Short Communication

^{14}C characteristics of dissolved lignin along a forest soil profile

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Abstract

Lignin is a key component of soil dissolved organic carbon (DOC) and is recently suggested to track ^{14}C-young DOC components. However, direct evidence is still lacking to prove this hypothesis in the soil. Here, utilizing molecular radiocarbon dating, we present the first ^{14}C dataset on dissolved lignin through a Podzol soil profile. Dissolved lignin and hydroxy phenols had similar ^{14}C content as soil organic carbon (SOC) and DOC in the surface organic layer. However, in contrast to SOC, both DOC and dissolved lignin phenols exhibited consistent and higher Δ^{14}C values in the mineral soils. Coupled with lignin phenol concentration data, our results suggest that dissolved lignin comprises a key DOC component throughout this Podzol profile and is mainly supplied by surface leachates with young ^{14}C ages.

Keywords: Soil organic carbon, dissolved organic carbon, lignin phenols, hydroxy phenols, compound-specific radiocarbon analysis.

Dissolved organic carbon (DOC) is the most bioavailable and reactive fraction of soil organic carbon (SOC; Kalbitz et al., 2000), whose source and age are related to DOC dynamics and the potential stability of SOC (Moore et al., 2013). As such, dissolved organic matter from fresh litter, root exudates and pre-aged roots with decadal turnover times supplies ^{14}C-enriched DOC to the soil (Hansson et al., 2010; Wu et al., 2014), albeit with minimal influences on the leaching of slow-cycling SOC (Tu et al., 2011). By contrast, dissolved organic matter released from old soil organic matter (SOM) introduces relatively ^{14}C-depleted DOC (Hagedorn et al., 2004; Lee et al., 2018), representing a critical pathway for the loss of relatively stable SOC (Moore et al., 2013). Hence, delineating the above source and age variations of soil DOC is essential for accurately assessing DOC dynamics and its impact on SOC stability. As bulk DOC consists of complex components with varied sources and ages (Kaiser et al., 2004), it is difficult to fully understand patterns and drivers for its age variation. It is also challenging to routinely analyze DOC-^{14}C, especially for low-DOC samples. Therefore, there has been an interest in seeking a potential indicator of young (and vice versa, old) DOC components in the soil to track their relative variations with changing environment (Benk et al., 2018).

Plant-derived lignin moieties are an important component of forest soil DOC (Kalbitz et al., 2006). Litter accumulating on the forest floor is considered to be the primary source of DOC and dissolved lignin (Wu et al., 2014), conferring both a young age in surface soils (Trumbore et al., 1992). With increasing depths, lignin-derived compounds may be selectively retained by sorption to reactive iron minerals and become less abundant in deeper soils relative to microbial-derived products (Kalbitz et al., 2003a). In mineral soils, desorption and/or SOM degradation may also introduce old (non-lignin) components into DOC (Hagedorn et al., 2004; Jia et al., 2017; Lee et al., 2018), thereby increasing age offsets between dissolved lignin and DOC with depth. Somewhat in line with this postulation, lignin phenols have been shown to trace relatively young surface carbon pools in the arctic watersheds by molecular radiocarbon dating (Feng et al., 2013; 2017). Moreover, using ultra-high resolution mass spectrometry, Benk et al. (2018) found that lignin-derived phenolic compounds (especially dimers) were key molecular species associated with young ^{14}C age in terrestrial dissolved organic matter in the critical zone. However, radiocarbon dating of lignin phenols, first used in marine and riverine systems (Feng et al., 2013; 2017), has not been conducted in soils so far to provide direct evidence for this emerging hypothesis. Filling the gap will help to verify dissolved lignin phenols as a tracer for young DOC in terrestrial environments and to improve our understanding of soil DOC dynamics.

Here we utilize a well-monitored site in a Norway spruce forest at the Long-term Forest Ecosystem Research (LWF) station of Beatenberg, Switzerland (46°43′N, 07°46′E; Schaub et al., 2011), where soil DOC is regularly sampled and easily accessible along an organic-rich profile. We employ the recently developed method of radiocarbon dating dissolved lignin (Feng et al., 2017), and present a benchmark study to analyze the ^{14}C content of dissolved lignin relative to bulk DOC and SOC along an 80-cm soil profile. By comparing ^{14}C offsets among these components, we attempt to examine source variations (from fresh litter, pre-aged ^{14}C-enriched roots with decadal turnover times and old SOM) in DOC and dissolved lignin with depth. We also demonstrate the use of compound-specific ^{14}C analysis in testing dissolved lignin as a potential tracer for young DOC components in soils (Benk et al., 2018).

Soils at the study site are classified as Podzols with a sandy texture and have a thick organic layer (20 cm; van der Voort et al., 2017). Bulk soils were collected using a soil corer from four depths in November 2012: 20 cm above the mineral soil surface (‘surface sample’; denoted depth of –20 cm) and at –5, 30 and 60 cm (all referred to as ‘subsurface samples’; Graf Pannatier et al., 2011; 2012). The first two depths were in the organic soil layer. Soil solutions were sampled periodically from July 2011 to October 2012 at the same depths using the established facilities including zero-tension lysimeters (for the surface sample) and ceramic suction cups (for the subsurface samples; Graf Pannatier et al., 2011; 2012). In total, 23 solution samples were collected, including 11 samples with ample quantities for lignin phenol quantifications. Samples collected in May 2012 from the depths of –20, 30 and 60 cm were further used for ^{14}C analysis of individual phenols.

Soil solutions were filtered through pre-washed 0.45-µm filters, stored in plastic bottles and kept in the fridge in the dark at 4ºC before analysis. The filtrates and bulk soil samples were acidified with hydrochloric acid to remove inorganic carbon. A subset of the filtrate was kept frozen before DOC measurement on a Shimadzu TOC-V organic carbon analyzer. Another subset (~5–8 mL) was freeze-dried using an oil-free vacuum-pump powered freeze dryer (Christ, Alpha 1-4 LO plus). The freeze-dried filtrates and bulk soil samples were then measured on the Mini radioCArbon DAting System (MICADAS) accelerator mass spectrometry (AMS) system coupled to an elemental analyzer (Wacker et al., 2010) for ^{14}C contents of DOC and SOC, respectively. All ^{14}C data were reported as $\Delta^{14}C$ (‰). Lignin and hydroxy phenols were released using alkaline copper oxide oxidation (Feng et al., 2015) with individual phenols isolated by high-performance liquid chromatography (Feng et al., 2017) and measured as CO_2 for ^{14}C content on the Mini radioCArbon DAting System (MICADAS; Wacker et al., 2013). Radiocarbon contents were corrected against procedural blanks as described in Feng et al. (2017). Further details on analytical methods and blank assessment can be found in the Supplementary Information (SI) and Fig. S1.

Along the soil profile, DOC concentrations decreased with depth whereas lignin and hydroxy phenols exhibited large variability in abundance (Table 1). Dissolved lignin and hydroxy phenols showed positive correlations with DOC concentrations in the subsurface ($P < 0.05$) but not surface samples (Fig. 1), suggesting that lignin is a key component of DOC in the mineral soils. Our results contrast with the down-profile decrease of lignin phenols relative to carbohydrates in the DOC of two forest soils (Kaiser et al., 2004). These differences may be attributed to (i) the strong leaching process and low content of reactive minerals (i.e., clay, iron and aluminum oxides) in the examined Podzols (van der Voort et al., 2017), preventing strong sorption and retention of lignin in the upper soils (Kalbitz and Kaiser, 2008), and/or (ii) preferential biodegradation of non-phenolic DOC components (such as carbohydrates and proteins) during transport to depth (Kalbitz et al., 2003b), leading to relatively consistent contribution of lignin to bulk DOC.

The $\Delta^{14}C$ values of bulk SOC showed clear evidence for the presence of 'bomb ^{14}C' (Trumbore, 2009), peaking at the bottom of the organic layer (~167‰ at –5 cm) and decreasing to –173‰ at 60 cm (Fig. 2a), reflecting rapid accumulation of organic carbon at this site (van der Voort et al., 2018). In contrast to SOC, DOC displayed relatively constant $\Delta^{14}C$ values throughout the profile during the sampling period (74~108‰; Fig. 2a and Table S1). These

DOC-Δ^{14}C values, similar to those reported for DOC collected at the same site during May-September 2015 (van der Voort et al., 2017), are close to that of surface SOC (~82‰). Our observations are similar to those reported for soils from an old Norway spruce forest where surface soil DOC was more ^{14}C-enriched than the atmosphere (Karltun et al., 2005). Given that atmospheric CO_2 had a Δ^{14}C value of ~38‰ during our sampling years (Levin et al., 2013), DOC emanating from recently synthesized litter and root exudates was unlikely to make a large contribution to soil DOC at our study site. By the same token, degradation products of old SOM in the mineral soils are likely minor contributors to DOC at depth. Instead, leachates from the surface organic layers appeared to the main source of DOC throughout the examined profile.

Similar to bulk DOC, individual lignin phenols showed similar and positive Δ^{14}C values (78–180‰) throughout the profile (Fig. 2b and Table S2). For the surface sample, hydroxy phenols potentially derived from proteins, tannin-like compounds and/or demethylation of lignin (Goñi et al., 2000) exhibited higher Δ^{14}C values (108–180‰) than that of DOC (96‰), indicating that non-phenolic DOC components with somewhat lower ^{14}C contents (likely from newly synthesized organic matter) comprise a larger proportion of DOC in the organic layer. This interpretation is consistent with the contrasting relationships between DOC and dissolved phenol concentrations in the subsurface versus surface samples (Fig. 1). For subsurface samples, dissolved lignin phenols exhibited higher Δ^{14}C than bulk SOC or solvent-extractable lipids isolated from the same depth (van der Voort et al., 2017), suggesting minor contribution from SOM decomposition.

Overall, we conclude that lignin is a key DOC component in the Podzol subsurface soil and remains relatively young throughout the examined profile, providing first direct evidence for the recent postulation that dissolved lignin phenols may trace ^{14}C-young DOC in terrestrial (soil) settings (Feng et al., 2017; Benk et al., 2018). Leachates from the surface serve as the main source of DOC and dissolved lignin in the deeper soil while contributions from pre-aged roots and SOM decomposition are minimal at our site. However, these patterns need to be further confirmed using dissolved lignin ^{14}C analysis in deeper soil horizons that were not available at this study site and for soils without thick organic layers and/or contain more reactive minerals to interact with lignin and/or experience less intensive leaching. We postulate that larger ^{14}C offsets may be found between (young) dissolved lignin and (old) bulk DOC in the latter soils due to higher inputs from aged non-lignin components (such as microbial carbon or black carbon) to bulk DOC at depth. If this is the case, quantification of dissolved lignin phenols may open an analytical window for assessing the relative variation of young terrestrial DOC in complex systems when the expensive and sensitive ^{14}C analysis is not allowed. Coupled with bulk DOC measurement, this advance will also facilitate the evaluation of old DOC release from terrestrial settings (such as the arctic rivers) and improve our understanding of DOC dynamics.

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Table 1. Concentrations of dissolved organic carbon (DOC), lignin and hydroxy phenols in the Podzol soil profile at three sampling times.

Depth (cm)	DOC	Lignin phenols[1]		Hydroxy phenols[2]	
	(mg L^{-1})	(μg L^{-1})	(mg g^{-1} DOC)	(μg L^{-1})	(mg g^{-1} DOC)
2011/07					
–20	64.0	86.7	1.4	53.1	0.8
–5	41.6	219.4	5.3	92.7	2.2
30	28.3	59.0	2.1	29.7	1.1
60	26.4	83.4	3.2	36.3	1.4
2011/10					
–20	52.6	416.8	7.9	85.6	1.6
–5	59.5	235.0	4.0	104.1	1.8
30	37.3	141.0	3.8	47.7	1.3
60	36.6	189.4	5.2	58.3	1.6
2012/05					
–20	38.8	219.4	5.7	41.0	1.1
–5	n.a.	n.a.	n.a.	n.a.	n.a.
30	22.7	68.2	3.0	15.4	0.7
60	26.5	110.7	4.2	19.3	0.7

n.a.: not available.

[1] Lignin phenols include eight monomers: vanillyl, syringyl and cinnamyl phenols.

[2] Hydroxy phenols include *p*-hydroxybenzaldehyde, *p*-hydroxyacetophenone, and *p*-hydroxybenzoic acid.

Figure captions

Fig. 1. Correlations of dissolved organic carbon (DOC) with dissolved lignin (a) and hydroxy phenols (b) in the Podzol profile. Blue dashed line shows linear regression for the subsurface samples (n = 8).

Fig. 2. The Δ^{14}C values of dissolved organic carbon (DOC; sampled from July 2011 to October 2012 and May-September of 2015; a) and individual phenols isolated from soil solutions in May 2012 (b) in comparison to bulk soil organic carbon (SOC; sampled in September 2012). Black dotted line shows the changing pattern of SOC Δ^{14}C with depth. Errors represent propagated analytical error of ^{14}C measurement (with procedural blanks considered). $^{\dagger}\Delta^{14}$C values of DOC for May-September of 2015 are obtained from van der Voort et al.(2017); $^{\#}$*p*-hydroxybenzaldehyde and *p*-hydroxyacetophenone were combined for ^{14}C measurement; *vanillin and acetovanillone were combined for ^{14}C measurement at 30 cm.

Figure 1

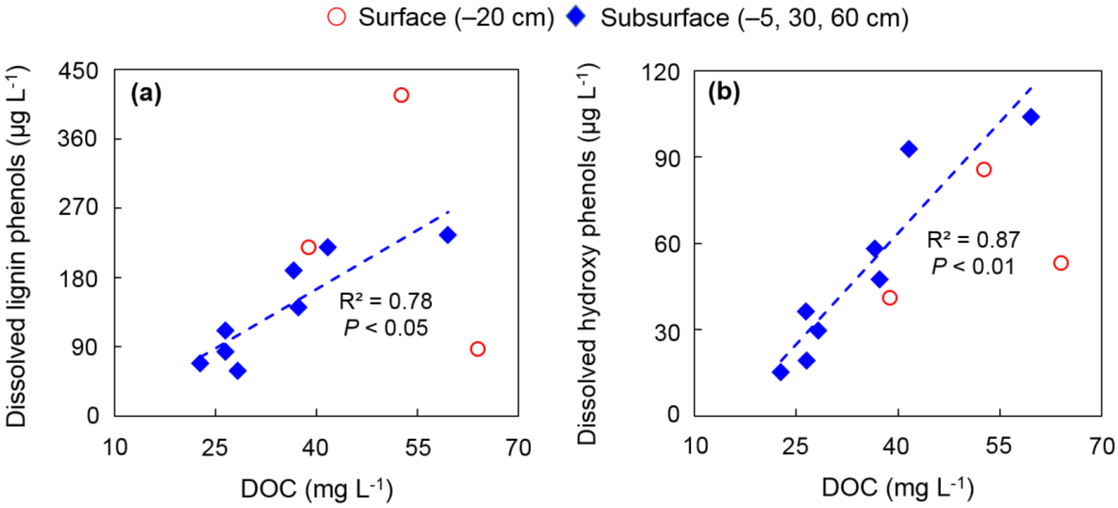
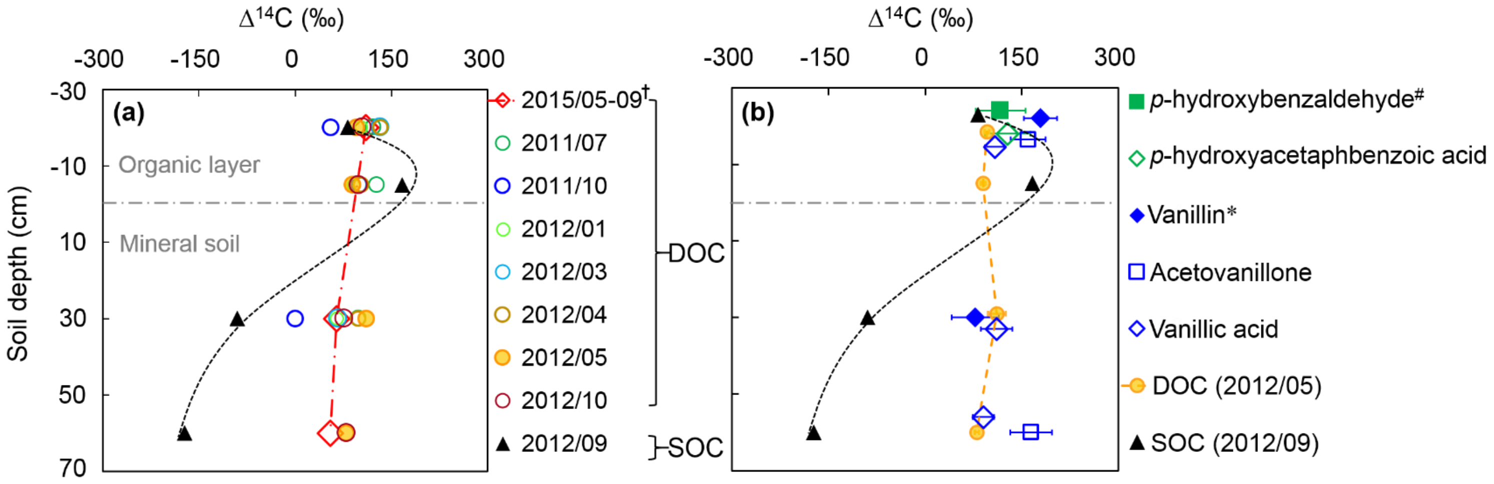

○ Surface (–20 cm) ◆ Subsurface (–5, 30, 60 cm)
(a)
Dissolved lignin phenols (µg L-1)
450
360
270
180
90
0
10
25
40
55
70
DOC (mg L-1)
R² = 0.78
P < 0.05
(b)
Dissolved hydroxy phenols (µg L-1)
120
90
60
30
0
10
25
40
55
70
DOC (mg L-1)
R² = 0.87
P < 0.01

Figure 2


Δ14C (‰)
-300
-150
0
150
300
-30
-10
10
30
50
70
Soil depth (cm)
(a)
Organic layer
Mineral soil
2015/05-09†
2011/07
2011/10
2012/01
2012/03
2012/04
2012/05
2012/10
2012/09
DOC
SOC
(b)
p-hydroxybenzaldehyde#
p-hydroxyacetaphbenzoic acid
Vanillin*
Acetovanillone
Vanillic acid
DOC (2012/05)
SOC (2012/09)