



Lignin Dimers as Potential Markers for ^{14}C -young Terrestrial Dissolved Organic Matter in the Critical Zone

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The origin, molecular composition and fate of dissolved organic matter (DOM) provides essential information that links surface and subsurface processes and explores the functioning of the Critical Zone. Therefore it is important to identify specific marker compounds that provide information on the temporal and spatial linkages in the Critical Zone. Here, we used ultra-high resolution mass spectrometry and accelerator mass spectrometry in order to identify markers for fast transport of surface-derived DOM through the Critical Zone. We assessed the molecular composition and radiocarbon age of solid phase extracted DOM (SPE-DOM) from forest top soils. The ^{14}C ages of SPE-DOM in our study were similar to that of bulk DOM. Calibrated ages ranged from 0 to 49 years and the within-site variability was larger than between sampling sites. Spearman rank correlation between the ^{14}C ages and the DOM composition identified 129 sum formulae that were significantly correlated to ^{14}C age. We found that molecular entities with younger ^{14}C ages had lower molecular weight, higher unsaturation and less oxygen and heteroatoms than those associated with older ^{14}C ages. A chemical library search suggested that phenylpropanoids and compounds, which are known to be lignin derived, are key molecular species for terrestrial DOM with young ^{14}C ages. Among them, lignin dimers emerged as prominent surface-derived compounds that can potentially be used as markers for fast transport of water and DOM into the subsurface and groundwater.

Keywords: FT-ICR-MS, radiocarbon, lignin, markers, carbon cycle

INTRODUCTION

Processes that are taking place in the Critical Zone (CZ) are mirrored in the chemical composition of dissolved organic matter (DOM) in surface-, soil- and groundwater (Wickland et al., 2007; Kaiser and Kalbitz, 2012; Gabor et al., 2014; Roth et al., 2015; Seifert et al., 2016). Major sources of DOM in terrestrial ecosystems include young carbon inputs from the decomposition of plant litter and leaching of microbially modified (Kallenbach et al., 2016), aged soil organic matter (SOM). Composition and fate of DOM are strongly influenced by the ecosystem as a whole (Nebbioso and Piccolo, 2013; Miller et al., 2016) and the discovery of terrestrial ecosystem-specificity of DOM spectra highlights their close connection (Roth et al., 2014). As a result, tracing surface signals over time has emerged as a key objective in CZ research (Küsel et al., 2016). Specific markers for ^{14}C young and surface-derived inputs could help to trace surface signals into the subsurface. Their

degradability could further make the occurrence of these marker compounds in groundwater an indicator for fast transport of water and DOM through the CZ.

The search for compositional marker molecules that identify ecosystem constitution and source characteristics (Kujawinski et al., 2009; Roth et al., 2014; Dubinenkov et al., 2015; Kothawala et al., 2015) is strongly aided by the integration of non-targeted Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). FT-ICR-MS allows study of the molecular composition of DOM in unprecedented detail, providing chemical information about thousands of mass-resolved peaks due to accurate determination of their exact mass (Hertkorn et al., 2013).

Correlating the molecular composition of DOM with ^{14}C ages of the respective samples has led to the identification of degradation states of marine DOM on the basis of individual sum formulae (Flerus et al., 2012). However, marine derived marker compounds cannot be readily applied to terrestrial systems due to terrestrial ecosystem heterogeneity and DOM representing a snapshot of ecosystem activities (Roth et al., 2014). Generally, marine DOM is composed of a wide range of compounds ranging from labile carbohydrates to refractory black carbon (Moran et al., 2016), while terrestrial DOM contains linear terpenoids and carboxyl-rich-alicyclic molecules in high proportions (Hertkorn et al., 2006; Arakawa and Aluwihare, 2015). Thus, new markers applicable for terrestrial ecosystem processes and characteristics are required.

To achieve traceability of ^{14}C young DOM through the subsurface, potential markers for CZ DOM must link to the major constituents of plant biomass. Plant litter is generally composed of the biopolymers cellulose and lignin as well as monomeric natural compounds such as carbohydrates and terpenes (Thomson, 1993). During the process of decomposition lignin was suggested to be comparatively recalcitrant in a variety of environments (Melillo et al., 1982; Hedges et al., 1985; Akin and Benner, 1988). However, more recent investigations highlight that lignin structures are not recalcitrant (Gleixner et al., 2002; Schmidt et al., 2011; Waggoner et al., 2017). Lignin phenols were further shown to be young components in both sediments and DOM of arctic rivers (Feng et al., 2013, 2015, 2017). Potential pathways for the removal of lignin structures include photochemical alteration (Waggoner et al., 2017), microbial degradation (Ward et al., 2013) in aquatic systems as well as sorption onto minerals (Kaiser et al., 2004; Hernes et al., 2013) and microbial degradation in soils (Gleixner et al., 2002). As a consequence, lignin-derived compounds may be used for tracing fast flow processes of recent biomass through the CZ.

In non-targeted ultra-high resolution studies of DOM current limitations for the identification of new and specific marker compounds arise from lack of information on the structure of specific molecules and the multitude of structural isomers per molecular formula (Zark et al., 2017). Our efforts were therefore targeted at receiving direct molecular information from the sum formulae level on the basis of chemical databases and annotated information on their biogeochemical relevance (Degtyarenko et al., 2008).

In this study we explored the feasibility of combining ultra-high resolution molecular composition data with ^{14}C ages to identify marker compounds for the transport of water and DOM in the CZ from the surface into the subsurface. We hypothesize that (1) leaching from plant biomass governs the molecular composition of ^{14}C -young carbon inputs and (2) non-recalcitrance of lignin molecules allows selection of potential ^{14}C -young marker compounds that relate to their plant litter source.

MATERIALS AND METHODS

Sampling

Soil water samples were collected in March, May, and November of 2005 at three different long term monitoring sites in Germany. The first site is a deciduous old-growth forest in the Hainich National Park [51°04'46"N, 10°27'08"E, 440 m above sea level (a.s.l.)], Thuringia (Knohl et al., 2003; Gleixner et al., 2009; Tefs and Gleixner, 2012). The forest is unmanaged and dominated by European beech (*Fagus sylvatica*, 65%) and ash (*Fraxinus excelsior*, 25%). Understorey vegetation includes *Alium ursinum*, *Mercurialis perennis*, and *Anemone nemorosa*. The soil column consists of a 5 to 15 cm deep A horizon which is followed by a clayish T horizon on 50 to 60 cm deep cambisol. The second site is a maple (*Acer pseudoplatanus*), pine and spruce (*Pinus sylvestris* and *Picea abies*) mixed forest stand near the village of Thann (49°19'24"N, 12°27'37"E), Bavaria. A 5 to 7 cm deep organic layer overlays a sandy cambisol developed on granite parent material (Sachse et al., 2009). The third site is the Wetzstein forest (50°27'13"N, 11°27'27"E, 785 m a.s.l.) in Thuringia. This managed forest consists mostly of ca. 50 year old Norway spruce (*Picea abies*) trees on an acidic and carbonate-free podzol (Schulze et al., 2005; Moyano et al., 2008; Kindler et al., 2011). Setup and sampling was conducted with permission of the national park administration, the land owner and the forest management administration, respectively, for the sites. The diversity in vegetation and soil parameters within the sample set results in a relatively wide coverage of top soil DOM conditions. Together with the replicated sampling throughout the growing season we aim at providing overarching trends in DOM composition not biased through site-specific effects.

Sample Preparation and FT-ICR-MS Analysis

The water samples were taken during a regular biweekly sampling scheme using glass ceramic suction plates with a pore size of 1–1.6 μm which were permanently installed at 5 cm soil depth. If samples were unavailable at 5 cm on a specific sampling location, we used samples from 10 cm depth. Bottles connected to the suction plates were evacuated to 20 kPa such that free flowing soil water was sucked through the ceramic plate into the bottle until pressure equilibrium was reached. As a result of the biweekly sampling scheme, each sampling time point integrates the water flow of 2 weeks. Water samples were immediately freeze-dried in the lab and stored at room temperature in the dark. After re-dissolution, DOM was subjected to solid phase extraction using

PPL (styrene-divinylbenzene polymer) cartridges according to previously established protocols (Dittmar et al., 2008), with an average extraction efficiency of 63%. A procedural blank of ultrapure water (pH 2) was stored and extracted together with samples.

FT-ICR-MS analysis was performed at the University of Oldenburg, Germany (Roth et al., 2015). The FT-ICR-MS (Bruker Solarix, 15 T) measurement was conducted in negative electrospray ionization mode at a continuous injection flow of 120 $\mu\text{L}/\text{h}$ and ESI needle voltage of -4 KV . 500 scans of m/z 150–2000 and 0.25 s ion accumulation time were combined into one spectrum. Peaks with signal/noise (S/N) > 3 and within 150–800 m/z were used for further analysis. Ions with m/z > 800 were not detected. Peaks found in the procedural blank were removed from the peak list of the samples. For molecular formula assignment the elements C, H, O, N, and S ($N \leq 2$, $S \leq 1$) were utilized in accordance with previous studies (Herzsprung et al., 2012; Riedel et al., 2012). Compounds with successful molecular formula assignment were considered for further data interpretation (Supplementary Table S4).

Radiocarbon Analysis

PPL extracts were dried into tin capsules for combustion to CO_2 in an elemental analyzer, graphitization and subsequent radiocarbon analysis on a 3 MV Tandemron ^{14}C -AMS at the MPI-BGC in Jena, Germany together with size-matched modern (Oxalic Acid II) and ^{14}C -depleted standard materials (Steinhof et al., 2004, 2010). The $\delta^{13}\text{C}$ values of the samples were determined online in the AMS. Age calibration was performed on the background corrected fraction modern [F^{14}C ; (Reimer et al., 2004; Steinhof, 2013)] values using OxCal 4.3 (Bronk Ramsey, 2009) and the NH1 bomb 2013 curve (Hua et al., 2013). Resulting median calAD dates were subtracted from the year of sampling (2005) to give an age estimate.

^{14}C System Blank of SPE-DOM

A system blank test was conducted using DOM from the Wetzstein site that was (i) non-extracted, (ii) PPL extracted, (iii) PPL extracted, dried and re-dissolved in ultrapure water once, and (iv) re-dissolved three times. For (i) DOM material from the Wetzstein site was de-carbonified by acidifying with HCl and bubbling with N_2 in order to remove inorganic carbon from affecting the $^{14}\text{C}/^{12}\text{C}$ DOM signal. For (ii) the same original material as in (i) was extracted onto PPL cartridges and eluted with methanol (Dittmar et al., 2008). For (iii) and (iv) aliquots of the eluate from (ii) were dried and re-dissolved in ultrapure water in order to break any methyl ester bonds that might have formed to the methanol solvent and would skew the $^{14}\text{C}/^{12}\text{C}$ isotopic ratio.

Statistical Analysis and Correlation

Spearman rank correlation was employed on relative intensities of mass peaks with respect to the ^{14}C calibrated ages of the samples. Rank correlation was used to account for known competitive ionization efficiencies that affect the ion intensities of the individual masses (Hertkorn et al., 2008; Herzsprung et al., 2012). Only mass peaks present in at least 8 out of 10 samples

were considered for correlation in order to identify bulk trends. Statistical data analysis and correlation was done in R 3.4.1 using the R package “vegan” 2.4–5. Principal component analysis (PCA) was performed on the matrix of relative intensities of m/z values per sample, which was centered and scaled by the standard deviations. The environmental parameters pH, cal ^{14}C age, DOC concentration and length of the growing season were fitted to the PC values as vectors. For characterization of molecular entities matching of sum formulae with the ChEBI database was performed using the web interface at <http://www.ebi.ac.uk/chebi/> (Hastings et al., 2016). We chose the ChEBI database for its focus on “small” chemical compounds that have biological relevance, its links and integration with similar chemical databases [(for example PubChem (Kim et al., 2016) and KEGG (Kanehisa et al., 2017))], free availability and general ease of use for the extraction of annotated information and chemical structures.

RESULTS AND DISCUSSION

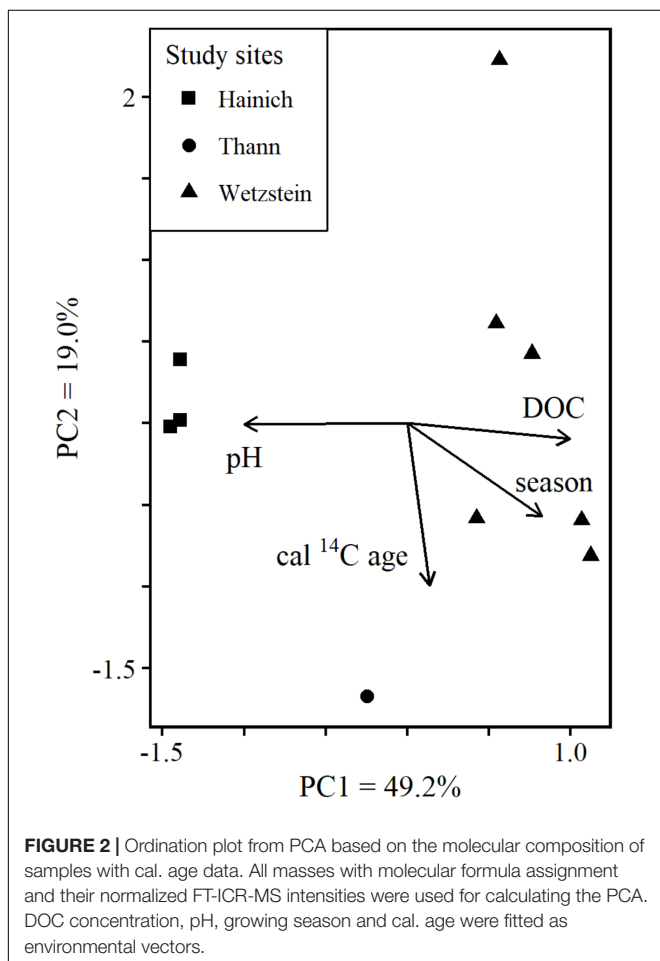
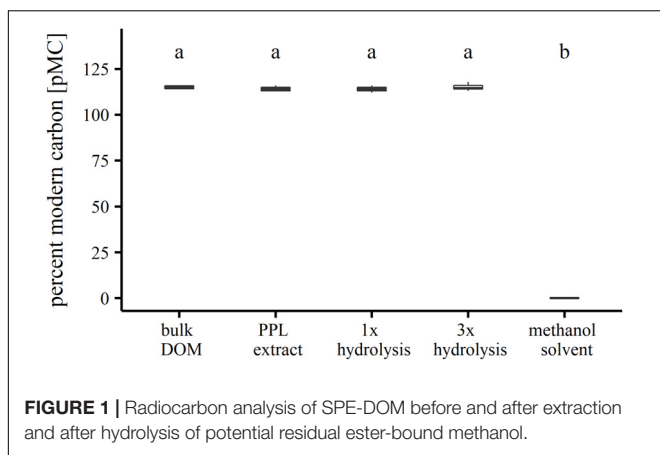
^{14}C System Blank of SPE-DOM

Solvent residues in carbon extracts resulting from elution steps can influence the $^{14}\text{C}/^{12}\text{C}$ ratio in organic matter samples. As for PPL extraction, the methanol solvent could esterify free carboxyl groups in the organic matter (McIntyre and McRae, 2005), thereby introducing ^{14}C -depleted carbon to the sample, which persists even after evaporating to dryness. Depending on the abundance of carboxyl groups in the sample, the percentage of modern carbon would be expected to be significantly reduced especially if samples are comprised entirely of modern carbon whereas commercially bought methanol is usually produced from fossil carbon sources. Since methyl ester bonds are highly susceptible to hydrolysis, re-dissolution in water could help to remove potential solvent residues. Testing our extraction method for changes of $^{14}\text{C}/^{12}\text{C}$ before and after extraction as well as after hydrolysis of residual ester bound methanol yielded highly similar percentages of modern carbon (pMC; Figure 1). A one-way analysis of variance (ANOVA) revealed that there was no significant difference ($\alpha = 0.05$) between the groups. We conclude that the $^{14}\text{C}/^{12}\text{C}$ ratios of SPE-DOM in our study are not influenced by residual ester bound MeOH and likely represent the $^{14}\text{C}/^{12}\text{C}$ ratios of bulk DOM.

Age Correlation of Molecular Entities

Principal component analysis (PCA) using the exact masses of molecules with annotated sum formulae summarized 68.2% of the variability in the molecular data by the first two principal components (PC): PC1 49.2%, PC2 19.0%. The PCA produced a well-separated clustering reflecting the 3 sampling sites (Figure 2). The fitted environmental parameters DOC concentration and pH were correlated with PC1. Cal ^{14}C age was correlated to PC2. In a previous assessment none of the considered environmental parameters were correlated to PC2 (Roth et al., 2015). Our results show that cal ^{14}C age can help to interpret additional variability in DOM composition.

The radiocarbon activity of the samples ranged from 102 to 117 percent Modern Carbon (pMC). All samples were “modern”



and the calibrated ages ranged between 0 and 49 years (Table 1). The within-site variability was higher than between sampling sites, suggesting that radiocarbon ages in this study are not biased through site-effects.

From here on we refer to negative/positive correlation with age of a molecular formula in cases when the rank relative intensity distribution of the respective sum formula was significantly descending/ascending between samples from low cal ^{14}C age

to samples with higher cal ^{14}C age, respectively. We observed distinct differences in the m/z ranges as well as H/C and O/C elemental ratios between the group of negatively and positively correlated compounds (Figure 3). The masses of negatively correlated formulae ranged from 250 to 450 m/z while the positively correlated formulae ranged from 450 to 650 m/z . This indicates that inputs into soil water DOM containing recently assimilated carbon are likely of lower molecular weight compared to molecular entities comprised of ^{14}C -older carbon. The full distribution of masses and respective rank correlation strength values further suggests a continuous mass-age relation rather than fractionated sub pools of young and old DOM (Supplementary Figure S1). The finding of lower molecular weight compounds being associated with more recent SPE-DOM is consistent with results from an assessment of marine SPE-DOM aging trends (Flerus et al., 2012). Previous studies of marine DOM size fractions have suggested decreasing molecular weight with increasing age (Kaiser and Benner, 2009; Benner and Amon, 2015). Yet, direct comparisons between SPE-DOM and studies using size-fractionation cannot be drawn due to the differences in sample preparation and resulting observed mass range.

In the forest environment one of the most prevalent sources of young carbon is plant litter. Consequently, lignin-derived molecules associated with the young carbon inputs are expected. In the van Krevelen diagram indeed many of the negatively correlated sum formulae can be estimated to represent lignin-type structures from their elemental composition [H/C: 0.6 – 1.7, O/C: 0.1 – 0.6; (Antony et al., 2014)]. A search within the ChEBI library revealed that the group of negatively ^{14}C -age correlated formulae could possibly be mostly comprised of methoxylated phenylpropanoid-derivatives (Table 2 and Supplementary Tables S1, S2). Lignin dimers were prominent suggestions among flavonoids, coumarins and stilbenes, all of which result from the phenylpropanoid pathway (Vogt, 2010). This suggests that the group of negatively correlated compounds represents inputs of plant biomass derived molecules that feature recently assimilated carbon and are likely leaching from litter decay processes.

Positively ^{14}C -age correlated sum formulae were more saturated and more oxygenated, their molecular weight was higher and in some cases they contained nitrogen or sulfur as heteroatoms (Table 2). From the elemental composition in the van Krevelen diagram the positively correlated sum formulae plot in the tannin-like area [H/C: 0.5 – 1.5, O/C: 0.6 – 1.2; (Antony et al., 2014)]. However, database search did not select tannin-type structures for positively correlated sum formulae even though tannin structures are annotated in the ChEBI library and in contrast structures of glycosylated phenylpropanoid derivatives were suggested (Supplementary Tables S1, S3). The tannin area in the van Krevelen diagram lies aside the lignin and carbohydrate areas [H/C: 1.5 – 2.2, O/C: 0.6 – 1.2; (Antony et al., 2014)] and consequently the structure suggestions are very likely (Figure 3).

The observed ^{14}C -age related trends regarding the elemental composition and structure suggestions for the molecular entities differ from aging trends in marine DOM, likely due to largely different timescales of DOM aging. There, the composition

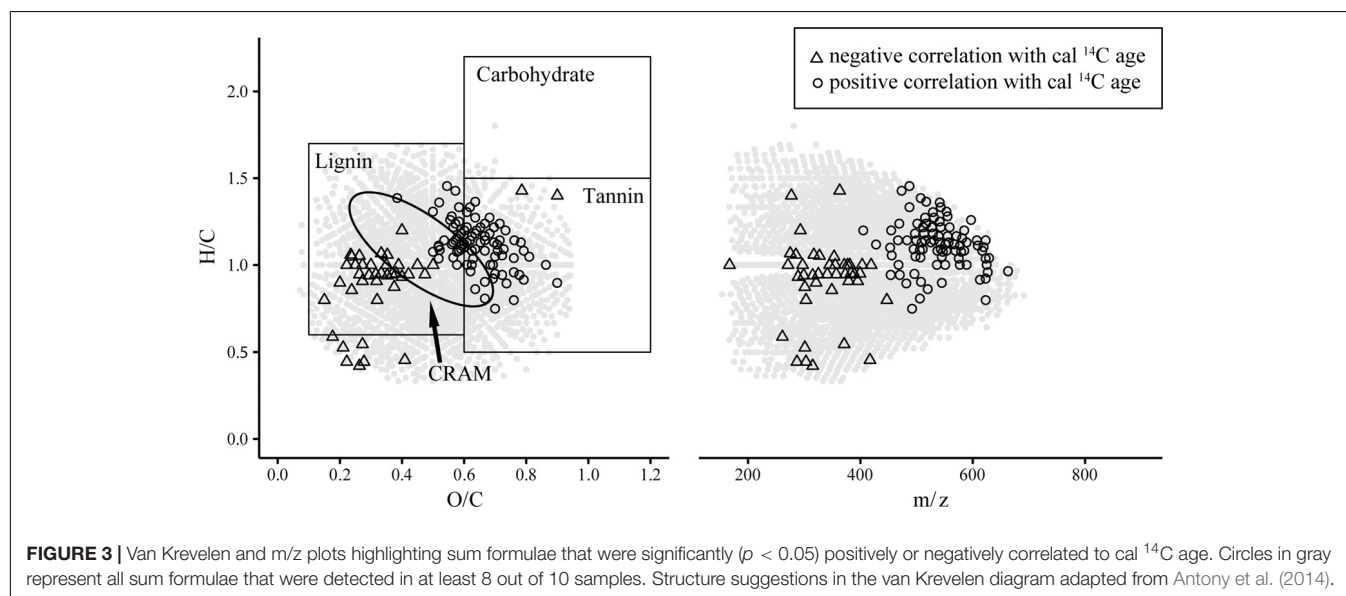
TABLE 1 | Overview of the sample sites regarding pH, DOC concentration, length of the growing season and radiocarbon ages of SPE-DOM.

| Site | Plot number designation | Sampling depth (cm) | Sampling month | pH | DOC (mg/L) | Length of the growing season (days) | Percent modern carbon (pMC) | ± std. dev. | Median cal. age (years) |
|-----------|-------------------------|---------------------|----------------|-----|------------|-------------------------------------|-----------------------------|-------------|-------------------------|
| Hainich | 2 | 5 | March | 5.3 | 12.1 | 11 | 117.427 | 0.396 | 17 |
| | 4 | 5 | March | 5.5 | 9.2 | 11 | 109.416 | 0.415 | 5 |
| | 2 | 5 | May | 5.0 | 17.4 | 55 | n.d. | | |
| | 4 | 5 | May | 5.0 | 15.7 | 55 | 108.939 | 0.460 | 4 |
| | 2 | 5 | November | 5.0 | 27.6 | 237 | n.d. | | |
| | 4 | 5 | November | 5.0 | 20.9 | 237 | n.d. | | |
| Thann | 1 | 5 | March | 4.5 | 55.5 | 8 | m.f. | | |
| | 1 | 5 | May | 4.5 | 89.7 | 70 | n.d. | | |
| | 1 | 5 | November | 4.3 | 42.7 | 235 | 113.842 | 0.512 | 13 |
| Wetzstein | 1 | 5 | March | 4.0 | 69.1 | 0 | n.d. | | |
| | 3 | 10 | March | 4.0 | 120.5 | 0 | 110.583 | 0.375 | 9 |
| | 1 | 5 | May | 4.3 | 96.0 | 41 | 113.203 | 0.412 | 12 |
| | 3 | 5 | May | 4.3 | 64.4 | 41 | 111.045 | 0.387 | 9 |
| | 3 | 10 | May | 4.0 | 151.9 | 41 | 105.572 | 0.401 | 0 |
| | 1 | 5 | November | 4.0 | 61.9 | 199 | 102.014 | 0.443 | 49 |
| | 3 | 5 | November | 4.0 | 100.1 | 199 | 106.953 | 0.403 | 1 |

n.d., not determined due to insufficient signal intensity.

m.f., measurement failed during run.

Standard deviation indicates instrument error range. Age calibration performed using OXcal with the NH1 bomb 2013 curve (Hua et al., 2013), denoted as years before 2005 (year of sampling).



converged toward carboxyl rich alicyclic molecules (CRAM) (Hertkorn et al., 2006; Lechtenfeld et al., 2014). Marker compounds related to marine DOM degradation state (Flerus et al., 2012) were entirely absent within the data set of this study.

From our assessment of the suggested structures of molecular entities (Supplementary Tables S2, S3) that were associated with ^{14}C young and old carbon samples we identified that plant-derived phenylpropanoid compounds were core structural units for both groups, although it is probable that there are other structural units that may be suppressed during ionization (Nebbioso et al., 2010).

Molecular entities associated with ^{14}C -young or old DOM were characterized by a high degree of methoxylation and glycosylation, respectively. This finding is consistent with a previous investigation of plant-derived SOM (Gleixner et al., 2002).

While demethylation is a long known processes during lignin degradation (Jin and Kirk, 1990), it has recently been reported that glycosylation of phenylpropanoids greatly affects their bioavailability (Le Roy et al., 2016). In this study we cannot disentangle if the observed glycosylated phenylpropanoids remain from the original substrate or are

TABLE 2 | Molecular information from FT-ICR-MS and correlation with cal. ^{14}C age.

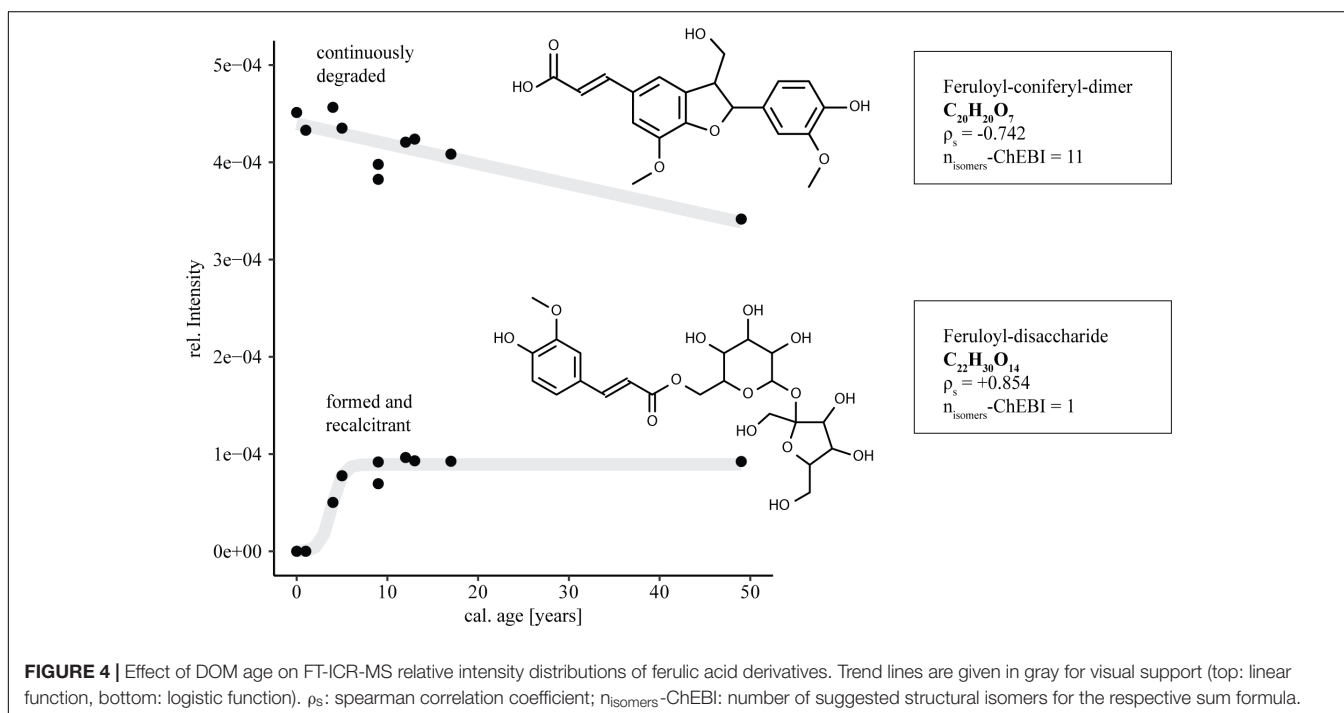
| | Neg. correlation with ^{14}C age | Pos. correlation with ^{14}C age |
|------------------------------|---|---|
| H/C | 0.95 | 1.12 |
| O/C | 0.32 | 0.63 |
| DBE | 11 | 11 |
| DBE/C | 0.58 | 0.48 |
| DBE-O | 5 | -3 |
| AI | 0.50 | 0.24 |
| mw | 334.11 | 538.13 |
| # sum formulae CHO | 42 | 72 |
| # sum formulae CHON | 0 | 14 |
| # sum formulae CHOS | 0 | 1 |
| # total suggested structures | 261 | 60 |
| % aromatic | 95 | 85 |
| % methoxyl-containing | 57 | 18 |
| % glycoside-containing | 2 | 88 |

Sum formulae are grouped based on their elemental composition: carbon, hydrogen and oxygen (CHO), nitrogen (CHON) or sulfur (CHOS). Median values are given for molecular characterization indices derived from the elemental ratios and double bond equivalents (DBE) as well as for the aromaticity index [AI; (Koch and Dittmar, 2016)] and molecular weight (mw).

synthesized in the environment. Yet, a combination of both processes is probable. Glycosylated phenylpropanoids are widely present in plants and glycosylated lignin oligomers can be stored in leaf vacuoles (Dima et al., 2015). Due to the increased solubility in water compared to their respective aglycones, the glycosides might be preferentially leached during

biomass decomposition and accumulate in DOM over time. Furthermore, glycosylation of phenylpropanoids increases their stability by weakening their nucleophilic character as well as their tendency to polymerize. Because polymerization and reactions of phenylpropanoids with biomolecules are potentially harmful if they happen in cells, active glycosylation of these compounds could be a way for organisms to tolerate elevated levels of otherwise toxic lignin-derived molecules. This detoxification effect and the solubility increase are especially crucial for lignin decomposers since degradation products resulting from the activity of extracellular enzymes must be taken up by the mycelium (Jeffries, 1994; Sanchez, 2009).

The introduction of ^{14}C young molecules derived from lignin degradation, their detoxification and stabilization can provide a framework for ^{14}C age related marker compounds. The differing evolution of individual molecular entities over time is highlighted in the relative intensity distributions of two possible ferulic acid derivatives (Figure 4). Their final identification needs of course further structural investigation. However, the preliminary identification of $\text{C}_{20}\text{H}_{20}\text{O}_7$ and $\text{C}_{22}\text{H}_{30}\text{O}_{14}$ is likely as ferulic acid is a key component of lignocellulose (de Oliveira et al., 2015). On the one hand glycosmistic acid, a lignin dimer comprised of a ferulic acid and a coniferyl alcohol substructure showed linearly decreasing relative intensities with respect to increasing age of the samples, suggesting continuous degradation. On the other hand ferulic acid in its form as a disaccharide showed strongly increasing relative intensities. From not being detected in two samples with zero and one year of cal ^{14}C -age, the ferulic acid disaccharide entity built up to stable levels within ~ 10 years. The observed build-up of persisting lignin derivatives supports recent advances in dissolved lignin analysis, suggesting



lability-based pools of lignin-derived molecules (Feng et al., 2017).

The decrease in the relative intensities of the feruloyl-coniferyl-dimer highlights the potential usability of lignin dimer molecules as fast flow markers for ^{14}C young, surface-derived DOM inputs in the CZ. High abundances of this new marker in ground water could indicate fast transport of water and DOM through the subsurface. As aromatic organic matter is known to interact with mineral surfaces, the potential applicability of the proposed molecular markers in groundwater will depend on the mineral structure of the respective subsurface environment (Klotzbücher et al., 2016). Recent investigations show that a considerable proportion of lignin-derived phenols might be irreversibly sorbed to minerals (Kaiser and Guggenberger, 2000; Hernes et al., 2013), thereby limiting the potential traceability of lignin-derived markers into groundwater. Low pH conditions, as are present in sandy forest soils, can further favor strong OM sorption to mineral surfaces (Kleber et al., 2015). Therefore further studies focusing on subsurface DOM are required in order to decide which of the proposed potential marker compounds are applicable in the respective pedologic settings.

CONCLUSION

^{14}C age is a strong driver of DOM composition in the CZ. ^{14}C -young DOM molecules linking to inputs from decaying plant biomass could be identified by correlating individual sum formulae to ^{14}C ages of SPE-DOM. Since this approach was applied only on water from forest top soils, it is important to expand this method to other CZ compartments. Groundwater DOM studies are needed to verify the applicability of lignin derived molecules as potential marker compounds for fast DOM transport.

We propose lignin derived molecules as potential markers for ^{14}C young DOM in the CZ as a result of their prominence in chemical database suggestions for highly ^{14}C age correlated sum formulae and their direct link to the forest environment. Still, they represent only a fraction of all ^{14}C correlated DOM compounds in our study. In general, chemical transformation of phenylpropanoids so far seems to be an underestimated factor when considering aging and transport of terrestrial DOM. Their bioavailability and detoxification in the environment emerged as a potential key driver in early DOM evolution. However, further research using multidimensional mass spectrometric experiments is needed to verify the

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molecular identifications based on our database searches (e.g., Ball and Aluwihare, 2014). Further recent methodological advances such as liquid chromatography coupling (Hawkes et al., 2018) and ion fragmentation (Osterholz et al., 2015; Zark and Dittmar, 2018) have been successfully applied in DOM research and can be applied in context to this study. Possible follow-up studies using targeted approaches could be designed similarly to recent investigations of root exudates with UPLC/ESI-QTOFMS, which also detected $\text{C}_{20}\text{H}_{20}\text{O}_7$, the sum formula of the feruloyl-coniferyl-dimer highlighted in **Figure 4** (Strehmel et al., 2014).

Overall, our findings support recent advances in biomarker research, proposing degradability of lignin derived molecules and using resulting leachates as marker compounds. Applying our methodology in groundwater and tracing the already identified potential markers will further improve our knowledge on transport and evolution of DOM in the subsurface.

AUTHOR CONTRIBUTIONS

V-NR and GG conceived the study idea. SB managed the samples and analyses and wrote the manuscript. SB and YL analyzed the data. SB, YL, V-NR, and GG interpreted the data and improved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/feart.2018.00168/full#supplementary-material>

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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