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Response of dissolved organic matter in the forest floor to long-term manipulation of litter and throughfall inputs

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Abstract Dissolved organic matter (DOM) contributes to organic carbon either stored in mineral soil horizons or exported to the hydrosphere. However, the main controls of DOM dynamics are still under debate. We studied fresh leaf litter and more decomposed organic material as the main sources of DOM exported from the forest floor of a mixed beech/oak forest in Germany. In the field we doubled and excluded aboveground litter input and doubled the input of throughfall. From 1999 to 2005 we measured concentrations and fluxes of dissolved organic C and N (DOC, DON) beneath the Oi and Oe/Oa horizon. DOM composition was traced by UV and fluorescence spectroscopy. In selected DOM samples we analyzed the concentrations of phenols, pentoses and hexoses, and lignin-derived phenols by CuO oxidation. DOC and DON concentrations and fluxes almost doubled instantaneously in both horizons of the forest floor by doubling the litter input and DOC concentrations averaged 82 mg C l^{-1} in the Oe/Oa horizon. Properties of DOM did not suggest a change of the main DOM source towards fresh litter. In turn, increasing ratios of hexoses to pentoses and a larger content of lignin-derived phenols in the Oe/Oa horizon of the *Double litter* plots in comparison to the *Control* plots indicated a priming effect: Addition of fresh litter stimulated microbial activity resulting in increased microbial production of DOM from organic material already stored in Oe/Oa horizons. Exclusion of litter input resulted in an immediate decrease in DOC concentrations and fluxes in the thin Oi horizon. In the Oe/Oa horizon DOC concentrations started to decline in the third year and were significantly smaller than those in the *Control* after 5 years. Properties of DOM indicated an increased proportion of microbially and throughfall derived compounds after exclusion of litter inputs. Dissolved organic N did not decrease upon litter exclusion. We assume a microbial transformation of mineral N from throughfall and N mineralization to DON. Increased amounts of throughfall resulted in almost equivalently increased DOC fluxes in the Oe/Oa horizon. However, long-term additional throughfall inputs resulted in significantly declining DOC concentrations over time. We conclude that DOM leaving the forest floor derives mainly from decomposed organic

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material stored in Oe/Oa horizons. Leaching of organic matter from fresh litter is of less importance. Observed effects of litter manipulations strongly depend on time and the stocks of organic matter in forest floor horizons. Long-term experiments are particularly necessary in soils/horizons with large stocks of organic matter and in studies focusing on effects of declined substrate availability. The expected increased primary production upon climate change with subsequently enhanced litter input may result in an increased production of DOM from organic soil horizons.

Keywords Dissolved organic matter · Field experiment · Forest soil · Hexoses · Lignin · Litter · Pentoses · Phenols

Introduction

The increase in CO_2 of the atmosphere has provoked research on the role of soils in the global C cycle and the role of dissolved organic matter (DOM). Dissolved organic matter is a significant component of the organic C either stored in the mineral soil (Neff and Asner 2001; Michalzik et al. 2003; Kalbitz et al. 2005) or exported to the hydrosphere (Bishop et al. 2004; Roulet and Moore 2006). Despite large research efforts during the last decade estimates of the main controls on DOM production in soils are uncertain (Kalbitz et al. 2000; McDowell 2003). Organic matter in the forest floor is the main source of DOM entering mineral horizons of forest soils. However, it is still under debate whether freshly fallen leaf litter or the more decomposed parts of the forest floor contribute most to DOM in the mineral soil. Answering this question may help to explain the observed increase in concentrations of dissolved organic carbon (DOC) in many surface waters across Europe and North America (Evans et al. 2006). Increased primary production caused by elevated CO_2 and high nitrogen deposition is discussed as one possible mechanism (Findlay 2005; Roulet and Moore 2006) assuming that enhanced input of fresh organic matter results in increased DOM production.

Laboratory experiments and field observations suggested fresh organic material from the Oi horizon is the most important source of DOM (Qualls et al. 1991; Huang and Schoenau 1996; Currie and Aber 1997; Michalzik and Matzner 1999). More recently, equal contribution or even larger importance of decomposed organic material was observed in field experiments with manipulations of litter input or/and by using carbon isotopes (Fröberg et al. 2003; Park and Matzner 2003; Hagedorn et al. 2004; Fröberg et al. 2005; Fröberg et al. 2007). Such field experiments have the potential to allow for quantification of different organic sources to DOM leached from the forest floor.

Fröberg et al. (2005) estimated that 20% of DOC leached from the forest floor of a Norway spruce forest originated from the Oi horizon whereas 80% was produced in the more decomposed parts of the forest floor. However, in this study the different forest floor horizons were completely removed and the annual needle litterfall was added once a year. Therefore, the reported proportions may not fully relate to natural conditions with equal distribution of aboveground litterfall during the year. In another coniferous forest characterized by a thin O horizon (2 cm), Yano et al. (2004) suggested the upper mineral soil (0–10 cm) as the most important DOM source.

To the best of our knowledge, there is only one study on the dynamics of C and N in forest floor percolates of deciduous forests after manipulating the litter input. In a mixed beech/oak forest, Park and Matzner (2003) found increased concentrations of dissolved organic carbon and nitrogen (DON) after doubling the litter input but no significant changes when litterfall was excluded over a period of 2 years. However, it remains unclear how long DOC and DON concentrations remain unchanged upon exclusion of aboveground litter input. Also, it is unclear if the increase in DOC and DON is an initial or a long-lasting response to the doubling of the litter input.

Changes in the source of DOM upon manipulating the litter input should result in changes in its composition. Dissolved organic matter from freshly fallen leaf litter contains more carbohydrates and less aromatic moieties than DOM from more decomposed parts of the forest floor (Kalbitz et al. 2003a). Kalbitz et al. (2004) observed an increasing aromaticity and complexity of DOM produced from the Oi to the Oa horizon due to increased release of lignin-derived molecules. We expect a larger proportion of plant-derived carbohydrates upon increased litter input because of the increased contribution of fresh litter-derived compounds to DOM.

Litter manipulation will also change the turnover of the forest floor. Addition of litter, representing an easily-decomposable substrate can stimulate the co-metabolic lignin degradation (Haider and Guggenberger 2005). Accelerated lignin degradation results in accelerated production of DOM, rich in lignin-derived compounds, in decomposing litter (Kalbitz et al. 2006). In turn, litter exclusion might reduce the contribution of aromatic, lignin-derived compounds to DOM because of limited substrate availability.

Spectroscopic and chemical properties of DOM may allow tracking of changes in the turnover of organic matter induced by changes in the litter input (Kalbitz et al. 2006). Analysis of phenols, UV and fluorescence spectroscopy were used to estimate the contribution of lignin-derived compounds to DOM (Kalbitz et al. 2003b, 2006). The content of lignin-derived phenols and their degree of oxidation as analyzed by the CuO oxidation method gives additional information on the oxidative degradation of lignin (Hedges and Ertel 1982, Kögel 1986). Analyses of pentoses were used to estimate the contribution of plant-derived carbohydrates to DOM whereas the proportion of hexoses hints at microbially produced components because they represent 70–90% of the microbial aldoses (summarized in Oades 1984).

Water is another key driver of DOM fluxes from the forest floor and within the soil profile (Tipping et al. 1999; Kalbitz et al. 2000; Michalzik et al. 2001). Many studies indicate that concentrations hardly change with water fluxes (Kalbitz et al. 2000; Michalzik et al. 2001) resulting in larger DOC and DON fluxes at larger water fluxes. However, in soils and horizons with small stocks of potentially water-soluble organic matter such as in Oi horizons (Park and Matzner 2003) or in peaty gleys (Tipping et al. 1999) larger water fluxes did not cause larger DOC fluxes. Evidence for the relationships between hydrology and DOM fluxes comes from field observations or laboratory experiments. We know only two field experiments with experimental manipulation of water input (Tipping et al. 1999; Park and Matzner 2003). The long-term response of DOM fluxes to experimentally enlarged water fluxes in the field, however, has not been addressed so far.

Here we determined the long-term response of concentrations and fluxes of dissolved organic C and N in the forest floor of a mixed beech/oak forest to experimentally changed litter and water input. We studied fresh litter or more decomposed organic material as the potential main sources of DOM exported from the forest floor by determining concentrations and fluxes of DOC and DON as well as spectroscopic and chemical properties of DOM. We hypothesized that increased litter input results in increasing concentrations and fluxes of DOC and DON and the opposite effect by decreased litter input. Furthermore, we hypothesized that changes in DOM properties would indicate a larger contribution of freshly fallen litter to DOM production by increasing the aboveground litter input. We also hypothesized that DOC and DON concentrations will not change with increasing water fluxes resulting in increased DOC and DON fluxes at plots with increased throughfall addition.

Material and methods

Study site

The experiment was conducted at the 1.3 ha 'Steinkreuz' site (49°52′ N, 10°27′ E) within the Steigerwald landscape conservation area, Bavaria, Germany. The site is a hardwood stand composed of European beech (*Fagus sylvatica* L.) and sessile oak (*Quercus petraea* (Matt.) Liebl.), both approximately 140 years old. Elevation of the 55 ha 'Steinkreuz' catchment ranges from 400 to 460 m. The annual mean temperature is 7.5°C and annual precipitation averages approximately 750 mm. Sandy to loamy Haplic Cambisols (ruptic, dystric, humic) (WRB 2006) have developed from underlying sandstone. The forest floor has an average thickness of 4 cm and is moder type comprising distinct Oi and Oe horizons and a patchy and thin Oa horizon. Some soil properties and the C and N stocks are listed in Table 1. A detailed description of the site has been published by Gerstberger et al. (2004).

Experimental setup

Treatment plots (2 × 2 m each, a total of 16 plots) were established in April 1999 with four replicated plots for each treatment and a minimum 1 m distance in between. Details were given by Park and Matzner (2003). Briefly, four plots were randomly assigned to one of the following treatments: *Control* (normal

Table 1 Soil properties and C storage at the deciduous forest "Steinkreuz" (Gerstberger et al. 2004)

Horizon	Thickness (cm)	Sand	Silt (%)	Clay	TOC[a] (%)	TOC/TON[a]	TOC[a] stocks (Mg C ha^{-1})	pH ($CaCl_2$)	CEC[b] ($mmol_c*kg^{-1}$)
Oi	1				44.5	21.8	2.0	4.7	568
Oe	1.5				41.3	18.9	12.8[c]	4.5	565
Oa	0.5				20.5	17.8		3.4	172
Ah	5	47	39	14	6.69	15.6	34.9	3.2	88
Bw1	19	55	35	10	1.09	18.2	27.1	3.8	45
Bw2	26	52	38	10	0.35	17.5	7.1	3.9	32
Bw3	30	57	33	10	0.16	8.0	4.1	4.0	41

[a] Total organic carbon and total organic nitrogen (Foss-Heraeus CHN-O-Rapid)

[b] Cation Exchange Capacity measured as the sum of cations in a 0.1M NH_4Cl extract

[c] Sum of Oe and Oa horizon

litter inputs), *No litter* (exclusion of aboveground litter inputs), *Double litter* (doubling aboveground litter inputs), and *Double throughfall* (doubling throughfall inputs). Beginning in September 1999 aboveground litter inputs have been excluded from the *No litter* plots by placing a roof-shaped litter trap composed of a wooden frame, cover net and side nets. The cover net was evaluated to have minimal water retention and under normal rainfall events only small portions (<5%) of troughfall might have been excluded by the litter trap itself (Park and Matzner 2003). From September to December, the period with the main litterfall, fresh litter taken from the *No litter* plots was added to the *Double litter* plots. Any large branches that fell on litter traps were discarded. In the first 2 years, the roofs were placed over the plots for the entire snow-free period (April to December). Afterwards, roofs were just placed over the plots during the period of the main litterfall in order to keep the plots as less disturbed as possible.

To date (7 years of litterfall; fall 1999–2005) we added 28,400 kg dry matter of litter per ha to the *Double litter* plots corresponding to an average annual litterfall of 4,060 kg ha^{-1}. The total amount of added litter was equal to 13,430 kg C ha^{-1} and 332 kg N ha^{-1}. The same amounts were excluded from the *No litter* plots. These amounts of litter collected by roof-shaped litter traps covering the *No litter* plots were almost the same as the annual input of leaf litter sampled by 10 usual litter traps at the same site (Park and Matzner 2003). The total litter input collected from these traps including woody debris, fruits, and flower parts was 5,900 kg ha^{-1} yr^{-1}. About half of this difference can be explained by litter input during winter when roofs were not installed above the *No litter* plots due to snowfall (Park and Matzner 2003). Large woody debris, fruits and flower parts which could not be collected by the roof-shaped litter traps, litterfall in spring and summer before the roofs were installed and the spatial variability of litterfall are supposed to be responsible for differences in amounts of litterfall sampled by the two different systems.

Throughfall inputs were doubled in a circular area (approximately 1 m^2) within the *Double throughfall* plots using an automatic sprinkling system. The sprinkler sprayed throughfall water collected in an adjacent sampler (1 × 1 m) to the plots during rainfall. This real-time throughfall addition doubles not only water volumes but also inputs of all the chemical components. These manipulations were only possible during the snow-free period from April to November/December. In 2001, the sprinkler only worked from April to June because of technical problems.

Zero-tension lysimeters with a surface area of 707 cm^2 were installed underneath the Oi and Oe/Oa horizon in spring 1999 (one per horizon and plot). Since the Oa horizon was not always distinct and the depth of whole organic horizons was very variable, we cut a rectangular block of forest floor, using a steel frame and a knife (Park and Matzner 2003). The forest floor block was separated from mineral soil materials and firmly put on the lysimeter now placed in the hole (Park and Matzner 2003). The construction of the lysimeters did not allow rooting of the forest floor material by the trees. However, this should be of minor importance because most of the roots at this site are located in the mineral soil.

Throughfall was collected using 12 samplers installed in an area next to the treatment plots. The 12 replicates were pooled into four composite samples.

In spring 2000, we installed temperature probes in the Oi and the Oe/Oa horizon of each plot recording temperature at 30-min intervals. Our treatments did not change temperatures. Therefore, data were not presented.

Sample collection and laboratory analyses

Throughfall and forest floor percolates were taken biweekly from November 1999 to October 2001. From November 2001, forest floor percolates were sampled once a month while throughfall sampling frequency was not changed. Immediately after collection, the solution samples were measured for pH and electrical conductivity and then filtered through a pre-rinsed (deionized water) cellulose-acetate membrane filter (0.45-μm pore size). The filtered samples were stored at 2°C prior to analysis.

Subsequent analysis included (i) dissolved organic C by infrared detection of CO_2 after high-temperature combustion (High-TOC), (ii) total dissolved N by chemiluminescence detection of NO_x after high-temperature combustion (High-TOC), (iii) NH_4^+ by colorimetric flow injection analysis (Lachat FIA), and (iv) NO_3^- and Cl^- by ion chromatography (Dionex 2000i-SP). Dissolved organic N (DON) was calculated as the difference between total N and mineral N.

All forest floor percolates (1999–2005) and throughfall samples from 2002 were analysed for specific UV absorbance at 280 nm (UVIKON 930, BIO-TEK Instruments) and fluorescence. The specific UV absorbance at 280 is an estimate of the aromaticity of dissolved organic matter from various sources (Traina et al. 1990; McKnight et al. 1997; Kalbitz et al. 2003a). Furthermore, we recorded emission fluorescence spectra (SFM 25, BIO-TEK Instruments) and calculated humification indices (Zsolnay et al. 1999). These indices increase with increasing content of aromatic nuclei, with increasing degree of condensation and/or conjugation, and with increasing C/H ratios of the organic molecules (Zsolnay 2003), or in simple words with increasing complexity of the organic molecules. Solutions used for the spectroscopic measurements were immediately frozen after sampling. The C content of the solutions was adjusted to 10 mg DOC l^{-1} before analyses to ensure comparability of all DOM samples. Further details of fluorescence measurements are given by Kalbitz and Geyer (2001).

In summer and fall 2002 and 2004, aliquots of forest floor percolates of the *Control*, the *No litter* and *Double litter* plots were combined into three-monthly samples and freeze dried. These three-monthly samples represented the following seasons: summer 2002, fall 2002, summer 2004, fall 2004. Seasons were defined according to the calendar.

The freeze-dried aliquots of forest floor percolates were analyzed for phenols, pentoses, hexoses after re-dissolution in ultra pure water. Concentrations of hexoses and pentoses were determined using anthrone-sulfuric acid and orcinol-ferric chloride hydrochloric acid, respectively (Brink Jr et al. 1960; Chantigny et al. 2007). Phenols were determined using the Folin-Ciocalteau reagent (Mejbaum 1939; Swain and Hillis 1959; Chantigny et al. 2007). We also determined the DOC content in these samples again in order to relate all of the results to the exact C basis. In all, we determined these properties in 86 forest floor leachates (4 seasons, 2 horizons, 3 treatments [*Control*, *No litter*, *Double litter*], 4 replications; in 10% of the samples the amount of solution was too little for these analyses).

The contents of lignin-derived phenols and their degree of oxidation were determined on 30 mg of freeze-dried aliquots using alkaline CuO oxidation at 170°C for 3 h (Hedges and Ertel 1982) followed by solid-phase extraction with C-18 columns (Mallinckrodt Baker Corp., Phillipsburg, NJ, USA). Phenols were eluted with ethyl acetate, dried under N_2, and derivatized with a 1:1 mixture of pyridine and N,O-bis(trimethysilyl)-trifluoroacetamide. The trimethylsilyl derivatives were separated and quantified by a gas chromatograph equipped with a flame ionization detector (GC-2010, Shimadzu Corp., Kyoto, Japan) and an SPB-5 fused silica capillary column (30 m length, 0.25 mm inner diameter, 0.25-μm film; Supelco, Bellefonte, PA, USA). Lignin phenols were identified and quantified according to the retention times and response factors of external phenol standards. Ethylvanillin was added as an internal recovery standard prior to the CuO oxidation and phenylacetic acid before derivatization. To increase the recoveries, 50 mg glucose was added prior to the CuO oxidation (Amelung et al. 1999). Recoveries were 80% for ethylvanillin and 106% for phenylacetic acid of the initial spike level. CuO oxidation yields phenolic monomers (vanillyl, syringyl and cinnamyl units) that derive from lignin. Vanillyl (V) and Syringyl (S) units may be present as aldehydes, carboxylic acids and ketones. Cinnamyl (C) compounds are *p*-coumaric acid and ferulic acid. It should be noted that the original method is designed to break off the intact insoluble lignin polymer (Benner et al. 1990). However, DOM is per definition already soluble. Therefore, CuO oxidation products of DOM comprise monomers as well as oligomers that derive from the enzymatic depolymerization of lignin (Guggenberger and Zech 1994; Kaiser et al. 2004).

The sum of these phenolic compounds (V + S + C) is indicative of the lignin content. The degree of decomposition of the lignin can be estimated from the acid/aldehyde ratios of the vanillyl and/or syringyl units (Kögel-Knabner et al. 1988). Because of the high sample demand, we had to pool the 4 spatial replicates of some samples (*Control* of the Oi horizons in fall 2004, *No litter* of the Oi horizon in summer and fall 2002, *No litter* of the Oe/Oa horizon in summer 2002 and fall 2004). Altogether we analyzed 68 DOM samples by cupric oxidation method.

Calculations and statistics

Mean annual throughfall fluxes of DOC and DON were calculated from the concentrations and water volumes of the four composite samples on a biweekly base. Water fluxes of forest floor leachates were estimated by assuming that they represented 80 (Oi horizons) and 75% (Oa horizons) of the throughfall (Solinger et al. 2001; Park and Matzner 2003). These proportions were derived from the mean proportions measured 1996–1998 at this site using tension plate lysimeters with reasonable water-collection efficiency (Solinger et al. 2001). Similar proportions have been found for the Oi (80%) and the Oa horizon (72%) of a forest floor under Norway spruce (Kalbitz et al. 2004). We used the average water fluxes of the 12 throughfall samplers for water flux calculations of all lysimeters. Annual fluxes of Cl^- with throughfall were on average only 15% larger than those calculated for Oi and Oe/Oa horizons indicating the validity of our approach. We did not use the volumes collected in the zero-tension lysimeters because they generally did not exceed 50% of throughfall volumes, which seems to be a clear underestimation of the percolation water. Like throughfall fluxes, annual fluxes of DOC and DON in forest floor leachates were calculated from estimated water fluxes (see above) and concentrations of each lysimeter based on biweekly or monthly data. Missing concentrations of DOC and DON were estimated by calculating the means of samples collected at the dates immediately before and after. Annual concentrations and fluxes in the forest floor are the mean values of 4 lysimeters per treatment.

Concentrations of Cl^- were similar in the different treatments and horizons of the forest floor (1.2–1.6 mg l^{-1}). Therefore, we assume that the different water-collection efficiencies of individual lysimeters did not influence element concentrations (Park and Matzner 2003). Thus, the estimated flux proportions were uniformly applied for the calculation of annual water fluxes for all treatments, including the *Double throughfall* plots where the inputs were assumed to be twice those of the *Control* during snow-free period.

The concentrations and fluxes of the measured solutes were not normally distributed. Therefore, differences between treatments were tested by the parameter-free Kruskal–Wallis rank test. In addition, the Mann–Whitney U-test was performed to determine differences between two mean values. For these analyses, we used n = 4, the number of lysimeters per treatment and horizon. The additionally measured DOM properties in 2002 and 2004 had a larger temporal than spatial variability. Furthermore, some of the spatial replications had to be combined because of the high sample demand (see above). Therefore, we calculated mean values and standard error using single data of each lysimeter. Linear regression analyses were performed to detect changes over time of concentrations and properties of DOM. For all statistical analyses we used a *P*-value of 0.05 unless otherwise noted.

Results

DOC and DON concentrations and fluxes in the control treatment

Mean DOC and DON concentrations are well within the range of deciduous forest floor horizons in temperate forests (Michalzik et al. 2001) with slightly larger concentrations in the Oe/Oa than in the Oi horizon (Figs. 1, 2; average concentrations, Oi: 38 mg C l^{-1}, 1.4 mg N l^{-1}; Oa: 43 mg C l^{-1}, 1.6 mg N l^{-1}). Over the 6-year experimental period, mean annual DOC and DON concentrations varied to some extent. Largest DOC concentrations were observed in 2000 and 2005 whereas DOC concentrations were smallest in 2002.

The mean annual DOC and DON fluxes in the Oe/Oa horizon (176 kg C ha^{-1} yr^{-1}, 6 kg N ha^{-1} yr^{-1}, Table 2) are in the lower range of those in hardwood forests of the temperate zone as reported by Michalzik et al. (2001). Like concentrations, DOC and DON fluxes were slightly smaller in the Oi horizon than in the Oe/Oa horizon. Annual DOC and DON fluxes were particularly dependent on water fluxes with small fluxes in the unusually dry year 2003. In this year the annual throughfall input was only 361 mm in comparison to 535–754 mm in the other years. Despite small DOC concentrations in 2002, DOC fluxes were above average because of large water fluxes (754 mm).

Effects of litter manipulation

DOC and DON concentrations and fluxes

Doubling the aboveground litter input resulted in a rapid response in both horizons of the forest floor. The DOC and DON concentrations and fluxes almost doubled compared to the *Control* (Figs. 1–3; Table 2). On average of 2000–2005, doubling the litter input resulted in 68 and 82 mg C l^{-1} in the Oi and Oe/Oa horizon, respectively, whereas DON concentrations reached 2.7 and 2.8 mg N l^{-1} in the Oi and Oe/Oa horizon, respectively. That means DOC and DON concentrations increased by 39 mg C l^{-1} and 1.2 mg N l^{-1} in the Oe/Oa horizon. This increase was something smaller in the Oi horizon. Despite a large variability of the data (Fig. 3), the effect was statistically significant in most of the years (Figs. 1, 2, Table 2). DOC fluxes in the Oe/Oa horizon increased slightly more (93%) than in the Oi horizon where the mean annual fluxes were only 70% larger than in the *Control* treatment (Table 2). In coniferous forests, Fröberg et al. (2005) and Lajtha et al. (2005) found similar results with elevated DOC concentrations underneath the forest floor after increasing leaf litter input.

After 2 years of litter exclusion no distinct Oi horizon was found and exclusion of aboveground litter input resulted in a rapid decrease in DOC concentrations and fluxes in the Oi horizon (Figs. 1, 3, Table 2). This effect was statistically significant in most of the years (e.g. fluxes: Table 2) and the DOC fluxes in Oi percolates of 86 kg C ha^{-1} yr^{-1} resembled those in throughfall. In contrast, DOC concentrations and fluxes in the Oe/Oa horizon

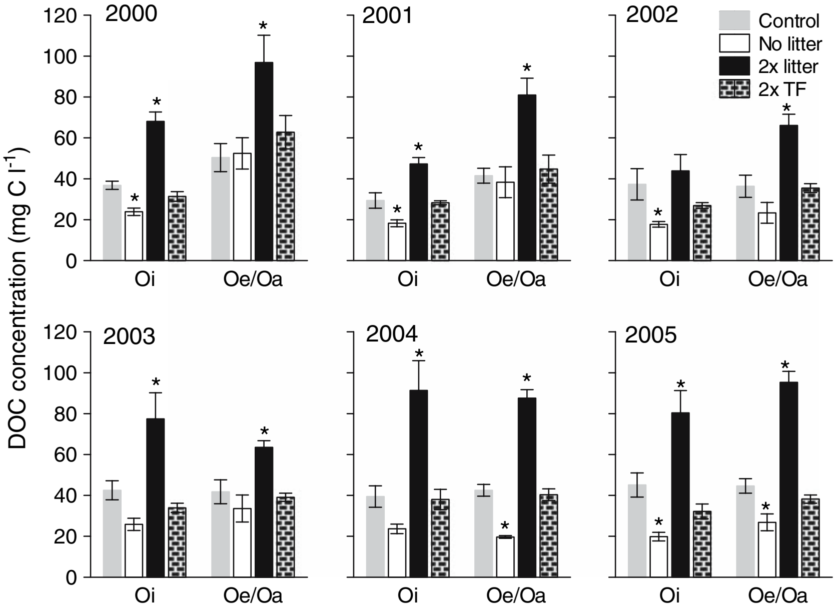

Fig. 1 Mean annual DOC concentrations in Oi and Oe/Oa horizons of the forest floor upon manipulation of litter (excluding aboveground litter: No litter; doubling aboveground litter: 2× litter) and throughfall (doubling throughfall: 2× TF) input; annual mean and standard error of 4 lysimeters for 2000–2005; asterisk indicates statistically significant deviation from the control in the respective horizon

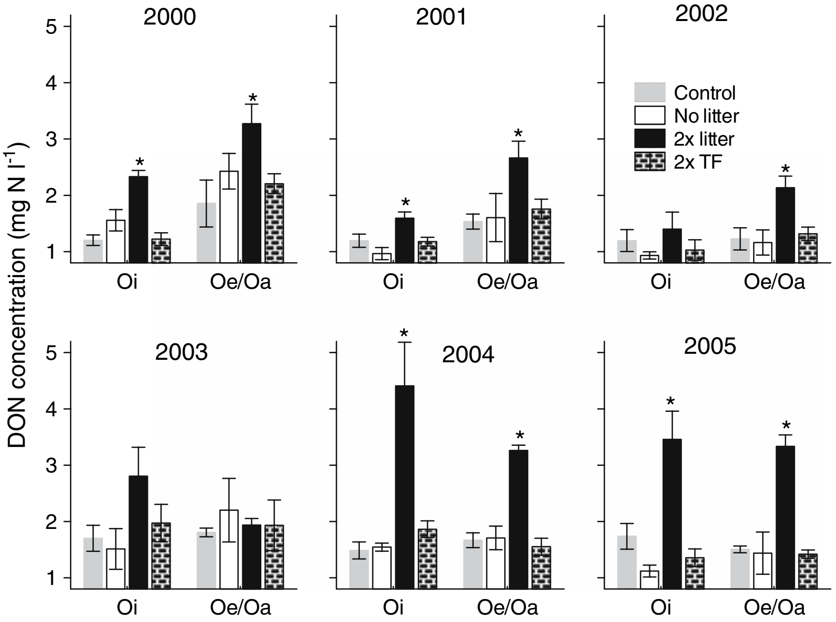

Fig. 2 Mean annual DON concentrations in Oi and Oe/Oa horizons of the forest floor upon manipulation of litter (excluding aboveground litter: No litter; doubling aboveground litter: 2× litter) and throughfall (doubling throughfall: 2× TF) input; annual mean and standard error of 4 lysimeters for 2000–2005; asterisk indicates statistically significant deviation from the control in the respective horizon

Table 2 Annual fluxes of dissolved organic carbon (DOC) and nitrogen (DON) in throughfall and in the forest floor after long-term litter and throughfall manipulation (No litter: without aboveground litter input, 2× litter: doubling the aboveground litter input, 2× TF: doubling the throughfall input)

	2000	2001	2002	2003	2004	2005	Mean of 6 years
DOC (kg C ha^{-1})							
Throughfall	78 (3)	110 (5)	63 (4)	47 (2)	92 (3)	74 (7)	77
Oi: Control	141 (11)	151 (19)	204 (42)	115 (17)	173 (21)	182 (19)	161
Oi: No litter	89* (8)	81* (7)	94* (6)	65 (8)	108 (13)	80* (11)	86*
Oi: 2× litter	254* (19)	239* (17)	249 (43)	190* (31)	397* (66)	318* (50)	274*
Oi: 2× TF	202* (17)	164 (5)	249 (12)	158 (12)	274 (30)	221 (28)	211*
Oe/Oa: Control	199 (26)	207 (19)	196 (28)	105 (13)	176 (13)	174 (15)	176
Oe/Oa: No litter	216 (35)	193 (40)	130 (27)	83 (16)	99* (9)	109* (21)	138
Oe/Oa: 2× litter	371* (60)	415* (36)	370* (33)	156* (9)	370* (18)	353* (15)	339*
Oe/Oa: 2× TF	424* (64)	269 (42)	310* (16)	172* (8)	275* (21)	248* (20)	283*
DON (kg N ha^{-1})							
Throughfall	4.5 (0.3)	5.1 (0.2)	3.5 (0.1)	2.8 (0.1)	3.6 (0.2)	3.9 (0.4)	3.9
Oi: Control	4.2 (0.5)	5.9 (0.6)	6.1 (0.8)	4.6 (0.7)	6.1 (0.5)	7.1 (0.8)	5.7
Oi: No litter	5.0 (0.8)	4.0* (0.3)	4.5 (0.4)	3.6 (1.0)	6.1 (0.4)	4.6 (0.5)	4.6
Oi: 2× litter	7.7* (0.3)	7.8* (0.5)	7.8 (1.6)	6.6 (1.1)	17.9* (3.5)	13.7* (2.2)	10.3*
Oi: 2× TF	7.6* (0.6)	6.5 (0.3)	7.8 (1.1)	8.6* (1.7)	13.2* (1.5)	9.9 (1.1)	8.9*
Oe/Oa: Control	5.9 (1.1)	7.4 (0.8)	6.1 (0.9)	4.4 (0.1)	6.5 (0.6)	6.2 (0.2)	6.1
Oe/Oa: No litter	7.6 (1.0)	7.4 (2.4)	6.1 (1.6)	4.9 (1.1)	6.8 (1.1)	6.2 (1.7)	6.5
Oe/Oa: 2× litter	10.3* (1.3)	13.7* (1.3)	10.6* (0.9)	4.9 (0.4)	13.4* (0.5)	12.6* (0.6)	10.9*
Oe/Oa: 2× TF	13.9* (0.8)	10.0 (1.0)	11.0* (0.9)	8.7* (2.2)	10.2* (0.9)	9.4* (0.8)	10.5*

Annual fluxes are the means of the fluxes of the 4 lysimeters/samplers per treatment. Standard error is given in brackets. Statistically significant differences to the Control are indicated by an asterisk remained constant in the first 2 years of the experiment and did not differ from those of the *Control*. In the third year of the experiment, DOC concentrations and fluxes in the Oe/Oa horizon started to decline by exclusion of litterfall and we found a significant trend of decreasing DOC concentrations with time (Fig. 3). However, the effect could not be statistically proven by annual concentrations and fluxes until year 5 (2004). After 6 years of litter exclusion, average DOC concentrations of 27 mg C l^{-1} and annual DOC fluxes in the Oe/Oa horizon were still about 50% larger than those in throughfall. Dissolved organic N responded less to the decreased litter input than did DOC. In the Oi horizon, DON fluxes were significantly smaller than those of the *Control* treatment in 2001 only. Even after 6 years, mean annual DON concentrations in the Oe/Oa horizon did not differ between the *Control* and the *No litter* treatment (Fig. 2).

Spectroscopic properties of DOM and concentrations of hexoses and pentoses

The specific UV absorbance and the indices deduced from fluorescence spectra of DOM did not differ significantly between the *Control* and the *Double litter* treatment in both horizons of the forest floor in most of the years (Figs. 4, 5). The spectroscopic parameters decreased significantly by exclusion of aboveground litter input indicating a smaller contribution of aromatic and complex compounds to DOM in comparison to the *Control* (Kalbitz et al. 2006). Particularly in the Oe/Oa horizon the differences between the *Control* and the *No litter* treatment became more pronounced with time (Fig. 6).

The concentrations of sugars were highly variable and no significant differences among treatments were found (Fig. 7). Sugars comprised between 2 and 17% of the total DOC. We found significantly larger concentrations of sugars in 2004 in comparison to 2002 in all treatments that coincided with larger DOC concentrations. The hexoses to pentoses ratios did not differ between horizons and treatments except for larger ratios in the Oe/Oa horizon of the *Double litter* treatment compared to the *Control* (Fig. 7). This shift in sugar composition indicates an increase in the contribution of microbial products to DOM by doubling the litter input.

Total contents of phenols and lignin-derived phenols as determined by CuO oxidation method

The mean concentrations of phenols were 0.10–0.13 g C per g DOC and did not vary significantly among treatments and forest floor horizons.

The contribution of lignin-derived phenolic CuO oxidation products (VSC) to DOM in Oi and Oe/Oa horizons was similar (30–38 mg C g C^{-1}) and did not vary between the seasons and 2002 and 2004. It decreased slightly in the Oi horizon by exclusion of litter input and increased significantly in the Oe/Oa horizon after doubling the litter input (Fig. 8). The VSC content of DOM from our site was about half of the VSC content of decaying beech litter (Kalbitz et al. 2006) but in the same range as in the solid Oa material (Rumpel et al. 2002).

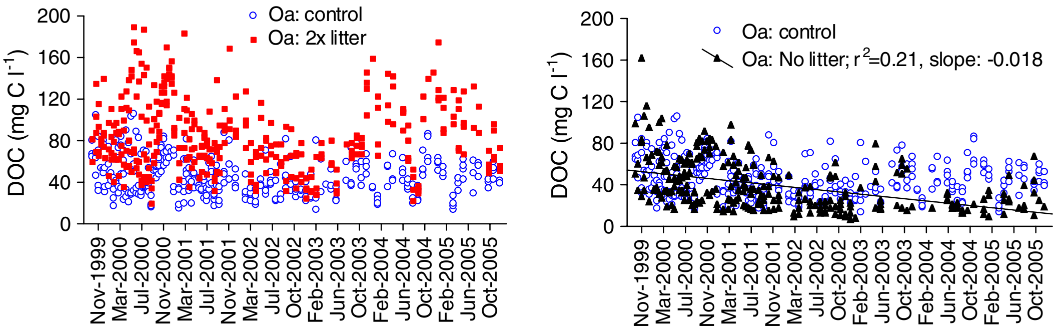

Fig. 3 DOC concentrations in the Oe/Oa horizon upon manipulation of litter input (excluding aboveground litter: No litter; doubling aboveground litter: 2× litter) from fall 1999 until the end of 2005 (single data of each lysimeter); linear regression between DOC concentration and time at the No litter treatment indicates significantly declining DOC concentration by exclusion of aboveground litter input

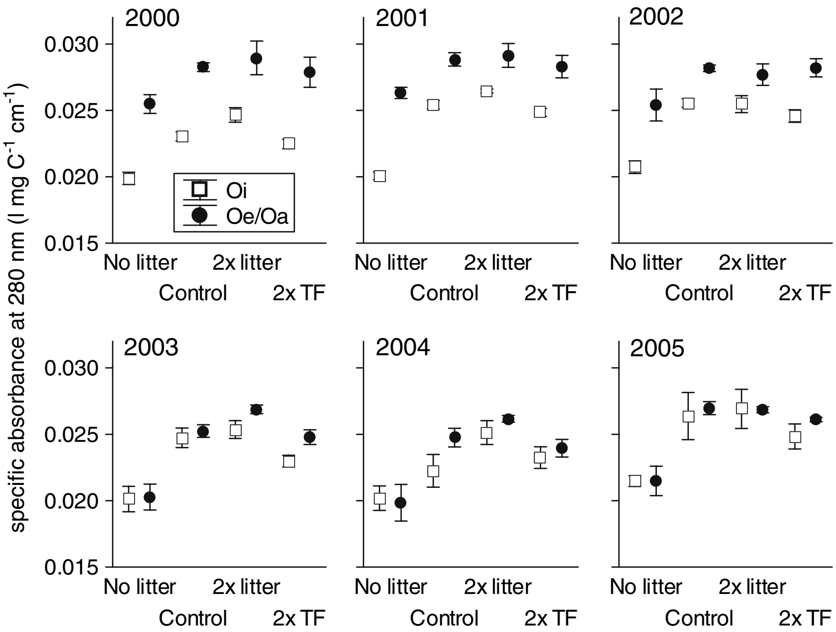

Fig. 4 Mean annual specific UV absorbance at 280 nm of DOM in Oi and Oe/Oa horizons of the forest floor upon manipulation of litter (excluding aboveground litter: No litter; doubling aboveground litter: 2× litter) and throughfall (doubling throughfall: 2× TF) input; annual mean and standard error of 4 lysimeters for 2000–2005

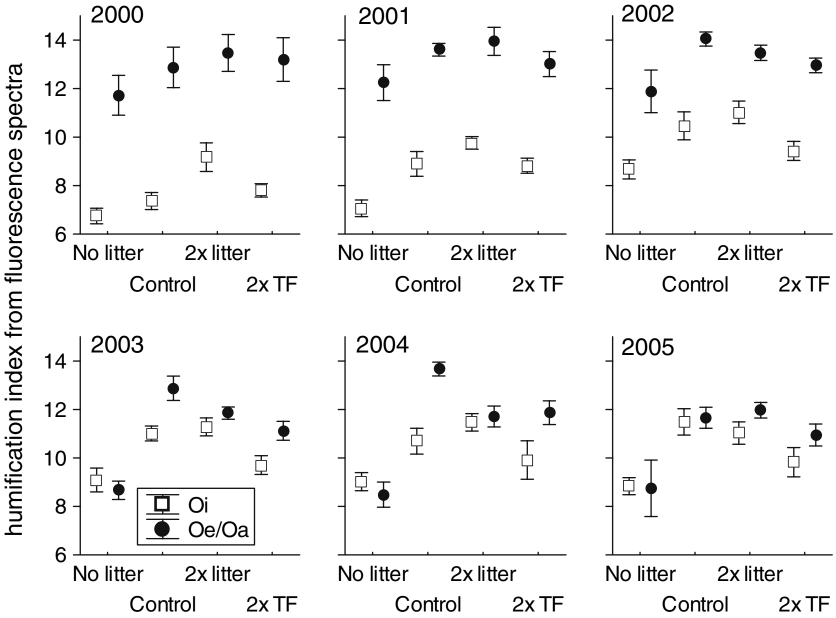

Fig. 5 Mean annual humification indices deduced from fluorescence emission spectra of DOM in Oi and Oe/Oa horizons of the forest floor upon manipulation of litter (excluding aboveground litter: No litter; doubling aboveground litter: 2× litter) and throughfall (doubling throughfall: 2× TF) input; annual mean and standard error of 4 lysimeters for 2000–2005

The acid-to-aldehyde ratios of the vanillyl (Fig. 8) and syringyl units were smaller in the *Double litter* treatment than in the *Control* indicating less enzymatic lignin oxidation (Kögel-Knabner et al. 1988). However, the effect was only significant at a probability level of 10% in the Oe/Oa horizon. The smaller degree of lignin oxidation at the *Double litter* plots compared to the *Control* plots is supported by significantly larger ratios of syringyl to vanillyl units in the Oe/Oa horizon (Fig. 8) since syringyl phenols are easier degradable than vanillyl counterparts (e.g., Bahri et al. 2006). These ratios were also larger after exclusion of litterfall in comparison to the *Control*. Smaller ratios of syringyl to vanillyl units in the Oe/Oa than in the Oi horizon indicate the expected larger lignin degradation at later stages of organic matter decomposition (Fig. 8).

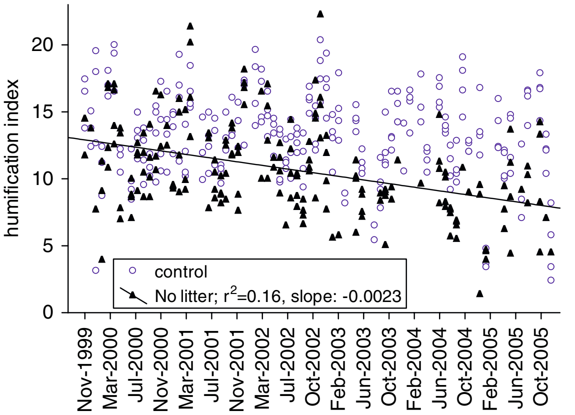

Fig. 6 Humification indices deduced from fluorescence emission spectra in the Oe/Oa horizon upon manipulation of litter input (excluding aboveground litter: No litter) from fall 1999 until the end of 2005 (single data of each lysimeter); linear regression between humification indices and time at the No litter treatment indicates significantly declining indices by exclusion of aboveground litter input

Effects of throughfall manipulation

On average, annual water fluxes could be increased by 61% in both horizons of the forest floor in the *Double throughfall* treatment compared to those of the *Control*. DOC and DON concentrations did not vary significantly between the *Control* and *Double throughfall* treatment (Figs. 1, 2). However, annual DOC concentrations in the Oi horizon were smaller for *Double throughfall* compared to the *Control* whereas this effect could not be observed for DON.

Because of unchanged concentrations and larger water fluxes DOC and DON fluxes were larger after increasing throughfall input compared to the *Control* in both horizons of the forest floor (Table 2). In the Oi horizon, this effect was statistically significant for DON only in three of the 6 years with an average increase of DON fluxes by 59%. In contrast, DOC fluxes were significantly larger compared to the *Control* in the first year only (Table 2). In the Oe/Oa horizon, increased water fluxes resulted in statistically larger DOC and DON fluxes than those in the *Control* in almost all years. DOC fluxes increased by the same magnitude as water fluxes (61%) whereas this increase was slightly larger for DON (70%).

In most of the years, spectroscopic properties did not indicate changes in the composition of DOM after doubling the throughfall input (Figs. 4, 5). However, we found a tendency of smaller humification indices deduced from fluorescence spectra of DOM from the *Double throughfall* treatment in both horizons compared to the *Control* in the last 3 years of the experiment.

Discussion

Main source of DOM in the forest floor—effects of increased litter input

Larger DOC and DON concentrations and fluxes at the plots with *Double litter* input and smaller DOC concentrations and fluxes by exclusion of aboveground

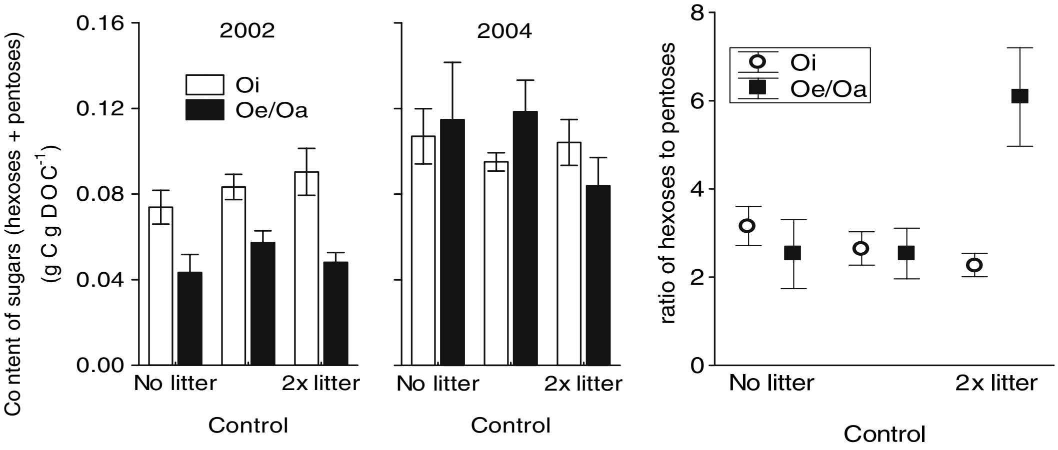

Fig. 7 Content of sugars and ratios of hexoses to pentoses in DOM from Oi and Oe/Oa horizons of the forest floor upon manipulation of litter input (excluding aboveground litter: No litter; doubling aboveground litter: 2× litter); mean and standard error comprising both seasons (summer, fall) and years (ratios of hexoses to pentoses)

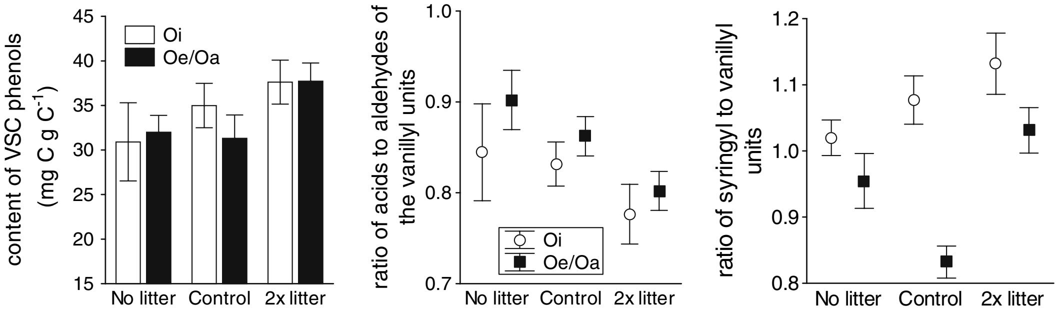

Fig. 8 Content of lignin-derived phenols (VSC), ratio of acids to aldehydes of the vanillyl units, and ratio of syringyl to vanillyl units of DOM samples from the Oi and Oe/Oa horizon as determined by CuO oxidation upon manipulation of litter input (excluding aboveground litter: No litter; doubling aboveground litter: 2× litter); mean and standard error comprising both seasons (summer, fall) and years (2002, 2004)

litter input compared to the *Control* might be clear indications that DOM in the forest floor derives mainly from fresh litter. However, this view is superficial. The specific UV absorbance (0.01 l mg C^{-1} cm^{-1}; Scheel et al. 2007) and humification indices deduced from fluorescence spectra (4.7; Kalbitz et al. 2003a) of percolates from fresh litter from the study site were less than 40% of the values of DOM in the Oe/Oa horizon and between 40 and 70% of those in the Oi horizon (Figs. 4, 5). Therefore, we should expect substantially decreasing values of these spectroscopic properties if fresh litter becomes more important as a DOM source by increasing the litter input. However, the spectroscopic properties did not change significantly by increasing the litter input and, therefore, did not indicate changes in DOM sources. Similar results were reported by Fröberg et al. (2005) at a Norway spruce site.

If additional DOM does not mainly derive from the added litter where does it come from? Increasing the addition of litter also means increasing the amount of easily degradable organic matter as indicated by larger C respiration at plots with increased litter additions in comparison to control plots (Bowden et al. 1993; Boone et al. 1998; Park and Matzner 2003). These studies were done in deciduous forests. In a coniferous forest carbon respiration was even larger than expected from the increased C input (Sulzman et al. 2005). Therefore, the authors concluded that an addition of easily degradable C source resulted in a positive priming effect with enhanced decomposition of more recalcitrant soil organic matter components. In a laboratory experiment, Cleveland et al. (2007) found increased microbial respiration from soil organic matter after addition of litter-derived DOM. Increased microbial activity in soils often results in an accelerated production of DOM (reviewed by Kalbitz et al. 2000; Kalbitz et al. 2004). Therefore, we propose that large DOC and DON concentrations and fluxes by increased litter addition are the result of priming effects with enhanced microbial DOM production from organic matter already stored in the forest floor. Our findings coincide with recent results of Fröberg et al. (2007) who found an almost complete loss of litter-derived C beneath the Oa horizon in a ^{13}C labeling study. Furthermore, Park et al. (2002) observed increasing DOM production from forest floor material after addition of easily available substrates like glucose and cellulose.

Increasing ratios of hexoses to pentoses in DOM of the Oe/Oa horizon (Fig. 7) are further indications that enhanced DOM production by increased litter input occurs mainly in the Oe/Oa horizon by stimulated microbial activity. Furthermore, microbially derived hexoses comprised a larger portion of DOM than plant derived pentoses in both horizons of the forest floor.

Lignin degradation is assumed to be an important process for DOM production (Kalbitz et al. 2006). Results of the CuO oxidation method revealed a larger contribution of lignin-derived phenols to DOM by doubling the litter input compared to the *Control* in both horizons of the forest floor. UV and fluorescence data and total contents of phenols were not different between the *Control* and the *Double litter* treatment. Obviously, these methods are not sensitive enough to detect the rather small increase in lignin degradation products in DOM at the *Double litter* plots.

Our data also indicated that the lignin-derived phenols additionally released at the *Double litter* plots in comparison to the *Control* plots are characterized by lesser side chain oxidation (lower acid-to-aldehyde ratios of vanillyl and syringyl units; Kögel-Knabner et al. 1988) and lesser degradation of syringyl units (larger ratio of syringyl to vanillyl units [S/V ratio]). Selective losses of syringyl units were observed during organic matter decomposition (Kögel 1986; Goñi et al. 1993; Guggenberger et al. 1994) and it was suggested that syringyl phenols are more degradable than vanillyl ones (Ertel and Hedges 1984; Dignac et al. 2005; Bahri et al. 2006). On the other hand, syringyl units should be less soluble than vanillyl ones (Benner et al. 1990) resulting in smaller S/V ratios of leached organic matter in comparison to the bulk phase. This is supported by our data with smaller S/V ratios of DOM samples (0.8–1.1; Fig. 8) than those of beech litter (1.3; Kalbitz et al. 2006). Therefore, elevated S/V ratios at the *Double litter* plots cannot be explained by a simple solubilization from the added litter. Instead we can assume that lignin degradation is stimulated at the *Double litter* plots but it is less complete than at the *Control* treatment.

Main source of DOM in the forest floor—effects of decreased litter input

Our data indicated that DOM production declines if the organic matter pool in the Oe/Oa horizon as the main DOM source is not replenished by continuous litter input. Furthermore, DOM production declined earlier in horizons with small stocks of organic matter by exclusion of litter input. Therefore, we found an immediate decrease of DOC and DON fluxes in the Oi horizon where only 2,000 kg C ha^{-1} are stored after exclusion of about 1,900 kg C ha^{-1} litterfall. In contrast about 13,000 kg C ha^{-1} are stored in the Oe and Oa horizons (Table 1). In these horizons, DOC production started to decrease in the third year after preventing a litterfall of 5,858 kg C ha^{-1} comprising 40% of C stocks in the forest floor (Table 1). Fröberg et al. (2005) diminished the input of organic matter by about 20% of the C stocks in the forest floor by removing the litter layer and by preventing the annual litterfall. They did not measure significantly declined DOC concentrations. In their study, DOC concentrations declined significantly after additionally removing of the Oe horizon, i.e. after removing more than 50% of the C stocks of the forest floor. Obviously, DOC production will only decrease if substantial amounts of C do not reach the soil. Probably, DOC production decreases if litterfall exclusion amounts to more than 40% of the stored C.

Decreasing specific UV absorbance and humification indices deduced from fluorescence spectra refer to a smaller contribution of aromatic compounds to DOM by exclusion of litterfall compared to the *Control*. Both DOC concentrations and the index deduced from fluorescence spectra exhibited the same decrease over the 6 years of the study (Fig. 6). However, results from the CuO oxidation did not indicate a smaller proportion of lignin-derived phenols in DOM in the Oe/Oa horizon by exclusion of aboveground litter input (Fig. 8). Taking into account the small DOC concentrations, lignin degradation is obviously not able to deliver the same amounts of soluble organic compounds because of the decreased input of organic matter over long periods. Lignin-derived compounds of DOM at the *No litter* plots were slightly more oxidized than those of the *Control* plots (larger acid-to-aldehyde ratios of vanillyl and syringyl units). Therefore, we can assume that the remaining organic matter in the Oe/Oa horizon as the main source of DOM is largely decomposed including lignin. Larger ratios of syringyl to vanillyl units in the Oe/Oa horizon of the *No litter* plots in comparison to the *Control* should not be interpreted as lesser lignin degradation because of the observed larger oxidative change. Instead, vanillyl units were preferentially leached from the Oe/Oa horizon over the time course of the manipulation experiment since vanillyl units are more soluble than syringyl units (see above). Therefore, we can expect an enrichment of syringyl units in the Oe/Oa horizon at the *No litter* plots resulting in a relative increase of syringyl units to produced DOM.

Surprisingly, exclusion of litter input did not result in smaller DON concentrations and fluxes in both horizons of the forest floor in comparison to the *Control*. Throughfall input of DON was too small to account for the remaining DON concentrations and fluxes in the Oi and Oe/Oa horizon. Probably, inorganic N delivered by throughfall and by the large N mineralization after exclusion of litterfall (large NO_3–N fluxes; Table 3) is microbially immobilized in the Oi and Oe/Oa horizon and consequently transferred into the DON pool. This process is rather fast. Seely and Lajtha (1997) observed large portions of an applied ^{15}N tracer in the DON pool after 2 days although only nitrate was added. Therefore, microbially derived compounds rich in N may be more important for DOM after exclusion of litter input although we did not find increasing contents of hexoses or increasing ratios of hexoses to pentoses. Probably, total contents of hexoses and pentoses are not specific enough to indicate these changes in DOM composition by exclusion of litterfall. Furthermore, the impact of throughfall-derived DOM on DOM in the forest floor should increase upon exclusion of aboveground litter input. This assumption is supported by similar DOC concentrations and fluxes in throughfall and in the Oi horizon of the *No litter* plots.

Calculation of net release of DOC and DON from the Oi and Oe/Oa horizon—does it make sense?

In many studies the net release of DOM from different soil horizons was calculated by subtracting the flux data in the respective soil horizon by flux data from the overlaying one (e.g., Solinger et al. 2001; Park and Matzner 2003). Our findings indicate that this approach does not provide appropriate data to illustrate the importance of the respective soil horizon for DOM production. It does not take into account DOM loss by biodegradation and sorption and new microbial production in each horizon. However, DOM biodegradation is quite efficient for litter derived organic matter (Kalbitz et al. 2003a; Kiikkilä et al. 2006). Furthermore, DOM will also be produced during its degradation (Kalbitz et al. 2003b). Small or negative net fluxes in the Oa horizon as measured by Qualls et al. (1991) and Michalzik and Matzner (1999) should therefore not be interpreted that fresh litter represents the most important source for DOM in the forest floor and the mineral soil. Instead of net fluxes we should consider changes in DOM composition as indications for its source.

Table 3 Mean annual fluxes of NH_4–N and NO_3–N (kg N ha^{-1}) in throughfall and in the forest floor after long-term litter and throughfall manipulation (No litter: without aboveground litter input, 2× litter: doubling the aboveground litter input, 2× TF: doubling the throughfall input)

	NH_4–N	NO_3–N
Throughfall	7.0	6.2
Oi: Control	3.6	12.5
Oi: No litter	6.0*	17.2*
Oi: 2× litter	3.7	7.9*
Oi: 2× TF	6.6*	18.8*
Oe/Oa: Control	5.1	14.8
Oe/Oa: No litter	10.5*	33.6*
Oe/Oa: 2× litter	3.5	7.4
Oe/Oa: 2× TF	5.4	18.3

Means were calculated from the annual fluxes of 2000–2005 which are the means of the fluxes of the 4 lysimeters/samplers per treatment. Statistically significant differences to the control are indicated by an asterisk

Role of water fluxes for DOM dynamics

Similar mean annual DOC and DON concentrations in the *Control* and *Double throughfall* treatment showed that DOC and DON concentrations are not dependent on water fluxes. In turn, the strong dependence of annual DOM fluxes on water fluxes illustrate the dominating role of water fluxes for leaching of DOM from the forest floor. However, fluxes of DOC increased in the same magnitude as water fluxes by doubling the input of throughfall only beneath the Oe/Oa horizon characterized by much larger stocks of organic matter than the Oi horizon (Table 1). Obviously, the litter horizon contains too little organic matter which can be solublized by adding more water. We assume a similar effect as by exclusion of aboveground litterfall: Additional leaching of DOM by increasing water fluxes requires large stocks of organic matter. However, decreasing DOC concentrations over time indicate exhaustion of the pool of potential DOM in the Oe/Oa horizon which is in contrast to all previous studies (see introduction). We found significantly declining DOC concentrations in the Oe/Oa horizon at the *Double throughfall* plots (Fig. 9; $r = -0.37$) although the annual means were statistically similar even in 2005 (*Control*: 45 mg C l^{-1}, 2× TF: 38 mg C l^{-1}).

DOM properties did not indicate substantial changes in DOM sources at the *Double throughfall* plots supporting our idea that the additional DOM mainly derives from stored organic matter in the forest floor. However, declining DOC concentrations over time in the Oe/Oa horizon coincided with slightly decreasing humification indices deduced from fluorescence spectra. Therefore we can assume that the importance of lignin-derived compounds for DOM production will decrease after exhausting the pool of potential dissolved organic matter.

The observed dilution effect in the Oi horizon and declining concentrations over time in the Oe/Oa horizon was not found for DON. The increased addition of inorganic N at the *Double throughfall* plots might result in increased DON production (cf. discussion; main source of DOM in the forest floor—effects decreased litter input).

Effects of time—are long-term studies necessary?

Our data indicate that mean annual DOC and DON concentrations and fluxes can be determined with reasonable accuracy within 1 or 2 years if extreme weather conditions do not persist for longer periods. Also seasonality of DOC and DON concentrations and fluxes and of DOM properties can be studied within such time frames because we observed similar seasonal patterns in every year.

Observed effects of litter and throughfall manipulation depend largely on the time of the study period. Changes after addition of easily-decomposable organic matter by fresh litter were quicker than those after diminished inputs. Obviously, large stocks of organic matter in the forest floor can compensate the missing input of fresh organic matter for a certain time. In turn, additional input of easily-decomposable organic matter enhances the turnover of organic matter stored in Oe and Oa horizons considerably which results in immediately increasing DOC and DON production. However, the fast response to additional litter inputs is limited if the stocks of organic matter in the forest floor are too large. Even after 3 years, DOC concentrations increased only slightly in the Oa horizon of a Norway spruce forest with about 60 t C ha^{-1} stored in the forest floor after doubling aboveground litter input (Huang et al. 2008).

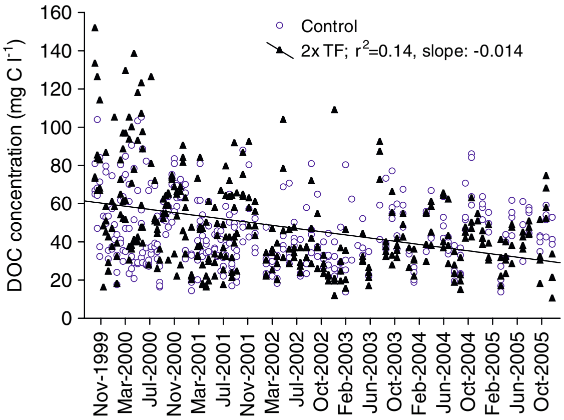

Fig. 9 DOC concentrations in the Oe/Oa horizon upon doubling of throughfall input (2× TF) from fall 1999 until the end of 2005 (single data of each lysimeter); linear regression between DOC concentration and time at the double throughfall treatment indicates significantly declining DOC concentration

The time aspect is also of large importance when looking at the effects of doubling the input of throughfall. Annual mean DOC and DON concentrations were statistically not different from the *Control* even after 6 years of manipulation. However, the time series of DOC concentration indicate depletion of potential DOM within 6 years.

Our long-term study also revealed some evolution of organic horizons within the experiment. DOC and DON concentrations of the Oi horizons approximated those of the Oe/Oa horizon in each treatment during the 6 years of the experimental period (Figs. 1, 2). The spectroscopic parameters describe the transformation of the Oi into an Oe/Oa horizon very well. The specific UV absorbance and the humification index were significantly larger for DOM from the Oe/Oa than for the Oi horizon at the beginning of the experiments but became similar over time (UV absorbance in 2003; fluorescence in 2005). Thus the composition of DOM became uniform in the former Oi and Oe/Oa horizons within 4–6 years illustrating the time necessary for the transformation of the Oi into an Oe/Oa horizon.

Conclusions

Dissolved organic matter leaving the forest floor derives mainly from decomposing organic material stored in Oe/Oa horizons. Leaching of organic matter from fresh litter is of less direct importance. However, it delivers labile organic matter into Oe/Oa horizons stimulating microbial DOM production. This conclusion is consistently supported by all of our findings: Doubling the litter input results in an increased microbial activity which is responsible for increased DOM production from decomposed organic material stored in Oe/Oa horizons. This fact could be experimentally proven by an increased portion of microbially derived sugars and a slightly larger contribution of lignin-derived compounds to DOM in the Oe/Oa horizon after doubling the litter input because the composition of fresh leaf leachates would suggest the opposite. Furthermore, exclusion of litter input has to exceed 40% of the C stocks in the forest floor before DOC production starts to decline. Then, lignin degradation in Oe/Oa horizons is not able to maintain DOC production at the same level. An increasing contribution of throughfall components and microbially derived compounds cannot compensate this decline. The long-term increasing addition of throughfall did not only demonstrate the great importance of water fluxes for DOM fluxes. The small but significant exhaustion of the pool of potentially water-soluble compounds in Oe/Oa horizons after long-term throughfall manipulation also refers to the main source of DOM, organic matter stored in Oe/Oa horizons.

Determinations of DOM properties indicative of its sources are particularly valuable to gain deeper insights into DOM processing. Although this approach does not allow an exact quantification of the various DOM sources, it clearly demonstrates the dominance of microbial processes and the importance of lignin degradation for DOM production. The application of carbon isotopes to trace the fate of applied C quantitatively (Hagedorn et al. 2004; Fröberg et al. 2007) in combination with analysis of the applied tracer in various DOM components would allow both the quantification of sources and processes.

The sources of DOC and DON leaving the forest floor are not identical. Transformation of inorganic N from deposition and from N mineralization in the forest floor to DON might increase if the input of C by litter declines. At such circumstances an increasing contribution of microbial products to DOM and increasing oxidative degradation of lignin refer to a microbiological process. The biotic transformation of inorganic N into DON and its subsequent transport into the mineral soil might be an important pathway of N sequestration in soils.

Climate change, direct effects of increased atmospheric CO_2 concentrations, and high N deposition will result in increasing net primary production throughout Europe in the future (Morales et al. 2007). Therefore, we can assume increasing input of C into the forest floor by litter with the direct consequence of increasing DOM production in the forest floor. This DOM produced from decomposed organic matter is fairly stable against microbial decay (Kalbitz et al. 2003a). Thus, we hypothesize that increasing DOM production in the forest floor as a result of enhanced plant productivity can partly explain increasing DOC concentrations in surface waters. This mechanism is of particular relevance in catchments with shallow soils where adsorption of DOM in the mineral soil is of low importance.

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