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Manuscript title

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Running title

Pedogenic horizons sampling for an accurate forest SOC assessment

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Abstract

In soil organic carbon (SOC) survey the role of sampling approach is crucial and should not be underestimated. In this sense, the appropriateness of soil sampling by pedogenic horizons (PGH) and fixed-depth layers (FDL) in forest is still field of debate. The present work aimed to a) study the spatial variability of SOC concentrations and C stocks through PGH and FDL samplings in uneven–aged sweet chestnut, European beech and Norway spruce forests of Apennine chain (Italy); b) clarify the major advantages and drawbacks of sampling by PGH and FDL. In a representative area (18×18 m) of each forest, a soil profile was investigated and 8 additional sampling points were selected. The sampling was performed both by PGH and FDL (0–15 and 15–30 cm). For each forest, no SOC content and C stock differences in 0–30 cm soil depth were found between PGH (58.8±5.3 g kg^{-1} and 103±7 g ha^{-1}, respectively) and FDL (52.7±4.3 g kg^{-1} and 117±9 Mg ha^{-1}, respectively) sampling. However, under Norway spruce, PGH sampling pointed out that C was greatly stored in Oe and Oa horizons (51.1 *vs* 146 Mg ha^{-1} in the 0-30 cm layer). The higher coefficient of variation obtained when soil horizons were considered (from 19.2 to 72.8% for PGH and from 16.5 to 25.7% for FDL) suggested that PGH sampling preserved the information about the spatial variability within an ecosystem. The information loss about SOC vertical and spatial distribution would indicate the inability of FDL sampling to support decision–making plans addressed for sustainable use of soil resource.

Keywords

Mini–sampler; soil horizons; SOC stock; forest soil; cambisols

1. Introduction

The report published by the Intergovernmental Panel on Climate Change in 2018 (IPCC, 2018) highlighted the urgent need for actions on global scales to prevent the increase of the mean air temperature of 1.5°C compared to the pre-industrial levels. At the global scale, soils are the largest organic carbon (C) sink in terrestrial ecosystems, containing approximately 1500 Pg C in 1 m of depth which is 2–3 folds the amount of C found in plant biomass and atmosphere (Batjes, 2014; Vicente-Vicente et al., 2016). Therefore, while a slight decrease of the soil organic C (SOC) content can lead to significant changes in atmospheric CO_2 (IPCC, 2013), the increase of C sequestration in form of SOC can have an important role on mitigation of the current global warming (Minasny et al., 2017).

Globally, forest ecosystems cover approximately more than 4 billion hectares (FAOSTAT, 2020) and the most recent estimates reported that they store about 650 Pg of C, of which 44 % in the biomass, 11 % in dead wood and litter, and 45 % into the soil (FAO, 2010). In Italy, forested areas amount for about 10 million hectares (FAOSTAT, 2020) and their soils store from 80 to 130 Mg C ha^{-1} to 1 m depth (Caddeo et al., 2019). Italian forests are mainly distributed in mountainous areas (Cesaro and Romano, 2017), where soils are often characterized to be poorly developed, shallow and skeletal due to limited pedogenic processes and high soil erosion rates (FAO, 2015; Guerra et al., 2020; Alewell et al., 2008). Further, mountain soils are recognized to have a high sensitivity to climate change (Hagedorn et al., 2010), which make them highly vulnerable to SOC decline, erosion and further soil degradation (Conforti et al., 2013; Bojko and Kabala, 2016), which, in turn, can affect the ecosystem functions supplied by forest soil such as biomass production, nutrient and water supply, C sequestration, and biodiversity conservation (Vicente-Vicente et al., 2019; Baveye et al., 2016).

SOC amount is the result of a combination of various environmental factors as well as vegetation composition and climate. To this regard, several studies pointed out that higher SOC concentrations occur under broadleaf than coniferous forests (e.g., Cha et al., 2019; Massaccesi et al., 2020) and

SOC stock is negatively correlated with temperature and positively correlated with precipitation (e.g., Choudhury et al., 2016; Jobbágy and Jackson, 2000; Massaccesi et al., 2020). Further, also parent material can affect the size of organic C pool stored in soil. According to Yang et al. (2018) and Mao et al. (2020), limestone–derived soils tend to promote C stock due to the presence of calcium and magnesium which link the organic compound to soil minerals. In acidic soils, SOC is stabilized in organic- mineral complexes through the binding action of Fe and Al ions (Su et al., 2021).

SOC plays a key role in the functioning of forest ecosystems by regulating nutrient cycling, water storage and availability, erosion and flooding, and supporting biota habitat (Brang et al., 2006; Adhikari and Hartemink, 2016; Minasny and McBratney, 2018; Rabbi et al., 2018). Because of the large number of environmental functions provided by SOC, its adequate monitoring is the first step towards preserving SOC and thus limiting soil degradation processes.

Several studies were performed in European forests to give a large–scale view of the current state and changes in forest soils (e.g., Wellbrock and Bolte, 2019; Hiederer et al., 2011). At local scale, however, the measurement of SOC concentration and, consequently, the determination of C stock pose several critical points in their assessment, and the soil sampling approaches are still field of debate. Indeed, with the exception of the organic layers, namely the O horizons, for which there is a general agreement on sampling by horizons (e.g., Koga et al., 2020; Lull et al., 2020), in forest ecosystems mineral soil samples can be collected both by horizons (e.g., De Feudis et al., 2021b; Massaccesi et al., 2020; Valtera and Šamonil, 2018) or by fixed-depth layers (Canedoli et al., 2020; Gross et al., 2018; Tesfaye et al., 2016). The ICP Forest (Cools and De Vos, 2020) designed a manual for the monitoring of the main soil properties based on both sampling types. The choice to sample by pedogenic horizons (PGH) or by fixed depth layers (FDL) is driven by the research aims and the advantages and disadvantages of the two approaches. Soil sampling by FDL is usually employed for modelling and mapping (e.g., De Feudis et al., 2021a; Orton et al., 2016; Sulieman et al., 2018). The major strength of soil sampling by FDL is related to its greater ease execution. It does not need experts for the delineation of the soil horizons, it is less time consuming compared to the sampling by PGH and is, therefore, cheaper. Sampling by FDL is statistically efficient because not all soil profiles have all the same morphology, although it does not give any information about type and thickness of the soil horizons. The sampling by PGH is useful when vertical and lateral changes of soil properties (e.g., nutrients and SOC) are studied (Grüneberg et al., 2010; Shahbazi et al., 2019; Sharififar et al., 2019), or when soil processes regulating properties and functions are investigated. Additionally, the soil sampling by PGH provide the same data that can be obtained collecting samples by fixed depth, because during the pedological survey the thickness and depth of each horizon are recorded.

In the present study we determined the soil C concentration and stocks in three widespread forest ecosystems, (chestnut, beech and spruce forests) sampling by both PGH and FDL and considering the upper 30 cm of soil. We choice to investigate the soil down to 30 cm of depth because it has been reported that the SOC in the topsoil is noticeably responsive to global climate change and human disturbances (Don et al., 2011; Grüneberg et al., 2014; Li et al., 2017) and such interval is worldwide used for the soil organic carbon monitoring (Makipaa et al., 2012; IPCC, 2003; Hiederer et al., 2011). In this context, it is important to highlight that, though the upper part of the soil (i.e., 0 – 10 cm) has higher SOC concentration compared to the subsoil (e.g., Massaccesi et al., 2020; De Feudis et al., 2021b), the latter can considerably contribute to the C stocks of the whole soil (Grüneberg et al., 2010). The main aims of this study were a) to study the spatial variability of SOC concentrations and C stocks through PGH and FDL samplings in the three forest ecosystems investigated; b) to clarify the major advantages and drawbacks of sampling by soil horizons and fixed depths. We hypothesized that: a) SOC concentration and C stock values do not change in 0–30 cm soil depth between the two sampling approaches; b) PGH sampling allows to keep information about horizontal and vertical SOC distribution.

2. Materials and methods

2.1. Study sites description

Three forest types that are widespread distributed in the Italian mountain areas (Cislaghi et al., 2021), uneven–aged sweet chestnut (*Castanea sativa* Mill.), European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* (L.) H. Karst.) forests, were selected as study sites (Figure 1) in the northern Apennine chain (Italy). The three study sites had similar lithology made of stratified arenaceous–pelitic turbidites (Bertolini et al., 2005) belonging to the Miocene period, with feldspars, micas and quartz as the main minerals (Vittori Antisari et al., 2013). In all sites, the soils were classified as Dystric Cambisols (FAO, 2014).

The sweet chestnut forest was located at about 700 m above sea level (a.s.l.), with a North–Northwest exposure (315°N) and a slope ranging from 12 to 18%. The mean annual air temperature and precipitation were 10.0 °C and 1403 mm, respectively. The European beech and Norway spruce forests were placed at about 1300 m a.s.l., with a Northeast exposure (47°N) and a slope ranging from 19 to 24% for the beech forest, and a South–Southwest exposition (225°N) and a slope ranging from 10 to 12% for the spruce one. The mean annual air temperature was 6.9 °C and the mean annual precipitation was 2098 mm for both forest type.

The chestnut forest had a density of 448 plants ha^{-1} and the average tree diameter at breast height (DBH) was of 31 cm, 24 cm and 10 cm for 39, 30 and 31 % of total plants, respectively. The beech forest had a density of 1105 plants ha^{-1} and the average tree DBH was of 34 cm, 22 cm and 12 cm for 25, 40 and 35 % of total plants, respectively. The spruce forest had a density of 608 plants ha^{-1} and the average tree DBH was of 54 cm and 42 cm for 27 and 73 % of total plants, respectively.

2.2. Soil sampling design

In each site, an area of 324 m^2 (18 m × 18 m) was selected. In order to obtain comparable data among the forest types, we selected investigation areas with healthy plants, no herb coverage, no invasive plants intrusion, similar slope and no evident erosive soil processes. At the centre of the selected area, a representative soil profile was described down to BC or C horizon (Figure 2). The morphological features (i.e., depth, thickness, structure, etc.) of organic and mineral horizons were described according to Schoeneberger et al. (2012) and are reported in Table S1 of the Supplementary Materials. In each area, the Oi horizon has been collected within one 100×100 cm sampling frame that was placed near the point where the soil profile was excavated. Soil samples were collected from the profile both by considering all the pedogenic horizons (PGH) and by fixed-depth layers at 0-15 cm (comprising the thin Oe horizon) and 15–30 cm (FDL) (Figure 2). Further, 8 additional sampling points were chosen at a distance of 6 m from the soil profile and 6 m from one to each other (Figure 3). In such sampling points, two soil cores from 0 to 30 cm of depth were collected through metallic mini–samplers made by two half-cylinders (height 30 cm, diameter 4 cm, weight 700 g) (Figure 3) that were hammered into the soil starting from Oe horizon. Once in the laboratory, one of the two soil cores collected in each sampling point was divided according the recognized pedogenic horizons, whereas the other was divided in two samples by depth (0–15 cm and 15–30 cm) (Figure 2).

2.3. Bulk density determination

2.3.1 Bulk density for Oi and Oe horizons

In each area, one sample from Oi horizon and one sample from Oe horizon were collected and their thickness were measured. The collected samples were oven–dried at 105 °C and then their mass was weighted. The bulk density (BD) of Oi and Oe horizons was calculated taking into account the 105°C oven–dried collected mass (g), the sampling area (cm^2) and the horizon thickness (cm).

Conversely to Oi and Oe, the Oa horizon was hard to identify in the field and it was classified as an organic horizon only after it was analysed for its C content (130 g kg^{-1}, on average). Therefore, the BD of the Oa horizon was calculated by a pedotransfer function (see section 2.3.3).

The measured BD of the organic horizons under Sweet chestnut, Norway spruce and European beech forests were 0.028, 0.084 and 0.068 g cm^{-3} for Oi and 0.062, 0.099 and 0.157 g cm^{-3} for Oe, respectively. The calculated mean BD for the Oa horizon, present only under Norway spruce, was 0.73 g cm^{-3}.

2.3.2 Bulk density for 0–15 and 15–30 soil layers of the representative soil profile

Undisturbed soil samples were collected from each soil profile by a steel core (diameter 5 cm; height 5 cm) at 0–5, 5–10, 10–15, 15–20, 20–25 and 25–30 cm depth starting from the upper limit of Oe horizon. The undisturbed soil samples collected by steel-core were oven-dried at 105 °C, weighed, and the dry weight was divided by the volume of the cores. The obtained density values were adjusted by subtracting the mass and the volume of skeleton to determine the BD of the fine earth fraction. The mass and the volume occupied by roots inside the steel cores accounted less than 1% and were considered negligible. Then, to obtain the BD of the 0–15 cm depth layer, the BD values of the samples collected at 0–5, 5–10, 10–15 cm depths were averaged. Similarly, the BD of 15–30 cm depth layer was obtained by averaging the BD values of the samples collected at 15–20, 20–25 and 25–30 cm depths. The BD values of 0–15 cm and 15-30 cm depth layers were then used to develop the pedotransfer function (PTF) that was used for the calculation of the BD values of the soil samples collected both by PGH and FDL.

2.3.3 Pedotransfer function (PTF) development for estimating soil bulk density

The BD values of the soil samples collected both by PGH and FDL were calculated by the following PTF:

$$BD = a + b \times \log(sand) + c \times \log(SOC) \quad [1]$$

where a = 3.80429, b = -0.42052, c = -0.08518.

The PTF used for the present study was developed taking into account sand and SOC contents because of their major influence on BD (Chen et al., 2018). The obtained PTF had an adjusted R^2 = 0.74 and $P < 0.001$ both for log(sand) and log(SOC).

The PTF was developed by merging two datasets: a set of data obtained from the samples collected by depth within the soil profile of the three study areas, as previously described, and a set of additional data obtained from a Dystric Cambisol under chestnut forest located close to the sites understudy and with a similar lithology, which it is known to have relevant influence on soil BD (Patton et al., 2019), and soil type.

The BD values of both datasets (Table S2 of the Supplementary Materials) are in accordance with those reported in literature for similar pedological conditions (Heinze et al., 2018; De Feudis et al., 2020a; De Feudis et al., 2021b).

2.4. Particle size distribution and organic carbon content and stock

The soil samples collected from the soil profiles and by mini–samplers were air–dried and sieved through a 2–mm mesh. The particle size distribution was determined by the pipette method (Gee and Bauder, 1986). SOC content was determined by loss–on–ignition method according to Schulte and Hopkins (1996) and Cambardella et al. (2001).

The C stock [Mg ha^{-1}] of each horizon and fixed depth layers was calculated by the following equation (FAO, 2019):

$$Cstock = SOC \times BD \times (1 - Vg) \times th \times 0.1 \qquad [2]$$

where *SOC* is the organic carbon content (g kg^{-1}), *BD* is the mass of the fine earth per volume of fi-ne earth (g fine earth cm^{-3} fine earth), *Vg* is the coarse fragment content (Ø > 2 mm) expressed as percent by volume evaluated on the soil profiles, *th* is the thickness of the considered soil horizon or fixed depth layer (cm), *0.1* is the conversion factor for converting mg C cm^{-2} to Mg C ha^{-1}.

To test the data transferability from soil horizon sampling procedure to soil fixed depth layers, the data of SOC content and stock from soil horizons were also expressed referring to soil fixed depth as weighted average based on soil horizon thickness.

2.5. Statistical analysis

The graphical analysis of residuals was used to verify the normality and homoscedasticity of the data (Schützenmeister et al., 2012). If the assumptions of normality and homoscedasticity were not satisfied, the data were transformed according to the Box and Cox (1964) procedure. Then, within each soil horizon, one–way analysis of variance (ANOVA) was carried out to assess the effect of forest type on soil horizons thickness, SOC content, C stock and amount of sand, silt and clay. On data from soil horizons and fixed depth layers, two–way ANOVA was applied to assess the effect of soil sampling approach (soil horizons *vs* fixed depth layers) and forest type on SOC content and C stock.

The comparison between the means was performed with Tukey's honest significant differences with a significance level of 0.05. In order to evaluate the short–scale variability of C stock in each study site, the coefficient of variation (CV) was calculated (Rosemary et al., 2017) for soil horizons and 0–15 and 15–30 cm soil depth layers. The statistical analyses were performed using the R 4.1.1 statistical software.

3. Results

3.1. Pedogenic horizons in 0–30 cm soil depth

Soils of the selected study sites showed a similar soil horizon sequence (Oe, A, AB and Bw) in the upper 30 cm of soil with the exception of Norway spruce forest where also an Oa horizon was detected (Table S1 of the Supplementary Materials and Figure 4).

All study sites had a sandy loam texture (Figure 5). The A and AB horizons of soils under chestnut and Norway spruce forests showed the lowest sand content and the highest silt content (Figure 5a, b). The amount of clay did not differ among the three forest types (87 and 111 g kg^{-1} on average, for A and AB horizons, respectively; Figure 5c). In Bw horizon, soil under chestnut forest showed the lowest sand content and the highest silt content (369 and 485 g kg^{-1}, respectively; Figure 5a, b). Clay in Bw horizon showed some differences between chestnut and spruce forests (Figure 5c); in particular we found higher clay content under the former forest type than in the latter one (146 and 107 g kg^{-1}, respectively).

SOC concentration always showed the lowest values under chestnut forest (Figure 6a). Norway spruce and European beech forests showed similar SOC content in Oe and Bw horizons, while higher SOC content was found in beech than in Norway spruce forest for A and AB horizons (Figure 6a).

Taking in consideration the total amount of organic carbon stored in each soil horizon, chestnut forest showed the lowest C stock in Oe (3.2 Mg ha^{-1}), A (14.9 Mg ha^{-1}) and AB (16.4 Mg ha^{-1}) horizons. No differences occurred between Norway spruce and beech forests where the C stock in Oe, A and AB horizons were on average 9.0, 31.0 and 41.6 Mg ha^{-1}, respectively (Figure 6b).

3.2. Soil organic carbon stock and content in 0–15 and 15–30 cm soil depths

In Figure 7, the values of SOC content and C stock observed in 0–15 and 15–30 cm soil depths sampled through the fixed depth interval approach and those calculated using the data of each identified soil horizon were reported. At both soil depths, soil under chestnut forest showed the lowest SOC content, while no SOC content differences occurred between Norway spruce and European beech forests (Figure 7a, b). For C stock in 0–15 cm soil depth some few differences between the two sampling approaches were observed (Figure 7c). Through the FDL sampling, the lowest C stock was found in soil under chestnut forest, while the highest one under Norway spruce forest. Through the calculated values from PGH sampling, the lowest C stock was observed under chestnut forest, but no differences occurred between European beech and Norway spruce forests.

For C stock in 15–30 cm soil depth, through both sampling approaches, the highest C stock was observed in soil under Norway spruce forest (Figure 7d).

3.3. Short–scale variability of soil organic carbon stock

The coefficient of variation (CV) showed higher values when the soil sampling was performed considering the soil horizons compared to the values obtained through dividing soil samples in the two depth intervals (Table 1). The differences of the CV between the two approaches were pronounced for the surface layer. The CV tended to decrease from Oe to Bw horizon in chestnut and Norway spruce forests. Under beech forest the CV values did not change with exception of AB horizon where an increase was observed. Taking in consideration the fixed depth layers, the CV decreased in Norway spruce forest and negligible differences were found for chestnut and beech forests (Table 1).

Comparing the study sites, the Oe horizon showed the highest CV in chestnut forest, while the lowest one was observed in beech forest. The CV in A horizon showed the trend Norway spruce > chestnut > beech, but no differences occurred for the AB horizon. In Bw horizon the CV showed the highest value under beech forest while the lowest one under chestnut forest. Compared to the horizons, the CV values of 0–15 and 15–30 cm layers showed weaker differences among the study sites. For the 0–15 cm layer the highest CV was found in Norway spruce forest and the lowest one in chestnut forest. The trend beech > Norway spruce > chestnut trend was observed for the 15–30 cm soil depth (Table 1).

4. Discussion

4.1. Quantity of soil organic carbon storage by horizon sampling

As showed by the soil sampling by PGH, in the 0–30 cm soil depth interval below Oi horizon, the Oe horizon was present in all sites as well as A–AB–Bw sequence in mineral soil (Figure 4). Under Norway spruce, the Oa horizon underlie the Oe one. Under Norway spruce forest, the sampling by

PGH pointed out how the organic material tends to accumulate on soil surface as organic horizon likely due to the chemical recalcitrance to microbial attack of coniferous plant residues (Cha et al., 2019; Díaz-Pinés et al., 2011). To this regard, in the literature is reported a higher C:N ratio of Norway spruce litter layer compared to that of beech and chestnut forests (Cremer et al., 2016; Sariyildiz and Anderson, 2005) indicating a lower degradability of conifers compared to broadleaves (Massaccesi et al., 2020) and explaining the highest storage of organic C in the 0–30 cm of soil depth under Norway spruce, as observed also in a study conducted in similar ecozone by Vittori Antisari et al. (2015).

The lower C stock in the Oe, A and AB horizons under chestnut forest compared to Norway spruce and European beech forests could be also attributed to climatic factors (Figure 6b). In fact, compared to the chestnut forest, Norway spruce and European beech forests were located at higher altitude where a lower mean air temperature limited the microbial activity and promote SOC storage (De Feudis et al., 2019; Massaccesi et al., 2020). Although in chestnut forest we observed the lowest C stock (Figure 7c, d), PGH approach allowed to observe a similar thickness of A and AB horizons (Figure 4). This fact would indicate that chestnut forest promoted organic carbon deepening (De Feudis et al., 2020b; Gautam et al., 2017), independently from soil organic matter turnover, likely due to the high appetibility of chestnut residues for soil fauna and microbes (Anderson, 1973). The deepening of organic matter in soil can be considered a positive aspect because it could prevent soil degradation due to the presence of stable SOC forms in depth (e.g., Kramer et al., 2017; Soucémarianadin et al., 2018).

Compared to Norway spruce forest, located at similar altitude, beech forest had a lower C stock in 0–30 cm soil depth (Figure 7c, d), although in A and AB horizons the concentration of SOC was higher in beech forest than in Norway spruce soils (Figure 6a). The lower soil C stock under beech has been mainly attributed to the high amount of skeleton found between 10 and 30 cm depth (see Table S1 of the Supplementary Materials) which lowered the C stock (Rytter, 2012).

Sampling by PGH allowed us to detect that a large part of the soil organic pool of Norway spruce forest had a substantial proportion of weakly stabilised components, as observed by the soil horizonation. In fact, the presence of a Oa horizons, rich in SOC scarcely associated to mineral particles, made the spruce ecosystem more susceptible than broadleaves forest to SOC reduction following natural or anthropic disturbances (climatic change, wildfires, forest cutting and land use change) and, hence, soil resilience decline (Bonetti et al., 2017).

4.2. Comparison between sampling methods

In the three forest types, the lack of differences observed for C stock in 0–30 soil depth between the sampling by PGH and by FDL (Figure 7c, d) demonstrated the reliability of both methodologies for SOC estimation. Our findings are in contrast to those observed by Francaviglia et al. (2017) in vineyards and by Parras-Alcántara et al. (2015) in Mediterranean natural areas. Both studies reported a higher C stock estimation through FDL sampling than through PGH sampling. The authors attributed the higher C stock by FDL to the mixing of horizons through such sampling approach which affects BD and gravel content. In this sense, because of the pivotal role of the amount of skeleton and BD on C stock estimation, in the present study the relative volume occupied by the skeleton was considered and the BD was calculated by PTF built–up through data of soils located in the same study area. It is noteworthy that to neglect the relative volume occupied by stones and gravels in C stock calculation cause the overestimation of the amount of organic C stored in soil (Rytter, 2012; Wang et al., 2018), although some authors reported skeleton itself contain organic C (Agnelli et al., 2002, 2008) and should be considered for C stock estimation (Corti et al., 2002). However, since one of the aims of the present study was to compare two soil sampling approaches for C stock estimation, and skeleton was found only under beech forest within the 0–30 soil depth, we evaluated the relative volume occupied by skeleton but not its organic C content.

The data on SOC storage, calculated by both PGH and FDL sampling, suggested the key combined effect of the organic residues' quality (Sariyildiz, 2003; 2008) and climate (Massaccesi et al., 2020;

Kumar et al., 2021) on C stock. The sampling by PGH pointed out that under Norway spruce forest the organic material tends to accumulate on soil surface as organic horizon. Such results are in according to previous studies (e.g., Labaz et al., 2014; Kuznetsova et al., 2019) which found a greater organic horizon thickness under coniferous than under broadleaved forests. The findings obtained through the sampling by FDL would indicate a positive effect of conifers on SOC storage (Figure 7c, d), but the "reading" of the soil horizonation, as occurred with PGH sampling, allowed to assess that SOC under spruce forest was greatly stored in the organic horizons (Oe and Oa), where it accounted for about one third of the 0-30 cm C stock (Figure 6b), because of the recalcitrant nature of the spruce litter. The noticeable storage of organic matter in the organic horizons rather than in the mineral ones, as occurred for beech and chestnut forests, makes the spruce forest susceptible to loose relevant amount of SOC in case of wildfires (Poirier et al., 2014), which have increased dramatically in frequency and extent in the European Mediterranean region (Oliveira et al., 2018). Further, the poorly degradable litter together with the siliceous substrate of our study sites might promote the formation of Mor humipedon (Zanella et al., 2018) which is more vulnerable to erosion compared to Mull, Moder and Amphi humipedon types (Pintaldi et al., 2018). Hence, in a view of SOC monitoring, our findings demonstrated that the sampling by PGH draws a better picture of SOC distribution along depth and its potential susceptibility to external factors leading to degradation.

Besides the large information about soil processes that the sampling by PGH can provide (Jiang et al., 2021), through this approach also the information about the spatial variability within each study site is preserved. In fact, we noted higher values of CV when soil horizons were considered compared to fixed depth intervals and this difference was marked for the 0–15 cm soil depth interval (Table 1). The loss of information on spatial variability would indicate the low reliability of FDL sampling to support decision–making plans addressed for sustainable use and preservation actions of soil and forest resource (Denton et al., 2017; Kirchen et al., 2017). Indeed, accurate information about soil variability is important in environmental modelling and prediction (Zeraatpisheh et al., 2019; Rosemary et al., 2017; Taghizadeh-Mehrjardi et al., 2016).

5. Conclusion

The overall data of the present study suggested that both the soil sampling by PGH and by FDL could be considered reliable for C stock examination in forest ecosystems. However, it is necessary to specify that carefulness should be paid for soil BD and skeleton amount when C stock is calculated. Our findings highlighted that, although both sampling approaches resulted appropriate to evaluate the effects of biotic and abiotic factors (e.g., vegetation and climate, respectively) on C stock, the sampling by PGH provides information about SOC processes. In fact, through the identification and characterization of soil horizons it is possible to predict how and how much the considered biotic and abiotic factors influence C stock and pools. The present study showed that the evaluation of soil properties (i.e., C stock) through soil depth layers provided a lower information about spatial variability inside the selected areas compared to the same evaluation through soil horizon sampling. Our results drawn a clear picture where sampling by PGH is appreciable in order to gain knowledge useful both for scientific purposes and a sustainable land use management of natural ecosystems.

Conflict of interest

The authors declare no conflict of interest.

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Table 1. Coefficient of variation to describe the spatial variability of C stock calculated both for each identified soil horizon (Oe, Oa, A, AB and Bw) and soil depth layer (0–15 and 15–30 cm) of the plots selected inside chestnut, Norway spruce and European beech forests.

Forest type	Oe	Oa	A	AB	Bw	0–15	15–30
				%			
Chestnut	72.8		36.2	49.2	19.2	17.7	16.5
Norway spruce	65.3	50.3	60.4	49.8	26.0	25.7	16.9
European Beech	35.5		27.7	46.1	35.1	21.0	19.5

Figure captions

Figure 1. Map of the study area showing the sampling locations (Sweet chestnut, Norway spruce and European beech forests).

Figure 2. Collection of samples from 0–30 cm of soil depth for soil profile and for the 8 additional sampling points by horizon (on upper left) and fixed 0–15 and 15–30 cm (on upper right), and pictures of the representative soil profiles (on the bottom) dug under Sweet chestnut, Norway spruce and European beech forests.

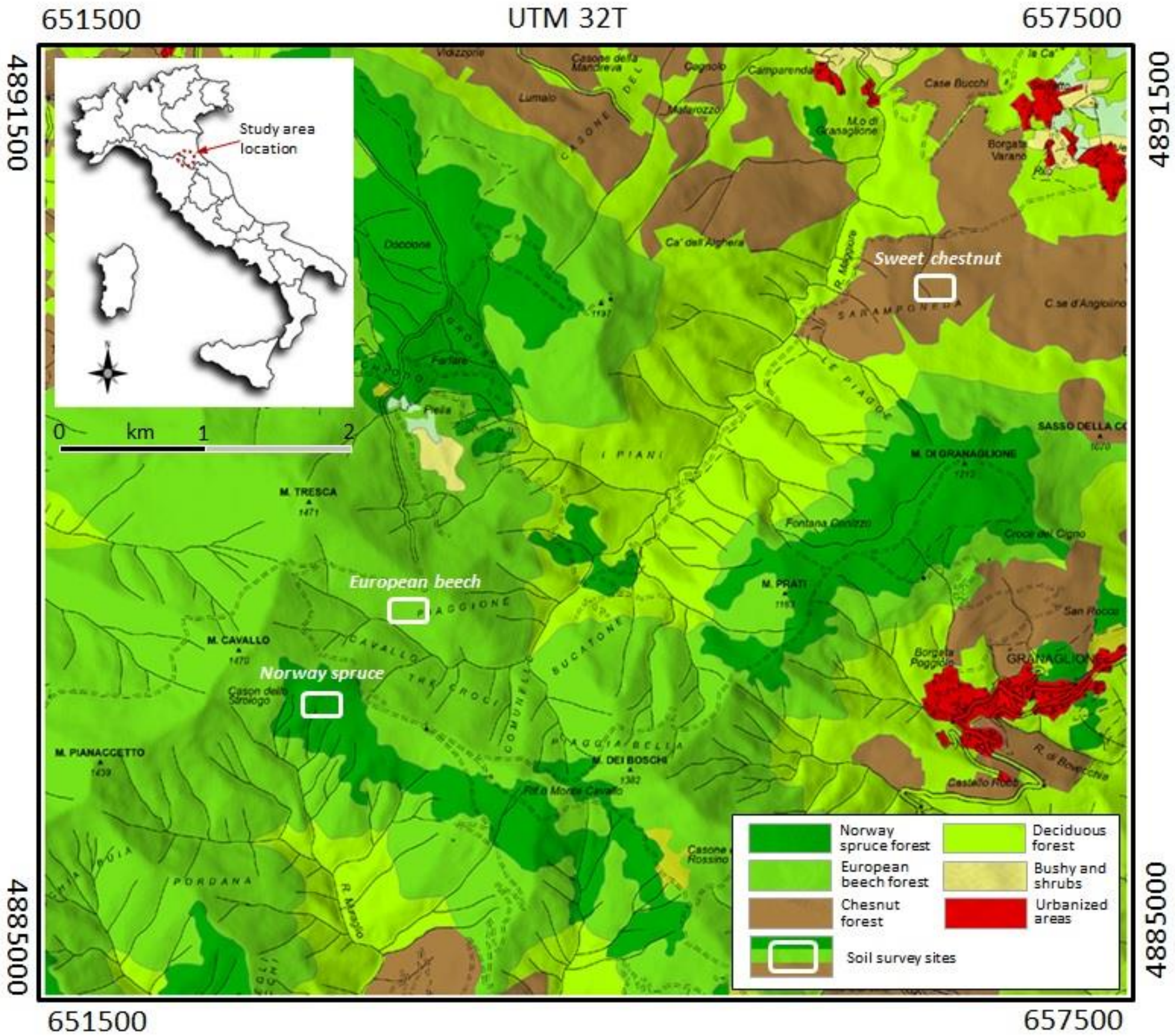
Figure 3. Sampling design scheme carried out in each study site (left side) and the engaged mini–sampler (right side). On left, the full circle indicates the representative soil pit, the empty circles are sampling points where the mini samplers were engaged.

Figure 4. Mean thickness of soil horizons identified in 0–30 cm depth interval under chestnut (dark grey bars), Norway spruce (white bars) and European beech (light grey bars) forests. Error bars indicate standard errors (n=9). Different letters, where included, indicate significant differences among forest types for each soil horizon (One-way ANOVA and Tukey HSD test, $p \leq 0.05$).

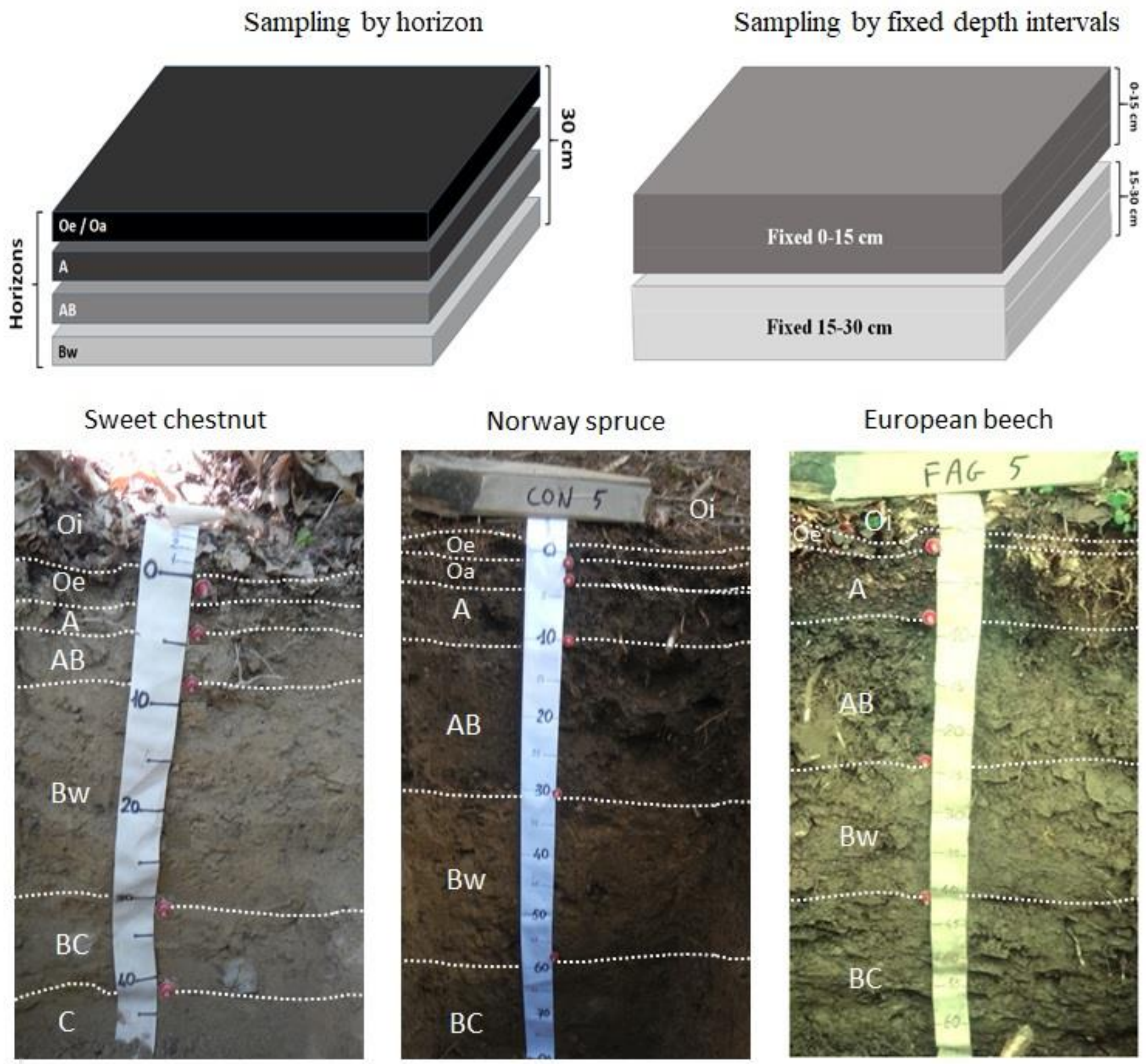
Figure 5. Sand (a) silt (b) and clay (c) contents in mineral horizons identified in 0–30 cm soil depth interval under chestnut (dark grey bars), Norway spruce (white bars) and European beech (light grey bars) forests. Error bars indicate standard errors (n=9). Different letters, where included, indicate significant differences among forest types for each soil horizon (One-way ANOVA and Tukey HSD test, $p \leq 0.05$).

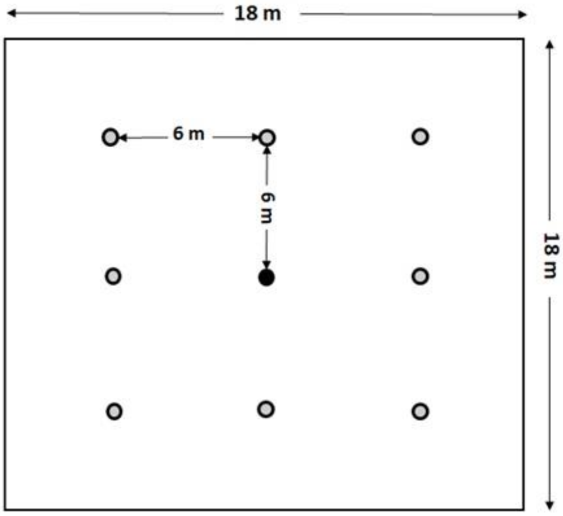
Figure 6. (a) Soil organic carbon (SOC) content and (b) C stock in soil horizons identified in 0–30 cm depth interval under chestnut (dark grey bars), Norway spruce (white bars) and European beech (light grey bars) forests. Error bars indicate standard errors (n=9). Different letters, where included, indicate significant differences among forest types for each soil horizon (One-way ANOVA and Tukey HSD test, $p \leq 0.05$).

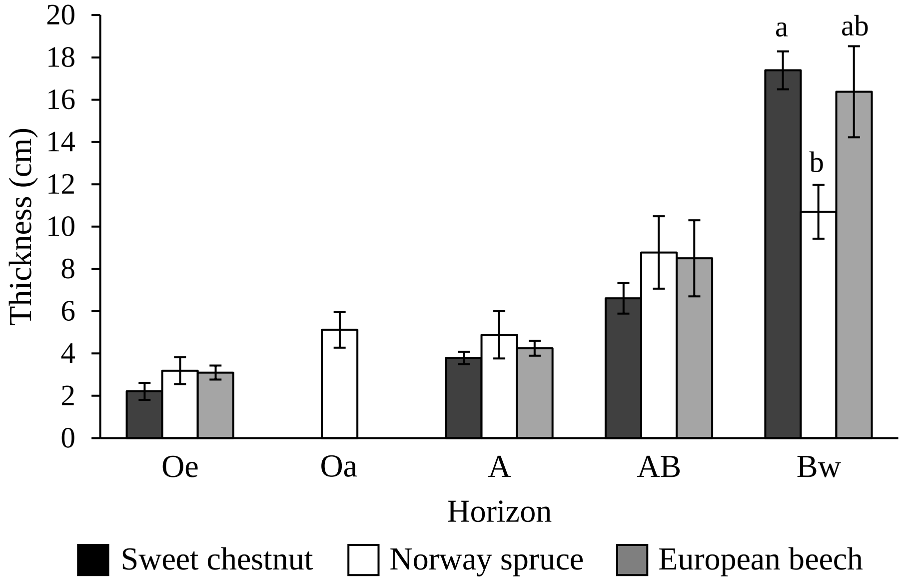
Figure 7. Soil organic carbon (SOC) content in (a) 0–15 and (b) 15–30 cm soil depth layers, and C stock in (c) 0–15 and (d) 15–30 cm soil depth layers, under chestnut, Norway spruce (Spruce) and European beech (Beech) forests obtained through fixed depth layers (grey bars) and soil horizons (white bars) sampling approaches. Error bars indicate standard errors (n=9). Different letters, where included, indicate significant differences among the means (Two-way ANOVA and Tukey HSD test, $p \leq 0.05$). p values of forest type × sampling approach: 0.5829, 0.4810, 0.7279 and 0.6897 for barplot a, b, c and d, respectively.

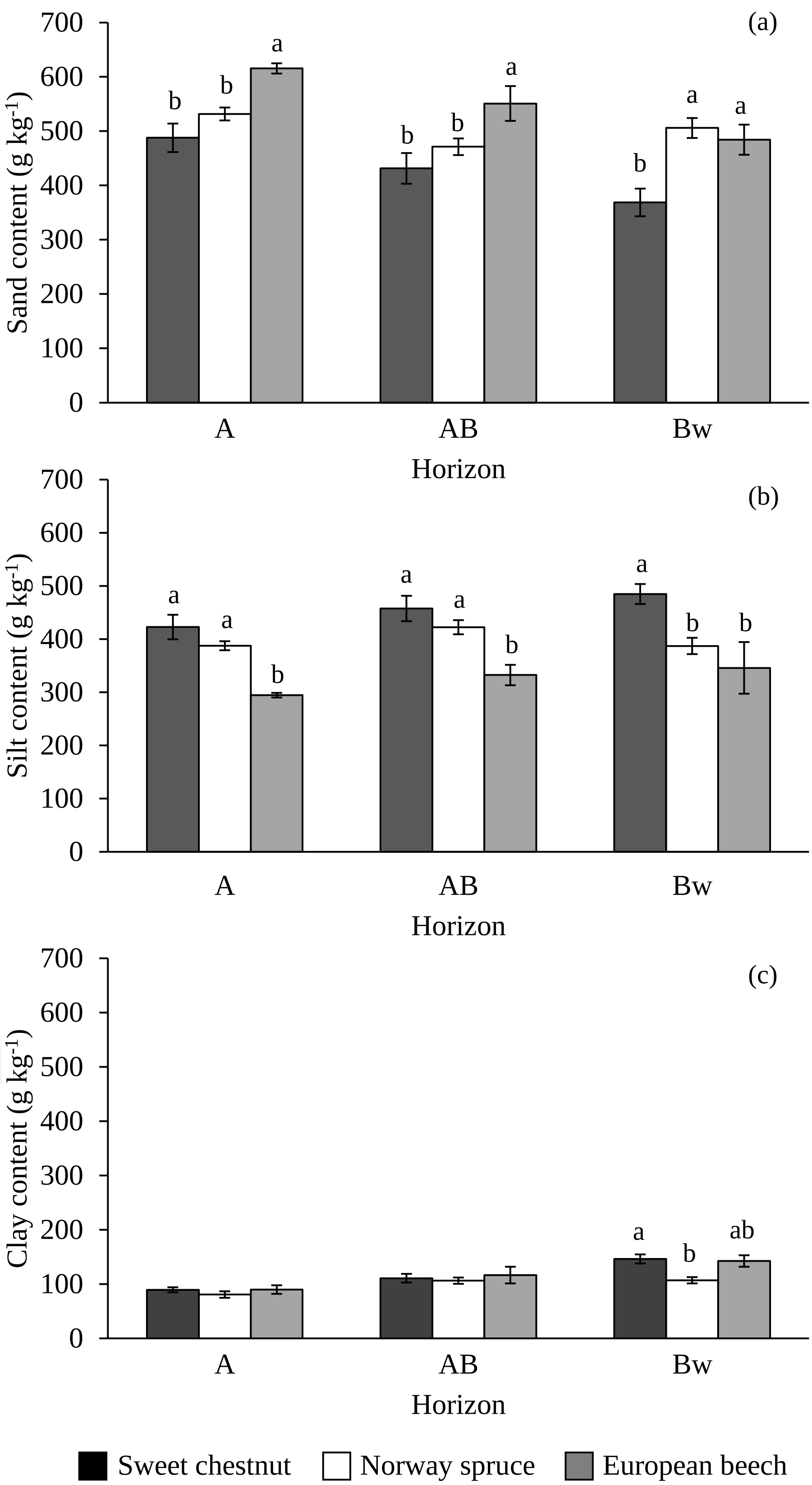


651500
UTM 32T
657500
4891500
4885000
Study area location
0 km 1 2
Sweet chestnut
European beech
Norway spruce
M. TRESCA
M. CAVALLO
M. PIANACCETTO
M. DI GRANAGLIONE
M. PRATI
M. DEI BOSCHI
GRANAGLIONE
SASSO DELLA CROCE
Norway spruce forest
European beech forest
Chesnut forest
Deciduous forest
Bushy and shrubs
Urbanized areas
Soil survey sites

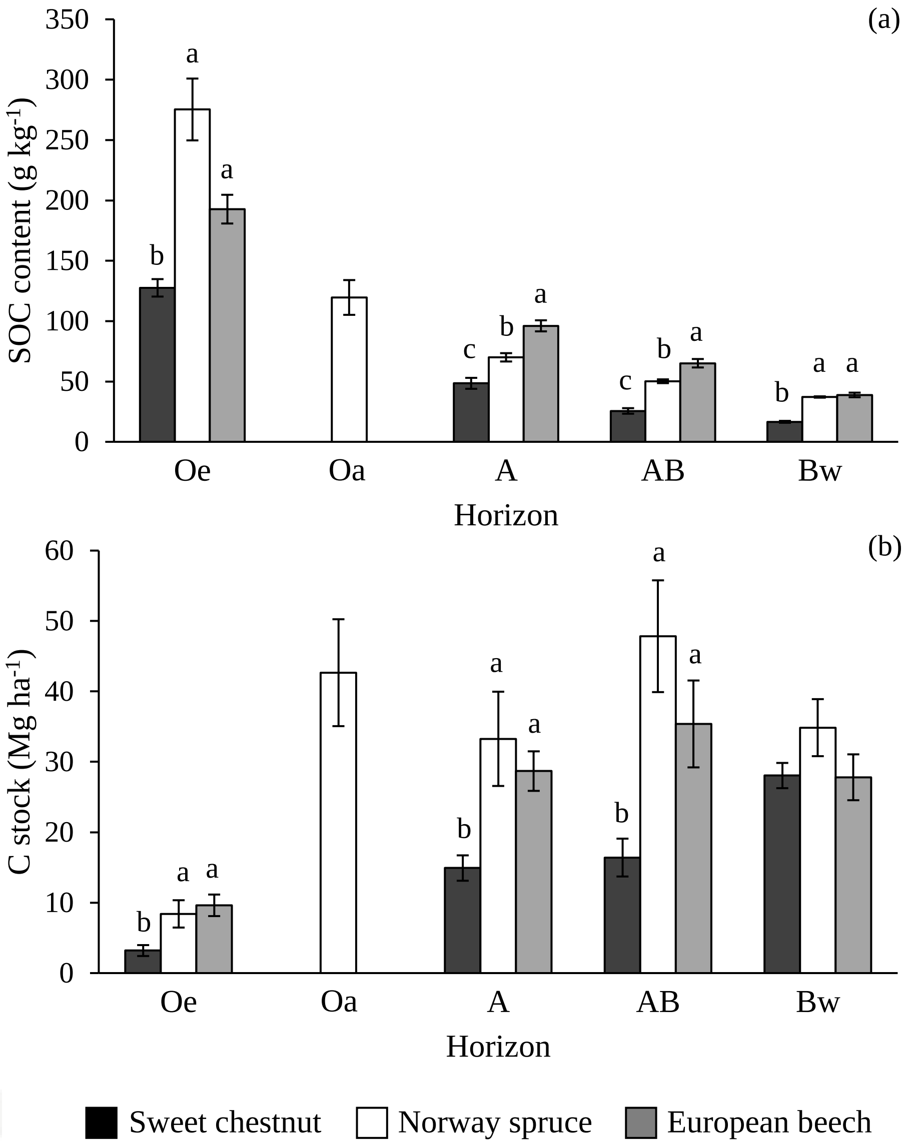

Sampling by horizon
Sampling by fixed depth intervals
30 cm
Horizons
Oe / Oa
A
AB
Bw
0-15 cm
15-30 cm
Fixed 0-15 cm
Fixed 15-30 cm
Sweet chestnut
Norway spruce
European beech
Oi
Oe
A
AB
Bw
BC
C
CON 5
Oi
Oe
Oa
A
AB
Bw
BC
FAG 5
Oi
Oe
A
AB
Bw
BC

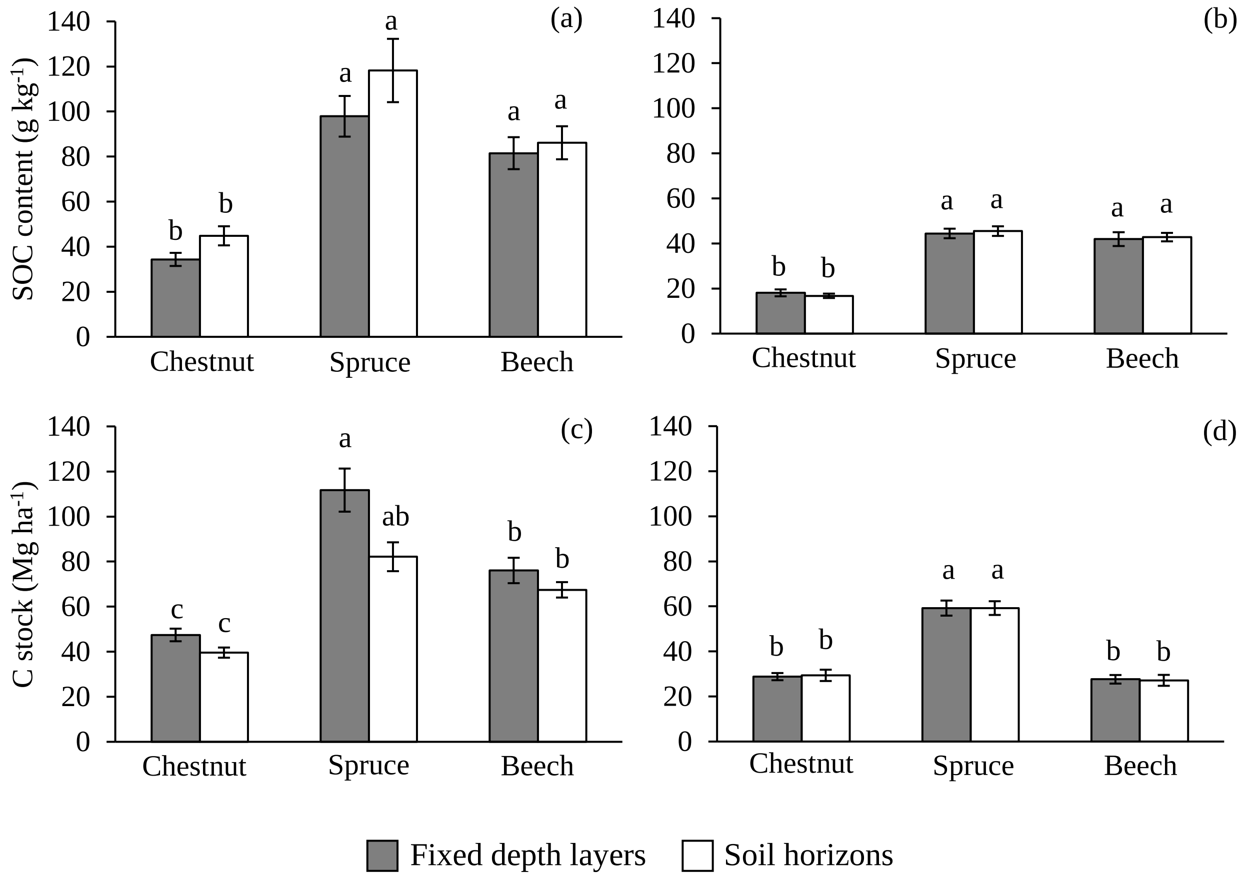

18 m
6 m
6 m
18 m


Thickness (cm)
20
18
16
14
12
10
8
6
4
2
0
a
ab
b
Oe
Oa
A
AB
Bw
Horizon
Sweet chestnut
Norway spruce
European beech



(a)
Sand content (g kg-1)
0
100
200
300
400
500
600
700
b
b
a
b
b
a
b
a
a
A
AB
Bw
Horizon
(b)
Silt content (g kg-1)
a
a
b
a
a
b
a
b
b
A
AB
Bw
Horizon
(c)
Clay content (g kg-1)
a
b
ab
A
AB
Bw
Horizon
Sweet chestnut
Norway spruce
European beech


(a)
SOC content (g kg-1)
350
300
250
200
150
100
50
0
Oe
Oa
A
AB
Bw
Horizon
(b)
C stock (Mg ha-1)
60
50
40
30
20
10
0
Oe
Oa
A
AB
Bw
Horizon
Sweet chestnut
Norway spruce
European beech


(a)
(b)
(c)
(d)
SOC content (g kg-1)
C stock (Mg ha-1)
Chestnut
Spruce
Beech
Fixed depth layers
Soil horizons