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Litter contribution to soil organic carbon in the agriculture abandons processes

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Abstract

Mechanisms of litter decomposition, translocation and stabilization into soil layers are fundamental processes in ecosystem functioning as it regulates the cycle of soil organic matter (SOM), CO_2 emission into the atmosphere, carbon sequestration into the soil. In this study, it was investigated the contribution of litters of different stages of Mediterranean secondary succession on Carbon sequestration, analyzing the role of earthworms on translocation of SOM into soil profile. For this purpose $\delta^{13}C$ difference between meadow C_4-C_{soil} and C_3-C_{litter} were used in a field experiment. Four undisturbed litters of different stages of succession were collected (45, 70, 100 and 120 since agriculture abandon) and placed on the top of isolated soil cores.

The litter contribution to C stock was affected by plant species and increased with the age of the stage of secondary succession. The soil organic carbon after 1 year since litter position increased up to 40 % in comparison to no litter treatment in soil with litter of 120 years since abandon.

The new carbon derived from C_3-litter was decomposed and transferred into soil profile thanks to earthworms and dissolved organic carbon leaching. After 1 years the carbon increase attributed to earthworm activity ranged from 6 to 13 % in soil under litter in field abandoned since 120 and 45 years, respectively.

1 Introduction

The major input of vegetative C to forest soil is represented by litter and hence, changes in litter inputs are likely to have important consequences for soil C dynamics (Sayer et al., 2007). Generally, it has been recorded that an accumulation of litter corresponds to an increase of the carbon storage in the soil; for instance, following the processes of abandonment has been recorded an accumulation of litter and a consequent increase in carbon content of soil (Costa and La Mantia, 2005).

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Mechanisms of litter decomposition, translocation and stabilization into soil layers, are therefore, fundamental processes in ecosystem functioning as it regulates the cycle of soil organic matter (SOM), CO_2 emission into the atmosphere, carbon sequestration into the soil and nutrients mineralization (Maisto et al., 2011; Smolander et al., 2008; Fioretto et al., 1998, 2005).

The decomposition of litter is affected by the quality of the residues (Smith et al., 2008), that determines different decadiment rates. Soluble substances and labile compounds of litter are rapidly degraded in early stages of decomposition by fast growing microorganisms that may require a high concentration of nitrogen (Swift et al., 1979). Cellulose and lignin, the most abundant components of forest litter, are slowly decomposed.

The mainly responsible for the decomposition of litter are bacteria and fungi forming up to 90 % of the soil microbial biomass. Generally, fungi are the most abundant primary decomposers at the soil–litter interface in terrestrial ecosystems, and therefore play an important role in the global carbon cycle (Dix and Webster, 1995; Schimel et al., 1999). The invertebrates can, also, in soil ecosystem functioning, including C cycle.

On the hand, several author attributed to earthworm the role to creating a favorable conditions for microbial activity, through the fragmentation of litter and mixing of organic matter with soil mineral portion (Tiunov et al., 2001; Wurst et al., 2004).

Earthworms have also been shown to affect the amount and distribution of SOM and to increase the rates of SOM decomposition. Earthworms transport large quantities of C from the surface of the soil to the lower horizons, effectively mixing the soil and increase humification rates and overall decomposition rates significantly (Lee, 1985; Alban and Berry, 1994; Edwards and Bohlen, 1996; Burtelow et al., 1998; Li et al., 2002; Pulleman et al., 2005; Fonte et al., 2007).

On the other hand, Alban and Berry (1994) and Burtelow et al. (1998) found that earthworm invasion resulted in C loss in the upper soil layer.

Other fundamental processes for the stabilization of SOM is the leaching of fresh litter compound and recently formed as dissolved organic matter (DOM) from organic

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layers to mineral soil and the sorption of DOM onto mineral surfaces (Sollins et al., 1996; Kaiser and Guggenberger, 2000; Kalbitz and Kaiser, 2008). In case of prolonged leaching, the litter can become more recalcitrant to decomposition, as a consequence of the significant loss of soluble organic compounds, readily degradable (Mangenot and Tuotain, 1980).

Another mechanism of translocation of SOM in the deeper layer could be is the role of soil invertebrates and the complex soil food web in soil C dynamics is receiving increased attention, but these biotic interactions have rarely been incorporated into general models of soil C turnover (Fitter et al., 2005; Huang et al., 2010).

In this study, the objectives were (i) determine the contribution of litter in soil ecosystems as C sink, (ii) analyzing mechanisms of C translocation from litter to soil, (iii) individuate through isotopic analysis the amount of C leached and the probably role of earthworms in this process.

2 Material and method

2.1 Experimental layout, soil and litter sampling

The experiment was carried out in the fields of the Department of Agricultural and Forestry Sciences, University of Palermo, Italy (38°06′ N, 13°20′ E, 50 m a.s.l.). The soil is an Aric regosol according to World Reference Base for Soil Resources (WRB, 2006), shallow, rich in limestone with a pH value of 7.61, with a ~~sandy loam~~ texture (53.9 % sand, 22.6 % ~~loam~~ and 23.4 % clay) and organic matter content of 1.40 %. The climate is semiarid Mediterranean with a dry period of 4–5 months (mean temperature: minimum 13.7 °C, maximum 22.1; mean annual rainfall: 531 mm).

The field plot used in the experiment was a *Cynodon* meadow. The soil under *Cynodon* is a C_4 soil under isotopic steady state, since it has been covered by *Cynodon* (C_4 photosynthetic pathway plant) for more than 15 years. The $\delta^{13}C$ of the experi-

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mental soil was −14.5 ± 1.8. *Cynodon* meadow was established with an interspecific Bermudagrass hybrid (*C. dactylon* x *C. transvalaalensis*), cv *Tifway 419.*

Agronomic management of the turf grass included monthly fertilizer application from April to October of 50 kg ha^{-1} of N, 10 kg ha^{-1} of P and 40 kg ha^{-1} of K. Irrigation was carried out during the spring-summer season with a sprinkler system in order to reinstate evapotranspiration (determined by a Class A evaporimeter and rainfall). The turf grass was maintained at a height of 30–35 mm using a reel lawn mower 2–3 times weekly. The cuttings were removed without grasscycling or mulching.

Plastic cores (n. 30), 20 cm diameter and 40 cm height, were installed in the meadow soil, after carefully grasses removal in March 2013 (Fig. 1). The cores were 30 cm buried, leading 10 cm surface collar. In 15 of the whole of installed cores, a grid (0.1 mm) was placed on top of the soil core to avoid the earthworms crossing. Undisturbed different litters (4 litters of C_3 plant) were placed on top of soil. In all, 30 cores were placed (5 litters treatments (4 litters + 1 no litter) × 2 grid (grid and no grid) × 3 replicas).

Litters were collected with cores (20 cm diameter) in 4 different successional stages of a secondary succession in Pantelleria island, Italy (Sicily, 36°44′/36°50′ N, 11°57′/12°03′ E). The selected stages for litter collection were: Maquis 45 years since abandon (L45), Maquis 70 years since abandon (L70), Maquis 100 years since abandon (L100) and Forest 120 years since abandon (L120). Abandonment age of the sampled successional stages was determined by evaluating aerial photographs taken during 1955 and 1968 (produced by Istituto Geografico Militare, Florence) and 1987 (Regione Siciliana). The sampled areas were located in direct proximity to each other and were characterized by comparable abiotic conditions (aspect, slope, soil type, rock outcrop, stone cover, etc.). Litters types are described in Table 1.

Soil samples were collected in February 2014. The 30 cm soil core was divided in four subsamples (each 7.5 cm soil thickness). The soil was dried, 2 mm sieved and the organic fragments were removed.

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2.2 Litter analysis

Dry biomass weight and its chemical composition (ADL – acid detergent lignin, NDF – neutral detergent fibre, cellulose) were determined for each collected litter.

The litter respiration rates (mg CO_2 day^{-1} dry litter) were measured during incubation experiment, using an alkali absorption in a closed chamber method. Three replicates in each litter treatment with three blank samples were measured. Ten grams of litter were placed inside 1 l glass bottle. A 30 mL 0.1 N NaOH solution was used to trap CO_2 evolved inside the bottle. The CO_2-trapped solution was measured was titrated HCl solution using phenolphthalein and methyl-orange as color indicator. During the 7 days of incubation, CO_2 measurements were done after 24, 48, 60, and 96 h and 1 week from the start of incubation. Twenty-four hours before the CO_2 sampling, all flasks were ventilated for 30 min with fresh air and then sealed with rubber stoppers. The C mineralization rate was expressed as mg CO_2-C g^{-1} TOC day^{-1} and was fitted to the following first-order decay function:

$$\text{Mineralized C} = C_r e^{-kt} \quad (1)$$

where C_r is the readily mineralizable C at time zero (i.e. the intercept value), k is the decay rate constant and t is the time. The amount of total C mineralized was calculated through the linear interpolation of two neighbouring measured rates and the numerical integration over time as reported in the following equation:

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

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

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2.3 Chemical analysis

For each soil sample the C content and $\delta^{13}C$ abundance were measured. $\delta^{13}C$ isotopic signature of litter biomass was, also, analysed. For SOC and 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, δ^{13}CV-PDB = −26.43 ‰). IA-R001 is traceable to IAEA-CH-6 (cane sugar, δ^{13}CV-PDB = −10.43 ‰). IA-R001, IA-R005 (Iso-Analytical Limited beet sugar standard, δ^{13}CV-PDB = −26.03 ‰), and IA-R006 (Iso-Analytical Limited cane sugar standard, δ^{13}CV-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:

$$\delta\,(‰) = \frac{R_s - R_{st}}{R_{st}} \cdot 1000 \tag{3}$$

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

2.4 Data analysis

Natural abundance of $\delta^{13}C$ was used to determine the proportion of C in SOC that was derived from the new C input (C_3-C). These proportions were calculated by the mixing equation (Gearing, 1991) separately for grid and no grid plots:

$$\text{New Carbon Derived} = f\,(\text{NCD})\,(\%) = \frac{(\delta^{13}C_{new} - \delta^{13}C_{old})}{(\delta^{13}C_{litter} - \delta^{13}C_{old})} \tag{4}$$

where NCD is the fraction of new C derived, $\delta^{13}C_{new}$ is the isotope ratio of the soil sample, $\delta^{13}C_{litter}$ is the isotope ratio of different litters, and $\delta^{13}C_{old}$ is the isotopic ratio of the previous vegetation (*Cynodon*).

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Carbon derived from worms was calculated as differences between NCD in grid and no grid treatments.

The mass of new carbon additions was calculated according to Eq. (5).

$$\text{New Carbon } (\text{g kg}^{-1}) = C_{soil}\ (\text{g kg}^{-1}) \times (1 - \text{New Carbon Derived}) \quad (5)$$

TI[illegible]D of the $\delta^{13}C$ and C values was calculated for each depth and treatment (Duncan test).

What about statistical tests? It should be included some information the specific tests used

3 Results

3.1 Litter characteristics

The plant litter collected under the stages of secondary succession differed in the total weight and C content. The weight of litter biomass was highest in L120 with values of $1113 \pm 90\,\text{g m}^{-2}$, followed by L100, L45 and L70 with values of $1027 \pm 77\,\text{g m}^{-2}$, 915 ± 104 and $946 \pm 82\,\text{g m}^{-2}$, respectively. The C content of litter was highest in L45 and decreased with the increase of the age of abandon (Fig. 2); however, the L120 contributed to highest C litter input (total C litter/core) due to the higher weight in comparison to other litters of the stages of secondary succession.

The results of litter incubation experiment showed the lowest cumulative CO_2 emission for L45 and L100 ($32\,\text{mg}\,CO_2\text{-C}\,\text{g}^{-1}$), followed by L70 ($35\,\text{mg}\,CO_2\text{-C}\,\text{g}^{-1}$) and L120 ($40\,\text{mg}\,CO_2\text{-C}\,\text{g}^{-1}$).

The MRT was significantly lower for L120 in comparison to others litters, which were not significantly different among them for MRT value (Table 2). These findings were confirmed by C_{extr} which was higher in L120 in comparison to others litters, showing, therefore, an highest mineralizable organic carbon fraction in L120 (Table 2).

The composition of litter was not statistically differen[illegible]hong successional stages regarding Cellulose and ADL content (Table 2). The NDF value was, instead, significantly higher in L120 in comparison to litters of other successional stages.

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3.2 Soil carbon content and distribution

The total amount of SOC differed under the two treatments (grid and no grid) and litters age. The SOC was significantly higher in soil where L120 was placed on the top of soil cores, followed by the other litter treatments (Table 3). Comparison between grid and no grid treatment showed highest C content in soil cores without grid for all litters.

After one year from litter position, on average (by soil layers) the SOC under L120 increased in comparison to no litter treatment by 26 and 40 % in grid and no grid treatment, respectively.

Such C increase after litter position was smaller in grid treatment for the other litters (L45, L70 and L100) with a value of about 12 %. In no grid treatment after one year of litter position, the SOC increased in comparison to no-litter treatment by 22, 23 and 15 % in soil under L100, L70 and L45, respectively. SOC decreased with soil depth, but on average the difference between the first and the deepest soil layer was more pronounced in no grid treatment (Table 3).

3.3 ^{13}C isotopic signature in soil profile

Soil δ^{13}C value significantly changed after litter positioning. The baseline is represented by soil without litter, where the δ^{13}C values ranged between -14 ± 0.3 ‰ and -16 ± 0.4 ‰ in the top and deepest soil layer, respectively. After litter position, δ^{13}C was depleted due to C_3 litter input. The most depleted soil was L120 with on average (grid and no grid treatment) values of −18.6 and −21.6 ‰ in the top and deepest soil layer, respectively (Fig. 3). For the others litters treatments the value ranged between −15.0 and −20.5 ‰.

The effect of litter input on C stock was highlighted by estimates of C derived from litter (C_3 plant) in the meadow soil (C_4 soil). After 1 year since litter position, the C originated from litter input was 32.4, 34.2, 38.5 and 49.8 % of total SOC in L45, L70, L100 and L120, respectively.

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The new C derived from litter was lower for all litter treatments in soil with grid, therefore, it was attribute to earthworms the litter decomposition and incorporation into SOC. The portion of C_3-C in soil with grid was, in fact, 12.4, 23.1, 23.4 and 40.7 % of total C in L45, L70, L100 and L120, respectively (Fig. 4). The difference of C_3-C between no grid and grid treatment assess the role of earthworm to litter incorporation into soil. Considering only the C_3-C of SOC for each litter treatment, it was highlighted that the contribution of earthworm to incorporation of new C_3-SOC was in percentage highest in L45, decreased with the age of litter and for each treatment decreased with soil depth.

4 Discussion

4.1 Litter contribution to SOC stock

Previous studies in Pantelleria island demonstrated the potential of land cover to change the C steady state level (Novara et al., 2014). Such hypothesis was confirmed by the present experiment, where the effect of plant litter contribution to SOC stock was isolated by other soil and environmental parameters. In line with several reports in other ecosystem (Lal, 2005), it has been recorded that the SOC stock depends on C litter input, as well as on litter quality. The incubation experiment of litters, showed differences in C_{extr}, litter composition (NDF %) and consequently C litter mineralization rate. The litter of L120 had a higher amount of extractable C, in comparison to other litters, and it was easily decomposed and transferred to SOC pool. The faster mineralization rate of L120 could be attributable both to a different composition of plant species (lower content of sclerofille) and t variation of microclimatic condition due to higher accumulation layer on soil surface. Concerning the effect of plant species on litter mineralization rate, several studies found a lower litter decomposition rate in *Q. ilex* in comparison to other Mediterranean species, like *Myrtus* and *Cistus* (Berg et al., 1996; Fioretto et al., 2005). Likewise, Maisto et al. (2011) found a slower decomposition of *Q.*

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ilex in comparison to *Ph. angustifolia*, while no significative difference of decomposition rate were recorded between *Q. ilex* and *Pistacia lentiscus*. In these studies the lower decomposition of *Q. ilex* was attributed to higher lignin content. Our results confirmed those of other research regard to the higher lignin content of *Q. ilex*, but it was not tightly associated to lower decomposition rate. In fact, L120, where the main species is *Q. ilex*, was the litter with an higher decomposition rate. Therefore, other aspects could explain differences in decomposition rates, like the percentage of a species in each stage of succession, the age of litter, and the thickness of litter.

4.2 Influence of earthworm on soil carbon

Plant litter is the principal sources of SOM in soil under secondary succession. The transformation of C litter into SOM is determined by decomposition of plant biomass and incorporation into soil profile. The responsible of this mechanisms are bacteria and fungi, forming up to 90 % of the soil microbial biomass (Dix and Webster, 1995; Schimel et al., 1999) and faunal groups. Our observations highlighted the annual contribution to SOM derived from litter and it was discriminated the activity of decomposer thanks to difference of isotopic signature between previous SOC-C and the new C_3-C input originated from litter. The ^{13}C litter recovery in the soil profile was highest in L120 (89 %), followed by L45 (63 %), L100 (60 %) and L70 (52 %). On the hand, it was highlighted the activity of microbial biomass in soil samples where the grid was placed between litter and soil. In this case, the new C_3-C represented the C-pool originated by fungi and bacterial decomposition, transferred in soil depth, mainly through dissolved organic carbon. Such decomposition and incorporation activity contributed to C increase up to 77.6 g core^{-1} year^{-1} in L120 treatment (Fig. 5). On the other hand, the difference between soil core with and without litter furnished information about the contribution of earthworm to litter decomposition and incorporation into soil. In several studies, the introduction of earthworm in cold temperate forests resulted a decline of SOC (Bohelen et al., 2004; Alban and Berry, 1994). The observation of present study, instead suggested that earthworms have the potential to increase SOC. After 1 year,

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earthworm activity increased SOC by 13.5, 11.3, 11.1 and 5 %, in L120, L100, L70 and L45, respectively. The activity of earthworm to recovery in soil C released from litter could be attribute to different mechanisms: (i) mixing undecayed particulate C into soil, (ii) create preferential flowpaths in soil increasing nutrient transportation, (iii) protection of C in soil aggregates created by earthworm feeding (Bohlen et al., 2004; Fahey et al., 2013).

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What about conclusion? It should be included

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Table 1. Characteristics of litters collected in Pantelleria island.

Successional stages	Years since abandon	Vegetation (Main species)	Soil Use during XX century	Current use
1	45	High-maquis (~~*Pistacia lentiscus,*~~ *Quercus ilex*, *Phillyrea latifolia*, *Calicotome infesta*, *Erica arborea*, *Cistus salvifolius)*	No use after abandon	No use
2	70	Maquis-forest (*Quercus ilex*, *Pistacia lentiscus*, *Phillyrea latifolia*)	Coppice	No use
3	100	Forest (*Quercus ilex*, *Pistacia lentiscus*)	Coppice	No use
4	120	Forest (*Quercus ilex*, *Smilax aspera*)	High forest	No use

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Table 2. Biomass composition (% of dry biomass) of litters in different stages of secondary succession (L45, L70, L100 and L120). Abbreviations: ADF = acid detergent fibre, NDF = neutral detergent fibre, MRT = mean residence time. Standard deviation was calculated on 3 replicates.

Litter	C_{extr} (mg kg^{-1})	MRT days	R^2	Cellulose	ADL	NDF
L45	154.1 ± 13.3	25.0 ± 1.2	0.92	19.0 ± 3.9	28.9 ± 2.5	44.6 ± 5.6
L70	163.2 ± 11.3	26.0 ± 1.4	0.86	17.6 ± 5.6	24.1 ± 4.6	39.3 ± 4.0
L100	150.7 ± 12.0	26.0 ± 2	0.90	18.2 ± 4.8	30.5 ± 2.7	44.4 ± 6.4
L120	217.0 ± 17.5	22.0 ± 1.0	0.92	19.9 ± 2.6	31.4 ± 3.9	51.4 ± 2.6

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Table 3. Average of soil organic carbon (%) at different soil depths. L45, L70, L100 and L120 indicate the soil cores where different litters were placed at the bottom. For each treatment different letters indicate differences for $P \leq 0.05$.

	soil depth (cm)	no litter	L45	L70	L100	L120
grid	0–7.5	1.5	1.8	1.8	1.8	1.9
	7.5–15	1.4	1.5	1.6	1.5	1.7
	15–22.5	1.3	1.6	1.3	1.4	1.6
	22.5–30	1.2	1.1	1.3	1.2	1.5
	Average	1.3a	1.5b	1.5b	1.5b	1.7c
no grid	0–7.5	1.6	1.9	1.8	2.0	2.4
	7.5–15	1.4	1.9	1.9	2.0	2.1
	15–22.5	1.3	1.6	2.1	1.6	1.7
	22.5–30	1.2	0.9	0.9	1.1	1.4
	Average	1.4a	1.6b	1.7b	1.7b	1.9c

SED
7, 595–616, 2015

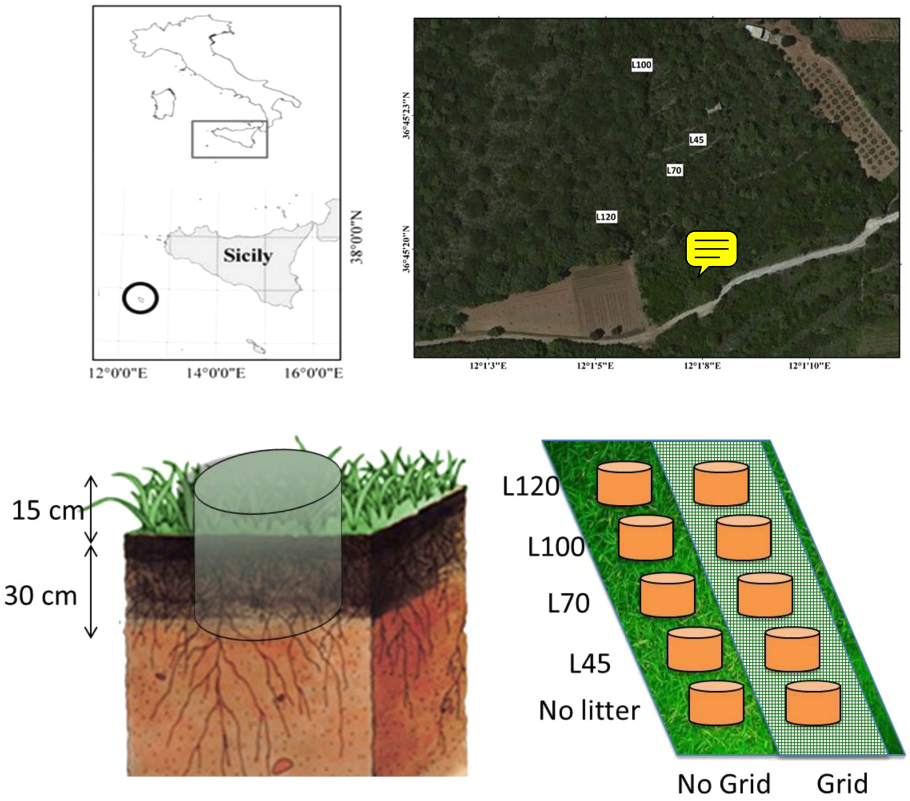

Figure 1. Sampling area of litter in Pantelleria secondary succession (numbers represent litter in field abandoned since 120, 100, 70 and 45 years, respectively) and experimental design in meadow field. Numbers indicate the age since abandon.

SED
7, 595–616, 2015

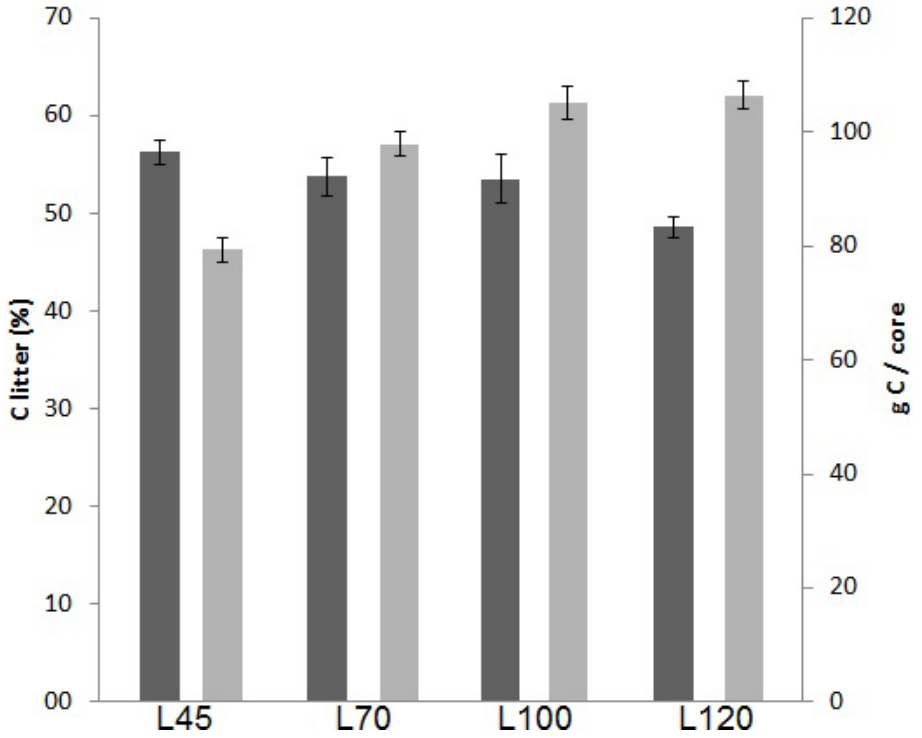

Figure 2. C litter content (%) (black columns) and C litter input (g) for each core (grey columns) in L45, L70, L100 and L120 treatments.

SED
7, 595–616, 2015

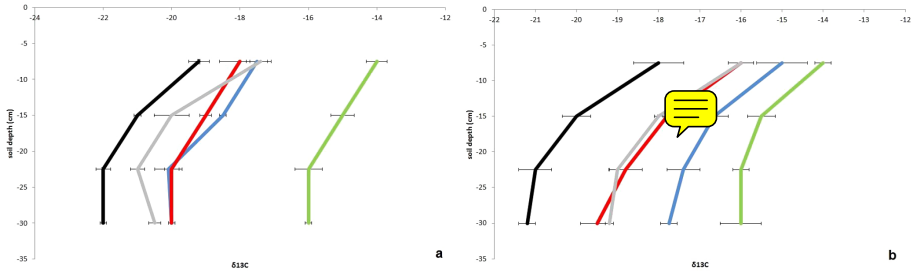

Figure 3. $\delta^{13}C$ value at different depth in no grid **(a)** and grid **(b)** treatment. The green line represents no litter treatment, while blue, red, grey and black represent litter in field abandoned since 120, 100, 70 and 45 years, respectively.

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7, 595–616, 2015

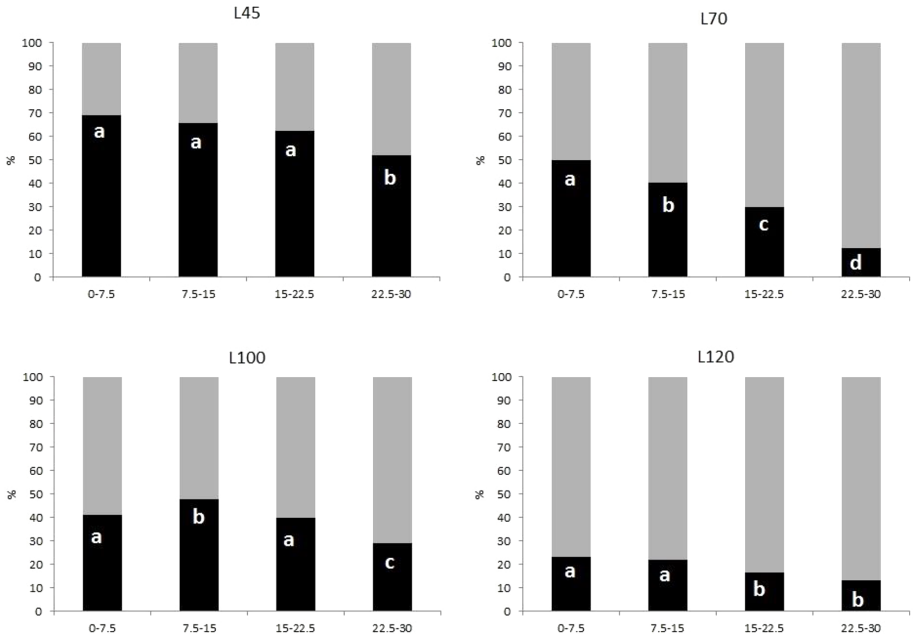

Figure 4. Contribution (%) of worm activity (black columns) and DOC (grey columns) in C_3-C portion at different soil depth. For each portion different letters indicate differences for $P \leq 0.05$.

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7, 595–616, 2015

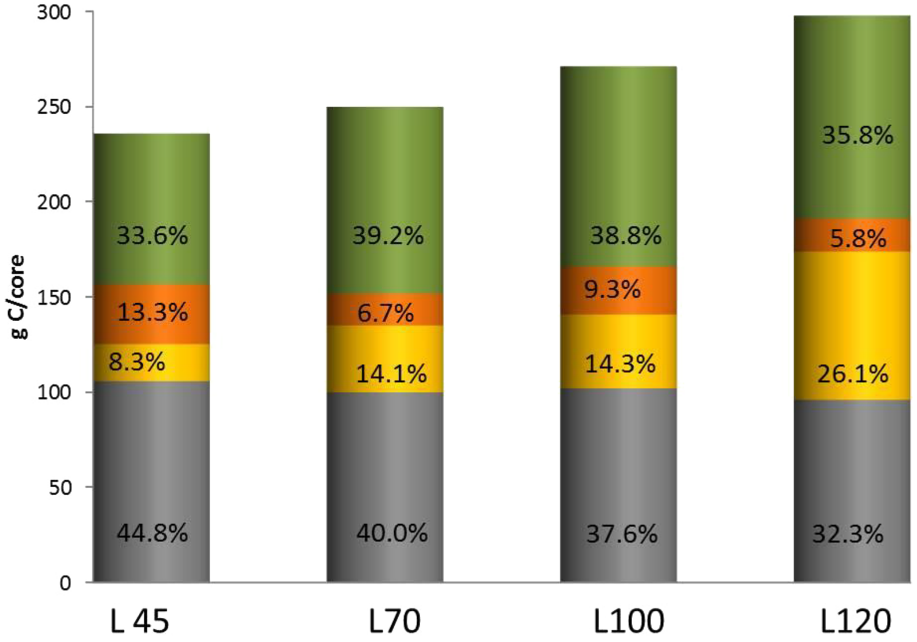

Figure 5. C content in each core (L45, L70, L100 and L120) originated from C_4-SOC (grey columns), C_3-SOC from worm activity (yellow columns), C_3-SOC from DOC leaching (orange columns) and C litter (green columns).

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7, 595–616, 2015