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Carbon dynamics of soil organic matter in bulk soil and aggregate fraction during secondary succession in a Mediterranean environment

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ABSTRACT

Clarifying which factors cause an increase or decrease in soil organic carbon (SOC) after agricultural abandonment requires integration of data on the temporal dynamics of the plant community and SOC. A chronosequence of abandoned vineyards was studied on a volcanic island (Pantelleria, Italy). Vegetation in the abandoned fields was initially dominated by annual and perennial herbs, then by *Hyparrhenia hirta* (L.) Stapf, and finally by woody communities. As a consequence, the dominant photosynthetic pathway changed from C_3 to C_4 and then back to C_3. Conversion of a plant community dominated by one photosynthetic pathway to another changes the $^{13}C/^{12}C$ ratio of inputs to SOC. Using the time since abandonment and the shift in belowground $\delta^{13}C$ of SOC relative to the aboveground $\delta^{13}C$ plant community, we estimated C_3-C and C_4-C changes during secondary succession.

SOC content (g kg^{-1}) increased linearly ($R^2 = 0.89$ and 0.73 for 0–15 and 15–30 cm soil depth) with the age of abandonment, increasing from 12 g kg^{-1} in cultivated vineyards to as high as 26 g kg^{-1} in the last stage of the succession. $\delta^{13}C$ increased in the bulk soil and its three aggregate fractions (>250, 250–25, and <25 μm) during succession, but the effect of soil depth and its interaction with succession age were significant only for soil aggregate fractions. Polynomial curves described the change in $\delta^{13}C$ over the chronosequence for both depths. $\delta^{13}C$ in the bulk soil had increased from −28‰ to −24‰ by 35 years after abandonment for both depths but then decreased to −26‰ at 60 years after abandonment (corresponding with maturity of the woody plant community). Overall, the results indicate that abandoned vineyards on volcanic soil in a semi-arid environment are C sinks and that C storage in these soils is closely related to plant succession.

1. Introduction

Because of human activities, the concentration of atmospheric CO_2 is increasing rapidly while the long-term storage capacity of terrestrial and ocean ecosystems is declining (Canadell et al., 2007). Understanding the role played by soil in global C dynamics requires estimation of soil carbon (C) stocks. Because more C is stored in the soil than in vegetation or the atmosphere (Eswaran et al., 2000), changes in soil C content could greatly affect the atmosphere (Lal, 2004).

The effects of the conversion of native vegetation to cropland or pasture on C storage are well known (Del Galdo et al., 2003; Desjardins et al., 2004; Romkens et al., 1999). In contrast, less is known about the dynamics of soil organic carbon (SOC) after agricultural abandonment, and this is especially true for Mediterranean areas (Alberti et al., 2011; La Mantia et al., 2007). Soil carbon dynamics after the abandonment of cultivated land is connected to the development of the natural vegetation through secondary succession processes (Kosmas et al., 2000; Martinez-Fernandez et al., 1995; Van Rompaey et al., 2001). There is some evidence that abandonment of agricultural land and the subsequent regeneration of forests through secondary succession may return C storage to the pre-agricultural levels, although the rate of recovery depends on the time one considers and whether the land was previously used for crops or pasture (Guo and Gifford, 2002; Post and Kwon, 2000).

A major factor affecting the dynamics of SOC after the abandonment of an agricultural land is climate (Alberti et al., 2011). Jinbo et al. (2007) found that abandonment led to an increase in SOC in a favourable (medium rainfall and high temperatures that supported high primary productivity) climate but a decrease in SOC in an unfavourable climate. This finding, however, was not confirmed by other experimental evidence. For example, abandonment under climatic conditions that limit primary production (as in Mediterranean climate) caused an increase in the soil C stock (Alberti et al., 2011). Similarly, a lack

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of soil disturbance in a semi-arid environment resulted in an increase of C in the soil surface (Alvaro-Fuentes et al., 2009).

Furthermore, the increase of SOC content is determined by the incorporation of new organic matter in the coarse fraction and the reduction of mineralisation processes in the finest ones (Barbera et al., 2012; Ouédraogo et al., 2005). While during the early stages of secondary succession the increase of carbon stock is mainly due to anthropic disturbance reduction, in the older stages of succession it is caused by an increasing plant productivity, which is generally found along secondary succession in mesic Mediterranean conditions, and which has been confirmed also during old field succession on Pantelleria Island (La Mantia et al., 2007, 2008). It is not clear whether SOC content can continue to increase as a function of time since agricultural abandonment or whether there is some limit. Whether SOC accumulates or decreases greatly depends on how interactions between climate and soil type affect SOC mineralisation rates and/or accumulation in soil.

SOC mineralisation can be reduced within soil aggregates in comparison to bulk soil, and the formation and turnover of soil aggregates are affected by agricultural abandonment and other changes in land management (Schimel, 1995). There is still lack of knowledge on how management conditions and their abandonment affect aggregate formation and the protection of SOC. Generally, micro-aggregates (<250 μm) (Tisdall and Oades, 1982) are relatively stable and are bound by persistent polysaccharide-based glues produced by roots and microbes and by calcium bridges. On the other hand, micro-aggregates are bound into macro-aggregates (>250 μm) by a network of roots and hyphae. Therefore, macro-aggregate stability is thought to respond more rapidly to changes in land use. Several authors have reported that the aggregate stability and SOC content of stable macro-aggregates were higher in native grassland than in cultivated fields (Cambardella and Elliot, 1992; Six et al., 2002). Others reported a higher mean residence time of SOC for reduced-tilled soils (Collins et al., 1999; Six et al., 2002), and the increased residence time was attributed to an increased physical protection of soil aggregates in the absence of disturbance.

As noted, the physical protection of SOC provided by aggregates favours SOC accumulation whereas cultivation tends to break aggregates apart and therefore increases SOC mineralisation. The inclusion of SOC in soil aggregates and the mineralisation of SOC in broken aggregates are accompanied by changes in the chemical structure of SOC. These processes have been studied with the aid of $\delta^{13}C$ analysis (Buzek et al., 2009; Desjardins et al., 1994, 2004, 2006; Wookey et al., 2002).

After agricultural abandonment, old fields are spontaneously colonised by various plants, a gradual process during which different plant communities develop (secondary succession). In general, annual and perennial herb communities dominate early and are then partially or completely replaced by perennial grasses, shrubs, and/or trees. If during succession there is a switch between C_3 and C_4 photosynthetic pathways, the $^{13}C/^{12}C$ ratio ($\delta^{13}C$) of inputs to SOC is modified. Using the time since perturbation and the shift in belowground $\delta^{13}C$ of SOC relative to the aboveground $\delta^{13}C$ of the plant community, researchers can estimate the SOC turnover rate (Wolf et al., 1994). SOC turnover rates have been estimated when C_4-dominated natural grasslands were converted to C_3 annual crops and perennial pastures (Balesdent et al., 1990; Follet et al., 1997), or when C_3-dominated natural forests and grasslands were converted to C_4 annual crops and perennial pastures (Balesdent et al., 1987; Gregorich et al., 1995; Jastrow et al., 1996), but no data are available for secondary successions characterised by a C_3–C_4–C_3 pathway. When separated on the basis of chemical composition, aggregate size, or particle density, SOC aggregate fractions have been shown to differ in age and thus in turnover rate (Balesdent et al., 1990; Jastrow et al., 1996).

The present study analyses the change in soil carbon stock along a secondary succession after agricultural abandonment describing differences in SOC turnover rates along succession for all soil aggregate fractions. The study of soil carbon dynamics linked to spontaneous secondary succession processes has been identified as a priority in policy-oriented research since data on natural post-abandonment soil evolution is lacking (Zanchi et al., 2007). Furthermore, the present study evaluates SOC turnover using changes in the natural abundance of $\delta^{13}C$. This evaluation contributes to the knowledge on aggregate formation and the protection of SOC under different management systems, and provides data on SOC turnover along a C_3–C_4–C_3 succession pathway.

2. Materials and methods

2.1. Study and sampling area

The study was carried out in cultivated and abandoned terraced vineyards on Pantelleria Island (Italy), which is situated in the rift of the Sicilian Channel (83 km^2 surface area; 36°44′ N, 11°57′ E) (Fig. 1a). Pantelleria is of volcanic origin, its highest summit is *Montagna Grande* (836 m a.s.l.), and its surface rocks are mainly acidic, silicic vulcanites (pantellerites and trachytes). The most frequent soils are Lithosols, Regosols (mainly escalic), and Cambisols (mainly vitric). The island is semi-arid (Thornthwaite and 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 25.6 °C (August) to 11.7 °C (January).

Except for the most recent lava flows, Pantelleria has over the centuries been almost entirely terraced for agriculture. Currently, only about 20% of these terraces is still cultivated with grapevine, caper, and olive trees (Rühl, 2007), while the rest have been abandoned by agriculture. This abandonment has continued since the 1950s; it first affected the most inaccessible and marginal areas, and then the more accessible ones. Because of secondary succession dynamics, natural vegetation has spread spontaneously into the old fields.

The rapidity and course of these colonisation processes depend on various factors such as exposure (Rühl et al., 2006), the presence of favourable microsites for germination and growth of woody species (safe-site-effect), and the distance of seed sources from the old field (neighbourhood-effect; Rühl and Schnittler, 2011). Thus, the current landscape of Pantelleria is characterised by a mosaic of patches of old fields differing in abandonment age and degree of woody colonisation.

As a consequence of these differing factors, succession dynamics on Pantelleria can be slow or fast but in any case lead to the formation of the same maquis-wood community dominated by *Quercus ilex* L. (Rühl et al., 2006). For the first 5 years after abandonment, these fields are dominated by annual and perennial herbs. Under uncultivated conditions and perennial herb encroachment, the grape vines persist for some years, but their biomass productivity is very low. Then, the cover of woody species increases rapidly, so that shrub communities are present about 15–20 years after abandonment, with the most common species being *Arbutus unedo* L., *Cistus* L. sp. pl., *Erica arborea* L., *Erica multiflora* L., *Phillyrea latifolia* L., *Pistacia lentiscus* L., and *Q. ilex*. Old fields characterised by slow succession dynamics, in contrast, are south-facing and/or are located far from woody seed sources; in these fields, colonisation by woody species is slower and often follows an intermediate stage that is dominated by the C_4 grass *Hyparrhenia hirta*. The period of the *Hyparrhenia* grassland stage depends on the distance of woody seed sources. Apart from the community dominated by *H. hirta*, all other communities occurring during local secondary succession are dominated by C_3 plants.

The sampling area was situated on a south-facing slope where still-cultivated and abandoned terraced vineyards were present in close proximity to each other (no more than 50 m). Environmental factors such as geological substrate, soil type (in terms of soil texture), exposure values, etc., can be regarded as homogeneous for all samples.

Soil samples were taken in seven terraces which represent stages of secondary succession of vine old fields (chronosequence approach). In

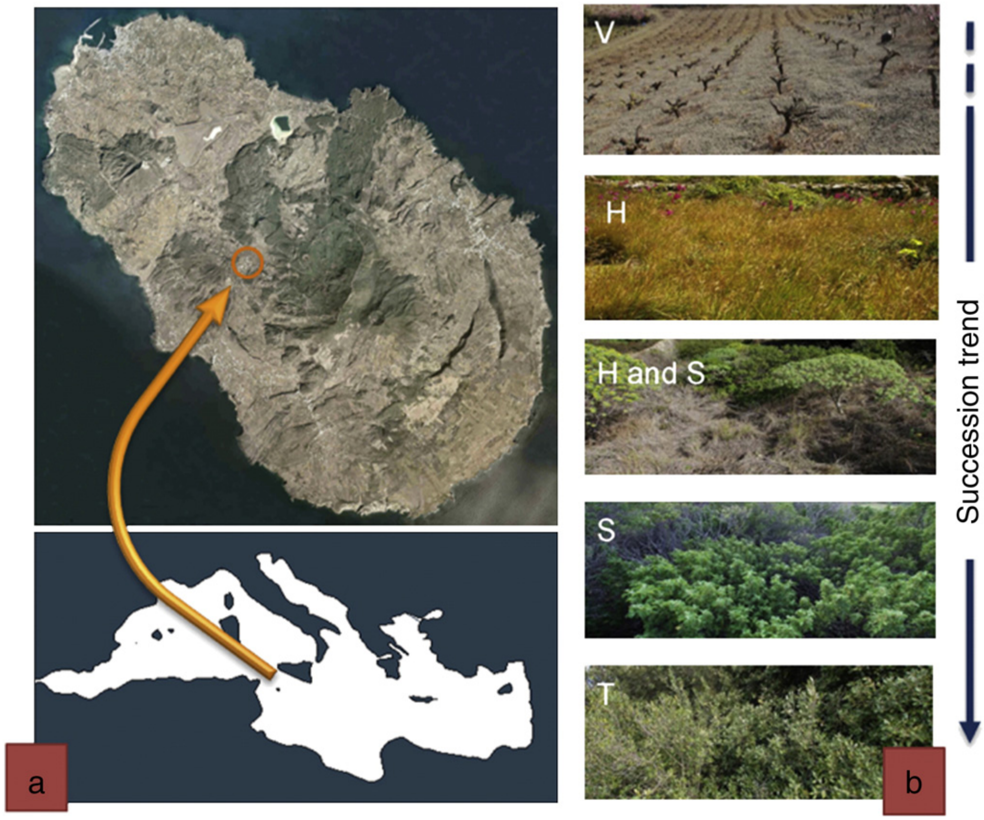

Fig. 1. Aerial photograph of Pantelleria Island (a) and plant succession after vineyard abandonment (V, vineyard; H, *Hyparrhenia hirta*; S, shrubs; T, trees). H is a C4 community and V, S, and T are C3 communities (b).

fact, the selected terraces host different plant communities (herb community, *Hyparrhenia* grassland, *Hyparrhenia* grassland with shrubs, shrubland, and maquis) as a function of their abandonment age and their distance from woody seed sources (Table 1 and Fig. 1b). The abandonment age of the terraces was determined using aerial photographs taken in 1954, 1968, 1979, 1987, 1992, and 2000 (Rühl, 2007; Rühl and Schnittler, 2011; Rühl et al., 2005, 2006).

2.2. Soil sampling and processing

In each of the seven terraces, three soil samples were collected at 0–15 cm and 15–30 cm depth, respectively. Soil samples were taken randomly in an area of about 20 m^2 in terraces covered by uniform vegetation, and in the transition zone between *Hyparrhenia* and shrub cover in those terraces characterised by a mosaic of species (terraces 3 and 6).

The soil was air-dried and passed through a 2-mm sieve. The organic fragments (plant material and root residues) were removed using electrostatic method (Kuzyakov et al., 2001). To reduce the error tolerance to less than ±5%, 2–4 kg of soil (Hitz et al., 2002) were collected per sample. The soil texture was 60% sand, 25% silt, and 15% clay, and did not statistically differ among soil samples and depths. Because of its volcanic origin, soil contained no carbonate. The pH was 6.8 ± 0.3.

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

$$C\,stock\left(\text{Mg ha}^{-1}\right) = BD * C\,conc * D * CF\,coarse \qquad (1)$$

where *C conc* is carbon content (g/100 g), *BD* is bulk density (Mg m^{-3}), *D* is depth thickness (m), and *CF coarse* is a correction factor (1 − (Gravel % + Stone %)/100).

Wet aggregate fractions, without prior chemical dispersion, were isolated by mechanical shaking of 100 g of air-dried fine soil on a column of sieves with pore sizes >250, 250–25, and <25 μm using a Shaker AS 200 Sieve (RETSCH analytical, 203-mm sieves) (amplitude of 2 cm, frequency of 1.6 Hz and a water flux of 2 l min^{-1}). After the physical fractionation, we distinguished three main aggregates of soil based on size: >250 μm (macro-aggregates, of particulate organic matter), 25–250 μm (micro-aggregates), and <25 μm (silt and clay fraction).

Table 1
Abandonment age, distance of woody seed source, and vegetation type present in the studied fields. Close and distant woody seed sources were <50 m and >100 m, respectively, from the sampled field.

Terrace	Abandonment (years)	Seed source of woody species	Vegetation
1	Cultivated	Close	Vineyard
2	15	Close	*Hyparrhenia hirta* grassland (without woody individuals)
3	28	Close	Mosaic of 50% *Hyparrhenia hirta* grassland mixed with 50% shrub cover (*Euphorbia dendroides*, *Cistus creticus*, *Phillyrea latifolia*, *Quercus ilex*)
4	30	Close	Shrubland of *Cistus creticus* and *Euphorbia dendroides*
5	35	Distant	*Hyparrhenia hirta* grassland (without woody individuals)
6	40	Distant	Mosaic of 50% *Hyparrhenia hirta* grassland mixed with 50% shrub cover (*Phagnalon saxatile*, *Phillyrea latifolia*, *Rubus ulmifolius*)
7	60	Close	*Quercus ilex* and *Phillyrea latifolia* maquis

Table 2
Results of statistical analysis for the effects of succession (years of abandonment), soil depth, and their interaction on SOC (g kg^{-1} of bulk soil) and $\delta^{13}C$ for bulk soil and soil fractions. Single and double asterisks indicate $P<0.05$ and 0.01, respectively in ANOVA, ns indicates $P>0.05$.

	Bulk soil			Soil fractions					
				>250 μm		250–25 μm		<25 μm	
Source of variance	SOC	$\delta^{13}C$	N	SOC	$\delta^{13}C$	SOC	$\delta^{13}C$	SOC	$\delta^{13}C$
Succession (S)	**	**	**	**	**	**	**	**	**
Depth (D)	*	ns	**	*	**	**	**	**	**
S×D	ns	ns	**	**	**	**	**	**	**

2.3. Chemical analysis

For each soil aggregate fraction, the relative weight distribution, the C content, and $\delta^{13}C$ abundance were measured. Total N was measured only for the bulk soil. SOC and N content were measured using an elemental analyser (NA1500 Carlo Erba, Milan, Italy). For the $\delta^{13}C$ analysis, an EA-IRMS (elemental analyser isotope ratio mass spectrometry) was used. The reference material used for analysis was IA-R001 (Iso-Analytical Limited 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 samples for the analysis. The International Atomic Energy Agency (IAEA), Vienna, distribute IAEA-CH-6 as a reference standard material.

The results of the isotope analysis are expressed as a δ value (‰) relative to the international Pee Dee Belemnite standard as follows:

$$\delta(‰) = \frac{Rs - Rst}{Rst} * 1000 \quad (2)$$

where $\delta=\delta^{13}C$, $R={}^{13}C/{}^{12}C$, s = sample, and st = standard.

2.4. Data analysis

Distinct vegetation types present along succession were considered: 1) annual C_3 herb communities (still cultivated fields), 2) perennial C_4 *H. hirta* grasslands, 3) perennial C_4 *H. hirta* grasslands colonised by C_3 shrubs, 4) C_3 shrublands, and 5) C_3 *Q. ilex* high maquis (Table 1). We defined that the term new species refers always to the new vegetation type colonising a terrace with time with respect to the old vegetation type which previously dominated. For example, when the present vegetation type is perennial C_4 *H. hirta* grassland, we defined annual C_3 herb communities of the still cultivated fields as the previous vegetation type and *H. hirta* as the new colonising species.

In this way, natural abundance of $\delta^{13}C$ was used to determine the proportion of C in SOC that was derived from the new vegetation type (C_3 or C_4) and how much C remained from the previous vegetation type in each soil aggregate fraction. These proportions were calculated by the mixing equation (Gearing, 1991):

$$New\ carbon\ derived(Ncd)(\%) = \frac{\left(\delta^{13}C\ New - \delta^{13}C\ old\right)}{\delta^{13}C\ biomass\ new\ species - \delta^{13}C\ old} \quad (3)$$

and

$$\text{Old carbon derived (Ocd)}(\%) = 1 - \text{Ncd} \quad (4)$$

where Ncd is the fraction of soil C derived from the new vegetation type, $\delta^{13}C_{new}$ is the isotope ratio of the present soil sample, $\delta^{13}C_{biomass\ new\ species}$ is the isotope ratio of the colonising species, and $\delta^{13}C_{old}$ is the isotopic ratio of the soil of the precedent vegetation type. Species biomass $\delta^{13}C$ was measured for cultivated vineyards (27.5 ± 2.1‰), *H. hirta* grassland (11.1 ± 1.2‰) and shrubland (27.1 ± 1.6‰); the mass balance equation was used to estimate $\delta^{13}C$ for the other vegetation types.

The mass of new carbon additions was calculated both for bulk soil and each soil fraction (Eq. (5)).

$$\text{New Carbon}\left(\text{Mg C ha}^{-1}\right) = \text{C soil (Mg ha}-1) * \text{Ncd}\ (\%) \quad (5)$$

2.5. Statistical analysis

The data for mass content, total soil C, new vegetation type-derived C, C content, and C content for each fraction were analysed by analysis of variance (ANOVA) for a completely randomised block. Differences between means were tested with the LSD test at $P<0.05$. SAS statistical programs were used (SAS Institute, 2001). Time since the abandonment was regressed against new and old carbon derived in the same plot both for bulk soil and its fractions.

3. Results

3.1. Total C and N

SOC content (g kg^{-1}) increased linearly ($R^2=0.89$ and 0.73 for 0–15 and 15–30 cm soil depth) with the age of abandonment; it increased from 12 g kg^{-1} in the cultivated vineyard (terrace 1) to 26 g kg^{-1} in the vineyard abandoned 60 years earlier (terrace 7) (Table 2, Fig. 2a). There was no interaction between age and depth,

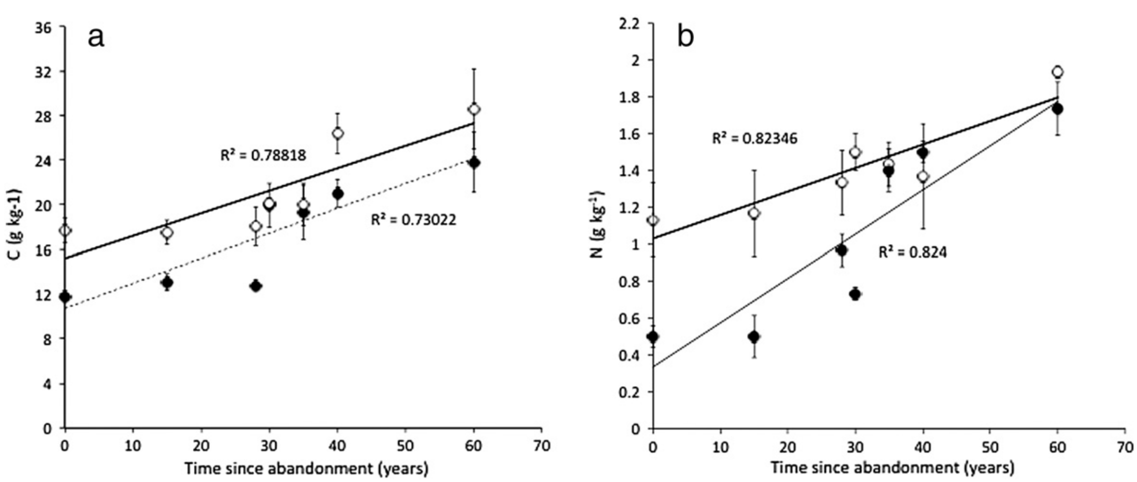

Fig. 2. SOC (g kg^{-1}) (a) and total nitrogen (g kg^{-1}) (b) along the chronosequence at the two depths (○ 0–15 cm; ● 15–30 cm).

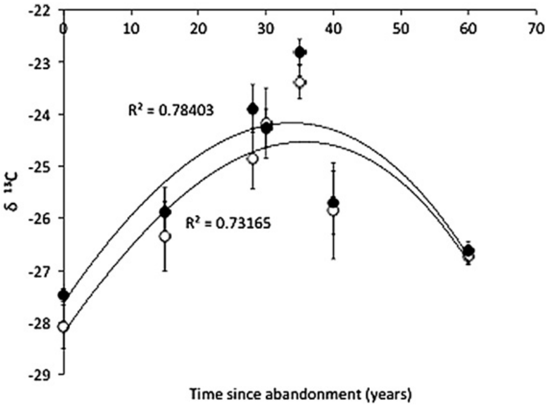

Fig. 3. δ^{13}C values along the chronosequence for the two depths (○ 0–15 cm; ●15–30 cm).

and SOC content was greater near the surface (0–15 cm) than deeper (15–30 cm) in the soil (Fig. 2a); after 60 years of abandonment, SOC content was 28 g kg^{-1} at 0–15 cm and 26 g kg^{-1} at 15–30 cm.

Interestingly, we found a noteworthy difference in SOC between the two terraces of the vegetation type perennial C_4 *H. hirta* grasslands colonised by some individuals of C_3 shrubs (in average 18 vs 27 g kg^{-1} in 0–15 and 13 vs 21 g kg^{-1} in 15–30, respectively, for the terraces abandoned 28 and 40 years ago).

Nitrogen content also increased with abandonment age, but with a highly significant interaction between depth and age such that the two regressions converged 60 years after abandonment (Table 2, Fig. 2b).

3.2. δ^{13}C values in bulk soil and fractions

δ^{13}C values in bulk soil and in the three fractions were significantly affected by succession (years since abandonment) (Table 2). Depth and its interaction with succession were significant for the fractions but not for the bulk soil (Table 2).

Changes in δ^{13}C values for the two depths over the course of the secondary succession were described by highly significant polynomial curves ($R^2 = 0.82$ for 0–15 cm and 0.73 for 15–30 cm) (Fig. 3). δ^{13}C values in the bulk soil increased from −28‰ at time of abandonment to −24‰ at 30 years after abandonment for both soil depths. δ^{13}C values subsequently decreased to −26‰ 60 years after abandonment, corresponding to the maturity of the woody community. A similar trend occurred for both macro and micro-aggregates at both depths (Tables 3 and 4). The silt–clay aggregate fraction did not reduce variability along the chronosequence and maintained the same trend of bulk soil, macro and micro-aggregate fractions.

3.3. Soil and C mass of the three soil fractions

The mass of macro-aggregates (>250 μm) was greater than that of the two smaller aggregate fractions and did not change substantially with abandonment age at either depth (Fig. 4). In contrast, the mass of the micro-aggregates (25–250 μm) increased while that of the smallest aggregate fraction (<25 μm) decreased during the 60 years after abandonment. For the two depths, 500 and 450 g kg^{-1} of the smallest aggregate fraction were measured, respectively, in the still cultivated vineyard.

Following abandonment, SOC content in the 0–15 soil layer changed more in the intermediate soil aggregate fractions (25–250 μm) than in the larger or smaller aggregates; in the intermediate aggregate, SOC content jumped from <5 g kg^{-1} of C in old fields abandoned less than 35 years ago to >10 g kg^{-1} of C in old fields abandoned more than 35 years ago (Table 3). The SOC content of macro-aggregates, except for vineyard in the 15–30 soil layer, did not show any statistical differences underlining the high stability of SOC in this fraction.

3.4. SOC derived from the new vegetation type in bulk soil and in aggregate fractions

The effect of change of vegetation (from C_3 to C_4 and again to C_3) was easily explained by estimates of carbon derived from soil in the chronosequence. At both depths in the bulk soil, the proportion of carbon derived from the new vegetation type increased linearly ($R^2 = 0.83$ for 0–15 and $R^2 = 0.78$ for 15–30; Fig. 5). The quantity of old SOC was greater than that of new SOC until year 40 but the opposite was true at year 45 and later in the chronosequence. The trend was similar for macro-aggregates (>250 μm) (Fig. 5) except that the quantity of new carbon exceeded old carbon about 35 years after abandonment. In the other two aggregate fractions, the two lines either intersected or at least met much later after abandonment (Fig. 5). The effect of succession (from C_3 to C_4 and from C_4 to C_3) on C stock was highlighted by the estimates of C derived from *H. hirta* and from vineyard (Fig. 6). The contribution of new C for each stage of succession in the soil fractions decreased proportionally with aggregate size (Fig. 6). In the smallest aggregate fraction, new C addition was inconsequential and did not statistically differ over time.

After 15 years of cultivation abandonment 90% of the C had a vineyard origin and the C_3-C content slowly decreased with a rate of 0.13 Mg ha-1y^{-1}. In 15 years after abandonment C_4-C contribution to SOC from *H. hirta* was of 2.5 Mg ha^{-1} in 0–15 cm soil depth and 2 Mg ha^{-1} in 15–30 cm. The rate of C4-C increase (0.16 Mg ha^{-1} y^{-1}) was higher than C_3-C decrease, determining a SOC increase and at the same time the C derived from previous vegetation was preserved.

In the intermediate stage (after 28 years of abandonment) the C_3-C remained constant in comparison to previous stage, while C_4-C increased with a rate of 0.42 Mg ha^{-1}.

In the last stage of succession (after 60 years) the rate of C_3-C increase was slightly higher than C_4-C rate of decrease. We observed that the rate of C_4-C change (increase or decrease) after vegetation conversion was slower in comparison to C_3-C portion. In general the C_3-C enrichment was faster in top soil than sub soil. On the contrary, under *H. hirta* grassland the C_4-C increase was highest in the 15–30 cm depth.

Table 3
SOC (g C kg^{-1} bulk soil) and δ^{13}C in bulk soil and fractions in the seven terraces of the chronosequence at 0–15 cm depth. Values in a column followed by different letters are significantly different ($P \leq 0.05$).

	Bulk soil				Soil fractions >250 μm				250–25 μm				<25 μm			
Terrace	SOC		δ^{13}C		SOC		δ^{13}C		SOC		δ^{13}C		SOC		δ^{13}C	
1	17.7	b	−28.08	a	7.0	b	−28.40	a	3.5	b	−28.10	a	7.2	a	−27.90	a
2	16.2	b	−26.34	b	8.9	b	−26.70	b	5.1	b	−27.11	a	2.2	b	−25.70	a
3	18.1	ab	−24.85	c	11.5	a	−24.26	c	4.2	b	−26.00	a	2.4	b	−25.77	a
4	20.1	ab	−24.18	c	13.8	a	−23.64	c	4.9	b	−24.61	a	1.4	b	−24.59	b
5	20.0	a	−23.39	c	7.6	b	−23.49	c	10.0	a	−23.34	b	2.2	b	−23.93	b
6	26.4	a	−25.74	b	10.0	a	−26.86	b	11.5	a	−26.70	a	4.9	b	−26.49	a
7	28.7	a	−26.62	b	11.0	a	−27.10	b	15.0	a	−26.36	a	2.6	b	−25.98	a

Table 4
SOC (g C kg^{-1} bulk soil) and δ^{13}C in bulk soil and fractions in the seven terraces of the chronosequence at 15–30 cm depth. Values in a column followed by different letters are significantly different (P≤0.05).

					Soil fractions											
	Bulk soil				>250 μm				250–25 μm				<25 μm			
Terrace	SOC		δ^{13}C		SOC		δ^{13}C		SOC		δ^{13}C		SOC		δ^{13}C	
1	11.7	b	−27.48	a	5.2	b	−26.96	a	2.5	b	−27.32	a	4.0	a	−27.26	a
2	13.1	b	−25.88	b	9.5	a	−25.05	a	2.7	b	−24.96	b	0.9	b	−24.69	b
3	12.7	ab	−23.90	c	8.5	a	−24.68	a	3.5	b	−25.75	b	0.7	b	−25.65	b
4	19.9	ab	−24.27	c	13.3	a	−23.10	b	2.9	b	−24.44	b	3.7	a	−24.46	b
5	19.3	a	−22.81	c	9.3	a	−22.50	b	6.3	a	−22.95	b	3.7	a	−23.81	b
6	21.0	b	−25.70	b	9.3	a	−27.21	a	9.9	a	−26.58	a	1.8	a	−26.51	a
7	23.8	b	−26.62	b	13.1	a	−27.07	a	7.1	a	−26.26	a	3.6	a	−26.25	a

4. Discussion

In the semi-arid Mediterranean environment, where climate is one of the major driving forces for determining both rate of vegetation community turnover within secondary succession, and the period necessary to complete conversion (new steady state), knowledge of C stock and the resilience of the soil must be increased. The results of the current study indicate that, following abandonment of vineyards on volcanic soil in a semi-arid Mediterranean area, the soil acts as a C sink. This finding is consistent with previous reports on semi-arid environments (Desjardins et al., 1994; Lisboa et al., 2009). We found a noteworthy difference in SOC between two terraces of the same vegetation type (*Hyparrhenia* grassland mixed with shrubs) but of different abandonment age (28 vs 40 years), which indicates that for SOC accumulation abandonment age is a more important factor than vegetation type.

Furthermore, the current study documented an increase in total soil nitrogen with time since abandonment, and this finding is consistent with other studies of succession in Mediterranean regions (Bonet, 2004; Robertson and Vitousek, 1981). Studies in semi-arid regions showed that C losses may occur only if there is a substantial decrease in soil N stock and that the rate of change in both of these stocks is related to annual rainfall (Halliday et al., 2003; Kirschbaum et al., 2008).

Secondary succession is often accompanied by changes in soil structure that affect C storage. In the last stages of a succession, the increase in litter and roots can favour the formation of macro-aggregates and therefore an increase in the total C stock (Tisdall and Oades, 1982). The volcanic soil in the current study contained a large percentage of sand, which may explain why macro-aggregates increased only slightly in the chronosequence and were not associated with the increase in SOC. In contrast, the increase in SOC was associated with the substantial increase in the intermediate aggregates (the micro-aggregates, 25–250 μm). Previous reports indicated that both the micro-aggregate and silt–clay fractions contribute to SOC increase and stabilisation (Six et al., 2002). In the present study, the increase in SOC was not associated with the silt–clay fraction (<25 μm), but it has to be considered that we observed a steep decline of this aggregate fraction along the chronosequence because of its incorporation in the micro-aggregate fraction. With the agricultural abandonment of a field ceases the disturbance of soil by rotary tillage that breaks apart micro- and macro-aggregates.

Furthermore, according to Six et al. (2002), the micro-aggregate fraction was more enriched than the other aggregate fractions in new carbon at the end of the studied chronosequence. In the current study, reduction in quantity of silt–clay aggregates did not apparently compromise carbon accumulation; in fact, the finest aggregate fraction was stabilized into micro-aggregate determining also a decrease of the mineralisation coefficient of this fraction. A previous report indicated that the silt–clay fraction seems to be very important in maintaining the oldest soil C stock (Del Galdo et al., 2003).

In the chronosequence of the current study, new carbon gradually replaced old carbon in the different soil aggregate fractions. New carbon became dominant about 40 years after abandonment in the macro-aggregates and after nearly 50 years in the micro-aggregates. In the silt–clay aggregate fraction, however, new and old carbon were nearly equivalent 60 years after abandonment. According to Marin-Spiotta et al. (2009), the organic carbon in macro-aggregates and micro-aggregates are relatively unprotected and are consequently more sensitive to the influence of vegetation dynamics after land abandonment. The current results demonstrate that the SOC sequestration potential is lower for C_4 than for woody C_3 plant communities, which is due to the lower primary productivity and the high lignin content of C_4 *H. hirta*

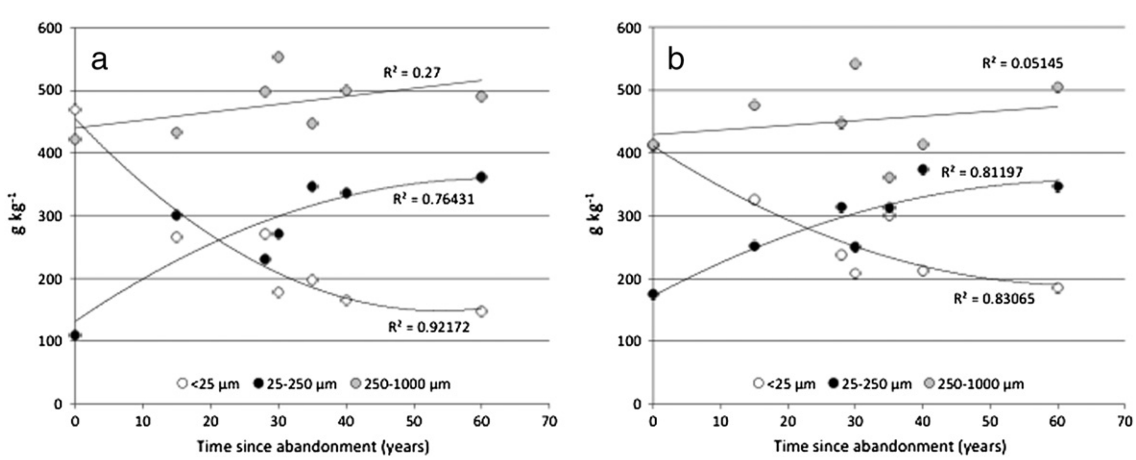

Fig. 4. Masses (g kg^{-1}) of three soil fractions along the chronosequence at 0–15 cm (a) and 15–30 cm (b) soil depth.

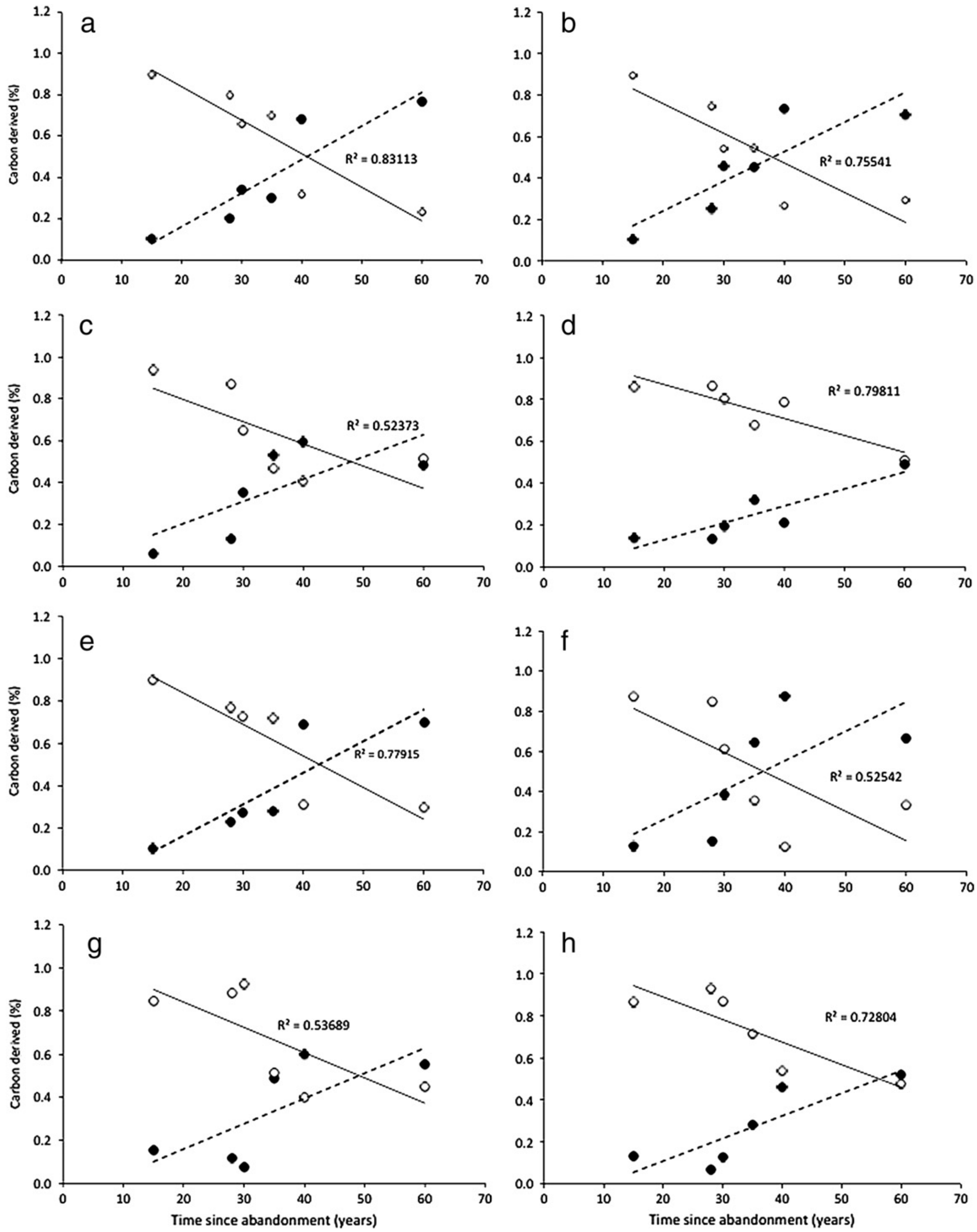

Fig. 5. C_4-C ● and C_3-C ○ carbon derived (%) for bulk soil and the three fractions at two depths along the chronosequence in the 0–15 cm layer (a, b, c and d) and in the 15 – 30 cm layer (e, f, g and h respectively).

(Wynn and Bird, 2007). The biomass (aboveground and belowground, dry matter) of *H. hirta* is 4.92 t ha^{-1}, while for high maquis have been reported values ranging from 72.35 to 121.47 t ha^{-1} (both unpublished data, T. La Mantia). Litter mass of *H. hirta* is 1.56 t ha^{-1} y^{-1} (Lodge et al., 2005), while litterfall of high maquis is 5.98 t ha^{-1} y^{-1} (Arianoutsou, 1989).

5. Conclusions

In this study, we evaluate the SOC incorporation in bulk soil and aggregate fraction after vineyard abandonment and consequent natural vegetation encroachment, using an approach based on natural differences in δ^{13}C of plants with C_3 and C_4 photosynthesis. Our findings have some implications for the understanding of carbon turnover and organic matter stabilisation in semi-arid Mediterranean environments. The study of a C_3-C_4–C_3 succession pathway has enabled us to put into evidence the quantity and the step of organic C incorporation in the different soil aggregate fractions and soil depths. The replacement of cultivation with other species and/or abandonment and renaturalisation are interesting practices aimed to the carbon stock improvement.

The results have shown that, following abandonment of vineyards on volcanic soil in a semi-arid Mediterranean area, the soil acts as a C sink, increasing the C stock from 12 g kg^{-1} in cultivated vineyards to as high as 26 g kg^{-1} after 60 years of abandonment. The carbon increase during the

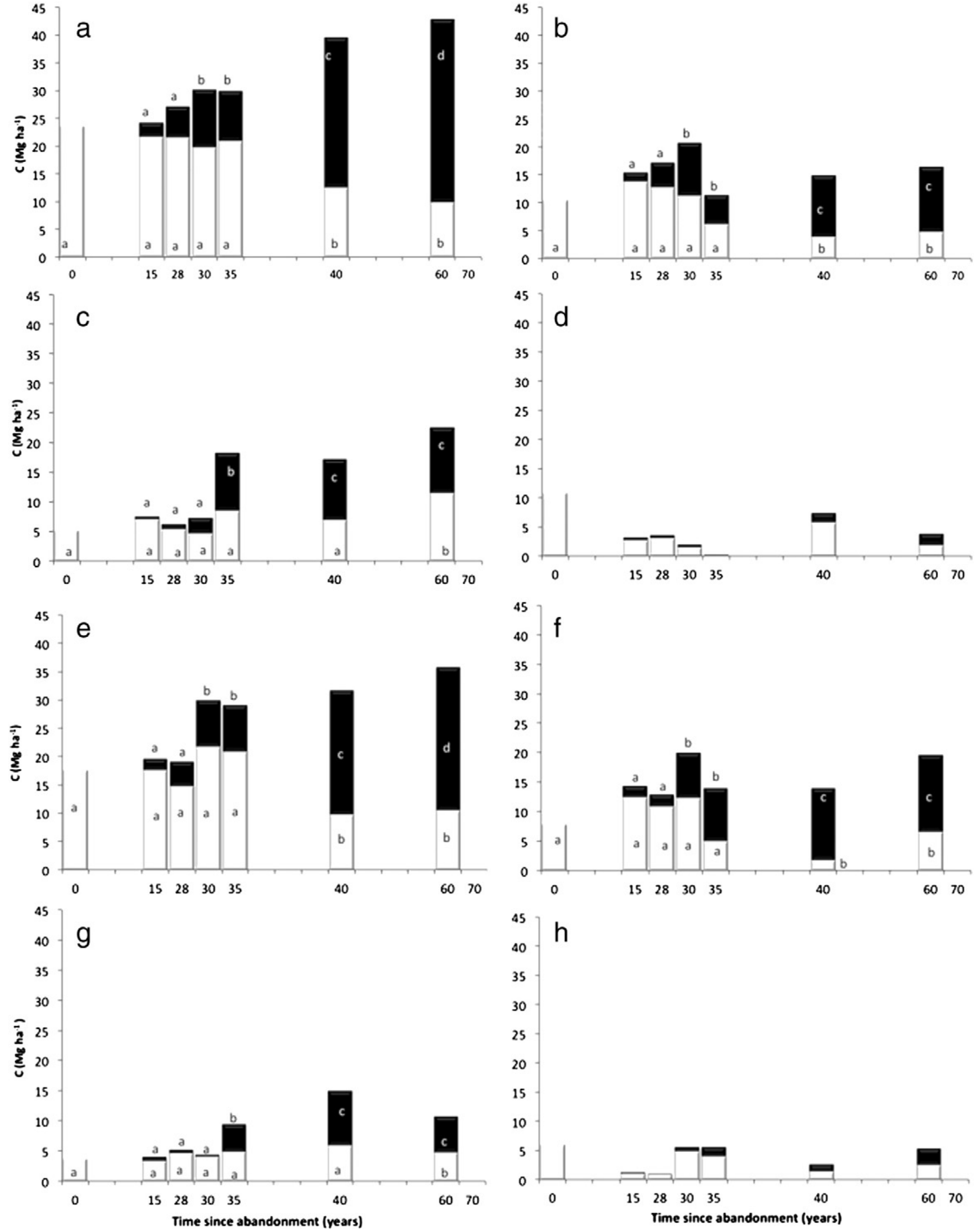

Fig. 6. C_4-C ● and C_3-C ○ carbon (Mg ha^{-1}) for bulk soil and the three fractions at two depths in the chronosequence in the 0–15 cm layer (a, b, c and d) and in the 15–30 cm layer (e, f, g and h respectively). Sections of bars with different letters are significantly different among samples ($P \leq 0.05$); no significant differences were found among samples in the <25 μm fraction.

secondary succession was accompanied by changes in soil structure, favouring the formation of micro-aggregates from the silt–clay aggregate fraction. Most of the carbon derived from vineyard was replaced by new carbon derived from *H. hirta* vegetation after 40 years in the macro-aggregate fraction and after 60 years in the micro-aggregate fraction. The current study verified the use of stable carbon isotopes to explore and date vegetation change across landscape after abandonment.

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References

Alberti, G., Leronni, V., Piazzi, M., Putrella, F., Cairota, P., Peressotti, A., Pissi, P., Valentini, R., Pristina, L., La Mantia, T., Novara, A., Rühl, J., 2011. Regional Environmental Change 11 (4), 917–924.

Alvaro-Fuentes, J., Cantero-Martinez, C., Lopez, M.V., Paustian, K., Denef, K., Stewart, C.E., Arrue, J., 2009. Soil aggregation and soil organic carbon stabilization: effects of management in semiarid Mediterranean agroecosystems. Soil Science Society of America Journal 73, 1519–1529.

Arianoutsou, M., 1989. Timing of litter production in a maquis ecosystem of North-Eastern Greece. Acta oecologica Oecologia Plantarum 10 (4), 371–378.

Balesdent, J., Mariotti, A., Guillet, B., 1987. Natural 13C abundance as a tracer for studies of soil organic matter dynamics. Soil Biology and Biochemistry 19, 25–30.

Balesdent, J., Marioti, A., Guillet, B., 1990. Natural 13C abundance as a tracer for soil organic matter dynamics studies. Soil Biology and Biochemistry 19, 25–30.

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.

Bonet, B., 2004. Secondary succession of semi-arid Mediterranean old-fields in south-eastern Spain: insights for conservation and restoration of degraded lands. Journal of Arid Environments 56, 213–233.

Buzek, F., Paces, T., Jackova, I., 2009. Production of dissolved organic carbon in forest soils along the north–south European transect. Applied Geochemistry 24, 1686–1701.

Cambardella, C.A., Elliot, E.T., 1992. Particulate soil organic matter changes across a grassland cultivation sequence. Soil Science Society of America Journal 56, 777–783.

Canadell, J.G., Quere, C.L., Raupach, M.R., Field, C.B., Buitenhuis, E.T., Ciais, P., Conway, T.J., Gillett, N.P., Houghton, R.A., Marland, G., 2007. Contributions to accelerating atmospheric CO_2 growth from economic activity, carbon intensity, and efficiency of natural sinks. Proceedings of the National Academy of Sciences of the United States of America 104, 18866–18870.

Collins, H.P., Blevins, R.L., Bundy, L.G., Christenson, D.R., Dick, W.A., Huggins, D.R., Paul, E.A., 1999. Soil carbon dynamics in corn-based agroecosystems: results from carbon-13 natural abundance. Soil Science Society of America Journal 63, 584–591.

Del Galdo, I., Six, J., Peressotti, A., Cotrufo, F., 2003. Assessing the impact of land-use change on soil C sequestration in agricultural soils by means of organic matter fractionation and stable C isotopes. Global Change Biology 9, 1204–1213.

Desjardins, T., Andreux, F., Volkoff, B., Cerri, C.C., 1994. Organic-carbon and C-13 contents in soils and soil size-fractions, and their changes due to deforestation and pasture installation in Eastern Amazonia. Geoderma 61, 103–118.

Desjardins, T., Barros, E., Sarrazin, M., Girardin, C., Mariotti, A., 2004. Effects of forest conversion to pasture on soil carbon content and dynamics in Brazilian Amazonia. Agriculture, Ecosystems and Environment 103, 365–373.

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 C-13 abundance and particle size fractionation. Soil Biology and Biochemistry 38, 2753–2761.

Eswaran, H., Reich, P.F., Kimble, J.M., Beinroth, F.H., Padmanabhan, E., Moncharoen, P., 2000. Global carbon stocks. In: Lal, R., Kimble, J.M., Eswaran, H., Stewart, B.A. (Eds.), Global Change and Pedogenic Carbonate. CRC Press, Boca Raton, FL, pp. 15–25.

Follet, R.F., Paul, E.A., Leavitt, S.W., Halvorson, A.D., Lyon, D., Peterson, G.A., 1997. Carbon isotope ratios of Great Plains soils and in wheat-fallow systems. Soil Science Society of America Journal 61, 1068–1077.

Gearing, J.N., 1991. The Study of Diet and Trophic Relationships Through Natural Abundance 13C. In: Coleman, D.C., Fry, B. (Eds.), Carbon isotope techniques. Academic press, San Diego, pp. 201–218.

Gregorich, E.G., Ellert, B.H., Monreal, C.M., 1995. Turnover of soil organic matter and storage of corn residue carbon estimated from natural 13C abundance. Canadian Journal of Soil Science 75, 161–167.

Guo, L.B., Gifford, R.M., 2002. Soil carbon sequestration and land-use change: a meta analysis. Global Change Biology 8, 345–360.

Halliday, J.C., Tate, K.R., McMurtrie, R.E., Scott, N.A., 2003. Mechanisms for changes in soil carbon storage with pasture to *Pinus radiata* land-use change. Global Change Biology 9, 1294–1308.

Hitz, C., Egli, M., Fitze, P., 2002. Determination of the sampling volume for representative analysis of alpine soils. Zeitschrift für Pflanzenernährung und Bodenkunde 165, 326–331.

Jastrow, J.D., Boutton, T.W., Miller, R.M., 1996. Carbon dynamics of aggregate associated organic matter estimated by carbon-13 natural abundance. Soil Science Society of America Journal 60, 801–807.

Jinbo, Z., Changchun, S., Shenmin, W., 2007. Dynamics of soil organic carbon and its fractions after abandonment of cultivated wetlands in northeast China. Soil & Tillage Research 96, 350–360.

Kirschbaum, M.U.F., Guo, L.B., Gifford, R.M., 2008. Why does rainfall affect the trend in soil carbon after converting pastures to forests? Possible explanation based on nitrogen dynamics. Forest Ecology and Management 255, 2990–3000.

Kosmas, C., Gerontidis, S.T., Marathianou, M., 2000. The effect of land use change on soils and vegetation over various lithological formations on Lesvos (Greece). Catena 40, 51–68.

Kuzyakov, Y., Biriukova, O., Turyabahika, F., Stahr, K., 2001. Electrostatic method to separate roots from soil. Journal of Plant Nutrition and Soil Science 164, 541–545.

La Mantia, T., Oddo, G., Rühl, J., Furnari, G., Scalenghe, R., 2007. Variazione degli stock di carbonio in seguito ai processi di abbandono dei coltivi: il caso studio dell'isola di Pantelleria (Tp). Foresta@ 4 (1), 102–109 http://www.sisef.it/forest@/show.php?id=433, Last Access: 28 May 2012.

La Mantia, T., Rühl, J., Pasta, S., Campisi, D., Terrazzino, G., 2008. Structural analysis of woody species in Mediterranean old fields. Plant Biosystems 142 (3), 462–471.

Lal, R., 2004. Soil carbon sequestration impacts on global climate change and food security. Science 304, 1623–1627.

Lisboa, C., Conant, R.T., Haddix, M.L., Cerri, E., Cerri, C., 2009. Soil carbon turnover measurement by physical fractionation at a forest-to-pasture chronosequence in the Brazilian Amazon. Ecosystems 12, 1212–1221.

Lodge, G.M., McCormick, L.H., Harden, S., 2005. Grazing studies of a *Hyparrhenia hirta* (Coolatai grass) pasture in northern New South Wales. Australian Journal of Experimetal Agriculture 45, 1603–1611.

Marin-Spiotta, E., Silver, W.L., Swanston, C.W., Ostertag, R., 2009. Soil organic matter dynamics during 80 years of reforestation of tropical pastures. Global Change Biology 15, 1584–1597.

Martinez-Fernandez, J., Lopez-Bermudez, F., Martinez-Fernandez, J., Romero-Diaz, A., 1995. Land use and soil-vegetation relationships in a Mediterranean ecosystem: El Ardal, Murcia, Spain. Catena 25, 153–167.

Ouédraogo, E., Mando, A., Stroosnijder, L., 2005. Effects of tillage, organic resources and nitrogen fertiliser on soil carbon dynamics and crop nitrogen uptake in semi-arid West Africa. Soil and Tillage Research 91 (1–2), 57–67.

Post, W.H., Kwon, K.C., 2000. Soil carbon sequestration and landuse change: processes and potential. Global Change Biology 6, 17–327.

Robertson, G.P., Vitousek, P.M., 1981. Nitrification potentials in primary and secondary succession. Ecology 62, 376–386.

Romkens, P.F.A.M., van der Plicht, J., Hassink, J., 1999. Soil organic matter dynamics after the conversion of arable land to pasture. Biology and Fertility of Soils 28, 277–284.

Rühl J., 2007. Vegetation dynamics on abandoned terraces of Sicily: the course and driving factors of succession. Unpublished PhD thesis. Greifswald: University of Greifswald. 221 pp. Available from URL http://ub-ed.ub.uni-greifswald.de/opus/volltexte/2007/409/. Last Access: 1 April 2012.

Rühl, J., Schnittler, M., 2011. An empirical test of neighbourhood effect and safe-site effect in abandoned Mediterranean vineyards. Acta Oecologica 37, 71–78.

Rühl, J., Pasta, S., La Mantia, T., 2005. Metodologia per lo studio delle successioni secondarie in ex coltivi terrazzati: il caso di studio delle terrazze di Pantelleria (Canale di Sicilia). Foresta@ 2 (4), 388–398 http://www.sisef.it/forest@/pdf/Ruhl_324.pdf, Last Access: 28 May 2012.

Rühl, J., Pasta, S., Schnittler, M., 2006. A chronosequence study of vegetation dynamics on vine and caper terraces of Pantelleria Island (Sicily). Arch. f. Natursch. & Landschaftsforsch. 45, 71–90.

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

Schimel, D.S., 1995. Terrestrial ecosystems and the carbon cycle. Global Change Biology 1 (419), 77–91.

Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: implications for c-saturation of soils. Plant and Soil 241, 155–176.

Thornthwaite, C.W., Mather, J.R., 1955. The Water Balance. Publications in Climatology VIII(1). Drexel Institute of Climatology, Centerton, N.J., pp. 1–104.

Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soil. Journal of Soil Science 33, 141–164.

Van Rompaey, A.J.J., Govers, G., Van Hecke, E., Jacobs, K., 2001. The impacts of land use policy on the soil erosion risk: a case study in central Belgium. Agriculture, Ecosystems and Environment 83, 83–94.

Wolf, D.C., Legg, J.O., Boutton, T.W., 1994. Isotopic Methods for the Study of Soil Organic Matter Dynamics. SSSA. Methods of Soil Analysis: Part 2. Microbiological and Biochemical Properties: Soil Sci. Soc. Am., Book Series, vol. 5, pp. 865–906 (Madison, WI, Chapter 39).

Wookey, P.A., Bol, R.A., Caseldine, C.J., Harkness, D.D., 2002. Surface age, ecosystem development, and C isotope signatures of respired CO_2 in an alpine environment, north Iceland. Arctic, Antarctic, and Alpine Research 34, 76–87.

Wynn, J.G., Bird, M.I., 2007. C4-derived soil organic carbon decomposes faster than its C3 counterpart in mixed C3/C4 soils. Global Change Biology 13, 2206–2217.

Zanchi, G., Thiel, D., Green, T., Lindner, M., 2007. Afforestation in Europe. EFI, Joensuu, Finland. http://www.ieep.eu/assets/298/wp4_nd_afforestation_in_europe.pdf, Last Access: 18.04.2012.