The linkage of ^{13}C and ^{15}N soil depth gradients with C:N and O:C stoichiometry reveals tree species effects on organic matter turnover in soil

Marcel Lorenz · Delphine Derrien · Bernd Zeller · Thomas Udelhoven ·
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Abstract The knowledge of tree species dependent turnover of soil organic matter (SOM) is limited, yet required to understand the carbon sequestration function of forest soil. We combined investigations of ^{13}C and ^{15}N and its relationship to elemental stoichiometry along soil depth gradients in 35-year old monocultural stands of Douglas fir (*Pseudotsuga menziesii*), black pine (*Pinus nigra*), European beech (*Fagus sylvatica*) and red oak (*Quercus rubra*) growing on a uniform post-mining soil. We investigated the natural abundance of ^{13}C and ^{15}N and the carbon:nitrogen (C:N) and oxygen:carbon (O:C) stoichiometry of litterfall and fine roots as well as SOM in the forest floor and mineral soil. Tree species had a significant effect on SOM δ^{13}C and δ^{15}N reflecting significantly different signatures of litterfall and root inputs. Throughout the soil profile, δ^{13}C and δ^{15}N were significantly related to the C:N and O:C ratio which indicates that isotope enrichment with soil depth is linked to the turnover of organic matter (OM). Significantly higher turnover of OM in soils under deciduous tree species depended to 46% on the quality of litterfall and root inputs (N content, C:N, O:C ratio), and the initial isotopic signatures of litterfall. Hence, SOM composition and turnover also depends on additional—presumably microbial driven—factors. The enrichment of ^{15}N with soil depth was generally linked to ^{13}C. In soils under pine, however, with limited N and C availability, the enrichment of ^{15}N was decoupled from ^{13}C. This suggests that transformation pathways depend on litter quality of tree species.

Keywords Stable isotopes · Microbial turnover · Litter · Roots · Common garden experiment · Recultivated forest soil

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Introduction

Soils in forest ecosystems bear a high potential as carbon (C) sinks in the mitigation of climate change (Pan et al. 2011). Tree species identity plays a crucial role in the C cycle of these ecosystems, e.g. by fueling soil with bio- and necromass, respectively (Augusto et al. 2015). The total amount of C that is stored in the forest floor and mineral soil is affected by the dominating tree species (Mueller et al. 2015). Furthermore, stoichiometric ratios like the carbon:nitrogen (C:N) ratio of soil organic matter (SOM) are influenced by the forest stand (Cools et al. 2014; Lorenz and Thiele-Bruhn 2019). Ecological stoichiometry using elemental ratios is a suitable tool to assess SOM and its turnover (Manzoni et al. 2010; Zechmeister-Boltenstern et al. 2015). For this, the C:N ratio is commonly used (Stevenson 1994). Also the oxygen:carbon (O:C) ratio bears high potential to characterize SOM because it reflects the state of oxidation of SOM (Beyer et al. 1998). Furthermore, the abundance of stable isotopes (^{13}C and ^{15}N) in soils also provide a powerful tool for investigating spatial and temporal SOM dynamics (Ehleringer et al. 2000; Brüggemann et al. 2011; Craine et al. 2015). Variations in the isotopic composition are useful for tracing carbon sources and fluxes between plants, microorganisms and soils, thus serving to elucidate the impact of plant inputs on SOM formation (Balesdent et al. 1987). A combination of both approaches, ecological stoichiometry and stable isotopes, in soil depth gradients promises to get deeper insights into the turnover of SOM. To our knowledge, this has not been used before to characterize the turnover of tree species dependent organic matter (OM) in the soil.

Typically, ^{13}C and ^{15}N show trends of enrichment with increasing soil depth that were related to aging and turnover of OM (Nadelhoffer and Fry 1988; Billings and Richter 2006; Trumbore 2009). Several SOM turnover and stabilization mechanisms were identified that can lead to a variation of the natural abundance of ^{13}C. Litter with lower $\delta^{13}C$ values from aboveground plant materials triggers the topsoil, while the contribution of ^{13}C-enriched root inputs to SOM $\delta^{13}C$ increases with increasing soil depth (Bird et al. 2003). Root inputs encompass C release from plant roots to soil including: (1) root cap and border cell loss, (2) necromass from root cells and tissues, (3) C flow to root-associated, soil living symbionts (e.g.

mycorrhiza), (4) gaseous losses, (5) root exudates, and (6) mucilage (Jones et al. 2009). During the microbial metabolism of C, preferentially ^{13}C-depleted molecules will be respired by microorganisms and the remaining SOM will be ^{13}C-enriched (Lerch et al. 2011). In general, microorganisms are ^{13}C-enriched compared to plant material or bulk SOM (Dijkstra et al. 2006) and the contribution of microbial derived C increases with the extent of OM turnover (Boström et al. 2007). Additionally, OM associated with soil minerals is characterized by increased $\delta^{13}C$ values compared to free or occluded light OM fractions (Schrumpf et al. 2013). The association of OM with minerals is an important mechanism for its stabilization in soil (von Lützow et al. 2007). The prevalence of SOC decrease and $\delta^{13}C$ increase with depth in well-drained forest soils has prompted the use of the gradient of SOC plotted against $\delta^{13}C$ as a proxy for SOM turnover (Acton et al. 2013). Consequently, depth-related interconnection of $\delta^{13}C$ and SOC describes the rate of change in ^{13}C natural abundance along a decay continuum from fresh litter inputs to more decomposed SOM (Garten et al. 2000).

The absolute enrichment of ^{15}N over soil depth can be determined as the difference between the maximum enrichment of ^{15}N in the mineral soil and the litter bearing OL horizon (Hobbie and Ouimette 2009). The development of ^{15}N with soil depth is related to N cycling processes that are coupled to SOM turnover (Emmett et al. 1998). Similar to $\delta^{13}C$ values, organo-mineral associations (Kramer et al. 2017) and the accumulation of ^{15}N enriched microbial biomass in more transformed SOM (Wallander et al. 2009) can drive the $\delta^{15}N$ patterns within soil. Furthermore, the type and degree of mycorrhizal associations (Hobbie and Högberg 2012), enzymatic hydrolysis (Silfer et al. 1992), N losses after ammonification, nitrification and denitrification (Högberg 1997; Pörtl et al. 2007), atmospheric depositions (Vallano and Sparks 2013) and mixing of soil N through bioturbation (Wilske et al. 2015) contributes to the ^{15}N enrichments along the soil profile. Both $\delta^{13}C$ and $\delta^{15}N$ are mechanistically linked through the decomposition and microbial processing of SOM (Nel et al. 2018), thus highlighting the suitability of both parameters to determine the degree of organic matter turnover in soil.

In natural mixed forest ecosystems it is difficult to track down a tree species effect on SOM status; therefore common garden experiments, where

different tree species were planted in adjacent blocks at the same time on similar soil, were established to study tree species effects (Reich et al. 2005; Vesterdal et al. 2013). Important insights into the relationship between tree species and the cycling of soil C and other nutrients in forest ecosystems were gained from common garden experiments (e.g. Mueller et al. 2012; Gurmesa et al. 2013). Nevertheless, such experiments are often handicapped by a previous land-use conversion, e.g. from arable land or from clear felled forests (Vesterdal et al. 2008). Old SOM from former land-use types often makes the interpretation of the effects of tested species and their SOM on SOM dynamics rather difficult (Balesdent et al. 2018). Callesen et al. (2013) revealed in a common garden experiment that the patterns of $\delta^{15}N$ in soil profiles reflected the former arable land-use type. Comparable with common garden experiments, differently afforested soils at post-mining sites provide a unique opportunity for understanding mechanisms in SOM formation (Frouz et al. 2009). In particular, sites that are free from old C sources can be suitable but investigations on such sites are rare. Due to this, further research is required to clarify if $\delta^{13}C$ and $\delta^{15}N$, and thus the SOM status in organic forest floor horizons (litter—OL, fragmented—OF, humified—OH) and mineral soil differs between tree species.

This research was conducted on a post-mining site, where previous accumulation of plant or coal material are negligible (Lorenz & Thiele-Bruhn 2019). We studied monocultural stands of Douglas fir (*Pseudotsuga menziesii*), black pine (*Pinus nigra*), European beech (*Fagus sylvatica*) and red oak (*Quercus rubra*) that were grown for 35 years under identical soil and geomorphological conditions to assess tree species effects on the SOM status. In more detail, we investigated the natural abundance of ^{13}C and ^{15}N in combination with C:N and O:C stoichiometry of litterfall and root inputs (determined as belowground phytomass) as well as SOM in depth gradients of forest soils to answer the following questions:

(1) Do litterfall and root inputs differ in their isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$ between tree species?

(2) Is there a tree species effect on $\delta^{13}C$ and $\delta^{15}N$ in the depth gradients starting from the OL horizon down to 10–30 cm of mineral soil?

(3) Are stable isotope contents in the depth profiles related to the stoichiometry (C:N and O:C ratio) of the bulk soil?

(4) Varies the decomposition of OM and the stabilization of it in soil significantly between tree species and if yes, are litterfall and/or root properties important for these processes?

Materials and methods

Study site

The study was conducted at the afforested spoil heap 'Sophienhöhe', located in the northwest of the lignite open-cast mine 'Hambach' in the Rhineland, Germany (N 50° 56.11′, E 6° 26.56′). There, boundary conditions regarding soil, climate, topography and management were highly similar, equivalent to a common garden experiment. The Regosols at the investigated sites developed on the same sandy gravelly parent material (Lorenz and Thiele-Bruhn 2019). The carbonate-free parent material that was used for the spoil heap recultivation had a C content of $0.20 \pm 0.05\%$ and a C/N molar ratio of 7.5 ± 1.2 (Table S1). Therefore, a relevant impact of old or fossil carbon from former land use types and the introduction of coal from lignite mining was excluded (Lorenz and Thiele-Bruhn 2019). The investigation was carried out in monocultural stands of Douglas fir (*Pseudotsuga menziesii*), black pine (*Pinus nigra*), European beech (*Fagus sylvatica*) and red oak (*Quercus rubra*) that were afforested in 1982 on the western slopes of the spoil heap. Within 35 years after the start of the afforestation organic layers had developed that were classified as Moder (Zanella et al. 2018) with slight differences between tree stands. Dependent on the thickness of the OH layer, Dysmoder was the dominant humus form that coexisted in some patchy sections with Eumoder under Douglas fir, beech and oak, while under pine solely Dysmoder had developed (Lorenz and Thiele-Bruhn 2019).

Sampling scheme and sample preparation

Each species stand is subdivided in six to ten plots with a size of 1780 ± 660 m^2 by skid trails established in slope line. For each of the four stands, five plots were

selected for sampling of the forest floor, mineral soil, roots and litterfall (Fig. 1). Sampling points (light grey circles) within one plot were located at least 25 m away from forest roads and in the upper parts of the middle slopes. In April 2016, forest floor samples were taken with a steel frame (20 cm × 20 cm) and carefully separated into the organic litter, fragmented, and humified horizon, OL, OF and OH, respectively, according to Zanella et al. (2018). Afterwards, bulk soil samples were taken at three different depths (0–5 cm, 5–10 cm, 10–30 cm) from excavated 50 cm × 50 cm × 50 cm pits. To ensure representativeness, forest floor and soil samples (grey rectangles) were taken from four positions and samples from similar depths were subsequently pooled. In total 60 forest floor samples and 60 mineral soil samples were collected (five per depth in each stand, Table S2), transported and stored at 4 °C for further preparation. Forest floor samples were dried at 60 °C and visible roots were carefully sorted out. Mineral soil samples were passed through a 2 mm sieve, roots were removed and the soil samples were dried at 60 °C. All samples were ground and homogenized using a ball mill (Retsch MM400, Retsch GmbH, Haan, Germany).

We performed root sampling 2 years later, in April 2018 and distinguished roots from different horizons.

To do so, we collected five replicate samples of forest floor and mineral soil (dark grey circles in Fig. 1) with a distance of 1 m around a tree within each of the five plots per species stand. Forest floor roots were collected using a steel frame (20 cm × 20 cm). Underneath, mineral soil roots were collected using a root auger with a diameter of 8 cm (Eijkelkamp Soil & Water, Giesbeck, Netherlands). The cores of mineral soil were divided into the three subsamples (0–5 cm, 5–10 cm. 10–30 cm). In total, we collected 400 root samples (25 per depth in each species stand, Table S2) that were transported and stored at 4 °C. In the laboratory, the forest floor samples were spread out in plastic bowls and roots were carefully separated using a tweezer. The roots were carefully washed to remove adherent soil particles. The mineral soil samples were put into plastic bowls and immediately washed with water to separate roots. Roots with a diameter ≤ 5 mm were dried at 105 °C to determine dry weights and a subset of 80 samples (five per depth per species) was homogenized using a ball mill (Retsch MM400, Retsch GmbH, Haan, Germany) for further chemical analysis.

In each of the five plots per species stand the litterfall was collected using litter traps made with nylon mesh (0.5 mm mesh size) that was fixed on a wooden frame (1 m × 1 m). Litter traps were
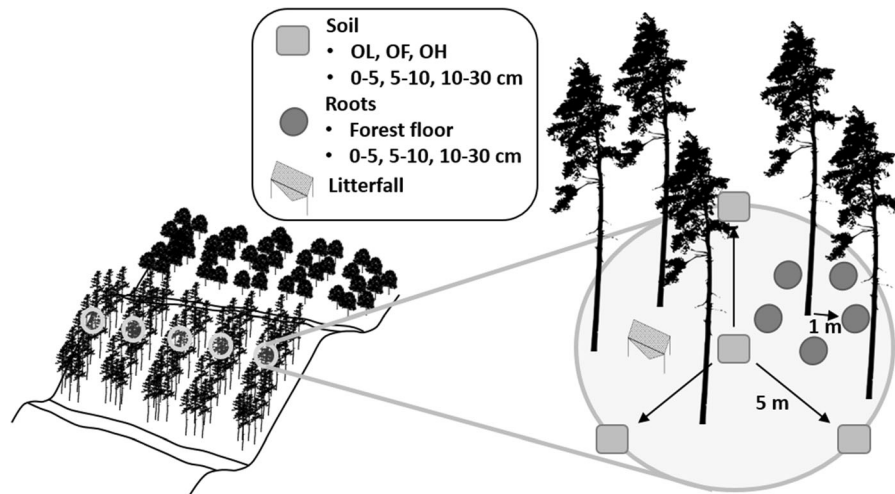

Fig. 1 Sampling design at the study site 'Sophienhöhe'. 35 year-old afforested monocultural stands of Douglas fir (*Pseudotsuga menziesii*), black pine (*Pinus nigra*), European beech (*Fagus sylvatica*) and red oak (*Quercus rubra*) were investigated. They are located on the western exposed slopes (inclination: 22.2° ± 2.2°) of the spoil heap. Each stand is subdivided in six to ten plots with a size of 1780 ± 660 m² by skid trails established in slope line. For each of the four tree stands, five plots were selected for sampling (light grey circles) of the forest floor (grey rectangles), mineral soil (grey rectangles), roots (dark grey circles) and litterfall (litter trap)

installed 1 m above the soil surface and located in the plot near the central soil sampling point (Fig. 1). In the timespan from July 2016 to June 2017 the litter traps were monthly emptied. In the laboratory, the 240 samples were immediately separated into foliar and non-foliar fractions and dried at 60 °C to determine dry weights (Ukonmaanaho et al. 2016). The foliar fraction of the 12 monthly samples of each litter trap were pooled into one mixed sample. Consequently, five litterfall samples per tree species resulted in a total number of 20 litterfall samples (Table S2) that were homogenized using a ball mill (Retsch MM400, Retsch GmbH, Haan, Germany) for further chemical analysis.

Laboratory analysis

Total contents of C, N and O were determined using an Elemental Analyser EA3000 (HEKAtech GmbH, Wegberg, Germany). Soil samples were acidic and free of carbonate (Lorenz and Thiele-Bruhn 2019), thus the measured total C content represents organic C. The contents of the elements were used to calculate the molar C:N and O:C ratios. The stable isotopes ^{13}C and ^{15}N were determined by an IsoPrime 100 isotope ratio mass-spectrometer (IsoPrime Corporation, Cheadle, UK) and vario ISOTOPE cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Stable isotope compositions are reported in delta notation (δ^{13}C ‰ and δ^{15}N ‰) relative to Vienna Pee-Dee Belemnite (VPDB) for C, using the international reference materials IAEA-CH-7 ($-$ 32.151‰ VPDB SD $\pm$ 0.05‰) as a standard, and relative to atmospheric N_2 for N, using IAEA-N-1 ($+$ 0.4‰ air N2 SD $\pm$ 0.2‰), IAEA-N-2 ($+$ 20.3‰ air N2 SD $\pm$ 0.2‰) and USGS32 ($+$ 180‰ air N2 SD $\pm$ 1‰) as standard according to Eq. (1):

$$\delta_{sample} = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \cdot 1000, \tag{1}$$

where R represents the ratio of ^{13}C/^{12}C or ^{15}N/^{14}N, respectively. The measurement error of δ^{15}N was approximately 0.2‰ and < 0.1‰ for δ^{13}C.

Data processing and statistics

The relationship between the prevalent vertical decrease of SOC and increase of δ^{13}C in depth profiles was used as a natural indicator of SOC turnover (Acton et al. 2013). The slope (β) of the linear regression ($y = a + \beta x$) between the mean δ^{13}C values and their respective log-transformed C concentrations (mg C g^{-1}) was calculated and is referred as $\beta_{\delta13C}$ value. The distribution of ^{15}N along soil depth profiles was compared between tree stands using the soil enrichment factor ($\varepsilon_{soil}{}^{15}$N). It is defined as absolute enrichment between the OL horizon and the 10–30 cm mineral soil layer (Hobbie and Ouimette 2009) and was calculated following Eq. (2):

$$\varepsilon_{soil}{}^{15}N(‰) = \delta^{15}N_{10-30cm} - \delta^{15}N_{OL}. \tag{2}$$

The following statistical analyses were conducted separately for litter inputs (litterfall and roots) and each depth starting from the OL horizon down to 10–30 cm with the R statistical package version 3.3.2. (R Core Team 2016). Boxplots and one-way analysis variance (ANOVA) as pretests were carried out to inspect the data structure. The residuals of ANOVA were tested for normality and homoscedasticity using the Shapiro–Wilk test respectively Levene's test. Accordingly, normal distributed and homoscedastic data were tested for significant differences between tree species by one-way ANOVA followed by the Tukey's honest significant difference (HSD) post hoc test. Significant differences between tree species for normal distributed but heteroscedastic data were tested using Welch-ANOVA followed by a pairwise t test with Bonferroni–Holm correction. In case data was not normal distributed but homoscedastic the Kruskal–Wallis test was applied followed by the Dunn test. Variance analyses and necessary pretests were performed with a significance level of $p < 0.05$. The results are presented in arithmetic mean $\pm$ standard deviation (SD) for the different tree stands. To characterize relationships between isotopic (δ^{13}C, δ^{15}N,) and stoichiometric (C:N, O:C) parameters regression analyses with linear and logarithmic functions were done. Additionally, multiple linear regression models were generated to analyze explaining variables for $\beta_{\delta13C}$ and $\varepsilon_{soil}{}^{15}$N values. For this purpose, C, N, C:N, O:C, δ^{13}C, δ^{15}N, as well as the biomass of litterfall and roots were used as independent variables. To simplify the complexity of the model, parameters were stepwise eliminated that decrease the quality of the regression model by assessing R^2 and p values. Finally, the most

appropriate models that comprise only parameters with significant portions of the explainable variance were used to discuss driving factors for $\beta_{\delta13C}$ and $\varepsilon_{soil}{}^{15}N$ values. The results of the regression analyses were described as significant in cases where $p < 0.05$.

Results

Litter inputs

The total annual foliar litterfall in pine stands was significantly higher compared to beech stands, whereas under Douglas fir and oak intermediate amounts were reached (Table 1). The ^{13}C content of litterfall were significantly highest in Douglas fir stands and declined in the sequence Douglas fir > pine ≥ oak > beech. Also, $\delta^{15}N$ of litterfall was significantly higher in Douglas fir stands compared to the other tree species. The litterfall of coniferous species was characterized by significantly higher C:N ratios compared to deciduous forest stands. The O:C ratio of litterfall decreased in the order oak > beech > pine > Douglas fir.

In contrast to the litterfall, substantially and in part significantly higher root biomasses were detected in the upper 30 cm of soil under deciduous tree species than under conifers (Table 1). In general, the roots of all tree species were significantly enriched in ^{13}C by $4.56 \pm 0.99‰$ and ^{15}N by $3.01 \pm 0.61‰$ compared to the litterfall. Douglas fir roots were characterized by significantly higher $\delta^{13}C$ values compared to those of pine and beech, while the significantly lowest $\delta^{13}C$ values were determined in oak roots. Similar differences between tree stands were found for $\delta^{15}N$ of roots as well as for litterfall $\delta^{15}N$. Obviously, the isotopic signatures of roots in the forest floors did not differ from roots in deeper soil horizons but the tree species effect was similarly pronounced in each soil depth. In contrast to the litterfall, the C:N ratio of beech and oak roots was significantly higher compared to the coniferous species (Table S3). The O:C ratio of roots was highest in the oak stand similar to the litterfall.

Depth profiles of bulk soil $\delta^{13}C$ and $\delta^{15}N$ and their relationship to stoichiometry patterns

The parent material for soil recultivation was characterized by a $\delta^{13}C$ value of $-29.69 \pm 0.13‰$ and a $\delta^{15}N$ value of $-0.89 \pm 0.09‰$ (Table S1). In general, with increasing soil depth an enrichment of ^{13}C and ^{15}N was observed, while the contents of C and N decreased (Fig. 2). A small deviation from this pattern occurred for ^{13}C in the forest floor horizons, where a depletion or no significant variation from OL to OH horizon was detected. $\delta^{13}C$ varied in a range from $-29.37‰$ (oak, OF) to $-26.19‰$ (Douglas fir, 10–30 cm). The coniferous species Douglas fir and pine caused significantly higher $\delta^{13}C$ values in the forest floor compared to beech and oak. In the mineral soil lowest $\delta^{13}C$ values were found in the oak stands ($-28.13‰$ to $-27.32‰$), while in the Douglas fir stands highest $\delta^{13}C$ values from $-26.91‰$ to $-26.19‰$ were measured (Table S4). Throughout the soil profile, a significant effect of tree species on $\delta^{13}C$ was detected (Fig. 2b).

Compared to $\delta^{13}C$, $\delta^{15}N$ varied in a wider range from $-6.93‰$ (pine, OL) to $0.54‰$ (beech, 10–30 cm) and depth gradients were more pronounced. The forest floor horizons (OL, OF, OH) of Douglas fir showed significantly higher $\delta^{15}N$ values compared to the other tree species (Fig. 2d). In the first two mineral soil layers (0–5 cm, 5–10 cm) the $\delta^{15}N$ values of all tree species converged, while at a depth from 10 to 30 cm under beech and Douglas fir significantly higher values were determined compared to oak. Consequently, a tree species effect on $\delta^{15}N$ was found in the forest floor as well as the deepest investigated mineral soil layer from 10 to 30 cm.

Regression analyses revealed that $\delta^{13}C$ and $\delta^{15}N$ were related to the C:N and O:C ratio (Table 2). The relationships of these two stoichiometric ratios of the bulk soil were stronger with $\delta^{15}N$ than with $\delta^{13}C$ and were better described by a logarithmic equation rather than by a linear equation (Tables 2 and S5). Along the soil profile from OL to 10–30 cm depth the C:N ratio decreased in a range from 52.8 (oak, OL) to 15.7 (beech, 10–30 cm), while the O:C ratio increased from 0.40 (Douglas fir, pine, OL) to 3.07 (beech, 10–30 cm) (Table S4). With increasing soil depth the decline of C:N was exponentially correlated to an enrichment of ^{15}N (Fig. 3a). Different slopes of the regression lines showed that the relationship between C:N and $\delta^{15}N$ was differently pronounced dependent on the tree species. Similarly close regressions were determined for the relationship between O:C and $\delta^{15}N$ (Fig. 3b). Yet, curves exponentially increased, showing ^{15}N enrichment with increasing O:C ratio.

Table 1 Biomass and isotopic composition of litterfall and root inputs of different tree species, 35 years after afforestation on a post-mining site

		Litterfall	Roots (general)	Roots in			
				Forest floor	0–5 cm	5–10 cm	10–30 cm
Biomass [t ha⁻¹]	Douglas fir	3.59 ± 0.99ab	9.01 ± 1.83ab	0.37 ± 0.19bc	4.23 ± 1.72a	1.38 ± 0.54a	3.04 ± 0.66
	Pine	4.54 ± 0.73b	6.73 ± 1.89a	0.09 ± 0.05a	1.30 ± 0.44a	1.68 ± 1.02a	3.66 ± 1.59
	Beech	3.30 ± 0.34a	18.41 ± 6.88bc	0.61 ± 0.10c	4.75 ± 2.40a	4.29 ± 2.77b	8.76 ± 6.51
	Oak	3.43 ± 0.38ab	22.70 ± 7.22c	0.29 ± 0.18ab	10.66 ± 3.19b	4.59 ± 3.98b	7.16 ± 3.18
	p values	**0.0366**	**0.0011**	**0.0003**	**0.0011**	**0.0169**	0.0797
	n	25	400	100	100	100	100
$\delta^{13}C$ [‰]	Douglas fir	− 31.25 ± 0.11c	− 26.43 ± 0.57c	− 26.14 ± 0.43c	− 26.57 ± 0.55c	− 26.50 ± 0.69c	− 26.52 ± 0.65c
	Pine	− 32.19 ± 0.34b	− 27.49 ± 0.52b	− 27.06 ± 0.21b	− 27.47 ± 0.57b	− 27.61 ± 0.43b	− 27.83 ± 0.56ab
	Beech	− 33.17 ± 0.25a	− 27.63 ± 0.41b	− 27.64 ± 0.43b	− 27.89 ± 0.29b	− 27.67 ± 0.49b	− 27.32 ± 0.28bc
	Oak	− 32.06 ± 0.46b	− 28.89 ± 0.56a	− 28.88 ± 0.58a	− 29.25 ± 0.41a	− 28.90 ± 0.63a	− 28.51 ± 0.50a
	p values	**0.0010**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.0001**	**0.0001**
	n	25	100	25	25	25	25
$\delta^{15}N$ [‰]	Douglas fir	− 5.22 ± 0.65c	− 2.54 ± 1.39c	− 2.19 ± 1.76c	− 2.13 ± 1.62c	− 2.69 ± 1.19b	− 3.18 ± 1.02b
	Pine	− 8.25 ± 0.58ab	− 5.91 ± 0.82b	− 6.05 ± 0.66ab	− 5.45 ± 0.79a	− 6.08 ± 1.01a	− 6.07 ± 0.89a
	Beech	− 7.79 ± 0.22b	− 4.10 ± 1.57b	− 4.89 ± 0.40bc	− 3.65 ± 2.33bc	− 3.19 ± 1.88ab	− 4.66 ± 0.45ab
	Oak	− 8.74 ± 0.37a	− 5.42 ± 0.98a	− 6.31 ± 0.58a	− 4.71 ± 0.73ab	− 5.63 ± 1.06a	− 5.02 ± 0.87a
	p values	**< 0.0001**	**< 0.0001**	**0.0014**	**0.0126**	**0.0052**	**0.0005**
	n	25	100	25	25	25	25

Root properties are given on horizon level as well as sum (biomass) and on average ($\delta^{13}C$, $\delta^{15}N$) of roots in the forest floor and mineral soil, irrespective of soil horizons (*"Roots general"*). Values are mean ± SD. Differences between tree species are marked by different letters. Significant *p* values (< 0.05) are highlighted in bold font style. The total number of samples (n) per parameter and substrate comprises all four tree species
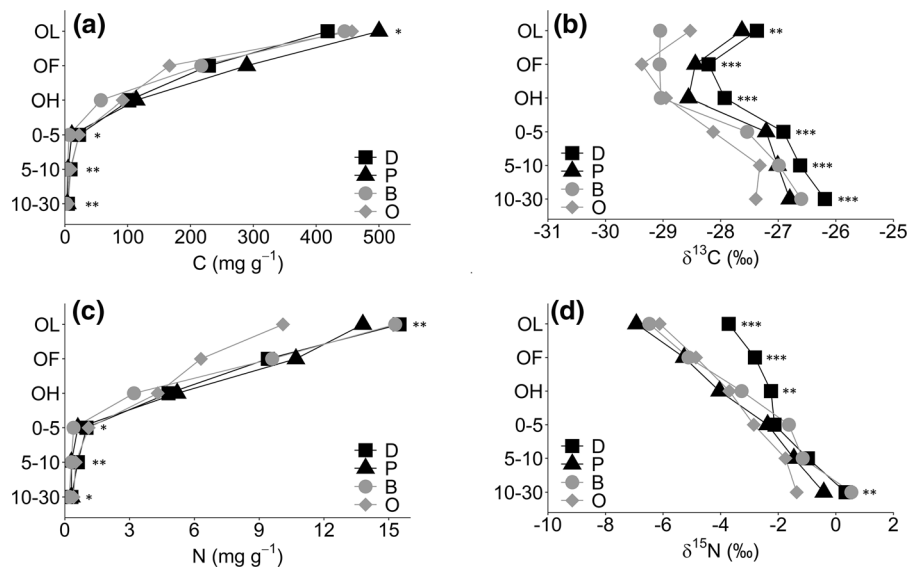
Fig. 2 Depth gradients of C (**a**), δ^{13}C (**b**), N (**c**) and δ^{15}N (**d**) from the OL horizon to 10–30 cm. Coniferous species Douglas fir ("D") and pine ("P") are presented by black symbols and the deciduous species beech ("B") and oak ("O")

by grey symbols. Significant differences between tree species are marked with "*" ($p < 0.05$), "**" ($p < 0.01$), "***" ($p < 0.001$). Detailed information about statistical differences can be found in Table S4

Table 2 Results of the regression analyses between isotopic and stoichiometric ratios

	Function	R^2	*p values*
δ^{13}C vs. C/N			
Douglas fir	y = − 0.181 ln(x) − 1.763	0.38	**0.0003**
Pine	y = − 0.252 ln(x) − 3.745	0.32	**0.0011**
Beech	y = − 0.191 ln(x) − 2.295	0.56	**< 0.0001**
Oak	y = − 0.248 ln(x) − 3.740	0.30	**0.0018**
δ^{13}C vs. O/C			
Douglas fir	y = 0.538 ln(x) + 14.472	0.55	**< 0.0001**
Pine	y = 0.595 ln(x) + 16.336	0.51	**< 0.0001**
Beech	y = 0.550 ln(x) + 15.463	0.76	**< 0.0001**
Oak	y = 0.402 ln(x) + 11.131	0.45	**< 0.0001**
δ^{15}N vs. C/N			
Douglas fir	y = − 0.141 ln(x) + 2.889	0.79	**< 0.0001**
Pine	y = − 0.124 ln(x) + 2.796	0.85	**< 0.0001**
Beech	y = − 0.105 ln(x) + 2.772	0.88	**< 0.0001**
Oak	y = − 0.180 ln(x) + 2..662	0.75	**< 0.0001**
δ^{15}N vs. O/C			
Douglas fir	y = 0.363 ln(x) + 0.521	0.85	**< 0.0001**
Pine	y = 0.234 ln(x) + 0.711	0.86	**< 0.0001**
Beech	y = 0.263 ln(x) + 0.763	0.90	**< 0.0001**
Oak	y = 0.244 ln(x) + 0.602	0.80	**< 0.0001**

Significant *p* values (< 0.05) are highlighted in bold font style

$\beta_{\delta13C}$ values and $\varepsilon_{soil}{}^{15}$N and the contribution of litterfall and root inputs

The $\beta_{\delta13C}$ values for beech (− 1.14) were significantly more negative compared to pine (− 0.64) and Douglas fir (− 0.81) by a factor of 1.8, 1.4 respectively (Fig. 4a). For the oak stands intermediate $\beta_{\delta13C}$ values (− 0.87) were determined. Values for $\varepsilon_{soil}{}^{15}$N differed up to a factor of 1.7 with significantly higher values under beech (7.02‰) and pine (6.52‰) compared to oak (4.76‰) and Douglas fir (4.05‰) (Fig. 4b). Apparently, $\beta_{\delta13C}$ values differed systematically between coniferous and deciduous species, while $\varepsilon_{soil}{}^{15}$N depended more on individual tree species.

Using multiple linear regression analyses, the impact of litterfall and root properties on both indices, $\beta_{\delta13C}$ and $\varepsilon_{soil}{}^{15}$N, was assessed. In total, 49% and 74% of the variability in $\beta_{\delta13C}$ and $\varepsilon_{soil}{}^{15}$N was represented by the explaining variables (Table 3). Higher $\beta_{\delta13C}$ values were associated with litterfall that was characterized by higher δ^{13}C values and lower δ^{15}N values. Furthermore, root C:N played a significant role for $\beta_{\delta13C}$. Litterfall with lower C:N ratios and more negative δ^{15}N values were related to higher $\varepsilon_{soil}{}^{15}$N. Additionally, higher root δ^{13}C values and lower O:C ratios of roots were associated with higher $\varepsilon_{soil}{}^{15}$N.
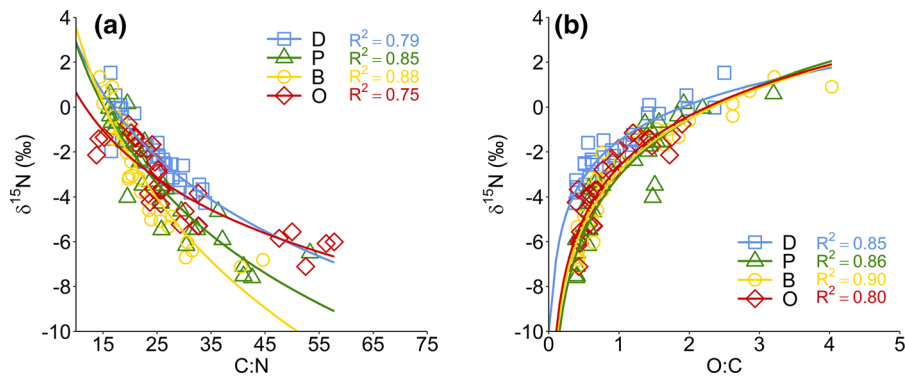
Fig. 3 Relationship between $\delta^{15}N$ and the molar ratios of C:N and O:C. Douglas fir ("D") and pine ("P") are presented by blue and green symbols, beech ("B") and oak ("O") are represented by yellow and red symbols. Detailed information about statistics of the logarithmic and linear relationships can be found in Table 2 and Table S5
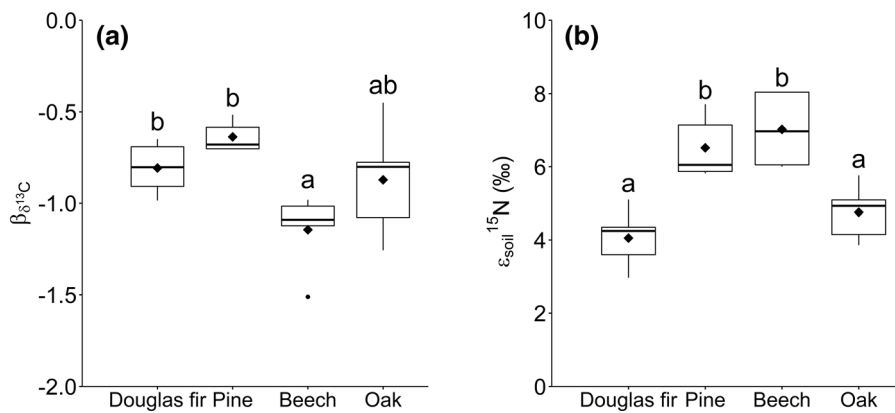

Fig. 4 Boxplots of $\beta_{\delta13C}$ (slopes of the linear regression between $\delta^{13}C$ and log C) (**a**) and $\varepsilon_{soil}^{15}N$ (SOM ^{15}N enrichment from OL to 10–30 cm) (**b**) of investigated forest stands. Black rhombuses represent mean values

Table 3 Final models with explaining variables of the multiple linear regression analysis for the determination of factors influencing $\beta_{\delta13C}$ and $\varepsilon_{soil}^{15}N$

	Model parameter		R^2	p values
$\beta_{\delta13C}$	Litterfall $\delta^{13}C$ + litterfall $\delta^{15}N$ + root C:N		0.46	**0.0046**
$\varepsilon_{soil}^{15}N$	Litterfall C:N + litterfall $\delta^{15}N$ + root O:C + root $\delta^{13}C$		0.74	**< 0.0001**
Proxy	Explaining variables	Coefficients		p values
$\beta_{\delta13C}$	Intercept	5.63		**0.0245**
	Litterfall $\delta^{13}C$	0.21		**0.0131**
	Litterfall $\delta^{15}N$	− 0.09		**0.0337**
	Root C:N	− 0.01		**0.0114**
$\varepsilon_{soil}^{15}N$	Intercept	42.74		**0.0002**
	Litterfall C:N	− 0.04		**0.0361**
	Litterfall $\delta^{15}N$	− 1.14		**< 0.0001**
	Root O:C	− 18.61		**0.0086**
	Root $\delta^{13}C$	1.19		**0.0089**

Significant p values (< 0.05) are highlighted in bold font style

Discussion

Isotopic signatures of litter inputs

In all investigated forest stands roots were consistently enriched in ^{13}C and ^{15}N compared to the litterfall (Table 1). Several post-photosynthetic allocation mechanisms can lead to an enrichment of ^{13}C in heterotrophic plant organs compared to leaves (Cernusak et al. 2009). For example, a greater allocation of depleted C to lignin and lipid pools and an export of less depleted carbohydrates to roots result in an enrichment of ^{13}C in belowground organs (Hobbie and Werner 2004; Badeck et al. 2005). The observed significant impact of tree species on root and litterfall $\delta^{13}C$ is caused by a complex interplay of physiological differences between the tree species and their response to environmental conditions, which has been thoroughly reviewed by Dawson et al. (2002). The higher $\delta^{13}C$ values of the here investigated coniferous species in comparison to deciduous trees (Table 1) are mainly caused by a higher intrinsic water-use-efficiency, lower stomatal conductance and lower photosynthetic rates (Brooks et al. 1997). It must be noted that the $\delta^{13}C$ of different plant parts varies on diurnal, seasonal and annual to interannual time scales (Brüggemann et al. 2011). Here we use the $\delta^{13}C$ of the annual litter inputs as reference points to evaluate the decomposition of OM along soil profiles (Bowling et al. 2008).

The generally lower $\delta^{15}N$ values of litterfall compared to roots are in line with findings of other studies (Högberg et al. 1996; Templer et al. 2007). This pattern can be assigned to fractionation during N transformation and transport within the plant that leads to an assimilation of ^{15}N-depleted N in leaves and ^{15}N-enriched N in roots (Pardo et al. 2013). Moreover, the formation of mycorrhizal symbioses is one of the most important factors influencing the $\delta^{15}N$ signature of leaves. The here investigated tree species Douglas fir, pine, beech and oak are well known to form symbioses with ectomycorrhizal (EM) fungi (Wang and Qiu 2006). ^{15}N-enriched N compounds are preferentially retained by the fungal biomass, while ^{15}N-depleted N compounds are transported to their host plant (Craine et al. 2009). The biggest difference between root and litterfall $\delta^{15}N$ was determined for beech (3.69 ± 0.96‰) followed by oak (3.33 ± 0.52‰). This range is in agreement with differences of ∼ 4‰ observed by Hobbie and Colpaert (2003). According

to them, the amount of ectomycorrhizal mass included with the roots also determines the enrichment of roots in ^{15}N compared to foliar tissues. The threefold higher root biomass of beech and oak compared to Douglas fir and pine (Table 1) can therefore be responsible for the highest differences between the plant organs at these stands. Analyzing the abundance of mycorrhizal fungi in symbioses with the investigated tree stands was beyond the scope of the study. However, it is reported that the EM fungal biomass does not vary significantly between beech and conifers in temperate forests but the mechanisms behind the regulation of EM fungal biomass are highly complex (Awad et al. 2019). Additional to mycorrhizal associations, the variability in plant $\delta^{15}N$ depends on the form of soil N that plants predominantly acquire (Vallano and Sparks 2013). Denitrification and nitrification both discriminate against ^{15}N because ^{15}N-depleted nitrate can be leached from the soil, resulting in ^{15}N-enrichment of the remaining N that can be taken up by plants (Hobbie and Högberg 2012). High nitrate concentrations of soils under Douglas fir (Zeller et al. 2019) can account for the significantly highest litterfall $\delta^{15}N$ values at the study site 'Sophienhöhe'. Beech, with the second highest $\delta^{15}N$ values for litter inputs, is recognized as a tree species that promotes nitrification in soils (Andrianarisoa et al. 2010). However, a more profound investigation of specific N cycling processes in the plant-soil system that potentially influence the natural abundance of $\delta^{15}N$ is beyond the scope of this study.

Depth profiles of $\delta^{13}C$ and $\delta^{15}N$ and their relationship to stoichiometry patterns

Within 35 years after afforestation distinct depth profiles of $\delta^{13}C$ developed in all investigated forest stands confirming findings of Brunn et al. (2017), who demonstrated that three decades after afforestation are sufficient to yield such profiles. The gradients from OL to OH in our investigated forest stands were characterized by a decrease or at least no alteration of $\delta^{13}C$ (Fig. 2b). Within the early stages of OM decomposition water-soluble substances and non-lignified carbohydrates are degraded, while the proportion of lignin residually increases (Berg 2008; Osono et al. 2008). Lignin is characterized by lower $\delta^{13}C$ values compared to bulk foliar $\delta^{13}C$, while cellulose and sugars are characterized by higher values (Bowling et al. 2008). Therefore, a selective preservation of

lignin, and lignin building blocks, respectively (Suárez-Abelenda et al. 2015), which cannot be attacked by the vast majority of decomposers, can lead to the depletion of ^{13}C downwards through the organic horizons under Douglas fir, pine and oak. No significant depletion of ^{13}C was found under beech. It appears that beech maybe belongs to the group of tree species, where the carbohydrate-dominated early stage of litter decomposition is so marginal that it has no measurable impact on δ^{13}C values (Berg and McClaugherty 2014). The fact that the sampling campaign was in April and the litterfall predominately occurred in October and November corroborates the assumption. The time difference between litterfall and organic-layer sampling can also be responsible for the high extent of ^{13}C enrichment from litterfall to OL material. In the mineral soil horizons, however, δ^{13}C of bulk SOM increased with increasing depth. A relevant contribution of atmospheric ^{13}C-depleted CO_2 (Francey et al. 1999), to OM at the soil surface can be excluded because the 35 years old afforested sites are rather young. Instead, ^{13}C-depleted litter from aboveground plant materials accumulates at the soil surface, while the contribution of OM that derives from ^{13}C-enriched roots to SOM formation increases with soil depth (Bird et al. 2003). Correspondingly, in our study roots were on average higher with ^{13}C by $4.56 \pm 0.99‰$ compared to the litterfall. Furthermore, the kinetic fractionation of C isotopes during the maturation of SOM leads to an enrichment of ^{13}C with increasing depth (Wynn et al. 2006). Within the microbial metabolism of C sources preferentially ^{13}C-depleted CO_2 is respired by microorganisms, while the remaining SOM including the soil microbial biomass becomes enriched in ^{13}C (Werth and Kuzyakov 2010). Thus, microorganisms fractionate during the C assimilation and/or preferentially use ^{13}C-enriched substrates (Schwartz et al. 2007). Especially in mineral soils of forests ^{13}C-enriched microbial-derived OM has a larger share of bulk SOM δ^{13}C values than lignin or aliphatic biopolymers (Dümig et al. 2013). Throughout the soil profile, δ^{13}C of SOM was affected by tree identity with consistently highest values in Douglas fir stands and lowest values in oak stands. In contrast, Marty et al. (2015) found a negative impact of the percentage of conifers in Canadian forests on δ^{13}C values in mineral horizons. They assume that this was caused by lower microbial activity and/or lower SOM degradation at sites dominated by conifers. Yet,

in our study the differences in the SOM δ^{13}C values among tree species reflect the isotopic signatures of the OL horizon that in turn strongly correlated with litterfall δ^{13}C. This explains why OM under Douglas fir with highest δ^{13}C values in litterfall and roots exhibited the highest δ^{13}C values throughout the soil profile, while they were lowest under oak.

In coincidence with the ^{13}C enrichment with increasing soil depth, gradients of δ^{15}N from the OL down to the 10–30 cm layer of mineral soil had developed in all forest stands during 35 years of afforestation (Fig. 2d). The depth distribution of SOM δ^{15}N mainly results from an interplay of input signatures and losses that occur during decomposition processes (Craine et al. 2015). The accumulation of ^{15}N-depleted plant litter on the soil surface determines the gradient from the significantly lower δ^{15}N values of forest floor horizons to the ^{15}N-enriched mineral soil. Thus, the highest δ^{15}N values in the forest floor horizons under Douglas fir reflect the highest δ^{15}N values of the litterfall and roots of all tree species that in turn were determined by the nitrate concentrations of Douglas fir soils (Zeller et al. 2019). The lowest forest floor δ^{15}N values that were observed in the pine stand are in accordance with other studies revealing that conifer-dominated sites were ^{15}N-depleted compared to deciduous species (Pardo et al. 2007). In contrast to the δ^{13}C depth gradients, δ^{15}N increased consistently throughout the soil profile following a curve that is typical for N-limited forest ecosystems dominated by EM fungi (Hobbie and Ouimette 2009). The clearly higher δ^{15}N values under beech and Douglas fir compared to oak and pine are in accordance with observations made in a common garden experiment in Poland (Angst et al. 2019). With increasing depth and ongoing decomposition, SOM becomes preferentially ^{15}N-enriched due to microbial activity coupled with an increasing proportion of ^{15}N-enriched microbial derived compounds (Lerch et al. 2011). The individual SOM δ^{15}N depth gradients of tree species converged in the upper two mineral soil horizons (0–5, 5–10 cm) and diverged again with increasing depth implying that SOM turnover differed under the influence of tree species. Additionally, tree species and their mycorrhizal symbionts, respectively, also contributes to the δ^{15}N depth profiles by their N uptake from soil (Handley and Raven 1992; Callesen et al. 2013). The type of mycorrhizal association mainly drives the form of N acquisition of temperate

tree species (Liese et al. 2017) and leads to differences in ^{15}N enrichments in soil profiles between EM and arbuscular mycorrhizal fungi dominated systems (Hobbie and Högberg 2012). The here investigated tree species are all dominated by EM fungi with similar fungal biomasses (Wang and Qiu 2006; Awad et al. 2019) and thus, the N transfer from the soil to the host plant is presumably not significantly different between tree species. Anyhow, the turnover of SOM and the N uptake by plants are highly interrelated and therefore both mechanisms will have contributed to the δ^{15}N depth profiles.

The negative relationship between δ^{15}N and soil C:N ratio may result from increasing loss of ^{15}N-depleted N in the form of nitrate leaching or denitrification as consequence of decreasing N retention (Marty et al. 2019). However, this option is likely subordinate because N-limited temperate forests are characterized by a largely closed internal N cycle, where N-losses are generally low due to a high competition for this growth-limiting resource (Rennenberg et al. 2009). Rather, the relationship of δ^{15}N to the soil C:N is best explained by the increase in OM decomposition with increasing soil depth. The relationship between δ^{13}C and soil C:N ratio, that is also negative, supports this assumption (Baisden et al. 2002). The well-known decline of the C:N ratio with increasing depth (Marín-Spiotta et al. 2014) is mostly attributed to OM decay because substrate that accumulates at the soil surface has significantly higher C:N ratios compared to decomposers and their products (Manzoni et al. 2010; Paul 2016). The slopes of the specific regression lines (Fig. 3a) were more driven by the enrichment of ^{15}N with increasing depth than by the C:N ratio. Steeper slopes in beech and pine stands were associated with the significantly higher ε_{soil}^{15}N values of beech and pine compared to oak and Douglas fir. Nonetheless, the significant relationship between δ^{15}N and the C:N ratio emphasizes the potential of ^{15}N depth gradients as proxy for OM decay. Kramer et al. (2017) found that changes in organo-mineral associations can drive depth trends of C:N and δ^{15}N more than the microbial decay. However, this effect was largely reduced, since forest stands with uniform mineral phase were investigated in this study. Furthermore, the O:C ratio that represents the state of chemical oxidation (Fan et al. 2018) of SOM was also significantly related to δ^{15}N and δ^{13}C throughout the soil profile. Oxidation is accompanied with microbial

breakdown and depolymerization of plant residues followed by assimilation of C in microbial biomass as well as mineralization at the same time (Lehmann and Kleber 2015). Thus, with increasing depth the rise of the O:C ratio indicated the progressive oxidative degradation of OM and correlated with the enrichment of ^{15}N and ^{13}C in SOM. All this led to the assumption that the depth trends of SOM δ^{15}N and δ^{13}C resulted mainly from the decomposition of OM.

$\beta_{\delta13C}$ values and ε_{soil}^{15}N and the contribution of litterfall and root inputs

In well-drained forest soils, like our study site 'Sophienhöhe', the linear regression function of δ^{13}C and the logarithm of SOC with soil depth, termed as $\beta_{\delta13C}$ value, is a suitable indicator of isotopic fractionation during decomposition (Brunn et al. 2014). Physical soil mixing processes that could also have a contribution to the isotopic fractionation with soil depth (Acton et al. 2013) can be excluded because during field surveys no earthworms or signs of significant bioturbation processes were found (Lorenz and Thiele-Bruhn 2019). However, steeper regression slopes, and more negative $\beta_{\delta13C}$ values respectively, indicate higher rates of ^{13}C enrichment through the soil depth profile and enhanced organic matter turnover (Garten 2006; Wang et al. 2018). Tree species had a significant effect on $\beta_{\delta13C}$ values. The most negative $\beta_{\delta13C}$ values and therefore the highest rates of SOM turnover were determined in beech forest stands, while reduced SOM turnover at coniferous sites was indicated by less negative $\beta_{\delta13C}$ values. This is in accordance with the view that turnover rates, especially in the early-stage of decomposition, of deciduous species litter are generally higher compared to conifers (Augusto et al. 2015 and references in there). Long-term studies (> 10 years) suggest that there are also significant differences in the remaining masses after decomposition between tree species (Harmon et al. 2009; Prescott 2010). This can be addressed to significantly higher N contents and lower C:N ratios in the litterfall of beech and oak (Table S3), because it is well documented that these parameters correlate well with decomposition (Fernandez et al. 2003; Laganière et al. 2010; Vesterdal et al. 2012). Our findings are confirmed by other studies revealing that more negative $\beta_{\delta13C}$ values were related to higher N contents and lower C:N ratios of litterfall (Garten et al. 2000;

Garten 2006; Wang et al. 2015). The multiple linear regression analysis, which included different quantity and quality properties of the litter inputs, pointed out that the isotopic signatures of litterfall and root C:N account for nearly half of the variation (46%) in $\beta_{\delta13C}$ values. This implies on the one hand that the initial isotopic composition of the aboveground litter plays a crucial role, for evaluating the ^{13}C enrichment in soil depth profiles in the context of SOM turnover. Camino-Serrano et al. (2019) figured out that litter $\delta^{13}C$ is the key to predict and model $\delta^{13}C$ depth profiles. On the other hand, the C and N stoichiometry of root biomass seems to be of high importance for $\beta_{\delta13C}$ values in forest soils. Belowground inputs are still less researched but knowledge is growing that these inputs have a significantly contribution to OM formation (Angst et al. 2018; Poirier et al. 2018). For example, Kramer et al. (2010) demonstrated that root-derived C is the major ($> 60\%$) source of C for microbes in temperate deciduous forest soils, while also in boreal forest soils 50 to 70% of the stored C derives from roots and root-associated microorganisms (Clemmensen et al. 2013). However, 54% of the variation in $\beta_{\delta13C}$ values cannot be explained with the here investigated properties of litterfall inputs and root material suggesting that SOM decomposition depends additionally on other factors.

The use of bulk SOM $\delta^{15}N$ as proxy for turnover and stabilization of SOM bears an uncertainty due to factors like the variability of the initial abundance of ^{15}N in litter inputs (Högberg 1997). Thus, we evaluated soil enrichment factors ($\varepsilon_{soil}{}^{15}N$) to level out the different isotopic signatures of litter inputs. Like the $\beta_{\delta13C}$ values, $\varepsilon_{soil}{}^{15}N$ values were significantly affected by tree species but the differences between tree species are not consistent for $\varepsilon_{soil}{}^{15}N$ and $\beta_{\delta13C}$ values. If mainly microbial turnover of SOM would drive both parameters in similar trajectories, this would result in a negative relationship between both indices. This applies for Douglas fir, oak and in particular for beech with oppositely high or low $\beta_{\delta13C}$ and $\varepsilon_{soil}{}^{15}N$ values, respectively (Fig. 4). However, soils under pine were characterized by highest $\beta_{\delta13C}$ as well as high $\varepsilon_{soil}{}^{15}N$ values. This indicates that in pine stands ^{13}C and ^{15}N enrichments with soil depth are decoupled from each other and not systematically interlinked. Nel et al. (2018) reported on a general link between $\delta^{13}C$ and $\delta^{15}N$ on global scale and with soil depth but they also mentioned that this link varied with

local influences from biota, disturbance and environmental conditions. Multiple linear regression analysis revealed that litterfall C:N and $\delta^{15}N$ as well as root O:C and $\delta^{13}C$ account for 74% of the variability in $\varepsilon_{soil}{}^{15}N$. This highlights the importance of litterfall and root inputs for the ^{15}N enrichment with soil depth. The general view that the higher decomposition rates of litter with high N content and low C:N ratio (Garten et al. 2000) are associated with both, high ^{15}N and ^{13}C enrichment does not match with the finding of high ^{15}N enrichment in soils under pine. This must be caused by other processes. The high C:N ratio of the N-poor litterfall (Table S3) suggests that the N supply in the upper 30 cm of soil in the pine stands is limited. The significantly lowest root biomasses of the typical deep rooting tap root system (Burylo et al. 2011) combined with its low turnover rates (Yuan and Chen 2010) will also provide not much N. Under such N-limited conditions EM fungi are able to oxidize OM primarily as N source rather than as a source of metabolic C (Lindahl and Tunlid 2015). Thus, EM fungi in the pine stands can compete directly with decomposers for soil N resources and exacerbate the N-limitation of free-living decomposers (Averill 2016). Therefore, the soil microbial community could adapt their N-utilization strategy to an efficient re-use of organic N derived from their own bio- and necromass. The multiple recycling of these N sources will lead to an ongoing enrichment of ^{15}N without higher rates of decomposition and could end up in the discrepancy between $\beta_{\delta13C}$ and $\varepsilon_{soil}{}^{15}N$ values. Furthermore, it has been reported that under conditions with low C availability, N-containing organic compounds can be primarily used as source of C and energy. Consequently, dissimilated ^{15}N-depleted N will be exported and the microbial cell gets enriched relative to its source (Dijkstra et al. 2008). Consequently, both mechanisms, the effective recycling of microbial derived N under N-limited conditions as well as the dissimilation of ^{15}N-enriched N, when the relative availability of C is low, could lead to high $\varepsilon_{soil}{}^{15}N$ and low $\beta_{\delta13C}$ values in pine stands.

Conclusion

The post-mining site "Sophienhöhe" represented a suitable site to characterize the influence of tree species on the natural abundance of ^{13}C and ^{15}N in soil

depth gradients. As an advantage to many common garden experiments, an interference from old C sources originating from former land use was negligible. Additionally, 35 years after the afforestation were sufficient to generate tree species-specific depth gradients. Hence, evidence was provided that differences in isotopic signatures of SOM originated from the input of plant litter and its decomposition products.

The significantly different δ^{13}C and δ^{15}N values in the OM of forest floor and mineral soil reflected the signatures of the litter inputs (litterfall and roots) that were tree species specific. Along the soil profile, both isotopes were significantly related to the C:N and O:C ratio indicating that the enrichment of ^{13}C and ^{15}N with increasing soil depth is driven by processes that presumably can be assigned to microbial decomposition of OM. Consequently, when ^{13}C and ^{15}N of bulk SOM are used to evaluate decomposition and stabilization of OM, the isotopic signatures of litter inputs should be considered as well. Differences in $\beta_{\delta13C}$ values indicated different turnover of SOM between tree species with higher decomposition rates in deciduous forest stands compared to conifers. The quality of litterfall and root inputs (N content, C:N, O:C ratio) as well as the initial isotopic signatures of litterfall contributed to the regulation of OM decomposition. Yet, 54% of the variance in $\beta_{\delta13C}$, and 26% in $\varepsilon_{soil}{}^{15}$N respectively, cannot be explained with the here investigated litterfall and root properties showing that SOM decomposition depends additionally on other—presumably microbial driven—factors. The correspondence of $\varepsilon_{soil}{}^{15}$N values with $\beta_{\delta13C}$ values in three of the four investigated forest stands (Douglas fir, beech, oak) suggests that the ^{13}C and ^{15}N enrichment with increasing depth followed similar principles. However, the conditions under pine did not follow the systematic link between ^{13}C and ^{15}N enrichment. This is presumably due to specific N cycling mechanisms mediated by microorganisms that were adapted to conditions of limited N availability and the relatively low availability of C.

It is concluded that typical pattern of ^{13}C and ^{15}N enrichment with increasing soil depth are due to maturation and ongoing turnover of SOM. However, under the influence of tree species the enrichment of both isotopes did not follow similar trajectories in general because of microorganisms that can create specific utilization strategies depending on the litter quality. It was possible to obtain this finding by combining stable isotope analysis with the classical determination of stoichiometry ratios (C:N, O:C).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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