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Soil Greenhouse Gases Emissions in Mediterranean Forage Systems

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CONTENTS

INTRODUCTION

Soil Greenhouse Gases Emissions from Agricultural Soils

The agricultural soils occupy about the 37% of land. The impacts of their management on Greenhouse Gases (GHG) emissions become in the last years a focus point of agronomic research (IPCC, 2013). The most important GHGs determining the Global Warming Potential (GWP) of the agroecosystems are the carbon dioxide (CO_2), the nitrous oxide (N_2O) and the methane (CH_4) (Smith *et al.*, 2008).

The increase of CO_2 concentration in the atmosphere is partly attributable to human activities (Smith *et al.*, 2008), among which the agriculture is one of the most impacting. The soils can result as sinks or sources of C. The factors that mostly affect soil C exchanges, and consequently the soil CO_2 emissions, are the climatic conditions and the agronomic management. The abiotic factors that mostly influence the seasonal variability of soil CO_2 fluxes are the soil temperature and water content (Davidson *et al.*, 1998; Janssens *et al.*, 2003). Other soil chemical and physical characteristics, as total N, bulk density, porosity and water retention capacity affect mainly the spatial variability of CO_2 emissions (Luan *et al.*, 2012). The best agronomic practices leading to enhance soil organic carbon and the land uses such as pastureland seem crucial in regulating soil C sequestration (Smith *et al.*, 2008).

In the last years the scientific community has shown an increasing interest on the factors that regulate soil N_2O emissions from agroecosystems (Parton *et al.*, 2001; Schulze *et al.*, 2009; Butterbach-Bahl *et al.*, 2013). The N_2O is a very powerful GHG due to a 100-years GWP of 298 times higher than CO_2 (Myhre *et al.*, 2013). It was estimated that about 84% of N_2O emissions due to anthropic activities derive from agricultural activities (Butterbach-Bahl *et al.*, 2013). The N_2O emissions from soil are distinguished by the occurrence of "hot spots" and "hot moments", as well as for very high temporal and spatial variability (Groffman *et al.*, 2006). The main factor affecting N_2O emissions is the soil reactive N availability (organic and mineral N compounds except N_2), which is strongly related with fertilization practices in agroecosystems. The environmental factor that affect spatial and temporal variability of N_2O emissions is the soil water content, which in turn regulates the oxygen availability for microbial nitrification and denitrification processes (Davidson *et al.*, 2000). The denitrification process is in turn strongly affected by the increase of soil temperature (Schaufler *et al.*, 2010).

In the Fifth Assessment Report of the IPCC (Myhre *et al.*, 2013) it is reported that CH_4 has a 100-year GWP of 34 time higher than CO_2. In the agroecosystems, under non-flooded conditions, the soil CH_4 fluxes could be relevant when the organic materials added with fertilizations decompose in low-oxygen availability conditions (Chadwick *et al.*, 2000; Smith *et al.*, 2008). As well as CO_2, the soil can be a sink or source of CH_4, depending on the balance between the production by methanogenic bacteria and the consumption by the methanotrophic ones (Le Mer and Roger, 2001).

The agricultural systems addressed to provide animal feed have a substantial role in the global computation of soil GHG emissions. In Mediterranean environment, the forage systems have a considerable relevance for their impacts in terms of surface and socio-economic implications (EIP-AGRI, 2016; Kipling *et al.*, 2016). Furthermore, these systems are characterized by an high level of heterogeneity and different levels of intensification (Porqueddu *et al.*, 2016; Sanz-Cobena *et al.*, 2017).

The Mediterranean extensive agroecosystems

In extensive agricultural systems under semi-arid climate, the water deficit in the dry season affect the primary production (Caballero *et al.*, 2009) and the soil ability to store C (Lal, 2004). In these systems, the management options that allow to enhance the soil C sequestration play a relevant role among the mitigation strategies of GHG emission and on determining the GWP. For this reason, under Mediterranean conditions it becomes crucial to investigate the agricultural practices increasing soil organic C. Grasslands have a crucial role in the global C cycle, because of their contribution to C sequestration in the biosphere (Lal, 2004; Ciais *et al.*, 2010), but they are known to be uncertain components of the global C balance (Scurlock and Hall, 1998; Schulze *et al.*, 2010). Permanent grasslands contributes to the stability of their functions and services, e.g. C-cycle regulation (Lai *et al.*, 2014). The soil C input in grassland, which mostly consist of recalcitrant material, is higher than cropping systems (Dawson and Smith, 2007) and similar to that observed in forestry systems (Smith *et al.*, 2008). Furthermore, the soil organic C content and its quality can be affected by the soil management (Seddaiu *et al.*, 2013; Lai *et al.*, 2014). The CO_2 efflux from soil, also referred to as soil respiration (SR), is a core component of the total biosphere C cycle. In Mediterranean semi-natural environments, the SR is mostly related to seasonal variation of both soil temperature and water content (Rey *et al.*, 2002).

The Mediterranean intensive agroecosystems

The intensive forage cropping systems are characterized by high inputs levels. Under Mediterranean conditions, the organic and mineral fertilizations, soil tillage, irrigation and residues management play a relevant role in determine the GHG emissions (Sanz-Cobena *et al.*, 2017). The business-as-usual management options imply high dry matter biomass removal and readily mineralizable C supply (Wang *et al.*, 2014). Furthermore, decomposition rates are associated with the high soil temperature (e.g. Lai *et al.*, 2017). The soil N cycle is also affected by the peculiar pedoclimatic conditions in Mediterranean environment. This involves in N_2O emission patterns different from soils under temperate climate (Aguilera *et al.*, 2013).

Although it is demonstrated that an efficient use of organic fertilizers can contribute to mitigate GHG emissions increasing also the soil C sequestration (Calleja-Cervantes *et al.*, 2017), the manure features can differently affect the soils resulting in a sink or source of GWP (Peters *et al.*, 2011; Maillard and Angers, 2014). Furthermore, the high fertilization levels are frequently associated to irrigation, which enhances the soil water content, thus creating environmental conditions that favour mineralization processes (Tejada *et al.*, 2009; Mancinelli *et al.*, 2010). Therefore, the effectiveness of organic fertilizers to enhance the soil organic C is still debated (Powlson *et al.*, 2011; Lai *et al.*, 2012). In intensive cropping systems, the high irrigation and N inputs create favourable soil conditions to N_2O emissions, which are in fact strongly related to available N and water (Aguilera *et al.*, 2013; Sanz-Cobena *et al.*, 2017). The use of organic instead of mineral fertilizers is commonly considered a good mitigation option to reduce soil N_2O emissions (Aguilera *et al.*, 2013; Cayuela *et al.*, 2017). The importance of this strategy becomes crucial if it is considered the impact of the industrial Haber-Bosh process of N fixation to obtain mineral fertilizers (Crutzen *et al.*, 2008). Nevertheless, at field scale, the heterogeneity of organic fertilizers can differently affect the N_2O emissions from soil. For instance, liquid manures as the slurry can have impacts similar to mineral fertilizers due to their high N-NH_4^+ content (Meijide *et al.*, 2010). The fertilization strategies have an impact also in determining CH_4 fluxes from soil, although in non-flooded conditions it occurs at a lesser extent. The impact of organic fertilizations can result in both sink or source of GWP deriving from CH_4 emission or uptake (Sanchez-Martin *et al.*, 2010), mostly depending on fertilizer ability on modifying the oxygen availability for microbial processes (Le Mer and Roger, 2001; Merino *et al.*, 2004).

Overall aims

The importance of Mediterranean grasslands in terms of primary production and soil C sequestration requires the implementation of tools that are able to assess the impacts of different management options. A modelling approach allows to assess with different temporal and spatial scales how the interactions of the multiple environmental and management factors affect the soil GHG emissions from grasslands (Kipling *et al.*, 2014; Snow *et al.*, 2014; Sándor *et al.*, 2016). From these scientific findings it comes the need to implement the parametrization of biogeochemical models which is appropriate for Mediterranean environments, also in the perspective of assessing climate changes impacts (Kipling *et al.*, 2016).

The interaction of multiple factors affecting the impacts of fertilization on GHG emissions under Mediterranean conditions requires further deepening on the role of different management strategies (Aguilera *et al.*, 2013; Cayuela *et al.*, 2017; Sanz-Cobena *et al.*, 2017). Therefore, it becomes crucial to investigate the different management options through experimental field research, in order to identify an array of mitigation practices. This approach can provide elements to better understand the processes which today appear to have controversial impacts. Furthermore, an important focus for agricultural research is to identify trade-off practices which are oriented both to mitigate the GHG pollution and to guarantee the agronomic sustainability of the cropping systems.

The overall aim of the PhD dissertation was to analyse the processes and the management options that affect the soil C cycle and GHG emission patterns in Mediterranean forage systems at different levels of intensification. For extensive agroecosystems, a biogeochemical grassland-specific model parametrization was performed, in order to assess its applicability to simulate the C fluxes under Mediterranean conditions. For intensive forage systems, the impacts of different fertilization options on soil GHG emissions were assessed, in order to identify options that ensure adequate production levels and at the same time can mitigate the field-scale GWP.

The thesis is articulated into two chapters, as listed below:

1. Modelling pasture production and soil temperature, water and carbon fluxes in Mediterranean grassland systems with the Pasture Simulation Model.
2. Global Warming Potential of a Mediterranean Irrigated Forage System: Implications for Designing the Fertilization Strategy.

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CHAPTER 1

Modelling pasture production and soil temperature, water and carbon fluxes in Mediterranean grassland systems with the Pasture Simulation Model.

Modelling pasture production and soil temperature, water and carbon fluxes in Mediterranean grassland systems with the Pasture Simulation Model

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Abstract

Grasslands play important roles in agricultural production and provide a range of ecosystem services. Modelling can be a valuable adjunct to experimental research in order to improve the knowledge and assess the impact of management practices in grassland systems. In this study, the PaSim model was assessed for its ability to simulate plant biomass production, soil temperature, water content, total and heterotrophic soil respiration in Mediterranean grasslands. The study-site was the extensively-managed sheep grazing system at the Berchidda-Monti Observatory (Sardinia, Italy), from which two datasets were derived for model calibration and validation, respectively. A new model parameterization was derived for Mediterranean conditions from a set of eco-physiological parameters. With the exception of heterotrophic respiration (Rh), for which modelling efficiency (EF) values were negative, the model outputs were in agreement with observations (e.g. EF ranging from ~0.2 for total soil respiration to ~0.7 for soil temperature). These results support the effectiveness of PaSim to simulate C cycle components in Mediterranean grasslands. The study also highlights the need of further model development to better represent the seasonal dynamics of Mediterranean annual species-rich grasslands and associated peculiar Rh features, for which the modelling is only implicitly being undertaken by the current PaSim release.

Keywords: Grassland production; Mediterranean pastures; Model calibration; PaSim; Sheep grazing systems; Soil respiration

Introduction

Grasslands and rangelands, which play a crucial role on supporting livestock systems and providing a wide variety of ecosystem services (EIP-AGRI, 2016), cover about 50% of European Mediterranean areas, which represent the largest region in the world characterized by a Mediterranean-type climate (Cosentino *et al.*, 2014). In the Mediterranean region, as well as in other parts of the world with similar climate, the grassland vegetation is dominated by C_3 annual plant species characterized by a variety of traits that ensure their persistence through self-reseeding under varying rainfall and temperature patterns (Roggero and Porqueddu, 1999; Bagella *et al.*, 2013; Porqueddu *et al.*, 2016). Mediterranean grasslands are almost exclusively secondary prairies, shaped by a range of anthropogenic practices, often belonging to mixed agro-ecosystems characterized by the presence of shrubs and trees (Bagella *et al.*, 2016). Mediterranean grassland growing season starts in autumn, with plant germination, and ends in late spring, followed by summer senescence. Within this period, ranging from 180 to 270 days depending on weather and altitude, typically two maximum growth rates are observed: the first one occurring in mid-autumn and the second in April (Cavallero *et al.*, 1992). Annual production is strongly constrained by summer drought duration and, even to a greater extent, by the variability of autumn and spring precipitation regimes (Gea-Izquierdo *et al.*, 2009; Golodets *et al.*, 2015).

Grasslands have a crucial role in the global C cycle because of their contribution to C sequestration in the biosphere (Lal, 2004; Ciais *et al.*, 2010). The Soil Respiration (SR), is a core component of the total C cycle. Under Mediterranean climatic conditions it is possible to observe wide SR temporal variations due to the dynamics of both soil temperature (Soil T) and soil water content (SWC) (Rey *et al.* 2002). Consistently, from winter to early spring soil T was observed to act as main driver of SR while, during summer SWC mostly controls this process (Almagro *et al*. 2009; de Dato *et al*. 2010; Oyonarte *et al*. 2012; Lai *et al.*, 2014). Although in humid periods SR variability can be explained by an exponential relationship between Soil T and SR (Davidson *et al.*, 1998), under drought conditions SWC turns into the most important factor affecting SR dynamics (Almagro *et al.*, 2009; Correia *et al.*, 2012; Oyonarte *et al.*, 2012). In fact, during the summer period, SR is constrained by water-limited microbial activity (Davidson and Janssens, 2006). Because of long dry periods occurring in Mediterranean environments, water deficit is considered in this region as the main limiting factor of the inter-annual variation of terrestrial C ecosystem exchanges, as it causes large reductions in primary productivity and affects SR (Reichstein *et al.*, 2002). Moreover, in Mediterranean agro-silvo-pastoral systems not only abiotic factors could affect SR dynamics, but also soil management and land use (Costa *et al.*, 2013).

The Pasture Simulation model (PaSim, https://www1.clermont.inra.fr/urep/modeles/pasim.htm, accessed on 1 January 2017), originally developed by Riedo *et al.* (1998), deals with grassland vegetation and major soil processes (water, C and N cycling) on a plot-scale configuration and performs analysis of management options, through the control of fertilizer application, irrigation, cutting and grazing. The model was used to simulate temperate grasslands in France to assess climate change impacts (Graux *et al.*, 2013; Vital *et al.*, 2013; Lardy *et al.*, 2014) and the global warming potential of forage-based livestock systems (Graux *et al.*, 2011; Graux *et al.*, 2012). A generic parameterization of the model was established for regional-scale analyses of C and water cycles in Europe (Ma *et al.*, 2015), where it generally performs comparatively better than the competing models (e.g. Sándor *et al.*, 2016). These studies contributed to the recognition of PaSim as a suitable tool to reproduce biophysical and biogeochemical processes of managed grasslands. However, a detailed application of the model for Mediterranean grasslands has not previously undertaken.

The aim of this study was to evaluate whether PaSim could be reasonably used to estimate C fluxes (i.e. biomass and soil respiration) and soil biophysical components (i.e. water and temperature) in a Mediterranean grassland system. Considering the characteristics of these agro-ecosystems, the objective of the study was to provide a framework for the quantitative assessment of the processes driving C cycle and water balances, as influenced by management practices.

Materials and Methods

Study site

The study site is located in the Long Term Observatory of Berchidda-Monti (NE Sardinia, Italy) (40° 49' N / 9° 17' - 9° 19' E; 287-325 m a.s.l.). The site is representative of Sardinian grassland-based dairy sheep farming systems and shares several commonalities with the agro-silvo-pastoral systems widespread in the Mediterranean Basin, in particular in the Iberian Peninsula (Caballero *et al.*, 2009). In Sardinia, permanent grasslands are the most common land use, occupying over 700,000 ha, i.e. ~30% of total area of the island (Ravenna, 2013). In the study site, the mean annual rainfall is 632 mm, of which 70% falls between October and May, inclusive. The mean annual temperature is 14.2°C and the aridity index (mean annual precipitation divided by mean annual reference evapotranspiration) is 0.53. According to USDA (2010), the dominant soil type is a Typic Dystroxerept, with sandy loam texture in the first soil horizon, derived from a granitic substratum (Carmignani *et al.*, 2012).

The Pasture Simulation model

PaSim (Riedo *et al.*, 1998) simulates water, C and N cycling in grassland systems in a sub-daily (1/50 of a day) time step. Microclimate, soil biology and physics, vegetation, grazing herbivores and management practices are interacting modules. Simulations are not spatially resolved (e.g. patchiness is not considered) because each simulated plot is assumed to be homogeneous and input/output data are assumed to be representative of the entire field. Photosynthetic-assimilated C is either allocated dynamically to one root and three shoot compartments (each of which consisting of four age classes) or lost through animal metabolism and ecosystem respiration. Accumulated aboveground biomass is either cut or grazed, or enters a litter pool. Management includes organic and mineral N fertilizer application, mowing and grazing, with parameters set by the user or optimized by the model. Some more details about model processes are provided in the supplementary material.

Model input data

Two grassland fields (Table 1) within two private farms in the Berchidda-Monti Observatory were identified (BM1 and BM2, having an area of 3.094 and 2.187 ha, respectively), for which suitable datasets were available to set up distinct model input files and parameterize the model.

The meteorological dataset was compiled for the period 2008-2014 from two weather stations located at the study site, owned by the Regional Environmental Protection Agency of Sardinia - Meteo-climatic Department (http://www.sar.sardegna.it). The soil profile was sampled according to horizons and depth, and analysed for particle-size distribution, bulk density and pH. Soil sampling and analyses methods were described by Seddaiu *et al.* (2013). Soil hydraulic properties (saturated soil water content, saturated hydraulic conductivity, field capacity and permanent wilting point) were estimated from the measured soil texture and soil organic matter according to Saxton and Rawls (2006). The grassland management was

Table 1 Location, grazing management and soil properties of the grassland study sites.

	Location			Grazing		Soil[2]									
Site ID	Latitude	Longitude	Elevation (m a.s.l.)	Average stocking rate (LSU^1 ha^{-1})	Yearly average duration ($d\ yr^{-1}$)	Soil depth (m)	Sand (%)	Silt (%)	Clay (%)	Bulk density ($Mg\ m^{-3}$)	Saturated soil water content ($m^3 m^{-3}$)	Saturated hydraulic conductivity ($mm\ d^{-1}$)	Field Capacity ($m^3 m^{-3}$)	Permanent Wilting Point ($m^3 m^{-3}$)	pH
BM1	40.81	9.29	287	2.10±1.01	102±74	0.93	64	13	23	1.59	0.40	695	0.18	0.08	5.2
BM2	40.82	9.32	319	1.79±0.83	135±69	1.40	68	13	19	1.57	0.41	704	0.17	0.09	5.8

[1] Livestock Standard Unit: standard measurement unit allowing the aggregation of various categories of livestock (Allen *et al.*, 2011).
[2] Values are weighted averageof all the six soil layers considered by the model

monitored at field scale during the observational periods through systematic interviews with farmers. In this way, it was possible to build a dataset of daily animal stocking rate. Daily animal stocking rates were mimicked by model management requirements approximated at 10 grazing events per year, by aggregating the days close to each other when grazing occurred for only a few hours per day and calculating in these periods the weighted stocking rates.

Model parametrization and evaluation

Field data resources were segregated into two groups: a sample (BM1) was used to estimate model parameters (calibration set); an independent sample (BM2) was used to validate model results (validation set).

The biophysical and biogeochemical data of the grassland systems in BM1 and BM2 was mainly represented by measurements of soil temperature at -0.10 m (soil T, °C), soil water content at -0.03 m (SWC, $m^3\ m^{-3}$), and total and heterotrophic soil respiration (SR and Rh, respectively, kg $C\text{-}CO_2\ m^{-2}\ d^{-1}$). In addition, data on grassland dry matter (DM) production (kg DM $m^{-2}\ d^{-1}$) from both grazed and ungrazed plots (GRDM and NGDM, respectively) were available. The model was calibrated based on a set of influential parameters (Table 2) identified in previous studies (Ben Touhami *et al.*, 2013; Ma *et al.*, 2015).

Pasture production was measured from February 2009 to May 2010, and from February 2013 to May 2014 at monthly intervals by cutting the herbage sward inside and outside randomly positioned fences (10 m *

10 m), so as to quantify herbage biomass without grazing (NGDM) and the grazed herbage on offer (GRDM), respectively. To determine biomass DM, samples (*n*=3) were taken on 0.5 m^2 areas, immediately stored in plastic bags kept cool, and then brought to the lab within 4 hours to be dried in a ventilated oven at 65°C until achieving constant weight.

Hourly measurements of soil T and SWC were taken with a WatchDog 1000 Series Micro Station (Spectrum Technologies, Inc., IL, USA, http://www.specmeters.com), equipped with a WaterScout SM 100 Soil Moisture Sensor and Soil Temperature Sensor for soil T and SWC, respectively.

A portable, closed chamber, soil respiration system (EGM-4 with SRC-1, PP-Systems, Hitchin, UK, http://ppsystems.com) was used to measure *in situ* SR and Rh, between 8:30 and 13:00 (solar time) in order to collect data representative of daily means (Almagro *et al.*, 2009 and citations therein). The measurements were carried out from 1st August 2013 to 5th August 2014 at weekly to monthly intervals (*n*=2), depending on grassland growth phases and weather conditions (Rey *et al.*, 2002). Measurements of Rh were made in trenched plots (Hanson *et al.*, 2000), in which soil was isolated with a PVC cylinder (0.40 m diameter and 0.25 m height) open at both ends (Lai *et al.*, 2012) and inserted into the soil profile till 0.20 m depth according to Unger *et al.* (2009).

For the calibration purpose, 10 model parameters were modified within their plausible ranges (Table 2) through a trial-and-error process comparing the model predictions with observational data to ensure realistic representation of a variety of outputs. They govern: i) canopy morphology and phenological features, in particular maximum specific leaf area, thermal sum for the transition from reproductive to vegetative phase, parameters describing the canopy height development and root-shoot turnover rates, and a parameter of root distribution in different soil layers; ii) canopy physiological features, in particular parameters describing CO_2 absorption rates at both vegetative and reproductive stages; and iii) soil biological activity, in particular a parameter governing soil respiration.

The agreement of model outputs with observational data of soil T, SWC, SR, Rh, GRDM and NGDM was assessed through a set of indices and by graphical reports applied to both calibration and validation sets. The multiplicity of aspects to be accounted for a multi-perspective assessment of model performance requires the use of a variety of metrics for model evaluation (Bellocchi *et al.*, 2010). These metrics (Table 3) include the goodness-of-fit R^2 (coefficient of determination) which assesses the linear dependence between modelled and observed data and the proportion of the total variation explained by the model, and a set of metrics such as the mean differences (BIAS), the percent relative root mean square error (RRMSE), the coefficient of residual mass (CRM), the modelling efficiency (EF) and the index of agreement (d) which assess quantitative differences.

In order to test the robustness of the obtained PaSim parametrization, model performance were assessed also for a set of ancillary observed data. The parametrization was tested on: i) grassland DM production under N fertilizer conditions; ii) the DM intake from pasture of grazing animals; iii) the C and N input from grazing animals. These ancillary data were collected in both BM1 and BM2 and overall metrics were calculated on both sites. The ancillary observations methods, results and relative metrics values are reported as supplementary material.

The RStudio extension of R-language computing environment (version 3.1.1) (R Core Team, 2014) was used to perform linear regression analysis between observed and simulated data for each output. The regression significance was tested using the "anova" function of the software base package. Residuals analysis were performed by combining both BM1 and BM2 datasets for each output. Standardized residuals were obtained by dividing differences between simulations and observations by standard deviation.

Table 2 Summary of the PaSim parameters considered for calibration. For each parameter, two sets of values are reported: reference values or ranges of values from previous studies (Ben Touhami *et al.*, 2013; Ma *et al.*, 2015) and values obtained from the calibration performed in this study.

Parameter	Description	Unit	Values		
			Proposed		Calibration
			Min	Max	
Maximum specific leaf area	Maximum value of specific leaf area, defined as the ratio of leaf area to dry weight, used to derive canopy leaf area from leaf biomass	$m^2\ kg^{-1}$ DM	28.0	35.0	30.0
The normalization factor for development	This parameter (dividing the sum of thermal units) normalizes the developmental stage index in such a way that the value 1 marks the transition from the reproductive to the vegetative stage	K-d	604	814	700
Canopy Height Parameter 1	This parameter expresses the leaf area index for which canopy is half the maximum height	$m^2\ m^{-2}$	4.0		2.0
Canopy Height Parameter 2	This is flowering plant height, with highest leaf not elongated[1]	m	0.3	0.7	0.3
The root turnover parameter	The root turnover rate at 20°C	d^{-1}	0.0096	0.0144	0.0144
The shoot turnover parameter	The shoot turnover rate at 20°C	d^{-1}	0.0360	0.0540	0.0250
Light-saturated leaf photosynthetic rate for reproductive stage	They represent the influence of developmental stage on the light-saturated leaf photosynthetic rate (defined as standard conditions of temperature, atmospheric CO_2 concentration), which is a component of the rate of canopy photosynthesis.	µmol C $m^{-2}\ s^{-1}$	15.0	22.5	14.0
Light-saturated leaf photosynthetic rate for vegetative stage			10.0	15.0	10.0
Temperature dependence factor of the soil respiration	It multiplies the temperature-dependent function to estimate soil respiration	-	0.7	2.0	0.7
Relative root distribution	This parameter fixes the fraction of structural dry root dry matter in each soil layer[2]	%	0.095 0.297 0.238 0.145 0.195 0.300		0.050 0.450 0.470 0.010 0.010 0.010

[1] The obtained parameter is an average value of measurements performed at the same time of DM samplings.
[2] The obtained root distribution is an assumption made according to model soil layers, which are specified in supplementary material.

Table 3 Index of model performance used in model assessment.

Performance metric	Equation	Unit	Value range and purpose	References
R^2, Coefficient of determination of the linear regression estimates vs measurements	$R^2 = \frac{\sum_{i=1}^{n}(P_i - O_i) \cdot (O_i - \bar{O})}{\sqrt{\sum_{i=1}^{n}(P_i - \bar{P})^2 \cdot \sum_{i=1}^{n}(O_i - \bar{O})^2}}$	-	$0 \leq R^2 \leq 1$ The best values are close to 1	
BIAS, mean difference of simulations and observations	$BIAS = \frac{\sum_{i=1}^{n}(P_i - O_i)}{n}$	Unit of the variable	$-\infty < BIAS < +\infty$ The best values are close to 0. Negative values: underestimation; positive values: overestimation	(Addiscott and Whitmore, 1987)
RRMSE, Relative root mean square error	$RRMSE = \frac{\sqrt{\frac{\sum_{i=1}^{n}(P_i - O_i)^2}{n}}}{\bar{O}} \times 100$	-	$0 \leq RRMSE \leq +\infty$ The best values are close to 0	(Fox, 1981)
CRM, Coefficient of residual mass	$CRM = \frac{\sum_{i=1}^{n} O_i - \sum_{i=1}^{n} P_i}{\sum_{i=1}^{n} O_i}$	-	$-\infty < CRM < +\infty$ Positive values: underestimation; negative values: overestimation	(Loague and Green, 1991)
EF, Modelling efficiency	$EF = \frac{\sum_{i=1}^{n}(O_i - \bar{O})^2 - \sum_{i=1}^{n}(P_i - O_i)^2}{\sum_{i=1}^{n}(O_i - \bar{O})^2}$	-	$-\infty < EF \leq +1$ The best values are close to 1	(Greenwood *et al.*, 1985)
d, Index of Agreement	$d = 1 - \frac{\sum_{i=1}^{n}(P_i - O_i)^2}{\sum_{i=1}^{n}(\lvert P_i - \bar{O}\rvert + \lvert O_i - \bar{O}\rvert)^2}$	-	$0 < d < 1$ The best values are close to 1	(Willmott and Wicks, 1980)

P, predicted value
O, observed value
n, number of P/O pairs
i, each of P/O pairs
$\bar{O}$, mean of observed values
$\bar{P}$, mean of predicted values

Results

At both study sites, observed GRDM and NGDM (Figure 1) showed the highest values in spring, for which interannual means were 3.67±0.54 and 4.37±0.64 Mg DM ha^{-1} in BM1, and 2.69±0.35 and 4.29±0.57 Mg DM ha^{-1} in BM2 for GRDM and NGDM, respectively. In summer, DM production was null. The average observed and simulated DM growth rates in NGDM are showed in Figure 2.

Soil T dynamics in BM1 ranged from 8.9°C in February to 25.7°C in August, and in BM2 from 8.3°C in December to 26.7°C in August. Annual mean values were 17.5 and 17.8°C, respectively in BM1 and BM2 (Figure 3). Compared to Soil T, SWC showed the opposite dynamics in both BM1 and BM2 datasets. Maximum values were observed in autumn-winter, when SWC reached 0.32 m^3 m^{-3} at both sites, while minimum SWC values were observed in summer: 0.08 m^3 m^{-3} in BM1 and 0.05 m^3 m^{-3} in BM2.

At both BM1 and BM2 SR and Rh (Figure 4) showed a two-peak profile.. Maximum SR values were: 3.31±0.49 g C-CO_2 m^{-2} d^{-1} observed on 11th September 2013 and 6.45±0.43 g C-CO_2 m^{-2} d^{-1} on 23rd April 2014 at BM1, and 2.62±1.38 g C-CO_2 m^{-2} d^{-1} on 11th September 2013 and 6.98±1.93 g C-CO_2 m^{-2} d^{-1} on 20th May 2014 at BM2. Minimum SR values were observed on 27th September 2013, when fluxes were 1.18±0.20 g C-CO_2 m^{-2} d^{-1} at BM1 and 1.05±0.20 g C-CO_2 m^{-2} d^{-1} at BM2. Maximum Rh values were: 2.49±0.52 g C-CO_2 m^{-2} d^{-1} on 8th November 2013 and 3.31±0.43 g C-CO_2 m^{-2} d^{-1} on 10th April 2014 at BM1, and 2.10±1.11 g C-CO_2 m^{-2} d^{-1} on 8th November 2013 and 3.37±0.29 g C-CO_2 m^{-2} d^{-1} on 23rd April 2014 at BM2. Minimum Rh fluxes were observed on 27th September 2013, when CO_2 fluxes were 0.56±0.23 g C-CO_2 m^{-2} d^{-1} at BM1 and 0.85±0.00 g C-CO_2 m^{-2} d^{-1} at BM2.

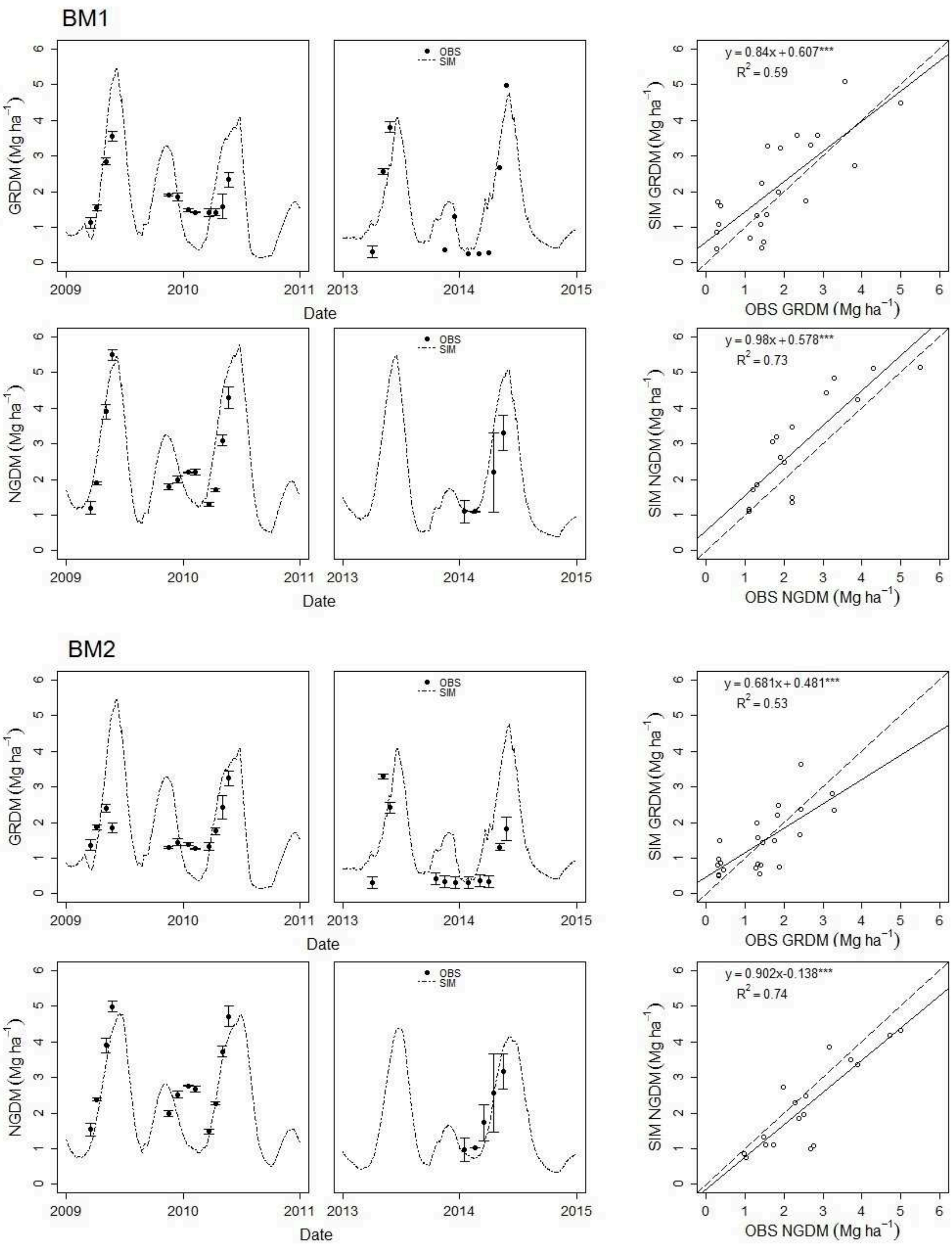

Figure 1 Calibration (BM1) and validation (BM2) results for GRDM (DM biomass in grazed plots, Mg DM m^{-2}) and NGDM (DM biomass in ungrazed plots, Mg DM m^{-2}) and relation between observed (OBS) and simulated (SIM) data. Bars indicate the observed data standard error (*n*=2). (***: P<0.001)

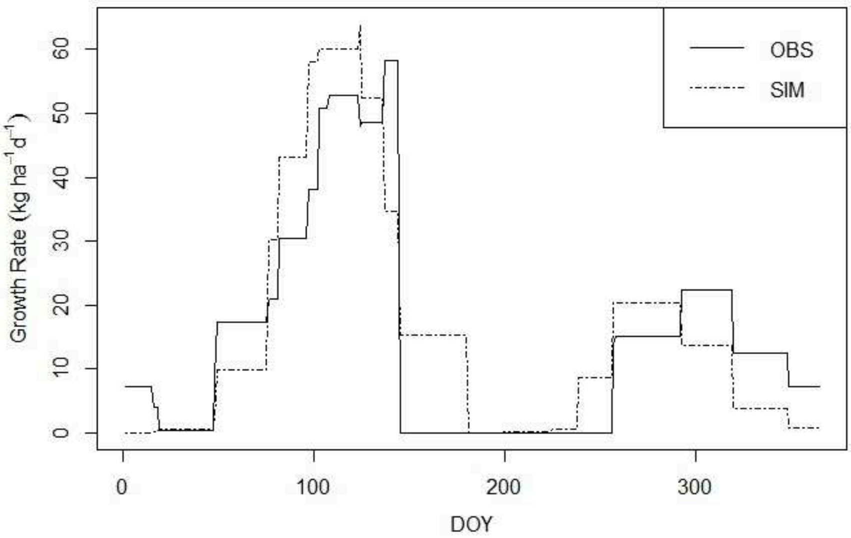

Figure 2 Observed and simulated average values of DM Growth Rate in NGDM. DOY= day of year; OBS = observed data; SIM = simulated data

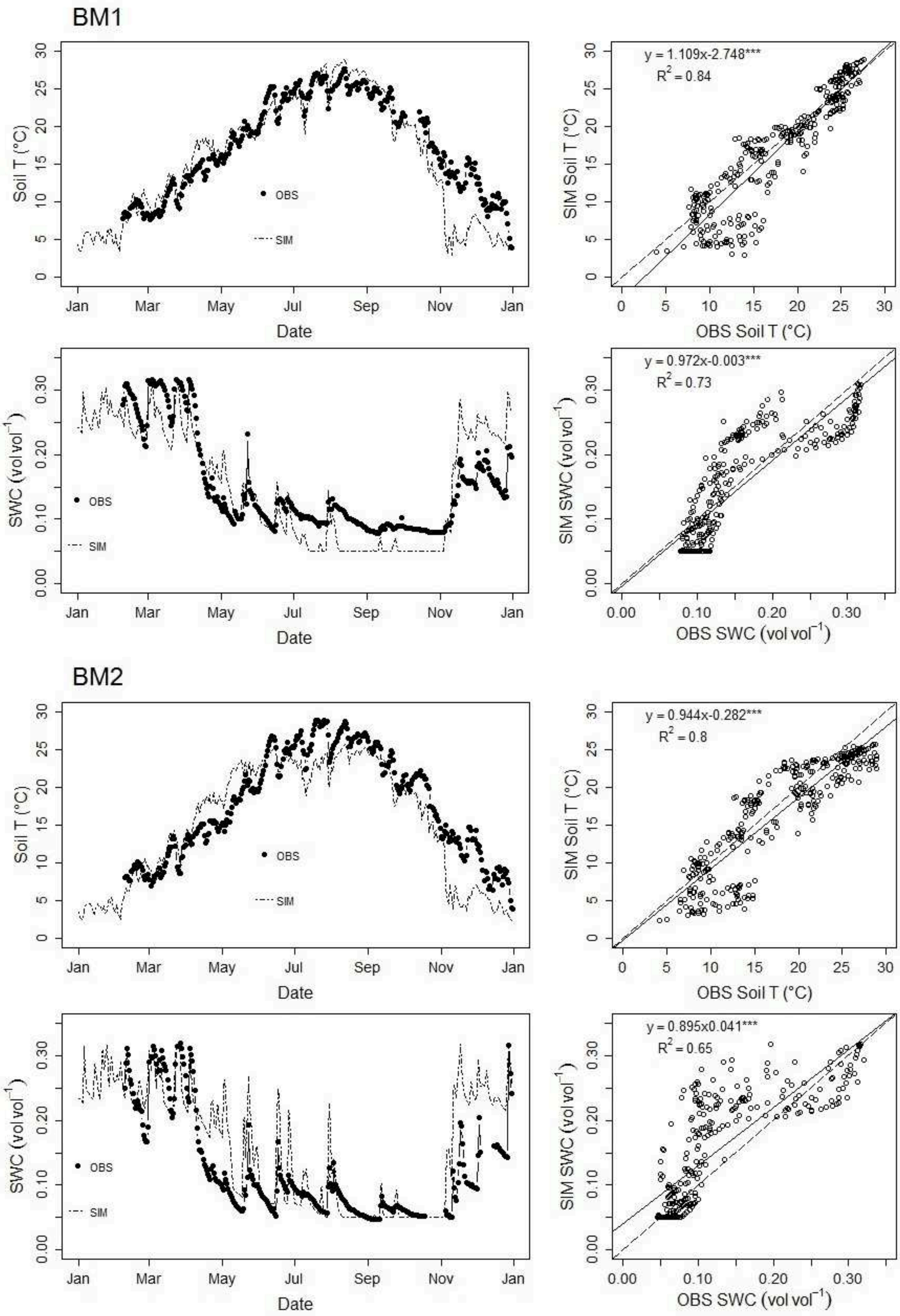

Figure 3 Calibration (BM1) and validation (BM2) results for Soil T (Soil Temperature, °C) and SWC at 0.03 m (Soil Water Content, m^3m^{-3}) and relation between observed (OBS) and simulated (SIM) data. (***: P<0.001)

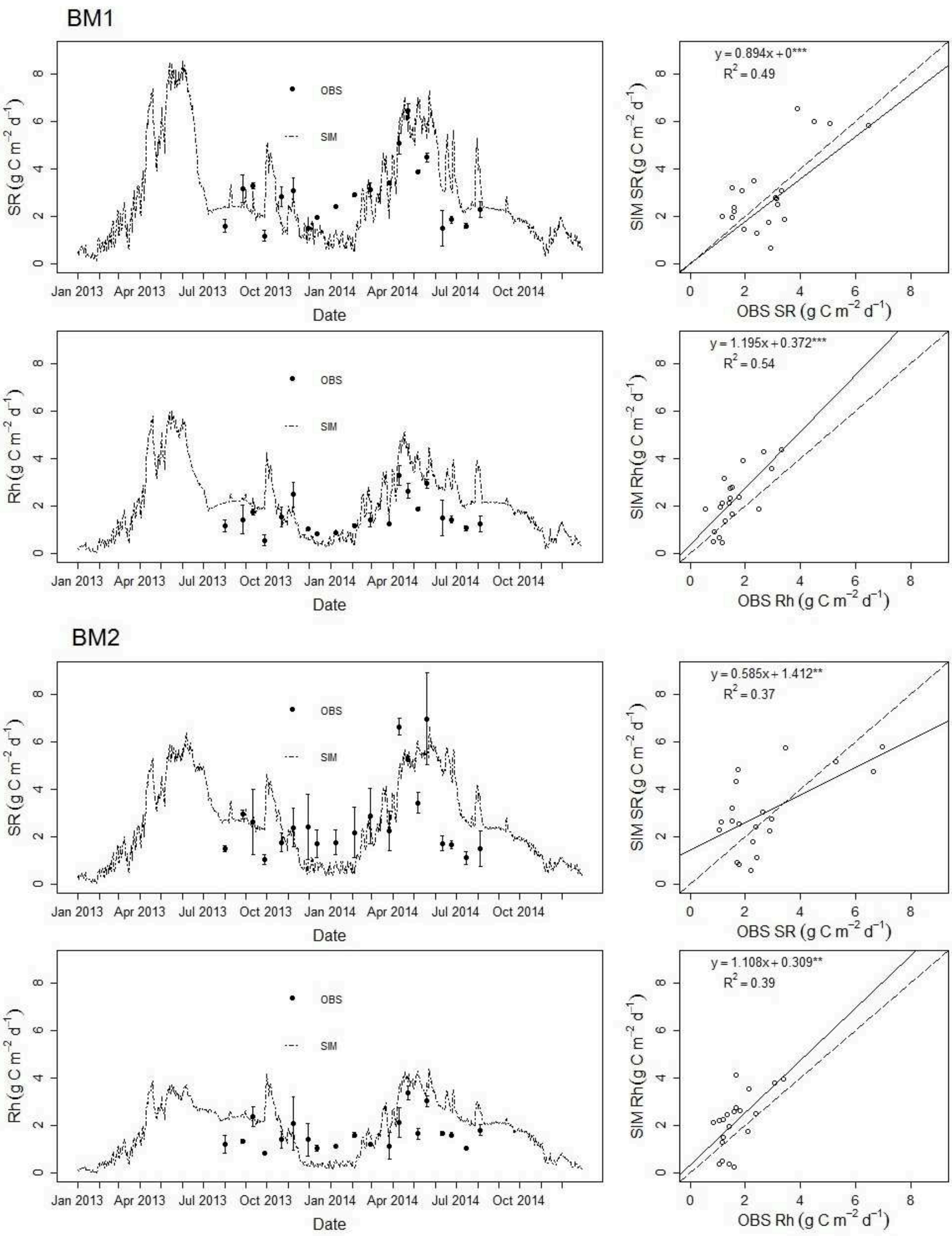

Figure 4 Calibration (BM1) and validation (BM2) results for SR (Soil Respiration, g C m^{-2} d^{-1}) and Rh (Heterotrophic Respiration, g C m^{-2} d^{-1}) and relation between observed (OBS) and simulated (SIM) data. Bars indicate the observed data standard error (n=2). (***: P<0.001; **: P<0.01)

Table 4 Values of the metrics used to assess model performance in both calibration (Cal) and validation (Val) datasets

Observed Data	Years of available data	BIAS		RRMSE		CRM		EF		d		R^2	
		Cal	Val	Cal	Val	Cal	Val	Cal	Val	Cal	Val	Cal	Val
Soil T	2014	-0.834	-1.275	18.44	19.68	0.05	0.07	0.73	0.74	0.94	0.94	0.84	0.80
SWC	2014	-0.007	0.028	29.91	47.12	0.05	-0.22	0.64	0.44	0.92	0.87	0.73	0.65
NGDM	2009-2010-2014	0.53	-0.39	37.38	27.97	-0.22	0.15	0.47	0.59	0.88	0.90	0.73	0.74
GRDM	2009-2010-2013-2014	0.32	0.02	51.80	44.86	-0.18	-0.02	0.42	0.49	0.86	0.85	0.59	0.53
SR	2013-2014	0.16	0.29	41.59	54.15	-0.05	-0.11	0.16	0.22	0.82	0.77	0.49	0.37
Rh	2013-2014	0.68	0.48	66.27	62.13	-0.43	-0.29	-1.17	-1.54	0.72	0.66	0.54	0.39

GRDM = DM biomass in the grazed plots; NGDM = DM biomass in ungrazed plots; soil T = soil temperature; SWC = soil water content; SR = soil total respiration; Rh = soil heterotrophic respiration.

Deviations from published ranges or values were observed for four calibrated parameters (Table 2). The obtained leaf area index when canopy is half the maximum height (2.0 m^2 m^{-2}) was set lower than the default value (4.0 m^2 m^{-2}). Also the shoot turnover parameter (0.0250 d^{-1}) and the light-saturated leaf photosynthetic rate for reproductive stage (14.0 µmol C m^{-2} s^{-1}) were lower than minimum values as from previous studies (0.0360 d^{-1} and 15.0 µmol C m^{-2} s^{-1}, respectively). The parameters of relative root distribution in each soil layers were also different from the original values.

The calibrated model substantially matched the observed data, with the exception of Rh (Table 4). Overall, the best performance was observed for soil T and SWC simulations, with EF ranging from ~0.4-0.7. Best metrics were generally obtained with the calibration dataset, with a few exceptions such as the RRMSE values calculated for NGDM and GRDM, which were better with the validation set. The performance was not satisfactory when the model was used to simulate Rh, as shown by the negative EF values with both calibration and validation datasets. Relationships between observed and simulated data in both BM1 and BM2 for Soil T, SWC, GRDM, NGDM and SR were always significant at $P<0.001$ with high R^2 values, in particular for the calibration phase (Figures 1, 3 and 4; Table 4). Less significant relationships were found between observed and simulated Rh (Figure 4; Table 4). An analysis of the standardized residuals (Figure 5) showed seasonal patterns, which differed depending on the output. In particular, deviations from 95% confidence bounds highlight underestimation of soil T towards the end of the year and overestimation of SWC roughly uniformly over the year. Values outside 95% boundaries were observed in late spring (from day of year 80 to 171) and summer period (from day of year 171 to 265) for SR and Rh outputs.

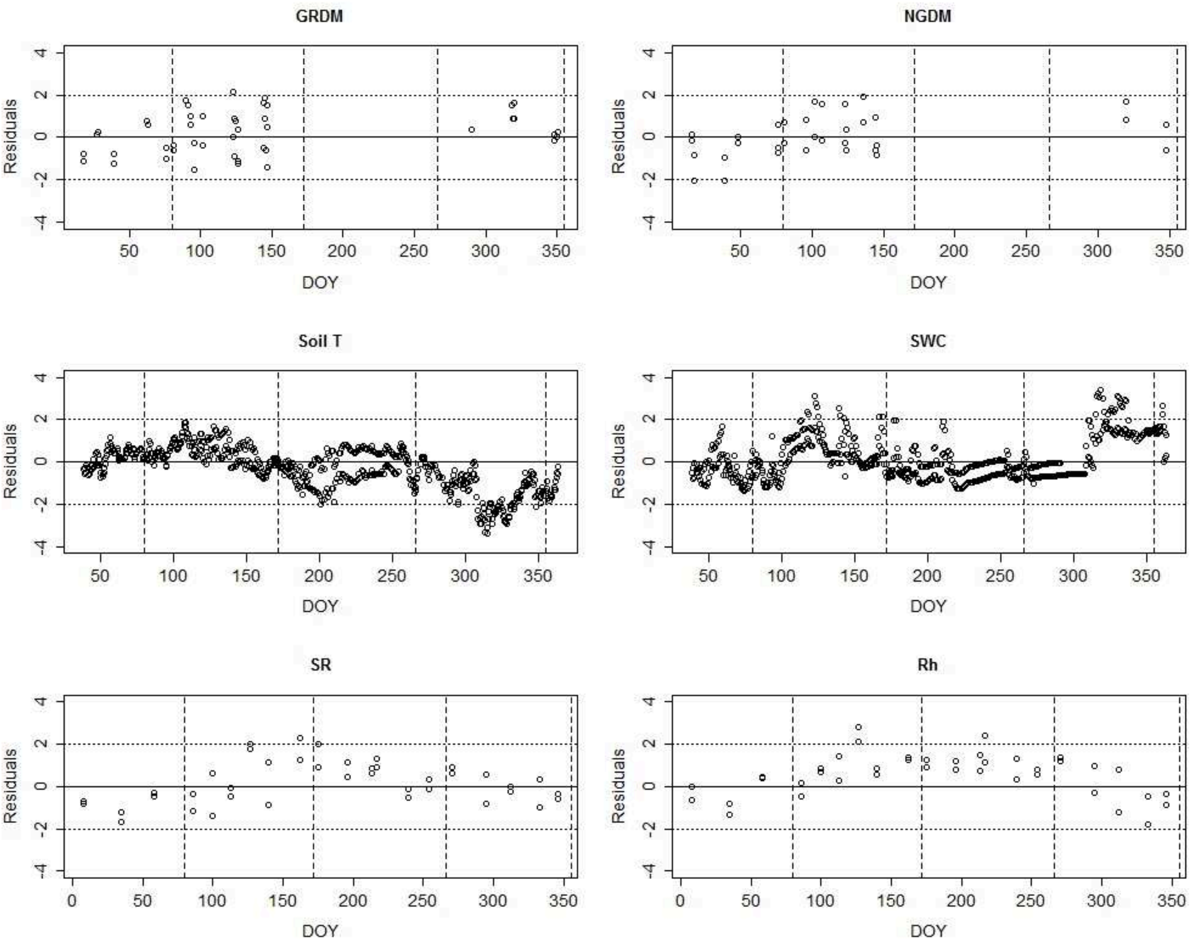

Figure 5 Standardized residuals analysis. Dashed vertical lines highlight seasons. Dashed horizontal lines indicate 95% confidence bounds. GRDM = DM biomass in the grazed plots; NGDM = DM biomass in ungrazed plots; soil T = soil temperature; SWC = soil water content; SR = soil total respiration; Rh = soil heterotrophic respiration; DOY = day of year

Discussion

For NGDM, performance metrics showed satisfactory results with both calibration and validation datasets. RRMSE values in the range ~28-37% are comparable to those reported for multi-location simulations in France with PaSim (Graux *et al.*, 2011). The simulation of GRDM (with RRMSE around 50%) was also satisfactory, considering that this output is affected by higher uncertainties than NGDM. In this study, PaSim showed an overall satisfactory capability to simulate dairy sheep post-grazing effects on grassland growth and after-grazing regrowth. This was obtained despite the time-based approximation required to set the PaSim management input file, which only allows for 10 grazing events per year to be prescribed, that does not reflect the business-as-usual grazing regimes of dairy sheep semi-extensive farming systems in Mediterranean regions. In fact, these systems, differently from continental cattle grazing systems usually characterized by larger pasture areas for livestock management, are often carried out in small areas, where animals graze over a few hours per day with different grazing regimes related to forage availability, phenological plant stages, sheep physiology, nutritional needs and farm typology (Molle *et al.*, 2008). Despite the satisfactory overall simulation of the cumulated biomass, limitations in the simulation of the grassland production dynamics were observed in autumn season. In autumn the simulated grass growth started about 25 days earlier than was observed in the field. Moreover, the simulated maximum grassland growth rate occurred 20 days earlier than the field observations. This difference between simulated and observed data was attributed to the limitations of PaSim in simulating the mechanistic process underlying plant growth, which is meant to reflect the dynamics of perennial plant species. In fact, when optimal conditions of soil T and SWC occur after the season break summer, the model simulates plant growth as if the sward was composed of well-established perennial species. Under Mediterranean conditions, autumn grassland re-growth is related to the germination of new seedlings of annual species, hence plant growth rates are slower than expected for perennials for the same soil T and SWC conditions.

Overall, the evaluation metrics obtained for soil T and SWC simulations were comparable with those obtained in a previous study undertaken using PaSim across a range of European grasslands (Ma *et al.*, 2015). In autumn, soil T was underestimated, as highlighted by residuals analysis. This was consistent with a greater simulated than observed aboveground biomass, as a lower soil cover would have resulted into less shading and hence higher daily mean soil T due to the effect of vegetation cover on soil temperature. The marked fluctuations of the simulated SWC can be attributed to the high variability inherent to the water balance in the topsoil (Li *et al.*, 2015), where most influential biological and biophysical processes occur showing greater temporal and spatial variability (Cambardella *et al.*, 1994).

The model performance indices for SR were acceptable, considering the high spatial variation inherent to this variable (Oyonarte *et al.*, 2012), as also observed previously in the Berchidda-Monti Observatory (Lai, 2011). The spatial variability of soil C fluxes affects the model ability to simulate SR and Rh dynamics (Mekonnen *et al.*, 2016). The seasonal fluctuations and amount of total SR were satisfactorily represented by the model. RRMSE values were similar to those obtained by Correia *et al.* (2012), who compared alternative statistical models in a multi-site analysis in the Mediterranean basin. For SR, the values of R^2 and EF found in this study were similar to those reported in the meta-analysis performed by Chen *et al.* (2010) on yearly simulated values for a broad variety of climate areas and land uses including Mediterranean grassland areas. Under Mediterranean conditions, the effects of soil T on both SR and Rh can be flattened by the inhibitory effect of low SWC on C-CO_2 fluxes from soil, when SWC limits microbial activity (Davidson *et al.*, 1998; Rey *et al.*, 2002). When comparing observed and simulated data at both BM1 and BM2, it emerged that the ability of PaSim to simulate soil respiration strongly depends on soil T. The effect of SWC becomes apparent when examining the autotrophic component of SR: in fact, the simulated SR during the summer drought period is almost completely due to Rh. This demonstrates the ability of PaSim to satisfactory simulate drought-stress effects on plant growth, although the modelled mechanisms of senescence do not entirely reflect the peculiarities of Mediterranean grasslands. The analysis of residuals highlighted a considerable underestimation of soil T dynamics and an overestimation of SWC in autumn. Despite this, SR and Rh residuals in autumn were within the 95% confidence intervals. The role of soil T in determining underestimation of the annual soil C-CO_2 effluxes was not substantial. The negative EF values observed in the validation phase for Rh also could be due to differences between within-model conceptualisation of Rh and what it is actually measured in the field. In fact, the model considers C losses from soil due to soil heterotrophic biota as the sum of C-CO_2 respiration from five C pools derived from the CENTURY model (Parton *et al.*, 1993). The pools are characterized by different levels of C recalcitrance, with the addition of C-CO_2 losses from root exudates decomposition. The field Rh measurements performed in this study only allowed C-CO_2 fluxes to be observed due to the most recalcitrant soil C. These differences, although quantitatively not important, might have generated a possible misalignment of observed and simulated data, thus adding further uncertainty to the prediction of the soil respiration processes.

The deviations of some PaSim parameters from published ranges or values, as obtained by calibration, reflect the profound differences between specific features of annual species mostly composing Mediterranean grassland communities and perennial species typical of continental grassland swards. The parameter of relative root distribution was adjusted in order to feature the root allocation in a vegetation cover mostly composed by annual species, having a shallower root system than perennial plants. Regarding light-saturated leaf photosynthetic rates, lower calibrated value was obtained in the reproductive stage than the one suggested as minimum, while for the vegetative stage the calibrated value corresponded to the specified minimum limit. Considering that under N fertilization conditions the calibrated parameters gave satisfactory estimates of biomass (as reported in supplementary material) such low values, yet outside the range of values previously set, likely reflect the biology of annual plants. It is known that grasses of the same genus may have different light-saturated photosynthesis rates in relation to their different biological cycles. As reported by Charles-Edwards *et al.* (1974) in a multi-environment trial, the perennial species *Lolium perenne* L. showed higher photosynthesis rates compared to the annual *Lolium multiflorum* Lam. With the obtained calibration, the shoot turnover parameter stretched to about 40 days, which is longer than the corresponding range of acceptable values (from about 18 to 27 days) for the conditions in which the model was developed. The life cycle of annual species is based on morphological and physiological features, which are expressed by the duration of the cycle and the allocation of assimilates, supporting the accumulation of resources for flowering and seed ripening (e.g. Schippers *et al.*, 2001). The annual plants tend to invest mainly in seeds while abandoning the mother plant at an early stage (for annual self-reseeding). Alternatively, the perennial plants tend to invest mainly in a long-lived and competitive adult individuals while producing fewer seeds. Consequently, shoot turnover tends to be slower in annual plants than in perennial species, since the latter allocate more resources for the growth of new leaves, so as to maximize photosynthetic efficiency. Furthermore, in Mediterranean environmental conditions, if the dry season lasts too long (e.g. absence of precipitation in late summer and autumn), water deficits may negatively affect the capacity of plants for C assimilation as a result of lower photosynthetic rates due to less developed leaf areas and shorter life span of the dominant annual plants with respect to perennials (Jongen *et al.*, 2011).

In conclusion, this work highlights the potential of PaSim to reasonably simulate grassland production dynamics and soil CO_2 fluxes under Mediterranean conditions. In particular, the results suggest that PaSim could be a useful tool to simulate annual C balances in Mediterranean grasslands. The limitations detected during the model assessment (e.g., shift of biomass production in autumn and summer Rh overestimation) indicate that PaSim requires further improvement to better represent the typical growth patterns of Mediterranean grassland ecosystems. Further model improvement could be targeted to better represent the peculiar dynamics of a grassland sward with a prevalence of annual species and the processes that control the turnover of organic matter and the associated Rh emissions during dry periods.

This study provides a basis for the development of scenario analyses and vulnerability assessment studies in relation to climate change, and to evaluate synergies and trade-offs between adaptation and mitigation strategies. This could also be achieved by exploiting the model capability to simulate annual biomass production under different management options such as livestock rates and animal types (cattle or sheep) and, in addition, under different cutting and fertilizer application regimes

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Supplementary material

Brief description of the main model features relevant to the study

To model grassland phenology a base temperature is used above which plant development proceeds proportionally to temperature until an optimum level may be reached. A condition is the occurrence of mean soil temperature higher than or equal to the base temperature (K). The temperature sum is calculated by accumulating daily differences between the mean air temperature and the base temperature. A developmental stage index is initialized to 0 at the 1st of January (northern hemisphere) and calculated by a normalized temperature sum on a range of 0 to 2, where value 1 marks the end of ear emergence and the transition from the reproductive to the vegetative stage. The phenological development of the canopy is not only determined by air temperature but it is also influenced by the cutting regime, so that a second reproductive period can be an option during the growing season if a cutting event occurs long before the end of the vegetative stage. The stage of "ear at 10 cm" is a key parameter for shoot and root growth rates, and plays a role on the allocation of assimilates between aerial and root parts, as well as among aerial sub-compartments (laminas, sheaths and stems, ears). During the vegetative stage, the fraction of shoot structural growth partitioned to the lamina is set to a constant value and there is no partitioning to ears. Otherwise, the fractions partitioned to lamina, sheath and stem, and ears depend on the developmental stage (e.g. the fraction of shoot structural growth partitioned to the lamina decreases linearly until the end of ear emergence). The production of new biomass is accompanied by new sheath and stem area, and new lamina, respectively depending on specific sheath and stem area (constant) and specific leaf area (decreasing from its maximum based on plant substrate C). Two parameters - light-saturated leaf photosynthetic rate for reproductive and vegetative stages - represent the influence of developmental stage on the light-saturated leaf photosynthetic rate, as a basis to calculate the rate of canopy photosynthesis, given by the sum over all canopy layers of leaf photosynthetic rate, which in turn depends on the C concentration in structural dry matter.

Respiratory fluxes are associated with growth (shoot and root), maintenance and root N uptake. Other sinks are: root exudation of C, estimated as a fraction of plant substrate C and, in case of grazing, the C substrate removed by the animals, estimated by stocking rate of animals and animal intake. The fractional N concentration of plant structural dry matter is assumed to be constant, the N concentration of newly produced structural dry matter allowing for a changing C/N ratio of the plant structural dry matter. Senescence is the terminal phase in the development of plant compartments. Each compartment (consisting of four age classes) turns over a given rate. This gives each age class an output which enters the next class without loss of C or N. The output from the oldest pool provides a structural dry mass flux, part of which is recycled while the rest enters the litter. The turnover rate is modified by temperature and water stress.

A parameter of the shape of the relative root dry matter distribution in different soil layers calculates the proportion of roots at each of six horizons. The soil organic C content is divided into five pools with different turnover times ranging from 0.5 to 1500 years: the litter in decomposition over the total soil depth splits into its structural and substrate components, supplying the structural and metabolic soil pools respectively; other three compartments with different decomposition rates include active, slow and passive pools, consisting of the microbial biomass, refractory components of litter and highly humified organic compounds respectively. An additional component is given by the amount of C released as exudates from roots into the soil, estimated as a fraction of plant substrate C. Total heterotrophic soil respiration is the result of multiple respiration fluxes in each pool (and accompanied by transfers from active to slow, from slow to passive, and from passive back to active pools). The effects of temperature, lignin, soil texture, and soil water content on decomposition of C pools are estimated by constant decomposition rates.

Table 1S Soil properties of the grassland study sites as expressed in PaSim site specific input file.

Site	Layer	Soil depth (m)	Sand (%)	Silt (%)	Clay (%)	Bulk density (Mg m^{-3})	Saturated soil water content (m^{3}m^{-3})	Saturated hydraulic conductivity (mm d^{-1})	Field Capacity (m^{3}m^{-3})	Wilting Point (m^{3}m^{-3})	pH
BM1	1	0.02	65.0	21.0	13.0	1.53	44.5	934	19.4	9.8	5.0
	2	0.16	65.0	21.0	13.0	1.53	44.5	934	19.4	9.8	5.0
	3	0.35	65.0	21.0	13.0	1.53	44.5	934	19.4	9.8	5.0
	4	0.54	63.0	23.0	13.0	1.64	38.1	552	17.4	8.6	5.3
	5	0.73	63.0	23.0	13.0	1.64	38.1	552	17.4	8.6	5.3
	6	0.93	63.0	23.0	13.0	1.64	38.1	552	17.4	8.6	5.3
BM2	1	0.02	61.0	25.0	14.0	1.47	0.45	784	20.8	5.0	5.1
	2	0.10	61.0	25.0	14.0	1.47	0.45	784	20.8	5.0	5.1
	3	0.40	65.0	20.0	15.0	1.49	0.44	738	20.8	11.2	5.3
	4	0.66	66.0	20.0	14.0	1.51	0.43	753	19.7	10.5	5.6
	5	1.00	68.0	20.0	12.0	1.59	0.4	738	17	8.8	5.9
	6	1.40	72.0	15.0	13.0	1.70	0.36	601	14.2	7.7	6.2

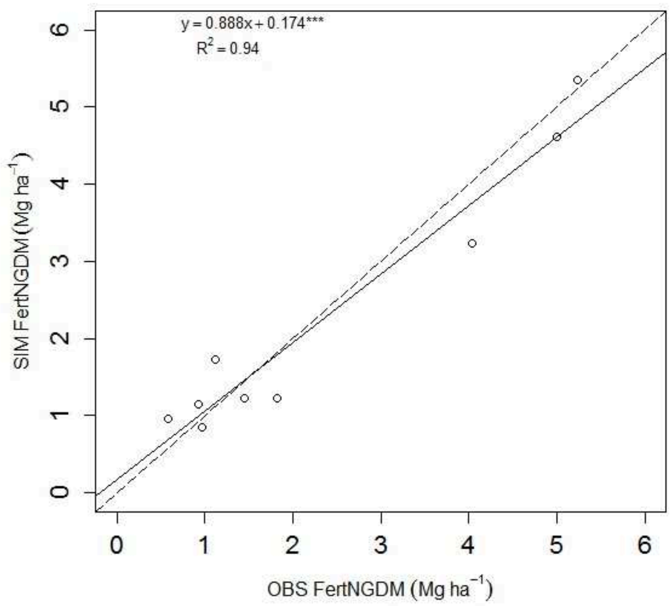

Figure 1S Relation between observed (OBS) and simulated (SIM) data in fertilized ungrazed plots (FertNGDM, Mg DM ha^{-1}). (***: P<0.001)

Table 2S Monthly average stocking rate, DM intake from animals, C and N input from faeces in the grassland study sites (2009-2014). Mean values (if n>1) are followed by the standard errors.

	BM1					BM2				
	Average stocking rate (LSU ha^{-1} d^{-1})	Duration (d)	DM intake[1] (Mg ha^{-1})	C input[2] from faces (kg ha^{-1})	N input[2] from faeces (kg ha^{-1})	Average stocking rate (LSU ha^{-1} d^{-1})	Duration (d)	DM intake[1] (Mg ha^{-1})	C input[2] from faces (kg ha^{-1})	N input[2] from faeces (kg ha^{-1})
Available data period	2009-2014	2009-2014	Jan 2013-May 2014	Jan 2013-May 2014	Jan 2013-May 2014	2009-2014	2009-2014	Jan 2013-May 2014	Jan 2013-May 2014	Jan 2013-May 2014
Jan	0.71±0.34	14±6	0.04	31.4	1.3	0.56±0.14	14±4	0	0	0
Feb	0.7±0.22	15±5	0.02	32.4	1.3	0.08±0.07	5±5	0.01	5.9	0.2
Mar	0.79±0.39	9±5	0.02	20.4	0.8	0.6±0.09	13±4	0.05	38.3	1.5
Apr	0.81±0.29	15±6	0.41±0.23	58.0±48.0	2.3±1.9	1.24±0.28	24±4	0.04	3.6	0.1
May	0.6±0.27	13±6	0.23	13.1	0.5	0.64±0.21	12±5	0	0	0
Jun	0.38±0.22	10±6	0.05	5.5	0.2	0.56±0.33	15±7	0	0	0
Jul	0.36±0.36	5±5	0	0	0	0.25±0.18	8±5	0	0	0
Aug	0.36±0.36	5±5	0	0	0	0.07±0.07	5±5	0	0	0
Sep	0	0	0	0	0	0.07±0.07	5±5	0	0	0
Oct	0	0	0	0	0	0.07±0.07	5±5	0.07	28.2	1.1
Nov	0.01±0.01	0±0	0	0	0	0.67±0.14	13±5	0.05	22.7	0.9
Dec	0.71±0.23	17±6	0.04	0.15	0.006	0.18±0.15	6±5	0	0	0

[1] An experiment using the mobile cages method (Frame, 1981) was conducted in order to assess the effects of grazing on grassland features. The external-cages cuttings were used in this work to obtained the GRDM values. The sheep monthly DM intake was calculated from 2013 to 2014 by the difference between the internal and external cuttings at the same sampling date. The monthly intake was adjusted considering the daily livestock stocking rate.
[2] The C and N input from faeces were calculated on the basis of total daily animal DM intake, resulting from the sum of DM intake from grazing and supplemented feeds, that were monitored together whit livestock stocking rate data. The animal faeces were calculated as suggested by Van Soest (1994), assuming an average digestibility coefficient of 0.7. Faeces samples were collected and analysed in order to determine their average C and N content by using a CHN analyser.

Table 3S Values of the metrics used to assess model performance in ancillary datasets

Observed Data	Years of available data	BIAS	RRMSE	CRM	EF	d	R^2
FertNGDM[1]	2014	0.39	19.15	0.04	0.93	0.98	0.94
DM intake	2013-2014	0.05	11.90	-0.48	0.61	0.91	0.72
C input from faeces	2013-2014	10.11	94.65	0.16	0.53	0.83	0.55
N input from faeces	2013-2014	0.41	96.03	-0.07	0.52	0.86	0.57

FertNGDM = DM biomass in fertilized ungrazed plots

[1] Measuraments carried out during the last year of observation, at the same time of NGDM. The total N amount was 64 kg N ha^{-1}, that was supplied in two fertilization dates: i) 7th November 2013 (18 kg N ha^{-1} from diammonium phosphate); ii)18th March 2014 (46 kg N ha^{-1} from urea)

CHAPTER 2

Global Warming Potential of a Mediterranean Irrigated Forage System: Implications for Designing the Fertilization Strategy.

Global Warming Potential of a Mediterranean Irrigated Forage System: Implications for Designing the Fertilization Strategy.

Abstract

The aim of this study was to analyse the soil Greenhouse Gases (GHG) emissions and the net Global Warming Potential (GWP) in a Mediterranean irrigated forage system under different fertilization strategies. Three N fertilization treatments were compared for two years in a double-crop rotation of silage maize and Italian ryegrass for hay: cattle slurry (SL), solid fraction of the slurry (SO) and mineral fertilizer with a nitrification inhibitor (MN). The soil CO_2, N_2O and CH_4 fluxes were highly influenced by the interaction between treatment and date. The maximum values of GHG emissions were observed after fertilizations, to a different extent depending on the fertilizer used. The C losses from soil respiration were higher in SO than SL and MN, however the C sequestration was higher in SO than in the other treatments. The N_2O fluxes where higher in SL than in SO, while the MN had intermediate values. No differences were observed in cumulative CH_4 emissions. The SO resulted as a sink of net GWP, while the SL a source. The MN treatment showed generally a source behaviour. The SO seemed to have a higher potential in terms of reducing GHG emissions by maintaining adequate levels of agronomic efficiency. This study put in evidence how different organic fertilizers can have contrasting impacts on GHG emissions providing some insights on their different potential mitigation roles under Mediterranean conditions.

Keywords: Maize-based agroecosystems; GHG Emissions; Cattle Slurry; Slurry Separation; Nitrification Inhibitor

Introduction

The most recent studies highlighted that agriculture is responsible of about 10-12% of total anthropogenic Greenhouse Gases (GHG) emissions, above all through soil CO_2, N_2O and CH_4 fluxes (Smith *et al.*, 2014). Nevertheless, agricultural sector can be pivotal in influencing the soil organic carbon (C) sequestration and C changes which are widely recognized as crucial for counteracting climate change (Follett, 2001; Farina *et al.*, 2011).

Under Mediterranean conditions, the total C losses form soils can be attribute to the CO_2 efflux due to the autotrophic and heterotrophic metabolic activities (Hanson *et al.*, 2000; Follett, 2001), also referred as Soil Respiration (SR), which are regulated from the interaction of multiple factors. Although it is recognized that the soil water content (SWC) and temperature (T) are the main driver of seasonal C changes in semi-arid rainfed environments (Davidson *et al.*, 1998), high N inputs and irrigation influence soil C dynamics in intensive cropping systems. The use of organic fertilizers in agroecosystems is recognized as increasing the soil organic carbon (SOC) stocks in the soil (Bertora *et al.*, 2009; Maillard and Angers, 2014) but research findings on their effectiveness are often contrasting depending among other factors on the origin and type of supplied fertilizer (Peters *et al.*, 2011; Lai *et al.*, 2017). Therefore, it is needed to investigate on the role of different organic fertilizers to achieve soil GHG emissions mitigation and, at the same time, the maintenance of agro-ecosystems productivity (Smith *et al.*, 2008; Sanz-Cobena *et al.*, 2017).

Under irrigated cropping systems, a high amount of N inputs are usually supplied to the soil to ensure high crop production. In these systems, although a high primary productivity can enhance the C sequestration rate, favourable conditions for N_2O production are also created, which are in turn strongly related to water and fertilization management (Halvorson *et al.*, 2010; Cayuela *et al.*, 2017). The denitrification processes are mainly linked to the organic N and total C supplied with the organic fertilizers (Vallejo *et al.*, 2006; Aita *et al.*, 2015; Tellez-Rio *et al.*, 2015), while the nitrification is linked to the mineral fertilizers use (Meijide *et al.*, 2007; Hube *et al.*, 2017). The N fertilizers, particularly the organic ones, can vary considerably in composition, resulting in differences in terms of N_2O emissions (Meijide *et al.*, 2007). High organic and soluble C contents in organic substrate enhance total emissions of N_2O, while high C:N organic fertilizers can have a reduction impact (Vallejo *et al.*, 2006). The use of nitrification and urease inhibitors showed a N_2O fluxes depleting potential from 30 to 50% (Huérfano *et al.*, 2015), indicating a mitigation efficiency in both rainfed and irrigated systems with a possible indirect effect on reducing denitrification (Sanz-Cobena *et al.*, 2017). In irrigated cropping systems, denitrification is the main responsible of N_2O fluxes, which are positively correlated with the soil water condition (Vallejo *et al.*, 2005; Alluvione *et al.*, 2010; Álvaro-Fuentes *et al.*, 2017).

The role of fertilization strategies in influencing CH_4 fluxes in Mediterranean non-flooded systems is debated. Soon after the application into the soil, organic fertilizers can result both as a source of CH_4 (Guardia *et al.*, 2016) or sink (Meijide *et al.*, 2007). Sanchez-Martin *et al.* (2010) reported opposite effects displayed by organic (sink) and mineral (source) fertilizers. Furthermore, high C fertilizers can bring changes in soil porosity resulting in creation of anaerobic microsites suitable for methanogenesis (Le Mer and Roger, 2001).

The analysis of the literature highlights a lack of studies and contrasting findings on the influence of fertilization on Global Warming Potential (GWP) in not-flooding cropping systems under Mediterranean irrigated conditions (e.g., Aguilera *et al.*, 2013). Under drip irrigation, Forte *et al.* (2017) found that the use of compost in maize resulted in a slightly higher net GWP than the urea fertilization, while vetch green manure showed a GWP sink capacity. In irrigated maize, Guardia *et al.* (2017) reported that the use of pig slurry compared to urea may prove to be a good mitigation strategy, although a better agronomic efficiency can be obtained when slurries are added with the nitrification inhibitors (DMPP). Furthermore, Hube *et al.* (2017) found a significant effect of dicyandiamide in mitigate GHG emissions in a oat cropping systems. Comparing organic and mineral fertilizers under different tillage options, Plaza-Bonilla *et al.* (2014) suggested that in a rainfed monoculture of barley the best strategy to mitigate the net GWP is to combine intermediate rates of organic or mineral fertilizers with no tillage. Overall, in maize-based cropping systems the use of organic fertilizers resulted as a sink of GWP also by using green manure from barley straw (Cuello *et al.*, 2015), wheat-straw biochar (Zhang *et al.*, 2012) and wastewater (Fernández-Luqueño *et al.*, 2010).

The hypothesis of this study was that the use of organic instead of mineral fertilizers with nitrification inhibitor could be considered as a suitable GWP mitigation option when the characteristics of the organic substrates allow to enhance the C sequestration and to reduce the N reactive availability to N_2O creation while maintaining a good productivity. In a Mediterranean irrigated maize-based cropping system, different sources of N as organic (cattle slurry and its solid fraction after separation) or mineral (ammonium sulphate nitrate with nitrification inhibitor) fertilizers were tested in order to assess their impact on: (i) the seasonal dynamics of CO_2, N_2O and CH_4 fluxes from the soil and their relationship with abiotic factors such as soil water content and temperature; (ii) the soil C sequestration rate, as calculated through both the SOC variation along the study period and the difference between C inputs (fertilizers and crop residues) and heterotrophic respiration;(iii) net GWP and the agronomic efficiency related to GHG emissions

Materials and Methods

Study Site and Experimental Design

The field experiment was conducted in a private farm located in the Arborea district (Sardinia, Italy) (3 m a.s.l., 39°47'45" N, 8°33'25"E). The Arborea district is characterized by an intensive dairy cattle livestock system, representing the most important human activity of the area (Demurtas *et al.*, 2016). The area was designated as Nitrate Vulnerable Zone, therefore it is subjected to the European Directive 91/676/EEC prescriptions. The climate is Mediterranean: the mean annual temperature (1959–2012) is 16.7 °C. The average annual rainfall is 568 mm, 73% of which occurs between October and March, with an annual aridity index (rainfall/reference evapotranspiration) of 0.49 (semi-arid area). The soil was classified as *Psammentic Palexeralfs* (USDA, 2010), with sand content of 96% and soil organic matter of 1.4% in the 0.40 m layer. The typical forage cropping system is based on the double-crop rotation of silage maize (*Zea mays* L.) grown from June to September and a winter hay crop, typically Italian ryegrass (*Lolium multiflorum* Lam.) grown from October to May.

Two double-crop rotations of silage maize and Italian ryegrass from July 2014 (maize seeding) to May 2016 (ryegrass mowing) were considered in the present study. The experimental design was a completely randomized design with three different N fertilization treatments and four replicates and a plot size of 5.2 m x 19.2 m. The treatments reflected the business as usual management options in the district and they were chosen in agreement with the representatives of the local farmers' cooperative. The treatments were: i) cattle slurry (SL); ii) solid fraction of cattle slurry, obtained after mechanical separation (SO); iii) mineral fertilizer (MN), using ammonium sulphate nitrate with nitrification inhibitor (3,4 DMPP, ENTEC® 26, EuroChem Agro). For each treatment, the target N rate was 130 kg N ha^{-1} for ryegrass and 315 kg N ha^{-1} for maize, which should correspond to the expected N uptake by the studied crops. The irrigation frequency and the water supply were managed by the farm owner. Details on the cropping system management along the experiment are reported in Table 1.

Table 1. Crops management practices along the experiment.

Crop	Date	Operation	Details	Measure
Maize	*5th June 2014*	Pre-seeding Fertilization		
		Soil Tillage	Ripping - Milling Rotary Harrowing	0.40 m depth
		Seeding	680 FAO class maize hybrid (72-A, Maisadour Semences, FR)	7.5 seeds m^{-2}
	6th June 2014	Weed Pre-Emergence Control	Mesotrione, S-Metolachlor and Terbuthylazine (Lumax®, Syngenta)	
	9th September 2014	Harvest		
Ryegrass	*14th October 2014*	Pre-seeding Organic Fertilization		
		Soil tillage	Milling Rotary Harrowing Rolling	0.25 m depth
		Seeding		
	4th March 2015	Topdressing Mineral Fertilization		
	5th May 2015	Hay Mowing		
Maize	*4th June 2015*	Pre-seeding Fertilization		
		Soil Tillage	Plowing Rotary Harrowing	0.40 m depth
		Seeding	680 FAO class maize hybrid (72-A, Maisadour Semences, FR)	7.5 seeds m^{-2}
	5th June 2015	Weed Pre-Emergence Control	Mesotrione, S-Metolachlor and Terbuthylazine (Lumax®, Syngenta)	
	8th September 2015	Harvest		
Ryegrass	*29th October 2015*	Pre-seeding Organic Fertilization		
		Soil Tillage	Plowing Rotary Harrowing	0.40 m depth
		Seeding		
	5th March 2015	Topdressing Mineral Fertilization		
	7th May 2016	Hay Mowing		

Measurements

Weather, Fertilizers and Soil

Air temperature (°C), rainfall (mm), relative humidity (%), radiation (MJ m^{-2} d^{-1}) and wind speed (km h^{-1}) were daily measured starting from 21st October 2014, by a weather station (WatchDog 2000 Series, Spectrum Technologies Inc., IL, USA) placed in close proximity of the experimental field (Figure 1). Reference evapotranspiration during the observed period was calculated according to FAO paper 56 (Allen *et al.*, 1998). The mean temperature (17.2 °C), the maximum (27.3 °C, on June) and the minimum daily mean temperature (12.3 °C, on February), the total rainfall (619 mm, of which 547 mm from October to March) and the total reference evapotranspiration (1312 mm, aridity index of 0.47) were consistent with the pluriannual means (Figure 1).

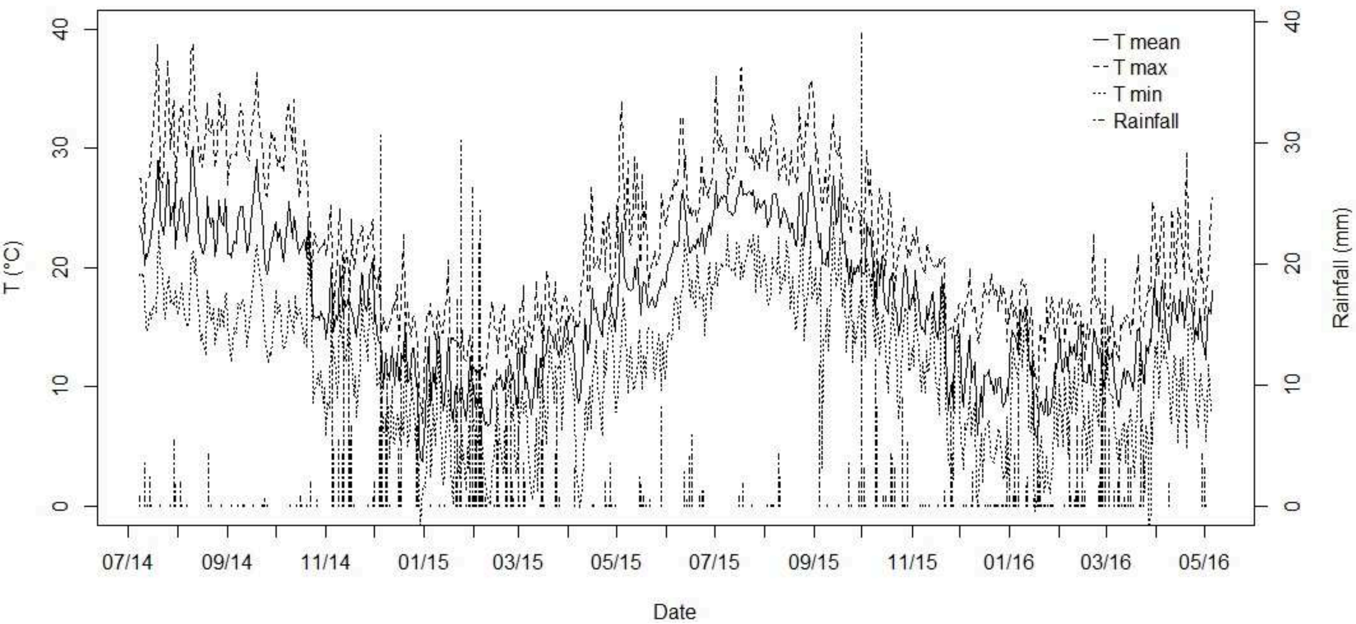

Figure 1 Air temperature and rainfall dynamics in the observed period. T mean: daily mean air temperature (°C); T max: daily maxim air temperature (°C); T min: daily minimum air temperature (°C); Rainfall: daily cumulated rainfall (mm).

Before fertilizations, organic fertilizers were sampled in order to determine C, N, N-NH_4^+, N-NO_3^- contents. Total C and N contents were analysed by an elemental analyser, while the N compounds were analysed using the Kjeldhal method. The rate of organic fertilizers (i.e. slurry and solid fraction of slurry) were calculated on the basis of the average

N contents measured previously (Demurtas *et al.*, 2016). The amount of N actually supplied was calculated ex-post for both crops.

Soil samples were collected (0÷0.2 m and 0.2÷0.4 m soil depths) at the beginning of the trial and after each harvest events of both crops for a total of six sampling dates. Soil total C and N were determined by an elemental analyser (LECO Corporation, MI, USA). Soil texture and inorganic C content were measured following the guidelines of the Italian Ministry of Agriculture and Forestry (Mipaaf, 2014). Bulk density (BD, Mg m^{-3}), field capacity (FC, %) and wilting point (WP, %) were obtained from Pedotransfer functions (Saxton and Rawls, 2006). The functions were based on soil texture and organic C (SOC), obtained by subtracting the inorganic C from the total C. The FC was recomputed considering the soil water content threshold beyond which the decrease of soil moisture is not significant from an agronomic point of view (Lai *et al.*, 2012; Demurtas *et al.*, 2016). The SOC and N (Mg ha^{-1}) contents at 0.40 m depth were obtained by multiplying their contents with BD and the soil layer thickness.

Soil GHG emissions

Soil GHG emissions were measured from daily to bimonthly from 8th June 2014 to 7th May 2016. In detail, CO_2 emissions were daily measured throughout the study, while N_2O and CH_4 fluxes were measured on twenty-eight sampling dates form 4th November 2014 to 12th November 2015. Measures were carried out applying a Closed Automatic Chamber technique (Parkin and Venterea, 2010; Smith *et al.*, 2010).

Daily SR (µmol CO_2 m^{-2} s^{-1}) measurements were carried out hourly during the whole period 2*n* per treatment, by weekly changing the plots in which the measurements were performed. More, the SR were measured 4*n* in 62 sampling dates. Measurements were performed with a LI-8100 coupled with a LI-8150 Multiplexer system (LICOR Inc., Lincoln, NE, USA) Infra Red Gas Analyser. The system was connected to a LI-8100-104 Long Term Chambers (LICOR Inc., Lincoln, NE, USA). Chambers were placed on PVC soil collars 0.11 m high and 0.21 wide, inserted in the soil from 0.02 to 0.05 m depth. In order to minimize the error due to the circadian dynamics, 4*n* measures were performed from 9:00 to 13:00 CET+1 standard time.

Fluxes of N_2O (kg N_2O ha^{-1} d^{-1}) and CH_4 (kg CH_4 ha^{-1} d^{-1}) were measured in 28 sampling dates trough the same instrumental system used for SR measurements, taking air samples from a trace gas sampler, consisting in a hydraulic "T" device. The samplers, equipped with silicon septa, were placed in the "IN" air circuit from chamber to analyser. Thirty ml air samples were taken by the circuit by a 60 ml polyethylene syringe and injected into 12 ml pre-evacuated vials (12 ml Soda Glass Vials - Flat Bottomed 739W, Labco Exetainer®, Lampeter, UK) at 0.10, 10 and 20 minutes after chamber closing. Concentrations of N_2O and CH_4 in air samples were analysed by a gaschromatograph (Agilent 7890 A, Agilent Technologies, Santa Clara, CA, USA) equipped with an Electron Capture Detector (Rapson and Dacres, 2014) and Flame Ionization Detector, respectively. Fluxes were calculated according to linear or non-linear increase of gas concentration in time within the chamber headspace (Hutchinson and Mosier, 1981). Gas fluxes were determined as follows:

$$F_{GHG} = \frac{dC}{dt}\, MC\, \frac{V}{S}$$

where *dC/dt* is the gas concentration increasing per time unit, *MC* is the gas mass coefficient (kg m^{-3}) and *V/S* is the system volume and collar surface ratio (m^3 m^{-2}).

Soil Temperature and Water Content

Soil temperature (T, °C) and water content (SWC, %) were measured hourly at 0.10 m soil depth during both crop cycles in two plots per treatment. In addition, T and SWC were measured simultaneously to the GHG measurements. Measurements of soil T were performed by a LI-8150-203 Soil Temperature Thermistor, while SWC by a LI-8150-202 ECH2O Soil Moisture Probe (both LICOR Inc., Lincoln, NE, USA). Both sensors were connected to the LI-8100-104 chambers and data were stored in the LI-8100 storage device. Values of SWC were used to calculate the Water Filled Pore Space (WFPS, vol vol^{-1}) as follows:

$$WFPS = \frac{SWC}{1 - \frac{BD}{\rho_p}}$$

where SWC is the measured soil moisture value, BD is the bulk density and ρ_p is the soil mineral component density, that is considered as constant (2.65 Mg m^{-3}).

The WFPS index is not soil type dependent and is considered as the most important soil moisture index used to explain the soil aerobic or anaerobic potential (Doran *et al.*, 1990).

Cumulative GHG Emissions and GWP calculation

A reference year was selected to calculate the cumulative GHG emissions, in order to obtain annual-based values for the GWP assessment. For SR, the considered period was from 14th October 2014 to 14th October 2015, while cumulative N_2O and CH_4 fluxes were calculated from 14th November 2014 to 12th November 2015. The cumulative CO_2 emissions (Mg CO_2 ha^{-1}) were calculated as the sum of the average daily SR values (kg CO_2 ha^{-1} d^{-1}). Daily SR values within plots in which SR was not measured were estimated as a function of soil T, according to the model SR = a exp (b T) (Davidson *et al.*, 1998), by using the average T daily value within each treatment. During the inter-cropping periods when the LI-8100 system had to be moved from the field in order to allow tillage and fertilization operations, the model was applied using soil temperature data measured by a soil thermistor coupled to the weather station. Cumulative N_2O and CH_4 emissions were determined by integrating the area under the fluxes variation between subsequent sampling dates.

The net GWP was calculated using two different methods, as performed by Sainju *et al.* (2014) for a short-term net GWP assessment. The two methods were based on: i) soil heterotrophic respiration (Rh) (Mosier *et al.*, 2006) (GWPr); ii) SOC sequestration rate (Mg C ha^{-1} yr^{-1}) in the considered year (Robertson and Grace, 2004) (GWPs).

The GWPr (Mg CO_2 equivalents ha^{-1} yr^{-1}) was calculated as follows:

GWPr = cumulative N_2O fluxes + cumulative CH_4 fluxes + (Cumulative CO_2 fluxes - C input from previous crop residues - C input from fertilizers).

All quantities were expressed in terms of CO_2 equivalents. For N_2O and CH_4, emissions were calculated by multiplying their cumulative values by respectively 298 and 34 (IPCC, 2013).

The CO_2 equivalents of cumulative SR were estimated by applying to cumulative CO_2 emissions a monthly Rh/SR ratio (annual average of 0.57, 0.48 and 0.68 for SL, MN and SO, respectively) as observed in a previous study conducted in the same environment (Lai *et al.*, 2017).

The CO_2 equivalents of the C inputs from crop residues were calculated starting from Total Aboveground Biomass (TAB) of ryegrass and maize. The TAB was measured on 5th May 2015 and on 8th September 2015 for ryegrass ad maize, respectively, in correspondence with the grass cutting for hay production and the silage maize harvest. In ryegrass, TAB measurements were carried out by cutting the grass from a 0.5 m^2 area within each plot.

Measurements of TAB in maize were performed by cutting 10 plants randomly chosen along a 10 m in each plot and determining the mean plant density. For both crops, biomass samples were oven-dried at 65 °C until reaching constant weight, in order to obtain the DM values. Biomass sub-samples were taken at the same time and dried at 40 °C, until reaching constant weight. The dried sub-samples were then addressed to CHN analysis, in order to measure biomass C content. The crops residues were calculated as a sum of aboveground crop residues, root biomass and rhizodepositions, that were estimated as proposed by Lai *et al.* (2017). The CO_2 equivalents of C inputs from fertilizers were calculated by multiplying their C concentration by their amount supplied. The C inputs from crop residues and fertilizers were converted in CO_2 equivalents by multiplying their value by CO_2/C stoichiometric ratio (3.67). The difference between soil C input and output represented the estimated C sequestration based on SR fluxes in the reference year.

The GWPs was calculated as follows:

GWPs= Cumulative N_2O fluxes + Cumulative CH_4 fluxes – Soil C sequestration.

For each treatment, the soil C sequestration (Mg CO_2 ha^{-1}) was determined from the slope of the linear model of SOC at 0.40 m soil depth over the period in a daily time-step. With the purpose to obtain an annual-based C variation, the linear model slope values, which represented the daily SOC sequestration rate, were multiplied by 365. In order to obtain CO_2 equivalents, SOC was finally multiplied by 3.67.

The GHG Eco-Efficiency index (GHGe) was calculated by dividing the GWP by the total aboveground biomass, both for GWPr (GHGer) and GWPs (GHGes).

Statistical Analysis

Rstudio application of R software (version 3.1.1) (RCoreTeam, 2014) was used to perform data analysis. The effect of the interaction between treatments and dates on SOC, total soil N, WFPS, T, GHG emissions and SOC was tested using the generalized least squares (“gls”, package “nlme”) model (Pinheiro *et al.*, 2015), through which different variance and covariance matrices of the dataset were tested, according to Onofri *et al.* (2016). Least-square means (“lsmeans”, package “lsmeans”, by Lenth (2015)) within dates were computed on the best fitted gls model. The linear model (“lm”) was used to test the significance of the linearized SR = a e^{bT} within crops and treatments. Differences between regression models were tested following Soliani (2005). Linear regression analysis was also performed to test the effect of WFPS on SR. Pearson's correlation analysis was performed in order to test the association between N_2O or CH_4 fluxes and WFPS and soil T for each combination of the treatments and crops. The effect of treatments on cumulated GHG emissions, GWPr, GWPs, GHGir and GHGis was tested by computing the ANOVA ("anova") of fitted linear models. If ANOVA was significant, the Student-Newman-Keuls ("SNK.test", package "agricolae", by de Mendiburu (2014)) post-hoc test was performed in order to discriminate means between treatments. Differences between GWP estimation methods were tested by comparing the means of the same treatment with the Student's t-Test ("t.test"). Data were opportunely root- or asin- or log-transformed in case of not normal residuals distribution. The significance of statistical computations was evaluated at $P<0.05$ unless otherwise stated.

Results

Fertilizers and Soil characteristics

The C and N inputs and fertilizers characterization are reported in Table 2. The average C concentration of organic fertilizers was 1.1% (range 0.5%÷1.6%) for SL and 9.1% (7.7÷10.7) for SO. The average total N content was 0.22% (0.19÷0.26) and 0.38% (0.33÷0.50) respectively for SL and SO. The mean $N\text{-}NH_4^+$ concentration was 125.9 (113.5÷142.5) mg kg^{-1} in SL and 85.1 (65.0÷102.5) mg kg^{-1} in SO. The mean $N\text{-}NO_3^-$ concentration was 5.1 (1.8÷9.5) mg kg^{-1} and 3.8 (3.0÷4.5) mg kg^{-1} in SL and SO, respectively.

A significant interaction between date and treatment ($P<0.001$) was observed for SOC content. Significant SOC differences between treatments were observed at the end of the first cropping cycle after the ryegrass cutting on May 2015 and at the end of the experiment, on May 2016. In September 2015 and in May 2016, SOC was always higher in SO than SL and MN (Table 3). The treatments did not affect soil total N content, as shown in Table 3.

Table 2. Fertilizers composition and total C and N input

Crop		C Input (kg ha^{-1})	Total N (kg ha^{-1})	N-NO_3^- (kg ha^{-1})	N-NH_4^+ (kg ha^{-1})	Mineral N / Total N	C:N	N supply – N target (kg ha^{-1})
Maize								
5th June 2014	SL	1719.9	300.4	11.1	166.3	0.59	5.7	-14.6
	SO	8426.7	391.8	3.5	51.2	0.14	21.7	76.8
	MN	-	315.0	90.8	224.1	1.00	-	0
4th June 2014	SL	642.1	244.7	4.6	143.7	0.61	2.6	-70.3
	SO	6570.6	231.1	2.7	64.9	0.29	28.4	-83.9
	MN	-	315.0	90.8	224.1	1.00	-	0
Ryegrass								
14th October 2014	SL	771.5	107.1	0.8	62.6	0.59	7.2	-22.9
	SO	2548.9	105.6	1.1	28.4	0.28	24.1	-24.4
4th March 2015	MN	-	130.0	37.5	92.5	1.00	-	0
29th October 2015	SL	505.5	97.8	2.9	59.2	0.63	5.0	-32.2
	SO	2445.4	105.8	1.0	27.2	0.27	23.1	-24.2
5th March 2016	MN	-	130.0	37.5	92.5	1.00	-	0

SL: Slurry; MN: Mineral; SO: Solid fraction of slurry.

Table 3. Mean values (± standard error) of Soil Organic C (SOC) and total N content at 0.40 m soil depth in 5 sampling dates during the study.

Date	Treatment	SOC (Mg ha^{-1})		N (Mg ha^{-1})	
5th June 2014	SL	47.1±0.4	NS	NA	
	MN	46.9±0.4	NS	NA	
	SO	46.1±0.5	NS	NA	
25th September 2014	SL	45.6±2.1	NS	5.9±0.1	NS
	MN	46.4±1.1	NS	5.9±0.0	NS
	SO	49.4±0.2	NS	6.4±0.2	NS
5th May 2015	SL	45.1±0.8	ab	5.2±0.3	NS
	MN	41.0±2.2	b	5±0.3	NS
	SO	49.8±2.9	a	5.5±0.5	NS
23rd September 2015	SL	45.3±1.6	b	4.4±0.4	NS
	MN	42.6±0.5	b	4.3±0.2	NS
	SO	53.6±1.5	a	5.1±0.4	NS
6th May 2016	SL	47.1±1.2	b	3.2±0.4	NS
	MN	45.4±0.6	b	3.1±0.3	NS
	SO	51.4±1.3	a	3.7±0.4	NS

Means followed by the same letters within sampling date are not significantly different according to the Least Square Means test (P<0.05).
NS: not significant.
SL: Slurry; MN: Mineral; SO: Solid fraction of slurry.

WFPS, Soil T and GHG emissions

A significant interaction (P<0.001) between date and treatment in WFPS dynamics was observed. Differences between treatments within dates were observed in 12 out of 56 sampling dates, 75% of which during the first year ryegrass crop. Overall, daily WFPS average values ranged from 0.11 to 0.94 vol vol^{-1}. The lower daily WFPS mean values were observed during the maize-ryegrass intercrop, at the end of summer 2014 (0.15 vol vol^{-1}), while WFPS was on average higher in the 2014-15 ryegrass crop, during which its mean value was 0.70 vol vol^{-1}. Overall, daily WFPS never fell below the WP during the observation period. Furthermore, the 60% of observed WFPS values were higher than the FC threshold (Figure 2).

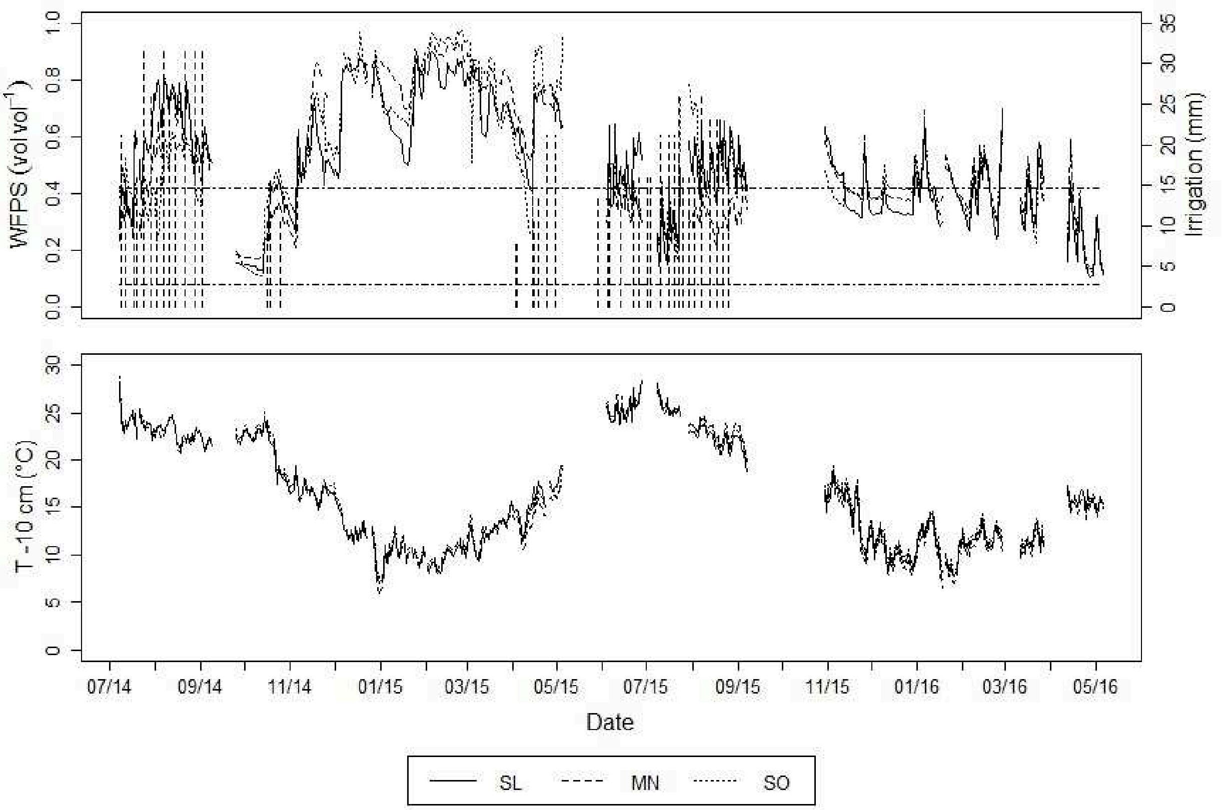

Figure 2. Dynamics of Water Filled Pore Space (WFPS, vol vol^{-1}), Irrigation (mm) and Soil Temperature at -10 cm (T -10 cm, °C). Horizontal chain lines in the first box indicate the Field Capacity (WFPS = 0.42) and the Wilting Point (WFPS = 0.08).

Soil T was strongly influenced by the season (P<0.001), while not significant effects of treatments were observed. On average, daily soil T ranged from 6.7 °C to 28.5 °C, with an average value of 16.7 °C. The maximum daily soil T value was observed in SO on 8th July 2014 (28.9 °C), while the minimum was observed in MN on 2nd December 2015 (5.3 °C), as reported in Figure 2.

The SR was higher in SO than SL and MN during the whole maize crop in 2014, on 80% of sampling dates during the first year of ryegrass and on 38% of sampling dates in the second year of ryegrass. Both organic fertilizers showed higher SR than MN (SO>SL>MN) in two sampling dates during the first ryegrass cycle in autumn (11th December) and winter (16th January). Higher SR values in MN than SL and SO were observed at the end of June in the second year of maize, while no differences between MN and organic treatments (SL≤MN≤SO) were observed in both ryegrass cycles at the first sampling date after MN fertilization. No differences between treatments were observed in the first year in the day before ryegrass sowing, from two to fifteen days after MN fertilization and at the ryegrass cutting. In the second year, no differences were observed during the first month after maize sowing, at the end of June (27th), from 5th August to maize harvest, from the end of January to 15th February and nine days after MN fertilization. Peaks of SR values were observed after the first year of ryegrass and second year of maize fertilizations. The SR value in SO (23.1±6.6 µmol m^{-2} s^{-1}) was higher than in SL (10.0±2.1 µmol m^{-2} s^{-1}) and MN (6.3±0.2 µmol m^{-2} s^{-1}) in ryegrass, while differences between SL (26.3±4.2 µmol m^{-2} s^{-1}), MN (13.3±2.5 µmol m^{-2} s^{-1}) and SO (13.2±0.9 µmol m^{-2} s^{-1}) after maize fertilizations were not significant. Minimum SR values in ryegrass crop were observed at 3rd February 2015 (0.3±0.1, 0.2±0.0 and 0.6±0.2 µmol m^{-2} s^{-1} in SL, MN and SO respectively). During maize crops, lower SR rates were observed at 8th September 2014 in SL an MN (2.4±0.4 and 1.7±0.1 µmol m^{-2} s^{-1} in SL and MN, respectively) and at 27th August 2015 in SO (3.0±1.5 µmol m^{-2} s^{-1}) (Figure 3). The treatments significantly influenced the relationship between soil T and SR (Figure 4). No significant relationships between WFPS and SR were observed. The SR dynamics was significantly influenced by the interaction between treatment and date (P<0.001).

A significant interaction (P<0.001) between treatment and sampling date was observed for N_2O fluxes. After maize fertilizations (5th July 2015), higher rates of N_2O fluxes were observed in SL (0.10±0.01 kg ha^{-1} d^{-1}) and SO (0.08±0.02 kg ha^{-1} d^{-1}) than MN (0.02±0.01

kg ha^{-1} d^{-1}). The day after, maximum values in SL (0.55±0.24 kg ha^{-1} d^{-1}) and SO (0.44±0.17 kg ha^{-1} d^{-1}) were observed. In the following days, N_2O fluxes in MN reached maximum values on 9th June (0.22±0.04 kg ha^{-1} d^{-1}) and 10 days after fertilizations, when N_2O flux in MN (0.10±0.02 kg ha^{-1} d^{-1}) was higher than SO (0.01±0.0 kg ha^{-1} d^{-1}). The differences among treatments were not significant until twelve days after SL and SO supply. From one month after fertilizations until the end of July, fluxes in SL were higher than in SO, while differences between treatments were not observed in the last month of maize crop. On 12th October 2015, higher N_2O fluxes in SL (0.07±0.02 kg ha^{-1} d^{-1}) were observed. Maximum N_2O fluxes rates in ryegrass were observed after MN fertilization. The days after MN supply (5-6th March 2015), fluxes in MN (0.08±0.02 – 0.40±0.03 kg ha^{-1} d^{-1}) were higher than in SL and SO, which values were quantitatively negligible (fluxes<0.01 kg ha^{-1} d^{-1}) (Figure 3). No significant correlations were observed between soil T and N_2O fluxes. Negative significant correlations (Table 4) were observed in ryegrass between WFPS and N_2O emissions in SL and SO.

The CH_4 flux dynamics were strongly affected (P<0.001) by the interaction between date and treatment. In each treatment, both CH_4 uptake and emissions were observed. Values of CH_4 fluxes ranged from -0.55±0.56 g CH_4 m^{-2} d^{-1} to 28.36±7.43 g CH_4 m^{-2} d^{-1}, observed on 5th June 2015 (after maize fertilization) in SL and MN, respectively. On this date, the CH_4 fluxes in SL were significantly higher than MN and SO (0.01±0.01 g CH_4 m^{-2} d^{-1}). Differences between treatments were observed also after MN fertilization on 4th March 2015, when CH_4 fluxes were higher in MN (0.24±0.10 g CH_4 m^{-2} d^{-1}) than in SL and SO (-0.03±0.03 and 0.03±0.03 g CH_4 m^{-2} d^{-1} in SL and SO, respectively). Differences between treatments were also observed in winter, on 23rd December 2014 (MN>SO), and in spring on 20th March 2015 (MN>SL). In both cases, values of CH_4 fluxes were quantitatively negligible (Figure 3). In the MN treatment during ryegrass cycle, the soil temperature inversely affected the CH_4 fluxes (-0.50), while WFPS was positively correlated (0.67), as shown in Table 4.

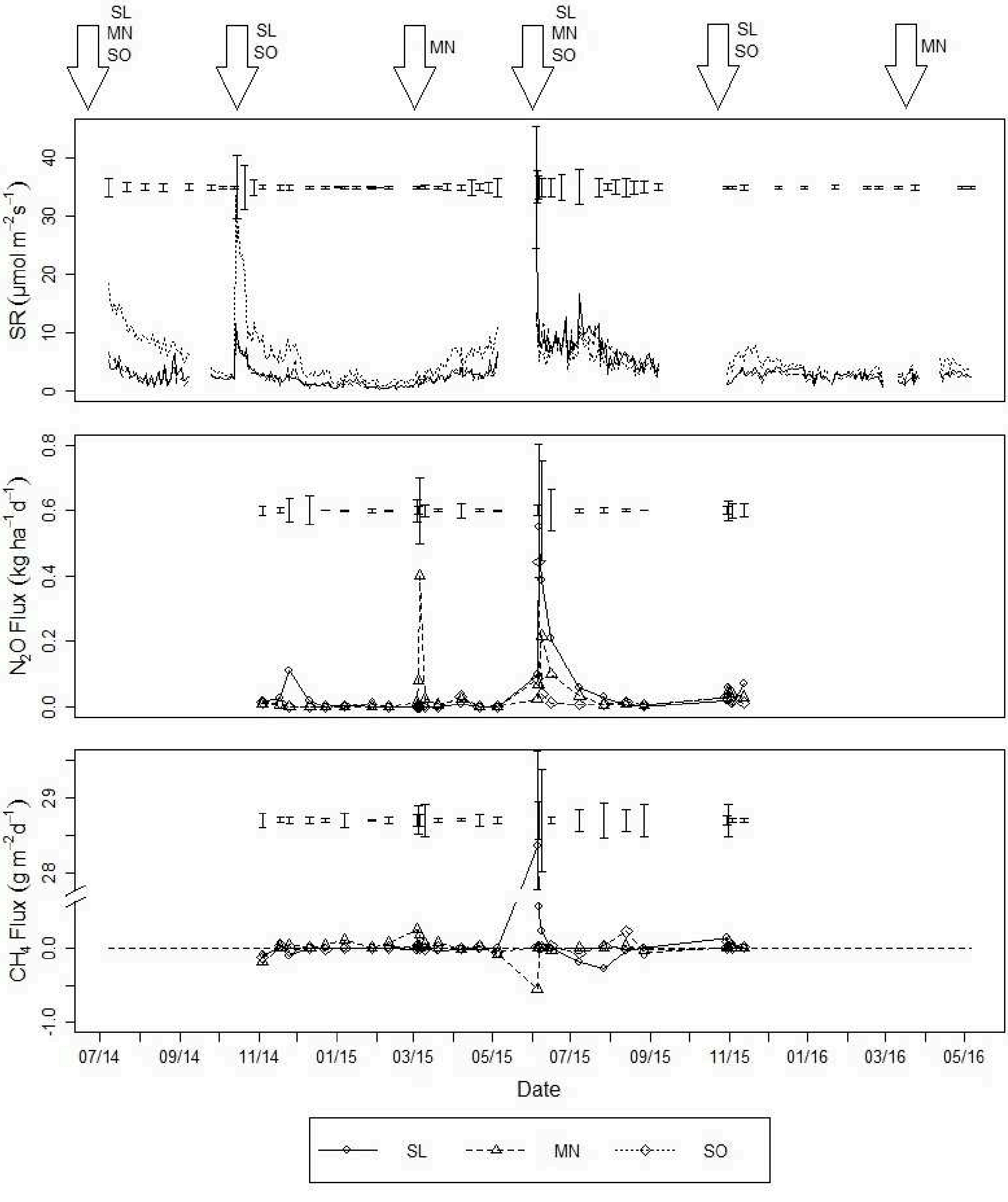

Figure 3 Soil Respiration (SR, µmol m^{-2} s^{-1}), N_2O (kg ha^{-1} d^{-1}) and CH_4 (g m^{-2} d^{-1}) fluxes. Bars represent the differences between back-transformed least square means confidence intervals (0.5%÷95%). The arrows on top indicate the fertilization events.

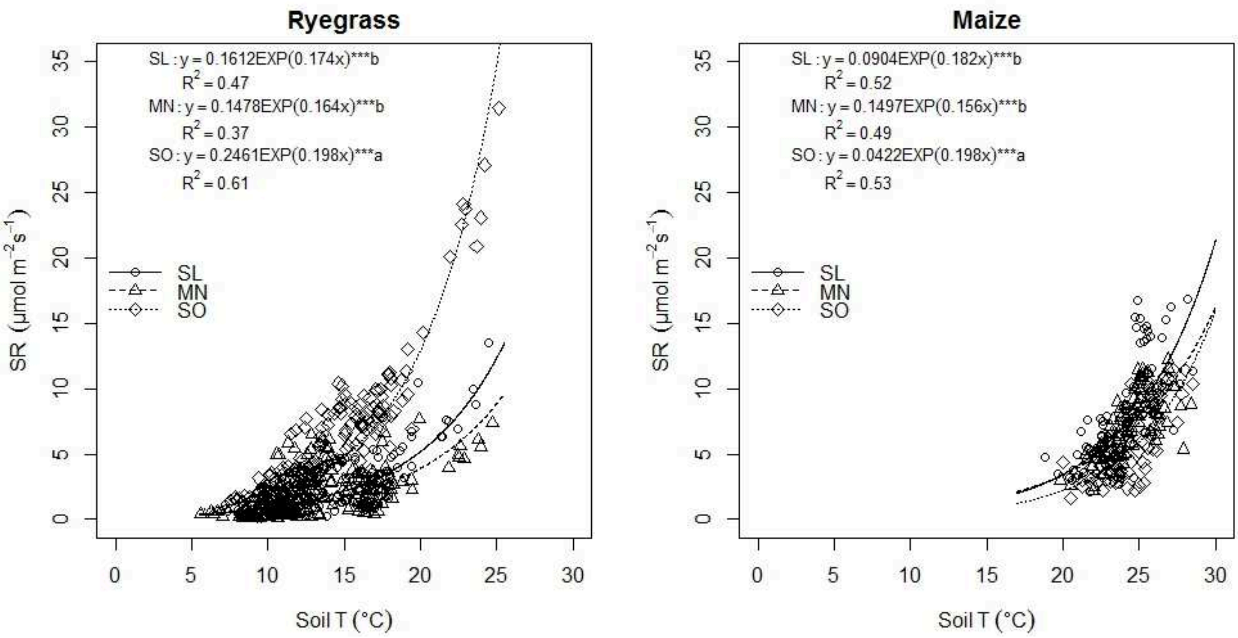

Figure 4 Relationship between soil temperature at -10 cm (Soil T, °C) and Soil Respiration (SR, µmol m^{-2} s^{-1}) in ryegrass and maize crop. Models followed by the same letter are not significantly different (P<0.05) according to Soilani (2005). SL: Slurry; MN: Mineral; SO: Solid fraction of slurry. ***P<0.001.

Table 4. Pearson's Correlation Index between log-transformed N_2O and CH_4 fluxes and Soil T and WFPS in maize and ryegrass crop. ***: P<0.001; **: P<0.01; *: P<0.05; NS: not significant.

		N_2O		CH_4	
Crop	Treatment	Soil T	WFPS	Soil T	WFPS
Maize	SL	0.11 ^{NS}	0.50 ^{NS}	-0.21 ^{NS}	-0.02 ^{NS}
	MN	0.22 ^{NS}	0.53 ^{NS}	-0.23 ^{NS}	0.09 ^{NS}
	SO	0.11 ^{NS}	0.50 ^{NS}	-0.21 ^{NS}	-0.02 ^{NS}
Ryegrass	SL	0.43 ^{NS}	-0.52 *	-0.14 ^{NS}	0.18 ^{NS}
	MN	-0.16 ^{NS}	0.08 ^{NS}	-0.51 *	0.68 **
	SO	0.35 ^{NS}	-0.70 ***	-0.33 ^{NS}	0.30 ^{NS}

SL: Slurry; MN: Mineral; SO: Solid fraction of slurry.

Cumulative GHG and GWP

The yearly cumulative CO_2 emissions (Mg CO_2 ha^{-1} yr^{-1}) were 30% significantly higher in SO (70.5±1.5 Mg CO_2 ha^{-1} yr^{-1}) than in SL and MN (Figure 5). Under SO fertilization, the 68% of CO_2 emissions occurred during the ryegrass crop, while for SL and MN respectively 60% and 61% of emissions occurred during maize. The cumulative N_2O fluxes

(Figure 5) were higher in SL (11.5±5.2 kg N_2O ha^{-1} yr^{-1}) than in SO (3.4±1.8 kg N_2O ha^{-1} yr^{-1}). In addition, emissions in MN (6.5±1.4 kg kg N_2O ha^{-1} yr^{-1}) were higher than in SO. As shown in Figure 3, for all treatments most of the emissions occurred along the first month after maize fertilization (42%, 47% and 41% in SL, MN and SO respectively). For SO, 72% of the first month emissions occurred in the first four days after fertilizer supply. Furthermore, the 20% of total fluxes in MN occurred after fertilizations in March during ryegrass crop. The treatments had no effect on cumulated CH_4 fluxes. Values of cumulated CH_4 fluxes (Figure 5) were not different to 0 according to the Student's one sample t-test.

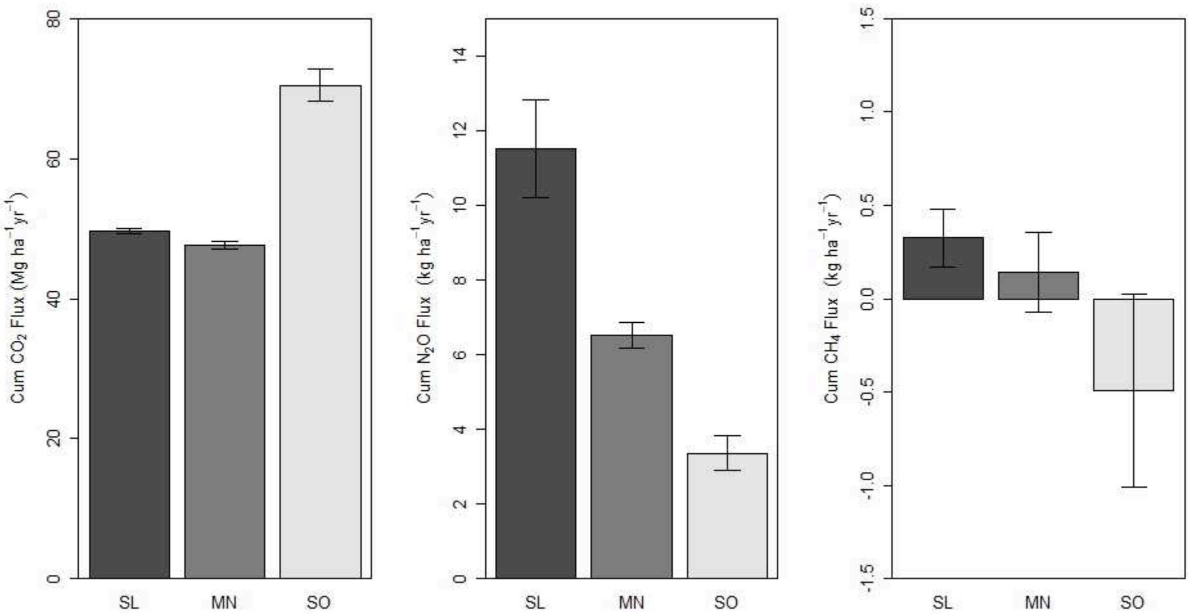

Figure 5 Cumulative emissions of CO_2 (Mg ha^{-1} yr^{-1}), N_2O (kg ha^{-1} yr^{-1}) and CH_4 (kg ha^{-1} yr^{-1}). Bars indicate the standard errors of the means (n=4).

In the reference year for net GWP assessment, a lower ryegrass TAB in SL (6.9±2.3 Mg DM ha^{-1}) than MN (10.3±1.2 Mg DM ha^{-1}) and SO (10.2±2.4 Mg DM ha^{-1}) was observed, while differences between treatments were not observed in maize (average TAB of 18.6±0.2 Mg DM ha^{-1}). Overall, the C inputs from crop residues were significantly higher in MN (6.6±0.6 Mg C ha^{-1} yr^{-1}) and SO (6.4±1.1 Mg C ha^{-1} yr^{-1}) than in SL (5.0±0.6 Mg C ha^{-1} yr^{-1}). Values of C input from residues and fertilizers expressed as CO_2 equivalents are reported in Table 5.

The SR-based C sequestration (Mg C ha^{-1}) was higher in SO (2.96±0.77 Mg ha^{-1}) than in MN (0.17±0.47 Mg ha^{-1}). Under SL treatment, a negative value of C sequestration (-1.73±0.29 Mg ha^{-1}) different from SO and MN was observed.

In the SO treatment, a soil C sequestration rate of 3.60±1.85 Mg C ha^{-1} yr^{-1} was observed, while in SL (-0.46±0.96 Mg C ha^{-1} yr^{-1}) and MN (-0.65±0.42 Mg C ha^{-1} yr^{-1}) soil C losses were observed, as reported in Table 5 expressed as CO_2 equivalents.

The treatments significantly influenced the net GWPr, that was higher in SL than MN, and negative in SO (Table 5). The SL and MN treatment showed a positive GWPs, significantly different compared to the negative values observed in SO. Net GWP were not significantly different between the two assessment methods.

The GHG Eco-Efficiency index from GWPr was higher in SL (39.7%). Lower index was observed in MN (5.2%), that was significantly different from SO (-35.1%). Considering GWPs for the calculation, the GHG Eco-Efficiency index was significantly lower in SO (-44.5%) than those observed in SL and MN (20.9% and 15.3%, respectively).

Table 5. CO_2 Equivalents and net GWP computation based on soil respiration (GWPr) and soil C sequestration (GWPs).

	SL		MN		SO	
GWP compounds	CO_2 Equivalents (Mg CO_2 ha^{-1} yr^{-1})					
N_2O	3.43±0.78	a	1.94±0.21	b	1.00±0.27	c
CH_4	0.011±0.010	a	0.005±0.014	a	-0.017±0.035	a
SR	30.03±0.40	b	23.71±0.57	c	46.26±3.26	a
Crop Residues	-18.50±1.02	a	-24.32±1.16	b	-23.64±1.96	b
Organic Fertilizers	-5.19±0.30	a	0		-33.47±0.50	b
SR based C sequestration*	6.35±1.07	a	-0.61±1.70	b	-10.85±2.82	c
Soil C Sequestration	1.71±3.51	a	2.40±1.53	a	-13.21±6.81	b
GWPr	9.79±1.41	a	1.34±1.87	b	-9.86±3.05	c
GWPs	5.15±1.98	a	4.34±0.94	a	-12.23±3.49	b

Means followed by the same letter indicates no significant differences between treatments according to the Student-Newman-Keuls test (P<0.05).
SL: Slurry; MN: Mineral; SO: Solid fraction of slurry.

Discussion

Soil GHG emissions

The average daily WFPS value ranged from 0.11 to 0.94 vol vol^{-1} in the study period, and no significant effects of WFPS were observed on SR. These findings were in agreement with the hypothesis that in irrigated systems seasonal SR dynamics are not limited by the WFPS. Under Mediterranean climate, Lado-Monserrat *et al.* (2014) observed significant WFPS effects on SR under a matric potential of -100 kPa, corresponding to a WFPS of 0.08 vol vol^{-1}. During the experiment, irrigation allowed to keep WFPS at non-limiting thresholds for most of maize and ryegrass cycles. For this reason WFPS dynamics were different than those ordinarily observed in rainfed Mediterranean agroecosystems, where the WFPS showed generally an inverse dynamics with respect to soil T (Almagro *et al.*, 2009). At the same time, our results confirmed what was already observed by Lai *et al.* (2017) in the same environmental conditions.

The response of SR to soil T was higher in SO during both ryegrass and maize crop. The observed SR dynamics are consistent to what reported in the previous studies conducted in the same experimental site (Lai *et al.*, 2012; Lai *et al.*, 2017). Overall, SO showed higher SR fluxes during almost the whole observation period, evidencing differences in SR between organic fertilizers. The different responses to soil T between SO and MN are in agreement to what reported by Mancinelli *et al.* (2010) and Florio *et al.* (2016), both comparing conventional and organic fertilization options. This finding could be explained by the higher sensitivity of SR to soil T when SWC in not limiting for microbial activities (Janssens *et al.*, 2003), which suggested different responses in relation to C input from fertilizers, litter and root biomass (Chen and Tian, 2005). Though the MN treatment showed the lower SR emission rates, a share of measured soil CO_2 efflux could be attributable to the supply of livestock effluents for decades before the beginning of the experiment.

Although a limited influence of C inputs on organic matter decomposition rates and consequently on the SR sensitivity to soil T is expected (Fang *et al.*, 2005), the differences between SO and SL could be explained considering the differences between organic substrates in terms of water content and N mineral content. Although no significant effects of WFPS on SR were observed, the early response of SL supply may be associated to a lack of oxygen for mineralization processes, by creating a temporary anaerobic microsites and reducing gases diffusivity (Bowden *et al.*, 2004; Ding *et al.*, 2007). On the other hand, during the whole crop cycles the higher mineral N content in SL could have inhibited the mineralization, as observed under no limiting soil N conditions (Carreiro *et al.*, 2000).

A not significant effect of soil T on N_2O emissions was observed during the experiment, while the WFPS showed a negative correlation with N_2O fluxes for the organic treatments. The lack of significant effect of soil T is consistent to what reported by Schindlbacher *et al.* (2004), who however observed an indirect soil T effect with high WFPS values, which led denitrification to occur. In ryegrass, a share of N_2O emission could be attributed to denitrification, which begins to be prevalent above a WFPS value of 0.60 vol vol^{-1} (Davidson *et al.*, 2000). For this reason, the negative correlation between WFPS and N_2O emissions could be linked to the lower N-NO_3^- supplied with organic fertilizers, which might have caused in turn a lower nitrate availability as substrate for denitrification (Sanz-Cobena *et al.*, 2014). The average observed N_2O fluxes after maize fertilizations are in agreement to those observed by Alluvione *et al.* (2010) using mineral and organic fertilizers similar to SL in terms of C input and C:N ratio. During the maize cycle, the N_2O fluxes showed a peak on the day after SL and SO fertilizations, while maximum fluxes in MN were observed a week later. Observed N_2O fluxes in MN are consistent to those observed in maize under Mediterranean conditions (Louro *et al.*, 2015; Forte *et al.*, 2017). This behaviour could be related to the presence of a nitrification inhibitor in the mineral fertilizer supplied, as observed in a study carried out in Mediterranean maize-based systems by Ranucci *et al.* (2011). At the same time, in SL the N_2O fluxes remained relevant from one month after fertilizer supply, consistently with what observed using pig slurry in a maize-wheat system in Brazil by Aita *et al.* (2015). The different responses of organic fertilizers in terms of duration of relevant N_2O fluxes can be explained by the combined effects of ammonium and oxygen availability for microbial activity. As already reported, in maize the average WFPS value across treatments never overcames the threshold of 0.60 vol vol^{-1}. A WFPS value around 60% is considered as the soil water condition beyond which anaerobic processes such denitrification begin to be prevalent (Davidson *et al.*, 2000). In this experiment, the amount of NH_4^+, which represented on average 96% of mineral N from organic fertilizers, was on average 55% higher in SL than SO. Consequently, the N_2O emissions from organic fertilizers were higher in SL than SO as a result of combined soil nitrifying environment and higher NH_4^+ availability (Bateman and Baggs, 2005; Louro *et al.*, 2013). During the ryegrass cycle,

N_2O fluxes were significantly higher in MN after fertilizer topdressing in March. This finding is consistent with that observed by Huérfano *et al.* (2015) for a single application of mineral fertilizer in winter wheat crop. Despite of a no significant effect of WFPS on N_2O fluxes, the significant increase of N_2O emission after the MN fertilization could be associated to the high N-NO_3^- supplied, that, in combination with favourable WFPS conditions, could have likely enhanced denitrification processes.

A significant correlation of CH_4 fluxes between both soil T and WFPS was observed in ryegrass for the MN treatment. The MN treatment resulted as a sink of CH_4 on 75% of sampling dates during the ryegrass cycle. Moreover, higher positive fluxes in MN than SL and SO were observed after topdressing fertilization, although the absolute rates were not relatively high. The inhibitory effect of soil T could be a secondary factor of the most important effect of WFPS, as a result of a lower CH_4 uptake (Merino *et al.*, 2004). After maize fertilizations, higher soil CH_4 fluxes were observed in SL than in the other treatments. After SL application in maize, the effect of the treatment lasted up to four days, while with the autumn fertilization before ryegrass sowing fluxes in SL were higher only the day after the fertilizer distribution. This result is consistent with that observed by other scholars after SL supply in different agro-ecosystems (Jones *et al.*, 2005; Louro *et al.*, 2013). The WFPS condition in the days following fertilization suggested a hostile environment for the microbial anaerobic processes that induce the methanogenesis. Nevertheless, peaks of CH_4 fluxes testified the occurrence of transitory anaerobic conditions, which is explainable as a consequence of SL application. The organic fertilizers had qualitative differences in terms of DM content higher in SO (20.3%) than SL (2.8%). The addiction of a high-water content substrate such as in the case of SL (97.2% of water content) could have caused a temporary creation of saturated microsites that promoted methanogenic activities. Plaza-Bonilla *et al.* (2014), after pig slurry supplying in a barley monoculture in the Aragon region (Spain), also attributed CH_4 emissions after fertilization to the ammonium inhibitory effect on methane monooxygenase (Le Mer and Roger, 2001), produced by soil metanotrophic microbial communities. Furthermore, methane might originate from animal slurry, also inside dissolved or as originated by degradation of manure fatty acids (Chadwick *et al.*, 2000).

Soil C sequestration and Global Warming Potential

The average yearly SOC increased of 5.9% (Mg C ha^{-1}) in SO, which corresponds to an average increase in the soil C concentration of 1.45 g C kg^{-1}. Alluvione *et al.* (2013) found similar results in a maize-based cropping system fertilized with compost. The increase in SOC observed in SO can be attributed to the higher C input (Kong *et al.*, 2005; Aguilera *et al.*, 2015) than SL. In fact, SL had a lower C input from fertilizers and from crop residues. This latter finding was explained with the lower SL efficiency as N fertilizers (e.g. Ceotto *et al.*, 2014).

The soil C changes and the GHG emissions observed in the present study indicated that SL and MN were net sources of GWP while SO was a net sink, independently of the estimation method. However, the impact of fertilization systems in terms of GWP was different depending on the estimation method. The GWPr was higher in SL than MN, while no differences between SL and MN treatments considering GWPs were observed. These results are consistent with findings reported by other scholars (e.g. Fornara *et al.*, 2016). The higher GWP estimated considering SR (GWPr) in SL than MN could be associated to the higher C losses in SL that were not compensated by the C inputs to the soil. Whereas, in MN the C input from residues was significantly higher than SL, as already reported by Lai et al. (2017), as a consequence of higher TAB (Demurtas, 2014), that almost totally restored the lower C losses through respiration. The SOC sink behaviour of SO was interpreted as associated to its relative high C:N and N content, that characterizes organic fertilizers and it was recognized to play a crucial role for mitigating GHG emissions under Mediterranean conditions (Grignani *et al.*, 2012; Forte *et al.*, 2017). The differences between SO and SL in terms of GWP were attributed to the separation processing of slurry, that, although it determines C and mineral N losses with respect to slurry DM (Peters *et al.*, 2011), produces a high N-organic fertilizer with higher C content than the original SL. Moreover, the different influence on the net GWP between SO and SL can be explained also by a higher C input from residues of SO than SL and lower N_2O emissions than the other treatments. In fact, the mineral-N:total-N ratio was on average 60% and 29% in SL and SO, respectively. The contribution of C sequestration to the net GWPr was respectively 65% and 43% in SL and MN, while N_2O fluxes in MN had a higher impact (57%) than in other treatments consistently to what reported by Aguilera *et al.* (2015). When GWP was computed considering the SOC sequestration rate (GWPs), differences between treatments were only partially confirmed. However, GWPs estimates have to be taken with some caution. In fact, although the hourly SR measurements would result in a robust measure of daily variability, the estimate of SOC variation in time could be affected by an error due to the spatial variability that could hide the temporal variability, as already suggested by Mosier *et al.* (2006) and confirmed in a long-term (>20 years) experiment by Jiang *et al.* (2017). These authors reported a SOC increase with continuous mineral N fertilizations, with a significant higher C concentration in soil micro-aggregates. These findings suggested that the effect on C sink of the mineral fertilizers could be appreciated after a long time span, which is required for the stabilization of the C compounds from crop residues.

As the GWP, positive values of GHGe indicate a net source of GWP while negative GHGe values correspond to a net sink of GWP to the soil. Considering GWPr, the calculated GHGe suggested a non-optimal fertilization management that resulted in a net source of GWP observed in SL and MN, while environmental benefits could be achieved by using SO. The already discussed differences between GWPr and GWPs estimates, that reflect also a different GHGe, suggested a cautionary interpretation of results for MN. The efficiency of MN could be anyhow improved through fertilization strategies oriented to the reduction of N_2O emissions.

Conclusion

The results of this study confirmed the hypothesis that different sources of organic N can show a completely different behaviour in determining soil GHG emissions and net GWP. The results highlight a good predisposition of the SO fertilizers to be an effective fertilization strategy in terms of GHG emissions mitigation, by maintaining at the same time good levels of agronomical efficiency.

A higher SR sensitivity to soil T seasonal variation was observed in SO, resulting in higher cumulative CO_2 emissions. However, the SO resulted a soil C sink system, as opposite to SL, which appeared as a source of C despite of lower cumulative CO_2 emissions. Furthermore, the results in MN evidenced a tendency toward neutrality with respect to soil C exchanges, which suggested the need for further research on soil C changes over time using MN fertilizers. According to what has been reported in this study, the use of SO can be considered a good fertilization strategy in order to mitigate N_2O emissions. The higher

N-NH_4^+ content and the favourable WFPS conditions for nitrification after maize fertilization resulted in higher cumulative N_2O emissions in SL than in MN and SO. The nitrification inhibitor could have reduced the N_2O emissions of MN fertilizer, for which further investigations are required in order to assess the impacts of different MN fertilization strategies. In these experimental conditions, CH_4 fluxes were not quantitatively relevant to influence the net GWP. The maximum observed rate in SL suggested that different techniques of slurry supply could be investigated in order to mitigate their impacts in terms of CH_4 emissions.

The SL and MN fertilization options were sinks of net GWP, irrespectively of the GWP estimation method, while the SL was a source of net GWP. The absence of differences between SL and MN in net GWPs still suggests that further studies are needed to assess the long period consequences on C sequestration of the use of mineral fertilizers. In the studied cropping system, the most important components determining the GWP were the soil C balance terms. The higher crop productivity with SO and MN caused a higher C return to soil. The net GWP balance item that mostly affected the differences between treatments was the lower C input from fertilizers and from residues in SL.

The GHGe values evidenced the agronomical efficiency of SO, which allowed to maintain adequate TAB production and satisfactory yields in the cropping system. On the contrary, the SL showed important limitations in terms of impacts on GWP and agronomic efficiency of the fertilization. The results also suggest that with high C inputs, GWPr could be a good tool to assess the net warming impacts of fertilizers.

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OVERALL CONCLUSIVE REMARKS

The research carried out have contributed to gain insights on some processes controlling GHG emissions and soil C sequestration of forage systems under Mediterranean conditions and on some agricultural practices that are most promising to mitigate climate change. The field scale experiments and the modelling approaches proved to be both relevant for enhancing our understanding of soil GHG effluxes and to test the efficiency of some possible mitigation strategies.

In the first chapter, it was demonstrated that the PaSim model proved to be reasonably reliable for assessing the impact of management options on Mediterranean grasslands C exchanges. For the specific agri-environmental context of the experimental areas of this study, the model could be linked with existing datasets of soil features and grassland productivity, in order to perform regional scale analyses e.g. on the impacts of management options on C sequestration. Furthermore, the model could be used as a valid tool in supporting farmers' management choices from the medium to the long term, by simulating different livestock rates, N fertilizations and cuttings. The future projections provided by the model could be valuable also to all the stakeholders involved in the livestock sector, as a tool for supporting the decision-making processes at different scales. The obtained parametrization of the model is valuable to perform studies on soil C changes under similar environmental conditions opening to potential application for other Mediterranean grasslands that are highly worldwide spread. A model improvement is however needed in order to better represent peculiar features of Mediterranean pastures. The model was in fact originally developed for very different environmental and management contexts to those investigated in this study. The performance of PaSim under Mediterranean conditions could be improved for example by considering the characteristics of the floristic composition of Mediterranean grassland communities, which are mostly composed by annual species with a high percentage of self-reseeding legumes. This last aspect also highlights the need to foresee in a future model improvement the possibility to consider the phosphorous fertilizations, which are recognized to be crucial aspect for legume productivity in Mediterranean pastures. Furthermore, it is not of secondary importance to enhance the capability of the model to include more complex management practices that are very common in Mediterranean extensive livestock farms, such as the rotational regimes with hay crops, cereal crops and meadows. Regarding the livestock management, an important model improvement could be reached by allowing to insert in the management input file of PaSim more grazing events than the current release, which allows only ten events per year. To achieve the model improvement performing specific experimental studies, e.g. on impacts of management and/or climate change on autumn pasture regrowth, may have to be carried out.

This study provides a basis for the development of scenario analyses and vulnerability assessment studies in relation to climate change and different management options, by evaluating also synergies and trade-offs between adaptation and mitigation strategies. The model can be also considered a good tool to evaluate at different scales the impacts of climate and management on a wide range of ecosystem services, including agricultural production, that are provided by Mediterranean pastures.

In the second chapter, the effectiveness of different fertilization strategies on GWP mitigation was highlighted as well as that in the specific experimental conditions a trade-off between agronomic efficiency and environmental sustainability goals, in terms of soil GHG emissions, can be achieved. The results of this study suggest that a potential improvement of the GHG Eco-efficiency of organic fertilizers such as cattle slurry could be achieved by adopting technological innovations, as the separation process, that allows to achieve good levels of agricultural efficiency and at the same time can contribute to mitigate the impacts of fertilization on soil GHG emissions. Another mitigation strategy to be further tested is the combined use of slurry and mineral fertilization. The reduction of GHG emissions could be also further widened by adding nitrification inhibitors to organic substrates. An analysis of the solid fraction of slurry production chain may provide more accurate information about the effectiveness of this fertilizer to mitigate the GWP.

The findings of this study can be of a certain importance for similar contexts characterized by semi-arid Mediterranean climates and intensive irrigated maize-based forage systems. However, caution is required when generalizing these findings since the GWP of fertilization systems is highly context-sensitive as demonstrated by the contrasting results reported by several scholars working under Mediterranean conditions in intensive agro-ecosystems, depending for example on the different quality traits of organic matter supplied with fertilizers.

The research findings represent a key information base for supporting the decisional processes toward the design of sustainable fertilization strategies. In order to better evaluate the agri-environmental effectiveness of different fertilization strategies it becomes crucial to assess the soil C dynamic through years. Further studies could be focused e.g. on the long-term assessment of soil C sequestration in relation to different N sources, with particular attention toward the use of organic fertilizers. The creation of robust mid and long-term datasets is in fact of great importance to develop and improve the existing biogeochemical model, in order to perform long terms analysis of the impacts of different fertilization options on the net GWP under a range of environmental and management conditions. In this respect, huge efforts have been devoted in the last decade for developing knowledge hubs aiming to data gathering and standardization and for model improvements across Europe and worldwide (e.g. MACSUR project, JPI FACCE, European Union, www.macsur.eu; AGMIP project, www.agmip.org). These knowledge hubs projects have proved to be an excellent approach to improve scientific knowledge for designing adaptation and mitigation strategies to climate change and they could be also important for the standardization and sharing of field methodologies and experimental designs in the domain of agro-ecosystem research.

The bottom-up approach used in this study, with the active involvement of farmers in the design of the experimental treatments, could be considered a worthwhile approach to develop agricultural practices that are sound scientific based and at the same time are considered meaningful and feasible at farming systems scale.

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