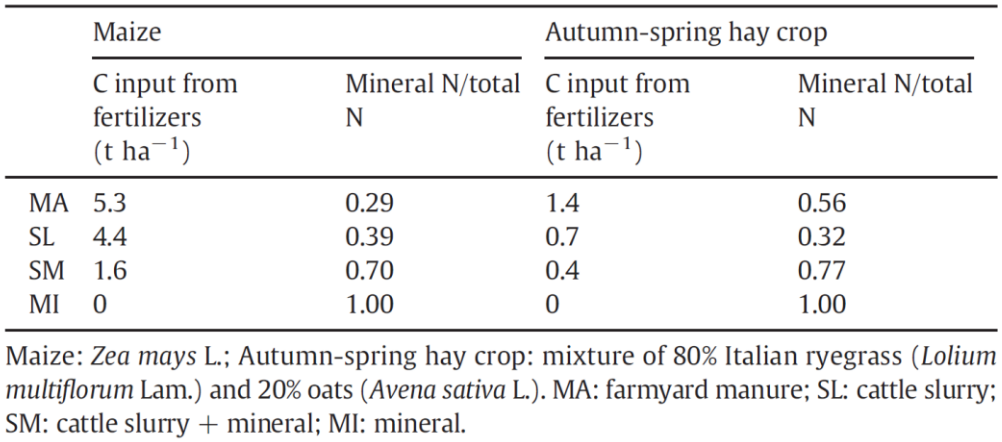
Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.catena.2016.12.013. These data include the graphical abstract and the Google map of the area described in this article.

Tables

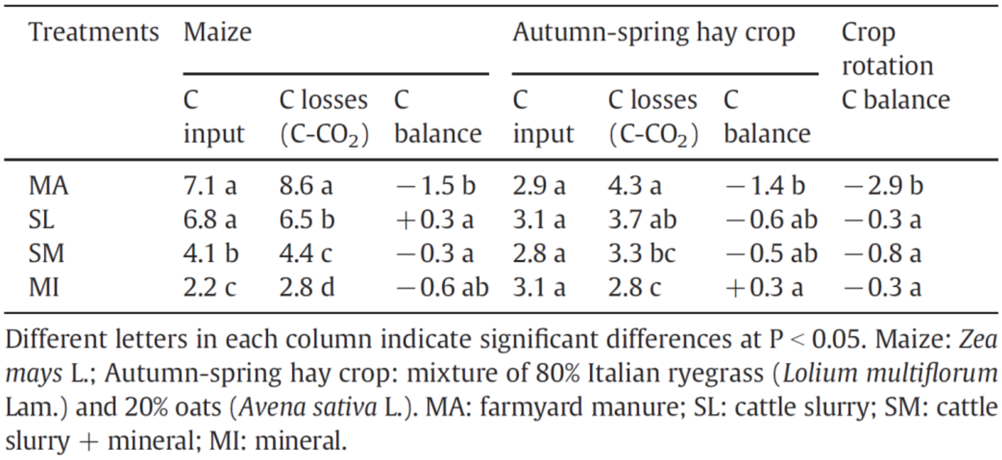
Table 1 Soil carbon (C) inputs and mineral nitrogen (N)/total N ratio provided by the different fertilizers during the two crop phases within the monitoring period.



	Maize		Autumn-spring hay crop	
	C input from fertilizers (t ha^{-1})	Mineral N/total N	C input from fertilizers (t ha^{-1})	Mineral N/total N
MA	5.3	0.29	1.4	0.56
SL	4.4	0.39	0.7	0.32
SM	1.6	0.70	0.4	0.77
MI	0	1.00	0	1.00

Maize: *Zea mays* L.; Autumn-spring hay crop: mixture of 80% Italian ryegrass (*Lolium multiflorum* Lam.) and 20% oats (*Avena sativa* L.). MA: farmyard manure; SL: cattle slurry; SM: cattle slurry + mineral; MI: mineral.

Table 2 Total soil carbon (C) inputs (fertilizers+crop residues), C-CO_2 losses and C balance during the maize crop phase, the autumn-spring hay crop phase and the entire crop rotation. The C inputs, losses and balances were computed at the harvest of each crop. Data are expressed as t ha^{-1}.



Treatments	Maize			Autumn-spring hay crop			Crop rotation
	C input	C losses (C-CO_2)	C balance	C input	C losses (C-CO_2)	C balance	C balance
MA	7.1 a	8.6 a	−1.5 b	2.9 a	4.3 a	−1.4 b	−2.9 b
SL	6.8 a	6.5 b	+0.3 a	3.1 a	3.7 ab	−0.6 ab	−0.3 a
SM	4.1 b	4.4 c	−0.3 a	2.8 a	3.3 bc	−0.5 ab	−0.8 a
MI	2.2 c	2.8 d	−0.6 ab	3.1 a	2.8 c	+0.3 a	−0.3 a

Different letters in each column indicate significant differences at $P < 0.05$. Maize: *Zea mays* L.; Autumn-spring hay crop: mixture of 80% Italian ryegrass (*Lolium multiflorum* Lam.) and 20% oats (*Avena sativa* L.). MA: farmyard manure; SL: cattle slurry; SM: cattle slurry + mineral; MI: mineral.

Figures

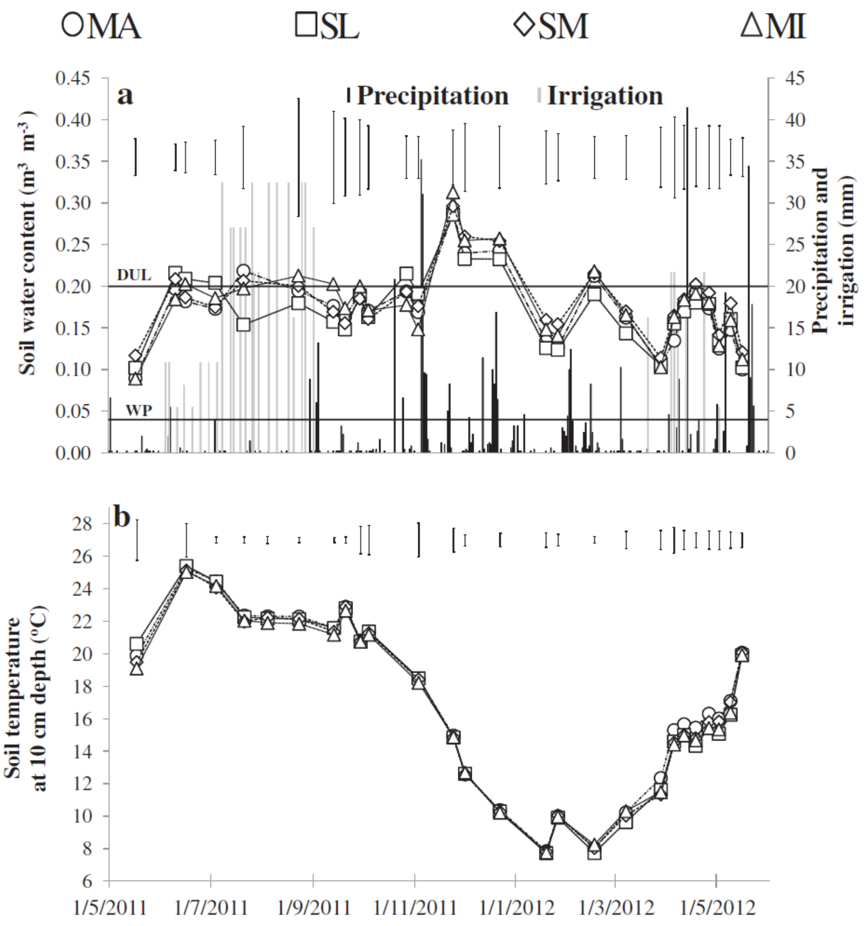


Figure 1 Dynamics of the soil water content (a) and soil temperature (b) measured, respectively, over 0–20 cm top soil and at 10 cm depth, within the monitoring period (May 2011–May 2012). Dates are expressed as day/month/year. Vertical bars represent least significant differences for treatment comparisons within sampling dates at $P < 0.05$. DUL: drained upper limit; WP: wilting point. MA: farmyard manure; SL: cattle slurry; SM: cattle slurry + mineral; MI: mineral.

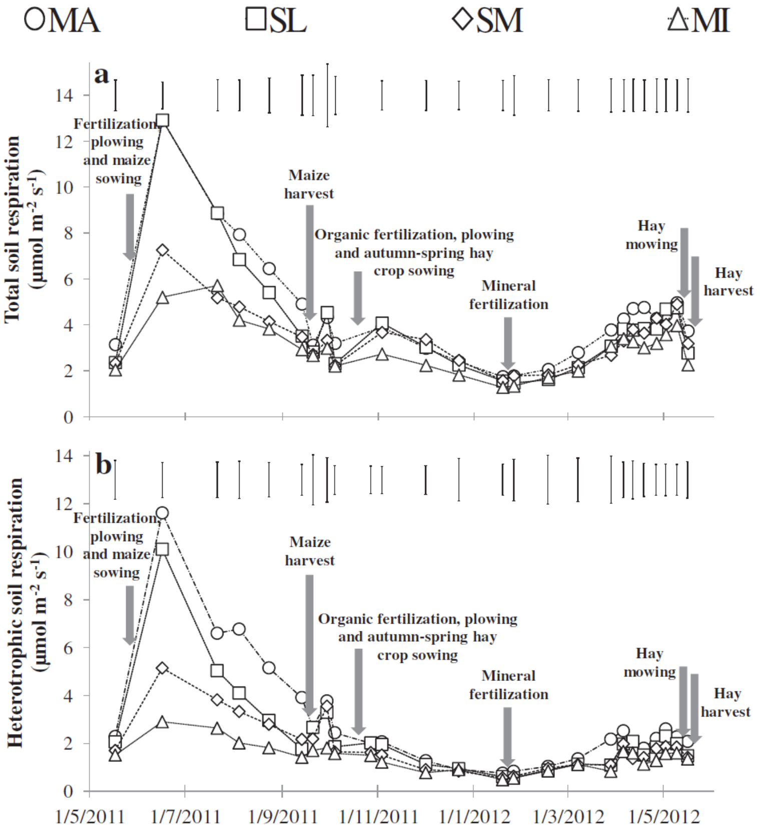


Figure 2 Dynamics of the total soil respiration (a) and heterotrophic soil respiration (b) in the monitoring period (May 2011–May 2012). Dates are expressed as day/month/year. Vertical bars represent the least significant difference values for treatment comparison within the sampling date at $P < 0.05$. Maize: *Zea mays* L.; Autumn-spring hay crop: mixture of 80% Italian ryegrass (*Lolium multiflorum* Lam.) and 20% oats (*Avena sativa* L.). MA: farmyard manure; SL: cattle slurry; SM: cattle slurry + mineral; MI: mineral.

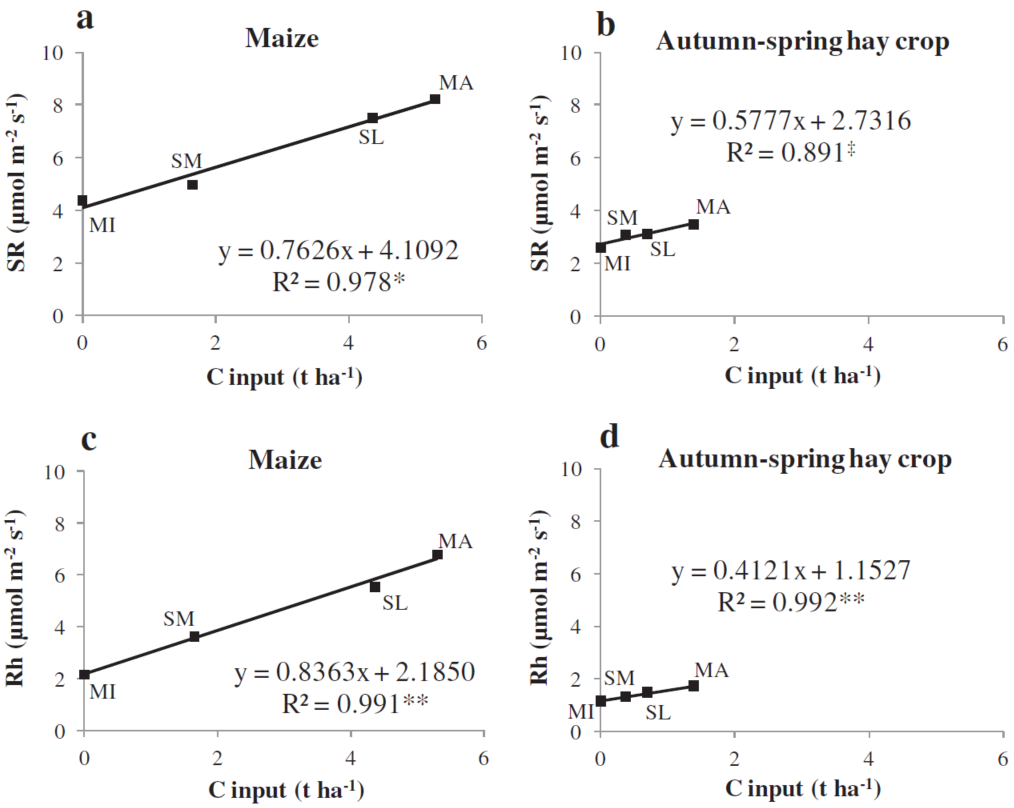


Figure 3 Relationship between the fertilizer carbon (C) input and the mean total (SR) and heterotrophic (Rh) soil respiration of measurements made from fertilization to harvest during each crop phase. Maize: *Zea mays* L.; Autumn-spring hay crop: mixture of 80% Italian ryegrass (*Lolium multiflorum* Lam.) and 20% oats (*Avena sativa* L.).MA: farmyard manure; SL: cattle slurry; SM: cattle slurry+ mineral; MI: mineral. ‡P < 0.10; *P < 0.05; **P < 0.01.

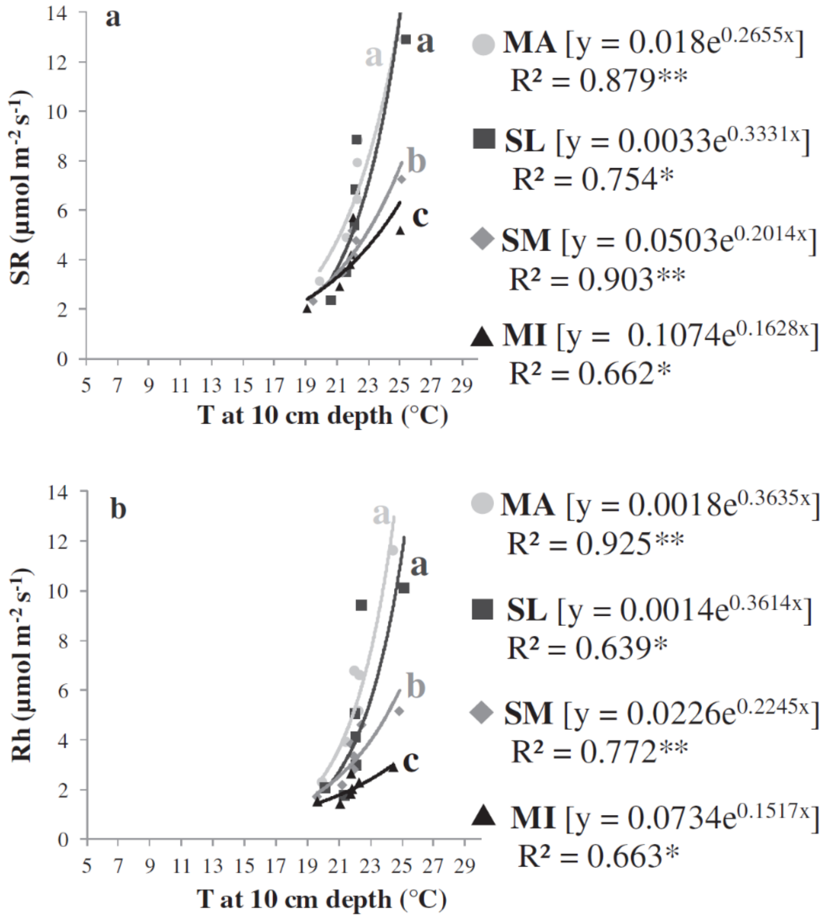


Figure 4 Relationships between the soil temperature (T) at a 10 cm depth and (a) the total soil respiration (SR) or (b) heterotrophic soil respiration (Rh) in the four treatments during the maize crop (*Zea mays* L.) phase. Different letters indicate differences between regression lines at $P < 0.05$. Asterisks indicate the significance: ***$P < 0.001$; **$P < 0.01$;*$P < 0.05$.MA: farmyard manure; SL: cattle slurry; SM: cattle slurry + mineral; MI: mineral.

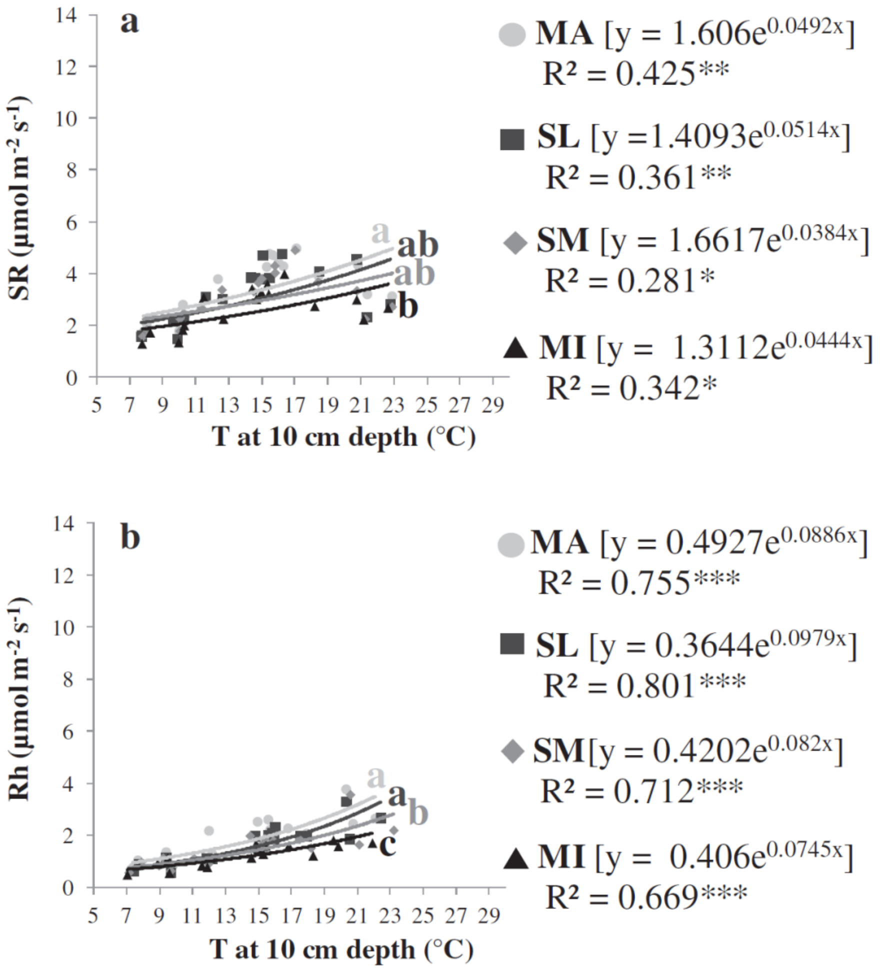


Figure 5 Relationships between the soil temperature (T) at a 10 cm depth and (a) total soil respiration (SR) or (b) heterotrophic soil respiration (Rh) during the autumn-spring hay crop (80% *Lolium multiflorum* Lam. and 20% *Avena sativa* L.) phase. Different letters indicate differences between regression lines at $P < 0.05$. Asterisks indicate the significance: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$. MA: farmyard manure; SL: cattle slurry; SM: cattle slurry + mineral; MI: mineral.

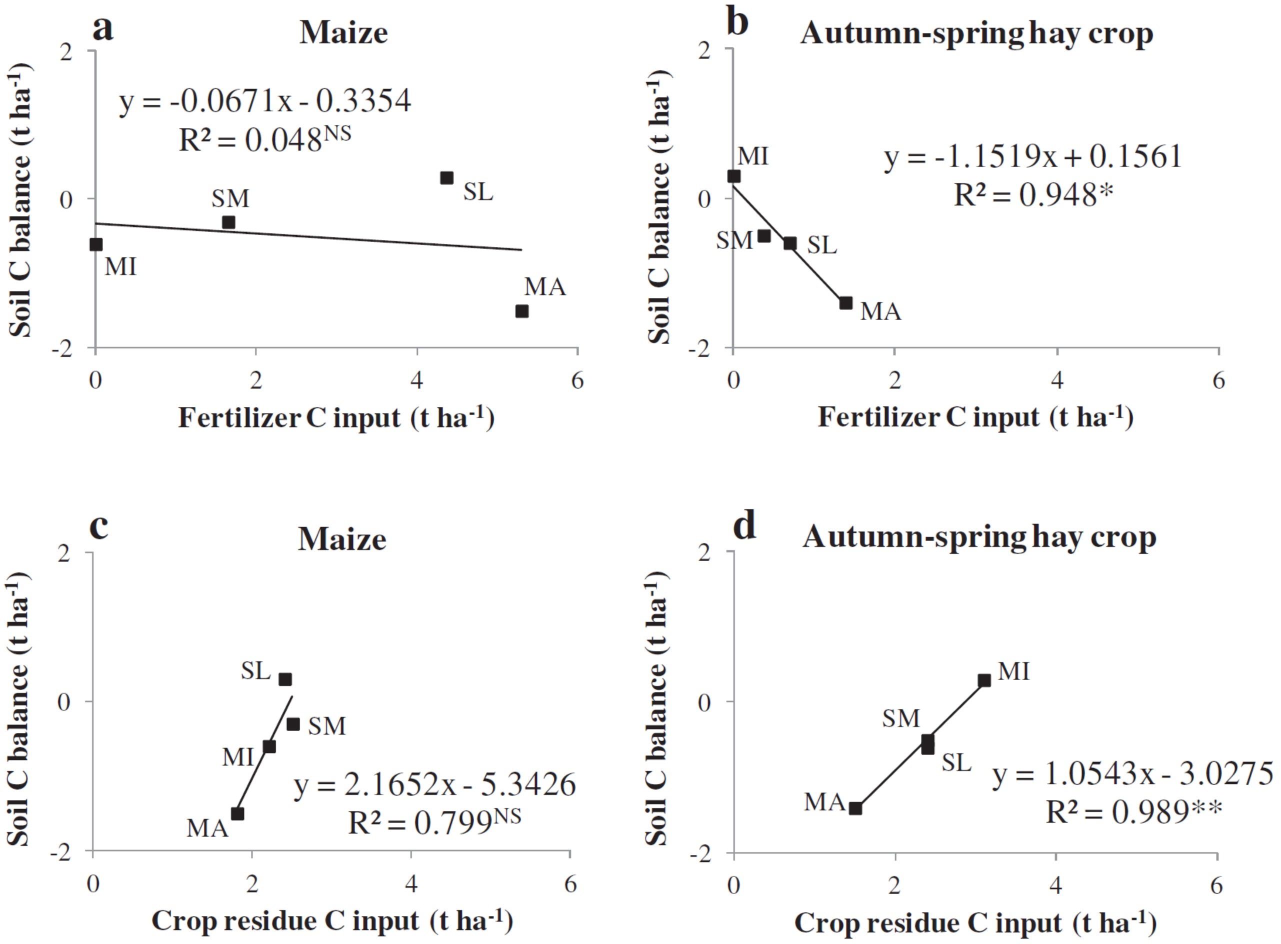


Figure 6 Relationship between fertilizer or crop residue carbon (C) inputs and the carbon balance of each crop. Maize: *Zea mays* L.; Autumn-spring hay crop: mixture of 80% Italian ryegrass (*Lolium multiflorum* Lam.) and 20% oats (*Avena sativa* L.). MA: farmyard manure; SL: cattle slurry; SM: cattle slurry + mineral; MI: mineral. NS not significant; $^*P < 0.05$; $^{**}P < 0.01$.

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