Soil Biology & Biochemistry 67 (2013) 133–139

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Processes controlling the production of aromatic water-soluble organic matter during litter decomposition

Thimo Klotzbücher [a,*], Klaus Kaiser [a], Timothy R. Filley [b], Karsten Kalbitz [c]

[a] Soil Sciences, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany
[b] Department of Earth and Atmospheric Sciences and the Purdue Climate Change Research Center, Purdue University, West Lafayette, IN, USA
[c] Earth Surface Science, Institute for Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 13 March 2013
Received in revised form
19 July 2013
Accepted 3 August 2013
Available online 20 August 2013

Keywords:
Dissolved organic matter
Water soluble organic matter
Litter decomposition
Lignin degradation
^{13}C-TMAH thermochemolysis
Litter bag experiment

ABSTRACT

Dissolved organic matter (DOM) plays a fundamental role for many soil processes. For instance, production, transport, and retention of DOM control properties and long-term storage of organic matter in mineral soils. Production of water-soluble compounds during the decomposition of plant litter is a major process providing DOM in soils. Herein, we examine processes causing the commonly observed increase in contribution of aromatic compounds to WSOM during litter decomposition, and unravel the relationship between lignin degradation and the production of aromatic WSOM. We analysed amounts and composition of water-soluble organic matter (WSOM) produced during 27 months of decomposition of leaves and needles (ash, beech, maple, spruce, pine). The contribution of aromatic compounds to WSOM, as indicated by the specific UV absorbance of WSOM, remained constant or increased during decomposition. However, the contribution of lignin-derived compounds to the total phenolic products of ^{13}C-labelled tetramethylammonium hydroxide (^{13}C-TMAH) thermochemolysis increased strongly (by >114%) within 27 months of decomposition. Simultaneous changes in contents of lignin phenols in solid litter residues (cupric oxide method as well as ^{13}C-TMAH thermochemolysis) were comparably small (−39% to +21% within 27 months). This suggests that the increasing contribution of lignin-derived compounds to WSOM during decomposition does not reflect compositional changes of solid litter residues, but rather the course of decomposition processes. In the light of recently published findings, these processes include: (i) progressive oxidative alteration of lignin that results in increasing solubility of lignin, (ii) preferential degradation of soluble, non-lignin compounds that limits their contribution to WSOM during later phases of decomposition.

1. Introduction

Despite extensive research over the last decades, we still have a limited understanding of the processes controlling the formation and storage of soil organic matter (SOM). Recent studies suggest that dissolved organic matter (DOM) in soils (i.e., release into soil water, transport, immobilization by adsorption or co-precipitation, mineralization) plays an important role for the long-term retention of SOM (Kramer et al., 2012; Kaiser and Kalbitz, 2012). Hence, improved knowledge about these processes might be the key for better understanding SOM and its response to environmental changes.

Decomposition of plant litter in the forest floor is a major source of DOM in forest soils (e.g., Kalbitz et al., 2000). Forest floor-derived DOM contains varying amounts of aromatic compounds (Kaiser et al., 2001; Kalbitz et al., 2006; Hansson et al., 2010), which originate either from lignin, tannins, or low molecular components (e.g., simple phenols, phenolic acids, and flavonoids; Hättenschwiler and Vitousek, 2000). They can affect soil processes via different mechanisms, e.g., by interacting with metals or proteins (Kraus et al., 2003; Scheel et al., 2008). Aromatic acids strongly bind to certain minerals, which might be a major mechanism for long-term sequestration of organic matter in soils (Kramer et al., 2012). Typically, the contribution of aromatic compounds to water-soluble organic matter (WSOM) is larger in extracts of decomposed than of fresh litter (e.g., Kalbitz et al., 2006; Hansson et al., 2010). One explanation is that lignin accumulates in solid litter during the first months of decomposition and then becomes increasingly degraded at later phases (Berg, 2000), which results in increasing release of soluble, lignin-derived aromatics (Kalbitz et al., 2006). However,

* Corresponding author. Martin-Luther-Universität Halle-Wittenberg, Naturwissenschaftliche Fakultät III, Institut für Agrar- und Ernährungswissenschaften, Bodenkunde/Bodenbiogeochemie, Von-Seckendorff-Platz 3, 06120 Halle (Saale), Germany. Tel.: +49 345 5522535.
E-mail address: thimo.klotzbuecher@landw.uni-halle.de (T. Klotzbücher).

0038-0717/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.soilbio.2013.08.003

recent work suggests that the long assumed accumulation of lignin during litter decomposition has been overestimated, i.e., lignin does not accumulate or only to a small extent (Preston et al., 2009; Klotzbücher et al., 2011; Wallenstein et al., 2012). Hence, the following questions remain unanswered: (How) does lignin degradation relate to changes in aromatic WSOM? Does WSOM reflect compositional changes of solid litter residues or rather does it reflect changes in decomposition processes? A limitation of the currently available data is that a molecular characterization of aromatic WSOM during different phases of litter decomposition is missing; i.e., the importance of lignin and of other aromatic litter components as sources of WSOM has not been addressed so far. To date, discussions on the contribution of lignin to WSOM are based on unspecific estimates of aromatic compounds.

Here, we analysed the specific UV absorbance at 280 nm (a measure of total aromatic compounds) and aromatic biomolecules, using ^{13}C-TMAH thermochemolysis (Filley et al., 1999), of WSOM from needle and leaf litters at different stages of decomposition. Our approach allows for estimating relative changes in contributions of (i) lignin-derived phenols, (ii) non-lignin derived phenols, and (iii) non-aromatic compounds to WSOM during decomposition. The study builds on results from a litter decomposition experiment showing changes in lignin concentrations of solid litter residues and in the biodegradability of WSOM (Don and Kalbitz, 2005; Kalbitz et al., 2006; Klotzbücher et al., 2011). In combination with published results, the present study allows for elucidating the relationship between lignin degradation and the production of aromatic WSOM during different phases of the decomposition of needles and leaves.

2. Materials and methods

2.1. Samples

The plant litter samples investigated were derived from a litter bag experiment (for details see Don and Kalbitz, 2005) and included leaf or needle litter 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.). These were exposed to degradation in a 160-yr old Norway spruce forest and at a nearby 2-yr old clear-cut site in June 2001 in the Fichtelgebirge in northeast Bavaria, Germany (775 m above sea level, 50°08′35″ N, 11°52′10″ E). Annual precipitation in the area is 1100 mm and the annual mean temperature 5 °C. Soils are Podzols, which developed on granitic parent material. The organic layer is mor type and about 9 cm thick. We used samples of freshly fallen litter and litter from litter bags after 3, 12, and 27 months. Litter bag incubation over 27 months resulted in mass loss of 26% (beech leaves), 44% (maple leaves), 54% (spruce needles), 56% (pine needles) and 58% (ash leaves) of the initial litter mass (mean values of all spatially replicated litter bags; Kalbitz et al., 2006). For present study, litter material from all litter bags (forest and clear cut site) was combined into one composite sample per litter type and sampling date (0, 3, 12, 27 months). For Scots pine, not enough fresh litter material (0 months) remained for analysis and so is not included.

For the production of WSOM samples, litter was equilibrated in polyethylene bottles with ultrapure water (litter:water ratio of 1:20) for 24 h at 4 °C, and then the suspensions were filtered to <0.45 μm. For analysis of (DOC) and the UV absorbance at 280 nm, we extracted sub-samples of 1 g litter (dry weight, 3 replicates); for ^{13}C-TMAH thermochemolysis analysis, we extracted a 10 g subsample as a larger amount of WSOM was necessary for the analysis. Then, filtrates were analysed for dissolved organic carbon (DOC) and UV absorbance at 280 nm or were freeze-dried for ^{13}C-TMAH thermochemolysis.

We compare results of ^{13}C-TMAH thermochemolysis of WSOM with those of the solid litter residues from which the WSOM was extracted (i.e., the solid litter residues were analysed after the WSOM was removed). The results for the solid litter residues were taken from Klotzbücher et al. (2011) where the ^{13}C-TMAH thermochemolysis method was compared with other approaches to analyse lignin in solid litter residues.

2.2. DOC analysis

Concentrations of DOC were analysed using high-temperature combustion and subsequent determination of CO_2 (High-TOC, Elementar Analysensysteme, Hanau, Germany).

2.3. Litter carbon (litter-C) analysis

Carbon contents of solid litter samples was analysed with a Vario EL CNS analyser (Elementar, Hanau, Germany).

2.4. UV absorbance at 280 nm

Specific absorbance of UV at 280 nm ($SUVA_{280}$) was analysed (Cary 1E, Varian, Palo Alto, CA) to estimate the aromaticity of WSOM. $SUVA_{280}$ was shown to linearly increase with aromatic C content as determined by solution ^{13}C nuclear magnetic resonance spectroscopy (Scheel et al., 2007).

2.5. ^{13}C-TMAH thermochemolysis procedure

Thermochemolysis in the presence of tetramethylammonium hydroxide (TMAH thermochemolysis) allows for analysing lignin in environmental samples (Challinor, 1995). It releases a suite of phenolic acids, which then are determined by gas chromatography. Conventional TMAH thermochemolysis is not selective for lignin, i.e., other phenol sources such as tannins release identical thermochemolysis products (Nierop and Filley, 2008). The problem can, however, be overcome by using ^{13}C-labelled TMAH, which allows for distinguishing phenols derived from lignin and phenols derived from non-lignin sources (Filley et al., 2006). In comparison to cupric oxide (CuO) oxidation, the most commonly used method to study molecular properties of lignin (Thevenot et al., 2010), ^{13}C-TMAH thermochemolysis requires very small sample amounts (Filley, 2003). It is therefore ideal for studying small samples, such as WSOM.

Briefly, freeze dried WSOM was weighed into 3 mm × 3 mm Pt buckets (typically 150–300 μg), containing eicosane as internal standard. Then, 3.5 μl of ^{13}C-TMAH (25% in water, prepared according to Filley et al., 1999) were added. Sample buckets were placed in the sample holder on top of the pyrolysis unit (Pyr-4a, Shimadzu Corp. Kyoto, Japan), and kept there for 15 min at room temperature and under a He stream, allowing the ^{13}C-TMAH solution to soak into the sample. Thereafter, the buckets were dropped into the heated zone of the pyrolyser, which was maintained at 350 °C. The injector base of the pyrolysator was maintained at 320 °C and a split ratio of 10:1 was used. Products were analysed with a Shimadzu GC17A–QP5050A GC–quadrupole MS system, collecting ions of a m/z ratio between 40 and 550. Compounds were separated on a fused silica column (SPB-1, Supelco, Bellefonte, PA, USA; 30 m length, 0.25 mm i.d., 0.25 μm film thickness). GC oven temperature programme was 60 °C (1 min) to 140 °C at 10 °C min^{-1}, then to 300 °C (held for 20 min) at 6 °C min^{-1}. Two analytical replicates were run for each sample.