Fate of lignin in forest soils

Klotzbücher, T.J.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 1

General Introduction
General Introduction

1.1 Background

Dynamics of organic matter in soils

The decomposition of organic matter in soils plays a fundamental role for the functioning of ecosystems. It controls (1) the mineralization of carbon and other essential nutrients making them re-available for plants and (2) the quantity and quality of organic matter stored in soils, which affect important chemical, physical and biological soil properties (Swift et al. 1979). Decomposition also controls the carbon (C) balance of terrestrial ecosystems and thus plays an important role for research on global climate change. The amounts of organic C stored in mineral soils globally were estimated to be ~1500 Pg, which is larger than the amounts of C stored in vegetation (~560 Pg) and the atmosphere (~750 Pg) (Schlesinger and Andrews 2000). Any changes in decomposition processes might therefore significantly affect the atmospheric CO₂ level and thus accelerate or retard global warming.

The primary resources for organic matter decomposition are plant-derived and include leaves, needles, fruits, woody parts and roots; secondary resources include tissues of microorganisms and animals, whereas later are quantitatively of minor importance (Zech and Guggenberger 1996). Decomposition alters the composition and properties of the organic materials as individual compounds differ in stability and due to transformations into often more stable organic matter (Berg and McClaugerty 2008).

In general, C storage in soils depends on organic matter input and turnover times. A global average turnover time of 32 years for organic C stored in the top 1 m of soils was estimated by Raich and Schlesinger (1992). The average turnover times can vary largely between
regions and ecosystems and are controlled by basic soil forming factors like climate, vegetation, parent material, topography, and time (Jenny 1941; Trumbore 1997). Furthermore the turnover times of organic C stored within a soil differ largely from less than a day to millennia. The share of stable organic C with turnover times of >100 years was thereby proposed to make 10-50% of the organic C in mineral soils (Trumbore 1997). The reasons for the formation of stable organic matter in soils are still not fully understood. The most widely discussed stabilization mechanisms include (1) preservation of recalcitrant compounds (i.e. organic compounds that resist decomposition because of their material properties), (2) interaction with surfaces (minerals, other organic matter) and metal ions and (3) spatial inaccessibility of organic matter for microbes and enzymes (Sollins et al. 1996; von Lützow et al. 2006). In particular the concept of selective preservation of recalcitrant compounds will play an important role for this thesis because lignin is often proposed as an example for a recalcitrant compound.

**Lignin degradation and its significance for research on soil organic matter**

Lignin is a major organic component of plant cell walls of vascular plants, ferns and club mosses (Kögel-Knabner 2002). Structurally lignin is a heterogeneous phenolic macromolecule synthesized by polymerization from the three primary sources coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (monolignons) (Ralph et al. 2004). They give in the lignin macromolecule so-called syringyl units (derived from sinapyl alcohol), guaiacyl (or vanillyl) units (derived from coniferyl alcohol) and p-hydroxyphenyl units (derived from coumaryl alcohol). The phenolic monomers are linked by a variety of ether and C-C bonds (Kögel-Knabner 2002). The macromolecular structure of lignin can largely
vary between plant species and parts of a plant (i.e. abundance of different monomeric units and types of bonds) and can therefore be used as biomarker for the botanical origin of organic matter (e.g. Otto and Simpson 2006).

It is generally thought that fungi (wood rotting basidiomycetes and ascomycetes) play the major role for lignin degradation (Kirk and Farrell 1987; Osono 2007). They produce the extracellular enzymes necessary for depolymerization and solubilization of lignin derived organic matter, which is thought to be prerequisite for further decomposition processes like uptake by microbial cells to produce biomass or CO$_2$ (Kirk and Farrell 1987). It is further assumed that lignin degradation occurs mainly in oxic environments, although some studies also report of lignin degradation under anoxic conditions (Thevenot et al. 2010). Lignin degradation was also found to be a co-metabolic process, i.e. wood rotting fungi require the supply of another easily degradable growth substrate (e.g. cellulose, glucose) for lignin degradation (Kirk et al. 1976). This can be understood that lignin itself does not yield sufficient energy to maintain its own degradation (Osono 2007).

The fate of lignin in soils is of great importance for research on the dynamics of soil organic matter. Lignin is among the most abundant biopolymers on earth, making about 20% of the plant litter input to soils (Thevenot et al. 2010). Therefore, any changes in rates or pathways of lignin degradation might directly influence quantities and properties of organic matter stored in soils. Furthermore lignin degradation is thought to control the degradation of other litter components. Lignin physically protects most of the cellulose and hemicellulose of plant cell wall from enzymatic hydrolysis (Kirk and Farrell 1987, Osono 2007). This important C and energy source for microorganisms can thus only be accessed upon lignin degradation. It is also believed that lignin degradation controls the access to
other essential nutrients like N and P (Sinsabaugh and Schah 2011). Lignin itself is traditionally assumed to be among the recalcitrant components of plant litter (Berg 2000; Derenne and Largeau 2000; Ruiz-Dueñas and Martínez 2009). The long-term stabilization of lignin in soils is however uncertain (Thevenot et al. 2010). Research of recent years suggested that lignin does not contribute to stable organic matter in mineral soils (Kiem and Kögel-Knabner 2003; Dignac et al. 2005; Heim and Schmidt 2007). It was thus proposed that a selective preservation of lignin is only important during early litter decomposition phases but not during later phases when rates of lignin degradation might be same or even higher than rates of overall litter decomposition (von Lützow et al. 2006; Marschner et al. 2008).

1.2 Research questions and outline of the thesis

General outline

The overarching objective of this thesis is to improve knowledge on controls of lignin degradation in the forest floor and mineral soils of temperate forests. In a first part (chapters 2 and 3) lignin degradation and related processes are studied in decomposing leaf and needle litters. In a second part (chapters 4 and 5) the effects of altered litter inputs on lignin degradation and related processes are studied. Chapter 6 synthesizes the major findings of the research presented in this thesis. In the following main research questions are introduced and the experimental approach is shortly outlined. More specific questions and hypotheses are introduced and presented in the individual chapters.
1.2.1 Lignin degradation in decomposing leave and needle litters (chapters 2 and 3)

How stable is lignin during different phases of litter decomposition?

In studies on litter decomposition ‘lignin’ was traditionally determined as acid unhydrolyzable residue (AUR; e.g. Klason lignin, van Soest method). The use of nuclear magnetic resonance spectroscopy however revealed that the AUR contains besides the lignin macromolecule also compounds originating from cutin, waxes, condensed tannins and transformation products formed during litter decomposition (Zech et al., 1987; Preston et al., 2009). Hence, the feasibility of using AUR contents to evaluate the stability of the lignin macromolecule in litter residues is largely uncertain.

In this thesis alternative analytical methods which are specific for the lignin macromolecule are used (CuO oxidation method, Hedges and Ertel 1982; \(^{13}\)C-TMAH thermochemolysis, Filley et al. 1999). An overall aim of the first part of the thesis is to test with the \(^{13}\)C-TMAH and CuO method widely used standard concepts on lignin degradation, which base on lignin concentrations estimated by AUR analysis. In particular a model originally proposed by Berg and Staaf (1980) is questioned (chapter 3). It predicts (1) preservation of lignin during early phases of litter decomposition because more easily degradable compounds get used over lignin, whereas (2) enhanced lignin degradation occurs in later decomposition phases when only lignin and lignified compounds (i.e. cell wall carbohydrates protected by lignin) are present in the litter. The model seems to be in conflict with other studies suggesting that lignin degradation by fungi is a co-metabolic process (Kirk et al. 1976). According to the concept of co-metabolism one would expect that lignin degradation is
large upon sufficient supply of easily degradable compounds, but hampered during later phases of litter decomposition when easily degradable compounds decline.

*Which relationships occur between lignin degradation, dissolved organic matter and CO₂ production during leave and needle decomposition?*

Dissolved organic matter (DOM) is a small but important fraction of the organic matter in forest floors or mineral soils. For example, DOM is a main path for the transport of carbon/energy sources and essential nutrients within soil profiles (Zech et al. 1996; Kalbitz et al. 2000). Knowledge on the controls of DOM fluxes and properties thus can help to understand the patterns in which microbial processes occur in soils.

The relationship between DOM and CO₂ production in the forest floor and mineral soils is poorly understood. As microorganisms live in aquatic environments, DOM is potentially the most bioavailable organic matter in soils (Marschner and Kalbitz 2003). Several studies found correlations between amounts of DOM and CO₂ evolution from mineral soils suggesting DOM might be used as a measure for easily degradable C (Burford and Bremner 1975; Rees and Parker 2005; Zhao et al. 2008). However such correlations are not generally found in mineral soils (Lundquist et al. 1999). Laboratory incubations have shown that DOM of different origin (i.e. litter, forest floor, agricultural soils, peat) can vary largely in resistance against biodegradation (Kalbitz et al. 2003a), suggesting DOM is to varying extent rather refractory by-product of decomposition than C and energy source for microorganisms (Kalbitz et al. 2003; Marschner and Kalbitz 2003a; Kalbitz and Kaiser 2008).
General Introduction

Decomposition processes in the forest floor are a major source for DOM in soils (Kalbitz et al. 2000), but little is known about the relationship between DOM production and CO₂ evolution during the decomposition of needle and leave litters (Hansson et al. 2010). Herein, the relationship between DOM production and CO₂ evolution during different phases of needle and leave decomposition are studied. Furthermore, patterns for DOM production and CO₂ evolution are related to lignin degradation. This offered the opportunity to examine the role of easily degradable organic compounds for lignin degradation.

Outline and approach

Chapter 2 reports of a litter decomposition experiment (leaves or needles of ash, beech, maple, pine, spruce) conducted for 27 months in a German spruce forest (litterbag method). For the first time the ¹³C-TMAH thermochemolysis method is used to follow lignin degradation in a range of different litter types. The results are compared to those of the CuO oxidation and van-Soest method, an approach to determine the acid unhydrolzable residue (AUR) of plant litter. It will be shown that molecular lignin degradation patterns (¹³C-TMAH thermochemolysis, CuO oxidation method) reveal a different picture on lignin degradation during leave and needle decomposition than the traditional approach of AUR analysis.

Chapter 3 reports of a two-years laboratory incubation experiment using litter of different decomposition degree derived from the litterbag experiment (i.e. field exposure for 0, 3, 12 and 27 months before laboratory incubation). Laboratory incubation allows to extend the field experiment and to examine under controlled conditions if/how patterns of lignin degradation (CuO method) differ between early and later phases of the decomposition
process. Furthermore, laboratory incubation enables to study relationships between lignin degradation, production of DOM and evolution of CO₂ during litter decomposition.

1.2.2 Litter input effects on lignin degradation in forest soils (chapters 4 and 5)

An important challenge for research on soil organic matter is to evaluate the response of decomposition processes to climate change. Increasing atmospheric CO₂ levels and heat contents will likely result in enhanced plant productivity in many temperate and boreal regions (Norby et al. 2005; Heimann and Reichstein 2008). When organic C storage in soils increases with plant productivity, it could help to slow down the current increase in atmospheric CO₂ levels. There is however evidence that altered plant litter fluxes change decomposition processes in soils (Fontaine et al. 2004; Sulzman et al. 2005; Sayer 2006; Kalbitz et al. 2007; Crow et al. 2009 a,b). The effects of litter input on organic matter decomposition are however poorly understood in detail. The main goal of the second part of this thesis is to improve knowledge on the effects of litter inputs on lignin degradation in the forest floor and mineral topsoils (i.e. 0-20 cm depth) of temperate forests. Thereby it will be discussed whether litter inputs affect lignin degradation by changing the fluxes of easily degradable co-substrates and/or by changing the properties of the soil microbial community.

Outline and approach

Samples from long-term litter manipulation experiments (i.e. doubling/exclusion of litter fall and/or exclusion of root litter for several years) conducted in different temperate forests
are used. In chapter 4 the effects of 6 years litter manipulation on the fluxes and properties of DOM in forest floor leachates at a spruce site in Germany (‘Coulissenhieb’) are shown. Possible changes in lignin degradation processes in the forest floor are examined by following the contribution of lignin-derived compounds to DOM. In chapter 5 the effects of litter manipulation on lignin degradation in mineral soils (0-20 cm depth) of 2 deciduous temperate sites are assessed: the Steinkreuz site in Germany (beech/oak) and the Bousson site in Pennsylvania (maple/cherry). For these sites, data on soil C contents and distribution over density fractions, traits of the microbial community, and fluxes of dissolved organic matter (DOM) can be used to unravel whether changes in lignin properties (CuO method) are caused by altered input of recent lignin or lignin degradation.