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RESEARCH ARTICLE

Tree species affect the vertical distribution of soil organic carbon and total nitrogen

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Abstract

Background: Forest soils are considered sinks for atmospheric C. Many studies revealed that tree species and their mycorrhizal association affect forest floor and topsoil organic C (OC) and total N, while the knowledge of their effect on subsoil OC and N is still scarce.

Aims: We aimed to identify (1) tree species and mycorrhizal association effects on forest floor, topsoil (0–30 cm) and subsoil (30–80 cm) OC and N stocks and vertical distribution and (2) drivers for soil OC and N distribution.

Methods: We sampled forest floor, topsoil and subsoil under *Fagus sylvatica* L., *Quercus robur* L., *Acer pseudoplatanus* L. and *Fraxinus excelsior* L. in four Danish common garden experiments along a gradient in soil texture and determined OC and N stocks.

Results: Total N (forest floor + soil) was higher under oak than beech, while total OC was unaffected by species. Forest floor C and N were higher under oak and beech, both ectomycorrhizal species (ECM), compared to under maple and ash, which are both arbuscular mycorrhizal species (AM). Relatively more OC and N were transferred to the topsoil under AM than ECM species, and this could be explained by greater endogeic earthworm biomass in AM species. In contrast, a higher proportion of OC was stored under ECM than AM species in the subsoil, and here OC correlated negatively with anecic earthworms. Subsoil N was highest under oak.

Conclusions: Tree species and in particular their mycorrhizal association affected the vertical distribution of soil OC and N. Tree species differences in topsoil OC and N were not mirrored in the subsoil, and this highlights the need to address the subsoil in future studies on AM- versus ECM-mediated soil OC and N stocks.

KEYWORDS
broadleaved tree species, earthworms, forest floor, mycorrhizal association, soil pH, temperate forest

1 | INTRODUCTION

Temperate forests (vegetation plus soil) currently act as a carbon sink of 0.7 Pg C year^{-1} (Pan et al., 2011) and contribute to mitigating the atmospheric CO_2 increase due to anthropogenic CO_2 emissions. Changes in soil OC are driven by inputs from vegetation versus outputs from, for example heterotrophic respiration. In addition, changing characteristics of the N cycle, such as the input of N or whether the trees prefer NH_4^+ and/or NO_3^- as a nutrient source, indirectly affect the net ecosystem exchange (Schulze, 2000), for example by altering the tree biomass production. It remains uncertain how management practices and tree species selection affect this functioning as a C sink, especially in the subsoil (Lorenz et al., 2022). A better understanding of such effects under current climatic conditions is a prerequisite for reliable predictions of this C sink under a changing climate (IPCC, 2019; Nabuurs et al., 2007). Here, we focus on tree species effects on the vertical distribution of soil OC and N stocks under current climatic conditions.

Tree species affect forest floor OC and N stocks (Fleck et al., 2019; Langenbruch et al., 2012; Peng et al., 2020; Steffens et al., 2022; Vesterdal et al., 2013). These stocks have been found to be higher under coniferous than broadleaved tree species. Among the broadleaved tree species, for example maple and ash (both associated with arbuscular mycorrhizal fungi: AM) have a thinner forest floor than for example beech and oak (both ectomycorrhizal: ECM). However, tree species effects on topsoil OC and N stocks are less clear. Approximately 60% of the studies that compared topsoil OC stocks under several tree species observed tree species effects on topsoil OC stocks, while the other 40% did not (Boca et al., 2014; Langenbruch et al., 2012; Mayer et al., 2020; Peng et al., 2020; Vesterdal et al., 2013). The ranking of tree species with increasing topsoil OC and N stocks tended to be opposite to the ranking for the forest floor (Fleck et al., 2019; Peng et al., 2020; Rodeghiero et al., 2018; Steffens et al., 2022; Vesterdal et al., 2013). This pattern indicates a trade-off between forest floor and mineral soil OC and N stocks, which again may imply that tree species affect the persistence of the stored OC and N stocks (Prescott & Vesterdal, 2021; Vesterdal et al., 2013).

Tree species effects on forest floor and topsoil OC and N stocks were related to aboveground litter quantity and quality, and to belowground C inputs, for example, by root exudations or root mortality (Feng et al., 2022; Langenbruch et al., 2012; Rasse et al., 2005; Vesterdal et al., 2013). Litter high in lignin is more resistant to decomposition, forming a thicker forest floor (Berg, 2000). In addition, the litter decay rate is slower under tree species with a high lignin:N (Melillo et al., 1982) and the topsoil OC stocks were smaller under tree species with a high leaf litter lignin:N ratio (Mueller et al., 2015).

Epigeic earthworms live and feed in the forest floor, and their abundance is directly linked to litter quality on the one hand and to environmental conditions (such as moisture) on the other hand (Curry & Schmidt, 2007; Schelfhout et al., 2017). In return, the feeding behaviour of the epigeics directly affects litter breakdown in the forest floor. Previous studies indicated higher topsoil acidification under tree species that form a thick forest floor (De Schrijver et al., 2012; Langenbruch et al., 2012), and as opposed to epigeic earthworms, the burrowing earthworms (endogeic and anecic) are very sensitive to soil acidification. As a consequence, their abundance and biomass differ among tree species (Schelfhout et al., 2017). Burrowing earthworms are effective in bioturbation and, hence, promote the transport of C and N from the forest floor into the soil. While the endogeic species (soil-dwellers) live and feed within the topsoil, the anecic earthworms (deep-burrowers) feed on leaf litter in the forest floor and pull down freshly fallen litter deep into the soil within their large vertical burrows (Curry & Schmidt, 2007).

The mycorrhizal association of tree species has been reported to affect topsoil and subsoil OC and N stocks (Peng et al., 2020; Wu et al., 2022) as well as the C and N cycling within the soil (Craig et al., 2018; Eagar et al., 2022; Phillips et al., 2013). Moreover, the mycorrhizal association strongly affects the microbial community within the soil, for example the fungal diversity and the saprotrophic fungal abundance and diversity were greater in soils of AM-associated trees than in soils of ECM-associated trees (Eagar et al., 2022). However, total fungal biomass, fungal growth and the fungi:bacteria ratio were higher under ECM than AM tree species (Heděnec et al., 2020).

In the subsoil, tree species and their mixture affect OC via root biomass and turnover (Dawud et al., 2016) or via higher accumulation of microbial residues in AM- than ECM-dominated soils (Craig et al., 2018). Carbon concentrations in subsoils are rather low, suggesting a potential for sequestration given the right conditions (Cotrufo & Lavallee, 2022; Hassink et al., 1997; Mayer et al., 2020; Six et al., 2002). Furthermore, Rumpel and Kögel-Knabner (2011) reported that subsoil OC was more stabilised as compared to topsoil OC. Despite the large importance of subsoils as a C reservoir, it remains unclear whether tree species effects could be extrapolated from the topsoil, and which processes are responsible for differences in subsoil OC and N stocks (Lorenz et al., 2022).

The objective of the present study was to identify tree species and/or mycorrhizal type-related differences in forest soil OC and N stocks, and the vertical distribution of these stocks from forest floor via the mineral topsoil to the mineral subsoil until main rooting depth (80 cm depth). We investigated general patterns across several common garden sites along a wide gradient in clay content. We hypothesised that tree species affect forest soil OC and N stocks and their vertical distribution through differences in (1) litter quality, (2) mycorrhizal association and (3) earthworm abundance and functional group. Specifically, we expected that the effect of soil-dwelling endogeic earthworms on OC and N stocks would be mostly limited to the topsoil, while the effect of the deep-burrowing anecic species on OC and N stocks would be significant in the subsoil. We expected that topsoil and subsoil processes are connected by the bioturbation of the anecic earthworms. Therefore, we hypothesised that tree species effects on topsoil OC and N stocks will be mirrored in the subsoil, but to a decreasing extent with increasing soil depth.

2 | MATERIALS AND METHODS

2.1 | Common garden experiment with four tree species

This study was conducted in a common garden experiment replicated at four sites across Denmark (Table 1). Climate and soil conditions varied between the sites, as well as the previous land-use: arable land (Kragelund and Mattrup) and beech forest for at least 200 years (Viemose and Wedellsborg). Each site contained monoculture stands of the four tree species under study that were planted in adjacent plots of each about 0.25 ha in 1973, except for Kragelund, where stands were smaller and trees were planted in 1961. The studied tree species were European beech (*Fagus sylvatica* L.), pedunculate

TABLE 1 Climatic conditions, management and soil properties at the six Danish common garden experimental sites (Vesterdal et al., 2008; Danish Meteorological Institute [precipitation], Horizon boundaries from Callesen [2003]; N deposition average from 2005 to 2015 [J. L. Bak, personal communication], based on regional modelling of N deposition [Ellermann et al., 2018] adjusted for local agricultural emissions according to Bak et al. [2018])

Site	Location	Mean prec. 2004–2015 (mm year^{-1})	Ann. temp.(°C)	*N* deposition in open land (kg ha^{-1} year^{-1})	Soil type (WRB), parent material	Lower horizon boundaries (cm)	Land use history	Soil texture	BS (%) of parent material	Management
Viemose	55°01′N, 12°09′E	633	8.1	8.3	Luvisol over glacial till	A: 11; E: 43; B: 101	Beech forest, afforestation in 1973	Loamy sand, ~14% clay	84	Thinning every 4th year since 1987, last thinned in 2015
Wedellsborg	55°24′N, 9°52′E	761	7.8	11.7	Luvic Phaeozem over glacial till	A: 35; AE: 56; B: 93	Beech forest, afforestation in 1973	Sandy loam, ~22% clay	100	Thinning every 4th year since 1987, last thinned in 2015
Mattrup	55°57′, 9°38′E	825	7.5	16.0	Luvisol over glacial till	A: 24; EA: 34; B: 115	Afforestation of arable land in 1973	Loamy sand, ~16% clay	76	Thinning every 4th year since 1987, last thinned in 2015
Kragelund	56°10′N, 9°25′E	825	7.5	13.7	Arenic Alisol from Aeolian sand over glacial till	A: 40; B: 89	Afforestation of arable land in 1961	Sand, ~4% clay	18	Last thinned in 2015

Abbreviation: BS, base saturation.

TABLE 2 Information on mycorrhizal association (ECM: ectomycorrhizal, AM: arbuscular mycorrhiza), foliar litter chemistry, earthworm biomass and topsoil pH measured in $CaCl_2$ of the four tree species under study

Species	Mycorrhizal association	Foliar litter chemistry[a] C:N	Lignin:N	Ca (mg g^{-1})	Earthworm biomass[b] in forest floor plus mineral topsoil (g m^{-2}) Endogeics	Anecics	Epigeics	Soil pH 0–5 cm	5–15 cm
Beech	ECM	37.3^{a} (2.8)	25.3^{a} (4.0)	11.4^{a} (0.8)	6.8 (6.5)	5.5 (2.7)	2.6 (2.0)	3.6 (0.1)	3.7 (0.1)
Oak	ECM	31.4^{bc} (1.3)	17.1^{b} (1.3)	10.4^{a} (0.7)	5.2 (1.9)	4.2 (1.8)	9.5 (4.0)	3.9 (0.1)	3.8 (0.1)
Ash	AM	25.1^{d} (2.3)	10.1^{c} (1.6)	20.7^{b} (2.5)	6.9 (4.9)	44.4 (28.7)	6.7 (5.9)	4.4 (0.3)	4.2 (0.1)
Maple	AM	27.1^{cd} (1.1)	10.4^{c} (1.0)	19.3^{b} (1.7)	13.2 (6.0)	23.2 (8.2)	8.4 (3.6)	4.0 (0.2)	4.0 (0.2)

Note: Displayed are means ($n = 6$ for litter chemistry, and $n = 4$ for earthworms) with standard error of the mean in brackets. Different superscript lowercase letters indicate significant differences between the species ($n = 4$, $p < 0.05$).

[a] Vesterdal et al. (2008, 2012).

[b] Schelfhout et al. (2017).

oak (*Quercus robur* L.), sycamore maple (*Acer pseudoplatanus* L.) and European ash (*Fraxinus excelsior* L.). The tree species differed in their foliar litter chemistry, mycorrhizal association, earthworm community and topsoil pH (Table 2; Harley & Harley, 1987; Schelfhout et al., 2017; Vesterdal et al., 2008, 2012). The stands were thinned approximately every 4 years. See Vesterdal et al. (2008) for a full description of the study design.

2.2 | Field sampling

The forest floor was sampled just before the start of the dormant season in September 2016 by collecting 10 samples with a cylinder (diameter: 20 cm) evenly distributed across each species plot. The samples were then pooled to one composite sample per plot. Humus forms were characterised according to Zanella et al. (2011).

The mineral soil was sampled from November 2015 until March 2016 with a split-tube auger designed for undisturbed soil sampling (diameter: 5 cm). We took 15 topsoil (0–5, 5–15, and 15–30 cm) cores and five subsoil (30–50 and 50–80 cm) cores evenly distributed across each plot. The 15 and five samples, respectively, from each depth increment were pooled to one composite sample per plot. Here, we present total stocks for topsoil (0–30 cm) and subsoil (OC: 30–80 cm; N: 30–50 cm). In the depth increment 50–80 cm, the N concentrations were below detection limit in the majority of samples. Therefore, we present subsoil N stocks and C:N ratios only to a depth of 50 cm.

2.3 | Sample preparation for analyses

The forest floor samples were air-dried at room temperature until constant weight. Grasses, herbs and mosses were removed. Subsequently, the samples were divided into foliar (i.e., leaf litter material) and non-foliar (i.e., fruits, twigs, branches) compartments and weighed separately. After well mixing the sample, a subsample of the foliar forest floor was ground—depending on the volume of the sample either with the disk vibration mill (Scheibenschwingmühle-TS, Siebtechnik, Mülheim an der Ruhr, Germany) or with the mixer mill (MM 400, Retsch, Haan, Germany)—to fine material. The non-foliar part of the forest floor was very heterogeneous, and, hence, the complete sample was ground.

The fresh mineral topsoil samples were weighed and subsequently sieved to 2 mm. A subset was used for deriving the moisture content and the remaining part was air-dried at room temperature until constant weight. The subsoil samples were air-dried until constant weight and subsequently sieved to 2 mm. Coarse material (gravel and stones) was weighed. A subsample of each sieved soil sample was ground to fine material in the disk vibration mill (Scheibenschwingmühle-TS). All ground forest floor and mineral soil samples were subsequently dried at 105°C for 24 h. Bulk density of the fine mineral soil (<2 mm) of the individual depth increments was derived similarly to the method in Vesterdal et al. (2008).

2.4 | Chemical analyses

All forest floor and mineral soil samples were analysed by dry combustion (Elementar VarioMax analyser, Hanau, Germany) to derive C and N contents. We assumed all measured C to be organic down to 50 cm depth, because pH measured in $CaCl_2$ was below 5.5. In 50–80 cm depths, the pH value was above 5.5 at Viemose and Wedellsborg. A quick test with 10% H_2SO_4 indicated a small concentration of $CaCO_3$ in four of these samples (Viemose: oak, Wedellsborg: oak, maple, beech). These four samples had organic matter removed by alkaline H_2O_2 treatment after Falster et al. (2018). Subsequently, the samples were dried and 20 mg weighted into 12-mL glass vials. To these soil samples, 3 mL of H_3PO_4 (75%, 1:1 diluted) was added. A blank vial (no soil, just acid) was treated accordingly. The vials were sealed and kept overnight at room temperature. The produced CO_2 in the vials was measured at the GC Agilent 7890A (Santa Clara, Ca, USA). It was corrected by the CO_2 measured in the blank vial and considered as the inorganic C in the soil samples (all below 1%) and subtracted from the total C measured at the Variomax in order to derive the OC of these four samples.

2.5 | Stock calculations and statistical analyses

Forest floor OC and N stocks were calculated by multiplying the forest floor mass per area with the respective OC and N concentration. Mineral soil OC (0–30 and 30–80 cm) and N (0–30 and 30–50 cm) stocks were calculated by multiplying the depth (cm) with the fine soil density (g cm^{-3}).

To detect possible differences in (1) OC and N concentrations and stocks, (2) C:N ratio and (3) depth distribution of OC and N between the four tree species and two mycorrhizal associations across sites, we used Analysis of Variance (Anova) with 'site' as random factor followed by Tukey's test ($p < 0.05$). In case residuals were not (almost) normally distributed (Shapiro–Wilk test, $p < 0.05$) and/or variances were not homogenous (Levene, $p < 0.05$), data were transformed using the natural logarithm, the square root or the reciprocal (Tables S1 and S2). If no transformation of the data was possible, a Kruskal–Wallis rank sum test followed by pairwise comparison using the Wilcoxon rank sum test was conducted instead. These statistics were conducted by R 3.6.0 using the packages '*lme4*' Version 1.1–21 (Bates et al., 2015), '*lmer*Test' Version 3.1-1 (Kuznetsova et al., 2017), '*car*' Version 3.0-6 (John Fox, 2019), '*multcompView*' Version 0.1-8 (Graves et al., 2019) and '*lsmeans*' Version 2.30-0 (Lenth, 2016). We report mean and standard error for observed variables if not noted otherwise.

Pearson correlations were conducted to check whether OC, N and C:N were correlated with the litter lignin:N ratio and/or the biomass of the three earthworm groups (epigeics, endogeics, anecics; Table 2) using IBM SPSS Statistics 27. Displayed correlations were significant at $p < 0.05$.

3 | RESULTS

3.1 | Stocks of OC, N and C:N ratio

Mean total OC stocks ranged from 73 to 106 Mg ha^{-1} irrespective of tree species (Figure 1A). Total N stocks were highest under oak and lowest under beech (Figure 1B).

Forest floor OC stocks were higher under beech and oak (both ECM) than under ash and maple (both AM), while topsoil OC stocks were unaffected by species or mycorrhizal association. Subsoil OC stocks under ECM species were by trend higher than under AM species (Figure 1A).

Forest floor N stocks were highest under beech and lowest under ash, while higher topsoil N stocks were found under AM than ECM species. Subsoil N stocks were highest under oak and lowest under maple and beech (Figure 1B).

Forest floor C:N ratio tended ($p < 0.1$) to be wider under AM than ECM species (Figure 2). Topsoil (0–30 cm) and subsoil (30–50 cm) C:N ratio did not differ among species or mycorrhizal association. It averaged 11.3 in the topsoil and 9.7 in the subsoil. However, in the top 5 cm of mineral soil (Table S2), the C:N ratio was wider under ECM (12.9 ± 0.5) than under AM species (11.6 ± 0.4), and on a species level, it increased in the following order: ash (11.4 ± 0.8) = maple (11.8 ± 0.6) < oak (12.5 ± 0.7) < beech (13.4 ± 0.7).

3.2 | Vertical distribution of OC and N

In the forest floor, a higher proportion of OC and N was stored under ECM (OC: 5.4% ± 0.7%; N: 2.6% ± 0.4%) than under AM species (OC: 2.0% ± 0.5%; N: 1.0% ± 0.4%), while in the mineral topsoil it was vice versa (ECM < AM). Under ECM species, 58.1% ± 1.4% of OC and 68.3% ± 1.3% of N and under AM species, 64.5% ± 2.4% of OC and 73.6% ± 1.7% of N were stored in the topsoil. In the subsoil, it was reversed again (Figure 1C,D): A higher proportion of OC and N was stored in the subsoil under ECM (OC: 36.5% ± 1.3%; N: 29.1% ± 1.3%) than AM species (OC: 33.4% ± 2.1%; N: 25.5% ± 1.5%).

At tree species level, a higher proportion of N was stored in the forest floor under beech than under maple and ash (Figure 1D). In the topsoil, highest proportion of OC and N were found under maple and lowest under oak. The proportion of OC and N stored in the subsoil did not differ significantly among tree species (Figure 1C,D).

Total OC stocks were positively correlated with topsoil OC stocks ($r = 0.85$), and total N stocks were strongly positively correlated with topsoil N stocks ($r = 0.98$) and with subsoil N stocks ($r = 0.75$). Topsoil and subsoil OC stocks were uncorrelated (Figure 3A), while topsoil and subsoil N stocks were positively correlated across sites (Figure 3B).

3.3 | Correlations of OC, N and C:N ratio with foliar litter lignin:N ratio and earthworm biomass

Total N stocks were positively correlated with the biomass of endogeic earthworms ($r = 0.61$), while total OC stocks did not correlate with any of the tested parameters.

Forest floor OC and N stocks were strongly positively associated to litter lignin:N ratio (OC: $r = 0.81$, N: $r = 0.76$). The anecic earthworms were negatively correlated with forest floor OC (Figure 4A) and N stocks ($r = 0.57$), while epigeic earthworms were uncorrelated with forest floor OC and N stocks. Topsoil OC (Figure 4C) and N ($r = 0.64$) stocks correlated positively with the biomass of endogeic earthworms. In the subsoil, the OC stocks were negatively associated with the biomass of anecic earthworms (Figure 4E), while no significant correlation between earthworms and subsoil N stocks could be detected.

Forest floor C:N ratio was positively associated with the biomass of anecic earthworms (Figure 4B). Top- and subsoil C:N ratio correlated negatively with the biomass of endogeic earthworms (Figure 4D,F).

4 | DISCUSSIONS

4.1 | Forest floor

Forest floor OC and N stocks were higher under beech and oak than under ash and maple (Figure 1). This was in line with several published

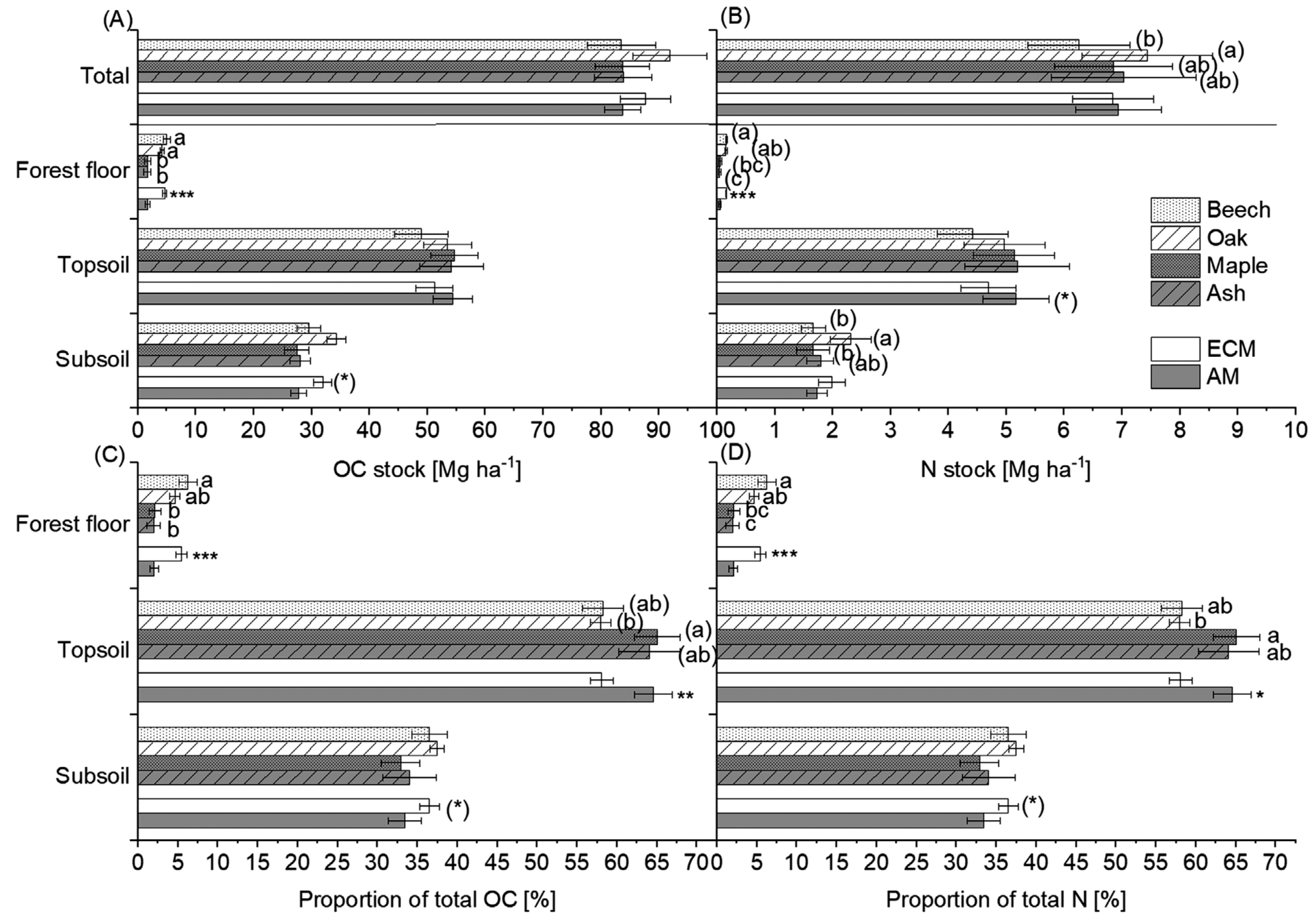

FIGURE 1 Stocks of organic carbon (OC) and total nitrogen (N) and its relative contribution to total OC and N stocks in the different soil layers under four species and two mycorrhizal groups, respectively, displayed as means ($n = 4$ in the case of tree species, $n = 8$ in the case of mycorrhizal group) with standard error. Topsoil: 0–30 cm; subsoil: 30–80 cm (OC) and 30–50 cm (N). Total: sum of forest floor, topsoil and subsoil OC and N stock. ECM: species associated with ectomycorrhiza; AM: species associated with arbuscular mycorrhiza. Different lowercase letters indicate significant differences between species at $p < 0.05$ in the respective layer and if in parentheses, they indicate trends at $p < 0.1$. Asterisks indicate higher values in this mycorrhizal group compared to the others at different levels of significance: (*)$p < 0.1$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

findings (Langenbruch et al., 2012; Vesterdal et al., 2013; Vesterdal et al., 2008). It is related to the more recalcitrant litter of the ECM versus the AM species (Mayer et al., 2020; Phillips et al., 2013) and with this a higher turnover time (Keller & Phillips, 2019). Non-foliar forest floor had a significantly higher C:N ratio (≈ 45) compared to foliar forest floor (≈ 25) irrespective of tree species (Table S2). The slightly lower C:N ratio ($p < 0.1$) in total forest floor under ECM than AM species (Figure 2) can therefore mainly be attributed to the higher proportion of foliar forest floor to total forest floor under ECM (mean: ≈ 72%) than AM (mean: ≈ 51%) species. We expect this to be related to the longer turnover time of foliar material in ECM forest floor as previously reported from the common garden experiments (Vesterdal et al., 2008). As opposed to the foliar forest floor material in ECM species, foliar material in AM forest floors will most likely preferably be transferred to mineral soil by bioturbation, where it will be further processed (Curry & Schmidt, 2007; De Wandeler et al., 2016; Schelfhout et al., 2017). We did find support for this mechanism in connection with anecic earthworms. This group of earthworms is known to feed on the forest floor and to pull down the freshly fallen leaves into the soil within large vertical burrows (Curry & Schmidt, 2007), and their biomass indeed had a negative impact on forest floor OC and N. Epigeic earthworms live and feed mainly in the forest floor (Curry & Schmidt, 2007; Schelfhout et al., 2017), but did not show any correlation with forest floor OC and N stocks. Epigeic and anecic earthworms are both positively affected by litter quality (De Wandeler et al., 2016), and anecic earthworms may have limited further growth and activity of the epigeic population due to competition for the same food source.

4.2 | Mineral soil

The transition from topsoil to subsoil at 30 cm soil depth roughly matched the thickness of the organic matter-rich A horizons at most sites (except Viemose) (Callesen, 2003). The different thicknesses of the A horizons could have contributed to site-specific differences in topsoil and subsoil C and N stocks. These site effects were taken into account in the statistical analyses. For comparability, we chose to calculate C and N stocks for the same depths' increments in all sites and

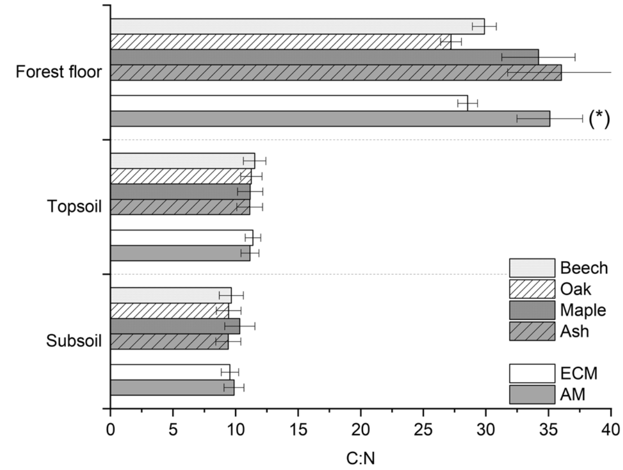

FIGURE 2 Ratio of organic carbon and total nitrogen (C:N) in the different soil layers under four species and two mycorrhizal groups, respectively, displayed as means ($n = 4$ in the case of tree species, $n = 8$ in the case of mycorrhizal group) with standard error. Topsoil: 0–30 cm; subsoil: 30–50 cm. ECM: species associated with ectomycorrhiza; AM: species associated with arbuscular mycorrhiza. The asterisk in parentheses indicates a by trend wider C:N ratio in the respective mycorrhizal group compared to the other at $p < 0.1$.

not for genetic horizons. Before checking the tree species effects on C and N stocks in the increments 0–30 and 30–80 cm, we calculated tree species effects (Table S2) and correlations for smaller soil increments (0–5, 5–15, 15–30, 30–50, and 50–80 cm). These analyses indicated that the choice of 30 cm for the boundary at all sites was suitable to show the differences in tree species effects between topsoil and subsoil.

4.2.1 | Organic C stocks

The tree species did not affect stocks of OC in the topsoil, but the proportion of the total OC that was stored in the mineral topsoil was higher for AM than ECM species, that is opposite to tree species ranking from lowest to highest forest floor OC stock (Figure 1). This supports the trade-off in OC distribution suggested in reviews (Mayer et al., 2020; Prescott & Vesterdal, 2021; Vesterdal et al., 2013), that is tree species forming a thicker forest floor tend to have lower topsoil OC stocks and vice versa. Our results indicate that anecic earthworm species ensured a transport of litter-C towards the topsoil (Don et al., 2008; Huang et al., 2020; Schelfhout et al., 2017), while the endogeic soil-feeding species, which had higher mean values under AM than ECM species, protected this organic matter from microbial decomposition by forming aggregates in the soil (Angst et al., 2019; Knowles et al., 2016). Marhan and Scheu (2006) published indications that earthworms can reduce microbial mineralisation of fresh organic matter in C-unsaturated soils. At our sites, the trees had been planted slightly >40 years prior to sampling, and we expect that the sites had not yet reached steady state in respect to soil OC (Mayer et al., 2020).

Subsoil OC stocks were uncorrelated to topsoil OC stocks across sites (Figure 3), indicating differences between topsoil and subsoil processes affecting OC stocks. In contrast to the topsoil, subsoil OC stocks were higher under ECM than AM tree species (Figure 1). The negative correlation of anecic earthworm biomass with subsoil OC stocks (Figure 4) leads us to hypothesise that (1) anecic earthworms transport leaf litter into the subsoil, which would likely increase OC stocks within a few millimetres around the worm holes (Don et al., 2008) and (2) the mineralisation of subsoil OC by soil microbes would be stimulated by the availability of easily decomposable AM leaf litter (Fontaine et al., 2007; Hoang et al., 2017) and by the increased aeration of the subsoil via anecic burrows. In fact, several studies found a negative influence of earthworms on mineral soil OC stocks (Lubbers et al., 2013), which could be attributed to a positive influence of earthworms on microbial community, biomass and activity (Groffman et al., 2015; Hoang et al., 2017; Hoeffner et al., 2018), especially in the subsoil (Hoang et al., 2017).

In addition to the effect of earthworms on mineral soil OC stocks, differences in root C inputs, root vertical and horizontal distribution and root turnover might have contributed to differences in topsoil and subsoil OC stocks between tree species and/or mycorrhizal association (Keller et al., 2021; Rasse et al., 2005). For example, Keller et al. (2021) found significantly higher root-derived C inputs into the top 15 cm of the mineral soil under AM than ECM tree species. This might additionally have contributed to the higher proportion of OC in the topsoil under AM than ECM species. However, it does not explain the higher subsoil OC stocks under ECM species. It is very likely that a multitude of processes related to roots, aboveground litter quality, earthworms and microorganisms interact with each other, thus complicating interpretations. Future research is necessary to disentangle the individual and combined effects.

Whole profile OC stocks to 80 cm depth were in the mean range of earlier published values for similar European soils (De Vos et al., 2015) and they were not affected by tree species after >40 years (Figure 1). The OC stocks in the entire soil profile across sites could be fairly well estimated from topsoil OC stocks. However, because tree species affected the vertical distribution of OC within the soil (Figure 1), tree species ranking from lowest to highest topsoil OC stocks cannot be extrapolated to the subsoil (Figure 3A). Therefore, the subsoil needs to be sampled alongside, when the aim is to identify tree species specific OC stocks in the rooted soi.

4.2.2 | Total N stocks

The higher topsoil N stocks under AM than ECM species (Figure 1) were in line with previous findings (Langenbruch et al., 2012; Oostra et al., 2006; Vesterdal et al., 2008), which could be related to the higher amount of N returned to the soil with the litterfall under AM species. High-quality AM leaf litter and root exudates are rapidly mineralised. This results in a fast transformation of N from organic forms to inorganic forms, which is the dominant source for AM plants. In contrast, the slower decomposition of the litter from ECM trees leads to

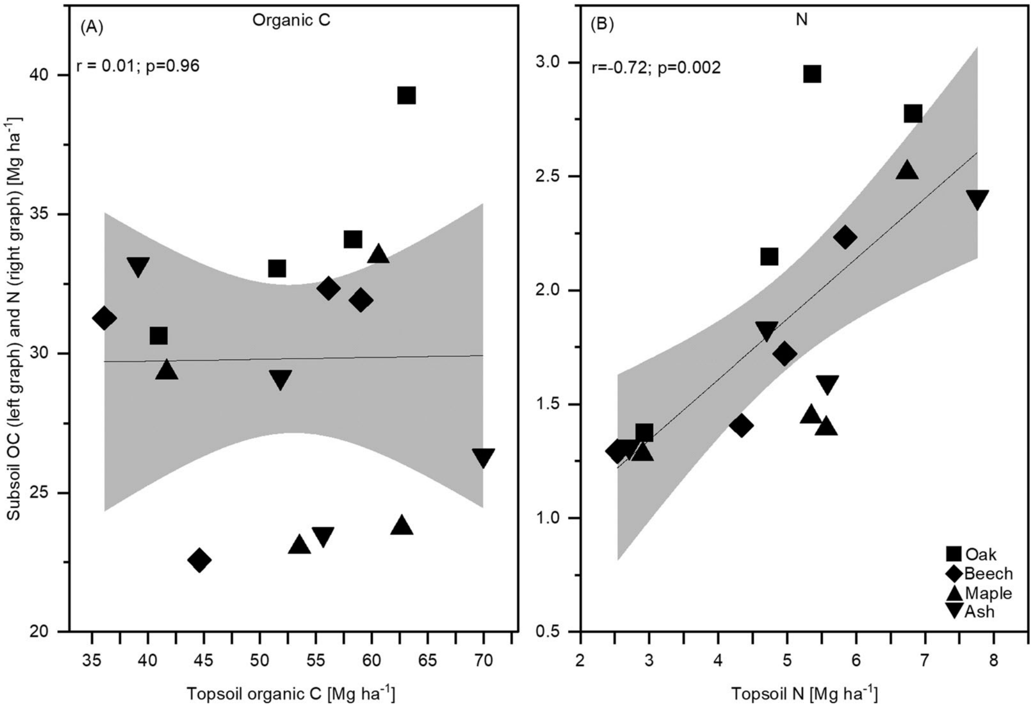

FIGURE 3 Pearson correlation between (A) topsoil (0–30 cm) and subsoil (30–80 cm) organic C stocks and between (B) topsoil (0–30 cm) and subsoil (30–50 cm) N stocks at the individual species plots. Stocks are presented in Mg ha^{-1}.

a greater accumulation of soil organic matter (especially in the forest floor) and with this more N remains in organic forms, where it can be taken up by the ECM hyphae (Phillips et al., 2013).

The positive correlation of endogeic earthworms with topsoil N supports earlier findings that earthworm mixing of soil and litter stimulated a sink for N in microbial biomass and into more permanent soil organic matter (Cotrufo et al., 2013; Groffman et al., 2015; Xue et al., 2022).

Tree species differently affected subsoil N stocks than topsoil N stocks. Subsoil N stocks were highest under oak (an ECM species) and lowest under beech (also an ECM species) and maple (an AM species; Figure 1). The mycorrhizal type may not be the main factor in control of subsoil N. The differences in subsoil N stocks in our study (Figure 1) might be related to a combination of (1) enhanced microbial activity due to earthworm activity in the subsoil (Xue et al., 2022), which was higher under AM than ECM species, (2) the effect of earthworm activity on soil N cycling and plant N acquisition (Blume-Werry et al., 2020; van Groenigen et al., 2014) that was driven by leaf litter quality (Yang et al., 2015), (3) the different N economies of AM versus ECM species (Phillips et al., 2013), (4) different root N concentrations among species (Hobbie et al., 2010; Kubisch et al., 2015) and root order (Kubisch et al., 2015; Li et al., 2010) and (5) tree species-related differences in vertical distribution of fine root biomass (pers. comm. Arndal, 2016). The results from the present study do not enable us to disentangle these possible influencing factors, and we suggest that more targeted research on the mechanisms in control of subsoil N stocks is needed. As for OC, our results clearly show that tree species effects on topsoil N stocks cannot be extrapolated to the subsoil.

Whole profile N stocks (Figure 1) fell within the range of earlier published values for German forest soils (Fleck et al., 2019). Highest subsoil N stocks under oak and lowest subsoil N stocks under beech were the main contributors to the tree species ranking from lowest to highest whole profile N stocks. The AM species showed intermediate whole profile N stocks (0–50 cm), because the higher N stocks under AM species in the topsoil and the lower N stocks under AM species in the subsoil cancelled each other out. Our results indicated that across sites whole profile N stocks could be estimated from known topsoil N stocks. However, to accurately assess tree species effects on N stocks in the entire soil profile, it is necessary to sample the subsoil.

4.2.3 | C:N ratio

At our study, tree species did not affect topsoil (0–30 cm) or subsoil (30–50 cm) C:N ratio significantly (Figure 2). Former studies found that tree species effects on soil C:N ratio decrease with increasing soil depths (Cools et al., 2014; Heděnec et al., 2020; Langenbruch et al., 2012; Vesterdal et al., 2008) and were absent in the subsoil (Cools et al., 2014). Accordingly, in the very top 5 cm of mineral soil, we found that C:N ratio was smallest under AM tree species and highest under beech (Table S2). A narrower C:N ratio under the same soil pre-conditions indicates a higher proportion of microbially processed and/or mineral-associated organic matter, while a wider C:N ratio indicates a higher proportion of particulate or fresh organic matter (Song et al., 2014). In support of this, Heděnec et al. (2020) found higher microbial biomass under AM than ECM tree species. Furthermore, the fungi:bacteria ratio

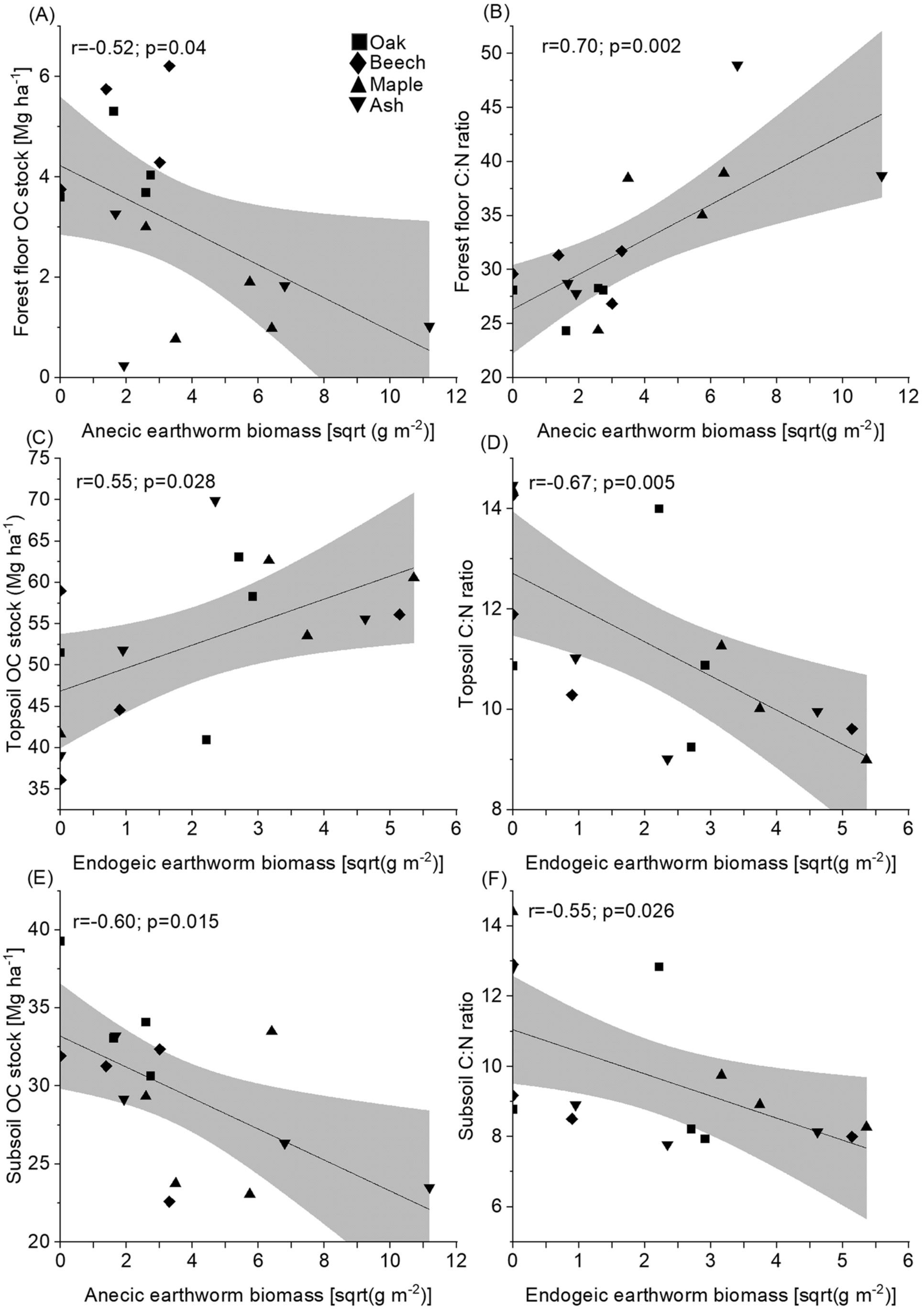

FIGURE 4 Nonlinear relationship of earthworm biomass to organic C and C:N ratio in the different soil layers for endogeics or anecics, respectively. The red line indicates the linear regression line and the red shade the 95% confidence interval. The earthworm biomass is presented as square-root transformed.

positively correlated to soil C:N ratio, which is in line with a generally lower fungi:bacteria ratio under AM species (lower C:N ratio) than ECM species (wider C:N ratio) (Heděnec et al., 2020). Furthermore, some bacterial groups were found to profit from the presence of endogeic earthworms (Medina-Sauza et al., 2019), which were also more abundant in AM tree species. The negative correlation of the C:N ratio in the subsoil with endogeic earthworm biomass (Figure 4) seemed to be mainly driven by site-related differences in subsoil N status.

5 | CONCLUSIONS

Mycorrhizal association strongly affected the vertical distribution of OC and N within the profile, that is in the forest floor, the mineral topsoil and the mineral subsoil. Different processes probably controlled OC and N stocks and their vertical distribution among the different layers as mediated by the different mycorrhizal associations. This was supported by a missing correlation between topsoil and subsoil OC stocks. Under ECM species, higher forest floor and subsoil OC and N stocks were observed than under AM species, while in the mineral topsoil it was vice versa. Burrowing earthworms were more abundant under AM than ECM species. Endogeic earthworms correlated positively with topsoil OC and N stocks, because they incorporate fresh organic substrates and support the formation of aggregates in which the soil OC and N may be protected against microbial breakdown.

We suggest that the lower subsoil OC stocks in AM species with high abundance of anecic earthworms may be attributed to stimulated microbial mineralisation of subsoil OC due to a (1) better aeration of the subsoil through the earthworm burrows and (2) the downward transport of high-quality litter, that is easily decomposable organic matter and nutrients. We recommend testing this hypothesis in future studies in order to improve the understanding of the underlying mechanisms affecting subsoil OC stocks.

Whole profile N stocks were slightly higher under oak than under beech. However, contrary to our hypothesis, tree species and mycorrhizal association did not affect whole profile OC stocks from forest floor to 80 cm depth of mineral soil. Our results indicate that at site level, topsoil OC and N stocks allowed a fair estimate of total stocks. However, if the goal is to quantify tree species and/or mycorrhizal association effects on total OC and N stocks, the entire root zone must be considered, because the parameters and processes affecting topsoil OC and N stocks cannot be extrapolated to the subsoil.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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