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Stable organic carbon pool rises in soil under chestnut (*Castanea sativa* Mill.) forest for timber production after 15 years since grafting onto satin-cut stumps

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Abstract
Forest soils represent an important terrestrial organic carbon sink and the management practices could affect this C pool. The purpose of the present study was to investigate the effect of a forest restoration on the quantity and quality of the soil organic C (SOC). Since the widespread distribution of European chestnut (*Castanea sativa* Mill.) trees in European temperate forests, a 15 years old chestnut forest for timber production was selected on Northern Apennine chain (Italy) which was established on a clear–cut old chestnut stand. The soil sampling was performed in November 2004 (t0, before grafting), 2008 (t1) and 2019 (t2) through the digging of minipits down to 10–30 cm. In each minipit the surface and subsurface horizons were sampled and analysed for the determination of the total organic C content (TOC), and the C of fulvic acids (FA–C), humic acids (HA–C), humin (humin–C) and non–humic substances. Then, the total organic C stock (OCstock), the humification index (HI) and the humification rate (HR) were calculated. From t0 to t2 the mean thickness of subsurface horizon increased likely due both to the tree roots development and to the increased canopy cover due to tree growth which probably reduced the soil erosion. Generally, both TOC content and OCstock did not change over time. The FA–C and HA–C concentrations, instead, more sensitive to the change of management practises, generally reduced over time. This trend was also confirmed by the decline of HR. The humin–C content increased indicating an accumulation of the most recalcitrant SOC pool over time. Hence, the restoration of chestnut stands in mountainous areas, beyond to be a valid economic practice, has beneficial effects on the soil capacity to storage stable C.

Keywords
chestnut forest, soil organic carbon, organic matter pools, forest restoration, mountain soil

Introduction

Soil is the most important terrestrial organic carbon sink (Lal, 2008) and forest soil contain around 39% of global soil carbon (Janzen, 2004). Furthermore, several studies supported the importance of soil in the CO_2 sequestration and its potential feedback to climate change (e.g., Albaladejo et al., 2013; Six et al., 2002). Because of its importance in the physical, chemical and biological functions of the soil, soil organic carbon (SOC) is the major index of soil quality (Obalum et al., 2017). The SOC concentrations are affected by land use (e.g., Massaccesi et al., 2018; Poeplau et al., 2011; Redmile-Gordon et al., 2020), climate (e.g., De Feudis et al., 2019; Jobbágy and Jackson, 2000; Koven et al., 2017), erosion (e.g., Borrelli et al., 2016; Fissore et al., 2017) and management practices (e.g., Grüneberg et al., 2019; Santini et al., 2019). Among silvicultural practices, clear–cutting is commonly used in forest ecosystems (Ma et al., 2013; Zhao et al., 2019), and, as a consequence, damage to soil surface and change of the SOC content have been often reported (e.g., Hyvönen et al., 2016; Zhao et al., 2019). However, if it is well managed applying good forest practices able to guarantee a sustainable ecosystem development, after clear–cutting operations new forest stands are established which significantly promote SOC accumulation over time (Shao et al., 2019). Until

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now, however, there is still a lack of certainty about the direction and magnitude of SOC accumulation after reforestation likely due to the limited number of long–term experiments. Furthermore, to better understand the SOC dynamics and transformations over time it could be highly helpful to study the different SOC pools (Wang et al., 2012). SOC in fact includes several components which have a different susceptibility to biological decomposition and, therefore, differ in their degree of stabilization. SOC can be divided into a labile fraction whose lifespan in the soil ranges from few weeks to months, and a more stable pool whose lifespan ranges from years to centuries (Trumbore, 2009). Other than the need of knowledge on the C amount in labile and more stable pools, it should be relevant to evaluate the relationships among the SOC pools and between SOC pools and total amount of SOC. This is in order to gain information on the degradation degree of the organic matter (Ciavatta et al., 1989) and thus on the SOC dynamics over time in managed forest soils.
Among the forest tree species, chestnut is one of the most important broadleaf species that can be found either in natural or semi–natural temperate forests of the northern hemisphere spanning from Asia to North America via Europe (Lang et al., 2007). Specifically, in Europe chestnut stands cover around 2.5 million hectares mainly concentrated in Italy, France, Italy, Spain, Portugal, and Switzerland (Conedera et al., 2016). Despite their wide distribution, during the second half of XX century, numerous chestnut stands were abandoned due to cultural, economic and ecological modifications (Pezzi et al., 2011). However, the increased demand for high-quality timber of broadleaf species which is taking place in the last decades is promoting the conversion of the abandoned chestnut stands to chestnut coppices stands for the production of valuable roundwood with large calibre through long rotations (Patrício et al., 2019).
In this context, the main aim of the present study was to investigate the effect of the recovery of chestnut forests for timber production on the SOC dynamics over time. Specifically, we studied the quantity and quality of SOC pools at the grafting time with wood varieties of clear–cut old chestnut trees (i.e., initial time) and after 4 and 15 years (short- and long-term, respectively). We hypothesized that during the chestnut growth there is *a*) an increase of SOC content and stock; *b*) a decrease of the most labile SOC pool contents; *c*) an increase of the most stable SOC pool concentrations.

Materials and methods

Study area location and soil sampling

The study was carried out within the experimental chestnut forest located in Granaglione (Italy) in the northern part of the Apennine chain (44°08′ N, 10° 57′ E). The study site covers an area of 1.2 ha at around 700 m above sea level and it is exposed to North–East with an average slope of 15 %. In this area the rainfall has an annual average of 905 mm with July as the driest month (42 mm) and November as the wettest one (113 mm). The mean annual air temperature is 12.2 °C, with July as the warmest month (22.0 °C) and January as the coldest one (2.5 °C). The parent material is sandstone, which belongs to the Miocene period with feldspars, micas and quartz as the main minerals (Vittori Antisari et al., 2013).
In order to restore the old abandoned chestnut (*Castanea sativa* Mill.) forest, in the summer 2004 the stand was clear–cut. Then, around 180 stumps ha^{-1} were grafted and the restored chestnut forest was managed for timber production. The soil sampling was performed in 2004 (t0, before grafting), 2008 (t1) and 2019 (t2) through the digging of minipits down to 10 – 30 cm (Fig. 1).

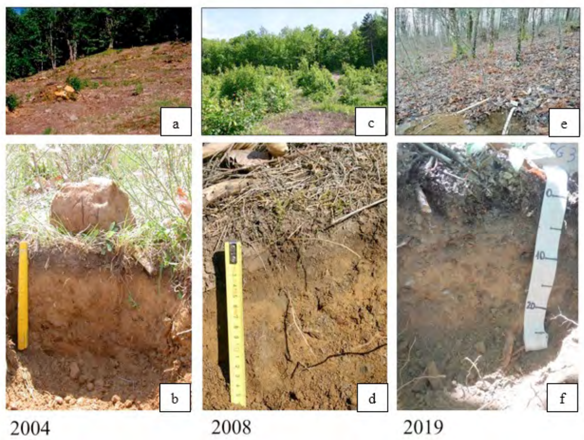

Figure 1. *Images of study sites and their representative soil profiles in the investigated years (2004, 2008, 2019). Specifically, study site and representative soil profile after clear–cut of a chestnut forest (a and b, respectively), and after 4 (c and d, respectively) and 15 (e and f, respectively) years since the restoration with a wood chestnut forest.*

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In each minipit the surface and subsurface horizons (A and AC or Bw horizons, respectively) were sampled and their thickness was recorded. The soil samples were air–dried and sieved to < 2 mm, and an aliquot of each soil sample was further ground for organic C pool determination. In Table 1 are reported the main soil properties of the pedogenic horizons at the beginning (2004) and at the end (2019) of investigated period. According to IUSS Working Group WRB (2015), the soil in 2004 was classified as Dystric Protic Skeletic Regosol (Loamic, Humic) while in 2019 the soil was classified as a Dystric Brunic Skeletic Regosol (Loamic, Humic).

Year	Horizon	Depth	Sand	Silt	Clay	SOM	pH	CEC
		cm	------------%------------			g kg^{-1}		$cmol_{(+)} kg^{-1}$
2004	A	0 – 3	55 ±1	33 ±1	13 ±1	153 ±12	4.97 ±0.11	22.68 ±3.37
	AC	3 – 9	50 ±3	37 ±3	13 ±1	41 ±1	5.07 ±0.08	8.39 ±1.78
	C	9 – 10+	44 ±3	39 ±4	17 ±1	18 ±1	5.01 ±0.08	5.17 ±1.64
2019	A	0 – 5	67 ±2	30 ±2	4 ±0	102 ±21	4.15 ±0.13	10.22 ±2.56
	Bw	5 – 16	44 ±3	46 ±1	10 ±2	31 ±7	4.59 ±0.10	6.47 ±0.59
	BC	16 – 30+	47 ±5	41 ±2	12 ±3	16 ±1	4.75 ±0.05	6.71 ±0.75

Table 1. *Depth and averages ± standard errors of sand, silt, clay and soil organic matter (SOM) contents, pH and cation exchange capacity (CEC) of pedogenic horizons of soil profiles digged at the beginning (2004) and at the end (2019) of the investigated period in restored wood chestnut (Castanea sativa* Mill.*) forest. For horizon depth, the average values of upper and lower limit is reported.*

Soil analysis

Total organic C (TOC) was determined by wet oxidation at 160 °C with 1/3M $K_2Cr_2O_7$ according to Springer and Klee (1954). The content of organic carbon was calculated by back–titration with a solution of 0.2 M $FeSO_4$. The total extractable organic carbon (TEC) was performed in 250 mL centrifuge tube with 100 mL of 0.1 N NaOH/0.1 N $Na_4P_2O_7$ added to 10 g of soil sample. The suspension was shaken for 24 h at 65 °C, under N_2 atmosphere. Afterwards, the suspension was centrifuged and filtered through Whatman 42 filter paper. In new 250 mL centrifuge tube, the obtained TEC was fractioned into humic acids (HA) and fulvic acids (FA) by precipitation of HA at pH < 2 using 1 M HCl. After precipitation, the tubes containing HA and FA were centrifuged. While the precipitate (HA) was redissolved in 50 mL 0.5 M NaOH, the supernatant (FA) was purified by solid chromatography using polyvinylpyrrolidone (PVP) resin (Ciavatta et al., 1990) and the non-humified organic matter (NH) was discarded. Since the NH organic molecules, such as carbohydrates, peptides and amino acids, which were coextracted in the alkaline solution, were not retained by the PVP resin in an acid environment (Sequi et al., 1986), the C of the NH (hereafter called NH–C) fraction was obtained by subtracting the C content of the HA and FA from that of TEC. The organic C content of TEC, HA (hereafter called HA–C) and FA (hereafter called FA–C) pools was measured according to Springer and Klee (1954). The non–extractable C (humin–C) was obtained by subtracting the C content of TEC from that of TOC.

Statistical analysis and calculations

The relative amounts of SOC fractions (RFA, RHA, RNH and Rhumin) were calculated as follows:

$$RFA = 100 \times FA–C / TOC \quad [1]$$

$$RHA = 100 \times HA–C / TOC \quad [2]$$

$$RNH = 100 \times NH–C / TOC \quad [3]$$

$$Rhumin = 100 \times humin–C / TOC \quad [4]$$

Humification parameters, such as humification index (HI) and humification rate (HR) were calculated as follows (Ciavatta et al., 1990):

$$HI = NH–C / (HA–C + FA–C) \quad [5]$$

$$HR = 100 \times (HA–C + FA–C) / TOC \quad [6]$$

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Further, for each horizon the organic C stock (OCstock) was calculated according to the following equation:

$$\text{Ocstock (Mg ha}^{-1}) = \text{bulk density (Mg m}^{-3}) \times \text{horizon thickness (m)} \times \text{TOC (kg kg}^{-1}) \times 10^4 \text{ m}^2 \text{ ha}^{-1} \quad [7]$$

The bulk density values were determined by pedotransfer function according to Leonavičiutė (2000) because of its high predictive potential of bulk density (Casanova et al., 2016).
The statistical analyses were carried out using R version 3.5.2. In order to evaluate the effect of sampling time and soil horizon on the measured parameters, two–way ANOVA was performed. The normality and homoscedasticity of the data were verified by graphical analysis of residuals and transformed if necessary. LSD post–hoc test was used to calculate significant differences among the means and the significance threshold was set at $p < 0.05$.

Results

The recovery of clear–cut abandoned chestnut forest increased the thickness of subsurface horizon over the time, while the surface horizon thickness showed a slight increasing trend even if it was not statistically meaningful (Fig. 2a).
In contrast to our first hypothesis, we did not observe differences both for TOC content and OCstock over the time (Fig. 2b, c).
Nevertheless, as expected, while FA–C, HA–C and NH–C concentrations reduced over time, humin–C conversely showed the highest values in t2 (Fig. 3a, b, c and d, respectively). Specifically, the FA–C content (Fig. 3a) decreased in both soil horizons since t0 till t2, for HA fraction this trend was observed in surface horizon while in subsurface horizon the decline was found between t1 and t2 (Fig. 3b). For the NH fraction (Fig. 3c), instead, in surface horizon the lowest values were found in t2, while in subsurface horizon some differences occurred between t1 and t2, in particular, higher NH–C concentrations were detected in t1 than in t2.
Unlike FA, HA and NH fractions, humin–C did not show differences in surface horizon, while an increase was observed between t0 and t1 for the subsurface horizon (Fig. 3d). Taking in account the relative amount of SOC pools, RFA decreased between the first and the second sampling time for both soil horizons (Fig. 4a). The RHA (Fig. 4b), for the surface horizon showed the highest values in t0, while for the subsurface horizon we observed a decreasing trend over time but with meaningful differences between t0 and t2. The RNH (Fig. 4c), instead, showed the lowest values always in t2. Conversely, for Rhumin (Fig. 4d), in surface horizon we observed an increasing trend over the whole period (i.e., since t0 till t2), while in subsurface horizon the increase was observed between t1 and t2.

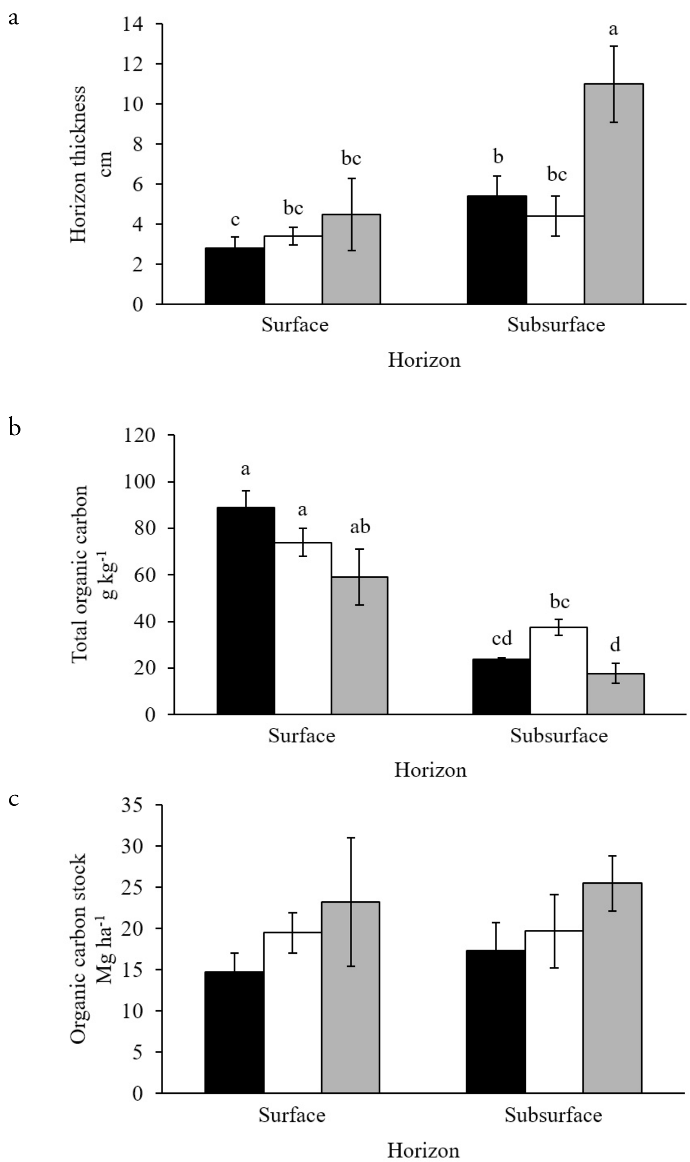

Figure 2. *Thickness (a), total organic carbon content (b) and organic carbon stock (c) in surface and subsurface horizons sampled in 2004 (black bars), 2008 (white bars) and 2019 (grey bars), under chestnut stand for timber production established in 2004 in a clear–cut abandoned chestnut forest. Error bars represent standard errors of the means. Different letters above the bars represent statistically differences among the means according to the least significant difference at the 5% probability level.*

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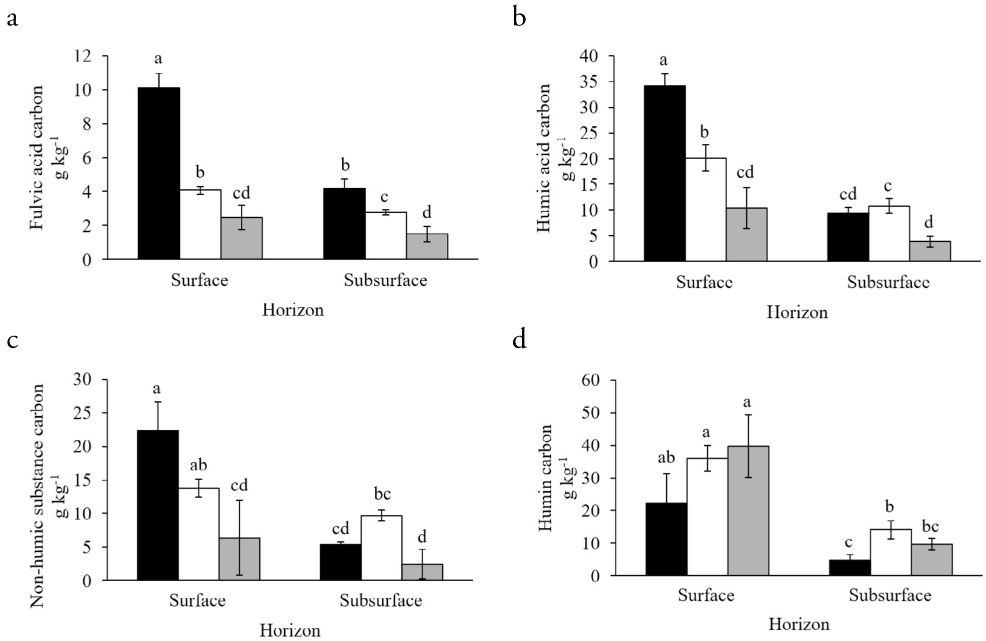

Figure 3. *Fulvic acid carbon (a), humic acid carbon (b), non–humic substance carbon (c) and humin carbon (d) concentrations in soils, sampled from surface and subsurface horizons in 2004 (black bars), 2008 (white bars) and 2019 (grey bars), under chestnut stand for timber production established in 2004 in a clear–cut abandoned chestnut forest. Error bars represent standard errors of the means. Different letters above the bars represent statistically differences among the means according to the least significant difference at the 5% probability level.*

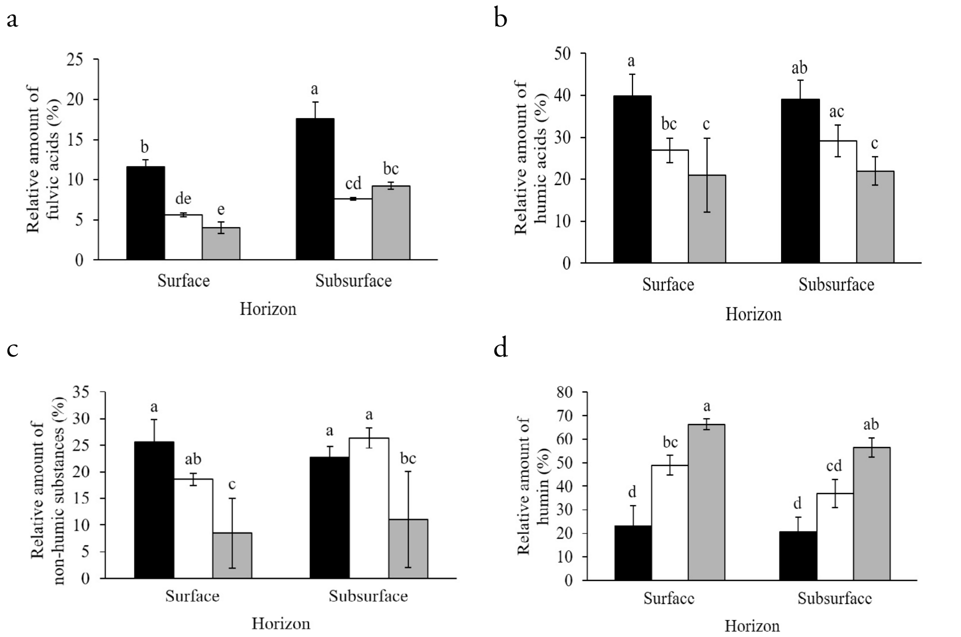

Figure 4. *Relative amount of fulvic acids (a), humic acids (b), non–humic substances (c) and humin (d) in soils, sampled from surface and subsurface horizons in 2004 (black bars), 2008 (white bars) and 2019 (grey bars), under chestnut stand for timber production established in 2004 in a clear–cut abandoned chestnut forest. Error bars represent standard errors of the means. Different letters above the bars represent statistically differences among the means according to the least significant difference at the 5% probability level.*

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For the humification parameters, in surface horizon the HI (Fig. 5a) showed an increase from t1 to t2, while in subsurface horizon we observed an increase from t0 to t1 and a decline from t1 to t2 but with values that still remain higher compared to the first sampling time. Unlike HI, in both soil horizons the HR showed the highest values in t0 (Fig. 5b).

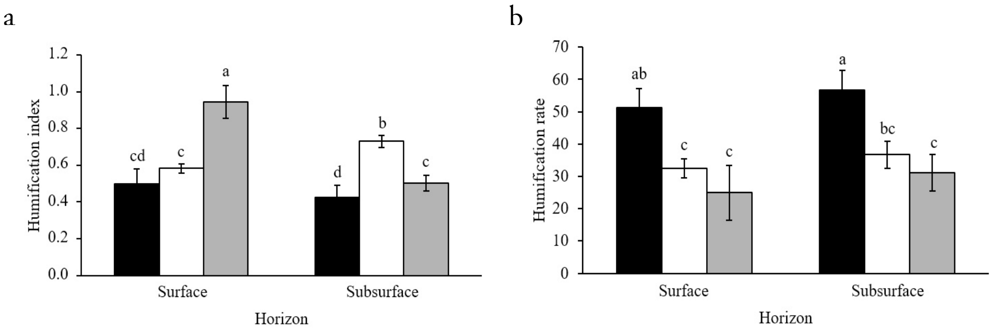

Figure 5. *Humification index (a) and humification rate (b) of soils, sampled from surface and subsurface horizons in 2004 (black bars), 2008 (white bars) and 2019 (grey bars), under chestnut stand for timber production established in 2004 in a clear–cut abandoned chestnut forest. Error bars represent standard errors of the means. Different letters above the bars represent statistically differences among the means according to the least significant difference at the 5% probability level.*

Discussion

The restoration of clear–cut old abandoned chestnut forest had a pronounced effect on the thickness of the considered subsoil horizon. The relative rapid (15 years) changing of subsurface horizon thickness seems to be in contrast to the general knowledge about the soil formation processes which define the pedogenesis as a slow process (Targulian and Krasilnikov, 2007). The accelerated development of the subsurface horizon can be attributed to the high influence of root activities on pedogenic processes (Jenny, 1958). In our study, actually, the density and depth of fine roots strongly increased in subsoil (data not shown), and it is well known that chestnut tree roots are mainly concentrated within 50 cm soil depth and the most of them are fine roots (Dazio et al., 2018; Frey et al., 2006) which are responsible for water and nutrient uptake and, as a consequence, of soil formation processes. In addition, the presence of tree roots together with the wood chestnut forest growth could have reduced the soil erosion and promoted soil development (Gyssels et al., 2005; Křeček et al., 2019; Sun et al., 2018; Świtoniak, 2014). The greater soil development in 2019 compared to the beginning of the experiment is also supported by the evolution of a Bw horizon in subsurface layer since this latter develops later compared to the upper horizon (Dümig et al., 2011). However, according to previous studies (Aubert et al., 2004; Nikodemus et al., 2013) which investigated the effect of forest growth and development on soil properties, no modifications of thickness were found for the surface horizon.

In contrast to several studies that reported a positive correlation between forest age and SOC content (e.g. Adeli et al., 2019), in our case TOC concentration and stock generally did not change over time. The unchanged TOC content over time could be attributed to the reduced soil erosion and increase root activities which probably promoted the vertical redistribution of organic C. Moreover, the apparently weak influence of wood chestnut forest recovery on the total amount of organic matter can be also attributed to the fact that the total organic C, as a whole, does not promptly respond to the management changes (Blair et al., 1995). Unlike TOC content and OCstock, the amounts of organic C of the obtained organic matter fractions (FA, HA, NH and humin) strongly varied over time confirming their higher sensitivity to the management practices (Duval et al., 2016). Specifically, the organic C content of the humic substances showed a declining trend from t0 to t2 with a more pronounced effect for the FA fraction probably due to both the low pH and high amount of sand in our investigated soils. In fact, the decrease of both FA–C content and RFA can be attributed to the high solubility of this fraction in acidic condition (Stevenson, 1994) and the low water retention typical of sand-rich soils (e.g., Ceballos et al., 2002) which allowed this pool to be easily leached. The HA–C concentrations and their relative proportions mirrored the FA pool trend in surface horizon, while

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few differences occurred in subsurface horizon. The diverse behaviour of HA–C in the two considered soil horizons can be attributed to the low solubility of HA in acidic environments (Stevenson, 1994). In particular, the low solubility of this fraction allowed its leaching but not enough to remove the HA also from the subsurface horizon during the experimental period. However, the generally higher organic C concentrations of humified substances at t0 compared to the following sampling times can be inherited by the previous land use or, at least partly, attributed to the decomposition of clear–cut logging residues. This latter hypothesis can be supported by the concentrations of the NH–C which showed higher amount at t0. Indeed, the NH fraction, which includes labile compounds easily utilized as substrates by soil microorganisms (Schmidt et al., 2011), mainly derives from the decomposition of plant litter (Kaiser and Kalbitz, 2012). As regards of NH faction, it is interesting also to note that HI generally increased over the time, and in particular in both horizons the HI values at t2 were higher than those at t0. This should mean that, over time, the contribution of easy available organic compounds increase with respect the humified ones. This was in agreement with the increase of rhizodepositions due to the enhancement of root activities (Barron-Gafford et al., 2005), to which was also attributed the thickening of subsurface horizon. Despite the irrelevant effect of chestnut forest restoration on the total amount of organic C, it was interesting to observe the increase of humin–C enrichment in TOC highlighting the pivotal role of chestnut restoration on soil organic matter stability. Indeed, humin is considered the most resistant organic fraction to decomposition among the humic substances (Almendros and González-Vila, 1987) and the less sensible to the environmental changes (De Feudis et al., 2019). The increase of Rhumin over the time, in particular in subsurface horizon, could be related to the probably chestnut roots development. Specifically, roots might have simulated the soil microbial activities (Paterson, 2003) which in turn promoted on one side the SOC decomposition rate and on the other the mineral weathering. As a consequence, the humin–C can accumulate (Guimarães et al., 2013). The stabilization of the soil organic matter with chestnut stand restoration is further supported by the decline of HR which indicates the accumulation of more recalcitrant compounds to the microbial attack (Bonifacio et al., 2008) due to interactions with the mineral phase.

Conclusions

The establishment of a chestnut stand for timber production through the restoration of clear–cut old abandoned chestnut forests positively affected the soil quality. The present study pointed out the positive key role of recovery of forest on soil development through the deepening of the organo–mineral horizons. Furthermore, despite the 15 years of chestnut woodland weakly affected the total amount of SOC (TOC content and OCstock), likely due to its poor sensibility to the environmental modifications, a meaningful increase of the more stable SOC pool was observed. The highest accumulation of stable SOC fraction, stabilized by mineral interaction, compared to the more labile humified ones was also confirmed by the decrease of the humification rate. Hence, the triggering of these changes can represent important traits for the revaluation of the chestnut grove because, beyond its economic benefits, it can provide beneficial effects on the soil quality of mountainous areas.

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