



Soil carbon dynamics as affected by long-term contrasting cropping systems and tillages under semiarid Mediterranean climate



Vito Armando Laudicina, Agata Novara, Luciano Gristina, Luigi Badalucco*

Dipartimento di Scienze Agrarie e Forestali, Università degli Studi di Palermo, Viale delle Scienze, Edificio 4, I-90128 Palermo, Italy

ARTICLE INFO

Article history:

Received 1 March 2013

Received in revised form 29 August 2013

Accepted 4 September 2013

Keywords:

Labile carbon pools

Field CO₂ flux

Carbon stock

Wheat monoculture

Wheat–faba rotation

Tillage

ABSTRACT

In a dryland Mediterranean agrosystem (Sicily, Italy) a comparative study was carried out among two crop systems (wheat/wheat and wheat/bean) after 19 years under three most used tillage managements (conventional, dual layer and no-tillage), in order to ascertain the effects of those experimental factors, single and combined, on various soil organic C pools (total and extractable organic C, microbial biomass C, basal respiration). Field CO₂ fluxes from soil, throughout a year, were also determined. Moreover, C input and output were assessed, as well as microbial and metabolic quotients. Tillage management more than cropping system affected the soil organic C stored in the first 15 cm of soil. After 19 years, no-tillage caused a 3.6 Mg ha⁻¹ increase of C content in wheat/faba rotation while of 5.6 Mg ha⁻¹ in wheat monoculture. The higher soil total organic C content in wheat monoculture was ascribed to a lower quality of residues supplied (higher both C/N ratio and acid detergent fibre (ADF) content). Moreover, wheat/bean rotation increased soil microbial biomass C, basal respiration and microbial quotient, thus suggesting that crop rotation more than tillage management was the driving factor in improving soil biochemical indicators.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Reduced tillage is regarded as one of the most effective conservation agricultural practices to decrease CO₂ emissions from soil and sequester C into it (Curtin et al., 2000; Laudicina et al., 2011; Luo et al., 2010). On the other hand, conventional tillage causes an increase in soil CO₂ evolution, although many authors (Roberts and Chan, 1999; Ellert and Janzen, 1999; Reicosky and Archer, 2007) noted that such an increase usually occurs for few days after machine operations. However, Luo et al. (2010) highlighted that the role of adopting no-tillage in sequestering C is greatly regulated by cropping systems. As a consequence, it is important to emphasise on both quantity and quality of crop residue inputs, as well as on tillage management.

Long-term field experiments have shown a direct linear relationship between C added as residues and C matter accumulated in soil (Cole et al., 1993; Duiker and Lal, 1999). However, in semiarid environments, where quantitative differences in residues among cropping systems are low, their quality becomes a determinant for soil C dynamics (Yadav et al., 2008). Curtin et al. (2000) have demonstrated the advantage of cereals over legumes in achieving maximum soil C sequestration rates. Indeed, in a semiarid area of Canada, they found that annual C added to soil ranged from 1.6 Mg C ha⁻¹ in average for the black lentil to 2–3 times that

amount for wheat crop. Similarly, in Argentina soybean, which produced 1.2 Mg ha⁻¹ residues, resulted in a net loss of soil C while corn, with 3.0 Mg ha⁻¹ residues, significantly lessened the loss of soil C from the system (Studdert and Echeverria, 2000). Guo et al. (2009), comparing crop performance in soil CO₂ emissions, found leguminous crop residues having more potential than wheat, although they improved soil quality and crop productivity. Those results confirmed that the quality of residue inputs, which generally is inversely related to the residue C/N ratio, can strongly affect CO₂ emissions (Al-Kaisi and Yin, 2005) by changing the microbial decomposition rate of residues (Kuo et al., 1997; Sainju et al., 2002). Therefore, residue C to N ratio is crucial in soil organic matter decomposition modelling (Melillo et al., 1989). Moreover, recent studies (Dubeux et al., 2006; Stubbs et al., 2009) have demonstrated as also the acid detergent fibre (ADF) is useful to predict plant material decomposition rates. Long-term field experiments provide reliable opportunities to investigate directly the effects of management on soil quality properties that do not respond rapidly to change, such as soil organic C (SOC), but not confounded by land use and management changes themselves (Hopkins et al., 2011). Such experiments provide benchmark values against which the effect of wider environmental changes can be examined (Hopkins et al., 2009).

The objectives of the present study, carried out in a representative dryland agrosystem, were to evaluate the long-term (19 years) effects of two contrasting crop systems (wheat–wheat vs. wheat–faba) under three different tillage managements (conventional tillage, dual layer and no-tillage) on (i) soil organic C labile

* Corresponding author. Tel.: +39 09123897052; fax: +39 091484035.
E-mail address: luigi.badalucco@unipa.it (L. Badalucco).

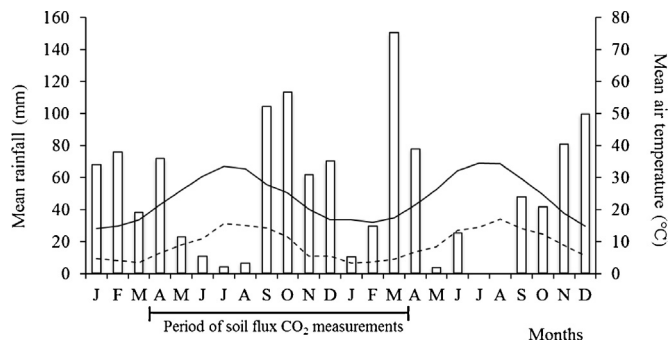


Fig. 1. Climatic trend from January 2008 to December 2009. Histograms are mean monthly rainfall, continuous and broken lines are, respectively, maximum and minimum mean monthly temperature. Soil CO_2 flux measurements were carried out from April 2008 to March 2009.

pools, and (ii) field and potential soil CO_2 emissions, to relate all them with both quantity and quality of residues input in cropped soils of the Mediterranean environment.

2. Materials and methods

2.1. Study site

The experimental work was carried out from April 2008 to April 2009 at the Pietranera farm of the University of Palermo, located in the central-southern Sicily, Italy, ($37^\circ 30' \text{N}$, $13^\circ 31' \text{E}$), 178 m above sea level. The climate at the experimental area is semi-arid Mediterranean, with a mean annual precipitation of 552 mm and a mean annual air temperature of 15.9°C . Climatic data were collected from January 2008 to December 2009 by a weather station located 500 m far from the experimental field (Fig. 1). The soil used was a fine-clayey, calcareous, mixed, xeric Chromic Haploxerert, with a slope of 7%. Main soil properties, determined before setting up the experimental field and referring to 0–15 cm depth, were: clay $471 \pm 51 \text{ g kg}^{-1}$, silt $225 \pm 26 \text{ g kg}^{-1}$, sand $304 \pm 28 \text{ g kg}^{-1}$, total organic C $18.1 \pm 0.5 \text{ g kg}^{-1}$, total N $1.3 \pm 0.1 \text{ g kg}^{-1}$ and pH 8.1 ± 0.1 .

2.2. Experimental design

The experimental design, established in 1991, was a strip-plot with two replications. Since 1952 up to 1991, the experimental area was cropped with a durum wheat/vetch crop rotation under a deep ploughing regime. Two crop sequences (wheat monoculture and wheat/bean rotation) were assigned as horizontal treatments and three tillage systems as vertical treatments. The plot size was 370 m^2 ($18.5 \times 20.0 \text{ m}$). Tillage managements were: (i) conventional tillage (CT), consisting of ploughing to a depth of 30 cm followed by one or two shallow (10–15 cm depth) harrowing operations before sowing; (ii) dual-layer (DL), consisting of a chisel ploughing to a depth of 40 cm coupled with a mouldboard ploughing to a depth of 15 cm and followed by one shallow harrowing operation; (iii) no-tillage (NT). Although some studies suggest that soil C content across the profile could be driven by differences in incorporation depth of residues depending on tillage treatments (Sun et al., 2011), here only the 0–15 cm soil layer was investigated as most tillage operations involved the 0–15 cm soil layer, with conventional ploughing (up to 30 cm) more invasive than chisel plowing. To minimise the annual climatic variability effects, all crops had a yearly-based rotation. Seed density was 350 seeds m^{-2} in rows 16 cm apart for wheat (*Triticum durum*) and 40 seeds m^{-2} in rows 70 cm apart for faba bean (*Vicia faba*). Following normal farm practices, fertilisation was carried out every year by applying in wheat/bean (WB) rotation 92 kg ha^{-1} of P_2O_5 and 82 kg ha^{-1}

of urea-N before seeding wheat, while 18 kg ha^{-1} of urea-N and 46 kg ha^{-1} of P_2O_5 before seeding faba. In wheat monoculture (WW), 92 kg ha^{-1} of P_2O_5 and 120 kg ha^{-1} of urea-N before seeding were applied. In WW monoculture, weed control was carried out by using in post-emergence Tribenuron-methyl herbicide (at the 3rd to 4th leaf stage). In no tilled plots of WB rotation, weeds were controlled prior to sowing by glyphosate [N-(phosphonomethyl) glycine]. Further details about experimental site and agronomic techniques are reported in Giambalvo et al. (2012).

2.3. Carbon and acid detergent fibre (ADF) input

During the 19 years since the experiment was established, the C inputs consisted mainly of root biomasses and marginally of shoots left after the harvest, both in wheat monoculture and in wheat/bean rotation. The cumulative C supplied by root biomass (CCRB) both for wheat and bean was calculated according to Kong et al. (2005) and Chung et al. (2008) as follows:

$\text{CCRB} (\text{Mg ha}^{-1}) = 0.22 \times \text{CAB} (\text{Mg ha}^{-1}) \times \text{root carbon} (\%)$, where CAB is the cumulative aboveground biomass and 0.22 is a conversion factor for root to shoot ratio used for both wheat and legumes, assumed as grains (IPCC, 2006).

The yield biomasses of both wheat and bean crops were estimated by weighing one square metre of aboveground biomass after the harvest for each plot. The C content of root and shoot residues was assumed for both species equal to 43% (Kong et al., 2005; Gan et al., 2009; Jensen et al., 2010). Root and shoot biomasses were also analysed for total N by Kjeldahl digestion (Bremner, 1996) and for acid detergent fibre (ADF) content (Van Soest, 1963). Then, the ADF input was calculated by multiplying the C input by ADF content.

2.4. Soil sampling and analyses

Soils were sampled just after the harvest carried out in June 2009. Three soil samples (0–15 cm), each composed by five soil sub-samples, were collected in each plot. Soil samples were sieved at 2 mm and split in two aliquots, the first being air-dried and the second remoistened to 50% of water holding capacity (WHC). Soil chemical analyses were carried out on air-dried aliquots whereas soil biochemical analyses on remoistened aliquots. Total organic C (TOC) was determined using a Carlo-Erba CHN analyser. Soil bulk density was measured using the tube core method (Blake and Hartge, 1986). Microbial biomass C was determined by the substrate (glucose) induced respiration method (SIR), according to Anderson and Domsch (1978). Briefly, 10 g soil aliquots, remoistened at 50% water holding capacity (HWC), were weighed into bottles and added with a glucose solution to have a final glucose concentration of 2 mg g^{-1} dry soil; the final soil moisture corresponded to 120% of WHC, in order to optimally spread the substrate within soil samples. The bottles with soil were conditioned for 0.5 h, sealed with butyl rubber stoppers, and incubated for 2 h at 22°C . SIR was converted to microbial biomass C (MBC) using a conversion factor of 40 (Anderson and Domsch, 1978). The glucose concentration and the duration of the incubation period in the SIR assay, in order to reach the soil maximal linear respiratory response had been determined by previous tests. A short-term (36 days) aerobic incubation procedure was used to determine the potential of the soils to mineralise organic C (Nannipieri et al., 1990). Soil samples at 50% WHC were pre-incubated for a week at room temperature; then, readjusted the moisture by water addition, they were incubated at 22°C in 200 cm^3 air-tight glass bottles. The CO_2 evolved was measured by sampling an aliquot of gas from the bottles by using a syringe and injecting it into a gas-chromatograph (Trace GC, Thermo Electron) equipped with a thermal conductivity detector (TCD). After air sampling, stoppers were removed for half hour to

balance the atmosphere inside and outside the bottles. The containers were capped again and incubated under the same conditions. This procedure, after the first sampling at day 1, was repeated at 3- or 4-day intervals for 36 days of incubation. The C mineralisation rate was expressed as $\text{mg CO}_2\text{-C g}^{-1}\text{ TOC d}^{-1}$ and was fitted to the following first-order decay function:

Mineralised C = $C_r e^{-kt}$, where C_r is the readily mineralisable C at time zero (i.e. the intercept value), k is the decay rate constant and t is the time. C mineralisation rate of the first day of incubation was used as a measure of basal respiration and to calculate the metabolic quotient ($q\text{CO}_2$), an index of the soil stress/disturbance status (Anderson and Domsch, 1993) expressed as $\text{mg C-CO}_2 \text{ h}^{-1} \text{ g}^{-1} \text{ MBC}$.

Gravimetric water content of soil samples was determined by weight difference between fresh and dried (24 h at 105°C) sample. Soil water filled pore space (WFPS) and soil temperature were measured at each time of CO_2 efflux measurements. WFPS was calculated according to the following formula:

$$\text{WFPS} = \frac{\text{SWC} \times \text{BD} \times 100}{(1 - \text{BD}/\text{AD})}$$

where, SWC is the gravimetric soil water content, BD is the soil bulk density and AD is the soil absolute density (2.65 g cm^{-3}). Soil temperature was measured at 15 cm depth using a digital thermometer 16 times per plot. Soil analyses were carried out in triplicate.

2.5. Soil CO_2 fluxes in the field

In the field soil CO_2 flux was measured at 36 different days from April 2008 to April 2009, using a portable infrared gas analyser (PP System—Ciras/1) connected to a soil respiration chamber, i.e. a closed gas flow system consisting of a 0.854 dm^3 cylinder with a 95 mm diameter opening, and covering a soil surface of 0.71 dm^2 . The chamber edge simply rested into the soil. For all the six treatments and the 2 replicated plots, at each measurement day, CO_2 was monitored 12 consecutive times between 9.00 and 11.00 a.m. To exclude the effect of the single crop within the two crop systems, the measures of CO_2 flux were carried out always on *T. durum*.

The soil C outputs were calculated through the linear interpolation of two neighbouring measured fluxes and the numerical integration over time, as reported in the following equation:

$$\text{CO}_2 - \text{C flux (kg ha}^{-1} \text{ d}^{-1}) = \sum_i^n \left[(X_i + X_{i+1}) \times \frac{N}{2} \right] + \dots + \left[(X_{n-1} + X_n) \times \frac{N}{2} \right]$$

where i is the date of first measurement of CO_2 rate; n is date of the last measurement of CO_2 rate; X is the CO_2 rate averaged among 24 (12 daily per 2 replicated plots) measurements, and N is the number of days between the two consecutive averaged CO_2 rates.

2.6. Statistical analysis

Two-way analysis of variance (ANOVA) was performed with tillage (conventional, dual layer and no-tillage) and crop system (wheat/wheat, WW, and wheat/bean, WB) as experimental factors. Residual maximum likelihood variance components were also performed to determine which of the two factors or their interaction accounted for the majority of the variation in each of the measured variable. All statistical analyses were carried out using SPSS 13.0. Soil CO_2 field flux data were analysed by using the repeated measures procedure in the general linear model.

3. Results

3.1. Soil C and N

Total organic C ranged from 18.3 in DL plots to 21.2 g kg^{-1} in WW/NT plots (Table 1). Both tillage and crop systems affected soil TOC concentration (17% and 19% of variance explained, respectively). No-tillage accumulated the highest amount of C in both crop systems, followed by CT and DL. With regard to crop systems, the C concentration was significantly higher in soil with WW monoculture (21.2 g kg^{-1}) than in those with WB rotation (19.6 g kg^{-1}). Extractable C ranged from 63.1 (WB) to 66.7 (WW) mg kg^{-1} in CT plots to 103.8 (WB)– 107.0 (WW) mg kg^{-1} in NT ones, and was affected only by tillage management (82% of explained variance). Total N values ranged from 1.0 g kg^{-1} in WB/CT and WW/DL to 1.4 g kg^{-1} in WW/CT (Table 1). Total N was mainly influenced by the interaction crop system $\times$ tillage (55% of explained variance), and marginally by the two single factors. Consequently, most variance of TOC/TN ratio was explained by the interaction crop system $\times$ tillage. Carbon stored in the first 15 cm of soil slightly differed among treatments and followed the same trend of TOC concentration, being higher in WW monoculture and in NT plots (Table 2). Indeed, it ranged between 28.9 and 35.2 Mg ha^{-1} in WB/CT and WW/NT plots, respectively. Tillage was the main factor affecting C stock followed by the crop system, while the interaction of the two factors did not affect it. Carbon stock was highest in NT plots compared to tilled ones (Table 2).

3.2. Carbon and ADF inputs

Carbon input did not show significant differences among investigated plots (Table 2) while, on the contrary, the C/N ratio of biomasses supplied by roots was significantly different. Indeed, the average ($n=18$) C/N ratio of faba bean residues was 11 ± 1.5 , whereas that of wheat roots was 42 ± 3.0 . Also the ADF content showed significant differences among supplied root biomasses being 4.8 and 4.2 g kg^{-1} for wheat and faba bean crops, respectively. As a consequence, the ADF input was higher in wheat monoculture than in WB rotation (Table 2).

3.3. Soil CO_2 flux and C output

Soil CO_2 fluxes significantly changed during the observation period and were affected by crop system and tillage, and by their interaction (respectively, 29%, 42% and 13% of variance explained; Fig. 2). Moreover, significant correlations between CO_2 flux and soil temperature occurred in four (Fig. 3) of the six investigated treatments, but not for wheat monoculture under conventional tillage and dual layer. Conversely, no significant ($P < 0.05$) correlation at all was found between CO_2 flux and gravimetric water content or WFPS throughout the six treatments. Finally, even the multiple regression between CO_2 flux (dependent variable) and soil temperature and gravimetric water content or WFPS (independent variables) was not significant.

CO_2 fluxes were generally highest in CT plots for wheat monoculture, while in DL plots for WB rotation. In NT plots, CO_2 fluxes were generally lowest, regardless of cropping system, whereas from August to October they tended to be higher in WB rotation, regardless of soil tillage management (Fig. 2). Moreover, during the observation period CO_2 fluxes showed higher oscillations in tilled plots compared to no tilled ones. The amount of C output calculated by interpolating CO_2 flux measurements carried out during the trial period, ranged from 3.1 in WB plots to $2.0 \text{ Mg ha}^{-1} \text{ y}^{-1}$ in WW/NT plots (Table 2).

The C output was from 0.1 to $1.3 \text{ Mg C ha}^{-1} \text{ y}^{-1}$ higher than C input, suggesting a net C loss from all investigated treatments.

Table 1

Chemical properties of the studied agrosystem soil in response to different management practices. Percentages of variance explained for any variable by each experimental factor (crop system and tillage) and by their interaction are also reported.

Crop system	Tillage	Bulk density (g cm ⁻³)	TOC (g kg ⁻¹)	Cextr (mg kg ⁻¹)	TN (g kg ⁻¹)	TOC/TN
WW	CT	1.04 Aa	20.8 Aa	66.7 Aa	1.4 Aa	15.1 Aa
WW	DL	1.08 ABa	18.3 Ba	86.8 Ba	1.0 Ba	17.9 Ba
WW	NT	1.11 Ba	21.2 Aa	107.0 Ca	1.3 Aa	15.8 Aa
WB	CT	1.04 Aa	18.6 ABb	63.1 Aa	1.0 Ab	18.0 Ab
WB	DL	1.07 ABa	18.3 Aa	85.8 Ba	1.1 Aa	16.3 Ba
WB	NT	1.10 Ba	19.6 Bb	103.8 Ca	1.1 Ab	17.6 ABa
Crop system (%)		NS	19**	NS	15**	7*
Tillage (%)		NS	17**	82***	5*	NS
Crop system × tillage (%)		NS	NS	NS	55**	71***

WW, durum wheat monoculture; WB, durum wheat–faba–bean rotation; CT, conventional tillage; DL, dual layer; NT, no-tillage.

Different capital letters indicate significant differences ($P < 0.05$), within the same crop system, among tillage managements. Different lowercase letters indicate significant differences ($P < 0.05$), within the same tillage management, between crop systems.

TOC, total organic C; TN, total nitrogen; C extr, extractable C in 0.5 M K₂SO₄.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

However, as outlined before, in C input root exudates are not included and it is well known that they may supply at least an equal or greater C amount than that supplied together by hypogeous and epigeal biomasses C. For example, the calculated amount of C input for wheat may range from 0.48 to 3.2 Mg C ha⁻¹ y⁻¹ (Kuzyakov and Schneckenberger, 2004; Martin and Puckridge, 1982; Whipps, 1990).

3.4. Biochemical soil properties

Soil microbial biomass C (MBC) and microbial quotient (MBC/TOC) were, at the same tillage management, higher in WB rotation (Table 3). Within crop systems, MBC and MBC/TOC were higher in NT plots and lower in CT ones. Crop system was the experimental factor with the highest proportion of the explained variance for biochemical properties (Table 3). Basal respiration (BR) ranged from 13.5 to 22.2 mg C kg⁻¹ d⁻¹ (Table 3). Within each crop system, BR increased by decreasing tillage intensity (NT > DL > CT), whereas at the same tillage management it showed higher values in WB plots. However, tillage and crop system affected BR at similar extent, since the explained variance percentage of BR was comparable. The microbial basal respiration rate per unit of microbial biomass carbon (specific respiration or metabolic quotient, qCO_2) ranged between 2.7 and 1.8 mg CO₂–C g⁻¹ MBC h⁻¹ (Table 3) and was inversely correlated with MBC/TOC ($R^2 = 0.894$; $P < 0.01$; $n = 12$). Within each crop system, the metabolic quotient showed higher values in CT plot while lower ones in NT plot. Regardless

of tillage management, the WB rotation showed lower qCO_2 values than WW monoculture. The decay rate constant (k) was higher in WB plots, ranging from 0.064 and 0.116 d⁻¹ (Table 3). Indeed, most variance of k was explained by the crop system. Moreover, within wheat monoculture, tillage did not affect the decay rate constant whereas, within WB rotation, it was inversely related to tillage intensity (CT < DL < NT).

4. Discussion

4.1. Total C pool

The effects of tillage and crop system after 19 years on C stored in the first 15 cm of soil were comparable and relatively small, with the sum of variance percentages explained by the two factors being less than 50% (Table 2). The slight effect of tillage management on C stock could be due to the high clay soil content (471 g kg⁻¹) that protected soil organic matter from decay, also when soils are subjected to intensive tillage managements. The highest amount of C stock in NT plots compared to tilled ones agrees with reviews that report no-tillage management promoting TOC storage, especially over long term experiments (Franzuebbers, 2005). As regards crop system, WW plots tended to have higher amount of C stored in the first 15 cm than WB soil. Although the C input was the same among plots (Table 2), the higher amount of C stored in WW plots could be ascribed to the quality of residues returned to soils. Indeed, wheat residues had the highest C to N ratio and ADF content compared to

Table 2

Soil C stock, input and output in response to different management practices. Percentages of explained variance for each variable by each experimental factor (crop system and tillage) and by their interaction are also reported.

Crop system	Tillage	C stock at 15 cm (Mg ha ⁻¹)	C input (Mg ha ⁻¹ y ⁻¹)	C output (Mg ha ⁻¹ y ⁻¹)	ADF input (Mg ha ⁻¹ y ⁻¹)
WW	CT	32.5 ABa	1.8 Aa	2.8 Aa	0.89 Aa
WW	DL	29.6 Ba	1.9 Aa	2.4 Bb	0.90 Aa
WW	NT	35.2 Aa	1.9 Aa	2.0 Ca	0.90 Aa
WB	CT	28.9 Bb	1.8 Aa	3.1 Aa	0.84 Ab
WB	DL	29.5 Ba	1.9 Aa	3.1 Aa	0.87 Bb
WB	NT	32.5 Ab	1.9 Aa	2.3 Ba	0.87 Bb
Crop system		19**	NS	29***	18*
Tillage		26**	NS	42***	7*
Crop system × tillage		NS	NS	13*	NS

WW, durum wheat monoculture; WB, durum wheat and faba bean crop system; CT, conventional tillage; DL, dual layer; NT, no-tillage.

C input is the amount of C supplied by root and shoot biomasses (average of 19 years); C output is the amount of CO₂–C flux from soil surface (i.e. root respiration included) during a year (April 2008–April 2009).

Different capital letters indicate significant differences ($P < 0.05$), within the same crop system, among tillage managements. Different lowercase letters indicate significant differences ($P < 0.05$), within the same tillage management, between crop systems.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table 3
Biochemical properties of soil in response to different management practices. Percentages of variance explained for any variable by each experimental factor (crop system and tillage) and by their interaction are also reported.

Crop system	Tillage	MBC (mg kg ⁻¹)	BR (mg C kg ⁻¹ d ⁻¹)	MBC/TOC (%)	Metabolic quotient (mg CO ₂ -C g ⁻¹ MBC h ⁻¹)	k (d ⁻¹)
WW	CT	208.3 Ab	13.5 Ab	1.00 Ab	2.7 Aa	0.064 Bb
WW	DL	272.6 Bb	15.8 Bb	1.49 Bb	2.4 Ba	0.068 Bb
WW	NT	329.5 Cb	18.7 Cb	1.55 Bb	2.4 Ba	0.071 Bb
WB	CT	293.3 Aa	17.0 Aa	1.59 Aa	2.4 Aa	0.098 Aa
WB	DL	391.1 Ba	18.8 Ba	2.14 Ba	2.0 Bb	0.107 Ba
WB	NT	509.2 Ca	22.2 Ca	2.61 Ca	1.8 Bb	0.116 Ca
Crop system		48***	48***	55***	51***	91***
Tillage		27**	40***	20**	24***	3*
Crop system × tillage		10*	NS	NS	NS	3*

WW, durum wheat monoculture; WB, durum wheat and faba bean rotation; CT, conventional tillage; DL, dual layer; NT, no-tillage.

Different capital letters indicate significant differences ($P < 0.05$), within the same crop system, among tillage managements. Different lowercase letters indicate significant differences ($P < 0.05$), within the same tillage management, between crop systems.

MBC, microbial biomass C; BR, basal respiration; TOC, total organic C; k , decay rate constant.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

bean residues. These results agree with those of Guo et al. (2009) and confirmed that the quality of residue inputs, which generally is inversely related to the residue C/N ratio, can strongly enhance CO₂ emissions (Al-Kaisi and Yin, 2005) by speeding up the decomposition rate of faba residues by microbial biomass since it has a C/N ratio closer to that of faba than wheat residues (Kuo et al., 1997; Sainju et al., 2002). In other words, with faba a higher N availability (and immobilisation) by microbial biomass is concomitant to a higher C immobilisation, which is functionally preceded by the faba

C mineralisation (CO₂ evolution). Thus, in WW monoculture crop residues result less mineralised, i.e. more stable, against decomposition than in WB rotation, where faba residues occur at alternate years.

4.2. Labile C pools and C use efficiency

No-tillage after 19 years increased the labile C pools, i.e. microbial biomass C (MBC), extractable C and basal respiration. These findings agree with those of Balota et al. (2004), Gonzalez-Chavez et al. (2010) and Laudicina et al. (2011) that reported increases of the same pools in soils subjected to minimum or no-tillage managements. Indeed, the higher values of extractable C in NT soil is probably due to the lowest accessibility of the soil organic matter to decomposer organisms (Dungait et al., 2012). Hence, being less accessible to soil microorganisms, labile C may accumulate in soil. The larger microbial biomass in no-tilled or minimally tilled soils, compared to soils conventionally tilled, could be ascribed to the fact that surface litter can lower soil temperature while increasing soil aggregation and water and carbon contents (Doran, 1980). Thus, no-tilled soils are not only high in available substrates but also wetter, cooler and less variable with respect to their temperature and moisture regimes. These conditions stimulate the proliferation of soil microorganisms, particularly in surface layers (Balota et al., 2004; Ferreira et al., 2000). Moreover, the highest basal respiration in NT soil likely derives from the combined effects of the increased other pools, i.e. extractable C and MBC, functionally linked to it. Finally, while conventional tillage from one side may increase the accessibility of organic substrates to microbial biomass, from the other may mechanically kill it and/or make worse its habitat (Laudicina et al., 2011).

Comparing crop system effect on C mineralisation, we found WB rotation having more potential in enhancing CO₂ emissions than WW monoculture and this, again, agrees with its higher quality (lower both C/N ratio and ADF content) of faba residues compared to wheat residues.

Metabolic ($q\text{CO}_2$) and microbial quotients (MBC/TOC) are inter-dependent but inversely related indexes that, respectively, reflect the efficiency of the C use and the proportion of the total organic C present as microbial C (Badaluco et al., 2010). The trend of these two quotients suggested a more efficient C use and a high available C for soil microorganisms in WB rotation and with reduced or no-tillage. These quotients have been also used as stress indicators for soil microbial biomass (Anderson and Domsch, 1989; Laudicina et al., 2012). Our findings suggest that both WB rotation and reduced tillage induced a minor stress of soil microbial biomass compared to conventional wheat in monoculture. Anderson and

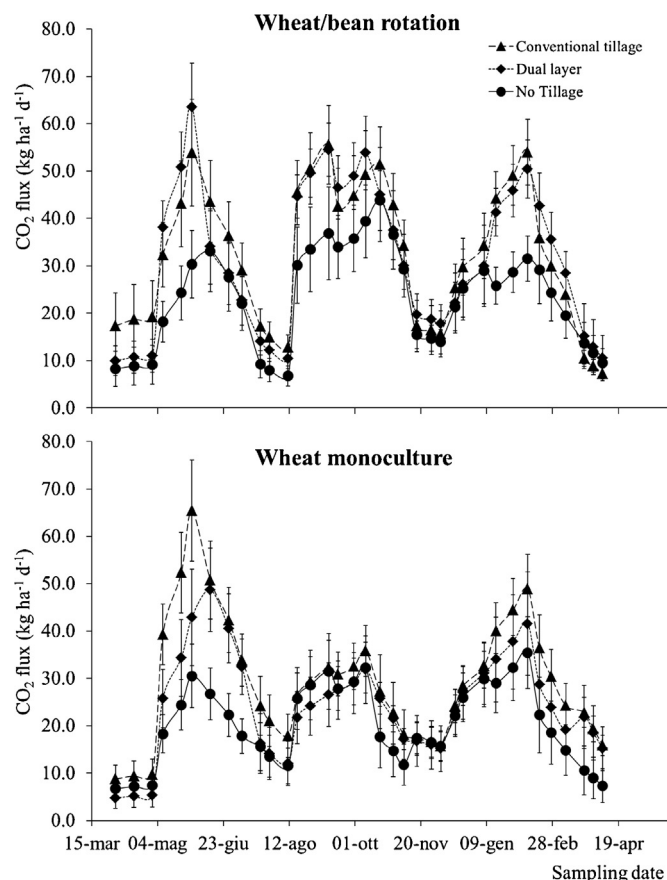


Fig. 2. Soil CO₂ fluxes (kg ha⁻¹ d⁻¹) during the trial period. Error bars are standard deviations ($N = 24$; 12 consecutive measurements × 2 plots). Soil CO₂ fluxes were significantly affected by sampling date ($P < 0.001$). Crop system, tillage and their interaction explained 29 ($P < 0.001$), 42 ($P < 0.001$) and 13% ($P < 0.01$) of soil CO₂ flux variance, respectively (two-way ANOVA, see Section 2 for details).

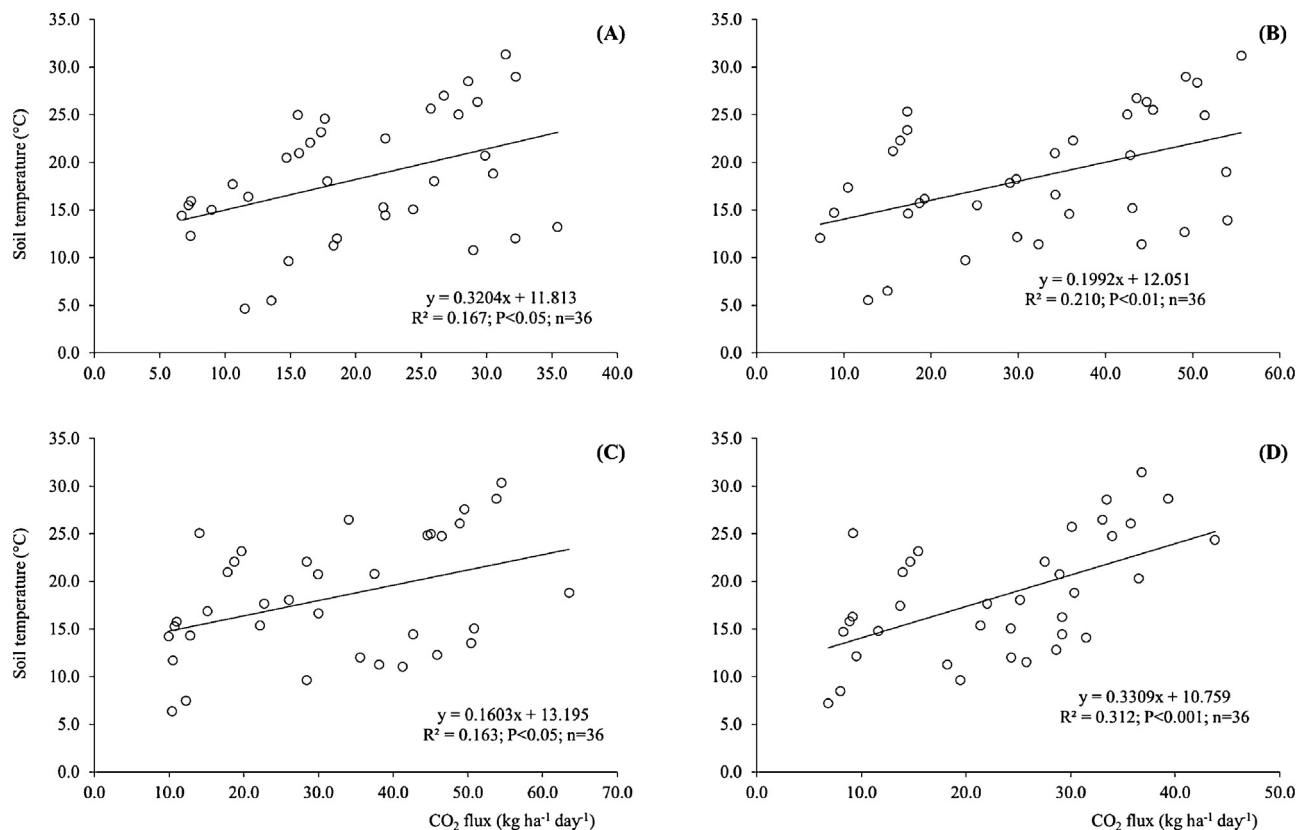


Fig. 3. Linear relationships between average daily CO₂ fluxes and soil temperatures measured at 15 cm depth in wheat monoculture plot under no-tillage (A), and in wheat/bean rotation plots under conventional tillage (B), dual layer (C) and no-tillage (D).

Domsch (1989) reported that agrosystems including leguminous crops encourage higher MBC and MBC/TOC but lower qCO_2 than monoculture systems. This difference is attributed to the high input of organic residues in both variety and quantity (rhizosphere effect) under wheat–faba rotation (Anderson and Domsch, 1989; Hungria and Stacey, 1997; Ferreira et al., 2000). Indeed, legume roots may release into the rhizosphere flavonoids facilitating both rhizobial (N fixation) and mycorrhizal symbioses (Cesco et al., 2012).

4.3. C output

Many biotic factors, such as root and microbial respiration (Kuzakov, 2006), and soil organic matter content (Frank et al., 2006), mainly contribute to soil CO₂ flux. As, in turn, biotic factors are strongly dependent on abiotic factors, such as soil temperature and moisture, significant relationships among soil CO₂ flux and those abiotic factors would have been expected, but in our work this was not the case, especially for soil moisture (WFPS). However, time effect on soil CO₂ flux was consistent with previous research showing strong seasonal variation in soil respiration (Lee et al., 2009). The dependence of CO₂ fluxes on sampling time could be explained by changes in soil temperature, although a univocal trend did not emerge and in two plots no significant correlations was found between soil CO₂ flux and temperature. The lack of significant correlation between soil CO₂ flux and temperature in two treatments is not surprising. It is well known that soil temperature may be a key abiotic factors driving soil CO₂ flux (Akinremi et al., 1999; Qi and Xu, 2001), also in extreme environments (Ball et al., 2009; Parsons et al., 2004; Xie et al., 2009). However, as reported in other studies, temperature is not always correlated with soil CO₂ flux (Werner et al., 2006; Salimon et al., 2004). Such contrasting results might be ascribed to the interaction between soil

temperature and moisture causing a reduction or cancelling their single effects. For example, the amount of CO₂ dissolved in soil solution depends on soil moisture content and, at the same moisture content, the amount of soil CO₂ dissolved in the soil solution may change depending on soil temperature because the CO₂ solubility in water is inversely related to temperature (Wilhelm et al., 1977). Therefore, the widest ranges in both soil temperature and moisture reached during the year of field observations, typical of the Mediterranean climate, could have confounded the main role of biotic factors to CO₂ fluxes.

Tillage was the experimental factor affecting CO₂ flux at the greatest degree. This increase in CO₂ flux likely resulted from the increased accessibility of soil organic matter to microbial decomposition. Tillage may promote the microbial oxidation of TOC basically for two reasons: (1) it helps the fragmentation of macro-aggregates and increases the surface area for soil microbes to attack and decompose the originally physically aggregate-protected soil organic matter (Beare et al., 1994; Six et al., 1999); (2) the incorporation of crop residues into soil by tillage leads to more favourable physical conditions (e.g. moisture and temperature) for their decomposition than on the soil surface (Aulakh et al., 1991; Coppens et al., 2007), thus providing nutrients and energy for microbial growth and further enhancing TOC decomposition, more inert C included (Fontaine et al., 2007).

5. Conclusions

In a semiarid Mediterranean environment, tillage management more than cropping system affected the soil organic C stored in the first 15 cm of soil. After 19 years, no-tillage caused a 3.6 Mg ha⁻¹ increase of C content in wheat/faba rotation while of 5.6 Mg ha⁻¹ in wheat monoculture.

The higher increase of soil C stored in wheat monoculture compared to wheat/faba rotation can be ascribed to a lower residues quality, i.e. lower C/N ratio and higher ADF content of wheat supplied biomasses compared to faba ones. The lower substrate quality reduced organic matter mineralisation so carrying to a greater C accumulation in wheat monoculture. In the long-term period (19 years), cropping system more than tillage management affected most soil biochemical properties such as labile C pools. Finally, the wheat/bean rotation had beneficial effects on soil biological activity parameters and on C use efficiency.

Acknowledgements

This work was funded by the Italian Ministry of Education, Universities and Research (MIUR) through the 2-year programme “Bilancio del carbonio negli agroecosistemi. Determinazione dei ritmi e limiti di saturazione del carbonio del suolo”, which started in 2007, and by the University of Palermo (Italy).

References

- Akinremi, O.O., McGinn, S.M., McLean, H.D.J., 1999. Effects of soil temperature and moisture on soil respiration in barley and fallow plots. *Can. J. Soil Sci.* 79, 5–13.
- Al-Kaisi, M.M., Yin, X.H., 2005. Tillage and crop residue effects on soil carbon and carbon dioxide emission in corn-soybean rotations. *J. Environ. Qual.* 34, 437–445.
- Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for measurement of microbial biomass in soils. *Soil Biol. Biochem.* 10, 215–221.
- Anderson, J.P.E., Domsch, K.H., 1989. Ratios of microbial biomass carbon to total carbon in arable soils. *Soil Biol. Biochem.* 21, 471–479.
- Anderson, T.H., Domsch, K.H., 1993. The metabolic quotient for CO₂ (q_{CO_2}) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. *Soil Biol. Biochem.* 25, 393–395.
- Aulakh, M.S., Doran, J.W., Walters, D.T., Mosier, A.R., Francis, D.D., 1991. Crop residue type and placement effects on denitrification and mineralization. *Soil Sci. Soc. Am. J.* 55, 1020–1025.
- Badalucco, L., Rao, M., Colombo, C., Palumbo, G., Laudicina, V.A., Gianfreda, L., 2010. Reversing agriculture from intensive to sustainable improves soil quality in a semiarid South Italy soil. *Biol. Fertil. Soils* 46, 481–489.
- Ball, B.A., Virginia, R.A., Barrett, J.E., Parsons, A.N., Wall, D.H., 2009. Interactions between physical and biotic factors influence CO₂ flux in Antarctic dry valley soils. *Soil Biol. Biochem.* 41, 1510–1517.
- Balota, E.L., Colozzi-Filho, A., Andrade, D.S., Dick, R.P., 2004. Long-term tillage and crop rotation effects on microbial biomass and C and N mineralization in a Brazilian Oxisol. *Soil Tillage Res.* 77, 137–145.
- Beare, M.H., Hendrix, P.F., Coleman, D.C., 1994. Water-stable aggregates and organic-matter fractions in conventional-tillage and no-tillage soils. *Soil Sci. Soc. Am. J.* 58, 777–786.
- Blake, G.R., Hartge, K.H., 1986. Bulk density. In: Klute, A. (Ed.), *Methods of Soil Analysis, Part I. Physical and Mineralogical Methods*, second ed. ASA/SSSA, Madison, WI, pp. 363–375.
- Bremner, J.M., 1996. Nitrogen-Total. In: Sparks, D.L. (Ed.), *Methods of Soil Analysis, Part 3-Chemical Methods*. SSSA Book Series No 5. SSSA and ASA, Madison, WI, pp. 1085–1121.
- Cesco, S., Mimmo, T., Tonon, G., Tomasi, N., Pinton, R., Terzano, R., Neumann, G., Weisskopf, L., Renella, G., Landi, L., Nannipieri, P., 2012. Plant-borne flavonoids released into the rhizosphere: impact on soil bio-activities related to plant nutrition. A review. *Biol. Fertil. Soils* 48, 123–149.
- Chung, H., Grove, J.H., Six, J., 2008. Indications for soil carbon saturation in a temperate agroecosystem. *Soil Sci. Soc. Am. J.* 72, 1132–1139.
- Cole, C.V., Flach, K., Lee, J., Sauerbeck, D., Stewart, B., 1993. Agricultural sources and sinks of carbon. *Water Air Soil Pollut.* 70, 111–122.
- Coppens, F., Garnier, P., Findeling, A., Merckx, R., Recous, S., 2007. Decomposition of mulched versus incorporated crop residues: modelling with PASTIS clarifies interactions between residue quality and location. *Soil Biol. Biochem.* 39, 2339–2350.
- Curtin, D., Wang, H., Selles, F., McConkey, B.G., Campbell, C.A., 2000. Tillage effects on carbon fluxes in continuous wheat and fallow-wheat rotations. *Soil Sci. Soc. Am. J.* 64, 2080–2086.
- Doran, J.W., 1980. Soil microbial and biochemical changes associated with reduced tillage. *Soil Sci. Soc. Am. J.* 44, 764–771.
- Dubeux, J.C.B., Sollenberger, L.E., Interrante, S.M., Vendramini, J.M.B., Stewart, R.L., 2006. Litter decomposition and mineralization in bahiagrass pastures managed at different intensities. *Crop Sci.* 46, 1305–1310.
- Duiker, S.W., Lal, R., 1999. Crop residue and tillage effects on carbon sequestration in a Luvisol in central Ohio. *Soil Tillage Res.* 52, 73–81.
- Dungait, J.A.J., Hopkins, D.W., Gregory, A.S., Whitmore, A.P., 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. *Global Change Biol.* 18, 1781–1796.
- Ellert, B.H., Janzen, H.H., 1999. Short-term influence of tillage on CO₂ fluxes from a semi-arid soil on the Canadian Prairies. *Soil Tillage Res.* 50, 21–32.
- Ferreira, M.C., Andrade, D.S., Chueire, L.M.O., Takemura, S.M., Hungria, M., 2000. Effects of tillage method and crop rotation on the population sizes and diversity of bradyrhizobia nodulating soybean. *Soil Biol. Biochem.* 32, 627–637.
- Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature* 450, 277–280.
- Frank, A.B., Liebig, M.A., Tanaka, D.L., 2006. Management effects on soil CO₂ efflux in northern semiarid grassland and cropland. *Soil Tillage Res.* 89, 78–85.
- Franzleubbers, A.J., 2005. Soil organic carbon sequestration and agricultural greenhouse gas emissions in the southeastern USA. *Soil Tillage Res.* 83, 120–147.
- Gan, Y.T., Campbell, C.A., Janzen, H.H., Lemke, R.L., Basnyat, P., McDonald, C.L., 2009. Carbon input to soil from oilseed and pulse crops on the Canadian prairies. *Agric. Ecosyst. Environ.* 132, 290–297.
- Giambalvo, D., Ruisi, P., Saia, S., Di Miceli, G., Frenda, A.S., Amato, G., 2012. Faba bean N₂ fixation, and weed infestation in a long-term tillage experiment under rainfed Mediterranean conditions. *Plant Soil* 360, 215–227.
- Gonzalez-Chavez, M.A., Aitkenhead-Peterson, J.A., Gentry, T.J., Zuberer, D., Hons, F., Loeppert, R., 2010. Soil microbial community, C/N, and P responses to long-term tillage and crop rotation. *Soil Tillage Res.* 106, 285–293.
- Guo, Z.L., Cai, C.F., Li, Z.X., Wang, T.W., Zheng, M.J., 2009. Crop residue effect on crop performance, soil N₂O and CO₂ emissions in alley cropping systems in subtropical China. *Agrofor. Syst.* 76, 67–80.
- Hopkins, D.W., Waite, I.S., McNicol, J.W., Poulton, P.R., Macdonald, A.J., O'Donnell, A.G., 2009. Soil organic carbon contents in long-term experimental grassland plots in the UK (Palace Leas and Park Grass) have not changed consistently in recent decades. *Global Change Biol.* 15, 1739–1754.
- Hopkins, D.W., Waite, I.S., O'Donnell, A.G., 2011. Microbial biomass, organic matter mineralization and nitrogen in soils from long-term experimental grassland plots (Palace Leas meadow hay plot, UK). *Eur. J. Soil Sci.* 62, 95–104.
- Hungria, M., Stacey, G., 1997. Molecular signals exchanged between host plants and rhizobia: basic aspects and potential application in agriculture. *Soil Biol. Biochem.* 29, 819–830.
- IPCC, 2006. Guidelines for national greenhouse gas inventories, volume 4: agriculture, forestry and other land use. In: N₂O Emissions from Managed Soils, and CO₂ Emissions from Lime and Urea Application. <http://www.ipccnggip.iges.or.jp/public/2006gl/vol4.html> (accessed 07 November 2012), Chapter 11.
- Jensen, E.S., Peoples, M.B., Hauggaard-Nielsen, H., 2010. Faba bean in cropping systems. *Field Crop Res.* 11, 203–216.
- Kong, A.Y.Y., Six, J., Bryant, D.C., Denison, R.F., van Kessel, C., 2005. The relationship between carbon input, aggregation, and soil organic carbon stabilization in sustainable cropping systems. *Soil Sci. Soc. Am. J.* 69, 1078–1085.
- Kuo, S., Sainju, U.M., Jellum, E.J., 1997. Winter cover crop effects on soil organic carbon and carbohydrate in soil. *Soil Sci. Soc. Am. J.* 61, 145–152.
- Kuzyakov, Y., 2006. Sources of CO₂ efflux from soil and review of partitioning methods. *Soil Biol. Biochem.* 38, 425–448.
- Kuzyakov, Y., Schneckenberger, K., 2004. Review of estimation of plant rhizodepositions and their contribution to soil organic matter formation. *Arch. Agron. Soil Sci.* 50, 115–132.
- Laudicina, V.A., Badalucco, L., Palazzolo, E., 2011. Effects of compost input and tillage intensity on soil microbial biomass and activity under Mediterranean conditions. *Biol. Fertil. Soils* 4, 63–70.
- Laudicina, V.A., Barbera, V., Cristina, L., Badalucco, L., 2012. Management practices to preserve soil organic matter in semiarid Mediterranean environments. In: Björklund, P.A., Mello, F.V. (Eds.), *Soil Organic Matter: Ecology, Environmental Impact and Management*. Nova Science Publishers, New York, pp. 39–61.
- Lee, J., Hopkins, J.W., van Kessel, C., King, A.P., Evatt, K.J., Louie, D., Rolston, D.E., Six, J., 2009. Tillage and seasonal emissions of CO₂, N₂O and NO across a seed bed and at the field scale in a Mediterranean climate. *Agric. Ecosyst. Environ.* 129, 378–390.
- Luo, Z., Wang, E., Sun, O.J., 2010. Can no-tillage stimulate carbon sequestration in agricultural soils? A meta-analysis of paired experiments. *Agric. Ecosyst. Environ.* 139, 224–231.
- Martin, J.K., Puckridge, D.W., 1982. Carbon flow through the rhizosphere of wheat crops in South Australia. In: Galbally, I.E., Freney, J.R. (Eds.), *The Cycling of Carbon, Nitrogen, Sulphur and Phosphorus in Terrestrial and Aquatic Ecosystems*. Australian Academy of Science, Canberra, pp. 77–81.
- Melillo, J.M., Aber, J.D., Linkins, A.E., Ricca, A., Fry, B., Nadelhoffer, K.J., 1989. Carbon and nitrogen dynamics along the decay continuum—plant litter to soil organic-matter. *Plant Soil* 115, 189–198.
- Nannipieri, P., Ceccanti, B., Grego, S., 1990. Ecological significance of biological activity in soil. In: Bollag, J.M., Stotzy, G. (Eds.), *Soil Biochemistry*, 6. Marcel Dekker, New York, pp. 293–355.
- Parsons, A.N., Barrett, J.E., Wall, D.H., Virginia, R.A., 2004. Soil carbon dioxide flux in Antarctic dry valley ecosystems. *Ecosystems* 7, 286–295.
- Qi, Y., Xu, M., 2001. Separating the effects of moisture and temperature on soil CO₂ efflux in a coniferous forest in the Sierra Nevada mountains. *Plant Soil* 237, 15–23.
- Reicosky, D.C., Archer, D.W., 2007. Moldboard plow tillage depth and short-term carbon dioxide release. *Soil Tillage Res.* 94, 109–121.
- Roberts, W.P., Chan, K.Y., 1999. Tillage-induced increases in carbon dioxide loss from soil. *Soil Tillage Res.* 17, 143–151.

- Sainju, U.M., Singh, B.P., Yaffa, S., 2002. Soil organic matter and tomato yield following tillage, cover cropping, and nitrogen fertilization. *Agron. J.* 94, 594–602.
- Salimon, C.I., Davidson, E.A., Victoria, R.L., Melo, A.W.F., 2004. CO₂ flux from soil in pastures and forests in southwestern Amazonia. *Global Change Biol.* 10, 833–843.
- Six, J., Elliott, E.T., Paustian, K., 1999. Aggregate and soil organic matter dynamics under conventional and no-tillage systems. *Soil Sci. Soc. Am. J.* 63, 1350–1358.
- Stubbs, T.L., Kennedy, A.C., Reisenauer, P.E., Burns, J.W., 2009. Chemical composition of residue from cereal crops and cultivars in dryland ecosystems. *Agron. J.* 101, 538–545.
- Studdert, G.A., Echeverria, H.E., 2000. Crop systems and nitrogen fertilization to manage soil organic carbon dynamics. *Soil Sci. Soc. Am. J.* 64, 1496–1503.
- Sun, B., Hallett, P.D., Caul, S., Daniell, T.J., Hopkins, D.W., 2011. Distribution of soil carbon and microbial biomass in arable soils under different tillage regimes. *Plant Soil* 338, 17–25.
- Van Soest, P.J., 1963. Use of detergents in the analysis of fibrous feeds II. A rapid method for the determination of fiber and lignin. *J. Assoc. Off. Agric. Chem.* 46, 829–835.
- Werner, C., Zheng, X., Tang, J., Xie, B., Liu, C., Kiese, R., Butterbach-Bahl, K., 2006. N₂O, CH₄ and CO₂ emissions from seasonal tropical rainforests and a rubber plantation in Southwest China. *Plant Soil* 289, 335–353.
- Whipps, J.M., 1990. Carbon economy. In: Lynch, J.M. (Ed.), *The Rhizosphere*. Wiley, Chichester, pp. 59–97.
- Wilhelm, E., Battino, R., Wilcock, R.J., 1977. Low-pressure solubility of gases in liquid water. *Chem. Rev.* 77, 219–262.
- Xie, J.X., Li, Y., Zhai, C.X., Li, C.H., Lan, Z.D., 2009. CO₂ absorption by alkaline soils and its implication to the global carbon cycle. *Environ. Geol.* 56, 953–961.
- Yadav, R.S., Yadav, B.L., Chipa, B.R., 2008. Litter dynamics and soil properties under different tree species in a semi-arid region of Rajasthan, India. *Agrofor. Syst.* 73, 1–12.