



Dynamics of soil organic carbon pools after agricultural abandonment



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ABSTRACT

Abandonment of agricultural land and the subsequent recolonization by natural vegetation is known to cause increases in C contents, contributing to reduction in atmospheric CO₂ concentrations. Assessment of the possible mitigation of CO₂ excess requires understanding the SOC dynamics, the origin of C pools and the pathways of their transformation. The aims of this work were to assess, by using the $\delta^{13}\text{C}$ signature, the changes of old and new organic C in total (soil organic carbon, SOC) and labile (microbial biomass C, MBC, dissolved organic C, DOC, CO₂ efflux from soil) pools after vegetation change from vineyard (C₃) to grassland (C₄) under semiarid Mediterranean climate. Colonization of abandoned vineyard by the perennial C₄-grass *Hyparrhenia hirta* after 15 or 35 years increased topsoil C stocks by 13% and 16%, respectively. Such an increase was attributed to new above- and below-ground biomass C input from *H. hirta*. The maximal incorporation of new C was observed in MBC, whereas the DOC derived mainly from old SOC. Based on $\delta^{13}\text{C}$ values of SOC, MBC, DOC and CO₂ in C₃ soil and in soils after 15 and 35 years of C₄ grass colonization, ^{13}C fractionation per se from changes in isotopic composition by preferential utilization of substrates with different availability was separated. MBC in C₃–C₄ soil used more recent (^{13}C -enriched) versus old C (relatively ^{13}C -depleted) sources. The $\Delta^{13}\text{C}$ by decomposition of SOC to CO₂ ($\delta^{13}\text{C}$ of CO₂ minus $\delta^{13}\text{C}$ of SOC) was higher than $\Delta^{13}\text{C}$ by microbial respiration (CO₂ minus MBC), demonstrating that under semiarid climate, soil microorganisms do not always preferentially decompose the most available SOC pools. The use of $\delta^{13}\text{C}$ signature of SOC after C₃–C₄ vegetation change combined with soil incubation is a powerful tool to assess the exchange between old and new C in pools of various availability.

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1. Introduction

Until recently, extensive areas of arable land had been abandoned in many countries worldwide (Kalinina et al., 2011; Ramankutty, 2006), predominantly due to non-profitable agricultural and forestry management of marginal areas. As a consequence, the soils of these abandoned sites have undergone a process of self-restoration without any direct human impact. Preliminary studies of post-agrogenic soils undergoing self-restoration showed that such soils, as well as the vegetation, were developing towards their natural composition (Kalinina et al., 2011; Nicodemus et al., 2012; Novara et al., 2013a). The dynamics of these changes depend on climatic zones, soil genesis, previous land use history, and the existence of wild plant seeds nearby (Paul et al., 2002). Soil organic carbon (SOC) accumulates during self-restoration processes. Previous studies carried out in semiarid Mediterranean environment showed that abandonment of agricultural land and the subsequent recolonization by natural vegetation caused increases in C contents,

contributing to the reduction in atmospheric CO₂ concentrations (Novara et al., 2012, 2013b). The assessment of possible mitigation of excess CO₂ (i.e. after the conversion to perennial vegetation or application of sustainable practices) requires knowledge on SOC dynamics for various pedoenvironments, since C sequestration is soil and site specific (Lal, 2004; Novara et al., 2012). However, understanding SOC dynamics is complex because SOC comprises various heterogeneous pools with different turnover rates and stability (Flessa et al., 2000; Laudicina et al., 2013). Quantitative analysis of SOC dynamics requires tracing the origin of C pools and the pathways of their transformation (Flessa et al., 2000). To distinguish among C pools and to determine their contribution to soil CO₂, isotopic tracer techniques are today widely applied. A vegetation change from C₃ to C₄ plants results in different isotopic composition of new and old C pools, which allows for their separation (Balesdent et al., 1987). Depending on the different photosynthetic pathways, specific isotopic ^{13}C fractionations occur during CO₂ assimilation, leading to a distinct isotopic composition of C₃ and C₄ plants (Farquhar et al., 1989). Therefore, when growing C₄ plants on soil originally formed under C₃ vegetation (or vice versa), old (C₃-derived) and new (C₄-derived) C can be differentiated based on their isotopic differences (Balesdent et al., 1987).

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Most of the abandoned land in the Mediterranean area undergoes a recolonization with *Hyparrhenia hirta* (*H. hirta*) grassland. This species is widely distributed in the Mediterranean area (Botha and Russell, 1988), North Africa and the Middle East (Chejara et al., 2008) from sea level up to 600 m above sea level. *H. hirta* is a typical C₄-species of degraded areas quickly growing after disturbance: this means that *H. hirta* grasslands are very suitable for studying turnover and stabilization of SOC after a cultivation of C₃ plant such as vines.

Our study highlighted the changes in total and labile soil organic C pools following recolonization by natural vegetation of abandoned agricultural land in a semiarid Mediterranean area using the $\delta^{13}\text{C}$ signatures. The aims of this work were (i) to investigate both the exchange rates between old and new C pools (total, dissolved, microbial biomass and respired CO₂) and C use efficiency after agricultural abandonment (vineyard) and *H. hirta* recolonization, and (ii) to study the dynamics of C pools by discerning between fractionation per se versus substrate preferential utilization.

2. Materials and methods

2.1. Study area and experimental set-up

Cambisol soil (WRB, 2007) was sampled in April 2012 in a cultivated and abandoned terraced vineyard in Pantelleria (36°44' N, 11°57' E; Italy; Fig. 1). The climate is semiarid Mediterranean (Thornthwaite and Mather, 1955) with an annual mean precipitation of 409 mm and monthly average temperatures ranging from 11.7 °C in January to 25.6 °C in August (30 year average). In Pantelleria agriculture has been carried out on terraced land due to the high slope. Most of these terraces were abandoned in different ages and this permits chronosequence studies. Successional stages with different abandonment ages are in close proximity and present in different terraces.

Soil samples were taken in three distinct terraces using a chronosequence approach: vineyard, *H. hirta* grassland 15 years after vineyard abandonment (*H. hirta* 15) and *H. hirta* grassland 35 years after vineyard abandonment (*H. hirta* 35). The distance among the terraces was 50 m at most and homogeneous in geological substrate, soil texture and exposure. *H. hirta* 15 and 35 vegetation covers are stable over time and the evolution of succession is slow due to the south-exposed slope (Litav, 1972).

The composition of vegetation in *H. hirta* grassland is represented by 99% of *H. hirta* in terms of covered ground. Other species are annual plants with a short biological cycle and consequently their contribution to $\delta^{13}\text{C}$ can be considered negligible.

Vineyard was managed according to local agricultural practices, with soil tilled and uncovered by vegetation since 1954. The abandonment age was determined using aerial photographs taken in 1954, 1968, 1979, 1987, 1992 and 2000 (Rühl et al., 2005). For each successional stage, three adjacent non-randomized sampling areas were selected. In each sampling area, three soil samples were collected at 0–15 cm giving a total of 27 soil samples. After carefully removing fine roots and other plant, each soil sample was sieved to <2 mm diameter

Contemporary to soil sampling (early spring), both for *H. hirta* 15 and 35, biomass of three 1 m² squares was collected, dried and grinded for C isotopic analysis.

2.2. Mineralisation of soil organic carbon

A short-term (51 days) aerobic incubation procedure was used to determine the potential of the soil samples to mineralize organic C (Nannipieri et al., 1990). Soil samples (10 g) at 50% of their water holding capacity were pre-incubated for five days at room temperature; then, after readjustment of the moisture, they were incubated at 25 °C in 125 cm³ air-tight glass bottles. The evolved CO₂ was measured by sampling an aliquot of gas from the bottles using a syringe and injecting it into a gas-chromatograph (Trace GC, Thermo Electron) equipped with a thermal conductivity detector. CO₂ measurements were done after 1, 4, 10, 31 and 51 days from the start. Twenty-four hours before the CO₂ sampling, all flasks were ventilated for 30 min with fresh room air and then immediately sealed with rubber stoppers. Thus, evolved CO₂ from soil was allowed to accumulate for 24 h and then sampled. The C mineralisation rate was expressed as mg CO₂-C g⁻¹ TOC day⁻¹ and was fitted to the following first-order decay function:

$$\text{Mineralised C} = \text{Cr} e^{-kt} \quad (1)$$

where Cr is the readily mineralizable 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 (qCO₂), an index of the soil stress/disturbance status (Anderson and Domsch, 1993) expressed

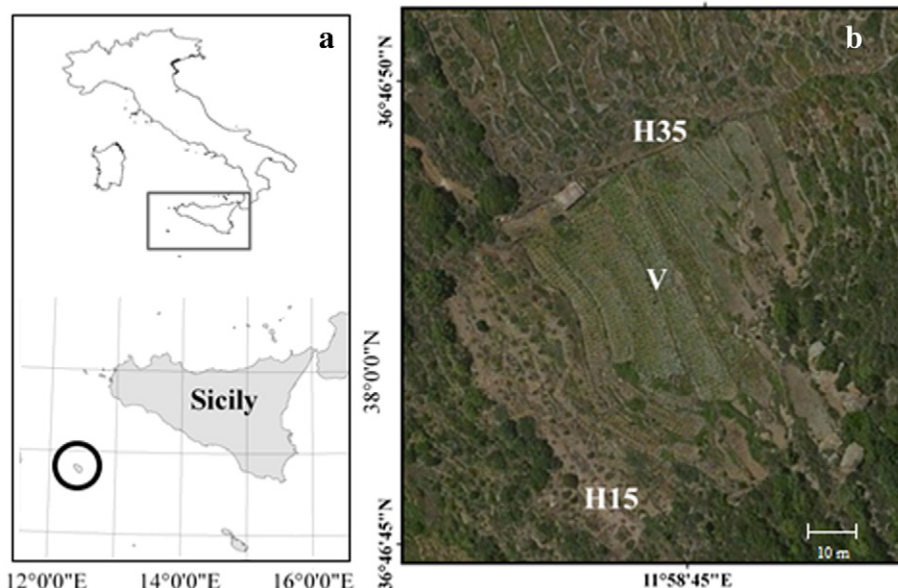


Fig. 1. (a) Pantelleria island in the black circle and an aerial photograph (b) of the study area (*Hyparrhenia hirta* 15 (H15), *Hyparrhenia hirta* 35 (H35) and vineyard (V) terraces.

as $\text{mg CO}_2\text{-C g}^{-1} \text{MBC h}^{-1}$. The amount of total C mineralized during incubation was calculated through the linear interpolation of two subsequent measured rates and the numerical integration over time as reported in the following equation:

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

where i is the date of the first measurement of $\text{CO}_2\text{-C}$ rate, n is the date of the last measurement of $\text{CO}_2\text{-C}$ rate, r is the $\text{CO}_2\text{-C}$ rate expressed as $\text{mg CO}_2\text{-C kg}^{-1} \text{dry soil day}^{-1}$, and d is the number of days between the two consecutive CO_2 rate measurements.

2.3. Soil analyses

Contemporary to CO_2 measurements, microbial biomass C (MBC), DOC and SOC were analyzed as well as their $\delta^{13}\text{C}$. Microbial biomass C was determined by the slightly modified fumigation-extraction (FE) method (Vance et al., 1987). Moist (50% of water holding capacity) soil aliquots (equivalent to 10 g oven-dry soil) were fumigated for 24 h at 25 °C with ethanol-free chloroform. After removing the chloroform by repeated evacuations, the fumigated soil was extracted with 40 ml 0.05 M K_2SO_4 for 30 min on a horizontal shaker and the suspension was filtered through Whatman 42 filter paper (nominal pore size 2.5 μm). An equivalent amount of non-fumigated soil was similarly extracted at the time when fumigation commenced. Both fumigated and non-fumigated extracts were analyzed for organic C by hot acid dichromate oxidation. Microbial biomass C was calculated as EC/k_{EC} , where EC is organic C extracted from fumigated soil minus that extracted from non-fumigated soil and k_{EC} is 0.45 (Jenkinson et al., 2004). An aliquot of both unfumigated and fumigated K_2SO_4 extracts was dried at 60 °C for isotope ratio mass spectrometry analyses (Brant et al., 2006). The concentration of K_2SO_4 -extractable C of non-fumigated soil (DOC) was used as an indicator of available C (Haynes, 2005; Laudicina et al., 2013).

2.4. Isotopic analysis

The $\delta^{13}\text{C}$ analyses of *H. hirta* biomass, DOC, MBC, CO_2 and SOC pools (samples replicated twice) were carried out by an EA-IRMS (Elemental Analyser Isotope Ratio Mass Spectrometry, Carlo Erba Na 1500, model Isoprime 2006, Manchester, UK) (0.1‰ repeatability). The reference material used for analysis was IA-R001 (Iso-Analytical Limited wheat flour standard, $\delta^{13}\text{C}_{\text{CV-PDB}} = -26.43\%$). IA-R001 is traceable to IAEA-CH-6 (cane sugar, $\delta^{13}\text{C}_{\text{CV-PDB}} = -10.43\%$). IA-R001, IA-R005 (Iso-Analytical Limited beet sugar standard, $\delta^{13}\text{C}_{\text{CV-PDB}} = -26.03\%$), and IA-R006 (Iso-Analytical Limited cane sugar standard, $\delta^{13}\text{C}_{\text{CV-PDB}} = -11.64\%$) were used as quality control samples for the analysis. The International Atomic Energy Agency (IAEA), Vienna, distributes IAEA-CH-6 as a reference standard material. 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.

2.5. Calculations

The $\delta^{13}\text{C}$ value of MBC was estimated as the $\delta^{13}\text{C}$ value of the DOC extracted from the fumigated soil sample minus that extracted from the unfumigated soil sample, as:

$$\delta^{13}\text{C}_{\text{MBC}} = \left(\delta^{13}\text{C}_{\text{DOCf}} * \text{DOC}_f - \delta^{13}\text{C}_{\text{DOCnf}} * \text{DOC}_{\text{nf}} \right) / (\text{DOC}_f - \text{DOC}_{\text{nf}}) \quad (3)$$

where $\delta^{13}\text{C}_{\text{DOCnf}}$ and $\delta^{13}\text{C}_{\text{DOCf}}$ are the $\delta^{13}\text{C}$ values of the organic C in the K_2SO_4 unfumigated and fumigated soil extracts, respectively and DOC_{nf} and DOC_f are the values of organic carbon in the K_2SO_4 extracts of unfumigated and fumigated soils, respectively.

The proportion of C derived from *H. hirta* within the investigated C pools (SOC, CO_2 , MBC and DOC) was calculated using a mixing model:

$$\text{New carbon derived (Ncd)} = \frac{\delta^{13}\text{C}_{\text{new}} - \delta^{13}\text{C}_{\text{old}}}{\delta^{13}\text{C}_{\text{biomass new species}} - \delta^{13}\text{C}_{\text{old}}} \quad (4)$$

and

$$\text{Old carbon derived (Ocd)}(\%) = 100 - \text{Ncd} \quad (5)$$

where Ncd is the fraction (%) of C derived from the present vegetation type, $\delta^{13}\text{C}_{\text{new}}$ is the isotope ratio of the C pool under *H. hirta*, and $\delta^{13}\text{C}_{\text{old}}$ is the isotopic ratio of the C pool in previous vegetation type (vine biomass). $\delta^{13}\text{C}_{\text{biomass new species}}$ was calculated based on $\delta^{13}\text{C}$ value of *H. hirta* biomass ($\delta^{13}\text{C} = 11.1 \pm 1.2\%$) and corrected for isotopic fractionation during humification by subtracting the difference between $\delta^{13}\text{C}$ of C_3 vegetation and $\delta^{13}\text{C}$ of SOC of the C_3 soil (Blagodatskaya et al., 2011). Soil under vineyard, without $\text{C}_3\text{-C}_4$ vegetation change was used as the reference soil to estimate $\delta^{13}\text{C}$ shift between the pools caused by isotopic fractionation.

Turnover of SOC (mean resident time in years, MRT) was determined by taking the reciprocal of the first order rate constant k (Eq. (6)) according to Balesdent and Mariotti (1996) and Dorodnikov et al. (2011).

$$k = \frac{-\ln(1 - \text{Ncd})}{\text{Years Since Disturbance}} \quad (6)$$

The mass of new carbon additions was calculated for bulk soil as follow:

$$\text{New Carbon (Mg C ha}^{-1}) = \text{C soil (Mg C ha}^{-1}) * \text{New carbon derived} / 100 \quad (7)$$

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

$$\delta^{13}\text{CO}_{2\text{measured}} = f * \delta^{13}\text{CO}_{2\text{atm}} + (1 - f) * \delta^{13}\text{CO}_{2\text{sample}} \quad (8)$$

such that:

$$\delta^{13}\text{CO}_{2\text{sample}} = \left(\delta^{13}\text{CO}_{2\text{measured}} - f * \delta^{13}\text{CO}_{2\text{atm}} \right) / (1 - f) \quad (9)$$

where $\delta^{13}\text{CO}_{2\text{measured}}$ is the measured isotopic ratio of CO_2 accumulated in the jars, f is the contribution of atmospheric CO_2 concentration ($450 \mu\text{l l}^{-1} \text{CO}_2$) to total CO_2 measured, $\delta^{13}\text{CO}_{2\text{sample}}$ is the undiluted isotopic ratio of soil sample, and the $\delta^{13}\text{CO}_{2\text{atm}}$ is the isotopic ratio of measured atmospheric (laboratory air) CO_2 .

The following mixing model was used to determine the portions of CO_2 deriving from $\text{C}_3\text{-C}$ and $\text{C}_4\text{-C}$:

$$\frac{[\text{CO}_2]_{\text{m}} * \delta^{13}\text{C}_{\text{m}} = x * [\text{CO}_2]_{\text{v}} * \delta^{13}\text{C}_{\text{v}} + (1 - x) * [\text{CO}_2]_{\text{H}} * \delta^{13}\text{C}_{\text{H}}}{[\text{CO}_2]_{\text{m}} = x * [\text{CO}_2]_{\text{v}} + (1 - x) * [\text{CO}_2]_{\text{H}}} \quad (10)$$

where x is the CO_2 production from vineyard soil, $[\text{CO}_2]_{\text{m}}$ is the measured CO_2 ; $[\text{CO}_2]_{\text{v}}$ is the vineyard soil CO_2 concentration and $[\text{CO}_2]_{\text{H}}$ represents the *H. hirta* soil CO_2 concentration. Values of -27.5% and -13.3% were used as $\delta^{13}\text{C}$ of vineyard and *H. hirta* grassland, respectively.

2.6. Statistical analysis

$\text{C}_3\text{-C}$ and $\text{C}_4\text{-C}$ contents of SOC, DOC and MBC under vineyard, *H. hirta* 15 and *H. hirta* 35 were analyzed by analysis of variance (ANOVA). Differences among means were tested with the LSD test at $P < 0.05$. SAS statistical programs were used (SAS Institute, 2001).

3. Results

3.1. Content and $\delta^{13}\text{C}$ isotopic signature of SOC and DOC

Soil organic C content significantly increased after 35 years since vineyard abandonment from 21.9 g kg^{-1} to 25.5 g kg^{-1} (Fig. 2). Carbon isotopic signature of SOC was affected by $\delta^{13}\text{C}$ of plant residues entering the system through litterfall and roots. The $\delta^{13}\text{C}$ values of SOC were lower in the vineyard, followed by *H. hirta* 15, and *H. hirta* 35 (Fig. 3). The $\delta^{13}\text{C}$ of SOC was enriched by 3.6‰ after further 15 years of *H. hirta* establishment because of SOC mineralization (C_3) from previous land cover and increase of C_4 -C originated from new vegetation. The C_4 -C in SOC increased by 3.37 g kg^{-1} and 9.03 g kg^{-1} after 15 and 35 years of *H. hirta*, respectively. The C_3 -C of SOC slightly decreased after the first 15 years since vineyard abandonment (less than 0.6 g kg^{-1} of C), while it dropped notably (4.89 g kg^{-1} of C) after 35 years of *H. hirta*.

From vineyard to *H. hirta* 15, the rate of C_3 -C decrease ($0.04 \text{ g kg}^{-1} \text{ year}^{-1}$) was 5 times slower than C_4 -C increase ($0.22 \text{ g kg}^{-1} \text{ year}^{-1}$). From *H. hirta* 15 to *H. hirta* 35, the rate of C_3 -C decrease was $0.24 \text{ g kg}^{-1} \text{ year}^{-1}$ and the rate of C_4 -C increase was $0.28 \text{ g kg}^{-1} \text{ year}^{-1}$.

Dissolved organic C ranged from 55.8 to 58.1 mg kg^{-1} and did not show significant differences among the land covers (Table 1). On the contrary, the ratio DOC/SOC was highest in vineyard soil (0.26%) compared to *H. hirta* soils between which no differences occurred (0.22% and 0.23% in *H. hirta* 15 and *H. hirta* 35, respectively). The DOC in soils under *H. hirta* had mainly a C_3 -C origin (Fig. 2), suggesting by difference that C_4 -SOC was more stable than C_3 -SOC. The $\delta^{13}\text{C}$ values of DOC was lowest in soil under vineyard, followed by *H. hirta* 15 and *H. hirta* 35 (Fig. 3; Table 2). The ratio DOC/SOC of C_3 -C portion was higher than C_4 -C portion in both *H. hirta* land covers (Table 3). The depletion rate of C_3 -DOC was $0.49 \text{ mg kg}^{-1} \text{ y}^{-1}$ and $0.30 \text{ mg kg}^{-1} \text{ y}^{-1}$ calculated after 15 and 35 years from vineyard abandonment (linear decrease is assumed), whereas the rate increase of C_4 -DOC was $0.43 \text{ mg kg}^{-1} \text{ y}^{-1}$ and $0.35 \text{ mg kg}^{-1} \text{ y}^{-1}$ if estimated after 15 and 35 years. This indicated that until 15 years of *H. hirta* colonization the main source of DOC was the old SOC, while from 15 to 35 years the average contribution of *H. hirta* exceeded that of vines.

3.2. Content and $\delta^{13}\text{C}$ signature of MBC

Microbial biomass C was significantly affected by land cover (Table 1). It ranged from 239 to 295 mg kg^{-1} and showed higher values in *H. hirta*, whereas the microbial quotient (MBC/TOC) was not affected by land cover (Table 1). In comparison to vineyard, $\delta^{13}\text{C}$ values of MBC were enriched by 3.6‰ and 6.8‰ after 15 and 35 years of *H. hirta* colonization, respectively. Under *H. hirta* 15, MBC consisted of 76% of C_3 -C, while under *H. hirta* 35 the C_3 -C portion of MBC decreased to 55% (Fig. 2). The amounts of both C_3 -C and C_4 -C in MBC were correlated with the $\delta^{13}\text{C}$ values of SOC, but although MBC was mainly of C_3 -C

origin, the ratio $\text{MBC-C}_3/\text{SOC-C}_3$ was lower than $\text{MBC-C}_4/\text{SOC-C}_4$, regardless of the duration of *H. hirta* colonization (Table 3). The latter ratio was higher than 1.0 both for *H. hirta* 15 and *H. hirta* 35. This result suggested the preferential utilization of the recent C_4 -C input by microorganisms, i.e. the input shift from old C_3 -C to more recent C_4 -C.

3.3. Changes of C pools and their $\delta^{13}\text{C}$ signature during incubation

Microbial biomass C and DOC contents at the beginning (day 1) and at the end (day 51) of incubation did not differ significantly (Fig. 4). The $\delta^{13}\text{C}$ values of all pools (SOC, MBC, DOC and CO_2) ranged between -29.18 and -22.35 in C_3 soil, between -26.84 and -18.38 in *H. hirta* 15 and between -24.12 and -17.23 in *H. hirta* 35 (data not shown). In all soils, the variations in $\delta^{13}\text{C}$ signature of C pools during the incubation were significant only for CO_2 .

In soil under C_3 - C_4 vegetation, microorganisms consumed prevalently C_3 -C of SOC during incubation and the contribution of C_4 -C to SOC increased at the last stage (51 days) of incubation from 13.7% to 19.7% and from 35.4% to 36.0% in *H. hirta* 15 and *H. hirta* 35, respectively (Fig. 6). On the contrary, the contribution of C_4 -C to CO_2 decreased at the last stage from 16.5% to 5.7% and from 18.6% to 7.7% in *H. hirta* 15 and *H. hirta* 35, respectively.

The contribution of C_4 -C to MBC showed significant changes during incubation only for *H. hirta* 15 (Fig. 6).

Under *H. hirta* 35, the C_4 -C portion of MBC was constant during incubation and therefore most of the CO_2 from C_3 sources was released by decomposition of SOC and not by reutilization of MBC. The C_3 and C_4 portions of DOC remained quite constant during incubation in both *H. hirta* soils, even if the DOC amount decreased during the incubation. These findings suggested that DOC composition was independent of MBC turnover, and there was no preferential decomposition of soluble organics.

3.4. Sources of CO_2 efflux from soil

At the first day of incubation, the largest CO_2 emission rate was recorded from vineyard soil, followed by *H. hirta* 15 and *H. hirta* 35 (Fig. 5). The total C mineralized from soil over the 51 days of incubation was greater in vineyard and *H. hirta* 35 soils (1283 ± 35 and $1342 \pm 61 \text{ mg CO}_2\text{-C kg}^{-1}$, respectively) compared with *H. hirta* 15 soil ($1204.7 \pm 37.5 \text{ mg CO}_2\text{-C kg}^{-1}$). The $\delta^{13}\text{C}$ of the evolved CO_2 during the incubation ranged between -27.2% and -22.3% in the vineyard, from -21.7% to -18.4% in the *H. hirta* 15 soil and from -19.1% to -17.3% in the *H. hirta* 35 soil. The $\delta^{13}\text{C}$ values of CO_2 from all soils were strongly depleted during the first week of incubation. According to Eq. (10), the contribution of recent C_4 to CO_2 flux during the 51 days of incubation decreased from 16.5% at the beginning to 5.6% at the end of incubation in *H. hirta* 15 soil, while it decreased from 18.5 to 7.7% in *H. hirta* 35 soil (Fig. 6).

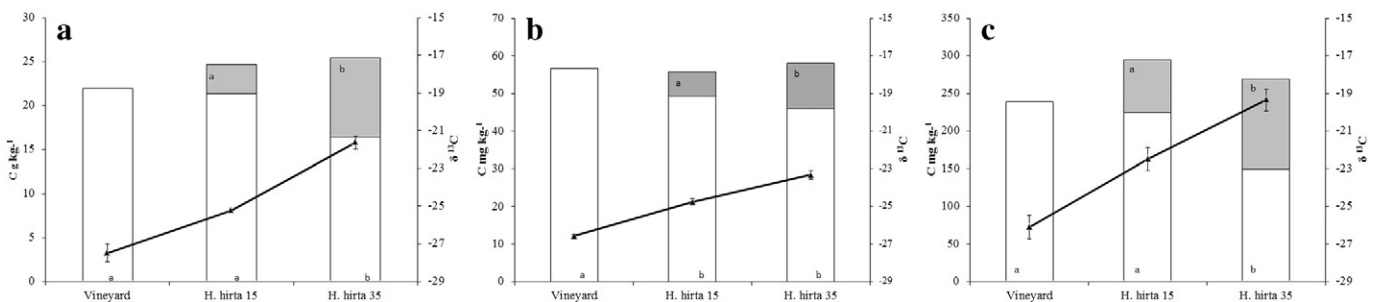


Fig. 2. C_3 -C (white columns) and C_4 -C (gray columns) content of soil organic carbon (SOC) (a), dissolved organic carbon (DOC) (b) and microbial biomass carbon (MBC) (c) under vineyard, *Hypertheca hirta* 15 and *Hypertheca hirta* 35. Triangles indicate $\delta^{13}\text{C}$ values and bars are standard deviations ($n = 9$). Values followed by the same letter are not significantly different at $P \leq 0.05$ (LSD test).

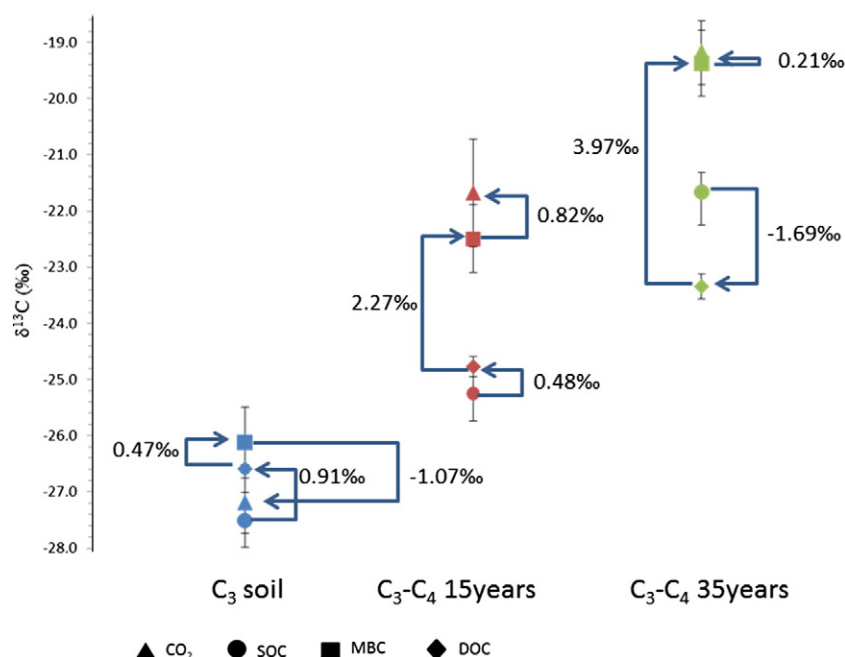


Fig. 3. ^{13}C discrimination processes between soil organic carbon (SOC) and the other soil organic carbon pools: dissolved organic carbon (DOC), microbial biomass carbon (MBC), and SOC-derived CO_2 for C_3 (vineyard), $\text{C}_3\text{-C}_4$ 15 years (*Hyparrhenia hirta* 15) and $\text{C}_3\text{-C}_4$ 35 years (*Hyparrhenia hirta* 35) soils. The arrows indicate the main fate of C of different pools.

3.5. Turnover of SOC

The MRT of SOC originated from vine residues, calculated according to Eq. (6) after 15 and 35 years, was about 102 ± 5 and 80 ± 4 years, respectively. It was calculated on the basis of isotope mixing model of Balesdent et al. (1987) and assuming exponential decomposition of SOC. Mean residence time calculated for different periods, but in the same pedoclimatic conditions, was expected to be similar, instead, the two MRT values differed by more than 20 years. Compared to the exponential model, the MRT calculated based on $\delta^{13}\text{C}$ showed that after 15 years since abandonment, the C cycle was slower than the theoretical model and from 15 to 35 years the decomposition was faster. The MRT calculated by the second approach, based on released CO_2 (Eq. (1)), was 24, 45 and 42 days for vineyard, *H. hirta* 15, and *H. hirta* 35, respectively. These values reflect the MRT of easily available C in soil that was involved in decomposition during 51 days.

4. Discussion

4.1. SOC content after *H. hirta* recolonization

The SOC content measured in the secondary succession showed that in a semiarid environment, the abandonment of cultivated land increased the organic matter content: by 13% after 15 years since vineyard abandonment; while from 15 to 35 years after abandonment, the SOC still increased slightly but not significantly. Similar results, although in a different soil type, were obtained in our previous studies, where the

C content increased due to fast colonization by *H. hirta* grassland after abandonment of olive plantations (Novara et al., 2013a). The higher belowground root biomass of grassland in comparison to cultivated soil was one of the main reasons of soil C differences. Moreover, intensive agricultural practices used for cultivation of olive and vines cultivation in the Mediterranean region have a negative impact on soil C sequestration when compared with semi-natural vegetation. A similar increase of C stocks in soils after cropland abandonment was reported recently for large areas of abandoned lands (Kurganova et al., 2013). The significant increase of the SOC after 15 years from abandonment is mainly attributable to new C input from *H. hirta*. In fact, the $\text{C}_3\text{-C}$ content under *H. hirta* 15 is similar to SOC under vineyard, while a significant contribution of $\text{C}_4\text{-C}$ portion was recorded. In the second step of succession, SOC content remained constant, but further $\text{C}_3\text{-C}$ was replaced by new $\text{C}_4\text{-C}$.

The rates of C increase or decrease with different isotopic composition ($\text{C}_3\text{-C}$ or $\text{C}_4\text{-C}$) during secondary succession explain the potential of land cover to change the C steady state level (West and Six, 2007).

After abandonment of vineyard, the C sink capacity of soils primarily increased likely due to higher fine root biomass from developing *H. hirta*, with consequent higher C input by rhizodepositions, and due to the reduction of soil disturbance (i.e. tillage). Moreover, the soil under *H. hirta* 15 was completely covered by vegetation and, consequently, the light absorption, the surface temperature and the water evaporation of soil decreased compared to bare soil under vineyard. Such different micro-environmental conditions lead to better soil physical and chemical properties for microbial communities, which further improved the C substrate utilization (Gang et al., 2012; Laudicina

Table 1

Biochemical properties of soil determined after 1 day of incubation at 50% of its water holding capacity and 25 °C in response to different land uses. Different letters indicate significant differences ($P < 0.05$) among land uses.

| Land use | DOC | MBC | Basal respiration | MBC/SOC | Metabolic quotient |
|--------------------|---------------------|---------------------|--|---------|---|
| | mg kg ⁻¹ | mg kg ⁻¹ | mg CO ₂ -C kg ⁻¹ day ⁻¹ | % | mg CO ₂ -C g ⁻¹ MBC h ⁻¹ |
| Vineyard | 56.6 a | 239.4 a | 66.5 c | 1.1 a | 1.17 b |
| <i>H. hirta</i> 15 | 55.8 a | 295.2 b | 48.5 b | 1.2 a | 0.69 a |
| <i>H. hirta</i> 35 | 58.1 a | 269.5 ab | 37.4 a | 1.1 a | 0.58 a |

DOC, dissolved organic carbon; MBC, microbial biomass carbon; SOC, total soil organic carbon.

Table 2

$\delta^{13}\text{C}$ and portions of old ($\text{C}_3\text{-C}$) and new ($\text{C}_4\text{-C}$) C in mineralized C (after 1 day of soil incubation at 50% of its water holding capacity and 25 °C), microbial biomass carbon (MBC) and dissolved organic carbon (DOC) pools after first day of incubation.

| Land use | DOC | | MBC | | Mineralized C | | | | |
|--------------------|-------------------------|----------------|----------------|-------------------------|----------------|----------------|-------------------------|----------------|----------------|
| | $\delta^{13}\text{C}$ ‰ | $\text{C}_3\%$ | $\text{C}_4\%$ | $\delta^{13}\text{C}$ ‰ | $\text{C}_3\%$ | $\text{C}_4\%$ | $\delta^{13}\text{C}$ ‰ | $\text{C}_3\%$ | $\text{C}_4\%$ |
| Vineyard | -26.57 | | | -26.10 | | | -27.17 | | |
| <i>H. hirta</i> 15 | -24.75 | 88 | 12 | -22.48 | 76 | 24 | -21.65 | 83 | 17 |
| <i>H. hirta</i> 35 | -23.32 | 79 | 21 | -19.35 | 55 | 45 | -19.14 | 64 | 36 |

et al., 2012). After 15 years of *H. hirta* the soil reached a nearly steady state level with regard to the total C content, while $\text{C}_3\text{-C}$ portion decreased due to disappearance of $\text{C}_3\text{-C}$ input.

4.2. Labile C pools and C use efficiency

The lower MBC in vineyard soil could be ascribed to the previous tillage, although it could be also due to lower C input by turnover of roots in agricultural soil compared to natural vegetation. Since tillage causes the disruption of aggregates, it exposes previously protected microorganisms to adverse conditions (Alvarez et al., 1995; Balota et al., 2004; Laudicina et al., 2011). This was confirmed by the higher basal respiration at the beginning of incubation in vineyard soil, determined by a higher accessibility of organic C following the aggregate disruption. The absence of significant differences among the three land covers in MBC/SOC and also in DOC suggests that vineyard affected stable and labile C pools at the same extent. However, the C use efficiency by microorganisms was significantly different among land covers. The higher metabolic quotient in soil under vineyard denotes a lower C use efficiency of microbial biomass and/or a higher disturbance compared to soil under *H. hirta*. This could also be due to a shift in microbial communities as it has been demonstrated that the absence of tillage favors a higher ratio of fungi to prokaryotes (Bailey et al., 2002; Laudicina et al., 2011). Fungi have a lower energy requirement for maintenance than prokaryotes, and thus immobilize substrate-C and incorporate it into microbial biomass C more efficiently (Alvarez et al., 1995; Haynes, 1999), with less C respired. The increase in MBC, combined with the decrease of basal respiration after the abandonment of vineyard with the subsequent soil colonization from *H. hirta*, suggested that the microbial community under *H. hirta* was utilizing more efficiently the C substrates (Sparling, 1997).

Although the DOC pool was, as expected, very small in comparison to the SOC (Laudicina et al., 2013; van Hees et al., 2005), it provides the main source of C for soil microorganisms and, together with MBC, may be the most dynamic component of soil C (Pelz et al., 2005). Small variations in the DOC pool were relevant for C dynamics under the different land uses analyzed. Vineyard soils showed the highest ratio between the DOC and SOC contents. In fact, due to continuous tillage, it was prone to higher mineralization rates, in comparison to soil under natural vegetation. The higher decomposition of SOC implied the higher release of organic molecules at lower molecular weight, due to better soil aeration and increased aggregate breaking (Tisdall and Oades, 1982). The DOC deriving from vine residues (litter and dead

Table 3

Ratio between $\text{C}_3\text{-C}$ and $\text{C}_4\text{-C}$ portion of mineralized C (after 1 day of soil incubation at 50% of its water holding capacity and 25 °C), microbial biomass carbon (MBC) and dissolved organic carbon (DOC) pools with the total soil organic carbon (SOC) after the first day of incubation.

| Land use | DOC/SOC | | MBC/SOC | | Mineralized C/SOC | |
|--------------------|--------------|--------------|--------------|--------------|-------------------|--------------|
| | C_3 | C_4 | C_3 | C_4 | C_3 | C_4 |
| <i>H. hirta</i> 15 | 1.02 | 0.85 | 0.88 | 1.77 | 0.89 | 1.71 |
| <i>H. hirta</i> 35 | 1.23 | 0.59 | 0.86 | 1.26 | 0.99 | 1.01 |

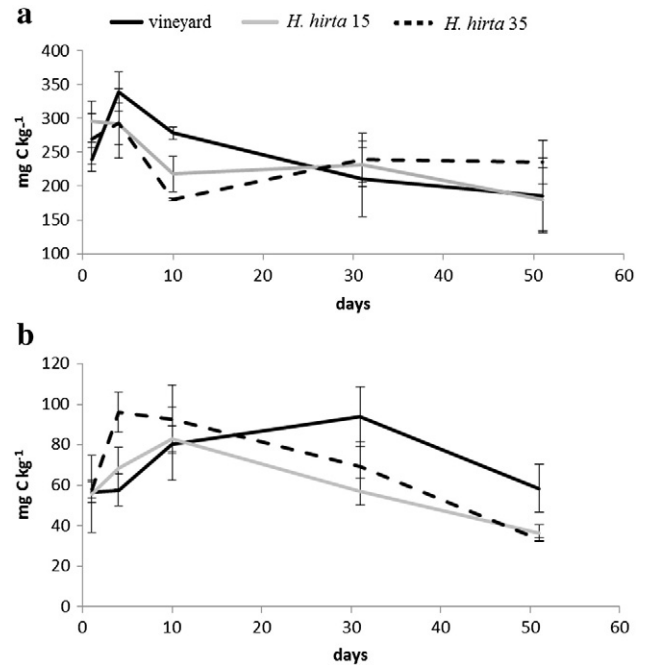


Fig. 4. Microbial biomass carbon (MBC) (a) and dissolved organic carbon (DOC) (b) during the incubation experiment in soil under vineyard (black line), *Hypparrhenia hirta* 15 (gray line) and *Hypparrhenia hirta* 35 (dotted line). Bars are standard deviations ($n = 9$).

roots) was likely the main source of DOC in *H. hirta* soils. Similar small contributions of DOC derived from recent vegetation have been observed in labeled litter experiments under organic layers and in mineral soils of mature forest ecosystems (Fröberg et al., 2007; Guelland et al., 2013; Kammer et al., 2012; Müller et al., 2009). These small inputs of C from more recent vegetation to recent DOC imply both its strong retention onto mineral soil colloidal surfaces and its fast biodegradation (mineralization and incorporation into MBC) (Kaiser et al., 1996; Kalbitz et al., 2003). In our study, the small portion of $\text{C}_4\text{-DOC}$ from *H. hirta* was attributable to higher mineralization and incorporation into MBC as compared to $\text{C}_3\text{-DOC}$. Our assumption is confirmed by the higher $\text{C}_4\text{-C}/\text{C}_3\text{-C}$ ratio in MBC and CO_2 in comparison to that in DOC.

4.3. Dynamics of C pools after vineyard abandonment

The contribution of ^{13}C fractionation per se and preferential utilization of recent versus old C was analyzed by comparing the $\delta^{13}\text{C}$ values during the incubation of both C_3 (vineyard) and $\text{C}_3\text{-C}_4$ soils. The

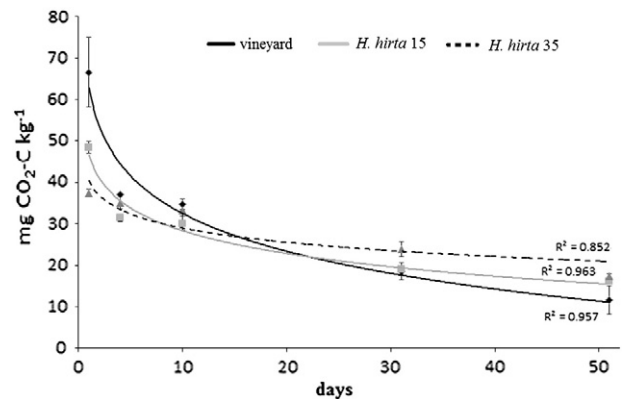


Fig. 5. CO_2 flux rates during the 51 days of incubation in vineyard (black line), *Hypparrhenia hirta* 15 (gray line) and *Hypparrhenia hirta* 35 (dotted line). Bars are standard deviations ($n = 9$). Data were fitted to the following first-order decay function: Mineralized C = $C_r e^{-kt}$ where C_r is the readily mineralizable C at time zero, k is the decay rate constant and t is the time.

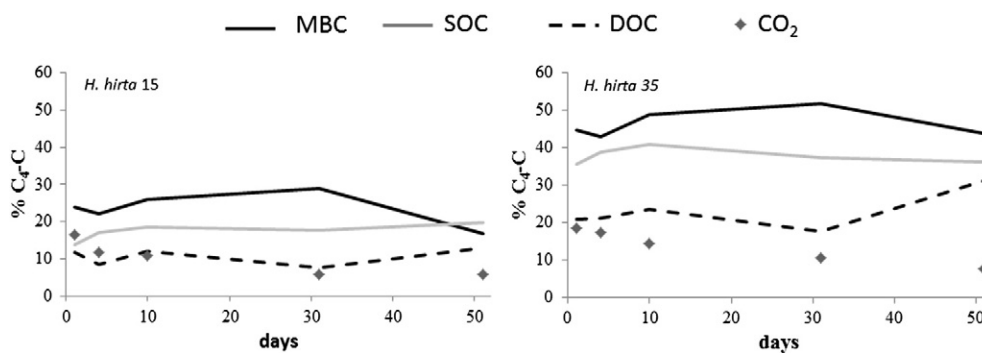


Fig. 6. Relative contribution of recent C_4 to soil organic carbon (SOC), CO_2 rate, microbial biomass carbon (MBC) and dissolved organic carbon (DOC) during the 51 days of incubation of *Hyparrhenia hirta* 15 (*H. hirta* 15) and *Hyparrhenia hirta* 35 (*H. hirta* 35) soils.

differences in $\delta^{13}C$ values among SOC, MBC, DOC and CO_2 in C_3 soil are related solely to ^{13}C fractionation, because this soil is under isotopic steady state (Werth and Kuzyakov, 2010). On the contrary, the soil under *H. hirta* is not under isotopic steady state and, therefore, the differences of $\delta^{13}C$ values among the pools are the results of both ^{13}C fractionation and preferential microbial utilization of recent versus old C. Comparison of $\delta^{13}C$ differences between the respective pools in C_3 and C_3 – C_4 soils allows us to separate the effects of ^{13}C fractionation and those of preferential utilization of substrates with a different C age.

The maximal range of difference in $\delta^{13}C$ among C pools under vineyard soil was smaller (1.38‰) than that under *H. hirta* 15 (3.58‰) and under *H. hirta* 35 (4.18‰) (Fig. 3). At the start of incubation, the CO_2 from soil under vineyard was enriched in comparison to SOC, indicating that during mineralization ^{13}C enriched substances from SOC were used. Consequently, MBC also gets enriched. The ^{13}C enrichment of MBC compared to SOC in C_3 soil (vineyard) was 1.38‰, a value comparable with those of other studies where the ^{13}C enrichment of MBC ranged from 0.9‰ (Blagodatskaya et al., 2011) to 1.1‰ (De Troyer et al., 2011).

In C_3 – C_4 soils, besides ^{13}C fractionation, preferential substrate utilization contributed to a much higher range in $\delta^{13}C$ differences among the C pools. This caused a ^{13}C enrichment of MBC in comparison to SOC of +2.75‰ and +2.28‰ for *H. hirta* 15 and *H. hirta* 35, respectively (Fig. 3). Consequently, the MBC in C_3 – C_4 soils used more recent (^{13}C -enriched) versus old C (relatively ^{13}C -depleted) sources.

In C_3 – C_4 soils of our study, $\Delta^{13}C$ (CO_2 -SOC) was higher than $\Delta(CO_2$ -MBC). This is in disagreement with results found by Blagodatskaya et al. (2011) and Werth and Kuzyakov (2010) obtained for soils under humid climates. The discrepancy could be ascribed to the various mechanisms included in microbial consumption and respiration of SOC developed under different climate conditions. In arid zones, soil microorganisms do not always preferentially decompose the most available SOC pools: during periods with high temperature and sufficient soil moisture they are also able to utilize recalcitrant SOC (Werth and Kuzyakov, 2010).

The $\Delta(CO_2$ -SOC) was highest at the first stage of succession, where SOC was still composed of 86% C_3 -C (Fig. 2). The absolute $\delta^{13}C$ values of CO_2 increased from *H. hirta* 15 to *H. hirta* 35 contemporary to the increase of the C_4 -C portion of SOC.

The effect of preferential utilization of young substrates was more pronounced in *H. hirta* 15 than in *H. hirta* 35, as confirmed by the value of $\Delta(CO_2$ -SOC) (Fig. 3). This is because the smaller portion of young C (which has C_4 signature) in *H. hirta* 15 led to higher differences in the substrates utilized by microorganisms. Under *H. hirta* 35, a depletion of $\delta^{13}C$ in the DOC pool compared to SOC was registered. This indicates that the available C_4 -C portion of DOC was involved in MBC turnover and, therefore, it was enriched by ^{13}C . The same happened in the soil of *H. hirta* 15, but the magnitude of this process on $\delta^{13}C$ value

of DOC was less relevant, due to lower C_4 -C portion of SOC. Moreover, it was supposed that from 15 to 35 years since vineyard abandonment, the old C_3 -C carbon was not only completely mineralized but was also decomposed to soluble monomers, and therefore the DOC got depleted in $\delta^{13}C$. This result is confirmed by the decreased portion of C_3 -C from *H. hirta* 15 to *H. hirta* 35 (Fig. 2).

5. Conclusions

This study highlighted the effects of vegetation succession after cessation of agricultural use on C dynamics in soil. Based on $\delta^{13}C$ signature of C pools in C_3 soil and C_3 – C_4 soils of two different ages, the pathways and contribution of each pool to C sequestration and turnover were estimated. The SOC content measured in the secondary vegetation succession showed that, in a semiarid environment, the abandonment of cultivated vineyard increased organic matter content by 13% after 15 years. The significant increase of SOC after 15 years of abandonment was mainly attributable to new above- and below-ground biomass C input from *H. hirta*, as highlighted by the contribution of C_3 -C and C_4 -C to SOC. The higher metabolic quotient in soil under vineyard denotes a lower C use efficiency of microbial biomass and/or a higher disturbance compared to soil under *H. hirta*. The increase in MBC, combined with the decrease of basal respiration after the abandonment of vineyard soil, suggested that the soil under *H. hirta* was becoming more stable.

Comparing the $\delta^{13}C$ values during the incubation of C_3 soil (vineyard) and C_3 – C_4 soils, the contribution of ^{13}C fractionation and preferential utilization of recent versus old C was analyzed. Although MBC in C_3 – C_4 soil used more recent (^{13}C -enriched) versus old C (relatively ^{13}C -depleted) sources, the $\Delta^{13}C$ by decomposition of SOC to CO_2 ($\delta^{13}C$ of CO_2 minus $\delta^{13}C$ of SOC) was higher than $\Delta^{13}C$ by microbial respiration (CO_2 minus MBC). This finding could demonstrate that under semiarid climate, soil microorganisms do not always preferentially decompose the most available SOC pools: during periods with high temperature and sufficient soil moisture they might be able to utilize recalcitrant SOC.

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