Higher subsoil carbon storage in species-rich than species-poor temperate forests

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Abstract
Forest soils contribute ca. 70% to the global soil organic carbon (SOC) pool and thus are an important element of the global carbon cycle. Forests also harbour a large part of the global terrestrial biodiversity. It is not clear, however, whether tree species diversity affects SOC. By measuring the carbon concentration of different soil particle size fractions separately, we were able to distinguish between effects of fine particle content and tree species composition on the SOC pool in old-growth broad-leaved forest plots along a tree diversity gradient (1-, 3- and 5-species). Variation in clay content explained part of the observed SOC increase from monospecific to mixed forests, but we show that the carbon concentration per unit clay or fine silt in the subsoil was by 30–35% higher in mixed than monospecific stands indicating a significant species identity or species diversity effect on C stabilization. Underlying causes may be differences in fine root biomass and turnover, in leaf litter decomposition rate among the tree species, and/or species-specific rhizosphere effects on soil. Our findings may have important implications for forestry offering management options through preference of mixed stands that could increase forest SOC pools and mitigate climate warming.

Keywords: carbon saturation, tree diversity, particle size fractionation, subsoil, soil organic carbon

1. Introduction
At the global scale, soils store approximately 1,500 Pg organic carbon (C) to a depth of one meter (Eswaran et al 1993, Jungkunst et al 2012). According to Dixon et al (1994), more than half of this amount (790 Pg C) is found in forest soils, which is roughly as much as is presently stored in the atmosphere in form of the greenhouse gases carbon dioxide and methane. Hence, forests play a key role in global carbon storage and cycling (Lal 2005, Bonan 2008, Janssens et al 2003). Many forests also harbour a rich biodiversity, in particular tropical and temperate forests (Millenium Ecosystem Assessment 2005). Since vast forest areas are managed and tree species are selected, there might be the option that both C storage and biodiversity can actively be promoted together. Certainly, it would be a desirable win-win strategy in forestry.
if increases in, or at least the maintenance of, the biodiversity would be accompanied by enhanced C storage (or at least the preservation of the existing stocks) (Kessler et al. 2012).

The SOC pool of soils and its quality varies with climate, soil type and forest community type and it is also influenced by stand characteristics such as stand age, stem density, species composition, management regime and forest continuity (Baritz et al. 2010, Jandel et al. 2007, Ladegaard-Peterson et al. 2005, Lal 2005, Pregitzer and Euskirchen 2004, Leuschner et al. 2013). If mixed forests would store more SOC than pure stands, this would be an important incentive to promote mixed stands in plantation forestry and in the management of existing forests in order to mitigate greenhouse gas emissions by land use. Recent ecological research has produced increasing evidence of marked tree species differences in rooting patterns (Jacob et al. 2013, Meinen et al. 2009), root turnover and rhizodeposition (Cesárz et al. 2013), traits with a possible influence on soil C sequestration, which seem to be species-dependent (’species identity effect’). However, tree species diversity (’tree diversity effect’) could also play a role, e.g. through a more complete exploration of the soil by root systems in more diverse stands (Jacob et al. 2013), priming effects on soil biota (Weintraub et al. 2007), or through a higher diversity of organic compounds released via exudation. The multitude of possible influencing factors makes it difficult, however, to separate assumed species identity and species diversity effects on SOC from co-varying factors such as soil clay content.

Biotic controls on C enrichment in soil may be investigated experimentally in plantations with variable tree species composition, but this approach examines immature stands with non-equilibrium soil systems. Therefore, comparative observations in old-growth mixed forests are the only approach to investigate biotic effects on SOC pools in soil in a quasi-equilibrium state. Clearly, this approach has a number of shortcomings, notably the existence of co-varying factors that may be difficult to separate from species identity or diversity effects, and the difficulty of finding sufficient stands with adequate species composition (Leuschner et al. 2009).

Soil texture, in particular clay content, has a large influence on soil water dynamics and soil biogeochemistry. This is particularly valid for soil carbon storage, which is promoted by the formation of clay-humus complexes. The higher the specific surface area and surface charge of soil minerals, the higher is their contribution to SOC storage. Due to the ability of small-sized particles (in particular clay and fine silt, < 6.2 µm) to protect SOC from decomposition by various physico-chemical mechanisms (Heitkamp et al. 2012a, von Lützow et al. 2008), SOC contents generally increase with clay and fine silt contents (Körshens et al. 1998). Thus, C is mainly bound to the smallest fraction, i.e. clay and fine silt (Kiem and Kögel-Knabner 2002, Christensen 2001). For example, Christensen (2001) found <10% of SOC to be associated with the sand fraction, 20–40% with the silt fraction, and 50–75% with the clay fraction in an agricultural soil. The major part of the sand-associated SOC belongs to the active pool, which has a high turnover rate. The turnover of C associated with silt and clay is much lower and this fraction is therefore kept longer locked away from the atmosphere (Heitkamp et al. 2012a).

Various studies have reported significant tree species effects on SOC (e.g. Binkley and Valentine 1991, Diaz-Pines 2011, Guckland et al. 2009, Jandel et al. 2007, Pregitzer and Euskirchen 2004, Schulp et al. 2008, Vesterdal et al. 2012, Leuschner et al. 2013), but the majority found stock changes only in the organic layer and uppermost mineral soil. In a common garden experiment, Vesterdal et al. (2008) showed that the C stocks in the forest floor and mineral soil began to differentiate already 40 years after planting of different tree species. They showed that SOC stocks in the mineral soil (0–30 cm) were 10–20% lower in monospecific beech, as compared to other monospecific broadleaved tree species. However, 40 years are still too short for a reliable evaluation of SOC dynamics, especially in the persistent fractions (i.e. C associated with clay and silt) that are not readily returned to the atmosphere within a few years.

We start from the observation of Guckland et al. (2009) in Hainich Forest (Central Germany) that mixed beech stands store significantly more SOC in the mineral soil than pure beech stands. The authors concluded that higher SOC stocks were caused by higher clay contents, rendering identification of possible species or diversity effects impossible. We hypothesise that (1) there is an additional species identity and/or diversity effect on SOC storage. (2) that this effect can be demonstrated by analysing the C concentrations on clay and silt sized particles directly.

2. Materials and methods

2.1. Study area

The study was conducted in a relatively species-rich temperate broad-leaved forest in Hainich National Park (Thuringia, Germany; 51°04’46”N, 10°27’08”E; 440 m above sea level), where human impact was low in the past 60 years and any form of management was absent since 1997 with the declaration of a national park. Mean annual precipitation was 670 mm and mean annual temperature 7.5 °C (Guckland et al. 2009). Soils were classified as Luvisols (WRB 2007) that developed on Pleistocene loess layers of variable thickness (60–120 cm) overlying Triassic limestone. The organic layer varies with tree species composition across the forest. An O horizon developed between the litter and mineral A horizon under monospecific European beech (Fagus sylvatica L.) stands, whereas the O horizon is weakly developed in the mixed stands (Guckland et al. 2009). The trees in the canopy layer are 80–150 yr old. Besides beech, which is the dominant species of the natural forest vegetation in the area, three maple species (Acer platanoides L., A. pseudoplatanus L., A. campestre L.), European ash (Fraxinus excelsior L.), lime (Tilia cordata Mill.) and T. platyphyllos Scop.), hornbeam (Carpinus betulus L.), oak species (Quercus robur L. and Q. petraea Lœbl.) and elm (Ulmus glabra Huds. em. Moss) are present in the forest (Jacob et al. 2010a). Variation in species composition and stand structure are mainly the consequence of differences in ownership and management regimes in the past that favoured lime, maple, hornbeam and oak over beech in those forest patches that were managed with selective cutting systems.
or coppice with standards regimes. Historical data indicate that the Hainich forest was continuously covered by forest at least since the early middle ages, even though more or less intensive logging activities most likely have occurred through the centuries (Grossmann 2006).

2.2. Experimental design

In the north-eastern section of the national park, nine plots (50 m x 50 m) with variable tree species composition and tree diversity were chosen for study. The plot design consists of a tree diversity gradient (dominance of 1 species, 3 species or 5 species) with European beech being increasingly diluted by other species (Guckland et al 2010); the three diversity levels (DL1, DL2 and DL3) were replicated threefold. The 1-species plots (DL1) were dominated to 89% by beech (according to basal area), the 3-species plots (DL2) consisted of a mixture of beech (59%), ash (13%) and lime (T. cordata and T. platyphyllos, 19%), while the 5-species plots (DL3) contained mixed stands of beech (41%), ash (31%), lime (15%), hornbeam (4%) and maple (A. pseudoplatanus, 9%).

The Shannon diversity index (based on basal area) increased from ~0 to ~1.8 (Leuschner et al 2009). While climate and bedrock were well comparable among the plots, soil properties are influenced by the variable species composition of the plots and its effect on litter chemistry, and by local differences in particle size distribution of the bulk soil material. The content of fine particles (clay, fine silt) was on average higher in the mixed plots (DL2, DL3) than in the monospecific beech plots (DL1, table 2).

2.3. Soil analysis

Soil sampling was conducted at three randomly selected points in each plot. Additionally, a soil pit was dug in close vicinity of every plot for soil classification. In September 2011, mineral soil samples of both the A and E horizons were taken in three small soil pits (30 cm x 30 cm x 40 cm; length x width x depth). Contrary to Guckland et al (2009), who studied soil carbon on the same plots, we took the samples in soil depths defined by the borders of the A and E horizons and did not adopt static sampling depths in order to account for local soil heterogeneity. Similar to Guckland et al (2009), we sampled to a maximum depth of 30 cm which allows comparison. Since the E horizon was always below the A horizon (and not only below an organic O horizon), we are using the terms ‘subsoil’ and ‘E horizon’ synonymously. The fresh soil material was sieved (2 mm) and dried at 45 °C for 24 h afterwards. To separate different particle size fractions, a combination of the sieving and sedimentation procedure was applied as suggested by Cambardella and Elliott (1993), Heitkamp et al (2012a) and von Lützow et al (2007). The protocol was modified in order to conduct a soil texture analysis and a particle size fractionation within the same step and to measure the C concentrations of the different size fractions separately. Afterwards, the results were checked against the usually applied soil texture analysis after the DIN ISO 11277 (2002) protocol; the agreement was good. The deviation between the two methods was not larger than that between two replicate measurements according to the DIN method. The sand fraction (Sa, 2000—63 µm) was obtained by wet sieving, while two silt fractions, coarse and medium silt (cSi, 63—6.3 µm) as well as fine silt (fSi, 6.3—2 µm) and the clay fraction (Cl, < 2 µm) were determined by using the pipette method after a pre-treatment with 0.1 M sodium hexametaphosphate solution to disperse the particles. Bulk soil samples and particle size fractions were ground and analysed for total C and N by dry combustion (Leco TruSpec, St. Joseph, MI, USA). To avoid confusion, the term ‘SOC’ will be used when relating C contents or stocks to the bulk soil while the term ‘Corg’ is used to express concentrations of organic C of the particle size fractions. To determine bulk soil pH, dried sieved soil samples were mixed with deionized water at a ratio of 1–5 (DIN ISO 10390 1997). The SOC stocks (kg m⁻²) of the A and E horizon to a depth of 30 cm were calculated according to Baritz et al (2010) by multiplying the SOC concentration of the bulk soil (in g kg⁻¹) with bulk soil density (for individual plots, as published in Guckland et al 2010) and the thickness of the soil horizons (table 1). For characterizing the change in SOC concentration with variation in soil texture, dimensionless enrichment factors (EFc) were calculated following Schulten and Leinweber (2000) who related the Corg concentration of a given particle fraction to the SOC concentration of the bulk soil (both in g kg⁻¹). The relations were described with exponential equations. Due to the large proportion of Corg associated with the clay and fine silt fractions (together 53.8% (±1.8 SE) and 75.4% (±1.3 SE) in the A and E horizon, respectively; table 2), the focus of the analysis was put on these two particle size fractions.

2.4. Statistical analysis

All basic calculations were performed with Microsoft Excel 2010 software (Microsoft Corporation, Redmond, USA). More complex statistical analyses were carried out with PASW Statistic 18 (IBM SPSS Statistics) and Statistica 7 software (StatSoft (Europe) GmbH). Significance was determined at p < 0.05 throughout. Data were tested for normality (Shapiro–Wilk test, p > 0.05) and homogeneity of variances (Levene test, p > 0.05). In case of normality and homogeneity of variances, ANOVAs were conducted to test for significant differences in soil chemical and physical parameters between the three diversity levels (DL1, DL2, DL3), followed by the Tukey test, or by the LSD test, if homogeneity of variances was not given. In cases of non-normal distribution, data were log- or root-transformed and tested with post hoc tests (Tukey or LSD) again. In a few cases, neither normality nor homogeneity of variances could be achieved and the non-parametric Kruskal–Wallis test was used for detecting significant differences between means.

Pearson correlation analysis was used to characterize the relationships between various investigated soil properties. The relationship between tree diversity (Shannon index) and the Corg concentrations in the clay and fine silt fractions was analysed by linear regression analysis. A multiple stepwise linear regression analysis (MLRA) with stepwise forward variable selection was conducted to characterize the influence of bulk density, horizon thickness and SOC concentration on the SOC stocks. Insignificant (p > 0.05) predictors were dropped from the model.
3. Results

3.1. Carbon storage

Considering a depth of 0–30 cm, total SOC stocks were 5.0 (±0.3 SE) kg m\(^{-2}\) in the monospecific DL1 stands, 8.3 (±0.5 SE) kg m\(^{-2}\) in the DL2 stands and 10.0 (±1.0 SE) kg m\(^{-2}\) in the DL3 stands. The DL1 stands had significantly smaller SOC concentrations (g kg\(^{-1}\)) in the lower horizon than the mixed stands (DL2 and DL3) (7.4, 14.6 and 13.6 for DL1, DL2 and DL3, respectively), but did not differ in the A horizon (47.5, 48.5 and 53.1 for DL1, DL2 and DL3, respectively) (table 1). The SOC stocks in the A and E horizons were significantly larger in the mixed stands [DL2, DL3] than in the monospecific beech stands [DL1], but no significant difference existed between the two mixed stand categories in the A horizon (table 1).

The results of the MLRA show that 95.7% of the total variance of the C stocks in the A horizon is explained by a combination of the three variables thickness of the soil horizon (explaining 55.7%), SOC concentration (31.3%) and bulk density (8.7%). In the E horizon, the largest proportion of variance was explained by the SOC concentration (90.2%). Thickness and bulk density did not significantly influence the variance in the E horizon and thus were excluded from the model. Therefore, it is justified to refer in the discussion about subsoil stocks mainly to SOC concentrations.

3.2. Soil texture effects on SOC

The clay and fine silt content was significantly higher in the soils of the mixed stands (DL2, DL3) compared to monospecific beech stands (DL1) in both soil horizons (table 2). A large proportion (49–79%) of SOC was associated with the clay and fine silt fractions (table 2). The relationship between SOC concentration and the clay content or fine silt content was strong and highly significant in both horizons across the nine plots (A horizon: \(r_{CI} = 0.60, p < 0.001, r_{SI} = 0.53, p = 0.002\); E horizon: \(r_{CI} = 0.91, p < 0.001, r_{SI} = 0.88, p < 0.001\)).

The enrichment factor (EF\(_C\)) decreased exponentially with increases in clay or fine silt content (figure 1). Correlation coefficients between EF\(_C\) and fine particle content in the A horizon showed a moderate to close relationship for the fine silt and clay fraction, whereas strong relationships were detected for the E horizon. Comparison of our relationships with a reference fit taken from Schulten and Leinweber (2000) showed close agreement; an exception was the fine silt fraction of the A horizon (figure 1). Comparison of the EF\(_C\) values between the A and E horizons showed a significantly lower EF\(_C\) in the topsoil horizon for the clay and the fine silt fraction, irrespective of the diversity level. The same result of a lower C enrichment per clay mass in the A horizon was obtained in nearly all cases when the EF\(_C\) values were calculated only for the plots of a certain diversity level; an exception was the fine silt fraction in the DL2 stands where no significant difference between A and E horizons was found.

3.3. Tree species identity and diversity effects on SOC

Significantly higher concentrations of C\(_{org}\) were detected within the clay and silt fraction of the E horizon in the two mixed stand categories [DL2, DL3] than in the DL1 plots. In the A horizon, C\(_{org}\) associated with the fine silt fraction did not differ significantly between the three diversity levels, whereas the C\(_{org}\) concentration of the clay fraction tended to be highest in the DL1 plots (table 2).

A linear regression analysis between Shannon index (based on the trees’ basal area) and C\(_{org}\) concentration of the clay and fine silt fraction revealed a close positive relationship in the E horizon reflecting the higher enrichment factors in the mixed stands, whereas no significant relationships were detected in the A horizon (figure 2).

4. Discussion

4.1. Carbon storage

Total stocks of SOC in the 0–30 cm profile were similar to those reported by Guckland et al. (2009) for the Hainich forest. Both studies agree that the SOC stocks were higher in mixed stands (DL2, DL3) than in the monospecific beech stands (DL1, table 2). Despite general agreement, the SOC stocks in the topsoil horizon of our study were slightly higher than

| Thickness (cm) | pH (H\(_2\)O) | SOC concentration (g kg\(^{-1}\)) | SOC stock (kg m\(^{-2}\)) | C/N ratio |
|---------------|-------------|---------------------------------|---------------------------|-----------|
| A horizon     |             |                                 |                           |           |
| DL1           | 5 (0.3)\(^a\) | 4.4 (0.06)\(^a\)              | 47.5 (3.8)\(^a\)          | 2.6 (0.2)\(^a\) | 15.5 (0.5)\(^b\) |
| DL2           | 7 (0.2)\(^a\) | 4.9 (0.05)\(^b\)              | 48.5 (4.2)\(^a\)          | 3.6 (0.2)\(^a\) | 13.0 (0.2)\(^a\) |
| DL3           | 10 (0.2)\(^b\) | 5.7 (0.10)\(^c\)             | 53.1 (4.4)\(^b\)          | 6.5 (0.7)\(^b\) | 14.4 (0.7)\(^b\) |
| E horizon     |             |                                 |                           |           |
| DL1           | 25 (0.3)\(^b\) | 4.6 (0.09)\(^a\)             | 7.4 (0.4)\(^a\)           | 2.4 (0.1)\(^a\) | 15.60 (0.5)\(^b\) |
| DL2           | 23 (0.2)\(^b\) | 5.1 (0.13)\(^ab\)            | 14.6 (1.4)\(^b\)          | 4.7 (0.4)\(^c\) | 10.34 (0.2)\(^a\) |
| DL3           | 20 (0.2)\(^a\) | 6.0 (0.14)\(^b\)             | 13.6 (1.2)\(^b\)          | 3.5 (0.4)\(^b\) | 12.14 (0.6)\(^a\) |
Table 2. Particle size distribution and carbon storage in the particle size fractions for the A and E horizons of soil samples taken from plots with different diversity levels (DL1, DL2, DL3). (Note: means and standard errors in brackets, small letters indicate significant differences between diversity levels \((p < 0.05), n = 3\); the values increase with from the letter ‘a’ to ‘c’. Note that throughout the text the abbreviation ‘C\(_{org}\)’ is used for C concentrations of particle size fractions, whereas ‘SOC’ is related to bulk soil C stocks or concentrations.)

|       | A horizon |       |       |       |       |       |       |
|-------|-----------|-------|-------|-------|-------|-------|-------|
|       | Sa 2000–63 \(\mu\)m | cSi 63–6.3 \(\mu\)m | fSi 6.3–2 \(\mu\)m | Cl < 2 \(\mu\)m | Sa 2000–63 \(\mu\)m | cSi 63–6.3 \(\mu\)m | fSi 6.3–2 \(\mu\)m | Cl < 2 \(\mu\)m |
| DL1   | 55 (3.5)\(b\) | 703 (6.0)\(b\) | 123 (4.1)\(a\) | 119 (1.7)\(a\) | 30 (1.5)\(b\) | 687 (4.9)\(b\) | 127 (2.7)\(a\) | 156 (2.8)\(a\) |
| DL2   | 35 (1.5)\(a\) | 563 (43.9)\(a\) | 171 (17.6)\(b\) | 232 (26.7)\(b\) | 23 (1.0)\(a\) | 533 (49.6)\(a\) | 163 (7.9)\(b\) | 282 (43.0)\(b\) |
| DL3   | 40 (3.2)\(a\) | 586 (26.1)\(a\) | 155 (7.4)\(b\) | 218 (16.5)\(b\) | 23 (1.0)\(a\) | 572 (24.5)\(a\) | 148 (7.2)\(b\) | 256 (17.7)\(b\) |

|       | Proportion of C\(_{org}\) of particle size classes on total SOC |       |       |       |       |       |
|-------|---------------------------------------------------------------|-------|-------|-------|-------|-------|
| DL1   | 23 (1.1)\(b\) | 29 (1.9)\(a\) | 29 (1.0)\(a\) | 20 (0.9)\(a\) | 13 (0.7)\(b\) | 17 (0.6)\(a\) | 34 (0.3)\(a\) | 37 (1.3)\(a\) |
| DL2   | 17 (1.4)\(a\) | 28 (4.0)\(a\) | 30 (3.9)\(a\) | 24 (2.0)\(b\) | 8 (1.0)\(a\) | 14 (2.3)\(a\) | 34 (1.9)\(a\) | 44 (2.5)\(b\) |
| DL3   | 17 (0.7)\(a\) | 25 (1.5)\(a\) | 31 (0.8)\(a\) | 26 (0.8)\(b\) | 8 (0.6)\(a\) | 13 (1.4)\(a\) | 33 (0.5)\(a\) | 46 (1.1)\(b\) |

|       | C\(_{org}\) concentration of particle size classes |       |       |       |       |       |
|-------|---------------------------------------------------|-------|-------|-------|-------|-------|
| DL1   | 167 (9.4)\(a\) | 17 (2.2)\(a\) | 97 (7.5)\(a\) | 69 (5.6)\(b\) | 33 (3.1)\(a\) | 2 (0.1)\(a\) | 19 (0.9)\(a\) | 17 (0.8)\(a\) |
| DL2   | 201 (8.4)\(b\) | 23 (3.8)\(a\) | 78 (9.0)\(a\) | 47 (3.7)\(a\) | 48 (3.1)\(b\) | 4 (0.7)\(b\) | 27 (0.9)\(b\) | 24 (0.8)\(b\) |
| DL3   | 213 (8.2)\(b\) | 21 (3.0)\(a\) | 97 (3.9)\(a\) | 59 (1.1)\(b\) | 46 (3.0)\(b\) | 3 (0.8)\(b\) | 29 (1.2)\(b\) | 24 (0.6)\(b\) |
Figure 1. Carbon enrichment factors ($EF_c$, $C_{org}$ concentration fraction/SOC concentration bulk soil) in relation to the relative abundance of (a) the fine silt fraction (b) the clay fraction in the soil of the A horizon, (c) the fine silt fraction, and (d) the clay fraction of the E horizon. Own data: data points ($n = 27$) indicate plots of the three different diversity levels (white = DL1, grey = DL2, black = DL3). Reference: own data related to the exponential fit of Schulten and Leinweber (2000).

Figure 1. Carbon enrichment factors ($EF_c$, $C_{org}$ concentration fraction/SOC concentration bulk soil) in relation to the relative abundance of (a) the fine silt fraction (b) the clay fraction in the soil of the A horizon, (c) the fine silt fraction, and (d) the clay fraction of the E horizon. Own data: data points ($n = 27$) indicate plots of the three different diversity levels (white = DL1, grey = DL2, black = DL3). Reference: own data related to the exponential fit of Schulten and Leinweber (2000).

4.2. Soil texture effects

Despite applying a different approach than Guckland et al (2009), we confirmed that the higher SOC stocks under the more species-rich DL2 and DL3 stands were caused by the higher abundance of the clay and fine silt fractions, especially in the E horizon. This effect relates to the specific surface area which increases from large to small particles and thus enhances SOC interactions with small-sized soil mineral particles (Baldock and Skjemstad 2000, Ransom et al 1998) as was found in a broad range of soil types (Korschens et al 1998).

Our results further show that the $EF_c$ value decreases exponentially with increasing clay or fine silt contents, probably due to a higher amount of unoccupied reactive surfaces with an increase of the clay and silt contents (Schulten and Leinweber 2000). Amelung et al (1998) and Schulten and Leinweber (2000) observed a similar negative exponential relationship between $EF_c$ and clay content. Furthermore, the
EFₐ values were higher in subsoil than in topsoil, pointing to a higher relative importance of mineral-associated SOC in subsoil horizons. However, our results indicate that biotic factors also contribute to the observed increase in the SOC stocks from the monospecific to the mixed stands, as will be elaborated in the next section 3.

4.3. Causes of the elevated SOC stocks under the mixed stands

In contrast with SOC concentration, the SOC stocks in the A horizon increased from the pure to the mixed plots. The main reason for the increase was an increase in the thickness of the A horizon, which likely is a consequence of the slower turnover of beech leaf litter as compared to the other broad-leaved species present (Jacob et al. 2010b, Vesterdal et al. 2008), slowing humification and the downward transport of organic matter through bioturbation and percolating water (Augusto et al. 2002, Langenbruch et al. 2011, Reich et al. 2005). This explanation is corroborated by the higher C stocks in the O layers found in pure beech stands (ca. 0.9 kg m⁻²) in the Hainich forest as compared to the mixed stands (ca. 0.4 kg m⁻²; Guckland et al. 2009); similar patterns were reported from studies in other temperate forests (e.g. Vesterdal et al. 2008). It should be mentioned that the differentiation between the lowermost organic layer and the A horizon in the mineral topsoil is not always distinct in the Hainich forest which introduces a certain bias in the analysis of SOC stock differences, as was also reported for other locations (Don et al. 2012).

When applying the C saturation model (Heitkamp et al. 2012b, Stewart et al. 2007) to the Hainich forest, the highest Cₐ concentrations in the clay and fine silt fraction should occur in the species-poor DL1 stands, where the clay and fine silt contents were lowest. The reason for this expectation is the notion that small-sized particles have a finite capacity for sorption of organic matter. When the saturation deficit is small, i.e. a high proportion of sorption sites is occupied, further C input (under steady-state assumptions) will increase the C concentration to a smaller extent, because less C will
be stabilized by organo-mineral interactions. In soils with the same mineralogy, as it is the case in our study, $C_{\text{org}}$ concentrations in the fine fractions should therefore be very similar, when SOC concentrations approach saturation (Six et al 2002, Stewart et al 2007). This appears to be valid in the A horizon, where a weak tendency toward slightly higher $C_{\text{org}}$ concentrations in the clay and silt fraction in the DL1 stands was detected (table 2), probably caused by the lower clay content in these stands. The small differences to the species-richer DL2 and DL3 stands were expected, because the SOC content and the C input were high in the A horizon. Thus, the level of organic C in the soil is probably near saturation in these stands (Six et al 2002, Stewart et al 2007) and the sorbate concentrations on the mineral surfaces must be high, irrespective of any biotic factors that could have an effect on sorption processes.

In a similar manner as for the A horizon, we assumed that the $C_{\text{org}}$ concentration of the clay and fine silt fractions was rather high in the E horizon of the DL1 stands because it is relatively clay-poor. However, in contradiction to the C saturation model, the $C_{\text{org}}$ concentrations in the clay and fine silt fraction were significantly higher in the E horizon in the two mixed stand categories (DL2, DL3) than in DL1. This strongly implies that the higher SOC pools in the mixed stands are not only a consequence of the higher mass proportions of clay and fine silt in the soil (soil texture effect) but are also caused by the higher $C_{\text{org}}$ concentrations in the clay and fine silt fraction in these stands. This is indicative for a tree species identity or a species diversity effect on soil carbon storage in this broad-leaved forest. A given amount of clay in the E horizon contained $\sim 30\%$ less $C_{\text{org}}$ per mass in the DL1 stands than in the species-richer DL2 and DL3 stands. In the fine silt fraction, the difference was 30–35%. Thus, it appears that higher species richness, a dilution of beech abundance or the presence of lime, ash, hornbeam or maple, resulted in higher $C_{\text{org}}$ concentrations in the clay and fine silt fractions of the subsoil of the mixed stands, which caused higher SOC stocks in this horizon. Unfortunately, our study design with a dilution gradient of beech abundance cannot distinguish between a possible species diversity effect, i.e. an effect of tree species numbers, and a species identity effect, which is related to specific traits of certain species and their possible influence on SOC stabilization. Nevertheless, our finding of an apparently higher C stabilization potential in the subsoil of mixed forests than in pure beech stands may have important implications for forest management and its role in mitigating global warming.

The causal explanation of a species identity or diversity effect on SOC storage must remain mostly speculative. Whereas the higher SOC stocks in the A horizon of the mixed stands due to greater horizon thickness can probably be explained by reduced decomposition and bioturbation in the beech-dominated DL1 stands as compared to the more diverse stands, explanations for the higher SOC contents in the subsoil remain somewhat elusive. Especially in the subsoil, in situ processes and mechanisms of C stabilization remain poorly understood (Rumpel and Kögel-Knabner 2011). Comparative SOC inventories under stands on similar bedrock evidence considerable tree species differences in the size of SOC pools. For example, Leuschner et al (2013) found by 30% higher SOC pools under beech than under pine forests on similar sandy soils in northern Germany. We speculate that species differences in SOC storage could exist also between beech, lime, maple, ash and hornbeam in the Hainich forest. Possible mechanisms of tree species effects on SOC stabilization include: (1) the amount of sorptive transferred into the subsoil is lower under beech than in mixed stands because less bioturbation of and less transport of dissolved organic C does occur (Augusto et al 2002, Reich et al 2005), (2) the properties of the sorptive derived from different tree species are different because the chemical composition of leaf and root litter and root exudates may differ among the species (Cesarz et al 2013), or (3) the functional traits of the microbial community differ considerably (Thoms et al 2010, Thoms and Gleixner 2013). Thoms et al (2010) showed that amounts of total phospholipid fatty acids (PLFA) in 0–20 cm soil depths increased with decreasing abundance of beech from DL1 to DL3. Moreover, PLFA markers for mycorrhizal fungi were significantly more abundant in DL3 stands. A key role might be played by fine root turnover, which is an important source of SOC (Rumpel and Kögel-Knabner 2011) and may be related to the above-mentioned points. Detailed studies on fine root biomass and root turnover in the plots of this study by Meinen et al (2009) and Jacob et al (2013 and unpubl. results) showed that fine root turnover is significantly higher in the DL2 and DL3 stands than in the DL1 stands, even though standing fine root biomass was not different. Thus, the supply of dead root mass should be higher in the mixed stands which could well contribute to higher $C_{\text{org}}$ concentrations in the fine particle fraction. Tree species differences in the amount and composition of rhizodeposition (Cesarz et al 2013) and in the decomposition rate of leaf litter (Jacob et al 2010b) might also underlie a tree species effect on SOC storage. Irrespective of the responsible mechanisms, the assumed higher SOC storage in the subsoil of mixed broad-leaved forests may be of particular relevance because the organo-mineral complexes in deeper horizons are typically more stable than in topsoil horizons.

Although we cannot completely explain the exact mechanisms and processes underlying our finding, it has important implications for forest management in the context of climate change adaptation and mitigation by soil carbon gains or at least SOC preservation. First, subsoil can serve as an important C sink due to the generally low degree of C saturation. Second, subsoil SOC is known to be more effectively stabilized as compared to topsoil or litter layer C (Rumpel and Kögel-Knabner 2011). Therefore, potential losses of SOC from subsoil induced by warming will lag in time and provide a temporal buffer. Since SOC increases soil aggregation and water holding capacity, higher SOC contents in the subsoil might mitigate the effects of future drought events in mixed forests as compared to monospecific beech stands.

5. Conclusion

For the first time, we could show that mixed beech forests are superior with respect to $C_{\text{org}}$ stabilization in the clay and
fine silt fractions of the subsoil as compared to monospecific beech forests, pointing at a pronounced species identity and/or diversity effect on soil carbon storage in an old-growth forest with near steady-state soil conditions. Clearly, the main effect leading to almost two-times higher SOC stocks is caused by differences in texture, but an additional effect of species identity and/or diversity is evident by means of particle size fractionations and the SOC saturation model. Supplemental experiments under controlled conditions have to clarify possible underlying mechanisms of species identity and diversity effects on SOC pools. To underpin our findings on the positive effect of mixed forests (in comparison to pure beech stands), we suggest considering SOC quality in further studies, e.g., the distribution of SOC among pools of different stability. This will be particularly important when focussing on the subsoil, where differences in SOC composition may considerably affect the C stabilization or destabilisation process by interaction with small-sized particle size fractions.

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