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Mid-term (30 years) changes of soil properties under chestnut stands due to organic residues management: An integrated study

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Abstract: Chestnut plantations are worldwide distributed and they are often subjected to intensive management practices such as the removal of the organic residues from the soil surface. The present study aimed to investigate the effect of such practices on soil properties at different depths and on nutrient contents in chestnut leaves. To reach our goal, 6 pits down to 30 cm soil depth were dug in European chestnut (Castanea sativa Mill.) stands where the organic residues such as burrs, leaves and pruned materials are yearly removed (REM), and 9 pits in stands where the organic residues are chopped and left on soil surface (CONS). Both practices began about 30 years ago. The nutrient contents in leaves were assessed on chestnut trees close to each pit. Our findings showed a more intense soil development in CONS than in REM. At soil surface, CONS had thicker mineral horizon than in REM probably due to the protection acted by the organic residues against erosion. In subsoil, organic residues conservation promoted the organic C (soil organic C, water-extractable organic C, fulvic-like C and soil organic C stock) accumulation which further promoted horizon development. Since the role of soil organic matter (SOM) as source of nutrients, the subsoil of CONS study sites had a greater stock of Ca, P and S and a higher exchangeable Ca content than REM. Furthermore, the higher SOM and nutrient contents in CONS subsoil compared to REM promoted the microbial respiration. The organic residues conservation effects on the surface soil horizon properties were, instead, negligible. Despite the generally better soil conditions in CONS than in REM, no differences were observed for the leaf nutrient contents likely due to the chestnut trees adaptability also to the scarcely fertile soils. Overall, our findings highlighted the importance of organic residues conservation for the improvement in deeper soil horizons of the chemical and biological fertility in chestnut plantations. Furthermore, this research pointed out to pay more attention on subsoil since it is a good indicator of the changes caused by external factors.

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Highlights

- Organic residues removal (ORR) effect on chestnut stand soil properties was tested
- ORR limits the organic C content and nutrient stocks (C, N, P and S) in subsoil
- CONS management promoted the development of AB and Bw horizons
- The limited organic C deepening due to ORR penalised the subsoil microbial activity
- Organic residues should be conserved to maintain soil quality in chestnut stands

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Mid-term (30 years) changes of soil properties under chestnut stands due to organic residues management: An integrated study

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Abstract

Chestnut plantations are worldwide distributed and they are often subjected to intensive management practices such as the removal of the organic residues from the soil surface. The present study aimed to investigate the effect of such practices on soil properties at different depths and on nutrient contents in chestnut leaves. To reach our goal, 6 pits down to 30 cm soil depth were dug in European chestnut (*Castanea sativa* Mill.) stands where the organic residues such as burrs, leaves and pruned materials are yearly removed (REM), and 9 pits in stands where the organic residues are chopped and left on soil surface (CONS). Both practices began about 30 years ago. The nutrient contents in leaves were assessed on chestnut trees close to each pit. Our findings showed a more intense soil development in CONS than in REM. At soil surface, CONS had thicker mineral horizon than in REM probably due to the protection acted by the organic residues against erosion. In subsoil, organic residues conservation promoted the organic C (soil organic C, water–extractable organic C, fulvic–like C and soil organic C stock) accumulation which further promoted horizon development. Since the role of soil organic matter (SOM) as source of nutrients, the subsoil of CONS study sites had a greater stock of Ca, P and S and a higher exchangeable Ca content than REM. Furthermore, the higher SOM and nutrient contents in CONS subsoil compared to REM promoted the microbial respiration. The organic residues conservation effects on the surface soil horizon properties were, instead, negligible. Despite the generally better soil conditions in CONS than in REM, no differences were observed for the leaf nutrient contents likely due to the chestnut trees adaptability also to the scarcely fertile soils. Overall, our findings highlighted the importance of organic residues conservation for the improvement in deeper soil horizons of the chemical and biological fertility in chestnut plantations. Furthermore, this research pointed out to pay more attention on subsoil since it is a good indicator of the changes caused by external factors.

Keywords: chestnut management; mountain soil; organic carbon dynamics; litter floor, soil development

1. Introduction

Chestnut plantations for nut production are widely distributed throughout the world (Conedera et al., 2016). Among the European countries, Italy is the largest sweet chestnut producer and importer with about 52,000 Mg (FAOSTAT, 2017). In Italy managed chestnut stands showed a decrease from the second half of the twentieth century to the present time principally due to the rural depopulation (Zlatanov et al., 2013), to the presence of more competitive cultures, such as corn and potatoes, and to the spread of the chestnut blight (*Chryphonectria parasitica* [Murr.] Barr) and ink (*Phytophthora cambivora* [Petri] Buism.) diseases (Pezzi et al., 2011). More recently, a pivotal role in the chestnust stands abandonment could have played by the reduced precipitations due to the climate change (Pieri et al., 2017) which have promoted the spread of the chestnut diseases (Waldboth and Oberhuber, 2009). However, in the last decades old chestnut groves have been recovered, also by replacement of coppices with plantations (Beccaro et al., 2009), with an increase of both socio-economic and environmental interest (Martín et al., 2017).

Chestnut stand agroecosystems represent today an important landscape component in the mountainous regions of the European Mediterranean basin. The management of the chestnut stands in a sustainable way is essential to improve or to maintain the soil quality in these areas. Soil organic matter (SOM) is the most frequently used indicator of soil quality, as it affects some soil biological, physical and chemical properties, followed by nutrient content and soil microbial biomass and respiration (Zornoza et al., 2015). Unfortunately, studies concerning the effect of above ground biomass management on SOM are missing for the chestnut ecosystems. Conversely, taking in account other tree species, several are the studies monitoring the influence of forest harvesting on soil organic carbon pool (e.g., Homann et al., 2001; Nave et al., 2010). For example, previous studies (James and Harrison, 2016; Johnson and Curtis, 2001; McDonnell et al., 2013; Zetterberg et al., 2016) highlighted how the intense harvesting practices aimed to remove most of the tree biomass significantly reduce the amounts of C and other essential nutrients such as Ca^{2+},

Mg^{2+} and K^+ in soil. However, in forest ecosystems another key driver of the soil C is the litter layer (Maes et al., 2019). Indeed, litter removal practices could alter the pool sizes of organic compounds and nutrients, and influence the rates of biogeochemical processes which could potentially compromise soil fertility (Xiong et al., 2008). Several studies have found that the presence of plant litter can significantly increase both the soil resistance to flowing water erosion and the water holding capacity (Giménez and Govers, 2008; Liu et al., 2017). Furthermore, the litter floor could be incorporated into the mineral soil horizons through the soil fauna activities (Yavitt et al., 2015), increasing soil aggregate stability, SOM amount and stabilisation, and decreases soil bulk density (Mandal et al., 2004; Mitchell et al., 2018). Since the positive relationship between soil microbial biomass and SOM (van Leeuwen et al., 2017) the removal or alteration of soil organic matter inputs due to the different forest management might change the size and the activity of the soil microbial biomass.

Because of the role of soil on plant health, changes of the soil properties could affect plant nutrient contents. For example, a reduced plant nutritional status was found in soil characterized by poor SOM (Neilsen et al., 2014) and nutrient contents (Demchik and Sharpe, 2000) and microbial biomass (Van Der Heijden et al., 2008).

In order to implement those agroforestry practices able to preserve or improve tree health and soil quality, we tested the effect of 30 years chestnut organic residues management practices on soil thickness, amount and quality of SOM, nutrient content and microbial biomass and its activity of top- and subsoil layers, and on nutrient concentrations in chestnut tree leaves.

To achieve this goal, we selected chestnut stands where the organic residues such as burrs, leaves and pruned materials are yearly removed, and chestnut stands with more conservationist practices where organic residues are chopped and left on the soil surface. It was hypothesized that the organic residues removal *i*) declines *solum* thickness; *ii*) reduces the contents of soil organic carbon and of SOM pools, and the soil organic carbon stock; *iii*) decreases the stocks of N, Ca, Mg, Na, K, Fe, P and S and the availability of Ca, Mg, Na and K; *iv*) has negative impact on soil microbial biomass and respiration; *v*) reduces the nutrient contents in plants.

2. Materials and methods

2.1. Study sites

The study sites included four farms (Figure 1 and Table 1) with uneven-aged chestnut (*Castanea sativa* Mill) stands for fruit production. The chestnut stands were recovered about 30 years ago through a more or less severe pruning of the plants, according to the age of the trees and to the severity of parasitic attacks, necessary to stimulate the growth of a new and vigorous canopy. Furthermore, the recovery actions included the selection of the healthiest trees, to maintain a tree spacing of about 10 × 10 m, whose dry, senescent, sick parts and overlapping branches were yearly removed in order to open the canopy and allowing a better fruits production on sunny branches (Figure 1). Therefore, the farms included uneven-aged chestnut stands whose average size of trees was about 30-35 cm reflecting the 40-years old age of shoots. In all study sites the chestnut production was about 40 kg tree^{-1}.

The study sites are located on the northern Apennine chain in Italy (Figure 1), their locations and climatic characteristics are reported in Table 1. In all investigated sites, the chestnut plantations stand at an altitude ranging from 650 to 750 m above sea level, they are exposed to south, south–east and on slopes with an inclination ranging from 15° to 30°. In all study sites the soil developed from sandstone with similar elemental compositions (ISPRA, 1999).

2.2. Experimental design

The four farms involved in this study were selected in order to compare the soil properties of chestnut stands in which the organic residues removal (ORR) has been made or not. In two farms, the chestnut stands were subjected to yearly ORR, hereafter called REM, due to the use of small blowing devices during the fruits harvesting which remove from the soil surface both fruits and other organic residues. In two other farms, management was more conservationist which consist to leave on the soil surface the organic residues, hereafter called CONS, which are chopped. In CONS study sites the fruits harvesting was manual or using nets put on trees. Both ORR and the more conservationist practices began at the recovery time of the chestnut stands, therefore about 30 years ago, and concerned the whole surface of the stands.

The soils were classified as Typic Udorthents for REM study sites and Typic Dystrudepts for CONS ones (Soil Survey Staff, 2014). The description of representative soil profiles are reported in Table S1 of the supplementary materials (Regione Emilia Romagna, 2000).

In spring 2017, fifteen pits down to 30 cm soil depth were dug (6 pits for REM and 9 for CONS). Since the high soil variability that generally occurs in mountainous areas (Hoffmann et al., 2014), a different number of pits were dug for each study area to get a better representation of the soil on the entire fields. Each pit was dug at a distance of at least 1 m away from the stem of the trees.

2.3. Sampling and analysis

In each dug pit, each identified genetic horizon down to 30 cm of soil depth has been sampled by collecting at least 2 kg of sample and, once in the laboratory, it was air dried and sieved (<2 mm). For the determination of the bulk density (BD), additional soil samples were taken using steel cylinder cores with an internal diameter of 5.0 cm and 5.0 cm height from the 0-10, 10-20 and 20-30 cm soil depth.

In order to evaluate the effect of ORR practice on nutrient contents in chestnut leaves, the uppermost fully expanded leaves and without visible injury symptoms were collected from randomly selected branches. Specifically, from 20 to 30 leaves were picked from each tree. The leaves were collected from 4 healthy trees located close to each mini-pit and mixed to composed one sample per pit. The leaf samples were oven-dried at 60 °C until constant weight and ground.

The soil particle size distribution was obtained by the pipette method after dispersion of the sample with a sodium hexametaphosphate solution (Gee and Bauder, 1986). The soil samples collected for the determination of BD were oven-dried at 105 °C for 24 h, and then weighed (Blake and Hartge, 1986). The dry weight was thereafter divided by volume of the cores. The obtained BD was adjusted by subtracting the mass and the volume of skeleton and roots in order to determine the BD of the fine earth fraction.

The soil pH was determined potentiometrically in a 1:2.5 solution ratio in both deionized water (pH_{H_2O}) and 1M KCl (pH_{KCl}) suspension. Total organic C (TOC) and total nitrogen (TN) were determined by a CHN elemental analyser (EA 1110 Thermo Fisher, USA) without to be pre–treated with hydrochloric acid due to the absence of carbonates. The total amount of Ca, Mg, Na, K, Fe, Mn, P and S were determined by inductively coupled plasma optical emission spectrometer (ICP–OES, Ametek, Spectro Arcos, Germany) after aqua regia extraction (Vittori Antisari et al., 2014).

The cation exchange capacity (CEC) and the exchangeable cation contents were determined according to the method proposed by Orsini and Rémy (1976) and modified by Ciesielski and Sterckeman (1997) using 0.017 M hexamminecobalt(III)chloride as extracting solution and measuring the amounts of Co and exchangeable cations by ICP–OES.

Different soil C pools characterized by increasing persistence in soil were sequentially separated according to Agnelli et al. (2014) with some modifications. Briefly, a 1:10 solid:water suspension was shaken on a horizontal shaker for 16 hours at 25°C, centrifuged and the surnatant was separated from the precipitate. From the supernatant two C pools have been further separated by sieving it at 53 μm: the particles >53 μm which represented the particulate organic matter (POM), namely plant and animal residues at different stages of decomposition, and the surnatant <53 μm which was further filtered through 0.45 μm filter paper and it represented the water–extractable organic matter (WEOM), namely the more mobile and labile organic matter pool. To the remaining precipitate into the centrifugation tubes, 0.1M NaOH solution was added and the samples were shaken for 24 hours at 25°C and then again centrifuged. The NaOH extract was passed through a 0.45 μm polycarbonate filter, while the remaining precipitate, containing the humin–like (Hum) compounds was washed using deionized water to remove the excess of Na until the pH of the rinsed solution was ≤ 7. The

0.45 μm filtered NaOH extract was acidified to about pH 1.5 with 6M HCl and allowed to settle overnight to separate humic acid–like (HA) and fulvic acid–like (FA) compounds and centrifuged. To remove the excess of Na from the obtained fractions, the surnatant (FA) was dialyzed through 1000 Da cut–off membranes (Spectra/Por® Dialysis membrane) against distilled water, while the residual (HA) was washed with 0.002 M HCl. Both purified fractions were freeze–dried. The POM and Hum fractions were instead dried at 40 °C. The organic C (OC) and N contents of POM, FA, HA and Hum were determined by a CHN elemental analyser (EA 1110 Thermo Fisher, USA). The OC and N contents of WEOM were recorded by a TOC–V CPN total organic carbon analyzer (Shimadzu, Japan).

The C and N concentrations in leaf samples were measured by the CHN elemental analyser (EA 1110 Thermo Fisher, USA). While, for the determination of Ca, Mg, K, Mn, Na, P and S, the ground leaves were mineralized in 4:1 HNO_3 (65 % v/v): H_2O_2 (30% v/v) solution in a microwave oven (START D Microwave Digestion System; Milestone Inc., Sorisole, Bergamo, Italy). The concentrations of the selected elements in the extracts were measured by ICP–OES.

The soil respiration during 28 days at 25 °C of incubation was determined according to Falsone et al. (2015) after conditioning of the samples at 60 % of their water holding capacity (WHC) and pre–incubating them for 3 days at 4 °C. After 1–3–7–10–14–21–28 days the beginning of incubation, the amount of CO_2 emitted from incubated soils was measured by alkali (1 M NaOH solution) absorption of the evolved CO_2 and titration of the residual OH^- with a standardized HCl solution. While the soil basal respiration (SBR) of each soil sample was computed as the average of the values measured during the incubation period, the cumulative soil basal respiration (RCUM) was expressed as the total amount of CO_2 evolved during the 28 days of incubation.

Soil microbial biomass–C (Cmic) and microbial biomass–N (Nmic) were measured on soil samples at 60% of WHC using chloroform fumigation extraction method with 0.5 M K_2SO_4 solution (Vance et al., 1987). Both fumigated and non–fumigated extracts were analysed using a TOC–V CPN total organic carbon analyzer (Shimadzu, Japan). Cmic was calculated as E_C*2.64, where E_C = difference between organic C extracted from fumigated soils and organic C extracted from non–fumigated soils. Nmic was calculated as E_N*2.22, where E_N = difference between total N extracted from fumigated soils and total N extracted from non-fumigated soils (Vance et al., 1987).

The metabolic (qCO_2) and mineralization (qM) quotients were calculated according to the following equations (Eq. 1 and 2):

$$qCO_2 = 100 * SBR/Cmic \quad (1)$$

$$qM = 28 \text{ days cumulative respiration (RCUM)/TOC} \quad (2)$$

2.4. Data analysis

In order to evaluate the effect on soil of the organic residues conservation or removal, the results of the chemical and biological properties are presented separating epipedon (superficial layers) and endopedon (deeper layers), in particular, epipedon (Epi) included superficial A horizons (i.e., A, A1, A2), while endopedon (Endo) included other deeper transition and mineral horizons (i.e., AC, AB, Bw, BC and C).

The stock of OC, TN and nutrients are presented for fixed soil–depth intervals (0–10, 10–20 and 20–30 cm), taking into account the BD value of each soil layer at fixed depth and the OC, TN and nutrients content of each genetic horizon averaged on the basis of horizon thickness.

Two–way analysis of variance was performed to assess the effect of chestnut stand management on the selected physical, chemical and biological parameters and the variation of the selected variables with soil depth. Instead, for the plant samples, one–way analysis of variance was carried out to assess the effect of chestnut stand management on the leaf nutrient concentrations. Since the graphical analysis of residuals showed the absence of normality and homoscedasticity, the all the data were processed by the Kruskal-Wallis rank sum test to identify statistically significant differences and Holm test was performed as multi–comparison test ($p < 0.05$). The results presented are based on mean values and their standard error. The data were analyzed using R software (R Core Team, 2016).

3. Results

3.1. Morphological properties of chestnut stand soils

Our results showed the differences in the *solum* thickness and horizons sequences between REM and CONS study sites.

The mean thickness of superficial A horizons (i.e., A, A1, A2), called Epi horizons, was in fact of 5.1 and 9.7 cm in REM and CONS, respectively. The deeper mineral horizons (i.e., AC, AB, Bw, BC and C), called Endo horizons, showed a mean thickness of 12.4 and 20.2 cm in REM and CONS, respectively (Table 2). Both in REM and CONS Epi horizons were thinner than Endo ones ($p<0.05$), and between both practices, CONS allowed a thicker Epi than REM ($p<0.05$). Similarly, for Endo, CONS showed a larger thickness compared to REM. However, it needs to take in mind that the horizons sequence down to 30 cm was A/AC/C in REM soils, while was A1/A2 or AB or Bw or BC in CONS ones. The horizons sequence of *solum* thus showed weak development in REM because of the lack of B horizons, and furthermore the thickness of *solum* was lower in REM than in CONS ones ($p<0.05$).

3.2. Physicochemical properties of chestnut stand soils

The occurrence of differences in particle size distribution, BD values, pH, CEC and concentration of exchangeable bases between Epi and Endo in the soils of REM and CONS study sites has been determined.

Our findings showed that the soils of the selected chestnut orchards have sandy–loam/loam texture, with lower amount of clay in CONS than in REM (Table 2). Furthermore, REM soils had similar particle distribution along the soil depth; in CONS soils, instead, Endo horizons had higher silt content than Epi ones ($p<0.05$). The bulk density, on average, ranged from 1.02 to 1.47 g cm^{-3}, but significant differences neither between soil depth nor between soil management practices have been found.

The pH_{H2O} values ranged from 4.8 to 5.4, the pH_{KCl} ranged from 3.2 to 4.1 with some differences in CONS where the Endo showed higher values compared to Epi (Table 2).

In both managements, the CEC did not change between Epi and Endo, however, the soils under REM management showed higher CEC than those under CONS (Figure 2I). Regarding to the exchangeable bases (Ca, Mg, K, Na), the ExCa always showed higher values in Epi than in Endo (Figure 2II), the ExK had higher concentrations in Epi than in Endo only in REM (Figure 1III), and no differences occurred between Epi and Endo for ExMg and ExNa (Figure 2IV, V). Comparing the two management practices, among the exchangeable bases, only the ExCa showed some differences, in particular higher ExCa values were found in Endo of CONS sites compared to Endo of REM (Figure 2II). As expected, the base saturation (BS) values were higher in Epi compared to Endo but only in REM soils (Figure 2VI). In CONS soils, the BS values were similar between Epi and Endo, and no differences occurred between the two management practices (Figure 2VI).

3.3. SOM pools of chestnut stand soils

As expected, in both chestnut managements, most of the studied pools of C and N cycles (i.e., total, labile, humic–like and humin substances) were higher in the Epi than in the Endo (Figure 3, Figure 4, Figure S1). Comparing the two chestnut stand managements, some differences occurred for the Endo. Specifically, the concentrations of TOC and of organic C related to WEOM and FA were higher in CONS than in REM (Figure 3I, III, Figure 4II). Similarly, the contents of TN and nitrogen related to WEOM were greater in CONS than in REM (Figure 3II, IV).

3.4. Microbial biomass amount and respiration of chestnut stand soils

Regarding to the soil microbial biomass, in both chestnut stand managements Cmic, Nmic, SBR and RCUM values were higher in Epi than in Endo, while no differences occurred for qR and qM (Figure 5I–VI). Through the comparison of the two chestnut stand managements, some differences occurred for RCUM and qM. In particular, the RCUM was higher in Endo of CONS than in REM (Figure 5IV), the qM was higher in CONS than in REM both for Epi and Endo (Figure 5VI).

3.5. Soil organic C and nutrients stocks, and nutrient concentrations in chestnut tree leaves

The stock of elements of organic origin (i.e., C, N, P and S) generally decreased along soil depth, and in deeper layers (10–20 and 20–30 cm) in CONS chestnut stands had higher values than in REM (Table 3). Nutrients mainly derived by parent material (Ca, Mg, Na, K and Fe) did not instead show significant differences between the two different managements (Table 3). However, a certain Mg and Fe redistribution occurred in deeper layers (10-20 cm and 20-30 cm) of CONS soils, having higher stock values than in 0-10 cm layer, while in REM soils Na stocks was higher in deeper layers than in 0-10 cm (Table 3).

Although some differences in soil elements stock occurred, the concentrations of C, N, Ca, Mg, K, Mn, Na, P and S in chestnut leaves did not differ between the two type of organic residue managements (Table 4).

4. Discussion

4.1. Organic residues conservation promotes soil thickness and development in chestnut stands

In the present study, the ORR practice reduced the thickness of superficial A horizons (5.1 *vs* 9.7 cm in REM and CONS, respectively) which could be attributed to higher soil erosion that probably takes place in the former than in the latter study sites. Indeed, as reported by several works (e.g., Dick et al., 1997; Křeček et al., 2019; Liu et al., 2017) the presence of the litter layer limits the surface runoff and soil erosion. The highest soil erosion that likely occurs in REM study sites could also limit the thickness of *solum* due to losses of pedogenized material (Mokma et al., 1996). Consequently, the formation of more developed B horizons or transitional AB ones was prevented in REM soils. Our findings are in accordance to Świtoniak (2014) and Jankauskas and Fullen (2002) which, through a pedological investigation of soils subjected to different erosion severity, observed weakly developed soil horizon sequences in eroded sites. In contrast, the reduced erosion in CONS study sites might promoted the development of their soils allowing to define the CONS soils as Inceptisol while those of REM as weakly developed Entisols.

4.2. Organic residues conservation promotes soil organic matter content and stock in chestnut stands

As reported in several works carried out on Italian mountains soils (e.g., De Feudis et al., 2017; Vittori Antisari et al., 2016), the TOC content was higher in organo–mineral horizons (Epi) than in deeper ones due to input of OC from the litterfall (Boča and Miegroet, 2017). However, the ORR practice reduced the soil OC content, in particular we found lower TOC content in Endo of REM than in CONS, in agreement with early studies (e.g., Achat et al., 2015; Tian et al., 2010). This fact might suggest how the ORR limits the incorporation of the organic matter in the subsoil preventing any vertical redistribution of organic C. Conversely, the conservation of the organic residues allows it. Specifically, our findings indicated that the incorporation of the organic matter in the subsoil of CONS could be assigned to the redistribution along depth of WEOM-C and FA-C fractions which showed higher concentrations in Endo of CONS than in that of REM. Both the water–soluble and the fulvic–like substances represent an important source of organic C for the deeper layers of the soils (Aran et al., 2001; Kaiser and Kalbitz, 2012).

Because of the importance of SOM on the development of the subsoil horizons even in poorly developed soils such as Entisols and Inceptisols (Falsone et al., 2012), the deepening of the organic substances in CONS, together with the limited soil erosion, likely promoted the development of AB and Bw horizons.

Although the lower clay content in CONS than in REM, the OC stock was larger in 10–30 cm soil depth section of CONS than in that of REM. This fact emphasizes the leading role of organic matter input amount and rate on soil OC stock (Orgill et al., 2017). Since the subsoil OC generally has a higher residence time compared to topsoil (Jenkinson et al., 2008; Trumbore, 2009), the ORR seemed to reduce the role of soil as stable sink of C reducing the OC stocks below 10 cm of soil depth.

The modification of TOC content and OC stock only in Endo and in 10–30 cm soil depth interval, respectively, might indicate a high sensibility of subsoil OC to management practices. The high sensibility of subsoil OC to external factors, such as management practices, is in accordance with recent studies (Jia et al., 2019; Leuschner et al., 2014; Mobley et al., 2015; Steinmann et al., 2016) which observed meaningful changes of subsoil OC stock caused by climate warming, reforestation and soil managements such as tillage and fertilization.

4.3. Organic residues conservation increases soil nutrient contents in chestnut stands

The similar parent material on which the studied soils developed explains the similar stock of nutrients, with exception of P and S, which exhibited higher values in 10 – 30 soil depth section of CONS than of REM probably due to the higher amount of organic matter. Indeed, concerning P, in forest soils most of the soil P is made up of organic P forms (Adams et al., 2018; De Feudis et al., 2016). As the organic P is the most important P source in forest ecosystems (Turner et al., 2014) and the total P concentration generally decline over time (Chen et al., 2015), litter recycling is essential to avoid an accelerated loss of soil P. The nutrient recycling process seemed to affect also S. In fact, similarly to P, the total amount of S in 10 – 30 cm soil depth interval was higher in CONS than in REM. As for P, the organic S compounds represent the most abundant S forms in soils (e.g., Likens et al., 2002; Vannier and Guillet, 1994). Regarding to the other nutrients, such as exchangeable Mg, K and Na, their amounts did not change between the two managements. Exchangeable Ca, instead, had higher contents in Endo of CONS than in that of REM, following the trend of OC content. This is in agreement with Vittori Antisari et al. (2013) who reported that SOM is an important source of available Ca in chestnut stands. The importance of Ca biocycling was further stressed by the lack of differences of Ca stock. In fact, in mountain acid soils the redistribution of Ca-mineral derived is often prevented by the limited Ca-mineral weathering

(Ghobadi and Mousavi, 2014), and available Ca derived mainly from litter recycling (Haynes and Swift, 1986; Johnson et al., 1994). Although the higher amounts of some nutrients in CONS than in REM, the lack of differences occurring for the nutrient contents in plant leaves would suggest the unresponsive of chestnut to soil fertility and, therefore, the ability of chestnut plants to grow also on poor sandy-rich soils (Oosterbaan, 1998). The ability of chestnut trees to healthily grow also in soils characterized by low amount of nutrients is confirmed by Ribeiro et al. (2019) which did not find differences in leaf nutrient contents between fertilized and unfertilized *Castanea sativa* plants.

4.4. Organic residues conservation improves soil microbial respiration in chestnut stands

The RCUM in Endo of CONS study sites was higher than in REM likely due to the higher C content associated to WEOM (WEOM-C). In fact, because of the most labile organic compounds represent the main substrate for soil microbial communities (Rees and Parker, 2005) and changes of WEOM-C quantity affect both microbial activities and composition (Drenovsky et al., 2004), the enhance of WEOM-C can promote the heterotrophic soil respiration (Rees and Parker, 2005). Furthermore, since the positive effect of WEOM-C on soil microbial activities, the higher qM in CONS than in REM might be attributed to the priming effect, namely a short–term change in microbial mineralization of SOC in response to labile OC inputs (Kuzyakov, 2010). Unlike RCUM and qM, both the microbial biomass and qCO_2 have shown similar values between the two chestnut orchard managements. The lack of differences about microbial biomass and qR might be attributed to the fact that they are driven not only by the amount of TOC but also by other soil properties such as BD, soil moisture and temperature, and C:N ratio (Ren et al., 2018; Stefanowicz et al., 2016; van Leeuwen et al., 2017; Xu et al., 2007) which, in our case, were similar among the study sites.

4.5. Overview of the effects of organic residues management on soil properties in chestnut stands

In the present study, the soil erosion occurring in the mid-term (30 years) in REM study sites due to the periodic ORR might promoted the thinning of the A horizons and prevented the development of

B or transitional AB horizons. Moreover, ORR practice reduced the SOM content in subsoil of REM study sites because this practice impeded the inclusion of the organic material in soil mineral horizons. Therefore, because of the importance of SOM on the development of soil horizons, the limited amount of organic residues left on soil surface in REM could have further prevented the development of B or transitional AB horizons.

The presence of chopped organic residues on soil surface of CONS promoted a greater soil OC stock than in REM. However, it is noteworthy to highlight that the differences about soil OC stock were observed in subsoil layers indicating how the conservation of the organic residues enhanced SOM stabilization. Because of the role of organic matter on soil aggregation, fertility and to store water (de Paul Obade and Lal, 2016), the decline of OC content and stock in subsoil due to ORR practice could instead cause a reduction of soil quality. For example, the higher OC stock in Endo horizons of CONS than in that of REM resulted in a higher amount of those nutrients, such as P and S, whose cycling is related to SOM. Moreover, our findings showed that the conservation of the organic residues on soil surface improves the soil also from the biological point of view. In fact, the higher SOM content and, therefore, the higher amount of labile OC in CONS than in REM increased the activity of soil microbes which are important player on the soil nutrient cycling.

Hence, a chestnut stand management based on the conservation of the litter floor should be supported in order to improve the soil quality and, in particular, to increase the volume of *solum*, the SOM and nutrient contents and the soil microbial respiration. However, though not considered in the present work, CONS management could have some negative effects on the spreading of some chestnut diseases. For example, the increase of SOM content could benefit the proliferation of the ink disease (*Phytophthora cinnamomi*) because of its saprotrophic nature (Fonseca et al., 2004). Furthermore, since in CONS study sites the organic residues are chopped, this practice could increase the production costs. Finally, since the ORR and the chipping of the litter floor are yearly performed, both studied managements could have some negative effects on the proliferation of *Torymus sinensis* Kamijo. In fact, in order to maintain this biological control agent of the chestnut gall wasp, the plant material bearing galls (branches, suckers) should not be removed from the stand at least for two years (Ferracini et al., 2015). More researches need however on the assessment of the phytosanitory consequences and on the role that the SOM and microbial activity can have on the enhancement of chestnuts disease resistance.

5. Conclusion

In the mid-term (30 years), the common practice of ORR in recovered century chestnut stands has negative impacts on soil quality. In particular, compared to chestnut stands where the organic residues are chopped and left on soil surface, the ORR practices prevent the development of AB and Bw soil horizons. The limited soil development in REM was likely due to an accelerated soil erosion leading to loss of pedogenized material, and to a negligible incorporation of organic matter from soil surface into the subsoil limiting mineral weathering. Furthermore, the reduced stock of P, S, and organic C and the reduced amount of exchangeable Ca, cumulative basal respiration and mineralization quotient in subsoil of the chestnut stands subjected to ORR practice might suggest that the conservation of the organic residues is necessary to maintain the soil chemical and biological fertility in mountainous ecosystems. The improved soil quality, together with the unchanged nutrient contents in chestnut leaves, would suggest that the switch from the common management practices in more conservative ones matches with the aims of those agroforestry practices addressed to preserve or improve both tree health and environmental quality. Furthermore, the present study highlighted the higher sensibility of the subsoil to the management practices compared to the topsoil. Therefore, for the studies about the effect of management practices in forests ecosystems located in mountainous areas, we suggest to pay more attention on subsoil properties since it is a good indicator of the changes caused by external factors.

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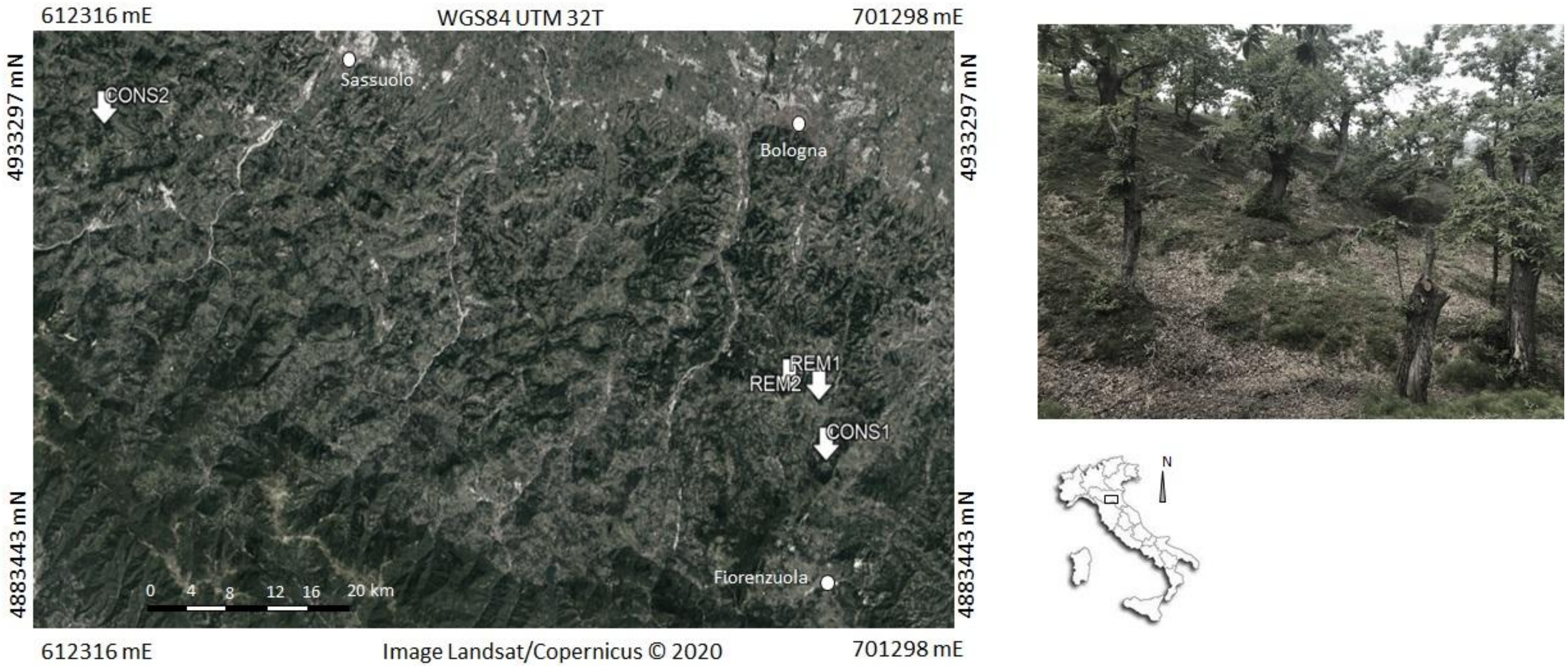
Figure 1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N

Figure 2

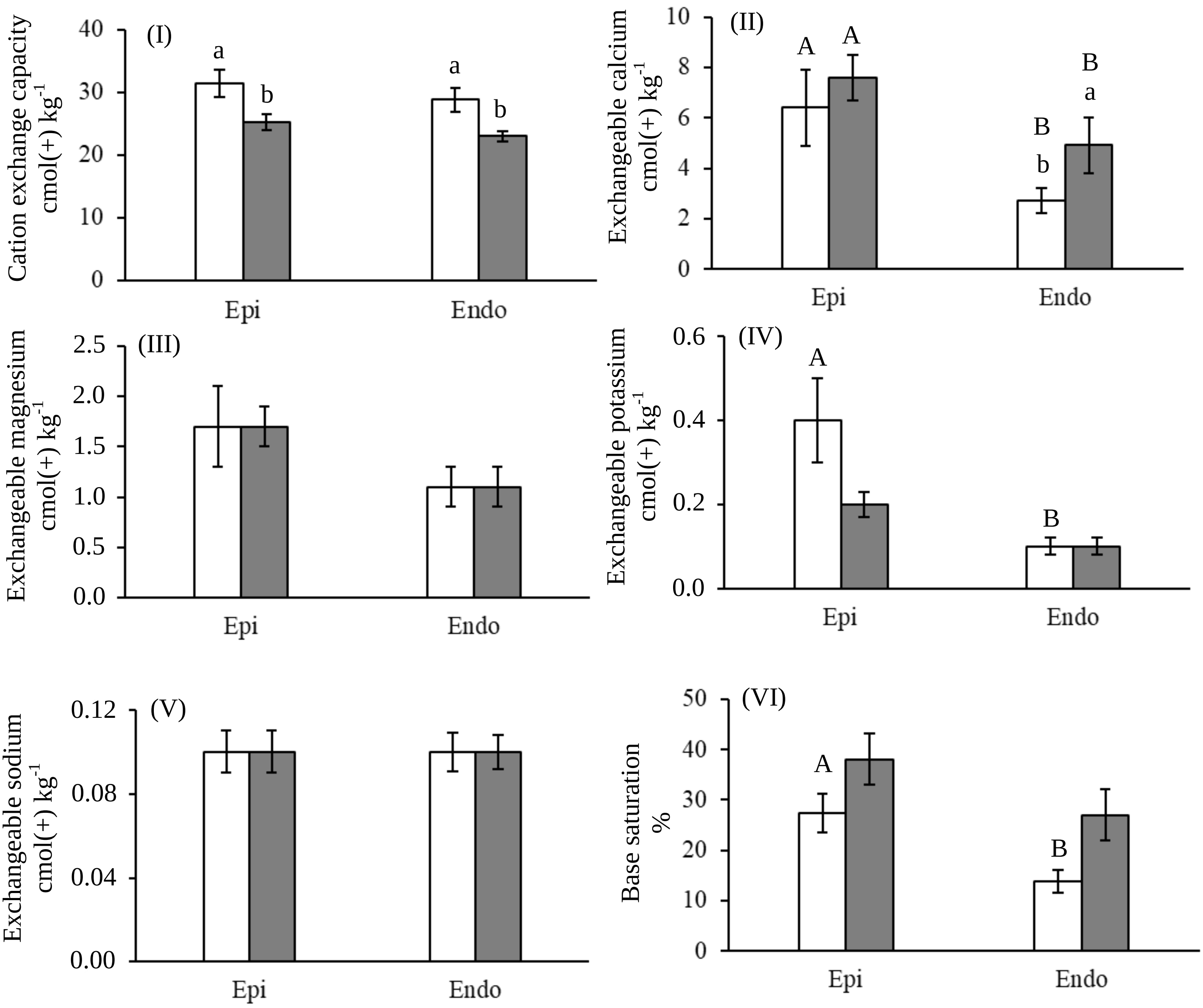

(I)
Cation exchange capacity cmol(+) kg-1
0
10
20
30
40
a
b
a
b
Epi
Endo
(II)
Exchangeable calcium cmol(+) kg-1
0
2
4
6
8
10
A
A
B
b
B
a
Epi
Endo
(III)
Exchangeable magnesium cmol(+) kg-1
0.0
0.5
1.0
1.5
2.0
2.5
Epi
Endo
(IV)
Exchangeable potassium cmol(+) kg-1
0.0
0.2
0.4
0.6
A
B
Epi
Endo
(V)
Exchangeable sodium cmol(+) kg-1
0.00
0.04
0.08
0.12
Epi
Endo
(VI)
Base saturation %
0
10
20
30
40
50
A
B
Epi
Endo

Figure 3

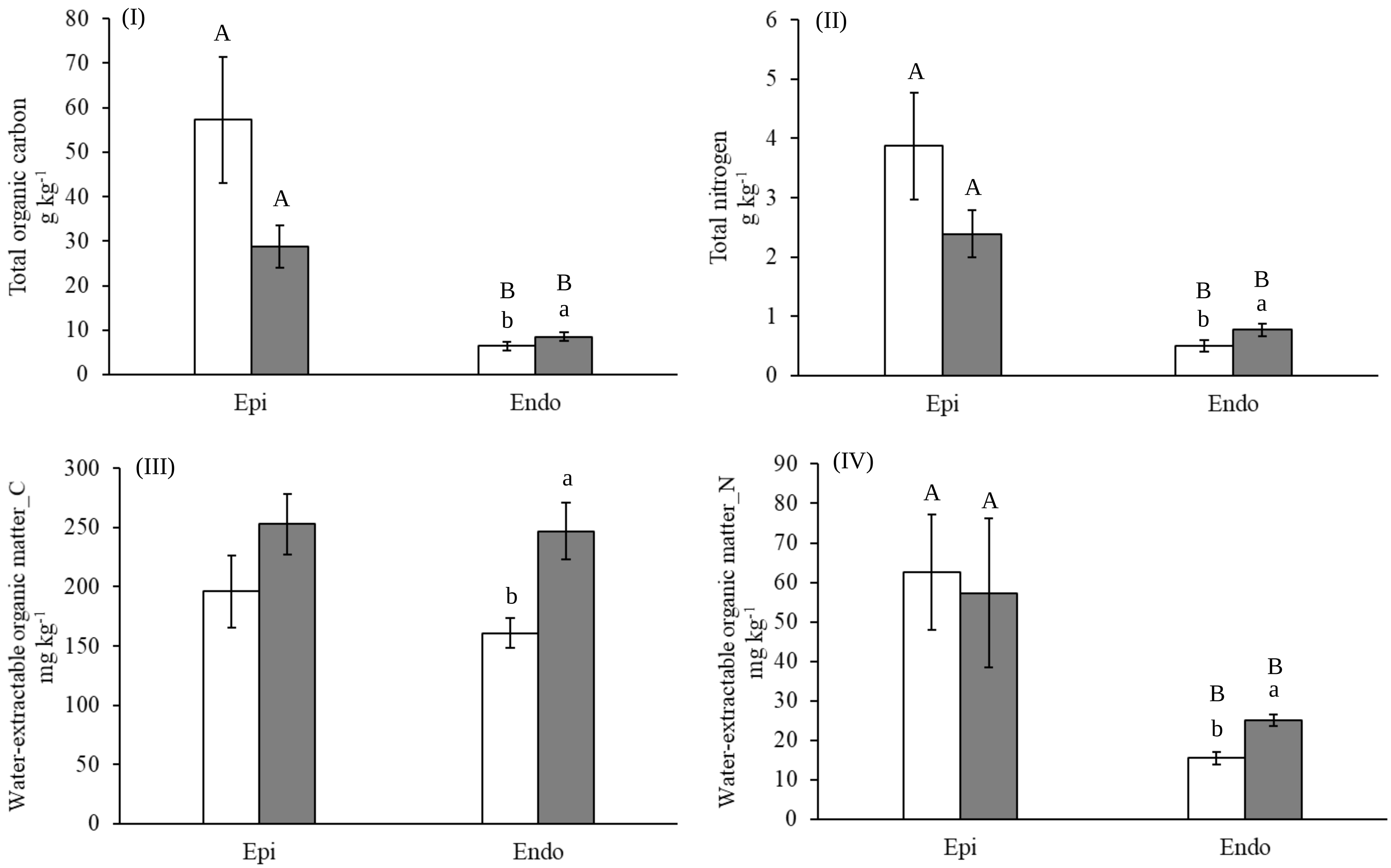
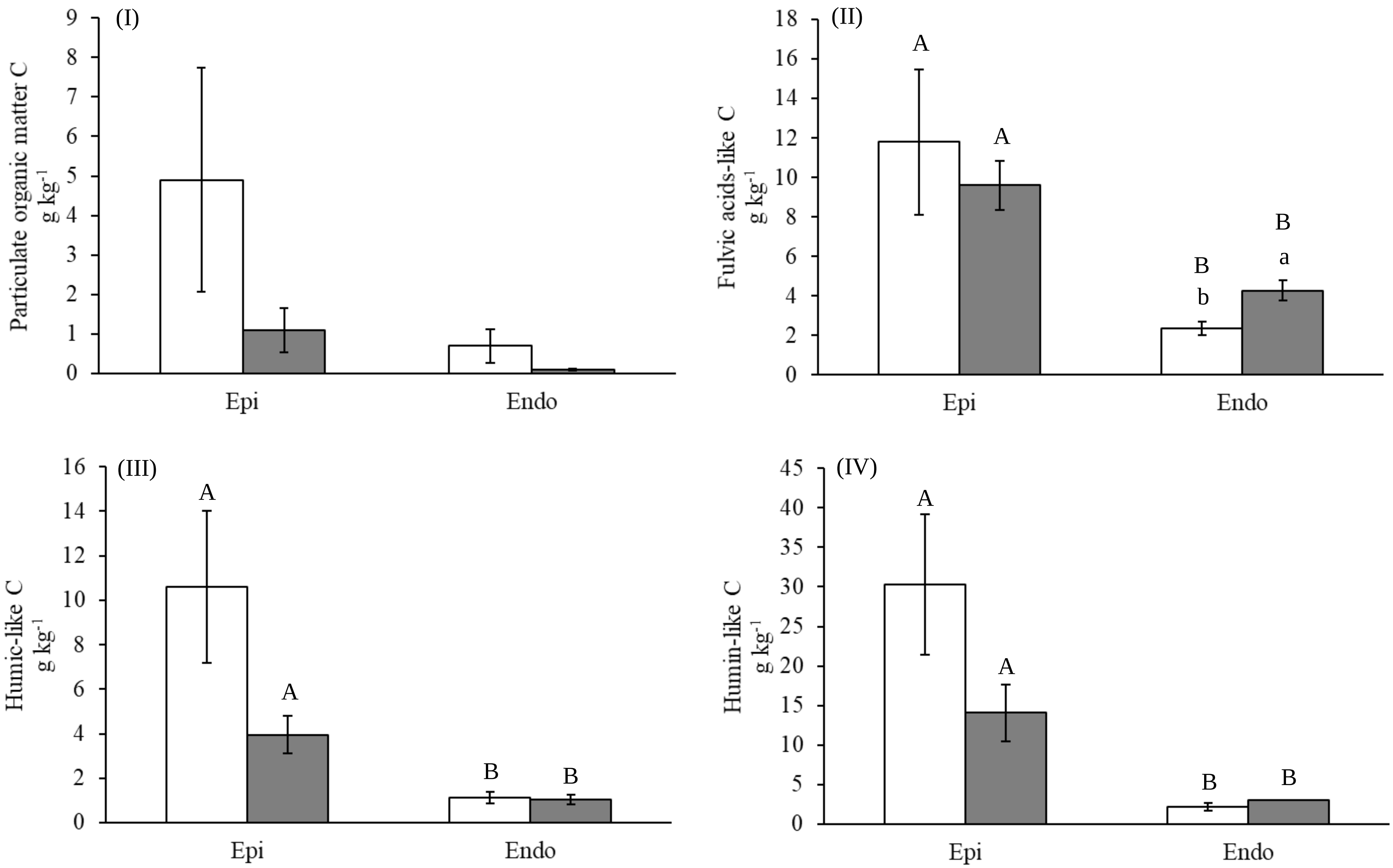
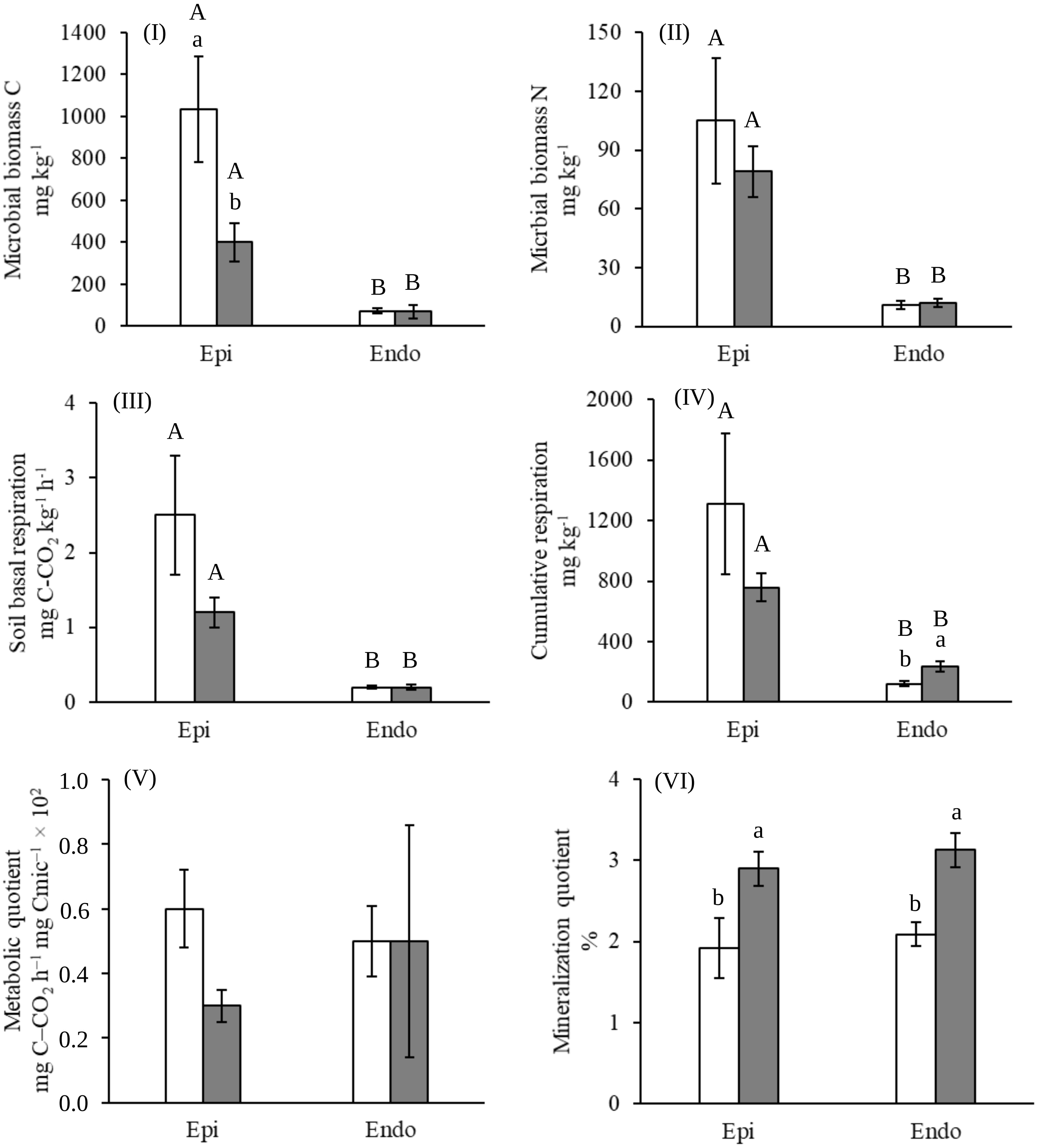

(I)
Total organic carbon
g kg-1
80
70
60
50
40
30
20
10
0
A
A
B
b
B
a
Epi
Endo
(II)
Total nitrogen
g kg-1
6
5
4
3
2
1
0
A
A
B
b
B
a
Epi
Endo
(III)
Water-extractable organic matter_C
mg kg-1
300
250
200
150
100
50
0
b
a
Epi
Endo
(IV)
Water-extractable organic matter_N
mg kg-1
90
80
70
60
50
40
30
20
10
0
A
A
B
b
B
a
Epi
Endo

Figure 4



(I)
Particulate organic matter C
g kg-1
9
8
7
6
5
4
3
2
1
0
Epi
Endo
(II)
Fulvic acids-like C
g kg-1
18
16
14
12
10
8
6
4
2
0
A
A
B
b
B
a
Epi
Endo
(III)
Humic-like C
g kg-1
16
14
12
10
8
6
4
2
0
A
A
B
B
Epi
Endo
(IV)
Humin-like C
g kg-1
45
40
35
30
25
20
15
10
5
0
A
A
B
B
Epi
Endo

Figure 5



(I)
Microbial biomass C mg kg-1
1400
1200
1000
800
600
400
200
0
A
a
A
b
B
B
Epi
Endo
(II)
Micrbial biomass N mg kg-1
150
120
90
60
30
0
A
A
B
B
Epi
Endo
(III)
Soil basal respiration mg C-CO2 kg-1 h-1
4
3
2
1
0
A
A
B
B
Epi
Endo
(IV)
Cumulative respiration mg kg-1
2000
1600
1200
800
400
0
A
A
B
b
B
a
Epi
Endo
(V)
Metabolic quotient mg C–CO2 h−1 mg Cmic−1 × 10²
1.0
0.8
0.6
0.4
0.2
0.0
Epi
Endo
(VI)
Mineralization quotient %
4
3
2
1
0
b
a
b
a
Epi
Endo

Figure captions

Figure 1. The localization of investigated sites (REM1, REM2, CONS1 and CONS2) (on left side) and a recovery chestnut stand where it is visible the 10 x 10 m tree spacing (on right side). REM and CONS study sites are chestnut (*Castanea sativa* Mill.) stands with removal and conservation of organic residues, respectively.

Figure 2. Cation exchange capacity (I), exchangeable calcium (II), magnesium (III), potassium (IV) and sodium (V), and base saturation (VI) in epipedon (Epi) and endopedon (Endo) of soils under chestnut (*Castanea sativa* Mill.) stands with removal (REM; white bars) and conservation (CONS; grey bars) of organic residues. Error bars are the standard errors. According to Kruskal–Wallis test, within each soil layer, different lowercase letters indicate significant differences (p<0.05) between the two managements (REM *vs* CONS), instead, within each management different uppercase letters indicate significant differences (p<0.05) between epipedon and endopedon (Epi *vs* Endo).

Figure 3. Amounts of total organic carbon (I), total nitrogen (II), C associated to water–extractable organic matter (III) and N associated to water–extractable organic matter (IV) in epipedon (Epi) and endopedon (Endo) of soils under chestnut (*Castanea sativa* Mill.) stands with removal (REM; white bars) and conservation (CONS; grey bars) of organic residues. Error bars are the standard errors. According to Kruskal–Wallis test, within each soil layer, different lowercase letters indicate significant differences (p<0.05) between the two managements (REM *vs* CONS), instead, within each management different uppercase letters indicate significant differences (p<0.05) between epipedon and endopedon (Epi *vs* Endo).

Figure 4. Amounts of C associated to particulate organic matter (I), and to fulvic–like (II), humic–like (III) and humin–like (IV) substances in epipedon (Epi) and endopedon (Endo) of soils under chestnut (*Castanea sativa* Mill.) stands with removal (REM; white bars) and conservation (CONS;

grey bars) of the organic residues. Error bars are the standard errors. According to Kruskal–Wallis test, within each soil layer, different lowercase letters indicate significant differences (p<0.05) between the two managements (REM *vs* CONS, instead, within each management different uppercase letters indicate significant differences (p<0.05) between epipedon and endopedon (Epi *vs* Endo).

Figure 5. Content of microbial biomass C (I) and N (II), basal respiration (III), total amount of CO_2 evolved during basal respiration experiments (IV), metabolic (V) and mineralization (VI) quotients in epipedon (Epi) and endopedon (Endo) of soils under chestnut (*Castanea sativa* Mill.) stands with removal (REM; white bars) and conservation (CONS; grey bars) of the organic residues. Error bars are the standard errors. According to Kruskal–Wallis test, within each soil layer, different lowercase letters indicate significant differences (p<0.05) between the two managements (REM *vs* CONS), instead, within each management different uppercase letters indicate significant differences (p<0.05) between epipedon and endopedon (Epi *vs* Endo).

Table 1
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Table 1. The study sites locations and climatic characteristics

Study site	Coordinates (UTM 32 T)		MAAT	MAP
	m E	m N	° C	mm
REM 1	686305	4904858	11.7	1136
REM 2	686751	4904078	11.7	1136
CONS 1	690032	4898682	11.0	1251
CONS 2	618314	4927009	10.9	883

MAAT = mean annual air temperature; MAP = mean annual precipitations.

Table 2
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Table 2. Thickness, amounts of sand, silt and clay, and pH in water (pH_{H_2O}) and in KCl (pH_{KCl}) values measured in epipedon (Epi) and endopedon (Endo) of soils under chestnut (*Castanea sativa* Mill.) stands with removal (REM) and conservation (CONS) of the organic residues. Standard error is reported in parentheses. According to Kruskal–Wallis test: *i)* within each management, the uppercase letters indicate significant differences (p<0.05) between soil layers (Epi *vs* Endo); *ii)* within each soil layer, the lowercase letters indicate significant differences (p<0.05) between the two managements (REM *vs* CONS).

Layer	Management	Thickness	Sand	Silt	Clay	pH_{H_2O}	pH_{KCl}
		cm		g kg^{-1}			
Epi	REM	5.1 Bb	564	382	152 a	4.9	3.5
		(0.7)	(67)	(51)	(21)	(0.2)	(0.2)
Epi	CONS	9.7 Ba	522	370 B	89 b	4.8	3.2 B
		(1.8)	(20)	(15)	(11)	(0.1)	(0.2)
Endo	REM	12.4 Ab	545	322	132 a	4.9	3.4
		(1.8)	(58)	(46)	(16)	(0.1)	(0.1)
Endo	CONS	20.2 Aa	467	461 A	71 b	5.4	4.1 A
		(2.1)	(13)	(13)	(3)	(0.1)	(0.1)

Table 3

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Table 3. Stock of organic carbon (OC), N, Ca, Mg, Na, K, Fe, P and S measured at 0 – 10, 10 – 20, 20 – 30 cm soil depth intervals under chestnut (*Castanea sativa* Mill.) stands with organic residues removal (REM) and conservation (CONS). Standard error is reported in parentheses. According to Kruskal–Wallis test: *i*) within each management, different uppercase letters indicate significant differences among the soil depth intervals (p<0.05); *ii*) within each soil depth interval, different lowercase letters indicate significant differences (p<0.05) between the two managements (REM *vs* CONS).

Depth	Management	OC	N	Ca	Mg	Na	K	Fe	P	S
						kg ha^{-1}				
0 – 10	REM	37183 A	2702 A	1319	3834	313 B	3716	16056	223	207 A
		(8159)	(598)	(255)	(890)	(17)	(642)	(3308)	(36)	(53)
0 – 10	CONS	30734 A	2338 A	2463	4357 B	346	4845	19435 B	295	186
		(3154)	(285)	(349)	(277)	(22)	(304)	(959)	(18)	(17)
10 – 20	REM	9581 Bb	739 Bb	1224	5002	407 A	4720	20897	159 b	84 Bb
		(2202)	(149)	(189)	(1044)	(32)	(801)	(3883)	(21)	(10)
10 – 20	CONS	17242 Ba	1389 Ba	2446	5537 A	393	5552	25697 A	280 a	138 a
		(2390)	(159)	(458)	(215)	(32)	(351)	(1448)	(27)	(12)
20 – 30	REM	6875 Bb	639 Bb	1441	6009	426 A	5431	24214	150 b	72 Bb
		(1354)	(134)	(207)	(1439)	(46)	(1099)	(5288)	(21)	(10)
20 – 30	CONS	13668 Ba	1159 Ba	2393	6027 A	405	5970	27837 A	269 a	118 a
		(1113)	(71)	(448)	(358)	(35)	(421)	(1562)	(14)	(8)

Table 4
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Table 4. Mean ± standard error of C, N, Ca, Mg, Na, K, Fe, P and S contents in chestnut (*Castanea sativa* Mill.) leaves collected from chestnut stands with organic residues removal (REM) and conservation (CONS).

	REM	CONS
C ($g\ kg^{-1}$)	411 ± 11	416 ± 5
N ($g\ kg^{-1}$)	21.0 ± 1.0	23.7 ± 1.7
Ca ($g\ kg^{-1}$)	4.31 ± 1.12	5.22 ± 078
Mg ($g\ kg^{-1}$)	1.94 ± 0.09	1.73 ± 0.08
Na ($g\ kg^{-1}$)	0.10 ± 0.02	0.19 ± 0.01
K ($g\ kg^{-1}$)	5.63 ± 0.19	7.03 ± 0.55
Fe ($g\ kg^{-1}$)	0.10 ± 0.00	0.13 ± 0.02
P ($g\ kg^{-1}$)	1.34 ± 0.17	1.05 ± 0.07
S ($g\ kg^{-1}$)	0.99 ± 0.07	1.48 ± 0.22

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***Declaration of Interest Statement**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: