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Spatial distribution and chemical composition of soil organic matter fractions in rhizosphere and non-rhizosphere soil under European beech (*Fagus sylvatica* L.)

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ABSTRACT

Little is known about how trees and their roots may influence the spatial distribution and chemical composition of soil organic matter (SOM) in subsoils with subsequent effects on soil organic carbon (SOC) storage and turnover. The aim of this study was to assess the impact of individual trees and their root system on the spatial distribution and chemical composition of SOM fractions and the storage of SOC in subsoils.

A Dystric Cambisol was sampled along three vertical replicate transects (3.15 m in length, 2.00 m in depth) in a regular grid (45 cm horizontal spaces, 25 cm vertical spaces) at increasing distance from three individual mature European beech trees (*Fagus sylvatica* L.). Soil OM fractions were obtained from rhizosphere soil and bulk soil samples taken at 10 and 85 cm depth increments by a combined density and particle size fractionation. Carbon and nitrogen measurements were performed, and the chemical composition of the SOM fractions was further characterized by solid state cross polarization magic angle spinning ^{13}C nuclear magnetic resonance spectroscopy.

The distance from the individual trees had no influence on the SOC contents and stocks or the chemical composition of the SOM fractions. This was ascribed to the dense and even rooting at 0–40 cm depth across all sampled distances. Instead, the SOC contents and stocks highly differed between 10 cm depth (11.4 g SOC kg^{-1}), where particulate organic matter (POM) dominated, and 85 cm depth (0.5 g SOC kg^{-1}), where clay associated SOC dominated. These differences seemed to be strongly influenced by the roots of the trees which were almost completely absent from depths ≥60 cm. Elevated SOC contents in the rhizosphere soil (40.1 g SOC kg^{-1}) were ascribed to root exudates in the root's vicinity and a very high amount (109.3 g kg^{-1}) of fresh POM (alkyl/O/N alkyl C ratio of 0.8). The data revealed that, besides root exudates, also root derived POM contributed significant amounts of SOC to the soil.

Although only low amounts of the clay fraction were found at 85 cm depth (22.8 g clay kg^{-1}), it accounted for high amounts of SOC and played a crucial role for the storage of SOM. The relatively high SOC stocks at 40–200 cm depth (1.4 kg C m^{-2}) compared to the SOC stocks at 0–40 cm depth (3.8 kg C m^{-2}) indicate that also sandy forest subsoils with low SOC contents have to be considered in terrestrial carbon inventories.

1. Introduction

Subsoils have received more attention in recent years (e.g., Eusterhues et al., 2005; Schöning and Kögel-Knabner, 2006; Fontaine et al., 2007) because a substantial amount of soil organic carbon (SOC – C in soil derived from organic constituents) can be stored in subsoil horizons (Rumpel et al., 2002; Jobbágy and Jackson, 2000). Forest soils are of particular interest because globally up to 70% of all SOC is stored in them (Jobbágy and Jackson, 2000) and a considerable amount thereof in the subsoil (Lorenz and Lal, 2005; Jobbágy and Jackson, 2000). However, little quantitative information is available on the SOC contents and stocks, and the chemical composition of soil organic matter (SOM – the entirety of dead matter derived from plants and animals, and their organic transformation products) in subsoil (Rumpel and Kögel-Knabner, 2011).

The distance from a tree can have a substantial influence on soil chemical (Lodhi, 1977; Koch and Matzner, 1993; Spielvogel et al., 2014) and physical properties (Chang and Matzner, 2000b) as well as on the microbial community structure and activity (Saetre and Bååth, 2000; Goemoeryova, 2004) and, therefore, on SOC storage and turnover. For example, Chang and Matzner (2000a,b) found an increased channeling of dissolved organic carbon (DOC), increased water content, and a higher N-mineralization rate near the stem base of European beech trees. Spielvogel et al. (2014) found a pronounced gradient in

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lipid root biomarker concentrations with distance from beech trees. In another study SOC stocks have been found to be unaffected by the distance from individual trees (Schöning et al., 2006). However, all of these studies focused on bulk soil properties. Soil sampling designs in most studies have only involved samples being collected from different soil horizons at one horizontal distance from a tree (e.g., Rumpel et al., 2004; Eusterhues et al., 2005; Schrumpf et al., 2013). To the best of our knowledge, variations in the properties of functionally defined SOM fractions that are important for stabilization and turnover of SOC with distance from individual trees using a dense sampling grid have not been studied previously.

The storage of SOC in forest subsoils is thought to be mainly driven by rhizodeposition (Rasse et al., 2005; Tefs and Gleixner, 2012). Rhizodeposits are root exudates and root litter (Kuzyakov and Domanski, 2000). Most studies involving the rhizosphere have focused on enzyme activities (Brzostek et al., 2013), microbial biomass and community structure in rhizosphere soil (Koranda et al., 2011), or the influence of rhizodeposition on C turnover using carbon dioxide (CO_2) efflux measurements (Dijkstra and Cheng, 2007; Schenck et al., 2012). To the best of our knowledge, SOC contents in combination with the chemical composition of root-derived particulate organic matter (POM) and other functional SOM fractions in rhizosphere soil have not been studied.

The aim of this study was to assess the impact of individual mature European beech trees on the spatial distribution and chemical composition of SOM fractions, and evaluate the role of rhizosphere soil fractions for input and storage of SOC in subsoil. The hypothesis was that a measurable influence of individual trees on the measured chemical parameters existed, that decreased as the distance to the trees' stem bases increased. Soil samples were collected in a regular sampling grid from the profile walls of three transects, each of which started at a European beech tree. Rhizosphere soil and soil samples from 10 cm and 85 cm depth were subjected to a combined density and particle size fractionation. Beside C and N measurements of all samples, the chemical composition of the clay and POM fractions was further characterized by cross-polarization magic angle spinning ^{13}C nuclear magnetic resonance (CPMAS ^{13}C NMR) spectroscopy. Additionally, the specific surface area (SSA) of representative samples of the clay fraction was determined.

2. Materials and methods

2.1. Study area and soil sampling

The study was carried out at the Grinderwald which is located northwest of Hannover (52° 34′ 22″ N 9° 18′ 51″ E), Germany. Climate data were obtained from a German Meteorological Service monitoring station (Nienburg). The mean annual precipitation and temperature for the period 1981–2010 were 762 mm and 9.7 °C, respectively. Parent materials were Pleistocene glaciofluvial sandy deposits from the Saale glacial stage (Bundesanstalt für Bodenforschung, 1973). The predominant soil type in the study area was an acid (pH 3.4–4.5), sandy (77.3% sand, 18.4% silt and 4.4% clay) Dystric Cambisol (IUSS Working Group WRB, 2014) and the humus form was moder. The phyllosilicate mineralogy was characterized by XRD measurements. It revealed the presence of chlorite, mixed-layer minerals, kaolinite, and illite, whereas smectites were absent. The study area was covered with an even-aged European beech (*Fagus sylvatica* L.) forest established in 1916 (Forstamt Nienburg, 2010). Mean stem density was 407 stems ha^{-1}, the mean diameter at breast height was 26.3 cm, and the mean basal area was 27.1 m^2 ha^{-1}. A mature beech forest was chosen, because aim was to study a climax forest association which commonly occurs in Germany. In addition, European beech is the most abundant tree species in Central Europe (Geßler et al., 2007).

Three transects, each 2.00 m deep and 3.15 m long, were dug on flat terrain in June 2013 using a mechanical digger, each starting at the stem base of a mature beech tree. We oriented the transects North, South, and West facing, respectively, to avoid a systematic bias by cardinal direction. The depth was chosen to assure that the parent material below the B-horizons had been reached. To follow the spatial influence of a single tree on SOM properties, the direction of each transect was chosen to avoid the stem base of neighboring trees being reached. Furthermore, the locations of the transects were chosen so that they all had comparable soil and vegetation properties, i.e., soil texture and no vegetation cover other than European beech. Composite soil samples (each ~1 kg) and volumetric samples (taken using steel cylinders; diameter: 8.5 cm, height: 6.0 cm) were collected from the wall of each transect in a regular grid pattern with 45 cm horizontal spaces and 25 cm vertical spaces (Fig. 1). To ensure comparable volumetric sampling throughout the whole grid using the same steel rings unbiased by differing topsoil thicknesses, the uppermost sampled depth increment was set to 10 cm depth. The volumetric samples were used for the determination of the bulk density. A total of 192 soil samples were collected, 64 from each transect. Due to the sampling approach, the reported parameters are mean values for a specific soil increment (radius of 4.25 cm). Approximately 50 g of the organic layer were collected above the horizontal grid points. Leaf litter was randomly collected next to the profile walls of each transect. Fine roots (diameter ≤ 2 mm) were manually extracted from the volumetric soil samples taken from the profile walls. One composite rhizosphere soil sample was taken from each transect, predominantly from the uppermost, densely and evenly rooted 0–40 cm and at deeper soil depths where roots were present, close to the tree stems (Figs. 1 and 2). Rhizosphere soil was defined as soil adhering to the roots after they had been shaken (Cieslinski et al., 1998; Gomes et al., 2003). The uppermost sampled depth increment at 10 cm depth was compared with the fourth sampled depth increment at 85 cm depth (Fig. 1). According to the WRB 2014 soil classification system, the AE horizon at the investigated soil ended at 2 cm depth and the first sampled depth increment at 10 cm depth was already located in the Bsw horizon. We consider subsoil as being the soil that is located below the A and E horizons (cf. IPCC, 2000). Consequently, the sampled depth increment at 10 cm was referred to as "$subsoil_{10}$" and the depth increment at 85 cm depth was referred to as "$subsoil_{85}$". The term "non-rhizosphere soil" refers to both the $subsoil_{10}$ and $subsoil_{85}$.

2.2. Fine root biomass and necromass

Roots were manually separated from the volume samples in the laboratory and cleaned in a sieve of 250 μm mesh size using deionized water (DI). Only fine roots (diameter ≤ 2 mm) could be detected in the samples, coarse roots (>2 mm diameter) were absent. By inspection under a stereo microscope, the extracted rootlets were distinguished in living (biomass) and dead (necromass) fine roots following the criteria root color, elasticity, and cohesion of cortex, periderm and stele (e.g., Hertel et al., 2013; Hertel and Leuschner, 2002; Persson, 1978). The root biomass and necromass was dried for 48 h at 70 °C and weighed.

To keep the analysis viable, fine roots >10 mm length were extracted from all samples but fine roots <10 mm length were only extracted from representative samples. While the inclusion of only fine roots >10 mm length and the negligence of fine roots <10 mm length allows to quantify the majority of living fine root mass (>95%), it fails to account for the mass of dead fine roots with sufficient accuracy, since a large proportion of fine root necromass consists of root fractions <10 mm length (Bauhus and Bartsch, 1996; Leuschner et al., 2001). In order to correct the fine root necromass for fine roots <10 mm length, we extrapolated the mass of dead fine roots <10 mm length of 30 representative samples per transect using soil depth-specific regression equations that relate the mass of fine dead roots <10 mm length to fine dead roots >10 mm length. These regression equations were

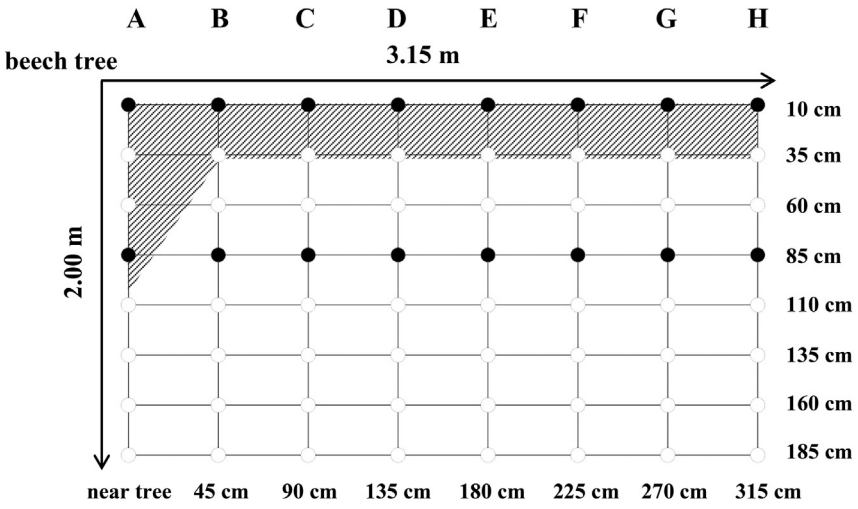

Fig. 1. Sampling grid applied to each transect wall (n = 64 samples per transect). Composite and volumetric soil samples (using steel cylinders; 8.5 cm diameter, 6 cm height) were taken. The black dots (n = 16 per transect) indicate the samples that were subjected to the combined density and particle size fractionation. The shaded area displays the regions from which the rhizosphere soil was collected. The letters above the graph represent the labels of the horizontal sampling spots, A being nearest to the tree. The distance between sampling spots were 45 cm in the horizontal and 25 cm and in the vertical, starting at a depth of 10 cm.

established applying a method introduced by van Praag et al. (1988) and modified by Hertel (1999).

2.3. Combined density and particle size fractionation

Bulk soil samples were air dried and gently passed through a 2 mm sieve. $Subsoil_{10}$ at sampling spots A to H (uppermost sampled depth increment at 10 cm), $subsoil_{85}$ (fourth sampled depth increment at 85 cm) (Fig. 1), and rhizosphere soil from each transect were fractionated. Aim was to separate the combined fine silt and clay fractions because these are thought to contribute to the long-term stabilization of SOM (Mueller et al., 2009; Rumpel and Kögel-Knabner, 2011).

A 30 g aliquot of air dried and sieved bulk soil was saturated with a sodium polytungstate (SPT) solution (TC Tungsten Compounds, Grub am Forst, Germany) adjusted to a density of 1.8 g cm^{-3}, and subsequently ultrasonicated at an energy of 600 J ml^{-1} to break up soil aggregates and release the POM occluded within aggregates (oPOM). The samples were cooled during the ultrasonication treatment to reduce changes in SOM composition by heating the solution (Mueller et al., 2012b). Preliminary tests were performed using soil samples from the study site with densities of 1.6 and 1.8 g cm^{-3}, and ultrasonication energies of 400, 600 and 800 J ml^{-1} to select experimental settings that separate the POM and mineral soil fractions most effectively. The results of the preliminary tests were evaluated against a particle size analysis of the respective samples, the C/N ratios, and reflectance light microscopy of the different fractions in order to ensure that the chosen parameters were appropriate. After ultrasonication, the POM fraction was removed using a water jet pump. The POM fraction was purged with DI until the electrical conductivity of the eluted water was below 5 μS, freeze-dried, and stored for further analysis. The remaining mineral residue was purged with DI until the conductivity of the eluted water was below 50 μS and wet sieved to obtain combined coarse and medium sand (200–2000 μm), fine sand (63–200 μm) and coarse silt (20–63 μm) fractions. The mineral soil that passed through all three sieves, i.e. medium silt, fine silt and clay, was subjected to sedimentation to separate the medium silt (6.3–20 μm) from the combined fine silt and clay fraction (< 6.3 μm). The mean recovery rate of the combined density and particle size fractionation on a mass basis was 98.4%. All of the fractions were freeze-dried and stored for further analysis. The coarse, medium, and fine sand fractions were

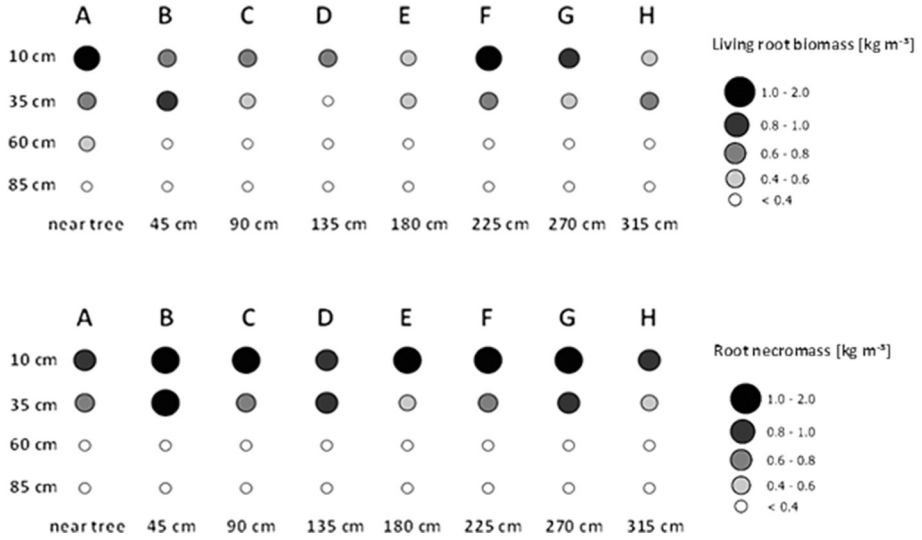

Fig. 2. Mean living and dead fine root concentration [kg m^{-3}] down to a depth of 85 cm. The letters above the plots are the labels of the horizontal sampling spots with A being nearest to the tree. n = 3 for each grid point.

referred to as the "sand fraction", the coarse and medium silt fractions were referred to as the "silt fraction", and the combined fine silt and clay fraction was referred to as the "clay fraction".

2.4. Determination of carbon and nitrogen contents

The C and N contents in the bulk soil were determined by weighing an aliquot of a soil sample into a ceramic cup and analyzing the sample by dry combustion with a VARIO MAX CNS analyzer (Elementar Analysensysteme, Hanau, Germany). The C and N contents in the mineral soil fractions and the POM were measured using an EA elemental analyzer (EuroVector, Milan, Italy). Both analyzers had a detection limit of 0.02% total C. The mineral soil fractions that were coarser than medium silt were finely ground prior to analysis. The pH value of the soil did not exceed 4.5 clearly indicating the absence of carbonates. Thus, the total C contents measured were equal to the SOC contents. All C and N measurements were run in duplicate.

2.5. Specific surface area measurements

The specific surface area of representative samples of the clay fraction of the $subsoil_{10}$ and $subsoil_{85}$ from each transect was measured by the multi-point BET method (Brunauer et al., 1938) using an Autosorb-1 analyzer (Quantachrome, Syosset, NY, USA). Nitrogen adsorption data at 11 points were obtained in the partial pressure range 0.05–0.3 in liquid nitrogen. Prior to measurement, the samples were outgassed at 40 °C for at least 16 h to remove water. A total removal of SOM from the samples by further chemical pretreatments was omitted. Thus, the free surface areas of the clay fractions that were not obscured by SOM were measured.

2.6. ^{13}C CPMAS NMR spectroscopy

The leaf litter (n = 3), fine roots (n = 3), organic layer material (n = 24), POM (n = 24) and clay fractions (n = 24) of the $subsoil_{10}$, POM (n = 3) and clay fractions (n = 3) of the rhizosphere soil, and the clay fractions of the $subsoil_{85}$ (n = 4) (marked in Fig. 1) were subjected to solid state ^{13}C CPMAS NMR spectroscopy. The POM and mineral associated SOM were analyzed as these fractions represented the largest SOC pool. Measurements were performed using a Bruker Avance^{III} 200 Spectrometer. An aliquot was weighed into a zirconoxide rotor that was spun at 5.0 kHz with a recycle delay time of 0.4 s for the clay fractions and 1 s for leave litter, roots, organic layer and the POM fractions. For the POM fractions, 4000 counts were acquired and more than 6 million counts were acquired for the clay fractions. Since SOC contents were very low in the clay fractions from the $subsoil_{85}$ and HF treatment of the samples (cf. Schmidt et al., 1997) was no option for us due to a loss of SOC and a possible alteration in SOC chemistry (Gonçalves et al., 2003; Rumpel et al., 2006), only four reasonable spectra could be obtained for the clay fractions of the $subsoil_{85}$. The spectra were processed with a line broadening of 50 Hz, phase adjusted and baseline corrected. Peaks were separated into four integration areas, 0–50 ppm (alkyl-C), 50–110 ppm (O/N-alkyl-C), 110–160 ppm (aromatic-C), and 160–220 ppm (carboxylic-C) (Kögel-Knabner et al., 1992).

The signals in the NMR spectra can be assigned to major chemical compound classes. O/N-alkyl C can be ascribed to amide C of proteins and the C2, C3, and C5 in polysaccharide molecules. The main signal at 30 ppm in the alkyl C region can be assigned to C in long chain aliphatic components from lipids, waxes, and other aliphatic biomacromolecules (Kögel-Knabner et al., 1992). Cellulose, hemicellulose, and proteins in plant residues are relatively easily decomposable, whereas aliphatic structures are thought to be more resistant to degradation. Thus, the ratio between alkyl C and O/N-alkyl C can be used as indicator for the degree of decomposition of OM (Baldock et al., 1997). Lignin, often detected in plant derived SOM, is indicated by signals at 56, 119, 130 and 150 ppm. High intensities at 130 ppm could also indicate the presence of pyrogenic C. The main peak around 175 ppm is assigned to carboxyl and amide groups in different compounds (Kögel-Knabner, 1997).

2.7. Statistics

Means and standard deviations (SD) of the field replicates were calculated using Microsoft Excel 2013 for Windows (Microsoft, Redmond, WA, USA). Correlation analysis (reported using the Pearson product–moment correlation coefficient, r) and all other statistics were carried out using the R 3.0.3 software for Windows (R Core Team, 2013). The Shapiro–Wilk test was used to determine whether the data were normally distributed. Significant differences were tested using the one-way analysis of variance (ANOVA) or the Kruskal Wallis test. If not explicitly mentioned, all statistical analyzes were regarded as being significant when $p < 0.05$. Neither ANOVA nor Kruskal Wallis test revealed any significant differences between the transects regarding SOC contents and stocks in the bulk soil and the fractions. Thus, the three transects were regarded as being replicates. Because there were also no significant differences between the horizontal sampling spots A to H, we refer to one mean value for each the $subsoil_{10}$ and $subsoil_{85}$ calculated from all three transects of sampling spots A to H.

The bulk soil densities were calculated from the weight of the dried soil volume samples relative to the volume of the steel cylinders used to collect the samples. Coarse particles (>2 mm) were removed from the mineral soil during the sieving process (cf. chapter 2.3) and the bulk densities were adjusted accordingly. Soil OC stocks were calculated for 1 m^2 and a layer thickness of 1 cm from the SOC contents, soil densities and the amount (g [kg $soil^{-1}$]) of the respective soil fractions for the $subsoil_{10}$ and the $subsoil_{85}$. Soil OC stocks were also calculated for the depth layers 0–40 cm and 40–200 cm, representing the densely rooted upper soil layer and the lower soil layer with low root density. Soil OC stocks for the rhizosphere soil were not calculated due to missing soil densities. Carbon enrichment factors (E_c) were calculated using Eq. (1) (Guggenberger et al., 1994; Christensen, 2001; Rumpel et al., 2004).

$$E_c = g\ C\ kg^{-1}\ fraction / g\ C\ kg^{-1}\ whole\ soil \quad (1)$$

The E_c values were calculated for the soil samples obtained from 10 cm and 85 cm depth.

3. Results

3.1. Fine root biomass and necromass

The fine root biomass and necromass did not show any significant differences between the sampling spots A to H and no significant correlations could be detected between the distance from the tree and the amount of the root biomass or necromass (Table A.1). Instead, both showed significant negative correlations with an increasing depth ($r = -0.67$ and $r = -0.86$, respectively) and were less than 0.4 kg m^{-3} at depths of 60 and 85 cm (Fig. 2). The only exception was at sampling point "A" at 60 cm depth, where the average living root biomass was greater than 0.4 kg m^{-3}.

3.2. Amount of recovered soil fractions, SOC contents and stocks

Unexpectedly, no significant correlations were found between the distance from the tree and the amount of recovered soil fractions, the SOC contents, and stocks (Table A.1). We thus focused our results on the comparison of vertical differences between average values for $subsoil_{10}$ and $subsoil_{85}$ (cf. Section 2.7), and on differences between rhizosphere and non-rhizosphere soil.

The amount of the sand fraction was significantly higher in the $subsoil_{85}$ compared to the $subsoil_{10}$ (Table 1). The amount of the clay and

Table 1
Mean +/− SD recovered mass, soil organic carbon (SOC) content, carbon to nitrogen ratio (C/N), SOC stock, and carbon enrichment factor (E_c) of the unfractionated bulk soil and soil organic matter (SOM) fractions (here referred to as "sand", "silt", "clay" and "POM") from the $subsoil_{10}$, $subsoil_{85}$ and rhizosphere soil. Significant differences in SOM fraction or the bulk soil between the $subsoil_{10}$, $subsoil_{85}$ and rhizosphere soil are indicated by lowercase letters. The superscript † symbols mark observations that are not significantly different when comparing the individual SOM fractions to each other within the $subsoil_{10}$, $subsoil_{85}$ or rhizosphere soil.

		$Subsoil_{10}$	$Subsoil_{85}$	Rhizosphere soil
Recovered mass [g (kg soil)$^{-1}$]	Sand	639.5 ± 14.2b	900.8 ± 26.6a	584.7 ± 11.8c
	Silt	285.2 ± 11.8a	76.4 ± 23.1b	264.9 ± 26.3a
	Clay	59.9 ± 3.9a	22.8 ± 4.2c	41.0 ± 4.0b
	POM	15.3 ± 2.3b	n.d.	109.3 ± 34.3a
SOC content [g C (kg fraction)$^{-1}$]	Bulk soil	11.4 ± 1.3b	0.5 ± 0.2c	40.1 ± 9.0a
	Sand	0.3 ± 0.1a	0.2 ± 0.1b	0.4 ± 0.1a
	Silt	2.7 ± 0.9a	1.5 ± 0.6b	4.0 ± 0.9a
	Clay	53.2 ± 6.4b	7.8 ± 1.7c	84.0 ± 4.5a
	POM	392.1 ± 18.1b	n.d.	424.7 ± 3.9a
C/N	Bulk soil	24.1 ± 3.1b	7.5 ± 1.7c	28.5 ± 1.4a
	Sand	n.d.	n.d.	n.d
	Silt	n.d.	n.d.	17.3 ± 2.3†
	Clay	15.9 ± 1.3a	8.1 ± 1.6c	14.3 ± 0.3b†
	POM	48.5 ± 5.9a	n.d.	26.9 ± 2.1b
SOC stock [g m^{-2}]	Bulk soil	132.4 ± 23.4a	8.1 ± 3.0b	n.d.
	Sand	2.6 ± 0.7a	3.2 ± 1.7a†	n.d.
	Silt	9.9 ± 3.9a	1.6 ± 0.8b	n.d.
	Clay	41.3 ± 8.3a	3.2 ± 1.3b†	n.d.
	POM	78.5 ± 13.3	n.d.	n.d.
E_c	Sand	0.03 ± 0.01b	0.4 ± 0.2a	0.01 ± 0.00c
	Silt	0.2 ± 0.1b	3.4 ± 2.4a	0.1 ± 0.0c
	Clay	4.8 ± 0.6b	17.3 ± 6.7a	2.4 ± 0.4c

N = 24 for $subsoil_{10}$, $subsoil_{85}$ & organic layer; n = 3 for leaves, roots & rhizosphere soil; n.d. = not determined.

silt fractions of the $subsoil_{10}$ was more than twofold the amount of the respective fractions of the $subsoil_{85}$. Particulate OM was not detected in the $subsoil_{85}$ (Table 1).

The rhizosphere soil had the lowest amount of the sand fraction, an amount of the silt fraction comparable to the $subsoil_{10}$, and an intermediate amount of the clay fraction (Table 1). Interestingly, a six times higher amount of the POM fraction was obtained from the rhizosphere soil (109.3 ± 34.3 g kg^{-1}) compared to the $subsoil_{10}$ (15.3 ± 2.3 g kg^{-1}).

The bulk $subsoil_{10}$ and fractions of the $subsoil_{10}$ had considerably higher SOC contents than the bulk $subsoil_{85}$ and the corresponding fractions (Table 1). The SOC contents of the clay fraction of the $subsoil_{10}$ were less variable (CV = 0.12) than those of the $subsoil_{85}$ (CV = 0.22). The differences in SOC contents between the rhizosphere soil and the non-rhizosphere soil were pronounced, especially regarding the bulk soil (Table 1). The rhizosphere soil had a more than three times higher SOC content compared to the bulk $subsoil_{10}$. Similarly, the SOC contents of the clay and POM fractions of the rhizosphere soil were also significantly higher than those of the non-rhizosphere soil. Apart from differences between the non-rhizosphere and rhizosphere soil, the clay and POM fractions always had the highest SOC contents, in contrast to the sand and silt fractions.

Similar to the SOC contents, the SOC stocks of the bulk $subsoil_{10}$ and its particle size fraction <63 μm were significantly higher than the SOC stocks of the bulk $subsoil_{85}$ and the corresponding fractions (Table 1). Although very low in mass, the clay fraction of the $subsoil_{85}$ accounted for 3.2 ± 1.3 g C m^{-2} (39.5%) of the bulk $subsoil_{85}$ SOC stocks (Table 1). This corresponds to a high E_c value for the clay fraction of the $subsoil_{85}$ (Table 1), when compared to the clay fractions of the $subsoil_{10}$ and rhizosphere soil. Despite these higher E_c values, there was a trend towards a higher specific surface area not covered by SOM of the clay fraction of the $subsoil_{85}$ (29.3 ± 5.3 m^2 g^{-1}) compared to the clay fraction of the $subsoil_{10}$ (18.6 ± 8.1 m^2 g^{-1}). Notably, the SOC stocks at deeper soil layers (40–200 cm) (1.4 ± 0.1 kg C m^{-2}), characterized by low amounts of root bio- and necromass, represented almost one third of the SOC stocks of the whole soil from 0 to 200 cm depth. The densely rooted soil at 0–40 cm depth accounted for 3.8 ± 0.9 kg C m^{-2} (~two thirds of the SOC stocks of 0–200 cm depth).The C/N ratios differed significantly between the $subsoil_{10}$, $subsoil_{85}$ and rhizosphere soil (Table 1). The C/N ratios of the $subsoil_{85}$ were significantly lower compared to those of the $subsoil_{10}$. Interestingly, the C/N ratio of the POM fraction of the rhizosphere soil (26.9 ± 2.1) was about half the C/N ratio of the POM fraction of the $subsoil_{10}$ (48.5 ± 5.9). The C/N ratios and OC contents of the leaves and the roots were significantly higher than the C/N ratios and OC contents of the organic layer (Table 3).

3.3. ^{13}C CPMAS NMR spectra

A significant correlation between the distance from the tree and the chemical compound classes could not be detected (Table A.1). Instead, differences between the $subsoil_{10}$ and $subsoil_{85}$, and between the non-rhizosphere and rhizosphere soil were observed.

In the clay fraction of the $subsoil_{85}$, the carboxyl and the aromatic C were higher compared to the corresponding compound classes of the clay fraction of the $subsoil_{10}$. This indicates a relative enrichment of aromatic compounds like lignin in $subsoil_{85}$. The relatively high O/N alkyl C peak of the clay fraction of the $subsoil_{85}$ points towards an accumulation of carbohydrates and proteins.

The NMR spectra of the clay and POM fractions of the $subsoil_{10}$ and the rhizosphere soil were dominated by alkyl C and O/N-alkyl C (Table 2, Figs. 3 and 4). Carboxyl and aromatic C together accounted for less than 30% of the sum of integrated peak areas. In most cases, O/N-alkyl C was significantly higher than alkyl C. This indicates the presence of high amounts of presumably more labile carbohydrates. Strikingly, the O/N-alkyl C of the POM fraction of the $subsoil_{10}$ was significantly lower than the alkyl C of the same fraction. This resulted in higher alkyl/O/N-alkyl C ratios in the POM fraction of the $subsoil_{10}$ (1.6 ± 0.4) compared to the POM fraction of the rhizosphere soil (0.8 ± 0.1).

The spectra of the leaves, roots and organic layer material (Fig. 5) were dominated by O/N alkyl C, which accounted for approximately two thirds of the sum of integrated peak areas of leaves and roots (Table 3). This was indicative for a high amount of polysaccharides and resulted in very low alkyl/O/N-alkyl C ratios. Higher amounts of alkyl C in the organic layer resulted in alkyl/O/N-alkyl C ratios of 0.7 ± 0.1.

4. Discussion

4.1. Impact of individual trees on SOM composition, SOC contents and stocks

In contrast to our hypothesis, the SOC contents and stocks of the bulk soil and the soil fractions were independent of the distance to individual trees. The same was observed for the chemical composition of SOM evaluated by ^{13}C NMR spectroscopy. For POM, this was probably because the beech roots and leaves, from which the POM is derived, have both been found to contain considerable amounts of similar alkanes, alcohols and carboxylic acids (Mueller et al., 2012a). This might render it difficult to identify effects on major chemical compound classes caused by a tree, although differences in monomeric composition could exist (cf. Spielvogel et al., 2014).

Moreover, the fine roots of the trees were evenly distributed in the horizontal and used all of the soil to the depth increment of 35 cm but were low in abundance at deeper soil layers (Fig. 2). Because roots are highly important for the input of OC to the soil (Rasse et al., 2005), we ascribe the non-existence of horizontal

Table 2
Relative peak intensities and alkyl/O/N alkyl C ratios of the clay and POM fractions of the $subsoil_{10}$, $subsoil_{85}$ and rhizosphere soil determined by solid state ^{13}C NMR spectroscopy. Significant differences between the $subsoil_{10}$, $subsoil_{85}$, and rhizosphere soil are indicated by lowercase letters. The superscript † symbols mark observations that are not significantly different when comparing the chemical compound classes to each other within the clay or POM fraction from $subsoil_{10}$, $subsoil_{85}$ or rhizosphere soil. Standard deviation (SD) of field replicates after ±.

	$Subsoil_{10}$		$Subsoil_{85}$		Rhizosphere soil	
	Clay	POM	Clay	POM	Clay	POM
Carboxyl C	12.6 ± 1.8b	7.9 ± 0.8a	22.7 ± 7.3a†	n.d	9.7 ± 0.9a	6.5 ± 0.3b†
Aromatic C	14.9 ± 1.1b	16.4 ± 2.4a	28.5 ± 5.1a†	n.d	12.6 ± 2.1b	15.3 ± 1.4a†
O/N alkyl C	36.9 ± 2.9b†	29.3 ± 3.9b	30.1 ± 5.0c†	n.d	49.8 ± 1.3a	43.1 ± 2.1a
Alkyl C	35.2 ± 4.5a†	46.4 ± 6.1a	17.5 ± 8.1b†	n.d	27.8 ± 2.3b	34.7 ± 2.8b
Alkyl/ O/N alkyl C	1.0 ± 0.2a	1.6 ± 0.4a	0.6 ± 0.2b	n.d	0.6 ± 0.1b	0.8 ± 0.1b

N = 24 for $subsoil_{10}$ & organic layer; n = 4 for $subsoil_{85}$; n = 3 for leaves, roots & rhizosphere soil; n.d. = not determined.

trends in NMR spectra and SOC contents and stocks mostly to the distribution of the fine roots.

4.2. Changes in chemical composition, SOC contents and stocks of the SOM fractions with depth

Although individual trees did not have a horizontal influence on the investigated parameters, we measured a significant vertical difference between $subsoil_{10}$ and $subsoil_{85}$ regarding the amount of the recovered fractions, the SOC contents and stocks, and the chemical composition of SOM (Tables 1 and 2). We assume that the spatially varying inputs of OM derived from the fine roots and above-ground litter were a main driver of these differences. Our data suggest a high input of OM in the densely rooted upper soil layers (to the depth increment of 35 cm depth) (Fig. 2) whereas the concentration of root bio- and necromass was low in deeper soil layers.

The chemical composition of the SOM fractions was dominated by alkyl and O/N-alkyl C, whereas carboxylic and aromatic C accounted for a smaller amount, as was also observed by others (Rumpel et al., 2002; Mueller et al., 2009). Beech roots and leaves had wide C/N and narrow alkyl/O/N-alkyl C ratios, indicating a low degree of decomposition. A relative increase of alkyl C and a decrease of O/N-alkyl C from plant inputs to the organic layer and the POM fraction of the $subsoil_{10}$ (Tables 2 and 3; Figs. 3 and 5) accompanied by decreasing C/N ratios can be ascribed to the decomposition of carbohydrates like cellulose and hemicellulose. Simultaneously, aliphatic components accumulate during decomposition relative to other compounds. These observations agree with the results of other studies (e.g., Quideau et al., 2001; Schöning and Kögel-Knabner, 2006).

Notably, alkyl/O/N-alkyl C ratios of the POM fraction of the $subsoil_{10}$ were very high (Table 2). This has also been observed for oPOM by Mueller et al. (2009) and suggests that the POM fraction in this study had already reached an advanced stage of decomposition. This indicates either that the aggregate turnover was rapid or very little macro-aggregation occurred, reducing physical protection (Six et al., 2000, 2002; Swanston et al., 2005). The high sand contents, especially in the $subsoil_{85}$, suggest a minor degree of macro-aggregation. Particulate OM can therefore be assumed to be readily available to the decomposition

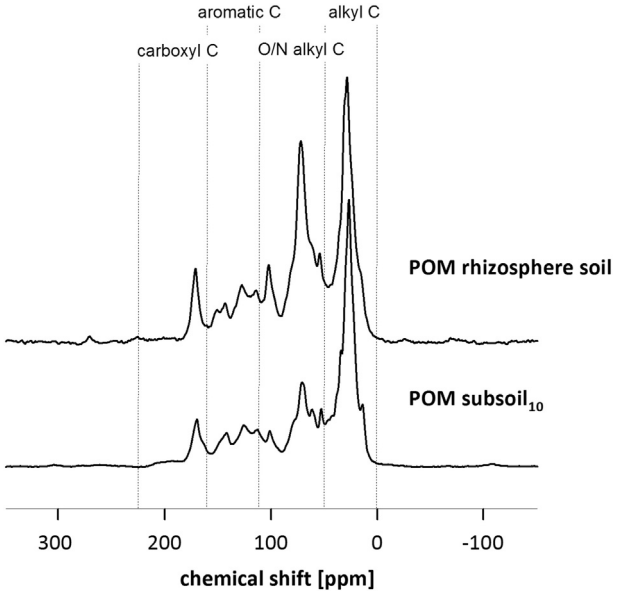

Fig. 3. ^{13}C CPMAS NMR mean spectra of the POM fractions of the rhizosphere soil (calculated from three spectra) and the $subsoil_{10}$ (calculated from 24 spectra).

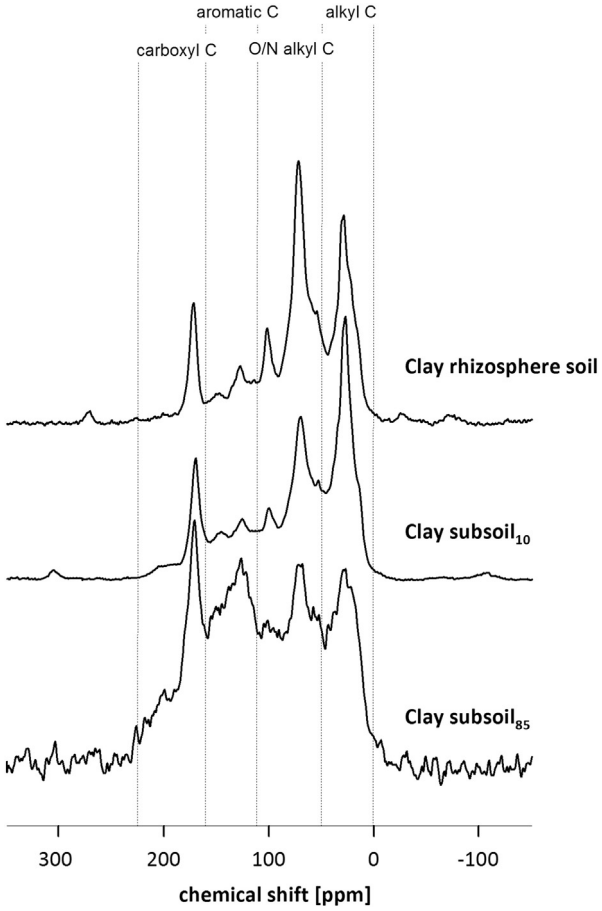

Fig. 4. ^{13}C CPMAS NMR mean spectra of the clay fractions of the rhizosphere soil (calculated from three spectra), $subsoil_{10}$ (calculated from 24 spectra) and $subsoil_{85}$ (calculated from four spectra).

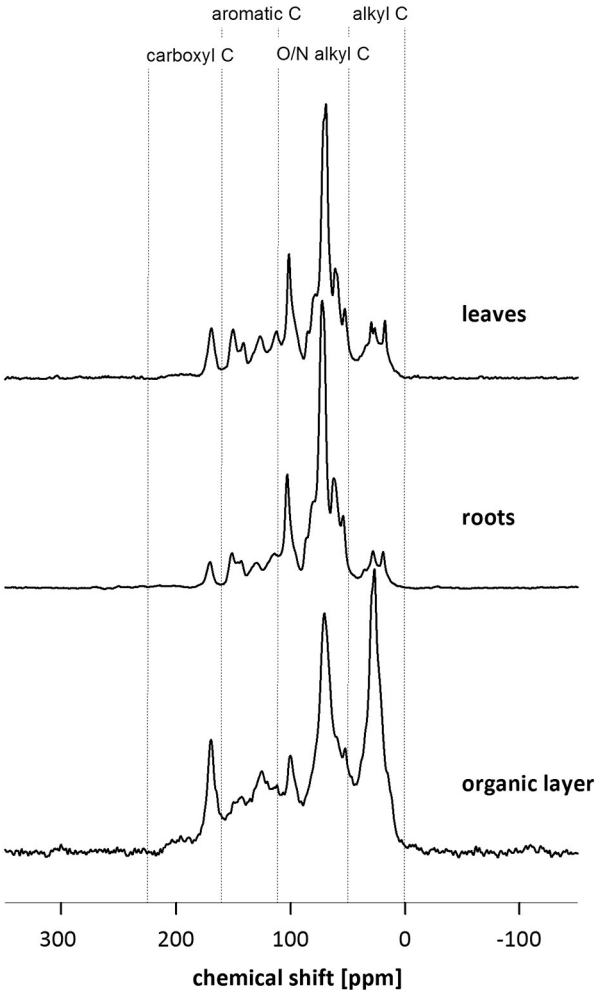

Fig. 5. ^{13}C CPMAS NMR mean spectra of the leaves, fine roots (each calculated from three spectra) and the organic layer material (calculated from 24 spectra) from all transects.

by microorganisms. This can be seen as an important reason for the absence of POM in the subsoil$_{85}$, together with a limited bioturbation and a large root litter input confined to depths < 40 cm.

The SOC contents and stocks of the bulk soil were drastically lower in the subsoil$_{85}$ compared to the subsoil$_{10}$. Similar trends have also been observed by others (Rumpel et al., 2004; John et al., 2005; Schöning and Kögel-Knabner, 2006). This was accompanied by a lower mass of the clay fraction of the subsoil$_{85}$ (Fig. 3). While the POM fraction was virtually absent in the subsoil$_{85}$, the clay fractions were enriched in SOC compared to the subsoil$_{10}$ (Table 3). A similar enrichment was also found by Rumpel et al. (2004) in the B horizons of a Dystric Cambisol under European beech. In contrast to our results, E_c values for the clay fraction determined by Rumpel et al. (2004) were about four times lower than E_c values detemined in our study. Clay was thus more important in stabilizing SOC by organo-mineral association in the sandy soils investigated in this study compared to soils with a lower sand content such as investigated by Rumpel et al. (2004). This conclusion was further corroborated by the SOC stocks (Table 1). The clay fraction of the subsoil$_{85}$ accounted for a considerable amount of the SOC stocks, although the mass of this fraction was only 22.8 ± 4.2 g kg^{-1} (Table 1). The SOC stocks at 40–200 cm depth were almost one third of the SOC stocks of the whole soil from 0 to 200 cm depth (Table 1). This is remarkable because the POM fraction, which accounted for the highest SOC stock in the subsoil$_{10}$, (Table 1) was absent from the subsoil$_{85}$. Most of SOC in the subsoil$_{85}$ was thus associated with the clay fraction.

The clay fraction of the subsoil$_{85}$ provided more free surface area not covered by SOC than the clay fraction of the subsoil$_{10}$. In addition, SOC contents of the clay fraction of the subsoil$_{85}$ were more variable than those of the clay fractions of the subsoil$_{10}$. This indicates that the amount and spatial variability of the SOM inputs to the deeper soil layers, rather than the availability of free sorption surfaces, were decisive for the quantity and spatial distribution of SOC stored in the clay fractions of deeper subsoil layers. Our data set indicates a drastic change from POM dominated SOC pools in the upper soil layers to SOC almost exclusively associated with clay in deeper soil layers.

4.3. Rhizosphere soil

The rhizosphere soil had three times higher SOC contents compared to the bulk subsoil$_{10}$. The fractionation approach suggests that this may be due to two different SOM contributions from the roots. First, the higher SOC contents of the clay fraction of the rhizosphere soil compared to the clay fraction of the non-rhizosphere soil (Table 1) were probably due to root exudates. These induce high microbial activity and the formation of microbial extracellular polymeric substances (EPS) in the direct vicinity of the roots (Kuzyakov, 2002; Koranda et al., 2011; Bengtson et al., 2012). Secondly, our data pointed towards a high and frequent supply of the rhizosphere soil with fresh POM. This was evidenced by a six times higher amount of the POM fraction derived from the rhizosphere soil compared to the amount of the POM fraction derived from the surrounding subsoil$_{10}$ (Table 1). Further, the POM fraction of the rhizosphere soil was significantly less processed than that of the subsoil$_{10}$ as indicated by lower alkyl/O/N-alkyl C ratios (Table 2).

Until now, root exudates have been considered to be the largest (Dennis et al., 2010) and most important contributor of SOC inputs to soils from roots (Kuzyakov et al., 2007). Our results suggest that root derived POM may also contribute considerable amounts of OC to the SOC pool.

5. Conclusions

In contrast to other studies, neither the SOC contents and SOC stocks nor the gross chemical composition of the SOM determined by ^{13}C CPMAS NMR spectroscopy were affected by the distance from *F. sylvatica* L. We ascribed this to the uppermost soil layers being densely and evenly rooted across all distances.

The trees caused significant vertical differences with POM dominated SOC pools in the upper soil layers, and SOC pools that were dominated by organo-mineral associations with the clay fraction in the deeper soil layers. Our results imply that these differences were strongly influenced by the roots of the trees. The SOC contents of the rhizosphere soil were more than three times as high as the SOC contents of the subsoil$_{10}$. This was ascribed to root exudates as well as to a high and frequent supply of the rhizosphere soil with fresh POM. We conclude that, besides

Table 3
Mean +/− SD organic carbon (OC) content, carbon to nitrogen ratio (C/N), chemical compound classes (carboxyl C, aromatic C, O/N alkyl C, alkyl C) and alkyl/ O/N alkyl C ratio of the leaves, fine roots and organic layer. Significant differences of the OC contents, C/N ratios or peak intensities between the leaves, roots and the organic layer are indicated by lowercase letters.

	Leaves	Roots	Organic layer
OC content [g (kg fraction)$^{-1}$]	453.1 ± 1.1a	484.8 ± 10.9a	112.9 ± 55.1b
C/N	37.7 ± 2.0a	93.8 ± 33.6a	24.0 ± 1.0b
Carboxyl C	5.9 ± 0.5b	3.6 ± 0.4c	10.6 ± 0.9a
Aromatic C	18.6 ± 0.3b	15.4 ± 1.9b	18.7 ± 0.4a
O/N alkyl C	62.2 ± 0.9a	71.3 ± 5.9a	40.5 ± 1.6b
Alkyl C	13.3 ± 0.3b	9.4 ± 4.3b	30.2 ± 1.4a
Alkyl/O/N alkyl C	0.2 ± 0.01b	0.1 ± 0.1b	0.7 ± 0.1a

root exudates, also root derived POM may contribute considerable amounts of SOC to the rhizosphere soil. The clay fractions in the vicinity of roots showed higher SOC contents and higher proportions of O/N alkyl C with respect to non-rhizosphere soil. This points to the rhizosphere as a hotspot for the formation of organo-mineral associations.

The clay fraction was specifically important for SOC storage at the deeper subsoil, where a low amount of organo-mineral associations comprised almost 40% of the bulk soil SOC stocks.

Soil OC stocks of deeper soil layers (40–200 cm) represented roughly one third of the total SOC stocks (0–200 cm depth). This indicates that sandy subsoils with low SOC contents have to be considered in C inventories and may be integral parts of the SOC pool.

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Appendix A

Table A.1
P-values for the statistical correlation between the distance from the individual beech trees and the respective parameter.

		$Subsoil_{10}$	$Subsoil_{85}$
Recovered mass	Sand	0.40	0.47
	Silt	0.41	0.53
	Clay	0.74	0.33
	POM	0.53	n.d.
SOC content	Bulk soil	0.91	0.32
	Sand	0.68	0.36
	Silt	0.39	0.46
	Clay	0.89	0.08
	POM	0.10	n.d.
C/N	Bulk soil	0.70	0.051
	Sand	n.d.	n.d.
	Silt	n.d.	n.d.
	Clay	0.43	0.21
	POM	0.77	n.d.
SOC stock	Bulk soil	0.83	0.32
	Sand	0.14	0.21
	Silt	0.07	0.93
	Clay	0.40	0.71
	POM	0.85	n.d.
E_c	Sand	0.35	0.88
	Silt	0.21	0.83
	Clay	0.36	0.65
POM	Carboxyl C	0.45	n.d.
	Aromatic C	0.84	n.d.
	O/N alkyl C	0.57	n.d.
	Alkyl C	0.60	n.d.
	Alkyl/O/N alkyl C	0.53	n.d.
Clay	Carboxyl C	0.62	0.98
	Aromatic C	0.88	0.94
	O/N alkyl C	0.46	0.79
	Alkyl C	0.83	0.86
	Alkyl/O/N alkyl C	0.82	0.76
Root biomass		0.73	0.98
Root necromass		0.70	0.49

Df = 22 for all correlations except for the NMR data of the clay fraction from the $subsoil_{85}$ (df = 2).

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