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Disentangling the sources, chemical composition, and spatial distribution of soil organic matter in topsoil and subsoil under European beech

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Zusammenfassung

Unterböden speichern große Mengen an Kohlenstoff (C) und sind somit hoch relevant für den terrestrischen Kohlenstoffkreislauf. Überraschenderweise hat die Forschung den Unterboden in der Vergangenheit vernachlässigt und die Anzahl der Studien nahm erst in den letzten Jahren signifikant zu. Dies hat zur Folge, dass die empirische Datengrundlage bezüglich der Menge, chemischen Zusammensetzung und räumlichen Verteilung von organischem Kohlenstoff (OC) im Unterboden gering ist. Weiterhin wurden die Quellen von organischer Bodensubstanz (SOM) – oberirdische oder unterirdische OC Einträge von verschiedenen Pflanzenteilen − weitgehend von den Untersuchungen ausgeschlossen, obwohl sie wesentlich die Stabilisierung oder Mineralisierung von SOM und des darin enthaltenden organischen Bodenkohlenstoffs (SOC) bestimmen. So wurde in einer der wenigen durchgeführten Studien ein schnellerer Abbau von laubbürtigem verglichen mit wurzelbürtigem SOC gefunden. Die Unterscheidung der Quellen des SOC geschah mit den Biopolymeren Cutin und Suberin. Deren Abbauverhalten und Eignung als Biomarker, besonders im Falle von Suberin, wurden allerdings noch nicht ausreichend untersucht. Weiterhin wurde der Einfluss der Vegetation (durch bspw. Wurzelraum oder Laubabwurf von Bäumen) vernachlässig, obwohl dieser große Bedeutung für die räumliche Verteilung des SOC aus unterschiedlichen Quellen haben kann.

Diese Arbeit wurde im Rahmen der Forschergruppe FOR1806 "The Forgotten Part of Carbon Cycling: Organic Matter Storage and Turnover in Subsoils (SUBSOM)" durchgeführt, die durch die Deutsche Forschungsgemeinschaft finanziert wurde. Übergeordnetes Ziel dieser Arbeit war es, die oben beschriebenen Missstände direkt zu adressieren und die Menge und räumliche Verteilung von SOC (besonders im Unterboden), dessen Stabilisierung, chemische Zusammensetzung und Quellen in Abhängigkeit zur Distanz von individuellen Bäumen zu untersuchen.

Um räumliche Heterogenitäten abzudecken, wurden Bodenproben in einem gleichmäßigen Raster (3.15 m lang und 2.00 m tief mit 45 cm Horizontal- und 25 cm Vertikalabständen zwischen den Probenahmepunkten) in drei Profilgruben genommen. Das Ende jeder Profilgrube wurde direkt am Stammfuß angelegt, sodass das andere Ende vom jeweiligen Baum wegzeigte und der Einfluss der Bäume auf die untersuchten Parameter evaluiert werden konnte. Um die Ziele dieser Arbeit zu erreichen, wurde ein umfassendes methodisches Vorgehen gewählt:

(1) Kohlenstoff und Stickstoffmessungen (N) an Gesamtböden und C,N und ^{13}C Kernspinresonanzspektroskopie (NMR) Messungen an Korngrößen- und Dichtefraktionen von Gesamt- und Rhizosphärenboden wurden durchgeführt, um

Stabilisierungsmechanismen, Menge und chemische Zusammensetzung von SOM/SOC in verschiedenen Bodentiefen zu untersuchen (Studie I).

(2) Um den Abbau und die Anwendbarkeit von Cutin- und Suberinmonomeren als Biomarker für ober- und unterirdische Quellen von SOC zu bewerten, wurde eine kurze Inkubation von Buchenblättern und –wurzeln und von Fichtennadeln und –wurzeln durchgeführt. Dabei wurde die Degradation von Pflanzenmaterial und Monomeren verfolgt und mögliche Einflussfaktoren auf den Abbau wurden statistisch evaluiert (Studie II).

(3) Um Quellen des SOC zu identifizieren wurde eine Kombination aus den in Studie II untersuchten Biomarkern, lösungsmittellöslichen Lipiden sowie ^{14}C Messungen verwendet.

Die Ergebnisse von Studie I zeigten, dass die Entfernung zu den individuellen Buchen keinerlei Einfluss auf die Menge und die chemische Zusammensetzung der SOM hatte. Eine horizontal gleichmäßige Durchwurzelung des Bodens, große Mengen an SOC und wenig abgebauter partikulärer organischer Substanz im Rhizosphärenboden deuteten auf eine essentielle Bedeutung von Wurzeleintrag für die beobachteten Muster hin. Diese Annahme wurde weiter bekräftigt von stark abnehmenden SOC Gehalten und Vorräten mit zunehmender Tiefe und gleichzeitig abnehmender Wurzelbio- und –nekromasse. Allerdings kann ein gleichmäßiger Eintrag von Laub ebenfalls zu einer Abwesenheit von horizontalen Trends bezüglich der untersuchten Parameter führen. Um diese Möglichkeit abzudecken, empfahl es sich ober- und unterirdische Quellen des SOC zu unterscheiden. Die Biopolymere Cutin und Suberin schienen dafür am besten geeignet. Da es allerdings immer noch substanzielle Wissenslücken bezüglich des Abbaus der beiden Polymere gibt (besonders bei Suberin), wurde Studie II durchgeführt, um den Abbau von Cutin und Suberin und ihre Eignung als Biomarker zu evaluieren.

Die Ergebnisse von Studie II bestätigten die Anwendbarkeit von Cutin und Suberin für die Unterscheidung von ober- und unterirdischen Quellen von SOC, da sich einzelne Monomere mit gleichen Raten abbauten. Weiterhin implizierten die Ergebnisse keine höhere Stabilität von Suberin im Vergleich zu Cutin, obwohl für gewöhnlich eine relative Akkumulation von Suberin in Waldböden beobachtet wird. Inhärente Eigenschaften wie der relative Ligningehalt, die Kettenlänge der jeweiligen Monomere und die Art (verschiedene funktionelle Gruppen) der Monomere hatten einen signifikanten Einfluss auf das Abbauverhalten von Cutin und Suberin. Allerdings wurden in etwa zwei Drittel der Variation in Lipidkonzentrationen über die Zeit nicht durch die getesteten Parameter erklärt. Dies bedeutet, dass die Degradation von Cutin und Suberin zusätzlich durch noch nicht quantifizierte externe Faktoren bestimmt wird.

Studie III wurde basierend auf den Ergebnissen der Studien I und II mit Hilfe von Cutin und Suberin Biomarkern durchgeführt, um die Bedeutung von SOC aus ober- und unterirdischen Quellen an unterschiedlichen Punkten der Profile näher zu differenzieren. Die Interpretation der Daten wurde weiterhin unterstützt von lösungsmittellöslichen Lipiden und ^{14}C Messungen. Die Ergebnisse von Studie III spiegelten diejenigen von Studie I wider, da weder distanzabhängige Unterschiede bei Cutin/Suberin und den lösungsmittellöslichen Lipiden, noch bei ^{14}C Gehalten festgestellt wurden. Stattdessen zeigte der Boden eine starke vertikale Zonierung in Abhängigkeit von (^{14}C) Gehalten und Herkunft des SOC. Es wurde eine (i) Wurzel und Laub beeinflusste Zone (10 und 35 cm Tiefe; B Horizont) von einer (ii) Wurzel beeinflussten Zone (60 bis 110 cm Tiefe, C Horizonte) unterschieden. Zone (i) war beeinflusst von großen Mengen an frischem Suberin und lösungsmittellöslichen Lipiden. Dies bekräftigt die Bedeutung von wurzelbürtigem SOC und impliziert, dass laubbürtiger SOC noch weit in den Unterboden hinein von Bedeutung sein kann. Frischer SOC in Zone (ii) war wurzelbürtig. Allerdings deuteten sehr geringe ^{14}C Gehalte auf einen größeren Anteil von sehr altem SOC hin, der eventuell vom Ausgangsmaterial ererbt war. Da SOC Vorräte unterhalb von 35 cm noch sehr hoch waren, leistet dieser alte SOC einen wichtigen Beitrag zum SOC-pool in tiefen Bodenschichten. Diese Ergebnisse deuten weiterhin darauf hin, dass es essentiell ist, den Unterboden in C Inventuren miteinzuschließen, auch wenn SOC Gehalte geringer sein sollten als im Oberboden.

Summary

Subsoil stores high amounts of carbon (C) and is thus of significant importance to terrestrial C cycles. Surprisingly, subsoil has only recently attracted the notice of soil scientists resulting in a scarcity of studies on the amount, chemical composition, and spatial distribution of subsoil organic carbon (OC). Furthermore, the source of soil organic matter (SOM) in subsoil – aboveground or belowground inputs from different plant parts − has widely been ignored although it is crucial for stabilization or mineralization of SOM and the soil organic carbon (SOC) contained within. For example, one of the few studies conducted so far found a faster cycling of leaf compared to root derived compounds in soil. The differentiation of SOC from different sources was achieved using the biopolymers cutin and suberin, despite the fact that their decay and suitability as biomarkers, especially regarding suberin, have not yet been sufficiently characterized. Furthermore, the influence of the vegetation (through leaf fall or the extent of the rooting zone of a tree) has mostly been excluded from investigation although it may determine the spatial distribution of the SOC from aboveground and belowground sources.

Within the Research Unit FOR1806 "The Forgotten Part of Carbon Cycling: Organic Matter Storage and Turnover in Subsoils (SUBSOM)" of the Deutsche Forschungsgemeinschaft (DFG), the aims of this thesis were to directly address the above mentioned short-comings and investigate the amount and location of SOC (especially in subsoil) and its stabilization, chemical composition, and sources as dependent on the distance to individual trees.

To cover spatial variabilities, soil samples were taken in a regular sampling grid (3.15 m in length and 2.00 m in depth with 45 cm horizontal and 25 cm vertical sampling increments) applied to the profile walls of three replicate transects. Each transect increased in distance to individual mature European beech trees. This sampling design enabled to study the influence of the individual trees on the investigated parameters. The aims of this thesis were approached by a combination of methods:

(1) Carbon and nitrogen (N) measurements on bulk soil and C, N, and ^{13}C nuclear magnetic resonance (NMR) spectroscopy measurements on density and particle size fractions from bulk soil and rhizosphere soil (Study I) were performed. These analyses allowed disentangling stabilization mechanisms, amount and chemical composition of SOM/SOC stored at different depths in the soil.

(2) To test the suitability of cutin and suberin derived monomers as biomarkers for above and belowground plant inputs, a short-term laboratory incubation of spruce and beech needles/leaves and roots was performed. The decay of plant material and monomers was monitored and possible factors that might influence the decomposition were statistically evaluated (Study II).

(3) A multi-biomarker approach (solvent extractable and hydrolysable lipids biomarkers) that built upon the results of study II in connection with ^{14}C measurements was applied to bulk soil samples in order to identify sources and apparent age of SOC stored at different depths in subsoil (study III).

The findings of study I showed that the distance to the individual beech trees did not have a significant influence on the chemical composition and amount of SOM/SOC. A horizontally even rooting of the soil, high amounts of C and little decomposed particulate organic matter in rhizosphere soil indicated the importance of root inputs for the observed patterns. These findings were further corroborated by highly decreasing SOC contents and stocks with increasing soil depth that coincided with a considerable decrease of the root biomass and necromass. However, horizontally even inputs of leaf litter may likewise have been responsible for the absence of distant dependent trends. Thus, distinguishing SOC from aboveground and belowground sources helps to evaluate the importance of SOC from different sources at different locations in the soil profiles. The biopolymers cutin and suberin appeared to be most suitable for this purpose. However, because there are still considerable uncertainties regarding the decay of both biopolymers, study II was conducted in order to eliminate these shortcomings and re-evaluate their suitability as biomarkers.

The findings of study II confirmed the suitability of cutin and suberin for the differentiation of aboveground and belowground plant inputs to soil because specific monomers decreased uniformly during decay. Furthermore, the data did not indicate a higher stability of suberin compared to cutin, despite the fact that suberin has often been observed to accumulate in forest soil relative to cutin. Inherent chemical properties like the relative lignin content, chain-length and lipid type significantly influenced the decay pattern of both biopolymers but approximately two thirds of the variation in lipid concentrations over time was not accounted for by the tested factors. Thus, the decomposition of cutin and suberin has to be additionally modulated by a not yet quantified external factor.

Based on the findings of studies I and II, study III was conducted to evaluate the importance of SOM from aboveground and belowground sources at different locations in the soil profiles using the biomarkers cutin and suberin. Data interpretation was aided by solvent-extractable lipids and ^{14}C measurements. The results of study III mirrored the patterns detected in study I. The distance to the individual beech trees did not have a significant influence on lipid concentrations or ^{14}C contents. Instead, a pronounced vertical zonation of the subsoil was detected with a (i) root- and leave-affected zone (upper subsoil, 10 and 35 cm depth, B horizons) and a (ii) root-affected zone (deeper subsoil, 60 to 110 cm depth, C horizons). Zone (i) was dominated by high amounts of fresh suberin and leave-derived solvent-extractable lipids. These findings confirm the importance of root OC input to soils and indicate that leaf-derived SOC may still be relevant below the A horizons of a soil. Roots

were an important source for fresh SOC in zone (ii). However, very high apparent ^{14}C ages point to a greater proportion of old SOC probably inherited from the parent material. Because SOC stocks were still considerably high below 35 cm depth, this old SOC has to be considered as an important contributor to the SOC pool in deep subsoils. Furthermore, these findings demand the inclusion of subsoils in carbon inventories, even if SOC contents are low.

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Abbreviations

AMS	accelerated mass spectrometry
ANOVA	analysis of variance
ASE	accelerated solvent extraction
C	carbon
CPI	carbon preference index
CPI_{alk}	carbon preference index for alkanes
CPI_{FA}	carbon preference index for fatty acids
CPMAS	cross-polarization magic angle spinning
fPOM	free particulate organic matter
GC/MS	gas chromatography/mass spectrometry
HF	hydrofluoric acids
N	nitrogen
NMR	nuclear magnetic resonance
OC	organic carbon
oPOM	occluded particulate organic matter
PCA	principal component analysis
POM	particulate organic matter
ppm	parts per million
P_{RML}	proxy for root/microbial- vs. leaf-derived C
SOC	soil organic carbon
SOM	soil organic matter
SSA	specific surface area

List of publications and contributions

First authored research articles

<u>Study I</u>:

Angst, G., Kögel-Knabner, I., Kirfel, K., Hertel, D., Mueller, C.W. (2016): Spatial distribution and chemical composition of soil organic matter fractions in rhizosphere and non-rhizosphere soil under European beech (Fagus sylvatica L.). Geoderma (264), 179 – 187.

Contribution: I carried out the field work, laboratory analysis, data evaluation and wrote the manuscript.

Summary: The knowledge about the amount, location, and chemical composition of carbon (C) in subsoil is scarce, despite the fact that subsoil C is an essential part of the global C cycle. In study I, the soil organic C (SOC) contents and stocks, and chemical composition of soil organic matter fractions from topsoil and subsoil were investigated. Soil samples were taken in a regular sampling grid (45 cm horizontal and 25 cm vertical spaces) applied to the profile walls of three replicate transects (3.15 in length and 2.00 deep), each of which started at the stem base of a mature European beech tree. By using such an approach, it was possible to evaluate the influence of the distance from individual trees, which was thought to have a major impact on the investigated soil parameters. Bulk soil and rhizosphere soil samples were subjected to a combined density and particle size fractionation. The C and nitrogen contents were determined, and the chemical composition revealed by ^{13}C nuclear magnetic resonance spectroscopy of the clay and particulate organic matter fractions. Surprisingly, the distance to the beech trees did not have any influence on the investigated parameters. Instead, a strong vertical gradient was detected with increasing contents of the sand fraction along with strongly decreasing SOC contents and stocks from 10 to 85 cm depth. These patterns were mainly ascribed to root carbon inputs due to a dense and even rooting of the upper soil layers, whereas concentrations of the root biomass and necromass were very low below 35 cm depth. A hint to a considerable importance of root carbon inputs was derived from a six times higher amount of little decomposed particulate organic matter of rhizosphere soil compared to non-rhizosphere soil. Despite the fact that SOC contents were low in deeper soil layers, the SOC stocks in 40 – 200 cm depth were one third of those measured in the whole profile (0 – 200 cm depth). In this regard, the clay fraction was enriched in C, which supported its importance for the stabilization of SOC by the formation of organo-mineral associations. The results further demand to include subsoil in C inventories, even if SOC contents are low.

Study II:

Angst, G., Heinrich, L., Kögel-Knabner, I. Mueller, C.W. (2016): The fate of cutin and suberin of decaying leaves, needles and roots – inferences from the initial decomposition of bound fatty acids. Organic Geochemistry (95), 81 – 92.

Contribution: I carried out the field work, laboratory analysis, data evaluation and wrote the manuscript.

Summary: The biopolymers cutin and suberin have readily been used to distinguish aboveground from belowground sources of SOC. Yet, still little is known about their fate during decomposition. Study II was conducted to investigate the decay of cutin and suberin monomers in relation to the source plant material and evaluate potential influences of inherent chemical properties on decomposition. European beech leaves, Norway spruce needles and roots of the respective tree species were incubated for 84 days under controlled laboratory conditions. The mass loss of the plant material was monitored and the initial, non-incubated and incubated materials (at 14, 28, 42 and 84 days of incubation) were subjected to C, nitrogen, and ^{13}C nuclear magnetic resonance spectroscopy measurements, and to a sequential extraction procedure to release constituting monomers of cutin and suberin. The results indicated that cutin and suberin monomers are readily decomposed without any indication of suberin being more resistant to decay than cutin. However, related to the source plant material, cutin and suberin monomers decomposed more slowly, indicating that the assumption of a similar decay of biopolymer and plant material might lead to an underestimation of the turnover of root- and shoot-derived soil organic matter. It still remains valid, though, to sum up individual cutin or suberin monomers to single biomarkers because biopolymer specific monomers decomposed with similar rates. Because the decay of both cutin and suberin monomers leveled off towards the end of the incubation and the relative lignin content of the plant materials explained a considerable portion of the variation in lipid concentrations over time, we proposed a two-phase model of the decay for the two biopolymers: In the first phase, only cutin or suberin that is not associated with lignin is decomposed and the decay of the biopolymers is rapid. In the second phase, only cutin or suberin that is associated with lignin remains, and the biopolymers decomposed with the initially slow decay rate of lignin. However, the evaluated inherent chemical properties of the plant material (relative lignin content derived from the NMR data, C chain length of each monomer, and lipid type defined by different functional groups) explained about one third of the variation in lipid concentrations over time. Thus, the decay of cutin and suberin has to be additionally modulated by a now yet quantified external factor.
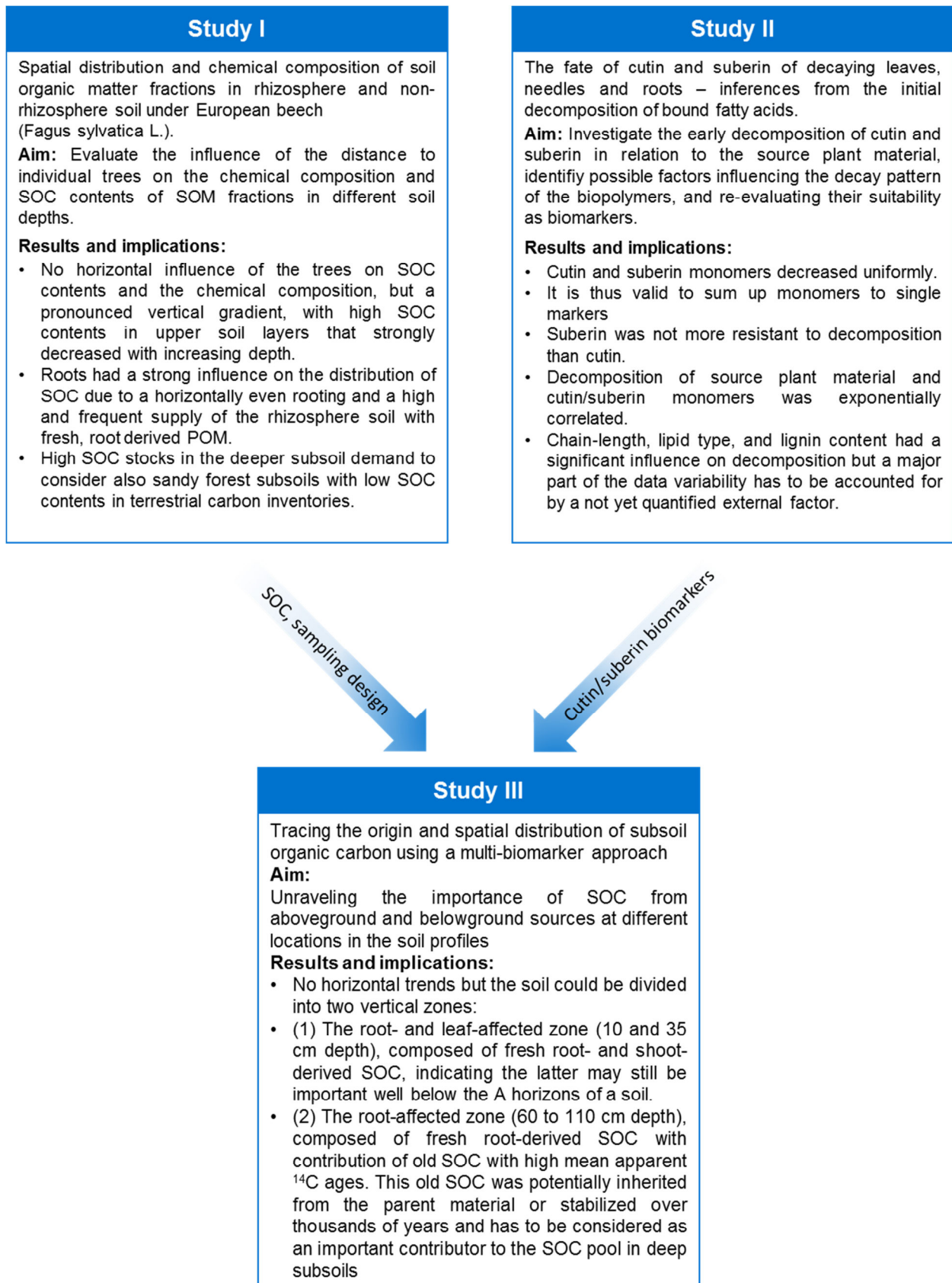
Study III:

Angst, G., John, S., Mueller, C.W., Kögel-Knabner, I., Rethemeyer, J. (2016): Tracing the origin of subsoil organic carbon using lipid biomarker and ^{14}C analyses.

Contribution: S. John and I equally contributed to the publication by carrying out the field work, laboratory analysis, data evaluation and jointly writing the manuscript.

Summary: Aboveground and belowground sources of SOC have rarely been studied despite the fact that the origin of SOC may drive its turnover and stabilization. Study III was conducted to reveal the contributions of SOC from aboveground and belowground sources at different location in a Dystric Cambisol under mature European beech trees. Soil samples were taken in a regular grid (down to 110 cm depth) applied to the profile walls of three transects each of which started at the stem base of a mature European beech tree. It was thus possible to trace the distant dependent influences of the beech trees on the distribution of the SOC from different sources. The sources of the SOC were distinguished by solvent-extractable and hydrolysable (cutin and suberin) lipid biomarkers aided by ^{14}C measurements. The distance to the trees had no measurable effect on the investigated parameters. Instead, a pronounced vertical zonation of the subsoil was detected. High contributions of leaf- and root-derived SOC in the upper subsoil (down to 35 cm depth; root- and leaf-affected zone) indicate that leaf-derived SOC may still be important below the A horizon of a soil. This leaf-derived SOC was likely transferred from the topsoil via bioturbation, since the cutin markers that were found at 35 cm depth possess a low water solubility and a transfer by water thus appears to be unlikely. Fresh SOC in the deeper subsoil (35 to 110 cm depth) was almost exclusively derived from roots (root-affected zone). However, very high apparent ^{14}C ages at the deeper subsoil indicate large contributions of considerably old SOC stabilized over long periods of time or inherited from the parent material. This old SOC has to be considered as an important contributor to SOC pools in deep subsoils.

Dissertation at a glance

Study I

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

Aim: Evaluate the influence of the distance to individual trees on the chemical composition and SOC contents of SOM fractions in different soil depths.

Results and implications:

- No horizontal influence of the trees on SOC contents and the chemical composition, but a pronounced vertical gradient, with high SOC contents in upper soil layers that strongly decreased with increasing depth.
- Roots had a strong influence on the distribution of SOC due to a horizontally even rooting and a high and frequent supply of the rhizosphere soil with fresh, root derived POM.
- High SOC stocks in the deeper subsoil demand to consider also sandy forest subsoils with low SOC contents in terrestrial carbon inventories.

Study II

The fate of cutin and suberin of decaying leaves, needles and roots – inferences from the initial decomposition of bound fatty acids.

Aim: Investigate the early decomposition of cutin and suberin in relation to the source plant material, identifiy possible factors influencing the decay pattern of the biopolymers, and re-evaluating their suitability as biomarkers.

Results and implications:

- Cutin and suberin monomers decreased uniformly.
- It is thus valid to sum up monomers to single markers
- Suberin was not more resistant to decomposition than cutin.
- Decomposition of source plant material and cutin/suberin monomers was exponentially correlated.
- Chain-length, lipid type, and lignin content had a significant influence on decomposition but a major part of the data variability has to be accounted for by a not yet quantified external factor.

SOC, sampling design

Cutin/suberin biomarkers

Study III

Tracing the origin and spatial distribution of subsoil organic carbon using a multi-biomarker approach

Aim:

Unraveling the importance of SOC from aboveground and belowground sources at different locations in the soil profiles

Results and implications:

- No horizontal trends but the soil could be divided into two vertical zones:
- (1) The root- and leaf-affected zone (10 and 35 cm depth), composed of fresh root- and shoot-derived SOC, indicating the latter may still be important well below the A horizons of a soil.
- (2) The root-affected zone (60 to 110 cm depth), composed of fresh root-derived SOC with contribution of old SOC with high mean apparent ^{14}C ages. This old SOC was potentially inherited from the parent material or stabilized over thousands of years and has to be considered as an important contributor to the SOC pool in deep subsoils

1. State of the art and objectives

1.1. Soil organic carbon and its fate

An integral characteristic of soil is its ability to store organic carbon (OC) over periods more than several thousands of years (Post et al., 1982) making it the largest terrestrial C reservoir in the global carbon cycle (globally 2344 Pg C in the top 3 m, Jobbágy and Jackson, 2000; Rumpel and Kögel-Knabner, 2011). Organic C that is stabilized in soils is withdrawn from the atmosphere and may not contribute to the greenhouse effect (Lal and Bruce, 1999). The most important source of soil OC (SOC - C in soil derived from organic constituents) is plant derived organic matter (OM). Aboveground sources of soil OM (SOM - the entirety of dead matter derived from plants and animals, and their organic transformation products) are aerial plant tissues like leave/needle litter (Kögel-Knabner, 2002). With ongoing time, the aboveground plant inputs become incorporated into the organic layer and mineral soil by the soil fauna (Pulleman et al., 2005). Belowground inputs are root derived litter or root exudates (Dennis et al., 2010) which are directly supplied to the soil *in situ*. The chemical composition and amount of aboveground and belowground inputs are highly variable and depend on land-use and plant species (Kögel-Knabner, 2002). For example, data compiled from different studies indicate that temperate forests have an average root-to-shoot ratio of 2.5, whereas temperate grasslands have a root-to-shoot ratio of 3.7 (Kögel-Knabner, 2002). Generally, leaf, needle, and root litter is composed of polysaccharides and lignin with additional contributions of different lipids, proteins, or tannins (Kögel-Knabner, 2002; von Lützow et al., 2006) differing in amount and composition depending on plant species (Mueller et al., 2012). Root exudates are primarily composed of low molecular weight organic compounds that are easily available to microorganisms (Kuzyakov et al., 2007) resulting in a higher microbial activity in the soil directly adjacent to roots (Farrar et al., 2003). The plant inputs supplied to the soil are subsequently mechanically and chemically altered and finally mineralized to CO_2 by the microbial community or stabilized within the soil. Three commonly accepted mechanisms have been identified that lower SOM bioavailability and thus lead to a stabilization of SOC: (i) resistance against decomposition because of the inherent, chemical composition of the respective SOM compound, (ii) a decreased bioavailability due to the development of soil aggregates (most importantly micro-aggregates (Six et al., 2002b)) separating substrate from decomposer organisms, and (iii) the stabilization of potentially available SOM in organo-mineral associations by chemical surface reactions (Christensen, 2001; von Lützow et al., 2006). The extent of the individual stabilization mechanisms is highly variable and dependent on soil type and environmental factors like temperature, humidity, pH, or oxygen availability (Schmidt et al., 2011) which in turn influence the microbial community and soil development. Regarding mechanism (i), it has been suggested that

chemical recalcitrance may only be important over short periods of time and cannot be responsible for the long-term stabilization of SOM because the microbial community was shown to be capable of decomposing any organic compound in the presence of an easily available C source (Marschner et al., 2008). This inference is supported by recent studies that showed that lignin, which should be relatively recalcitrant due to its aromatic nature (Mikutta et al., 2006), did not accumulate in particulate organic matter (POM) in soil (Vancampenhout et al., 2012) relative to e.g., the lipids cutin and suberin (Carrington et al., 2012; Filley et al., 2008). These lipids are predominantly composed of aliphatic acids (Mueller et al., 2012), which may be relatively easily degraded (Kögel-Knabner, 2002; Tegelaar et al., 1989). Thus, the interaction of SOM compounds with mineral surfaces thereby creating organo-mineral associations and the spatial inaccessibility due to the formation of aggregates seem to be crucial factors responsible for the persistence of SOM (Schmidt et al., 2011) rather than a presumed inherent chemical recalcitrance.

Recent studies indicate a different stability of SOM compounds derived from either aboveground or belowground sources (Pisani et al., 2015). For example, Crow et al. (2009) studied the effect of the removal of aboveground and belowground inputs in a deciduous forest. Within several years, root-derived C dominated and C mineralization decreased, suggesting that root-derived compounds were a source of SOC with greater relative stability, whereas aboveground leaf litter was the source of the most actively cycling C. Whether this observation was due to a different chemical composition and thus preferential stabilization of root-derived compounds on mineral surfaces remains unclear. However, this example clarifies that the source of SOC may have a significant importance for the stabilization or mineralization of SOC (Rasse et al., 2005).

The distribution of aboveground and belowground sources in soil is highly dependent on the prevailing vegetation. The OM input to soils by trees is influenced by litter fall and the extent of the rooting zone (Jandl et al., 2007). In SOC studies though, the distribution of SOC and the differentiation of its origin in relation to the prevailing vegetation has rarely been investigated (Spielvogel et al., 2014). The results of the few studies with a spatially coordinated sampling design are inconsistent. For example, Schöning and Kögel-Knabner (2006) found a small scale spatial variability of SOC stocks but no clear relation to the distance from individual trees. In contrast, Spielvogel et al. (2014) differentiated SOC by its origin and found pronounced vertical and horizontal gradients in lipid biomarker distributions mainly controlled by the rhizosphere of individual trees. However, in most studies, samples are collected at one spot only varying in soil depth. This approach may fail to depict possible spatial heterogeneities. This may be especially crucial in subsoils, where the few studies conducted so far found a significantly higher spatial variability of C and ^{14}C contents in subsoil compared to topsoil (Chabbi et al., 2009; Schöning et al., 2006). These spatial

heterogeneities question the reliability of subsoil C stocks, microbial biomass and chemical composition measures mostly derived from only one sample per soil horizon (e.g., Kaiser and Zech, 2000; Rumpel et al., 2004; Vancampenhout et al., 2012). A sound empirical basis regarding these parameters is crucial though for C models and management practices (Campbell and Paustian, 2015).

1.2.   Investigations of subsoil are scarce. Is it important anyway?

Although SOC contents at greater depth are generally lower than those measured in topsoil (Rumpel and Kögel-Knabner, 2011), a significant amount of SOC may be stored well below the first meter of the soil profile (global average of 842 Pg C in 1-3 m depth to 1502 Pg C in the first meter of the soil) (Jobbágy and Jackson, 2000). Furthermore, temporal changes in subsoil OC have not been well investigated but may be highly significant for the global C cycle (Richter and Billings, 2015). It follows, that subsoil is indeed important and relevant to SOC storage and dynamics. The scarcity of studies investigating subsoil may be due to the fact that its relevance has only been recognized in recent years. Also, the access to subsoils is commonly more complicated than that to topsoils, which may be a reason for the majority of subsoil studies reviewed by Post and Kwon (2000) and West and Post (2002) only having had a median sampling depth of 30 cm. The ^{14}C contents of bulk SOC generally decrease with increasing depth (Flessa et al., 2008; Rethemeyer et al., 2005; Rumpel et al., 2004) indicating that SOC stored in subsoils may be inherited from the parent material or stabilized over long periods of time (Lorenz and Lal, 2005; Rumpel and Kögel-Knabner, 2011). The major part of the SOC in subsoils has been detected in the mineral soil fraction <6.3 µm (fine silt and clay) (Hassink et al., 1997; Rumpel et al., 2004). An enrichment of SOC in these fractions with increasing depth in connection with low ^{14}C contents indicate their importance for subsoil OC stabilization (Kaiser et al., 2002; Rumpel et al., 2004). The chemical composition of these fine silt and clay fractions revealed by thirteen C (^{13}C) cross polarization magic angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectroscopy showed a dominance of alkyl C that might represent preserved plant-derived lipid components like cutin and suberin or microbial-derived SOC (Rumpel et al., 2004). Despite an enrichment of SOC in the fine mineral fractions of subsoils, specific surface area (SSA) measurements indicate that subsoil horizons have a minor portion of their surface covered with OM when compared to topsoils (Kaiser and Guggenberger, 2003). Some authors therefore regarded subsoils as having the potential to sequester additional C by inputs of root derived or dissolved OC (Lorenz and Lal, 2005; Lorenz et al., 2007). Fontaine et al. (2007) though, showed that the addition of fresh organic matter to subsoil resulted in the stimulation of the microbial community which in turn led to an intensified respiration of SOC. On the other hand, Salomè et al. (2010) did not observe an elevated C mineralization in subsoils after the addition of an easily available C source. They ascribed the lack of a priming effect to a limited contact

between degraders or exo-enzymes and substrate either by stabilization mechanism (ii) (cf., section 1.1.) or by the diffusion of e.g., root exudates away from zones of microbial activity (Salomè et al., 2010).

The inconsistency of these findings might be a result of the input pathways of OM to subsoils. Input is usually confined to locations where roots and preferential flow paths are present or where bioturbation takes place, while topsoils receive a spatially more even input of OM (Rumpel and Kögel-Knabner, 2011). Along the input pathways, OM and the microbial activity are located (Chabbi et al., 2009). As a result, SOC contents and also the amount of microbial biomass may highly vary in an only small volume of soil. The negligence of these spatial heterogeneities in most sampling designs may be the reason why the importance of SOC of different origin is controversially discussed and no clear consensus has been found yet. For example, Rasse et al. (2005) supported the notion that SOC is mostly root derived and increasingly so with increasing soil depth. In contrast, other studies regard DOC mostly derived from aboveground litter and the humus layer (Kalbitz et al., 2000) as most important pathway through which SOC is transferred to subsoil.

As highlighted above, results on the chemistry, amount, and origin of SOC that may determine its stabilization or mineralization are still far from being consistent, but of integral importance to the C cycle. These issues need to be addressed in a spatially resolved sampling design, encompassing vertical and horizontal heterogeneities.

1.3.   Methods to unveil the chemical composition and origin of soil organic carbon

1.3.1.   Differentiating functional SOM fractions using combined density and particle size fractionation

The separation of a soil sample into sub-fractions by physical means is based on the idea that different fractions of a soil represent differently stable functional SOM compartments with differing decay behavior (von Lützow et al., 2007). Further analyses may reveal the chemical composition of these more or less refractory SOM fractions. There have been a lot of fractionation protocols mixing different methods and parameters without any standardized protocol having been developed so far (Elliott and Cambardella, 1991) due to the need for a soil specific optimization of the procedure (Christensen, 2001). A common approach is to separate the bulk soil into aggregates (aggregate fractionation; e.g., Six et al., 2002a) or primary mineral particles (particle size fractionation) of different size. Often, POM fractions characteristic for specific aggregate or particle size fractions are also separated by density fractionation. Hence, the soil is subjected to different dispersion, sieving, density and sedimentation procedures. In the soil studied in this thesis, a minor degree of aggregation could be assumed due to a very high sand content (cf. chapter 2.1). Thus, the use of a combined density and particle size fractionation to study the amount of C and the chemical

composition of differently stable SOM fractions was regarded as superior compared to an aggregate fractionation. The reader is referred to other references for a detailed description regarding aggregate fractionation procedures (Six et al., 2002a).

Many studies combining particle size and density fractionations directly disperse the soil and subsequently separate different fractions based on their size and degree of organo-mineral interaction (Torn et al., 2009). Dispersion of a sample is mostly achieved by ultrasonication. A potential problem of this method is the redistribution of OM among fractions during the application of the ultrasound (Elliott and Cambardella, 1991; Mueller et al., 2012). To evaluate the feasibility of the fractionation and adjust the applied ultrasonication energies, the obtained fractions are often compared to the results of more classical particle size analyses (Elliott and Cambardella, 1991). A POM fraction is either separated prior to or after dispersion and removed from the mineral soil by saturation with a high density liquid (e.g., sodiumpolytungstate) resulting in the flotation of the lighter POM (often termed light fraction), whereas the heavier mineral soil (often termed heavy fraction) descends (Christensen, 2001). The density of the liquid is often adjusted to values between 1.6 and 2.2 g/m³ (Baldock et al., 1992; Crow et al., 2007; Glaser et al., 2000; Mikutta et al., 2009; Mueller et al., 2009) depending on the purpose of the study (Glaser et al., 2000). The POM may be separated into free (fPOM) and POM occluded within soil aggregates (oPOM) depending on the used procedure and these fractions may be further separated by size (Mueller et al., 2009; Wagai et al., 2009). The POM fraction is generally composed of plant and animal residues characterized by a rapid turnover (Glaser et al., 2000) and its bioavailability increases from fPOM to oPOM (von Lützow et al., 2007) and with decreasing size (Mueller et al., 2009). The mineral soil is separated into fractions of different size where the concentrations and stability of OM commonly increase with decreasing size of the respective mineral fraction (Christensen, 2001). However, the chemical composition and stability of OM in different fractions may be highly soil type specific and dependent on the fractionation scheme.

1.3.2. Elucidating the chemical composition of SOM by ^{13}C CPMAS NMR spectroscopy

Solid state ^{13}C CPMAS NMR spectroscopy has become a well-established tool to elucidate the chemical composition of a soil sample. A great advantage of ^{13}C NMR spectroscopy is the possibility to gain information on the chemical composition of a sample as a whole, while chemical extractions only release part of the total SOM (Kögel-Knabner, 1997). To gain an NMR spectrum, the respective sample is placed in an external static magnetic field that forces the nuclei spins to align themselves among different energy levels. These energy levels are different for nuclei in different chemical and physical environments. Changes in the spin of the energy levels can be induced by the application of an additional electromagnetic

field and detected as a resonance signal at a specific resonance frequency in a spectrum (Kögel-Knabner, 2002). To make measurements comparable, the resonance frequency is given as chemical shift (ppm) relative to a reference standard. To minimize anisotropic interactions between nuclei in solid material, the samples are spun with high speed around a "magic angle" (54.74°) relative to the external magnetic field (Hennel and Klinowski, 2005). An NMR spectrum can be divided into different chemical shift regions indicative for different functional groups of, in this case, plant materials and SOM (conventionally, 0-50 ppm (alkyl C), 50-110 ppm (O/N-alkyl C), 110-160 ppm (aryl C), and 160-220 ppm (carboxyl C); Kögel-Knabner et al., 1992)). Limiting factors in NMR spectroscopy of soil are the very low C contents of especially subsoil samples making it difficult to obtain a reasonable signal-to-noise ratio or the presence of paramagnetic materials leading to overlapping resonance lines (Kögel-Knabner, 1997). These problems may be overcome by treating the samples with hydrofluoric acid (HF) prior to measurement thereby removing mineral matter and concentrating OM. While older studies did not detect major changes to the chemical composition or a mass loss of SOM upon HF treatment (Preston and Newman, 1995; Schmidt et al., 1997), more recent studies found a considerable loss of C and N, and alterations in the chemical composition of saccharides and lignin (Gonçalves et al., 2003; Rumpel et al., 2006).

The very first attempt to investigate the chemical composition of soil humic substances using NMR spectroscopy was made by Barton and Schnitzler (1963) by comparing NMR spectra with wet chemical analyses of organic matter extracted from a podzolic subsoil horizon. Since a subsequent study by Wilson et al. (1981), who applied ^{13}C CPMAS NMR spectroscopy to whole soil, this technique has become a major tool for the investigation of SOM chemical structure (e.g., Golchin et al., 1996; Kögel-Knabner, 1997). Several indicators for the degree of degradation of plant material and SOM have been developed from NMR spectra. The ratio between alkyl C and O/N-alkyl C has most frequently been applied. It is based on the observation that aliphatic components (alkyl C) are more resistant to degradation than cellulose, hemicellulose, and proteins (O/N alkyl C) in plant residues and relatively accumulate during decomposition (Baldock et al., 1997). A recent study indicated that the interpretation of this ratio may be problematic when applied to SOM in mineral associated fractions as specific interactions of C and N with the mineral soil matrix modulate mineralization dynamics (Bimüller et al., 2014). More recently, Bonanomi et al. (2013 and 2011) introduced the ratio between the integration regions of 52-57 (methoxyl C of lignin) and 70-75 (C2, C3, and C5 of carbohydrates) ppm, which correlated well with the decay rates of plant residues. Nelson and Baldock (2005) applied a mathematical model (denominated "mixing model") for the processing of NMR spectra that enables the user to infer the chemical composition of natural organic materials in a more diversified way by estimating the content

of different biomolecule components, i.e., carbohydrates, proteins, lignin, aliphatics, char, and pure carbonyl. The application of ^{13}C NMR spectroscopy to functional subsoil OM fractions (as described in section 1.3.1) provides insight into their chemical composition and may thus contribute to a better understanding of the nature of differently stable subsoil OM.

1.3.3. Revealing the source of SOM using lipid biomarkers

Lipids are organic substances that are insoluble in water but extractable with organic solvents. This heterogeneous group of substances is present in plants, soils, and microorganisms (Kögel-Knabner, 2002) where plants represent the major source of lipids (Bull et al., 2000). Depending on the mode of occurrence (solvent-extractable lipids or hydrolysable lipids), chain-length, and functional groups, lipid monomers or groups of lipid monomers may be used to identify SOM from plant sources throughout the soil profile (e.g. Spielvogel et al., 2014). With specific biomarkers, also the aboveground or belowground plant origin of SOM can be distinguished (Crow et al., 2009). For this purpose, the hydrolysable lipid biopolymers cutin and suberin are most suitable. Cutin is present exclusively in leaves/needles of plants composing the macromolecular frame of the cuticle, whereas suberin is part of bark and roots constituting the periderm layer of plants (Kögel-Knabner, 2002; Kolattukudy, 1981). Thus, the biopolymers are specific for either aboveground or belowground sources of SOM. Cutin and suberin are mostly comprised of *n*-carboxylic, x,ω-hydroxy carboxylic and alkanedioic acids (Mendez-Millan et al., 2010) with differing chain lengths and relative abundance in each polymer (Mueller et al., 2012). However, an irrevocable assignment of different monomers to either cutin or suberin is still lacking due to the chemical similarities and a varying chemical composition of the biopolymers depending on plant species, and morphology and life-span of roots and leaves (Mueller et al., 2012). A local calibration (i.e. comparing the lipid composition of leaves and roots at a given study site) is thus meaningful.

Many studies suppose an equal decay of individual cutin/suberin derived monomers as well as an equal decay of source plant material and cutin/suberin derived monomers. These assumptions are pre-requisite to link the concentration of the two biopolymers to root and shoot derived SOM and its turnover. However, the decay of cutin monomers has only been reported in one study (Riederer et al., 1993) and the decomposition of suberin monomers has not been reported so far. These shortcomings need to be addressed prior to using cutin and suberin as biomarkers.

Many authors also used solvent-extractable lipids to trace SOM of plant origin or to infer information about paleo-vegetation at the respective site (Bush and McInerney, 2013). For example, *n*-alkanes are preserved in soil over long periods of time (Ficken et al., 1998) and those with odd chain-lengths ($C_{21} - C_{33}$) are derived from plant waxes analogous to *n*-fatty

acids with a chain-length $>C_{20}$ (Eglinton and Hamilton, 1967; Eglinton et al., 1962). The differentiation of SOM from aboveground and belowground sources by solvent-extractable lipids is more complicated than that with cutin and suberin due to the unspecific occurrence of solvent-extractable lipids in different plant parts. However, depending on plant species, concentrations of *n*-alkanes or *n*-fatty acids may be sufficiently different in different plant organs (leaves/needles and roots) (e.g., Huang et al., 2011), so that a differentiation of aboveground and belowground sources of SOM is also possible by the use of solvent-extractable lipids.

By a combination of both solvent-extractable and hydrolysable lipid biomarkers, Nierop et al. (2006) were able to assess soil processes like leaching and bioturbation without direct measurements. Similarly, a combination of hydrolysable and solvent-extractable lipids was used in this thesis to evaluate the contribution of SOC from aboveground and belowground sources and to benefit from the complementary information this approach offers (Nierop et al., 2006).

1.4.   Hypothesis and research questions

The individual project "Disentangling the sources, chemical composition, and spatial distribution of soil organic matter in topsoil and subsoil under European beech" was carried out within the 1st phase of the research unit FOR1806 "The Forgotten Part of Carbon Cycling: Organic Matter Storage and Turnover in Subsoils (SUBSOM)" funded by the Deutsche Forschungsgemeinschaft at the Chair of Soil Science in Freising-Weihenstephan.

The main hypothesis was that the distance to individual mature European beech trees has a significant influence on SOM chemical properties and the amount of OC stored in the soil, resulting in a distinct chemical composition and origin of SOM that change with increasing depth and distance to the trees. To test the hypothesis, three research questions where established:

(i)   What is the amount, spatial distribution, and chemical composition of the OC stored in the soil?

(ii)   How do cutin and suberin biomarkers decompose and are the biopolymers suitable to distinguish aboveground from belowground sources of SOC?

(iii)   How high is the amount of SOC from different sources (aboveground and belowground) at different locations in the soil?

To cover spatial heterogeneities, a spatially coordinated sampling design taking into account the soil depth and the distance to individual beech trees was established. Each research question was addressed by an individual study:

Study I, "Spatial distribution and chemical composition of soil organic matter fractions in rhizosphere and non-rhizosphere soil under European beech (Fagus sylvatica L.)", was performed to investigate the distribution and chemical composition of SOM fractions and the amount of SOC in subsoil as affected by the distance to individual, mature European beech trees. Furthermore, the role of rhizosphere soil for the input and storage of SOC was evaluated. A density and particle size fractionation was combined with C, N, and ^{13}C CPMAS NMR spectroscopy measurements to answer research question (i).

Study II, "The fate of cutin and suberin of decaying leaves, needles and roots – Inferences from the initial decomposition of bound fatty acids", was performed to investigate the decomposition patterns of cutin and suberin derived monomers in relation to the respective source plant material, detect possible factors that influence the decay pattern, and re-evaluate the suitability of cutin and suberin as biomarkers which were to be utilized as markers for aboveground and belowground sources of SOM. A short term incubation of leave/needle and root material with four destructive sampling events was combined with a sequential lipids extraction procedure and ^{13}C CPMAS NMR measurements to answer research question (ii).

Study III; **"Tracing the sources and spatial distribution of organic carbon in subsoils usin a multi-biomarker approach"**, was performed to unveil the origin of SOC in different soil depths and distances from individual, mature European beech trees. A multi-biomarker approach (solvent extractable and hydrolysable lipids) was combined with ^{14}C measurements to answer research question (iii). This study built upon the results and findings of studies I and II.

2.   Materials and Methods

2.1.   Sampling for studies I, II and III

Soil samples for studies I and III were taken at the main sampling site of the research unit, the Grinderwald. It is a managed, even-aged European beech forest, established in 1916 and located north-west of Hannover (52° 34′ 22″ N 9° 18′ 51″ E). The site was chosen according to previous investigations that revealed the relative homogeneity in texture (sand 70 – 95 %, silt 4 – 30 %, clay 1 – 9 %) of the soil at the study area, which has been classified as Dystric Cambisol (WRB, 2014). It was thus possible to install three replicate transects at different spots within the study site that featured similar soil conditions. Each transect originated at the stem base of a mature European beech tree and was 3.15 m in length and 2.00 m in depth. Composite soil samples were taken from the profile walls of each transect in a regular grid, starting at the stem base of the beech in 10 cm depth with 45 cm horizontal and 25 cm vertical increments (Fig.1, n=64 per transect). Additionally, forest floor material above each sampling spot (n=24), leaf litter (n=3) and roots (n=3) were collected at each transect. Rhizosphere soil was sampled (n=3) within the rooting zone of the trees (down to ~40 cm) along the whole horizontal extent of the transects. The plant material was
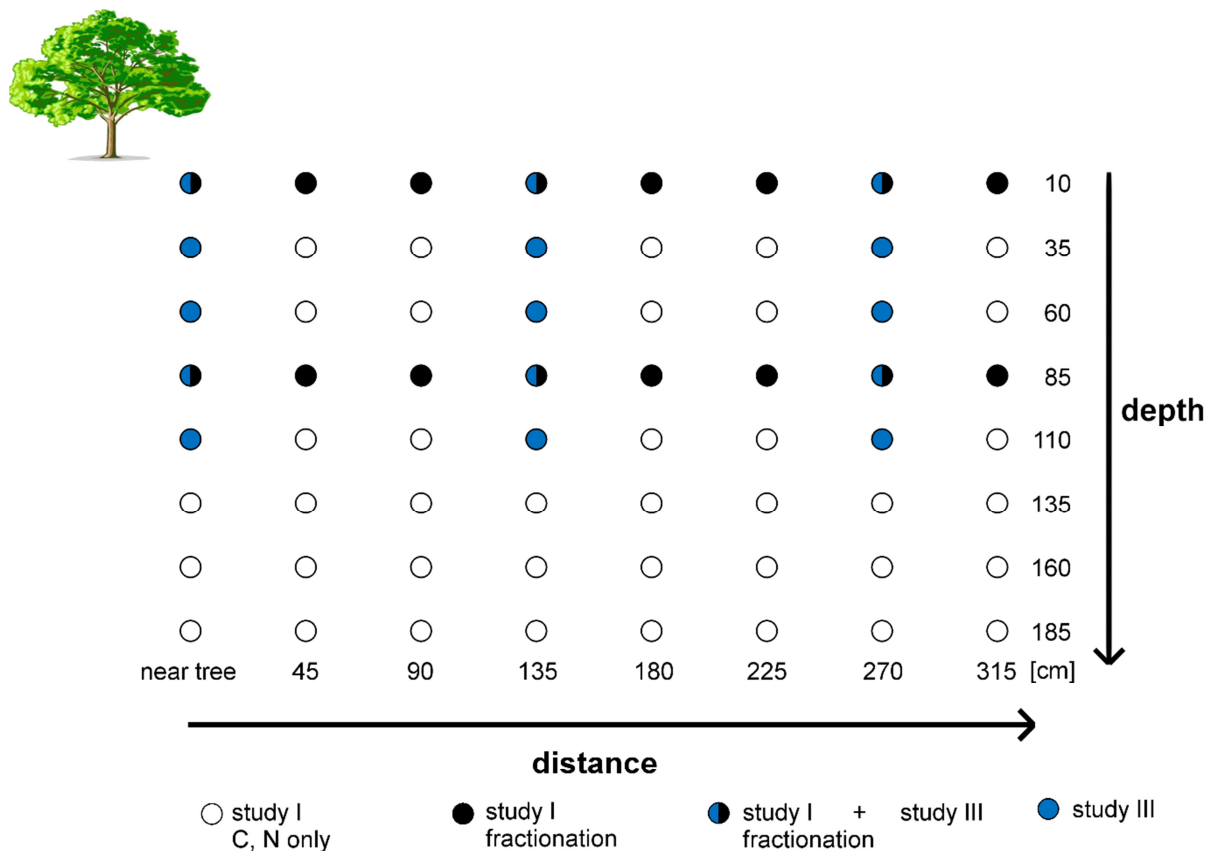
Fig. 1 Scheme of the spatially coordinated sampling design applied to three transects at the Grinderwald study area. Each point indicates one sampling spot. The colors of the points differentiate the analytical approaches applied to each sample in the different studies.

freeze-dried and ground for further analysis and the soil samples were air dried and sieved to < 2 mm.

For study II, plant material was sampled at the Kranzberger forest in October 2013 near Freising, Germany. Fine (diameter of < 2 mm) and coarse (diameter of > 2 mm) living roots were collected from the forest floor at both the beech and the spruce stand. Spruce needles and beech leaves of mature trees were sampled at the same site. Additionally, field moist forest floor material was collected in March 2014 at each stand to assure a high microbial activity (Bååth and Söderström, 1982) and a microbial decomposer community that is adapted to the respective plant litter material (Mooshammer et al., 2014; Wallenstein et al., 2013).

2.2.   Analytical Methods

2.2.1.   C and N measurements

All samples in this thesis, whether bulk soil, SOM fractions, or plant material, were analysed for their C and N content as a pre-requisite for further data processing and interpretation. An aliquot of each sample was measured in duplicate via dry combustion.

2.2.2.   Combined density and particle size fractionation and ^{13}C CPMAS NMR measurements for study I
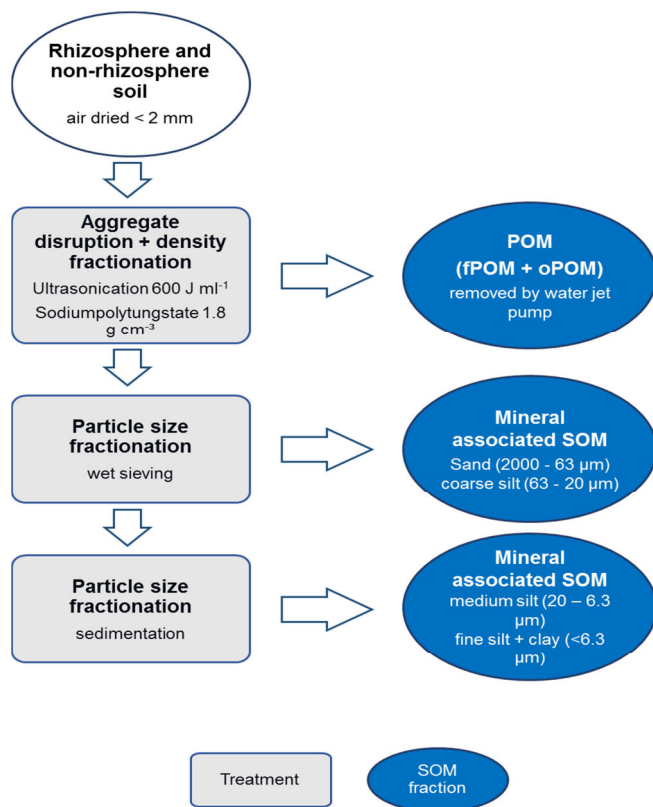
Fig. 2 Scheme of the combined density and particle size fractionation procedure used in study I

The sampling rows at 10 cm and at 85 cm depth of each transect (n=48) were chosen for fractionation in study I (Fig. 1). It enabled us to trace the impacts of the trees on the distribution, quantity, and chemical composition of SOM fractions while keeping the analytical work viable. Aliquots of air dried and sieved rhizosphere and non-rhizosphere soil were subjected to a combined density and particle size fractionation (Fig. 2) to obtain the POM fraction, sand fraction (2000 – 63 µm), silt fraction (coarse and medium silt, 63 – 6.3 µm), and clay fraction (fine silt and clay, <6.3 µm).

The plant material collected at the Grinderwald (roots, leaf litter), organic

layer material and POM and clay fractions were subjected to ^{13}C CPMAS NMR spectroscopy to reveal the chemical composition of the respective sample. 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).
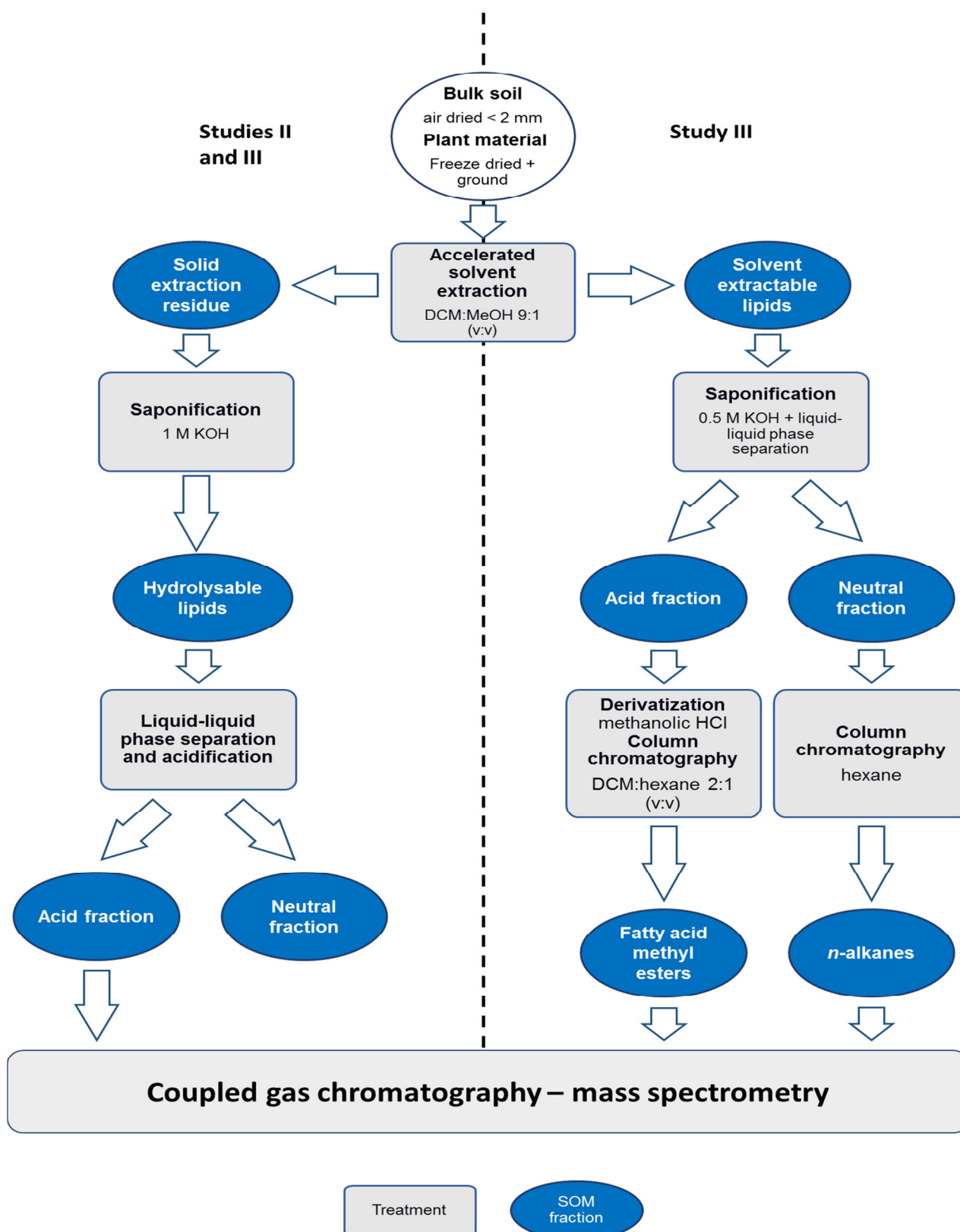

Fig. 3 Scheme of the sequential extraction procedure used in studies I and III. The left hand side of the scheme separated by the dashed line was used in both studies, the right hand side was used in study III only.

Roots were manually extracted from the soil samples and divided into biomass and necromass applying a procedure introduced by van Praag et al. (1988) and modified by Hertel (1999).

Bulk densities of the soil samples for the calculation of SOC stocks were kindly provided by Dr. Stefanie Heinze who took volume samples at the same spots from the sampling grid.

2.3.   Incubation experiment and analytical methods for study II

The plant materials collected at the Kranzberger forest (beech leaves and roots, spruce needles and roots) were cut into small fragments and 1 g of each root and leave/needle material was weighed into separate small litterbags (2x2 cm) and incubated embedded in fresh forest floor material of the respective tree species in individual 0.9 L glass jars for 84 days in the dark at a constant temperature of 20°C. Three jars per plant material were destructively sampled at 14, 28, 42 and 84 days of incubation giving a total of 48 incubated samples plus three replicate samples of each non-incubated initial leaf, needle and root material (n=12). All plant materials (initial and incubated) were ground and aliquots subjected to ^{13}C NMR spectroscopy and a sequential extraction procedure to release monomers specific to the lipid biopolymers cutin and suberin. The NMR measurements were conducted and integrated as described in section 2.2.2. In addition to the conventional processing of the spectra (cf. sections 1.3.2 and 2.2.2), two peaks were separately integrated to calculate the ratio between 52-57 and 70-75 ppm introduced by Bonanomi et al. (2011) that has been found to describe the decomposition of plant materials well (Bonanomi et al., 2013). To retrieve an estimate of different biomolecule components present in the samples during incubation (lignin, carbohydrates, lipids, proteins), we applied the molecular mixing model (Nelson and Baldock, 2005) to the NMR spectra.

To release cutin and suberin derived monomers, aliquots of each sample were extracted with organic solvents to release solvent extractable lipids. In a second step, the solid extraction residues were saponified to release hydrolysable lipids specific to cutin or suberin (Fig. 3). The acid fraction was separated from the extracts and measured using GC/MS. Cutin and suberin markers were then identified according to previously published specific monomers and their occurrence in plant material from the present study area. Factors that may influence the decomposition were evaluated using factorial ANOVA models.

2.4.   Analytical methods for study III

Study III was conducted in cooperation with the University of Cologne. Soil samples taken at the Grinderwald near the tree, at intermediate distance from the tree (135 cm), and far from the tree (270 cm) down to a depth of 110 cm at each transect (n=45, Fig. 1) were subjected to ^{14}C analysis and a multi biomarker extraction procedure to reveal origin and mean

residence times of the SOC. Data on root biomass and necromass already published in study I were involved to aid data interpretation. The ^{14}C contents were measured by accelerated mass spectrometry (AMS). Solvent extractable and hydrolysable lipids, extracted from the soil samples with organic solvents followed by saponification (cf. section 2.3), were measured using GC/MS. Analogous to study II, plant, leave, root, and microbial biomarkers were identified according to their occurrence in source plant material from the study area and previously published diagnostic monomers. The markers were: suberin and cutin markers for the differentiation of root and leaf derived carbon, odd chained n-alkanes C_{25}-C_{33} and n-fatty acids $>C_{20}$ as markers for plant derived SOC (due to highly differing concentrations of the solvent extractable lipids in beech leaves and roots, we could infer a leaf source of SOC where concentrations of n-alkanes C_{25}-C_{33} and n-fatty acids $>C_{20}$ in the soil were considerably high), P_{RML} (n-fatty acids C_{14}-C_{18}/$>C_{20}$) as a proxy for microbial/root vs. leaf derived SOC, n-fatty acids $C_{16:1}$ + $C_{18:1}$ as a marker for microbial derived SOC, and the carbon preference index (CPI) for n-alkanes (CPI_{alk}) and n-fatty acids (CPI_{FA}) as proxies for the degradation of n-alkanes and n-fatty acids, respectively. To facilitate data interpretation, the SOC contents, root biomass and necromass, ^{14}C contents, and solvent extractable and hydrolysable lipid biomarkers were analysed with two principal component analyses (PCA) – one for the densely rooted upper soil layers (10 – 35 cm depth, denominated "upper subsoil") and one for the less rooted deeper soil layers (65 – 110 cm depth, denominated "deeper subsoil").

3. Discussion

3.1. The spatial distribution and chemical composition of SOM is mainly influenced by roots?

In study I, the SOC contents and stocks and chemical composition of different SOM fractions were investigated using a combined density and particle size fractionation and ^{13}C CPMAS NMR spectroscopy. Statistical tests did not reveal any significant differences in the analyzed parameters with increasing distance to the individual trees, neither in the uppermost sampling row (10 cm depth) nor in the lower sampling row (85 cm depth; Fig. 1). Consequently, the data was combined to mean values for the three transects and each horizontal sampling spot for further analysis. The lack of horizontal differences was surprising because previous studies found distant dependent changes in soil chemical (Koch and Matzner, 1993; Lodhi, 1977; Spielvogel et al., 2014) and physical properties (Chang and Matzner, 2000), and regarding the microbial community structure and activity (Goemoeryova, 2004; Saetre and Bååth, 2000). Chabbi et al. (2009) stated that SOC in subsoil horizons is located at spatially distinct parts of the soil profile. The results of study I, however, point to a relatively even distribution of subsoil OC in the horizontal. This observation was likely due to a horizontally even rooting of the soil, independent of different amounts of roots at different soil depths. This assumption was corroborated by an about five times higher SOC content in rhizosphere compared to non-rhizosphere soil, due to a six times higher amount of fresh, little decomposed POM (Alkyl / O/N alkyl C ratio: 0.8) (Fig. 4). These data indicated that root OC inputs played a decisive role for the absence of significant horizontal variabilities in SOC contents and the chemical composition of SOM fractions. It furthermore points to an important contribution of root derived POM to SOC pools similar to root exudates, which have been assumed to be the largest (Dennis et al., 2010) and most important contributor of
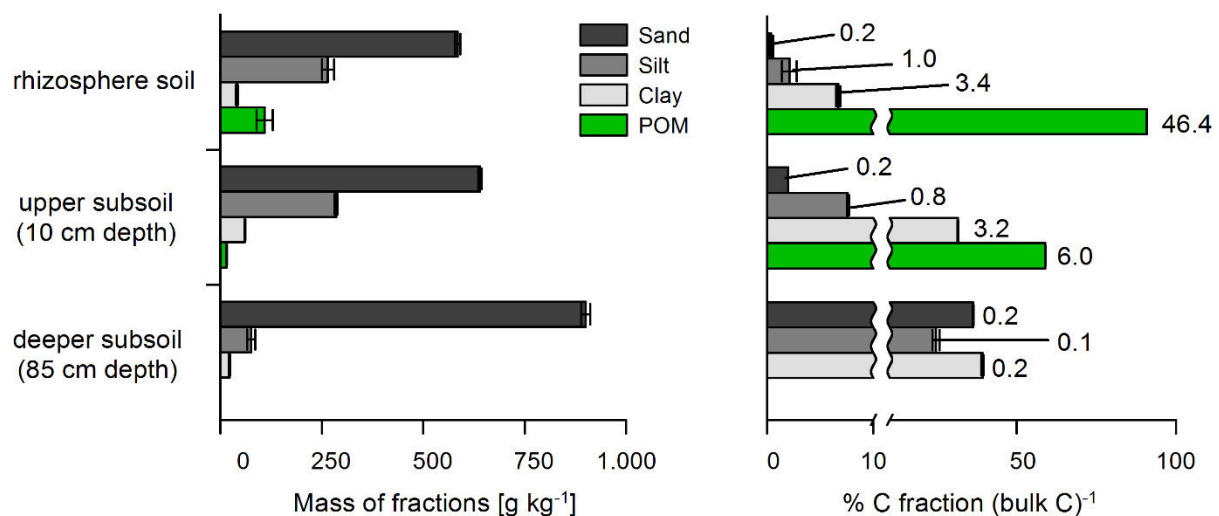

Fig. 4 Mass of the sand, silt, and clay fractions at the Grinderwald in rhizosphere soil, at 10 cm depth, and at 85 cm depth (left panel), and % C in each fraction related to the total C in bulk soil (right panel). Numbers to the right of the bars indicate absolute values in g C (kg bulk soil)$^{-1}$

SOC inputs from roots so far (Kuzyakov et al., 2007). The importance of root inputs was further reinforced by strong vertical gradients detected in the investigated parameters (especially decreasing amount and SOC contents of SOM fractions) with simultaneously decreasing concentrations of both the root necromass and biomass between the topsoil /upper subsoil (down to 35 cm depth) and deeper (60 to 110 cm depth) subsoil. Consequently, the SOC contents of all fractions at 85 cm depth were very low (Fig. 4). The POM fraction could not be detected anymore, and the contribution of the sand fraction to the total amount of the SOM fractions increased to approximately 900 g kg^{-1} (from 640 g kg^{-1} at 10 cm depth; Fig. 4). The clay fraction was highly enriched in SOC, considering the low contribution of this fraction to the total amount of SOM fractions recovered (2.3%). Related to the total SOC stocks in deeper soil layers (40 – 200 cm depth), on a percentage basis, the clay fraction accounted for higher SOC stocks than that from the depths of 0 – 40 cm. This finding points to the significance of clay for the stabilization of SOC by the formation of organo-mineral associations (Rumpel et al., 2004; Schrumpf et al., 2013), especially in the sandy soil encountered in this thesis. The absence of the POM fraction at 85 cm depth might have been due to low root litter inputs, a fast aggregate turnover or a generally low degree of aggregation, reducing physical protection (Six et al., 2000; Swanston et al., 2005). This inference was supported by high amounts of the sand fraction recovered in the upper and especially in the deeper subsoil, impeding the formation of aggregates (Fig. 4). Furthermore, the POM at 10 cm depth already showed an advanced stage of degradation evidenced by relatively high contents of alkyl C with respect to O/N-alkyl C, indicating that the POM was bioavailable and not protected from decay.

The results of study I implied a high importance of the roots and rhizosphere for the spatial distribution and chemical composition of SOC, horizontally and vertically. However, a horizontally even input of SOC by leaf litter may likewise have influenced SOC distribution and chemical composition. Thus, the differentiation of aboveground and belowground sources of SOC gave more detailed insights into the relative importance of leaf- and root-derived SOC in the whole soil profile. As described in chapter 1.3.3, the biopolymers cutin and suberin seem to be most appropriate for distinguishing aboveground from belowground sources. However, because there have been still considerable uncertainties regarding the decay of both biopolymers, study II was conducted in order to eliminate these shortcomings and re-evaluate the suitability of cutin and suberin as biomarkers.

3.2.   The decay of cutin and suberin monomers in relation to the source plant material and their suitability as biomarkers

In study II, the decay of cutin and suberin monomers was investigated by incubating leaf/needle and root material of European beech and Norway spruce for 84 days under controlled laboratory conditions. Beech and Spruce were chosen, because they constitute

the most abundant tree species in central Europe (Geßler et al., 2007). The calculated decomposition indices (alkyl / O/N alkyl C ratio, 52-57/70-75 ppm) reflected the commonly observed changes in chemical composition during the decay of the plant material (e.g., Bonanomi et al., 2013; Cepáková and Frouz, 2015; Lorenz et al., 2004, 2000). The mass loss of the root material was slower than that of the leaf material. Surprisingly, the mass of the spruce needles remaining at the end of the incubation was significantly lower compared to the remaining mass of the beech leaves, although spruce needles have been regarded as being more resistant to decomposition (Chapman et al., 1988; Johansson, 1995). This was ascribed to a combination of factors: (i) By using litterbags, the meso- and macrofauna was excluded which has been shown to be responsible for a considerable mass loss of beech leaves (Kammer et al., 2012; Staaf, 1987). (ii) The carbohydrates of beech leaves seemed to be more protected against decomposition than the carbohydrates of spruce needles, likely due to the association with lignin (Berg and McClaugherty, 2008; Melillo et al., 1982). (iii) The C/N ratio of the spruce needles was lower than that of the beech leaves, providing more favorable conditions for microbial growth. (iv) Finally, the home-field advantage may have been of importance since all plant materials were incubated in their natural environments where the microbial community is adapted and specialized to the decomposition of the respective plant material (Wallenstein et al., 2013). For example, Hobbie et al. (2006) showed that spruce needles decomposed faster than beech leaves when both plant materials were incubated in their home environment.

The loss in cutin and suberin monomeric concentrations was rapid already after 14 days of incubation but levelled off towards later sampling events. Suberin monomers did not decompose more slowly than cutin monomers. This was surprising because suberin has usually been found to accumulate in soils relative to cutin (Mueller et al., 2012; Spielvogel et al., 2014). Several factors were statistically tested that may have influenced the decay pattern of cutin and suberin. According to the established factorial ANOVA models, the factors chain length (number of C atoms in each monomer), lipid type (n-carboxylic acid, α,ω-alkanedioic acid, ω-hydroxy alkanoic acid, or mid-chain substituted hydroxy alkanoic acid) and lignin content (derived from the NMR molecular mixing model) explained about one third of the variation in lipid concentrations over time. The direction of the factor effects were as follows: the longer the chain length and the higher the relative lignin content, the higher the lipid concentrations left at the respective sampling event. The lipid concentrations increased in the order n-carboxylic acids < α,ω-alkanedioic acids < ω-hydroxy alkanoic acids < mid-chain substituted hydroxy alkanoic acids. Consequently, the presence of hydroxy alkanoic acids ≥C_{20} in suberin in contrast to the presence of hydroxy alkanoic acids ≤C_{18} in cutin should result in a slower decay of suberin relative to cutin. This, however, was not the case. A reason for a fast decay of suberin monomers may have been the smaller amount of mid-

chain substituted hydroxy alkanoic acids present in suberin compared to cutin. Regarding this lipid type, primary and secondary hydroxyl groups may be involved in cross-linking (Kolattukudy, 1980), while the predominantly suberin derived ω-hydroxy alkanoic acids may be involved in cross-linking only with one hydroxyl group. This may impede the degradation of mid-chain substituted hydroxy alkanoic relative to ω-hydroxy alkanoic acids due to their location in the polymeric network (Mendez-Millan et al., 2010; Naafs et al., 2005; Nierop and Verstraten, 2004; Nierop, 1998). Furthermore, the relative lignin content had a significant influence on the variation in lipid concentrations. In accordance with the decay pattern of cutin and suberin derived monomers, a two phase model has been proposed, where (i) cutin or suberin that is not associated with lignin (e.g., due to a surplus of cutin or suberin (Leuschner et al., 2003)) is readily consumed by microorganisms in early phases resulting in a rapid decrease of the respective polymer; (ii) in the second phase, only cutin and suberin associated with lignin remain, resulting in a decomposition that proceeds with the initially low decay rate of lignin (Berg, 2000). However, most of the variation in lipid concentrations (about two thirds) was not accounted for by the tested factors. Thus, the decay of cutin and suberin has to be additionally modulated by a not yet quantified external factor.

An important finding of study II was the high positive correlation of individual monomers during the course of the incubation, indicating that it is valid to sum up the cutin and suberin monomers to single markers for leaf and root derived OC. Furthermore, cutin and suberin both decomposed with similar rates. It is thus possible to relatively compare the abundances of each polymer. With respect to the source plant material, the concentrations of cutin and suberin monomers were exponentially correlated to the mass of the respective leaf/needle and root material. This suggests that isotopic studies making inferences about the turnover of SOM from different sources using cutin and suberin markers (e.g., Feng et al., 2010; Mendez-Millan et al., 2010) may underestimate the turnover of SOM.

A next step in the research of the decay of cutin and suberin or soil lipids in general may be the incubation of plant material in connection with OM-free artificial soil. It would then be possible to evaluate the influence of the mineral soil phase on the decomposition of the respective lipids. By applying a fractionation procedure to the artificial soil at the end of the incubation, it would further be possible to determine the association of certain lipids with different mineral soil compartments. This approach may enlighten the unknown factors behind the commonly observed higher concentrations of suberin compared to cutin derived compounds in soil (e.g., Mueller et al., 2012).

Because study II confirmed the suitability of cutin and suberin for distinguishing aboveground from belowground sources of SOM on a relative basis, the biopolymers were used in study III to get a more detailed insight into the importance of SOC from different sources at different locations in the soil profiles.

3.3.   Horizontally even distribution of SOC from leaves and roots independent of the distance to individual beech trees

In study III, the indications of study I were tested by using the lipid biopolymers cutin and suberin whose suitability as biomarkers for distinguishing aboveground and belowground sources of SOC has been confirmed by study II. Because leaf litter was highly enriched in *n*-alkanes and *n*-fatty acids compared to roots, these solvent-extractable lipids could be also used to identify SOC from aboveground sources. The ^{14}C contents of samples marked in Fig. 1 were supplied by Stephan John from the University of Cologne and aided data interpretation.

The observed spatial patterns of study I were also mirrored by the investigated parameters of study III. Neither solvent extractable and hydrolysable lipid biomarkers nor ^{14}C contents showed significant differences in the horizontal. The importance of root inputs was confirmed by high amounts of suberin markers in the upper subsoil. Similarly, high amounts of *n*-alkanes and *n*-fatty acids derived from plant leaf waxes (Huang et al., 2011) could be detected in the upper subsoil. These data point to a uniform and ubiquitous input of root and leaf derived SOC to the upper subsoil leading to the absence of a detectable distant dependent horizontal influence of the trees on the macromolecular (SOC contents/stocks and chemical compound classes derived from NMR spectra) as well as on the monomeric (solvent extractable and hydrolysable lipid biomarkers) chemical composition of SOM. Schöning et al. (2006) found a significant small scale variability of SOC stocks but no clear relation to the distance from individual beech trees similar to the results of study I. The authors suggested that SOC stocks in their study may have been influenced by inputs of the former vegetation. Regarding the upper subsoil, this explanation could be excluded due to high ^{14}C contents indicating the dominance of fresh SOC. However, in the deeper subsoil an influence of the former vegetation was presumable due to a shift of the origin and apparent ^{14}C ages of SOC with increasing soil depth.

3.4.   Vertical zonation of the subsoil as a function of SOM source and ^{14}C content

By joining the data of studies I and III, the soil at the Grinderwald study site was separated into two different zones: a zone influenced by root- and leaf-derived SOC (root- and leaf-affected zone) and a zone influenced by root-derived SOC (root-affected zone; Fig. 5).

3.4.1.   Root- and leaf-affected zone

The relatively high SOC contents in the upper subsoil (10 and 35 cm depth, root- and leaf-affected zone) in contrast to the deeper subsoil (60 and 110 cm depth, root-affected zone) consisted of predominantly fresh root- and leaf-derived OC from the recent beech vegetation. This inference was based on high concentrations of *n*-alkanes (C$_{25}$-C$_{33}$) and *n*-fatty acids

($>C_{20}$) in the upper subsoil mainly derived from leaves (Huang et al., 2011) as well as on high concentrations of suberin markers indicative for root derived compounds. In PCA_{10-35}, all these parameters were positively correlated to the SOC contents pointing towards their major contribution to SOC in the upper subsoil. Fresh plant derived SOC was also indicated by high amounts of POM that dominated the C-pools at 10 cm depth (Fig. 4; study I) with concurrently high concentrations of root biomass and necromass. High ^{14}C contents at 10 cm depth further confirmed the presence of predominantly fresh SOC. The microbial derived $C_{16:1}$ and $C_{18:1}$ fatty acids where low in concentration and uncorrelated to soil depth suggesting a ubiquitous occurrence but a minor importance of microbial compared to plant derived SOC. These results indicate that subsoils are still considerably influenced by the input of fresh SOC and leaf derived compounds may still be important below the A horizons of a soil. However, at 35 cm depth, apparent ^{14}C ages already increased to 810 ± 80 yrs BP indicating a contribution of relatively old SOC and/or decreasing concentrations of fresh plant derived SOC.

3.4.2.   Root affected zone

The decreasing trend of ^{14}C contents proceeded to the root-affected zone in the deeper subsoil (65 to 110 cm depth) where roots were an important source of fresh SOC. A considerable amount of SOC however had to be stabilized over long periods of time or
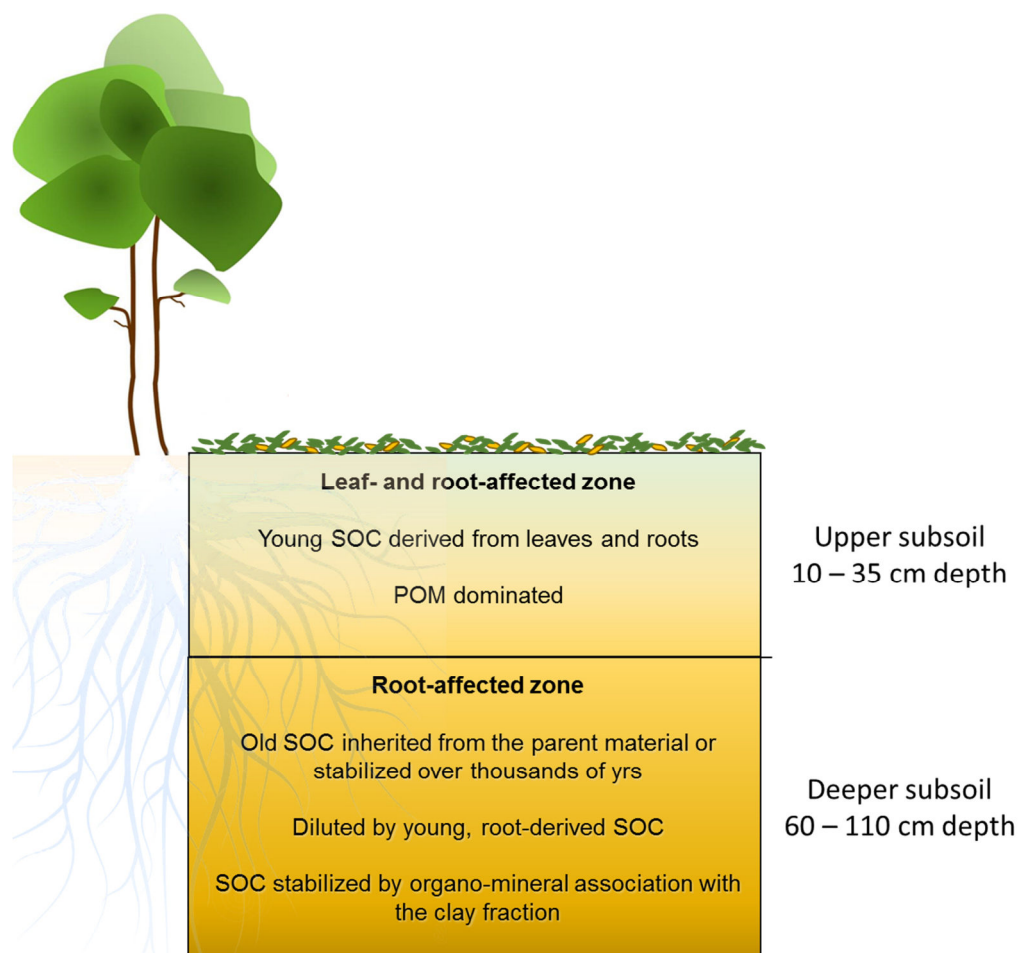
Fig. 5 Scheme of the two zones (leaf- and root-affected zone and root-affected zone) differentiated at the Grinderwald study site

inherited from the parent material due to very high apparent ^{14}C ages of up to 3860 ± 400 yrs BP, low concentrations of the root biomass and necromass, and a virtually absent POM fraction (Fig. 4). The PCA_{60-110} indicates that *n*-alkanes (C_{25}-C_{33}) and probably also *n*-fatty acids >C_{20} were old compounds because they were strongly negatively correlated to SOC and especially ^{14}C contents. This is in line with earlier studies that attested a high stability to long chain *n*-alkanes and used them as indicators for past vegetation (Andersson et al., 2011; Ficken et al., 2000, 1998). Another hint to relatively high residence times of *n*-alkanes was provided by the very low CPI_{alk} values in the deeper subsoil that indicated a high degree of degradation. However, compound specific ^{14}C analyses are needed to unambiguously identify the age of the investigated solvent-extractable lipids. Likewise, the very low CPI_{alk} values in the deeper subsoil could reflect the input of recent root derived SOC (Gocke et al., 2014) because they were highly similar to the CPI_{alk} values observed for beech roots. The dominance of root- in contrast to leaf-derived SOC was also clearly implied by the presence of suberin markers but the absence of cutin markers. Furthermore, the positive correlation of SOC and ^{14}C contents with the fine root necromass that was found to be no older than 20 yrs (Gaudinski et al., 2001; Gaul et al., 2009; Trumbore et al., 2006) indicates that the root necromass was a major source of fresh SOC. The P_{RML}, as a proxy for microbial/root vs. leaf derived SOC, points to the dominance of root/microbial derived C_{16} and C_{18} fatty acids compared to mostly leaf derived >C_{20} fatty acids. In this regard, microbial derived SOC seemed to be of minor importance because the concentrations of the microbial derived fatty acids ($C_{16:1}$, $C_{18:1}$) were low and strongly correlated with the plant derived fatty acids >C_{20}. This suggests that the $C_{16:1}$ and $C_{18:1}$ fatty acids in the deeper subsoil were rather derived from plant material from which trace amounts of these acids were released. Our data did not point towards an enrichment of subsoil OC in microbial derived compounds (e.g., Liang and Balser, 2008) but support the results by Rasse et al. (2005) who stated that fresh SOC inputs to the deeper subsoil are mainly root derived. However, similar to a statement by Rumpel and Kögel-Knabner (2011), very high apparent ^{14}C ages suggest a major contribution of very old SOC probably inherited from the parent material that may have been diluted by the younger, root derived SOC at the Grinderwald study site. Similarly, SOC may have been stabilized by the soil mineral fractions over longer periods of time (Rumpel et al., 2004; Six et al., 2002b). The great importance of the fine mineral soil for the stabilization of SOC was confirmed by the high carbon enrichment factors of the clay fractions in the deeper subsoil in contrast to the enrichment factors of the sand and silt fractions.

3.5.   Implications for C storage and allocation

Subsoil has previously been found to feature low SOC contents (Fierer et al., 2003; Salomè et al., 2010) and have a minor portion of the SSA covered with OM compared to topsoils (Kaiser and Guggenberger, 2003). Some authors therefore regarded subsoils as having the potential to sequester additional carbon (Lorenz and Lal, 2005; Lorenz et al., 2007). A suggested strategy for additional inputs of OC into deeper soil depths has been the planting of typically deep rooting plant species that would allocated root derived OC to the subsoil (Jobbágy and Jackson, 2000; Lorenz and Lal, 2005). The rooting system of European beech may reach high soil depths (Jandl et al., 2007) and the root biomass and necromass may be still substantially high below 0.6 m soil depth (Asche et al., 1995; Leuschner et al., 2003). The results of studies I and III did not confirm these inferences but indicate that the current beech vegetation at the Grinderwald influences SOC mainly in the upper subsoil. The OC input to the deeper subsoil was influenced by roots that are probably of less importance due to the very high mean apparent ^{14}C ages below 35 cm depth. These results suggest that site specific factors may essentially control the spatial growth of the rooting system and the allocation of SOC into deep subsoils cannot be achieved by planting typically deep rooting plant species. Despite the low SOC contents and amount of fresh SOC, SOC stocks at the deeper subsoil were still considerably high. The SOC stocks of bulk soil at 40 to 200 cm depth were roughly one third (1.4 ± 0.1 kg C m^{-2}) of those measured in the whole soil profile (0 to 200 cm depth, 5.2 ± 1.0 kg C m^{-2}). This requires to consider also deeper subsoil horizons in C inventories as they may be integral parts of the SOC pool.

Furthermore, the lipid data collected in study III indirectly allowed to draw conclusions about bioturbation processes in the soil profiles. Cutin derived acids generally possess a low water solubility (Nierop and Verstraten, 2004) and a translocation as DOC is thus unlikely. In study III, the cutin markers were abundant down to 35 cm depth, indicating that bioturbation has taken place even though soil conditions were rather unfavorable for soil faunal activity (e.g., low pH 3.4 – 4.5). Likewise, particulate leaf fragments could have been transported down the soil profile by percolating water (Ohta et al., 1986). However, a particulate transport is contradicted by the absence of cutin markers in the deeper subsoil. Here, the content of the sand fraction drastically increased in contrast to the other fractions, which should facilitate particulate transport by water. Thus, the absence of cutin markers at greater depths indicate the absence of bioturbation probably due to a low food quality (Marhan and Scheu, 2005).

4.   Conclusions and Outlook

The hypothesis of this thesis was that the distance to individual beech trees has a significant influence on the amount and chemical composition of SOC/SOM and that this influence results in a distinct chemical composition and origin of the SOM depending on where it is located in the soil profiles. Three research questions were established each of which was answered in an individual study. This approach made it possible to stepwise test the hypothesis.

Study I revealed that the tree had no influence in the horizontal, neither on the amount nor on the chemical composition of SOC. A horizontally even rooting of the soil, high amounts of C and little decomposed particulate organic matter in rhizosphere soil indicated the importance of root inputs for the observed patterns. These findings were further corroborated by highly decreasing SOC contents and stocks with increasing soil depth that coincided with a considerable decrease of the root biomass and necromass. To validate this indication and to evaluate the influence of leaf-derived SOC for the observed patterns, the aboveground and belowground sources of the SOC stored at different locations in the soil profiles were investigated. Because cutin and suberin are highly specific for leaf- and root-derived SOC/SOM, these biopolymers appeared to be ideal to distinguish the origin of SOC. However, there were still considerable uncertainties regarding the decomposition of both biopolymers.

In study II, a short-term incubation of leaf/needle and root material of two commonly occurring tree species in central Europe (European beech and Norway spruce) revealed that individual cutin and suberin monomers decreased uniformly. It thus remains reasonable to sum up individual monomers to single cutin and suberin markers. Furthermore, the results did not indicate a higher stability of suberin compared to cutin, despite the fact that root-derived compounds have often been observed to accumulate in forest soils relative to leaf-derived compounds. The decay of both biopolymers was significantly influenced by inherent chemical properties such as lignin content, chain-length or different functional groups of the monomers. However, about two thirds of the variation in lipid concentrations over time was not accounted for by the inherent chemical composition of the plant materials. The decomposition of cutin and suberin has to be additionally influenced by a not yet quantified external factor. A combination of the incubation of leaf/root material with artificial soil and the subsequent application of physical fractionation and chemical extraction techniques will generate new insights into the factors that govern the commonly observed higher concentrations of suberin compared to cutin in soil.

Based on the findings of studies I and II, study III was undertaken to unveil the amount of SOC from aboveground and belowground sources at different locations in the soil profiles, using cutin and suberin markers aided by solvent-extractable lipid biomarkers and ^{14}C

measurements. The distance to the tree did not have any detectable influence on the origin or ^{14}C content of SOC, mirroring the results of study I. Regarding the horizontal, the main hypothesis thus had to be rejected. However, a pronounced vertical gradient was detected and two vertical zones were distinguished. The SOC in the root- and leaf-affected zone (corresponding the B horizons at 10 and 35 cm depth) was a mixture of leaf- and root-derived SOC dominated by fresh POM that was to a large extent supplied by the roots of the trees. These findings highlight the importance of root OC input to soils and indicate that leaf-derived SOC may still be relevant below the topsoil A horizons. The SOC in the root-affected zone (corresponding to the depths of 60 to 110 cm, C horizons) was for the most part stabilized by the clay fraction. The roots were an important source for the input of fresh SOC. However, the high apparent ^{14}C ages (of up to 3850 yrs BP) at 60 to 110 cm depth suggest a major contribution of old SOC that was probably inherited from the parent material. Because SOC stocks were still considerably high below 35 cm depth, this old SOC has to be considered as an important contributor to the SOC pool in deep subsoils. Furthermore, these findings demand the inclusion of subsoils in carbon inventories, even if SOC contents are low.

Future studies should focus on input pathways that help to understand the evolution of allocation patterns of SOC from different sources such as observed in the present study.

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Acknowledgements

Appendix

Study I

Study II

Study III

Study I

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

Gerrit Angst, Ingrid Kögel-Knabner, Kristina Kirfel, Dietrich Hertel, Carsten W. Mueller
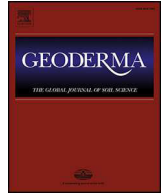
Spatial distribution and chemical composition of soil organic matter fractions in rhizosphere and non-rhizosphere soil under European beech (*Fagus sylvatica* L.)

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ARTICLE INFO

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

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
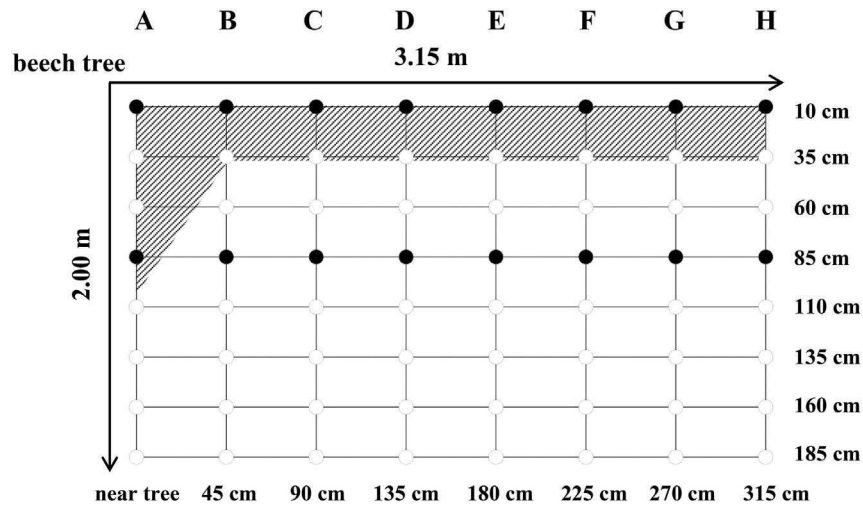
Fig. 1. Sampling grid applied to each transect wall (n = 64 samples per transect). Composite and volumetric soil samples (using steel cylinders; 8.5 cm diameter, 6 cm height) were taken. The black dots (n = 16 per transect) indicate the samples that were subjected to the combined density and particle 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
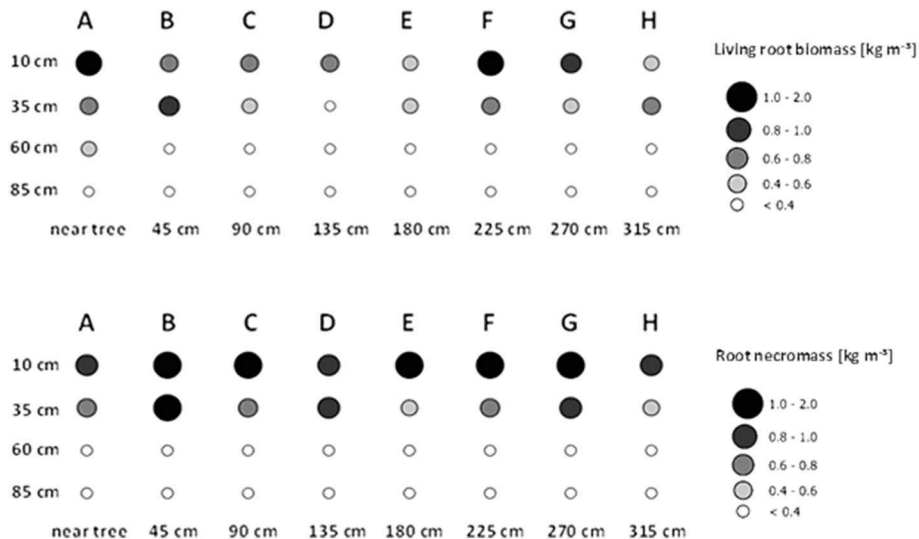

Fig. 2. Mean living and dead fine root concentration [kg m^{-3}] down to a depth of 85 cm. The letters above the plots are the labels of the horizontal sampling spots with A being nearest to the tree. n = 3 for each grid point.

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 zircon-oxide 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).

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

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
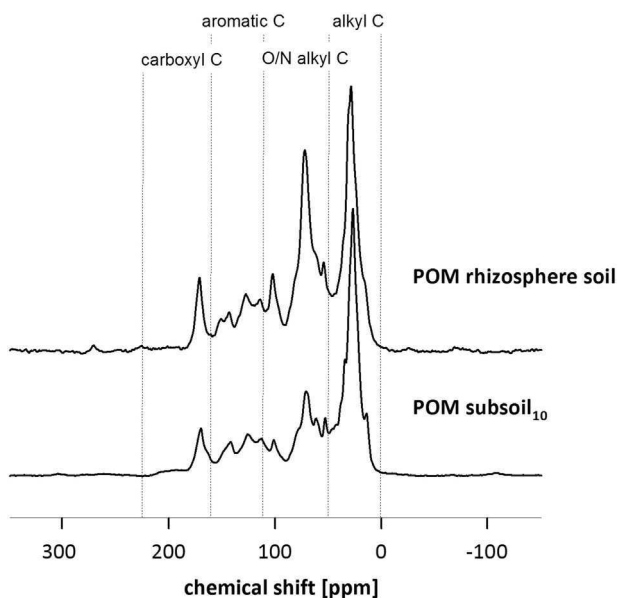

Fig. 3. ^{13}C CPMAS NMR mean spectra of the POM fractions of the rhizosphere soil (calculated from three spectra) and the subsoil$_{10}$ (calculated from 24 spectra).
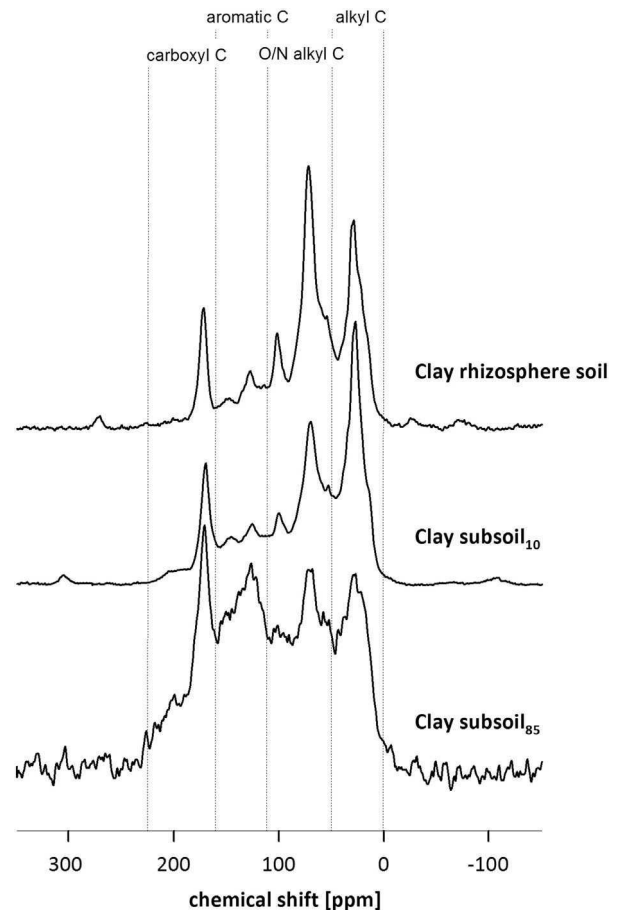

Fig. 4. ^{13}C CPMAS NMR mean spectra of the clay fractions of the rhizosphere soil (calculated from three spectra), subsoil$_{10}$ (calculated from 24 spectra) and subsoil$_{85}$ (calculated from four spectra).
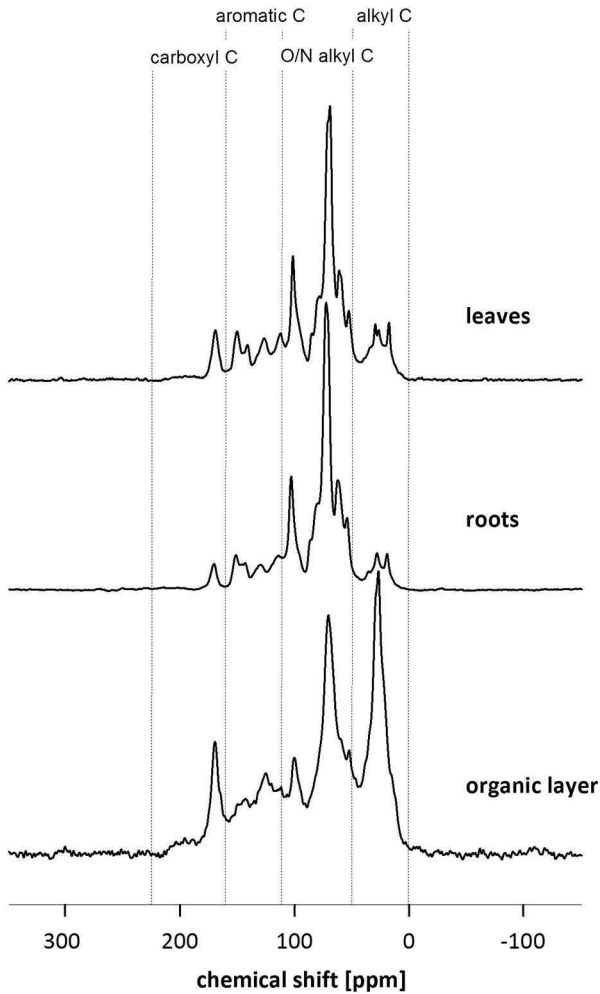
Fig. 5. ^{13}C CPMAS NMR mean spectra of the leaves, fine roots (each calculated from three spectra) and the organic layer material (calculated from 24 spectra) from all transects.

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

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

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 determined 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

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.

Acknowledgments

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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Study II

The fate of cutin and suberin of decaying leaves, needles and roots – Inferences from the initial decomposition of bound fatty acids

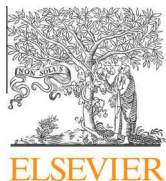Gerrit Angst, Lukas Heinrich, Ingrid Kögel-Knabner, Carsten W. Mueller

The fate of cutin and suberin of decaying leaves, needles and roots – Inferences from the initial decomposition of bound fatty acids

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ABSTRACT

The lipid biopolymers cutin and suberin are frequently used as biomarkers to distinguish above – from below-ground plant tissue input in soil. Despite a growing number of studies, still little is known about their fate during decomposition. The aim of this study was to investigate the decomposition of bound fatty acids with a special emphasis on cutin and suberin and to evaluate the effect of inherent chemical properties on decomposition. We incubated fresh leaves, needles and roots of European beech and Norway spruce for 84 days in a laboratory experiment. Cutin and suberin derived monomers were obtained by a sequential extraction procedure with subsequent GC-MS measurement. We monitored the mass loss of the plant materials, changes in chemical composition using solid-state ^{13}C NMR spectroscopy and, from this, calculated relative amounts of biomolecule components (i.e., relative lignin content). Our results suggest that both cutin and suberin biopolymers are readily decomposed without any indication of suberin being more resistant than cutin. The concentrations of cutin and suberin derived monomers were exponentially correlated to the mass loss of the respective plant material and rapidly decreased (beech: cutin: 47.4 ± 2.1%, suberin: 30.8 ± 5.5%; spruce: cutin: 31.2 ± 2.4%, suberin: 22.0 ± 4.8% of the initial concentration) at the beginning of the incubation, but leveled off towards the end. This indicates that studies which assume a similar degradation of biomarker and source plant material might underestimate the turnover of root and shoot derived soil organic matter. Beside the tested inherent chemical properties of the lipids (number of C atoms in each monomer, type and location of chemical functional groups), the relative lignin content explained a considerable portion of the variation in lipid concentrations over time. We thus propose a two phase model for the initial decomposition of cutin and suberin: (1) in early phases, cutin or suberin that is not associated with lignin is readily consumed by microorganisms resulting in a rapid decrease of the respective polymer. (2) After the first phase, only cutin or suberin associated with lignin remains, resulting in a decomposition that proceeds with the initially low decay rate of lignin. However, a substantial part of the variation in lipid concentrations was not accounted for by the tested factors. This suggests that the decomposition of cutin and suberin is additionally modulated by a not yet quantified external factor.

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1. Introduction

Because of their distinct occurrence in leaves/needles and roots/bark (Kolattukudy, 1980, 1981; Biggs et al., 1984; Kögel-Knabner, 2002), the lipid biopolymers cutin and suberin have recently been used as biomarkers to investigate the dynamics of shoot and root derived organic matter in soils (Mendez-Millan et al., 2011; Andreetta et al., 2013; Spielvogel et al., 2014). In this respect, suberin turned out to be a valuable tool for revealing the fate of root compounds in soils (e.g., Feng et al., 2010), which are supposed

to be the major contributor to soil organic matter especially in the subsoil (Rasse et al., 2005; Angst et al., 2016). However, there are still some uncertainties regarding the fate of cutin and suberin during decomposition.

First, whether cutin and suberin are chemically resistant to decomposition (Lorenz et al., 2007; Feng and Simpson, 2011; Carrington et al., 2012) or not (Tegelaar et al., 1989; Otto et al., 2005) is still under debate. Cutin and suberin are mostly composed of *n*-carboxylic, ω-hydroxy carboxylic and alkanedioic acids (Mendez-Millan et al., 2010) with differing chain lengths and relative abundance in each polymer (Mueller et al., 2012). These compounds are relatively easily hydrolyzed (Otto et al., 2005) by a range of microorganisms that are capable of producing hydrolyzing

enzymes (Kolattukudy, 1981). Nevertheless, both biopolymers are frequently preserved in soils (Kögel-Knabner et al., 1989; Rasse et al., 2005; Winkler et al., 2005). Especially root derived compounds have been found to occur in higher concentrations in mineral soils compared to leaf derived compounds (Mueller et al., 2012; Spielvogel et al., 2014). Whether this pattern is caused by differences in chemical properties between cutin and suberin, or by the preferential stabilization of suberin derived compounds remains unclear. Mueller et al. (2013) made a first attempt to quantify effects of plant lipid chemical properties (including those derived from cutin and suberin) on their concentrations in soil. They found a significant effect of chain length (number of C atoms in each lipid) and lipid type (location and type of chemical functional groups in each lipid) on lipid concentrations in mineral soil. Such effects of lipid chemical properties on the decomposition of cutin and suberin remain to be tested.

Secondly, it has been proposed that both cutin/suberin and source plant material decompose similarly (e.g., Mendez-Millan et al., 2010). This assumption is a prerequisite to link the concentration of the two biopolymers to root and shoot derived soil organic matter (SOM) and its turnover. Until now, experimental evidence for a similar degradation of cutin/suberin and source plant material is scarce (Riederer et al., 1993). The estimation of root and shoot derived SOM might thus be under- or overestimated depending on the relation between cutin/suberin and the respective source plant material during decomposition. These estimates might be further complicated by a potentially differing decomposition rate of individual cutin or suberin derived compounds. Experimental evidence for a uniform degradation of cutin derived monomers of beech has been provided by Riederer et al. (1993). In an incubation study, the authors found a rapid and uniform decrease of all five identified cutin monomers. To the best of our knowledge, no studies that directly monitor the decomposition of suberin monomers have been reported so far.

The first aim of this study was to investigate the initial decomposition of cutin and suberin monomers of leaves, needles and roots in relation to the respective source plant material. The decomposition was studied without any interference by the soil mineral phase. The second aim was to evaluate the effect of inherent chemical properties of bound fatty acids (including those derived from cutin and suberin) and the source plant material on the decomposition of both biopolymers.

We individually incubated fresh leaves, needles and roots of European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* L. Karst.) in litterbags within forest floor material of the respective tree species for 84 days in the laboratory. Bound fatty acids (including cutin and suberin derived monomers) were obtained from the samples by the application of a sequential extraction procedure with subsequent GC–MS measurement. The overall alteration in the chemical composition of the decomposing plant material was analyzed using solid state ^{13}C nuclear magnetic resonance (NMR) spectroscopy. To compare the decomposition of the biopolymers and the source plant materials, we monitored the mass loss of the incubated bulk plant materials and calculated decomposition indices from the NMR spectra, and from carbon (C) and nitrogen (N) measurements (alkyl/O/N alkyl C, 70–75/52–57 ppm and C/N ratio).

2. Materials and methods

2.1. Sampling of plant material and experimental design

The sampling of the plant and forest floor material for the incubation experiment was carried out at a pure European beech (*F. sylvatica* L.) and a pure Norway spruce (*P. abies* L. Karst.) stand at the Kranzberger forest near the city of Freising, Germany (48°24.512′N, 11°41.623′E, elevation 480–515 m a.s.l.). General information about the study area is shown in Table 1.

In October 2013, fine (diameter < 2 mm) and coarse (diameter > 2 mm) living roots were collected from the forest floor at both the beech and the spruce stand. Spruce needles and beech leaves of mature trees were sampled at the same site. Additionally, moist forest floor material was collected in March 2014 at each stand to assure a high microbial activity (Bååth and Söderström, 1982) and a microbial decomposer community that is adapted to the respective plant litter material (Wallenstein et al., 2013; Mooshammer et al., 2014). The water content of the forest floor material at the beech stand was 36.0 ± 2.7% and the water content of the forest floor material at the spruce stand was 40.6 ± 2.1%. Understory vegetation in both investigated stands was absent.

In the laboratory, the fresh root material was gently rinsed with deionized water to remove adhering soil particles. All plant materials were freeze dried. Prior to incubation, the plant materials were cut into small fragments < 1 cm^2 and 1 g of each root and litter material were weighed into separate small litterbags (2 × 2 cm) consisting of a polyamide membrane with a mesh size of 80 μm. The use of litterbags enabled us to investigate the microbial decomposition of pure plant material on a mass basis with minimal contaminations of forest floor material. Each litterbag was sealed with a nylon thread and incubated embedded in fresh forest floor material of the respective tree species in individual 0.9 L glass jars for 84 days in the dark at a constant temperature of 20 °C. The aerated jars were regularly hydrated with a sprayer to sustain the original field water content. Three jars per plant material were destructively sampled at 14, 28, 42 and 84 days of incubation giving a total of 48 incubated samples plus three replicate samples of each non-incubated initial leaf, needle and root material (*n* = 12). After removal from the jars, the material from the litter bags was freeze dried, weighed and stored for further analysis.

2.2. Carbon and nitrogen measurements

Carbon and N measurements of non-incubated plant material (*n* = 12) and incubated plant material at each sampling date (*n* = 48) were performed on an elemental analyzer (EuroVector, Milan, Italy) by dry combustion. An aliquot of 1–2 mg of each sample was ground and used for analysis. All measurements were run in duplicate.

2.3. Nuclear magnetic resonance spectroscopy

Solid state ^{13}C cross polarization magic angle spinning (CPMAS) NMR spectroscopy was performed on each sample (*n* = 60) to analyze the chemical composition of leaves, needles and roots. Measurements were performed using a Bruker Avance III 200 Spectrometer. The samples were spun at 5.0 kHz with a recycle delay time of 1 s. The spectra were processed with a line broadening of 50 Hz, the phase was adjusted and the 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–

Table 1
General parameters of the two investigated stands (European beech and Norway spruce) at the Kranzberger forest.

	European beech	Norway spruce
Soil type	Haplic Luvisol	Haplic Luvisol
Humus form	Mulltype moder	Moder
C/N	19.2 ± 0.2	22.8 ± 0.2
pH$_{H2O}$	5.1	4.7
Water content (%)	36.0 ± 2.7	40.6 ± 2.1

220 ppm (carboxylic C) (Kögel-Knabner et al., 1992). Since cellulose, hemicellulose and proteins (O/N alkyl C) in plant residues are relatively easily decomposable and aliphatic structures (alkyl C) are thought to be more resistant to degradation, the ratio between alkyl C and O/N-alkyl C can be used as indicator for the degree of decomposition of plant material and organic matter (Baldock et al., 1997).

In addition to the conventional separation into four integration regions, we applied the 'molecular mixing model' (Nelson and Baldock, 2005) to derive an estimate of the lignin content during the course of the incubation. The spectra were separated into seven integration areas, carbonyl (210–165 ppm), O-aromatic (165–145 ppm), aromatic (145–110 ppm), O_2-alkyl (110–95 ppm), O-alkyl (95–60 ppm), N-alkyl/methoxyl (60–45 ppm) and alkyl C (45–10 ppm). The molecular mixing model estimates the relative content of biomolecule components (in this study: carbohydrates, lignin, proteins and lipids) after determining the signal intensity of the compounds in each of the seven integration regions. The model is described in detail in Nelson and Baldock (2005).

In addition to the alkyl C to O/N-alkyl C ratio, the ratio between the integrated shift regions of 70–75 and 52–57 ppm was calculated (referred to as 70–75/52–57 ppm ratio), which correlates well with the decay rates of plant residues (Bonanomi et al., 2013). The integration area of 70–75 ppm corresponds to the C2, C3 and C5 of carbohydrates, and the integration area of 52–57 ppm corresponds to the methoxyl C of lignin (Bonanomi et al., 2011).

2.4. Sequential lipid extraction

In a first extraction step, free lipids were removed from the samples by accelerated solvent extraction (Dionex ASE 200, Dionex GmbH, Idstein, Germany). Each sample was weighed into a 11 ml stainless steel extraction cell with glass fiber filters applied at both ends. Free lipids were then extracted with dichloromethane (DCM)/methanol (9/1, v/v) at 17×10^6 Pa (Jansen et al., 2006) and a temperature of 75 °C (Wiesenberg et al., 2004; Jansen et al., 2006). The heating phase, as well as the static extraction time were set to 5 min. All samples were extracted twice under the same conditions.

In a second step, the pre-extracted leaf, needle and root residues were subjected to alkaline hydrolysis to release ester bound lipids (cutin and suberin derived monomers). The samples were weighed into Teflon lined bombs (Groteklaes GmbH, Jülich, Germany) and saponified with 1 M methanolic KOH solution at 100 °C for three hours (Spielvogel et al., 2014). After cooling, the supernatant KOH was transferred to separate vials. The soil residues were saturated with DCM/methanol (1/1, v/v), ultrasonicated, the supernatant combined with the KOH extracts, and the combined extracts were dried under a stream of nitrogen. The dry extracts were re-dissolved in deionized water and extracted with DCM by liquid-liquid extraction. To separate the acid fraction, the residual deionized water phase was adjusted to pH 1 using concentrated hydrochloric acid and extracted $3\times$ with DCM. The DCM phases were dried under nitrogen and stored in the freezer. Because cutin and suberin are mostly differentiated by their constituting acids (Otto and Simpson, 2006), only the acid fractions were considered in our analysis. It is worth noting that alcohols can also be part of cutin or suberin (Otto and Simpson, 2006), but as they may also be produced by microorganisms (Andreetta et al., 2013; Spielvogel et al., 2014), we did not include alcohols in our analysis.

2.5. GC–MS analyses

Prior to the GC–MS measurement, the dry acid fractions were re-dissolved in pyridine containing phenyl acetic acid for the calculation of the GC response factor. N,O-bis-(trimethylsilyl)-trifluoroa

cetamide (BSTFA) containing 1% trimethylchlorosilane was added as derivatization agent to transform hydroxy and carboxylic acid functions into their trimethylsilyl ether and ester derivatives. The samples were silylated at 70 °C for 1 h. The GC–MS analyses were performed on a Trace GC Ultra coupled to an ISQ mass spectrometer (ThermoFisher Scientific, Waltham, USA). The GC oven was run with a starting temperature of 90 °C held for 1 min, a subsequent heating phase to 130 °C at a rate of 30 °C/min, a heating phase to 200 °C at a rate of 7 °C/min and a heating phase to 320 °C at a rate of 3 °C/min. The isotherm of 320 °C was held for 15 min. Samples were injected in splitless mode with an injector temperature of 320 °C. The ISQ was operated in electron ionization mode and a scan mass range of 50–650 Da.

The compounds were identified by their fragmentation pattern assisted by the comparison with published mass spectra (Eglinton et al., 1968; Holloway and Deas, 1971, 1973; Hauff et al., 2010) and with a mass spectral library (NIST mass spectral search program, Standard Reference Program of the National Institute of Standards and Technology, USA). The compounds were quantified by applying calibration curves derived from different concentrations of an external standard consisting of n-hexadecanoic acid, n-octadecanoic acid, n-eicosanoic acid, n-tetracosanoic acid, 9-hexadecenoic acid, α,ω-decanedioic acid, α,ω-hexadecanedioic acid, ω-hydroxyhexadecanoic acid and 9,10,16-trihydroxy hexadecanoic acid, and normalized to the specific GC response factor, which was always close to 1. The measured concentrations of each acid were normalized to the organic carbon (OC) content of the respective sample. The sum of concentrations (in µg/g OC) of the respective indicator acids (see Section 2.6) (referred to as cutin and suberin derived monomers) and its change over time was used to track the decomposition of cutin and suberin of beech and spruce. In addition to the cutin and suberin monomers, we evaluated the decomposition of all extracted acids (in µg/g OC; referred to as lipid concentrations).

2.6. Identification of cutin and suberin derived monomers from released acids of leaves, needles and roots

The sequential extraction approach released several n-carboxylic acids, α,ω-alkanedioic acids, ω-hydroxy alkanoic acids and mid-chain substituted hydroxy alkanoic acids from the initial leaf, needle and root material (Table 2).

Acids that were released from both the beech leaves and spruce needles and correspond to previously suggested cutin specific monomers (Otto and Simpson, 2006; Mueller et al., 2012; Spielvogel et al., 2014), were the 8,9,10,ω-hydroxyhexadecanoic acids (subsumed under x,ω-C_{16}). Since we analyzed material from known sources and not complex mixtures (e.g., soil), we were also able to make use of non-specific acids that are either part of cutin or suberin (cf. Otto and Simpson, 2006), i.e. the ω-hydroxyhexadecanoic acid (ω-C_{16}), the 9,10,ω-trihydroxyoctadecanoic acid (9,10,ω-C_{18}) and the α,ω-hexadecanedioic acid (C_{16} DA) for both beech leaves and spruce needles and the ω-hydroxyoctadecenoic acid (ω-$C_{18:1}$) for beech leaves (Table 2).

Acids that were released from the incubated beech roots and correspond to previously suggested suberin specific monomers (Otto and Simpson, 2006; Mueller et al., 2012; Spielvogel et al., 2014) were the ω-hydroxy alkanoic acids with a chain length of C_{20} to C_{24} (ω-C_{20}, ω-C_{22}, ω-C_{24}). The α,ω-octadecanedioic acid (C_{18} DA) usually present in both cutin and suberin (Otto and Simpson, 2006) was only detected in roots and thus added to the suberin specific monomers in this study. The spruce roots released the same acids with the exception of ω-C_{24}. Non-specific acids detected were the C_{16} DA, ω-$C_{18:1}$, 9,10,ω-C_{18} and the ω-C_{16} acids for beech roots and the C_{16} DA and ω-C_{16} acids for spruce roots, respectively (Table 2).

Table 2
Overview of monomers released from the initial, non-incubated leaves, needles and roots of European beech and Norway spruce. Mean (±SE) of the monomers in µg/g OC. Specific cutin markers are highlighted by a dashed frame, specific suberin markers are bold-framed. Non-specific monomers for either cutin or suberin are gray-shaded.

	European beech		Norway spruce	
	Leaves	Roots	Needles	Roots
***n*-Carboxylic acids**				
Dodecanoic acid (n-C$_{12}$)	n.d.	n.d.	55.7 ± 3.9	n.d.
Tetradecanoic acid (n-C$_{14}$)	48.5 ± 13.0	n.d.	44.7 ± 3.9	n.d.
Hexadecanoic acid (n-C$_{16}$)	957 ± 256	198.9 ± 19.7	81.6 ± 9.5	46.1 ± 11.1
Heptadecanoic acid (n-C$_{17}$)	n.d.	n.d.	n.d.	23.0 ± 6.5
Octadecanoic acid (n-C$_{18}$)	87.1 ± 19.7	37.8 ± 3.9	n.d.	9.1 ± 1.4
Octadecenoic acid (n-C$_{18:1}$)	18.7 ± 7.8	19.6 ± 11.3	2.7 ± 2.2	n.d.
Octadecadienoic acid (n-C$_{18:2}$)	19.0 ± 5.9	37.1 ± 1.7	1.8 ± 1.5	n.d.
Eicosanoic acid (n-C$_{20}$)	7.7 ± 6.3	52.5 ± 3.4	n.d.	19.6 ± 3.3
Docosanoic acid (n-C$_{22}$)	27.0 ± 21.4	162.7 ± 14.4	n.d.	26.5 ± 5.1
Hexacosanoic acid (n-C$_{26}$)	19.2 ± 2.9	n.d.	n.d.	n.d.
Sum n-carboxylic acids	*1185 ± 333*	*508.6 ± 54.4*	*187 ± 40.6*	*124 ± 27.4*
ω-Hydroxy alkanoic acids				
ω-Hydroxyhexadecanoic acid (ω-C$_{16}$)	226.9 ± 35.8	2009 ± 266	2008 ± 66.7	240.7 ± 37.1
ω-Hydroxyoctadecenoic acid (ω-C$_{18:1}$)	27.0 ± 3.8	115.1 ± 35.4	n.d.	n.d.
ω-Hydroxyeicosanoic acid (ω-C$_{20}$)	n.d.	573.3 ± 56.6	n.d.	268.1 ± 39.0
ω-Hydroxydocosanoic acid (ω-C$_{22}$)	n.d.	1271 ± 156.9	n.d.	267.9 ± 38.4
ω-Hydroxytetracosanoic acid (ω-C$_{24}$)	n.d.	147.0 ± 18.2	n.d.	n.d.
Sum ω-hydroxy alkanoic acids	*253.9 ± 39.6*	*4116 ± 533*	*2008 ± 66.7*	*777 ± 115*
α,ω-Alkanedioic acids				
α,ω-Heptadioic acid (C$_7$ DA)	n.d.	11.5 ± 9.4	n.d.	n.d.
α,ω-Octadioic acid (C$_8$ DA)	n.d.	29.4 ± 0.9	n.d.	n.d.
α,ω-Nonadioic acid (C$_9$ DA)	98.5 ± 42.1	133.6 ± 2.9	n.d.	3.3 ± 0.1
α,ω-Decanedioic acid (C$_{10}$ DA)	17.9 ± 2.8	4.3 ± 3.5	n.d.	n.d.
α,ω-Undecanedioic acid (C$_{11}$ DA)	n.d.	n.d.	291.4 ± 8.1	n.d.
α,ω-Hexadecanedioic acid (C$_{16}$ DA)	65.7 ± 12.2	2563 ± 381	99.7 ± 5.7	232.6 ± 8.9
α,ω-Octadecanedioic acid (C$_{18}$ DA)	n.d.	492 ± 74.1	n.d.	196.0 ± 7.8
Sum α,ω-alkanedioic acids	*173.1 ± 57.1*	*3233 ± 472*	*391 ± 13.8*	*431.9 ± 16.8*
Mid-chain substituted hydroxy alkanoic acids				
x,ω-Dihydroxyhexadecanoic acid (x,ω-C$_{16}$)	1007 ± 133	n.d.	1320 ± 175	n.d.
9,10,ω-Trihydroxyoctadecanoic acid (9,10,ω-C$_{18}$)	366 ± 52.6	572 ± 150	366 ± 19.4	n.d.
Sum mid-chain substituted hydroxy alkanoic acids	*1373 ± 186*	*572.1 ± 150*	*1687 ± 194*	*0.0*
Sum of extracted aliphatic acids	*2985 ± 616*	*8430 ± 1210*	*4272 ± 315*	*1333 ± 159*

n.d. = not detected

2.7. Statistics

Means and standard errors (SE) were calculated using Microsoft Excel 2013 for Windows (Microsoft, Redmond, WA, USA). All other statistics were performed using the R 3.0.3 software for Windows (R Core Team, 2013). The Shapiro-Wilk and the Bartlett test were used to test the data for normality and homogeneity of variances, respectively. Depending on the tests' outcomes, significant differences between the sampling dates were tested using the one-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) post-hoc test or the Kruskal–Wallis test in connection with Dunn's post-hoc test. Correlation and regression (linear, exponential, logarithmic) analyses were separately conducted between the mass loss and the cutin/suberin monomers, the decomposition indices of the individual plant materials, and to test the relation between single cutin or suberin monomers during the incubation. We established ANOVA models with type III sums of squares to evaluate the influence of different factors on the decomposition of cutin and suberin specific monomers. We tested the influence of chain length (number of C atoms in each monomer), lipid type (n-carboxylic acid, α,ω-alkanedioic acid, ω-hydroxy alkanoic acid, or mid-chain substituted hydroxy alkanoic acid) and lignin content (derived from the NMR molecular mixing model) on the percentage of lipid concentrations left at each date of sampling. We established an ANOVA model including all three factors to analyze possible interactions between the factors and, subsequently, one-way ANOVA models to evaluate the main effects of single factors on lipid decomposition. We used all extracted acids (not necessarily cutin or suberin specific) and sampling dates for the ANOVA models. We added interaction terms for all factors and the dates of sampling because effects may have been confounded with 'time' by using data from different dates of sampling. All interaction terms were non-significant ($p > 0.12$) indicating that the observed effects were mechanistically related to lipid decomposition. The effect sizes were reported as eta-squared (η^2) values. Since we only had one lignin content per plant material and date of sampling, we categorized this variable into a factor with three factor levels (low, medium, high) using equal interval classification. We did not additionally test for the influence of species (beech or spruce) because we postulate that differences in species are reflected by the respective chemical composition of the extracted monomers (chain length and lipid type) from beech and spruce. Environmental factors like differing microbial communities or pH values of the forest floor as related to different tree species may influence the decay of lipids (van Bergen et al., 1998; Hackl et al., 2005). We did not include such factors in the ANOVA models because the autochthonous species specific forest floor materials featured similar C/N ratios, pH values and water contents (Table 1) and were incubated at constant laboratory conditions. Our approach thus specifically evaluated the influence of the inherent chemical characteristics of the plant materials on decomposition. All statistical analyzes were regarded as being significant at a level of $p < 0.05$.

3. Results

3.1. Change in mass and chemical composition

The mass loss was greatest for the spruce needles (38.7 ± 3.4%), followed by spruce roots (20.5 ± 1.3%), beech leaves (17.3 ± 0.5%) and beech roots (6.0 ± 3.0%) (Fig. 1). The change in OC was similar and correlated well ($r = 0.78$–0.99) with the mass loss of the incubated plant material (Fig. 1).

An overview of NMR spectra is given in Supplementary Fig. S1. From these data the alkyl/O/N-alkyl C ratio and the ratio of the signal intensities 70–75/52–57 ppm were calculated. The C/N and the 70–75/52–57 ppm ratios of all incubated plant materials significantly decreased during the incubation (Fig. 2). Concurrently, the alkyl/O/N-alkyl C ratios significantly increased (Fig. 2). Beech roots showed only a trend of increasing alkyl/O/N alkyl C ratios. The course of these decomposition indices during incubation was consistent with the mass loss of the incubated materials. The C/N, 70–75/52–57 ppm and the alkyl/O/N-alkyl C ratios of all incubated plant materials (except beech roots) significantly correlated with the mass loss of the respective material. The C/N and the 70–75/52–57 ppm ratios showed the strongest correlations ($r = -0.60$ to -0.92 and $r = -0.73$ to -0.97, respectively). The results of the mixing model (Fig. 3) showed no significant differences in relative lignin content in spruce needles and roots but significant differences in beech leaves and roots. However, when comparing initial lignin contents and lignin contents at the end of the incubation, no significant differences could be observed for either plant material. The relative content of carbohydrates significantly decreased during the course of the incubation. Interestingly, the beech roots differed from this pattern where the

Fig. 2. Mean (±SE) C/N, 70–75/52–57 ppm and alkyl/O/N alkyl C ratio of the incubated materials at the different sampling dates. Letters above the bars indicate significant differences between the sampling dates.

relative content of carbohydrates at the end of the incubation did not significantly differ from that of the non-incubated material. The relative content of lipids stayed constant (beech leaves and roots), significantly decreased (spruce needles), or even increased (spruce roots). The relative content of proteins significantly decreased in all plant materials except for the spruce roots, where it stayed constant and the beech roots, where it first increased but decreased again at the end of the incubation (Fig. 3).

3.2. Lipid concentrations and cutin/suberin derived monomers

The concentration of n-carboxylic, ω-hydroxy alkanoic, α,ω-alkanedioic, and mid-chain substituted hydroxy alkanoic acids significantly decreased over time (Fig. 4). The concentrations of ω-hydroxy alkanoic acids of beech leaves and mid-chain substituted acids of beech roots and spruce needles differed from this pattern and did not decrease significantly. Notably, the beech leaves and spruce needles showed significantly higher concentrations of
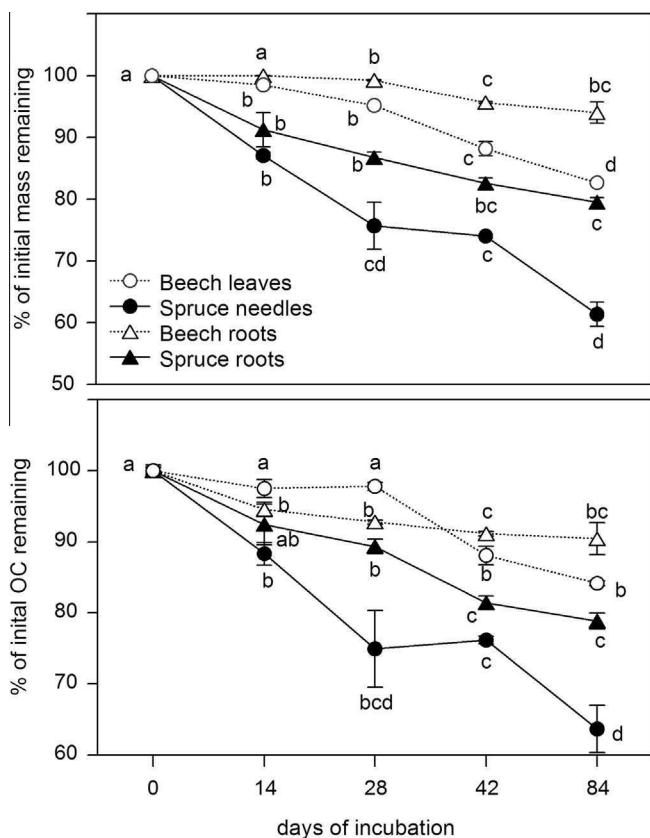

Fig. 1. Mean (±SE) % of initial mass and OC remaining of each incubated material at the different sampling dates. Letters indicate significant differences between the sampling dates.
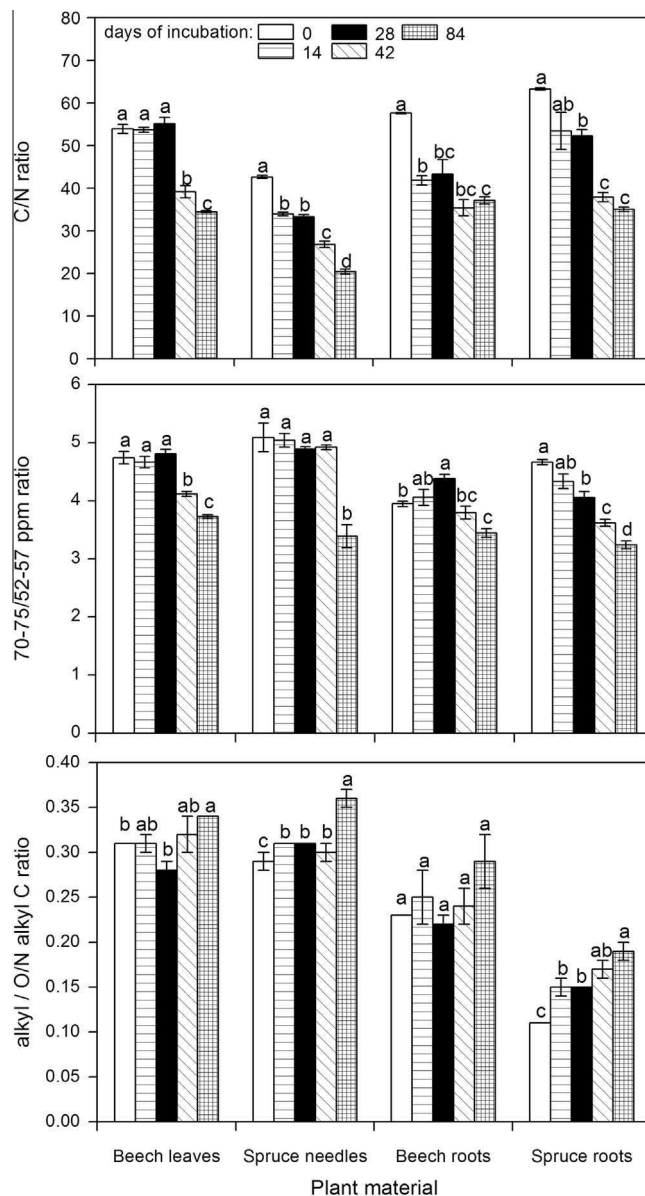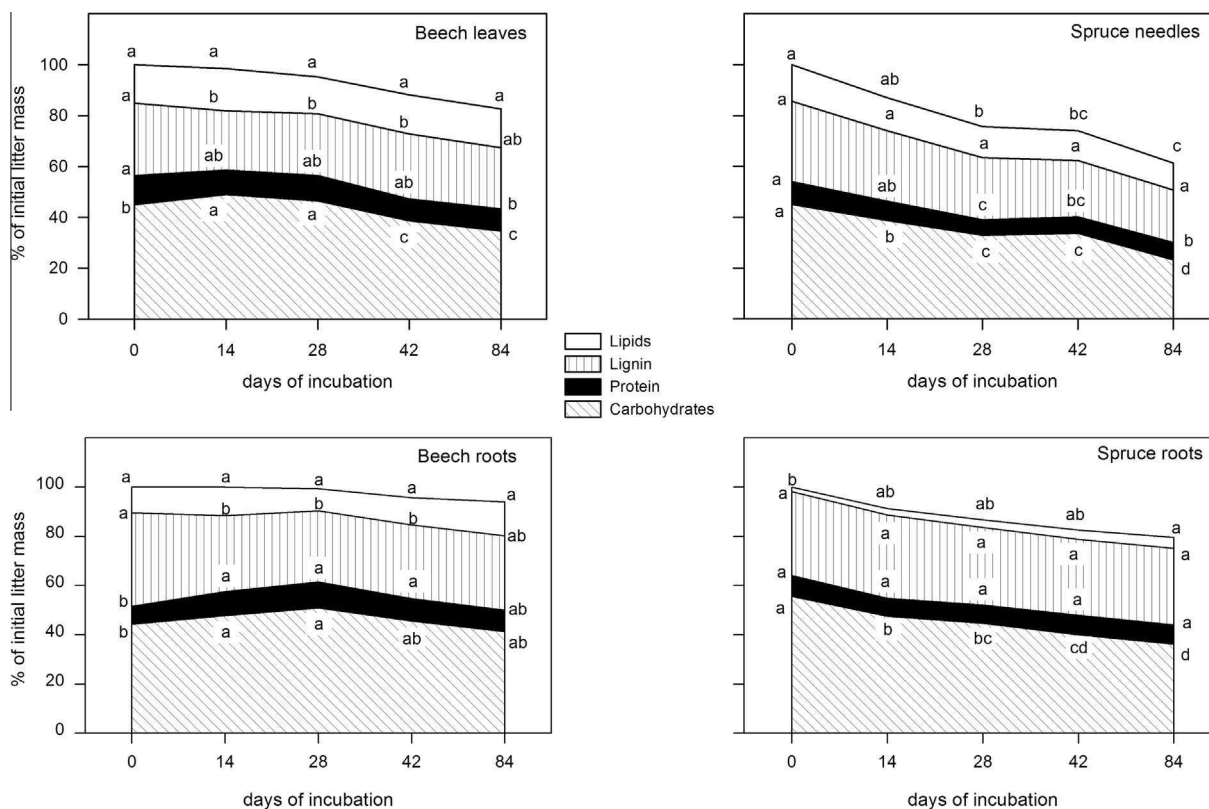
Fig. 3. Mean relative amount of lipids, lignin, protein and carbohydrates of the incubated materials at the different sampling dates normalized to the mass loss of the respective plant material. Letters near the bars indicate significant differences between the sampling dates. The data are derived from the mixing model that has been applied to the NMR spectra. The non-normalized data output of the mixing model is depicted in Supplementary Table S1.

mid-chain substituted hydroxy alkanoic acids ($p < 0.05$) than their respective root counterparts. The percentage of all acids remaining at the end of the incubation was $32.6 \pm 8.1\%$ and $19.0 \pm 4.7\%$ for the beech leaves and roots, and $43.3 \pm 10.8\%$ and $23.3 \pm 5.8\%$ for the spruce needles and roots, respectively. Interestingly, no mid-chain substituted hydroxy alkanoic acids were released from the spruce roots. The ANOVA model including all three factors revealed no significant interaction between chain length, lipid type and relative lignin content. Thus, the main effects could be directly interpreted from the ANOVA models with one factor. Most of the variability in lipid concentrations was accounted for by chain length and relative lignin content followed by lipid type (Table 3). The direction of the main effects was as follows: (1) the higher the chain-length, the higher the lipid concentrations at the respective sampling event; (2) The higher relative lignin contents present in the sample, the higher the lipid concentrations at the respective date of sampling; (3) Lipid concentrations increased in the order carboxylic acids < α,ω-alkanedioic acids < ω-hydroxy alkanoic acids < mid-chain substituted hydroxy alkanoic acids.

The concentration of the cutin and suberin monomers in the litterbags showed similar trends in all incubated materials, i.e., it decreased during the course of the incubation (Fig. 5) and significantly correlated with the mass loss of the respective plant material (Fig. 6). The best fit was achieved using simple exponential regressions, as evaluated by minimal values for p and maximal values for R^2. The consistency of data is also partly mirrored in the NMR data. For example, the low yield of suberin markers of spruce (Fig. 5b) corresponded well with the low relative amounts of lipids detected by the molecular mixing model (Fig. 3).

The concentration of cutin monomers of the beech leaves decreased after 14 days to 801.8 ± 36.3 µg/g OC ($47.4 \pm 2.1\%$ of the initial concentration) and stayed constant afterwards without

any trend of a further decrease. The concentration of cutin monomers of the spruce needles decreased stepwise. First, after 28 days to 2490 ± 61 µg/g OC ($65.6 \pm 1.6\%$ of the initial concentration), and secondly, after 42 days to 1184 ± 116 µg/g OC ($31.2 \pm 2.4\%$ of the initial concentration) (Fig. 5a and c). During decomposition, only the unspecific ω-$C_{18:1}$, $9,10,\omega$-C_{18} and C_{16} DA acids for beech and the $9,10,\omega$-C_{18} acid for spruce significantly correlated with the specific x,ω-C_{16} acids (Table 4).

The concentration of the suberin monomers of the beech roots decreased drastically after 14 days of incubation to 2386 ± 130 µg/g OC ($30.8 \pm 5.5\%$ of the initial concentration), with no significant decrease towards the end of the incubation. The concentration of the suberin monomers of the spruce roots decreased to 263.7 ± 57.3 µg/g OC ($22.0 \pm 4.8\%$ of the initial concentration) until the end of the incubation (Fig. 5b and d). The specific suberin monomers in this study (C_{18} DA, ω-C_{20}–ω-C_{24} for beech and C_{18} DA, ω-C_{20} and ω-C_{22} for spruce) showed highly linear correlations with each other during the course of the incubation (Table 5).

4. Discussion

4.1. Mass loss

The rapid mass loss of the leaves and needles in this study can be attributed to the high availability of easily decomposable substances in fresh plant material. Our results are similar to those of Girisha et al. (2003) for fresh pine needles and Aneja et al. (2004, 2006) who incubated fresh beech leaves and spruce needles. The decomposition indices (C/N, alkyl/O/N alkyl C and 70–75/52–57 ppm ratios) reflected the common changes in chemical composition during litter decay (e.g., Lorenz et al., 2000, 2004; Bonanomi et al., 2013; Cepáková and Frouz, 2015) at which the chemical
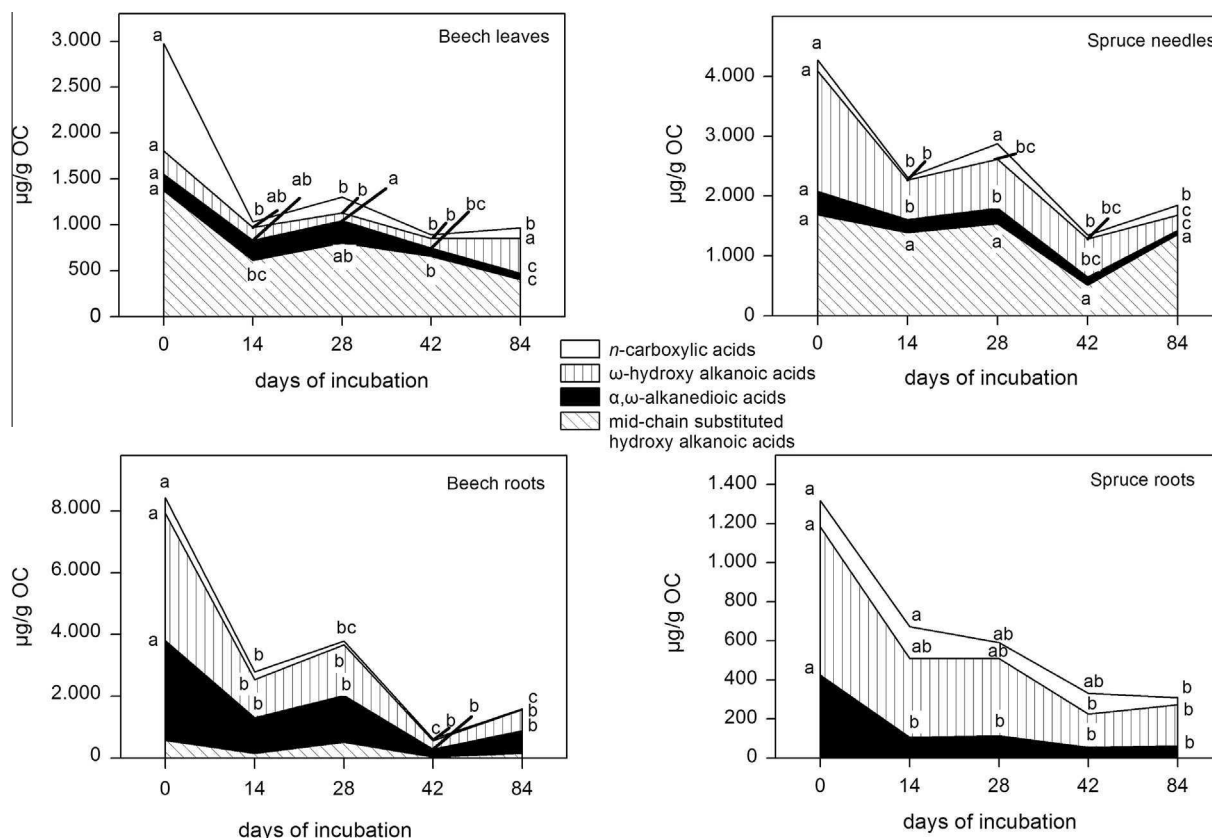
Fig. 4. Mean concentration (μg/g OC) of lipid types (n-carboxylic, α,ω-diacids, ω-hydroxy and mid-chain substituted hydroxy acids) of the incubated materials at the different sampling dates. Letters near the bars indicate significant differences between the sampling dates.

Table 3
Eta-squared (η^2) and p-values for the factors chain length (number of C-atoms in each monomer), lipid type (n-carboxylic acid, α,ω-alkanedioic acid, ω-hydroxy alkanoic acid or mid-chain substituted hydroxy alkanoic acid) and relative lignin content. The effect of the three factors on the lipid concentrations during decomposition was tested using ANOVA models with type III sums of squares ($n = 250$ for each model). The p-value for the model including all three factors was < 0.01.

Factor	ANOVA models			
	η^2 for the model with all factors	p-Value for the model with all factors	η^2 for models with one factor	p-Value for models with one factor
Chain length		< 0.01	0.15	< 0.001
Lipid type	0.31	< 0.01	0.06	< 0.01
Relative lignin content		< 0.001	0.12	< 0.001

changes captured by the 70–75/52–57 ppm ratio were most closely related to the mass loss of the plant materials. The 70–75/52–57 ppm ratio does not compare large integration areas (unlike the alkyl/O/N alkyl C ratio), but relies on distinct peaks in the ^{13}C NMR spectrum which are thought to be more related to the actual chemical change of decomposing plant material (Bonanomi, 2013).

Notably, beech and spruce roots clearly decomposed more slowly than their respective aboveground counterpart as was also observed by others for different sets of species (e.g., Bloomfield et al., 1993; Harmon et al., 2009). This may be due to their higher initial amounts of lignin compared to the leaf and needle material ($p = 0.004$ and $p = 0.04$, respectively) and/or their higher C/N ratios (Berg, 1984; Zhang et al., 2008) (Fig. 2) which may retard decomposition.

Interestingly, the mass of the spruce needles remaining at the end of the incubation was significantly lower compared to the remaining mass of the beech leaves ($p \leqslant 0.01$) (Fig. 1). Spruce nee-

dles have been commonly regarded as being recalcitrant to decomposition (Chapman et al., 1988; Johansson, 1995) and a faster decay of spruce compared to beech litter was only documented by few studies (Vesterdal, 1999; Albers et al., 2004; Sariyildiz et al., 2005). The reason for the retarded decay of the beech leaves might be simply due to the exclusion of the meso- and macrofauna from the litterbags which may otherwise be responsible for a considerable mass loss by removing leave fragments (Staaf, 1987; Kammer et al., 2012). Depending on mesh-size, litterbags may influence the accessibility of incubated material by meso- and/or macrofauna (Kampichler and Bruckner, 2009) and distort the actual decomposition process. However, for the aims of this study, the use of litterbags with a small mesh-size (80 μm) was crucial, because it enabled us to exclusively study the microbial decay (and thus the effect of chemical properties) of cutin/suberin and the respective plant materials on a mass basis. A possible loss of mass or the focal lipids (cutin and suberin monomers; discussed in Section 4.2) in soluble form (Rubino et al., 2010) should have
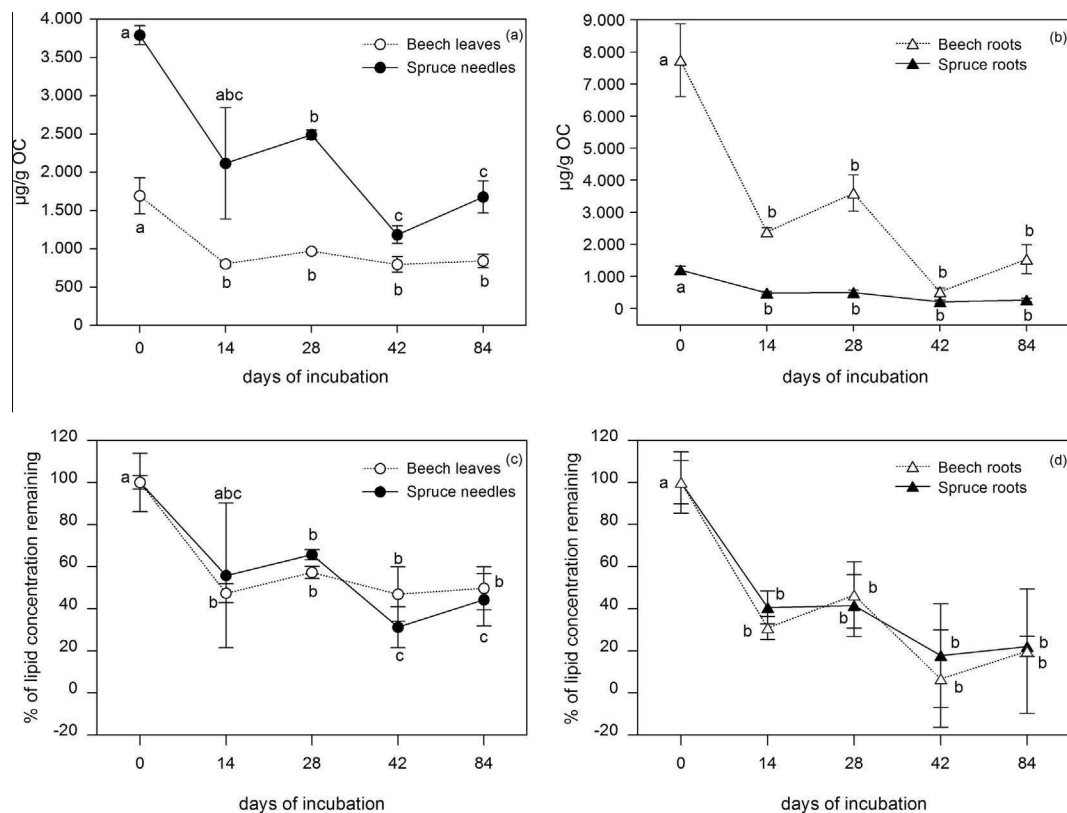
Fig. 5. Mean (±SE) amount of (a) cutin and (b) suberin monomers (in μg/g OC; cf., Table 2) of the incubated materials at the different sampling dates. Letters indicate significant differences between the sampling dates.
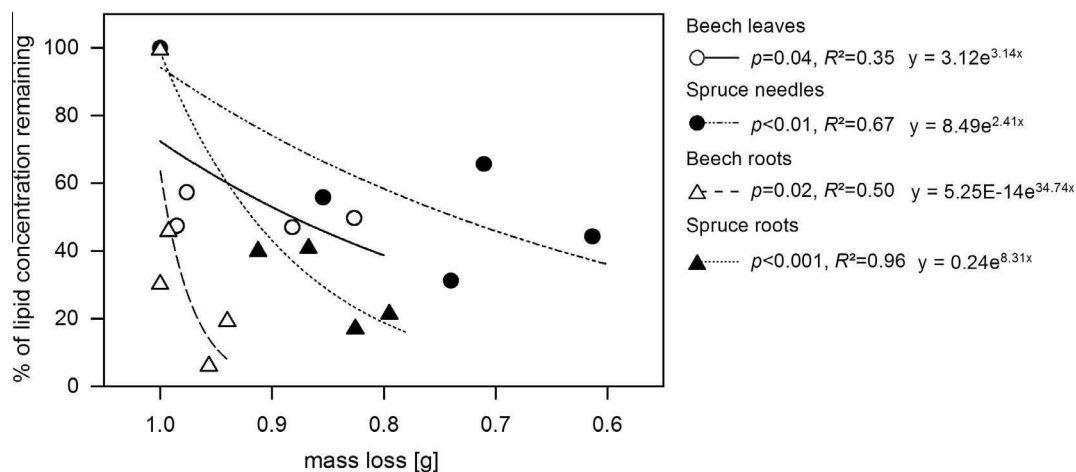
Fig. 6. Exponential regressions between the mass loss and the concentration of cutin and suberin monomers.

Table 4
Pearson's product moment correlation coefficient (r) of the correlation between individual cutin monomers of European beech leaves and Norway spruce needles. Cutin specific acids are gray-shaded.

	European beech leaves					Norway spruce needles			
	C_{16} DA	ω-C_{16}	x,ω-C_{16}	ω-$C_{18:1}$	9,10,ω-C_{18}	C_{16} DA	ω-C_{16}	x,ω-C_{16}	9,10,ω-C_{18}
C_{16} DA	1	n.s.	0.64	0.70	0.60	1	n.s.	n.s.	0.61
ω-C_{16}		1	n.s.	n.s.	n.s.		1	n.s.	0.53
x,ω-C_{16}			1	0.82	0.90			1	0.87
ω-$C_{18:1}$				1	0.85				
9,10,ω-C_{18}					1				1

Degrees of freedom = 10, $p < 0.05$, n.s. = not significant.

Table 5

Pearson's product moment correlation coefficient (r) of the correlation between the individual suberin monomers of European beech and Norway spruce roots. Suberin specific acids are gray-shaded.

	European beech roots								Norway spruce roots			
	C_{16} DA	ω-C_{16}	C_{18} DA	ω-$C_{18:1}$	9,10,ω-C_{18}	ω-C_{20}	ω-C_{22}	ω-C_{24}	C_{16} DA	C_{18} DA	ω-C_{20}	ω-C_{22}
C_{16} DA	1	0.99	0.99	0.82	0.73	0.97	0.96	0.96	1	0.99	0.86	0.87
ω-C_{16}		1	0.99	0.82	0.74	0.97	0.96	0.96		0.89	0.95	0.91
C_{18} DA			1	0.82	0.73	0.98	0.98	0.98		1	0.82	0.87
ω-$C_{18:1}$				1	0.94	0.76	0.75	0.74				
9,10,ω-C_{18}					1	0.65	0.64	0.64				
ω-C_{20}						1	0.99	0.99			1	0.97
ω-C_{22}							1	0.99				1
ω-C_{24}								1				

Degrees of freedom = 10, $p < 0.05$.

been minimal because we gently rewetted the forest floor material with a sprayer and the lipids in question are characterized by a low water solubility (Nierop and Verstraten, 2004). However, another reason for a slow decay of the beech leaves might be the association of carbohydrates with lignin which may hamper the decomposition of the more easily degradable carbohydrates (Melillo et al., 1982; Berg and McClaugherty, 2008). The relative content of carbohydrates of the spruce needles at the end of the incubation was significantly smaller than that of the beech leaves ($p < 0.001$) (Fig. 3) indicating that the carbohydrates of the beech leaves seemed to be more protected against decomposition. Another explanation might be the lower C/N ratio of the spruce needles providing more favorable conditions for microbial growth. Likewise, the 'home-field advantage' may be relevant, which describes that plant litter decomposes faster when placed in its natural environment – as was the case in the present study – due to an adapted and specialized microbial community (Wallenstein et al., 2013). This is supported by Hobbie et al. (2006) who showed that spruce needles decomposed faster than beech leaves when both materials were placed in their 'home' environment. Conclusively, a combination of the above discussed effects may be responsible for the faster decay of the spruce needles.

4.2. Decomposition of cutin and suberin derived monomers and bound fatty acids

The incubated plant materials released highly differing amounts of cutin and suberin monomers (Fig. 5) which generally corresponded to previously suggested cutin and suberin specific monomers reported in the literature (Otto and Simpson, 2006; Mueller et al., 2012). To the best of our knowledge, the behavior of suberin during decomposition has not been reported. Our data show that significant decreases of the cutin and suberin monomers occurred within 14 days of incubation (Fig. 5). Evidence for a relatively fast decay of cutin is supplied by previous incubation studies. For example, Riederer et al. (1993) incubated beech litter under laboratory conditions and monitored cutin decomposition over 446 days. After 83 days, roughly 42% of the investigated cutin monomers remained, which is in the same range as observed in the present study.

The decomposition of all extracted acids as well as of individual (data not shown) and summed cutin and suberin monomers seem to level off during the incubation with rapid decreases after 14 days and only slower decreases thereafter (Figs. 4 and 5). Similar results were found by Riederer et al. (1993) for cutin. The slowdown of the decrease in lipid concentrations is also reflected by the exponential relation between cutin/suberin monomers and the mass loss of the respective plant material (Fig. 6). This indicates that studies which assume a similar degradation of cutin/suberin monomers and source plant material may overestimate the amount of root and shoot derived organic matter and underesti-

mate its turnover at least at initial stages of decomposition. Still, representing bulk suberin quantitatively by summing up its specific monomers (e.g., Feng and Simpson, 2008) remains reasonable because concentrations of specific (and unspecific) monomers in this study showed a highly linear correlation during the incubation for both beech and spruce (Table 5). However, several unspecific cutin derived monomers were uncorrelated to the specific x,ω-C_{16} acids (Table 4) suggesting a different degradation of specific and unspecific cutin monomers.

To investigate possible controls of the inherent chemical properties of the cutin/suberin monomers and the plant material on the observed decomposition patterns we tested the effects of chain length (number of C atoms in each monomer), lipid type (n-carboxylic acid, α,ω-alkanedioic acid, ω-hydroxy alkanoic acid, or mid-chain substituted hydroxy alkanoic acid) and lignin content (derived from the NMR molecular mixing model). Chain length explained most of the variability in lipid concentrations (Table 3). Our results are in line with earlier studies that have shown that decomposition of lipids decreased as chain length increased (Moucawi et al., 1981; Amblés et al., 1989). This, in connection with the slower mass loss of the root compared to the leaf materials (cf. section 4.1), suggests that suberin derived monomers, containing also acids $\geqslant C_{20}$, should decompose more slowly than cutin derived monomers, containing acids $\leqslant C_{18}$. In fact, the percentage of suberin monomers left at the end of the incubation was lower than (for spruce) or not significantly different (for beech) from that of the cutin monomers (cf. Section 3.2 and Fig. 5c and d). Feng and Simpson (2008) also found a faster degradation of suberin compared to cutin derived aliphatic compounds in soil. They ascribed this observation to a higher degree of degradation and thus recalcitrance of cutin compared to suberin derived compounds at the time when the biopolymers are incorporated into soil organic matter. However, this explanation cannot be valid in the present study, as we incubated fresh leaf/needle and root material without the presence of mineral soil.

Since lipid type and relative lignin content also explained a portion of the variability in lipid concentrations (Table 3; cf., Section 3.2), the fast decay of suberin monomers might be partly explained by the smaller amount of mid-chain substituted hydroxy alkanoic acids (C_{16}, C_{18}) present in root material (Table 2). Cross-linking of this lipid type to other monomers may involve primary and secondary hydroxyl groups (Kolattukudy, 1980) while ω-hydroxy alkanoic acids of suberin may be involved in cross-linking only with one hydroxyl group. This may retard the degradation of mid-chain substituted hydroxy alkanoic relative to ω-hydroxy alkanoic acids due to their position in the polymeric network (Nierop, 1998; Nierop and Verstraten, 2004; Naafs et al., 2005; Mendez-Millan et al., 2010).

Furthermore, the occurrence of lignin in the cuticles of spruce and probably also of other plant species might have a protective function against biodegradation and lead to the selective preserva-

tion of cutin during decomposition (Kögel-Knabner et al., 1994). Schreiber et al. (1999) found that lignin and suberin always occur in combination with each other in endodermal and hypodermal cell walls of roots from monocotyledonous and dicotyledonous plant species. Similar results for the periderms of roots of Norway spruce and European beech were found by Leuschner et al. (2003). Accordingly, the observed decomposition pattern of the cutin and suberin monomers might be subdivided into two phases: (1) in early phases, that part of cutin or suberin that is not associated with lignin, e.g. due to a surplus of cutin or suberin compared to lignin (cf. Leuschner et al., 2003), is readily consumed by microorganisms resulting in a rapid decrease of the respective monomers (Fig. 5); (2) After the first phase, only cutin and suberin associated with lignin remains, resulting in a decomposition that proceeds with the initially low decay rate of lignin (Berg et al., 2000). In the second phase, cutin/suberin, lignin and associated compounds would therefore start to accumulate relative to other compounds (Berg and McClaugherty, 2008). In this respect, the amount of cutin and suberin associated with lignin could be more important than the mere quantity of lignin. Evidence for a constant relative lignin content during the incubation, which supports the above proposed decomposition phases, can be drawn from the molecular mixing model applied to the NMR data (Nelson and Baldock, 2005). When comparing initial relative lignin contents and relative lignin contents at the end of the incubation, no significant change could be observed (Fig. 3), indicating that lignin decomposes more slowly than other biomolecule compounds. The significant differences of relative lignin contents at 14, 28 and 42 days of incubation in beech leaves and roots can be ascribed to changes in other biomolecule components, probably reflecting the colonization of the plant material by microorganisms. The proposed two phases would also explain the patterns observed by Riederer et al. (1993) who found a retarded decrease of cutin monomers after a sharp decline in early stages of decomposition.

However, about two thirds of the variation in lipid concentrations was not accounted for by the three tested factors (chain length, lipid type, lignin content). Similar effects of chain length and lipid type on lipid concentrations in surface soils under different tree species were found by Mueller et al. (2013). They stated that stabilization of lipids in soil occurs by interaction with the soil mineral phase which might be responsible for the higher concentrations of root in contrast to shoot derived lipids observed in soils. These interactions should be strongly dependent on lipid functional groups (Kleber et al., 2007). Such organo-mineral interactions were absent in the present study, but an interaction of lipids with other organic molecules might also be a possible stabilization mechanism (Hajje and Jaffé, 2006).

Another factor that might have influenced the observed decomposition may be co-metabolism (Kuzyakov et al., 2000; Rinkes et al., 2014). As the incubation starts, high amounts of easily degradable OC (like sugars) are available to the microbes enabling them to decompose also more resistant compounds. When the source of labile OC is depleted the microbes are not able to degrade the more resistant materials anymore and the decomposition of cutin and suberin levels off. Unless associated with lignin, the high relative amounts of carbohydrates still present in each plant material at the end of the incubation, of which a considerable part should be easily degradable, contradict this hypothesis (Fig. 3). Other factors that might highly alter the decomposition of plant materials are significant differences in pH value (Moucawi et al., 1981; van Bergen et al., 1998) and, in connection with that, differences in microbial community (Hackl et al., 2005). However, because the species-specific forest floor materials used in this study had similar characteristics with respect to pH and C/N ratios (Table 1), and the patterns of the decline in the concentrations of cutin and suberin monomers were closely related in beech leaves/

roots and their respective spruce counterparts (Fig. 5c and d), these factors did not seem to be relevant in the present study.

5. Conclusions

The rapid decrease of cutin and suberin monomers in this study indicates that both biopolymers are readily decomposed in initial stages of decomposition without any trend of suberin being more resistant than cutin. Cutin/suberin monomers and the mass loss of the source plant material were exponentially correlated and the decrease of cutin and suberin monomers leveled off during the incubation. This suggests that the turnover of root and shoot derived soil organic matter may be underestimated when assuming a similar degradation of biomarker and source plant material. Furthermore, beside chain length (number of C atoms in each monomer) and lipid type (n-carboxylic acid, α,ω-alkanedioic acid, ω-hydroxy alkanoic acid, or mid-chain substituted hydroxy alkanoic acid), relative lignin content explained a considerable portion of the variation in lipid concentrations over time. Accordingly, we propose a two phase model for the initial decomposition of cutin and suberin: (1) in early phases, cutin or suberin that is not associated with lignin is readily consumed by microorganisms resulting in a rapid decrease of the respective polymer; (2) after the first phase, only cutin and suberin associated with lignin remains, resulting in a decomposition that proceeds with the initially low decay rate of lignin.

However, a substantial part of the variation in lipid concentrations was not accounted for by the tested inherent chemical properties of the extracted fatty acids and plant material. This suggests that the decomposition of cutin and suberin is additionally modulated by a not yet quantified external factor.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.orggeochem. 2016.02.006.

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Study III

Tracing the sources and spatial distribution of organic carbon in subsoils using a multi-biomarker approach

Gerrit Angst, Stephan John, Carsten W. Mueller, Ingrid Kögel-Knabner, Janet Rethemeyer

1 **Tracing the sources and spatial distribution of organic carbon in subsoils using a multi-**

2 **biomarker approach**

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12 Aboveground and belowground sources of soil organic carbon (SOC) have rarely been

13 differentiated despite the fact that they may determine the fate of plant-derived carbon in soil.

14 The aim of this study was to assess the contributions of organic carbon from aboveground and

15 belowground parts of beech trees to subsoil organic carbon in a Dystric Cambisol developed

16 from Pleistocene glacio-fluvial deposits. Samples were taken from a regular grid installed on

17 the profile walls of three replicate transects increasing in horizontal distance to individual beech

18 trees. Different sources of SOC were distinguished by solvent-extractable and hydrolysable

19 lipid biomarkers aided by ^{14}C analyses of mineral soil compartments <63 µm. The distance

20 from the trees had no effect on the investigated parameters. Instead, a vertical zonation of the

21 subsoil was detected. A high contribution of fresh leaf- and root-derived organic carbon to the

22 upper subsoil (leaf- and root-affected zone) indicate that leaf-derived SOC may still be of

23 considerable importance below the A horizon. In the deeper subsoil (root-affected zone), fresh

24 SOC was almost exclusively derived from the roots. Strongly increasing apparent ^{14}C ages

25 (3860 yrs BP) indicate considerable contribution of SOC that may be inherited from the

26 Pleistocene parent material.

Introduction

27

28 In recent years, the importance of subsoils for soil organic carbon (SOC) storage and the

29 terrestrial carbon cycles has increasingly been recognized[1,2]. Surprisingly, most of the studies

30 on SOC dynamics have been conducted in very shallow subsoils with a median sampling depth

31 of 20 cm[1], although a significant amount of SOC may be stored well below the first meter of

32 the soil profile[3]. The SOC stored at greater soil depths has been found to generally feature low

33 ^{14}C contents (corresponding to high mean apparent ^{14}C ages) that decrease with increasing

34 depth[2,4,5]. This finding suggests that the SOC stored there is either partly inherited from the

35 parent material or stabilized over longer periods of time[2,6]. Because subsoils are commonly

36 unsaturated in C and microbial activity has been found to be low[7,8], some authors regarded

37 subsoils as having the potential to sequester carbon[6,9]. However, the processes and factors that

38 are important to subsoil organic carbon (OC) stabilization still remain poorly investigated[2].

39 The main source of SOC is plant-derived organic matter. Aboveground sources of organic

40 matter are leaf/needle litter and partly tree bark[10]. With ongoing time, the aboveground plant

41 inputs become incorporated into the organic layer and mineral soil via the soil fauna[11].

42 Belowground inputs are root-derived litter or root exudates[12] that are directly supplied to the

43 soil *in situ*. Recent studies suggest that SOC from aboveground and belowground sources may

44 highly differ in its degradability[13]. In a litter manipulation experiment, root-derived compounds

45 have been found to be a source of SOC with greater relative stability, whereas aboveground

46 leaf litter was found to be the source of the most actively cycling C[14]. This points to the

47 importance of unravelling the origin and spatial distribution of SOC as it determines the fate of

48 plant-derived C, either as mineralized CO_2 or as stabilized SOC[15]. However, the sources and

49   distribution of SOC in subsoils have rarely been addressed and are still subject to considerable

50   uncertainty.

51   In forest soils, the input of SOC from aboveground and belowground vegetation parts may be

52   strongly dependent on the distance to the trees[16]. This spatial dimension has mostly been

53   overlooked, and there have only been a few studies that involved the factor 'distance' in their

54   sampling design. The studies performed so far yield no uniform results. For example, in one

55   study, a significant small-scale variability of SOC stocks was found but with no clear relation

56   to the distance from individual beech trees[17]. In another study, an influence of the distance to

57   individual beech trees on the chemical composition of soil organic matter fractions and SOC

58   contents was absent[18]. However, these studies did not differentiate aboveground and

59   belowground sources of SOC. To the best of our knowledge, there has been only one study to

60   date that distinguished plant sources of SOC in a spatially coordinated sampling design[19]. The

61   authors found strong horizontal and vertical gradients in SOC from different plant sources

62   mainly controlled by the rooting zone of individual trees.

63   An approach to distinguish aboveground and belowground sources involves the analysis of

64   hydrolysable lipid biomarkers that are distinct to either root or shoot plant materials. The

65   biopolymers cutin (leaf-derived) and suberin (root-/bark-derived) fulfil this requirement and

66   have increasingly been used to study the fate of shoot- and root-derived SOC, respectively[19–21].

67   Besides cutin and suberin, solvent-extractable lipids have been used to investigate the

68   contribution of root-, leaf-, and microbial-derived compounds to soil organic matter[22–25].

69   Although solvent-extractable lipids are not necessarily distinct to aboveground and

70   belowground sources of SOC, they can be ascribed to either plant material when concentrations

71   of n-alkanes or carboxylic acids highly differ in roots and leaves[26]. The combined use of

72   extractable and hydrolysable lipids has been shown to provide complementary information on

73   vegetation history and soil processes, such as leaching and bioturbation[27].

74 The aims of this study were to reveal the contributions to subsoil OC from aboveground and

75 belowground sources at different soil depths (down to 110 cm) and distances to individual beech

76 trees using solvent-extractable and hydrolysable lipid biomarkers in connection with [14]C

77 measurements. The dominance of root-derived OC as a source for subsoil OC was assumed by

78 previous studies for the same site, based on gradients in SOC and ^{14}C contents and the chemical

79 composition of soil organic matter fractions[5,18]. With the present data, we were able to verify

80 or falsify these previous assumptions.

81 **Results**

82 **Bulk parameters**

83 Because no horizontal differences in the investigated parameters could be detected, all data are

84 displayed as statistical means ± standard error of the mean (s.e.m.) summarised for all transects

85 and horizontal sampling spots at the respective depth (cf. Statistics and calculations section).

86 Concentrations of both root biomass and necromass showed significant vertical decreases (p <

87 0.01) between the densely rooted upper subsoil (0 and 35 cm depths, B horizons) and the deeper

88 subsoil (85 and 110 cm depths, C horizons) (Table 1;[18]).

89 The SOC contents displayed a similar pattern with high contents in the upper subsoil (10 cm:

90 11.6 g ± 1.1 OC kg^{-1} soil and 35 cm: 5.2 ± 0.5 g OC kg^{-1} soil) and significantly lower contents

91 at depths of 85 and 110 cm (<0.5 ± 0.1 g OC kg^{-1} soil; p < 0.01) (Table 1;[18,28]).

92 The radiocarbon contents (Table 1) decreased slightly in the upper subsoil at the depths of 10

93 and 35 cm from 0.988 ± 0.009 fMC (95 ± 75 yrs BP) to 0.905 ± 0.009 fMC (810 ± 80 yrs BP).

94 Below the 35 cm depth, strong decreases were determined, with values of 0.723 ± 0.026 fMC

95 (2650 ± 300 yrs BP) at the 60 cm depth, 0.624 ± 0.028 fMC (3860 + 400 vrs BP) at the 85 cm

96 depth and 0.652 ± 0.064 fMC (3750 ± 810 yrs BP) at the 110 cm depth.

97 **Lipid biomarkers**

Solvent-extractable lipid biomarkers

99 The concentration of solvent-extractable lipids highly differed between roots and leaves. The

100 most prominent differences were observed in the concentrations of the odd-numbered n-alkanes

101 C_{25}-C_{33} (equation (1), $332.4 \pm 6.6\ \mu g\ g^{-1}$ OC in leaves and $15.2 \pm 5.6\ \mu g\ g^{-1}$ OC in roots) and n-

102 fatty acids $>C_{20}$ (equation (2), $3596.1 \pm 231.7\ \mu g\ g^{-1}$ OC in leaves and $122.7 \pm 51.1\ \mu g\ g^{-1}$ OC

103 in roots), which were several orders of magnitude higher in leaves compared with those in roots

104 (Figure 1 and supplementary Table S1 online). These differences enabled us to develop the

105 P_{RML} ratio as a proxy for root-/microbial-derived SOC in contrast to mainly leaf-derived SOC

106 (cf. Methods section).

107 In the strongly rooted upper subsoil (10 and 35 cm depths), the contents of plant-derived n-

108 alkanes (C_{25}-C_{33}) decreased significantly (p = 0.02) between the 10 and 35 cm depths and then

109 remained constant with a slightly increasing trend below the 35 cm depth (Figure 2). The

110 dominant n-alkane in the upper subsoil was C_{27}. The distribution patterns changed with depth

111 towards longer chain lengths dominating at C_{29} and C_{31} below the 35 cm depth in the deeper

112 subsoil (Figure 3). In contrast, the plant-derived fatty acids $>C_{20}$ decreased from the 10 to 110

113 cm depths (Figure 2). The n-fatty acid distribution patterns were strongly dominated by C_{22} and

114 C_{24} (Figure 3). Below the 35 cm depth, the distribution patterns changed to being dominated by

115 C_{16} and C_{18}. The fatty acids mainly derived from microorganisms ($C_{16:1}$; $C_{18:1}$, equation (3))

116 decreased strongly from $57.3 \pm 20.1\ \mu g\ g^{-1}$ C at the 35 cm depth to $8.2 \pm 2.4\ \mu g\ g^{-1}$ C at the 110

117 cm depth.

118 The P_{RML} proxy (equation (4), Figure 4) established in this study showed generally narrow ratios

119 (leaf dominated) in the upper subsoil at the 10 and 35 cm depths in the range from 0.20 ± 0.01

120 to 0.43 ± 0.08 and wider ratios (root and/or microorganism dominated) at the depths from 60 to

121 110 cm (1.38 ± 0.35 to 1.97 ± 0.13).

122   The CPI_{Alk} for *n*-alkanes (equation (5)), as a proxy for the degree of degradation of these lipids,

123   decreased from 5.6 ± 0.4 (10 cm depth) to 2.8 ± 0.2 (60 cm depth) and remained constant below

124   the 60 cm depth (Figure 4). The low CPI_{Alk} values at the 60 to 110 cm depths were very similar

125   to those found in *n*-alkanes from beech roots (2.2 ± 0.5; Figure 4). A different trend could be

126   observed in the values of CPI_{FA} (equation (6)) for *n*-fatty acids. This index showed a decreasing

127   trend from the 10 cm depth (6.6 ± 0.2) to the 35 cm depth (5.2 ± 0.4), but strongly increased

128   below the 60 cm depth to a maximum value of 11.1 ± 0.2 (85 cm depth) in the deeper subsoil.

129   **Hydrolysable lipid biomarkers**

130   All hydrolysable lipid biomarkers (Supplementary Table S2 online) showed significant

131   differences ($p < 0.01$) between the densely rooted upper subsoil (10 and 35 cm depths) and the

132   less rooted deeper subsoil (85 and 110 cm depths) (Figure 2). The suberin (root markers) and

133   $\sum$CvS markers (plant markers; equations (8) and (9), see Methods section) decreased from

134   2127.9 ± 546.9 and 2127.3 ± 535.9 µg g^{-1} OC at the 10 cm depth to 288.1 ± 195.4 and $78.6 \pm$

135   64.0 µg g^{-1} OC at the 85 cm depth. Notably, the distribution of the suberin markers widely

136   resembled that of the $\sum$CvS markers. The concentrations of the cutin markers (leaf markers;

137   414.4 ± 188.6 µg g^{-1} OC at the 10 cm depth, equation (7)) were substantially lower than that of

138   the suberin and $\sum$CvS markers, and no cutin markers could be detected in depths greater than

139   35 cm (Figure 2).

140   **Principle component analysis**

141   The principle component analysis (PCA) was performed to evaluate the correlation of the lipid

142   biomarkers and the soil parameters in the strongly rooted upper subsoil (PCA_{10-35} for 10–35 cm

143   depths; Figure 5a) as opposed to the less rooted deeper subsoil (PCA_{60-110} for 60–110 cm depths;

144   Figure 5b). The first two principle components (PCs) together explained 63% of the variation

145   of the data (PC 1 = 39.7% and PC 2 = 23.3%; Figure 5a). Principal component 1 was mostly

146   influenced by the SOC and ^{14}C contents. Principal component 2 separated the solvent-

147   extractable (negative contribution) from the hydrolysable (positive contribution) lipid

148   biomarkers. The SOC and ^{14}C contents were strongly positively correlated with root biomass,

149   fatty acids >C_{20} and the *n*-alkanes (C_{25}-C_{33}) and to a lesser extent with root necromass and

150   hydrolysable lipid biomarkers identified in this study. Notably, the root necromass and

151   hydrolysable lipid biomarkers plotted together as well as the plant-derived fatty acids >C_{20} and

152   *n*-alkanes (C_{25}-C_{33}) (Figure 5a). A negative correlation could be detected between soil depth

153   and all other variables, with the exception of the unsaturated fatty acids ($C_{16:1}$; $C_{18:1}$), which

154   were widely uncorrelated to the investigated biomarkers and soil parameters.

155   The first two PCs (PC 1 and PC 2) of PCA$_{60-110}$ explained 62.7% of the variability of the dataset

156   (PC 1 = 45.9%, PC 2 = 16.8%; Figure 5b). Principal component 1 was mainly influenced by

157   the root necromass and biomass and soil depth. Principal component 2 was mostly influenced

158   by the solvent-extractable lipids (*n*-fatty acids and *n*-alkanes) and ^{14}C contents. The $\sum$CvS and

159   suberin markers were positively correlated with the root biomass and were most closely related

160   to the SOC contents, as was the case for the root necromass and ^{14}C contents. The analysed

161   solvent-extractable lipids were less correlated with the root necromass and biomass and

162   negatively correlated with the SOC and ^{14}C contents. Similar to PCA$_{10-35}$, the soil depth was

163   negatively correlated with most of the investigated parameters.

164   **Discussion**

165   The concentrations and homologue distribution patterns of the solvent-extractable lipids of the

166   beech leaves, which were dominated by *n*-fatty acids >C_{20} and the C_{27} *n*-alkane, were similar

167   to the results of previous studies that investigated the lipid composition of European beech

168   leaves[29,30]. To the best of our knowledge, the solvent-extractable lipid composition of European

169   beech roots has not been reported so far. The composition was dominated by C_{16} and C_{18}

170   homologues for fatty acids and C_{27} for *n*-alkanes (Figure 1). The most striking characteristic of

171   the solvent-extractable lipids in beech leaves and roots were their highly differing

172   concentrations in *n*-alkanes (C_{25}-C_{33}) and *n*-fatty acids ($>C_{20}$) (Figure 1 and supplementary

173   Table S1 online). These differences enabled us to (1) infer a leaf source of SOC where

174   concentrations of the *n*-alkanes (C_{25}-C_{33}) and *n*-fatty acids ($>C_{20}$) in the soil were considerably

175   high and (2) develop a proxy (P_{RML}) for the differentiation of leaf and root/microbial sources

176   of SOC.

177   The concentrations of suberin monomers released from the beech roots and the upper soil layers

178   were in the range of concentrations detected in a study that also investigated soil and plant

179   tissues in a European beech stand[19]. The concentrations of cutin monomers released from the

180   beech leaves in the present study were approximately four times lower than those observed in

181   the aforementioned study. This result may be due to the extraction of leaf litter in the present

182   study in contrast to the extraction of fresh leaves. However, the comparison of such data from

183   different studies is complicated because the lipid composition may change with the life span or

184   morphology of leaves and roots[31].

185   The statistical analysis of the data revealed no influence of the distance from the trees on the

186   solvent-extractable and hydrolysable lipids as well as on the ^{14}C contents of the SOC (cf. section

187   2.6). Instead, a pronounced vertical gradient could be detected with the largest decrease of the

188   investigated parameters between the densely rooted upper subsoil (10 and 35 cm, corresponding

189   to B horizons) and the less densely rooted deeper subsoil (60–110 cm, corresponding to C

190   horizons). These results reflect the findings of previous studies that investigated the chemical

191   composition and distribution of soil organic matter fractions in the same transects and ^{14}C

192   contents in one of the transects[5,18]. The authors did not find any horizontal trend but a similar

193   vertical gradient down to the 110 cm depth as was observed in the present study. The authors

194   hypothesised that OC inputs by roots likely played a dominant role for the observed patterns

195   because of a dense and even rooting of the upper subsoil (10 and 35 cm depths) and considerably

196 higher SOC contents of rhizosphere than that of bulk soil. This hypothesis could be confirmed

197 and expanded by the source identification of SOC in the present study.

198 The subsoil in the present study could be differentiated into two vertical zones, a 'leaf- and

199 root-affected zone' and a 'root-affected zone'.

200 The SOC in the leaf- and root-affected zone, corresponding to the upper subsoil (B horizons at

201 10 and 35 cm depths), was composed of a mixture of fresh leaf- and root-derived compounds,

202 evidenced by a positive correlation of SOC contents with the solvent-extractable and

203 hydrolysable lipids and root biomass and necromass (Figure 5a). The relatively high ^{14}C

204 contents at the 10 cm depth (0.988 ± 0.009 fMC; 95 ± 75 yrs BP) support the presence of SOC

205 from fresh sources (Table 1). The declining ^{14}C contents at the 35 cm depth (0.905 ± 0.009

206 fMC; 810 ± 80 yrs BP) indicate an increasing contribution of SOC derived from a relatively old

207 source and/or decreasing concentrations of fresh plant-derived SOC. In addition to root-derived

208 SOC, the strong correlation of the long-chain n-alkanes (C_{25}-C_{33}) and n-fatty acids ($>C_{20}$) with

209 SOC contents indicate the importance of leaf-derived SOC in the upper subsoil. This finding is

210 supported by the low values of P_{RML} (Figure 4). The low correlations of cutin markers with SOC

211 contents may be explained by an already advanced stage of decomposition of the leaf litter,

212 which is also reflected in the low concentrations of cutin monomers released from the extracted

213 leaves. Comparably low concentrations of the predominantly microbial-derived fatty acids

214 ($C_{16:1}$, $C_{18:1}$) that were uncorrelated with the SOC contents suggest a low contribution of

215 microbial-derived compared with plant-derived SOC (Figure 5a). Furthermore, the microbial-

216 derived fatty acids were uncorrelated to the soil depth, indicating a ubiquitous occurrence of

217 microbes in the upper subsoil.

218 The SOC in the root-affected zone, corresponding to the deeper subsoil (60–110 cm depths, C

219 horizons), was composed of relatively high amounts of old SOC (minimum values of $0.624 \pm$

220 0.028 fMC; 3860 ± 400 yrs BP) and smaller proportions of younger, mainly root-derived SOC.

9

221 The dominance of root- in contrast to leaf-derived SOC was clearly implied by the presence of

222 suberin along with the absence of cutin markers. Furthermore, dead fine roots were found to be

223 no older than 20 yrs[32–34]. Thus, the positive correlation of the fine root necromass with SOC

224 and ^{14}C contents (Figure 5b) indicates that the root necromass was a major source of fresh SOC

225 at greater soil depths (60–110 cm depths). Strongly increasing CPI_{FA} values from depths of 35

226 to 60 cm and below (Figure 4) also indicate the presence of fresh SOC in the C horizons. The

227 high values of P_{RML} (Figure 4) indicate the dominance of root-/microbial-derived C_{16} and C_{18}

228 fatty acids compared with mostly leaf-derived $>C_{20}$ fatty acids. The slightly hydrophilic short-

229 chain fatty acids (C_{16} and C_{18}) are either translocated from the upper soil layers or are produced

230 *in situ* by microorganisms or roots[35]. In this regard, microbial-derived SOC appeared to be of

231 minor importance because the concentrations of the microbial-derived fatty acids ($C_{16:1}$, $C_{18:1}$)

232 were low and strongly correlated with the plant-derived fatty acids $>C_{20}$, indicating that the

233 former were rather derived from plant material from which trace amounts of these acids were

234 released (Figure 1). This finding questions the assumption of subsoil OC being enriched in

235 microbial-derived SOC[36]. Surprisingly, the root biomass was almost uncorrelated to the SOC

236 contents, indicating that root exudates appeared to be of minor importance, probably because

237 of their higher lability in soils[37,38]. The weaker correlation of the root necromass to the suberin

238 markers in the depth range of 60 to 110 cm compared with the upper subsoil (10 and 35 cm

239 depths) may be explained by a higher stage of degradation of the root necromass, which was

240 most likely more depleted in suberin monomers. The high correlation of suberin and $\sum CvS$

241 markers in the depths of 60 to 110 cm indicates that the latter were most probably also root-

242 derived. Thus, our results support the notion of Rasse et al. (2005) that fresh SOC inputs to the

243 deeper subsoil are mainly root-derived.

244 However, a considerable amount of the SOC located at the depths of 60 to 110 cm was very old

245 and probably inherited from the parent material. Long-chain *n*-alkanes may contribute to the

246 older SOC pool at greater depth and were found to be relatively stable against

247 decomposition[39,40]. These long-chain *n*-alkanes may thus be an important indicator for past

248 vegetation[41–43]. The constant or slightly increasing concentrations of *n*-alkanes with increasing

249 soil depth (Figure 2), which were also observed by others[25,40], and the strong negative

250 correlation of *n*-alkanes with the ^{14}C contents support their contribution to old SOC. Similar

251 results were reported by others, who found an accumulation of aliphatics with soil depth that

252 were likely not derived from the current vegetation[44]. This inference is further corroborated by

253 very low values of CPI$_{Alk}$ (Figure 4), indicating a high degree of degradation and, in turn, a

254 relatively high residence time of the *n*-alkanes in the investigated subsoil. Notably, the CPI$_{Alk}$

255 in the depths of 60 to 110 cm was highly similar to the CPI$_{Alk}$ observed for the beech roots

256 (supplementary Table S1 online), suggesting that the CPI$_{Alk}$ in soil may also reflect a more

257 recent input of root-derived SOC[45]. Generally, the CPI$_{Alk}$ values calculated from long-chain *n*-

258 alkanes must be interpreted with caution because they may vary strongly in different plant

259 species from 0.039 to 99[46]. Another indication for SOC that is not derived from the present

260 vegetation is provided by a change in the distribution patterns of *n*-alkanes in the depths of 60

261 to 110 cm from a dominance of beech-derived C$_{27}$ *n*-alkanes to a dominance of C$_{29}$ and C$_{31}$ *n*-

262 alkanes (Figure 3). However, compound-specific radiocarbon analyses of *n*-alkanes are

263 required to undoubtedly prove the assumption that these lipids were considerably old.

264 Synthetically, all the data for the deeper subsoil indicate that most of the SOC located at these

265 depths likely originated from an old source and may potentially be inherited from the parent

266 material. Similarly, other authors stated that the very old apparent ^{14}C ages of some soils may

267 reflect the dilution of inherent geogenic carbon with younger SOC[2].

268 Our results have some important implications for C allocation in subsoils. Considerable

269 amounts of leaf-derived SOC (presence of cutin markers and low P$_{RML}$ values) were still found

270 in the B horizons of the soil profiles (down to 35 cm depth). This finding indicates that

271 bioturbation has occurred, even at the 35 cm depth in the subsoil, because cutin is characterised

272   by a low water solubility[47] and thus a translocation by water is unlikely. This finding is

273   surprising because the soil conditions were rather unfavourable for soil fauna (e.g. low pH 3.4–

274   4.5; cf. section 2.1) in the investigated Cambisol. The absence of cutin markers in the deeper

275   subsoil (60–110 cm depth) indicates that bioturbation no longer occurs at that depth, probably

276   due to a very low food quality[48]. Although not directly monitored, our data enabled us to obtain

277   information on bioturbation processes that may be important for the translocation of

278   considerable amounts of leaf-derived SOC into subsoils. In this regard, some authors proposed

279   sequestering SOC in subsoils by planting deep rooting plant species that would allocate root-

280   derived SOC to deep soil layers[3,6]. European beech may develop a deep rooting system[16], and

281   the amount of root biomass and necromass may still be considerably high at soil depths greater

282   than 0.6 m[49,50]. Our results do not confirm these hypotheses, but indicate that the recent tree

283   vegetation influences the SOC mainly in the uppermost subsoil horizons (down to the 35 cm

284   depth). The deeper subsoil receives considerably smaller inputs of fresh, root-derived organic

285   carbon that is likely of little importance in the present study because of very high mean apparent

286   ^{14}C ages below the 35 cm depth. Our results indicate that the allocation of SOC into deep soil

287   layers cannot be accomplished by simply establishing typical deep rooting plant species, but

288   that site-specific factors may essentially control the spatial growth of the rooting system.

289   In summary, we identified lipid biomarkers specific to European beech that enabled us to trace

290   SOC from leaf, root and microbial sources at different soil depths and distances from individual

291   trees. The distribution of lipid biomarkers was not influenced by the distance from individual

292   trees but by vertically stratified inputs of leaf- and root-derived SOC. Accordingly, we

293   distinguished two vertical zones. (1) The root- and leaf-affected zone (10 and 35 cm depth; Bsv

294   and Bv horizons) was composed of fresh root- and shoot-derived SOC, indicating that

295   contributions of leaf-derived SOC may be still important well below the A horizons of a soil.

296   (2) The root-affected zone (60 to 110 cm depth; ICv and IICv horizons) was composed of fresh

297 root-derived SOC, with an important contribution of relatively old SOC with high mean

298 apparent ^{14}C ages (up to 3860 yrs. BP). This old SOC was potentially inherited from the parent

299 material or stabilized over thousands of years and has to be considered as an important

300 contributor to the SOC pool in deep subsoils. Future studies should focus on input pathways of

301 SOC from different sources to help elucidate the evolution of SOC distribution patterns, such

302 as those observed in the present study.

303 **Methods**

304 **Study area and soil sampling**

305 The study was performed at the Grinderwald, a managed, even-aged European beech forest

306 (*Fagus sylvatica* L.) established in 1916, located northwest of Hannover (52° 34' 22" N, 9°18'

307 51" E), Germany. The predominant soil type was an acidic (pH 3.4–4.5), sandy (77.3% sand,

308 18.4% silt and 4.4% clay) Dystric Cambisol[51] developed from sandy glacio-fluvial deposits

309 (Saale glacial), the humus form was moder. The phyllosilicate mineralogy was characterised

310 by the presence of chlorite, mixed-layer minerals, kaolinite and illite, whereas smectites and

311 carbonates were absent. A more detailed description of the study area is given elsewhere[18].

312 Three 3.15 m long and 2.00 m deep transects were dug, each starting at the stem base of a

313 mature beech tree. The direction of each transect was chosen such that the stem base of

314 neighbouring trees was not reached to track the influence of a single tree on the spatial

315 distribution of selected soil properties. Composite soil samples (each ~1 kg) were taken next to

316 the tree (0 cm), at an intermediate distance from the tree (135 cm), and far from the tree (270

317 cm) down to a depth of 110 cm (starting at 10 cm depth with 25 cm depth increments, n = 45).

318 The first vertical sampling spot was set to 10 cm depth (Bsw horizon) to ensure a regular

319 sampling along the grid that is unbiased by varying topsoil thicknesses. This study thus

320 exclusively investigated subsoil samples. In addition, leaf litter (n = 3) and roots (n = 3) of

321 European beech were randomly collected from each transect. The soil samples were air-dried

322 and sieved to <2 mm, and the litter and root samples were freeze dried and finely ground. All

323 samples were subjected to a sequential extraction procedure to release solvent-extractable and

324 hydrolysable lipids. Data regarding root biomass, root necromass and SOC contents were partly

325 derived from previous studies at the same site[18,28]. Twelve data points regarding root biomass

326 and necromass were supplied by Kristina Kirfel (Albrecht von Haller Institute for Plant

327 Sciences, Georg-August-Universität Göttingen, Germany).

SOC analysis

329 Carbon measurements of all soil samples were performed using an elemental analyzer

330 (EuroVector, Milan, Italy) via dry combustion. An aliquot of 1–2 mg of each sample was

331 ground and used for analysis. All measurements were performed in duplicate. Because

332 carbonates were absent from the study area[18], all carbon contents were equal to the organic

333 carbon contents.

Radiocarbon analysis

335 Because the SOC content of the sand fraction was very low (≤ 0.3 g kg^{-1};[18]), this fraction was

336 removed by dry sieving (mesh size of 63 µm). All samples were processed using a modified

337 protocol published earlier[52]. Briefly, potentially present inorganic carbon was removed by

338 extraction with 0.5% HCl. The suspension was placed in a drying oven for one hour at 60 °C

339 and then left overnight at room temperature. The hydrochloric acid was removed by washing

340 with Milli-Q water to pH 5. The samples were dried at 60 °C and subsequently graphitized with

341 H_2 over an iron catalyst. The radiocarbon contents of the samples were then measured on a 6

342 MV Tandetron AMS (HVE, Netherlands) at the University of Cologne. The results of the ^{14}C

343 measurements were reported as fraction modern carbon (fMC) and as apparent conventional

344 ^{14}C ages in years before present (yrs BP), related to 1950.

Sequential liquid extraction procedure

Analysis of the solvent-extractable lipids

Lipids were extracted from ~20–50 g of bulk soil and 1.0–1.5 g of beech leaf and root material using accelerated solvent extraction (Dionex ASE 350, USA) with dichloromethane:methanol (9:1, 100 bar, 120 °C, 20 min). The extracts were saponified with methanolic KOH (0.5 M) and then separated into a neutral and an acid fraction by liquid–liquid phase separation (water:dichloromethane). The n-alkanes were separated from the dichlormethane phase by eluting with hexane using column chromatography (activated SiO_2; mesh size 60 µm). After acidification with concentrated HCl, the acid fraction was derivatised using methanolic HCl (95:5). Fatty acid methyl esters (FAMEs) were separated and purified over a SiO_2–Na_2SO_4 column with dichloromethane:hexane (2:1).

The n-alkanes and FAMEs were measured using a gas chromatograph equipped with a flame ionisation detector (GC-FID, 5890 series II plus, Hewlett Packard, USA equipped with DB-5MS column 50 m and 5 m pre-column, 0.2 mm ID, 0.33 µm df). Lipid identification and quantification was performed using external standard mixtures.

Analysis of the hydrolysable lipids

After pre-extraction of the solvent-extractable lipids, the soil/plant residues were subjected to alkaline hydrolysis to release hydrolysable lipids. The samples, 10 g of soil and 0.5 g of plant material, were saponified with methanolic KOH in teflon lined bombs at 100°C for 3 hours. The extracts were processed, qualified and quantified using GC/MS, following the procedure described elsewhere[53]. The amounts of aliphatic acids were normalised to the OC content of the respective sample (stated as µg g^{-1} OC).

Identification of lipid biomarkers for distinguishing aboveground, belowground and microbial sources of SOC

Solvent-extractable lipid biomarkers

370   The vegetation markers in this study were selected according to their occurrence in the analysed

371   beech leaves and roots (Figure 1, supplementary Table S1 online) and previously published

372   biomarkers[21].

373   Waxes derived from higher plants are commonly identified by large abundances of long-chain,

374   odd-numbered n-alkane homologues C_{21} to C_{33} and long-chain n-fatty acids $>C_{20}$[54,55]. These

375   compounds were also found to be the most abundant compounds in the beech leaves and roots

376   of the present study (Figure 1, supplementary Table S1 online). Notably, the concentrations of

377   the odd-numbered n-alkanes C_{25}-C_{33} and n-fatty acids C_{20}-C_{32} were several orders of magnitude

378   higher in leaves compared with those in roots (Figure 1). Similar results were obtained by others

379   for n-alkanes in the roots and leaves of different plant species[26]. We assume that considerably

380   high concentrations of the mentioned lipids (n-alkanes C_{25}-C_{33} and n-fatty acids C_{20}-C_{32}) in soil

381   are indicative of SOC being mainly derived from leaves:

382         $$n\text{-Alkanes } (C_{25}\text{-}C_{33}) = \Sigma\,(C_{25}\text{-}C_{33})_{odd} \tag{1}$$

383   and

384         $$n\text{-Fatty acids } >C_{20} = \Sigma_{FA}(C_{20}\text{-}C_{32}) \tag{2}$$

385   The n-fatty acids extracted from beech leaves and roots (Figure 1) showed not only large

386   differences in the concentrations but also in the distribution patterns of the homologues. The

387   beech leaves were dominated by n-fatty acids $>C_{20}$ (equation 2), whereas short-chain n-fatty

388   acids C_{14}-C_{18} were the most abundant compounds in beech roots dominated by C_{16} (Figure 1).

389   We thus used the ratio of short-chain n-fatty acids (C_{14}-C_{18}, derived from roots and/or

390   microorganisms) to long-chain n-fatty acids ($>C_{20}$, dominant in leaves in the present study)

391   expressed by the proxy termed P_{RML} (root-/microbial- vs. leaf-derived SOC) to differentiate

392   SOC derived from roots and/or microorganisms in relation to SOC derived from leaves:

393         $$P_{RML} = \Sigma_{FA}(C_{14}\text{-}C_{18}) \,/\, \Sigma_{FA}(>C_{20}) \tag{3}$$

16

394   Only trace amounts of mono-unsaturated fatty acids $C_{16:1}$ and $C_{18:1}$ could be detected in the

395   leaves and roots of the present study. Thus, these unsaturated compounds were used as

396   indicators of microbial-derived SOC according to the findings of a previous study[22]:

397   $$n\text{-Fatty acids } (C_{16:1}; C_{18:1}) = \Sigma_{FA}(C_{16:1}; C_{18:1}) \tag{4}$$

398   The degradation of plant material leads to decreasing abundances of odd-numbered n-alkanes

399   and decreasing even-numbered n-fatty acids. This can be identified by the carbon preference

400   index (CPI)[56], which reflects the odd-over-even and the even-over-odd predominance of n-

401   alkanes and fatty acids, as given below, respectively.

402   $$CPI_{Alk} = 0.5* [(\Sigma z\text{-}C_{21\text{-}31} \text{ odd} / \Sigma z\text{-}C_{20}\text{-}C_{30} \text{ even}) / (\Sigma z\text{-}C_{21\text{-}31} \text{ odd} / \Sigma z\text{-}C_{22}\text{-}C_{32} \text{ even})] \tag{5}$$

403   $$CPI_{FA} = 0.5* [(\Sigma z\text{-}C_{12\text{-}30} \text{ even} / \Sigma z\text{-}C_{11}\text{-}C_{29} \text{ odd}) / (\Sigma z\text{-}C_{12\text{-}30} \text{ even} / \Sigma z\text{-}C_{13}\text{-}C_{31} \text{ odd})] \tag{6}$$

404   The equations were slightly modified with z being the number of carbon atoms[57]. High CPI

405   values (>10) reflect the input of mainly fresh SOC, and low CPI values (<<10) indicate the

406   degradation of SOC[56].

Hydrolysable lipid biomarkers

408   Leaves and roots were characterised by different abundances and chain lengths of n-carboxylic,

409   ω-hydroxy alkanoic, α,ω-alkanedioic and mid-chain-substituted hydroxy alkanoic acids mainly

410   derived from cutin and suberin (supplementary Table S2 online). Because specific cutin- and

411   suberin-derived monomers were found to decompose at similar rates[53,58], we used the sum of

412   the respective monomers in soil to evaluate the contribution of aboveground vs. belowground

413   SOC.

414   The 8,9,10,ω-dihydroxy hexadecanoic acids (subsumed under x,ω-C_{16}) were used as markers

415   for leaf-derived SOC as they were not released from roots and correspond to previously

416   suggested cutin biomarkers[19,31,53].

417          $$\text{Cutin markers} = \sum(x,\omega\text{-}C_{16}) \tag{7}$$

418   The ω-hydroxy alkanoic acids with a chain length of C_{20}, C_{22} and C_{24} (ω-C_{20}, ω-C_{22} and ω-C_{24})

419   were used as markers for root-derived SOC as they were not released from leaves and

420   correspond to previously suggested suberin biomarkers[19,53]. The α,ω-octadecanedioic acid (C_{18}

421   DA), usually present in both cutin and suberin[21], was not detected in leaves and thus was added

422   to the specific root markers in this study:

423          $$\text{Suberin markers} = \sum(C_{18}\text{ DA};\omega\text{-}C_{20}; \omega\text{-}C_{22}; \omega\text{-}C_{24}) \tag{8}$$

424   The sum of the unspecific (part of cutin and suberin) monomers, i.e. ω-hydroxy hexadecanoic

425   acid (ω-C_{16}), α,ω-hexadecanedioic acid (C_{16} DA) and 9,10,ω-hydroxy octadecanoic acid

426   (9,10,ω-C_{18}), was used as a marker for plant-derived SOC (referred to as $\sum$CvS):

427          $$\sum\text{CvS} = \sum(\omega\text{-}C_{16}; C_{16}\text{ DA}; 9,10,\omega\text{-}C_{18}) \tag{9}$$

428   **Statistics and calculations**

429   Statistical means and s.e.m were calculated using Microsoft Excel 2013 (Microsoft, Redmond,

430   WA, USA). All other statistics (significant if $p < 0.05$) were computed using the R 3.0.3

431   software for Windows[59]. The data were analysed to identify significant differences among the

432   three different transects, including the horizontal (0, 135 and 270 cm distances) and vertical

433   (10–110 cm depths). First, the data were tested for normality and homoscedasticity using the

434   Shapiro–Wilk and Bartlett test, respectively. Depending on the outcomes of the tests, significant

435   differences were then evaluated using the one-way analysis of variance (ANOVA) or the

436   Kruskal–Wallis test. The Tukey honestly significant difference (HSD) and Dunn's test were

437   applied as post-hoc tests. In a previous study, significant differences between the transects

438   regarding the SOC contents, root biomass and necromass were not detected[18]. The same was

439   found for the ^{14}C contents and the solvent-extractable and hydrolysable lipids in this study.

440   Thus, we regarded the transects as being replicates. Subsequent analyses among the sampling

441 spots revealed that there were also no significant differences between the horizontal sampling

442 intervals at the respective depths. We therefore present our data summarised as one depth

443 function for each parameter (mean ± s.e.m.). Based on the results of previous studies[18,28], two

444 principle component analyses (PCAs) were performed to separately investigate the strongly

445 rooted upper subsoil (PCA$_{10-35}$, 10–35 cm depths, corresponding to B horizons (Table 1)) and

446 the less densely rooted deeper subsoil (PCA$_{60-110}$, 60–110 cm depths, including the ICv and

447 IICv horizons (Table 1)). The dataset of PCA$_{10-35}$ included 18 data points with 11 variables,

448 whereas the dataset of PCA$_{60-110}$ included 27 data points with 10 variables because cutin

449 markers were not detected in the 60 to 110 cm depths. All variables were standardised (centred

450 and scaled). The PCA was conducted using PAST 3.06 for Windows[60].

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621   **Author contributions statement**

622   G. A. and S. J. equally contributed to the manuscript by conducting laboratory work, data

623   analysis and interpretation, and jointly writing the manuscript. I. K. K., C. W. M. and J. R.

624   supervised the work, commented on and reviewed the manuscript.

625   **Additional information**

626   **Competing financial interest**

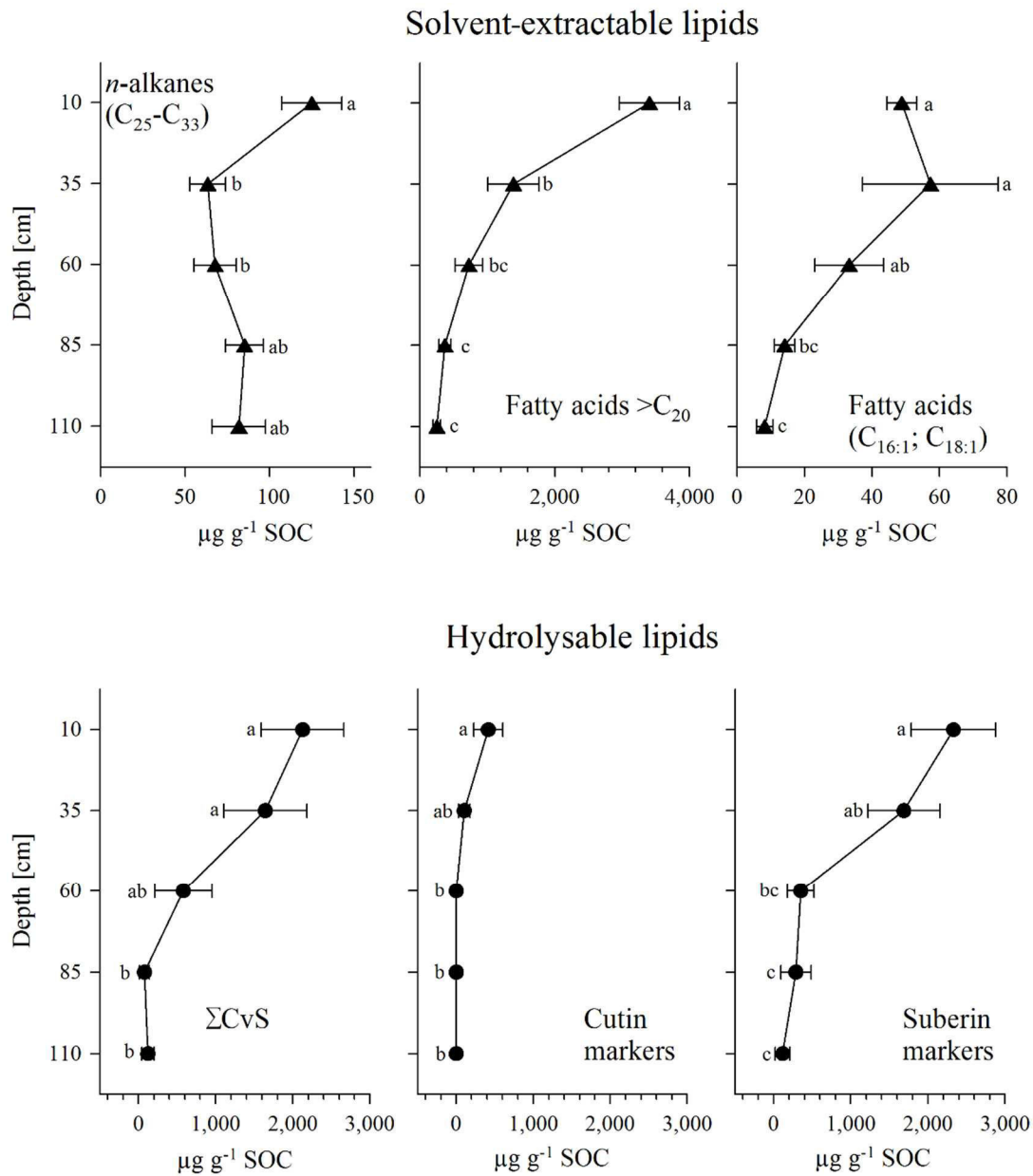627   The authors declare no competing financial interests.

628   **Figures**
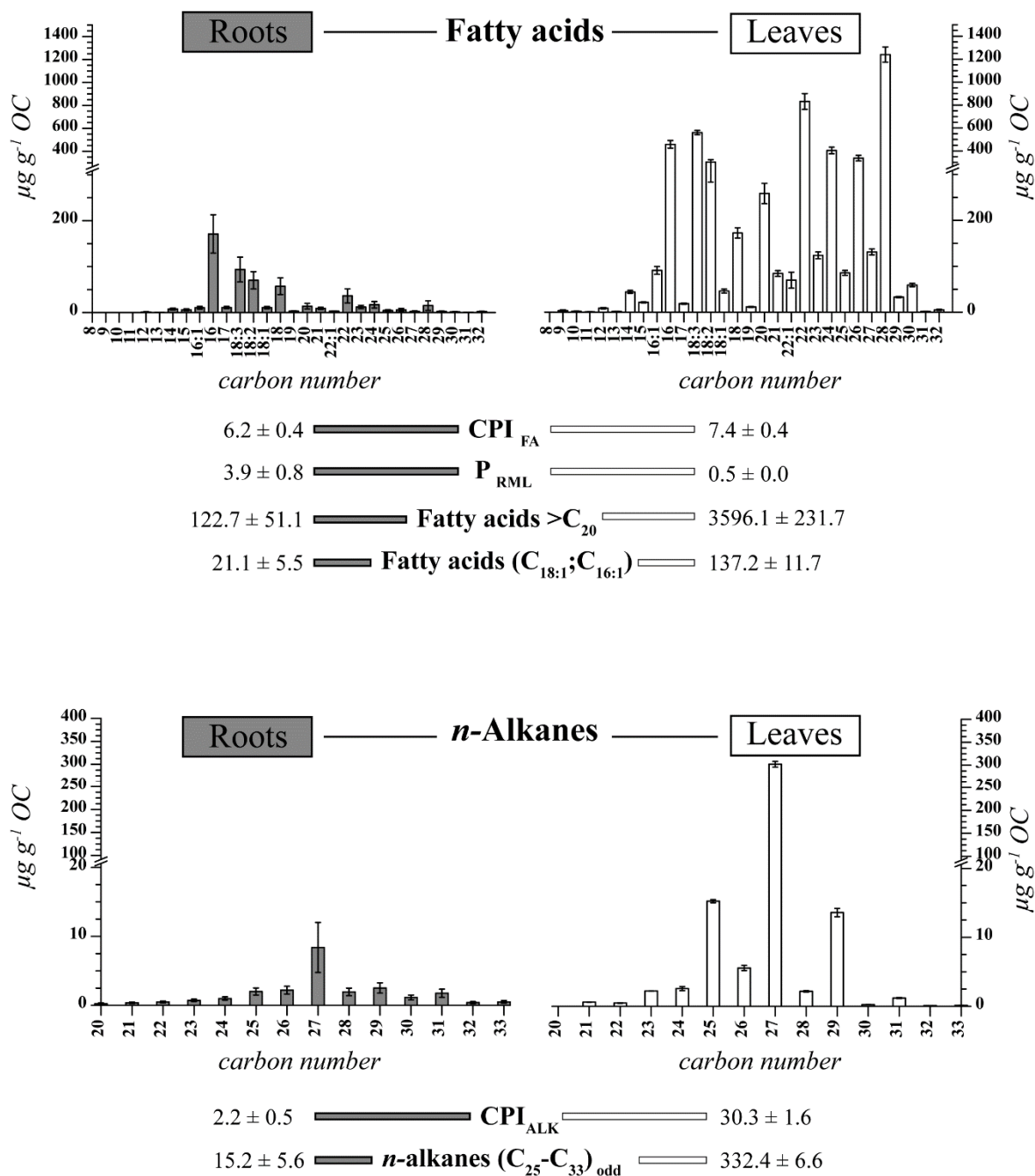
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630   Figure 1. Distribution patterns of the solvent-extractable lipids of the leaf (n = 3) and root (n =

631   3) material from the study area.

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Figure 2. Concentrations of the solvent-extractable and hydrolysable lipid biomarkers (mean of all transects and horizontal sampling spots ± s.e.m.) at different soil depths. Significant differences ($p < 0.05$) are indicated by different letters (a, b, c). n = 9 for each parameter and depth increment.
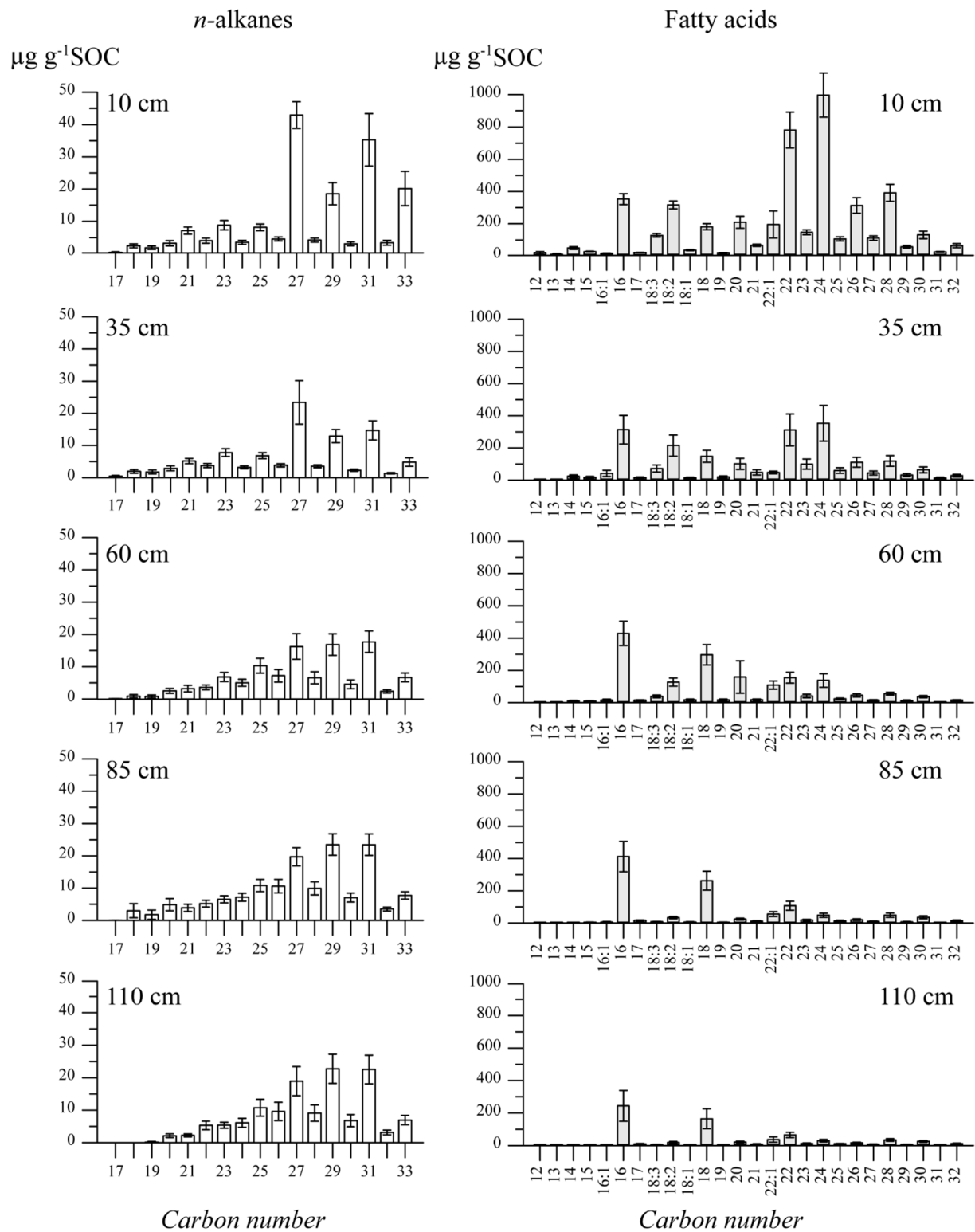
Figure 3. Distribution patterns of the solvent-extractable lipids at different soil depths (n = 9 for each soil depth and lipid type).

Figure 4. Mean of all transects and horizontal sampling spots ± s.e.m. of the carbon preference index (CPI) of *n*-alkanes (CPI$_{Alk}$) and *n*-fatty acids (CPI$_{FA}$), and the proxy for root-/microbial- vs. leaf-derived SOC (P$_{RML}$). Significant differences ($p < 0.05$) are indicated by different letters (a, b, c). n = 9 for each parameter and depth increment.

a.) $\text{PCA}_{10\text{-}35}$
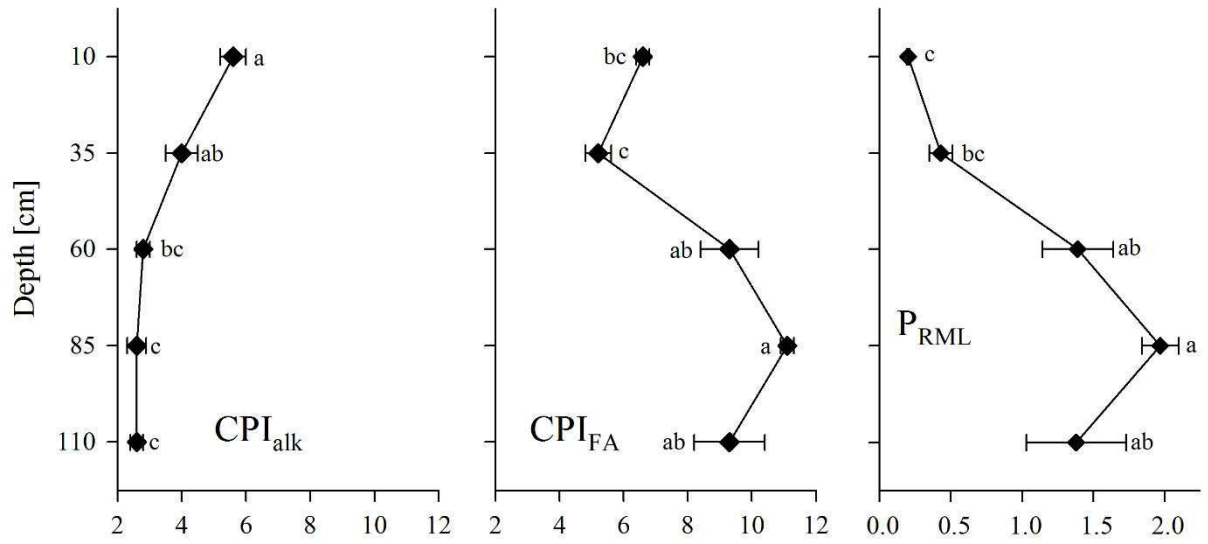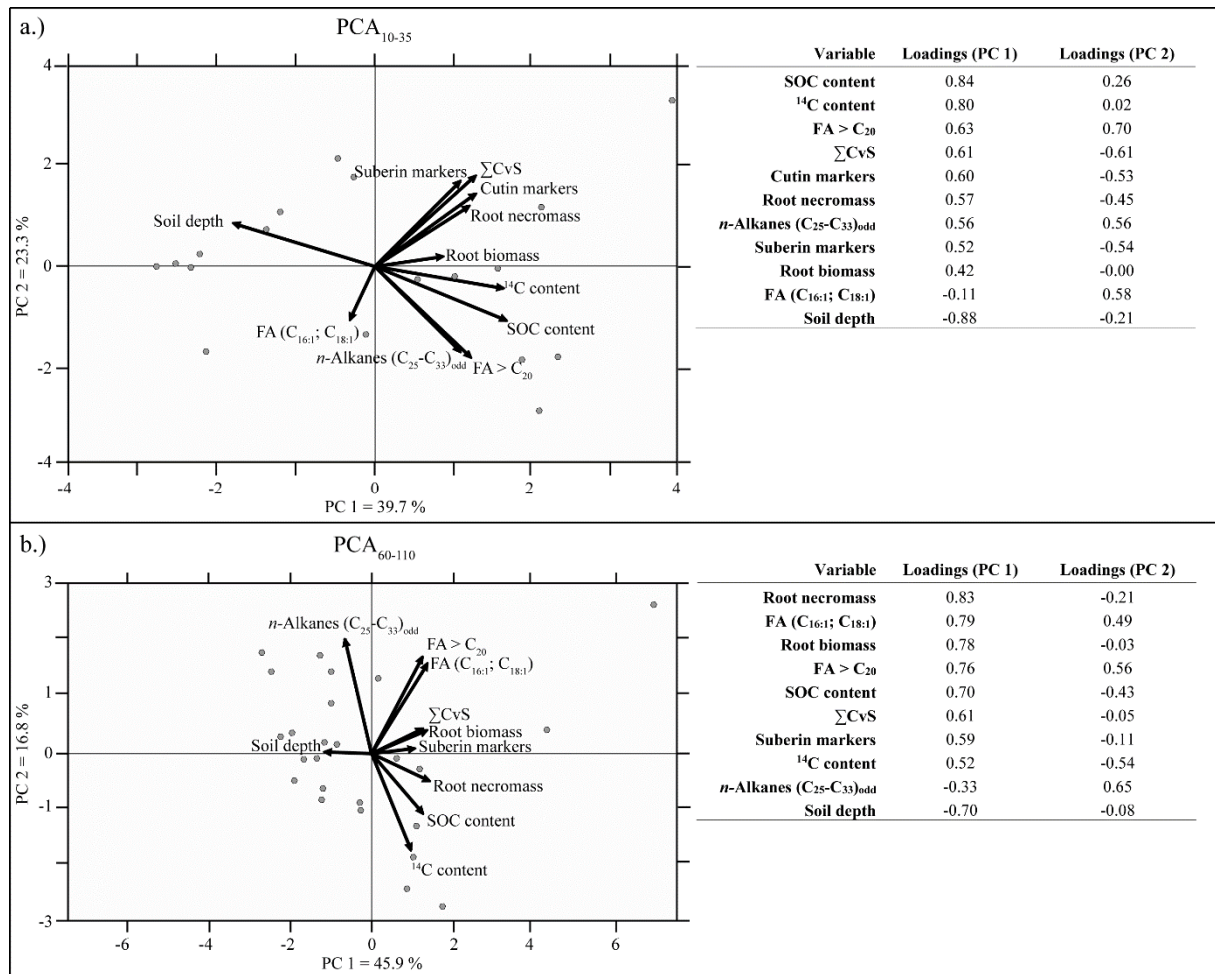
Variable	Loadings (PC 1)	Loadings (PC 2)
SOC content	0.84	0.26
^{14}C content	0.80	0.02
FA > C_{20}	0.63	0.70
$\sum$CvS	0.61	-0.61
Cutin markers	0.60	-0.53
Root necromass	0.57	-0.45
n-Alkanes (C_{25}-C_{33})$_{odd}$	0.56	0.56
Suberin markers	0.52	-0.54
Root biomass	0.42	-0.00
FA ($C_{16:1}$; $C_{18:1}$)	-0.11	0.58
Soil depth	-0.88	-0.21

b.) $\text{PCA}_{60\text{-}110}$

Variable	Loadings (PC 1)	Loadings (PC 2)
Root necromass	0.83	-0.21
FA ($C_{16:1}$; $C_{18:1}$)	0.79	0.49
Root biomass	0.78	-0.03
FA > C_{20}	0.76	0.56
SOC content	0.70	-0.43
$\sum$CvS	0.61	-0.05
Suberin markers	0.59	-0.11
^{14}C content	0.52	-0.54
n-Alkanes (C_{25}-C_{33})$_{odd}$	-0.33	0.65
Soil depth	-0.70	-0.08

Figure 5. Biplots of the principal component analyses (PCA) for a) the densely rooted upper soil layers ($\text{PCA}_{10\text{-}35}$, 10–35 cm depths), including 18 data points with 11 variables, and b) the less densely rooted deeper soil layers ($\text{PCA}_{60\text{-}110}$, 60–110 cm depths), including 27 data points with 10 variables (excluding cutin markers). The loadings (displayed as correlation coefficients) on PC1 and PC2 of the respective PCA are shown in the tables. FA = Fatty acids.

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662 **Tables**

663 Table 1. Soil parameters (mean of all transects and horizontal sampling spots ± s.e.m.) at

664 different soil depths: SOC contents, root biomass and necromass, ^{14}C contents and apparent [14]C

665 ages. Significant differences ($p < 0.05$) are indicated by different letters (a, b, c). n = 9 for each

666 parameter and depth increment.

Depth (cm)	Soil horizon	SOC content (g kg⁻¹)	Root biomass (kg m⁻³)	Root necromass (kg m⁻³)	[14]C content (fMC)	[14]C age (yrs BP)
10	Bsw	11.6 ± 0.4a	0.94 ± 0.30a	1.09 ± 0.10a	0.988 ± 0.009a	95 ± 75a
35	Bw	5.2 ± 0.5ab	0.53 ± 0.07ab	0.79 ± 0.19a	0.905 ± 0.009a	810 ± 80a
60	I Cv	1.3 ± 0.3bc	0.18 ± 0.09bc	0.13 ± 0.02ab	0.723 ± 0.027b	2650 ± 300b
85	II Cv	0.5 ± 0.0c	0.01 ± 0.01c	0.00 ± 0.00b	0.624 ± 0.028b	3860 ± 400b
110	II Cv	0.4 ± 0.0c	0.03 ± 0.02c	0.03 ± 0.03b	0.652 ± 0.064b	3750 ± 810b

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