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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

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

3

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27 **Abstract**

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

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

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54 **1. Introduction**

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

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

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

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

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

99 To achieve this goal, we selected chestnut stands where the organic residues such as burrs, leaves
100 and pruned materials are yearly removed, and chestnut stands with more conservationist practices
101 where organic residues are chopped and left on the soil surface. It was hypothesized that the organic
102 residues removal *i*) declines *solum* thickness; *ii*) reduces the contents of soil organic carbon and of
103 SOM pools, and the soil organic carbon stock; *iii*) decreases the stocks of N, Ca, Mg, Na, K, Fe, P

104 and S and the availability of Ca, Mg, Na and K; iv) has negative impact on soil microbial biomass
105 and respiration; v) reduces the nutrient contents in plants.

106

107 **2. Materials and methods**

108 *2.1. Study sites*

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

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

124

125 *2.2. Experimental design*

126 The four farms involved in this study were selected in order to compare the soil properties of
127 chestnut stands in which the organic residues removal (ORR) has been made or not. In two farms,
128 the chestnut stands were subjected to yearly ORR, hereafter called REM, due to the use of small
129 blowing devices during the fruits harvesting which remove from the soil surface both fruits and

130 other organic residues. In two other farms, management was more conservationist which consist to
131 leave on the soil surface the organic residues, hereafter called CONS, which are chopped. In CONS
132 study sites the fruits harvesting was manual or using nets put on trees. Both ORR and the more
133 conservationist practices began at the recovery time of the chestnut stands, therefore about 30 years
134 ago, and concerned the whole surface of the stands.

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

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

142

143 *2.3. Sampling and analysis*

144 In each dug pit, each identified genetic horizon down to 30 cm of soil depth has been sampled by
145 collecting at least 2 kg of sample and, once in the laboratory, it was air dried and sieved (<2 mm).
146 For the determination of the bulk density (BD), additional soil samples were taken using steel
147 cylinder cores with an internal diameter of 5.0 cm and 5.0 cm height from the 0-10, 10-20 and 20-
148 30 cm soil depth.

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

154 The soil particle size distribution was obtained by the pipette method after dispersion of the sample
155 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 ($\text{pH}_{\text{H}_2\text{O}}$) 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 hexaminecobalt(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 supernatant 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 supernatant <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 supernatant (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 HNO₃ (65 % v/v): H₂O₂ (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 CO₂ emitted from incubated soils was measured by alkali (1 M NaOH solution) absorption of the evolved CO₂ 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 CO₂ evolved during the 28 days of incubation.

Soil microbial biomass-C (C_{mic}) and microbial biomass-N (N_{mic}) were measured on soil samples at 60% of WHC using chloroform fumigation extraction method with 0.5 M K₂SO₄ solution (Vance et al., 1987). Both fumigated and non-fumigated extracts were analysed using a TOC-V CPN total organic carbon analyzer (Shimadzu, Japan). C_{mic} was calculated as $E_C \cdot 2.64$, where E_C = difference

208 between organic C extracted from fumigated soils and organic C extracted from non-fumigated
209 soils. N_{mic} was calculated as $E_N \times 2.22$, where E_N = difference between total N extracted from
210 fumigated soils and total N extracted from non-fumigated soils (Vance et al., 1987).

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

$$213 \quad qCO_2 = 100 \times SBR / C_{mic} \quad (1)$$

$$214 \quad qM = 28 \text{ days cumulative respiration (RCUM)} / TOC \quad (2)$$

215

216 *2.4. Data analysis*

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

222 The stock of OC, TN and nutrients are presented for fixed soil-depth intervals (0–10, 10–20 and
223 20–30 cm), taking into account the BD value of each soil layer at fixed depth and the OC, TN and
224 nutrients content of each genetic horizon averaged on the basis of horizon thickness.

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

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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.

260 The $\text{pH}_{\text{H}_2\text{O}}$ values ranged from 4.8 to 5.4, the pH_{KCl} ranged from 3.2 to 4.1 with some differences in
 261 CONS where the Endo showed higher values compared to Epi (Table 2).

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

272

273 *3.3. SOM pools of chestnut stand soils*

274 As expected, in both chestnut managements, most of the studied pools of C and N cycles (i.e., total,
 275 labile, humic-like and humin substances) were higher in the Epi than in the Endo (Figure 3, Figure
 276 4, Figure S1). Comparing the two chestnut stand managements, some differences occurred for the
 277 Endo. Specifically, the concentrations of TOC and of organic C related to WEOM and FA were
 278 higher in CONS than in REM (Figure 3I, III, Figure 4II). Similarly, the contents of TN and nitrogen
 279 related to WEOM were greater in CONS than in REM (Figure 3II, IV).

280

281 *3.4. Microbial biomass amount and respiration of chestnut stand soils*

282 Regarding to the soil microbial biomass, in both chestnut stand managements C_{mic} , N_{mic} , SBR
 283 and RCUM values were higher in Epi than in Endo, while no differences occurred for qR and qM
 284 (Figure 5I–VI). Through the comparison of the two chestnut stand managements, some differences

285 occurred for RCUM and qM. In particular, the RCUM was higher in Endo of CONS than in REM
286 (Figure 5IV), the qM was higher in CONS than in REM both for Epi and Endo (Figure 5VI).

287

288 *3.5. Soil organic C and nutrients stocks, and nutrient concentrations in chestnut tree leaves*

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

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

299

300 **4. Discussion**

301 *4.1. Organic residues conservation promotes soil thickness and development in chestnut stands*

302 In the present study, the ORR practice reduced the thickness of superficial A horizons (5.1 vs 9.7
303 cm in REM and CONS, respectively) which could be attributed to higher soil erosion that probably
304 takes place in the former than in the latter study sites. Indeed, as reported by several works (e.g.,
305 Dick et al., 1997; Křeček et al., 2019; Liu et al., 2017) the presence of the litter layer limits the
306 surface runoff and soil erosion. The highest soil erosion that likely occurs in REM study sites could
307 also limit the thickness of *solum* due to losses of pedogenized material (Mokma et al., 1996).
308 Consequently, the formation of more developed B horizons or transitional AB ones was prevented
309 in REM soils. Our findings are in accordance to Świtoniak (2014) and Jankauskas and Fullen
310 (2002) which, through a pedological investigation of soils subjected to different erosion severity,

311 observed weakly developed soil horizon sequences in eroded sites. In contrast, the reduced erosion
312 in CONS study sites might promoted the development of their soils allowing to define the CONS
313 soils as Inceptisol while those of REM as weakly developed Entisols.

314

315 *4.2. Organic residues conservation promotes soil organic matter content and stock in chestnut* 316 *stands*

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

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

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

337 seemed to reduce the role of soil as stable sink of C reducing the OC stocks below 10 cm of soil
338 depth.

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

345

346 *4.3. Organic residues conservation increases soil nutrient contents in chestnut stands*

347 The similar parent material on which the studied soils developed explains the similar stock of
348 nutrients, with exception of P and S, which exhibited higher values in 10 – 30 soil depth section of
349 CONS than of REM probably due to the higher amount of organic matter. Indeed, concerning P, in
350 forest soils most of the soil P is made up of organic P forms (Adams et al., 2018; De Feudis et al.,
351 2016). As the organic P is the most important P source in forest ecosystems (Turner et al., 2014)
352 and the total P concentration generally decline over time (Chen et al., 2015), litter recycling is
353 essential to avoid an accelerated loss of soil P. The nutrient recycling process seemed to affect also
354 S. In fact, similarly to P, the total amount of S in 10 – 30 cm soil depth interval was higher in
355 CONS than in REM. As for P, the organic S compounds represent the most abundant S forms in
356 soils (e.g., Likens et al., 2002; Vannier and Guillet, 1994). Regarding to the other nutrients, such as
357 exchangeable Mg, K and Na, their amounts did not change between the two managements.
358 Exchangeable Ca, instead, had higher contents in Endo of CONS than in that of REM, following the
359 trend of OC content. This is in agreement with Vittori Antisari et al. (2013) who reported that SOM
360 is an important source of available Ca in chestnut stands. The importance of Ca biocycling was
361 further stressed by the lack of differences of Ca stock. In fact, in mountain acid soils the
362 redistribution of Ca-mineral derived is often prevented by the limited Ca-mineral weathering

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

370

371 *4.4. Organic residues conservation improves soil microbial respiration in chestnut stands*

372 The RCUM in Endo of CONS study sites was higher than in REM likely due to the higher C
373 content associated to WEOM (WEOM-C). In fact, because of the most labile organic compounds
374 represent the main substrate for soil microbial communities (Rees and Parker, 2005) and changes of
375 WEOM-C quantity affect both microbial activities and composition (Drenovsky et al., 2004), the
376 enhance of WEOM-C can promote the heterotrophic soil respiration (Rees and Parker, 2005).
377 Furthermore, since the positive effect of WEOM-C on soil microbial activities, the higher qM in
378 CONS than in REM might be attributed to the priming effect, namely a short-term change in
379 microbial mineralization of SOC in response to labile OC inputs (Kuzyakov, 2010). Unlike RCUM
380 and qM, both the microbial biomass and qCO₂ have shown similar values between the two chestnut
381 orchard managements. The lack of differences about microbial biomass and qR might be attributed
382 to the fact that they are driven not only by the amount of TOC but also by other soil properties such
383 as BD, soil moisture and temperature, and C:N ratio (Ren et al., 2018; Stefanowicz et al., 2016; van
384 Leeuwen et al., 2017; Xu et al., 2007) which, in our case, were similar among the study sites.

385

386 *4.5. Overview of the effects of organic residues management on soil properties in chestnut stands*

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

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

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

405 Hence, a chestnut stand management based on the conservation of the litter floor should be
406 supported in order to improve the soil quality and, in particular, to increase the volume of *solum*, the
407 SOM and nutrient contents and the soil microbial respiration. However, though not considered in
408 the present work, CONS management could have some negative effects on the spreading of some
409 chestnut diseases. For example, the increase of SOM content could benefit the proliferation of the
410 ink disease (*Phytophthora cinnamomi*) because of its saprotrophic nature (Fonseca et al., 2004).
411 Furthermore, since in CONS study sites the organic residues are chopped, this practice could
412 increase the production costs. Finally, since the ORR and the chipping of the litter floor are yearly
413 performed, both studied managements could have some negative effects on the proliferation of
414 *Torymus sinensis* Kamijo. In fact, in order to maintain this biological control agent of the chestnut

415 gall wasp, the plant material bearing galls (branches, suckers) should not be removed from the stand
416 at least for two years (Ferracini et al., 2015). More researches need however on the assessment of
417 the phytosanitary consequences and on the role that the SOM and microbial activity can have on the
418 enhancement of chestnuts disease resistance.

419

420 **5. Conclusion**

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

438

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445

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682

Figure 1

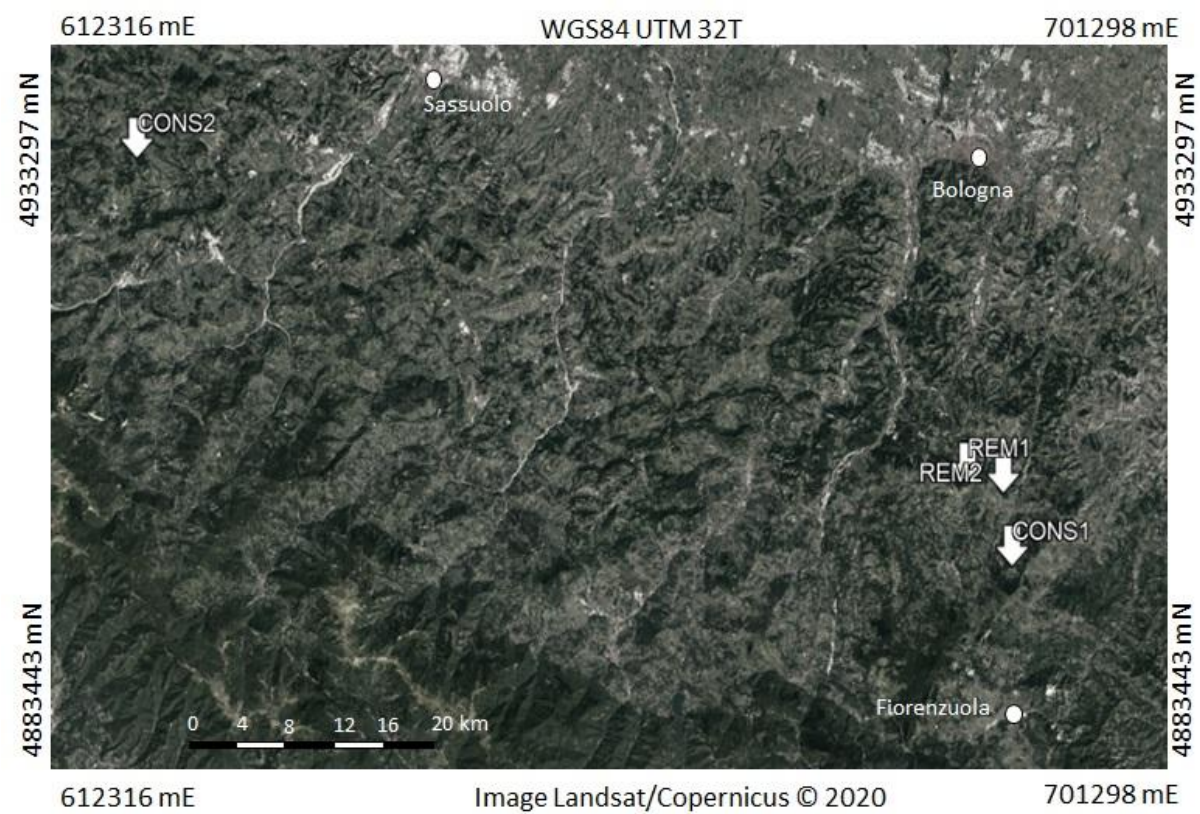


Figure 2

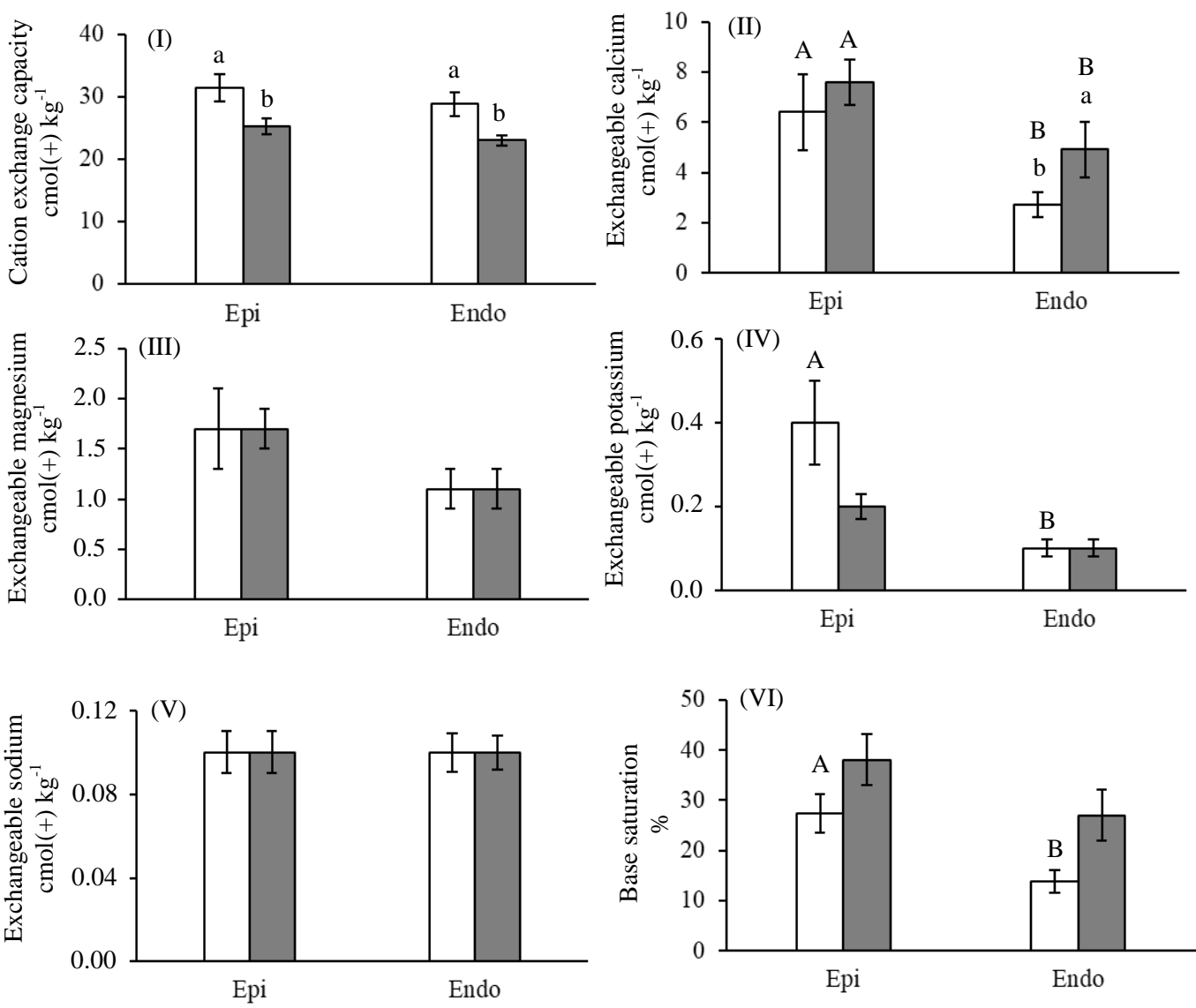


Figure 3

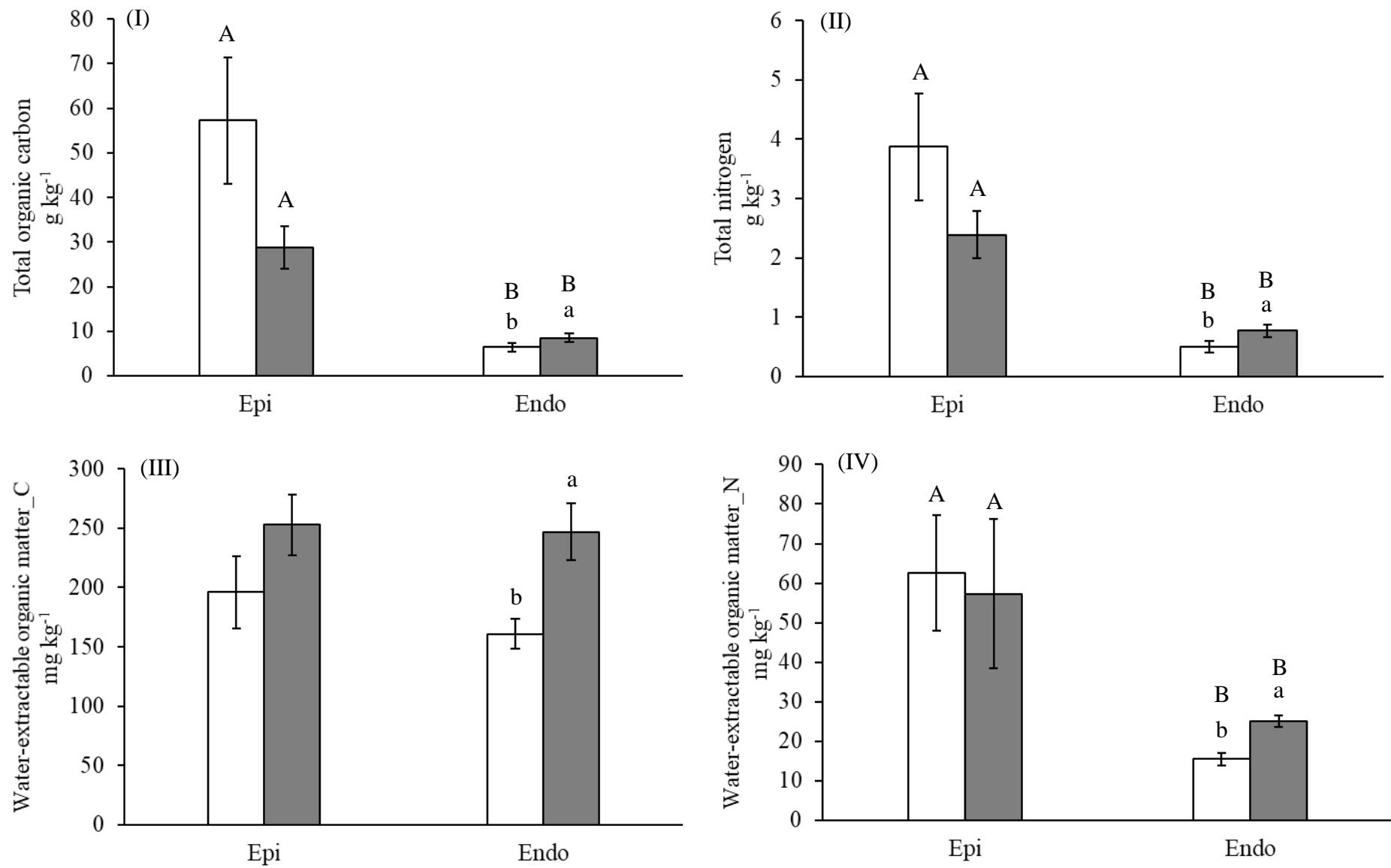


Figure 4

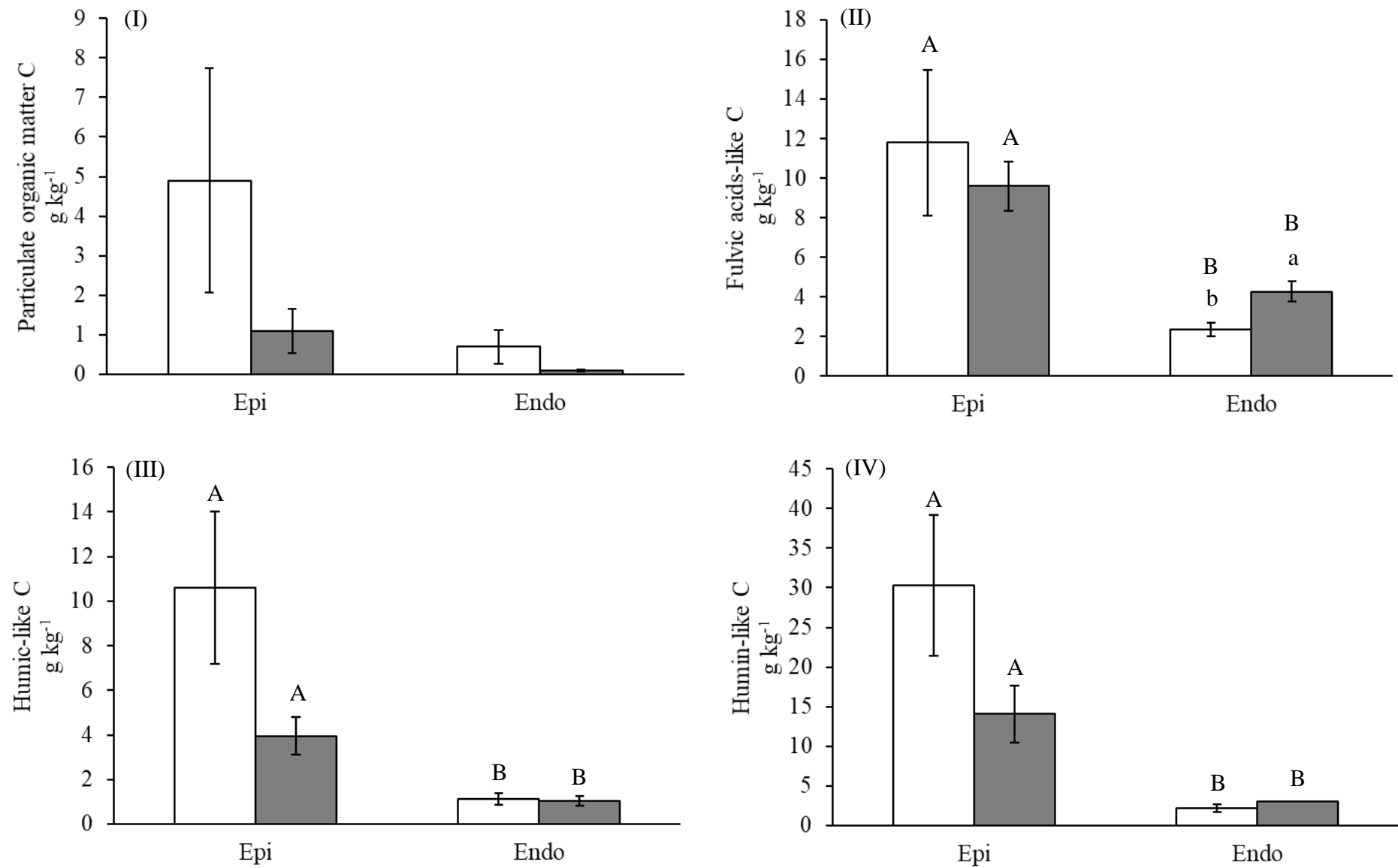


Figure 5

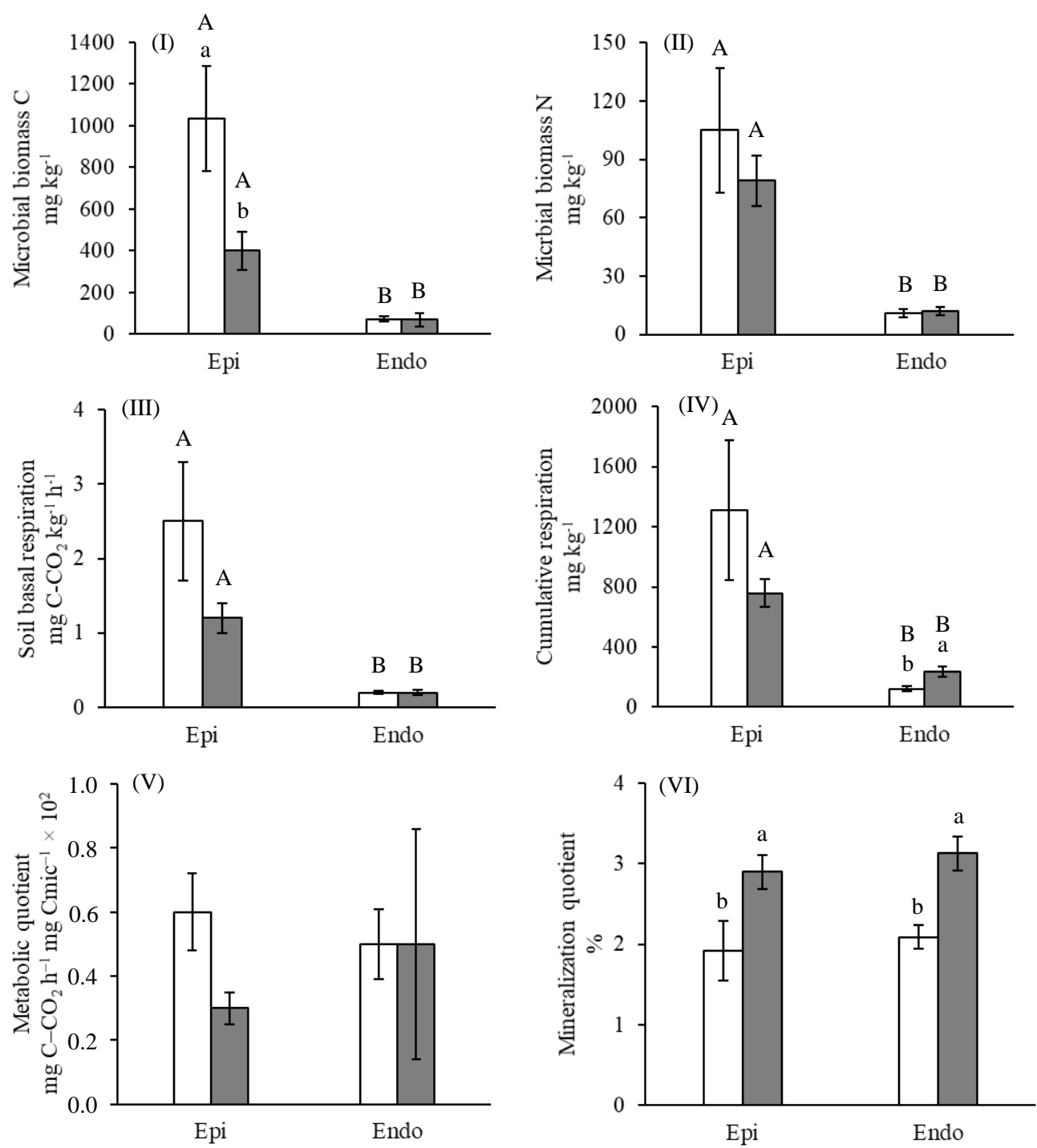


Figure captions

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 CO₂ 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. 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. Thickness, amounts of sand, silt and clay, and pH in water (pH_{H₂O}) 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 _{H₂O}	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 (8159)	2702 A (598)	1319 (255)	3834 (890)	313 B (17)	3716 (642)	16056 (3308)	223 (36)	207 A (53)
0 – 10	CONS	30734 A (3154)	2338 A (285)	2463 (349)	4357 B (277)	346 (22)	4845 (304)	19435 B (959)	295 (18)	186 (17)
10 – 20	REM	9581 Bb (2202)	739 Bb (149)	1224 (189)	5002 (1044)	407 A (32)	4720 (801)	20897 (3883)	159 b (21)	84 Bb (10)
10 – 20	CONS	17242 Ba (2390)	1389 Ba (159)	2446 (458)	5537 A (215)	393 (32)	5552 (351)	25697 A (1448)	280 a (27)	138 a (12)
20 – 30	REM	6875 Bb (1354)	639 Bb (134)	1441 (207)	6009 (1439)	426 A (46)	5431 (1099)	24214 (5288)	150 b (21)	72 Bb (10)
20 – 30	CONS	13668 Ba (1113)	1159 Ba (71)	2393 (448)	6027 A (358)	405 (35)	5970 (421)	27837 A (1562)	269 a (14)	118 a (8)

Table 4. Mean \pm 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 \pm 11	416 \pm 5
N (g kg ⁻¹)	21.0 \pm 1.0	23.7 \pm 1.7
Ca (g kg ⁻¹)	4.31 \pm 1.12	5.22 \pm 0.78
Mg (g kg ⁻¹)	1.94 \pm 0.09	1.73 \pm 0.08
Na (g kg ⁻¹)	0.10 \pm 0.02	0.19 \pm 0.01
K (g kg ⁻¹)	5.63 \pm 0.19	7.03 \pm 0.55
Fe (g kg ⁻¹)	0.10 \pm 0.00	0.13 \pm 0.02
P (g kg ⁻¹)	1.34 \pm 0.17	1.05 \pm 0.07
S (g kg ⁻¹)	0.99 \pm 0.07	1.48 \pm 0.22

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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: