Plant Soil (2014) 381:95–110
DOI 10.1007/s11104-014-2103-z

REGULAR ARTICLE

Distribution of cutin and suberin biomarkers under forest trees with different root systems

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Received: 29 July 2013 / Accepted: 25 March 2014 / Published online: 25 April 2014

Abstract
Background and aims Differences in chemical composition of root compounds and root systems among tree species may affect organic matter (OM) distribution, source and composition in forest soils. The objective of this study was to elucidate the contribution of species specific cutin and suberin biomarkers as proxies for shoot- and root-derived organic carbon (OC) to soil OM at different depths with increasing distance to the stems of four different tree species.
Methods The contribution of cutin- and suberin-derived lipids to OM in a Cutanic Alisol was analyzed with increasing soil depth and distance to the stems of *Fagus sylvatica* L., *Picea abies* (L.) Karst., *Quercus robur* L. and *Pseudotsuga menziesii* (Mirb.) Franco. Cutin and suberin monomers of plants and soils were analyzed by alkaline hydrolysis and subsequent gas chromatography–mass spectrometry.
Results The amount and distribution of suberin-derived lipids in soil clearly reflected the specific root system of the different tree species. The amount of cutin-derived lipids decreased strongly with soil depth, indicating that the input of leaf/needle material is restricted to the topsoil. In contrast to the suberin-derived lipids, the spatial pattern of cutin monomer contribution to soil OM did not depend on tree species.
Conclusions Our results document the importance of tree species as a main factor controlling the composition and distribution of OM in forest soils. They reveal the impact of tree species on root-derived OM distribution and the necessity to distinguish among different zones when studying soil OM storage in forests.

Keywords Biomarkers · Cutin · Suberin · Depth profile · Subsoil

Responsible Editor: Kees Jan van Groenigen.

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Introduction

The distribution of OC and OM accumulation may differ among tree species. Trees with deep root systems (e.g. European beech, Douglas fir) contribute substantially to the soil OM pool, since root litter input and turnover are major sources of soil OC besides root

exudates and dissolved OC (Farrar et al. 2003; Rasse et al. 2005; Schenk and Jackson 2005). Differences in chemical composition of root compounds and root systems among tree species affect OM sequestration in forest soils (Guo et al. 2005; Schenk and Jackson 2005). Thus, the total amount and the depth distribution of OC in forest soils are strongly influenced by tree community composition (Catovsky et al. 2002; Gleixner et al. 2005; Jandl et al. 2007). However, tree species-specific effects on soil OM input, distribution and composition are not well understood to date (Gleixner et al. 2005; Jandl et al. 2007).

Several studies have demonstrated the potential of cutin and suberin polymers to distinguish between the inputs of above- and belowground plant tissues to OM in grasslands and forest soils (Riederer et al. 1993; Nierop 2001; Naafs et al. 2005; Rasse et al. 2005; Mendez-Millan et al. 2011, Mueller et al. 2012). Cutin and suberin biopolymers were found to be more appropriate tracers of the various inputs of plant materials than lignin, since discrimination between above- and belowground litter as sources of OM was still possible, even in the most humified OM fraction (Nierop 2001). A selective enrichment of cutin- and suberin-derived moieties compared to lignin during the first steps of OM degradation in forest soils was also reported by Rumpel et al. (2002) and Winkler et al. (2005). In addition to their recalcitrance during initial organic matter decomposition cutin and suberin are accumulated in a stationary manner due to their insolubility in water and their macro-molecular structure that preserves them from leaching and transport downwards the soil profile (Nierop and Verstraten 2004). Moreover, root lipids were documented to substantially contribute to soil lipid accumulation beneath tree species, providing evidence that this might be typical of mineral soils in temperate forests (Mueller et al. 2012).

However, the use of cutin and suberin monomers as markers for above- versus belowground plant input to soil OM is hampered by the fact that the attribution of certain monomers as being cutin- or suberin-specific is still uncertain (Mueller et al. 2012). Kögel-Knabner et al. (1989) suggested the ratio of (9,10,ω-OH C_{18}+ 9,10-ep-ω hydroxyl C_{18}) – to – x,ω-OH C_{16} to roughly estimate the suberin vs. cutin contents in different horizons of forest soil stocked with Norway spruce and European beech. Monomers selected by Otto and Simpson (2006) to distinguish between above- and belowground plant input to grassland and forest soils of Western Canada included long-chain (C_{20}–C_{32}) ω-hydroxy acids and α,ω diacids and the 9,10-ep C_{18} diacid for suberin. Cutin biomarkers chosen in the study of Otto and Simpson (2006) were mid-chain substituted C_{14}, C_{15}, and C_{17} hydroxy acids and mono- and dihydroxy C_{16} acids and diacids. Some authors found only long-chain ω-hydroxy acids and α,ω diacids with chain lengths > 20 carbon atoms being indicative of suberin (Matzke and Riederer, 1991; Simpson et al., 2008). Other studies included also diacids with chain lengths < C_{20} into the group of suberin specific monomers (Kolattukudy, 2001; Kögel-Knabner, 2002; Amelung et al. 2008). These different cutin- and suberin-proxies were established with a relatively limited number of analyzed plant species and tissues. Therefore the different results may arise, partially, from insufficient knowledge of the differences in cutin and suberin composition among plant taxa (Mueller et al. 2012). Moreover, much of these previous studies analyzed the suberin of bark and cork or grass roots that likely differs from that of tree roots (e.g. Kögel-Knabner et al. 1989; Matzke and Riederer, 1991; Otto and Simpson 2006).

A recent study of Mueller et al. (2013) reported that about70% of the variation in soil lipids originates from the variation of monomer concentration and composition in the leaves and roots of the respective tree species. Thus more plant species specific biomarkers for cutin and suberin are required to assess to what extent above- versus belowground biomass input explain hydrolysable lipid concentration and distribution in soil.

The focus of this study was to improve our knowledge about the effect of four major temperate tree species (European beech, pedunculate oak, Norway spruce and Douglas fir) for soil OC accumulation using species specific cutin and suberin monomers as molecular biomarkers for above- and belowground OM input, respectively. The following specific objectives were addressed in detail:

(i) Analysis of tissue-specific monomer distribution, which are indicative of aboveground litter (leaves, needles) and root biomass of the four different tree species, respectively.
(ii) Determination of the contribution of suberin-derived aliphatic lipids to soil OM at different soil depths and tree-distance increments at sites, which are stocked with those different tree species.
(iii) Identification of cutin- versus suberin-derived biomarkers in soil OM at different soil depths and

interpretation of these results in terms of biomass origin.

Materials and methods

Study sites, sampling design, and sample pretreatment

Four adjacent sites were sampled, located in the prealpine loess region of Bavaria, Germany (48°18′ N 11°05′ E, 540 m a.s.l.). The climate at the sites is suboceanic (mean annual temperature: 7.7 °C) with an annual precipitation of about 930 mm.

The natural forest association at all sites was dominated by European beech (*Fagus sylvatica* L.) with some admixed pedunculate oak (*Quercus robur* L.), hornbeam (*Carpinus betulus* L.), and Scots pine (*Pinus silvestris* L.). However, for the last 80 years, tree species composition of the four different sites has been changed to either (i) pure European beech (*Fagus sylvatica* L.), (ii) pure Norway spruce (*Picea abies* (L.) Karst.), (iii) mixed deciduous forest, dominated by pedunculate oak (*Quercus robur* L.) and about 40 % European beech, or (iv) pure Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), respectively. Soils in the study area are Cutanic Alisols (IUSS Working Group Reference Base, 2006), formed from loess and tertiary fluvial deposits. Soils of all sites have the same parent material, mineralogical composition, and texture. A description of basic soil properties is given in Table 1.

Mature leaf and needle samples were manually cut from the twigs of five randomly selected trees per site in autumn, shortly before litter fall season. Soil and root samples were taken from three soil profiles of 2.5 m width and 0.8 m depth in direct vicinity of a living tree or the stump of a recently cut tree (Fig. 1). Sampling pits were subdivided in 16 mineral soil increments, each of 0.5 m width and 0.2 m height. Forest floor samples were taken directly above the mineral soil increments, using 300 mm x 300 mm metal frames per forest floor increment. From every mineral soil increment of all soil profiles, fine roots and soil samples were obtained from five stainless steel cores per soil increment (Fig. 1) with an inner diameter of 56 mm and a height of 40 mm. Roots from the forest floor were obtained from the respective metal frames (see above). Roots with a diameter between 5 mm and 10 mm were manually removed. Then, the soil samples were screened through a 2 mm sieve and roots>2 mm were separated. Due to the dense rooting in some of the uppermost mineral soil increments, samples were sieved up to four times to ensure most careful removal and separation of all visible roots. Coarser roots, with a diameter between 10 mm and 25 mm were manually cut on site out of the respective depth increment. All roots were separated into dead and alive components based on their color and physical integrity. All living roots per increment were combined into a single sample. All plant material was dried at 40 °C until constant weight, roughly shredded with a draw-knife and, subsequently, in a coffee mill. The weight of the washed and dried living roots was determined to calculate the root biomass in the different depth increments for the bulked samples of the five cores per increment. Bulk density of the soil samples was quantified with the biomass of the dry soil sample divided by the volume of the respective steel cores.

Basic soil chemical analyses

Soil pH values were measured on sieved subsamples in 0.01 M $CaCl_2$ at room temperature (soil:solution 1:2.5; w/w). Total C and nitrogen (N) concentrations of soil samples, roots, needles and leaves were determined in duplicate on fine-ground subsamples by dry combustion with a Vario EL CN Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Since all soil samples were free of carbonate, total C and OC concentrations were equivalent. Soil OC concentration was measured separately for the samples of each soil core of each depth increment (see above), consequently OC stocks were calculated separately for each soil core.

Sequential extraction and derivatization of lipid biomarkers

Ester-bound lipids of cutin and suberin were obtained from plant and soil samples by sequential chemical extraction (solvent extraction followed by alkaline hydrolysis). Samples of 1–5 g (plant material, forest floor) to 10–15 g (mineral soil) were solvent extracted as described by Otto and Simpson (2007) to remove solvent-extractable "free" lipids. The dry soil residues after solvent extraction were subjected to alkaline hydrolysis at 100 °C for 3 h in Teflon-lined bombs (Groteklaes GmbH, Jülich, Germany) with 20 ml of 1 M methanolic KOH to release ester-bound lipids. After cooling, the suspensions were centrifuged and

Table 1 Basic properties of the soils at the investigated sites. Mean values were calculated for the three profiles per site.

Layer	Depth (cm)	Bulk density (g cm^{-3})	Sand (g kg^{-1})	Silt	Clay	pH ($CaCl_2$)	OC (g kg^{-1})	N	C/N ratio
European beech-site									
Layer 1	0–20	1.03	340	450	210	5.4	44.4	2.26	19.88
Layer 2	20–40	1.13	340	480	180	5.4	20.3	1.40	14.55
Layer 3	40–60	1.11	280	480	240	5.7	11.1	0.92	13.97
Layer 4	60–80	1.24	290	380	330	6.4	8.30	0.87	10.70
Pedunculate oak-site									
Layer 1	0–20	1.12	430	410	160	4.4	27.7	1.52	19.13
Layer 2	20–40	1.21	390	400	210	5.0	16.6	1.10	15.17
Layer 3	40–60	1.24	360	420	220	4.8	6.10	0.53	10.50
Layer 4	60–80	1.29	300	530	180	5.5	4.62	0.49	9.06
Norway spruce-site									
Layer 1	0–20	1.13	360	460	180	4.1	22.4	1.32	17.69
Layer 2	20–40	1.23	290	510	190	4.2	14.0	1.06	13.44
Layer 3	40–60	1.20	260	490	250	4.6	7.93	0.65	11.01
Layer 4	60–80	1.32	370	420	210	4.9	5.09	0.60	8.54
Douglas fir-site									
Layer 1	0–20	1.04	390	430	180	4.5	29.1	1.78	16.42
Layer 2	20–40	1.15	270	450	290	4.7	17.1	1.23	14.24
Layer 3	40–60	1.20	240	410	340	5.7	9.28	0.81	12.10
Layer 4	60–80	1.32	230	430	340	5.7	5.91	0.68	9.66

the supernatant was transferred into a glass round bottom flask. The soil residues were extracted two more times by sonication for 15 min with 30 ml DCM:methanol (1:1; v/v) and centrifuged to obtain the supernatants. The three supernatants were combined and further processed as described by Otto and Simpson (2007), dried and stored for further processing. For derivatization, the extracts were re-dissolved in pyridine, containing phenyl acetic acid for calculating the GC response factor, and converted to their trimethyl-

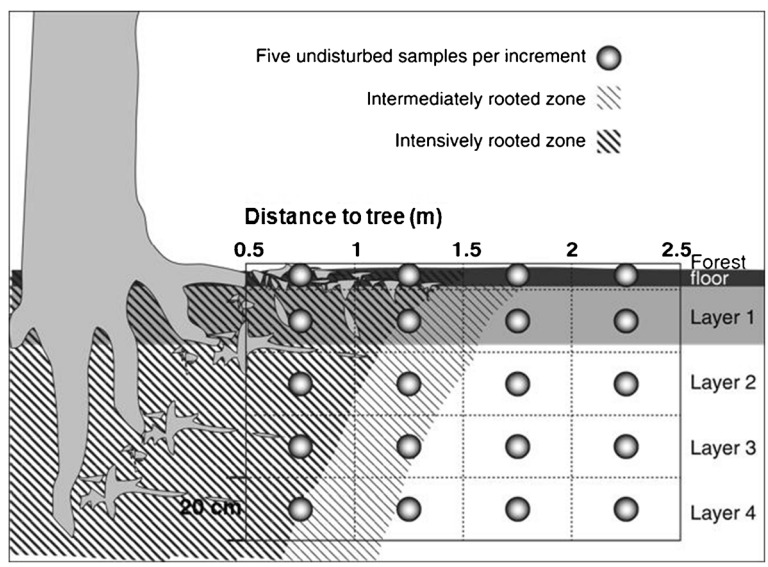

Fig. 1 Sampling design. Three soil profiles of 2.50 m length and 0.80 m depth were sampled at each site in direct vicinity of a living tree or the stump of a recently cut tree

silyl (TMS) esters and ethers by reaction with N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) containing 1 % trimethylchlorosilane (TMCS) at room temperature for 6 h.

Identification of cutin- and suberin-derived monomers

Gas chromatography–mass spectrometry analyses of the derivatized extracts were performed on an Agilent 6890 GC coupled to a quadrupole mass selective detector (5973, Agilent; EI at 70 eV, m/z 50–650 Da). Separation of the monomers was achieved on a Zebron ZB-5HT fused silica capillary column (60 m length, 0.32 mm ID, and 0.25 μm film thickness). The GC operating conditions were as follows: temperature was held at 90 °C for 1 min after injection, raised by 30 °C min^{-1} to 130 °C, then raised by 5 °C min^{-1} to 200 °C, then raised by 2 °C min^{-1} to 330 °C and finally isothermal held at 330 °C for 15 min. Helium was used as carrier gas at a flow rate of 1.2 ml sec^{-1}. Samples (1 μl) were injected splitless. The injector temperature was set at 300 °C.

Data were acquired and processed with the Chemstation G1701 CA software. Individual compounds were identified by comparison of their fragmentation pattern with published mass spectra (Draffan et al. 1968; Eglinton and Hunneman 1968; Holloway and Deas 1971, 1973; Hunneman and Eglinton 1972), with the NIST mass spectra library, and - if commercially available - with authentic standards.

Quantification of cutin- and suberin-derived monomers

Cutin- and suberin-derived monomers were quantified according to Mendez-Millan et al. (2011) with the following modifications: (i) hexacosanol, (ii) hexadecanoic acid, (iii) methyl 17-hydroxy heptadecanoate (for quantification of compounds that have been methylated already during base hydrolysis, or compounds that are naturally occurring as methylated compounds) and (iv) α,ω-hexadecanedioic acid were used as additional external quantification standards to (v) ω-hydroxy hexadecanoic acid for the quantification of *n*-alcohols, *n*-alkanoic acids, ω-hydroxy alkanoic acids and α,ω-alkanedioic acids, respectively.

Individual compounds were quantified by comparison of peak areas in the total ion current (TIC) with the TIC area of the external standard and corrected for the calculated GC response factor.

Geostatistical analyses

Depth distribution of OC stocks (kg m^{-2}) in direct vicinity of the trees at every site was calculated by first order local polynomial interpolation (LP). The search ellipse to create the maps was calculated from five georeferenced samples per depth increment (Fig. 1). Primarily, two interpolations using kriging and local polynomial were carried out. Semivariogram-based kriging showed an overestimation of OC stocks in the upper 20 cm, therefore the LP-interpolation was used. The point spacing of the interpolated raster datasets was 2 cm. A circular search ellipse with a diameter of 108 cm was used. The raster interpolation was computed with the software package Surfer (Golden Software).

Results

Contrasting content and composition of hydrolysable aliphatic lipids in plant tissues

Alkaline hydrolysis of the vegetation samples yielded a series of lignin-phenols and non-lignin phenols as well as aliphatic compounds commonly found in cutin and suberin: *n*-alcohols, *n*-alkanoic acids, ω-hydroxy alkanoic acids, α,ω-alkanedioic acids and mid-chain hydroxy alkanoic acids. The total amounts of aliphatic monomers identified in roots and leaves/needles of the trees are listed in Table 2. Among those monomers, our further analyses were targeted at ω-hydroxy alkanoic acids, α,ω-alkanedioic acids and mid-chain substituted alkanoic acids. Phenols, *n*-alcohols and *n*-alkanoic acids were released from both, above- and belowground plant organs. Furthermore, *n*-alkanoic acids and *n*-alcohols with chain lengths between C_{16} and C_{24} in soil can also be produced by microorganisms (Andreetta et al. 2013). Thus, we considered those compounds not to be indicative of the tissue source.

ω-Hydroxy alkanoic acids (ranging between 1,825 and 19,197 μg g^{-1} OC) with chain lengths between C_{12} and C_{26} were major compounds in the hydrolyzed extracts of all plant tissues. However, among them, C_{12} and C_{14} ω-hydroxy alkanoic acids were not detected in most of the root samples, with one exception of very low amounts of C_{14} ω-hydroxy alkanoic acid in European beech roots. Differences in C_{12} and C_{14} ω-hydroxy alkanoic acid concentration were apparent not only between leaves/needles and roots but also among tree

Table 2 Amounts of compounds identified after alkaline hydrolysis of the tree tissues (bdl, below detection limit). Mean values were calculated for the three trees per site.

Compounds	Norway spruce		European beech		pedunculate oak		Douglas fir	
	needles	roots	leaves	roots	leaves	roots	needles	roots
	($\mu g\ g^{-1}$ OC)							
n-Alkanoic acids								
n-$C_{10:0}$	bdl	bdl	bdl	bdl	25	bdl	bdl	bdl
n-$C_{12:0}$	318	21	31	bdl	54	bdl	56	25
n-$C_{14:0}$	357	15	232	63	39	22	74	82
n-$C_{16:0}$	1,998	497	4,446	1,009	1,944	1,095	934	595
n-$C_{17:0}$	15	bdl	32	29	20	22	bdl	54
n-$C_{18:2}$	bdl	bdl	976	116	1,727	35	bdl	bdl
n-$C_{18:1}$	457	154	2,142	200	812	65	355	1,083
n-$C_{18:0}$	215	1,193	920	16	157	48	278	409
n-$C_{20:0}$	88	1,485	282	140	374	82	bdl	111
n-$C_{22:0}$	179	829	382	806	10	108	138	151
n-$C_{24:0}$	223	448	114	731	512	347	618	500
n-$C_{26:0}$	122	19	588	223	bdl	82	342	39
n-$C_{28:0}$	bdl	bdl	140	6	bdl	47	82	14
n-$C_{30:0}$	bdl	bdl	bdl	bdl	bdl	bdl	30	22
n-$C_{32:0}$	bdl	bdl	bdl	bdl	bdl	bdl	17	bdl
Σ *n*-alkanoic acids	3,972	4,661	10,285	3,339	5,674	1,953	2,924	3,085
n-Alcohols								
n-$C_{16:0}$	bdl	304	49	36	13	73	bdl	91
n-$C_{18:0}$	bdl	282	68	39	8	1,259	bdl	620
n-$C_{20:0}$	32	bdl	246	604	640	804	bdl	bdl
n-$C_{22:0}$	bdl	bdl	651	1,462	52	574	bdl	147
n-$C_{24:0}$	84	850	bdl	881	927	120	123	983
n-$C_{26:0}$	bdl	bdl	bdl	12	150	28	bdl	bdl
n-$C_{28:0}$	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
n-$C_{30:0}$	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
Σ *n*-alcohols	116	1,436	1,014	3,034	1,790	2,858	123	1,841
ω-Hydroxy alkanoic acids								
ω-$C_{12:0}$	370	bdl	97	bdl	296	bdl	244	bdl
ω-$C_{14:0}$	2,537	bdl	60	15	bdl	bdl	4,042	bdl
ω-$C_{16:0}$	6,965	2,157	542	2,847	437	2,067	2,598	3,537
ω-$C_{17:0}$	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
ω-$C_{18:2}$	bdl	202	bdl	117	bdl	bdl	bdl	bdl
ω-$C_{18:1}$	488	6,082	751	3,552	1,631	9,821	967	9,287
ω-$C_{18:0}$	194	1,831	108	320	308	135	73	1,448
ω-$C_{20:0}$	255	3,368	96	654	186	709	737	1,923
ω-$C_{22:0}$	55	2,587	11	1,684	403	1,328	bdl	2,292
ω-$C_{24:0}$	bdl	295	20	576	bdl	466	bdl	341
ω-$C_{26:0}$	bdl	bdl	bdl	bdl	bdl	244	bdl	369
Σ ω-hydroxy alkanoic acids	10,864	16,522	1,825	9,765	3,261	14,770	8,661	19,197
α,ω-Alkanedioic acids								

Table 2 (continued)

Compounds	Norway spruce		European beech		pedunculate oak		Douglas fir	
	needles	roots	leaves	roots	leaves	roots	needles	roots
	(μg g^{-1} OC)							
α,ω-$C_{16:0}$	643	196	407	1,990	527	752	49	331
α,ω-$C_{18:0}$	bdl	152	bdl	575	bdl	140	bdl	95
α,ω-$C_{18:1}$	bdl	2,549	bdl	909	874	1,415	bdl	4,761
α,ω-$C_{20:0}$	bdl	1,108	bdl	114	bdl	2,163	bdl	2,139
α,ω-$C_{22:0}$	bdl	874	bdl	112	bdl	272	bdl	323
α,ω-$C_{24:0}$	bdl	18	bdl	81	bdl	232	bdl	bdl
α,ω-$C_{26:0}$	bdl	bdl	bdl	bdl	bdl	56	bdl	bdl
Σ α,ω-alkanedioic acids	643	4,897	407	3,781	1,401	5,030	49	7,649
Mid-chain hydroxy and epoxy alkanoic acids								
x-hydroxy $C_{14:1}$	171	bdl	bdl	bdl	bdl	bdl	217	bdl
x-hydroxy $C_{15:0}$	bdl	bdl	66	bdl	115	bdl	bdl	bdl
x,ω-dihydroxy $C_{15:0}$	85	bdl	bdl	bdl	bdl	bdl	59	bdl
x-hydroxy $C_{15:0}$ α,ω-dioic	140	bdl	bdl	bdl	bdl	bdl	112	bdl
x,ω-dihydroxy $C_{16:0}$	19,901	129	14,615	293	10,581	362	21,744	292
x-hydroxy $C_{16:0}$ α,ω-dioic	340	bdl	512	228	2,454	231	1,868	24
x,ω-dihydroxy $C_{18:1}$	708	511	1,289	121	1,196	1,714	646	396
x,ω-dihydroxy $C_{18:0}$	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
9,10,ω-trihydroxy $C_{18:0}$	1,821	3,621	10,518	4,814	9,141	4,448	830	1,786
9,10-epoxy ω-hydroxy $C_{18:0}$	2,658	189	4,471	2,612	7,098	805	1,679	1,881
9,10-epoxy $C_{18:0}$ α,ω-dioic	bdl	bdl	bdl	625	bdl	208	bdl	443
Σ mid-chain hydroxy and epoxy alkanoic acids	25,824	4,450	31,471	8,693	30,585	7,768	27,155	4,822
Σ alcohols and alkanoic acids	41,419	31,966	44,802	28,597	42,711	30,379	38,912	36,594

species. The needles of the two conifer species could be distinguished from the leaves of European beech and pedunculate oak by considerably larger amounts of C_{12} and C_{14} ω-hydroxy alkanoic acids, with oak leaves lacking C_{14} ω-hydroxy alkanoic acid completely. Mueller et al. (2012) also observed significant differences between the concentrations of C_{12} and C_{14} ω-hydroxy alkanoic acids in leaves of angiosperms and needles of gymnosperms and suggested high amounts of C_{12} and C_{14} ω-hydroxy alkanoic acids as diagnostic biomarkers for evergreen conifers from the Pinaceae family. Furthermore, this finding is also in agreement with the results of Goñi and Hedges (1990a) who analyzed the cutin composition of 67 plant species, among them several Pinaceae with high amounts of C_{14} ω-hydroxy alkanoic acid and various angiosperms, which contained only traces of C_{14} ω-hydroxy alkanoic acid or where lacking this acid completely. Acids with chain lengths between 16 and 20 carbon atoms dominated the compound class of ω-hydroxy alkanoic acids. Generally, the relative abundance of C_{16} to C_{20} ω-hydroxy alkanoic acids in leaves/needles was lower compared to the relative abundance in roots, with C_{16} and $C_{18:1}$ ω-hydroxy alkanoic acid being major components in all analyzed tissues. Long-chain ω-hydroxy alkanoic acids with ≥ 20 carbon atoms were also detected in all plant tissues, with a significantly ($p<0.05$) higher, relative abundance of these compounds in roots compared to leaves/needles. Moreover, ω-hydroxy alkanoic acids > C_{24} were lacking completely in aboveground tissue. Roots of the four tree species differed substantially with respect to the abundance and concentration of ω-hydroxy alkanoic acids with≥20 carbon atoms (Table 2). The concentration of those compounds was significantly ($p<0.05$) higher in the roots of the two conifer species with 4,925 μg g^{-1} OC (Douglas fir) and 6,250 μg g^{-1}

OC (Norway spruce) compared to the two broadleaved species with concentrations of 2,914 μg g^{-1} OC (European beech) and 2,747 μg g^{-1} OC (pedunculate oak), respectively.

Among the α,ω-alkanedioic acids, the C_{16} alkanedioic acid was released from leaves/needles and roots, while C_{18} to C_{26} alkanedioic acids occurred almost exclusively in root tissue, regardless of tree species. Concentration of C_{18} to C_{24} alkanedioic acids was significantly ($p<0.05$) higher in the roots of Douglas fir (7,318 μg g^{-1} OC) and Norway spruce (4,702 μg g^{-1} OC) compared to the roots of European beech (1,791 μg g^{-1} OC) and pedunculate oak (4,222 μg g^{-1} OC), respectively. The range in α,ω-alkanedioic acid composition was highest in pedunculate oak roots with α,ω-alkanedioic acids ranging from C_{16} to C_{26}, whereas Douglas fir roots contained only five different α,ω-alkanedioic acids with chain length between C_{16} and C_{22}.

Mid-chain substituted alkanoic acids represented the most prominent class of compounds in leaves and needles (between 62 % in spruce needles and 72 % in oak leaves), whereas their contribution to root suberin was considerably lower (between 13 % in Douglas fir roots and 30 % in beech roots). There was also a considerable amount of variability in the concentration of certain mid-chain substituted alkanoic acids among the four tree species (Table 2). Concentrations of x,ω-hydroxy hexadecanoic acids and x-hydroxy hexadecanedioic acids were nearly twice as high in the needles of the two gymnosperm species compared to the leaves of the two angiosperm species. In contrast, concentration of 9,10,ω-hydroxy octadecanoic acid was fivefold to tenfold higher in the leaves of European beech and pedunculate oak compared to the needles of Douglas fir and Norway spruce. Furthermore, some of the mid-chain substituted alkanoic acids that occurred in the gymnosperm needles were completely lacking in the angiosperm leaves and vice versa (Table 2).

Based on the results of the plant tissue analyses (Table 2), vegetation specific molecular markers for cutin and suberin were selected (Table 3), to assess per site to what extent above- versus belowground biomass input can explain the hydrolysable lipid distribution within the soil profile. Cutin and suberin specific biomarkers were designated based on the following criteria: (i) Selected monomers contribute at least 0.3 % of the overall input of ω-hydroxy alkanoic acids, α,ω-alkanedioic acids and mid-chain hydroxy alkanoic acids and (ii) their contribution to a certain plant tissue (leave/needle versus root) is at least sixfold higher compared to their contribution to other plant organs. Mueller et al. (2012) have shown that for some lipids, their abundance in soils is not consistent with their abundance in leaves/needles or roots of the overlying tree species. This diminishes the utility of those lipids as plant type or tissue type indicators. For example, Mueller et al. (2012) reported the concentration of $C_{18:1}$ ω-hydroxy alkanoic acid in soil beneath angiosperms being approx. twofold that in soil beneath conifers, despite the observation that these plant types had overlapping concentrations of this acid in both leaves and roots. Such a disconnection between the concentrations of $C_{18:1}$ ω-hydroxy alkanoic acid in plant tissues and soil is consistent with the results of our study. Pedunculate oak, Douglas fir and Norway spruce had considerably higher concentrations of $C_{18:1}$ ω-hydroxy alkanoic acid in their roots compared to European beech whereas all four tree species had similar concentration of this lipid in soil. Therefore, as a third criterion, only fatty acids were selected as tissue specific markers whose (iii) concentration in 0–0.2 m depth of the two innermost increments of the mineral soil was consistent with the differences we observed for the lipid composition of leaves/needles and roots of the four tree species (Table 4). Furthermore, epoxy alkanoic acids were excluded from the selection of vegetation specific molecular markers, since their quantification is hampered by the fact that part of the epoxy functions can be converted into vicinal diols and vicinal methoxy-alcohol groups during alkaline hydrolysis (Holloway and Deas 1973; Goñi and Hedges 1990b). The concentrations of hydrolysable lipids that fulfilled the criteria defined above were significantly higher ($p<0.05$) in the needles and roots of the two gymnosperms compared to the two leaves and roots of the two angiosperms.

Distribution of OC and N stocks and root and shoot biomarkers

Clear gradients of OC and N concentrations and stocks were found in soils with increasing vertical and horizontal distance from the stem of single European beech trees (Fig. 2), and also root biomass decreased with increasing distance from a given stem. Especially subsoil OC and N concentrations and stocks were significantly ($p<0.01$) higher close to the stems of European beech trees compared to distal soil compartments. Soil

Table 3 Selected vegetation specific molecular markers for cutin and suberin.

Norway spruce site	European beech site	pedunculate oak site	Douglas fir site
Cutin-specific molecular markers			
ω-$C_{12:0}$	ω-$C_{12:0}$	ω-$C_{12:0}$	ω-$C_{12:0}$
ω-$C_{14:0}$	x,ω-dihydroxy $C_{16:0}$	x-hydroxy $C_{15:0}$	ω-$C_{14:0}$
x-hydroxy $C_{14:1}$	x,ω-dihydroxy $C_{18:1}$	x,ω-dihydroxy $C_{16:0}$	x-hydroxy $C_{14:1}$
x-hydroxy $C_{15:0}$ α,ω-dioic		x-hydroxy $C_{16:0}$ α,ω-dioic	x-hydroxy $C_{15:0}$ α,ω-dioic
x,ω-dihydroxy $C_{16:0}$			x,ω-dihydroxy $C_{16:0}$
x-hydroxy $C_{16:0}$ α,ω-dioic			x-hydroxy $C_{16:0}$ α,ω-dioic
Suberin-specific molecular markers			
ω-$C_{20:0}$	ω-$C_{20:0}$	ω-$C_{24:0}$	ω-$C_{22:0}$
ω-$C_{22:0}$	ω-$C_{22:0}$	ω-$C_{26:0}$	ω-$C_{24:0}$
ω-$C_{24:0}$	ω-$C_{24:0}$	α,ω-$C_{18:0}$	ω-$C_{26:0}$
α,ω-$C_{18:0}$	α,ω-$C_{18:0}$	α,ω-$C_{20:0}$	α,ω-$C_{16:0}$
α,ω-$C_{18:1}$	α,ω-$C_{18:1}$	α,ω-$C_{22:0}$	α,ω-$C_{18:0}$
α,ω-$C_{20:0}$	α,ω-$C_{20:0}$	α,ω-$C_{24:0}$	α,ω-$C_{18:1}$
α,ω-$C_{22:0}$	α,ω-$C_{22:0}$		α,ω-$C_{20:0}$
	α,ω-$C_{24:0}$		α,ω-$C_{22:0}$

OC stocks up to 2.7 kg m^{-2} were found at a depth of 0.6 to 0.8 m in the innermost investigated zone of tree influence. The horizontal and vertical pattern of root biomass and OC stocks under Douglas fir trees largely resembles the pattern under European beech trees, whereas root biomass as well as OC and N stocks under Norway spruce decreases steeply between the first and the second depth increment (Fig. 2). In contrast to the clear vertical gradient, neither a distinct horizontal gradient in root biomass distribution, nor in OC and N concentrations and stocks in topsoil or subsoil was found with increasing distance from Norway spruce trees. Soil under pedunculate oak trees exhibited the most patchy depth and horizontal distribution of root biomass as well as OC and N stocks. As for the soil under Norway spruce, clear horizontal gradients in the concentrations and stocks of OC and N were not found, but decreasing contents and stocks of OC and N with soil depth that were restricted to the innermost investigated zone of tree influence (Fig. 2). Concentrations of suberin-specific monomers in the uppermost depth increment of the mineral soil (0–0.2 m) were on average significantly higher ($p<0.01$) under Douglas fir and Norway spruce compared to the concentrations under European beech and pedunculate oak (Table 4; Fig. 3b). This reflects the strong relationship between the concentrations of suberin-specific lipids in soil and in roots with >10 000 μg suberin monomers g^{-1} OC in the roots of the two conifers but only <5,000 μg suberin monomers g^{-1} OC in the roots of the two angiosperms. The relationship between the concentrations of cutin-specific monomers in the uppermost mineral soil increment and in leaves/needles was less pronounced but still significant ($p<0.01$).

The spatial pattern of suberin-specific monomers in soil differed considerably among the four tree species. Concentration and stocks of suberin-specific monomers was lowest in the outermost zone next to the European beech and Douglas fir trees, whereas suberin-specific monomers were the main components contributing to topsoil and subsoil OC stocks in the root-affected soil compartments next to these two trees (Fig. 3b, Fig. 4). The decrease of suberin-derived monomers with soil depth was more pronounced with increasing distance to the stem under both tree species. Under Norway spruce, the contribution of suberin-derived monomers to OC reflected the distinct vertical gradient of decreasing root biomass with soil depth. However, due to the low concentrations of suberin-specific compounds in some of the deeper profile compartments at the Norway spruce site, the margin of deviation of the measurements was comparably high for the samples of those soil compartments. The contribution of suberin-derived monomers to OC was highly variable in the profiles

Table 4 Concentrations of selected identified monomers after alkaline hydrolysis in the tree tissues and the two innermost increments of the mineral soil (0–0.2 m depth) (bdl, below detection limit). Mean values were calculated for the three trees and profiles per site.

Compounds	Norway spruce site			European beech site			pedunculate oak site			Douglas fir site		
	needles	roots	soil	leaves	roots	soil	leaves	roots	soil	needles	roots	soil
	($\mu g\ g^{-1}$ OC)											
ω-Hydroxy alkanoic acids												
ω-$C_{12:0}$	370	bdl	208	97	bdl	55	296	bdl	84	244	bdl	72
ω-$C_{14:0}$	2,537	bdl	841	60	15	13	bdl	bdl	bdl	4,042	bdl	1,419
ω-$C_{18:2}$	bdl	202	174	bdl	117	121	bdl	bdl	97	bdl	bdl	159
ω-$C_{18:1}$	488	6,082	3,980	751	3,552	3,647	1,631	9,821	4,026	967	9,287	3,318
ω-$C_{18:0}$	194	1,831	860	108	320	710	308	135	933	73	1,448	957
ω-$C_{20:0}$	255	3,368	2,117	96	654	497	186	709	657	737	1,923	1,275
ω-$C_{22:0}$	55	2,587	1,861	11	1,684	530	403	1,328	802	bdl	2,292	925
ω-$C_{24:0}$	bdl	295	170	20	576	199	bdl	466	185	bdl	341	148
ω-$C_{26:0}$	bdl	bdl	bdl	bdl	bdl	bdl	bdl	244	116	bdl	369	282
α,ω-Alkanedioic acids												
α,ω-$C_{16:0}$	643	196	322	407	1,990	1,511	527	752	476	49	331	270
α,ω-$C_{18:0}$	bdl	152	117	bdl	575	359	bdl	140	147	bdl	95	166
α,ω-$C_{18:1}$	bdl	2,549	1,113	bdl	909	526	874	1,415	1,265	bdl	4,761	2,999
α,ω-$C_{20:0}$	bdl	1,108	899	bdl	114	191	bdl	2,163	2,059	bdl	2,139	1,964
α,ω-$C_{22:0}$	bdl	874	647	bdl	112	42	bdl	272	108	bdl	323	207
α,ω-$C_{24:0}$	bdl	18	2	bdl	81	77	bdl	232	64	bdl	bdl	bdl
Mid-chain hydroxy and epoxy alkanoic acids												
x-hydroxy $C_{14:1}$	171	bdl	37	bdl	bdl	bdl	bdl	bdl	bdl	217	bdl	86
x-hydroxy $C_{15:0}$	bdl	bdl	bdl	66	bdl	41	115	bdl	95	bdl	bdl	bdl
x-hydroxy $C_{15:0}$ α,ω-dioic	140	bdl	45	bdl	bdl	bdl	bdl	bdl	bdl	112	bdl	52
x,ω-dihydroxy $C_{16:0}$	19,901	129	7,240	14,615	293	5,828	10,581	362	3,091	21,744	292	12,597
x-hydroxy $C_{16:0}$ α,ω-dioic	340	bdl	107	512	228	266	2,454	231	1,443	1,868	24	1,160
x,ω-dihydroxy $C_{18:1}$	708	511	397	1,289	121	416	1,196	1,714	1,383	646	396	325
ΣC	23,459	129	8,478	16,001	414	6,299	13,446	593	4,713	28,227	316	15,386
ΣS	310	10,638	6,924	127	4,705	2,421	0	3,517	2,679	49	10,651	6,961

next to pedunculate oak trees. However, the innermost soil compartment was characterized by a clear depth gradient of suberin-specific monomer concentrations, which strongly decreased with increasing soil depth. For each of the tree species, specific zones with larger stocks of suberin-derived lipids and zones with lower stocks of suberin-derived lipids can be clearly distinguished (Fig. 4). In case of European beech and Douglas fir trees, the zone with large stocks of suberin derived lipids captures an area up to 2.5 m in diameter and reaches a depth of more than 0.6 m next to the stem. In contrast, more than 64 % of the total stocks of suberin-derived lipids in the mineral soil under Norway spruce were found within the top 0.2 m of the soil profile and profiles next to pedunculate oak trees were characterized by large stocks of suberin-derived lipids only in the innermost soil compartment. In contrast to the tree species specific pattern of the suberin monomer contribution to soil OC within a soil profile, no clear tree specific pattern for the contribution of cutin-specific monomers to soil OC was found with increasing distance to the different trees (Fig. 3a). Contribution of cutin-specific monomers to soil OC decreased rapidly with soil depth under Douglas fir, Norway spruce, and pedunculate oak. Contrarily, the decrease of cutin-specific monomers with soil depth was less pronounced at the European beech site (Fig. 3a), even within the second depth increment (0.2–0.4 m), concentrations of

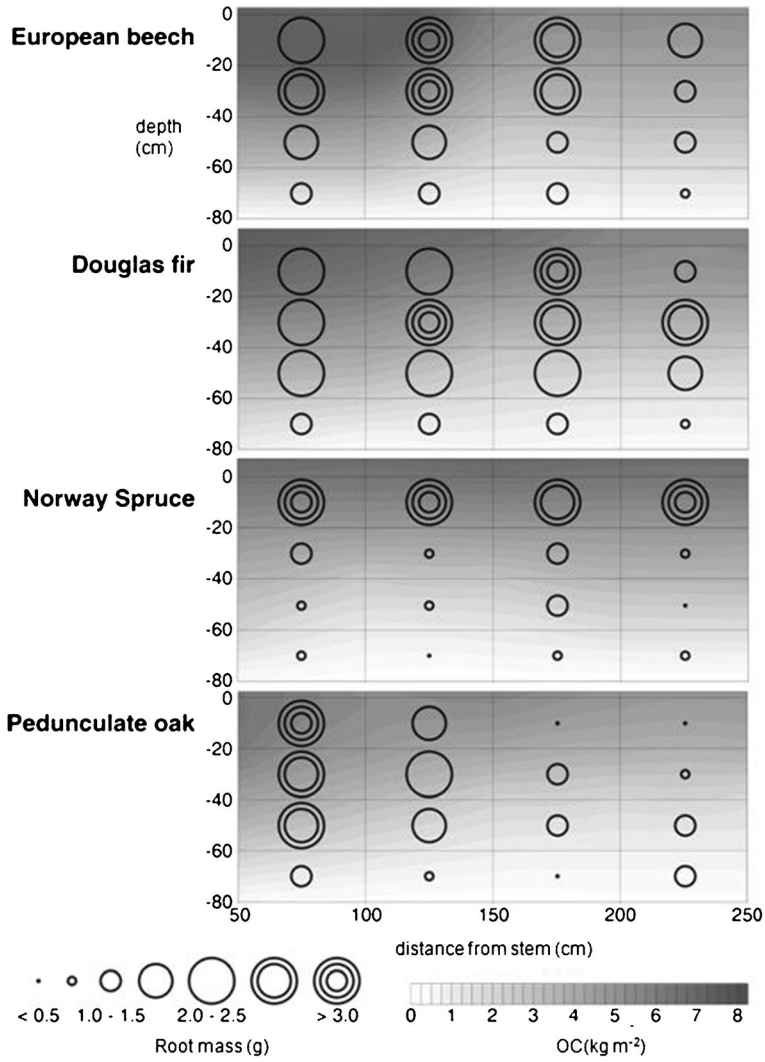

Fig. 2 Mean OC stocks and root biomasses of the three soil profiles per site. Depth distribution of OC stocks is calculated by local polynomial (Surfer, Golden Software) using five georeferenced samples per depth increment

cutin monomers were on average higher than 500 μg g^{-1} OC at the beech site (Fig. 3a). Consequently, stocks of cutin-specific monomers in the mineral soil next to European beech trees are up to 70 g m^{-2} higher compared to the soil compartments next to the other tree species (Fig. 4).

Discussion

Selection of root- and leaf/needle-specific biomarkers

The most challenging circumstance with respect to the selection of root- and leave/needle-specific biomarkers was the disconnection between plant tissue concentration and soil concentration for some of the ω-hydroxy alkanoic acids, α,ω-alkanedioic acids, and mid-chain substituted alkanoic acids (Table 4), e.g. the $C_{18:1}$ ω-hydroxy alkanoic acid, see above. Also for some other acids, concentrations in 0–0.2 m depth of the two innermost increments of the mineral soil differed substantially from those in plant tissues. The concentration of C_{18} ω-hydroxy alkanoic acid in the selected soil increments of 0–0.2 m depth was about the same beneath European beech (710 μg g^{-1} OC), pedunculate oak (933 μg g^{-1} OC) and Norway spruce (806 μg g^{-1} OC) despite the fact that its concentration in Norway spruce roots was fivefold to tenfold higher compared to its concentration in the roots of the two angiosperms (Table 4). The legacy of root litter input from Norway spruce could be responsible for the high concentrations of C_{18} ω-hydroxy alkanoic acid in the mineral soil beneath the

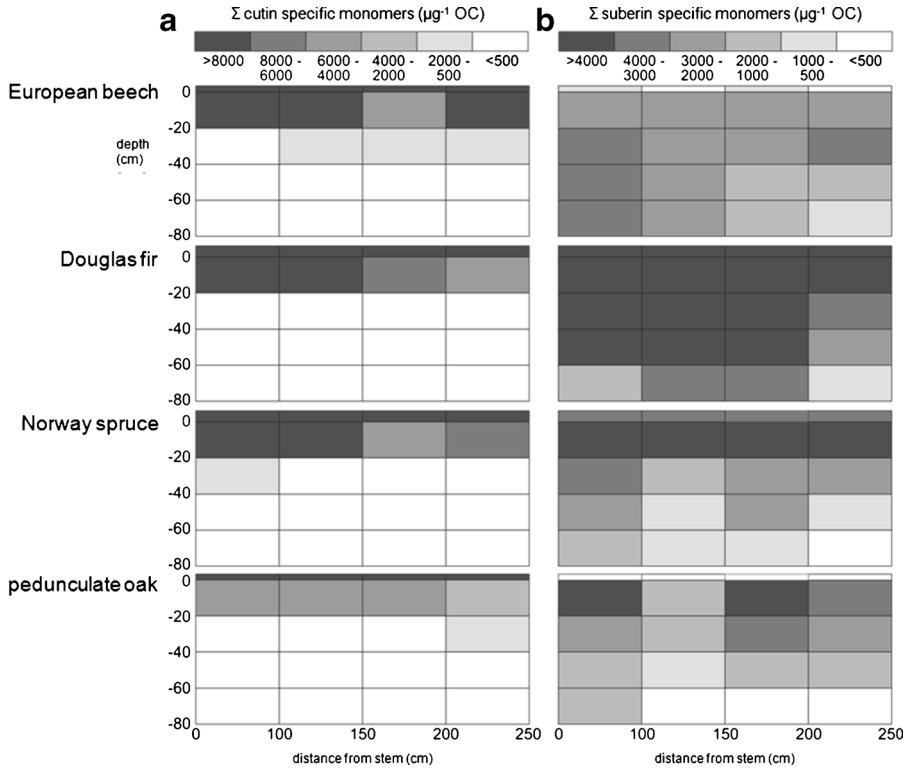

Fig. 3 Depth distribution of mean cutin-specific monomer concentrations (**a**), and mean suberin-specific monomer concentrations (**b**). Mean values were calculated from three soil profiles per site

two angiosperms, since both sites were stocked with Norway spruce before clear cutting 80 years ago. However, concentrations of C_{20} ω-hydroxy alkanoic acid in soil are consistent with the different concentrations of this acid in the roots of European beech, pedunculate oak, and Norway spruce (Table 4). Thus, current input from understory grasses and herbs could be another explanation for the discrepancy between the concentrations in tree roots and soil in case of the C_{18} ω-hydroxy alkanoic acid. The distorting influence of understory vegetation that may contribute substantially to the preserved lipid pool of a certain site was previously discussed by Nierop et al. (2006) and Mueller et al. (2012). In case of the sites of our study, the impact of understory vegetation might be of special importance at the pedunculate oak site, where the canopy structure is less dense. However, the understory consists of patches with a number of grasses and herbs whose lipid distribution in shoots and roots is so far uncharacterized. In addition to the discussed impact of understory grasses and herbs, and the legacy of preserved lipids from former vegetation, input from sources other than roots and leaves/needles (e.g. bark, seeds) were discussed by Mueller et al. (2012) as another factor that could also distort soil lipid composition relative to that of roots and leaves/needles. Finally, processes of OM decomposition and stabilization could exert more influence on the concentration of certain cutin- and suberin-specific monomers in soil than on other cutin- and suberin-derived compounds (Nierop et al. 2003; Mueller et al. 2012).

Spatial patterns of OC and N stocks as well as cutin and suberin distribution in soil as influenced by root system and tree distance

The pattern of suberin-derived lipids in soil strongly reflects the tree species-specific root systems (Fig. 4; Köstler et al. 1968): European beech and Douglas fir trees, which form a heart-shaped root system, develop an intensively-rooted, zone rich in suberin-derived lipids that dominates the soil profile next to the stem down to a depth of more than 0.6 m and within a distance to the stem of more than 2.5 m. The amount of OC and N cycled due to fine root production and decomposition within these intensively rooted soil

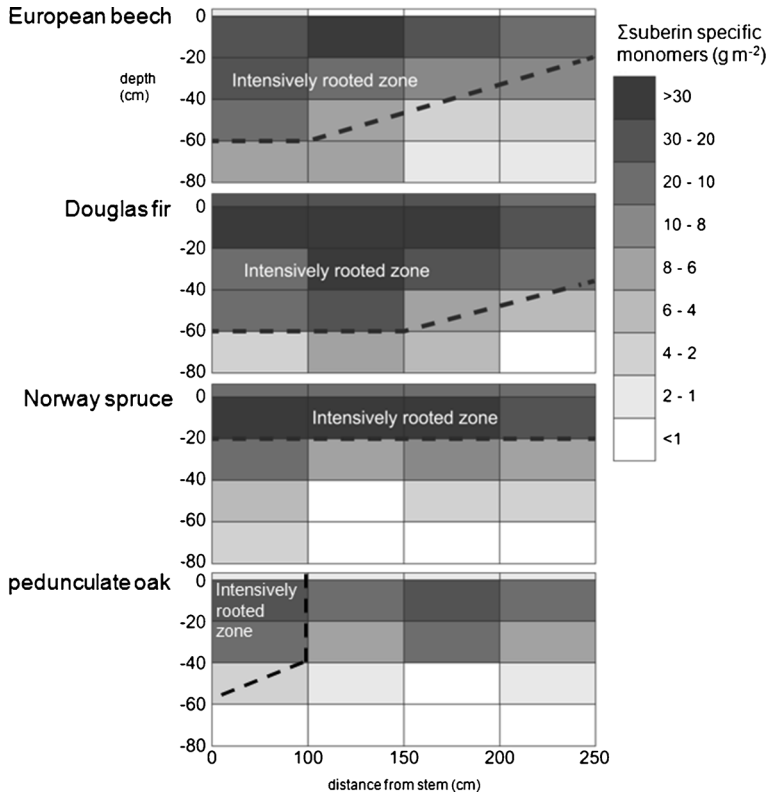

Fig. 4 Depth distribution of mean suberin-specific monomer stocks. Mean values were calculated from three soil profiles per site

compartments can be equal to or even higher than that circulated through aboveground litter fall (Fahey and Hughes 1994; Majdi and Andersson 2005). Hence, we assume that in this intensively-rooted inner zone, which is characterized by high stocks of suberin-derived lipids, soil OM formation is dominated by the accumulation of organic matter derived from the decomposition of root litter. Contrarily, OM formation in the outermost zone of lower stocks of suberin-specific lipids may be dominated by other processes, e.g. the leaching of dissolved OC from the forest floor and adsorption/(co-)precipitation processes with the mineral phase.

The lateral and horizontal extent of the intensively-rooted zone under Norway spruce differs substantially from that under European beech and Douglas fir trees. It corresponds to the shallow root system of this tree species and is in line with previous studies that found especially coarse roots in pure Norway spruce stands to be limited to the upper 0.2 m of a soil profile (Schmid and Kazda, 2002). Hence, root litter-derived compounds such as the selected suberin-specific compounds are less important for the formation of OM in deeper soil compartments of pure Norway spruce stands compared to sites, which are stocked with tree species that develop a heart-shaped root system. Subsoil OM under Norway spruce trees probably differs in its composition from root litter-dominated OM under European beech and Douglas fir trees due to their different sources. Moreover, there is increasing evidence that in mixed stands of Norway spruce and European beech the root system of Norway spruce is even more restricted to the topsoil than in Norway spruce monocultures (Schmid 2002; Schmid and Kazda 2002; Bolte and Villanueva 2006). In mixed stands, interspecific competition results in a vertical stratification of beech and spruce fine root systems with a shift in beech fine roots from upper to lower soil layers and a concomitant shift of Norway spruce fine roots from lower to upper soil layers (Bolte and Villanueva 2006). In such mixed stands, OM formation, composition and three-dimensional patterns may trace this vertical stratification with spruce-derived suberin monomers dominating the OM composition in the uppermost soil and beech-derived suberin monomers mainly contributing to the OM composition in the subsoil.

Pedunculate oak trees develop a deep but narrow taproot system to scavenge soil moisture and nutrients

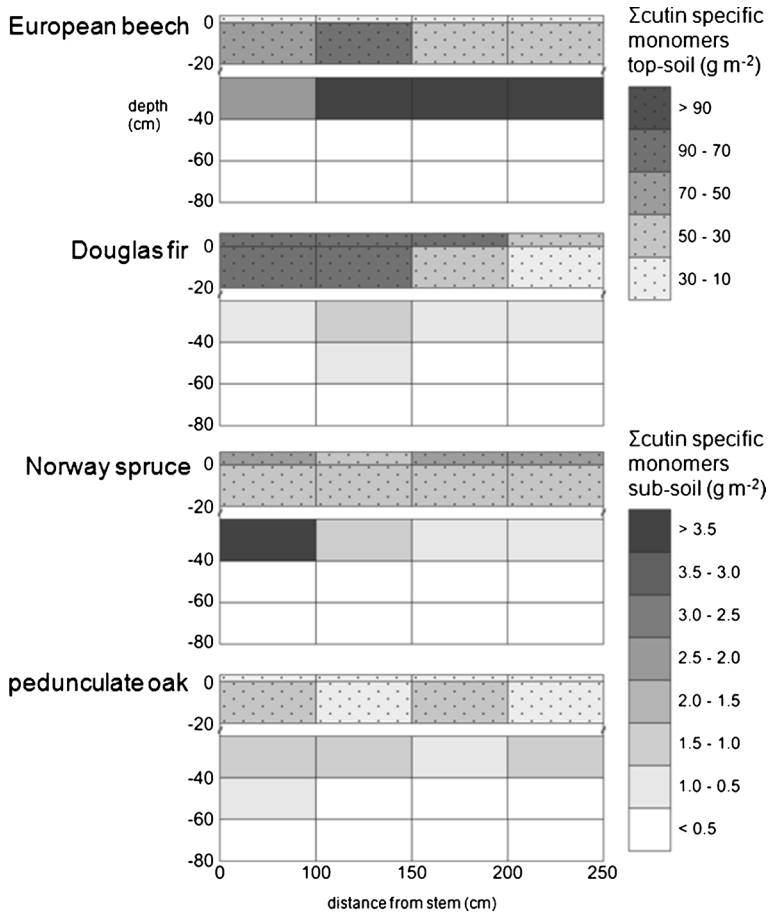

Fig. 5 Depth distribution of mean cutin-specific monomer stocks. Mean values calculated from three soil profiles per site

from deep soil compartments (Köstler et al. 1968). This root system is characterized by high OC, N and suberin monomer concentrations (Figs. 2, 3 and 4) in the innermost soil compartment (0 to 0.1 m distance to the stem). At distances of more than 0.1 m, patchiness of OC, N and alkaline hydrolysable aliphatic lipid stocks in the soil under pedunculate oak trees increases. This is most likely a result of the influence of understory grasses, herbs and shrubs that may contribute to the preserved lipid pool at the pedunculate oak site (see above) Moreover, also the mixed structure and tree species composition of this stand, which consists of about 60 % pedunculate oak and 40 % European beech, and has a mixed age structure likely contributes to the patchiness. The deep, but narrow root system of pedunculate oak dominates the suberin pattern in the soil compartment next to the oak stems, whereas with increasing distance other OM sources (e.g. of admixed beech or ground vegetation) are of evident importance. The roots of young admixed beech trees and shrubs in the understory of this mixed stand compete with the root system of the oak trees and likely exert a considerable influence on the horizontal and depth distribution of OC, N as well as alkaline hydrolysable aliphatic lipids. An increasing contribution of root litter from beech to soil OC with increasing distance to the oak trees at this site is also indicated by decreasing concentrations of C_{26} ω-hydroxy alkanoic acid and C_{26} alkanedioic acid but increasing amounts of C_{16} alkanedioic acid in part of the two outermost soil compartments next to the pedunculate oak trees. A previous study was conducted in a mixed European beech – sessile oak stand. Leuschner et al. (2001) reported that beech root biomass even increased with increasing distance from beech stems, whereas oak root biomass was not dependent on stem distance. Moreover, beech roots not only dominated within the soil compartments in direct vicinity of beech stems, but also in the soil compartments under adjacent oak trees. This might be a result of oak root growth suppression by European beech rather than attributing

them to inherent differences between the spatial root system and root biomass patterns of the two tree species (Leuschner et al. 2001). Thus, the spatial pattern of oak-derived suberin contribution to soil OM formation may differ substantially between mixed forest stands and monospecific oak woods. This assumption is supported by Zoth and Block (1992), who found that beech and oak trees tend to develop a heart root system if growing in monospecific forest stands.

In contrast to the distinct pattern of suberin-specific monomers within a soil profile that resembles the root system of the respective tree, no clear horizontal pattern of cutin-derived lipids was found with increasing distance to the different trees. However, vertical distribution of cutin-specific monomers differed between the sites. Substantial stocks of cutin-derived compounds in the second depth increment were apparent only at the European beech site. Contrarily, stocks of cutin-derived compounds decreased more rapidly with soil depth at the other sites (Fig. 5). A greater earthworm density and consequently higher degree of bioturbation could be one explanation for this observation. Lower bioturbation is in accordance with the more acidic, nutrient poor soil conditions at the Norway spruce, Douglas fir and peduculate oak sites compared to the European beech site. However, earthworms selectively ingest the leaf body but not the petioles (Suárez et al. 2006; Crow et al. 2009) and have esterase enzymes that degrade cutin and suberin polyesters (Nakajima et al. 2005). Thus, a higher abundance of earthworms, which at the one hand increases soil mixing can at the other hand also impact cutin and suberin composition and reduce the cutin concentration in the mineral soil (Crow et al. 2009). Therefore, other factors such as the input from understory species could also be responsible for the larger amount of cutin-specific compounds in the second depth increment of the mineral soil under European beech compared to the three other sites.

Conclusions

In summary, our results show that single trees contribute to the formation of particular patches in forest soils where OM is dominantly derived from the accumulation and decomposition of root litter. Within this zone, the tree specific root system strongly affects soil properties and OC cycling by the species-specific release of root litter.

This finding confirms the important role of tree root systems for soil OC biogeochemistry and sequestration in forest ecosystems. By contrast, the formation of OM in the mineral soil in more-extensively rooted areas between single trees is presumably dominated by other processes (e.g. leaching of DOC from the forest floor into the mineral soil). Hence, stand age, forest structure, tree species and tree distribution have to be considered when soil OC and N stocks as well as OM properties are studied as part of OC inventories.

Acknowledgements We gratefully acknowledge the invaluable help of G. Albert during sample preparation, pretreatment, hydrolysis, derivatization and GC analyses. We also want to thank the two anonymous reviewers for valuable comments on an earlier version of the manuscript. Funding for this study was provided by the German Science Foundation (DFG; Ko 1035/34-1).

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