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Turnover and availability of soil organic carbon under different Mediterranean land-uses as estimated by ^{13}C natural abundance

A. Novara[a,b], L. Gristina[a], Y. Kuzyakov[b], C. Schillaci[a], V. A. Laudicina[a] & T. La Mantia[a]

[a]*Dipartimento di Scienze Agrarie e Forestali, University of Palermo, Palermo, 90128, Italy , and* [b]*Soil Science of Temperate Ecosystems, Georg–August-University of Göttingen, Göttingen, 37077, Germany*

Summary

Soil organic matter (SOM) is an important factor in ecosystem stability and productivity. This is especially the case for Mediterranean soils suffering from the impact of human degradation as well as harsh climatic conditions. We used the carbon (C) exchange resulting from C_3-C_4 and C_4-C_3 vegetation change under field conditions combined with incubations under controlled conditions to evaluate the turnover and availability of soil organic C under different land-uses. The 40-year succession of *Hyparrenia hirta* L. (C_4 photosynthesis) after more than 85 years of olive tree (*Olea europaea* L.; C_3 photosynthesis) growth led to the exchange of 54% of soil organic C from C_3 to C_4 forms. In contrast, 21 years of vine (*Vitis vinifera* L.) growing after *H. hirta* decreased the organic C content to 57%. Considering this exchange and decrease as well as the periods after the land-use changes, we calculated the mean residence time (MRT) of soil C of different ages. The MRT of C under grassland dominated by *H. hirta* was about 19 years, but was 180 years under the vineyard. The rates of C accumulation under the *H. hirta* grassland were about 0.36 Mg C ha^{-1} year^{-1}. In contrast, the rates of C losses after conversion from natural grassland to a vineyard were 1.8 times greater and amounted to 0.65 Mg C ha^{-1} year^{-1}. We conclude that changes of land use from natural Mediterranean grassland to a vineyard lead to very large C losses that cannot be compensated for over the same periods.

Introduction

Soil organic matter (SOM) consists of various heterogeneous pools with different stability and turnover rates that play an important role in soil fertility and carbon (C) sequestration (Ussiri & Johnson, 2003). The level and composition of organic matter pools in soil is determined by the equilibrium between the factors affecting its formation and decomposition (Laudicina *et al*., 2011). Land-use and land-cover changes modify the turnover of C and the formation of SOM (Dinesh *et al*., 2003). Several studies have shown that plant species differ in their capacity to modify soil properties (Vinton & Burke, 1995; Cornelissen *et al*., 2004). Thus plant functional characteristics such as growth form, functional traits, biomass allocation, tissue chemistry and lifespan can affect significantly organic matter decomposition and nutrient dynamics in the soil (Carrera *et al*., 2009). The direction of changes, loss or gain of soil C stocks after land-use change, depends also on soil properties, climate and management (Novara *et al*., 2012). In order to assess and predict the effects of land-use change on C storage, quantitative knowledge of C stock magnitude and, especially, understanding of mechanisms of SOM stabilization for each specific environment are necessary.

Very few studies have focused on the effect of land-use change on turnover and availability of C in Mediterranean ecosystems (Gavrichkova *et al*., 2010). Mediterranean areas in recent centuries have been subjected to a substantial human impact, with intensive cultivation altering the structure and functions of soil, leading to erosion and degradation. Such strongly degraded lands have been removed from agricultural use because of reduced economic benefits and left for succession of (semi)natural vegetation developing to 'macchia' and 'garrigue' vegetation (Loumou & Giourga, 2003). Such semi-natural vegetation succession contributes to improvement of soil properties over the longer term and also changes the C stocks. Despite some studies on C sequestration after natural vegetation succession in abandoned land in other areas (Kurganova *et al*., 2010), Mediterranean areas are strongly under-represented.

To identify mechanisms controlling changes in C pools associated with land use and land cover, various physical

Correspondence: A. Novara. E-mail: agata.novara@unipa.it

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fractionation methods have been used (Jolivet *et al*., 2003). Physical fractionation studies of SOM have revealed that soil aggregation, reducing accessibility of microbial biomass to particulate organic matter, contributes to physical protection and thus stabilization of SOM (von Lützow *et al*., 2006). It was observed that a greater OM content and greater mineralization rates are usually associated with the macroaggregate fraction. On the other hand, the OM associated with microaggregates may be more protected physically and more recalcitrant biochemically (Dorodnikov *et al*., 2011). However, fractionation provides information on the net change in C only and not on the mineralization of organic matter originating from old vegetation and the incorporation of plant residue from the new land use (Shang & Tiessen, 2000). Alternatively, the turnover of SOM can be evaluated with approaches based on natural differences in $\delta^{13}C$ of plants with C_3 and C_4 photosynthesis (Schneckenberger & Kuzyakov, 2007). This isotopic approach permits calculation of organic C pools derived from old (in most studies, C_3) and new (in most studies, C_4) vegetation because the two types of vegetation differ in their $\delta^{13}C$ according to C_3 and C_4 photosynthetic pathways (Werth & Kuzyakov, 2008).

Our study highlighted the effects of land-use changes on SOC stocks and organic matter dynamics in Mediterranean ecosystems using natural differences in $\delta^{13}C$ of plants with C_3 and C_4 photosynthesis. In contrast to nearly all previous studies, we used land-use changes in both directions ($C_3 \rightarrow C_4$ and $C_4 \rightarrow C_3$). A unique research area with a history of vegetation change from a C_3-olive (*Olea europaea* L.) plantation to a C_4-*Hyparrenia hirta* L. grassland and from C_4-*H. hirta* grassland to a C_3-vineyard (*Vitis vinifera* L.) was chosen to follow changes in both directions. It is important to note that these vegetation changes occurred at the same location with identical climatic conditions and the same soil type. This is an important prerequisite for using $\delta^{13}C$ signatures to calculate mean residence time (MRT) of various SOM pools, this being the spatial variation of soil $\delta^{13}C$ in relation to C input and soil texture (Bai *et al*., 2012). *H. hirta* is widely distributed in the Mediterranean area (Botha & Russell, 1988), North Africa and the Middle East (Chejara *et al*., 2008) from sea level up to 600 m above sea level. It is a typical species of degraded areas after disturbance such as fire or intensive grazing with fast growth: this means that *H. hirta* grasslands are very suitable for studying turnover and stabilization of SOM in semiarid environments.

H. hirta produces much above- and below-ground biomass, especially under water limitation as is common in Mediterranean areas in summer. As is common for grasses, its contribution to below-ground C input is greater than that of herbs, shrubs and trees. Such accumulation and input of C into the soil under *H. hirta* is, furthermore, better stabilized during periods of microbial dormancy during summer. Moreover, natural grasses such as *H. hirta* strongly decrease and even stop erosion, and consequently contribute to maintenance of C stocks in the soil.

We hypothesize that such land-use changes in a typical Mediterranean environment affect C dynamics in soil and the natural recovery of soil fertility and functions. Our objectives were to (i) assess the change in SOC stocks when agricultural land-use is followed by native vegetation and *vice versa*, (ii) estimate SOM turnover during secondary succession in an agricultural abandoned land (C_3-C_4 soil) and under an agricultural crop (C_4-C_3 soil) and (iii) reflect on the importance of various soil organic fractions in the turnover and stabilization of SOM by common land uses in the Mediterranean.

Material and methods

Study and sampling area

The study was carried out on the south side of Inici Mountain, in Sicily, Italy (37°57′45°N, 12°51′34°E) (Figure 1). The area is semiarid (Thornthwaite & Mather, 1955) with a typical Mediterranean climate: most of the annual mean precipitation (409 mm) falls between October and February; monthly average temperatures range from 12°C (January) to 25° C (August). The underlying geology is the result of tectonic overlaying of various carbonate, carbonate-silicoclastic and terrigenous geological bodies, which are formed in the upper Triassic–middle Tortonian. The soil in the study area is classified as a Regosol (WRB, 2006) with 60% clay, 25% silt and 15% sand.

A secondary succession was selected on a south-facing gentle slope (2–5%) represented by an olive grove, H. *hirta* grassland and a vineyard (Figure 1). The area was completely covered by olives for at least 75 years until 1971, when a wildfire burnt half of the olive grove area. The burnt area was left to undergo secondary succession to H. *hirta* (C_4 photosynthesis) grassland and in 1990 half of the H. *hirta* grassland was converted to a vineyard. The dates of the fire event and the vineyard planting were determined using aerial photographs. Environmental factors such as geological substrate, soil texture, slope, soil type and aspect can be regarded as homogeneous for all the land covers in the succession. Three soil samples were collected at 0–15-cm depth for each stage of succession. To reduce the error tolerance to less than ± 5%, 24 kg of soil was collected per sample and mixed. The soil was air-dried and passed through a 2-mm sieve.

Soil analyses and aggregate-size fractionation

Wet aggregate-size fractions with no prior chemical dispersion, were isolated by mechanical shaking of 100 g, air-dried fine earth on a column with sieves of 1000, 250, 63 and 25 µm using a Shaker AS 200 Sieve (RETSCH analytical, Haan, Germany) (203-mm sieves, amplitude of 2 cm, frequency of 1.6 Hz and a water flux of 2 litres minute^{-1}). After the physical fractionation, we distinguished four main aggregate-size fractions: 1000–2000, 250–1000, 63–250 and < 63 µm.

For $\delta^{13}C$ analysis of CO_2-C, carbonates in NaOH were precipitated with 1 ml 5.5 M $CaCl_2$ (formation of a $CaCO_3$ precipitate) and immediately washed/centrifuged three times with degassed deionized water before being dried at 55°C for 24 hours.

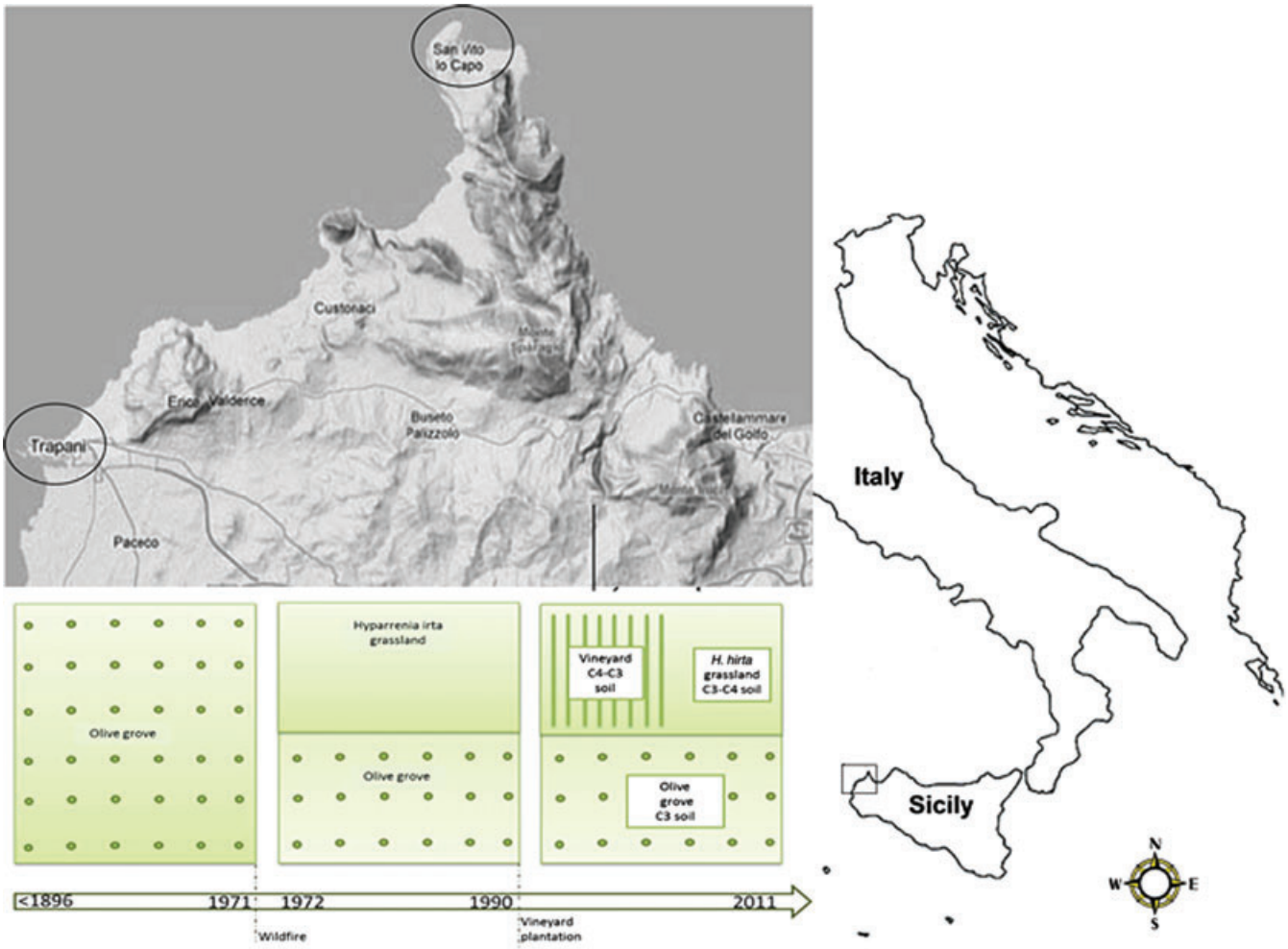

Figure 1 Schema of the experiment with three land-use types, secondary vegetation succession and periods of C_3 and C_4 residues input to study site in Sicily (IT).

For each aggregate fraction, the relative mass distribution, the C content and δ ^{13}C signature were measured. Soil OC content was measured using an elemental analyser (NA1500 Carlo Erba, Milan, Italy).

Soil C stock (Mg ha^{-1}) was calculated as:

$$C_{stock}\left(\text{Mg ha}^{-1}\right) = \text{BD} \times \text{Ccon x D} \times \text{CF}_{coarse}, \qquad (1)$$

where C_{con} is carbon content (%), BD is bulk density (Mg m^{-3}), D is depth thickness (m) and CF is a correction factor for stone content (1- [gravel % + stone %]/100). Bulk density was measured by using the volume of the collected sample and the mass of dry soil in the sample (Blake & Hartge, 1986).

Incubation procedure

In the three soil land-use types (an olive grove [C_3], H. *hirta* grassland [C_3-C_4] and a vineyard [C_4-C_3]) C mineralization dynamics was investigated on three sub-samples for each replicate (nine samples) by incubating 10 g soil at 50% WHC in 125-ml glass flasks at 25°C for 32 days. Soil respiration rate was determined seven times (over 32 days), measuring the CO_2 accumulated in the headspace of the flasks by a gas chromatograph equipped with a thermal conductivity detector (TCD). Twenty-four hours before the CO_2 sampling, all of the flasks were ventilated with fresh air and then sealed with rubber stoppers. Carbon dioxide sampling started 1 week after the start of the incubation to allow the soil to equilibrate after handling (Robertson *et al*., 1999). The amount of CO_2 evolved from each flask was calculated according to Zibilske (1994).

Stable carbon isotopic analysis and calculations of old and new C

The ^{13}C:^{12}C ratio of bulk soil, SOM pools and CO_2 released during incubation was measured using an EA-IRMS (elemental analyser isotope ratio mass spectrometer Carlo Erba Na 1500, model Isoprime (2006), Manchester, UK). The reference material

used for analysis was IA-R001 (Iso-Analytical Limited, Crewe, UK, wheat flour standard, $\delta^{13}C_{V\text{-}PDB} = -26.43‰$). IA-R001 is traceable to IAEA-CH-6 (cane sugar, $\delta^{13}C_{V\text{-}PDB} = -10.43‰$). IA-R001, IA-R005 (Iso-Analytical Limited beet sugar standard, $\delta^{13}C_{V\text{-}PDB} = -26.03‰$) and IA-R006 (Iso-Analytical Limited cane sugar standard, $\delta^{13}C_{V\text{-}PDB} = -11.64‰$) were used as quality control for the analysis. The C isotope results are expressed in delta (δ) notation and $\delta^{13}C$ values are reported in parts per thousand (‰) relative to the Vienna Pee Dee Belemnite (VPDB) standard.

Natural abundance of $\delta^{13}C$ was used to determine the proportion of C in SOM that was derived from the new crop and how much C remained from the previous crop in each soil aggregate size fraction. These proportions were calculated with the mixing equation (Gearing, 1991):

$$New\ carbon\ derived\ (Ncd) = \frac{\delta^{13}C_{new} - \delta^{13}C_{old}}{\delta^{13}C_{biomass\ new\ species} - \delta^{13}C_{old}} \quad (2)$$

and

$$Old\ \ Carbon\ \ derived\ (Ocd) = 1 - Ncd \quad (3)$$

where Ncd is the fraction of C derived from new vegetation (*H. hirta* and/or vines), $\delta^{13}C_{new}$ is the isotope ratio of the soil sample, $\delta^{13}C_{biomass\ new\ species}$ is the isotope ratio of the colonizing species ($-13.3 \pm 0.15‰$ for *H. hirta* and $28 \pm 0.09‰$ for vines) and $\delta^{13}C_{old}$ is the isotopic ratio of the previous vegetation type. The $\delta^{13}C$ values of soil under H. *hirta* are different from $\delta^{13}C$ of H. *hirta* biomass. The portion of old C derived under vines does not correspond to soil C_4-C amount. Under the vineyard, SOC contains residue of C_3-C from previous land use (olive grove). Therefore, we calculated the percentage of C_3-C and C_4-C of the old C derived using the proportions of C_3-C and C_4-C under the previous land-use.

Estimation of C turnover

The C turnover in the succession of natural vegetation was estimated with two approaches: (i) $\delta^{13}C$ isotopic signature shift in SOM after C_3-C_4 and C_4-C_3 vegetation change (Werth & Kuzyakov, 2008) and (ii) C mineralization during incubation experiments (Blagodatskaya *et al*., 2011).

For the first approach, the turnover of SOM (mean residence time in years, MRT) was determined as a reciprocal of the rate constant (k) of first order decay (Equation (4)) (Balesdent & Mariotti, 1996; Derrien & Amelung, 2011):

$$k = -\ln(1 - Ncd)\ /\text{years since disturbance.} \quad (4)$$

The MRT for C in soil under *H. hirta* and the vineyard was calculated as the weighted mean MRT of new and old SOM. The mass of new C added by plants to the soil was calculated both for bulk soil and for each aggregate-size fraction.

In the second approach, the amount of total C mineralized was calculated by the linear interpolation of two neighbouring measured rates and the numerical integration over time as reported in the following equation:

$$CO_2 - C = \sum_i^n \left[(r_i + r_{i+1}) \times \frac{d}{2}\right] + \ldots + \left[(r_{n-1} + r_n) \times \frac{d}{2}\right] \quad (5)$$

where i is the date of the first measurement of CO_2-C rate, n is the date of the last measurement of CO_2-C rate, r is the CO_2-C rate expressed as mg CO_2-C kg^{-1} dry soil and d is the number of days between the two consecutive CO_2 rate measurements (3, 4, 4, 7, 4, 7 and 7 days corresponding to the days after the incubation start of 3, 7, 11, 18, 22, 29 and 36, respectively). The C mineralization rate was expressed as mg C g^{-1} SOC day^{-1} and was fitted to the following first-order decay function:

$$\text{Mineralized C} = C_r e^{-kt} \quad (6)$$

where C_r is the readily mineralizable C at time zero (the intercept value), k is the decay rate constant and t is time.

Calculations

Corrections for dilution by atmospheric CO_2 in the incubation jars were made with the following equation:

$$\delta^{13}CO_{2\ measured} = f + \delta^{13}CO_{2\ atm} + (1 - f) \times \delta^{13}CO_{2\ sample}, \quad (7)$$

such that:

$$\delta^{13}CO_{2\ sample} = \delta^{13}CO_{2\ measured} - f \times \delta^{13}CO_{2\ atm} \quad (8)$$

where f is the fraction of the sample value contributed by atmospheric CO_2, which is calculated using a background concentration of 450 $\mu l\,l^{-1}$ CO_2 in the incubation jars. $\delta^{13}CO_{2\ measured}$ is the measured isotopic ratio, $\delta^{13}CO_{2\ sample}$ is the undiluted isotopic ratio of microbial respiration, and the $\delta_{13}CO_{2atm}$ is the isotopic ratio of measured atmospheric (laboratory air) CO_2.

Fractionation was determined solving the following equation system for x:

$$[CO_2]_m \times \delta^{13}C_m = x \times [CO_2]_O \times \delta^{13}C_O + (1 - x) \times [CO_2]_H \times \delta^{13}C_H$$
$$[CO_2]_m = x \times [CO_2]_O + (1 - x) \times [CO_2]_H \quad (9)$$

where x is the olive soil's CO_2 production, $[CO_2]_m$ is the measured CO_2, $[CO_2]_O$ is the olive soil CO_2 and $[CO_2]_H$ represents the *H. hirta* soil CO_2. Values of -27.5, $-13.3‰$ and $-28.0‰$ were used as $\delta^{13}C_O$, $\delta^{13}C_H$ and $\delta^{13}C_V$, respectively. The equivalent procedure was carried out for the second succession step (*H. hirta* compared with vineyard).

Statistical analysis

The data for total soil C, new crop-derived C, C stock and content for each fraction were analysed by analysis of variance (ANOVA) after assessing variance homogeneity. Differences between the means were tested with the LSD test at $P < 0.05$. Standard errors for regression parameters were calculated. SAS statistical software was used (SAS Institute, 2001).

Results

SOC stocks in bulk soil and aggregate fractions

Carbon content during secondary succession increased by more than 39% after conversion from an olive grove to *H. hirta* grassland. In contrast, the conversion of natural grassland to a vineyard after 21 years resulted in an SOC depletion of 32%. Among the land uses, the largest SOC content was found under *H. hirta* (25 ± 3 Mg ha^{-1}), followed by the olive plantation (18 ± 5 Mg ha^{-1}) and vineyard (17 ± 3 Mg ha^{-1}), showing a strong decrease in C content in the bulk soil under cultivation.

The aggregate fraction < 63 µm was the most abundant because of a large clay content: this was followed in order in the 63–250, 250–1000 and 1000–2000-µm fractions (Figure 2). In all fractions, statistically significant differences were found between *H. hirta* grassland and agricultural land use (olives and vines), except for the 250–1000-µm fraction. Under all land uses, the mass contribution of each aggregate size fraction to the bulk soil increased with the decrease in aggregate sizes. However, no significant differences were found for the two biggest aggregate fractions (Figure 2).

In the olive grove and vineyard the largest C content was recorded in the 63–250 µm fraction (12.1 and 14.1 mg C g^{-1}, respectively) while the largest content under H. *hirta* was measured in the 1000–2000-µm fraction (15.2 mg C g^{-1}) (Figure 3). On average more than 50% of bulk SOC was contained in the smallest fraction (< 63 µm) because of the combined effects of the largest amount of fine aggregate fractions in the soils and its greater C content.

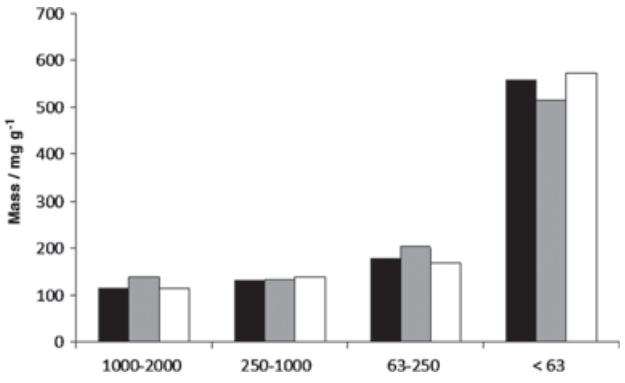

Figure 2 Mass of aggregate size fractions in soil under the olive grove (black columns), vineyard (white columns) and *H. hirta* grassland (grey columns).

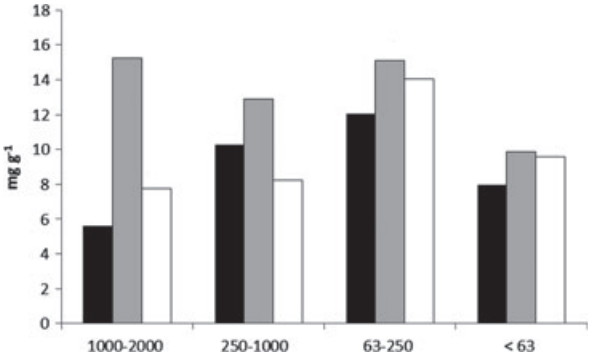

Figure 3 Carbon content in aggregate size fractions in soil under the olive grove (black columns), vineyard (white columns) and *H. hirta* grassland (grey columns).

$\delta^{13}C$ signature in bulk soil and aggregate size fractions

δ^{13}C values of bulk soil and aggregate size fractions were significantly ($P < 0.05$) affected by land use, and were largest in *H. hirta* grassland (C_3-C_4 soil), followed by the vineyard (C_4-C_3 soil) and the olive grove (C_3 soil) (Table 1 and 2). Under each land use, the differences among the fractions were small, except in the case of the smallest. The coarsest fraction had similar δ^{13}C values to that of plant residues, while in the smallest fraction the δ^{13}C value became less negative for cultivated C_3 soil and, in contrast, more negative in natural grassland. The average δ^{13}C values among the aggregate fractions were 0.73, 1.23 and 0.48‰ for the olive grove, *H. hirta* grassland and vineyard respectively.

The effect of land-use change (from C_3 to C_4 and from C_4 to C_3) on C stock was demonstrated by C derived from *H. hirta* and from vines (Table 1). After 40 years of *H. hirta* grassland, 80% of the C still originated from the olive grove, whereas in the vineyard only 6% of the C had a *H. hirta* origin after 21 years. The land-use change from olives to *H. hirta* led to an increase of 2.7% of C_4-C, but the stock of C derived from olives (C_3) was mostly preserved. In *H. hirta* grassland most of the C_3-C was contained in the smallest aggregates and therefore this portion was stable (Figure 4). The shift from *H. hirta* to vines led to the loss of 2.08 g kg^{-1} of C_3-C and 1.8 g kg^{-1} of C_4-C (Figure 4). Most of the C_3-C loss when vines followed *H. hirta* was recorded in the biggest aggregates.

Fluxes and sources of CO_2 by incubation

During 32 days of incubation, the largest CO_2 emission rate was recorded from the olive grove soil and ranged between 47 mg CO_2-C kg^{-1} day^{-1} (first day of incubation) and 14.7 mg CO_2-C kg^{-1} day^{-1} (after 32 days). This was followed by the soil under vines (from 37 to 13.05 mg CO_2-C kg^{-1} day^{-1}) and *H. hirta* (25.05 to 8.86 mg CO_2-C kg^{-1} day^{-1}) (Figure 5). The total C mineralized from soil over 1 month was greater under the vineyard and olive grove (592 ± 162 and 535 ± 82 mg kg^{-1}, respectively) than in the *H. hirta* soil (383 ± 27 mg kg^{-1}). The δ^{13}C of the CO_2 evolved during the incubation ranged between −26.5‰ and

Table 1 $\delta^{13}C$ (‰) and portion of C_3-C and C_4-C in bulk soil and aggregate size fractions

	$\delta^{13}C$			Carbon distribution			
	Olive grove	*H. hirta*	Vineyard	*H. hirta*		Vineyard	
	C_3 soil	C_3-C_4 soil	C_4-C_3 soil	C_3-C	C_4–C	C_4-C	C_3-C
Bulk soil	-27.14	-24.37	-26.81	0.20	0.80	0.064	0.936
1000–2000 µm	-27.14	-24.59	-26.81	0.18	0.82	0.063	0.937
250–1000 µm	-27.71	-24.58	-26.89	0.21	0.79	0.069	0.931
63–250 µm	-27.16	-23.97	-26.67	0.23	0.77	0.074	0.926
< 63 µm	-26.98	-25.20	-26.41	0.13	0.87	0.072	0.928

Table 2 Summary results for SOC (a) and δ13C from ANOVA (b)

(a) Source	Degrees freedom	Sum squares	Mean square	*F* ratio	*P*
Land use	2	1.8835	0.94176	4.02	0.02
Replicas	2	0.1520	0.07602	0.32	0.7
Aggregate fraction (residual)	428	2.0918	0.52295	2.23	0.09
(b) Source	Degrees freedom	Sum squares	Mean square	*F* ratio	*P*
Land use	2	69.8152	34.9076	72.96	0.00
Replicas	2	3.9760	1.9880	4.16	0.02
Aggregate fraction (residual)	428	0.1843	0.0460	0.10	0.04

−25.0‰ in the olive grove, from −26.0‰ to −23.5‰ in the vineyard soil and from −24.0‰ to −21.5‰ in the *H. hirta* soil (Figure 6). The $\delta^{13}C$ values of CO_2 from all soils were strongly depleted during the first week of incubation. The contribution of recent C_4 to CO_2 flux increased from 65% at the beginning to 91% at the last stage (32 days) of incubation in C_3-C_4 soil (Figure 7), while it ranged from 40 to 84% in C_4-C_3 soil. The ratio of C_3-C in evolved CO_2 to that in SOM was 0.37 for C_3-C_4 soil and 0.21 for C_4-C_3 soil (Figure 8).

Carbon turnover

Using the first approach, MRT of bulk soil was estimated as 19 and 183 years for *H. hirta* and olive grove SOM, respectively. The MRT increased with decreasing aggregate size under both land uses. In the fraction < 25 µm the MRT was 37 and 193 years, respectively, for the *H. hirta* and olive grove soils. The MRT calculated by the second approach, based on released CO_2, was 30, 17 and 22 days for *H. hirta* , olive and vineyard soils, respectively. These values reflect the MRT of easily available C in soil that was involved in decomposition within 1 month.

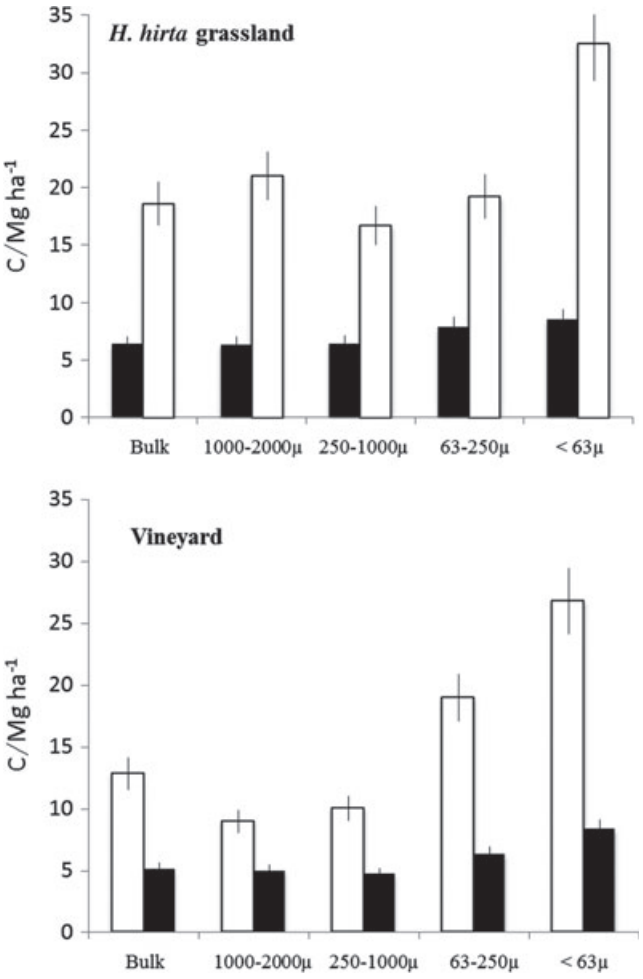

Figure 4 Content of C_3-C (white histograms) and C_4-C (black histogram) in bulk soil and for each particle size-fraction in *H. hirta* grassland and vineyard soils.

Discussion

Land-use change and C stocks

SOC content was very sensitive to land-use changes. Forty years after the abandonment of the olive grove and subsequent natural vegetation succession, the OC in bulk soil increased to 7 Mg C ha^{-1}. The opposite land-use change from natural vegetation to agriculture, resulted, after 21 years, in a decrease of 8 Mg C ha^{-1}. Our results confirm that agricultural use strongly modifies soil properties and especially C content (Yan *et al*., 2012): there is an increase in C content under natural vegetation and less mineralization than in agricultural soil (Novara *et al*.,

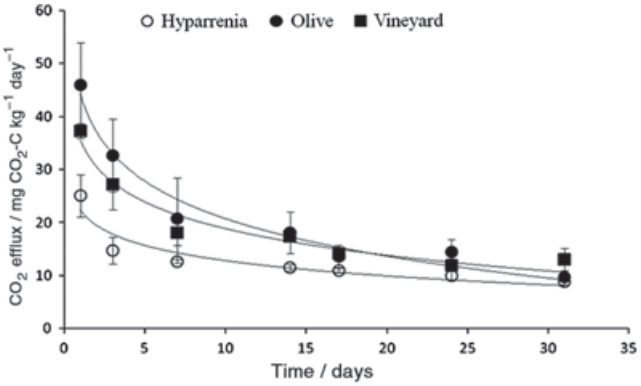

Figure 5 CO_2 efflux rates over 32 days of incubation for the *H. hirta* grassland, olive grove and vineyard.

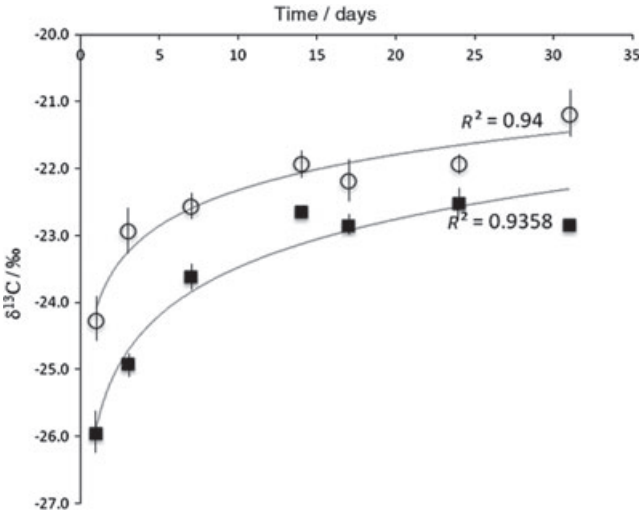

Figure 6 ^{13}C signature of the evolved CO_2-C during 32 days of incubation of C_3-C_4 (white dots) and C_4-C_3 (black dots) soils from the *H. hirta* grassland and vineyard, respectively.

2013). Soil management with continuous tillage of the vineyard and the olive grove in our study, contributed to the removal of spontaneous vegetation growth and more OM mineralization as a consequence of increased aeration, breaking aggregates (Barbera *et al*., 2012) and making more OM accessible to microbial activity (Solomon *et al*., 2002). As expected, the increase in C stock under natural vegetation was less than the C depletion after the vineyard plantation (Lal *et al*., 2004). The rate of C increase after abandonment of the olive grove was 0.36 Mg C ha^{-1} $year^{-1}$, while the C depletion rate was 0.65 Mg C ha^{-1} $year^{-1}$. These rates of C accumulation and losses in Mediterranean land use are faster than those described for temperate, boreal and steppe climates (Kurganova & de Gerenyu, 2008). The greater C depletion rate compared with the C increase results from intensive soil tillage in the vineyard that can also contribute to C losses by increasing erosion.

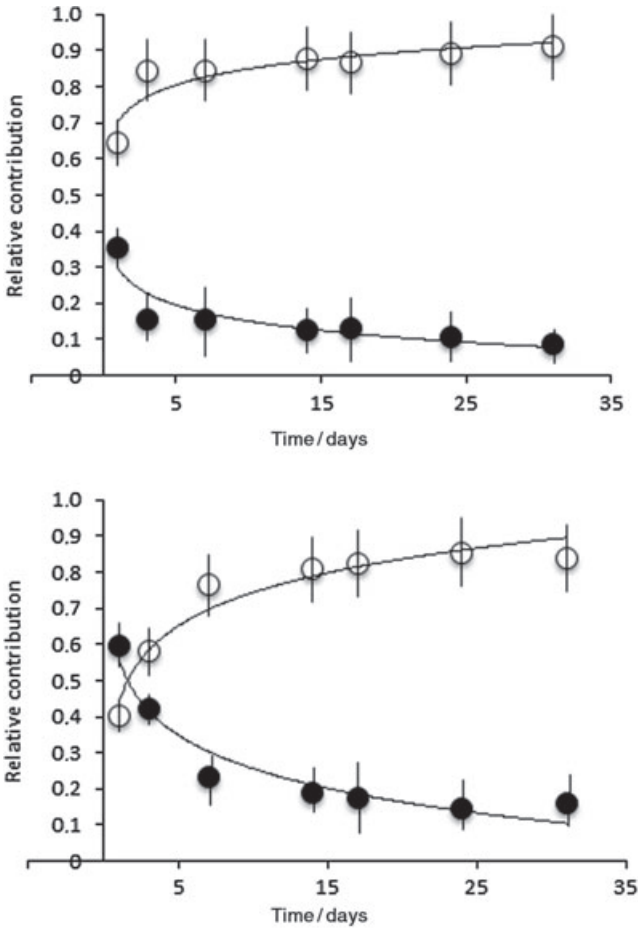

Figure 7 Relative contribution of C_4-C (white dot) and C_3-C (black dot) soil during 30 days of incubation of C_3-C_4 soil (a) and C_4-C_3 soil (b) from *H. hirta* grassland and vineyard, respectively.

Land use affected soil aggregate formation and destruction. In agricultural soil the aggregates are destroyed by cultivation (An *et al*., 2010), resulting in an increase in the amount of microaggregates. Soil under H. *hirta* had fewer microaggregates than the agricultural soil. This demonstrates that over 40 years, vegetation change and the consequent SOM increase strongly contribute to formation of macroaggregates. (Majumder *et al*., 2010). The distribution of SOC between aggregate size fractions in this Mediterranean soil was similar to the pattern observed previously in Sicilian clay soil (Barbera *et al*., 2010; Novara *et al*., 2012).

Land-use change from olives to H. *hirta* encouraged C stabilization in microaggregates and increased C content in the largest fraction through more litter production under *H. hirta* grassland and subsequent containment in macroagregates. The C stabilized in the smallest aggregates under *H. hirta* remained after 21 years of vineyard plantation, but C losses were noted in the largest aggregates. This is connected with the decreases in plant residues under vineyard management that are mainly allocated to the large aggregates (Dorodnikov *et al*., 2009).

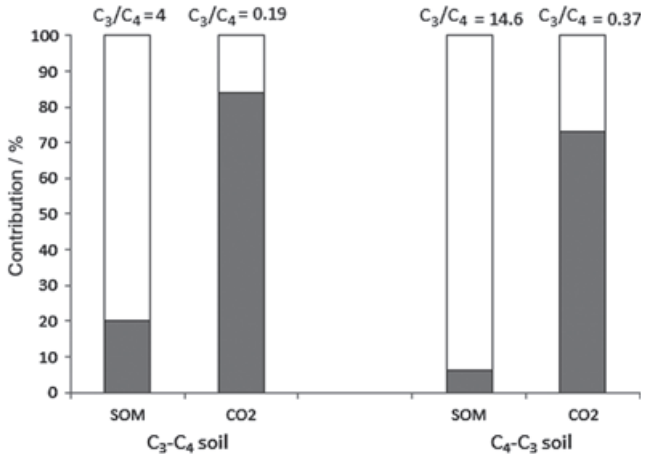

Figure 8 Comparison between the relative contribution of C_4-C (grey portion) and C_3-C (white portion) to SOM and CO_2 in C_3-C_4 soil (*H. hirta*) and C_4-C_3 soil (vineyard).

Stabilization of SOC in soil aggregate size fractions

Separation of SOC into aggregate size fractions in conjunction with the use of the natural ^{13}C abundance helped us to follow the mechanisms of SOM accumulation and turnover after olive grove-*H. hirta* grassland-vineyard succession. Before the fire (40 years ago), the OM input over a long period was solely of C_3 origin. Following the C_4 vegetation succession, bulk soil and all fractions were enriched in ^{13}C, showing the decay of C from olive origins and *H. hirta* C accumulation. After 40 years of natural vegetation development, 80% of C derived from the previous crop remained in the soil and most of this was in microaggregates ($< 63\,\mu m$) (Figure 4). This indicates that the smallest fraction contained more preserved OC and retained C derived from previous vegetation types for longer and more efficiently than the coarser fractions (Desjardins *et al*., 2006), through physicochemical stabilization. After the land-use change from *H. hirta* grassland to vineyard, most of the C losses were as C_3-C and more than 30% of this C_3-C was lost from the largest aggregates (Figure 4). These results clearly show (i) faster decomposition of new C_3-C compared with C_4-C derived from *H. hirta* and (ii) that there was not enough litter input to soil to restore the previous C content of the biggest aggregates.

CO_2 fluxes and their $\delta^{13}C$ signature during incubation

The ability of uncultivated soil to retain C was confirmed by CO_2 fluxes over 32 days of incubation. The cumulative CO_2 efflux in *H. hirta* soil was 32 and 40% less than that from the vineyard and olive grove soils, respectively (Figure 5). The $\delta^{13}C$ signature of evolved CO_2-C was depleted for all soils, but it was greater for C_3-C_4 and C_4-C_3 soil than for C_3 soil. Because the C_3 soil reached the isotopic steady-state after 85 years (Blagodatskaya *et al*., 2011), the difference in $\delta^{13}C$ between SOM and CO_2-C is related to ^{13}C fractionation or preferential use of ^{13}C-depleted substances such as lipids or lignin (Werth & Kuzyakov, 2010).

In the soils after the C_3-C_4 and C_4-C_3 vegetation change, not only ^{13}C fractionation but also preferential use of recent rather than old C contributed to $\delta^{13}C$ enrichment in CO_2. This means that less stabilized younger organic materials (having C_4 or C_3 signature for C_3-C_4 and C_4-C_3 soils, respectively) will be preferred for microbial utilization and consequently contribute strongly to CO_2 evolution.

The relative contribution of C_4-C to the evolved CO_2 was always more than 60% in C_3-C_4 soil, and increased during incubation as in other studies (Blagodatskaya *et al*., 2011). Although the relative contribution of C_4-C to SOC was less than the C_3-C contribution, this C_4 portion of organic matter in the *H. hirta* soil represents the new C, which was less stabilized and therefore more available for microbial use. The increase in the contribution of C_4-C during incubation can be related to microbial activity. It has been observed in short-term incubation studies that in the earlier stage of incubation the total microbial biomass decreased because of disturbance and the absence of new C inputs. After the initial period, the subsequent microbial population uses metabolites remaining after the first stage and, in this case, decomposition of microbial tissue led to an enrichment of the respired CO_2-C (Blagodatskaya *et al*., 2011).

The contribution of C_4-C to the total CO_2 efflux in C_4-C_3 soil was less than the C_3-C contribution in the first few days, but increased during the incubation period. However, the relative contribution of C_4-C was less in the C_3-C_4 soil than in the C_4-C_3 soil because of preferential use of new C (C_3-C). The ratio between C_3-C and C_4-C in CO_2 was, for both land uses, less than that in the SOM. The CO_2 emission had therefore originated mainly from H. *hirta* (Figure 8). The ratio between C_3-C in CO_2 and C_3-C in SOM was similar in the two soils, whilst that between C_4-C in CO_2 and C_4-C in SOM was greater in C_4-C_3 soil. The C of OM derived from H. *hirta* vegetation was therefore more degradable in C_4-C_3 soil.

Turnover of recent and old carbon

The C turnover in soil under *H. hirta* was about 10 times faster than in that under the olive grove. The reasons for this difference are: (i) the biomass of *H. hirta* is younger than that from olives, which was added to the soil more than four decades before, and therefore is more easily mineralizable; and (ii) the SOC in the olive grove was mainly stored in the smallest aggregates and was therefore more stable. According to Equation (6), the MRT of C decomposed to CO_2 within 1 month was 30 days for *H. hirta* soil and 17 days for olive grove soil. Results of MRT showed differences between the two estimation approaches: the MRT of *H. hirta* was less in the first approach and more in the second approach than that of the olive grove. The main difference is that the first approach, using $\delta^{13}C$ isotopic signature shift in SOM after C_3-C_4 vegetation change, estimates the MRT value only of C_4 organic matter, while the second approach estimated the MRT of C_3-C_4 soil under *H. hirta*. If only the portion C_4-C of total SOC in *H. hirta* is considered, the MRT

is approximately 6 days and therefore less than that for the olive grove soil.

Conclusion

This study highlighted the effect of land-use change on SOC stock and OM dynamics in a Mediterranean area using natural differences in $\delta^{13}C$ of plants with C_3 and C_4 photosynthesis. We demonstrated that (i) intensive agriculture (olives and vines) in the Mediterranean region has a negative impact on C sequestration when compared with semi-natural vegetation; (ii) vegetation change to *H. hirta* and consequent SOM increase strongly contribute to the formation of macroaggregates from microaggregates and (iii) turnover of SOM under *H. hirta* was quicker than under olives, but soils under natural vegetation emit less CO_2 and therefore are able to sequester more SOC. Consequently, we strongly recommend sustainable management practices to avoid SOM decrease and soil erosion. We suggest the application of organic fertilizers as well as promoting the growth of natural grasses in the winter period or burying pruning residues to contribute to C input and decrease erosion.

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References

An, S., Mentler, A., Meuer, H. & Blum, W.E.H. 2010. Soil aggregation, aggregate stability, organic carbon and nitrogen in different soil aggregate fractions under forest and shrub vegegation on the Loess Plateau, China. *Catena*, **81**, 226–233.

Barbera, V., Poma, I., Gristina, L., Novara, A. & Egli, M. 2012. Long-term cropping systems and tillage management effects on soil organic carbon stock and steady state level of C sequestration rates in a semiarid environment. *Land Degradation & Development*, **23**, 82–91.

Bai, E., Boutton, T.W., Liu, F., Wu, X.B., Hallmark, C.T. & Archer, S.R. 2012. Spatial variation of soil δ13C and its relation to carbon input and soil texture in a subtropical lowland woodland. *Soil Biology & Biochemistry*, **44,** 102–112.

Balesdent, J. & Mariotti, A. 1996. Measurement of soil organic matter turnover using 13 C natural abundance. In: Mass Spectrometry of Soils (eds T.W. Button & S. Yamasaki), pp. 83–111. Marcel Dekker, New York.

Barbera, V., Poma, I., Gristina, L., Novara, A. & Egli, M. 2012. Long-term cropping systems and tillage management effects on soil organic carbon stock and steady state level of C sequestration rates in a semiarid environment. *Land Degradation & Development*, **23,** 82–91.

Blagodatskaya, E., Yuyukina, T., Blagodatsky, S. & Kuzyakov, Y. 2011. Turnover of soil organic matter and microbial biomass under C3 – C4 vegetation change: consideration of 13C fractionation and preferential substrate utilization. *Soil Biology & Biochemistry*, **43,** 159–166.

Blake, G.R. & Hartge, K.H. 1986. Bulk density. In: *Methods of Soil Analysis*, *Part 1. Volume 9: Agronomy Monograph*, 2nd edn (ed A. Klute), pp. 363–375. American Society of Agronomy, Madison, WI.

Botha, C.E.J. & Russell, S. 1988. Comparison of photosynthetic temperature and light optima in selected C3 and C4 grasses from the E. Cape/Ciskei region. *South African Journal of Botany*, **54,** 133–136.

Carrera, A.L., Mazzarino, M.J., Bertiller, M.B., del Valle, H.F. & Carretero, E.M. 2009. Erratum to plant impacts on nitrogen and carbon cycling in the Monte Phytogeographical Province, Argentina. *Journal of Arid Environments*, **73,** 192–201.

Chejara, V.K., Kristiansen, P., Whalley, R.D.B., Sindel, B.M. & Nadolny, C. 2008. Factors affecting germination of Coolatai Grass (Hyparrhenia hirta). *Weed Science*, **56,** 543–548.

Cornelissen, G., Kukulska, Z., Kalaitzidis, S., Christanis, K. & Gustafsson, O. 2004. Relations between environmental black carbon sorption and geochemical sorbent characteristics. *Environmental Science & Technology*, **38,** 3632–3640.

Derrien, D. & Amelung, W. 2011. Computing the mean residence time of soil carbon fractions using stable isotopes: impacts of the model framework. *European Journal of Soil Science*, **62,** 237–252.

Desjardins, T., Folgarait, P.J., Pando-Bahuon, A., Girardin, C. & Lavelle, P. 2006. Soil organic matter dynamics along a rice chronosequence in north-eastern Argentina: evidence from natural 13C abundance and particle size fractionation. *Soil Biology & Biochemistry*, **38,** 2753–2761.

Dinesh, R., Chaudhuri, S.G., Ganeshamurthy, A.N. & Dey, C. 2003. Changes in soil microbial indices and their relationships following deforestation and cultivation in wet tropical forests. *Applied Soil Ecology*, **24,** 17–26.

Dorodnikov, M., Blagodatskaya, E., Blagodatsky, S., Marhan, S., Fangmeier, A. & Kuzyakov, Y. 2009. Stimulation of microbial extracellular enzyme activities by elevated CO_2 depends on aggregate size. *Global Change Biology*, **15,** 1603–1614.

Dorodnikov, M., Kuzyakov, Y., Fangmeier, A. & Wiesenberg, G.L.B. 2011. C and N in soil organic matter density fractions under elevated CO_2: turnover vs. stabilization. *Soil Biology & Biochemistry*, **43,** 579–589.

Gavrichkova, O., Moscatelli, M.C., Kuzyakov, Y., Grego, S. & Valentini, R. 2010. Defoliation decreases soil respiration and microbial activity in a Mediterranean grassland. *Agriculture, Ecosystems & Environment*, **136,** 87–96.

Gearing, J.N. 1991. The study of diet and trophic relationships through natural abundance ^{13}C. In: Carbon Isotope Techniques (eds D.C. Coleman & B. Fry), pp. 363–375, pp. 201–218. Academic Press, San Diego, CA.

Jolivet, L., Faccenna, C., Goffe, B., Burov, E. & Agard, P. 2003. Subduction tectonics and exhumation of high-pressure metamorphic rocks in the Mediterranean orogens. *American Journal of Science*, **303,** 353–409.

Kurganova, I.N. & de Gerenyu, V.O.L. 2008. Assessment and prediction of changes in the reserves of organic carbon in abandoned soils of European Russia in 1990–2020. *Eurasian Soil Science*, **41,** 1371–1377.

Kurganova, I.N., de Gerenyu, V.O.L., Shvidenko, A.Z. & Sapozhnikov, P.M. 2010. Changes in the organic carbon pool of abandoned soils in Russia (1990–2004). *Eurasian Soil Science*, **43,** 333–340.

Lal, R., Griffin, M., Apt, J., Lave, L. & Morgan, M.G. 2004. Managing soil carbon. *Ecology Science*, **304,** 393.

Laudicina, V.A., Badalucco, L. & Palazzolo, E. 2011. Effect of compost input and tillage intensity on soil microbial biomass and activity under Mediterranean conditions. *Biology & Fertility of Soils*, **47,** 63–70.

Loumou, A. & Giourga, C. 2003. Olive groves: "The life and identity of the Mediterranean". *Agriculture & Human Values*, **20,** 87–95.

Majumder, B., Ruehlmann, J. & Kuzyakov, Y. 2010. Effects of aggregation processes on distribution of aggregate size fractions and organic C content of a long-term fertilized soil. *European Journal of Soil Biology*, **46,** 365–370.

Novara, A., La Mantia, T., Barbera, V. & Gristina, L. 2012. Paired-site approach for studying soil organic carbon dynamics in a Mediterranean semiarid environment. *Catena*, **89,** 1–7. doi: 10.1016/J.Catena.2011.09.008.

Novara, A., Gristina, L., La Mantia, T. & Rhul, J. 2013. Carbon dynamics of soil organic matter in bulk soil and aggregate fraction during secondary succession in a Mediterranean environment. *Geoderma*, **193–194,** 213–221.

Robertson, G.P., Wedin, D., Groffman, P.M., Blair, J.M., Holland, E.A., Nadelhoffer, K.J., *et al*. 1999. Soil carbon and nitrogen availability. In: *Standard Soil Methods for Long-Term Ecological Research* (eds G.P. Robertson, D.C. Coleman, C.S. Bledsoe & P. Sollins), pp. 258–271. Oxford University Press, New York.

SAS Institute 2001. SAS/STAT, Release 8.01. SAS Institute, Cary, NC.

Schneckenberger, K. & Kuzyakov, Y. 2007. Carbon sequestration under Miscanthus in sandy and loamy soils estimated by natural 13C abundance. *Journal of Plant Nutrition & Soil Science*, **170,** 538–542.

Shang, C. & Tiessen, H. 2000. Carbon Turnover and Carbon-13 Natural Abundance in Organo-mineral Fractions of a Tropical Dry Forest Soil Under Cultivation. *Soil Science Society of America Journal*, **64**, 2149–2155.

Solomon, D., Fritzsche, F., Lehmann, J., Tekalign, M. & Zech, W. 2002. Soil organic matter dynamics in the subhumid agroecosystems of the Ethiopian highlands: evidence from natural C-13 abundance and particle-size fractionation. *Soil Science Society of America Journal*, **66,** 969–978.

Thornthwaite, C.W. & Mather, J.R. 1955. The Water Balance. *Publications in Climatology: Volume 8*. Laboratory for Climatology, Centerton, NJ.

Ussiri, D.A. & Johnson, C.E. 2003. Characterization of organic matter in a northern hardwood forest soil by 13C NMR and chemical methods. *Geoderma*, **111,** 123–149.

Vinton, M.A. & Burke, I.C. 1995. Interactions between individual plant species and soil nutrient status in short-grass-steppe. *Ecology*, **76,** 1116–1133.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., *et al*. 2006. Mechanisms for organic matter stabilization in temperate soils – a synthesis. *European Journal of Soil Science*, **57,** 426–445.

Werth, M. & Kuzyakov, Y. 2008. Determining root-derived carbon in soil respiration and microbial biomass using 14C and 13C. *Soil Biology & Biochemistry*, **40,** 625–637.

Werth, M. & Kuzyakov, Y. 2010. 13C fractionation at the root-microorganisms-soil interface: a review and outlook for partitioning studies. *Soil Biology & Biochemistry*, **42,** 1372–1384.

WRB 2006. World Reference Base for Soil Resources – A Framework for International Classification, Correlation and Communication. World Soil Resources Reports 103, International Union of Soil Sciences, ISRIC – World Soil Information and Food and Agriculture Organization of the United Nations, Rome.

Yan, Y., Tian, J., Fan, M., Zhang, F., Li, X., Christie, P., *et al*. 2012. Soil organic carbon and total nitrogen in intensively managed arable soils. *Agriculture, Ecosystems & Environment*, **159,** 102–110.

Zibilske, M.L. 1994. Carbon mineralization. In: *Methods of Soil Analysis. Part 2 – Microbiological and Biochemical Properties. Soil Science Society of America Book Series Number 5* (eds R.W. Weaver, S. Angle, P. Bottomley, D. Bezdicek, S. Smith, A. Tabatabai, *et al*.). Soil Science Society of America, Madison, WI. pp. 835–863.