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Soil Biology & Biochemistry 35 (2003) 1129–1142
Soil Biology & Biochemistry
www.elsevier.com/locate/soilbio

Changes in properties of soil-derived dissolved organic matter induced by biodegradation

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Received 2 October 2002; received in revised form 26 February 2003; accepted 10 March 2003

Abstract

Properties of dissolved organic matter (DOM) determine its biodegradation. In turn, biodegradation changes the properties of the remaining DOM, which may be decisive for the formation of stable organic carbon in soil. To gain information on both mechanisms and controlling factors of DOM biodegradation and the properties of biodegraded DOM, we investigated changes in the composition of 13 different DOM samples extracted from maize straw, forest floors, peats, and agricultural soils during a 90-day incubation using UV absorbance, fluorescence emission spectroscopy, FTIR-spectroscopy, ^{1}H-NMR spectroscopy, pyrolysis-field ionization mass spectroscopy (Py-FIMS), and ^{13}C natural abundance before and after incubation. Changes in the DOM properties were related to the extent of biodegradation determined by the release of CO_2. Increasing UV absorption and humification indices deduced from fluorescence emission spectra, and increasing portions of aromatic H indicated relative enrichment of aromatic compounds during biodegradation. This enrichment significantly correlated with the amount of DOC mineralized suggesting that aromatic compounds were relatively stable and slowly mineralized. ^{13}C depletion during the incubation of highly degradable DOM solutions indicated an enrichment of lignin-derived aromatic compounds. Py-FI mass spectra indicated increasing contents of phenols and lignin monomers at the expense of lignin dimers and alkylaromatics during incubation. This partial degradation of higher-molecular, lignin-derived DOM compounds was accompanied by relative increases in the proportions of lower-molecular degradation products and microbial metabolites. Carbohydrates, especially when abundant at high initial contents, seem to be the preferred substrate for microorganisms. However, four independent methods suggested also some microbial production of carbohydrates and peptides during DOM degradation. After incubation, the composition of highly degradable DOM samples became similar to relatively stable DOM samples with respect to aromaticity, carbohydrate content, and thermal stability. We conclude that DOM biodegradation seems to result in organic matter properties being a precondition for the formation of stable carbon. These structural changes induced by DOM biodegradation should also result in stronger DOM sorption to the soil matrix additionally affecting DOM stabilization.

Keywords: δ^{13}C; Degradation; Dissolved organic matter; Fluorescence spectroscopy; ^{1}H-NMR spectroscopy; Pyrolysis-mass spectrometry; Stabilization; UV/VIS spectroscopy

1. Introduction

All microbial uptake mechanisms require an aqueous environment (Metting, 1993). Therefore, dissolved organic matter (DOM) may be the key constituent for microbial

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doi:10.1016/S0038-0717(03)00165-2

degradation of organic matter in soils. Two recent studies showed that CO_2 evolution from soil samples can be largely explained by the decrease in water-extractable organic carbon (Marschner and Noble, 2000; Marschner and Bredow, 2002). The finding that biodegradability of DOM is similar to that of other fractions of soil organic matter (Kalbitz et al., 2003) suggests that biodegradation of soil organic matter is mediated by the aqueous phase.

Incubation studies in the laboratory showed that 4–93% of soil-derived DOM can be microbially decomposed (Kalbitz et al., 2003). Marschner and Kalbitz (2003) considered intrinsic DOM properties as control factors of DOM biodegradation. DOM with a large portion of carbon in the XAD-8-adsorbable fraction, rich in aromatic structures and complex molecules, and poor in carbohydrates is little biodegradable (Qualls and Haines, 1992; Jandl and Sollins, 1997; Jandl and Sletten, 1999; Volk et al., 1997; Amon et al., 2001; Kalbitz et al., 2003).

Evaluation of changes in composition of DOM during degradation could offer deeper insight into mechanisms and controlling factors of DOM biodegradation and could help to identify fractions of DOM preferentially decomposed. A recent study reported depletion in ^{14}C during biodegradation of riverine DOM which indicates that stable DOM compounds might be older than the degradable ones (Raymond and Bauer, 2001). Application of UV and fluorescence spectroscopy before and after incubation indicated an enrichment of aromatic moieties during biodegradation of DOM (Zsolnay and Steindl, 1991; Hongve et al., 2000; Moran et al., 2000; Parlanti et al., 2000; Pinney et al., 2000). Decomposition studies using litter or soil organic matter imply that these aromatic compounds should mainly derive from lignin (Nordén and Berg, 1990; Kögel-Knabner, 2002). Lignin is a large contributor to the residue of terrestrial biomass (Kögel-Knabner, 2002), and water-soluble aromatic lignin degradation products are also important DOM components (Guggenberger et al., 1994a).

Volk et al. (1997) and Amon et al. (2001) reported that dissolved carbohydrates and amino acids are preferentially utilized by microorganisms. However, carbohydrates can be bound to refractory DOM compounds (Guggenberger et al., 1994a; Jandl and Sollins, 1997), which could prevent their degradation, and carbohydrates like glucose can be transformed into stable DOM components by bacteria (Ogawa et al., 2001).

Detailed investigations on biodegradation induced changes of DOM properties are hampered by the decrease of the already low amounts of DOM in degradation experiments. Therefore, combining various techniques with low sample demand and addressing different DOM properties should be a promising strategy to overcome this problem. The use of UV, fluorescence, FTIR, and ^{1}H-NMR spectroscopy allows one to gain information about the aromaticity and complexity of DOM molecules, the contents of functional groups, carbohydrates, and simple organic compounds (Wilson et al., 1988; Senesi et al., 1989; Chin et al., 1994; McKnight et al., 1997; Zsolnay et al., 1999; Parlanti et al., 2000; Kalbitz et al., 2003). Analyzing ^{13}C natural abundance could also reveal some structural changes during DOM biodegradation because different classes of compounds have different $\delta^{13}C$ values (Lichtfouse, 2000). Pyrolysis-field ionization mass spectroscopy (Py-FIMS) was successfully applied to investigate the molecular composition and structural properties such as molecular weights and thermal stability of aquatic humic substances and DOM (Leinweber et al., 2001; Schulten et al., 2002).

The objectives of this study are to investigate changes in DOM properties during microbial DOM degradation as indicators of the mechanisms and controlling factors of DOM biodegradation. The tested hypotheses were

- Aromaticity and complexity of DOM molecules increase during DOM biodegradation whereas carbohydrates either decrease due to preferential degradation or increase as a result of microbial synthesis.
- DOM biodegradation results in a relative enrichment of lignin-derived moieties that are depleted in ^{13}C.
- DOM biodegradation affects the thermal behavior of whole DOM samples and individual compound classes with an assumed increased thermal stability of residual DOM.

2. Materials and methods

Samples were taken at two forest sites, one under Norway spruce (*Picea abies* (Karst.) L.) and another one under European beech (*Fagus sylvatica* L.), in a fen area, and from three agricultural soils (Table 1). In addition, maize straw was collected immediately after harvesting. The sampled material was sieved ($\leq$5 mm), visible roots and animals were removed, and the samples were thoroughly mixed and stored frozen. The maize straw and the beech litter were cut into pieces of 1–2 cm^2 in size before mixing. Production of DOM solutions was done by adding ultra-pure water to the solid material (solid/solution ratio: Table 1). The suspensions were equilibrated for 24 h, then filtered through 0.2 μm membranes (cellulose acetate; OE 66, Schleicher & Schuell). Prior to the experiment, aliquots of each solution were freeze dried for further analysis. Before incubation, DOM solutions with more than 20 mg C l^{-1} were diluted to avoid extensive growth of microorganisms. Details on sampling, preparation of DOM solutions, and the incubation experiment are given in Kalbitz et al. (2003). Seven replicates (700 ml) of each DOM solution were incubated in 1 l incubation flasks. A mixed inoculum extracted from Oa-spruce, Oi-beech, fen-4, and BL-manure was added (1% v/v) with no additional nutrient amendment. The incubation flasks were sealed, incubated in the dark at 20 °C for 90 days, gently shaken by

Table 1
Total organic carbon (TOC) and total organic nitrogen (TON) content of the solid material used for production of DOM solutions, the soil/solution ratio for extraction, the DOC content of the DOM solutions and the portion of DOC mineralized

Sample/site	Acronym	TOC (g kg^{-1})	TON (g kg^{-1})	Solid/solution ratio (w/w)[a]	DOC[b] (mg C l^{-1})	Mineralized DOC[c] (%)
Beech forest (Dystric Cambisol)						
Litter layer	Oi-beech	456	15.7	0.1	604	65.0
Fermented/Humified layer	Oa-beech	295	16.2	0.1	67.4	9.1
Spruce forest (Haplic Podzol)						
Litter layer	Oi-spruce	474	17.4	0.1	99.3	61.4
Fermented layer	Oe-spruce	435	21.2	0.1	149	93.4
Humified layer	Oa-spruce	312	13.9	0.1	38.1	7.2
fen area (Gleyic Mollisols and Terric Histosols)						
Arable soil	fen-1	43.3	3.0	1.5	19.8	7.8
Grassland soil	fen-2	48.3	3.6	0.2	18.4	9.4
Grassland in succession	fen-3	96.1	7.2	0.1	40.5	8.5
Almost natural forest	fen-4	383	26.8	0.1	75.7	5.3
Field sites of a long-term agricultural trial (Haplic Chernozem)						
Without any fertilization	BL-0	12.8	1.3	1.5	7.2	30.0
Mineral fertilization	BL-NPK	12.5	1.1	1.5	12.6	17.2
Organic fertilization	BL-manure	14.7	1.4	1.5	13.9	31.8
Maize straw	Maize straw	420	8.6	0.1	819	88.6

[a] Ratio applied to extract DOM.
[b] Measured DOC concentration in the extract.
[c] After 90 days; measured by CO_2 evolution (Kalbitz et al., 2003).

hand every day. The biodegradation of DOM was monitored by measuring the CO_2 evolution of each of three replicates (Kalbitz et al., 2003). The incubation flasks of these three replicates were opened after 6, 14, 54, and 90 days and an aliquot of the solution was sampled. Furthermore, the DOM solutions were aerated for 10 min using filtered compressed air. Solutions of the other four replicates (not sampled during the incubation) were filtered (0.2 μm) and freeze dried after the end of the experiment.

The filtered DOM solutions were analyzed before, during and after incubation for DOC concentrations (High TOC, Elementar; also for the unfiltered sample), UV absorbance at 280 nm (UVIKON 930, BIO-TEK Instruments) to estimate the aromaticity of DOM (Chin et al., 1994; McKnight et al., 1997), emission fluorescence (SFM 25, BIO-TEK Instruments) followed by calculations of humification indices (HIX_{em}; Zsolnay et al., 1999) as a measure for the complexity of the DOM molecules. For the UV and fluorescence measurements, all solutions were adjusted to 10 mg C l^{-1}, pH 7.7, and an electrical conductivity of 1000 μS cm^{-1} to ensure comparability. DOM solutions extracted from maize straw were only analyzed before and at the end of the incubation.

Freeze-dried samples of DOM before and after incubation were analyzed by the following methods:

- Liquid-state ^{1}H-NMR spectroscopy (Avance DRX 500, Bruker Analytik GmbH) to estimate the portions of H associated with O-containing functional groups (mainly carbohydrate H: 3.0–4.8 ppm) and with aromatic compounds (5.5–10.0 ppm) (Kaiser et al., 2002; Kalbitz et al., 2003).
- FTIR spectroscopy (BioRad® FTS 135; Ellerbrock et al., 1999).
- Isotope ratio mass spectrometry ($\delta^{13}C$; Delta^{plus}, ThermoQuest-Finnigan MAT, connected to an elemental analyzer, NA 2500, ThermoQuest-Fisons).
- Pyrolysis-field ionization mass spectrometry (Py-FIMS) of DOM samples from maize straw, Oi-spruce, and Oa-spruce.

For the FTIR and isotopic analysis of DOM after incubation we had to combine several replicates of the samples fen-1 and maize straw due to the low amount of material (fen 1: combining of all four replicates, maize straw: combining of each two replicates). All replicates of each sample were combined for ^{1}H-NMR spectroscopy and Py-FIMS.

For ^{1}H-NMR spectroscopy, a 15–50 mg aliquot of each sample was dissolved in 3 ml of 0.5 M NaOD and a portion of the solution was transferred into a 5 mm NMR tube (Kaiser et al., 2002). Conditions for ^{1}H-NMR were: spectrometer frequency, 500.13 MHz; homonuclear presaturation for solvent suppression; pulse delay, 1.0 s; acquisition time, 1.16 s; line-broadening factor, 2 Hz. Two hundred scans were accumulated for each sample. Chemical shifts were given relative to the resonance of

tetramethylsilane. Signal assignments to compound groups and single compounds were made according to the literature (Wilson, 1987; Wilson et al., 1988; Leenheer, 1994). Complementary ^{13}C-NMR was not applicable because of too small amounts of sample, especially after incubation.

In all cases except that of Oa-beech distinct signals at 3.69 and 3.76 ppm occurred in the spectra of DOM after incubation. These signals were mostly absent in the spectra before the experiment. These resonances may indicate an enrichment of methylaryl ethers (Wilson et al., 1988), which are typical lignin degradation products. However, also glycols give sharp signals in this region. Since the used filter membranes contained glycols, contamination of the samples could not be excluded although all filters were thoroughly washed with 250 ml ultra-pure water before use. The low DOC concentrations of samples after biodegradation could be responsible for the contamination becoming evident only in these samples. Therefore, we decided not to include the region between 3.65 and 3.80 ppm when integrating resonances typical of carbohydrates (3.0–4.8 ppm).

δ^{13}C values were determined on ground subsamples of freeze-dried DOM. 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). δ^{13}C value expresses the enrichment of ^{13}C in a sample relative to the ^{13}C of CO_2 prepared from a calcareous belemnite of the cretaceous Peedee formation, South Carolina. The analytical precision of the measurements was 0.1‰.

FTIR spectra of freeze dried DOM samples were recorded in a range of wave numbers between 3900 and 400 cm^{-1}. The freeze-dried DOM material (0.5 mg) was mixed with 80 mg KBr and finely ground using an agate mortar. The resulting mixture was dried for 12 h over silica gel in a desiccator. Afterwards pellets of 10 mm diameter were prepared at a pressure of 10,000 kg cm^{-2} for 10 min. All spectra were measured using a fixed resolution of 1 cm^{-1} and eight scans (Ellerbrock et al., 1999). To quantify the amount of carboxylic groups in each sample the sum of absorption bands at 1740–1700 cm^{-1} and 1640–1600 cm^{-1} were computed by measuring the length of lines drawn from the baseline to the maximum of each band using BIORAD WINIREZ software. The ranges and procedures are described in more detail by Celi et al. (1997). To quantify the amounts of C–O groups (polysaccharides), the same procedure was used for the bands in the region from 1100 to 1000 cm^{-1}.

For Py-FIMS, about 1 mg of sample was thermally degraded in the ion source of a modified Finnigan MAT 731 high performance mass spectrometer. The sample was heated in the high vacuum of the ion source from 110 to 700 °C at a heating rate of approximately 10 K per magnetic scan (three replicates). After 19 min of total registration time, about 60 magnetic scans were recorded for the mass range of 16–900 Da (single spectra). These were combined to obtain one thermogram of total ion intensity and an averaged mass spectrum. In addition, for each of the single scans the ion intensities of marker signals for 10 selected classes of chemical compounds found in soil organic matter were calculated. All Py-FIMS data were normalized per mg sample. Detailed descriptions of the Py-FIMS methodology and of statistical evaluations of sample weight and residue, volatilized matter, and total ion intensities have been published previously (Schulten, 1996). For the signal assignment to 10 classes of chemical compounds see Schulten and Leinweber (1999).

2.1. Statistics

We calculated linear regressions between the analyzed DOM properties and parameters describing the DOM biodegradation. These parameters (percentage of mineralized DOC after 90 days, calculated portions and mineralization constants of rapidly and slowly mineralizable DOC) are given in Kalbitz et al. (2003). Changes in DOM properties before and after incubation were tested for significance using a paired *t*-test.

3. Results

3.1. Extent of DOM biodegradation

Changes in DOM properties were evaluated in their relation to the extent of DOM biodegradation. Three distinct groups of different degradability could be identified: Biodegradation was high for DOM from weakly decomposed organic material (group 1: Oi-spruce, Oi-beech, Oe-spruce, maize straw). In these samples, between 61 and 93% of total DOC was mineralized after 90 days (Table 1). DOM extracted from agricultural soils (group 2: BL-0, BL-NPK, BL-manure) revealed moderate biodegradation (mineralization of 17–32% of total DOC), whereas DOM from peats and from Oa forest floor horizons (group 3: fen-1…4, Oa-spruce, Oa-beech) was relatively stable (mineralization of 5–9% of total DOC). Further details of the extent and temporal course of DOM biodegradation were published by Kalbitz et al. (2003).

3.2. UV and fluorescence data

The specific absorption at 280 nm (A 280) and the humification index deduced from fluorescence emission spectra (HIX_{em}) increased considerably during incubation of most of the DOM samples (Figs. 1 and 2, Table 2). Solutions of highly biodegradable DOM (group 1 in Table 2) exhibited much stronger increases than solutions of less degradable DOM. Nevertheless, even after incubation, the UV absorbance and HIX_{em} of the three sample groups remained different (see means of the three groups in

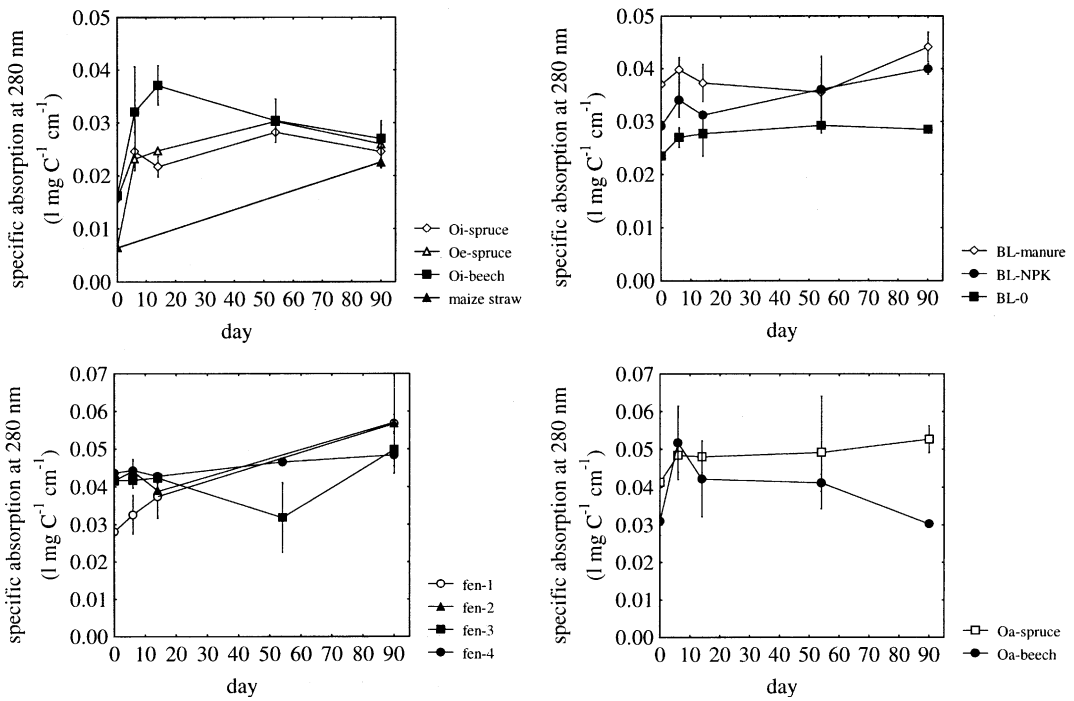

Fig. 1. Temporal changes in the specific absorption at 280 nm during DOM incubation (mean and standard deviation of three replicates).

Table 2). The increase in A 280 and HIX_{em} was positively correlated with the percentage of DOC mineralized after 90 days (A 280: $r^2 = 0.66$, HIX_{em}: $r^2 = 0.86$; $n = 39$; $p < 0.001$ in both cases). However, DOM solutions with intermediate biodegradation (group 2 in Table 2) showed a similar increase in A 280 as compared to the most stable DOM solutions (group 3 in Table 2). The HIX_{em} of DOM in group 2 did not change during incubation.

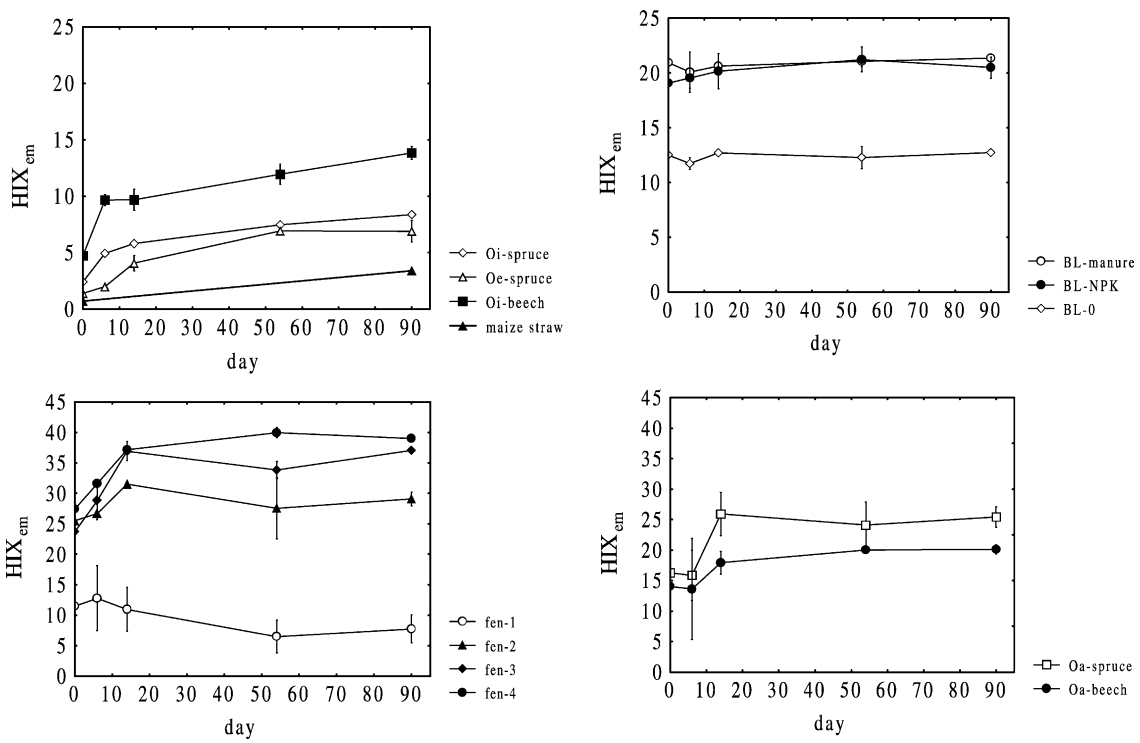

Fig. 2. Temporal changes in the HIX_{em} (humification index deduced from fluorescence emission spectra) during DOM incubation (mean and standard deviation of three replicates).

Table 2
Spectroscopic properties of DOM before and after a 90-day incubation experiment

	A 280[a]		ΔA 280[b] (%)	HIX_{em}[c]		ΔHIX_{em}[b] (%)	AR[d]		ΔAR[b] (%)	CH[e]		ΔCH[b] (%)
	Before	After		Before	After		Before	After		Before	After	
Group 1: DOM with high biodegradation												
Oi-beech	0.016	0.027	67	4.7	13.8	193	5.6	9.9	78	37.1	26.0	−30
Oi-spruce	0.016	0.025	57	2.4	8.4	246	6.2	11.6	88	39.6	19.1	−52
Oe-spruce	0.007	0.026	297	1.4	6.9	400	5.5	14.5	166	44.0	20.0	−55
Maize straw	0.006	0.023	252	0.7	3.4	386	5.4	15.9	194	33.5	21.5	−36
Mean	0.011	0.025	168	2.3	8.1	306	5.7	13.0	132	38.6	21.6	−43
Group 2: DOM with intermediate biodegradation												
BL-0	0.023	0.028	21	12.5	12.7	2	9.5	11.8	25	17.0	21.1	24
BL-NPK	0.029	0.040	37	19.1	20.5	7		9.1		20.8	18.4	−12
BL-manure	0.037	0.044	19	20.9	21.3	2	11.7	8.8	−25	24.2	17.1	−29
Mean	0.030	0.037	26	17.5	18.2	4	10.6	9.9	0	20.6	18.9	−5
Group 3: DOM with low biodegradation												
Oa-spruce	0.041	0.053	28	16.3	25.4	56	13.3	19.2	44	31.4	20.3	−35
Oa-beech	0.031	0.030	−2	14.0	20.1	43	10.5	10.7	2	24.4	14.0	−43
fen-1	0.028	0.057	102	11.4	7.7	−32	9.4	6.3	−33	28.8	26.8	−7
fen-2	0.042	0.057	36	25.5	29.1	14	10.2	15.4	50	13.9	18.3	32
fen-3	0.042	0.050	19	23.7	37.1	56	11.9	16.6	40	16.6	17.9	8
fen-4	0.044	0.048	11	27.4	39.0	42	13.1	14.0	7	17.7	13.4	−24
Mean	0.038	0.049	32	19.7	26.4	30	11.4	13.7	18	22.1	18.4	−12

[a] Specific absorption at 280 nm (l mg C^{-1} cm^{-1}; mean of three replicates).
[b] Increase in % during the course of DOM biodegradation (mean of three replicates).
[c] Humification index using emission fluorescence spectra (ratio of areas: 435–480 nm/300–345 nm; mean of three replicates) (Zsolnay et al., 1999).
[d] Aromatic H (% of H); ^{1}H-NMR (5.5–10.0 ppm).
[e] Carbohydrate H (% of H); ^{1}H-NMR, H associated with O-containing functionalities (3.0–4.8 ppm).

The changes in HIX_{em} of DOM solutions during incubation except those from arable soils were characterized by strong increases at the beginning and smaller increases later on. This was less pronounced for A 280, which showed larger fluctuations over time (Fig. 1). It is noteworthy that the A 280 of DOM solutions from the beech forest (Oi and Oa) increased initially, and considerably decreased at later stages of the experiment. After six days, the A 280 of DOM from the beech forest Oa almost doubled, but thereafter it decreased even below the initial value.

3.3. ^{1}H-NMR spectra

Most DOM solutions showed an increase in the abundance of aromatic H during incubation (Fig. 3, Table 2). Highly degradable DOM solutions (group 1 in Table 2) exhibited the strongest increase in aromatic H from about 6–13% (Table 2). Only in two solutions (BL-manure, fen-1), proportions of aromatic H decreased. The relative increase in aromatic H signals was closely correlated with the percentage of DOC mineralized after 90 days ($r^2 = 0.72$).

Highly degradable DOM solutions (group 1 in Table 2) and DOM from Oa-spruce and Oa-beech showed strong decreases in the portion of carbohydrate H during the incubation experiment. The relative abundance of carbohydrate H in most DOM samples from agricultural soils and from peats did not considerably change during the experiment. Changes in the content of carbohydrate H were neither related to the percentage of DOC mineralized

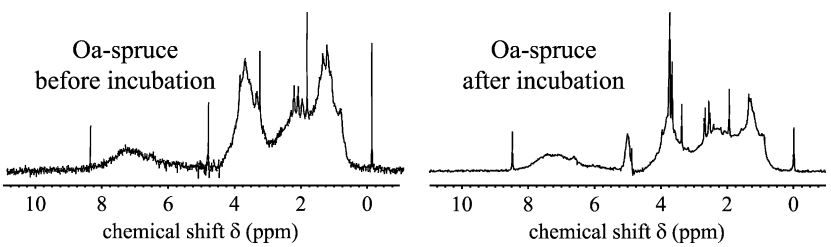

Fig. 3. ^{1}H-NMR spectra of one DOM sample before and after incubation.

nor to other variables describing DOM degradation. At the end of the experiment the relative portions of carbohydrate and aromatic H of the different sample groups approached a similar level (Table 2).

3.4. $\delta^{13}C$

Three out of four samples of highly degradable DOM samples were significantly ($p < 0.05$) depleted in ^{13}C after incubation (Fig. 4). In contrast, $\delta^{13}C$ values increased during incubation of samples with intermediate degradation (agricultural soils). The isotopic composition of C in DOM with low biodegradation changed only little during incubation. A small depletion in ^{13}C of about 0.4‰ after incubation was found for only two of these samples (Oa-spruce, fen-2) which were also characterized by relatively strong increases in aromatic H of about 5% (Table 2).

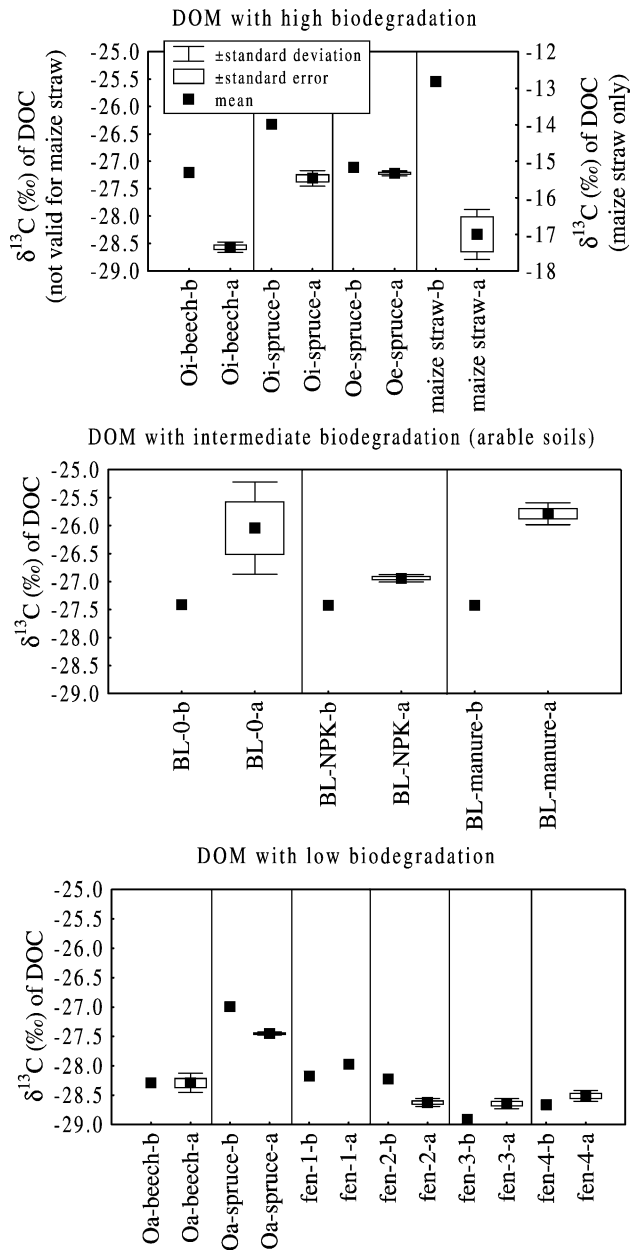

Fig. 4. $\delta^{13}C$ ratios of DOM before (b) and after (a) incubation.

3.5. FTIR spectra

Absorption at different wavenumbers could not be related to the extent of DOM degradation and did not show a clear trend during incubation. Polysaccharides have absorption bands at around 1100 cm^{-1} and Celi et al. (1997) quantified carboxylic groups by adding up the absorption at 1720 cm^{-1} (protonated) and 1600 cm^{-1} (deprotonated). Ratios of the absorption at 1100 cm^{-1} to the added-up absorption at 1720 cm^{-1} and 1600 cm^{-1} considerably increased for 11 of 13 DOM samples during incubation (Figs. 5 and 6) indicating an enrichment of polysaccharides relative to carboxylic groups during incubation.

3.6. Py-FI mass spectra

All DOM samples gave highly intensive Py-FI mass spectra, irrespective of source and biodegradability. The ion intensities in the lower mass range were larger for highly degradable DOM samples (Oi-spruce, maize straw) than for the relatively stable DOM sample (Oa-spruce, not shown). Difference Py-FI mass spectra were plotted to visualize differences between DOM samples before and after incubation (Fig. 7). Positive differences in signal intensities indicate relative enrichments during incubation. This was true for low-mass, non-specific signals (m/z 19, 23, 30, 39, 41), some signals of N containing compounds (m/z 57, 59), carbohydrates (m/z 96, 110), and phenols and lignin monomers (m/z 110, 124, 166, 208). For some of these signals there was no increase during incubation of the maize DOM sample. On the other hand, the relative signal intensities at higher m/z often decreased during incubation. This was especially pronounced for the relatively stable DOM sample (Oa-spruce). For this sample signal intensities of $m/z > 230$ decreased and of $m/z < 230$ increased following incubation (Fig. 7).

The abundances of 10 compound classes (Table 3) revealed higher contents of lipids, alkylaromatics, sterols, and free fatty acids, and lower contents of heterocyclic nitrogen compounds and peptides in the stable DOM sample (Oa-spruce) as compared with the two highly degradable samples. In all three samples incubation and biodegradation increased the proportions of peptides at the expense of lignin dimers (Table 3). In the samples from forest floor (Oi and Oa), the proportions of carbohydrates and phenols/lignin monomers, mainly heterocyclic N containing compounds and peptides increased, again, at the expense of lignin dimers. DOM from maize straw underwent different changes during incubations as monomeric and dimeric lignin building blocks and heterocyclic N containing compounds were decomposed and, in addition to peptides,

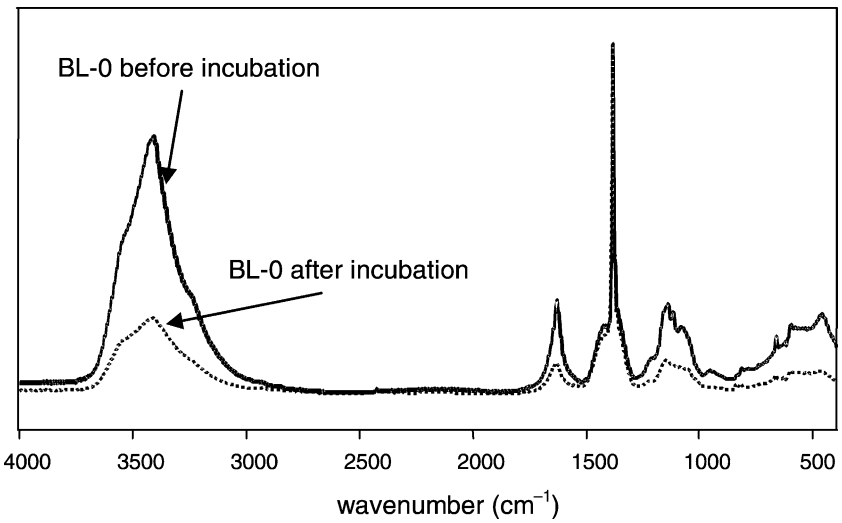

Fig. 5. FTIR spectra of one DOM sample before and after incubation.

lipids, sterols, and free fatty acids, all of them containing much alkyl structures, were newly formed (Table 3).

The thermograms of total ion intensity (TII; entire DOM samples) and of individual compound classes were relatively similar for the two highly degradable DOM samples before incubation. A first volatilization peak occurred between 250 and 300 °C and a second around 450 °C (Fig. 8). In contrast, the thermograms obtained from the relatively stable DOM sample (Oa-spruce) had a rather Gaussian-like shape with a peak at 350–400 °C before incubation (Fig. 9). Only carbohydrates had a first peak of volatilization at 290 °C and a second, smaller peak at about 400 °C.

Strong degradation of Oi-spruce DOM resulted in large changes of the thermal behavior. First, the TII thermograms in Fig. 8 indicate that the volatilization at lowest pyrolysis temperatures, starting from 150 °C, was reduced and the volatilization between 400 and 500 °C largely disappeared. Instead the onset temperature was

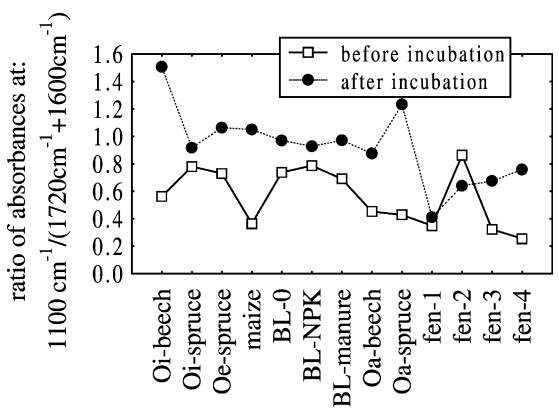

Fig. 6. Absorbance at 1100 cm^{-1} typical of polysaccharides divided by the added-up absorbances at 1720 and 1600 cm^{-1} typical of carboxylic groups of the DOM samples before and after incubation.

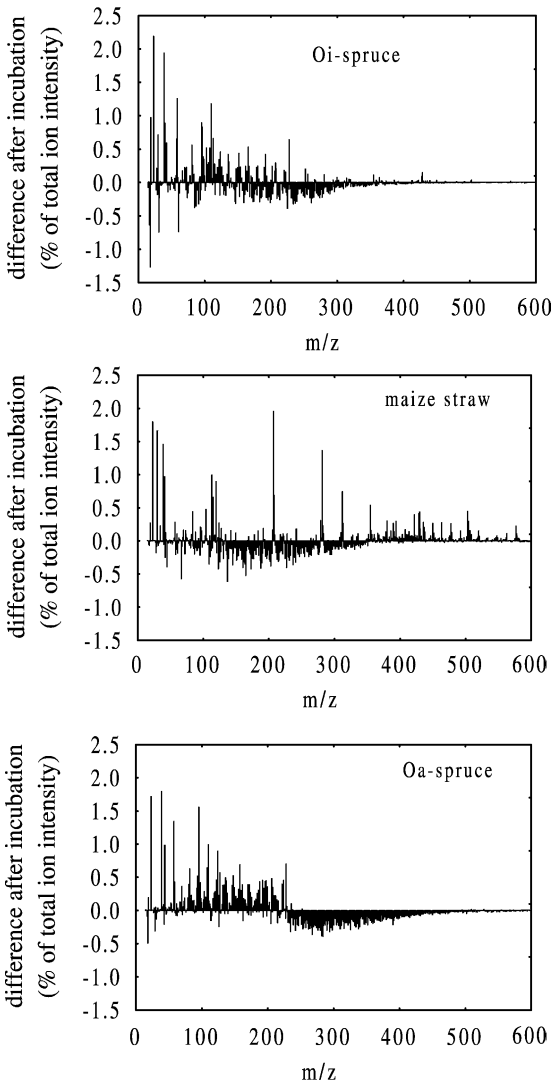

Fig. 7. Pyrolysis-field ionization mass spectra of three different DOM samples (abbreviations see Table 1): differences between spectra before and after the incubation (positive values indicate enrichment whereas negative values indicate depletion).

Table 3
Relative abundances of 10 important compound classes in DOM before and after DOM biodegradation (% of total ion intensity; carbohydrates = CHYD, phenols + lignin monomers = PHLM, lignin dimers = LDIM, lipids = LIPID, alkylaromatics = ALKY, heterocyclic nitrogen containing compounds = NCOMP, sterols = STEROL, peptides = PEPTI, suberin = SUBER, free fatty acids = FATTY; means of three analytical replicates)

Sample	CHYDR	PHLM	LDIM	LIPID	ALKY	NCOMP	STEROL	PEPTI	SUBER	FATTY
Oi-spruce-b	7.1	10.4	1.9	4.1	9.2	9.0	0.3	4.7	0.0	0.6
Oi-spruce-a	9.2	13.1	0.2	3.5	8.7	9.1	0.2	5.2	0.0	1.2
Oa-spruce-b	6.5	8.2	2.9	9.0	9.7	6.2	1.8	2.9	0.3	2.2
Oa-spruce-a	10.9	14.7	1.0	4.8	12.1	8.0	0.0	4.5	0.0	0.6
Maize straw-b	4.3	9.1	3.2	5.5	8.0	10.7	0.5	3.3	0.1	0.5
Maize straw-a	4.3	8.0	1.8	6.1	7.5	4.6	2.3	5.2	0.2	1.6

b:Sample before incubation, a: sample after 90 days of incubation.

shifted by +50 K and a peak of major volatilization appeared at 350 °C, which was 50 °C higher than that of DOM before incubation. The volatilization curves of the individual compound classes changed more or less similarly to TII except for lignin dimers, lipids, and free fatty acids which did not substantially change during incubation.

The more stable DOM sample (Oa-spruce) did not undergo strong changes in the bulk thermal stability during incubation (Fig. 9). Only for individual compound classes such as carbohydrates, N-containing compounds, peptides, phenols/lignin monomers and alkylaromatics a slight tendency of relative losses at lower pyrolysis temperatures and shifts of maximum volatilization by +80–100 K was observed. The thermograms of the Oi-spruce sample after incubation resembled the thermograms of the Oa-spruce sample before incubation.

4. Discussion

4.1. Relative enrichment of lignin-derived aromatic compounds by DOM biodegradation

Increasing values of UV absorption, HIX_{em}, portions of aromatic H, and contents of phenols and lignin monomers consistently indicate a relative enrichment of aromatic compounds during DOM biodegradation. These increases were linearly correlated with the extent of DOC mineralization supporting the idea that aromatic compounds are relatively stable against DOM biodegradation (Kalbitz et al., 2003). The close correlation with the increase in the HIX_{em} ($r^2 = 0.86$) suggests that stable molecules accumulating during DOM biodegradation are rather complex, possibly with an enhanced degree of conjugation and condensation (Senesi et al., 1989; Zsolnay et al., 1999). This is supported by the similarity in the temporal changes of CO_2 evolution (published by Kalbitz et al., 2003) and in HIX_{em} during the incubation (except for DOM samples derived from agricultural soils) (Fig. 2).

The stability of aromatic compounds and their relative enrichment during DOM degradation are partly questioned by the observation that alkyl compounds seem to be the main contributors to the stable fraction of C in the solid soil phase (Baldock et al., 1992). However, the reason for the stability of alkyl compounds seems to be inaccessibility rather than recalcitrance (Capriel et al., 1990; Sollins et al., 1996). Inaccessibility should be of minor importance for the incubation of solutes as presented here. Therefore, recalcitrant aromatic compounds seem to be the dominating fraction of stable DOM. This assumption is in agreement with findings of Almendros and Dorado (1999) and Yanagi et al. (2002) who studied biodegradation of soil-derived humic acids.

The depletion in ^{13}C observed for samples exhibiting strong degradation (Fig. 4) contradicts the often postulated preferential use of the lighter ^{12}C isotope by microorganisms during the decomposition of organic matter (e.g. Melillo et al., 1989). Recently, Ekblad et al. (2002) found that microbial ^{13}C discrimination during respiration is minor. Therefore, the observed ^{13}C depletion of incubated samples may reflect a relative enrichment of lignin-derived aromatic compounds which are known to be depleted in ^{13}C (Benner et al., 1987; Schulten and Gleixner, 1999). Also the enrichment of aromatic compounds shown above supports the idea of ^{13}C depletion induced by preferential accumulation of lignin-derived compounds. In samples with low degradation, changes in the $\delta^{13}C$ were small. However, ^{13}C depletion was also notable for two relatively stable DOM samples (Oa-spruce, fen-2) which also showed relatively strong increases in aromatic H content during incubation (Table 2).

Relatively high contents of lipids, sterols, and free fatty acids of the weakly degradable DOM determined by Py-FIMS indicate potentially stable compounds other than aromatic ones. However, the relative decrease of lipids during incubation especially evident for the weakly degradable DOM sample from Oa-spruce questions the assumption that lipids are stable against biodegradation.

The relative decrease of lignin dimers and alkylaromatics, and the increase in phenols and lignin monomers during incubation partly indicate degradation of lignin-derived compounds (Huang et al., 1998). This was further supported by the relative increases in the intensity of

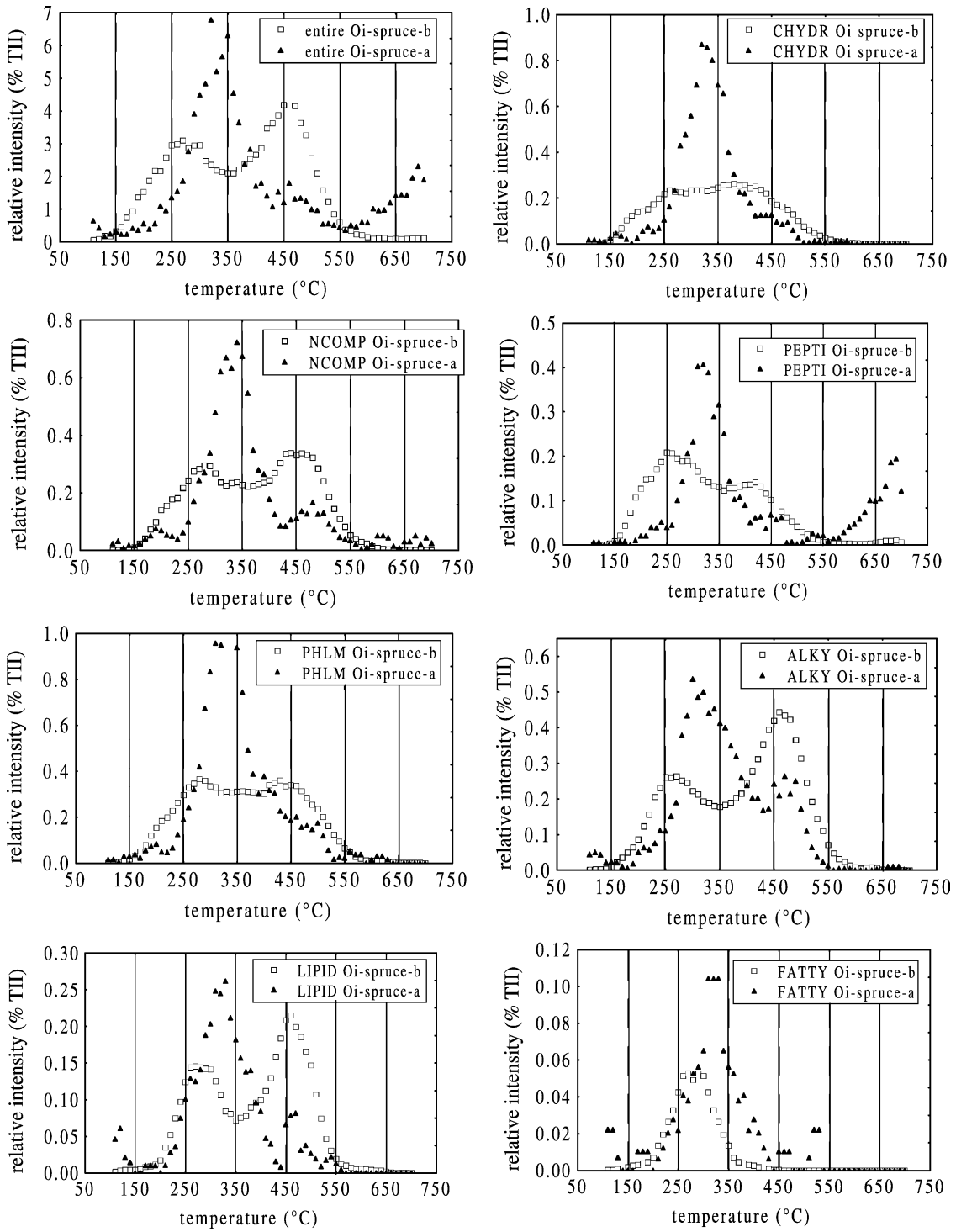

Fig. 8. Thermograms of total ion intensity (TII, entire DOM; upper left) and of the volatilization of compound classes of DOM from the Oi horizon under spruce (abbreviations see Table 3) before (b) and after (a) incubation.

compounds of lower masses (degradation products), and the decrease in compounds of high masses (more condensed lignin) in the Py-FI mass spectra. The changed thermograms of phenols, lignin monomers and of alkylaromatics of the highly degradable samples after incubation also indicate a shift in the molecular composition of these compound classes.

The data obtained by Py-FIMS indicate that DOM biodegradation results in a relative enrichment of monomeric lignin degradation products, and thus support

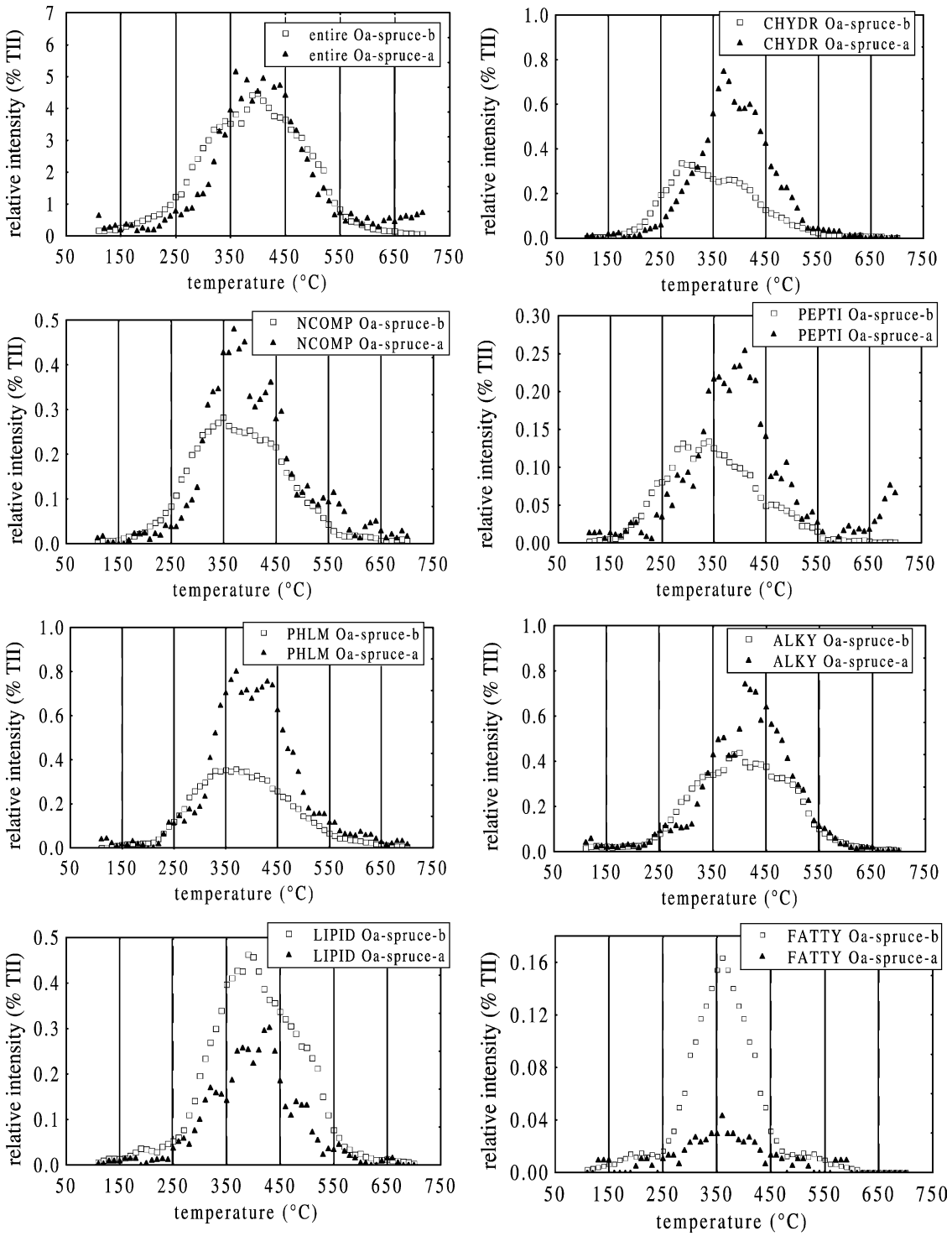

Fig. 9. Thermograms of total ion intensity (TII, entire DOM; upper left) and of the volatilization of compound classes of DOM from the Oa horizon under spruce (abbreviations see Table 3) before (b) and after (a) incubation.

the results obtained by UV, fluorescence, NMR, $\delta^{13}C$. Py-FIMS also indicates degradation of dimeric lignin-derived compounds. This was not expected, because incubation of DOM in solution with the chosen inoculation (Kalbitz et al., 2003) favored free living and loosely attached microorganisms like bacteria but not fungi which are supposed to be main degraders of lignin and lignin-derived compounds (Haider, 1992; Møller et al., 1999). Probably, the added surfaces in the incubation flasks (glass fiber filters) were sufficient for attachment of fungi

and development of mycelium. However, we did not examine the composition of the microbial community present in the samples.

4.2. Microbial formation of carbohydrates during DOM biodegradation

Based on the assumption that DOM biodegradation follows the same pattern as the decomposition of soil organic matter we hypothesized that carbohydrates will be degraded preferentially. A positive relationship between the content of carbohydrates of the samples at the beginning of incubation and the extent of DOM biodegradation (Kalbitz et al., 2003) supported this hypothesis. However, the ^{1}H-NMR spectra after incubation indicated a preferential use of carbohydrates only for highly degradable DOM samples and samples from the Oa layers with high initial contents of carbohydrates (on average 35%). For most of the other samples, carbohydrate contents changed little during incubation.

The increase in the absorbance at wavenumbers typical of polysaccharides ($\sim$1100 cm^{-1}) compared to the absorbance caused by carboxylic groups (Fig. 6) suggests higher stability of carbohydrates than of carboxyl groups, possibly due to preservation of carbohydrates bound to stable DOM compounds such as lignin (Guggenberger et al., 1994a; Volk et al., 1997). In contrast, oxidative microbial degradation of organic matter typically increases the negative charge and carboxyl groups of DOM (Guggenberger et al., 1994a). Therefore, relative increase in polysaccharides after incubation is likely to be caused by microbial formation. This assumption is confirmed by Ogawa et al. (2001) who reported production of refractory DOM due to bacterial use of labile compounds such as glucose and glutamate. Sollins et al. (1996) stated that many bacteria and fungi release diverse polysaccharides into their immediate environment. Biofilms that may have grown on the added glass fiber filters, are known to produce an extracellular polysaccharide matrix, which is another source of carbohydrates in the DOM samples (Lewandowski et al., 1994). It should be noted, however, that the increased absorption at around 1100 cm^{-1} might instead be due to enrichment in sulfate. Absorptions of sulfates range from 1114 cm^{-1} ($(NH_4)_2SO_4$) to 1119 cm^{-1} (Na_2SO_4).

Py-FI mass spectra indicated enrichments in typical microbial metabolites such as carbohydrates and peptides in samples derived from forest floor material already high in carbohydrates. Especially *m*/*z* 162 (levoglucosan) was significantly enriched in the Oi and Oa samples after incubation. This is a typical product of water cleavage from galactose and mannose (Schulten and Görtz, 1978) which are considered as indicators of microbial sugars (Guggenberger et al., 1994b). After incubation, shifts in the volatilization peaks of carbohydrates and peptides occurred even in the sample where thermograms of the entire sample (Oa-spruce; Fig. 9) before and after incubation were similar. This points out changes in the composition of carbohydrates. Microbial transformation seems to be a conclusive explanation. Also Huang et al. (1998) observed an accumulation of polysaccharides in mineral soil samples with increasing decomposition of organic matter, which could be attributed to microbially synthesized polysaccharides.

Further indication of microbial production of carbohydrates during DOM incubation is the observed increase of δ^{13}C in samples from agricultural soils that showed an intermediate extent of DOC mineralization, since polysaccharides and the microbial biomass are typically enriched in ^{13}C (Kracht and Gleixner, 2000; Lichtfouse, 2000). Also the unchanged HIX_{em} values of DOM samples from agricultural soils despite increasing aromaticity point to microbial transformation. HIX_{em} should increase with increasing aromaticity and complexity. However, HIX_{em} is low for microbial products (Zsolnay et al., 1999). The accumulation of both aromatic compounds (see above) and microbial products is a plausible explanation for lack of changes in HIX_{em} values during degradation of DOM from agricultural soils. The possible formation of microbial products did not result in increasing δ^{13}C during incubation of highly degradable DOM, because of the strong enrichment of lignin-derived compounds depleted in ^{13}C (see above). Additionally, we did not observe increasing δ^{13}C ratios for relatively stable DOM samples from Oa horizons and peats. For the samples from both Oa horizons, the ^{1}H-NMR spectra indicated decreases of the initially high contents of carbohydrates during incubation. According to ^{1}H-NMR, the peat samples showed hardly changed contents of carbohydrates. We hypothesize that only for DOM samples from agricultural soils, the microbial biomass production was sufficiently high to compensate for the enrichment of lignin-degradation products and thus to increase δ^{13}C.

In summary, the results obtained by different methods indicate degradation, transformation, and formation of carbohydrates during DOM biodegradation. At low initial contents of carbohydrates, other compounds such as lignin-derived moieties and lipids serve as energy and carbon sources for the microorganisms. However, this will also result in an accumulation of microbial metabolites such as carbohydrates and peptides. We can only speculate why these microbial products are not mineralized as fast as carbohydrates initially present in DOM before incubation. Possible explanations may be (i) the preservation of these compounds by bonding to stable DOM compounds like lignin or (ii) a changing of the microbial community during the incubation towards species adapted to the residual, more refractory DOM.

4.3. Mechanisms and controlling factors of DOM biodegradation—implications for the evolution of DOM properties in soil

Aromatic compounds, possibly deriving from lignin degradation, are likely to be the most stable fraction of DOM although partly degradation of these compounds occurred. The extent of biodegradation and relative enrichment in aromatic compounds seems to be a function of the initial aromaticity of DOM. During biodegradation, aromatic H increased to an average value of 13%, which resembles aromatic H contents of extractable soil organic matter (e.g. Six et al., 2001). Therefore, DOM biodegradation seems to be responsible for the evolution of organic matter properties being a precondition for the formation of stable carbon. Furthermore, these structural changes induced by DOM biodegradation should result in stronger DOM sorption to the soil matrix.

No evidence was found that dissolved lipids are stable. However, free fatty acids accumulated during DOM biodegradation, possibly due to the build-up of microbial biomass.

Besides preferential carbohydrate degradation in samples with high initial contents, microbial production of carbohydrates (and peptides) occurred especially for samples initially low in carbohydrates. The extent of degradation or production of certain compounds seems to be related to the initial distribution of potential carbon and energy resources. Nevertheless, DOM biodegradation is linked with microbial formation of soluble organic matter.

Biodegradation resulted in an approximation of initially very different DOM with respect to aromatic H, carbohydrate H, and thermal stability. That means after incubation, DOM from the Oi horizon strongly resembles DOM from the Oa horizon. Therefore, not all the DOM sampled in forest floor seepage is produced in the Oa horizon but might also originate from the Oi layer and is microbially altered during its migration through the forest floor. A possible consequence would be that DOC entering the mineral soil is much younger than estimated from the carbon age of the Oa horizon. Further research is necessary to quantify the relative contribution of different horizons to DOM in forest floor leachates.

Acknowledgements

Financial support came from the Deutsche Forschungsgemeinschaft and the German Ministry of Education and Research (BMBF).

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