Chapter 6

Synthesis
6.1 The traditional and current picture on lignin degradation in litter

The aim of this final chapter is to highlight and synthesize the most important research results and concepts presented in this thesis. In the following the major progress of the last decades on understanding the fate of lignin in litter residues and the contribution of results of the first part of this thesis (chapters 2 and 3) are shortly outlined.

In early literature it was assumed that stable organic matter in soils is formed directly from input of lignin, plant-derived waxes and fats which resist the decomposition processes (Waksman 1938; Umbreit 1962; cited in Lützow et al. 2006). Studies on plant litter decomposition described selective preservation of lignin during litter decomposition (Meentemeyer 1978; Berg and Staaf 1980; Melillo et al. 1982). Berg and Staaf (1980) presented a model on lignin degradation during different phases of litter decomposition. According to this model little lignin degradation occurs during early litter decomposition phases because easily degradable, non-lignified organic compounds are preferentially used over lignin. Enhanced lignin degradation was predicted in later litter decomposition phases when primarily lignified structures are left in the litter. The basic principles of this ‘traditional’ model are still widely referred to in recent research papers and text books (e.g. Moorhead and Sinsabaugh 2006; Berg and McCLougherty 2008). The model based on data on pine needle decomposition and proximate lignin analysis using contents of acid unhydroyzable litter residues (AUR), an approach which was used in most early studies on lignin in litter residues. The use of nuclear magnetic resonance spectroscopy (NMR) challenged the AUR approach showing that varying amounts of non-lignin organic matter are present in the AUR fraction (Zech et al. 1987).
Alternative analytical approaches (pyrolysis techniques, CuO method) were primarily applied to study lignin in soil profiles (Thevenot et al. 2010). Use of the CuO method in combination with $^{13}$C isotope analysis of lignin-derived phenols showed surprisingly fast turnover times of 5 to 38 years for the lignin in the top 25-30 cm of agricultural and grassland soils (Dignac et al. 2005; Heim and Schmidt 2007). Moreover, in mineral soils of temperate and boreal forests relatively low concentrations of lignin-derived phenols were found (Rumpel et al. 2002; Cerli et al. 2008; Crow et al. 2009; data of chapter 5). These results were in contrast to the long assumed preservation of lignin during litter decomposition. More recent review articles concluded that a selective preservation of lignin is only important in early litter decomposition phases; lignin is however not stabilized over the long-term in oxic mineral soils (von Lützow et al. 2006; Marschner et al. 2008).

In this thesis a ‘new’ conceptual model for lignin degradation in decomposing leaves and needles (chapter 3) was presented. It is supported by results of the litterbag incubation experiment (chapter 2) and the laboratory incubation experiment (chapter 3). The model challenges the above mentioned traditional model for lignin degradation during different phases of litter decomposition (Berg and Staaf, 1980). A first important aspect is that the new model predicts pronounced lignin degradation during early litter decomposition phases; the long proposed selective preservation of lignin during early litter decomposition phases is not found for leaf and needle litters. The method comparison shown in chapter 2 suggested that the stability of the lignin macromolecule during the first months and years of litter decomposition was overestimated in traditional studies using AUR as proximate lignin analysis. The AUR largely accumulated in needle and leave residues in the first 1-2 years of litterbag incubation, whereas data of the CuO oxidation method and $^{13}$C-TMAH
thermochemolysis (which are specific for the lignin macromolecule) suggested (1) pronounced chemical alterations of lignin (increasing acid-to-aldehyde ratios) and (2) no/little lignin accumulation in this period (lignin concentrations were estimated by yields of lignin-derived phenols). Furthermore, during laboratory incubation of the litter samples (chapter 3) similar patterns of lignin degradation were found for fresh and field exposed litter (i.e. up to 27 months exposure in litterbags), suggesting decomposition status of the litter was not the determining factor for lignin degradation in this experiment. In contrast, the availability of easily degradable compounds seemed to be a strong control for lignin degradation during the litter decomposition experiment presented in chapter 3. Laboratory incubation of the litter samples allowed to simultaneously study the production of dissolved organic matter (DOM; extractable from litter with cold water), CO$_2$ and lignin degradation (chapter 3). It was striking that similar pattern were found for all samples. Production of DOM and CO$_2$ as well as lignin degradation were large in the first weeks of incubation and diminished with incubation time. Furthermore, in the first weeks of incubation amounts of DOM and CO$_2$ production did not correlate, whereas in the later incubation period they positively correlated. This correlation suggested that (1) DOM was an indicator for readily decomposable organic matter, which (2) became a limiting factor for CO$_2$ production upon prolonged incubation of the litter samples in a closed system. In line with the concept of co-metabolic lignin degradation (Kirk the Farrell 1987) the limited availability of easily degradable organic matter as C and energy source was proposed to explain the little lignin degradation during the later period of the incubation experiments.

It needs to be noted that the $^{13}$C-TMAH and CuO method allowed to study the macromolecular structure of lignin in the litter residue, whereas the further fate of lignin-
derived organic matter was not assessed, including transformations into organic matter not identifiable as lignin anymore, leaching of dissolved lignin fragments or small lignin containing particles (a factor for open systems like litterbags) and mineralization. Hence, an important limitation of the proposed model is that it does not consider how complete the degradation of lignin-derived C is.

To conclude, in the first part of this thesis it was shown that temporal patterns of AUR contents during the first months and years of litter decomposition do not match temporal patterns of lignin degradation when followed with the $^{13}$C-TMAH and CuO method. Traditional concepts based on AUR can thus not be used to evaluate lignin degradation and its controls in decomposing leaves and needles. The conflict between traditional AUR-based concepts on lignin degradation and studies showing that lignin degradation is a co-metabolic process is solved when following molecular lignin degradation patterns ($^{13}$C-TMAH and CuO method). The molecular lignin degradation patterns ($^{13}$C-TMAH and CuO method) are in line with the concept of co-metabolic lignin degradation. Pronounced lignin degradation occurs during early litter decomposition phases when sufficient amounts of easily degradable compounds are present in the litter. It also follows that limited supply of easily degradable compounds might be an important stabilization mechanism for lignin in the lower parts of the forest soil or in mineral topsoils of certain forest soils. Environmental factors which change the fluxes of easily degradable compounds within forest floors and mineral topsoils might therefore affect lignin storage in soils. An example for such a factor is altered plant litter production. The effects of altered litter inputs on lignin degradation in different temperate forests were therefore studied in the second part of this thesis.
6.2 Relationship between C availability, DOM and lignin degradation

The following considerations on relationships between C availability, DOM and lignin degradation were important for data interpretation for the first part (chapter 3) and the second part of the thesis (see discussion below).

The question whether DOM is rather (a) C and energy source for microorganisms or (b) refractory by-product of decomposition needs to be considered in more detail. As pointed out above, the correlation between amounts of DOM and CO₂ production during later periods of the litter incubation in the laboratory (chapter 3) suggested DOM amounts were an indicator for easily degradable organic matter (chapter 3). This view is probably not generally valid for DOM in soils. Kalbitz et al. (2003a) showed that between 5 and 93% of dissolved organic C extracted from different organic materials (litter, forest floor, peat and agricultural soils) was mineralized to CO₂ in a 90-day incubation experiment. Furthermore, different compounds of DOM can largely differ in resistance, whereas aromatic components deriving from incomplete lignin degradation were among the most refractory components (Kalbitz et al. 2003b). Hence, DOM seems to consist to varying degrees of refractory by-products of decomposition. However, during the laboratory incubations of chapter 3 the amounts of DOM in the samples decreased with incubation time supporting the view that in this experiment DOM consisted largely of easily degradable compounds. In turn, no indication for accumulation of refractory DOM was found. This might partly be explained by the little lignin degradation after the first weeks of incubation as a possible source for refractory DOM. The lack of correlation between amounts of DOM and CO₂ production in the first weeks of litter incubation was proposed to be due to a large C
availability which was not limiting CO₂ production when large amounts of DOM were still present in the samples (chapter 3). It seems reasonable to assume that in systems which are not C limited, microorganisms will hardly use any additionally produced DOM, which thus appears as by-product of decomposition independent of the intrinsic DOM properties.

6.3 Litter input control on lignin degradation

In the second part of this thesis (chapter 4 and 5) the effects of litter input on lignin degradation processes in the forest floor and mineral soils were studied. The studied samples derived from field experiments with manipulated litter inputs over several years. In general the results showed that changes in litter fall input (1) affect amounts and quality of organic matter fluxes (dissolved organic matter, DOM; particulate organic matter, POM) in the forest floor and mineral topsoils, and (2) lignin degradation processes in the forest floor and mineral soil. In the following possible links between fluxes of organic matter and lignin degradation are discussed. In this synthesis it is particularly shown that many of the patterns found in the field can be explained by above discussed concepts linking status of C availability, DOM and lignin degradation.

Data presented in chapter 4 suggested that status of C availability in the forest floor is an important site property determining the response of DOM fluxes/properties and lignin degradation in the forest floor to increasing litter fall. The effects of 6 years litter fall manipulation on DOM fluxes and properties were studied at a spruce site (‘Coulissenhieb’). The results were compared to data from a similar experiment conducted at a beech/oak site (‘Steinkreuz’; Park and Matzner 2006; Kalbitz et al. 2007). Litter manipulation effects on
fluxes of dissolved inorganic nitrogen suggested that the forest floor of the beech/oak site was C limited whereas the forest floor of the spruce site was not C limited. On basis of results presented in chapter 4 and the study of Kalbitz et al. (2007) the following concept was proposed (see also Figure 4.4):

**Forest floor limited in C:**
- Additional litter fall increases the availability of easily degradable organic compounds as C/energy source in the forest floor.
- Increased availability of C/energy sources triggers co-metabolic lignin degradation in the forest floor.
- Enhanced lignin degradation increases the production of lignin-derived, refractory DOM.
  → As a result, increasing litter fall input results in enhanced fluxes of DOM with large contribution of refractory compounds from the forest floor to the mineral soil.

**Forest floor not limited in C:**
- Additional litter fall increases the availability of easily degradable organic compounds as C/energy source in the forest floor.
- The additional easily degradable compounds do not further promote organic matter decomposition.
- Litter addition does not affect co-metabolic lignin degradation in the forest floor.
  → Increasing litter fall input results in enhanced fluxes of DOM with large contribution of easily degradable compounds to the mineral soil.
Results presented in chapter 5 showed that litter addition to the C-limited forest floor (Steinkreuz) for 8 years did not affect the lignin properties (CuO) in the A and B horizon of the mineral soil, suggesting that the additional input of DOM from the forest floor upon litter addition (Kalbitz et al. 2007) did not affect lignin degradation in the mineral soil. Based on the concept proposed above, the reason for this might be that the DOM leached from the forest floor consisted primarily of refractory compounds which can not be used as C and energy source for co-metabolic lignin degradation (Kalbitz and Kaiser 2008). The overall available data for the Steinkreuz site thus suggested that increasing litter fall input will affect lignin degradation processes in the forest floor (Kalbitz et al. 2007) but not in the underlying mineral soil. The mineral soil samples from the Coulissenhieb site (forest floor not limited in C) were not yet analyzed for lignin (because of shorter manipulation times and yet small overall effects of litter manipulation on fluxes of DOM into the A horizon). Further studies are needed to reveal if prolonged increases in DOM input derived from fresh litter upon litter addition can trigger lignin degradation in the A horizon of this forest system.

The forest floor at Coulissenhieb is mor type. Decomposition processes in mor forest floors are typically not limited by C but by other essential nutrients (e.g. Ponge 2003). The forest floor at Steinkreuz is moder. Typically the nutrient supply is larger and C limitation can occur in such systems. Hence, forest floor type should be an important site property which determines the response of lignin degradation and DOM dynamics to increasing plant productivity. The development of different forest floor types is in turn determined by vegetation and climate (e.g. Ponge 2003). In forests with mor and (to lesser extent) moder forest floor types the decomposition of organic matter is relatively slow. Transport of
particulate organic matter (POM) from the forest floor to the mineral soil via bioturbation is of little importance (Ponge 2003). Thus a permanent forest floor is developed on top of the mineral soil. DOM fluxes are a main pathway for organic matter transport from the forest floor to the A horizons. Hence, changes in DOM fluxes/properties e.g. by altered litter input might be critical for many biological processes in the forest floor and mineral soil.

In chapter 5 also mineral soil samples (0-20 cm depth) from an experimental site with a mull type forest floor were studied (‘Bousson’). Decomposition of plant litter is typically quick in forests with a mull type forest floor. Bioturbation is an important transport path for POM from the forest floor to the mineral soil (Bernier 1998; Ponge 2003). Furthermore, already in the A horizon most of the organic C is associated with minerals (Zech et al. 1996). The results showed that lignin properties in the mineral soil were hardly affected despite 16 years of litter manipulation. Hence, data suggested that (1) no or little accumulation of recent lignin (added with the litter since start of manipulation) occurred with increasing litter input, (2) degradation of older lignin (already stored in the mineral soil before manipulation) did not change due to litter manipulation. The reason for the later effect might be that most of the lignin in the A horizon at sites with mull type forest floor is stabilized chemically by interaction with minerals (Miltner and Zech 1997).

Finally it needs to be noted that unexpected results were found when litter fall was excluded at the Steinkreuz site (chapter 5). Data suggested that litter exclusion led to increasing lignin degradation in the A horizon. Results furthermore suggested an enhanced input of POM from the forest floor and an enhanced fungi-to-bacteria ratio of the microbial community (amino sugar analysis). Apparently litter exclusion resulted in major shifts in the input pattern of organic matter from the forest floor to the A horizon (i.e. increasing
POM, decreasing DOM) which might be the reason for the changes within the microbial community composition. The results were explained according to the concept of co-metabolic lignin degradation: firstly, the enhanced POM input from the forest floor can also enhance the availability of easily degradable organic matter in A horizons (Fontaine et al. 2003); secondly, a microbial community with a higher contribution of fungi should be able to use the additional resources more efficiently for co-metabolic lignin degradation.

To conclude, the litter manipulation experiments offered the opportunity to reveal relationships between the quantity/quality of organic matter fluxes and lignin degradation processes in forest floors and mineral soils. In general, forest floor type seems to be a critical site property which determines the response of organic matter fluxes and lignin degradation processes to altered litter fluxes due to climate changes.

6.4. Implications

In this final part of the thesis some of the wider implications of the research on effects of plant litter input on lignin degradation will be proposed.

If soil carbon storage increases with increasing plant productivity of temperate forests, it might help to slow the increase in atmospheric CO₂ levels due to anthropogenic emissions. The results and concepts presented in the second part of this thesis suggest that type of forest floor might be used as an easily recognizable site property to evaluate how lignin storage in forest floors and mineral topsoils will respond to increasing litter fall inputs.

In forests with a mor-type forest floor, the storage of lignin might increases with increasing inputs of litter. Decomposition processes in such systems are not limited by easily
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degradable C sources but by the availability of other essential nutrients. Enhanced leaching of easily degradable organic matter from additional fresh litter will thus hardly affect co-metabolic lignin degradation.

In forests with moder-type forest floors, the storage of lignin will not (or only to a smaller extent) increase upon increasing litter inputs. The reason for this assumption is that enhanced leaching of easily degradable organic compounds seems to trigger co-metabolic degradation of lignin contained in decomposed plant residues which are stored in the lower parts of the permanent forest floor layer.

Also in forests with mull type forest floor, lignin storage will presumably not increase with litter input. Due to the pronounced mixing of fresh litter debris with ‘older’ organic material by bioturbation, input of easily degradable organic matter is not limiting lignin degradation processes in the forest floor and mineral topsoil (from this follows that altered inputs of easily degradable compounds upon changes in litter inputs should hardly affect lignin storage). In contrast to the soils with mor-type forest floors, also other essential nutrients are not limiting degradation processes. Interaction with minerals should be an important stabilization mechanism for lignin, but the unchanged lignin properties in the mineral topsoil at Bousson upon 16 years of litter fall addition (chapter 5) suggested that hardly any additional lignin was stabilized in the mineral topsoil.