14C and 13C characteristics of higher plant biomarkers in Washington margin surface sediments

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Received 8 June 2012; accepted in revised form 20 November 2012; available online 5 December 2012

Abstract

Plant wax lipids and lignin phenols are the two most common classes of molecular markers that are used to trace vascular plant-derived OM in the marine environment. However, their 13C and 14C compositions have not been directly compared, which can be used to constrain the flux and attenuation of terrestrial carbon in marine environment. In this study, we describe a revised method of isolating individual lignin phenols from complex sedimentary matrices for 14C analysis using high pressure liquid chromatography (HPLC) and compare this approach to a method utilizing preparative capillary gas chromatography (PCGC). We then examine in detail the 13C and 14C compositions of plant wax lipids and lignin phenols in sediments from the inner and mid shelf of the Washington margin that are influenced by discharge of the Columbia River. Plant wax lipids (including n-alkanes, n-alkanoic (fatty) acids, n-alkanols, and n-aldehydes) displayed significant variability in both δ13C (-28.3‰ to -37.5‰) and Δ14C values (-204‰ to +2‰), suggesting varied inputs and/or continental storage and transport histories. In contrast, lignin phenols exhibited similar δ13C values (between -30‰ and -34‰) and a relatively narrow range of Δ14C values (-45‰ to -150‰; HPLC-based measurement) that were similar to, or younger than, bulk OM (-195‰ to -137‰). Moreover, lignin phenol 14C age correlated with the degradation characteristics of this terrestrial biopolymer in that vanillyl phenols were on average ~500 years older than syringyl and cinnamyl phenols that degrade faster in soils and sediments. The isotopic characteristics, abundance, and distribution of lignin phenols in sediments suggest that they serve as promising tracers of recently biosynthesized terrestrial OM during supply to, and dispersal within the marine environment. Lignin phenol 14C measurements may also provide useful constraints on the vascular plant end member in isotopic mixing models for carbon source apportionment, and for interpretation of sedimentary records of past vegetation dynamics.

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1. INTRODUCTION

The synthesis, degradation, and storage of terrestrial organic matter (OM) form an important component of the global carbon cycle. Estimates of the flux of terrestrial organic carbon (OC) to the oceans imply that it must influence marine carbon budgets, especially on continental margins (Hedges et al., 1997; Masiello, 2007). The fate of terrestrial OM in the ocean is therefore one of the central questions that have continued to interest and challenge biogeochemists, and remains a fundamental constraint on (i) understanding the global carbon cycle (Hedges et al., 1997; Schlunz and Schneider, 2000; Burdige, 2005), and
(ii) interpreting the geologic sedimentary record with respect to reconstruction of biological evolution, sedimentary paleoenvironments and past climatic variations (McCaffrey et al., 1991; Rommerskirchen et al., 2006a; Ohkouchi and Eglinton, 2008). A key challenge for studying terrestrial OM in the marine environment is to trace it among the complex, heterogeneous assemblage of carbon-bearing constituents transported to, and produced in the sea. Prior attempts have utilized organic molecules specific to terrestrial higher plants (e.g., lignin-derived phenols and plant wax lipids). However, during their transport from plant source to sedimentary sink, these molecules are subject to biological and physiochemical processes that can substantially attenuate their flux and alter their chemical composition (Hermes and Benner, 2003). Despite this, isotopic information encoded in the carbon skeletons of these molecules is largely preserved, providing valuable insights into growth conditions, biological sources (C₃ versus C₄ plants) and reactivity of terrestrial OM accumulating in sediments (e.g., Gotí et al., 1997; Pearson et al., 2001; Smittenberg et al., 2006; Drenzek et al., 2007). For example, recent investigations on the 14C composition of organic compounds in marine sediments have revealed the importance of an additional continental OC source derived from the erosion of ancient sedimentary rocks or petrogenic sources (termed “relict OC”) in this paper) exposed at the Earth’s surface (Eglinton et al., 1997; Pearson et al., 2001; Drenzek et al., 2007). The contribution from this component may significantly influence sedimentary OC budgets (Drenzek et al., 2007), but minimally impacts the exchange of carbon between active reservoirs (Galy et al., 2008). Carbon isotopic (13C and 14C) characteristics of higher plant-derived organic molecules can thus provide important information on the sources of OC produced exclusively by the terrestrial biosphere, leading to improved estimates of continental OC fluxes in the ocean and to a better understanding of the ultimate fate of terrigenous OC in the marine environment.

Plant wax lipids and lignin phenols are the most commonly employed classes of molecular tracer for terrestrial OM in the marine environment (e.g., Prahl et al., 1994; review by Hedges et al., 1997; Gotí et al., 2000; Drenzek et al., 2007; Ohkouchi and Eglinton, 2008). While their origin is unequivocal, their transport pathways, storage times and modifications during land–ocean transfer are much less clear. Lignin is generally more abundant in the coarse particles that are rich in undegraded OM debris whereas plant wax lipids tend to be more enriched in mineral-bound OM (Wakeham et al., 2009). Hydrodynamic sorting processes are known to influence the dispersal and fate of mineral-associated OM versus plant debris during transport (Keil et al., 1994; Prahl et al., 1994; Gordon and Gotí, 2003; Huguet et al., 2008; Mead and Gotí, 2008; Vonk et al., 2006; Drenzek et al., 2007, 2009; Mollenhauer and Eglinton, 2007; Kusch et al., 2010; Gustafsson et al., 2011); while carbon isotopic (especially 14C) data on lignin phenols in marine sediments remains sparse (Gotí et al., 1997; Culp, 2012). Different groups of lignin phenols are reported to exhibit varying vulnerabilities to degradation in the environment; for instance, angiosperm-derived syringyl phenols and non-woody-tissue-derived cinnamyl phenols both show faster decay rates relative to vanillyl phenols (Hedges et al., 1988; Opsahl and Benner, 1995; Otto and Simpson, 2005). It is presently unknown whether individual lignin phenols exhibit any isotopic discrepancies that may reflect variations in their source or reactivity. It also remains unclear whether lignin and plant wax lipids exhibit similar 13C and 14C characteristics in drainage basins (i.e., with respect to provenance and dynamics) and if factors such as differing particle associations and turnover times may cause any isotopic discrepancies between them. Furthermore, in contrast to plant wax lipids, which are relatively trace constituents of terrestrial OM, lignin is one of the most abundant terrestrial biopolymers (Hedges et al., 1997; Kögel-Knabner, 2002), making it quantitatively more significant for use in isotopic mass balance-based source apportionment. Comparing the carbon isotopic characteristics of these two groups of terrestrial tracers may yield unique insights on the transfer and cycling of terrestrial OC in the ocean and provide further information on their utility in reconstructing paleoenvironmental conditions.

Compared to plant wax lipids, lignin phenols have remained a challenge to isolate and measure for 14C content. While successfully isolated by preparative capillary gas chromatography (PCGC), their separation requires derivatization with quite harsh and toxic reagents, and the efficiency of derivatization appears to suffer from competition with other reactants (McNichol et al., 2000). Adding derivative carbons to the relatively small monomeric lignin products from oxidative hydrolysis (8–10 carbons) also increases analytical error associated with isotopic analysis (Beramendi-Orosco et al., 2006; Corr et al., 2007). Direct separation of lignin phenols on high pressure liquid chromatography (HPLC) can circumvent this problem, which has been applied to plant tissues and lake sediments recently (Hou et al., 2010; Ingalls et al., 2010). Compared to terrestrial samples (plants, soils, lake and fluvial sediments), marine sediments represent challenging environmental matrices with myriad OC inputs and dilution of lignin residues with marine OM. In this paper, we evaluate an alternative HPLC-based method of isolating lignin phenols from marine sedimentary matrix for 14C analysis and compare the results with the PCGC-based isolation. We then use this method to compare and contrast the carbon isotopic composition of lignin phenols with those of plant wax lipids from two surface sediments collected from the Washington margin. The sediments in this region, which receive high inputs of terrestrial OM from the Columbia River, have been extensively characterized in terms of sedimentology and geochemistry (Hedges and Mann, 1979a; Nittrouer and Sternberg, 1981; Prahl et al., 1994; Hartnett et al., 1998), and provide a “classic location” for assessing vascular plant marker signatures on fluvially-influenced continental margins. To our knowledge, this study represents the first detailed investigation of both the
$^{13}$C and $^{14}$C compositions of the two major classes of these vascular plant molecular markers in marine sediments.

2. MATERIALS AND METHODS

2.1. Samples and bulk analysis

The mineralogy and geochemistry of the Washington margin have been well studied (White, 1970; Nittouer and Sternberg, 1981; Prahl et al., 1994; Hedges et al., 1999). Coastal surface sediments are dominated by fluvial inputs with steady supply and deposition of plant debris and coarse-grained sediment near the Columbia River mouth and over the mid-shelf over at least the last 400 years (Hedges and Mann, 1979a; Prahl et al., 1994). The sediment accumulation rate is approximately 400 cm/kyr close to the river mouth and ~300 cm/kyr in the mid-shelf (Coppola et al., 2007), with sediment mixed layer depths ranging from 20 to 30 cm over the shelf (Nittouer and Sternberg, 1981; Coppola et al., 2007). Coarse sand and silts are preferentially accumulated over the shelf while grain size progressively decreases with increasing distance from the Columbia River (Nittouer and Sternberg, 1981; Coppola et al., 2007). Vegetation in the drainage basin is dominated by C$_3$ plants and sediments over the Washington margin shelf contain a high abundance of terrestrial vascular plant OC with $^{13}$C-depleted stable carbon isotopic compositions ($-25.5^\circ$$_{oo}$), high C/N ratios and abundant higher plant biomarkers (Hedges and Mann, 1979a; Prahl et al., 1994; Hedges et al., 1999; Dickens et al., 2006).

Two large volume (ca. 350 g dry wt.) surface (<4 cm) sediment samples were collected using a grab sampler in 1993 during cruise W9308A (R/V Wenom) on the Washington margin. Station 1 (St 1, 46°15.12’N, 124°15.23’W) was at the inner shelf in close proximity to the mouth of Columbia River with a water depth of 74 m. Sediments at St 1 had a typical coarse sandy texture. Station 2 (St 2, 46°25.00’N, 124°20.03’W) was located at the mid shelf (water depth, 83 m) where the sediments were primarily composed of coarse silts. After collection, the samples were stored frozen in glass jars and subsequently freeze-dried.

An aliquot of bulk sediment was retained for elemental and isotopic analysis. The OC content of bulk sediments was determined on a Carlo Erba 1108 Elemental Analyzer (CE Elantech, Inc., NJ, USA) after removal of inorganic carbon with 2 N HCl solution. Stable carbon isotopic composition was determined by isotope ratio monitoring gas chromatography–mass spectrometry (Finnigan Delta-S mass spectrometer, see Fry et al., 1992 for details).

To validate an HPLC method to isolate lignin phenols for $^{14}$C analysis, we used three commercially available phenol standards (vanillin from Sigma, vanillic acid and aceto-vanillone from Acros) and standard plant tissues with a range of $^{14}$C contents that are pre-determined from the Fourth International Radiocarbon Intercomparison (FIRI) project (Scott et al., 2004) and the International Atomic Energy Agency (IAEA; Rozanski et al., 1992). Standard plant tissues included kauri wood (FIRI-I; the consensus fraction modern ($F_{\text{no}}$) value is 0.0033), subfossil wood from eastern Wisconsin (IAEA C-5; $F_{\text{no}}$ 0.2305), Belfast dendro-dated wood (FIRI-D; $F_{\text{no}}$ 0.5705), hohenheim wood (FIRI-H; $F_{\text{no}}$ 0.7574), and barley mash (FIRI-J; $F_{\text{no}}$ 1.1069). The wide range of $^{14}$C contents in these standard materials allowed us to assess the effect of procedural blanks on the measured $^{14}$C contents of isolated lignin phenols (see Section 2.8). Phenol standards were dissolved in methanol and plant tissues were ground to fine powders prior to analysis. The radiocarbon content of acid-treated bulk sediment and phenol standards was measured as described in Section 2.8.

For the subsequent chemical extractions and analyses, all glassware, SiO$_2$ and CuO powders (for lignin extraction) were pre-combusted at 450 °C for 5 h before use. Teflon bombs and vessels used for lignin extraction were soaked, washed, soaked in HCl (10%), and rinsed with Milli-Q water and dichloromethane (DCM):methanol (1:1) before use.

2.2. Extraction and purification of plant wax lipids

Dried sediments (~300 g) were Soxhlet-extracted with DCM:methanol (93:7, 2 h) to obtain a corresponding total lipid extract (TLE). The TLEs were spiked with a mixture of recovery standards (including C$_{25}$ n-alkane, C$_{19}$ n-alkanol, and C$_{19}$ n-alkanoic (fatty) acid) and transesterified with methanol (5% HCl, 70 °C for 12 h) of known isotopic composition to hydrolyze bound fatty acids and to form corresponding methyl esters. Lipid class sub-fractions (including hydrocarbon, fatty acid methyl esters (FAMEs), aldehyde/ketone, and alkanol) were obtained using SiO$_2$ gel flash chromatography, eluting with different polarity solvents (modified after Farrington et al., 1988). The hydrocarbon fraction was eluted with hexane and then further purified by AgNO$_3$ thin layer chromatography (TLC) and urea adduction (Marquart et al., 1968) to yield a fraction dominated by plant wax n-alkanes. FAMEs were eluted with ethyl acetate/hexane (10:90). Aldehyde/ketone and alkanol fractions were eluted with ethyl acetate/hexane (5:95 and 20:80, respectively) and further purified by urea adduction. n-Alkanols were converted to corresponding acetates after reaction with acetic anhydride in pyridine (65 °C, 15 min). Small aliquots (ca. 5%) of each fraction were reserved for gas chromatography–mass spectrometry (GC–MS) and gas chromatography–farm ionization detector (GC-FID) analysis (Section 2.4) and stable carbon isotopic analysis by isotope ratio monitoring gas chromatography–mass spectrometry (irn-GC–MS; Section 2.5). Individual lipids were isolated by PCGC for $^{14}$C analysis (Section 2.6).

2.3. Isolation of lignin phenols

Lignin phenols were released from the solvent-extracted sediments using CuO oxidation and isolated by both PCGC- and HPLC-based methods. For PCGC isolation, we used 10-mL Teflon-lined bombs for CuO oxidation. In order to process a large volume of sample simultaneously, we first treated solvent-extracted sediments (~150 g) with HCl (10% w/v, ~200 ml) and HF (40% w/v, ~25 ml) sequentially to reduce mineral content and sample volume.
The resulting residues (<5 g) were then solvent extracted (Section 2.2) again to remove any residual soluble material and subsequently subjected to alkaline CuO oxidation (2 g CuO, 150 °C, 1.5 h) to release lignin phenols (Hedges and Ertel, 1982; Goní et al., 1993). The lignin oxidation product (LOP) was spiked with a recovery standard (ethyl vanillin) and extracted with ethyl acetate after acidification to pH 2. To assess the concentration and 13C isotopic composition of LOP, an aliquot was derivatized with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and pyridine (70 °C, 1 h) and analyzed by GC-FID and irm-GC–MS as trimethylsilyl (TMS) derivatives, respectively. Based on the similar yield and composition of lignin phenols as compared to previous results in the same sedimentary region (Section 3.3), we do not think that HCl/HF treatment caused significant removal of lignin during the pretreatment. Due to the instability of TMS derivatives, isolation of individual lignin phenols by PCGC for 14C measurement required formation of more stable derivatives. We converted alkanol and acidic groups to methyl ethers and esters, respectively, using dimethyl sulfate (McNichol et al., 2000). Briefly, dried LOP was mixed with dimethyl sulfate in excess, 10–20 mg K2CO3, and 2 mL of dry acetone and stirred at 70 °C overnight. Unreacted dimethyl sulfate was then destroyed with a few drops (<1 mL) of 30% ammonium hydroxide solution by stirring for 1 h. The methylated phenols were extracted with diethyl ether, dried over sodium sulfate, and isolated by PCGC (see Section 2.6).

For the HPLC isolation of lignin phenols (Fig. 1), a second portion of the solvent-extracted sediments (~100 g) was first hydrolyzed with 1 M KOH in methanol (100 °C, 3 h) to remove hydrolysable lipids (Otto and Simpson, 2006, 2007). This step also removed some phenol moieties (including vanillin, vanillic acid, p-coumaric acid, and ferulic acid) that are present in the suberin macromolecule (Otto and Simpson, 2006). These phenols amounted to <4% of lignin phenols released by CuO oxidation (data not shown) and were not considered to represent “true” lignin (cf. Otto and Simpson, 2006, 2007). The residues were then subjected to CuO oxidation on a microwave system (MARS, CEM Corporation) following a modification of the method described by Goní and Montgomery (2000), which allowed for a larger quantity of sediments to be processed. Approximately, 20 g of sediment, 4 g of CuO, 0.6 g of ferrous ammonium sulfate, and 20 mL of N2-bubbled NaOH solution (2 M) were loaded into each of five vessels for one sample. Vessels containing all reagents but no sample were also included as “procedural blanks” along with each batch of sediment or standard plant tissue samples. All vessels were vacuum-purged with N2 four times and oxidized at 150 °C for 1.5 h. LOP was extracted with ethyl acetate after acidification to pH 2 and blown carefully to <100 μL under N2 for subsequent procedures (Section 2.7).

### 2.4. GC–MS and GC-FID analysis

Small aliquots of lipid sub-fractions (including n-alkanes, FAMEs, n-aldehydes, and n-alkanol acetates) and the TMS derivatives of lignin phenols were identified on an HP 5890 series II GC interfaced with a VG Autospec-Q mass spectrometer (MS). Lipids were separated on a CP-Sil-5-CB column (30 m × 0.25 mm i.d., film thickness, 0.25 μm) and phenols were separated on a J&W DB-1 column (60 m × 0.32 mm; film thickness, 0.25 μm) using He carrier gas (1 mL min⁻¹) and a temperature program from 50 °C (initial hold time, 0 min) to 320 °C at a rate of 6 °C min⁻¹. Spectra were obtained by scanning over the range 50–600 amu, with a cycle time of 1 s. Electron impact ionization (EI) at 70 eV was used for all analyses. Quantification was achieved on a GC-FID using the same columns and GC program by comparison with internal standards.

### 2.5. Stable carbon isotopic analysis by irm-GC–MS

Stable carbon isotopic measurements of lipid fractions and lignin phenols TMS derivatives were performed on an HP 6890 GC coupled with a Finnigan MAT DeltaPlus isotope ratio MS system. Instrumental conditions were described previously (Goni and Eglinton, 1994, 1996; Feakins et al., 2005). The mass-spectrometer was calibrated using deuterated n-alkane internal isotopic standards (co-injected with the sample) as well as external CO2 gas standards for each run. The δ13C values of fatty acids, n-alkanols, and lignin phenols were corrected for the derivative carbon based on isotopic mass balance and the associated errors were propagated. Uncertainty of δ13C values was typically ~0.4‰ for plant wax lipids and 0.1–1.2‰ for lignin phenols due to the large number of derivative carbons added.
2.6. Isolation of plant wax lipids and lignin phenols by PCGC

Individual plant wax lipids and methylated lignin phenols were isolated by PCGC for $^{14}$C analysis as described previously (Eglinton et al., 1996; McNichol et al., 2000). Briefly, plant wax lipids and methylated lignin phenols were separated on a 30-m “megabore” Rtx-1 (Restek; 0.53 mm i.d.; film thickness, 0.5 μm) and on a 60-m DB-5 fused silica column (0.53 mm i.d.; film thickness, 0.5 μm), respectively. Typically, >100 injections were required to isolate sufficient amounts (15–350 μg) of individual compounds. A small aliquot was used to check compound identity and purity by GC–MS.

2.7. Purification and isolation of lignin phenols by HPLC

Before HPLC isolation, lignin phenols were purified through two solid phase extraction (SPE) steps (Fig. 1). In details, the LOP (dissolved in <100 μL of ethyl acetate) was diluted to 10 mL with deionized water (pH 2), and loaded onto a Supelclean ENVY-18 SPE cartridge (Supelco, pre-conditioned with methanol and water). Lignin phenols were eluted with acetonitrile while neutral compounds and other impurities were retained on the cartridge (Lima et al., 2007). The purified LOP was blown under N2 to a volume of <0.5 mL and further separated on a self-packed amino SPE cartridge (0.5 g, Supelclean LC-NH2, Supelco, preconditioned with methanol) into phenolic aldehydes/ketones (eluting with methanol) and their corresponding acids (eluting with methanol/acetonitrile). Lignin phenols were re-dissolved in methanol and further purified individually on a Phenomenex Synergi Polar-RP column (0.53 mm i.d.; film thickness, 0.5 μm) and on a 60-m DB-5 fused silica column (0.53 mm i.d.; film thickness, 0.5 μm), respectively. Typically, >100 injections were required to isolate sufficient amounts (15–350 μg) of individual compounds. A small aliquot was used to check compound identity and purity by GC–MS.

An HPLC method was developed to isolate individual lignin phenols utilizing two LC columns with different selectivity in order to afford phenol separation at a much higher amount (up to 30 μg and average of 16 μg compound per injection) than PCGC without derivatization. Purified LOP fractions were separated on an Agilent 1200 HPLC system consisting of a degasser, a binary pump, an injection autosampler, coupled to a diode array detector (DAD), and a fraction collector, or a 6310 quadrupole MS system. The fraction containing phenolic aldehydes/ketones was first separated on a Phenomenex Synergi Polar-RP column (4.6 × 250 mm; 4 μm particle size) along with a Polar-RP SecurityGuard column (4.0 × 3.0 mm; 4 μm particle size). Phenols were eluted from the column using a binary gradient program (Table 1) of water/acetic acid (99.8:0.2; Solvent A) and methanol/acetonitrile (50:50; Solvent B). The column was maintained at 28 °C, and the initial conditions were 10% Solvent B at a flow rate of 0.8 mL/min for the first 3 min. The gradient program ramped to 15% Solvent B by 8 min, 20% by 15 min, held at 20% till 22 min, ramped to 25% by 27 min, held at 25% till 36 min, finally ramped to 100% by 37 min, and was held for 5 min at 100% to wash the column. Subsequently, the column was re-equilibrated in 10% Solvent B for 5 min between injections. Phenols were detected by DAD (280 nm) and MS (atmospheric pressure chemical ionization-negative ion mode, conditions described as in Hoffmann et al., 2007). Individual phenols were collected in 20-mL glass vials using time-based fraction collection from the beginning to the end of the time interval of each phenol UV peak. Phenols were recovered from the mobile phase through extraction with ethyl acetate at pH 2 and gently blown to <50 μL under N2. In order to remove impurities or phenols co-eluting on the Polar-RP column, all the isolated phenolic aldehydes/ketones were re-dissolved in methanol and further purified individually on a ZORBAX Eclipse XDB-C18 column (4.6 × 150 mm; 5 μm particle size) with a ZORBAX Eclipse C18 guard column (4.6 × 12.5 mm; 5 μm particle size) after Lobbes et al., 1999; Fig. 2a) using the same mobile phases and a slightly different gradient program (Table 1). In most cases, a total of eight injections (10 μL each) were conducted for each sample to collect approximately 40–300 μg of each phenol (i.e., ~20–150 μg C) for $^{14}$C measurement. Similarly, the fraction containing phenolic acids was separated on a ZORBAX Eclipse XDB-C18 column followed by further isolation on a Phenomenex Polar-RP column using similar binary gradient programs (Table 1; Fig. 2b). After isolation, lignin phenols were purified using a 5% deactivated SiO$_2$ column with ethyl acetate as the eluting solvent to remove potential column bleed. Recovery of phenols from the SiO$_2$ column was typically >90% and the overall recovery of phenols from the SPE and HPLC procedures was estimated around 60–80% by comparing phenol quantities before and after purification and isolation steps on the GC–FID. As also reported by Ingalls et al. (2010), the biggest loss of sample occurred during solvent removal processes due to the volatile nature of phenols. Although any isotopic fractionation that might occur during evaporation was corrected for with the $^{13}$C/$^{12}$C ratio during AMS measurement.

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$^a$ Phase of column washing.

$^b$ Phase of column equilibrium.
significant sample loss via solvent dry down should be avoided. Heating was therefore not used during N₂ blow-down when the solvent level was low. A small aliquot of purified phenols was removed and derivatized to check compound identity and purity by GC–MS as described previously (Supplementary Fig. S.1), and found to yield purities >99%. Procedural blanks from CuO oxidation and SPE purification were injected eight times on HPLC, collected at time intervals corresponding to the retention time of lignin phenols, and purified in the same way. A small aliquot of the resulting procedure blank was derivatized with BSTFA and pyridine and analyzed on GC–MS for its composition. No distinct peaks were observed in the GC–MS trace. The rest of the procedure blanks were combusted to CO₂ and quantified in a calibrated volume on the vacuum line (Section 2.8).

2.8. Radiocarbon measurement by accelerator mass spectrometry (AMS)

Quartz tubes and CuO catalysts were pre-combusted at 850 °C for 5 h one day before use. Decarbonated sediments, phenol standards, individual plant wax lipids and lignin phenols isolated from sediments and plant tissues, and HPLC-processed procedural blanks were transferred to pre-combusted quartz tubes using DCM:methanol (1:1) where necessary. After any solvents used in sample transfer were carefully removed under a gentle stream of N₂ gas, quartz tubes were sonicated in water for 1 min and gently blown again under N₂ gas without heat for 1 min to ensure complete dryness. The samples were subsequently combusted in evacuated pre-combusted quartz tubes in the presence of CuO at 850 °C for 5 h. Resulting CO₂ was

![HPLC chromatogram of lignin phenols isolated from the Washington margin surface sediment, St 1: (a) separation of phenolic aldehyde/ketones on Polar-RP column followed by XDB-C18 column; (b) separation of phenolic acids on XDB-C18 column followed by Polar-RP column. Shaded areas represent phenol peaks collected. Short names: pBl = 4-hydroxybenzaldehyde; pBn = 4-hydroxyacetophenone; Vl = vanillin; Sl = syringaldehyde; Vn = acetovanillone; Sn = acetosyringone; Vd = vanillic acid; Sd = syringic acid; pCd = p-coumaric acid; Fd = ferulic acid.](image-url)
dried, quantified on the vacuum line, and subsequently converted to graphite using standard methods (Pearson et al., 1998) for radiocarbon analysis with accelerator mass spectrometry (AMS) at the National Ocean Sciences Accelerator Mass Spectrometer (NOSAMS) facility at the Woods Hole Oceanographic Institution. Radiocarbon contents are reported as fraction modern carbon (Fm), $\Delta^{14}$C ($^{136}$C/O), and conventional $^{14}$C age (Stuiver and Polach, 1977). Errors associated with AMS measurement depend on the sample size, $^{14}$C content and instrument performance at the time of measurement, etc. The long-term average error associated with AMS measurement is typically about ±15 parts per thousand. The radiocarbon contents were corrected for the derivative carbon (where necessary) and procedural blanks using a mass balance approach. The associated errors were propagated in the results.

Procedural blanks as referred to in this paper include any background carbon originating from reaction vessels, SPE bonding materials, GC or LC column bleed, HPLC reagents (Milli-Q water), and/or background CO$_2$ on vacuum line. We made every attempt to reduce the procedural blank by pre-combusting glassware, quartz tubes, SiO$_2$ and CuO before use, pre-rinsing SPE cartridges, and purifying isolates with SiO$_2$ columns after PCGC or HPLC isolation. Based on our experience (Galy and Eglington, 2011), procedural blanks associated with the PCGC procedures (including extraction and combustion) carry 1.8 ± 0.9 μg of C with an $F_m$ of 0.44 ± 0.10. Procedural blanks associated with HPLC procedures were assessed separately in Section 3.2.3.1 using phenols purified from authentic standards and plant reference materials.

2.9. Isotopic mass balance model and statistics

We employed an isotopic mass balance model to assess the relative contribution of terrestrial (including soil and vascular plants), marine, and relic OC to bulk sediments following a procedure described previously (Pearson and Eglington, 2000; Drenzek et al., 2007). Briefly, the model is expressed in the following three equations:

$$f_T(\Delta^{14}C_T) + f_M(\Delta^{14}C_M) + f_R(\Delta^{14}C_R) = \Delta^{14}C_S$$

(1)

$$f_T(\delta^{13}C_T) + f_M(\delta^{13}C_M) + f_R(\delta^{13}C_R) = \delta^{13}C_S$$

(2)

$$f_T + f_M + f_R = 1$$

(3)

where $f$ is the fractional abundance and the subscripts T, M, R, and S are terrestrial, marine, relic OC, and bulk sediment sample, respectively. Among them, $\delta^{13}C_T$ and $\delta^{13}C_M$ have a value of −25.5‰ and −21.5‰, respectively, as determined by Hedges and Mann (1979a). The $\delta^{13}C_R$ and $\Delta^{14}C$ values of end members were constrained by the isotopic characteristics of analyzed biomarkers (Section 3.5). Comparison of isotopic values was tested using ANOVA or $t$ test and the difference was considered to be significant at the level of $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Bulk geochemical properties of the Washington margin sediments

Table 2 provides information on the bulk geochemical properties of the two Washington margin surface sediment samples studied. Similar to previous observations (Hedges and Mann, 1979a; Prahl and Carpenter, 1984; Prahl, 1985), the inner shelf sediment (St 1) had a lower OC content (0.40%) than the mid-shelf sample (St 2; 0.93%) due to the coarser-grained texture of the former. This trend is typical of Washington margin sediments, where coarse materials emanating from the Columbia River accumulate in the inner shelf whereas silts and finer particles with a higher OC content are preferentially transported farther from the source to the mid shelf and upper slope (Hedges et al., 1999; Coppola et al., 2007). Bulk OC had an identical $\delta^{13}C$ value of −25.3‰ at both stations, consistent with the C$_3$ terrestrial plant carbon signal (−25.5‰) supplied by the Columbia River (Hedges and Mann, 1979a; Prahl et al., 1994). Bulk OC in the surface sediment (0–4 cm) had a $\Delta^{14}C$ value of −195‰ for St 1 and 2, corresponding to a radiocarbon age of 1700 and 1140 years, respectively. These values are much more depleted than the $\Delta^{14}C$ values of surface dissolved inorganic carbon in the North Pacific Ocean in the 70s–90s (−0‰; Key et al., 2002) and the ages are significantly older than the deposition time of the sediments (approximately over 50–100 years of sampling time) based on the mixed layer depth (20–30 cm) and sedimentation rate of 400–300 cm/kyr across the region (Coppola et al., 2007), reflecting significant pre-aging of the bulk OC before its deposition into the sediments.

3.2. Molecular and isotopic characteristics of lignin phenols

3.2.1. Molecular composition

Eight “characteristic” lignin-derived phenolic monomers (A$_8$; Hedges and Mann, 1979a) were detected in high concentrations in the Washington margin sediments (Table 3), reflecting both the high abundance of lignin as a component of terrestrial plant biomass and the preferential accumulation of woody plant fragments (which have a high lignin content) from the mouth of Columbia River to mid shelf (Hedges and Mann, 1979a). Vanillyl phenols were the most abundant phenols and ratios of syringyl-to-vanillyl (S/V) and cinnamyl-to-vanillyl (C/V) phenols ranged at 0.19–0.30 and 0.04–0.05, respectively, comparable to the lignin phenol composition found at the nearby sites (Hedges and Mann, 1979a) and implying mixed inputs of angiosperm (minor) and gymnosperm (major) tissues (Hedges and Mann, 1979b; Prahl, 1985; Keil et al., 1998; Goni et al., 2000). Despite a general similarity in lignin composition, the acid-to-aldehyde ratio for syringyl phenols (Ad/...
than the bulk OC. This offset is slightly higher than the typ-
exception of acetosyringone; Fig. 3 a), 5–9
Bahri et al., 2006 ) which fractionated against plant bulk

<table>
<thead>
<tr>
<th></th>
<th>n-Alkanes</th>
<th>n-Fatty acids</th>
<th>n-Alkanols</th>
<th>n-Aldehydes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACL&lt;sub&gt;P&lt;/sub&gt;</td>
<td>CPI&lt;sub&gt;P&lt;/sub&gt;</td>
<td>ACL</td>
<td>ACL</td>
</tr>
<tr>
<td>St 1</td>
<td>60.7</td>
<td>0.19</td>
<td>0.04</td>
<td>0.24</td>
</tr>
<tr>
<td>St 2</td>
<td>51.3</td>
<td>0.30</td>
<td>0.05</td>
<td>0.27</td>
</tr>
</tbody>
</table>

n.a. = Not analyzed.

a Summed concentration of eight major lignin phenols (mg/g OC; Hedges and Ertel, 1982).
b Ratio of syringyl-to-vanillyl phenols.
c Ratio of cinnamyl-to-vanillyl phenols.
d Acid-to-aldehyde ratio of vanillyl phenols.
e Acid-to-aldehyde ratio of syringyl phenols.
f Summed concentration of n-alkanes C<sub>25,27,29,31,33,35</sub> (mg/g OC).
g Average chain length (ACL): concentration-weighted mean carbon chain length for plant wax lipids C<sub>21–31</sub> or C<sub>22–32</sub>.
h Carbon preference index (CPI) for n-alkanes C<sub>21–31</sub>.
i Summed concentration of n-fatty acids C<sub>24,26,28,30,32</sub> (mg/g OC).
j Summed concentration of n-alkanols C<sub>24,26,28,30,32</sub> (mg/g OC).
k Summed concentration of n-aldehydes C<sub>24,26,28,30,32</sub> (mg/g OC).

Al<sub>P</sub>, a lignin degradation indicator (Hedges et al., 1988; Opsahl and Benner, 1995), was higher at St 2 than St 1 (Table 3). This observation coincides with an enrichment of relatively undegraded woody debris (with a lower (Al<sub>P</sub>) ratio) in the coarse fractions that are deposited closer to the river mouth (i.e., St 1; Keil et al., 1994, 1998).

In addition to the eight monomers, three dimeric lignin phenols that are most abundant in gymnosperm wood (5-vanillovanillin, 5-vanilloacetovanillone, and dehydrovanillinvinic acid; Goni and Hedges, 1992) were detected in both sediments, albeit at much lower concentrations (<1.0 mg/g OC). Similar to previous studies (Prahl et al., 1994; Keil et al., 1998), p-hydroxybenzaldehyde, 3,5-dihydroxybenzoic acid (DHA), and dihydroxy C<sub>16</sub> fatty acid were also identified as LOP in both sediments. Among them, dihydroxy C<sub>16</sub> fatty acid is known to derive from higher plant cutin (Goni and Hedges, 1990), whereas the source of the hydroxybenzene compounds is less clear. p-Hydroxybenzaldehyde may derive from protein as well as lignin (Goni et al., 2000), and has been detected in algal extracts (Feng et al., unpublished results). DHA, a common LOP in sediments and soils but not of fresh vascular plant tissues, has been proposed to be a product of soil alteration processes but has also been detected in brown macroalgae (Prahl et al., 1994). DHA occurred in both sediment samples in a comparable abundance to lignin phenols (>1.0–1.3 mg/g OC) whereas p-coumaric acid and ferulic acid, gave similar isotopic results (ca. −23%/oo and −30%/oo, respectively), with the former being systematically more depleted.

### 3.2.3. Stable carbon isotope composition

The δ<sup>13</sup>C values of individual lignin-derived monomers fell between −30%/oo and −34%/oo for both stations (with the exception of acetosyringone; Fig. 3 a), 5–9%. 5–9% more depleted than the bulk OC. This offset is slightly higher than the typical δ<sup>13</sup>C offset between macromolecular lignin and bulk OC in plant tissues (2–6%/oo Benner et al., 1987). However, the δ<sup>13</sup>C values of lignin monomers fell within the range of δ<sup>13</sup>C values reported for C<sub>3</sub> plant lignin monomers (−31.1 ± 3.7%/oo, Goni and Eglinton, 1996; −32.9 ± 2.5%/oo, Bahri et al., 2006) which fractionated against plant bulk OC by as much as −9.8%/oo. No general trend was observed for the isotopic composition among the aldehyde, ketone, and acid monomers of vanillyl and syringyl phenols. Aceto-

### 3.2.3. Radiocarbon composition

#### 3.2.3.1. Assessment of lignin phenol <sup>14</sup>C measurement based on HPLC isolation

To assess the accuracy of radiocarbon measurement involving the HPLC isolation method, we first compared the measured <i>F</i><sub>m</sub> values of individual lignin
isolated from authentic standards and plant tissue reference materials with the nominal $F_m$ values of their corresponding bulk OC. The offset between the measured (not corrected for procedural blanks) and nominal $F_m$ values of lignin phenols ranged from $-0.0266$ to $+0.0267$ with an average of $-0.0021 \pm 0.0175$ (Table 4). Procedural blanks associated with HPLC procedures yielded $2 \pm 0.5 \mu g C$, similar values to those reported with HPLC isolation steps (Hou et al., 2010; Ingalls et al., 2010). We were unable to directly measure the radiocarbon content of our procedural blanks as sample sizes were too low. Instead, we indirectly estimated their $F_m$ value using

![Fig. 3](image-url)
Table 4
Mass and radiocarbon contents of lignin phenols isolated by HPLC relative to the nominal $F_m$ values of bulk OC.

<table>
<thead>
<tr>
<th>Source</th>
<th>Lignin phenol</th>
<th>Mass (µg C)</th>
<th>Measured values on phenols isolated by HPLC</th>
<th>Nominal $F_m$ of bulk OC&lt;sup&gt;b&lt;/sup&gt;</th>
<th>$\Delta F_m$ (AMS-corrected only)</th>
<th>$\Delta F_m$ (procedural blank-corrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AMS-corrected only</td>
<td>Procedural blank-corrected&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$F_m$</td>
<td>Error</td>
<td>$F_m$</td>
<td>Error</td>
</tr>
<tr>
<td>Commercial&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Vanilllic acid</td>
<td>182</td>
<td>0.0105</td>
<td>0.0005</td>
<td>0.0053</td>
<td>0.0018</td>
</tr>
<tr>
<td>Commercial&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Acetovanillnone</td>
<td>163</td>
<td>0.0297</td>
<td>0.0007</td>
<td>0.0241</td>
<td>0.0020</td>
</tr>
<tr>
<td>FRII-A</td>
<td>Vanilllin</td>
<td>224</td>
<td>0.0157</td>
<td>0.0005</td>
<td>0.0115</td>
<td>0.0015</td>
</tr>
<tr>
<td>C-5</td>
<td>Vanilllin</td>
<td>224</td>
<td>0.0246</td>
<td>0.0018</td>
<td>0.2402</td>
<td>0.0022</td>
</tr>
<tr>
<td>Acetovanillnone</td>
<td>34</td>
<td>0.2353</td>
<td>0.0027</td>
<td>0.2390</td>
<td>0.0079</td>
<td>0.5705</td>
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<tr>
<td>FRII-D</td>
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<td>0.5573</td>
<td>0.0018</td>
<td>0.5595</td>
<td>0.0035</td>
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<td>0.0018</td>
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<td>0.0034</td>
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<td>FRII-H</td>
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<td>184</td>
<td>0.7468</td>
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<td>0.7487</td>
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<td>FRII-J</td>
<td>Vanilllin</td>
<td>130</td>
<td>1.1191</td>
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<td>1.1291</td>
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<tr>
<td>Ferulic acid</td>
<td>226</td>
<td>1.0803</td>
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<td>Acetosyringone</td>
<td>78</td>
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<td>1.0995</td>
<td>0.0055</td>
<td>0.0233</td>
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<tr>
<td>p-Coumaric acid</td>
<td>82</td>
<td>1.0810</td>
<td>0.0023</td>
<td>1.0961</td>
<td>0.0052</td>
<td>0.0259</td>
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<tr>
<td>Commercial&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Vanilllin</td>
<td>152</td>
<td>1.1257</td>
<td>0.0076</td>
<td>1.1343</td>
<td>0.0081</td>
</tr>
</tbody>
</table>

<sup>a</sup> Procedural blank contains 2.0 ± 0.5 µg C with $F_m = 0.48 ± 0.10$.

<sup>b</sup> Nominal values were measured on authentic phenol standards (purchased from Acros or Sigma) and were pre-determined for bulk plant tissues. FRII-A, C-5, FRII-D, FRII-H, and FRII-J are plant tissues as international standards.

<sup>c</sup> Obtained from Acros.

<sup>d</sup> Obtained from Sigma.

a mass balance approach (Ziolkowski and Druffel, 2009), assuming that sedimentary and standard phenols were diluted with a constant amount of blank (2 ± 0.5 µg C) with a constant radiocarbon content which caused an offset between the measured and nominal $F_m$ values of the phenol standards that we measured ($\Delta F_m$; Table 4). A range of $F_m$ values (from 0.000 to 1.000) were tested to correct the measured $F_m$ values of all phenol standards (Table 4; Fig. 4). An $F_m$ value of 0.48 ± 0.10 was chosen for subsequent corrections of HPLC-based measurement, which decreased the $F_m$ offset to an average of 0.0000 ± 0.0131 (Table 4; Fig. 4), corresponding to a $\Delta^{14}C$ offset of 0 ± 13‰. The high uncertainty (±0.10) assigned to the $F_m$ value of HPLC procedural blank is similar to that of the PCGC blanks and most likely made it reasonable to compare the $\Delta^{14}C$ values of compounds isolated using different methods. Overall, syringyl and cinnamyl phenols exhibited an offset of −0.0073 ± 0.0002 and −0.0160 ± 0.0074 relative to their nominal $F_m$ values respectively, whereas vanillin phenols showed an offset of +0.0044 ± 0.0124 after blank corrections. These values are not considered to be significantly different (one-way ANOVA; $P = 0.73$), especially when the errors of measured $F_m$ values are taken into account (up to ±0.0090; Table 4). Different lignin phenols isolated from the same plant tissues had similar $F_m$ values (Table 4). The $F_m$ offset between individual phenols (within 0.0434, comparable to a $\Delta^{14}C$ offset of ~40‰) is comparable to that reported by Hou et al. (2010) and yet our measurement encompasses a broader array of lignin phenols. Although this variability is slightly larger than the uncertainties associated with processing (including extraction, HPLC isolation and combustion; 0 ± 13‰) and the average error of long-term AMS measurement (±15‰), it is sufficiently small to address questions concerning the cycling of lignin in the environment.

As compared to other published HPLC isolation methods of lignin phenols for radiocarbon measurement (Hou et al., 2010; Ingalls et al., 2010), our procedure has two important advantages. First, purification through two SPE cartridges greatly improves baseline separation on the subsequent HPLC analysis. In particular, the aldehyde/ketone fraction of LOP eluting from amino SPE was promising for lignin isolation on HPLC in that this fraction from both plant tissues and Washington margin sediments was almost colorless and yielded a flat baseline during HPLC-DAD (Fig. 2a). This is particularly important for complex environmental samples, from which inter-
fering non-lignin compounds are liberated during CuO oxidation (products of protein and carbohydrate hydrolysis, etc.). Second, SPE cartridges help to concentrate lignin phenols such that phenols of relatively lower abundances can be isolated fairly easily, enabling a broader array of lignin phenols to be targeted for radiocarbon measurement. Notably, we successfully isolated two lignin phenols (p-coumaric acid and ferulic acid) that had a very low abundance in the Washington margin sediments, demonstrating the effectiveness of our HPLC isolation method. Admittedly, two solvent dry-down steps were added by using two SPE cartridges in cleaning up extracts, which may increase the potential loss of lignin phenols through volatilization. Special care was taken in those steps to prevent complete removal of solvents and the recovery of phenols was quite satisfactory (Table S.2). We hence recommend the use of SPEs to purify samples and to protect HPLC columns.

3.2.3.2. $\Delta^{14}C$ values of lignin phenols isolated by HPLC and PGCG from Washington margin. Radiocarbon content was then compared for individual lignin phenols isolated from the Washington margin sediments using both PCGC and HPLC methods. Lignin phenols isolated by HPLC from the Washington margin sediments had $\Delta^{14}C$ values ranging from $-64^{\circ}/_{\text{oo}}$ to $-132^{\circ}/_{\text{oo}}$ at St 1 and from $-45^{\circ}/_{\text{oo}}$ to $-150^{\circ}/_{\text{oo}}$ at St 2 (Fig. 3a; Table S.1). Vanillic acid and vanillin were the most $^{13}C$-depleted phenols in St 1 and 2, respectively. The abundance-weighted $\Delta^{14}C$ values for three vanillyl phenols were $-107 \pm 3^{\circ}/_{\text{oo}}$ and $-134 \pm 4^{\circ}/_{\text{oo}}$ for St 1 and 2, respectively, more depleted than those of individual syringyl (by $41-57^{\circ}/_{\text{oo}}$) or cinamyl phenols ($40-89^{\circ}/_{\text{oo}}$) at the respective stations ($t$ test; $P < 0.05$). Vanillic phenols at St 1 were significantly more enriched in $^{14}C$ than those at St 2 ($t$ test; $P < 0.05$).

By comparison, lignin phenols isolated by PCGC displayed $\Delta^{14}C$ values ranging from $-13^{\circ}/_{\text{oo}}$ to $-105^{\circ}/_{\text{oo}}$ in St 1, and from $-23^{\circ}/_{\text{oo}}$ to $-116^{\circ}/_{\text{oo}}$ in St 2 (Fig. 3a; Table S.1). Values were similar for both stations and in both cases vanillin was the most $^{13}C$-depleted component. Because not all phenols were measured for $^{14}C$, we calculated the abundance-weighted $\Delta^{14}C$ values for the same phenols analyzed at both stations. Three vanillyl phenols and two syringyl phenols (acetosyringone and syringic acid) isolated by PCGC had an average $\Delta^{14}C$ value of $-86 \pm 7^{\circ}/_{\text{oo}}$ and $-17 \pm 21^{\circ}/_{\text{oo}}$, respectively at St 1 and $-105 \pm 16^{\circ}/_{\text{oo}}$ and $-50 \pm 13^{\circ}/_{\text{oo}}$, respectively at St 2. These values were statistically indistinguishable from St 1 and 2. Similar to the HPLC-based measurements, PCGC-isolated vanillyl phenols were significantly more depleted in $^{14}C$ than syringyl phenols at both stations (by 55–69$^{\circ}/_{\text{oo}}$; $t$ test; $P < 0.05$).

Overall, HPLC-based $\Delta^{14}C$ values of vanillyl phenols were 21–29$^{\circ}/_{\text{oo}}$ more depleted than PCGC-based values. Admittedly, sample pretreatment differed for the PCGC- and HPLC-isolated lignin phenols (HCl/HF treatment and alkaline hydrolysis before CuO oxidation, respectively). The $\Delta^{14}C$ offset is however not considered to be affected by the treatment procedures, because: (a) the concentration and composition of lignin phenols was similar to those measured previously in the Washington margin (Hedges and Mann, 1979a; Prahl, 1985; Prahl et al., 1994; Keil et al., 1998); (b) the HCl/HF treatment did not induce a depletion in the $\Delta^{14}C$ value of lignin phenols in the treated residues as is suspected for the acid-insoluble OC (Rumpel et al., 2008); and (c) even when we assume that phenols extracted by hydrolysis (which yielded 2–4$^{\circ}/_{\text{oo}}$ of their respective counterparts from the CuO oxidation) carry a modern $\Delta^{14}C$ value of 0$^{\circ}/_{\text{oo}}$, they would only increase the $\Delta^{14}C$ value of HPLC-isolated phenols by 4$^{\circ}/_{\text{oo}}$ much smaller than the offset between PCGC and HPLC-based $\Delta^{14}C$ values. Actually, a discrepancy of 21–29$^{\circ}/_{\text{oo}}$ is similar in size to the $\Delta^{14}C$ variability of individual phenols isolated from the same wood standards ($38^{\circ}/_{\text{oo}}$) and not considered to be significant, particularly when the average uncertainties of AMS measurement ($\pm15^{\circ}/_{\text{oo}}$) and blank assessment (0 $\pm 13^{\circ}/_{\text{oo}}$ for the HPLC method) are taken into account. As compared with the PCGC method, HPLC-based isolation of lignin phenols is preferred as it does not require derivatization and consumes far less instrument time (2 columns x 5 injections for HPLC versus >100 injections for PCGC).

3.3. Molecular and isotopic characteristics of plant wax lipids

3.3.1. Molecular composition

In comparison to lignin phenols, solvent extractable n-alkyl lipids were present in much lower concentrations in both sediments (Table 3). n-Alkanes were present in the range of C$_{19-35}$ and exhibited a marked odd-over-even carbon number preference (carbon preference index, CPI = $\sum_{21-31}$ odd-numbered n-alkanes/$\sum_{22-32}$ even-numbered n-alkanes of 3.1 and 4.2 at St 1 and 2, respectively). The average chain length (ACL) was 27.0 and 28.1 for n-alkanes at St 1 and 2, respectively, with n-C$_{29}$ n-alkane being the most abundant homologue. The concentration of plant wax n-alkanes ($\sum_{25-31}$ odd-numbered) was 0.08 and 0.09 mg/g OC at St 1 and 2, respectively (Table 3), consistent with previous reports (Prahl and Carpenter, 1984; Prahl, 1985, 1994). n-Alkanic (fatty) acids, n-alkanols, and n-aldehydes exhibited a strong even-over-odd carbon number predominance with C$_{24}$, C$_{26}$, and C$_{28}$ as the most abundant homologue for n-alkanoic acids, n-alkanols, and n-aldehydes, respectively. The ACL varied between 24.8 and 27.1 in both stations. These data are consistent with previous observations on the lipid composition of Washington margin coastal sediments (Prahl and Pinto, 1987) and indicate a predominant terrestrial input. Long-chain fatty acids ($\sum_{24-32}$ even-numbered) were the most abundant plant wax lipids in both sediments with a concentration of 0.18 and 0.12 mg/g OC at St 1 and 2, respectively (Table 3). $\sum_{24-32}$ even-numbered n-alkanols and n-aldehydes ranged from 0.06 to 0.09 mg/g OC.

3.3.2. Carbon ($^{13}C$, $^{14}C$) isotopic compositions and OC sources

Individual n-alkanes displayed $\delta^{13}C$ values between $-30^{\circ}/_{\text{oo}}$ and $-33^{\circ}/_{\text{oo}}$ (Fig. 3b), 5–8$^{\circ}/_{\text{oo}}$ more $^{13}C$-depleted than the bulk OC. Within homologous series, C$_{31}$ and C$_{33}$ n-alkanes exhibited the most depleted $\delta^{13}C$ values at both stations, indicating an origin predominantly from C$_3$ plant waxes for the longer chain homologues (Collister et al., 2019).
1994; Rommerskirchen et al., 2006b; Chikaraishi and Naraoka, 2007). n-Alkanes (C_{27-29,31}) that were characteristic of higher plant waxes had a similar radiocarbon content to the bulk OC, varying slightly within −100‰ to −125‰ at both stations (Fig. 3b). Their corresponding abundance-weighted δ^{13}C and Δ^{14}C values were −32.4‰ and −104±22‰, respectively, for St 1, and −32.5‰ and −122±15‰ for St 2, respectively. In sharp contrast, the summed Δ^{14}C values of shorter-chain C_{21-23,25} n-alkanes were significantly more depleted (−588‰ and −506‰, respectively), while the C_{22-24,26} homologues showed an even stronger depletion (−969‰ and −747‰, respectively), suggesting a predominant input from relict sources to C_{22,24,26} n-alkanes (particularly for St 1) and, to a less extent, to C_{21,23,25} n-alkanes (cf. Pearson and Eglinton, 2000; Pearson et al., 2001; Drenzek et al., 2007). Among these n-alkanes that showed signs of non-plant inputs, the even-numbered homologues had similar δ^{13}C values (ca. −32‰) to their odd-numbered counterparts in the C_{22-29} range, whereas shorter chain (C_{19-21}) homologues at St 1 had the most enriched values (−30.2‰ to −31.0‰).

In contrast to n-alkanes, even-numbered fatty acids exhibited a wider range of δ^{13}C values varying from −26.0‰ to −33.9‰, and a wide range of Δ^{14}C values between −204‰ and +179‰ (Fig. 3c). Short-chain fatty acids (C_{14,16,18}) had the highest δ^{13}C values (−26.0‰ to −26.9‰), −4.5‰ more depleted than marine planktonic OC (−21.5‰; Hedges and Mann, 1979a; Prahl et al., 1994) in the Washington margin. This isotopic offset is close to the fractionation between fatty acids and biomass (−4.3‰; Hayes, 1993; Schouten et al., 1998). C_{16} and C_{18} fatty acids also displayed the most enriched Δ^{14}C values between +4‰ and +179‰. These data collectively suggest a strong algal/bacterial contribution with a (greater than) modern radiocarbon age to short-chain fatty acids (Perry et al., 1979; Volkman et al., 1998). Longer-chain (C_{26-32}) homologues displayed a similar range of δ^{13}C values (−29.8‰ to −33.9‰) to long-chain n-alkanes (C_{21-33}), cutin marker (dihydroxy C_{16} fatty acid) and lignin phenols while C_{26} fatty acid displayed a similar radiocarbon content to bulk OC at both stations (Fig. 3c). The abundance-weighted δ^{13}C values of C_{26-28,30,32} fatty acids were −31.8‰ and −31.0‰ for St 1 and St 2, respectively. By comparison, C_{20,22,24} fatty acids displayed more enriched δ^{13}C (−28.2‰ to −29.0‰) and Δ^{14}C values (−73‰ to +74‰) than their longer homologues (Fig. 3c). Although long-chain (>C_{26}) saturated even-numbered fatty acids are usually considered to derive predominantly from vascular plant waxes, these lipids have also been identified in microalgae (Volkman et al., 1998 and references therein) and perhaps bacteria (Volkman et al., 1988; Gong and Hollander, 1997). The heavy δ^{13}C and Δ^{14}C isotopic data collectively suggest the contribution of modern planktonic OC to C_{22} and, to a less extent, C_{24} fatty acids.

Even-numbered C_{22-30} n-alkanols displayed δ^{13}C values from −29.9‰ to −34.3‰ at St 1 and were slightly more 13C-depleted (−29.7‰ to −37.5‰) at St 2 (Fig. 3d). In general, the values fell within the range reported for C_{3} plant wax n-alkanols (Bull et al., 2000; Rommerskirchen et al., 2006a). Similar to fatty acids, C_{22} and C_{24} n-alkanols exhibited more enriched δ^{13}C values (−29.7‰ to −31.1‰) than their longer homologues (C_{26-30}: −33.4‰ to −37.5‰) at both stations. However, C_{22} and C_{24} n-alkanols had a similar 14C content to plant wax n-alkanes, indicating a predominant input from terrestrial sources instead of modern marine biota such as microalgae, seagrasses, and cyanobacteria (Rommerskirchen et al., 2006a; Volkman et al., 2008). Furthermore, contrary to fatty acids, the longer homologues (C_{26-30}) of n-alkanols were more enriched in 14C, suggesting a shorter residence time or a greater contribution of fresher material. The observed 13C isotopic composition of long-chain n-alkanols may therefore reflect isotopic variation among plant wax lipids, where longer (>C_{26}) n-alkanols are reported to have more depleted δ^{13}C values than the C_{22} and C_{24} homologues in several plant species (Chikaraishi and Naraoka, 2007). The abundance-weighted δ^{13}C values of C_{22-30} n-alkanols were −32.4‰ and −34.5‰ for St 1 and 2, respectively, while the abundance-weighted Δ^{14}C value of these n-alkanols was −56±18‰ at St 1. Due to a limited sample size, only one composite sample of C_{22-30} even-numbered n-alkanols was measured for St 2, which had a more enriched Δ^{14}C value (−69‰) than plant wax n-alkanes, fatty acids and bulk OC in St 2.

The stable carbon isotopic composition of n-alkydehydes, which were only measured for St 2, ranged between −29.3‰ and −33.8‰ (Fig. 3e). Odd-numbered n-alkydehydes had relatively invariant δ^{13}C values (−31.8‰ to −33.8‰) that were similar to even-numbered n-alkanes. The n-alkydehydes have been suggested to be oxidation products of n-alkanes (Cardoso and Chicarelli, 1983; Stephanou, 1989) and hence may exhibit similar δ^{13}C values to the n-alkanes. By comparison, even-numbered n-alkydehydes were more enriched than their odd-numbered counterparts by up to 4.5‰ with the C_{30} homologue exhibiting the most enriched value (−28.3‰) and the C_{28} homologue showing the most depleted value (−33.6‰). Even-numbered long-chain n-alkydehydes are considered to derive mainly from terrestrial plants (Prahl and Pinto, 1987; Rieley et al., 1991; van Bergen et al., 1997) and our measured δ^{13}C values fall within the range reported for C_{3} plant wax n-alkydehydes (Collister et al., 1994). The abundance-weighted δ^{13}C value of C_{22-24,26,28,30} n-alkydehydes was −30.9‰ for St 2 and a composite sample of these n-alkydehydes had a similar Δ^{14}C value (−145‰) to plant wax n-alkanes and bulk OC at St 2 (Fig. 3e).

3.4. Comparing the carbon isotopic characteristics of higher plant biomarkers in Washington margin sediments

The δ^{13}C and Δ^{14}C contents of lignin phenols and various plant wax lipids revealed several interesting characteristics in the Washington margin sediments. Overall, lignin phenols displayed a relatively narrow range of Δ^{14}C values (corresponding to radiocarbon ages of ca. 300–1200 years) that were similar to, or younger than, bulk OC at both stations (Figs. 3 and 5). The coherence of 14C data for this suite of compounds lends confidence in the robustness of our method as a means of retrieving the isotopic characteristics of this terrestrial biopolymer. The corresponding age of lignin phenols suggests that this vascular plant compo-
The broader age span suggests varied stability and/or heterogeneity in their carbon sources, or more diverse transport pathways (such as eolian versus fluvial transport; Dahl et al., 2005) to the marine environment. Among plant wax lipids, long-chain n-alkanols displayed significantly higher $\Delta^{14}C$ values (ca. $-60^{\circ}/_{oo}$) than bulk OC or other lipid classes at both stations (Fig. 5), suggesting that this group of compounds exhibits a greater reactivity or has a shorter residence time in the environment before deposition into the Washington margin sediments. This finding is consistent with the faster degradation rate of long-chain n-alkanols as compared with long-chain n-alkanes and fatty acids during fluvial transport (van Dongen et al., 2008).

Alternatively, pollen of several dominating plant species (such as Pinus ponderosa) in the Pacific Northwest contains high concentrations of long-chain n-alkanols relative to other lipid classes (Pralh and Pinto, 1987), and pollen is widely distributed in the Washington margin shelf sediments (Hedges et al., 1999). Wind-borne pollen may supply the sediments with younger-age long-chain n-alkanols than other terrestrial lipids that are mainly delivered via fluvial transport. The contribution of pollen-derived OC to sediments is, however, not known. The other plant wax lipids (n-alkanes, fatty acids, and n-aldehydes) exhibited a similar radiocarbon content to the bulk OC and lignin phenols at St 2 (Fig. 5), suggesting a uniform origin and a similar transport and deposition pattern of terrestrial lipids and lignin at the mid shelf. This observation may be related to a narrower grain size distribution in the mid-shelf sediment of Washington margin, where fine particle-associated OC dominates bulk OC signatures (Coppola et al., 2007). In contrast, while plant wax fatty acids (C$\geq$26) displayed a similar $\Delta^{14}C$ value to the bulk OC at St 1, plant wax n-alkanes and lignin phenols showed higher $\Delta^{14}C$ values at this station (Fig. 5). Because the inner shelf Washington margin sediments contain a high proportion of coarse materials emanating from the Columbia River (Coppola et al., 2007), the younger radiocarbon age of plant wax n-alkanes and lignin phenols most likely reflected the contribution of woody and leafy debris (Hedges and Mann, 1979a) that is enriched with both groups of biomarkers. By comparison, C$\geq$26 fatty acid did not carry a strong plant debris $^{14}C$ signal, possibly because its abundance in plant debris relative to sediments is not as high as C$\geq$27,29,31 n-alkanes or lignin phenols (Table 3).

3.5. Constraining isotopic end members and their contributions in the Washington margin

Based on discussions above, we selected a range of values to constrain the $\delta^{13}C_{C}$ and $\Delta^{14}C$ values of end members in the isotopic mass balance model. Since even-carbon-numbered n-alkanes are not abundantly produced by extant terrestrial or marine biomass (Volkman et al., 1998; Romerskirchen et al., 2006b; Chikaraishi and Naraoka, 2007) and C$\geq$22,24,26 n-alkanes at St 1 had a $\Delta^{14}C$ value of $-969^{\circ}/_{oo}$, indicating a predominance of relict OC, relict OC in the mixing model assumes a similar range of $\delta^{13}C_{C}$ and $\Delta^{14}C$ values as those of even-numbered n-alkanes at St 1 from $-30^{\circ}/_{oo}$ to $-32^{\circ}/_{oo}$. Given that the sediments were collected in 1993, closer to the peak in $^{14}C$ stemming from above-
ground nuclear weapons testing (the so-called “bomb spike”), it might be expected that marine OC, which reflects surface ocean dissolved inorganic carbon isotopic characteristics, has a Δ14C value >0 (Pearson et al., 2000). However, surface sediments in the mixed layer (20–30 cm in depth) integrate 50–100 years of deposition across the study sites, and bioturbation further smoothes the bomb spike. Based on the radiocarbon content of C16,18 fatty acids (mainly of a planktonic origin) and C22,24,26 n-alkanes at St 1 (mainly derived from relict OC; Fig. 3), marine and relict OC are therefore assumed to carry Δ13C_M and Δ14C_M values of 0‰ and −1000‰, respectively. Terrestrial OC assumes a similar Δ14C value to plant wax n-alkanes and lignin vanillyl phenols (−115 ± 15‰). The contribution of each end member to the bulk OC varies only slightly (±2%) within the range of Δ13C_R and Δ14C_R values we adopted for the end members (see discussions in Drenzek et al., 2007). In general, this approach suggests that terrestrial, marine, and relict OC contribute 89 ± 2%, 2 ± 1% and 9 ± 2% (St 1) and 95 ± 2%, 2 ± 1%, and 3 ± 2% (St 2) of bulk sedimentary OC at these two sites on the Washington margin, respectively. This simple estimate is consistent with the predominance of terrestrial OM in the Washington margin sediments inferred previously (Hedges and Mann, 1979a; Prahl et al., 1994; Dickens et al., 2006), and highlights the utility of both lignin and plant wax δ13C and Δ14C data in source apportionment and for developing carbon budgets for coastal marine sediments. The small proportion of relict OC in the Washington margin sediments stands in sharp contrast with the high contribution of sedimentary rock derived OC in other systems where a similar approach has been applied (Drenzek et al., 2007, 2009), suggesting significant heterogeneity in OC sources and deposition patterns among different river systems.

4. CONCLUSIONS

This study examines compound-specific 13C and 14C data for various plant wax lipids and lignin phenols isolated from Washington margin shelf sediments. Plant wax lipids displayed a broader range of radiocarbon ages. Depending on the compound class, pre-aged soil components, relict carbon and microbial sources may contribute to the observed isotopic signatures. By comparison, lignin phenols displayed a narrower range of ages that reflected the origin and degradation characteristics of this terrestrial biopolymer. Interestingly, vanillyl phenols were on average ~500 years older than syringyl and cinnamyl phenols that degrade faster in soils and sediments. These isotopic characteristics, together with their high abundance and wide distribution in sediments, make lignin phenols a promising tracer of relatively recent terrestrial OM during the land–ocean transfer. The 14C composition of lignin phenols may hence provide a useful constraint on the vascular plant OC end member in mixing models and improve understanding of the marine OC budget.

ACKNOWLEDGMENTS

We thank Ann Pearson, John Ertel and Helen White for assistance in developing graphitization, CuO oxidation and methylation, and purification procedures, respectively. Carl Johnson, Leah Houghton, and NOSAMS are thanked for assistance with GC–MS, irm-GC–MS and AMS analyses. Jaap Sinninghe Damsté, Rienk Smittenberg and two other anonymous reviewers are greatly thanked for their constructive comments on the paper. Grants OCE-9907129, OCE-0137005, and OCE-0526268 (to T.I.E.) from the National Science Foundation (NSF) supported this research. X.F. thanks WHOI for a postdoctoral scholar fellowship and for postdoctoral support from ETH Zürich.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.gca.2012.11.034.

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Associate Editor: Jaap Sinninghe Damste