

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

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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 C₃-C₄ and C₄-C₃ 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 *Hypparhenia hirta* L. (C₄ photosynthesis) after more than 85 years of olive tree (*Olea europaea* L.; C₃ photosynthesis) growth led to the exchange of 54% of soil organic C from C₃ to C₄ 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

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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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ of plants with C_3 and C_4 photosynthesis. In contrast to nearly all previous studies, we used land-use changes in both directions ($\text{C}_3 \rightarrow \text{C}_4$ and $\text{C}_4 \rightarrow \text{C}_3$). A unique research area with a history of vegetation change from a C_3 -olive (*Olea europaea* L.) plantation to a C_4 -*Hyparrhenia 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 $\delta^{13}\text{C}$ signatures to calculate mean residence time (MRT) of various SOM pools, this being the spatial variation of soil $\delta^{13}\text{C}$ 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 ($37^\circ 57' 45''\text{N}$, $12^\circ 51' 34''\text{E}$) (Figure 1). The area is semiarid (Thorntwaite & 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 $\pm 5\%$, 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 $25\ \mu\text{m}$ 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 $2\ \text{litres minute}^{-1}$). After the physical fractionation, we distinguished four main aggregate-size fractions: 1000–2000, 250–1000, 63–250 and $< 63\ \mu\text{m}$.

For $\delta^{13}\text{C}$ 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.

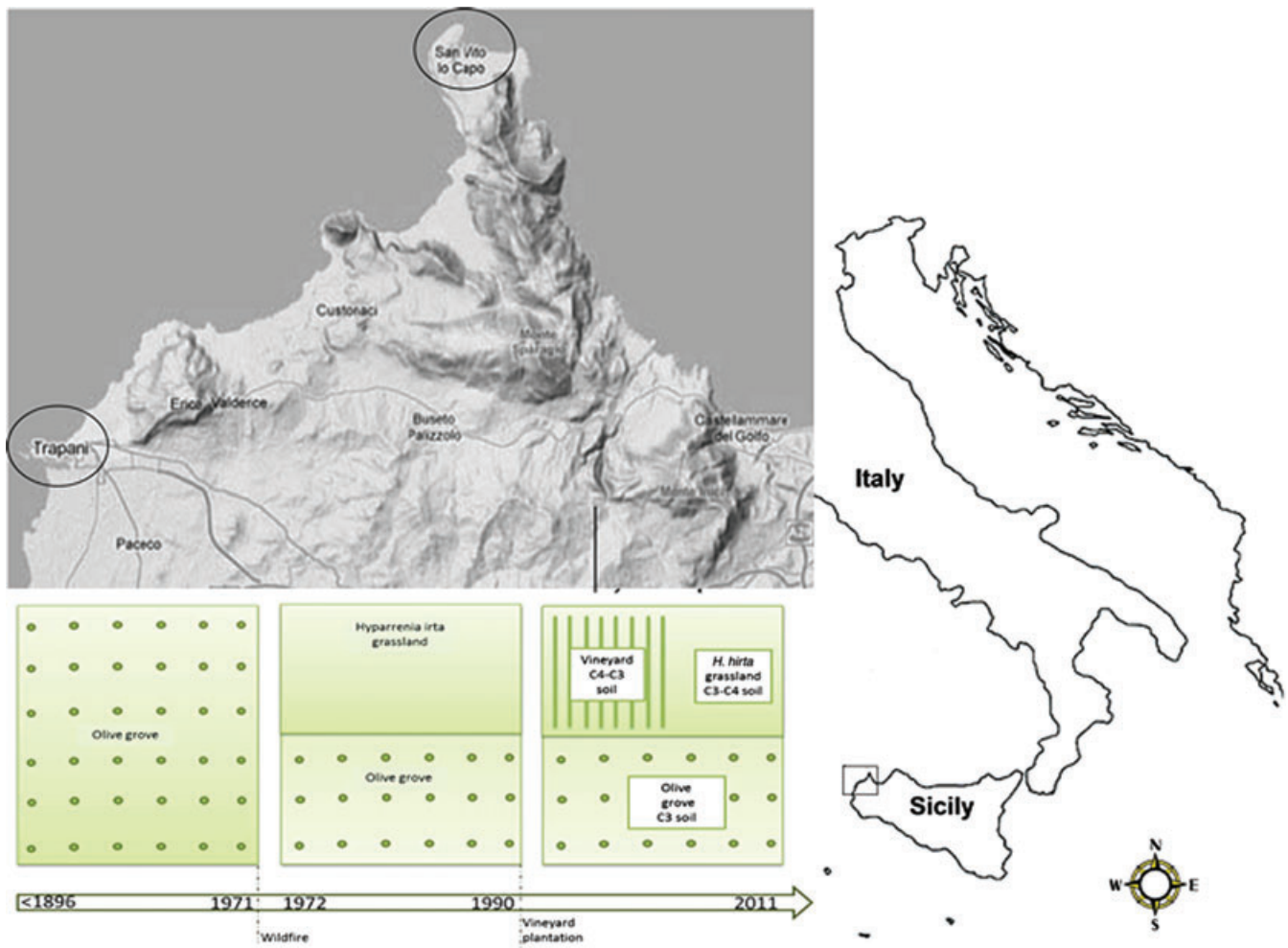


Figure 1 Schema of the experiment with three land-use types, secondary vegetation succession and periods of C₃ and C₄ residues input to study site in Sicily (IT).

For each aggregate fraction, the relative mass distribution, the C content and $\delta^{13}\text{C}$ 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:

$$C_{\text{stock}} (\text{Mg ha}^{-1}) = \text{BD} \times C_{\text{con}} \times D \times \text{CF}_{\text{coarse}}, \quad (1)$$

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 ($1 - [\text{gravel \%} + \text{stone \%}]/100$). 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 [C₃], *H. hirta* grassland [C₃-C₄] and a vineyard [C₄-C₃]) 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 CO₂ accumulated in the headspace of the flasks by a gas chromatograph equipped with a thermal conductivity detector (TCD). Twenty-four hours before the CO₂ 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 CO₂ evolved from each flask was calculated according to Zibilske (1994).

Stable carbon isotopic analysis and calculations of old and new C

The $^{13}\text{C}:^{12}\text{C}$ ratio of bulk soil, SOM pools and CO₂ 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, $\delta^{13}\text{C}_{\text{V-PDB}} = -26.43\text{‰}$). IA-R001 is traceable to IAEA-CH-6 (cane sugar, $\delta^{13}\text{C}_{\text{V-PDB}} = -10.43\text{‰}$). IA-R001, IA-R005 (Iso-Analytical Limited beet sugar standard, $\delta^{13}\text{C}_{\text{V-PDB}} = -26.03\text{‰}$) and IA-R006 (Iso-Analytical Limited cane sugar standard, $\delta^{13}\text{C}_{\text{V-PDB}} = -11.64\text{‰}$) were used as quality control for the analysis. The C isotope results are expressed in delta (δ) notation and $\delta^{13}\text{C}$ values are reported in parts per thousand (‰) relative to the Vienna Pee Dee Belemnite (VPDB) standard.

Natural abundance of $\delta^{13}\text{C}$ 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):

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

and

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

where Ncd is the fraction of C derived from new vegetation (*H. hirta* and/or vines), $\delta^{13}\text{C}_{\text{new}}$ is the isotope ratio of the soil sample, $\delta^{13}\text{C}_{\text{biomass new species}}$ is the isotope ratio of the colonizing species ($-13.3 \pm 0.15\text{‰}$ for *H. hirta* and $28 \pm 0.09\text{‰}$ for vines) and $\delta^{13}\text{C}_{\text{old}}$ is the isotopic ratio of the previous vegetation type. The $\delta^{13}\text{C}$ values of soil under *H. hirta* are different from $\delta^{13}\text{C}$ of *H. hirta* biomass. The portion of old C derived under vines does not correspond to soil C₄-C amount. Under the vineyard, SOC contains residue of C₃-C from previous land use (olive grove). Therefore, we calculated the percentage of C₃-C and C₄-C of the old C derived using the proportions of C₃-C and C₄-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) $\delta^{13}\text{C}$ isotopic signature shift in SOM after C₃-C₄ and C₄-C₃ 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):

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

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:

$$\text{CO}_2 - \text{C} = \sum_i^n \left[(r_i + r_{i+1}) \times \frac{d}{2} \right] + \dots + \left[(r_{n-1} + r_n) \times \frac{d}{2} \right] \quad (5)$$

where i is the date of the first measurement of CO₂-C rate, n is the date of the last measurement of CO₂-C rate, r is the CO₂-C rate expressed as mg CO₂-C kg⁻¹ dry soil and d is the number of days between the two consecutive CO₂ 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 CO₂ in the incubation jars were made with the following equation:

$$\delta^{13}\text{CO}_2 \text{ measured} = f + \delta^{13}\text{CO}_2 \text{ atm} + (1 - f) \times \delta^{13}\text{CO}_2 \text{ sample}, \quad (7)$$

such that:

$$\delta^{13}\text{CO}_2 \text{ sample} = \delta^{13}\text{CO}_2 \text{ measured} - f \times \delta^{13}\text{CO}_2 \text{ atm} \quad (8)$$

where f is the fraction of the sample value contributed by atmospheric CO₂, which is calculated using a background concentration of 450 $\mu\text{l l}^{-1}$ CO₂ in the incubation jars. $\delta^{13}\text{CO}_2 \text{ measured}$ is the measured isotopic ratio, $\delta^{13}\text{CO}_2 \text{ sample}$ is the undiluted isotopic ratio of microbial respiration, and the $\delta^{13}\text{CO}_2 \text{ atm}$ is the isotopic ratio of measured atmospheric (laboratory air) CO₂.

Fractionation was determined solving the following equation system for x :

$$\begin{aligned} [\text{CO}_2]_m \times \delta^{13}\text{C}_m &= x \times [\text{CO}_2]_O \times \delta^{13}\text{C}_O + (1 - x) \\ &\quad \times [\text{CO}_2]_H \times \delta^{13}\text{C}_H \\ [\text{CO}_2]_m &= x \times [\text{CO}_2]_O + (1 - x) \times [\text{CO}_2]_H \end{aligned} \quad (9)$$

where x is the olive soil's CO₂ production, $[\text{CO}_2]_m$ is the measured CO₂, $[\text{CO}_2]_O$ is the olive soil CO₂ and $[\text{CO}_2]_H$ represents the *H. hirta* soil CO₂. Values of -27.5 , -13.3‰ and -28.0‰ were used as $\delta^{13}\text{C}_O$, $\delta^{13}\text{C}_H$ and $\delta^{13}\text{C}_V$, 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* ($25 \pm 3 \text{ Mg ha}^{-1}$), followed by the olive plantation ($18 \pm 5 \text{ Mg ha}^{-1}$) and vineyard ($17 \pm 3 \text{ Mg ha}^{-1}$), showing a strong decrease in C content in the bulk soil under cultivation.

The aggregate fraction $< 63 \mu\text{m}$ was the most abundant because of a large clay content: this was followed in order in the $63\text{--}250$, $250\text{--}1000$ and $1000\text{--}2000\text{-}\mu\text{m}$ 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 $250\text{--}1000\text{-}\mu\text{m}$ 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 $63\text{--}250\text{-}\mu\text{m}$ fraction (12.1 and 14.1 mg C g^{-1} , respectively) while the largest content under *H. hirta* was measured in the $1000\text{--}2000\text{-}\mu\text{m}$ fraction (15.2 mg C g^{-1}) (Figure 3). On average more than 50% of bulk SOC was contained in the smallest fraction ($< 63 \mu\text{m}$) because of the combined effects of the largest amount of fine aggregate fractions in the soils and its greater C content.

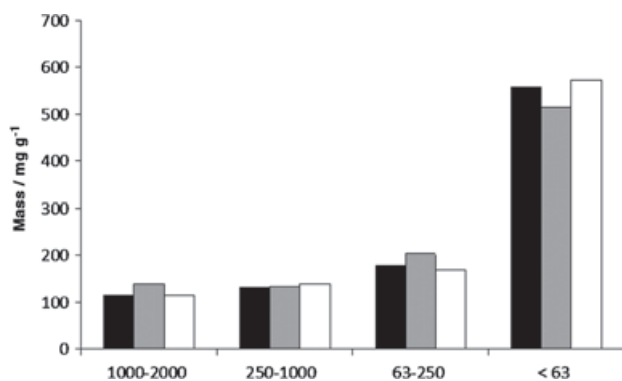


Figure 2 Mass of aggregate size fractions in soil under the olive grove (black columns), vineyard (white columns) and *H. hirta* grassland (grey columns).

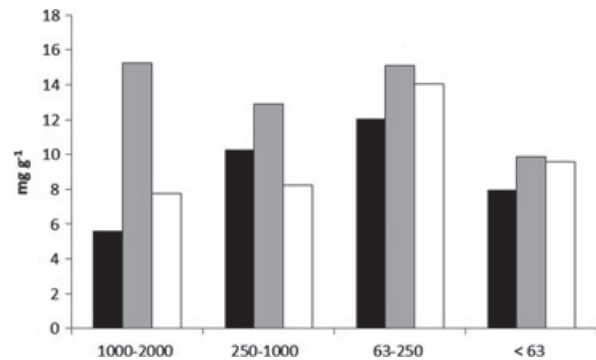


Figure 3 Carbon content in aggregate size fractions in soil under the olive grove (black columns), vineyard (white columns) and *H. hirta* grassland (grey columns).

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

$\delta^{13}\text{C}$ values of bulk soil and aggregate size fractions were significantly ($P < 0.05$) affected by land use, and were largest in *H. hirta* grassland ($\text{C}_3\text{--}\text{C}_4$ soil), followed by the vineyard ($\text{C}_4\text{--}\text{C}_3$ 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 $\delta^{13}\text{C}$ values to that of plant residues, while in the smallest fraction the $\delta^{13}\text{C}$ value became less negative for cultivated C_3 soil and, in contrast, more negative in natural grassland. The average $\delta^{13}\text{C}$ 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 $\text{C}_4\text{-C}$, but the stock of C derived from olives (C_3) was mostly preserved. In *H. hirta* grassland most of the $\text{C}_3\text{-C}$ 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 $\text{C}_3\text{-C}$ and 1.8 g kg^{-1} of $\text{C}_4\text{-C}$ (Figure 4). Most of the $\text{C}_3\text{-C}$ 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 $47 \text{ mg CO}_2\text{-C kg}^{-1} \text{ day}^{-1}$ (first day of incubation) and $14.7 \text{ mg CO}_2\text{-C kg}^{-1} \text{ day}^{-1}$ (after 32 days). This was followed by the soil under vines (from 37 to $13.05 \text{ mg CO}_2\text{-C kg}^{-1} \text{ day}^{-1}$) and *H. hirta* (25.05 to $8.86 \text{ mg CO}_2\text{-C kg}^{-1} \text{ day}^{-1}$) (Figure 5). The total C mineralized from soil over 1 month was greater under the vineyard and olive grove (592 ± 162 and $535 \pm 82 \text{ mg kg}^{-1}$, respectively) than in the *H. hirta* soil ($383 \pm 27 \text{ mg kg}^{-1}$). The $\delta^{13}\text{C}$ of the CO_2 evolved during the incubation ranged between -26.5‰ and

Table 1 $\delta^{13}\text{C}$ (‰) and portion of $\text{C}_3\text{-C}$ and $\text{C}_4\text{-C}$ in bulk soil and aggregate size fractions

	$\delta^{13}\text{C}$			Carbon distribution			
	Olive grove	<i>H. hirta</i>	Vineyard	<i>H. hirta</i>		Vineyard	
	C_3 soil	$\text{C}_3\text{-C}_4$ soil	$\text{C}_4\text{-C}_3$ soil	$\text{C}_3\text{-C}$	$\text{C}_4\text{-C}$	$\text{C}_4\text{-C}$	$\text{C}_3\text{-C}$
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 $\delta^{13}\text{C}$ from ANOVA (b)

(a) Source	Degrees freedom	Sum squares	Mean square	<i>F</i> ratio	<i>P</i>
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	<i>F</i> ratio	<i>P</i>
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 $\delta^{13}\text{C}$ 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 $\text{C}_3\text{-C}_4$ soil (Figure 7), while it ranged from 40 to 84% in $\text{C}_4\text{-C}_3$ soil. The ratio of $\text{C}_3\text{-C}$ in evolved CO_2 to that in SOM was 0.37 for $\text{C}_3\text{-C}_4$ soil and 0.21 for $\text{C}_4\text{-C}_3$ soil (Figure 8).

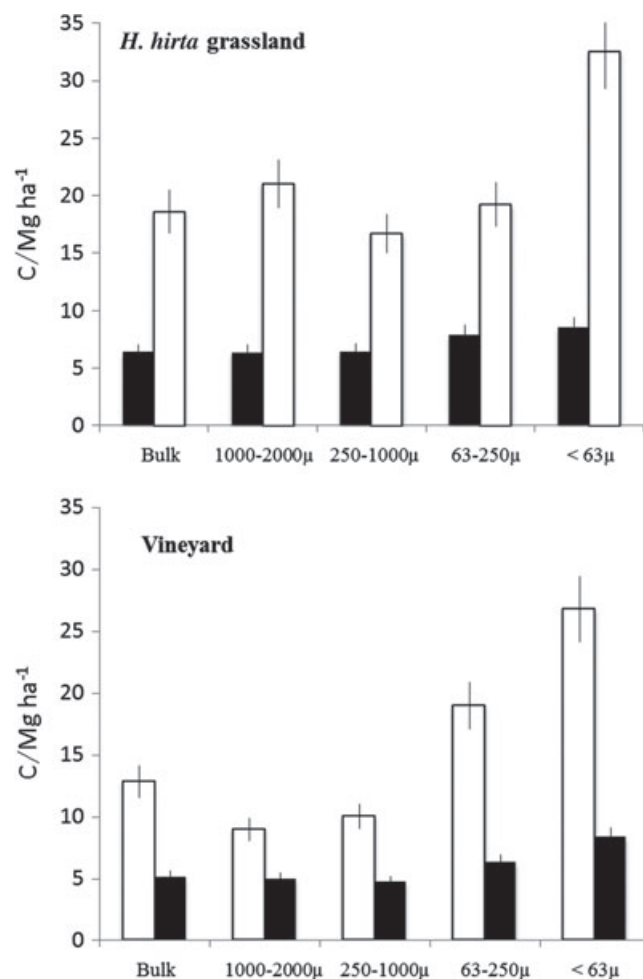
Carbon turnover

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.

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

**Figure 4** Content of $\text{C}_3\text{-C}$ (white histograms) and $\text{C}_4\text{-C}$ (black histogram) in bulk soil and for each particle size-fraction in *H. hirta* grassland and vineyard soils.

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.*,

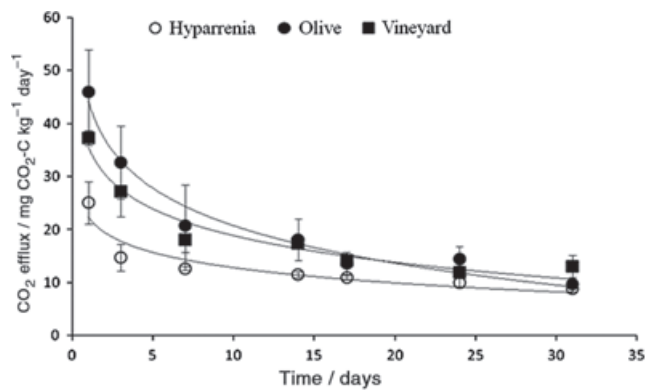


Figure 5 CO₂ efflux rates over 32 days of incubation for the *H. hirta* grassland, olive grove and vineyard.

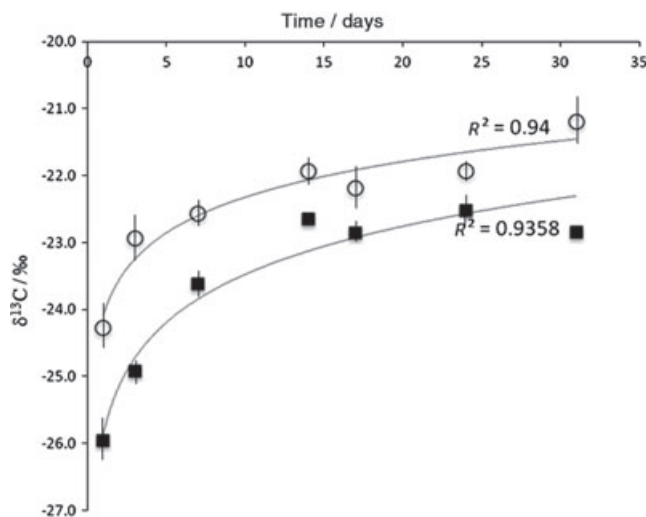


Figure 6 ¹³C signature of the evolved CO₂-C during 32 days of incubation of C₃-C₄ (white dots) and C₄-C₃ (black dots) soils from the *H. hirta* grassland and vineyard, respectively.

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.

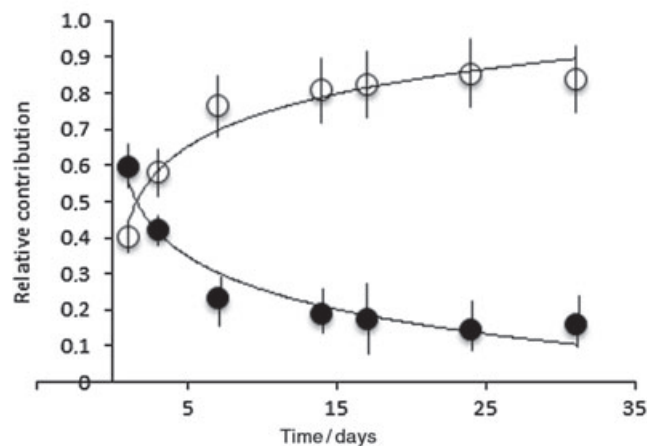
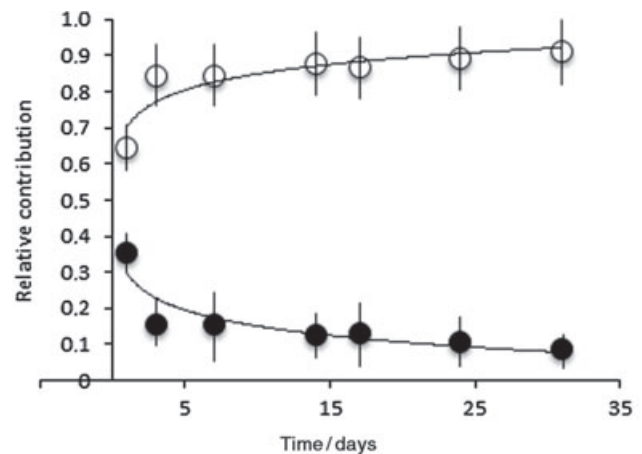


Figure 7 Relative contribution of C₄-C (white dot) and C₃-C (black dot) soil during 30 days of incubation of C₃-C₄ soil (a) and C₄-C₃ soil (b) from *H. hirta* grassland and vineyard, respectively.

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 macroaggregates. 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).

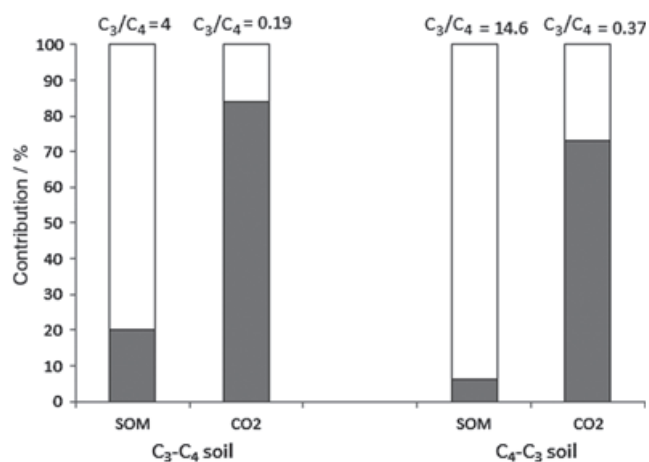


Figure 8 Comparison between the relative contribution of C₄-C (grey portion) and C₃-C (white portion) to SOM and CO₂ in C₃-C₄ soil (*H. hirta*) and C₄-C₃ soil (vineyard).

Stabilization of SOC in soil aggregate size fractions

Separation of SOC into aggregate size fractions in conjunction with the use of the natural ¹³C 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 C₃ origin. Following the C₄ vegetation succession, bulk soil and all fractions were enriched in ¹³C, 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 μm) (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 C₃-C and more than 30% of this C₃-C was lost from the largest aggregates (Figure 4). These results clearly show (i) faster decomposition of new C₃-C compared with C₄-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.

CO₂ fluxes and their δ¹³C signature during incubation

The ability of uncultivated soil to retain C was confirmed by CO₂ fluxes over 32 days of incubation. The cumulative CO₂ efflux in *H. hirta* soil was 32 and 40% less than that from the vineyard and olive grove soils, respectively (Figure 5). The δ¹³C signature of evolved CO₂-C was depleted for all soils, but it was greater for C₃-C₄ and C₄-C₃ soil than for C₃ soil. Because the C₃ soil reached the isotopic steady-state after 85 years (Blagodatskaya *et al.*, 2011), the difference in δ¹³C between SOM and CO₂-C is related to ¹³C fractionation or preferential use of ¹³C-depleted substances such as lipids or lignin (Werth & Kuzyakov, 2010).

In the soils after the C₃-C₄ and C₄-C₃ vegetation change, not only ¹³C fractionation but also preferential use of recent rather than old C contributed to δ¹³C enrichment in CO₂. This means that less stabilized younger organic materials (having C₄ or C₃ signature for C₃-C₄ and C₄-C₃ soils, respectively) will be preferred for microbial utilization and consequently contribute strongly to CO₂ evolution.

The relative contribution of C₄-C to the evolved CO₂ was always more than 60% in C₃-C₄ soil, and increased during incubation as in other studies (Blagodatskaya *et al.*, 2011). Although the relative contribution of C₄-C to SOC was less than the C₃-C contribution, this C₄ 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 C₄-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 CO₂-C (Blagodatskaya *et al.*, 2011).

The contribution of C₄-C to the total CO₂ efflux in C₄-C₃ soil was less than the C₃-C contribution in the first few days, but increased during the incubation period. However, the relative contribution of C₄-C was less in the C₃-C₄ soil than in the C₄-C₃ soil because of preferential use of new C (C₃-C). The ratio between C₃-C and C₄-C in CO₂ was, for both land uses, less than that in the SOM. The CO₂ emission had therefore originated mainly from *H. hirta* (Figure 8). The ratio between C₃-C in CO₂ and C₃-C in SOM was similar in the two soils, whilst that between C₄-C in CO₂ and C₄-C in SOM was greater in C₄-C₃ soil. The C of OM derived from *H. hirta* vegetation was therefore more degradable in C₄-C₃ 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 CO₂ 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 δ¹³C isotopic signature shift in SOM after C₃-C₄ vegetation change, estimates the MRT value only of C₄ organic matter, while the second approach estimated the MRT of C₃-C₄ soil under *H. hirta*. If only the portion C₄-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 $\delta^{13}\text{C}$ 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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