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Changes in dissolved lignin-derived phenols, neutral sugars, uronic acids, and amino sugars with depth in forested Haplic Arenosols and Rendzic Leptosols

KLAUS KAISER[1,2,*], GEORG GUGGENBERGER[1,2]
and LUDWIG HAUMAIER[1]
*[1]Institute of Soil Science and Soil Geography, University of Bayreuth, 95440 Bayreuth, Germany; Current address: [2]Institute of Soil Science and Plant Nutrition, Soil Biology and Ecology, Martin-Luther-University, Weidenplan 14, 06108 Halle (Saale), Germany; *Author for correspondence (Institute of Soil Science and Plant Nutrition, Soil Biology and Ecology, Martin-Luther-University, Weidenplan 14, 06108 Halle (Saale), Germany; e-mail: klaus.kaiser@landw.uni-halle.de; Fax: +49-345-5527116*

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Abstract. A major part of the dissolved organic matter produced in the organic layers of forest ecosystems and leached into the mineral soil is retained by the upper subsoil horizons. The retention is selective and thus dissolved organic matter in the subsoils has different composition than dissolved organic matter leached from the forest floor. Here we report on changes in the composition of dissolved organic matter with soil depth based on C-to-N ratios, XAD-8 fractionation, wet-chemical analyses (lignin-derived CuO oxidation products, hydrolysable sugars and amino sugars) and liquid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Dissolved organic matter was sampled directly beneath the forest floor using tension-free lysimeters and at 90 cm depth by suction cups in Haplic Arenosols under Scots pine (*Pinus sylvestris* L.) and Rendzic Leptosols under European beech (*Fagus sylvatica* L.) forest. At both sites, the concentrations of dissolved organic carbon (DOC) decreased but not as strongly as reported for deeply weathered soils. The decrease in DOC was accompanied by strong changes in the composition of dissolved organic matter. The proportion of the XAD-8-adsorbable (hydrophobic) fraction, carboxyl and aromatic C, and the concentrations of lignin-derived phenols decreased whereas the concentrations of sugars, amino sugars, and nitrogen remained either constant or increased. A general feature of the compositional changes within the tested compound classes was that the ratios of neutral to acidic compounds increased with depth. These results indicate that during the transport of dissolved organic matter through the soils, oxidatively degraded lignin-derived compounds were preferentially retained while potentially labile material high in nitrogen and carbohydrates tended to remain dissolved. Despite the studied soils' small capacity to sorb organic matter, the preferential retention of potentially refractory and acidic compounds suggests sorption by the mineral soil matrix rather than biodegradation to govern the retention of dissolved organic matter even in soils with a low sorption capacity.

Introduction

In the mineral horizons of forest soils, dissolved organic matter commonly decreases with depth (Michalzik et al. 2001). Sorption to mineral surfaces, that is, of aluminium and iron oxides–hydroxides, and, to a smaller extent, biodegradation are reasonable mechanisms to explain the changes in concentrations of dissolved organic carbon (DOC) during the transport through soil (Kalbitz et al. 2000). Typically, not only the DOC concentrations are affected but also the composition of dissolved organic matter changes. Compounds rich in carboxyls and aromatic moieties, which interact with the macroreticular resin XAD-8 at pH 2 (hydrophobic organic matter), are preferentially retained in soil while compounds high in carbohydrates, nitrogen, phosphorus, and sulphur, which do not interact with XAD-8 (hydrophilic organic matter), seem to be mobile and thus tend to be transported into the subsoil and exported from the rooting zone into surface and ground waters (Qualls and Haines 1991; Andersson et al. 1999; Nilsson et al. 2001; Kaiser et al. 2002).

Although these general aspects of changes in the chemical composition of dissolved organic matter during its movement in the mineral soil are well documented, little is known about probable effects on the molecular level. Biodegradation of dissolved organic matter is characterised by preferential metabolisation of carbohydrates (Amon et al. 2001; Kalbitz et al. 2003) leaving the residual material relatively enriched in lignin-derived compounds and newly synthesised microbial metabolites (Kalbitz et al. 2003). Biodegradation of organic matter shifts the composition of hydrolysable neutral sugars from pentoses to hexoses indicating consumption of plant-derived matter and production of microbial compounds (Murayama 1984). Amino sugars are microbial metabolites that increase during the biological processing of organic matter (Amelung et al. 2001). Oxidative metabolisation of dissolved lignin-derived compounds results in an increase in the content of carboxyl relative to the aldehyde functionalities (Ertel et al. 1986). Based on these findings, dissolved organic matter impacted by microbial decomposition should

- be enriched in lignin-derived phenols, especially of acidic ones,
- show a change from pentoses to hexoses,
- be enriched in amino sugars.

Laboratory experiments and field studies have shown that soils and minerals selectively sorb lignin-derived acidic phenols (McKnight et al. 1992; Kaiser et al. 1997). Also, it appears that sorption is selective for acidic compounds in general (Edwards et al. 1996; Kaiser and Zech 2000). In turn, carbohydrate-rich fractions of dissolved organic matter sorb only weakly to soils and minerals (Kaiser et al. 2002). On the molecular level, sorption can be assumed to result in organic matter remaining dissolved

- being depleted in lignin-derived phenols, especially of acidic ones,
- showing no change in composition of hydrolysable neutral sugars and amino sugars,
- being depleted in acidic sugars.

Consequently, analyses of specific compounds such as lignin-derived phenols and hydrolysable sugars and amino sugars dissolved in soil water sampled at different soil depths offer a good possibility to gain insight into processes that influence the composition of dissolved organic matter. Moreover, knowledge on dissolved organic compounds in the subsoil allows one to predict the fate and potential effects of dissolved organic matter transferred from soil into aquatic environments. Analyses of specific compounds have been proven to be useful in the evaluation of sources and controls on the production of dissolved organic matter in the forest floor (Guggenberger and Zech 1994; Michalzik and Matzner 1999; Kaiser et al. 2001a) and on sources, fate, and function of dissolved organic matter in terrestrial surface water and oceans (Ertel et al. 1986; Hedges et al. 1994; Opsahl and Benner 1997; Amon et al. 2001).

In this study, we compared the composition of dissolved organic matter in forest floor leachates and solutions from subsoils of Rendzic Leptosols under a deciduous (European beech, *Fagus sylvatica* L.) and Haplic Arenosols under a coniferous (Scots pine, *Pinus sylvestris* L.) forest. To achieve this goal, we analysed lignin-derived CuO oxidation products, hydrolysable neutral and acidic sugars, and hydrolysable amino sugars. Specifically, we aimed at using changes in the ratios between neutral and acidic compounds as possible indicators of selective retention processes during the transport of dissolved organic matter down the soil profile. Additionally, we determined C-to-N ratios of dissolved organic matter and applied liquid-state ^{13}C-NMR spectroscopy and δ^{13}C analysis to support the results of the wet-chemical analyses of specific compounds. Because the capacity of the studied soils to sorb dissolved organic matter is limited (Kaiser et al. 2001b) and the soils at the beech site were considered to be microbially highly active, we expected biodegradation to be of greater importance for the retention of dissolved organic matter than in the previously tested, pedogenetically more developed soils with large sorption capacities (Kalbitz et al. 2000; Michalzik et al. 2001).

Materials and methods

Field experiments

Sites. The experiments were carried out at two forested sites located on flat hilltops in NE-Bavaria, Germany. One site near Seybothenreuth (49°53′N, 11°41′E, 490 m above sea level), was covered with a 160-year-old Scots pine (*Pinus sylvestris* L.) forest. The soils, Haplic Arenosols (FAO–Unesco 1990), were derived from an Upper Triassic sandstone low in cementing agents. The mineral soils were strongly acid, low in nutrients and in organic C, and characterised by sandy texture with little variation in particle size (90% 0.5–1 mm). Soil aggregates were not present. The organic forest floor layer was mor-type. The vast majority of the fine roots of the trees and ground vegetation was in the forest floor layer. The mean annual precipitation during the experimental period (August 1997–November 1999) accumulated to 587 mm and the mean annual temperature was 8.3 °C. The second site located near Betzenstein (49°44′N, 11°23′E, 520 m above sea level) was in a 90-year-old European beech (*Fagus sylvatica* L.) stand. The soils were Rendzic Leptosols (FAO–Unesco 1990) deriving from Upper Kimmeridgian dolomite. They were strongly aggregated because of the loamy texture and the large contents of organic C. Flow funnels resulting from preferential weathering along cracks in the bedrock went through the whole profile. The organic forest floor layer was mull-type indicating strong biological activity. Despite these conditions thought to be favourable to rapid decomposition of litter, the mineral soil was covered with a litter layer of variable thickness throughout the entire experimental period. Trees and ground vegetation rooted exclusively in the mineral soil. The mean annual temperature during the study period was 8.2 °C and the mean annual precipitation was 956 mm. At both sites, snowfall contributed $<5\%$ of the precipitation during the experimental period. The clay mineralogy of all samples was dominated by illite with little contribution of kaolinite in soils at the pine site. More detailed information on the experimental sites, the organic forest floor layers, and the climatic conditions is given in Kaiser et al. (2001a). Chemical and physical properties of the mineral soils are summarised in Table 1.

Instrumentation and sampling. Three plots of 50 m × 50 m were selected at a minimum distance between each other of 200 m. Each plot was equipped with four stainless steel zero-tension lysimeters (Ø = 127 mm, mesh size 0.01 mm) directly beneath the organic forest floor layer. The zero-tension lysimeters were pre-washed with deionised water before installation. At 90 cm depth, four ceramic suction cups with a pore size $<40\,\mu m$ (Simplex K-100, UMS GmbH, München, Germany) were installed per plot using an auger and fitted to 2 l glass bottles. Suction cups were conditioned with a diluted water extract of the organic forest floor layer. The sampling equipment was equilibrated in the soil for 6 months and solution sampled during that time discarded. Suction cups removed from the soil after the end of the experimental period showed neither sorption nor release of organic or inorganic compounds in laboratory tests. On each plot, four tensiometers (Simplex T-100, UMS GmbH, München, Germany) were installed at 10, 20, 60, and 100 cm depth. Zero-tension lysimeters, suction cups, and tensiometers were placed at maximum distance between trees to avoid influence from stemflow. Tensions were measured weekly with a pressure conductor (Infield 5, UMS GmbH, München, Germany). The vacuum applied to the suction cups was regulated according to the measured tension of the soil water. We collected soil water from August 1997 to November 1999 at 7-day intervals during the growing season (April–November) and at least at 14-day intervals during the dormant season (December–March). The samples were measured for volume, filtered through 0.45 μm polysulfone membrane filters (Supor-450, Pall Gelman Science, Ann Arbor, MI), and then stored frozen ≤ -18 °C. In preliminary experiments, we found that this procedure had no effect on the concentrations and composition of dissolved organic matter. The amount of seepage water from the forest floor was calculated by normalising the volume of the sample to the area of the lysimeter.

Dissolved organic matter sample treatment

Aliquots of the solution samples were analysed for organic C using Pt-catalysed, high-temperature combustion (680 °C) followed by infrared detection of CO_2 (TOC-5050, Shimadzu Corp., Tokyo, Japan). Before determination of organic C,

Table 1. Properties of mineral soil horizons of Haplic Arenosols under a 160-year-old Scots pine (*Pinus sylvestris* L.) stand and Rendzic Leptosols under a 90-year-old European beech (*Fagus sylvatica* L.) forest (taken from Kaiser et al. 2001b). The chemical properties refer to the fine earth fraction (<2 mm). CEC = cation exchange capacity; CO_3-C = carbonate carbon; OC = organic carbon; TN = total nitrogen; Al_o, Fe_o = oxalate-extractable Al and Fe; Fe_d = dithionite–citrate–bicarbonate-extractable Fe. Samples were taken in October 1997.

Horizon	Depth (cm)	pH_{CaCl2}	CEC (mmol$_c$ kg^{-1})	CO_3–C (g kg^{-1})	OC (g kg^{-1})	TN (g kg^{-1})	Clay (g kg^{-1})	Al_o (g kg^{-1})	Fe_o (g kg^{-1})	Fe_d (g kg^{-1})
Haplic Arenosols										
A	0–5	2.9	21	0	15	0.3	20	0.1	0.1	0.3
C1	6–15	3.6	11	0	1	0.1	20	0.0	0.0	0.2
C2	16–95	3.6	9	0	1	0.1	20	0.1	0.1	0.4
Rendzic Leptosols										
A1	0–10	7.1	422	4	112	8.4	230	5.3	3.6	8.4
A2	11–25	7.3	284	24	69	5.6	210	4.7	3.3	7.7
C	26–95	7.6	63	70	11	0.9	70	1.4	1.6	5.0

the inorganic C was removed by adjusting the pH of the solution to 2 using concentrated H_3PO_4 and sparging with CO_2-free synthetic air at a flow rate of 50 ml min^{-1} for 2 min. No flocculation of organic matter was observed on acidification of the samples. Untreated samples were analysed for total N (photometric determination of NO_3^- after persulphate-UV digestion, SAN Plus, Skalar Analytical B.V., Breda, The Netherlands), NO_3^- and NH_4^+ (photometrically, SAN Plus). Dissolved organic N was calculated as the difference between total N and inorganic N.

A fraction of each sample (40 ml) was acidified to pH 2 with 1 M HCl, then pumped through small glass columns (4 ml) filled with Amberlite XAD-8 resin (Rohm & Haas Co., Philadelphia, PA) to determine the distribution of organic C and N among two operationally defined fractions, the so-called hydrophilic fraction and the so-called hydrophobic fraction (Aiken and Leenheer 1993; Raastad and Mulder 1999). The effluent of the columns, representing the hydrophilic fraction, was rapidly analysed for organic C and N (see above), and the amount of organic C and N in the hydrophobic fractions was calculated by difference between organic C and N in the bulk sample and in the hydrophilic fraction.

Volume-weighted portions of the bulk samples were combined into three-monthly samples representing the following periods: September – November 1997 (IV/97), December 1997 – February 1998 (I/98), March – May 1998 (II/98), June – August 1998 (III/98), September – November 1998 (IV/98), December 1998 – February 1999 (I/99), March – May 1999 (II/99), June – August 1999 (III/99), September – November 1999 (IV/99). The sample designation follows the meteorological definition of seasons. The combined samples were treated with a strongly acidic cation exchanger (AG-MP 50, BioRad Laboratories, Richmond, CA) in order to remove all other cations than H^+, and a portion was freeze-dried and then homogenised by grinding.

Calculation of fluxes

We calculated fluxes of dissolved organic C and N with seepage water from the organic forest floor layer into the mineral soil from the amounts of seepage water sampled by the zero-tension lysimeters beneath the forest floor layer and the concentrations therein.

Fluxes of C and N with the porewater in the mineral soil were calculated from porewater concentrations and simulated water fluxes. The simulation of water flux was carried out using soil water transport models (WHNSIM, Huwe and Totsche 1995; HYDRUS-2D, Simunek et al. 1996). Input variables for WHNSIM were meteorological data of the sites and physical properties of the soils. Root distribution and density was estimated from soil pits, and the evapotranspiration of the vegetation cover was estimated from literature (Peck and Mayer 1996; Köstner 2001) in conjunction with the meteorological data. In case of HYDRUS-2D, the water fluxes from the organic forest floor layer instead of precipitation were used as input data. The validation of the models was done according to measured soil water tensions. The simulated water fluxes were cross-checked by calculating the water fluxes from the measured tension gradients. All three methods gave comparable water fluxes for the mineral soil. To be consistent with previously published data we therefore based the calculation of the fluxes of dissolved organic C and N on the water fluxes simulated with WHNSIM.

Dissolved organic matter characterisation

Liquid-state ^{13}C-NMR spectroscopy. Freeze-dried three-month samples were analysed for C species using liquid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy. The ^{13}C-NMR spectra were obtained on an Avance DRX 500 spectrometer (Bruker Analytik GmbH, Karlsruhe, Germany) at 125.77 MHz. About 100–150 mg of the samples were dissolved in 3 ml of 0.5 M NaOD in a 10-mm NMR tube. At a pulse angle of 45°, 2.0-s pulse delay and inverse-gated proton decoupling, about 15,000 scans were accumulated for each sample. The signal-to-noise ratio was improved by using a line-broadening of 100 Hz. Chemical shifts were given relative to the resonance of tetramethylsilane and signal assignments were made according to literature (Orem and Hatcher 1987; Wilson 1987). The signal areas were calculated by electronic integration.

CuO oxidation products. The contents of lignin-derived phenols and their degree of oxidation in freeze-dried three-month dissolved organic matter samples were estimated using alkaline CuO oxidation at 170 °C for 2 h followed by solid-phase extraction. Phenols were determined as trimethylsilyl derivatives by gas chromatography. A detailed description of the analytical procedure is given in Kaiser et al. (2001a). Recoveries of lignin-derived phenols in all analyses were between 83 and 108% of an initial spike level. Analyses were carried out in duplicate.

Neutral and acidic carbohydrates. Individual neutral and acidic carbohydrates in freeze-dried three-month samples of dissolved organic matter were determined according to the method described by Amelung et al. (1996). For details on the measurement see Kaiser et al. (2001a). The recovery of carbohydrates in all analyses was between 72 and 112% of an initial spike level. Analyses were carried out in duplicate.

Amino sugars. Individual amino sugars (galactosamine, glucosamine, mannosamine, and muramic acid) in freeze-dried three-month samples of dissolved organic matter were analysed in duplicate by the method of Zhang and Amelung (1996). Details on the determination are given in Kaiser et al. (2001a). The recovery of amino sugars in all analyses was 78–115% compared to an initial spike.

Results and discussion

DOC concentrations and fluxes

Similar to other forest ecosystems, at the two study sites the concentrations and also the fluxes of DOC in the subsoils were considerably less than those in forest floor

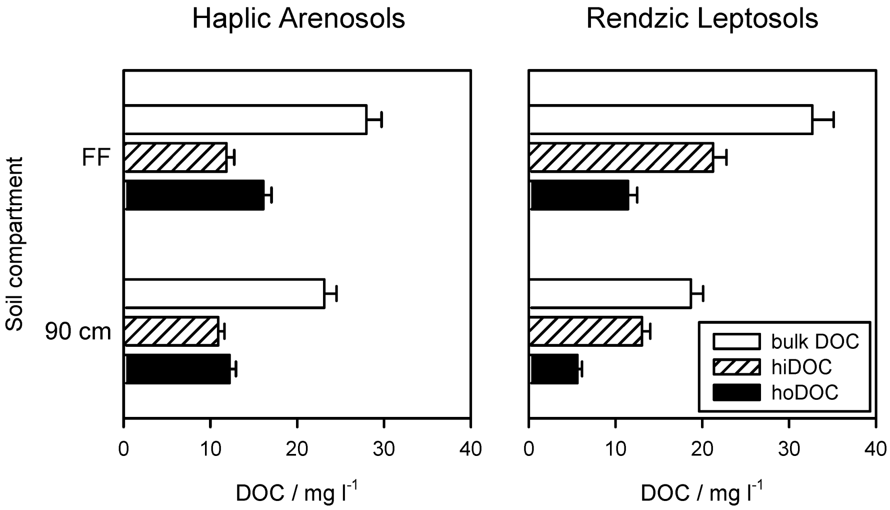

Figure 1. Mean concentrations of bulk dissolved organic carbon (bulk DOC) and of organic carbon in the hydrophilic and hydrophobic fractions of dissolved organic matter (hiDOC and hoDOC) in forest floor leachates (FF) and in soil solutions at 90 cm depth (90 cm) under a 160-year-old Scots pine (*Pinus sylvestris* L.) forest on Haplic Arenosols and under a 90-year-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols during the sampling period August 1997–November 1999. Error bars represent the standard error as measure of temporal variation ($n \geq 91$). Data taken from Kaiser et al. (2000).

Table 2. Mean annual fluxes of dissolved organic carbon (DOC) from the forest floor (FF) and in the mineral soil in 90 cm depth in Haplic Arenosols under Scots pine (*Pinus sylvestris* L.) and in Rendzic Leptosols under European beech (*Fagus sylvatica* L.) during the period August 1997–November 1999. In addition, percentages of the fluxes of organic carbon within the hydrophobic DOM fraction (hoDOC) are given ($n \geq 91$). Data taken from Kaiser et al. (2000).

Compartment	DOC (g m^{-2})	HoDOC (%)
Pine forest		
FF	8.81 ± 2.33	58
90 cm	4.62 ± 0.94	51
Beech forest		
FF	16.81 ± 5.46	32
90 cm	5.60 ± 1.05	25

leachates (Figure 1, Table 2). The decrease in DOC with depth, however, was far less than those reported for many Cambisols and Podzols under deciduous and coniferous forest stands (Michalzik et al. 2001). A possible reason for the relatively weak reduction in DOC with depth is the low sorption capacity of the Haplic

Arenosols and Rendzic Leptosols under study as compared with other soils (Kaiser et al. 2001b).

The reduction in DOC from the forest floor leachates down to the subsoil solutions was accompanied by a decrease in the percentage of hydrophobic DOC (Figure 1, Table 2). Preferential retention of hydrophobic DOC seems to be a common feature of the transport of dissolved organic matter through the mineral soil (Qualls and Haines 1991; Kaiser et al. 2002), most likely because hydrophobic dissolved organic matter sorbs far stronger to mineral surfaces than hydrophilic organic compounds (Qualls and Haines 1991; Andersson et al. 1999). The decrease in hydrophobic DOC was more pronounced in the Rendzic Leptosols (by 22%) than in the Haplic Arenosols (by 12%) which is in agreement with the stronger sorption of hydrophobic (and thus also of total) DOC by the Rendzic Leptosols found in laboratory experiments (Kaiser et al. 2001b).

Because hydrophilic fractions seem to be the most biodegradable portions of dissolved organic matter (Qualls and Haines 1991; Jandl and Sollins 1997), the preferential decrease of hydrophobic DOC and the relative accumulation of hydrophilic compounds in soil water suggest that not biodegradation but sorption to mineral surfaces was the major reason for the decrease in DOC with soil depth. Similar conclusions have been drawn also in previous studies (Qualls and Haines 1992; Andersson et al. 1999; Kaiser et al. 2002). Interestingly, even in Rendzic Leptosols which are usually considered to have a high microbial activity, the hydrophobic DOC was retained preferentially and thus sorption is most likely to control the retention of dissolved organic matter.

Composition of dissolved organic matter according to liquid-state ^{13}C-NMR spectroscopy

Parallel to the decrease in hydrophobic DOC, ^{13}C-NMR spectroscopy suggested a slight decrease in aromatic and carbonyl moieties with depth (Figure 2) which is in good agreement with previous findings on the preferential removal of aromatic C and carboxyl C from solution when dissolved organic matter interacts with Al and Fe oxides–hydroxides (McKnight et al. 1992, 2002; Kaiser et al. 1997). In balance for the decrease in carbonyl and aromatic C, either alkyl C (Haplic Arenosols) or O-alkyl C (Rendzic Leptosols) increased. For the Haplic Arenosol, the average ratio of aromatic C to alkyl C decreased from 1.05 ± 0.15 in the forest floor leachates to 0.74 ± 0.13 in the subsoil solutions. The respective values for the Rendzic Leptosol were 0.34 ± 0.14 and 0.29 ± 0.11. The changes with depth of the ratio of aromatic C to O-alkyl C were from 0.60 ± 0.17 to 0.54 ± 0.14 for the Haplic Arenosol and from 0.28 ± 0.13 to 0.18 ± 0.09 for the Rendzic Leptosol. Compared to the changes in the composition of dissolved organic matter upon sorptive interactions with Al and Fe oxides–hydroxides, the changes found here were small. This might be due to the relatively weak sorption/retention of DOC (see above) by the tested soils as compared with pure hydrous oxides phase studied by McKnight et al. (1992, 2002) and Kaiser et al. (1997).

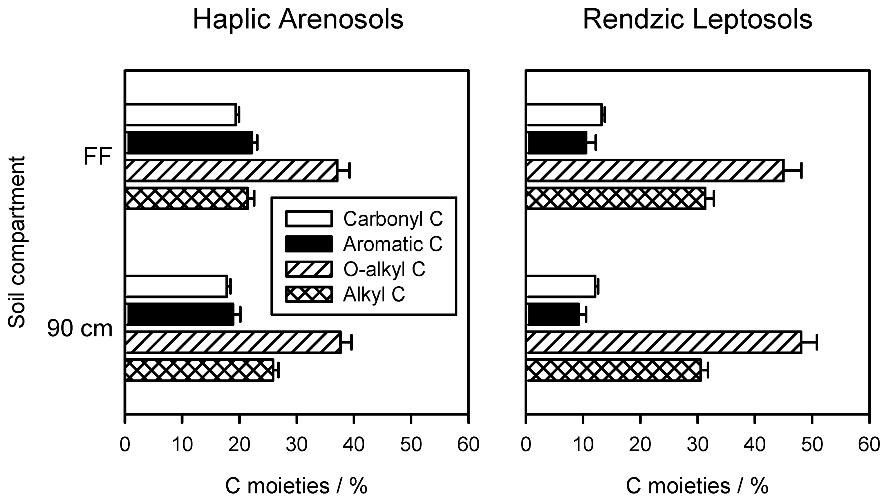

Figure 2. Mean distribution of carbon moieties in dissolved organic matter in forest floor leachates (FF) and in soil solutions at 90 cm depth (90 cm) under a 160-year-old Scots pine (*Pinus sylvestris* L.) forest on Haplic Arenosols and under a 90-year-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols during the sampling period August 1997–November 1999 as revealed by liquid-state ^{13}C nuclear magnetic resonance spectroscopy. The error bars represent the standard error as measure of the temporal variation ($n = 9$).

Lignin-derived phenols and hydrolysable sugars and amino sugars

In agreement with the decrease of hydrophobic DOC and aromatic C with soil depth, the content of lignin-derived CuO oxidation products in dissolved organic matter samples declined at both study sites (Figure 3). The strong affinity of lignin-derived acidic compounds for Al and Fe oxides–hydroxides (Jekel 1986; Kaiser et al. 2002) is a reasonable explanation for both the decrease in hydrophobic DOC and aromatic C with depth. Lignin decomposition products were most abundant in the hydrophobic fractions of dissolved organic matter at the study sites while the hydrophilic fractions seem to contain few or no lignin-derived compounds (Kaiser et al. 2001a). Moreover, the ratio of acid to aldehyde of the vanillyl units $[(Ac/Al)_V]$ decreased with soil depth suggesting a stronger retention of acidic phenols than of aldehydes (Figure 3). Because oxidative biodegradation of dissolved organic matter typically results in an increase of the $(Ac/Al)_V$ ratio (Ertel et al. 1986; Guggenberger and Zech 1994), sorption of acidic compounds seems to be a reasonable explanation of this finding. The decrease in acidic compounds agrees also with the decrease in carboxyl C (Figure 2). The decrease in lignin decomposition products was stronger in the Rendzic Leptosols that showed a stronger retention of hydrophobic DOC in the field (Figure 1, Table 2) and in laboratory sorption experiments (Kaiser et al. 2001b) than in the Haplic Arenosols. Increasing $\delta^{13}C$ ratios of dissolved organic matter with depth (Kaiser et al. 2001b) also suggest stronger

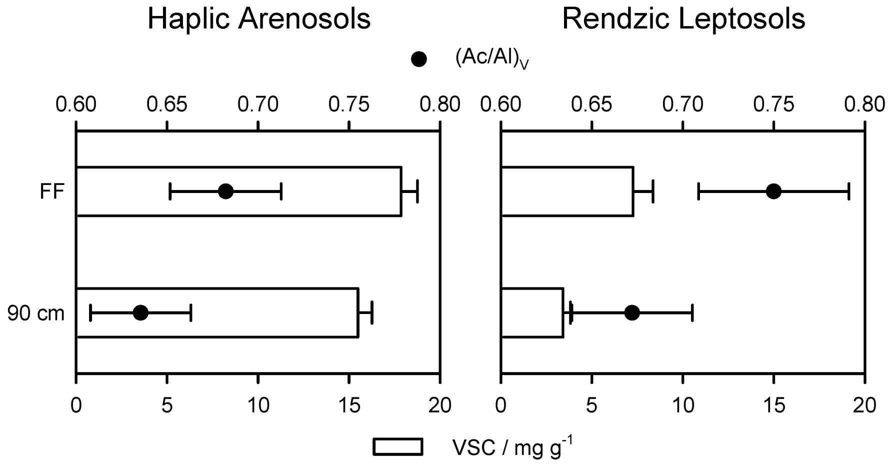

Figure 3. Mean contents of lignin-derived phenols (sum of vanillyl, syringyl, and cinnamyl CuO oxidation products, VSC) and mean acid-to-aldehyde ratios of the vanillyl units [$(Ac/Al)_V$] of dissolved organic matter in the forest floor leachates (FF) and in soil solutions at 90 cm depth (90 cm) under a 160-year-old Scots pine (*Pinus sylvestris* L.) forest on Haplic Arenosols and under a 90-year-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols during the sampling period August 1997–November 1999. The error bars represent the standard error as measure of the temporal variation ($n = 9$).

retention of compounds low in ^{13}C than of compounds rich in ^{13}C. Lignin typically shows a lower $\delta^{13}C$ than bulk organic matter (Benner et al. 1987).

In contrast to the lignin-derived phenols, the concentrations of carbohydrates in dissolved organic matter remained either constant (Haplic Arenosols) or increased (Rendzic Leptosols) with depth (Figure 4). This means that the carbohydrate concentrations were little affected during the passage of the mineral soil in the Haplic Arenosols. Carbohydrates were retained to a lesser extent than compounds such as lignin decomposition products in the Rendzic Leptosols which fits to the stronger relative retention of hydrophobic DOC by these soils compared to the Haplic Arenosols (Figure 1, Table 2) and to the increase in O-alkyl C (Figure 2). In agreement with the results on the distribution of aldehydes and acids among lignin-derived phenols (Figure 3), the ratio of neutral sugars to uronic acids increased with depth at both sites (Figure 4). This decrease in acidic compounds again indicates sorption as the most likely mechanism for the retention of dissolved organic compounds in the studied soils. In case of a significant contribution of biodegradation, the portions of acidic compounds should increase because of microbial oxidation of sugars.

The ratios of hexoses to pentoses remained largely constant with depth (results not shown) at both sites indicating that the composition of carbohydrates was unaffected during the transport of dissolved organic matter into the subsoil. The lack in shift from plant-derived pentoses to microbially-derived hexoses contrasts the idea of dissolved organic matter being affected by biodegradation or microbial

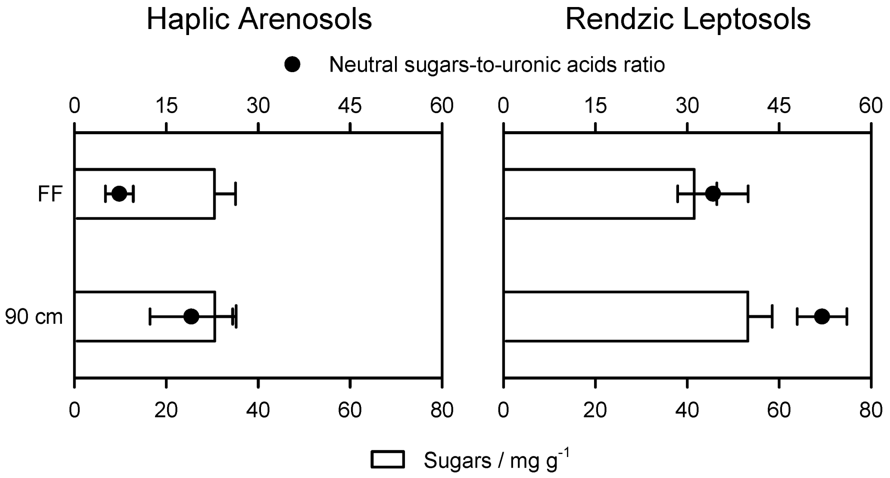

Figure 4. Mean total contents of hydrolysable sugars and mean neutral sugars-to-uronic acids ratios of dissolved organic matter in the forest floor leachates (FF) and in soil solutions at 90 cm depth (90 cm) under a 160-year-old Scots pine (*Pinus sylvestris* L.) forest on Haplic Arenosols and under a 90-year-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols during the sampling period August 1997–November 1999. The error bars represent the standard error as measure of the temporal variation ($n = 9$).

growth during its movement downwards through the soils. Also, there seemed to be neither preferential sorption nor preferential release by or from roots of certain neutral carbohydrates in the mineral soil.

The results for the amino sugars were comparable to those for neutral and acidic carbohydrates. In the Haplic Arenosols, the amino sugar concentrations of dissolved organic matter remained more or less constant with depth whereas the concentrations increased in the Rendzic Leptosols (Figure 5) because of the preferential retention of the hydrophobic fraction (Figure 1) which contains few amino sugars (Kaiser et al. 2001a). The ratios of neutral amino sugars to muramic acid increased with depth at both sites (Figure 5) which is in agreement with the preferential sorption of muramic acid observed in a laboratory sorption experiment (Kaiser and Zech 2000) and thus likely not indicative for changes due to biodegradation and/or microbial production.

In general, the results on changes in the composition of dissolved organic matter agreed well with those obtained for soils with a large capacity to sorb organic matter (Cambisols, Podzols) examined in a similar experiment (Guggenberger and Zech 1994).

Carbon-to-nitrogen ratios of dissolved organic matter

The interpretation of the DOC/DON ratios (Figure 6) of dissolved organic matter and its fractions contains uncertainties resulting from the fact that DON is

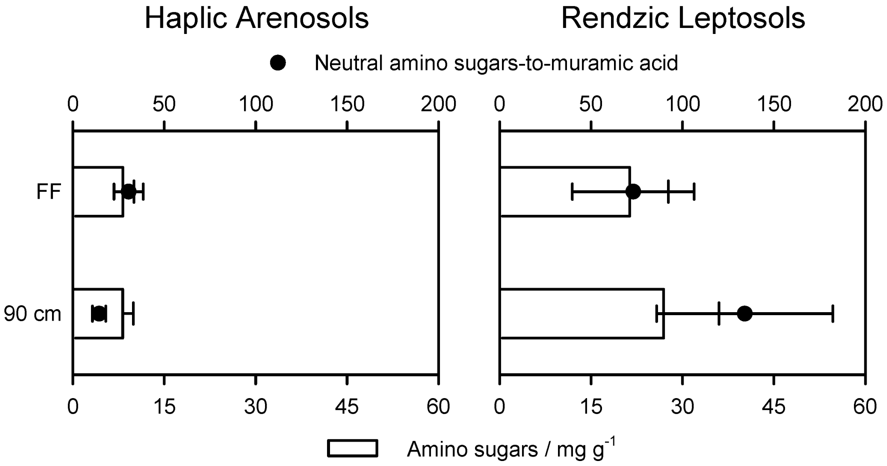

Figure 5. Mean total contents of hydrolysable amino sugars and mean neutral amino sugars-to-muramic acid ratios of dissolved organic matter in the forest floor leachates (FF) and in soil solutions at 90 cm depth (90 cm) under a 160-year-old Scots pine (*Pinus sylvestris* L.) forest on Haplic Arenosols and under a 90-year-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols during the sampling period August 1997–November 1999. The error bars represent the standard error as measure of the temporal variation ($n = 9$).

calculated by difference and so includes the errors of determination of total N, NO_3^- and NH_4^+. Moreover, DOC as well as DON in the hydrophobic fraction are also calculated by difference. Propagation of error therefore is likely. Despite the small standard errors found, much larger errors may be associated with the calculated DOC/DON ratios.

The DOC/DON ratio of bulk dissolved organic matter and of its fractions did not change with depth in the Haplic Arenosols (Figure 6) suggesting that compounds rich in N were neither preferentially retained nor excluded from sorption which is in agreement with the findings on amino sugars in these soils. However, amino sugars usually contain only a minor portion of dissolved organic nitrogen; the vast majority of organic nitrogen is within amino acids (Michalzik and Matzner 1999) which sorb strongly to Al and Fe oxides–hydroxides (McKnight et al. 1992). Amino sugars comprised at maximum 32% of dissolved organic nitrogen in the Haplic Arenosols. Consequently, the lack of changes in the DOC/DON ratios might indicate a weak retention of amino acids in the Haplic Arenosols which, however, contrasts the strong retention of muramic acid. One possible explanation could be a dominance of basic amino acids that are likely to be retained weakly in the Haplic Arenosols because of the low cation exchange capacity of the mineral horizons (Table 1). Another possible reason might be that amino acid-bound nitrogen exists mainly within neutral protein molecules or attached to other compounds that do not interact with mineral surfaces. Because protein sorbs strongly to various mineral surfaces (Fusi et al. 1989), attachment to mobile carbohydrates seems to be more reasonable.

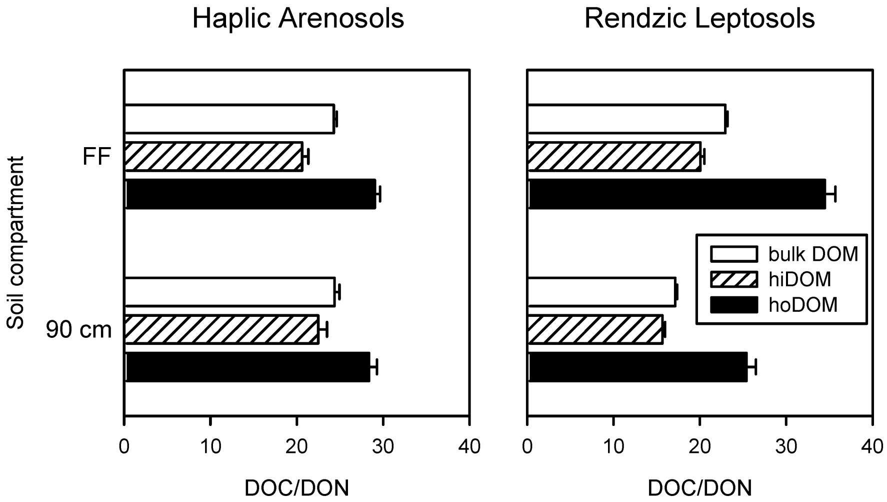

Figure 6. Mean DOC/DON ratios of bulk dissolved organic matter (bulk DOM) and its hydrophilic and hydrophobic fractions (hiDOM and hoDOM) in the forest floor leachates (FF) and in soil solutions at 90 cm depth (90 cm) under a 160-year-old Scots pine (*Pinus sylvestris* L.) forest on Haplic Arenosols and under a 90-year-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols during the sampling period August 1997–November 1999. The error bars represent the standard error as measure of the temporal variation ($n \geq 91$).

In the Rendzic Leptosols, the DOC/DON ratio of bulk dissolved organic matter as well as of the dissolved organic matter fractions decreased with depth (Figure 6) which suggests that dissolved organic compounds rich nitrogen were less strongly retained than compounds low in nitrogen. Similar observations have been made in field and laboratory experiments and attributed to the concentration of nitrogen in the weakly sorbing hydrophilic fraction of dissolved organic matter (Qualls and Haines 1991; Andersson et al. 1999; Kaiser and Zech 2000). Actually, the hydrophilic fraction contained on average 80% of dissolved organic nitrogen in the forest floor leachates and 85% in the subsoil solutions. Like in the Haplic Arenosols, amino sugars comprised only a minor proportion of dissolved organic nitrogen (<41%) which means that amino acids likely were the major contributors to dissolved organic nitrogen also in the Rendzic Leptosols. The weak retention of organic nitrogen combined with the high percentage of organic nitrogen in potentially strongly sorbing amino acids suggests that amino acids appear not in free form in soil water but probably attached to weakly sorbing compounds.

Conclusions

1. The results underline that, independent of the type of soil, the hydrophobic fraction of dissolved organic matter rich in lignin-derived compounds is less mobile than the hydrophilic fraction which comprises mainly carbohydrates.

The stronger retention of the hydrophobic fraction is likely due to the strong sorption of lignin-derived compounds by Al and Fe oxides–hydroxides.

The loss of lignin-derived compounds and consequently that of aromatic moieties possibly will reduce dissolved organic matter-mediated transport of hydrophobic organic contaminants in subsoils and also the export from soil to aquatic environments. However, this might be balanced by the increase in alkyl moieties that also can interact with organic contaminants.

2. Compared to lignin-derived compounds, carbohydrates remain either constant with depth or increase. In the latter case, this results in an enrichment of subsoil dissolved organic matter in potentially labile material which might serve as energy source for microbial processes when dissolved organic matter is exported into ground and surface waters. The increasing nitrogen concentration in dissolved organic matter with depth adds to the potentially high biodegradability of dissolved organic matter exported from soil.
3. From all compound classes tested, neutral constituents were retained less strongly than acidic ones which was probably due to stronger sorptive interactions between substances with acidic functional groups and mineral surfaces.

The effect on mineral weathering and metal mobilisation and transport of subsoil dissolved organic matter will be limited due to the reduced content of acidic groups.

The conclusions made here agree with previous work suggesting that sorption rather than biodegradation is controlling the decrease in dissolved organic matter with soil depth which has been observed in many forest soils. However, the used approach is unique as well as the studied soils are. Thus further experiments will be needed to justify the generality of the findings presented here.

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