Fate of lignin in forest soils

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Citation for published version (APA):

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Chapter 4

Effects of six years litter manipulation on dissolved C and N in the forest floor of a Norway spruce forest

With: Christoph Stepper, Emiel van Loon, Pedro Gerstberger and Karsten Kalbitz

To be submitted to *European Journal of Soil Science*
Abstract

Climate change likely affects the plant productivity of many temperate forests. Altered litter fall amounts in turn can affect the cycling of organic matter in the forest floor. We show how the production and properties of dissolved organic matter (DOM) and dissolved inorganic nitrogen (DIN) in forest floor leachates responded to 6 years doubling and exclusion of litter fall during the snow-free season at a temperate spruce site.

Litter exclusion did not change fluxes of dissolved organic C (DOC) and N (DON), thus 20% of the annual litter fall during winter was sufficient to maintain fluxes. Doubling litter inputs increased DOM fluxes already in the 2nd year. Cumulated fluxes of DOC over 6 years doubling litter input increased in average by 75% (Oi), 52% (Oe) and 25% (Oa leachate). UV and fluorescence spectroscopy indicated enhanced DOM leaching from fresh litter in Oa leachates starting after 3 years of doubling litter input, which potentially triggers energy-limited organic matter degradation in the mineral soil. Contrary to other studies, no indications for priming effects in the forest floor were found upon litter addition (i.e. enhanced DOM production from lignin degradation in the Oa horizon) probably because of the high C availability in the forest floor. Fluxes of DIN (NO$_3^-$-N and NH$_4^+$-N) did not change upon litter manipulation indicating the unexpected high C availability in the forest floor of this site. Generally, we assume C availability in the forest floor determines the kind of changes in DOM properties and DIN fluxes upon the expected increasing litter fall due to climate change.
4.1 Introduction

The decomposition of organic matter in forest floor and soils plays an fundamental role in the cycling of C and other essential nutrients in ecosystems. In temperate regions, plant productivity increases widely due to increasing temperature and atmospheric CO$_2$ levels (Norby, 2005; Heimann and Reichstein 2008). The response of organic matter decomposition in soils to altered plant litter inputs will largely affect soil properties and the future C balance of temperate forests (Fontaine et al. 2004; Crow et al. 2009a).

Dissolved organic matter (DOM) is thought to be involved in numerous biogeochemical soil processes, e.g., DOM might be an important C and energy source for microbial activity (Marschner and Kalbitz 2003) or control the mobility of metals in soils (Kalbitz et al. 2000). The effect of altered litter inputs on DOM in forest floor leachates differed between study sites. At a fir/hemlock forest in Northwest USA doubling the needle input for 4 years did not change the concentrations of dissolved organic C (DOC) in forest floor leachates (Lajtha et al. 2005), whereas at a beech/oak site in South Germany, a similar treatment of doubling aboveground litter increased DOC concentrations in the first treatment years (Park and Matzner 2003; Kalbitz et al. 2007).

Reasons for the different response of DOM to altered litter inputs are not yet understood. Following changes in DOM quality might help to understand the links between litter input and DOM fluxes. Kalbitz et al. (2007) showed that litter addition at the beech/oak site resulted in enhanced contribution of lignin derived compounds to DOM in forest floor leachates. The authors assumed that degradation of lignin stored in lower parts of the forest
Litter input effects on C and N in forest floor solutions

floor was enhanced upon litter addition, which produced refractory lignin-derived DOM and thus was a main reason for the enhanced DOM fluxes to the mineral soil.

Organic matter dynamics in the forest floor might also be closely linked to fluxes of dissolved inorganic nitrogen (DIN = NO$_3^-$-N and NH$_4^+$-N). At a beech/oak site in South Germany (Steinkreuz site), Park and Matzner (2006) found that concentrations of DIN in forest floor leachates were negatively related to litter inputs. The authors suggested that DIN was immobilized due to an increase in C availability for microorganisms upon litter addition. An inverse relationship between concentrations of dissolved organic C (DOC), which might measure bioavailable C, and concentrations of nitrate was recently shown for a wide range of fresh and marine waters (Taylor and Townsend, 2010).

Spruce forests are prevalent in temperate regions. Typically they develop thick, mor-type forest floors (Ponge 2003). Despite the slow rates of organic matter decomposition, DOM production in the forest floor is relatively large in such systems and an important input path of organic matter to the mineral soil (Currie and Aber 1997; Kalbitz et al. 2000). To the best of our knowledge, we report here of the only long-term litter manipulation treatment conducted in a temperate spruce forest. We report how DOM (amounts and properties) and DIN in forest floor leachates responded to doubling and exclusion of litter fall for 6 years. Following hypothesis are discussed: (1) Concentrations and fluxes of DIN in forest floor solutions decrease upon increasing litter input due to enhanced C availability and microbial N immobilization; (2) concentrations and fluxes of DOM increase with litter input because of additional DOM leached from fresh litter or enhanced lignin degradation in the forest floor. We followed changes in DOM properties with UV and fluorescence spectroscopy to
distinguish these processes. We further assessed changes in stocks of organic matter in the forest floor and mineral topsoil (0-10 cm depth) and compare them to DOM fluxes.

4.2 Materials and Methods

Study site

The litter manipulation experiment was conducted at the “Coulissenhieb” research site in the “Fichtelgebirge” area, Bavaria, Germany (58°08 N, 11°52 E). Detailed descriptions of the study area were provided by Michalzik and Matzner (1999) and Gerstberger et al. (2004). The forest stand is composed of 150-years old Norway spruce trees (Picea abies). Mean annual temperature is 5°C and annual precipitation is 1100 mm. The parent material at the site is granite. The soils are loamy and were classified as Albic Rustic Podzols (Kögel-Knabner et al. 2008). The forest floor is a mor-type of about 8.5 cm thickness and stratified into Oi, Oe and Oa horizons.

Design of litter manipulation experiment

All the necessary equipment for the litter manipulation experiment including plate lysimeters to collect soil solution was installed in august 2001. At the same site, fluxes of dissolved organic matter were measured and published by Michalzik and Matzner (1999) for the period 1995-1997 and Kalbitz et al. (2004) for the period 1999-2001. Hence we can use a long-term record of pre-treatment data. The manipulation started in spring 2002. Twelve research plots with an area of 2×2 m and a space of about 1 m in between the plots
were established. Spatial replicates (n=4) of Control plots, Litter Exclusion plots and Double Litter plots were randomly assigned. The Controls received the normal input of aboveground litter. At the Litter Exclusion plots aboveground litter fall was excluded. To this end, litter traps consisting of nylon grids (mesh size ~0.5 mm) were installed horizontally in 1 m above the ground. The traps covered the whole plot area. On the sides of the trap a nylon grid was installed vertically to prevent loss of collected litter due to wind. Litter material was manually collected from the litter traps in intervals of 6 weeks. The litter traps were only installed in the snow-free period, which made in average 80% of the year. Thus during the winter time the Litter Exclusion plots also received litter inputs. We used seasonal litterfall data by Berg and Gerstberger (2004) to calculate litter input at the Litter Exclusion plots during winter time. Averages for litter fall amounts during winter did not differ from values for the whole year. Accordingly, litter input at the Litter Exclusion plots was 20% of the annual litter input.

Litter collected at the Litter Exclusion plots was transferred to the Double Litter plots. Litter material consisted of needles, small branches and cones. Large branches were excluded. The litter was evenly applied to the whole area of the Double Litter plots.

Sampling and laboratory analysis of throughfall and forest floor leachates

Throughfall is a source of dissolved organic matter and dissolved inorganic N in the forest floor. We collected throughfall with 10 circular collectors (area of 314 cm$^2$) placed nearby the research plots. They were sampled biweekly to determine the volume for calculation of water fluxes (see below). Then two composite samples from 5 collectors each were used for chemical analysis (DOC, total dissolved nitrogen, NH$_4^+$, NO$_3^-$, Cl$^-$).
In August 2001, tension lysimeter plates (polyethylene) with a surface area of 176 cm² were installed underneath the Oi, Oe and Oa horizons of each plot (Kalbitz et al. 2004). Tension was applied each 3 min for 1 min, and the leachates were collected continuously in polypropylene bottles buried in a pit next to the plots. We collected leachates for chemical analysis starting in spring 2002, when the litter manipulation started and the litter traps were installed. From spring 2002 to the end of 2004 the forest floor leachates were sampled biweekly, thereafter monthly for laboratory analysis. In 2006 and 2007 we had technical problems with the lysimeters at one of the Double Litter plots. For this period the chemical data are available for 3 spatially replicated plots.

Sample solutions were filtered through a cellulose-acetate membrane filter with 0.45 μm pore size (Whatman, Kent, UK) and stored at either 2°C for a few days for analysis of dissolved organic carbon (DOC), total dissolved nitrogen, NH₄⁺, NO₃⁻, Cl⁻ or -20°C for later UV absorbance and fluorescence spectroscopic analysis.

Dissolved organic carbon and total nitrogen were analyzed using high-temperature combustion and subsequent determination of CO₂ and NOₓ (High-TOC, Elementar Analysensysteme, Hanau, Germany). NH₄⁺ was analyzed by colorimetric flow injection analysis (FIA-LAB, Medizin and Labortechnik Engineering, Bellevue, USA), NO₃⁻ and Cl⁻ were analyzed by ion chromatography (IC 25, Dionex Corporation, Sunnyvale, USA). Specific absorbance of UV at 280 nm (UVIKON 930, BIO-TEK Instruments, Winooski, USA) was analyzed to estimate the aromaticity of the DOM (Chin et al. 1994). Emission fluorescence spectra (SFM 25, BIO-TEK Instruments, Winooski, USA) were recorded to deduce a humification index (HIX; Zsolnay et al. 1999), a measure for the “complexity” of the dissolved organic matter. The HIX increases with increasing degree of condensation.
and/or conjugation, and with increasing C/H ratios of the organic molecules (Zsolnay 2003). For the UV and fluorescence measurements the samples were adjusted to 10 mg C l⁻¹ to ensure comparability of the values (Kalbitz et al. 2003a).

**Sampling and laboratory analysis of forest floor and mineral soil samples**

Forest floor and mineral soil was sampled in autumn 2008. Forest floor samples were taken with a 20×20 cm quadratic metal frame (n=12 per treatment; n=3 per plot). Samples were divided into Oi+Oe and Oa samples in the field. We decided to combine the Oi and Oe horizon because it was very hard to differentiate them in the field at the litter exclusion plots. Larger living roots were excluded already in the field. After removing the forest floor, the mineral soil was sampled with a cylindrical driller (diameter of 8 cm). We sampled in total n=16 cores per treatment (n=4 per plot). We separated samples from 0-5 and from 5-10 cm depth already in the field. For the 5-10 cm depth increment we could further use only n=12-14 cores per treatment, as in some cases large stones prevented sampling in 5-10 cm depth. In the laboratory all samples were dried at 40°C. Thereafter, Oi+Oe samples were homogenized by hand; they consisted of freshly fallen and fermented needles, small fruit structures and woody debris. The Oa samples consisted of humified organic material. The Oa and mineral soil samples were sieved to <2 mm for homogenization. Subsamples of the forest floor material were dried at 60°C to a constant weight, while subsamples of the mineral soil material were dried at 105°C over night to determine the water content of the samples for calculation of the stocks of C and N. Subsequently, subsamples were milled with a ball-mill and analyzed for C- and N-contents with a CNS analyzer (VarioEL, Elementar Analysensysteme, Hanau, Germany).
Calculations and Statistical analyses

Dissolved organic nitrogen (DON) was calculated as the difference between total N and inorganic N (\( \text{NH}_4^+ \)-N plus \( \text{NO}_3^- \)-N).

Annual throughfall fluxes of DOC, DON, \( \text{NH}_4^+ \)-N and \( \text{NO}_3^- \)-N were calculated from volumes and concentrations of throughfall collected biweekly. Annual fluxes of DOC, DON, \( \text{NH}_4^+ \)-N and \( \text{NO}_3^- \)-N were calculated from measured concentrations in forest floor leachates and volumes of throughfall water. Thereby, water fluxes from the Oi, Oe and Oa horizon were calculated as 80%, 80% and 72% of the water fluxes with throughfall. These data derived from measured mean ratios of the period 1999-2001 at the same site (Kalbitz et al. 2004). When concentration data were missing because not enough solution was obtained for analysis, concentrations were estimated by linear interpolation from data of samples collected at the dates before and after.

The annual means of the Cl\(^-\) concentration (2002-2007) were not significantly different between the litter manipulation treatments (\( p<0.05; \) one-way ANOVA; tested for each year). Chloride is widely used as tracer for soil water movement (White and Broadley 2001); under the assumption that the studied forest floor does not act as a sink or source of Cl\(^-\), our data suggest that litter manipulation did not influence water fluxes in the forest floor.

We tested the litter manipulation effects using the general linear model (GLM), considering treatment years as repeated measurements. In particular the treatment effects on mean annual DOC, DON and DIN concentrations and fluxes as well as mean annual UV280 and HIX values were evaluated with the repeated measurements model. One-way ANOVA was used to evaluate treatment effects on cumulated fluxes of DIN, DOC and DON as well as
the stocks of C and N and the C/N ratio in the forest floor and top 10 cm of the mineral soil. Statistical analysis was conducted on within plot means, which were calculated from values of multiple cores per plot. In the text we report effects as being significant if p was <0.05. To check for significant differences between individual treatments, post-hoc analysis was performed using Tukey’s HSD. We used the Statistica ’97 software (Statsoft, Hamburg, Germany) to conduct the statistical analyses.

4.3 Results

*Inputs of C and N upon litter manipulation*

The amounts of litter transferred from Litter Exclusion to the Double Litter plots from spring 2002 to end of 2008 were 14.4 Mg dry matter ha⁻¹ (2.1 Mg ha⁻¹ year⁻¹), equaling 6.7 Mg C ha⁻¹ (0.98 Mg C ha⁻¹ year⁻¹) and 0.19 Mg N ha⁻¹ (0.03 Mg N ha⁻¹ year⁻¹) (calculation based on C and N contents of fresh needle litter as determined by Berg and Gerstberger 2004). As the litter traps were installed for only 80% of the time (snow-free period), we calculated an annual input of 1.23 Mg litter-C ha⁻¹ year⁻¹ and 0.04 Mg litter-N ha⁻¹ year⁻¹ at the control plots from the amounts of litter transferred (Table 4.1), given that the average daily litter input during winter does not differ from average values of the whole year (see Methods section).
Table 4.1 Inputs of C and N [Mg ha\(^{-1}\) year\(^{-1}\)] with litter fall.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Litterfall input</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>N</td>
</tr>
<tr>
<td>Control</td>
<td>1.23</td>
<td>0.04</td>
</tr>
<tr>
<td>Litter exclusion</td>
<td>0.25</td>
<td>0.01</td>
</tr>
<tr>
<td>Double litter</td>
<td>2.21</td>
<td>0.07</td>
</tr>
<tr>
<td>Amounts transferred from litter exclusion to double litter plots</td>
<td>0.98</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Inputs of C and N with throughfall solution

Annual input of DOC with throughfall was in average 0.14 Mg C ha\(^{-1}\) year\(^{-1}\) (period 2003-2007, Table 2), making 11% of the C input via litter fall at the controls (Table 4.1). In comparison, the N input with throughfall made 86% of the N input via litter fall. Most of the N in throughfall (86%) was in form of dissolved inorganic N (DIN; sum of NH\(_4^+\)-N and NO\(_3^-\)-N), while DON formed only 14% of total N inputs with throughfall.

Concentrations and fluxes of dissolved inorganic nitrogen (DIN) upon litter manipulation

At the Litter Exclusion and Double Litter plots the concentrations of DIN (NO\(_3^-\)-N plus NH\(_4^+\)-N) were increased in Oi leachates when compared to Controls (Figure 4.1). The cumulative fluxes of DIN over the study period were increased by 40% (SE ±21%) at the Litter Exclusion plots and 48% (SE ±11%) at the Double Litter plots (Figure 4.2; Table 4.2). These increases were however not statistically significant. Also in leachates of the Oe and Oa horizon, cumulative fluxes of DIN were increased at the Litter Exclusion and Double Litter plots (Figure 4.2); these increases of mean values by 6-11% compared to the Controls were smaller than in Oi leachates and also not statistically significant.
Litter input effects on C and N in forest floor solutions

Concentrations and fluxes of dissolved organic C (DOC) and N (DON)

Average DOC concentrations of all measured samples increased at the Controls with depth from leachates of the Oi (39 ± 2 mg C l⁻¹; SE; n=4 plots) to leachates of the Oa horizon (57± 3 mg C l⁻¹). These values are in the range of those determined by Michalzik and Matzner (1999) in the period of autumn 1995 to autumn 1997 (Oi: 36 mg C l⁻¹; Oa: 39 mg C l⁻¹) and Kalbitz et al. (2004) in the period of summer 1999 to winter 2001 (Oi: 46 mg C l⁻¹; Oa: 53 mg C l⁻¹) at the same study site.

In leachates of the Oi and Oe horizon, DOC concentrations were significantly higher at the Double Litter than at the Control and Litter Exclusion plots (Figure 4.1). In the Oa horizon DOC concentrations were significantly increased at the Double Litter when compared to the Litter Exclusion plots. Cumulative fluxes of DOC during the study period (spring 2002 to end of 2007) increased upon doubling litter inputs by 75% (SE ± 8%) in the Oi, 52% (SE ± 16%) in the Oe and 25% (SE ± 3%) in the Oa horizon when compared to the Controls (Figure 4.2). These increases were significant for the Oi and Oe horizon.

Litter exclusion did not affect DOC concentrations when compared to the Controls (Figure 4.1). Also cumulative fluxes of DOC during the study period (spring 2002 to end of 2007) were not significantly affected by litter exclusion (Figure 4.2).

Effects of litter manipulation on concentrations and fluxes of DON were comparable to those for DOC (Figure 4.1, Figure 4.2, Table 4.2): Cumulative fluxes were significantly increased in the Oi and Oe horizons upon doubling litter inputs compared to Controls; litter exclusion showed no significant treatment effects on DON concentrations and fluxes.
Spectroscopic properties of DOM

Both, the specific UV absorbance at 280 nm (UV280) and the humification index deduced from fluorescence spectra (HIX) of DOM increased with forest floor depth at the control plots (Figure 4.3). We found no significant treatment effects of litter manipulation on the UV280 or HIX of DOM in forest floor leachates (Figure 4.3). However, in the period of 2004 to 2007, we found the trend that the UV280 and HIX values were increased in Oi leachates and decreased in the Oa leachates at the Double Litter plots. Also in the period 2004 to 2007, the UV280 values were decreased at the Litter Exclusion plots (Figure 4.3).

Table 4.2 Annual fluxes of dissolved organic C (DOC) and N (DON) and dissolved inorganic N (DIN) in the forest floor upon litter manipulation for the years 2003-2007. Shown are means of spatial replicates (n=4 or n=3 for Double Litter in 2006 and 2007 due to technical problems with one lysimeter). Note that data for 2002 is not given because the manipulation started in spring 2002, thus we do not have data for the whole year. Shown are p values of repeated measures ANOVA (GLM) (Tr.= Effects between litter treatments; Time= effects of time with treatment year regarded as repeated measurement).

<table>
<thead>
<tr>
<th></th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>Mean of 5 years</th>
<th>p values</th>
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<tr>
<td><strong>DOC Oi [kg C ha⁻¹]</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>168</td>
<td>219</td>
<td>232</td>
<td>235</td>
<td>229</td>
<td>216</td>
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</tr>
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<td>201</td>
<td>222</td>
<td>239</td>
<td>208</td>
<td>209</td>
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</tr>
<tr>
<td>2× Litter</td>
<td>246</td>
<td>364</td>
<td>336</td>
<td>422</td>
<td>574</td>
<td>389</td>
<td>Tr.×Time: 0.004</td>
</tr>
<tr>
<td><strong>DON Oi [kg N ha⁻¹]</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.4</td>
<td>7.7</td>
<td>6.0</td>
<td>5.5</td>
<td>4.9</td>
<td>5.9</td>
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<td>4.7</td>
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<td>14.3</td>
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<tr>
<td><strong>DIN Oi [kg N ha⁻¹]</strong></td>
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<td>51.0</td>
<td>42.5</td>
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Table 4.2 continued

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<td><strong>DOC Oa [kg C ha⁻¹]</strong></td>
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<tr>
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<td>476</td>
<td>416</td>
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</tr>
<tr>
<td><strong>DON Oa [kg N ha⁻¹]</strong></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
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<td>9.4</td>
<td>6.1</td>
<td>7.2</td>
<td>9.9</td>
<td>7.6</td>
</tr>
<tr>
<td>Litter excl.</td>
<td>6.3</td>
<td>10.6</td>
<td>9.1</td>
<td>7.6</td>
<td>8.4</td>
<td>8.4</td>
</tr>
<tr>
<td>2× Litter</td>
<td>5.7</td>
<td>11.6</td>
<td>14.2</td>
<td>10.4</td>
<td>10.2</td>
<td>10.4</td>
</tr>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>DIN Oa [kg N ha⁻¹]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
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<td>27.2</td>
<td>32.0</td>
<td>38.4</td>
<td>30.7</td>
<td>31.6</td>
</tr>
<tr>
<td>Litter excl.</td>
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<td>32.6</td>
<td>38.3</td>
<td>29.5</td>
<td>33.6</td>
</tr>
<tr>
<td>2× Litter</td>
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<td>35.7</td>
<td>30.5</td>
<td>41.6</td>
<td>29.2</td>
<td>35.1</td>
</tr>
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</table>
Figure 4.1 Concentrations of dissolved inorganic nitrogen (DIN=NO$_3^-$-N+NH$_4^+$-N), dissolved organic carbon (DOC) and nitrogen (DON) in forest floor leachates upon litter manipulation (annual mean values). Error bars indicate standard error of spatial replicates (n=4, or only n=3 for the Double Litter treatments in 2006 and 2007 due to technical problems). Significant effects of time, treatment or interactions of time and treatment are indicated on the upper left of the boxes (p<0.05, GLM). Between which treatments significant effects (p<0.05, Tukey HSD) were found is indicated on the upper right side (K=controls, OL= litter exclusion, 2L= Double Litter). Legend: (○) Controls; (△) Litter exclusion; (■) Doubled Litter Input.
Figure 4.2 Cumulated fluxes of dissolved inorganic nitrogen (DIN=NO$_3^-$-N+NH$_4^+$-N), dissolved organic carbon (DOC) and nitrogen (DON) in forest floor leachates upon litter manipulation for the period of spring 2002 until end of 2007. Error bars indicate standard error of spatial replicates (n=4, or only n=3 for the Double Litter treatments due to technical problems with one lysimeter in 2006 and 2007). Significant differences between treatments are indicated by different letters (p<0.05, Tukey HSD).
Figure 4.3 Specific absorbance at 280 nm and humification index deduced from fluorescence spectra of forest floor leachates upon litter manipulation (annual means 2002-2007). Error bars indicate standard error of spatial replicates (n=4, or only n=3 for the Double Litter treatments in 2006 and 2007 due to technical problems). Significant effects of time, treatment or interactions of time and treatment are indicated on the upper left of the boxes (p<0.05, GLM). Between which treatments significant effects were found is indicated on the upper right side (p<0.05, Tukey HSD). Legend: (☉) Controls; (△) Litter exclusion; (■) Doubled Litter Input.
The C and N stocks in the Oi+Oe and Oa horizon were not significantly changed at the Double Litter and Litter Exclusion plots compared to the Controls (Table 4.3). For the Oi+Oe horizon, the average values for C stocks increased by 20% upon doubling litter inputs and decreased by 19% upon litter exclusion, whereas the N stocks increased/decreased by 10% and 8%, respectively. The C/N ratio was significantly lower in the Oi+Oe horizon at the Litter Exclusion plots in comparison to the Controls and Double Litter plots.

In the mineral topsoil (0-5 and 5-10 cm depth increments), stocks of C and N tended to be lower at the Double Litter plots in comparison to Controls and Litter Exclusion plots. However, these differences in C and N stocks as well as C/N mass ratios were not significant between the treatments and Controls.
Table 4.3: Depth of the Oi+Oe and Oa horizon, carbon and nitrogen stocks in the forest floor and upper mineral soil and C/N ratio upon litter manipulation. Shown are values after 6.5 years of litter manipulation (spring 2002–autumn 2008). Standard error of spatial replicates in brackets (n=4 plots per treatment). Shown are p values of one-way ANOVA (GLM). Values followed by different letters are significantly different at p<0.05 (Tukey HSD).

<table>
<thead>
<tr>
<th>Depth of forest floor horizon</th>
<th>Depth of horizon</th>
<th>C-Stocks [Mg ha⁻¹]</th>
<th>N-Stocks [Mg ha⁻¹]</th>
<th>C/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Excl.</td>
<td></td>
<td></td>
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<tr>
<td>Double Litter</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Depth of Oi (Ea horizon)</td>
<td>0-5 cm depth</td>
<td>23.7 (0.4)</td>
<td>2.1 (0.2)</td>
<td>11.3</td>
</tr>
<tr>
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<td>5-10 cm depth</td>
<td>24.2 (0.3)</td>
<td>2.2 (0.3)</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td>0-5 cm depth</td>
<td>23.1 (0.2)</td>
<td>2.2 (0.3)</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td>5-10 cm depth</td>
<td>24.8 (0.3)</td>
<td>2.1 (0.1)</td>
<td>11.7</td>
</tr>
</tbody>
</table>

ANOVA (GLM). Values followed by different letters are significantly different at p<0.05 (Tukey HSD).
4.4 Discussion

*Changes in DIN fluxes upon litter manipulation*

We hypothesized that concentrations and fluxes of dissolved inorganic N (DIN) decrease with increasing litter input. This pattern was found at a similar litter manipulation experiment in a beech/oak forest in South Germany (Park and Matzner 2006; Kalbitz et al. 2007). The proposed rationale was that enhanced C availability upon litter addition triggered DIN immobilization by microbial uptake (Park and Matzner 2006). Fresh litter has relatively wide C/N ratios and releases high amounts of soluble and readily available C sources (Don and Kalbitz 2005). Also at the site studied here, the amounts of available C should have increased by litter addition; we found significantly increased concentrations of DOC in leachates of the Oi and Oe horizon (Figure 1). However, concentrations and fluxes of DIN were hardly affected and even tended to increase upon litter addition, suggesting C availability was not a limiting factor for N immobilization. In line, the C:N mass ratios of the forest floors were wider at the spruce site studied here (22.6-24.1, see Table 3) than at the beech/oak site (18.6, Park and Matzner 2006).

*Effects of litter addition on fluxes of DOC and DON in the forest floor*

Doubling litter inputs significantly increased the concentrations and fluxes of dissolved organic C (DOC) and N (DON) in Oi and Oe leachates (Figures 1 and 2). No significant increase in C and N stocks of the Oi+Oe horizon were found after 6 years doubling litter inputs (Table 3), suggesting DOC and DON fluxes increased faster than the stocks of overall C and N in the Oi+Oe horizon. Similar patterns were found at other sites: At a
Norway spruce forest in Sweden the addition of litter corresponding to 4 times of the annual litterfall increased DOC fluxes into the mineral soil by 35%, although the stocks of organic matter in the forest floor were not measurably affected (Fröberg et al. 2005); at the beech/oak site in South Germany, DOC fluxes significantly increased already in the second year upon doubling litter inputs (Kalbitz et al. 2007). In contrast, at a fir/hemlock forest in Northwest USA, DOC export into the mineral soil did however not significantly change after 3-4 years of doubling aboveground litter inputs (Lajtha et al. 2005).

Reasons for the different response of DOM to increasing litter inputs are not well understood. Kalbitz et al (2007) showed that litter addition led to enhanced contribution of lignin derived compounds to DOM leaving the forest floor at the studied beech/oak site. They assumed that enhanced lignin degradation might have been an important reason for the increase in DOM fluxes upon litter addition. We examined the UV280 and the HIX of the DOM in forest floor leachates as indicators for changes in source of DOM. The parameters increased when DOM was extracted from spruce litter of increasing decomposition stage, presumably due to increasing contribution of lignin-derived components to DOM (Don and Kalbitz 2005). We found a time trend of increasing UV280 and HIX ratios over the course of the experiment, which might in part be explained by an increasing decomposition stage of the organic material on top of the lysimeters (Figure 3). Doubling litter inputs showed no significant treatment effects on the parameters yet. However, we found that differences developed with time and might become more pronounced upon longer manipulation. Starting in the 3rd year of doubling litter inputs, the UV280 in Oi leachates increased (Figure 3). We assume this indicates decreased contribution of DOM from throughfall to Oi leachates due to increasing depth and C stocks.
Litter input effects on C and N in forest floor solutions

of the Oi horizon upon litter addition. The UV280 of throughfall at the site was in average 0.011 L mg C\textsuperscript{-1} cm\textsuperscript{-1} during the study period (unpublished data), thus lower than values for Oi leachates. Starting in the 3\textsuperscript{rd} year of litter addition, the UV280 and HIX values in Oe and Oa leachates decreased, suggesting DOM composition shifted towards higher contribution of recent litter as DOM source. Hence, we found no indications for enhanced lignin degradation in the lower parts of the forest floor upon litter addition. In line, the effect of increasing DOM fluxes upon litter addition decreased with forest floor depth (Figure 3).

The reasons for the enhanced DOM fluxes in the forest floor upon doubling litter inputs thus probably differed between study sites. As discussed above, C availability was presumably not a limiting factor in the forest floor at the spruce site. Hence, DOM leached from the added recent litter might not been used by microorganisms and contributed additionally to DOM in Oa leachates. On the other side, enhanced lignin degradation in the forest floor might only be important upon litter addition in C limited systems; lignin degradation was found to be a co-metabolic process requiring inputs of easily available and labile organic compounds (Kirk and Farrell 1987; Klotzbücher et al., 2011) as typically released by recent litter (Don and Kalbitz 2005).

Effects of litter exclusion on fluxes of DOC and DON in the forest floor

The exclusion of aboveground litter in the snow-free period for almost 6 years did not significantly affect the concentrations and fluxes of DOC and DON in the forest floor (Figure 1, Figure 2). The small litter input during the winter time, making 20 % (0.25 Mg C ha\textsuperscript{-1} y\textsuperscript{-1}) of the annual litter input (1.23 Mg C ha\textsuperscript{-1} y\textsuperscript{-1}), was thus sufficient to maintain DOC and DON fluxes even from the small Oi horizon, storing 3-4 Mg C ha\textsuperscript{-1} (Kalbitz et al. 2005).
Similarly, at other sites litter exclusion had to sum up to 20% (spruce site; Fröberg et al. 2005) - 40% (beech/oak site; Kalbitz et al. 2007) of the C stored in the forest floor to result in decreasing DOC fluxes. At our site, this number appeared to be much higher, as C excluded was almost twice the amounts of C stored in the Oi horizon. Obviously much larger amounts of litter have to be excluded than added to affect DOM fluxes. Hence, overall fluxes of DOM do not linearly relate to litter input or to changes in C stocks upon litter manipulation. Composition of the forest floor (younger vs. older organic matter) and/or altered decomposition processes apparently are more important controls for DOM fluxes.

One reason that DOM fluxes to the mineral soil remain stable upon litter exclusion might be that DOM in forest floor leachates derives mainly from decomposed organic matter stored in the forest floor, and only to a small portion from fresh litter (Hagedorn et al. 2004; Fröberg et al. 2005; Kalbitz et al. 2007; Müller et al. 2009). This is supported by the result that the UV280 and HIX of the DOM in forest floor leachates were not significantly affected when fresh litter was excluded (Figure 3). Note however that we found a trend of decreasing values in Oa leachates beginning in the 4th year of manipulation. These differences might become more pronounced with ongoing manipulation. Possibly, the microbial removal of DOM derived from the Oi and Oe horizon was reduced, resulting in a larger contribution of the Oi and Oe horizons for DOM in the Oa horizon. This would further explain why DOC and DON fluxes remained constant upon litter exclusion.
Changes in stocks of organic matter in the forest floor and mineral soil

The forest floor at the studied site is mor type, typically showing slow rates of organic matter decomposition. Mean turnover times of 4, 9 and 133 years for organic matter of the Oi, Oe and Oa horizons were calculated on basis of radiocarbon data by Schulze et al. (2009). Accordingly, we found that the differences in C stocks in the Oi+Oe horizon (mean values) between the manipulation treatments and the Controls did about resemble the amounts of carbon transferred with the litter in that period (6.7 Mg C ha\(^{-1}\)). Overall, our data suggest the increasing litter fall due to climate change will lead to accumulation of organic matter in the forest floor accompanied with increasing DOM export to the mineral soil. In the mineral soil, mean values for C and N stocks decreased at the Double Litter plots, but the difference to the other treatments was not significant (Table 3). The decrease might be due to enhanced degradation of soil organic matter upon enhanced inputs of DOM from the forest floor as energy source (Fontaine et al. 2004). However, longer manipulation times are necessary to verify these trends and speculated mechanisms.

4.5 Conclusions

We presented the first long-term data on effects of altered litter fall inputs on the dynamics of organic matter and nitrogen in temperate coniferous forests with a typical mor-type forest floor. Our data suggest the expected increasing litter fall due to climate change will increase DOM fluxes in the forest floor, whereas DIN fluxes will hardly change. Patterns differ from those found at other temperate forests sites and we assume the status of C availability in the forest floor is a critical factor for the response of DOM properties and
DIN to increasing litter fall (Figure 4.4). In case C availability is not a limiting factor, DOM leached from additional recent litter is not used by microorganisms and contributes to enhanced DOM export from the forest floor. In case of C limitation, DOM leached from additional recent litter will be used by microorganisms already in the forest floor; this can lead to enhanced lignin degradation in the forest floor, which increases leaching of refractory lignin-derived DOM.

Dissolved OM is a main pathway of organic matter input to the mineral soil at sites with mor type forest floors. Hence changes in DOM fluxes and properties might have significant effects on organic matter dynamics and related soil processes. For example, enhanced inputs of DOM derived from recent litter, which contains many labile compounds (Don and Kalbitz 2005), might accelerate the energy-limited decomposition of older organic matter stored in the mineral soil.
Figure 4.4 Effect of C availability in the forest floor on the response of dissolved organic matter (DOM) and dissolved inorganic nitrogen (DIN) in forest floor leachates to the expected increasing litter fall due to climate change. Results of measurements are summarized in boxes (results presented herein for a spruce site and by Kalbitz et al. 2007 for a beech oak forest in South Germany), while processes described outside the boxes is our interpretation of the data.
Acknowledgements

We would like to thank Uwe Hell for help in the field and many colleagues and students of the Department of Soil Ecology of the University of Bayreuth for help in sampling and sample preparation. For laboratory assistance we are grateful to the staff of the Central Analytical Department of BayCEER. Chiara Cerli, Sharon Mason, Jens Altmann, Suzanne Waaijers, Dan Asscheman, Sebastian Felizeter gave valuable comments to an earlier version of this manuscript. We gratefully thank the Deutsche Forschungsgemeinschaft (DFG) for financial support.