RESPONSE TO REVIEWER' COMMENTS (Manuscript SE-2014-131)

We have received the comments of the reviewers and we thank them for the helpful contribution to improve our manuscript. The original comment of the reviewers (black type) and our answers (red type) are reported below.

Reviewer 1

This manuscript represents a good advance related to the litter contribution to soil organic carbon in agricultural processes. The authors have done a great work to present all the methodological and analysis in a presentable and interesting way. However, some typographical mistake with the English version should be considered by the authors. Furthermore, the manuscript has been lacking the conclusion section and requires a more in-depth discussion. In any case, all of this could be solved. I consider that it is a good one manuscript to have in considered in Solid Earth after the revision processes.

Page 604 line 23 the reference was added

In the table 2 p-value was added

"Fig 3" was added in the chapter 3.3

The figure 1 was changed according to the comments.

In the figure 3 the size of number and letters was changed. Statistical information were added

The English was checked by native speaker

The conclusion were added

Reviewer 2

Comments to SED 7, 595-616 (2015) "Liitter contribution to soil organic carbon in the agriculture abandons processes" by Novara et al. Although the paper addresses relevant scientific issues within the aims of SE, at the moment their various sections are not well linked among them. Indeed, Introduction seems an assemblage of generic sentences which do not focus on precise topics.

Even research objectives must be formulated less vaguely. However, the most problematic section is M&M and the reason will be explained later, while the most difficult section to understand is "Results", also for the "cryptic" English. Finally, Discussion often results unrelated to reported results and lacks of Conclusions

The adopted experimental design is rather complex but some crucial points need to be clarified by Authors.

For example: 1) soil characterization (soil pH value is too low for high content in limestone; based on reported granulometry, the textural class is sandy-clay-loam;

The content of CaCO3 was added in material and method.
The texture was changed as sandy-clay-silty

2) the earthworm effect is only presumed, since no earthworm biomass has been measured and followed during the experiment;

We agree with the reviewer regard no measurement of earthworm biomass were done. The difference between the two treatments (grid and no grid) are not presumed but measured as described in M&M, data analysis chapter.

3) litter respiration (the described measurement procedure is not persuasive under many aspects: the used NaOH volume is not enough to trap all the CO2 declared being produced during 1-week incubation by litters; on the other hand, Authors seem not having replaced NaOH solution by fresh one before each trapping; the carbonate deriving by CO2 trapping was not apparently precipitated before titration of residual NaOH; methylorange indicator works at acidic pH ranges, which is not the case here);

The NaOH trap was placed inside the bottle 24h before titration for CO_2 measurement.
The methylorange is commonly used to tritation with HCl (strong acid)

4) there is no indication for calculating MRT,

It was added in M&M “The mean residence time in days, (MRT) was determined as a reciprocal of the rate constant (k) of first order decay (Equation 1).”

for defining Cextr and for determining it (Table 2);
The C extr was changed as readily mineralizable and it is explained in the equation 1

5) procedures for ADF, ADL, NDF determinations are not reported, and even their definitions, rather equivocal within literature, are lacking; moreover, based on performed analyses, particularly cellulose content, the quality of the 4 litters, so different for the duration of abandons, did not change, what is rather unexpected;

M&M was improved, a reference was added.

6) statistics is quite poor and significant differences among various experimental factors are not unequivocally deducible. More details were added in M&M

In conclusion, the deep reviewing of the manuscript is not possible, and even unrecommended, without properly answering the above questions.

Litter contribution to soil organic carbon in the processes of agriculture abandon

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Key words: ^{13}C isotopic signature; earthworm; SOC; litter**.**

Abstract

The mechanisms of litter decomposition, translocation and stabilization into soil layers are fundamental processes in the functioning of the ecosystem, as they regulate the cycle of soil organic matter (SOM) and CO_2 emission into the atmosphere. In this study the contribution of litters of different stages of Mediterranean secondary succession on Carbon sequestration was investigated, analyzing the role of earthworms in the translocation of SOM into soil profile. For this purpose δ^{13}C difference between meadow C4-C soil and C3-C litter were used in a field experiment. Four undisturbed litters of different stages of succession (45, 70, 100 and 120 since agriculture abandon) were collected and placed on the top of isolated C4 soil cores.

The litter contribution to C stock was affected by plant species and it increased with the age of the stage of secondary succession. 1 year after the litter position, the soil organic carbon increased up to 40% in comparison to soils not treated with litter after 120 years of abandon.

The new carbon derived from C3-litter was decomposed and transferred into soil profile thanks to earthworms and to the leaching of dissolved organic carbon. After 1 year the carbon increase attributed to earthworm activity ranged from 6% to 13% in the soils under litter of fields abandoned for 120 and 45 years, respectively.

Introduction

The major input of vegetative C to forest soil is represented by litter, 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, an accumulation of litter and a consequent increase in the carbon content of the soil has been recorded following the processes of abandonment (Costa and La Mantia, 2005).

Therefore, the mechanisms of litter decomposition, translocation and stabilization into soil layers are fundamental processes in the functioning of the ecosystem as they regulate 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 mineralization rates. Soluble substances and labile compounds of litter are rapidly degraded in the 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 decomposed slowly (Fioretto et al., 2005). Together with bacteria and fungi, invertebrates are responsible for the main functions of the soil ecosystems, including C cycle (Dix and Webster, 1995; Schimel et al., 1999). Several). Authors have attributed earthworm the role of creating 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 also affect both amount and distribution of SOM and cause anincrease in the rates of SOM decomposition. Earthworms, in fact, transport large quantities of C from the surface of the soil to the lower horizons, effectively mixing the soil and significantly increasing both the rates of the humification trough litter fragmentation and of the overall decomposition (Lee 1985; Alban and Berry 1994; Edwards and Bohlen 1996; Burtelow et al. 1998; Li et al. 2002, Pulleman et al., 2005; Steven et al, 2007). On the contrary, Alban and Berry (1994) and Burtelow et al. (1998) found that an earthworm invasion resulted in a C loss in the upper soil layer. On the contrary, Other fundamental processes for the stabilization of SOM are the leaching of fresh litter compound and of recently formed dissolved organic matter (DOM) from organic layers to mineral soil and the sorption of DOM into mineral surfaces (Sollins et al., 1996; Kaiser and Guggenberger, 2000; Kalbitz and Kaiser, 2008). In case of prolonged leaching, however, the litter can become more resistant to decomposition, as a consequence of the significant loss of soluble organic compounds, readily degradable (Mangenot and Tuotain, 1980).

In this study, the objectives were i) determining the role of litter in SOC sequestration; ii) analyzing the mechanisms of C translocation from litter to soil; iii) singling out the amount of C leached and the role of earthworms in this process through isotopic analysis.

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.l.s.). According to World Reference Base for Soil Resources (WRB, 2006), the soil used was shallow Aric regosol, rich in limestone (46% of $CaCO_3$) with a pH value of 7.61, with a sandy-clay-silty texture (53.9% sand, 22.6% silt and 23.4% clay) and organic matter content of 1.40%. The climate was semiarid Mediterranean with a dry period of 4–5 months (mean temperature: minimum 13.7 °C, maximum 22.1°C; mean annual rainfall: 531 mm).

The field plot used in the experiment was a *Cynodon* meadow. The soil under *Cynodon* was a C_4 soil under isotopic steady state, since it had been covered with *Cynodon* (C_4 photosynthetic pathway plant) for more than 15 years. The $\delta^{13}C$ of the experimental soil was -14.5±1.8. *Cynodon* meadow was established with an inter-specific Bermudagrass hybrid (*C. dactylon* x *C. transvalaalensis*), cv *Tifway 419.*

Agronomic management of the turf grass included monthly application of 50 kg ha^{-1} of N, 10 kg ha^{-1} of P and 40 kg ha^{-1} of K fertilizer from April to October. Irrigation was carried out during the spring-summer season with a sprinkler system in order to reinstate evapo-transpiration (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 a week. The cuttings were removed without grass-cycling or mulching.

Plastic cores (n. 30), 20 cm diameter and 40 cm height, were installed in the meadow soil, after a careful removal of the grasses in March 2013 (Fig. 1). The cores were 30 cm buried, with a 10 cm surface collar. In 15 of the 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 C3 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). Soil samples were collected in February 2014. The 30 cm soil core was divided in four sub-samples (each 7.5 cm soil thickness). The soil was dried, 2 mm sieved and the organic fragments were removed.

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). The 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) (La Mantia et al., 2008). 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.). The land covers where litters were placed are described in table 1.

2.2 Litter analysis

Dry biomass weight and its chemical composition (ADL- acid detergent lignin, NDF - neutral detergent fibre, cellulose) were determined using Van Soest sequential method for each collected litter (Van Soest et al., 1991).

The litter respiration rates (mg CO_2 day^{-1} dry litter) were measured during the incubation experiment, using a method of alkali absorption in a closed chamber. 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 the CO_2 which was released inside the bottle. The CO_2-trapped solution titrated with HCl solution using phenolphthalein and methyl-orange as colour indicator. During the 7 days of incubation, CO_2 measurements were done after 24, 48, 60, 96, and 1 week from the start of incubation. Twenty-four hours before the CO_2 sampling, all flasks were ventilated for 30 minutes with fresh air, NaOH trap was placed inside the bottle and then sealed with rubber stoppers. The C mineralization rate was expressed in mg CO_2-C g^{-1} TOC day^{-1} and was fitted to the following first-order decay function:

$$\text{Mineralized C} = C_r\, e^{-kt} \qquad \text{(Eq. 1)}$$

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:

$$CO_2 - C = \sum_i^n \left[(r_i + r_{i+1}) * \frac{d}{2}\right] + \cdots + \left[(r_{n-i} + r_n) * \frac{d}{2}\right] \qquad \text{(Eq. 2)}$$

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.

The mean residence time in days, (MRT) was determined as a reciprocal of the rate constant (k) of first order decay (Equation 1).

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 standard wheat flour, 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 standard beet sugar, δ^{13}CV-PDB = -26.03 ‰), and IA-R006 (Iso-Analytical Limited standard cane sugar, 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:

$$\delta(‰) = \frac{R_s - R_{st}}{R_{st}} * 1000 \quad \text{(Eq. 3)}$$

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

2.4 Data calculation

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

$$\text{New Carbon Derived} = \text{f(NCD)(\%)} = \frac{(\delta^{13}\text{Cnew-}\ \delta^{13}\text{Cold})}{(\delta^{13}\text{Clitter-}\delta^{13}\text{Cold})} \quad \text{(Eq. 4)}$$

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*).

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

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

$$\text{New Carbon (g kg}^{-1}) = \text{Csoil (g kg}^{-1}) * (1 - \text{New Carbon Derived}) \quad \text{(Eq. 5)}$$

The standard deviation of the δ^{13}C and C values were calculated for each depth and treatment. For the average value, Duncan test was used at $p<0.05$ (SAS software, 2001).

3. Results

3.1 Litter characteristics

The plant litter collected during the stages of secondary succession differed in the total weight and C content. The highest weight of litter biomass was in L120 with values of 1113±90 gm^{-2}, followed by L100, L45 and L70 with values of 1027±77 gm^{-2}, 915±104 gm^{-2} and 946±82 gm^{-2}, respectively. The highest C content of litter was in L45 and decreased with the increase of the age of abandon (Fig. 2); however, L120 contributed with the 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 (32mg CO_2-C g^{-1}), followed by L70 (35 mg CO_2-C g^{-1}) and L120 (40 mg CO_2-C g^{-1}).

The MRT (mean residence time) was not significantly different among litter ages, except for L120 (Table 2). These findings were confirmed by the readily mineralizable C which was highest in L120 (Table 2). The composition of litter was not statistically different among 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.

3.2 Soil carbon content and distribution

The total amount of SOC differed under the two treatments (grid and no grid) and time of abandon. The SOC was significantly higher in soils 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 of litter permanence, the SOC under L120 increased on average (0-30cm) by 26% and 40% in grid and no grid treatment respectively, in comparison to no litter treatment.

Such C increase was smaller in grid treatment for the other litters (L45, L70 and L100) with a value of about 12%. In no grid treatment, the SOC increased by 22%, 23% and 15% in soil under L100, L70 and L45 respectively, in comparison to no-litter treatment. SOC decreased with the increase of the 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 changed significantly after litter positioning (Figure 3). The baseline is represented by soil without litter, where the δ^{13}C values ranged between -14.0±0.3‰ and -16.0±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 average (grid and no grid treatment) values of -18.6‰ and -21.6‰ in the top and deepest soil layer, respectively (Figure 3). For the others litter 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 of litter permanence, 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.

The new soil C derived (C_3-SOC) was lower for all litter treatments in soil with grid. 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 (Figure 4). Considering only the C_3-C of SOC for each litter treatment, it was highlighted that the contribution of earthworms to the incorporation of new C_3-SOC was in percentage higher in L45, it decreased with the age of litter and it decreased for each treatment with the increase of the soil depth. The difference of C_3-C between no grid, grid treatment and depth assess the earthworm contribution to soil C increase and distribution.

4. Discussion

4.1 Litter contribution to SOC stock

Previous studies in the island of Pantelleria demonstrated the potential of land cover in the change of C stocks (Novara et al., 2014; Saiano et al., 2013). In fact, land abandon determines the increase in litter layer and SOC. In natural ecosystems, unlike ecosystem , on arable lands, litter is not incorporated into the soil. For this reason it was hypothesized that SOC increase is due to C leaching and/or to earthworm contribution. Such hypothesis was confirmed by the present experiment, where the effect of plant litter contribution to SOC stock was isolated from other soil and environmental parameters. In line with several reports in other ecosystems (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 readily mineralizable C, litter composition (NDF %) and consequently C litter mineralization rate. The litter of L120 had a higher amount of readily mineralizable C, in comparison to other litters, and it was easily decomposed and transferred to SOC pool. The faster mineralization rate of L120 could be attributed both to a different composition of plant species (lower content of sclerofille) (Gianguzzi, 1999) and to a variation in the micro-climatic conditions (Wang et al., 2010; Sheffer et al., 2015) due to a higher accumulation layer on the soil surface. As far as the effect of plant species on the litter mineralization rate is concerned, several studies found a lower litter decomposition rate in *Q. ilex* in comparison to other Mediterranean species, like *Myrtus* and *Cistus* (Berg at al., 1996; Fioretto et al., 2005). Likewise, Maisto et al., (2011) found a slower decomposition of *Q. ilex* in comparison to *Ph. angustifolia*, while no significant difference in the decomposition rate was recorded between *Q. ilex* and *Pistacia lentiscus*. In these studies the lower decomposition of *Q. ilex* was attributed to higher lignin content. Our results confirm those of other researches with regard to the higher lignin content of *Q. ilex*, but this was not tightly associated to lower decomposition rates. In fact, L120, where the main species was Q. ilex, was the litter with a higher decomposition rate. Therefore, other aspects could explain the differences in the 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 main source of SOM in soils under secondary succession. The transformation of C litter into SOM is caused by the decomposition of plant biomass and its incorporation into the 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 singled out the activity of decomposition through the difference of isotopic signature between previous SOC-C (C_4 soil) and the new C_3-C input originated from litter. The ^{13}C litter recovery in the soil profile was higher in L120 (89%), followed by L45 (63%), L100 (60%) and L70 (52%). Firstly , the activity of microbial biomass in soil samples where the grid was placed between litter and soil was highlighted. In this case, the new C_3-C represented the C-pool originated by fungi and bacterial decomposition, transferred into the 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 gave information about the contribution of earthworms to litter decomposition and incorporation into the soil. In several studies, the introduction of earthworms in cold temperate forests resulted in a decline of SOC (Bohelen et al., 2004, Alban and Berry 1994). The results of the present study instead suggest that earthworms have the potential to increase SOC. After 1 year, earthworm activity increased SOC by 13.5%, 11.3%, 11.1% and 5%, in L120, L100, L70 and L45, respectively. The effects of earthworm activity on the recovery of soil C released from litter could be attributed to different mechanisms: (i) the mixture of undecayed particulate C into the soil; (ii) the creation of preferential flowpaths in the soil increasing nutrient transportation; (iii) protection of C in soil aggregates created by earthworm feeding (Bohlen et al., 2004; Fahey et al., 2013).

Conclusions

This study highlights the effects of vegetation succession on C dynamics in soil after the termination of its agricultural use. Based on δ13C signature of C3-C of litter and C4-C of meadow soil, the annual contribution of vegetation input to C stock was estimated. Moreover, the effect of DOC leaching and earthworm activities on C storage in soil depth have also been evaluated.

Hence, in order to understand the ecosystem processes of C sequestration in semiarid environments a better understanding of the impact of above-ground biomass on soil community is still needed.

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Figure Caption

Figure 1. Sampling area of litter in Pantelleria secondary succession (numbers represent litter in field abandoned for 120, 100, 70 and 40 years, respectively) and experimental design in meadow field. Numbers indicate the age since abandon

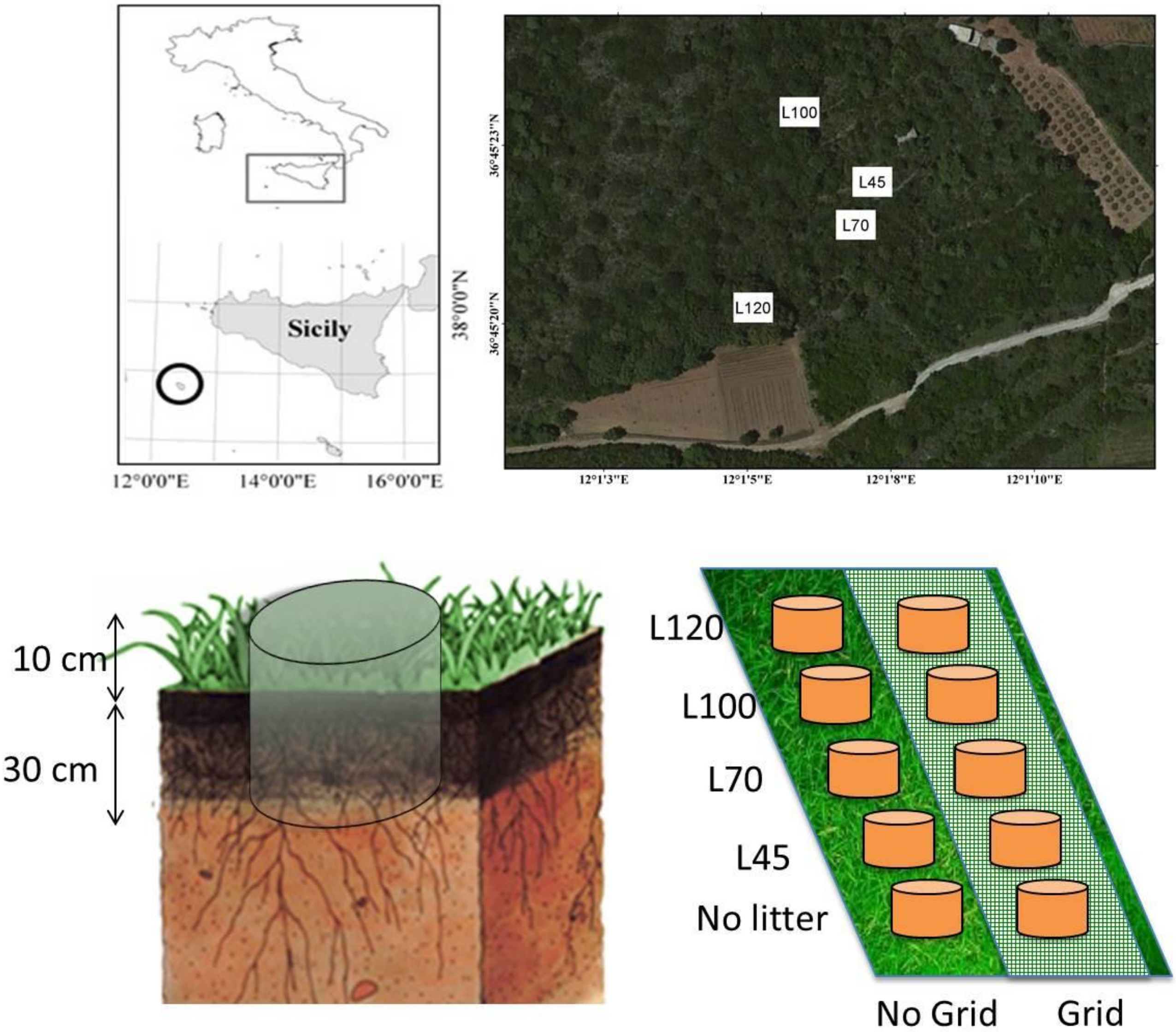

Figure 2. C litter content (%) (black columns) and C litter input (g) for each core (grey columns) in L45, L70, L100 and L120 treatments.

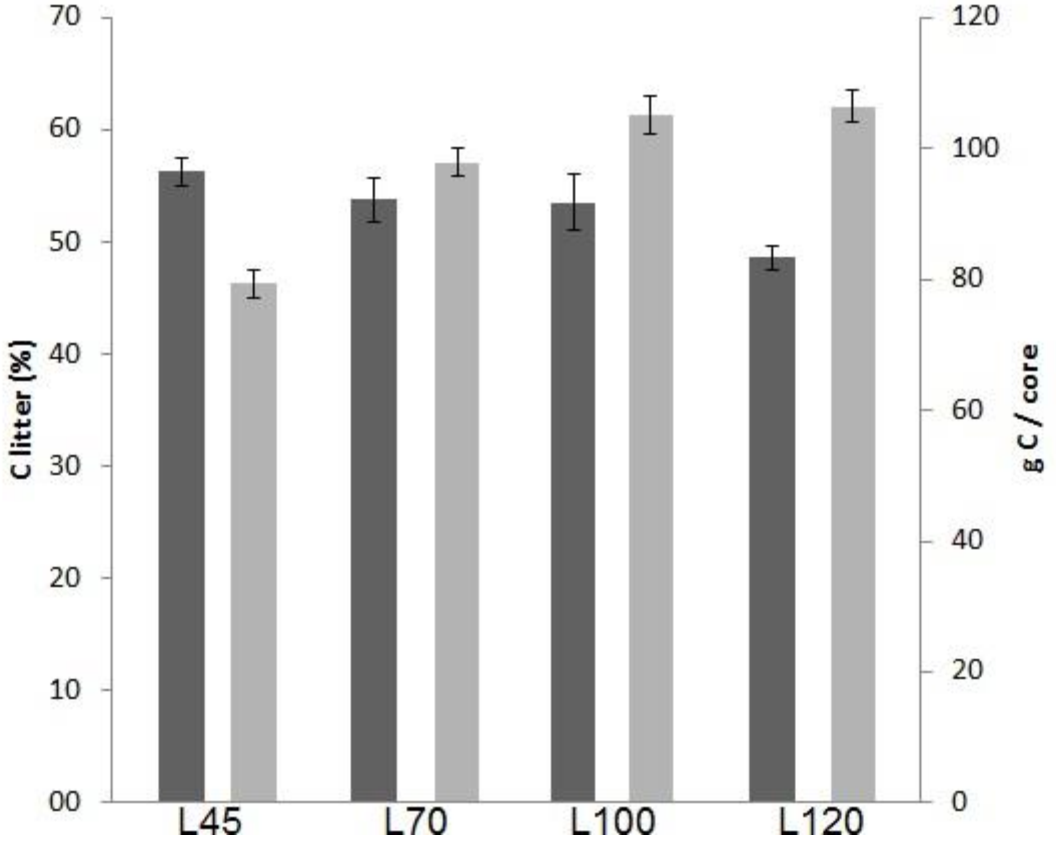

Figure 3. δ13C value at different depth in no grid (a) and grid (b) treatment. The green line represents no litter treatment, while blue, red, grey and black represent litter in fields abandoned for 120, 100, 70 and 40 years, respectively.

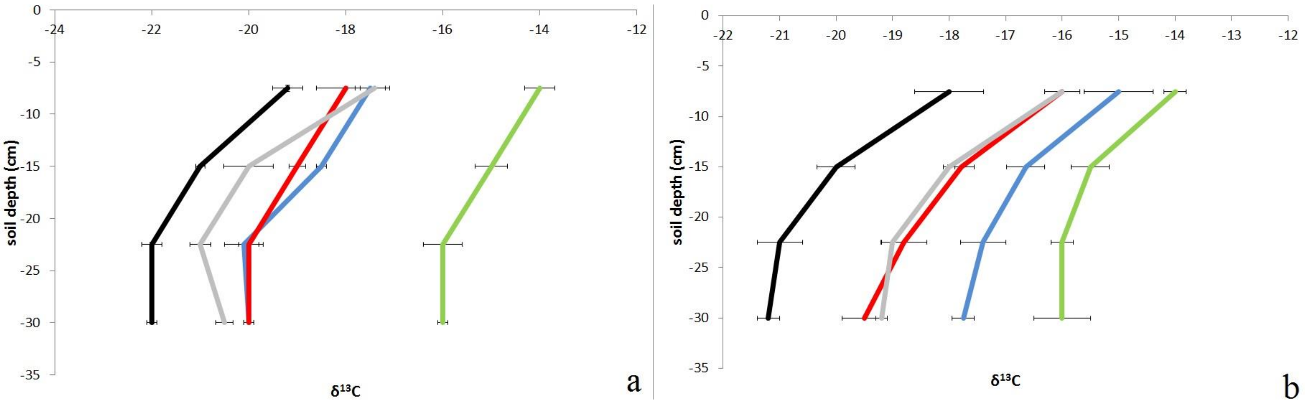

Figure 4. Contribution (%) of worm activity (black columns) and DOC (grey columns) in C_3-C portion at different soil depth. For each portion different letters indicate differences for $P \leq 0.05$.

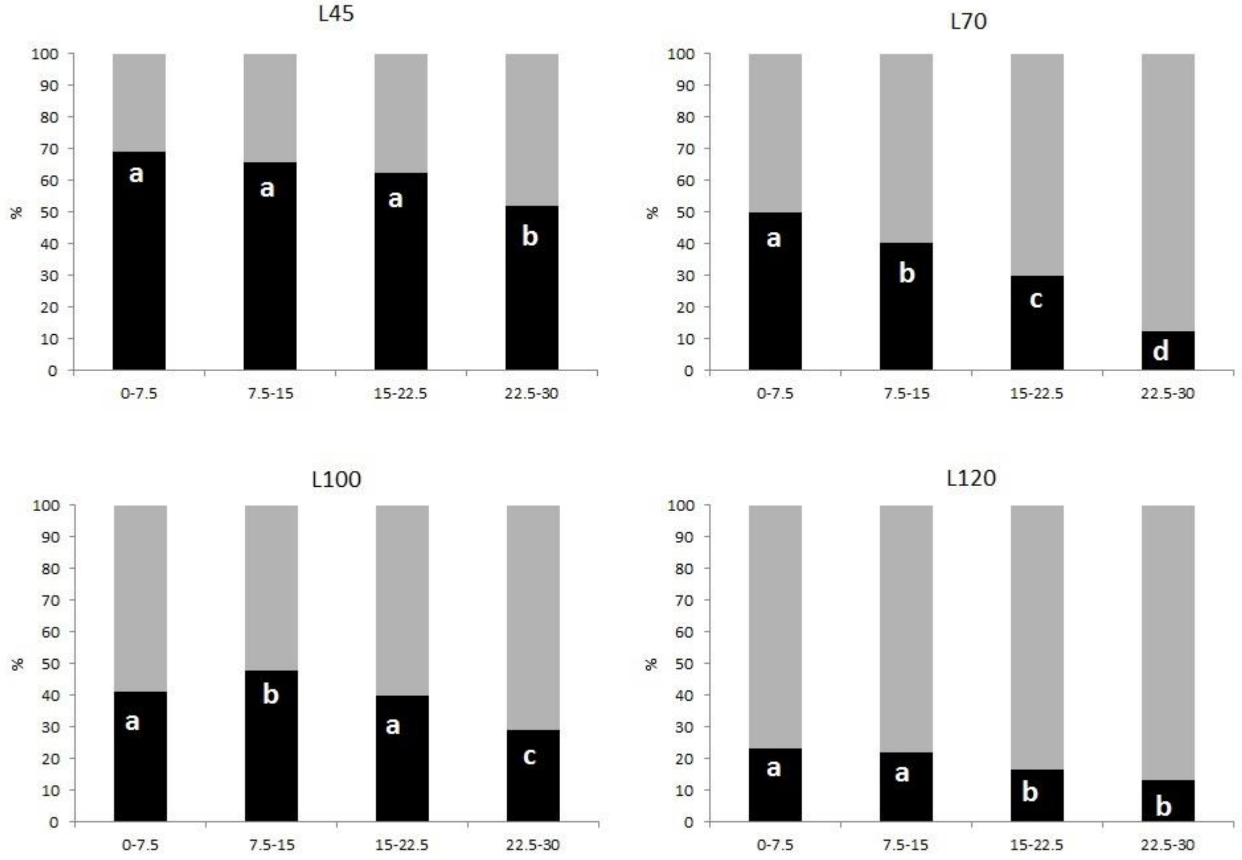

Figure 5. C content in each core (L45, L70, L100 and L120) originated from C4-SOC (grey columns), C_3-SOC from worm activity (yellow columns), C_3-SOC from DOC leaching (orange columns) and C litter (green columns).

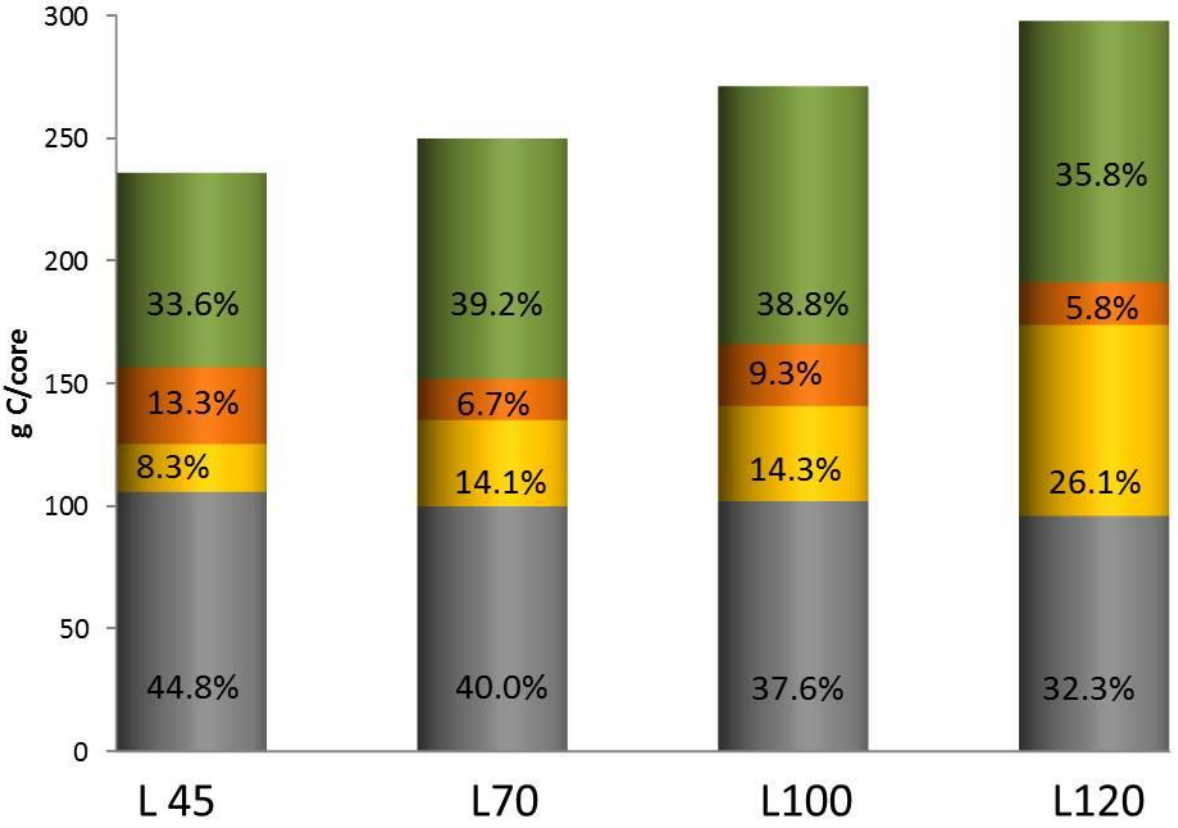

Table 1. Characteristics of litter collected in Pantelleria island.

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

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, C min= readily mineralizable carbon, MRT=mean residence time. In the same column different letters indicate differences for P≤ 0.05.

Litter	C min (mg kg^{-1})	MRT days	R^2	Cellulose	ADL	NDF
L45	154.1 c	25.0 a	0.92	19.0	28.9 b	44.6 b
L70	163.2 c	26.0 a	0.86	17.6	24.1 c	39.3 c
L100	150.7 b	26.0 a	0.90	18.2	30.5 a	44.4 b
L120	217.0 a	22.0 b	0.92	19.9	31.4 a	51.4 a

Table 3. Average of soil organic carbon (%) at different soil depths. For each treatment different letters indicate differences for P≤ 0.05.

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

Litter contribution to soil organic carbon in the processes of agriculture abandon

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Summary

Key words: ^{13}C isotopic signature; earthworm; SOC; litter.

Abstract

The mechanisms of litter decomposition, translocation and stabilization into soil layers are fundamental processes in the functioning of the ecosystem, as they regulate the cycle of soil organic matter (SOM) and CO_2 emission into the atmosphere. In this study, the contribution of litters of different stages of Mediterranean secondary succession on Carbon sequestration was investigated, analyzing the role of earthworms in the translocation of SOM into soil profile. For this purpose $\delta^{13}C$ difference between meadow C4-C soil and C3-C litter were used in a field experiment. Four undisturbed litters of different stages of succession (45, 70, 100 and 120 since agriculture abandon) were collected and placed on the top of isolated C4 soil cores.

The litter contribution to C stock was affected by plant species and it increased with the age of the stage of secondary succession. 1 year after the litter position, the soil organic carbon increased up to 40% in comparison to soils not treated with litter after 120 years of abandon.

The new carbon derived from C3-litter was decomposed and transferred into soil profile thanks to earthworms and to the leaching of dissolved organic carbon. After 1 year the carbon increase attributed to earthworm activity ranged from 6% to 13% in the soils under litter of fields abandoned for 120 and 45 years, respectively.

Introduction

The major input of vegetative C to forest soil is represented by litter, 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 in the presence of carbon in the soil; for instance, an accumulation of litter and a consequent increase in the carbon content of the soil has been recorded following the processes of abandonment (Costa and La Mantia, 2005).

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Therefore, the mechanisms of litter decomposition, translocation and stabilization into soil layers are fundamental processes in the functioning of the ecosystem as they regulate 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 mineralization rates. Soluble substances and labile compounds of litter are rapidly degraded in the 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 decomposed slowly (Fioretto et al., 2005). Togethertogether with bacteria and fungi, invertebrates are responsible forof the main functions of thesoil ecosystems, including C cycle (Dix and Webster, 1995; Schimel et al., 1999). Several), S authors have attributed earthworm the role of creat 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 also affectboth amount and distribution of SOM and cause anincrease in the rates of SOM decomposition. Earthworms, in fact, transport large quantities of C from the surface of the soil to the lower horizons, effectively mixing the soil and significantly increasing both the rates of the humidification trough litter fragmentation and of the overall decomposition (Lee 1985; Alban and Berry 1994; Edwards and Bohlen 1996; Burtelow et al. 1998; Li et al. 2002, Pulleman et al., 2005; Steven et al, 2007). On the contrary, Alban and Berry (1994) and Burtelow et al. (1998) found that an earthworm invasion resulted in a C loss in the upper soil layer. On the contrary, Other fundamental processes for the stabilization of SOM are the leaching of fresh litter compound and of recently formed dissolved organic matter (DOM) from organic layers to mineral soil and the sorption of DOM into mineral surfaces (Sollins et al., 1996; Kaiser and Guggenberger, 2000; Kalbitz and Kaiser, 2008). In case of prolonged leaching, however, the litter can become more resistant to decomposition, as a consequence of the significant loss of soluble organic compounds, readily degradable (Mangenot and Tuotain, 1980).

In this study, the objectives were i) determining the role of litter in SOC sequestration; ii) analyzing the mechanisms of C translocation from litter to soil; iii) singling out the amount of C leached and therole of earthworms in this process through isotopic analysis.

2 Material and Method

2.1 Experimental layout, soil and litter sampling

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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.l.s.). According to World Reference Base for Soil Resources (WRB, 2006), the soil used was shallow Aric regosol, rich in limestone (46% of CaCO3) with a pH value of 7.61, with a sandy-clay-siltysilty texture (53.9% sand, 22.6% silt and 23.4% clay) and organic matter content of 1.40%. The climate was semiarid Mediterranean with a dry period of 4–5 months (mean temperature: minimum 13.7 °C, maximum 22.1°C; mean annual rainfall: 531 mm).

The field plot used in the experiment was a *Cynodon* meadow. The soil under *Cynodon* was a C$_4$ soil under isotopic steady state, since it had been covered with *Cynodon* (C$_4$ photosynthetic pathway plant) for more than 15 years. The δ^{13}C of the experimental soil was -14.5±1.8. *Cynodon* meadow was established with an inter-specific Bermudagrass hybrid (*C. dactylon* x *C. transvalaalensis*), cv *Tifway 419*.

Agronomic management of the turf grass included monthly application of 50 kg ha^{-1} of N, 10 kg ha^{-1} of P and 40 kg ha^{-1} of K fertilizer from April to October. Irrigation was carried out during the spring-summer season with a sprinkler system in order to reinstate evapo-transpiration (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 a week. The cuttings were removed without grass-cycling or mulching.

Plastic cores (n. 30), 20 cm diameter and 40 cm height, were installed in the meadow soil, after a careful removal of the grasses in March 2013 (Fig. 1). The cores were 30 cm buried, with a 10 cm surface collar. In 15 of the 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 C3 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). Soil samples were collected in February 2014. The 30 cm soil core was divided in four sub-samples (each 7.5 cm soil thickness). The soil was dried, 2 mm sieved and the organic fragments were removed.

Litters were collected with cores (20 cm diameter) in 4 different consecutive 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). The abandonment age of the sampled consecutive stages was determined by evaluating aerial photographs taken during 1955 and 1968 (produced by Istituto Geografico Militare, Florence) and 1987 (Regione Siciliana) (La Mantia et al., 2008). The sampled areas were located in direct proximity to each other and were characterized by comparable abiotic conditions (aspect, slope, soil

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type, rock outcrop, stone cover, etc.). The land covers where litters were placed are described in table 1.

2.2 Litter analysis

Dry biomass weight and its chemical composition (ADL- acid detergent lignin, NDF - neutral detergent fibre, cellulose) were determined using Van Soest sequential method for each collected litter (Van Soest et al., 1991)).

The litter respiration rates (mg CO_2 day^{-1} dry litter) were measured during the incubation experiment, using a method of alkali absorption in a closed chamber. 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 the CO_2 which was released inside the bottle. The CO_2-trapped solution titrated with HCl solution using phenolphthalein and methyl-orange as colour indicator. During the 7 days of incubation, CO_2 measurements were done after 24, 48, 60, 96, and 1 week from the start of incubation. Twenty-four hours before the CO_2 sampling, all flasks were ventilated for 30 minutes with fresh air, NaOH trap was placed inside the bottle and then sealed with rubber stoppers. The C mineralization rate was expressed in mg CO_2-C g^{-1} TOC day^{-1} and was fitted to the following first-order decay function:

$$\text{Mineralized C} = C_r\, e^{-kt} \qquad \text{(Eq. 1)}$$

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:

(Eq. 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.

The mean residence time in days, (MRT) was determined as a reciprocal of the rate constant (k) of first order decay (Equation 1).

2.3 Chemical analysis

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For each soil sample the C content and δ^{13}C abundance were measured. δ^{13}C isotopic signature of litter biomass was also analysed. For SOC and the δ^{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 standard wheat flour, 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 standard beet sugar, δ^{13}CV-PDB = -26.03 ‰), and IA-R006 (Iso-Analytical Limited standard cane sugar, 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:

(Eq. 3)

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

2.4 Data calculation

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

(Eq. 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*).

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

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

$$\text{New Carbon (g kg}^{-1}) = \text{Csoil (g kg}^{-1}) * (1 – \text{New Carbon Derived}) \qquad \text{(Eq. 5)}$$

The standard deviation of the δ^{13}C and C values wereere calculated for each depth and treatment. For the average value, Duncan test was used at p<0.05 (SASSAS software, 20012001).

3. Results

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3.1 Litter characteristics

The plant litter collected during the stages of secondary succession differed in the total weight and C content. The highest weight of litter biomass was in L120 with values of 1113±90 gm^{-2}, followed by L100, L45 and L70 with values of 1027±77 gm^{-2}, 915±104 gm^{-2} and 946±82 gm^{-2}, respectively. The highest C content of litter was in L45 and decreased with the increase of the age of abandon (Fig. 2); however, L120 contributed with the 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 (32mg CO_2-C g^{-1}), followed by L70 (35 mg CO_2-C g^{-1}) and L120 (40 mg CO_2-C g^{-1}).

The MRT (mean residence time) was not significantly different among litter ages, except forto L120 (Table 2). These findings were confirmed by the readily mineralizable C which was st (Table 2). The composition of litter was not statistically different among consecutive stages regarding Cellulose and ADL content (Table 2). The NDF value was, instead, significantly higher in L120 in comparison to litters of other consecutive stages.

3.2 Soil carbon content and distribution

The total amount of SOC differed under the two treatments (grid and no grid) and time of abandon. The SOC was significantly higher in soils 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.RIVEDERE tab 3 per stat.

After one year of litter permanenceermanence, 0 the SOC under L120 increased on average (0-30cm) by 26% and 40% in grid and no grid treatment respectively, in comparison to no litter treatment. Such C increasewas smaller in grid treatment for the other litters (L45, L70 and L100) with a value of about 12%. In no grid treatment, the SOC increased by 22%, 23% and 15% in soil under L100, L70 and L45 respectively, in comparison to no-litter treatment. SOC decreased with the increase of the 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 changed significantly after litter positioning (Figure 3). 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 average (grid and no grid treatment) values of -18.6‰ and -21.6‰

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in the top and deepest soil layer, respectively (Figure 3). For the others litter 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 of litter permanence ermanenceC originated from litter input was 32.4%, 34.2%, 38.5% and 49.8% of total SOC in L45, L70, L100 and L120, respectively.

The new soil C derived (C3-SOC) was lower for all litter treatments in soil with grid. 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 (Figure 4). Considering only the C_3-C of SOC for each litter treatment, it was highlighted that the contribution of earthwormsearthworm to the incorporation of new C_3-SOC was in percentage higherhighest in L45, it decreased with the age of litter and it decreased for each treatment with the increase of the soil depth. The difference of C_3-C between no grid grid treatment and depth assess the earthworm contribution to soil C increaseincrease and distribution.

4. Discussion

4.1 Litter contribution to SOC stock

Previous studies in the island of Pantelleria demonstrated the potential of land cover in the change of C stocksstocks (Novara et al., 2014; Saiano et al., 2013). In fact, land abandon determines the increase in litter layer and SOC. In natural ecosystems, unlikeecosystem , on arable landsland, litter is not incorporated into the soil. For this reason it was hypothesized that SOC increase is due to C leaching and/or to earthworm contribution. Such hypothesis was confirmed by the present experiment, where the effect of plant litter contribution to SOC stock was isolated from other soil and environmental parameters. In line with several reports in other ecosystems (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 easily mineralizable C, 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 attributed both to a different composition of plant species (lower content of sclerofille) (Gianguzzi et al., 1999) and to a variation in the micro-climatic conditions (Wang et al., 2010; Sheffer et al., 2015) due to a higher accumulation layer on the soil surface. As far as the effect of plant species on the litter mineralization rate is concerned, several studies found a lower litter decomposition rate in *Q. ilex* in comparison to other Mediterranean species, like *Myrtus* and *Cistus* (Berg at al., 1996; Fioretto et al., 2005). Likewise, Maisto et al., (2011) found a slower decomposition of *Q. ilex* in comparison to *Ph. angustifolia*, while no significant difference in the decomposition rate was recorded between *Q. ilex* and *Pistacia lentiscus*. In these studies the lower

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decomposition of *Q. ilex* was attributed to higher lignin content. Our results confirm those of other researches with regard to the higher lignin content of *Q. ilex*, but this was not tightly associated to lower decomposition rates. In fact, L120, where the main species was Q. ilex, was the litter with a higher decomposition rate. Therefore, other aspects could explain the differences in the 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 main source of SOM in soils under secondary succession. The transformation of C litter into SOM is caused by the decomposition of plant biomass and its incorporation into the 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 singled out the activity of decomposition through thethe difference of isotopic signature between previous SOC-C (C$_4$ soil) and the new C$_3$-C input originated from litter. The ^{13}C litter recovery in the soil profile was higher in L120 (89%), followed by L45 (63%), L100 (60%) and L70 (52%). Firstly, the activity of microbial biomass in soil samples where the grid was placed between litter and soil was highlighted. In this case, the new C$_3$-C represented the C-pool originated by fungi and bacterial decomposition, transferred into the 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 gave information about the contribution of earthworms to litter decomposition and incorporation into the soil. In several studies, the introduction of earthworms in cold temperate forests resulted in a decline of SOC (Bohelen et al., 2004, Alban and Berry 1994). The results of the present study instead suggest that earthworms have the potential to increase SOC. After 1 year, earthworm activity increased SOC by 13.5%, 11.3%, 11.1% and 5%, in L120, L100, L70 and L45, respectively. The effects of earthworm activity on the recovery of soil C released from litter could be attributed to different mechanisms: (i) the mixture of undecayed particulate C into the soil; (ii) the creation of preferential flowpaths in the soil increasing nutrient transportation; (iii) protection of C in soil aggregates created by earthworm feeding (Bohlen et al., 2004; Fahey et al., 2013).

Conclusions

This study highlights the effects of vegetation succession on C dynamics in soil after the termination of its agricultural use. Based on δ13C signature of C3-C of litter and C4-C of meadow

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soil, the annual contribution of vegetation input to C stock was estimated. Moreover, the effect of DOC leaching and earthworm activities on C storage in soil depth have also been evaluated.

Hence, in order to understand the ecosystem processes of C sequestration in semiarid environments a better understanding of the impact of above-ground biomass on soil community is still needed.

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Picture

Caption

Picture 1. Sampling area of litter in Pantelleria secondary succession (numbers represent litter in field abandoned for 120, 100, 70 and 40 years, respectively) and experimental design in meadow field. Numbers indicate the age since abandon

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

Picture 3. δ13C 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 fields abandoned for 120, 100, 70 and 40 years, respectively.

Picture 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 $P \leq 0.05$.

Picture 5. C content in each core (L45, L70, L100 and L120) originated from C4-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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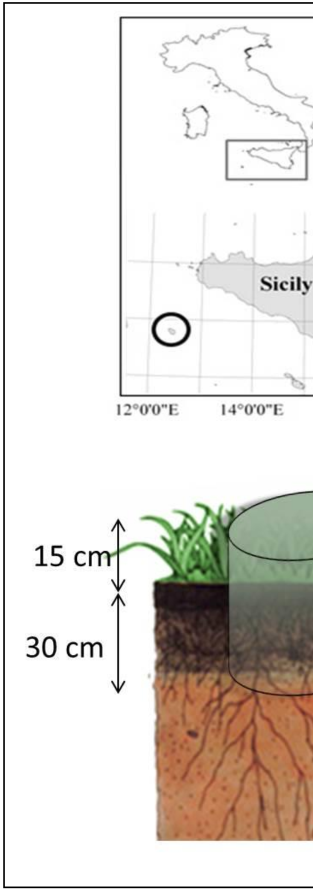

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