Biogeochemistry **55:** 103–143, 2001.

Seasonal variations in the chemical composition of dissolved organic matter in organic forest floor layer leachates of old-growth Scots pine (*Pinus sylvestris* L.) and European beech (*Fagus sylvatica* L.) stands in northeastern Bavaria, Germany

KLAUS KAISER*, GEORG GUGGENBERGER, LUDWIG HAUMAIER & WOLFGANG ZECH
*Institute of Soil Science and Soil Geography, University of Bayreuth, 95440 Bayreuth, Germany (*Author for correspondence, e-mail: klaus.kaiser@uni-bayreuth.de)*

Key words: amino sugars, δ^{13}C values, dissolved organic carbon, lignin decomposition products, liquid-state ^{13}C NMR, neutral and acidic carbohydrates, seasonal variations, XAD-8 fractionation

Abstract. Organic matter dissolved in the percolation water of forest soils contributes largely to element cycling and transport of natural and anthropogenic compounds. The way and extent to which these processes are affected depends on the amount and the chemical composition of soluble organic matter. Because the amount of soluble organic matter varies seasonally with changes in the microbial activity in soil, it seems reasonable to assume that there may be also seasonal changes in the chemical composition of dissolved organic matter. We examined dissolved organic matter in the seepage waters of organic forest floor layers over a 27-month period (1997–1999) in two forest ecosystems, a 160-year-old Scots pine (*Pinus sylvestris* L.) stand and a 90-year-old European beech (*Fagus sylvatica* L.) forest. The forest floor leachates were analysed for bulk dissolved organic C, C in hydrophilic and hydrophobic dissolved organic matter fractions, lignin-derived phenols (CuO oxidation), hydrolysable neutral carbohydrates and uronic acids, hydrolysable amino sugars, and stable carbon isotope composition. In addition, we studied the samples by use of liquid-state ^{13}C-nuclear magnetic resonance (NMR) spectroscopy.

For both investigated forest sites we found that the dissolved organic carbon concentrations in forest floor leachates were largest during summer. They peaked after rain storms following short dry periods (106–145 mg dissolved organic C l^{-1}). The proportions of C in the hydrophilic fractions were largest in winter and spring whereas in summer and autumn more C was found in the hydrophobic fraction. According to liquid-state ^{13}C-NMR spectroscopy, summer and autumn samples had larger abundances of aromatic and aliphatic structures as well as larger proportions of carboxyl groups whereas the winter and spring samples were dominated by resonances indicating carbohydrates. Wet-chemical analyses confirmed these results. Winter and spring samples were rich in neutral carbohydrates and amino sugars. The summer and autumn samples contained more lignin-derived phenols which were also stronger oxidised than those in the winter and spring samples. Seasonal changes of δ^{13}C values were found to reflect the changes in the chemical composition of dissolved organic matter. Most negative values occurred when isotopically light lignin-derived compounds were abundant and less negative values when carbohydrates predominated.

The different vegetation, age of the stands, and underlying mineral soils resulted in different concentrations of dissolved organic carbon and in differences in the distribution between hydrophobic and hydrophilic organic carbon. Despite of this, the results suggest that the trends in temporal variations in the composition of dissolved organic matter in forest floor seepage water were remarkably similar for both sites. Dissolved organic matter in winter and spring seems to be mainly controlled by leaching of fresh disrupted biomass debris with a large contribution of bacterial and fungal-derived carbohydrates and amino sugars. Dissolved organic matter leached from the forest floor in summer and autumn is controlled by the decomposition processes in the forest floor resulting in the production of strongly oxidised, water-soluble aromatic and aliphatic compounds. The chemical composition of dissolved organic matter in forest floor seepage water in winter and spring indicates larger mobility, larger biodegradability, and less interaction with metals and organic pollutants than that released during summer and autumn. Thus, the impact of dissolved organic matter on transport processes may vary throughout the year due to changes in its composition.

Introduction

Dissolved organic matter is an important constituent in the soil environment of forest ecosystems because of its manifold interactions with other solutes, colloids, and solid material (Kalbitz et al. 2000). It contributes to the mobilisation and transport of metals (Pohlman & McColl 1988), nutrients (Donald et al. 1993), acidity (Guggenberger & Kaiser 1998), and hazardous compounds (McCarthy & Zachara 1989). In addition, it changes the properties of soil surfaces by inducing weathering (Raulund-Rasmussen et al. 1998) and sorptive interactions (Murphy et al. 1990), and it controls the colloidal properties of particles (Kretzschmar & Sticher 1997). The extent of these interactions is a function of the concentration but also of the chemical composition of dissolved organic matter. Certain structural units and fractions are differently affected at sorption to the mineral surfaces (McKnight et al. 1992; Edwards et al. 1996; Kaiser et al. 1997; Meier et al. 1999; Moore & Matos 1999), at binding of metals and organic pollutants (Pohlman & McColl 1988; Kukkonen et al. 1990; Raber & Kögel-Knabner 1997), and by biodegradation (Qualls & Haines 1992; Boyer & Groffman 1996; Jandl & Sollins 1997). Consequently, the dissolved organic matter-related transport processes, e.g. of nutrients, are affected by changes in the composition of dissolved organic matter (Qualls & Haines 1991; Andersson et al. 1999).

Numerous studies on forest ecosystems have shown that the amount of dissolved organic matter released from forest floors and organic layers relates to seasonal changes (e.g., McDowell & Likens 1988; Scott et al. 1998;

Michalzik & Matzner 1999; Hongve 1999; Tipping et al. 1999). Besides to hydrological variations throughout the course of the year (e.g., Easthouse et al. 1992; Mulholland & Hill 1997; Hinton et al. 1998), these changes have been attributed to changes in the microbial activity (e.g., Guggenberger et al. 1998; Tipping et al. 1999). Based on observations made on dissolved organic matter in surface and stream waters (Clair et al. 1996; Stepanauskas et al. 2000), we hypothesised that varying microbial activity during the year may not only affect the amount of released dissolved organic matter but also its composition. Such changes may lead to changing bioavailability and changing interactions with other solutes, colloids, and mineral surfaces and thus may alter dissolved organic matter-induced soil processes significantly. First observations made on total amino N and amino sugar N in forest floor leachates of a Norway spruce forest indicates large seasonal fluctuations in the composition of dissolved organic N (Michalzik & Matzner 1999).

We tested this hypothesis by sampling dissolved organic matter of forest floor leachates throughout a 27-month period in two old-growth forest stands (Scots pine, European beech). The samples were characterised by means of XAD-8 fractionation (Leenheer 1981; Aiken & Leenheer 1993), liquid-state ^{13}C-NMR spectroscopy, wet-chemical analyses (lignin-decomposition products, neutral and acidic carbohydrates, amino sugars), and determination of stable carbon isotope composition.

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 with a maximum ground coverage by the canopy close to 70%. The ground vegetation was dominated by bilberry (*Vaccinium myrtillus* L.) with small patches covered with *Deschampsia flexuosa* (L.) Trin. and various moss species. 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 to 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 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 ground coverage by the forest canopy approached 95% in summer. Ground vegetation was little throughout the year and was dominated by *Anemone nemorosa* L. in early spring and later on by *Mercuralis perennis* L. The soils were Rendzic Leptosols (FAO–Unesco 1990) deriving from Upper Kimmeridgian dolomite. They were strongly aggregated due to the loamy texture and the large content 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 favourable conditions, 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. Basic properties of the soils are summarised in Table 1. The clay mineralogy of all samples was dominated by illite with little contribution of kaolinite in soils at the pine site.

Instrumentation and sampling. At each of the experimental sites, three plots of 50 × 50 m were selected. The minimum distance between these plots was 200 m. Each plot was equipped with four stainless steel zero-tension lysimeters (∅ = 127 mm, mesh size 0.01 mm) directly beneath the organic forest floor layer. The lysimeters were installed laterally from soil pits by inserting them into a 25 to 30 cm deep pre-cut slit and connected to 2-l glass bottles below them. The lysimeters were pre-washed with deionized water and equilibrated in the soil for six months before the beginning of the sampling period. Forest floor seepage water collected during that time was discarded. Lysimeters were placed at mean maximum distance between trees to avoid influence from stemflow. We sampled forest floor seepage water from the beginning of August 1997 to the end of November 1999 at 7-d intervals from April to November and at least at 14-d intervals from December to March. The leachate samples were measured for volume and filtered through 0.45-μm polysulfone membrane filters (Supor-450, Pall Gelman Science, Ann Arbor, MI) and stored frozen at – 18 to −30 °C. The amount of seepage water from the forest floor was calculated by normalising the volume of the sample to the area of the lysimeter.

The air temperature was measured 20 cm above the ground using electronic thermometers connected to data loggers (Delta-T Devices Ltd., Cambridge, UK). Temperature reading was at 15-min intervals. The amount of rainfall was determined using rainfall collectors made of plastic funnels

Table 1. Chemical characteristics of organic forest floor layers in a 160-yr-old Scots pine (*Pinus sylvestris* L.) forest on Haplic Arenosols and in a 90-yr-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols. Samples were taken in October 1997. Methods for determination of CuO oxidation products, of hydrolysable sugars and amino sugars, and of $\delta^{13}C$ values were the same as for dissolved organic matter samples. Σ VSC = sum of vanillyl, syringyl, and cinnamyl CuO oxidation products; $(ac/al)_V$ = acid-to-aldehyde ratios of the vanillyl units in CuO oxidation products; $(ac/al)_S$ = acid-to-aldehyde ratios of the syringyl units in CuO oxidation products; Σ sugars = sum of hydrolysable neutral and acidic carbohydrates; [(Man+Gal)/(Xyl+Ara)] = ratios of mannose plus galactose to xylose plus arabinose; [(Rha+Fuc)/(Xyl+Ara)] = ratios of rhamnose plus fucose to xylose plus arabinose; Σ amino sugars = sum of hydrolysable amino sugars; GluN/GalN = ratios of glucosamine to galactosamine

Site Horizon	Thickness cm	pH[1] ($CaCl_2$)	OC[2] mg g^{-1}	N[2] mg g^{-1}	Σ VSC mg g^{-1}	$(ac/al)_V$	$(ac/al)_S$	Σ sugars mg g^{-1}	[(Man+Gal)/ (Xyl+Ara)]	[(Rha+Fuc)/ (Xyl+Ara)]	Σ amino sugars mg g^{-1}	GluN/GalN	$\delta^{13}C$ ‰ PDB
Pine forest (mor-type forest floor)													
Oi	2	3.5	498	13	31.1	0.2	0.3	128	1.4	0.2	1.4	19.1	–29.1
Oe	4	3.0	476	13	23.6	0.3	0.4	98	1.3	0.3	4.7	3.5	–27.9
Oa	4	2.6	377	14	9.8	0.8	0.9	59	1.8	0.4	4.6	3.4	–26.7
Beech forest (mull-type forest floor)													
Oi	5	5.8	481	16	30.5	0.1	0.1	141	0.3	0.1	3.3	3.4	–30.7

[1] measured potentiometrically at a soil-to-solution ratio of 1:2.5 and 1 h reaction time.

[2] measured on ground subsamples with a CN analyser (NC 2500, ThermoQuest-Fisons, Milan, Italy).

(∅ = 200 mm) fitted to 2-l bottles. At both sites, 12 of those collectors were placed on clearings close to the experimental plots (<400 m) and the volume of rainwater was measured at the same intervals as the lysimeters were sampled. The amount of rain was calculated by normalising the volume of rainwater to the area of the rainfall collectors.

Dissolved organic matter sample treatment

Bulk organic matter in seepage water from organic forest floor layers. Bulk samples of seepage water 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, 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. A part of the bulk samples (20 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 and Haas Co., Philadelphia, PA) to determine the distribution of organic C among two operationally defined fractions, the so-called hydrophilic fraction and the so-called hydrophobic fraction (Aiken & Leenheer 1993). The effluent of the columns, representing the hydrophilic fraction, was analysed for organic C, and the amount of organic C in the hydrophobic fractions was calculated by difference between organic C in the bulk sample and in the hydrophilic fraction.

Three-monthly fluxes of organic C in the bulk samples and the hydrophilic and hydrophobic fractions of seepage water from the forest floor layers were calculated from the concentration data and the amounts of seepage water.

Volume-weighted portions of the bulk samples were combined into monthly and three-monthly samples. The three-monthly samples represented the following periods: September to November 1997 (IV/97), December 1997 to February 1998 (I/98), March to May 1998 (II/98), June to August 1998 (III/98), September to November 1998 (IV/98), December 1998 to February 1999 (I/99), March to May 1999 (II/99), June to August 1999 (III/99), September to 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.

Hydrophilic and hydrophobic dissolved organic matter fractions. Bulk dissolved organic matter samples combined by month or three months were separated into hydrophilic and hydrophobic fractions (Aiken & Leenheer

1993). The solutions were acidified to pH 2 with 1 *M* HCl, analysed for organic C, and pumped through large columns (200 ml) filled with XAD-8. In the effluent of the XAD-8 columns, representing the hydrophilic fraction, the organic C concentration was measured (see above). Dissolved organic C in the hydrophobic fraction was calculated by difference. After passage of the bulk dissolved organic matter samples, the columns were rinsed with water adjusted to pH 2 with 1 *M* HCl. The rinse water was combined with the hydrophilic fraction, freeze-dried and homogenised by grinding. The hydrophobic fraction was eluted from the columns with 0.1 *M* NaOH and the organic C concentration in the effluent was measured (see above). Mass-balance calculations showed that $>97\%$ of the C in the hydrophobic fraction was recovered from the XAD-8 resin. This indicated that the contribution of hydrophobic neutral compounds was little in the samples used and that therefore the hydrophobic fraction consisted mainly of acidic substances. The hydrophobic fractions were protonated with a strongly acidic cation exchanger (AG-MP 50), acidified to pH 2 with 1 *M* HCl, and finally freeze-dried and homogenised by grinding.

Characterisation of the chemical composition of dissolved organic matter

Liquid-state ^{13}C nuclear magnetic resonance spectroscopy. Freeze-dried bulk three-month samples and hydrophilic and hydrophobic dissolved organic matter fractions from IV/97 of both sites were analysed for chemical structures 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 to 150 mg of the samples was 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 (Wilson 1987; Orem & Hatcher 1987).

CuO oxidation products. The contents of lignin-derived phenols and their degree of oxidation in freeze-dried bulk three-month samples and in hydrophilic and hydrophobic dissolved organic matter fractions from IV/97 were estimated using alkaline CuO oxidation at 170 °C for 2 h (Hedges & Ertel 1982). Instead of the liquid–liquid extraction, a solid-phase extraction with C-18 columns (Mallinckrodt Baker Corp., Phillipsburg, NJ) was used. Phenols were eluted and derivatized with a 1:1 mixture of pyridine and N,O-bis(trimethysilyl)-trifluoroacetamide. The derivatives were separated and quantified by a gas chromatograph equipped with a flame ionisation detector (Hewlett-Packard 6890, Agilent Technologies, Inc., Palo Alto, CA) and an Ultra 2 fused silica column of 25 m length (Agilent Technologies, Inc., Palo Alto, CA). Ethylvanilline was added as internal standard prior to the CuO oxidation and phenylacetic acid before derivatisation. To increase the recoveries, 50 mg glucose was added prior to the CuO oxidation (Amelung et al. 1999a). Recoveries were between 85 and 108% of the initial spike level. Analyses were carried out in duplicate.

Neutral and acidic carbohydrates. Individual neutral and acidic carbohydrates in freeze-dried bulk three-month samples and in hydrophilic and hydrophobic dissolved organic matter fractions from IV/97 were determined according to the method described by Amelung et al. (1996). Freeze-dried organic matter samples were treated with 4 *M* trifluoroacetic acid at 105 °C for 4 h, then filtered through glass fibre filters, dried by rotary evaporation, re-dissolved in 3 ml of deionized water, and purified with Amberlite XAD-7 (Rohm and Haas Co., Philadelphia, PA) and Dowex 50W X8 (Dow Chemical Co., Midland, MI) resins. The carbohydrates were transformed into O-methyloxime trimethylsilyl derivatives, separated by capillary gas chromatography using an HP 5 fused silica column (Agilent Technologies, Inc., Palo Alto, CA) of 25 m length and N_2 as carrier gas. Separated compounds were detected by flame ionisation. Myo-inositol was added as internal standard prior to hydrolysis, and 3-*O*-methylglucose was added as internal standard prior to the derivatisation. The recovery of carbohydrates in all analyses was between 73 and 112% of the initial spike level. Analyses were carried out in duplicate.

Amino sugars. Individual amino sugars (galactosamine, glucosamine, mannosamine, and muramic acid) in freeze-dried bulk three-month samples and in hydrophilic and hydrophobic dissolved organic matter fractions from IV/97 were analysed using the method of Zhang and Amelung (1996). Briefly, the samples were hydrolysed with 6 *M* HCl at 105 °C for 8 h, then the hydrolysates were filtered and neutralised. After freeze-drying, the residues were re-dissolved in methanol, the resulting solutions were dried again by air, and then derivatised to aldononitrile acetates. These derivatives were separated by capillary gas chromatography using an HP 5 fused silica column (Agilent Technologies, Inc., Palo Alto, CA) of 25 m length and detected by flame ionisation. Myo-inositol was added as internal standard before hydrolysis and methyl-glucamine as recovery standard before derivatisation. The recovery of amino sugars in all analyses was 80 to 115% compared to the initial spike. Analyses were carried out in duplicate.

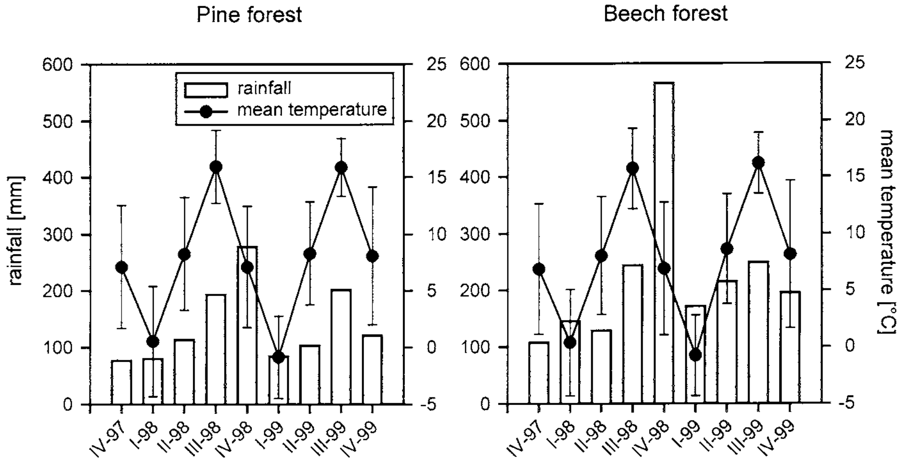


Figure 1. Sum of rainfall and mean air temperature 20 cm above ground at the two studied forested sites during the following three-month periods: September–November 1997 (IV/97), December 1997–February 1998 (I/98), March–May 1998 (II/98), June–August 1998 (III/98), September–November 1998 (IV/98), December 1998–February 1999 (I/99), March–May 1999 (II/99), June–August 1999 (III/99), September–November 1999 (IV/99). The error bars of temperature represent the standard deviation of each three-month period.

$\delta^{13}C$ values. The δ^{13}C values of freeze-dried one-month bulk samples were obtained with an isotope ratio mass spectrometer (Delta S, ThermoQuest-Finnigan MAT, Bremen, Germany) connected to an elemental analyser (NC 2500, ThermoQuest-Fisons, Milan, Italy). The isotope ratios were compared with that of reference CO_2 (Linde AG, Unterschleißheim, Germany) and calibrated against NBS19–limestone (National Institute of Standards and Technology, Gaithersburg, MD) and sucrose ANU (International Atomic Energy Agency, Vienna, Austria). The δ^{13}C expresses the enrichment of ^{13}C in a sample compared to CO_2 prepared from a calcareous belemnite of the cretaceous Peedee formation, South Carolina. Analyses were carried out in triplicate.

Results and discussion

Climatic conditions and water percolation through the forest floor

During the 27-month experimental period, the temperature at the two studied stands showed a similar course during the year (Figure 1). The mean temperatures during the summers (June–August) were between 15 and 16 °C and during the winters (December–February) around –1 °C. Mean temperatures

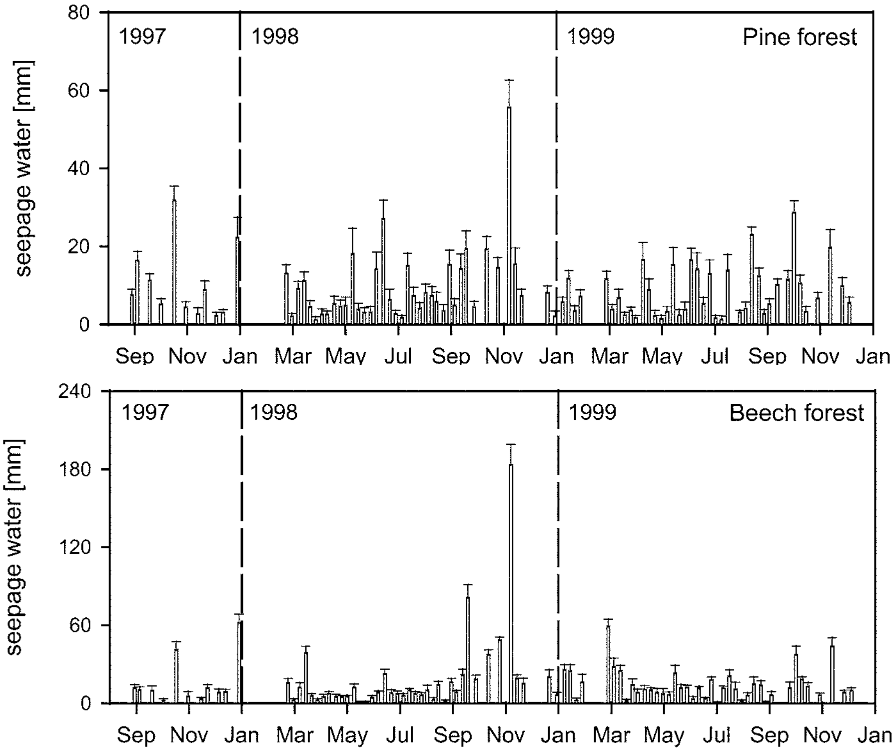


Figure 2. Amounts of seepage waters from the organic forest floor layers at the two studied sites during the sampling period August 1997 to November 1999. The error bars represent the standard deviation of at least three samples.

of spring (March–May) and autumn (September–November) seasons ranged from 4 to 8 °C. Usually in the study region there is a period with permanent frost of at least four weeks in January and February (Gerhard 1996) but in contrast to most previous years, the temperature was no longer than five subsequent days below 0 °C during the experimental period. Instead, in January, February, and March there were often daily freeze–thaw cycles. Rainfall in the study region is typically highest during summer while during the rest of the seasons precipitation is evenly distributed (Gerhard 1996). 1998 was an exception from the long-time trend with large rainstorm events during autumn. At the beech forest site, the autumn rainfall represented 50% of the total rain in 1998 (Figure 1). Snow contributed unusually little (<10%) to precipitation in winter 1997/1998 and 1998/1999.

The amount of seepage water from the organic forest floor layers of the two forested sites in general followed the course of rainfall (Figure 2). Though evapotranspiration can be considered to be larger during the growing season (May–October) than during the rest of the year, at the pine forest site we observed largest amounts of seepage water during summer. This we attributed to the high rainfall intensity at that time of the year when heavy rainstorms dominated the precipitation. In contrast, at the beech site the largest amounts of seepage water occurred during autumn. This was due to the almost complete ground coverage by the forest canopy during summer resulting in the interception of a large part of the rainfall ($\geq$30%) even during heavy rainstorms. After litterfall in autumn, the amount of seepage water was almost equal to the amount of rainwater. On an average, about 75% of the rainfall percolated through the organic forest floor layer at the beech forest site. At the pine forest site, seepage water equalled about 73% of the amount of rain. The large proportion of forest floor seepage water at the beech site was mainly due to October/November 1998 when huge amounts of rainfall infiltrated after the litterfall.

Dissolved organic carbon concentrations and fluxes

The course of the bulk dissolved organic C concentrations in the seepage waters from the organic forest floor layers at the pine (Figure 3) and at the beech site (Figure 4) revealed clear seasonal variations with largest concentrations in summer and smallest concentrations in winter. In agreement with previous studies (e.g., Guggenberger & Zech 1993; Tipping et al. 1999), we found significantly ($p < 0.001$) smaller amounts of dissolved organic C in forest floor leachates during the dormant season (November–April) than during the growing season (May–October). We assume that this seasonal variation depended on variations in moisture, temperature, and leaching conditions. According to Andersson et al. (2000) and Christ and David (1996a,b), these factors largely control the production of soluble organic C. The increase of dissolved organic C in summer was slightly more pronounced at the beech site with maximum concentrations of 145 mg l^{-1}. Maximum summer concentrations at the pine site were 106 mg C l^{-1}. The peak concentrations occurred after rain events following short dry periods in summer. Similar observations were made by Scott et al. (1998) and Tipping et al. (1999). The summer peaks in 1999 were smaller than those observed in summer 1998. The climatic conditions of both summer seasons were similar and, in contrast to Michalzik and Matzner (1999), the dissolved organic C concentrations in the forest floor seepage water were not correlated with the rainfall intensities ($p > 0.95$). Therefore we have no reasonable explanation for the observed differences. Increased dissolved organic C concentrations were also found during leaf sprout at the beech site. The concentrations during autumn 1998 were relatively small compared to autumn 1999. We attributed this to intensive leaching of potentially soluble material from the

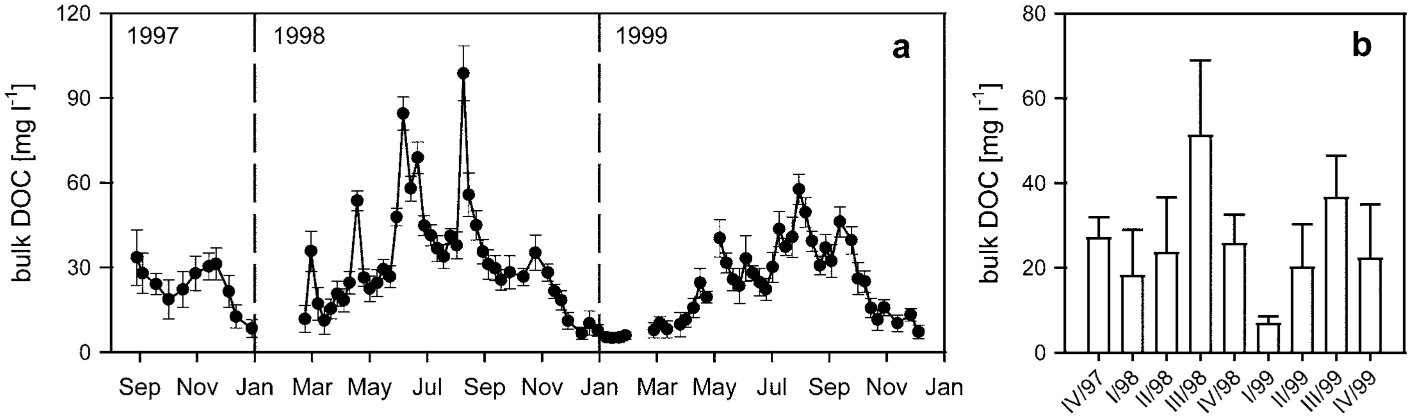


Figure 3. Course of the bulk dissolved organic carbon (DOC) concentrations in the seepage waters from the organic forest floor layer of a 160-yr-old Scots pine (*Pinus sylvestris* L.) forest on Haplic Arenosols during the sampling period August 1997 to November 1999. (a) Weekly or bi-weekly DOC concentrations. Error bars represent the standard deviation of at least three samples. (b) Mean bulk DOC concentrations during the following three-month 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 error bars represent the standard deviation of each three-month period (n = 6).

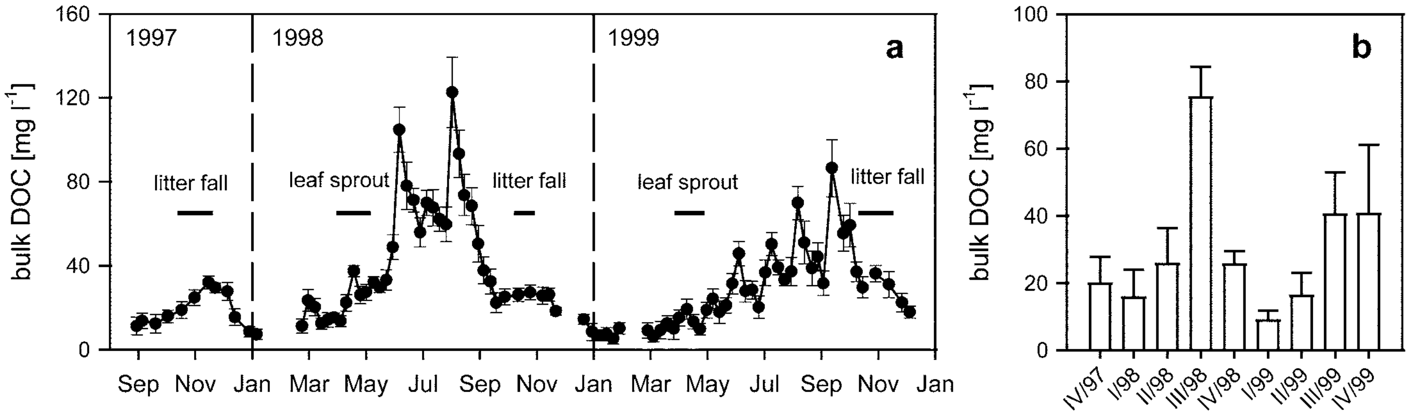


Figure 4. Course of the bulk dissolved organic carbon (DOC) concentrations in the seepage waters from the organic forest floor layer of a 90-yr-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols during the sampling period August 1997 to November 1999. (a) Weekly or bi-weekly DOC concentrations. Error bars represent the standard deviation of at least three samples. (b) Mean bulk DOC concentrations during the following three-month 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 error bars represent the standard deviation of each three-month period (n = 6).

organic forest floor layer upon the large amount of precipitation. In laboratory studies it was shown that intensive and repeated leaching of forest floor material reduced the concentrations dissolved organic C (Christ & David 1996b). Due to small amounts of snowfall during the experimental period, effects of snowmelt could not be studied. Snowmelt events generally result in large concentrations of dissolved organic C and N in soil as well as in stream water (e.g., Boyer et al. 1997; Michalzik & Matzner 1999).

Like the concentrations of bulk dissolved organic C, the distribution of C between the hydrophilic and the hydrophobic dissolved organic matter fractions varied during the course of the year (Figure 5). Mean proportions of C in the hydrophilic fraction at the beech site were largest during winter and spring representing 75 to 83% of total dissolved organic C. During summer and autumn, the mean proportions of C in the hydrophilic fraction decreased to 53 to 60%. The proportions of C in the hydrophilic fraction were generally lower at the pine site which is in agreement with the results of Hongve (1999) who found larger amounts of hydrophilic compounds being leached from deciduous than from coniferous litter. Similar to the beech site, maximum mean proportions of C in the hydrophilic fraction occurred in winter (41–49%), whereas minimum mean proportions of 36 to 39% were observed in autumn. Similar increases of hydrophilic compounds towards the dormant season have been reported for other forest ecosystems (Dai et al. 1996; Hongve 1999). Increased release of organic C with the hydrophilic fraction from forest floor material and mineral soil is usually observed after drastic changes of environmental conditions such as drying or freezing (Christ & David 1994; Guggenberger et al. 1998; Kaiser et al. 2001). We assumed that this may induce disruption and subsequent leaching of fresh microbial and plant debris upon contact with water. Because wet-dry cycles are more likely during summer enhanced proportions of hydrophilic matter during winter and spring suggest that there are processes other than simple disruption of organic debris involved in the release of the dissolved organic C during summer and autumn.

Due to large differences in the concentrations of dissolved organic C (Figure 3) and also the differences in the amount of seepage water (Figure 2), the fluxes of bulk dissolved organic C and of C in the hydrophilic and hydrophobic fractions of dissolved organic matter were much higher during summer and autumn than in winter and spring (Tables 2 and 3). Largest fluxes at the pine site occurred in summer while at the beech site the fluxes in autumn were above those in summer. We consider this to result from the litterfall in October and November and subsequent leaching of fresh leaf litter. Although the concentrations of dissolved organic C were small in autumn 1998 (Figures 3 and 4) the fluxes at that time were large at both sites. This

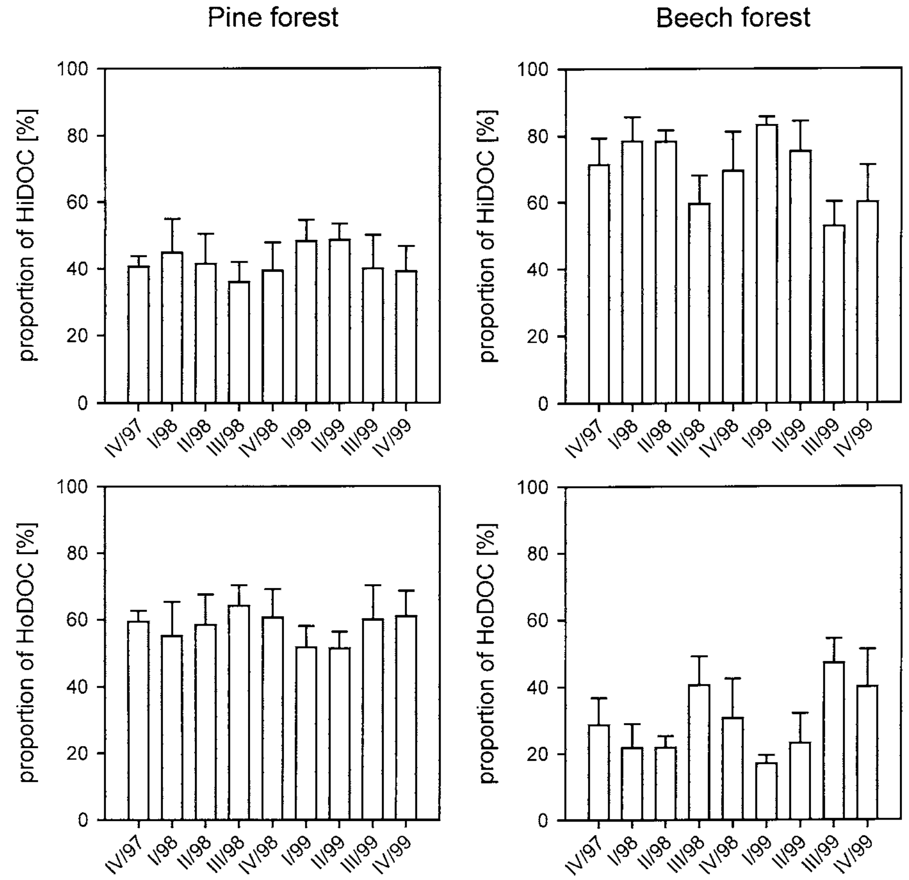


Figure 5. Mean proportions of dissolved organic carbon in the hydrophilic (HiDOC) and hydrophobic (HoDOC) fraction of seepage waters from the organic forest layers of the two studied sites during the following three-month periods: September–November 1997 (IV/97), December 1997–February 1998 (I/98), March–May 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 error bars represent the standard deviation of each three-month period (n = 6).

agrees well with the finding of Christ and David (1996b) who showed reduced concentrations but increased fluxes of dissolved organic C from forest floor material upon intensive leaching. The fluxes during summer and autumn of bulk dissolved organic C and C in the hydrophilic and hydrophobic fraction comprised about 79, 77, and 81% of the fluxes during the period from December 1997 to November 1999. The respective values for the beech site were 78, 74, and 86%. This underlines the importance of summer and autumn for the export of dissolved organic matter and especially for its hydrophobic

Table 2. Fluxes of dissolved organic carbon (DOC) in bulk samples and in hydrophilic and hydrophobic fractions of dissolved organic matter (HiDOC and HoDOC) in seepage waters from the forest floor of a 160-yr-old Scots pine (*Pinus sylvestris* L.) forest on Haplic Arenosols during the following sampling periods: 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). In addition, the annual fluxes for the periods December 1997 to November 1998 (1997/1998) and December 1998 to November 1999 (1998/1999) are given

Sampling period	C fluxes (g m^{-2})		
	bulk DOC	HiDOC	HoDOC
Seasonal fluxes			
I/98	0.36	0.17	0.19
II/98	1.59	0.59	1.00
III/98	5.05	2.28	2.77
IV/98	3.25	1.46	1.79
I/99	0.31	0.16	0.15
II/99	1.42	0.71	0.71
III/99	3.36	1.23	2.13
IV/99	2.36	0.88	1.48
Annual fluxes			
1997/1998	10.25	4.50	5.75
1998/1999	7.45	2.98	4.47

fraction from the forest floor and it implies that the reduced concentrations of organic C in winter and spring (Figure 3 and 4) are not by simple dilution but by reduced production of soluble organic matter.

At both experimental sites, the annual fluxes of dissolved organic C from the forest floor layers during the period December 1997 to November 1998 were higher than from December 1998 to November 1999 (Tables 2 and 3). The observed annual fluxes at the pine site (Table 2) were somewhat lower than those of other temperate coniferous forests (McDowell & Likens 1988; Guggenberger & Zech, 1993; Currie et al. 1996; Michalzik & Matzner 1999) which we assume was due to the small amount of rainfall during the experimental period. The fluxes at the beech site were above these values but within

Table 3. Fluxes of dissolved organic carbon (DOC) in bulk samples and in hydrophilic and hydrophobic fractions of dissolved organic matter (HiDOC and HoDOC) in seepage waters from the forest floor of a 90-yr-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols during the following sampling periods: 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). In addition, the annual fluxes for the periods December 1997 to November 1998 (1997/1998) and December 1998 to November 1999 (1998/1999) are given

Sampling period	C fluxes (g m^{-2})		
	bulk DOC	HiDOC	HoDOC
Seasonal fluxes			
I/98	1.22	0.95	0.27
II/98	2.13	1.66	0.45
III/98	7.92	4.90	3.02
IV/98	8.07	6.16	1.92
I/99	1.42	1.19	0.23
II/99	2.43	1.63	0.80
III/99	4.44	2.28	2.16
IV/99	6.05	3.48	2.57
Annual fluxes			
1997/1998	19.34	13.67	5.67
1998/1999	14.34	8.58	5.76

the range of values for temperate deciduous forests (Qualls et al. 1991; Currie et al. 1996; Qualls et al. 2000).

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

The liquid-state ^{13}C-NMR spectra of dissolved organic matter fractions in the seepage water from the organic forest floor layers of the two studied sites (Figure 6) exhibit distribution of C moieties typical of such fractions (Kaiser & Zech 1997; Guggenberger et al. 1998). The broad resonances indicate a predominantly heterogeneous structural composition of both fractions (Wilson 1987). Although being derived from different litter (Scots pine, European beech), the spectra of corresponding dissolved organic matters frac-

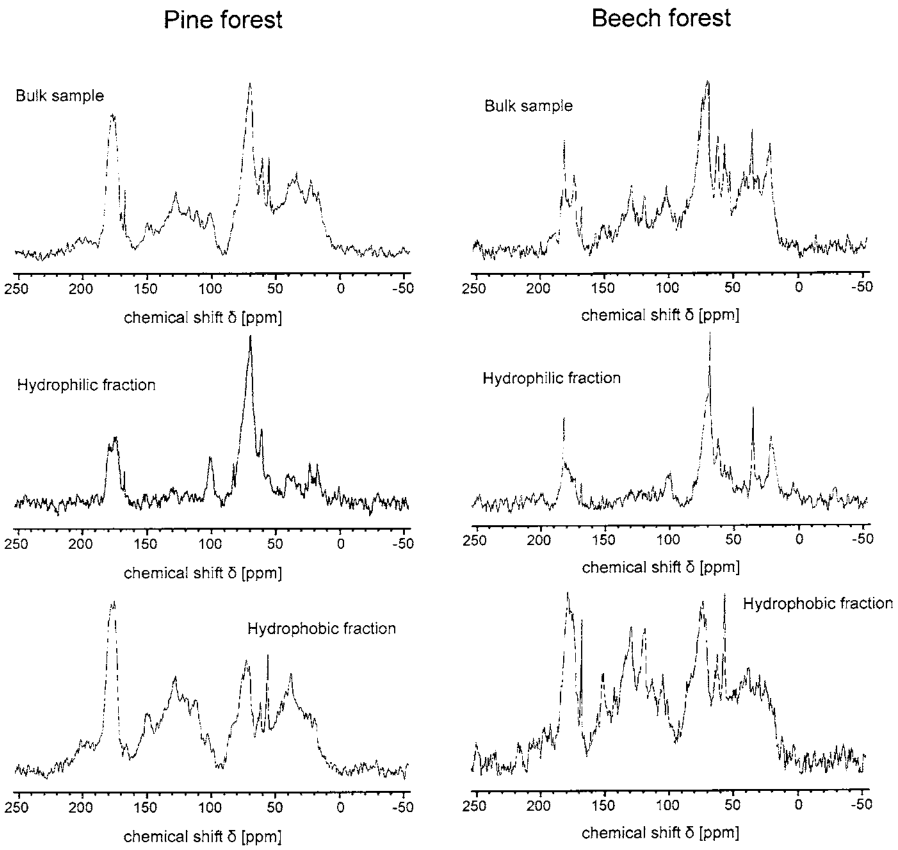


Figure 6. Liquid-state ^{13}C-NMR spectra of bulk samples of dissolved organic carbon and of its hydrophilic and hydrophobic fractions in combined three-month samples of forest floor seepage waters of a 160-yr-old Scots pine (*Pinus sylvestris* L.) forest on Haplic Arenosols and of a 90-yr-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols. Samples were collected from September to November 1997.

tions were similar. They also showed considerable similarities to dissolved organic matter fractions derived from the mor-type forest floor layer of a Norway spruce (*Picea abies* (L.) Karst.) forest (Kaiser & Zech 1997; Guggenberger et al. 1998). These similarities in the composition of fractions of dissolved organic matter of different origin suggested that the processes controlling their formation were largely similar.

The spectra of the hydrophilic fractions from both sites were dominated by resonances in the O-alkyl C region (60–110 ppm). This is consistent with observations in previous studies (Dai et al. 1996; Kaiser & Zech 1997) and indicates that the hydrophilic fractions were largely composed of carbo-

Table 4. Distribution of carbon among structural units of bulk samples of dissolved organic carbon (DOC) and of its hydrophilic and hydrophobic fractions (HiDOC and HoDOC) in combined three-month samples of forest floor seepage waters of a 160-yr-old Scots pine (*Pinus sylvestris* L.) forest on Haplic Arenosols and of a 90-yr-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols as revealed by liquid-state ^{13}C-NMR spectroscopy. Samples were collected from September to November 1997

Sample	C moieties (%)			
	carbonyl C 160–220 ppm	aromatic C 110–160 ppm	O-alkyl C 50–110 ppm	alkyl C 0–50 ppm
Pine forest				
bulk DOC	21	22	35	22
HiDOC	18	5	57	20
HoDOC	23	30	24	23
Beech forest				
bulk DOC	16	12	41	31
HiDOC	15	4	48	33
HoDOC	20	29	31	21

hydrates (Orem & Hatcher 1987). Next to the O-alkyl C region there were signals due to alkyl C (0–50 ppm) hinting at aliphatic compounds (Table 4). In addition, resonances in the carbonyl C region (160–220 ppm), mainly comprising carboxyl C (160–190 ppm), indicated oxidative transformation of organic matter. Resonances of aromatic C (110–160 ppm) were almost absent which suggested that there was little contribution of lignin-derived compounds. The lack of signals due to methoxyl C (56 ppm) confirmed this assumption. Overall, the spectra resembled CPMAS ^{13}C-NMR spectra of polygalacturonic acid and of microbial products (Golchin et al. 1996).

The spectra of the hydrophobic fractions were characterised by large proportions of aromatic C and smaller abundances of signals in the O-alkyl C region than in the spectra of the hydrophilic fraction (Table 4). Resonances around 150 ppm (O-aryl C) and 115 ppm (substituted aromatic C) as well as the sharp resonances at 56 ppm (methoxyl C) indicated that parts of the hydrophobic fractions likely derived from lignin decomposition (Wilson 1987; Preston et al. 1994). The large signals due to carboxyl C suggested that the hydrophobic fractions represented strongly oxidised organic material. The finding that hydrophobic dissolved organic matter represents strongly microbially altered lignin decomposition products is in line with previous studies using wet-chemical analyses and spectrometric means (e.g., Guggenberger et al. 1994a).

The ^{13}C-NMR spectrum of the bulk sample of dissolved organic matter in the forest floor seepage waters of the pine site represents a combination of the spectra of its fractions (Figure 6) which is in line with the distribution of carbon between the dissolved organic matter fractions in autumn 1997 (Figure 5). Due to the slightly higher proportions of carbon in the hydrophobic fraction (about 60%), the spectrum showed larger similarities to this fraction than to the hydrophilic one. In contrast, the spectrum of the bulk sample of dissolved organic matter in forest floor leachates of the beech site resembles much more its hydrophilic than its hydrophobic fraction (Figure 6). This is in agreement with observed dominance of the hydrophilic fraction at the beech site (Figure 5).

Figure 7 displays the liquid-state ^{13}C-NMR spectra of bulk dissolved organic matter in forest floor seepage waters collected at the pine site from winter 1997/98 to autumn 1999. Samples from winter and spring showed largest signal abundances in the O-alkyl C region indicating large contents of carbohydrates. Compared to O-alkyl C, the contributions of resonances due to carbonyl, aromatic and alkyl C were small (Table 5). The aromatic C region did not exhibit the typical signature of lignin-derived compounds and, moreover, the methoxyl C signal was small. We therefore assume that lignin decomposition products contribute little to dissolved organic matter released during winter and spring. Overall, these spectra resembled those of the hydrophilic dissolved organic matter fraction (Figure 6). This is consistent with the results of the fractionation showing large proportions of hydrophilic compounds during winter and spring (Figure 5). In summer and especially in autumn, signals due to aliphatic and aromatic structures strongly increased and the signals of carbohydrate structures were less dominant than in the winter and spring samples. More pronounced resonances of carboxyl C of the dissolved organic matter released in summer and autumn suggested a stronger oxidative transformation than in winter and spring. The signature of the aromatic region and the increased signal of methoxyl C in summer and autumn suggested that lignin-derived phenols and other soluble products of organic matter decomposition were present. Summer and especially autumn samples exhibited increasing similarity to hydrophobic dissolved organic matter fractions (Figure 6) which is in agreement with the larger contribution of this fraction to summer and autumn samples (Figure 5).

The ^{13}C-NMR spectra of bulk dissolved organic matter in forest floor seepage waters of the beech site in winter and spring were even more dominated by resonances in the O-alkyl C region than those from the pine site (Figure 8). They strongly resembled the spectra of hydrophilic organic matter

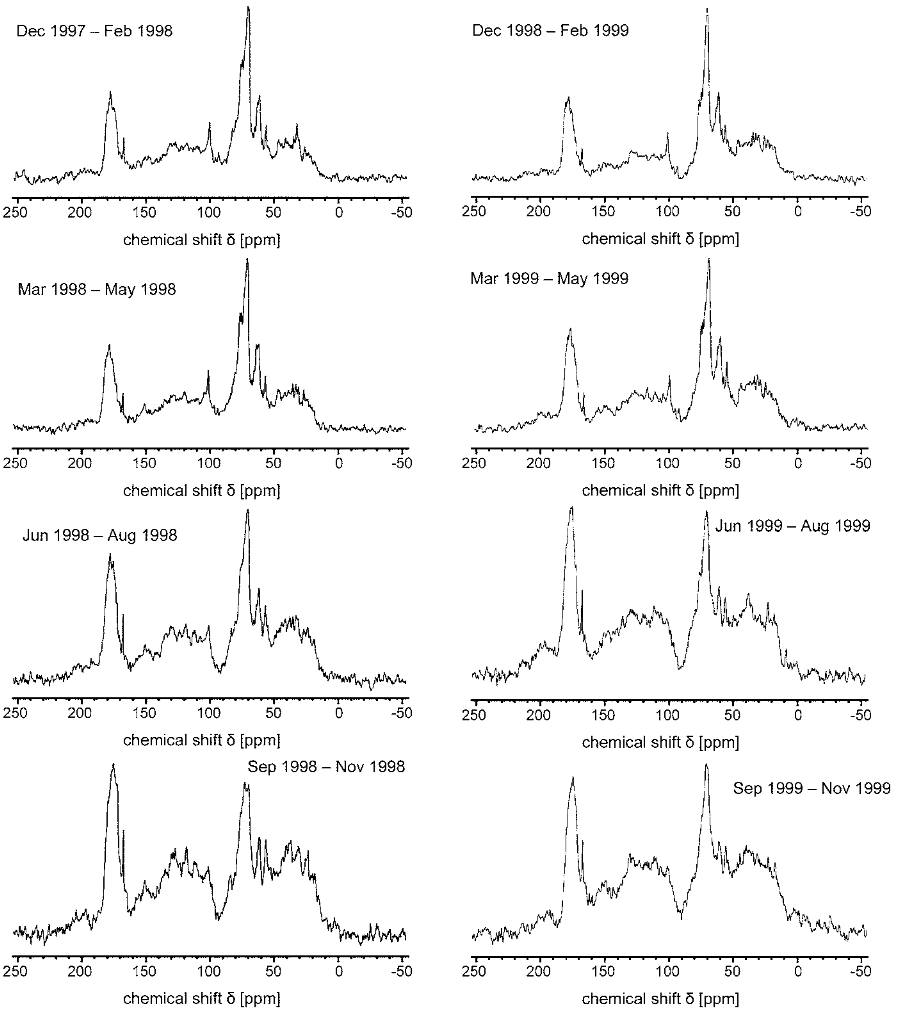


Figure 7. Liquid-state ^{13}C-NMR spectra of bulk dissolved organic carbon in combined three-month samples of seepage waters from the organic forest floor layer of a 160-yr-old Scots pine (*Pinus sylvestris* L.) forest on Haplic Arenosols. Samples were collected from winter 1997/98 to autumn 1999.

fractions (Figure 6). This corresponds well with the observed dominance of the hydrophilic fraction in winter and spring at the beech site (Figure 5). Contributions of carbonyl and alkyl C were also smaller than in the corresponding samples from the pine site and resonances due to aromatic systems were almost absent (Table 6). In summer and autumn, resonances in the O-alkyl C region dropped dramatically whereas those of aromatic and espe-

Table 5. Distribution of carbon among structural units of combined three-month samples of forest floor seepage waters of a 160-yr-old Scots pine (*Pinus sylvestris* L.) forest on Haplic Arenosols as revealed by liquid-state ^{13}C-NMR spectroscopy. Samples were collected during the following periods: 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). In addition, the mean distribution for the carbon moieties of winter–spring and summer–autumn samples are given with their standard errors (n = 4)

Sample	C moieties (%)			
	carbonyl C 160–220 ppm	aromatic C 110–160 ppm	O-alkyl C 50–110 ppm	alkyl C 0–50 ppm
Seasonal samples				
I/98	17	22	44	17
II/98	17	21	44	18
III/98	21	24	33	22
IV/98	20	26	30	24
I/99	18	19	44	19
II/99	18	20	43	19
III/99	22	24	32	22
IV/99	20	25	31	25
Half-year samples				
winter–spring	18 ± 1	20 ± 3	44 ± 1	18 ± 1
summer–autumn	20 ± 1	25 ± 1	32 ± 3	23 ± 2

cially of aliphatic structures strongly increased (Table 6). Also carboxyl C increased. The increase of aliphatic and aromatic structures at the expense of O-alkyl C went along with increasing proportions of hydrophobic organic matter in the forest floor seepage waters (Figure 5). But in contrast to the findings for the pine site, the summer and autumn samples showed no similarities to hydrophobic fractions which represented 40 to 47% of bulk dissolved organic matter (Figure 5). This suggests changes in the composition of the hydrophobic fractions during the course of the year at the beech site.

In contrast to all other samples, the spectra of summer and autumn samples of dissolved organic matter in the forest floor seepage water of the beech site exhibited a sharp signal at 35 ppm indicating the presence of a low-molecular-weight compound which we assigned to succinate by addition of a standard of succinic acid. ^{13}C and ^{1}H-NMR spectra of water extracts of the forest

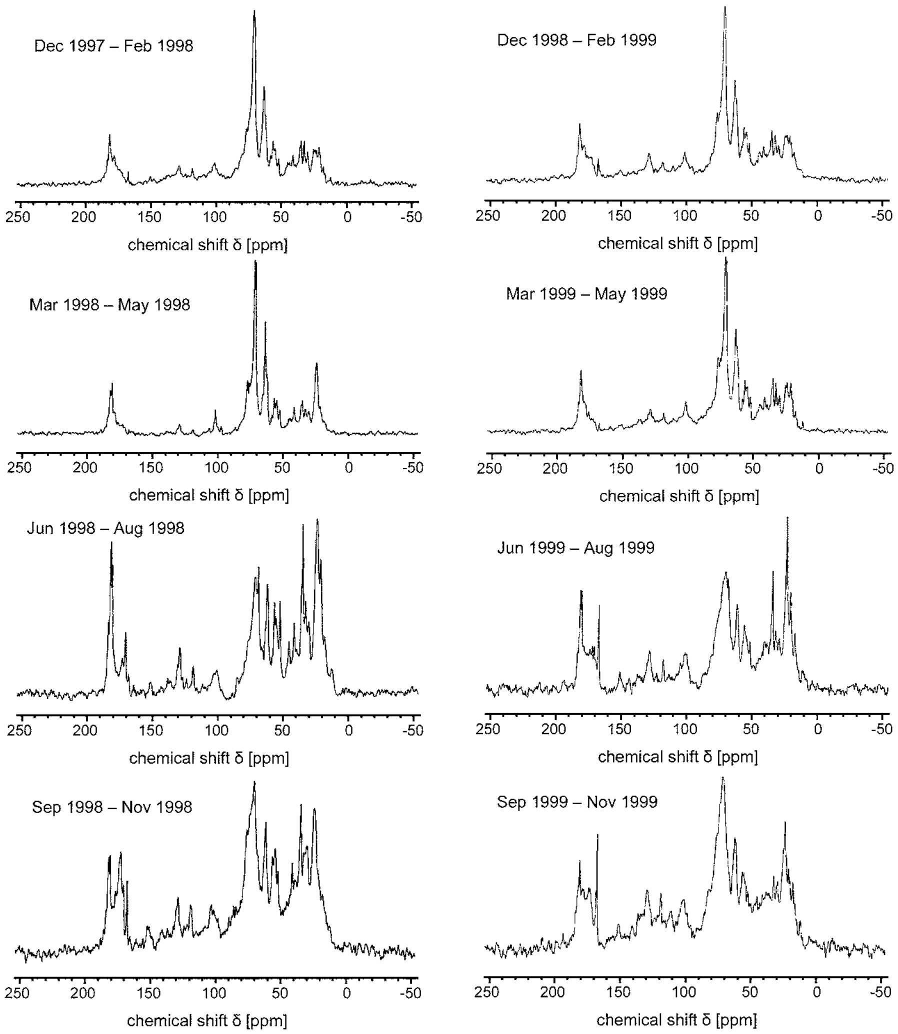


Figure 8. Liquid-state ^{13}C-NMR spectra of bulk dissolved organic carbon in combined three month samples of seepage waters from the organic forest floor layer of a 90-yr-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols. Samples were collected from winter 1997/98 to autumn 1999.

floor plants (mainly *Mercuralis perennis* L.) suggested that it was likely to result from leaching of the ground vegetation (results not shown here). The large abundance of succinate in the forest floor seepage water under beech accords with the finding of Bruckert (1970) who showed that succinic acid is the most abundant water-soluble low-molecular-weight acid in forest floor material under European beech (*Fagus sylvatica* L.).

Table 6. Distribution of carbon among structural units of combined three-month samples of forest floor seepage waters of a 90-yr-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols as revealed by liquid-state ^{13}C-NMR spectroscopy. Samples were collected during the following periods: 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). In addition, the mean distribution for the carbon moieties of winter–spring and summer–autumn samples are given with their standard errors (n = 4)

Sample	C moieties (%)			
	carbonyl C 160–220 ppm	aromatic C 110–160 ppm	O-alkyl C 50–110 ppm	alkyl C 0–50 ppm
Seasonal samples				
I/98	12	5	57	26
II/98	12	3	54	30
III/98	16	11	33	40
IV/98	15	13	37	35
I/99	12	6	55	27
II/99	11	10	54	28
III/99	15	13	37	35
IV/99	14	17	37	32
Half-year samples				
winter–spring	12 ± 1	6 ± 4	55 ± 2	28 ± 2
summer–autumn	15 ± 2	13 ± 3	36 ± 3	35 ± 5

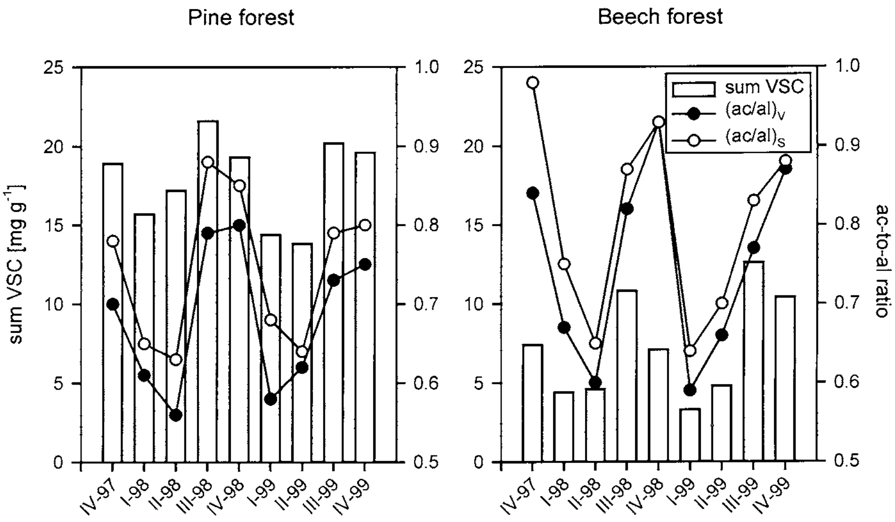
Lignin-derived phenols

The analyses of lignin-derived CuO oxidation products in the fractions of dissolved organic matter confirmed the results of ^{13}C-NMR spectroscopy. Lignin-derived phenols were not detectable in the hydrophilic fractions (Table 7) which agrees well with the lack of signals in the aromatic and phenolic region of the ^{13}C-NMR spectra of these samples (Figure 6, Table 4). In contrast, the hydrophobic fractions contained considerable amounts of lignin-derived phenols and thus mirrored the large abundances of signals in the aromatic and phenolic region of the ^{13}C-NMR spectra of hydrophobic fractions (Figure 6, Table 4). Compared to the forest floor materials (Table 1), the degree of oxidation of the lignin-derived compounds in the hydrophobic fractions as revealed by the acid-to-aldehyde ratios of the vanillyl and syringyl units was elevated indicating a strong oxidative transformation

Table 7. Chemical characteristics of hydrophilic and hydrophobic fractions of dissolved organic carbon (HiDOC and HoDOC) in combined three-month samples of forest floor seepage waters of a 160-yr-old Scots pine (*Pinus sylvestris* L.) forest on Haplic Arenosols and of a 90-yr-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols. Samples were collected from September to November 1997. Methods for determination of CuO oxidation products, of hydrolysable sugars and amino sugars, and of δ^{13}C values were the same as for forest floor samples. Σ VSC = sum of vanillyl, syringyl, and cinnamyl CuO oxidation products; $(ac/al)_V$ = acid-to-aldehyde ratios of the vanillyl units in CuO oxidation products; $(ac/al)_S$ = acid-to-aldehyde ratios of the syringyl units in CuO oxidation products; Σ sugars = sum of hydrolysable neutral and acidic carbohydrates; [(Man+Gal)/(Xyl+Ara)] = ratios of mannose plus galactose to xylose plus arabinose; [(Rha+Fuc)/(Xyl+Ara)] = ratios of rhamnose plus fucose to xylose plus arabinose; Σ amino sugars = sum of hydrolysable amino sugars; GluN/GalN = ratios of glucosamine to galactosamine. The contents of lignin-derived phenols, sugars, and amino sugars are given on ash-free dry-weight of samples

Sample	C[1] mg g^{-1}	N[1] mg g^{-1}	Σ VSC mg g^{-1}	$(ac/al)_V$	$(ac/al)_S$	Σ sugars mg g^{-1}	[(Man+Gal)/ (Xyl+Ara)]	[(Rha+Fuc)/ (Xyl+Ara)]	Σ amino sugars mg g^{-1}	GluN/GalN	δ^{13}C ‰ PDB
Pine forest											
HiDOM	378	12	<0.2	—	—	42	3.0	1.7	5.7	10.4	–24.8
HoDOM	412	5	35.4	0.7	0.8	10	1.8	0.6	1.8	9.1	–27.7
Beech forest											
HiDOM	366	16	<0.2	—	—	35	4.1	2.0	8.8	2.6	–26.0
HoDOM	417	5	27.0	0.8	1.0	13	1.6	0.8	3.7	2.9	–29.1

[1] measured on ground subsamples with a CN analyser (NC 2500, ThermoQuest-Fisons, Milan, Italy).


Pine forest

Beech forest

Figure 9. Contents of lignin-derived phenols (sum of vanillyl, syringyl, and cinnamyl CuO oxidation products, sum VSC) as well as the acid-to-aldehyde ratios of the vanillyl [(ac/al)$_V$] and syringyl [(ac/al)$_S$] units of combined three-month bulk dissolved organic matter samples of seepage waters from the organic forest floor layers of the two studied sites. Samples were collected during 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 contents are given on ash-free dry-weight of samples.

before solubilisation. These results confirmed previous work showing that the hydrophobic fraction comprises mainly water-soluble products of the oxidative degradation of lignin (Guggenberger & Zech 1994; Guggenberger et al. 1994a). Because lignin-derived degradation products were exclusively in the hydrophobic fraction we concluded them to be indicative for this fraction.

The contents of lignin-derived CuO oxidation products in bulk samples of dissolved organic matter in forest floor leachates largely accorded with the temporal variations as revealed by ^{13}C-NMR spectroscopy and dissolved organic matter fractionation. Though bulk dissolved organic matter in forest floor seepage waters of the pine site was much more enriched in lignin-derived compounds than the samples of the beech site, the seasonal course at both sites was similar (Figure 9). Lowest contributions of lignin-derived compounds were generally found during winter and spring, maximum release of such materials occurred during summer and autumn. This result suggested that the formation of water-soluble lignin decomposition products is enhanced under moist and warm conditions which was true at least for the summer seasons. Considering that the mean temperatures in autumn and spring were almost similar (Figure 1) it seemed that a part of the released organic matter might have been produced during summer and leached from the forest floor later in the year. Also Scott et al. (1998) and Tipping et al. (1999) assumed that dissolved organic matter released from peat materials in autumn was produced during summer.

The mean content of lignin-derived compounds in dissolved organic matter in seepage water of the pine forest floor was 17.9 mg g^{-1}, the respective value for the beech site was 7.3 mg g^{-1}. These values are smaller than the values for the Oi and Oe horizons (Table 1) indicating a preferential solubilisation of non-lignin materials.

The degree of oxidation of the lignin-derived compounds in the forest floor leachates as indicated by the acid-to-aldehyde ratios of the vanillyl and syringyl units also showed strong seasonal variations (Figure 9). In general, the values we found for dissolved organic matter were higher than those reported for bulk soil organic matter and organic matter in most soil particle-size separates (e.g., Guggenberger et al. 1994b; Amelung et al. 1999a) and also of the parent organic forest floor layers (Table 1). This underlines the strong oxidative alteration which is necessary to transform lignin into water-soluble decomposition products (Guggenberger & Zech 1994; Guggenberger et al. 1994a). An exception is organic matter of clay-sized particles which shows acid-to-aldehyde ratios similar to those found for dissolved organic matter. It seems therefore possible that a part of clay organic matter may result from sorption of dissolved organic matter (Amelung et al. 1999b; Kaiser & Guggenberger 2000). During winter and spring the ratios were much lower than during summer and autumn at both experimental sites, indicating that the compounds in the forest floor seepage waters in winter and spring were less microbially altered and oxidised than those released in summer and autumn. This finding confirms the results of ^{13}C-NMR spectroscopy showing larger abundances of carboxyl moieties in summer and autumn than in winter and spring (Figures 7 and 8).

Taking the fluxes of carbon into account (Tables 2 and 3), our results suggest that at both sites the export of lignin-derived degradation products with seepage water from the forest floor is taking place predominantly in summer and autumn. Export in winter and spring seems to be almost negligible.

Neutral and acidic carbohydrates

The amounts of hydrolysable carbohydrates in fractions of dissolved organic matter (Table 7) were in line with the abundances of signals in the O-alkyl C region of the ^{13}C-NMR spectra (Figure 6, Table 4). The hydrophilic factions had contents of carbohydrates which were about three times larger than those of the hydrophobic fractions. This in line with the results of Guggenberger and Zech (1994) and Guggenberger et al. (1994a).

Ratios of hexoses to pentoses inform about the possible source of carbohydrates because cellular plant polysaccharides are enriched in pentoses such as arabinose and xylose whereas microbial biomass is characterised by larger proportions of hexoses like galactose and mannose and of deoxy sugars like rhamnose and fucose (Moers et al. 1990). Therefore the ratios of mannose plus galactose to xylose plus arabinose [(Man+Gal)/(Xyl+Ara)] and of rhamnose plus fucose to xylose plus arabinose [(Rha+Fuc)/(Xyl+Ara)] can be used to distinguish between more plant-derived and more microorganisms-derived carbohydrate signatures of organic matter (Oades 1984; Murayama 1984). Compared to the forest floor materials (Table 1), the dissolved organic matter fractions showed elevated ratios of hexoses to pentoses. This suggests a strong microbial influence on the composition of dissolved organic matter, especially on the hydrophilic fractions which had higher ratios than the hydrophobic ones.

The contribution of hydrolysable carbohydrates to bulk dissolved organic matter leached from the forest floor of the two investigated sites showed a trend opposite to that of the yield in CuO oxidation products (Figure 10). Carbohydrates were clearly enriched in winter and spring samples and depleted in dissolved organic matter released during summer and autumn. At both sites, the carbohydrate contents in the winter and spring samples were up to three times higher than those of summer and autumn samples. Thus, wet-chemical analyses confirmed the results of ^{13}C-NMR spectroscopy which showed that carbohydrates dominated dissolved organic matter released in winter and spring. In addition, the contents of hydrolysable carbohydrates showed a similar trend as the proportions of the hydrophilic fraction (Figure 5). This is in agreement with the observation that hydrolysable carbohydrates were concentrated in hydrophilic fractions of dissolved organic matter (Table 7). We assumed the release of carbohydrates in winter and spring to result from leaching of disrupted fresh microbial debris and plant litter. The reduced carbohydrate contents of dissolved organic matter in summer and autumn samples pointed to preferential mineralisation. The contrasting trends in the release of carbohydrates and lignin decomposition products suggested that carbohydrates were utilised as the energy source for lignin decomposition.

The contents of carbohydrates (17–66 mg g^{-1}) in bulk dissolved organic matter of both forest sites were generally lower than those in the organic forest floor horizons (Table 1). This result was similar to the findings for lignin-derived phenols and indicated a different extent of solubilisation of constituents of the source materials. Another possible explanation of the

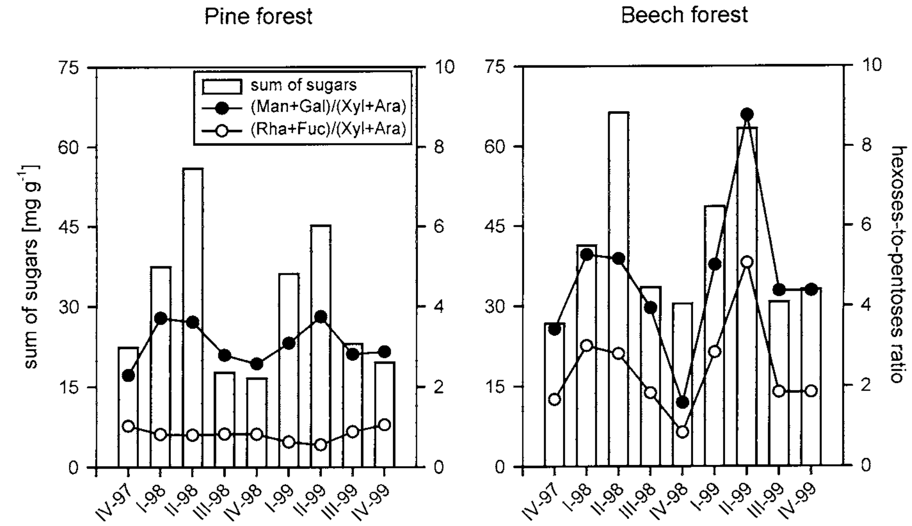


Figure 10. Total contents of hydrolysable neutral and acidic sugars (sum of arabinose, fucose, galactose, glucose, mannose, rhamnose, ribose, xylose, galacturonic acid, and glucuronic acid) as well as ratios of mannose plus galactose to xylose plus arabinose [(Man+Gal)/(Xyl+Ara)] and of rhamnose plus fucose to xylose plus arabinose [(Rha+Fuc)/(Xyl+Ara)] of combined three-month bulk dissolved organic matter samples of seepage waters from the organic forest floor layers of the two studied sites. Samples were collected during 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 contents are given on ash-free dry-weight of samples.

low yields of hydrolysable carbohydrates could be that the used method is not sensitive for sugar alcohols which might also contribute to water-soluble polysaccharides. All bulk samples, especially the ones from the beech site, had hexoses-to-pentoses ratios (Figure 10) higher than those found for organic matter in bulk soil samples and most particle-size separates (e.g., Guggenberger et al. 1994b; Amelung et al. 1999b). These high values indicate strong microbial impact on the carbohydrate composition. On an average, the [(Man+Gal)/(Xyl+Ara)] ratios were 3.1 at the pine site and 4.7 at the beech site. [(Man+Gal)/(Xyl+Ara)] ratios >2 indicate a predominance of microbial products (Oades 1984). Though the values in their study were lower (1.7–1.9), Guggenberger and Zech (1994) also came to the conclusion that the carbohydrates in forest floor leachates were mainly of microbial origin.

Compared to the organic forest floor layers (Table 1), bulk dissolved organic matter from both sites had much larger [(Man+Gal)/(Xyl+Ara)] and [(Rha+Fuc)/(Xyl+Ara)] ratios (Figure 10) which underlined the importance of microorganisms for the production of soluble organic matter in forest ecosystems (Guggenberger et al. 1994a, 1998).

The seasonal course of the [(Man+Gal)/(Xyl+Ara)] ratio was characterised by larger values in winter and spring than in summer and autumn at both sites. At the beech site, the [(Rha+Fuc)/(Xyl+Ara)] ratio showed the same seasonal trend as the [(Man+Gal)/(Xyl+Ara)] ratio while there were almost no seasonal changes at the pine site. The tendency of higher hexoses-to-pentoses ratios in winter and spring indicated enhanced release of microbially synthesised hexoses during these seasons, probably induced by the lysis of microbial biomass which has accumulated during summer and autumn. This finding agrees well with the observations that soil freezing and thawing induces release and leaching of microbially derived compounds (e.g., Schimel & Clein 1996). At little to no snow cover as it was in winter 1997/1998 and 1998/1999, even mild freezing results in an increase in solute leaching (Groffman et al. 1999). This effect may be intensified by the frequent freeze–thaw cycles during the experimental period.

Generally, the microbial activity is higher in summer than during the rest of the year and so it seemed not plausible that in summer the contribution of plant-derived pentoses was highest. We therefore assume that the changes in the hexoses-to-pentoses ratios may be connected to the enhanced release of lignin decomposition products in summer and autumn. Seelenfreund et al. (1990) found that water-soluble compounds released during the decomposition of wheat lignocellulose were enriched in hemicellulose-derived pentoses. Thus decomposition of lignocellulose could explain the decreasing hexoses-to-pentoses ratios in summer and autumn.

To get an idea about the oxidation degree of carbohydrates in dissolved organic matter, we calculated ratios of galactose and glucose to galacturonic and glucuronic acid, respectively (Table 8). We found largest values in winter and spring and smallest ratios in summer and autumn. This finding might suggest a stronger oxidation of released carbohydrates during summer and autumn which was consistent with the results of the ^{13}C-NMR spectroscopy (Figures 7 and 8) and the analyses of lignin-derived compounds (Figure 9). Because uronic acids are typical components of extracellular gums of bacterial biomass (Cheshire 1979), the lower ratios may hint at higher microbial activity in summer and autumn.

Though neutral and acidic carbohydrates were largely reduced in summer and autumn at both sites compared to lignin-derived compounds, this does not mean that their export from the forest floor was reduced. If we consider the large fluxes of C with seepage water in summer and autumn (Tables 2 and 3), it gets obvious that like for the lignin-derived phenols, export of carbohydrates was highest in summer and autumn.

Table 8. Ratios of galactose to galacturonic acid (Gal/GalA) and glucose to glucuronic acid (Glu/GluA) of bulk dissolved organic matter in combined three-month samples of forest floor seepage waters of a 160-yr-old Scots pine (*Pinus sylvestris* L.) forest on Haplic Arenosols and of a 90-yr-old European beech (*Fagus sylvatica* L.) forest on Rendzic Leptosols. Samples were collected during 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)

Sample	Pine forest		Beech forest	
	Gal/GalA	Glu/GluA	Gal/GalA	Glu/GluA
IV/97	1	8	4	7
I/98	11	68	7	52
II/98	10	47	6	41
III/98	<1	5	<1	3
IV/98	<1	6	4	5
I/99	12	80	12	65
II/99	4	53	7	46
III/99	1	7	5	10
IV/99	2	8	5	11

Amino sugars

Similar to the neutral and acidic carbohydrates, hydrolysable amino sugars were more abundant in the hydrophilic than in the hydrophobic fraction (Table 7).

The contents of hydrolysable amino sugars in bulk samples of dissolved organic matter from seepage waters of both sites (Figure 11) showed seasonal variations similar to those observed for neutral and acidic carbohydrates (Figure 10). The seasonal variations in amino sugar contents mirror the variations in the proportions of the hydrophilic fractions (Figure 5) which accords to the observed enrichment of amino sugars in hydrophilic dissolved organic matter (Table 7). The winter and spring samples were rich in amino sugars whereas samples from summer and autumn were relatively low in amino sugars. These seasonal variations were much more pronounced at the beech site than at the pine site. We assume that, like for the carbohydrates, the seasonal variations in the release of amino sugars were due to leaching of fresh biomass debris in winter and spring and accelerated microbial utilisation and mineralisation in summer and autumn.

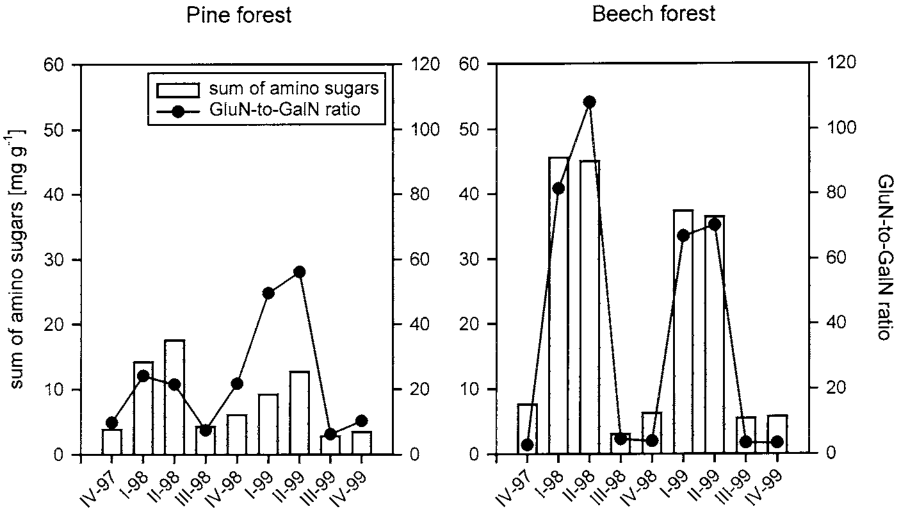


Figure 11. Total contents of hydrolysable amino sugars (sum of galactosamine, glucosamine, mannosamine, and muramic acid) as well as the ratio of glucosamine to galactosamine (GluN-to-GalN ratio) of combined three-month bulk dissolved organic matter samples of seepage waters from the organic forest floor layers of the two studied sites. Samples were collected during 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 contents are given on ash-free dry-weight of samples.

In contrast to lignin-derived compounds and carbohydrates which were depleted in dissolved organic matter compared to the forest floor layers, the amino sugars were strongly enriched in dissolved organic matter from the pine (mean value 8.2 mg g^{-1}) as well as from the beech site (mean value 21.4 mg g^{-1}). Because amino sugars in soils are mainly products of microorganisms this result was another proof for the microbial control on production of soluble organic matter in forests. The enrichment of amino sugars in dissolved organic matter was more pronounced for the beech forest which we assume to be indicative for the higher biological activity at this site.

The ratio of glucosamine to galactosamine is a measure of the relative contribution of fungal products compared to the products of bacterial biomass (Benzing-Purdie 1984; Kögel & Bochter 1985).The ratio was highest in winter and spring and dropped in summer and autumn at both sites (Figure 11). Thus, the microbial products in dissolved organic matter released from the forest floor layers were dominated by products from fungi in winter and spring and by bacterial products in summer and autumn. This result accords with the relatively strong enrichment of uronic acids in dissolved organic matter samples from summer and autumn (Table 8). The ratios of glucosamine to galactosamine measured at the beech site in winter and spring exceeded those of organic matter in bulk soil samples and particle-size separates (Zhang et al. 1999) by factors >60. We guess that this was the result of intense fungal growth on the fresh beech litter in late autumn. The fungal biomass decreased with the first frost and the resulting debris was subject to intense leaching thereafter. We assume a similar process to be responsible for the increase of the glucosamine-to-galactosamine ratios in winter and spring at the pine site. This increase, however, was smaller than that at the beech site.

The ratios of glucosamine to galactosamine of dissolved organic matter from both forest sites (Figure 11) were high above the respective ratios measured for most of the organic forest floor layers (Table 1). An exception was the Oi horizon from the pine site which had a glucosamine-to-galactosamine ratio of 19.1 due to the large abundance of fungal hyphae. The values of dissolved organic matter were mostly below this value during the experimental period which suggested that the composition of the dissolved organic matter in the forest floor seepage waters of the pine site was controlled by processes in the underlying Oe and Oa horizons.

Although the contents of amino sugars in dissolved organic matter were much higher in winter and spring than in summer and autumn, the major part of the export of amino sugars from the forest floor into the mineral soil occurred during summer and autumn because of the elevated C fluxes during these seasons (Table 2 and 3).

δ^{13}C values

The δ^{13}C values of bulk dissolved organic matter in seepage waters from the organic forest floor layers of both investigated sites showed seasonal variations which were characterised by less negative values during winter and spring and more negative values in summer and autumn samples (Figure 12). This means that samples from winter and spring were enriched in ^{13}C whereas the samples from summer and autumn were ^{13}C-depleted ($p < 0.01$). Assuming that ^{13}C enrichment is the result of increased metabolic transformation of organic matter (Blair et al. 1985) this result would be indicative for stronger microbial influence on dissolved organic matter release during winter and spring than in summer and autumn. However, such an assumption is neither consistent with known seasonal variations in microbial activity nor with the observed seasonal changes in the chemical composition of dissolved organic matter (see above). Therefore it seemed more likely that the measured variations of the δ^{13}C values of dissolved organic matter did reflect changes of the chemical composition (Ågren et al. 1996; Šantrůčková et al. 2000).

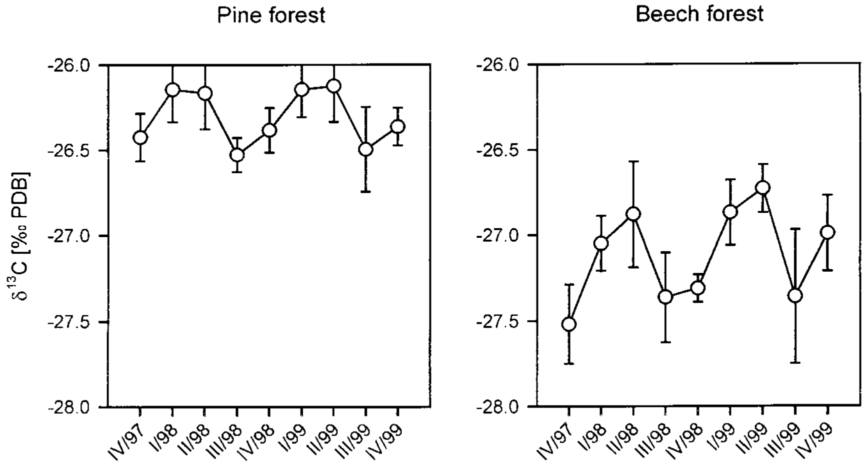


Figure 12. Mean δ^{13}C values of bulk dissolved organic carbon of combined one-month samples of seepage waters from the organic forest floor layers of the two studied sites. Error bars represent the standard deviation (n = 6) of each of the following sampling periods: September–November 1997 (IV/97), 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).

Summer and autumn samples were enriched in lignin-derived and aliphatic material which generally have lower δ^{13}C values than carbohydrates. In turn, the winter and spring samples were enriched in carbohydrates which tend to have less negative δ^{13}C values (Benner et al. 1987; Schleser et al. 1999). Consequently, the variations in the δ^{13}C values of bulk samples of dissolved organic matter of winter and spring and of summer and autumn may be connected to the variations in the proportions of hydrophilic and hydrophobic matter which had a different chemical composition as well as a different origin and thus differed in their isotopic compositions (Table 7).

Summary and implications

Summary

In summary, our results showed large seasonal variations in the amount of dissolved organic matter in seepage waters from organic forest floor layers. Fluxes in summer and autumn represented the predominant proportion of the annual export of organic C from the forest floor. Consequently, export of dissolved organic matter constituents such as lignin-derived phenols, carbohydrates, and amino sugars from the forest floor into the mineral soil was highest in summer and autumn.

These seasonal variations in the amount of dissolved organic matter were accompanied by changes in its chemical composition. The major differences occurred between winter and spring samples on the one hand and summer and autumn samples on the other hand. The winter and spring samples were more hydrophilic, richer in carbohydrates and amino sugars and poorer in aromatic and aliphatic moieties, carboxyl groups, and lignin-derived phenols. Samples from summer and autumn were more hydrophobic, and richer in aromatic and aliphatic structures, carboxyl groups, and lignin decomposition products. In turn, the proportions of carbohydrates and amino sugars were lower than in the winter and spring samples. The larger abundances of carboxyl groups and the higher oxidation degree of lignin-derived phenols indicated stronger oxidative transformation of the released dissolved organic matter in summer and autumn which we attributed to the higher microbial activity under warm and moist conditions. The parallel decrease of carbohydrates and amino sugars could be the result of their utilisation as energy source for decomposition processes. Higher abundances of uronic acids and the dominance of galactosamine pointed to larger impact of bacterial products on dissolved organic matter in summer and autumn. The release of carbohydrates in winter and spring was characterised by large proportions of hexoses which hinted at leaching of disrupted microbial biomass as the major contributor to dissolved organic matter during these two seasons. In addition, the composition of the released amino sugars suggested that fungi strongly contributed to the leached microbial biomass. Thus, the seasonal changes in the chemical composition of dissolved organic matter in seepage water of organic forest floor layers result from different mechanisms of mobilisation: simple leaching of disrupted fresh biomass debris in winter and spring and production of water-soluble metabolites during the oxidative decomposition in summer and autumn. The two sites differed largely in the vegetation (deciduous vs. coniferous), the age of the stands (90 vs. 160 years), the type of the forest floors (mull vs. mor), and in the underlying mineral soils (Rendzic Leptosols vs. strongly acidic Haplic Arenosols). This explains the differences in amounts and composition of the dissolved organic matter in the forest floor seepage waters at the two sites. However, the temporal changes in the composition of dissolved organic matter in forest floor leachates showed a very similar trend. This implied that despite the differences similar processes control the production and subsequent mobilisation of dissolved organic matter in the forest floors.

The seasonal changes in the stable isotope composition of carbon of dissolved organic matter were assumed to reflect changes in the chemical composition and not different degrees of metabolic transformation. According to these results, molecular markers such as lignin-derived phenols, carbohydrates, and amino sugars offer a deeper insight into the processes controlling the release of dissolved organic matter in forest ecosystems than the analysis of stable isotope composition does.

Implications

The seasonal variations in composition of dissolved organic matter released from the organic forest floor layers of the two old-growth Scots pine and European beech forests gives rise to the following implications:

1. Release in winter and spring is controlled by processes different from those in summer and autumn. During winter and spring leaching of carbohydrate-rich material from disrupted fresh microbial and plant debris dominates the dissolved organic matter release into forest floor seepage waters. Production of soluble organic matter in summer and autumn is much larger than in winter and spring. It is characterised by the enhanced microbially-mediated release of decomposition products of lignin and lignocellulose. At the same time, the release of carbohydrates is relatively reduced as compared to lignin-derived phenols, presumably due to the utilisation of sugars as an energy source for the co-metabolic lignin decomposition.
2. The considerable changes in the chemical composition of dissolved organic matter during the course of the year suggests variable impact on processes in soil and aquatic systems. Fluxes of dissolved organic matter from the forest floor are little in winter and spring but dissolved organic matter is dominated by hydrophilic carbohydrate moieties low in carboxyl groups. These compounds have the tendency to be more mobile in the soil environment and thus are more susceptible to leaching from soil down to the groundwater. Because of the predominance of labile structures, there they may serve as a potential C and energy source for microbial growth and denitrification (Boyer & Groffman 1996).

   In contrast, fluxes of dissolved organic matter from the forest floor in summer and autumn are much larger than those in winter and spring. However, the dissolved organic matter in these two seasons is rich in carboxyl groups and hydrophobic moieties. Its ability to interact with solutes, colloids, and the solid soil therefore is much larger than that of dissolved organic matter leached from the forest floor in winter and spring. Usually, this may strongly restrict its mobility in soil under conditions favourable for sorption onto solid soil surfaces such as enough available sorption sites and slow percolation of soil pore water. In case of limited retention upon rapid water movement and/or little sorption capacity of the mineral soil, enhanced dissolved organic matter-induced mobilisation and subsequent leaching of metals and hazardous hydrophobic organic compounds from soil and transport into the groundwater may take place. This could also reduce the bioavailability of nutrients and toxic substances to soil microorganisms and plant species rooting in the mineral soil.

Overall, the large seasonal variations in the composition of dissolved organic matter may complicate the prediction and evaluation of its influence on soil processes based on dissolved organic C data only. Therefore future studies on the environmental impact of dissolved organic matter should comprise at least some measures for its chemical composition. A simple method offering information on major changes in the dissolved organic matter composition is the fractionation with XAD-8 resin. Another possibility to gain rapid information on changes in the composition of dissolved organic matter could be the analysis of ^{13}C.

3. The changes in the chemical composition of dissolved organic matter during the two sampling years showed similar trends though they were not exactly the same. For example, the large amount of rainfall in autumn 1998 influenced the amounts as well as the composition of the dissolved organic matter released. Therefore the prediction of possible effects of dissolved organic matter in the soil environment is uncertain due to the influence of such singular events.

Acknowledgements

The study was funded by the Deutsche Forschungsgemeinschaft joint research program ROSIG and the University of Bayreuth. H. Ciglasch, J. Dilling, B. Glaser, T. Gonter, S. Peter, U. Roth, and A. Wetzel assisted during the field and laboratory work. We are grateful to the staff of the Bavarian Forest Administration at Bayreuth and Betzenstein for the help during the selection of the sites and the support during the sampling. We would also like to thank W. Amelung and R. Bol (IGER, North Wyke, UK) for helpful comments and discussions on the manuscript.

References

Ågren GI, Bosatta E & Balesdent J (1996) Isotope discrimination during decomposition of organic matter: a theoretical analysis. Soil Sci. Soc. Am. J. 60: 1121–1126

Aiken GR & Leenheer JA (1993) Isolation and chemical characterization of dissolved and colloidal organic matter. Chem. Ecol. 8: 135–151

Amelung W, Cheshire MV & Guggenberger G (1996) Determination of neutral and acidic sugars in soil by capillary gas-liquid chromatography after trifluoroacetic acid hydrolysis. Soil Biol. Biochem. 28: 1631–1639

Amelung W, Flach KW & Zech W (1999a) Lignin in particle-size fractions as influenced by climate. Soil Sci. Soc. Am. J. 63: 1222–1228

Amelung W, Flach KW & Zech W (1999b) Neutral and acidic sugars in particle-size fractions as influenced by climate. Soil Sci. Soc. Am. J. 63: 865–873

Andersson S, Nilsson I & Valeur I (1999) Influence of dolomite lime on DOC and DON leaching in a forest soil. Biogeochemistry 47: 297–317

Andersson S, Nilsson SI & Saetre P (2000) Leaching of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in mor humus as affected by temperature and pH. Soil Biol. Biochem. 32: 1–10

Benner R, Fogel ML, Sprague EK & Hodson RE (1987) Depletion of ^{13}C in lignin and its implication for stable isotope studies. Nature 320: 708–710

Benzing-Purdie L (1984) Amino sugar distribution in four soils determined by high resolution gas liquid chromatography. Soil Sci. Soc. Am. J. 48: 219–222

Blair N, Leu A, Munos E, Olsen J, Kwong E & des Marais D (1985) Carbon isotope fractionation in heterotrophic microbial metabolism. Appl. Environ. Microbiol. 50: 996–1001

Boyer JN & Groffman PM (1996) Bioavailability of water extractable organic carbon fractions in forest and agricultural soil profiles. Soil Biol. Biochem. 28: 783–790

Boyer EW, Hornberger GM, Bencala KE & McKnight DM (1997) Response characteristics of DOC flushing in an alpine catchment. Hydrol. Process. 11: 1635–1647

Bruckert (1970) Influence des composés organiques solubles sur la pédogenèse en milieu acide. Ann. Agron. 21: 421–452

Cheshire MV (1979) Nature and Origin of Carbohydrates in Soils. Academic Press, London

Christ MJ & David MB (1994) Fractionation of dissolved organic carbon in soil water: effects of extraction and storage methods. Comm. Soil Sci. Plant Anal. 25: 3305–3319

Christ MJ & David MB (1996a) Dynamics of extractable organic carbon in spodosol forest floors. Soil Biol. Biochem. 28: 1171–1179

Christ MJ & David MB (1996b) Temperature and moisture effects on the production of dissolved organic carbon in a spodosol. Soil Biol. Biochem. 28: 1191–1199

Clair TA, Sayer BG, Kramer JR & Eaton DR (1996) Seasonal variation in the composition of aquatic organic matter in some Nova Scotian brownwaters: a nuclear magnetic resonance approach. Hydrobiol. 317: 141–150

Currie WS, Aber JD, McDowell WH, Boone RD & Magill AH (1996) Vertical transport of dissolved organic C and N under long-term N amendments in pine and hardwood forests. Biogeochemistry 35: 471–505

Dai K‘oH, David MB & Vance GF (1996) Characterization of solid and dissolved carbon in a spruce-fir Spodosol. Biogeochemistry 35: 339–365

Donald RG, Anderson DW & Stewart JWB (1993) Potential role of dissolved organic carbon in phosphorus transport in forested soils. Soil Sci. Soc. Am. J. 57: 1611–1618

Easthouse KB, Mulder J, Christophersen N & Seip HM (1992) Dissolved organic carbon fractions in soil and stream water during variable hydrological conditions at Birkenes, southern Norway. Water Resour. Res. 28: 1585–1596

Edwards M, Benjamin MM & Ryan JN (1996) Role of organic acidity in sorption of natural organic matter (NOM) to oxide surfaces. Colloids Surf. A 107: 297–307

FAO–Unesco (1990) Soil Map of the World, Revised Legend. World Soil Resources Report 60, FAO, Rome

Gerhard E (1996) Klimaatlas von Bayern. Bayerischer Klimaforschungsverbund, München

Golchin A, Clarke P & Oades JM (1996) The heterogeneous nature of microbial products as shown by solid-state ^{13}C CP/MAS NMR spectroscopy. Biogeochemistry 34: 71–97

Groffman PM, Hardy JP, Nolan S, Fitzhugh RD, Driscoll CT & Fahey TJ (1999) Snow depth, soil frost and nutrient loss in a northern hardwood forest. Hydrol. Process. 13: 2275–2286

Guggenberger G & Kaiser K (1998) Significance of DOM in the translocation of cations and acidity in acid forest soils. Z. Pflanzenernähr. Bodenkd. 161: 95–99

Guggenberger G & Zech W (1993) Dissolved organic carbon control in acid forest soils of the Fichtelgebirge (Germany) as revealed by distribution patterns and structural composition analyses. Geoderma 59: 109–129

Guggenberger G & Zech W (1994) Composition and dynamics of dissolved carbohydrates and lignin-degradation products in two coniferous forests, N.E. Bavaria, Germany. Soil Biol. Biochem. 26: 19–27

Guggenberger G, Zech W & Schulten H-R (1994a) Formation and mobilization pathways of dissolved organic matter: evidence from chemical structural studies of organic matter fractions in acid forest floor solutions. Org. Geochem. 21: 51–66

Guggenberger G, Christensen BT & Zech W (1994b) Land-use effects on the composition of organic matter in particle-size separates of soil: I. Lignin and carbohydrate signature. Eur. J. Soil Sci. 45: 449–458

Guggenberger G, Kaiser K & Zech W (1998) Organic colloids in forest soils: 1. Biochemical mobilization in the forest floor. Phys. Chem. Earth 23: 141–146

Hedges JI & Ertel JR (1982) Characterization of lignin by gas capillary chromatography of cupric oxide oxidation products. Anal. Chem. 54: 174–178

Hinton HJ, Schiff SL & English MC (1998) Sources and flowpaths of dissolved organic carbon during storms in two forested watersheds of the Precambrian Shield. Biogeochemistry 41: 175–197

Hongve D (1999) Production of dissolved organic carbon in forested catchments. J. Hydrol. 224: 91–99

Hongve D, van Hees PAW & Lundström US (2000) Dissolved components in precipitation water percolated through forest litter. Eur. J. Soil Sci. 51: 667–677

Jandl R & Sollins P (1997) Water-extractable soil carbon in relation to the belowground carbon cycle. Biol. Fertil. Soils 25: 196–201

Kaiser K & Guggenberger G (2000) The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils. Org. Geochem. 31: 711–725

Kaiser K & Zech W (1997) Structure-dependent sorption of dissolved organic matter on soils and related minerals. In: Drozd J et al. (Eds) The Role of Humic Substances in the Ecosystems and in Environmental Protection (pp 385–390). IHSS – Polish Society of Humic Substances, Wroclaw

Kaiser K, Guggenberger G, Haumaier L & Zech W (1997) Dissolved organic matter sorption on subsoils and minerals studied by ^{13}C-NMR and DRIFT spectroscopy. Eur. J. Soil Sci. 48: 301–310

Kaiser K, Kaupenjohann M & Zech W (2001) Sorption of dissolved organic carbon in soils: effects of soil sample storage, soil-to-solution ratio, and temperature. Geoderma 99: 317–328

Kalbitz K, Solinger S, Park J-H, Michalzik B & Matzner E (2000) Controls on the dynamics of dissolved organic matter in soils: a review. Soil Sci. 165: 277–304

Kögel I & Bochter R (1985) Amino sugar determination in organic soils by capillary gas chromatography using a nitrogen-selective detector. Z. Pflanzenernähr. Bodenkd. 148: 260–267

Kretzschmar R & Sticher H (1997) Transport of humic-coated iron oxide colloids in a sandy soil: influence of Ca^{2+} and trace metals. Environ. Sci. Technol. 31: 3497–3504

Kukkonen J, McCarthy JF & Oikari A (1990) Effects of XAD-8 fractions of dissolved organic carbon on the sorption and bioavailability of organic micropollutants. Arch. Environ. Contam. Toxicol. 19: 551–557

Leenheer JA (1981) Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and wastewaters. Environ. Sci. Technol. 15: 578–587

McCarthy JF & Zachara JM (1989) Subsurface transport of contaminants. Environ. Sci. Technol. 23: 496–502

McDowell WH & Likens GE (1988) Origin, composition, and flux of dissolved organic carbon in the Hubbard Brook valley. Ecol. Monogr. 58: 177–195

McKnight D, Bencala KE, Zellweger GW, Aiken GR, Feder GL & Thorn KA (1992) Sorption of dissolved organic carbon by hydrous aluminum and iron oxides occurring at the confluence of Deer Creek with the Snake River, Summit County, Colorado. Environ. Sci. Technol. 26: 1388–1396

Meier M, Namjesnik-Dejanovic K, Maurice PA, Chin Y-P & Aiken GR (1999) Fractionation of aquatic natural organic matter upon sorption to goethite and kaolinite. Chem. Geol. 157: 275–284

Michalzik B & Matzner E (1999) Dynamics of dissolved organic nitrogen and carbon in a central European Norway spruce ecosystem. Eur. J. Soil Sci. 50: 579–590

Moers MEC, Baas M, de Leeuw JW, Boon JJ & Schenk PA (1990) Occurrence and origin of carbohydrates in peat samples from a red mangrove environment as reflected by abundances of neutral monosaccharides. Geochim. Cosmochim. Acta 54: 2463–2472

Moore TR & Matos L (1999) The influence of source on the sorption of dissolved organic carbon by soils. Can. J. Soil Sci. 79: 321–324

Mulholland PJ & Hill WR (1997) Seasonal patterns in streamwater nutrient and dissolved organic carbon concentrations: Separating catchment flow and in-stream effects. Water Resour. Res. 33: 1297–1306

Murayama S (1984) Changes in the monosaccharide composition during the decomposition of straws under field conditions. Soil Sci. Plant Nutr. 30: 367–381

Murphy EM, Zachara JM & Smith SC (1990) Influence of mineral-bound humic substances on the sorption of hydrophobic organic compounds. Environ. Sci. Technol. 24: 1507–1516

Oades JM (1984) Soil organic matter and structural stability: mechanisms and implications for management. Plant Soil 76: 319–337

Orem WH & Hatcher PG (1987) Solid-state ^{13}C NMR studies of dissolved organic matter in pore waters from different depositional environments. Org. Geochem. 11: 73–82

Pohlman AA & McColl JG (1988) Soluble organics from forest litter and their role in metal dissolution. Soil Sci. Soc. Am. J. 52: 265–271

Preston CM, Hempfling R, Schulten H-R, Schnitzer M, Trofymow JA & Axelson DE (1994) Characterization of organic matter in a forest soil of coastal British Columbia by NMR and pyrolysis-field ionization mass spectrometry. Plant Soil 158: 69–82

Qualls R & Haines BL (1991) Geochemistry of dissolved organic nutrients in water percolating through a forest ecosystem. Soil Sci. Soc. Am. J. 55: 1112–1123

Qualls R & Haines BL (1992) Biodegradability of dissolved organic matter in forest throughfall, soil solution, and stream water. Soil Sci. Soc. Am. J. 56: 578–586

Qualls RG, Haines BL & Swank WT (1991) Fluxes of dissolved organic nutrients and humic substances in a deciduous forest. Ecology 72: 254–266

Qualls RG, Haines BL, Swank WT & Tyler SW (2000) Soluble organic and inorganic nutrient fluxes in clearcut and mature deciduous forests. Soil Sci. Soc. Am. J. 64: 1068–1077

Raber B & Kögel-Knabner I (1997) Influence of origin and properties of dissolved organic matter on the partition of polycyclic aromatic hydrocarbons (PAHs). Eur. J Soil Sci. 48: 443–455

Raulund-Rasmussen K, Borggaard OK, Hansen HCB & Olsson M (1998) Effect of natural organic soil solutes on the weathering rates of soil minerals. Eur. J. Soil Sci. 49: 397–406

Šantrůčková H, Bird MI, Frouz J, Šustr V & Tajovský K (2000) Natural abundance of ^{13}C in leaf litter as related to feeding activity of soil invertebrates and microbial mineralisation. Soil Biol. Biochem. 32: 1793–1797

Schimel JP & Clein JS (1996) Microbial response to freeze-thaw cycles in tundra and taiga soils. Soil Biol. Biochem. 28: 1061–1066

Schleser GH, Frielingsdorf J & Blair A (1999) Carbon isotope behaviour in wood and cellulose during artificial aging. Chem. Geol. 158: 121–130

Scott MJ, Jones MN, Woof C & Tipping E (1998) Concentrations and fluxes of dissolved organic carbon in drainage water from an upland peat system. Environ. Int. 24: 537–546

Seelenfreund D, Lapierre C & Vicuna R (1990) Production of soluble lignin-rich fragments (APPL) from wheat lignocellulose by *Streptomyces viridosporus* and their partial metabolism by natural bacterial isolates. J. Biotechnol. 13: 145–158

Stepanauskas R, Laudon H & Jørgensen NOG (2000) High DON bioavailability in boreal streams during a spring flood. Limnol. Oceanogr. 45: 1298–1307

Tipping E, Woof C, Rigg E, Harrison AF, Ineson P, Taylor K, Benham D, Poskitt J, Rowland AP, Bol R & Harkness DD (1999) Climatic influences on the leaching of dissolved organic matter from upland UK moorland soils, investigated by a field manipulation experiment. Environ. Int. 25: 83–95

Wilson MA (1987) NMR Techniques and Applications in Geochemistry and Soil Chemistry. Pergamon Press, Oxford

Wilson MA, Collin PJ, Malcolm RL, Perdue EM & Cresswell P (1988) Low molecular weight species in humic and fulvic fractions. Org. Geochem. 12: 7–12

Zhang X & Amelung W (1996) Gas chromatographic determination of muramic acid, glucosamine, mannosamine, and galactosamine in soils. Soil Biol. Biochem. 28: 1201–1206

Zhang X, Amelung W, Yuan Y, Samson-Liebig S, Brown L & Zech W (1999) Land-use effects on amino sugars in particle size fractions of an Argiudoll. Appl. Soil Ecol. 11: 271–275