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Effect of coppice conversion into high forest on soil organic C and nutrients stock in a Turkey oak (*Quercus cerris* L.) forest in Italy

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Abstract

In forest ecosystems, a variety of abiotic and biotic soil forming factors drives soil organic matter (SOM) and nutrients cycling with a profitable outcome on climate change mitigation. As a consequence, type and intensity of forest management, through its impact on carbon (C) and nutrient soil stocks, can be considered as an additional soil forming force. In this study, we investigated the influence of the coppice conversion into high forest on pedogenesis and on soil C and nutrient (N, P, Ca, Mg, and K) stocks, fifty years later the beginning of the conversion-cycle. The trial was established in a Turkey oak forest historically managed under the coppice system in central Italy. Specifically, we considered tree population density (natural evolution – control, moderate thinning, heavy thinning) where soil samples were collected according to genetic horizon to estimate C, N, and P stocks both in the forest floor and at fixed depth intervals (0-30, 30-50 and 50-75 cm). Further, the stocks of exchangeable Ca, Mg, and K were also assessed for the mineral layers. The results showed that litter and the upper layer of mineral soil (0-30 cm) contained a similar quantity of C (about 74-83 Mgha^{-1}), independently of the trials and no differences were observed also in the whole soil stocks (about 192-213 Mg ha^{-1}). The comparison of the mean stocks calculated per 1-cm of thickness of organic (O), organo-mineral (OM), and mineral (M) layers, although it did not display any difference among trials (excepted for P and Mg), showed a similar capability of the organo-mineral horizons to store C and nutrients compared with the organic ones (e.g., about 6-12 Mg ha^{-1}, 0.3-0.5 Mg ha^{-1} and 0.5-1.5 kg ha^{-1} for C, N and P, respectively). Our findings showed that thinning operated on Turkey oak coppice did not affect soil capacity to store C and nutrients. These results suggested that the forest ecosystem itself is the main soil forming force and this is consistent with the target of adopting forest management able to control the global C cycle through the storage of SOM in the mineral soil rather than in forest floor, where SOM turnover is faster.

Keywords: forest soil**,** organic matter, rock fragments, pedogenetic horizons, coppice conversion into high forest, sustainable forest management

1. Introduction

Soil organic matter (SOM) plays key roles in terrestrial ecosystems, where it is involved in many processes of soil conservation. SOM is fundamental in stabilizing soil structures and reducing soil erosion, improving water-holding capacity, and releasing nutrients to plants, microorganisms, and soil fauna (Bot and Benites, 2005; Canedoli et al., 2020). In forest soils, the input of organic matter depends on litter production, mortality of fine roots, roots exudates, and shoots residues (Lehmann et al., 2015; Diao et al., 2020; Wu et al., 2020). Thus, depending on the interaction among the main soil forming forces (parent material, climate, living organisms, relief, and time; Jenny, 1941), a vastity of physicochemical and biological processes affects the transformation of plant-derived organic materials in SOM. During this transformation, SOM is stabilized by the formation of organo-metallic complexes with di- and trivalent cations (Kaiser et al., 2016), the formation of organo-mineral complexes with clay minerals (Kögel-Knabner et al., 2008, Barré et al., 2014; Gartzia-Bengoetxea et al., 2020), and the occlusion within aggregates (Schrumpf et al., 2013), favouring its preservation in the soil. Therefore, plant species, soil properties, and their interactions play a key role in determining the soil organic C (SOC) stock and, due to SOM elemental content, also in the biogeochemical cycles of nutrients like N, P, Ca, Mg, and K (Tiessen et al., 1994). For instance, estimations indicate that the mean world soil content to 1-m depth is 1462-1548 Pg for organic C and 133-140 Pg for total N (Batjes, 1996), more than the global content obtained combining vegetation and atmosphere (Lehmann et al., 2015; Mayer et al., 2020).

In forest ecosystems, forest management may impact on SOC and nutrient stock. In their review, Mayer et al. (2020) reported that management practices like site preparation, harvesting operations, removal of harvest residues, and removal of litter and biomass for fodder, fuel, or animal bedding have a negative impact on SOC stock capacity. Conversely, N addition, introduction of N-fixing plants, and herbivory regulation have a positive impact on SOC storage. Other practices like management of tree species diversity and periodical thinnings over the whole stand lifespan that are used to manage tree population density in high forest [which consists in a stand of trees, generally originated from seed , that develop a high, closed canopy (SAF, 2008)] and in the conversion into high forest of coppice systems seem not to interfere with the soil capacity to stock organic C under both broadleaves and conifers (Bravo-Oviedo et al., 2015; Prasad Dangal et al., 2017; Zhang et al., 2018; Mayer et al., 2020).

Coppicing represents the oldest form of systematic and sustainable use of forests. It is a very flexible system that requires a low energy input and has been adapted and modified according to the needs of rural societies, to whom coppice forests deliver small size wood primarily for energy (firewood and charcoal), agriculture, and small scale businesses. As a matter of fact, coppice forests characterize the European landscapes, especially in mountainous areas of central, east and southern

Europe. Due to rural migration and technical and economic restrictions, most of the coppice forests are today neglected or abandoned, representing a significantly underused natural resource (Unrau et al., 2018). In Italy, coppice forests cover 3.663 million hectares (Mairota et al., 2018)) and both evergreen and deciduous *Quercus* spp. make a significant share of the total cover (nearly 1.6 million ha). Following the crisis of the firewood and charcoal system, the conversion of coppices into high-forests is considered a sustainable forest management in many countries (Fabbio, 2016; Fabbio and Cutini, 2017; Cutini et al., 2021) due to the low-frequency soil disturbance that would favour the storage of SOC (Hölscher et al., 2001; Marchi et al., 2016). Therefore, the link between forest management and soil properties, with its specific capacity to determine SOC stock and climate change mitigation, has fostered a number of scientific researches (e.g., Caddeo et al., 2019; Zhang et al., 2019; Zhao et al., 2019; Lee et al., 2020), but scarce has been the interest on the effect of forest management on the soil stock of nutrients like N, P, Ca, Mg, and K, whose abundance and availability is key to soil fertility and biomass production.

The aim of this work was to assess the role of thinning performed for the conversion of a coppice forest into high forest on soil C, N, available P, and exchangeable Ca, Mg, and K stocks. The effect of periodical thinning vs no silvicultural intervention (namely, natural evolution following the suspension of periodical harvestings) was investigated in a Turkey oak (*Quercus cerris* L.) stand under conversion into high forest and managed as coppice up to 1949 (last coppicing).

To test the hypothesis that different forest managements can affect soil C and nutrients stocks, and to investigate on the contribution of each horizon to the whole soil stocks, we estimated: i) C, N, and P stored in the genetic horizons (ranked in organic, organo-mineral and mineral horizons) and at fixed depth intervals (0-30, 30-50, and 50-75 cm); and ii) exchangeable Ca, Mg, and K stored in the mineral soil (organo-mineral and mineral horizons) and at fixed depth intervals (0-30, 30-50, and 50-75 cm).

2. Materials and Methods

2.1. Environmental and historical background

The study was conducted in the Natural Reserve of Monterufoli-Caselli forest, Tuscany, Italy (Fig. 1), a Natura 2000 Site (SPA-SAC IT5170008 Complesso di Monterufoli). The whole reserve covers a gentle hilly environment and extends for 4,828 ha within altitudes spanning between 100 and 560 m. The mean annual precipitation is 750 mm and the mean annual air temperature is 13.5 °C. Geology of the area is rather complex being dominated by serpentinite and polygenic breccias (Paleocene), followed by calcareous sandstone interbedded with limestone (Cretaceous), pelitic marine sediments (Pliocene), silty-clay schists (Cretaceous), and quartzous sandstone interbedded with arenaceous limestone (Paleocene). The area was heavily influenced by human activities since ancient times. Central Italy, and especially Tuscany, was subject to an intense mining activity during Bronze and Iron ages. As reported by Cartocci et al. (2007), Tuscany can be considered one of the most important ancient metallurgical districts of Italy, with several active mining centres since the Iron Age. In the study area, considerable was the production of iron, pyrite, base metals, silver, antimony, mercury, and gold for millennia (Chiarantini et al., 2018). Because of this activity, there was the need of fuel for metal smelting, with the consequent exploitation of forests (especially oak forests of *Quercus cerris* L., *Quercus pubescens* Willd., and others) for charcoal production (Carrari et al., 2017).

2.2. Study area

The study was run in a long-term monitoring area located within the Caselli Forest. Forest stands consisted of Turkey oak (*Quercus cerris* L.) for about 90%, with broadleaves like *Fraxinus* spp., *Ulmus* spp., *Ostrya* spp., and *Quercus ilex* L. as subsidiary species. Under the coppice system, in Italy Turkey oak cover ≈675,000 ha, i.e. the 18.4% of coppice forests (Manetti et al., 2020). Leaf area index (LAI) ranged from 4.3 to 5.2 (Cutini, 1996). Here, last coppicing was performed in 1949; then, in 1969, a long-term experiment aimed at comparing the periodical thinning of standing crop *vs* its natural evolutive pattern to achieve coppice conversion into high forest was established. The main goal of the experiment was to verify stand dynamics as for its structural-compositional arrangement and functional traits of tree biomass. The treatments on the ground were the full release of the dominated layer, moderate thinning (MT) and heavy thinning (HT) with an average release of 1500 and 1100 stems ha^{-1} in the dominant layer, respectively. The coppice under natural evolution, in absence of any practice, was considered as control (CTR); here, the average full tree density was 4269 ha^{-1} (Fabbio and Amorini, 2006; Manetti and Gugliotta, 2006). Each trial was repeated four times according to a randomized blocks design. Within plots of several thousands of m^2, we selected a survey area of 900 m^2 all within a NNE-NNW exposure on slopes roughly ranging from 10 to 20% (Table S1, Supplementary Materials). A second thinning was implemented in 1989 releasing 715 and 1036 shoots ha^{-1} in HT and MT, respectively. Average stem density decreased to 3589 ha^{-1} in CTR. The arrangement of stand structure following the applied silviculture was a two-storied stand: the dominant layer mainly made of Turkey oak, and the dominated layer made the set of subsidiary broadleaved species.

Other inventories were performed in 1998 and 2004. In 2004, there were 578 shoots ha^{-1} in the dominant layer of HT and 869 shoots ha^{-1} in MT. Average full stem density was 3417 ha^{-1} in CTR. Main stand parameters are summarized in Table 1.

2.3. Sampling sites and soil sampling

The study site spanned from 337 to 345 m above sea level, on soil formed on calcareous sandstone interbedded with limestone. In each of the four plots of the three trials, in 2017 a survey was run to evaluate the spatial variability of surface stoniness, rock outcrops, slope, micro-topography, dominant vegetation, and understorey to select the location where to dig a soil profile. Then, a total of 12 profiles (1 profile • 4 plots • 3 trials) representative of each plot conditions were opened within locations with 12-15% slope and 90-95% soil cover. In each plot the soil profile was dug at ≈1 m from the stem (downslope position) of one of the oldest trees and until the depth of ≈1 m, except for lithic contact. For each profile, the organic horizons forming the forest floor were morphologically described per Baize et al. (2008) and sampled in an area of about 3 m^2 around the profile. The mineral soil was morphologically described per Schoeneberger et al. (2012) and sampled by genetic horizons. Soil morphologies provided of understorey composition (Frati et al., 2021) are reported in Table S1 of Supplementary Material. During the field operations, the collected samples were stored in a refrigerated bag and, once in the laboratory, they were allowed to air-dry. Thus, the mineral samples were sieved at 2 mm to separate the fine earth (< 2-mm fraction) from the skeleton (> 2-mm fraction).

2.4. Laboratory analysis

The bulk density of both fine earth and skeleton of each horizon was determined by soil cylinders. Specifically, two horizontal soil cores were collected from each mineral horizon by using cylinders of 503 cm^3 (height: 10.8 cm; diameter: 7.7 cm). In the laboratory, the collected sample was sieved at 2 mm and the volume of the skeletal particles was determined by water displacement after the particles were water-saturated (Corti et al., 1998). The volume of the fine earth was obtained by subtracting that of the skeletal particles from the total volume of the cylinder. Both fine earth and skeleton were then heated at 105°C and weighed. The content of large cobbles was estimated by the "percent of area covered" figure reported in Schoeneberger et al. (2012), and their bulk density determined as mentioned above. For the organic horizons, the bulk density was estimated by pedotransfer functions (De Nicola et al., 2014), which have been tested by other researcher in various Italian contexts (Brenna et al., 2010; Garlato et al., 2009a, b; Guermandi et al., 2013). These equations provide bulk density as a function of the percentage of estimated organic matter (OM = 2 • organic C) as follows:

1. For OM > 30%: bulk density (g cm^{-3}) = 0.00589 • organic C + 0.554;
2. For OM = 30–15%: bulk density (g cm^{-3}) = 0.00745 • organic C + 0.593;

3. For OM < 15%: bulk density (g cm^{-3}) = 0.00797 • organic C + 0.553.

Aliquots of 20 g of fine earth were used to determine the particle-size analysis after they were maintained submerged in deionised water for 24 h; sand was retrieved by wet sieving at 0.053 mm, while silt and clay were obtained by sedimentation. All the following analyses were performed on both fine earth and skeleton. The pH values were determined potentiometrically in water after one night of solid:liquid contact at 1:2.5 w:v ratio for the mineral samples and 1:8 w:v ratio for the organic samples (Cardelli et al., 2019). Total organic carbon (TOC) was estimated by K-dichromate digestion, heating the suspension at 180 °C for 30 minutes (Nelson and Sommers, 1996). Water-extractable organic matter (WEOM) was extracted after one night of the 1:10 solid:liquid suspension in an orbital shaker at 140 rpm and filtered through a Whatman 42 filters (Agnelli et al., 2014). The organic C content of the extract (WEOC, water-extractable organic carbon) was determined by titration (Nelson and Sommers, 1996). Total N (TN) was measured by a dry combustion analyser (EA-1110, Carlo Erba Instruments, Milan, Italy), while available P (P_{av}) was determined following the Olsen et al. (1954) method. Exchangeable Ca, Mg, and K were displaced by a 0.2 M $BaCl_2$ solution (solid:liquid ratio 1:10) and extracted after 10 min of shaking (Corti et al., 1997). The obtained suspensions were centrifuged and filtered through Whatman 42 filters. Elements were determined by atomic absorption with a Shimadzu AA-6300 spectrophotometer (Tokyo, Japan). For the skeletal fraction, pH, P_{av}, and exchangeable Ca, Mg, and K were determined on unground fragments, while TOC, WEOC, and TN were measured on ground aliquots (Ugolini et al. 1996; Corti et al., 1997; Corti et al., 2002).

2.5. Stock calculation

Soil rock fragments can contain considerable amounts of nutrients (Ugolini et al., 1996). In particular, as pointed out by Corti et al. (2002) and Cuniglio et al. (2009), calcareous skeleton may represent a large reservoir of C, N, and nutrient cations. Thus, considering the soil as made of fine earth only may result in significant overestimations of the soil nutrient budget. Therefore, C and nutrients (N, P, Ca, Mg, K) stocks were calculated for each genetic horizon taking into consideration both fine earth and skeleton contributions.

The amount of element stored in the fine earth and skeleton was calculated as following (De Nicola et al., 2014):

$$ES = EC \bullet BD \bullet TH \bullet CC \qquad (1)$$

where ES is the element stock (in Mg ha^{-1} for C, N, and exchangeable Ca, Mg, and K; in kg ha^{-1} for P_{av}), EC is the element concentration (g kg^{-1} for C and N; mg kg^{-1} for P_{av} and exchangeable Ca, Mg, and K), BD is the bulk density (kg dm^{-3}), TH is the horizon thickness (cm), and CC is the coefficient applied to normalize the units of measure (10^{-1} for C, N and Pav; 10^{-4} for exchangeable Ca, Mg, and K).

Thus, the total C and nutrient stored in each genetic horizon were determined as the weighed mean for the fine earth and skeleton contents:

$$ESTOT = [(ESfe \bullet FE\%) + (ESsk \bullet SK\%)] / 100 \qquad (2)$$

where ES_{TOT} is the total amount of element stored in the genetic horizon (in Mg ha^{-1} for C, N, and exchangeable Ca, Mg, and K; in kg ha^{-1} for P_{av}), ES_{fe} is the amount of element contained in the fine earth, FE% is the percentage of fine earth content in the horizon, ES_{sk} is the amount of element contained in the skeleton, SK% is the percentage of skeleton content in the horizon.

For each element, the amount stored by 1-cm thickness of the organic, organo-mineral, and mineral horizons was also calculated.

2.6. Statistical analysis

Because of the soil variability, profiles showed slight differences in the sequence of horizons. Thus, genetic horizons were grouped into soil layers based on their nature: forest floor (OLn, OLv, OFr, and OH horizons), organo-mineral (A and AB horizons), and mineral (Bw, Bg, BC, and Cr horizons). Properties of the soil layers were obtained by calculating the weighed mean of each property based upon the thickness of each horizon. The element stocks for the 0-30, 30-50, and 50-75 cm of soil were calculated considering the thicknesses of the organo-mineral and mineral soil horizons. To highlight differences in C and nutrient stocks, one-way ANOVA was performed along the soil layers and among soils under different forest managements. Prior to ANOVA, normality and homoscedasticity of the dataset were assessed using Shapiro-Wilk statistical test and by Levene's test at 5% significance level, respectively. Assumptions were not violated and Tukey's Honest Significant Difference (HSD) test with $P \leq 0.05$ was used to compare differences among means. Results of ANOVA (*F value* and *significance level*), showing the influence of management and depth on physical and chemical properties and elements stock in the surveyed soils are reported in Table S5 a/b of Supplementary Materials.

3. Results

3.1. Soil morphology, and physical and chemical properties in the three forest trials

Properties of the experimental site were similar in the three forest trials.

All the soils were classified as Humustepts (Soil Survey Staff, 2014). Soil morphology organized in soil layers is reported in Table 2 and Table S1 of Supplementary Material. The litter layer was on average 2 to 5 cm thick and was mainly made by Turkey oak leaves and branch fragments. Organo-minerals horizons showed a thickness spanning from 2 to 6 cm thick, and the soil structure was moderately to well-developed, in form of crumbs or subangular blocks; in the area massively frequented by wild boars (MT), the structure was platy. Mineral horizons showed poorly to well-developed structure mainly made of subangular and angular blocks. Gley B horizons (Bg) indicate periodical soil water saturation (Soil Survey Staff, 2014). The skeleton content in the three trials ranged from 0 to 50-60%, with the greatest contents in depth (Table 2).

The soil pH was sub-acid (ranging between 5.69 and 6.15), with no significant difference among layers and trials (Table 3). The particle-size distribution showed a coarser texture in the organo-mineral horizons (loam to sandy-loam textures) than in the mineral ones (silty clay and clay loam). No statistically significant difference ($P>0.05$) among the trials was observed. As expected, the largest contents of TOC, WEOC, TN, and P_{av} were in the litter and showed a decreasing trend with depth. Among the trials, no difference occurred for TOC, WEOC, and TN, whereas the organo-mineral horizons of HT displayed the highest P_{av} concentrations. The WEOC/TOC ratio showed very high values with respect to other reports (Corvasce et al., 2006; De Feudis et al., 2017), with statistically significant differences only in the MT trial, where the mineral horizons displayed the highest value. The C/N ratio showed a significantly decreasing trend with depth in all the trials, with no significant difference among them (Table 3, S2 and S3 of Supplementary Material for fine earth and skeleton data, respectively).

3.2. C and nutrient stocks in the three forest trials

In the three trials, litter and the upper 0-30 cm mineral layer contained similar quantity of C, which was higher than in the 30-50 and 50-75 cm mineral layers (Table 4). Other differences among litter and mineral layers were observed for TN in CTR, exchangeable Ca in HT, and K in HT and MT, always with the highest stock in the 0-30 cm layer. However, the stock of all elements showed no statistical difference among the trials. The contribution of the skeleton to the element stocks was negligible or null for C, TN, and P_{av}, but ranged from ≈1.5 to ≈11% for exchangeable K, and from ≈27 to ≈63% for exchangeable Ca and Mg (Table 4 and Table S4 of Supplementary Material).

The amount of the elements stored by 1-cm thickness of the organic, organo-mineral, and mineral horizons in the three trials is reported in Fig. 2. The quantity of C, TN, and P_{av} stored in 1 cm of litter was often similar to that of the organo-mineral horizons (except for P_{av} in CTR), and from three- to ten-fold higher than that of the mineral horizons (except for TN in CTR, where no significant difference was observed). For the exchangeable Ca, Mg, and K, the stock capacity of 1 cm of organo-mineral horizons was generally greater than in the mineral horizons but because the samples were small (Webster, 2001), the differences were not statistically significant except for Ca in MT, Mg in HT, and K in both HT and MT, where the variability was proportionally less than in the other cases. Contrasting the stock capacity per 1-cm thickness among the different trials, only P_{av} and exchangeable Mg showed significant differences, with the highest contents of the two elements in the organo-mineral horizons of HT (Table 3).

4. Discussion

4.1. Effect of thinning on soil morphology and physicochemical properties

The effect of thinning on the main pedological features appeared negligible in the studied forest. In the topsoil, which is the soil portion most sensitive to disturbances and management practices (Song et al., 2005), the effect of thinning could have been masked by the wild boar activity. In our case, the topsoil mixing due to wild boars seemed to have not substantially affected the soil morphology, probably because all trials have been characterized over time by their presence, albeit with different intensities.

Although the soils developed from calcareous parent material, the soil profiles displayed sub-acid pH values, indicating that soils have been subjected to a heavy decarbonation induced by several acidification processes (Haynes, 1990; Richter et al., 2007; Lemanceau et al., 2009; Chapin et al., 2011; Cocco et al., 2013; Corti et al., 2019). Because of this, and the long time needed to dissolve all carbonates (Cocco et al., 2013), these soils can be considered as highly weathered (e.g., Sundquist and Visser, 2003) and, consequently, it was not expected that the forest thinning could induce marked changes on soil pH in 50 years. Moreover, even when carbonates have been dissolved, acidification is buffered by clay and organic matter (Brady and Weil, 2017), which contribute to reduce pH changes. The soil texture is a parameter not responding quickly to environmental changes; in fact, it is similar for all the trials. Along the profiles, the texture was finer at depth than at the surface probably for the occurrence of lessivage, a process that requires long time to produce differences in terms of soil texture and drainage (Buurman et al., 1998; Quénard et al., 2011; Calabrese et al., 2018).

The decreasing of TOC, TN, and P_{av} with depth is a common trend in soil and especially in forest soils, where the majority of the biomass produced is added in form of litter (Mason and Zanner, 2005). While TOC and TN were not affected by thinning, as they respond slowly to changes (Bai et al., 2017), the different P_{av} content in the organo-mineral and mineral horizons of the three trials were considered an effect of the forest thinning. In fact, working on soils under *Fagus sylvatica* forests, Cardelli et al. (2019) reported that P liberation and activity of enzymes involved in the P cycle are higher in the organo-mineral (A) than in the organic (O) horizons because of the major content of decaying SOM and the consequently greater availability of P-bearing substances like nucleic acids, carbohydrates, proteins, and fatty acids. The major reduction of the canopy density in HT might have enhanced SOM degradation through the increased solar radiation and temperature (e.g., Gressel et al., 1996; Scharenbroch and Bockeim, 2007; Cheng et al., 2021)), with the subsequent higher release of P.

The WEOM content and the WEOM/TOC ratio did not change among the trials. This behaviour was unexpected because WEOM, which is composed of easily degradable molecules that represent the main C and energy source for the soil microbial community (De Feudis et al., 2019), is considered as an indicator of microbial activity (Gutiérrez-Girón et al., 2015), very sensitive to disturbances and management (Chantigny, 2003). However, since the WEOM is released following SOM mineralization (Bartos et al., 2020) and the two thinning intensities did not produce different litter thicknesses (Table 2) and soil TOC and TN concentrations (Table 3) in respect to the control, it would justify the similar WEOC contents and WEOC/TOC ratios found among the trials. Throughout the profiles, the decreasing content of WEOC and the parallel increase of WEOC/TOC ratio (statistically significant only in MT) confirmed the importance of this soluble fraction as energetic substrate for the organisms harbouring the deeper soil horizons. The increase of the WEOC/TOC ratio in the mineral layers, where the clay content is the highest, could be also due to adsorption of organics on the clay mineral lattices, with the formation of mobile organo-mineral complexes (Corvasce et al., 2006).

The magnitude of the values of the C/N ratio for the three trials agreed with those reported in other studies conducted on Mediterranean forests (Corral-Fernández et al., 2013; Cools et al., 2014). The decreasing trend of the C/N ratio with depth is a common trend in forest soils, where the litter is made by less degraded (and with higher C/N ratio) biomass than the organic molecules translocated into the deeper soil horizons after SOM decaying (Marinari et al., 2021).

4.2. Effect of thinning on the stocks of C and nutrients

In all the trials, SOC stock is similar in both litter (with a general thickness of 6-10 cm) and 0-30 cm layer, while the SOC stored below 30 cm depth amounted to 54-69% of that in the upper layer. The large amount of C stored in the sub-superficial mineral soil has a basic ecological relevance due to the role of forest soils as C sink and, hence, in the climate change mitigation. In the mineral layers, SOM is stabilized and protected from degradation (e.g., Ono et al., 2013; Yao et al., 2019) mostly because of clay minerals associations and oxygen limitation (e.g., Wattel-Koekkoek et al., 2003; Kleber, 2010). As a consequence, with increasing depth the C turnover rate slows down and the mean residence time of SOM (e.g., Trumbore, 2000; Wang and Chang, 2001) and decaying roots (e.g., Agnelli et al., 2014) tend to increase.

Regarding the different stocks observed along the depth for TN (in CTR) and exchangeable Ca (in HT) and K (in HT and MT), they appeared not related with the amount of roots or with the presence of leguminous species in the understorey that could have enriched the soil of N (Table S1 of Supplementary Materials). The knowledge on the influence of thinning on the stock of elements in the different layers along the soil depth is scarce but, working on a multi-centennial holm oak (*Quercus ilex* L.) forest in a pedoclimatic condition similar to that of our trials, Agnelli et al. (2016) found that many soil features, especially those not directly linked to the microbial activity, were rather homogeneous for each soil depth because of the long lasting pedogenesis. Since our trials were established under a forest cover as old as at least three millennia, we believe that, especially for exchangeable Ca and K, differences derived from spatial differences of parent material and skeleton content rather than to thinning experimentation started ≈50 years before this study.

The three trials showed no significant effect for none of the four (in case of C, N, and P_{av} stocks) or three (for exchangeable Ca, Mg, and K) layers considered. Thinning has been reported not to be able to produce changes on the organic C (and N) stock in naturally settled broadleaves and conifers stands (e.g., Bravo-Oviedo et al., 2015; Bai et al., 2017; Prasad Dangal et al., 2017; Zhang et al., 2018; Mayer et al., 2020). Opposite results were found in planted forests. For example, in their review Gong et al. (2021) took into consideration 77 articles on the effect of forest thinning on SOC stocks in the 0–30 cm mineral soil thickness across planted forests in China and concluded that a moderate thinning significantly increased SOC stocks with respect to both no-thinning and heavy thinning. Instead, working in a *Picea crassifolia* Kom. plantations, He et al. (2018) observed a decrease of the C stock with increasing thinning intensity, with a parallel increase of soil water storage. These reports reinforced the hypothesis that thinning cannot affect the stock of C and other elements in soils with long forest cover history, where pedogenesis has heavily homogenized the soil profile.

The 1-cm stock values confirmed that the organic and the organo-mineral horizons are able to stock similar amounts of C, TN, and P_{av}, while more in depth this ability is minor. While the concentration of C and TN in the organic and organo-mineral horizons was ascribed to their richness of SOM, the large concentration of P_{av} was attributed to the degradation of SOM, which released P from the organic structures (e.g., Pistocchi et al., 2018; Ni et al., 2021). With respect to 1 cm of mineral horizons, the ability of 1 cm of organo-mineral horizons to stock nutrient cations was statistically significant only for Ca in MT, Mg in HT, and K in HT and MT, but a generalized trend was observed in all cases, even though the differences were not significant because the samples were relatively small. In all these cases, the differences were attributed to the relatively fast SOM degradation occurring in the organo-mineral horizons (e.g., Pistocchi et al., 2018; Wang et al., 2019).

When contrasting the 1-cm stock capacity among the trials, thinning appeared to have an effect only on P_{av} and exchangeable Mg, which assumed the highest values in the organo-mineral horizons of HT. Also in this case, although not significant, the same appeared true at least for the potentially available Ca, while for the exchangeable K differences were probably disturbed by spatial variability of parent material and skeleton contribution. However, since a more intense thinning is expected to induce a diffuse higher soil water storage because of the resulted lower canopy density (He et al., 2018), it is probable that a larger water availability in the organic and organo-mineral horizons favoured a greater SOM mineralization with consequent release of nutrients (e.g., Vesterdal et al., 1995; Prescott, 2002; Chiti et al., 2015; Gross et al., 2018).

5. Conclusions

The soil physicochemical parameters and the stock of C and nutrients in the litter and in the 0-30, 30-50, and 50-75 cm layers under a multi-millennial Turkey oak forest cover, appeared slightly influenced by thinnings operated along the last 50 years. This result, which contradicts our research hypothesis, was achieved considering the contribution of the skeletal fraction that, especially in depth, was present in a considerable amount. The only parameters that appeared to be more affected by thinning were P_{av} and exchangeable Mg. The more intense thinning was able to increase the 1-cm storage of the organo-mineral horizons via a major SOM mineralization. Our results contrast with those reported for recently (decades) planted forests, especially if plantation occurs in former cultivated fields, where thinning has tangible effects on element storage. This is equivalent to saying that, after about three millennia of Turkey oak forest use, both forest cover and human activity are the main soil forming forces.

Our study also showed that organo-mineral and mineral horizons under the Turkey oak forest are able to store an amount of SOM similar to the litter layers. Since the SOM contained into the organo-mineral and mineral horizons has higher recalcitrance and, consequently, is less involved in the C turnover processes than that of the forest floor, it is mandatory to adopt forest managements strategies able to increase SOM in depth rather than in the superficial organic horizons, to affect positively the global C cycle.

Finally, considering that *i*) coppice stands under conversion into high forest via natural evolution and by means of periodical thinnings appeared to be equal as for soil ecosystem properties 50 years later, and that *ii*) the latter option is more profitable for environmental, socio-economic issues and recreational purposes, thinning implementation can be considered as a valuable solution, among the different and complementary strategies on the floor, to manage nowadays the original coppice area.

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