Tree Species Identity Drives Soil Carbon and Nitrogen Stocks in Nutrient-Poor Sites

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Abstract

The establishment of mixed forest stands can be seen as an option to improve soil organic carbon and N storages and to protect forest ecosystems from various impacts of climate change. We analyzed groups of pure mature European beech (*Fagus sylvatica*), Douglas fir (*Pseudotsuga menziesii*) and Norway spruce (*Picea abies*) stands as well as mixtures of beech with either Douglas fir or spruce along Northern Germany. To understand the potential effects of species mixtures on carbon and nitrogen storage, we examined the content and stocks of organic carbon and nitrogen in the O-horizon (organic layer) and mineral soil. The C stock of the organic layer was largely depending on tree species, whereas the C stock of the mineral soil varied among soil types. Total soil organic C (SOCt) stocks (Organic horizon + 0-30 cm soil depth) showed significant species identities and mixing effects were most obvious due to the high contribution of the SOC stocks in the organic layer (SOCo) to total SOCt. These contribution was site-dependent. Overall, under sandy soil conditions, conifers and mixed forests allocated 10% more SOC and N at the organic layer compared to loamy soils, whereas the SOC and N stocks under beech maintained the same proportion, independent of the site condition.

The interaction between species and sites was significant only for Douglas Fir and mixed Douglas Fir/beech stands, indicating that the effect of species on carbon and nitrogen varied across sites, being significantly high at sandy soils.

In summary, the higher potential for carbon storage in mixed-species forests compared to pure stands, along with the shift of carbon into more stable pools (such as from the Ol layer to the Oh layer), emphasizes the capacity of mixed forest to provide valuable ecosystem services while reducing the risk of unintended carbon losses.

Keywords: mixed forests; broadleaves; conifers; *Fagus sylvatica; Pseudotsuga menziesii; Picea abies;* SOC.

Introduction

Forest soils are complex ecosystems and their management, especially through the selection of trees with distinct species identities, may have a major impact on soil organic carbon (SOC) and nitrogen (N) dynamics and sequestration potentials (Jandl et al., 2007; Vesterdal et al., 2008).

Typical driving forces of such tree species identities include the type of foliage, the quality of leaf litter, indicated e.g. by the C/N ratio, or the type of rooting characteristic in forest soils. Thus tree species identity may have an important impact on the ecosystem level such as soil C stock and C/N ratios, particularly in the O-horizon and top mineral soil layers (Dawud et al., 2017; Vesterdal et al., 2013). Furthermore, the species diversity drives the fluxes of carbon and nitrogen in soils (Cepáková et al., 2016; Mueller et al., 2012; Vesterdal et al., 2002) and their role on SOC sequestration are been highlighted to improve the ecosystem functions and to cope with climate change scenarios.

Different patterns of C storage in soil profile and organic horizons has been reported for conifers and deciduous trees on temperate ecosystems (Bolte and Villanueva, 2006) . However, it is highly uncertain which processes are responsible for differences in soil C stocks in organic horizon and mineral soil. Differences in litter input (Díaz-Pinés et al., 2011), root distribution (Oulehle et al., 2007) as well as decomposition rates (Cools et al., 2014) have been suggested as the most likely explanation for tree species influence on soil C stocks. Often, Douglas fir (*Pseudotsuga menziesii menziesii*) and European beech (*Fagus sylvatica*) shows high fine root density in deeper soil layers (Bolte and Villanueva, 2006; Oulehle et al., 2007), increasing C allocation in mineral soil layers. In contrast, the native conifer Norway spruce (*Picea abies*), known as shallow-rooted tree species (Bolte and Villanueva, 2006) accumulate C in the upper mineral soil layers.

Compositionally and structurally diverse forests represent an important element of approaches to deliver a wide range of ecosystem goods and services (Ammer, et al., 2020). Moreover, the establishment and management of mixed stands is also discussed as an effective measure to adapt forests stands to climate change (Berg and McClaugherty, 2020; Frouz et al., 2009) and C sequestration in soil. Admixtures of beech in conifer forests (either with Douglas fir or Norway spruce) has been reported to modify C and N input through the fine root distribution in the soil profile (Vesterdal and Raulund-Rasmussen, 1998).

Additionally, the enhanced nutrient concentrations of beech litter increase litter decomposition of conifers needles (mixed stands)(Krishna and Mohan, 2017; Neumann et al., 2018; Vesterdal et al., 2008), followed by less C accumulation in the organic soil layers and shift to stable C pools in mineral soil. However, still inconsistent information on how mixed stands of beech with Douglas would affect forest soil C and N vertical distribution. Due the differences in litter input (Díaz-Pinés et al., 2011) as well as decomposition rates (Cools et al., 2014) its most likely that this mixture enhance the organic C stocks at organic layers as well. Thus, the deep root system reported to Douglas fir and beech and the high SOC on organic layer might suggest that the admixture of beech into Douglas fir stands potentially results in higher total C in the whole soil profile.

Therefore, the main objective of our study was to analyze the accumulation, vertical distribution as well as litter quality indices (C/N ratios) of the upper mineral C and N stocks of different pure and mixed stand types (pure European beech, pure Norway spruce, pure Douglas-fir, mixed European beech/Norway spruce and mixed European beech/Douglas-fir) along Northern Germany. We hypothesized that i) the admixture of beech to Douglas fir forests will increase C stocks at the mineral soil compared to respectively beech monocultures, ii) the OC stocks at litter layer will decrease under the admixture of beech to Douglas fir and, shift to stables C pools (H-layer and mineral soil), compared to respectively monocultures, and iii) on rather nutrient poor sites (sandy soil), species-identity effects on organic layer will be stronger pronunced than on rich soils (loamy soil).

Material and Methods

Study sites

We investigated seven sites in Lower Saxony, northern Germany. Each site contained quintet neighboring forest stands. Three of these stands were monospecific stands of European beech (*Fagus sylvatica*), Norway spruce (*Picea abies*), or Douglas fir (*Pseudotsuga menziesii*). The other two were mixed stands, one composed of spruce/beech and one composed of Douglas fir/beech.

Essential criteria for the selection of the sites were the presence of the respective quintets in a similar advanced silvicultural development status, altogether, we employed a multi-criteria approach, considering factors such as climate, soil type (based on German soil inventory), stand age, and existing research on land-use, as well as a relevant single tree admixture in the mixed stands.

However, on the basis of the available forest site mapping and also confirmed by Foltran et al. (2023), two groups with relatively homogeneous site conditions were distinguished in our data set, in particular via the texture data (Table 1), but also addressing the geological substrate classes, the correspondently developed soil types as well as the given range of water and nutrient levels (Foltran et al., 2023).

In the first region in southern Lower Saxony, we chose three sites (Dassel, Winnefeld, and Nienover). These three sites are located in the hills of the Solling plateau and lie between 300 and 450 m.a.s.l. They share similar climatic conditions, i.e. a mean annual air temperature of 7.2°C and mean annual precipitation of about 1040 mm/year. The age ranges between 45 and 90 years and the texture is relatively rich (23% of clay; Table 1). The soils at these locations are Dystric Cambisols (FAO, 2015) developed from Triassic sandstone material.

In the second region, located in the northern Lower Saxony, we selected four sites (Unterlüß, Nienburg, and Göhrde I and II). These sites are in lowland Lower Saxony and lie between 80 to 150 m.a.s.l. and all have similar climatic conditions, i.e., a mean annual air temperature of 8.4°C, mean annual precipitation of 720 mm/year. The age ranges between 53 and 130 years. Most frequent soil types are therefore Podzols (Table 1) and their transitional forms towards Cambisols.

With respect to nutrients and according to the official Lower Saxony forest site mapping and Foltran et al. (2023), the southern sites (loamy soils) are nutrient-rich, and the northern sites (sandy soils) are nutrient-poor.

Table 1. Soil classification from each plot is given following (FAO, 2014) and the German classification (BGR) (Düwel et al., 2007). Soil parent material was identified matching the plot coordinates with the German National inventory database (LBEG). Soil texture (10-30 cm*) was measured by the integral suspension pressure method (ISP) and determined by PARIO (Foltran et al., 2023).

Region	Sites	Soil (FAO-WRB, 2014)	German Classification (BGR)	Parent material (LBEG)	Clay %	Silt %	Sand %
Solling Region (SR)	Dassel	Dystric Cambisols from quartzitic sandstones and conglomerates with low base status	Podsolige Braunerde aus basenarmen quarzitischen Sandsteinen und Konglomeraten	Medium colored sandstone / sandstone, siltstone, claystone	21	53	26
	Winnefeld				23	57	20
	Nienover				23	57	20
Northern Region (NR)	Nienburg	Haplic Podzols / Dystric Regosols from dry dystrophic sand deposits	Eisenhumus-Podsol / Podsol-Regosol aus trockenen, nährstoffarmen Sanden	Drenthe stage of the Saale glaciation / sand, gravel /melt water deposits	7	13	80
	Unterlüß				6	15	79
	Göhrde II				6	15	79
	Göhrde I	Spodic Arenosols from dry dystrophic sand deposits	Podsol-Braunerde aus trockenen, nährstoffarmen Sanden	Warthe stage of the Saale glaciation / silt / clayey, sandy, gritty / basic moraine	3	24	73

WRB: Working Group World Reference Base
BGR:Bundesanstalt für Geowissenschaften und Rohstoffe
LBEG: Landesamt für Bergbau, Energie und Geologie

Soil sampling

In each forest stand type (50 m x 50 m) at all sites, 4 randomly selected points were chosen as representative sampling points. The selected points were oriented at stand-level, e.g., we standardized two meters minimal distance from the trees to avoid coarse roots. At each sampling plot, the forest floor was collected using a steel frame and sorted by identifiable foliar (O_L – Litter), non-foliar (O_F – decay layer), and non-identifiable and humified (O_H – Humus) layers of the organic layer. Mineral soil was sampled using a core auger (*d*=8 cm) and, separated at 0-5, 5-10, and 10-30 cm soil depth. Bulk soil density from each depth was calculated using soil metal rings (250 cm³) to further stock analysis.
Partly missing bulk density data due to frozen soil conditions, interfering tree roots or stones during sampling were estimated by Adams equation (ADAMS, 1973) adapted by CHEN et al. (2017). The approach uses SOM and pH as bulk density predictors.

Sample preparation and analysis

All mineral soil samples were oven-dried at 40°C until constant weight and sieved through 2 mm mesh, subsamples from the fine soil fractions (<2 mm diameter) were ground with a Retsch mortar grinder RM 200 (Retsch, Germany) for 10 min. The organic layer samples were dried at 60°C until constant weight, weighted and ball milled (MM2, Fa Retsch) for further analysis.

All the samples were analyzed for total C and N by dry combustion in a Leco CSN 2000 analyzer. There was no inorganic C ($CaCO_3$) within 30 cm depth in soils and all measured C were consequently considered to be organic.
The soil pH was measured in all mineral soil samples and are described in detail in the Foltran et al. (2023).

Calculations of Stocks

We estimated the soil bulk density from the oven-dried and moisture corrected (105 ◦C) fine soil mass and its volume. The fine soil volume was estimated from the difference between the volume of the soil corer and the volume of stones and roots. Carbon and N stocks in each layer were estimated from the organic horizon mass (O-layers) and soil bulk density (mineral soil), concentrations of C and N and depth of the individual soil depths for each sampling point.

Statistical Analyses

To address the non-independent nature of multiple horizons within one soil profile for this subset of data, we chose linear mixed effect models (Rasmussen et al., 2018). To estimate the effect of forest stand and site condition (loamy vs sandy), we fitted linear mixed models (LMMs) to log-transformed response variables (C and N stocks) and then applied planned contrasts (Piovia-Scott et al., 2019). All LMMs included forest stands (European beech, Douglas fir, Douglas fir/beech, Norway spruce, spruce/beech), site conditions (loamy and sandy sites), and soil depths (O_L, O_F, O_H [organic layers] and 0–5, 5–10 and 10-30 cm [mineral soil]) as fixed effects. Models were stepwise selected by likelihood ratio test, and minimal models included all main effects and the interaction of forest type and region.
The mean values for each site are given in the supplementary material (Table S3 and S4). There, the effect of the forest stand (pure or mixed forest) on the soil chemical parameters for individual depth was assessed by least significant difference test. As the assumption of normal distribution was not met (tested for with Shapiro Wilk test), we used the Kruskal Wallis test, followed by pairwise Mann-Whitney tests with a correction factor for multiple

pairwise testing, to identify statistically significant differences among forest stand and site conditions by individual depth.

All analyses were done in R 4.0.3 (R Core Team, 2020). We used the 'nlme' package to fit LMMs and the 'emmeans' package for planned contrasts. All mixed models met the assumptions of normality of residuals and homogeneity of variance.

Results

Organic horizon mass and soil density

No effects of forest stand and region on organic horizon masses (OHM) was observed. Instead, depth and the interactions Species:depth and Species:Region were consistency significant affecting the organic layer masses. The model was found to be statistically significant ($p < 0.001$; $r^2=0.397$), indicating that approximately 40% of the variation in the response variable (OHM) could be explained by the model (Figure S2).

The effect of depth was significant, with O_H having a positive effect ($p<0.001$) on OHM, its interaction term depth:Region shows that O_H layer shows negative effects at southern sites than northern sites ($p<0.001$). The interaction terms show that both mixed stands (Douglas fir/beech and spruce/beech) and O_H layer had significant and positive effects on OHM.

The mineral soil bulk density ranges from 0.8 to 1.4 g cm^{-3}. The southern sites showed significant lower soil bulk density ($p<0.001$) than northern sites, and Douglas fir and Douglas fir/beech showed significant lower bulk soil density ($p<0.001$) compared to beech. A significant interaction among forest stands and site conditions was observed (Figure 1), where Douglas fir and its mixture with beech (Douglas fir/beech) showed higher soil densities at loamy soils (Southern sites) than at sandy soils (Northern sites) (Table S1).

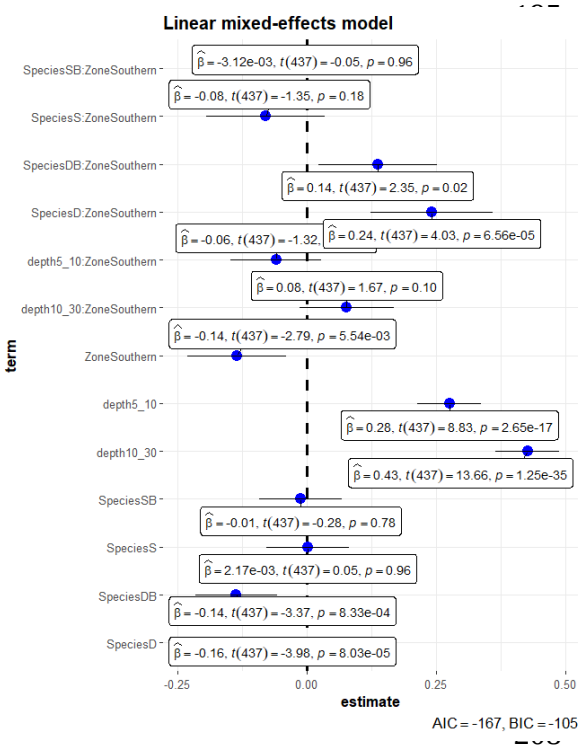

Figure 1. Linear mixed-effects model on soil bulk density. The figure (a) shows the estimate coefficient (β), t-test and significance *p* for each term included in the model. Species (D: Douglas fir, S: spruce; Be: beech; Douglas fir/beech: DB; and, spruce/beech: SB), Region (Southern sites [loamy soils] and Northern sites [sandy soils]) and depth (0-5, 5-10 and 10-30 cm]).

Organic-horizon and mineral soil C and N concentrations

We used a linear mixed model to investigate the relationship between carbon (C) and nitrogen (N) concentration and species, depth, and their interaction across the sites (Northern and Southern sites). The results showed that all five stands had a significant effect on carbon concentration. The highest carbon concentration was found in the O_H layer and decreased with depth. Furthermore, the effect of depth varied across species, with higher carbon concentrations found in the top layer of the organic horizon for all species except Douglas fir. In terms of the species effect, the carbon concentration was significantly higher under beech and mixed spruce/beech stands compared to conifers, Douglas fir and spruce. Except at the O_H layer where both conifers and mixed stands had higher concentrations of carbon than

beech stands. Moreover, the interaction between species and sites was significant only for Douglas Fir and mixed Douglas Fir/beech stands, indicating that the effect of species on carbon concentration varied across sites, being significantly high at sandy soils (Northern sites).

The forest stands also showed a significant effect on the N concentration. Specifically, the N concentration was higher for both mixture forests (Douglas fir/beech, spruce/beech), and spruce than for the pure beech stand. The depth also significantly affected the N concentration. Compared to the reference depth (10-30 cm), all other depths were significantly associated with higher N concentration. Additionally, the interaction between Species and Sites was also found to be significant, indicating that the effect of species on the N concentration varies depending on the site.

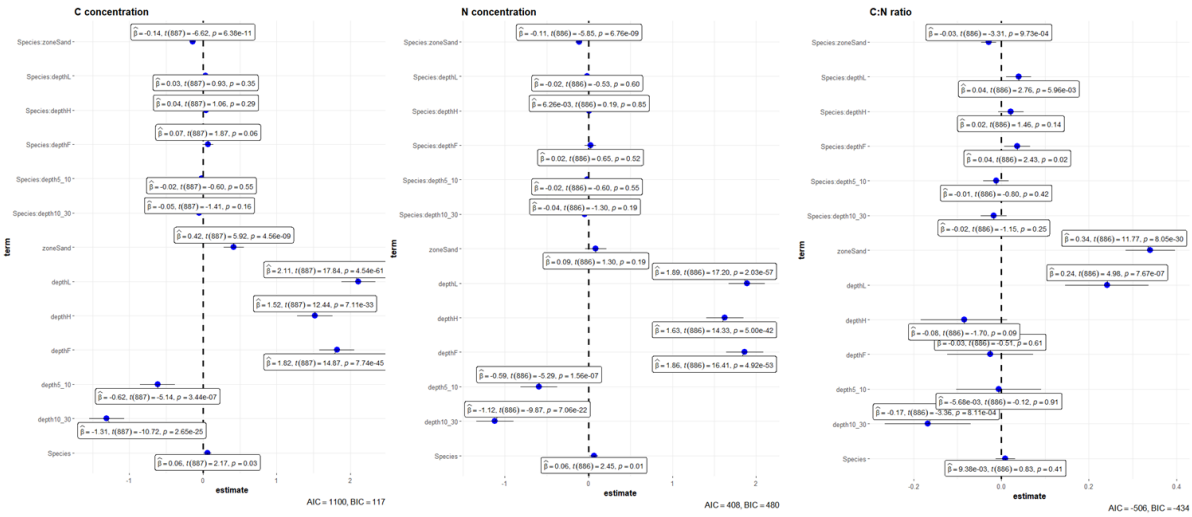

Figure 2. Linear mixed-effects model on Carbon (a), Nitrogen (b) concentration and CN ratio (c). The figure (a) shows the estimate coefficient (β), t-test and significance *p* for each term included in the model. Species (D: Douglas fir, S: spruce; Be: beech; Douglas fir/beech: DB; and, spruce/beech: SB), Region (Southern sites [loamy soils] and Northern sites [sandy soils]) and depth [O_L, O_F, O_H, 0-5, 5-10 and 10-30 cm]).

C and N stocks

The results indicated that C stock was higher in the beech stands at the Southern sites (loamy soils) (23.33 Mg ha^{-1}) than in Douglas-fir stands (19.60 Mg ha^{-1}). On the contrary, at Northern sites (sandy soils), Douglas-fir stands had higher C stock (23.46 Mg ha^{-1}) than beech stands (16.29 Mg ha^{-1}).

The multiple stepwise regression model explained 49% of the variance in C stock and was found to be statistically significant ($p < 0.001$). Species, soil conditions, and depth were significant predictors of C stock ($p < 0.05$) (Figure 2). The coefficient estimate for sandy soils was positive, indicating that C stock were higher in sandy soils compared to loamy soils. The interaction term between species and sandy soil was statistically significant ($p < 0.001$) and negative, indicating that the effect of species on C stock differed between sandy and loamy soils, and it was attenuated in sandy soils. There, Douglas fir and Douglas fir/beech showed the highest C stocks compared to beech.

The effect of species on C stock was stronger at litter layer (O_L). Additionally, the interactions between Species:depth and Species:sandy soils were found to be statistically significant ($p < 0.05$) with negative coefficients, suggesting that the effect of species on C stock was more pronounced in the litter layer at sandy soils.

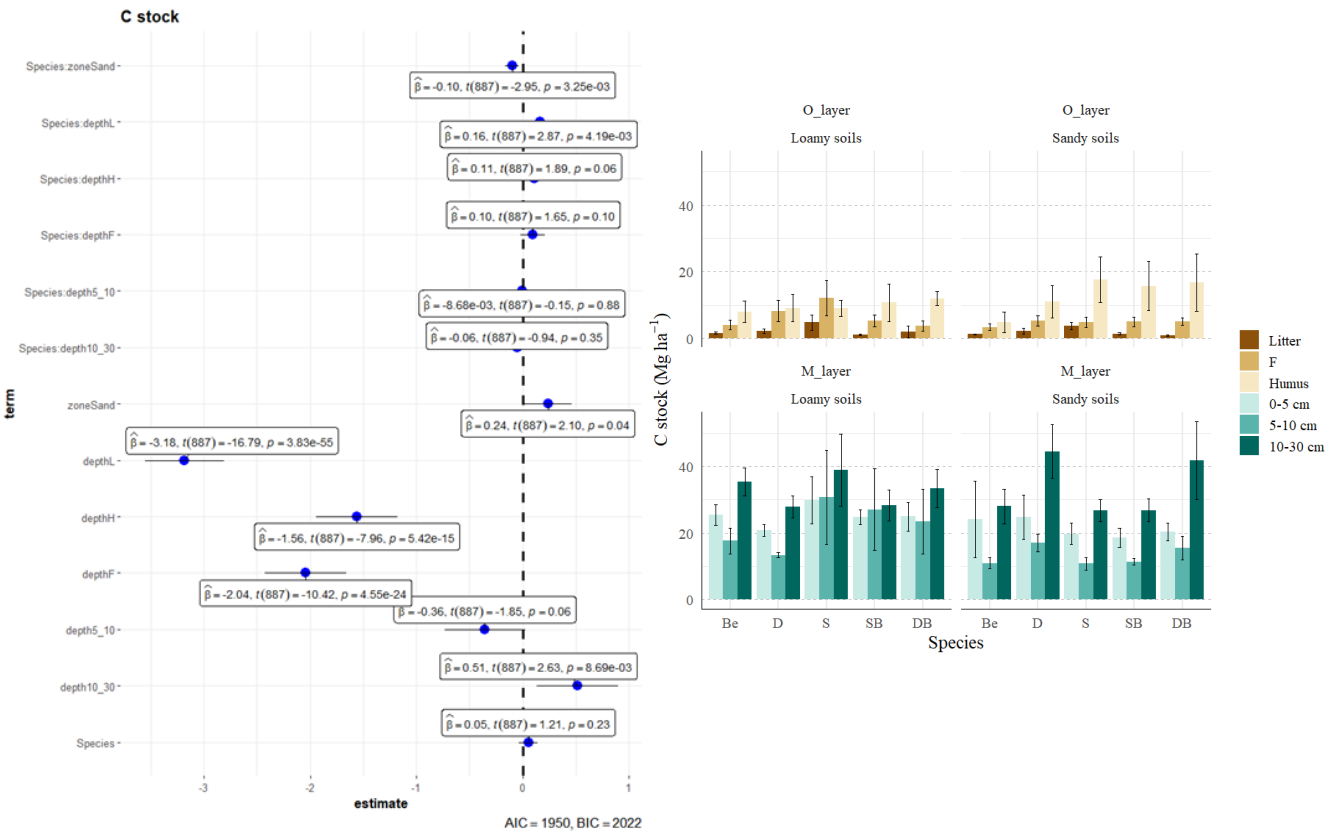

Figure 3. Linear mixed-effects model on Carbon stock (a) and Carbon stock (b; Mg ha^{-1}). The figure (a) shows the estimate coefficient (β), t-test and significance *p* for each term included in the model. Species (D: Douglas fir, S: spruce; Be: beech; Douglas fir/beech: DB; and, spruce/beech: SB), Region (Southern sites [loamy soils] and Northern sites [sandy soils]) and depth [L,F,H,0-5, 5-10 and 10-30 cm]). The figure (b) shows the C stock in the organic layer (brown bars) and mineral soil (green bars), and the standard error (n=16).

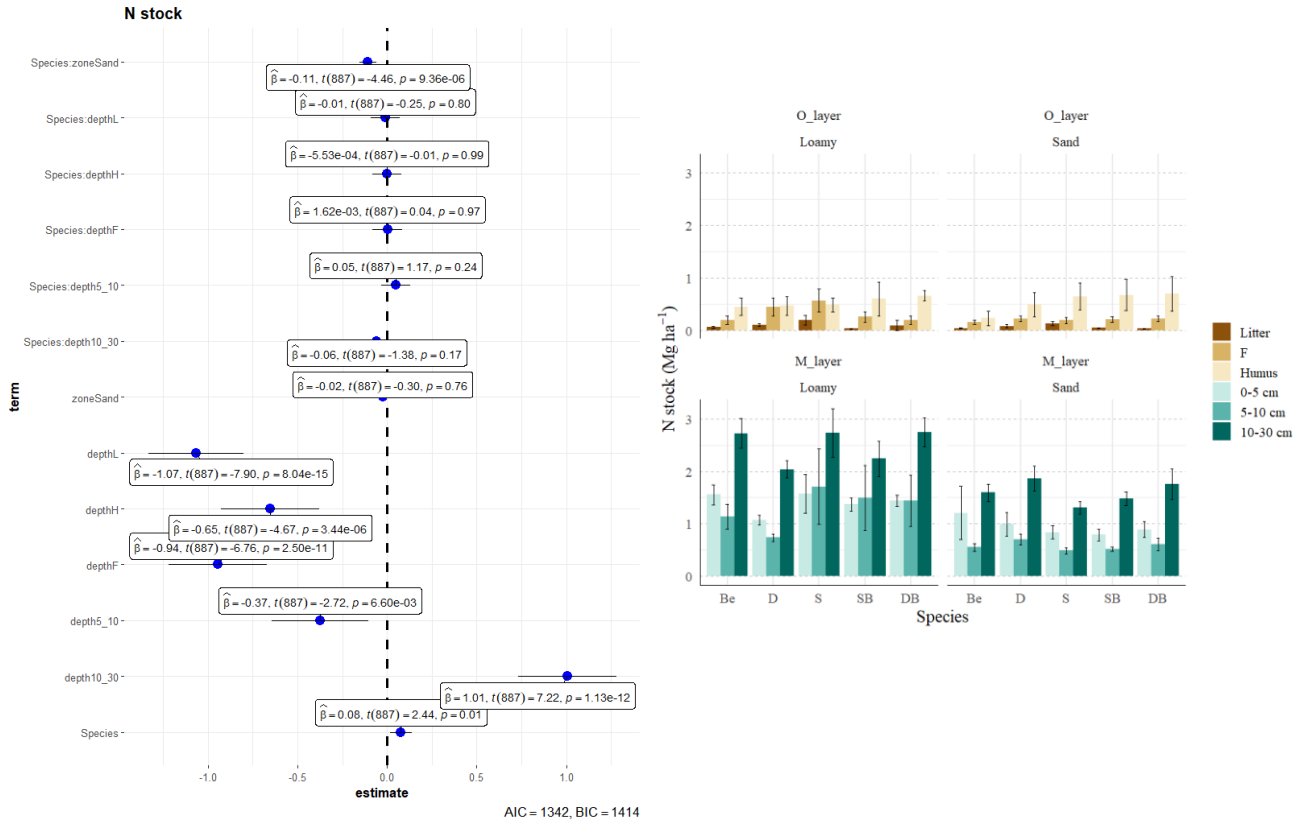

Figure 4. Linear mixed-effects model on Nitrogen stock (a) and Nitrogen stock (b; Mg ha^{-1}). The figure (a) shows the estimate coefficient (β), t-test and significance *p* for each term included in the model. Species (D: Douglas fir, S: spruce; Be: beech; Douglas fir/beech: DB; and, spruce/beech: SB), Region (Southern sites [loamy soils] and Northern sites [sandy soils]) and depth [L,F,H,0-5, 5-10 and 10-30 cm]). The figure (b) shows the N stock in the organic layer (brown bars) and mineral soil (green bars), and the standard error (n=16).

The model was statistically significant ($p < 0.001$) and explained 63.53% of the variance in N stock. The results showed that Species had a significant effect on N stock ($p = 0.02$). Aditionally, the interaction effect between Species and soil condition was also significant ($p > 0.001$), indicating that the effect of Species on N stock was attenueted in sandy soils compared to loamy soils. At the loamy soils (Southern sites), total N stocks were higher under spruce stands than under Douglas fir stands. In contrast, at the sandy soils (Northern sites), higher N stocks under Douglas fir stand than under spruce stand were observed (Figure 4).

At the southern sites, significantly larger N stocks were observed under mixed spruce/beech stand than Douglas/beech, whereas at nouthern sites, the opposite were observed (Douglas/beech > Spruce/beech).

At all sites, N stocks at organic layer under mixed beech–conifer stands (either Douglas/beech or spruce/beech), significantly exceeded beech stands. The effect of the mixtures showed the same site-dependent patterns as reported for SOC stocks, where the stocks under both beech–conifer mixtures were similar to those under the respective pure conifer stands.

The soil depth was also a significant predictor of N stock, with 10-30 cm showing the highest N stock ($p > 0.001$). However, none of the interaction terms between Species and depth were significant, indicating that the effect of Species on N stock did not vary across different soil depths.

SOC Distribution

The contribution of the organic layer stocks (SOC_{Org}) to the total stocks (SOCt) was site-dependent. At southern sites, the SOC_{Org} contributed between 15% and 22% for Douglas fir and spruce stands, respectively, whereas for beech it was only 8%. The mixture stands showed intermediate results, ranging from 10% (Douglas fir/beech) to 17% (spruce/beech). At northern sites, the contribution of SOC_{Org} on SOCt stock was strong pronounced. Under the spruce stand, 33% of the SOC stock is allocated to the organic horizon, whereas 20% was observed in the Douglas fir stand. The contribution of SOC_{org} to SOCt for mixed forests ranged between 23% (Douglas/beech) and 30% (spruce/beech) and only 10% for beech stands.

The N allocation corresponded to the SOC vertical distribution. At the northern sites, the relative contribution ranged from 20–26% for conifers and mixed forest to 11% at the beech forest, whereas at the southern sites, it ranged from 13–17% for conifers to only 6% under beech forest. At northern sites, mixture stands showed a clear contrast: at the spruce/beech stand, the contribution of organic layer N stock to the total N stock was 30%, while at the Douglas/beech stand, it was only 7%.

Overall, under sandy soil conditions, conifers and mixed forests allocated 10% more SOC and N at the organic layer compared to loamy soils, whereas the SOC and N stocks under beech maintained the same proportion (>90% at SOC_{ms}), independent of the site condition.

Effect of abiotic factors on C and N stocks

The effect of abiotic factors like soil pH on OC stock was investigated using linear mixed models (Figure 5). The increase of pH caused significant decreased (P<0.001) on SOC at mineral soil. The increase of 1 unit of pH (3.75 to 4.75) reduced in about 25 % the total SOC. Whereas, the opposite was observed for N stocks, where the increased of pH showed increases in N stocks.

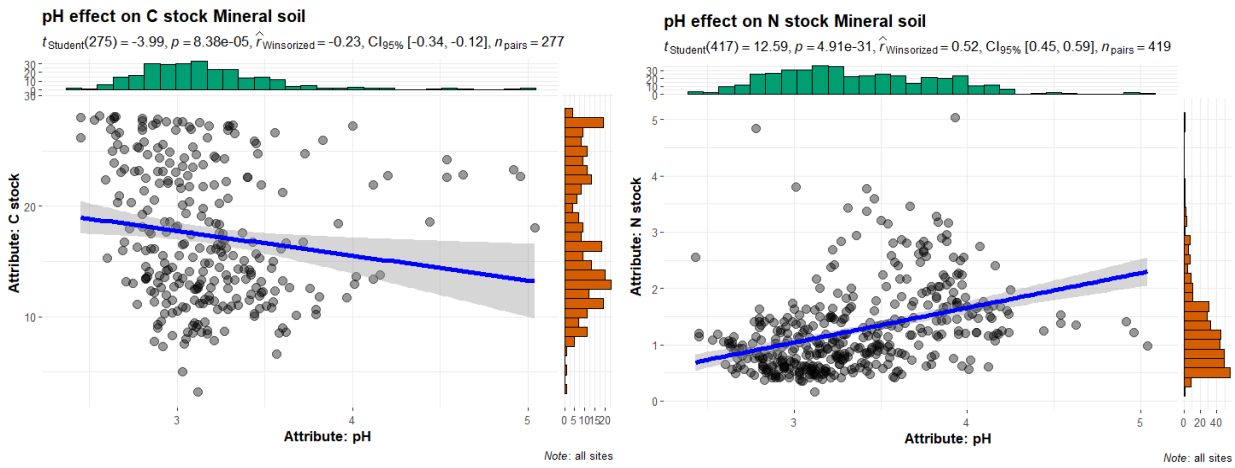

Figure 5. Relationship between the soil organic carbon (SOC) (a) and Nitrogen (b) on mineral soil. The grey line denotes a linear mixed-effect model (LMM; P < 0.05) with the predictor variables as fixed effect and species as random intercept terms. The dashed line represents a

95% confidence interval. All response variables (C stock, and N stock) were transformed with natural logarithm, whereas predictor variable (pH) were standardized by subtracting the mean and dividing by the standard deviation.

Discussion

Tree species effects

We observed significant effect of tree species on organic C and N stocks as well as its vertical distribution across all investigated sites. Overall, the organic-horizon (O) stocks of C and N were significantly higher under conifer forests (Douglas fir and spruce) than under beech (Figure 1). At the mineral soil (MS) the effects of tree species were site-dependent. The southern sites (loamy soil), beech and spruce stands presented higher C and N stocks than Douglas fir forest, confirming earlier results of Antisari et al. (2015) where smallest SOC stocks of the organic horizon under beech stand are accompanied by enhanced SOC stock in the mineral topsoil. In contrast, at northern sites (sandy soil), the beech stands showed the smallest OC stock at both layers (O and MS).

According to Neumann et al. (2018), broadleaves litter tends to decompose faster compared to conifer litter. This can be attributed to lower lignin and phenol concentrations, which promote rapid decomposition rates and efficient accumulation of mineral-associated organic matter, as reported by Rasse et al. (2005). In our study, we observed a higher carbon (C) input from the organic horizon to the mineral soil in the beech forest under loamy soil conditions, supporting the findings of previous research. The higher C allocation on mineral soil than organic horizon under beech than conifers has also been reported in common garden studies (Vesterdal et al., 2013). The differences in vertical distribution has been attributed to the associated community of macrofauna species in beech dominated forests (Achilles et al., 2021).

Therefore, the quality of the soil organic matter (SOM) might be significantly different under coniferous and deciduous trees (Jaffrain et al., 2007). The O_H layer under conifers results in a more recalcitrant and hydrophobic composition than that of O_H layer at beech stand (Thomas et al., 2014) . The low mineralization quotient consequently leads to larger C accumulation due the slow C turnover, as observed in our study under both conifers stands.

Besides the litter quality, rooting patterns and microbial activity among beech, Douglas fir and Norway spruce might change the C inputs between tree species. Simultaneous studies on the turnover of fine root biomasses carried out by Lwila et al. (2021) have shown that beech forests showed higher fine roots biomass than Douglas fir at the sandy soils, whereas the fine root necromass was higher for Douglas fir compared to beech. High microbial activity at the upper mineral soil is reported in our study area (Lu and Scheu, 2021), suggesting high rhizosphere carbon inputs at the beech forest stand compared to Douglas fir.

In general, conifer roots contain lower lignin concentrations than roots of beech (Thomas et al., 2014) (Newman and Hart 2006), which might lead to a lower longevity and a faster decomposition (Berger et al., 2002; Cremer et al., 2016). Additionally, fine roots necromass deliver a considerable amount of organic material (Cremer et al., 2016; Dawud et al., 2017) enhancing the root-derived SOM. Due the higher productivity of Douglas fir than beech (in particular in dry conditions as at northern sites) (Schmid et al., 2014; Schmidt et al., 2015) higher turnover rate of Douglas fir fine roots is expected and consequently fast fine root turnover might cause favorable changes on SOC stocks, as observed in our study and similar studies (Lorenz and Lal, 2014).

Mixed forest effect

Independent of the site conditions, the pure stands of beech showed relatively small total SOC stocks, whereas the pure conifer sites enhanced total SOC and N stocks, accumulated to a large extent in the organic layer. Furthermore, the admixture of conifers to beech enlarged the total SOC and N storage only in the northern sites, i.e. under sandy soil conditions, whereas in the southern sites, i.e., under loamy soil conditions, both mixtures of conifers to beech presented similar storages compared to pure beech. Overall, the carbon (C) stocks in the O_H layer, as well as the total soil organic carbon (SOC) and nitrogen (N) stocks, were generally similar between mixed beech-conifer stands and their respective pure stands. The C stocks at both mixed stands resembled those of beech stands at southern sites and approached the levels observed in conifer stands at northern sites.

The admixture of beech into conifers stands in nutrient-poor soil conditions, particularly observed at the northern sites, had significant effects on soil organic carbon (SOC) and

nitrogen (N) stocks, which aligns with the results reported by Dawud et al. (2017). In these soil conditions, both the mixture of Douglas fir and beech and pure Douglas fir stands exhibited higher SOC content in the humus layer (O_H) and the 5-30 cm soil depth. This can be attributed to the competitive advantage of beech fine roots, which have the ability to access deeper soil layers and exploit areas less occupied by competing species. In contrast, Douglas fir fine roots tend to be restricted to the topsoil (15-30 cm), resulting in a shift in the distribution of beech fine roots towards the subsoil. This phenomenon has been reported in previous studies (Hendriks and Bianchi, 1995; Schmid and Kazda, 2002). These observed shifts of root vertical distribution are known as below-ground complementarity effects, e.g., through vertical segregation of roots of different species which exploit soil at different depths allowing for reduced competition (Loreau M, 2001), has been suggested as a mechanism by which species mixtures store more C in deeper soil layers than monocultures (Bauhus et al., 2009; Forrester et al., 2006). Additionally, the admixture of beech leaf litter to the more recalcitrant needle litter induced a faster litter mass loss and consequently shift of SOC stock from O_L to O_H layer, i.e., similar decomposition rate (*k)* for mixed stands and pure beech and higher than Douglas fir and spruce forests, suggesting similar decomposability of litter in the mixed forest and in the beech forest (not published).

Thus, allocation of C in soils is an important ecosystem service provided by forests (Pretzsch et al., 2017). Owing to the potential of higher SOC storages in mixed-species forests compared to pure stands and to shift the C into stable pools i.e., the shift from O_L layers to O_H layer, enhance the capacity of mixed forest to provide ecosystem services e.g., improve soil functioning like soil biodiversity and nutrient availability and, furthermore the resilience of the forest ecosystem to cope with future climate change scenarios.

Abiotic effects

The persistence of SOC is largely due to complex interactions between SOC and its environment, such as reactive mineral surfaces, climate, water availability and soil acidity (Leifeld et al., 2013; Mikutta et al., 2009; Zhou et al., 2019). The most important factor in SOC stabilization at sites with high clay contents, as partly observed in our study, is probably the association with soil minerals, irrespective of forest type (Fierer and Jackson, 2006; Meier et al., 2020). Indeed, enhanced clay and silt content increased the SOC in the mineral soil at

our investigated sites and, in contrast decreased the SOC storage at organic horizon. Loamy soils are known to buffer influences by tree species more strongly than sandy soils (MEIER and LEUSCHNER, 2010) and this would explain why we see more effects of species on the northern sites dominated by sandy soils than at southern sites.

Therefore, differences in soil pH often go along with differences in soil mineralogy as well and the latter exerts control on the stabilization of mineral associated organic matter (Meier et al., 2020) and microbiota community (MEIER and LEUSCHNER, 2010). The accumulation of organic matter in soils is influenced by relatively low pH levels, which can reduce the decomposition of soil organic matter (SOM), as highlighted in the study by Meier et al. (2020). This, in turn, leads to noticeable effects of tree species on organic matter accumulation, as observed in our study. Interestingly, Meier et al. (2020) also suggested that soil acidity is associated with increased rates of root exudation in beech forests, particularly in the topsoil of glacial sandy soils found in the northern sites. This increased root exudation can be seen as an adaptation of the trees to low nutrient availability, a finding consistent with our study (Foltran et al., 2023). In these conditions, the majority of nutrient uptake occurs in the AE horizon, which is enriched with organic material, further explaining the adaptability of beech forests in response to varying nutrient availability.

Conclusion

Site dependent effects of tree species (European beech, Douglas fir and Norway spruce) on SOC and N stocks as well as on SOC and N concentration were observed. The SOC and N stocks generally were smallest in pure beech stands compared with Douglas fir and Norway spruce. The SOC and N stocks in mixed stands of beech with Douglas fir or Norway spruce are generally between those of the respective pure stands. However, the results indicate that the effects of admixture of beech into conifers stands (Douglas fir and spruce) are site-dependent At the Northern sites, the adaptability of Douglas fir under dry and poor-nutrient site conditions combined with high plasticity of beech promoted a favorable effect on total SOC and N, enhancing its storages as observed at pure Douglas fir and the mixture Douglas fir/beech. In contrast, at the Southern sites, spruce/beech showed higher SOC and N stocks than Douglas/beech.

The Douglas fir/beech stand mixture showed significant increases of total SOC under sandy soils (Northern sites). Overall, the higher potential for carbon storage in mixed-species forests compared to pure stands, along with the shift of carbon into more stable pools (such as from the O_L layer to the O_H layer), emphasizes the capacity of mixed forest to provide valuable ecosystem services while reducing the risk of unintended carbon losses.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by EF. The first draft of the manuscript was written by EF and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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