Fate of lignin in forest soils

Klotzbücher, T.J.

Citation for published version (APA):
Chapter 3

A new model for the fate of lignin in decomposing plant litter

With: Klaus Kaiser, Georg Guggenberger, Christiane Gatzek and Karsten Kalbitz

Accepted for publication in *Ecology*
New model for the fate of lignin in decomposing litter

Abstract

Lignin is a main component of plant litter. Its degradation is thought to be critical for litter decomposition rates and the build-up of soil organic matter. We studied the relationships between lignin degradation and the production of dissolved organic carbon (DOC) and of CO₂ during litter decomposition. Needle or leaf litter of 5 species (Norway spruce, Scots pine, Mountain ash, European beech, Sycamore maple) and of different decomposition stage (freshly fallen and up to 27 months of field exposure) was incubated in the laboratory for two years. Lignin degradation was followed with the CuO method.

Strong lignin degradation occurred during the first 200 incubation days, as revealed by decreasing yields of lignin-derived phenols. Thereafter lignin degradation levelled off. This pattern was similar for fresh and decomposed litter, and contrasts the common view of limited lignin degradation in fresh litter. Dissolved organic carbon and CO₂ also peaked in the first period of the incubation but were not interrelated. In the later phase of incubation, CO₂ production was positively correlated with DOC amounts, suggesting that bioavailable, soluble compounds became a limiting factor for CO₂ production. Lignin degradation occurred only when CO₂ production was high, and not limited by bioavailable carbon. Thus carbon availability was the most important control on lignin degradation. In turn, lignin degradation could not explain differences in DOC and CO₂ production over the study period.

Our results challenge the traditional view regarding the fate and role of lignin during litter decomposition. Lignin degradation is controlled by the availability of easily decomposable carbon sources. Consequently, it occurs particularly in the initial phase of litter decomposition and is hampered at later stages if easily decomposable resources decline.
3.1 Introduction

Decomposition of plant litter is a key process in ecosystems. It releases nutrients for plant growth, affects the built-up of soil organic matter, and controls fluxes of CO$_2$ from soils (Prescott 2005). Lignin is a major component of plant litter, and is considered to be relatively resistant against degradation (Kögel-Knabner, 2002). Lignin forms a protective shield around cellulose in plant cell walls. Lignified cellulose probably represents the largest part of cellulose in litter and can only be utilized when lignin is removed first (Osono 2007). Degradation of lignin is thus assumed to regulate litter decomposition. Berg and Staaf (1980) proposed a two phase model for the fate of lignin during litter decomposition. In the initial phase, litter mass loss is thought to be controlled by easily degradable compounds, such as water-soluble constituents and non-protected cellulose. Lignin is preserved and accumulates in the litter. In later decomposition phases, when litter is assumed to consist foremost of lignified structures (Couteaux et al. 1995), lignin degradation is enhanced and thought to be the major control on litter mass loss.

The described model is still referred to in textbooks (Berg and McClaugherty 2008) as well as in recent modelling studies on litter decomposition (Moorhead and Sinsabaugh 2006, Adair et al. 2008). To our knowledge, no alternative concepts for biotic lignin degradation in plant litter have been proposed. The model is based on data for pine litter decomposition (Berg and Staaf 1980; Berg and McClaugherty 2008). Its applicability to litter of other species is unclear (Berg and McClaugherty 2008). Uncertainties also arise as lignin was traditionally determined as acid unhydrolysable residue, which is known to contain lignin but also cutin constituents, surface waxes, condensed tannins and secondary compounds...
produced during litter decomposition (Johansson et al. 1986, Zech et al. 1987, Preston et al. 1997). Therefore, the general applicability of the Berg and Staaf (1980) model, since being based on such unspecific methods, seems questionable and needs to be tested using alternative approaches to study lignin such as the CuO oxidation method.

Recent research suggested that significant photodegradation of lignin can occur in early decomposition phases, which might be an important pathway for lignin loss in ecosystems where plant litter is exposed to high solar radiation, e.g., in arid and semi-arid regions (Day et al. 2007, Austin and Ballaré 2010). In many other ecosystems like forests, biotic processes should be the dominant drivers for lignin degradation. The study presented here addresses biotic lignin degradation in the absence of photodegradation.

Carbon turnover during litter decomposition proceeds via production of dissolved organic matter (DOM) and CO₂. Both processes might be closely related. Production of CO₂ requires biologically accessible carbon sources. As microorganisms require an aqueous environment, these sources should comprise soluble compounds (Marschner and Kalbitz 2003, Bengtson and Bengtsson 2007). Indeed, positive relationships between amounts of DOM and microbial biomass or respiration in mineral soils have been found (see review in introduction of Zhao et al. 2008). Whether production of DOM is crucial to CO₂ evolution during litter decomposition has not been addressed yet. To the best of our knowledge, the role of lignin degradation in both DOM and CO₂ production during litter decomposition has also not been studied. Data by Kalbitz et al. (2006) suggested that lignin degradation is the main control, and correlates positively with DOM production when litter mass loss exceeds 10–20%. In accordance with the model by Couteaux et al. (1995), it therefore seems
reasonable to assume that lignin degradation controls DOM and CO$_2$ production in later phases of litter decomposition.

Our study aimed at elucidating the interrelationship between lignin degradation, the production of DOM, and the production of CO$_2$ during litter decomposition. We conducted laboratory incubations over two years, using needle and leaf litter of five different species. The litter we used was pre-exposed for varying periods of time (0, 3, 12, 27 months) to decomposition in litterbags in the field (Kalbitz et al. 2006). This unique experimental setup, combining field and laboratory experiments, allowed for studying degradation of litter at different stages of decomposition covering up to 51 months. An advantage of the laboratory over the litterbag incubation is the possibility to simultaneously record DOM and CO$_2$ production. Production of DOM was assessed as concentrations of dissolved organic carbon (DOC) in water extracts. Lignin degradation was followed by using the CuO oxidation method (Hedges and Ertel 1982).

We tested the following hypotheses: (1) CO$_2$ production decreases with decomposition stage of the litter; (2) DOM is a measure for bioavailable carbon, thus, CO$_2$ production is positively related to DOM production during litter decomposition; (3) lignin degradation is stronger in decomposed than in fresh litter (according to the model by Berg and Staaf 1980); (4) lignin degradation is the primary control on DOM production when decomposed litter decays (it will relate positively to DOM and CO$_2$ production).
3.2 Materials and Methods

Litter samples

Air-dried litter samples of Sycamore maple (*Acer pseudoplatanus* L.), European beech (*Fagus sylvatica* L.), Mountain ash (*Sorbus aucuparia* L.), Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.) were used for the laboratory incubation. The samples were derived from a preceding litterbag experiment. Details on the origin of fresh litter samples and field conditions of the litterbag study are given in Don and Kalbitz (2005) and Kalbitz et al. (2006). Briefly, the litterbags were incubated in a 160 year old Norway spruce forest or at a nearby two-year old clear-cut site, both located in the Fichtelgebirge in North-East Bavaria, Germany (775 m a.s.l., 50°08’35’’N, 11°52’10’’E). Annual precipitation in the area is 1100 mm and the annual mean temperature is 5°C. Podzolic soils developed on granite parent material. The organic layer is mor-type and about 9 cm thick. For the laboratory incubation presented herein, we used fresh litter and litter exposed in the litterbags for 3, 12 and 27 months. At each sampling date (3, 12, 27 months), 12 spatial replicates for each species were collected at both the spruce and the clear-cut site. All spatial replicates from the two sites were combined and homogenized before laboratory incubation. Prior to laboratory incubation, the litter was cut into pieces of 0.5–1 cm diameter, except for spruce samples. Carbon concentrations, the C/N ratio, and the mass loss of samples during the litterbag study are given in Table 3.1.
**Procedure of the laboratory incubations**

Samples for the laboratory incubation experiments were prepared by mixing 1 g of litter with 40 g of pure quartz sand (dry mass). In total, 20 sets of samples were prepared, according to five plant species and four stages of exposure in the field (0, 3, 12, 27 months). Samples were re-wetted with ultrapure water to 60% maximum water holding capacity (approximately 8 mL per sample) and incubated in closed 200-mL plastic containers at 20°C in the dark. Throughout the experiment, the containers were opened regularly for about 30 minutes to aerate the samples. Intervals of aeration ranged from a 2–3 days at the beginning of the incubation when respiration rates were high to two weeks in later periods, according to decreasing respiration rates. During aeration, the water content was re-adjusted gravimetrically. We stirred the samples carefully with a spatula to prevent anoxic zones at the bottom of the containers; this was done weekly in the first month, biweekly during month 2 to 5 of incubation, and thereafter monthly.

The laboratory incubations were run for 716 days. We sampled five times, after 0, 33, 82, 176, 390, 716 days. Half of a sample was used to determine production of DOM, which was extracted with 100 mL of ultrapure water at 5°C for 24 hours. Water extracts were passed through 0.2-µm cellulose-acetate filters (OE 66; Whatman plc, Maidstone, UK), and then analyzed for concentrations of DOC. The other half of the sample was freeze dried and ground with a ball mill for carbon and lignin analysis. The sampling was destructive, hence a whole set of samples had to be prepared for each sampling date (60 samples: 5 species, 4 stages of pre-incubation in the field, 3 replicates). Not enough sample material of fresh pine litter was available, thus only two sets were prepared, which were sampled after 0 and 6 months.
Analytical methods

Evolution of CO$_2$ was recorded by placing the incubation containers with samples (n = 3 per sample type: 5 species, 4 stages of pre-incubation in the field) into a Respicond apparatus (Nordgren Innovations, Bygdeå, Sweden). The CO$_2$ analysis is based on hourly measurements of the electrical conductivity in 10 mL of 0.6 mol KOH solution placed inside the Respicond vessels (Nordgren 1988). Measurement periods lasted for at least 6 days, and at least 18 measurement periods per sample type were conducted over the 716 days of incubation. Incubation inside the Respicond vessels was at 20° C and in the dark, thus under the same conditions as for samples not used for CO$_2$ measurements. Cumulative CO$_2$ production over the incubation period was calculated on the basis of hourly CO$_2$ production rates. Rates during periods between measurements were calculated by averaging the production rates of the measurement periods before and after.

Concentrations of DOC were determined by near-infrared detection after high-temperature combustion (High-TOC, Elementar Analysensysteme GmbH, Hanau, Germany). Carbon content of solid samples was determined with a Vario EL CNS analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

Lignin-derived phenols were determined using alkaline CuO oxidation at 170°C for two hours (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(trimethylsilyl) trifluoroacetamide. The trimethylsilyl derivatives were separated and quantified using a gas chromatograph equipped with a mass-sensitive detector (GC/QP-2100, Shimadzu Corp., Kyoto, Japan) and an SPB-5 fused silica capillary column (30 m length, 0.25 mm inner
diameter, 0.25-mm film; Supelco, Bellefonte, PA, USA). Lignin phenols were identified by target ions and quantified according to response factors of external phenol standards. Ethylvanillin was added as an internal recovery standard prior to the CuO oxidation and phenylacetic acid before derivatization. CuO oxidation yields phenolic products (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) units include p-coumaric acid and ferulic acid. The sum of these phenolic compounds (V+S+C) is indicative of the lignin content, while the ratio between vanillic acid and vanillin ([Ac/Al]v) can be used as an indicator for the degradation state of the lignin in the litter (e.g., Otto and Simpson 2006).

Statistics

Reported results from statistical tests were obtained with Statistica 97 (StatSoft, Hamburg, Germany), including one-way ANOVA, followed by post-hoc analysis (Tukeys HSD test, results reported in text), paired t-tests (Table 3.2) and correlation analysis (Figure 3.5).
Table 3.1  Concentrations of carbon, C/N (mass ratios) and mass loss (% of initial mass) during the previous litterbag study of the samples used for the laboratory incubation study presented in this paper (5 tree species and 4 different periods of pre-exposure in the field: 0, 3, 12, 27 months). Data are drawn from Kalbitz et al. (2006).

<table>
<thead>
<tr>
<th>Field exposure (months)</th>
<th>Mountain ash</th>
<th>European beech</th>
<th>Sycamore maple</th>
<th>Norway spruce</th>
<th>Scots pine</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
<td>3</td>
<td>12</td>
<td>27</td>
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</tr>
<tr>
<td>C conc. [%]</td>
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<td>45</td>
<td>44</td>
<td>45</td>
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<tr>
<td>C/N ratio</td>
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<td>23</td>
<td>19</td>
<td>16</td>
<td>31</td>
</tr>
<tr>
<td>Mass loss [%]</td>
<td>0</td>
<td>28</td>
<td>41</td>
<td>58</td>
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</tbody>
</table>
3.3 Results

\textit{CO}_2 \textit{production}

During the 716 days of incubation, carbon loss in the form of \textit{CO}_2-C cumulated to 20–84\% of the initial litter carbon (Figure 3.1). Production of \textit{CO}_2 was generally highest in the first weeks of the incubation (Figures 3.1 and 3.2). Between 44 and 82\% of the overall \textit{CO}_2-C production (over 716 days) occurred during the first 200 days (28\% of the incubation time). After the initial respiration peaks, which were found for all samples, rates of \textit{CO}_2-C production remained at low and comparably constant levels beyond 200 days of incubation (Figure 3.2).

We found no consistent effect of the preceding field exposure on \textit{CO}_2 production during the laboratory incubation. In the case of Mountain ash and spruce litter, cumulative \textit{CO}_2 production over the entire incubation period of 716 days decreased with the length of the preceding field exposure, hence, with increasing decomposition stage of the litter at the beginning of the laboratory incubation. Contrarily, beech and maple litter exposed in the field for three months showed the highest carbon mineralization over the incubation period, and for pine it was litter exposed in the field for 12 months (Figure 3.1). We also found increasing mineralization of pine carbon during the first 200 days with longer field exposure.

\textit{Dissolved organic matter (DOM)}

Concentrations of DOC in water extracts showed high variations between the samples at \( t = 0 \) days, ranging between 6 (Mountain ash litter exposed in the field for 27 months) and 103
mg DOC/g C (fresh maple litter) (Figure 3.2). No consistent effect of the preceding field exposure on DOC concentrations at t=0 days of the laboratory incubation was found. In the case of Mountain ash, maple and pine, DOC concentrations were higher for fresh than for field-exposed samples, while for spruce litter differences were little and insignificant between fresh and pre-decomposed samples (one-way ANOVA). During the first 100–200 days of incubation, largely different temporal trends of DOC concentrations were found between samples. While DOC concentrations decreased consistently over time for most leaf litter samples, they were highest after 33 or 82 days of incubation for needle litter samples. Beyond 200 incubation days, temporal trends of DOC concentrations were similar for all samples; they slightly decreased or remained constant, mostly ranging at <20 mg DOC/g C. The largest DOC concentrations were then found for fresh Mountain ash litter, which released about 50 mg DOC/g C over the entire laboratory incubation period.

Lignin

The yield of VSC phenols is used as an indicator for lignin content in a sample. We present here VSC yield normalized to sample mass (mg VSC/g sample) to illustrate changes in absolute amounts of lignin in the samples. Patterns were similar for most samples (Figure 3.3). During the first 100–200 incubation days, VSC yields decreased, while after 82 days the decrease was on average 22% compared to t = 0, ranging from 1 to 50% decrease (Table 3.2). The decrease was found for fresh as well as for field-exposed litter, and no clear effect of the time of field exposure on extent of VSC decrease could be detected. After 100–200 days, VSC yields remained constant for most samples. For some leaf litter samples, however, the VSC yields increased towards the end of the incubation, which was 50
most pronounced for beech and Mountain ash litter exposed in the field for 12 or 27 months (Figure 3.3).

Ratios of vanillic acid to vanillin ([Ac/Al]v) were between 0.2 and 0.4 (Figure 3.3). No significant differences in [Ac/Al]v ratios between fresh and field-exposed litter were detected at the beginning of the experiment, except for spruce litter, for which the [Ac/Al]v increased from 0.3 by about 0.1 after 27 months of field exposure. The ratio was significantly higher for litter exposed in the field for 12 and 27 months than for fresh litter and litter exposed to decomposition in the field for three months (one-way ANOVA, Tukeys HSD test). The [Ac/Al]v ratios determined herein were larger than those found during the preceding litterbag study (Kalbitz et al. 2006). There, they were between 0.1 and 0.2, and increased slightly by about 0.1 for all litter species during the 27 months of field exposure. Values determined here, however, are well within the range found for fresh litter and partially decomposed material of Oe horizons (Rumpel et al. 2002, Otto and Simpson 2006). During incubation, [Ac/Al]v remained at constant levels, showing no distinct temporal trends.
Figure 3.1 Loss of CO$_2$-C per initially added carbon during 0–50, 50–200, 200–716 or 0–716 days of laboratory incubation of litter from five tree species. Shown are effects of exposure of the litter in litterbags prior laboratory incubation at a spruce stand in South Germany for 0, 3, 12, or 27 months (average of n = 3, ± standard deviation). Note that for fresh pine litter (0 months field exposure) CO$_2$-C production was not followed the whole period and only data for the 0–50 day period can be reported.
Figure 3.2 Temporal trends of (●) CO₂ production rates and (○) dissolved organic carbon (DOC) concentrations in water extracts during laboratory incubation of litter from five tree species, exposed in litterbags at a spruce stand in South Germany for 0, 3, 12, or 27 months (average of n = 3, ± standard deviation). For fresh pine litter, the data set is incomplete as not enough sample material was available.
Figure 3.3 Lignin degradation: temporal change of (●) absolute contents of VSC phenols in sample (mg g sample\(^{-1}\)) and (○) [Ac/Al]\(_v\) during laboratory incubation of litter from 5 tree species, exposed in litterbags at a spruce stand in South Germany for 0, 3, 12, or 27 months (average of n = 2 or 3, ± standard deviation). For fresh pine litter, the data set is incomplete as not enough sample material was available.
Table 3.2 Decrease in VSC content within the first 82 days of laboratory incubation (% change compared to day 0). Only in the first period of laboratory incubation VSC contents decreased (see Figure 3).

<table>
<thead>
<tr>
<th>Field exposure (months)</th>
<th>Mountain ash</th>
<th>European beech</th>
<th>Sycamore maple</th>
<th>Norway spruce</th>
<th>Scots pine</th>
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<tr>
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<td>27</td>
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</tbody>
</table>

p for change in VSC content 0-82 days (t-test)

|                      | .02          | .08           | .06            | .13           | .02         | .01          | .09           | .06           | .03           | nd           | .16          | .05          | .04           | .10           | nd           | .34          | .27          | .77          |

Means of n = 2 or 3, statistical significance for the difference of means after 0 and 82 days of incubation was tested with a paired t-test.

† nd = not determined due to missing values
3.4 Discussion

*Carbon mineralization: comparison with decomposition during field exposure*

During the preceding litterbag study the test samples were derived from, litter mass loss rates decreased with exposure time (Kalbitz et al. 2006), a trend typically found in litter decomposition studies. This was explained by rapid consumption of readily available carbon sources by microorganisms during the early phases of decomposition (Berg 2000). Thus, we hypothesized decreasing CO$_2$ production with advanced decomposition stage of the litter. However, we found no clear effect of field exposure on cumulative CO$_2$ production during the first 200 days of incubation (Figure 3.1). Also, litter exposed in the field for 12 or 27 months showed distinct peaks for CO$_2$ production during the first incubation weeks. The change in environmental conditions, when transferring litter from the field to the laboratory, seemed to amplify decomposition of pre-decomposed litter. One reason for this may be drying and re-wetting of the litter, which typically results in a release of easily available carbon sources, and thus can boost CO$_2$ production (Borken and Matzner 2009).

For pine litter, CO$_2$ production even increased with decomposition stage in the first 200 days of incubation. Couteaux et al. (1998) found similarly that pine needles exposed in the field for one year showed a higher CO$_2$ production than fresh needles during laboratory incubation. They suggested processes, such as the breakdown of intact cell walls by larger animals, possibly to be excluded during laboratory incubation of fresh pine litter. These processes were probably less relevant for the already pre-decomposed pine needles as
compared to fresh ones. To account for such effects, we compared mass loss during field exposure (Kalbitz et al. 2006) with CO₂ production from fresh litter in the laboratory (data not shown). The CO₂-C loss in the laboratory was, on average, 4% of initial carbon after six months for fresh pine litter, while mass loss in the field within five months represented 21% of initial mass. In contrast, for other litters, mass loss and CO₂-C loss were quantitatively comparable, or, in the case of fresh Mountain ash litter, CO₂-C loss in the laboratory made up on average 49% of initial carbon after six months, and was higher than mass loss in the field (36% of initial mass after 5 months). The meaning of comparing CO₂-C and mass loss is limited because carbon loss via leaching may contribute by 10–30% to mass loss of decomposing fresh litter (Hagedorn and Machwitz 2007). These comparisons, however, clearly show that differences in carbon mineralization from fresh litter as determined in the laboratory do not reflect the decomposability of litter in the field.

Dissolved organic matter (DOM): Relationship to carbon mineralization

It has been proposed that water soluble organic compounds can be among the most bioavailable forms of carbon in soils, as microorganisms take up dissolved compounds only (Marschner and Kalbitz 2003, Bengtson and Bengtsson 2007). Thus, DOM could be a major source for CO₂ production during litter decomposition. Positive relationships between DOM and microbial activity in mineral soils have been reported (Burford and Bremner 1975, Fang and Moncrieff 2005, Marschner and Bredow 2002, Zhao et al. 2009). However, such a relationship was not always found (Lundquist et al. 1999).

In our study, DOM production was assessed as concentrations of DOC in water extracts. Production of DOM was highly variable between samples during the first weeks of the
incubation (Figure 3.2). For many beech and maple litter samples, production was highest at the beginning of the experiment and thereafter strongly decreased. In contrast, the needle samples showed increasing DOM production during the first incubation weeks. At later stages of the incubation (>100 days), differences in time trends of DOM production diminished. Production was then generally small and decreased slightly over time (Figure 3.2). We found a positive relationship between DOC concentrations and rates of CO₂ production during the later stages of incubation (when combining data from all samples) (Figure 3.4). Such a relationship was not found for the first 100 days of incubation. This suggests that soluble carbon was not limiting CO₂ production in the first incubation period. Large amounts of soluble compounds were initially present in litter samples or formed in the first weeks, most likely due to drying–rewetting effects and excessive degradation (Figure 3.2). So, factors other than carbon availability were stronger regulators of CO₂ production. Under such conditions, DOM may be to a large part by-products or waste products of microbial activity, comprising organic components which are not utilized by microorganisms to produce biomass or CO₂. Thus, DOM may accumulate despite high respiration rates, resulting in increasing DOM production as found for many needle litter samples (Figure 3.2). Then, after 100–200 days, decomposition became carbon-limited as no further fresh organic matter was added during incubation, and a positive relationship between DOC concentrations and CO₂ production was found. Thus, under carbon-limited conditions water-soluble organic matter seems to directly drive CO₂ production, and it can be regarded as a measure of bioavailable carbon.
Lignin degradation: relationship to DOM and CO$_2$ production

Yields of VSC phenols upon CuO oxidation are correlated with the lignin content of a sample. Decreasing yields of VSC phenols are generally explained by progressing lignin degradation (Kögel 1986, Hedges et al. 1988). In our study, VSC yields normalized to sample mass showed similar temporal patterns for most samples (Figure 3.3). They decreased during the first incubation weeks, indicating loss of lignin. In a closed system, such as in our incubation study, the decrease of VSC can be due to transformation of lignin constituents into other compounds or ultimately mineralization. We cannot discriminate between these processes. In contrast to the first incubation weeks, VSC yields remained constant or even increased in some samples during the period >100–200 days. Constant VSC yield per sample mass suggests lignin degradation not exceeding mass loss. Increasing
VSC yields over time, a trend also reported by Sjöberg et al. (2004) for a laboratory incubation study with needle litter, indicate that other litter constituents degrade more rapidly than lignin. It is thought that lignin cleavage upon CuO oxidation is incomplete (Kögel 1986, Dignac et al. 2009) and that possibly only the outer parts of the lignin macromolecule are accessible with the method (Otto and Simpson 2006). Processes increasing the accessible surface may result in release of more VSC phenols. This could be due to, e.g., progressing depolymerisation. Therefore, incompletely degraded lignin possibly accumulated over time may also contribute to increasing VSC yields.

Acid-to-aldehyde ratios of CuO products are commonly used as indicators of lignin degradation. Increasing ratios are assumed to point at progressing oxidation of the lignin molecule (Kögel 1986). During our incubation study, Ac/Al ratios of vanillin units ([Ac/Al]v) showed no distinct temporal trends (Figure 3.3), thus gave no indications of changes in lignin degradation, at least not of side-chain oxidation.

Incubation time seemed to be more important for temporal trends in VSC yields than differences in the decomposition stage of the litter. No correlations between time of field exposure of litter and decrease in VSC yields during the first weeks of incubation were found (see Figure 3.3; Table 3.2). Our hypothesis that lignin degradation is stronger in decomposed than in fresh litter was not confirmed. Our results, thus, contradict the standard model for the fate of lignin during litter decomposition (Berg and Staaf 1980, Couteaux et al. 1995, Berg and McClougherty 2008). Also, our results question the idea, deduced from the model, that easily available and decomposable energy-rich compounds in fresh litter hamper lignin degradation (e.g., Moorhead and Sinsabaugh 2006, Herman et al. 2008). The contradicting results might result from differences in analytical approaches used.
model by Berg and Staaf (1980) is based on lignin determined as acid unhydrolysable residue (AUR). The typical accumulation of AUR during early litter decomposition phases might be due to accumulation of unhydrolysable compounds produced during litter decomposition (Johansson et al. 1986, Preston et al. 2009). Also, other field and laboratory incubation studies using the CuO method showed pronounced degradation of lignin in fresh litter already during the first months of decomposition (Johansson et al. 1986, Miltner and Zech 1997, Kalbitz et al. 2006). Hence, we assume that the stability of lignin during early decomposition phases has been overestimated due to the use of the unspecific AUR method.

The first weeks of incubation were characterized by high CO$_2$ production, thus high availability of carbon for microorganisms, as illustrated by the lack of a relationship between DOC concentrations and CO$_2$ (see discussion above). In the same period, VSC yields decreased, indicating strong lignin degradation. These results suggest that microorganisms produced lignin-degrading enzymes only when energy gain via respiration was high and enough carbon was available for metabolite production. This is not surprising, as production of extracellular enzymes for lignin degradation is highly energy- as well as carbon-demanding. Studies with pure fungi cultures showed that lignin degradation only occurred when other easily available carbon sources were present (Kirk and Farrell 1987). So far, the co-metabolic character of lignin degradation was not tested directly in more complex study systems such as litter decomposition experiments. After 100–200 days of incubation, the low energy production apparently limited lignin degradation in our experiment. Also in laboratory incubation studies on soils, with no easily available carbon sources present, hardly any lignin degradation was found (Bahri et al. 2008). According to
energy considerations, it is questionable if under conditions of limited availability of carbon, biotic degradation of any recalcitrant components can occur (Fontaine et al. 2004, Kemmitt et al. 2008).

Originally, we hypothesized lignin degradation to be the bottleneck for DOM and CO₂ production, at least from decomposed litter material. During the period after 100–200 incubation days, for which there are no indications of strong lignin degradation, it obviously cannot explain differences in DOM and CO₂ production between samples. Possibly the controls for rates of CO₂ production were mainly abiotic in this period. Recently, Kemmitt et al. (2008) also pointed out that when mineral soils are incubated without addition of fresh carbon sources, respiration remains constant over considerable periods. Since soil organic matter mineralization did not depend on microbial biomass or specific activity, they hypothesized that processes releasing bioavailable carbon in carbon-limited systems are abiotic. Such processes may, e.g., be diffusion or desorption (Kemmitt et al. 2008). Desorption should not have been of importance in our study system, as no sorptive minerals were present. Rather, regarding our original hypothesis, the causal relationship between lignin degradation, DOM and CO₂ production works the other way around: readily available carbon controls litter mass loss and lignin degradation. The relationship between DOC concentrations and CO₂ may, thus, be used as an indicator of lignin degradation.
3.5 Conclusions

We presented a framework describing the complex relationships between production of DOM, production of CO₂, and lignin degradation during decomposition of leaf and needle litter. The main conclusions drawn are the following (see also Figure 3.5):

- Dissolved organic matter directly influences CO₂ production when other sources of easily available carbon become limited. Then, DOM seems to represent bioavailable carbon, and processes yielding soluble organic carbon will control CO₂ production during litter decomposition. In contrast, no direct relationship between DOM and CO₂ occurs if bioavailable carbon is not limited, e.g., as a result of drying–rewetting effects or the input of fresh litter. Possibly, the DOM produced then is to a large part a by-product of litter decomposition, comprising incompletely degraded organic matter.

- Lignin degradation is mainly controlled by the availability of carbon sources. It will be hampered when bioavailable carbon becomes limited, e.g., at later decomposition stages, because energy and carbon are not sufficient to produce lignin degrading enzymes. In consequence, lignin degradation cannot be the regulator for DOM and CO₂ production under such conditions.

These conclusions further imply that lignin degradation in soils depends strongly on a continuous input of easily available energy and carbon sources.
New model for the fate of lignin in decomposing litter

Figure 3.5 Revised conceptual model for the fate of lignin during litter decomposition based on data presented herein. The traditional model was proposed in Berg and Staaf (1980) and based on data from the decomposition of Scots pine needles. In our model, lignin will be degraded if easily degradable OM is available.