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

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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 ^{13}C and ^{14}C 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 ^{14}C 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 ^{13}C and ^{14}C 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 $\delta^{13}C$ (-28.3 to -37.5 ‰) and $\Delta^{14}C$ values (-204 to +2 ‰), suggesting varied inputs and/or continental storage and transport histories. In contrast, lignin phenols exhibited similar $\delta^{13}C$ values (between -30 to -34 ‰) and a relatively narrow range of $\Delta^{14}C$ values (-45 to -150 ‰; HPLC-based mesurement) that were similar to, or younger than, bulk OM (-195 to -137 ‰). Moreover, lignin phenol ^{14}C 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 ^{14}C 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.

Key words: ^{14}C and ^{13}C composition, radiocarbon age, plant wax lipids, lignin phenols, Washington margin, marine carbon cycling, terrestrial organic matter

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 amongst 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 (Hernes 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 (C_3 versus C_4 plants) and reactivity of terrestrial OM accumulating in sediments (e.g., Goñi et al., 1997; Pearson et al., 2001; Smittenberg et al., 2006; Drenzek et al., 2007). For example, recent investigations on the ^{14}C 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 (^{13}C, ^{14}C) 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; Goñi 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 Goñi, 2003; Huguet et al., 2008; Mead and Goñi, 2008; Vonk et al., 2010) and hence may affect the distribution of lignin phenols versus plant wax lipids in the sediments. The ^{13}C and ^{14}C compositions of plant wax lipids have been investigated in a range of sedimentary environments (Jones et al., 1991; Huang et al., 1995; Pearson et al., 2001; Smittenberg et al., 2006; Drenzek et al., 2007; 2009; Mollenhauer and Eglinton, 2007; Kusch et al., 2010; Gustafsson et al., 2011); while carbon isotopic (especially ^{14}C) data on lignin phenols in marine sediments remains sparse (Goñi 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 et al., 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 ^{13}C and ^{14}C 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 ^{14}C 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 ^{14}C 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; Nittrouer 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 (Nittrouer 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 (Nittrouer 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 ‰), 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 *Wecoma*) 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 2N HCl solution. Stable carbon isotopic composition was determined by automated on-line combustion, followed by conventional isotope ratio 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 acetovanillone 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-A; the consensus fraction modern (F_m) value is 0.0033), subfossil wood from eastern Wisconsin (IAEA C-5; F_m: 0.2305), Belfast dendro-dated wood (FIRI-D; F_m: 0.5705), hohenheim wood (FIRI-H; F_m: 0.7574), and barley mash (FIRI-J; F_m: 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 soap washed, soaked in HCl (10 %), and rinsed with MilliQ 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, 72 h) to obtain a corresponding total lipid extract (TLE). The TLEs were spiked with a mixture of recovery standards (including C_{24} *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-flame ionization detector (GC-FID) analysis (Section 2.4) and stable carbon isotopic analysis by isotope ratio monitoring gas chromatography-mass spectrometry (irm-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; Goñi 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 ^{13}C 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 ^{14}C 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 K_2CO_3, 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 Goñi 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 N_2-bubbled NaOH solution (2 M) were loaded into each of 5 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 N_2 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 N_2 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 Delta^{plus} isotope ratio MS system. Instrumental conditions were described previously (Goñi and Eglinton, 1994, 1996; Feakins et al., 2005). The mass-spectrometor was calibrated using deuterated *n*-alkane internal isotopic standards (co-injected with the sample) as well as external CO_2 gas standards for each run. The $\delta^{13}C$ 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 $\delta^{13}C$ 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" R_{TX}-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 C, Supplementary Table S.1) 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 in ~0.5 mL of deionized water (pH 2), and loaded onto a Supelclean ENVI-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 N_2 to a volume of < 0.5 mL and further separated on a self-packed amino SPE cartridge (0.5 g, Supelclean LC-NH_2, Supelco, preconditioned with methanol) into phenolic aldehydes/ketones (eluting with methanol) and their corresponding acids (eluting with methanol:12 M HCl, 95:5), which have very similar retention times on subsequent HPLC analysis. Each fraction was then blown to < 50 μL under N_2 and re-dissolved in methanol for HPLC separation. Due to the high volatility of phenols, solvents were never completely removed during the extraction and purification steps to avoid sample loss. Recovery of phenols from the two-SPE cleanup procedure ranged from 65-110% (Supplementary Table S.2). Procedural blanks containing no sediments during CuO oxidation and standard plant tissues with pre-determined ^{14}C contents were processed in the same manner for method validation.

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 pore size) along with a Polar-RP SecurityGuard column (4.0 × 3.0 mm; 4 μm pore 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 N_2. 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 pore size) with a ZORBAX Eclipse C18 guard column (4.6 × 12.5 mm; 5 µm pore 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 8 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, significant sample loss via solvent dry down should be avoided. Heating was therefore not used during N_2 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 8 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 CO_2 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 N_2 gas, quartz tubes were sonicated in water for 1 min and gently blown again under N_2 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 CO_2 was 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 (F_m), $\Delta^{14}C$ (‰), 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 ‰. 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 (MilliQ 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 isolated compounds with SiO_2 columns after PCGC or HPLC isolation. Based on our experience (Galy and Eglinton, 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 relict OC to bulk sediments following a procedure described previously (Pearson and Eglinton, 2000; Drenzek et al., 2007). Briefly, the model is expressed in the following three equations:

$$f_T(\Delta^{14}C_T) + f_M(\Delta^{14}C_M) + f_R(\Delta^{14}C_R) = \Delta^{14}C_S \quad (1)$$

$$f_T(\delta^{13}C_T) + f_M(\delta^{13}C_M) + f_R(\delta^{13}C_R) = \delta^{13}C_S \quad (2)$$

$$f_T + f_M + f_R = 1 \quad (3)$$

where f is the fractional abundance and the subscripts T, M, R, and S are terrestrial, marine, relict 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 4.3). 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 and –136 ‰ 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 (Λ_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; Goñi et al., 2000). Despite a general similarity in lignin composition, the acid-to-aldehyde ratio for syringyl phenols $(Ad/Al)_s$, 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 $(Ad/Al)_s$ 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 8 monomers, three dimeric lignin phenols that are most abundant in gymnosperm wood (5-vanillovanillin, 5-vanilloacetovanillone, and dehydrovanillinvanillic acid; Goñi 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_{16} fatty acid were also identified as LOP in both sediments. Among them, dihydroxy C_{16} fatty acid is known to derive from higher plant cutin (Goñi and Hedges, 1990), whereas the source of the hydroxybenzene compounds is less clear. *p*-Hydroxybenzaldehyde may derive from protein as well as lignin (Goñi 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*-hydroxybenzaldehyde and dihydroxy C_{16} fatty acid were present in much lower concentrations (< 1.0 mg/g OC).

3.2.2. Stable Carbon Isotopic Composition

The $\delta^{13}C$ values of individual lignin-derived monomers fell between –30 and –34 ‰ for both stations (with the exception of acetosyringone; Fig. 3a), 5-9 ‰ more depleted than the bulk OC. This offset is slightly higher than the typical $\delta^{13}C$ offset between macromolecular lignin and bulk OC in plant tissues (2-6 ‰; Benner et al., 1987). However, the $\delta^{13}C$ values of lignin monomers fell within the range of $\delta^{13}C$ values reported for C_3 plant lignin phenols (–31.1 ± 3.7 ‰, Goñi and Eglinton, 1996; –32.9 ± 2.5 ‰, Bahri et al., 2006) which fractionated against plant bulk OC by as much as –9.8 ‰. No general trend was observed for the isotopic composition among the aldehyde, ketone, and acid monomers of vanillyl and syringyl phenols. Acetovanillone was the most ^{13}C-enriched phenol at both stations (–29.9 and –29.6 ‰ for St 1 and 2 respectively), while acetosyringone had exceptionally low $\delta^{13}C$ values (–43.3 and –44.6 ‰). Such an isotopic depletion in acetosyringone has not been observed in plant tissues (Goñi and Eglinton, 1996; Bahri et al., 2006) and appears inconsistent with an origin of C3 plants. We, therefore, suspect that acetosyringone co-eluted with an impurity during irm-GC-MS analysis. Syringic acid at St 2 exhibited a lower $\delta^{13}C$ value (–36.7 ‰) as compared to the other lignin monomers, and syringyl phenols generally were slightly more ^{13}C-depleted than vanillyl phenols at both stations. Cinammyl phenols, i.e., *p*-coumaric acid and ferulic acid, gave similar isotopic results (ca. –33 and –30 ‰ respectively), with the former being systematically more depleted. The abundance-weighted $\delta^{13}C$ values for the Λ_8 phenols (excluding acetosyringone) were –32.0 and –31.7 ‰ for St 1 and St 2 respectively, 6.2-6.5 ‰ more depleted than the bulk tissue of C_3 plants in the Columbia River drainage basin (–25.5 ‰; Hedges and Mann, 1979a), exhibiting an offset close to the reported fractionation between lignin and plant OC (2-6 ‰; Benner et al., 1987).

Three dimeric lignin phenols (5-vanillovanillin, 5-vanilloacetovanillone, and dehydrovanillinvanillic acid) had similar $\delta^{13}C$ values (–31.3 to –35.9 ‰). *p*-Hydroxybenzaldehyde yielded similar values to lignin phenols for both stations, suggesting a predominantly vascular plant origin. DHA was markedly depleted in ^{13}C at both stations and had a similar $\delta^{13}C$ value (ca. –42 ‰) to acetosyringone, possibly due to co-eluting impurities as well. Finally, cutin-derived dihydroxy C_{16} fatty acid yielded values (ca. –34 ‰) close to those of lignin phenols.

3.2.3. Radiocarbon Composition

3.2.3.1. Assessment of Lignin Phenol ^{14}C Measurement Based on HPLC Isolation

To assess the accuracy of radiocarbon measurement involving the HPLC isolation method, we first compared the measured F_m values of individual lignin phenols (34-281 µg C each, similar to the Washington margin sample size ranging from 22-235 µg C, Table 4 and Supplementary Table S.1) “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 ± 0.0175 (Table 4). Procedural blanks associated with HPLC procedures yielded 2 ± 0.5 µg 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 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 (ΔF_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 vanillyl 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 interfering 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 volatization. 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 PCGC 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 to –132 ‰ at St 1 and from –45 to –150 ‰ at St 2 (Fig. 3a; Table S.1). Vanillic acid and vanillin were the most ^{14}C-depleted phenols in St 1 and St 2, respectively. The abundance-weighted $\Delta^{14}C$ values for three vanillyl phenols were –107 ± 3 and –134 ± 4 ‰ for St 1 and St 2, respectively, more depleted than those of individual syringyl (by 41-57 ‰) or cinammyl phenols (40-89 ‰) at the respective stations (t test; $P < 0.05$). Vanillyl 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 to –105 ‰ in St 1, and from –23 to –116 ‰ in St 2 (Fig. 3a; Table S.1). Values were similar for both stations and in both cases vanillin was the most ^{14}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 ± 7 and –17 ± 21 ‰ respectively at St 1 and –105 ± 16 and –50 ± 13 ‰ respectively at St 2. These values were statistically indistinguishable between St 1 and St 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 ‰; t test; $P < 0.05$).

Overall, HPLC-based $\Delta^{14}C$ values of vanillyl phenols were 21-29 ‰ 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% of their respective counterparts from the CuO oxidation) carry a modern $\Delta^{14}C$ value of 0 ‰, they would only increase the $\Delta^{14}C$ value of HPLC-isolated phenols by 4 ‰, much smaller than the offset between PCGC and HPLC-based $\Delta^{14}C$ values. Actually, a discrepancy of 21-29 ‰ is similar in size to the $\Delta^{14}C$ variability of individual phenols isolated from the same wood standards (38 ‰) and not considered to be significant, particularly when the average uncertainties of AMS measurement (± 15 ‰) and blank assessment (0 ± 13 ‰ 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 × 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 $C_{19\text{-}35}$ and exhibited a marked odd-over-even carbon number preference (carbon preference index, CPI =

$\sum C_{21\text{-}31}$ odd-numbered *n*-alkanes/$\sum C_{22\text{-}32}$ 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 ($\sum C_{25\text{-}31}$ 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*-Alkanoic (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 ($\sum C_{24\text{-}32}$ 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). $\sum C_{24\text{-}32}$ 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 δ^{13}C values between –30 and –33 ‰ (Fig. 3b), 5 to 8 ‰ more ^{13}C-depleted than the bulk OC. Within homologous series, C_{31} and C_{33} *n*-alkanes exhibited the most depleted δ^{13}C values at both stations, indicating an origin predominantly from C_3 plant waxes for the longer chain homologues (Collister et al., 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 ‰ 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 ‰ for St 1 and 2, 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 $\delta^{13}C$ values (ca. –32 ‰) to their odd-numbered counterparts in the C_{22}-C_{29} range, whereas shorter chain (C_{19}-C_{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 $\delta^{13}C$ values varying from –26.0 to –33.9 ‰ and a wide range of $\Delta^{14}C$ values between –204 to +179 ‰ (Fig. 3c). Short-chain fatty acids (C_{14}, C_{16}, C_{18}) had the highest $\delta^{13}C$ 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 ‰; Hayes, 1993; Schouten et al., 1998). C_{16} and C_{18} fatty acids also displayed the most enriched $\Delta^{14}C$ values between +4 to +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}-C_{32}) homologues displayed a similar range of $\delta^{13}C$ values (–29.8 to –33.9 ‰) to long-chain *n*-alkanes (C_{21}-C_{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 $\delta^{13}C$ 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 $\delta^{13}C$ (–28.2 to –29.0 ‰) and $\Delta^{14}C$ values (–73 to +74 ‰) than their longer homologues (Fig. 3c). Although long-chain (>C_{20}) 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 ^{13}C and ^{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}-C_{30} *n*-alkanols displayed $\delta^{13}C$ values from –29.9 to –34.3 ‰ at St 1 and were slightly more ^{13}C-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 $\delta^{13}C$ values (–29.7 to –31.1 ‰) than their longer homologues (C_{26}-C_{30}; –33.4 to –37.5 ‰) at both stations. However, C_{22} and C_{24} *n*-alkanols had a similar ^{14}C 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}-C_{30}) of *n*-alkanols were more enriched in ^{14}C, suggesting a shorter residence time or a greater contribution of fresher material. The observed ^{13}C 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 $\delta^{13}C$ values than the C_{22} and C_{24} homologues in several plant species (Chikaraishi and Naraoka, 2007). The abundance-weighted $\delta^{13}C$ values of C_{22-30} *n*-alkanols were –32.4 and –34.5 ‰ for St 1 and St 2, respectively, while the abundance-weighted $\Delta^{14}C$ value of these *n*-alkanols was –56 ± 18 ‰ at St 1. Due to a limited sample size, only one composite sample of C_{22}-C_{30} even-numbered *n*-alkanols was measured for St 2, which had a more enriched $\Delta^{14}C$ value (–69 ‰) than plant wax *n*-alkanes, fatty acids and bulk OC in St 2.

The stable carbon isotopic composition of *n*-aldehydes, which were only measured for St 2, ranged between –29.3 and –33.8 ‰ (Fig. 3e). Odd-numbered *n*-aldehydes had relatively invariant $\delta^{13}C$ values (–31.8 to –33.8 ‰) that were similar to even-numbered *n*-alkanes. The *n*-aldehydes have been suggested to be oxidation products of *n*-alkanes (Cardoso and Chicarelli, 1983; Stephanou, 1989) and hence may exhibit similar $\delta^{13}C$ values to the *n*-alkanes. By comparison, even-numbered *n*-aldehydes 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*-aldehydes are considered to derive mainly from terrestrial plants (Prahl and Pinto, 1987; Rieley et al., 1991; van Bergen et al., 1997) and our measured $\delta^{13}C$ values fall within the range reported for C_3 plant wax *n*-aldehydes (Collister et al., 1994). The abundance-weighted $\delta^{13}C$ value of $C_{22, 24, 26, 28, 30}$ *n*-aldehydes was –30.9 ‰ for St 2 and a composite sample of these *n*-aldehydes had a similar $\Delta^{14}C$ 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 ^{13}C and ^{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 $\Delta^{14}C$ 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 ^{14}C 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 component is significantly pre-aged as a consequence of retention in either soils or upstream deposits of the Columbia River for hundreds of years. Furthermore, although this study only included two sites, our data suggest that the radiocarbon age of lignin phenols preserves the origin and degradation characteristics of this terrestrial biopolymer during land-ocean transfer as the age of lignin phenols appears to relate to their decay rate in the sediments. Vanillyl phenols were on average ~500 years older than syringyl and cinnamyl phenols in both sediments, suggesting a longer residence time of vanillyl phenols in soils or upstream deposits. This observation coincides with the faster decay of syringyl and cinnamyl relative to vanillyl phenols in soil and sedimentary environment (Hedges et al., 1988; Opsahl and Benner, 1995; Otto et al., 2005).

Unlike lignin phenols, carbon isotopic compositions reveal relict OC or algal/bacterial influences for some long-chain lipids in the Washington margin sediments such as $C_{21, 23, 25}$ *n*-alkanes and $C_{20, 22, 24}$ fatty acids. Although these lipids are usually considered to be of vascular plant origin, our data as well as reports on the Santa Monica Basin (Gong and Hollander, 1997; Pearson et al., 2001) and Beaufort Sea (Drenzek et al., 2007) suggest diverse origins in the marine environment. For comparative purposes, the abundance-weighted average $\delta^{13}C$ and $\Delta^{14}C$ values (where applicable) of lipids showing a predominance of C_3 vascular plant signals (including $C_{27, 29, 31}$ *n*-alkanes, $C_{26, 28, 30, 32}$ fatty acids, $C_{22, 24, 26, 28, 30}$ *n*-alkanols, and $C_{22, 24, 26, 28, 30}$ *n*-aldehydes) were compared with those of lignin phenols as represented by the most abundant vanillyl phenols isolated by HPLC (Fig. 5; Table S.1). As compared with lignin phenols, plant wax lipids exhibited higher variability in their average $\Delta^{14}C$ values, ranging from –60 to –200 ‰, corresponding to radiocarbon ages of 400-1800 years (Fig. 5). 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 ‰) 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 (Prahl 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_{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_{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 $C_{27, 29, 31}$ *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 $\delta^{13}C_R$ 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; Rommerskirchen et al., 2006b; Chikaraishi and Naraoka, 2007) and $C_{22, 24, 26}$ *n*-alkanes at St 1 had a $\Delta^{14}C$ value of –969 ‰, indicating a predominance of relict OC, relict OC in the mixing model assumes a similar range of $\delta^{13}C_R$ values as those of even-numbered *n*-alkanes at St 1 from –30 to –32 ‰. 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 $\Delta^{14}C$ value > 0 (Pearson et al., 2000). However, surface sediments in the mixed layer (20-30 cm in depth) integrate 50-100 yr of deposition across the study sites, and bioturbation further smoothes the bomb spike. Based on the radiocarbon content of $C_{16,\,18}$ fatty acids (mainly of a planktonic origin) and $C_{22,\,24,\,26}$ *n*-alkanes at St 1 (mainly derived from relict OC; Fig. 3), marine and relict OC are therefore assumed to carry $\Delta^{14}C_M$ and $\Delta^{14}C_R$ values of 0 and –1000 ‰, respectively. Terrestrial OC assumes a similar $\Delta^{14}C$ 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 $\delta^{13}C_R$ and $\Delta^{14}C_T$ 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 $\delta^{13}C$ and $\Delta^{14}C$ 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 ^{13}C and ^{14}C 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 ^{14}C 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.

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Tables

Table 1: Binary gradient of mobile phases of the HPLC method to separate lignin phenols. Solvent A: water/acetic acid (99.8:0.2); solvent B: methanol/acetonitrile (50:50); flow rate = 0.8 mL/min.

Phenomenex Polar-RP column		*ZORBAX Eclipse XDB-C18 column*	
Time (min)	**% Solvent B**	**Time (min)**	**% Solvent B**
0	10	0	10
3	10	3	10
8	15	8	15
15	20	15	20
22	20	20	20
27	25	25	25
36	25	26[1]	100
37[1]	100	30[1]	100
42[1]	100	31[2]	10
43[2]	10	36[2]	10
48[2]	10		

[1] Phase of column washing.

[2] Phase of column equilibrium.

Table 2: Bulk geochemical properties of the Washington margin surface sediment samples.

Station	Location	OC (%)	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	^{14}C age (yr)
1	Inner shelf	0.40	–25.3	–195	1700
2	Mid shelf	0.93	–25.3	–136	1140

Table 3: Composition of lignin phenols and lipids in the Washington margin surface sediment samples.

	Λ_8[1]	S/V[2]	C/V[3]	$(Ad/Al)_v$[4]	$(Ad/Al)_s$[5]	*n*-alkanes			*n*-fatty acids		*n*-alkanols		*n*-aldehydes	
						Σ[6]	ACL[7]	CPI[8]	Σ[9]	ACL	Σ[10]	ACL	Σ[11]	ACL
St 1	60.7	0.19	0.04	0.24	0.16	0.08	27.0	3.1	0.18	25.0	0.08	26.3	n.a.	n.a.
St 2	51.3	0.30	0.05	0.27	0.26	0.09	28.1	4.2	0.12	24.8	0.09	27.1	0.06	26.3

[1] Summed concentration of 8 major lignin phenols (mg/g OC; Hedges & Ertel, 1982).

[2] Ratio of syringyl-to-vanillyl phenols.

[3] Ratio of cinnamyl-to-vanillyl phenols.

[4] Acid-to-aldehyde ratio of vanillyl phenols.

[5] Acid-to-aldehyde ratio of syringyl phenols.

[6] Summed concentration of *n*-alkanes $C_{25, 27, 29, 31, 33, 35}$ (mg/g OC).

[7] Average Chain Length (ACL): concentration-weighted mean carbon chain length for plant wax lipids C_{21-31} or C_{22-32}.

[8] Carbon Preference Index (CPI) for *n*-alkanes C_{21-31}.

[9] Summed concentration of *n*-fatty acids $C_{24, 26, 28, 30, 32}$ (mg/g OC).

[10] Summed concentration of *n*-alkanols $C_{24, 26, 28, 30, 32}$ (mg/g OC).

[11] Summed concentration of *n*-aldehydes $C_{24, 26, 28, 30, 32}$ (mg/g OC).

n.a. = not analyzed.

Table 4: Mass and radiocarbon contents of lignin phenols isolated by HPLC relative to the nominal F_m values of bulk OC.

Source	Lignin phenol	Mass (μg C)	Measured values on phenols isolated by HPLC: AMS-corrected only F_m	AMS-corrected only error	Procedural blank-corrected[1] F_m	Procedural blank-corrected[1] error	Nominal F_m of bulk OC[2]	ΔF_m (AMS -corrected only)	ΔF_m (procedural blank -corrected)
Commercial[3]	Vanillic acid	182	0.0105	0.0005	0.0053	0.0018	0.0040	0.0065	0.0013
Commercial[3]	Acetovanillone	163	0.0297	0.0007	0.0241	0.0020	0.0030	0.0267	0.0211
FIRI-A	Vanillin	224	0.0157	0.0005	0.0115	0.0015	0.0033	0.0124	0.0082
C-5	Vanillin	199	0.2426	0.0018	0.2402	0.0022	0.2305	0.0121	0.0097
	Acetovanillone	34	0.2533	0.0027	0.2390	0.0079		0.0228	0.0085
FIRI-D	Vanillic acid	71	0.5573	0.0018	0.5595	0.0035	0.5705	-0.0132	-0.0110
	Acetovanillone	73	0.5540	0.0018	0.5561	0.0034		-0.0165	-0.0144
	Vanillin	191	0.5683	0.0040	0.5692	0.0042		-0.0022	-0.0013
FIRI-H	Vanillin	281	0.7468	0.0034	0.7487	0.0035	0.7574	-0.0106	-0.0087
	Syringaldehyde	184	0.7473	0.0046	0.7502	0.0048		-0.0101	-0.0072
FIRI-J	Vanillin	130	1.1191	0.0084	1.1291	0.0090	1.1069	0.0122	0.0222
	Ferulic acid	226	1.0803	0.0059	1.0857	0.0062		-0.0266	-0.0212
	Acetosyringone	78	1.0836	0.0026	1.0995	0.0055		-0.0233	-0.0074
	p-Coumaric acid	82	1.0810	0.0023	1.0961	0.0052		-0.0259	-0.0108
Commercial[4]	Vanillin	152	1.1257	0.0076	1.1343	0.0081	1.1213	0.0044	0.0130

[1] Procedural blank contains 2.0 ± 0.5 μg C with F_m = 0.48 ± 0.10.

[2] Nominal values were measured on authentic phenol standards (purchased from Acros or Sigma) and were pre-determined for bulk plant tissues. FIRI-A, C-5, FIRI-D, FIRI-H, and FIRI-J are plant tissues as international standards.

[3] Obtained from Acros.

[4] Obtained from Sigma.

Figure Captions

Fig. 1: Scheme of extraction and isolation of individual lignin phenols for radiocarbon measurement. Short names: Vl = vanillin; Sl = syringaldehyde; Vn = acetovanillone; Sn = acetosyringone; Vd = vanillic acid; Sd = syringic acid; *p*Cd = *p*-coumaric acid; Fd = ferulic acid.

Fig. 2: 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: *p*Bl = 4-hydroxybenzaldehyde; *p*Bn = 4-hydroxyacetophenone; Vl = vanillin; Sl = syringaldehyde; Vn = acetovanillone; Sn = acetosyringone; Vd = vanillic acid; Sd = syringic acid; *p*Cd = *p*-coumaric acid; Fd = ferulic acid.

Fig. 3: The $\delta^{13}C$ and $\Delta^{14}C$ values of individual lignin phenols (a) and lipids (b-e) in the Washington margin sediments (‰). All values are corrected for derivative carbon and procedural blanks with the errors propagated. Filled and open symbols represent samples in St 1 and 2, respectively. *The following data points for $\Delta^{14}C$ values are measured for composite samples of homologues in parentheses, with the point plotted at the most abundant homologue's chain length: C_{22} *n*-alkane ($C_{22, 24, 26}$), C_{25} *n*-alkane ($C_{21, 23, 25}$), St 2 C_{26} *n*-alkanol ($C_{22, 24, 26, 28, 30}$), C_{28} *n*-aldehyde ($C_{22, 24, 26, 28, 30}$). †Acetosyringone and di-hydroxybenzoic acid may have coelutes during irm-GC-MS analysis.

Fig. 4: Relationship between the F_m value of procedural blanks associated with the HPLC method

and the average offset between measured and nominal F_m values (ΔF_m) of phenol standards (listed in Table 4). The zero offset ($\Delta F_m = 0$) corresponds to an Fm of 0.48 for the HPLC method procedural blanks.

Fig. 5: Concentration-weighted average $\delta^{13}C$ and $\Delta^{14}C$ values of lignin phenols and plant wax lipids as compared with those of bulk OC in the Washington margin surface sediments (‰). All values are corrected for derivative carbon and procedural blanks with the errors propagated. Filled and open symbols represent samples in St 1 and 2, respectively. The $\delta^{13}C$ values are calculated for $C_{27, 29, 31}$ *n*-alkanes, $C_{26, 28, 30, 32}$ fatty acids, $C_{22, 24, 26, 28, 30}$ *n*-alkanols, $C_{22, 24, 26, 28, 30}$ *n*-aldehydes, and Λ_8 lignin phenols (except acetosyringone). The $\Delta^{14}C$ values of plant wax lipids is calculated or measured for $C_{27, 29, 31}$ *n*-alkanes, C_{26} fatty acid, $C_{22, 24, 26, 28, 30}$ *n*-alkanols, and $C_{22, 24, 26, 28, 30}$ *n*-aldehydes. *The $\Delta^{14}C$ values of lignin phenols are represented by the most abundant vanillyl phenols isolated by HPLC.

Fig. 1:

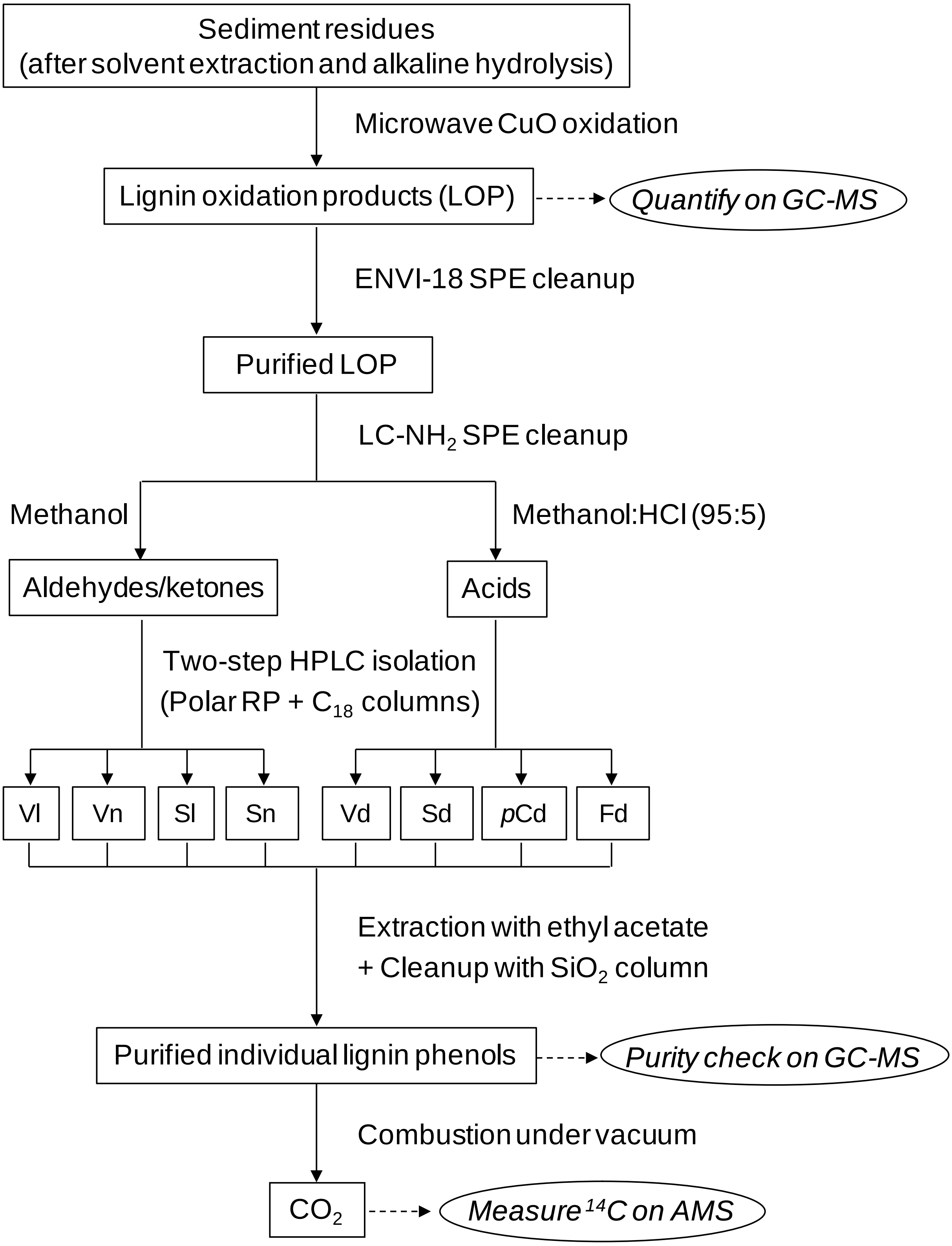

Sediment residues
(after solvent extraction and alkaline hydrolysis)
Microwave CuO oxidation
Lignin oxidation products (LOP)
Quantify on GC-MS
ENVI-18 SPE cleanup
Purified LOP
LC-NH_2 SPE cleanup
Methanol
Methanol:HCl (95:5)
Aldehydes/ketones
Acids
Two-step HPLC isolation
(Polar RP + C_{18} columns)
Vl
Vn
Sl
Sn
Vd
Sd
*p*Cd
Fd
Extraction with ethyl acetate
+ Cleanup with SiO_2 column
Purified individual lignin phenols
Purity check on GC-MS
Combustion under vacuum
CO_2
Measure ^{14}C on AMS

Fig. 2:

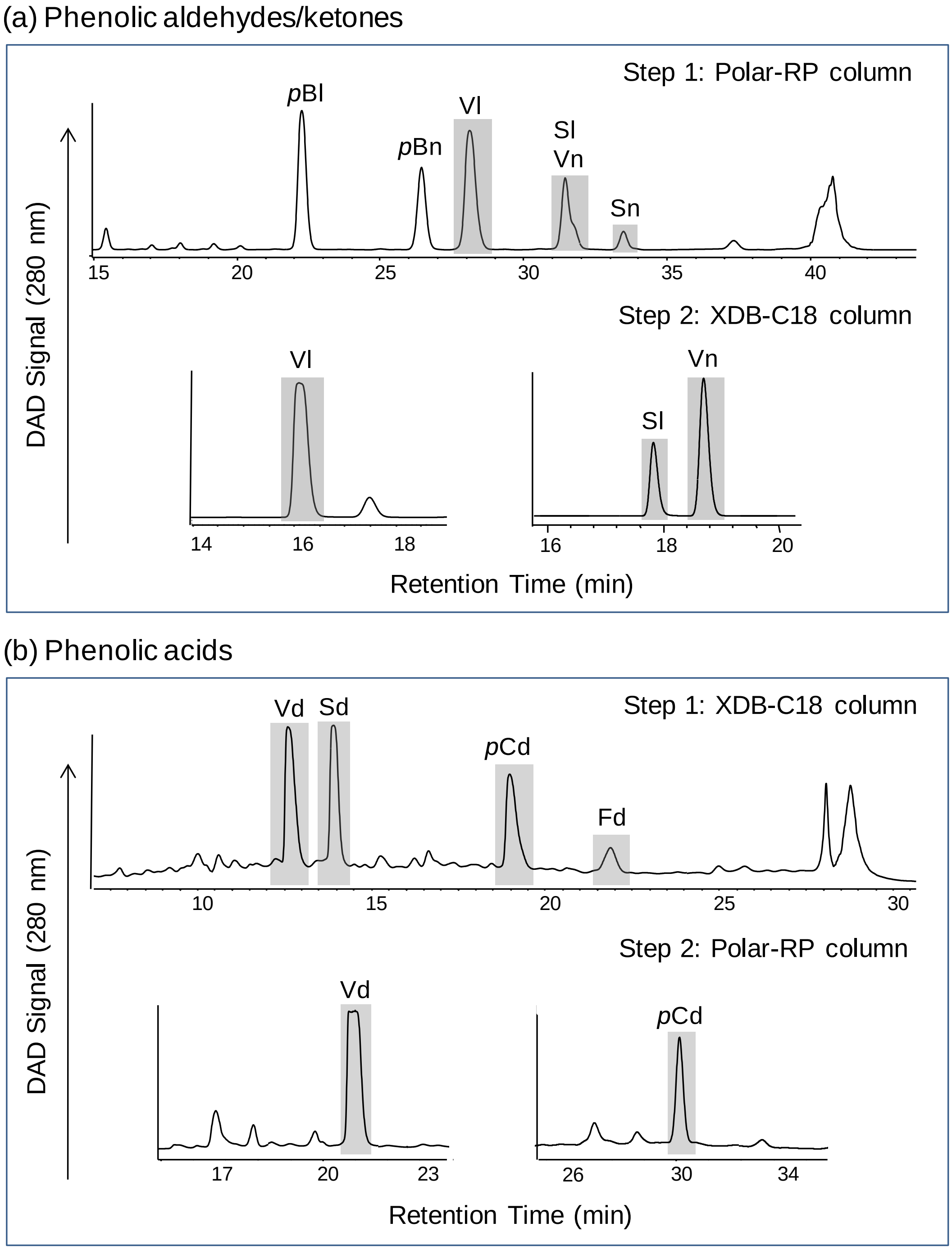
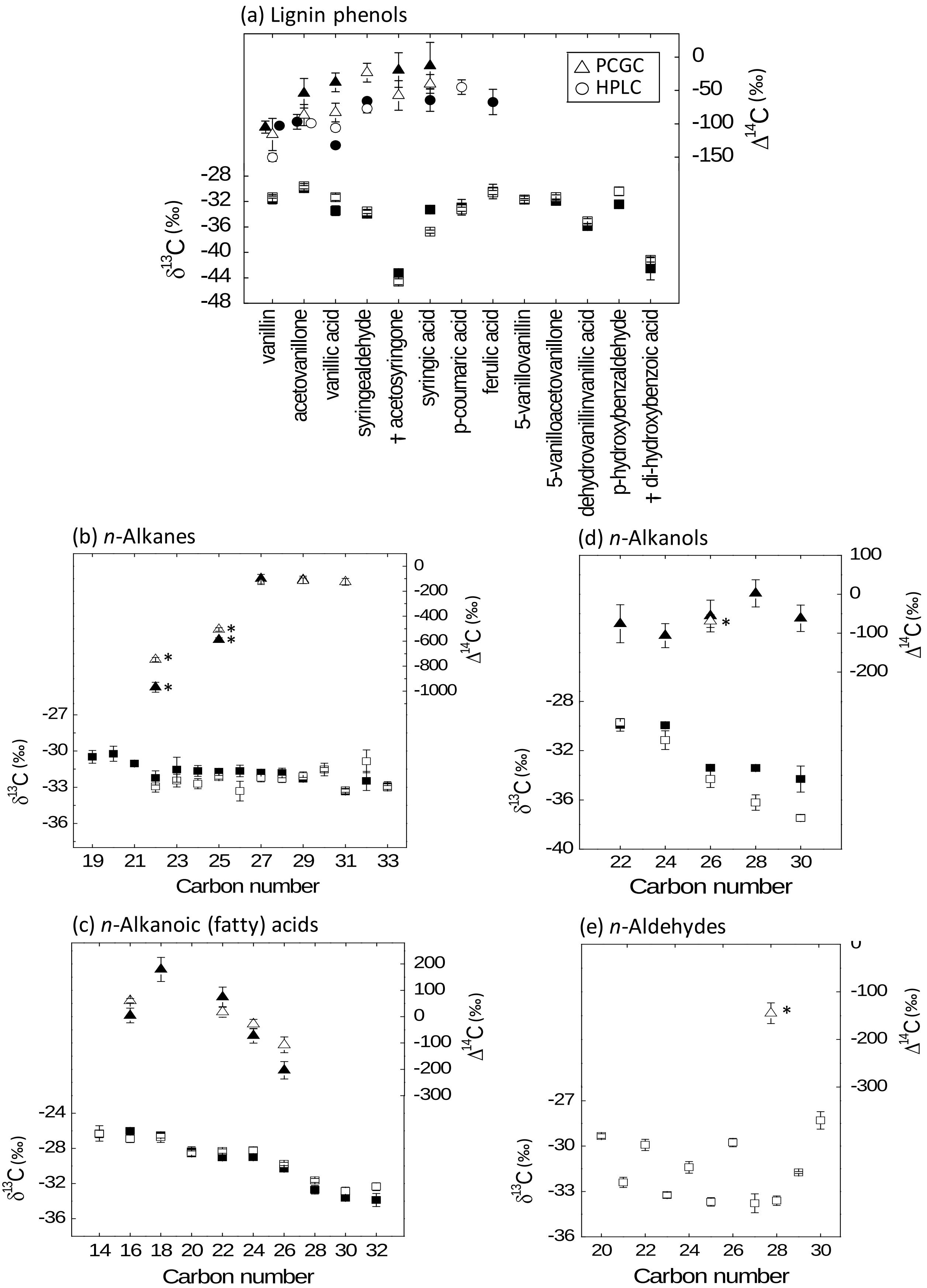
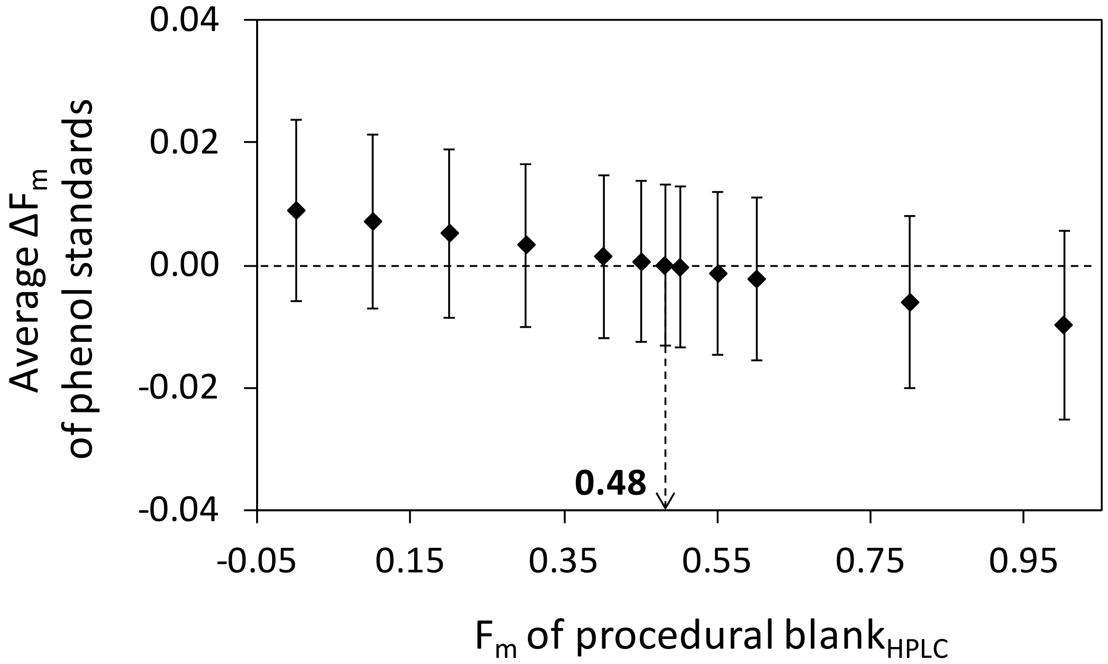

(a) Phenolic aldehydes/ketones
Step 1: Polar-RP column
pBl
pBn
Vl
Sl
Vn
Sn
15
20
25
30
35
40
Step 2: XDB-C18 column
Vl
14
16
18
Sl
Vn
16
18
20
DAD Signal (280 nm)
Retention Time (min)
(b) Phenolic acids
Step 1: XDB-C18 column
Vd
Sd
pCd
Fd
10
15
20
25
30
Step 2: Polar-RP column
Vd
17
20
23
pCd
26
30
34
DAD Signal (280 nm)
Retention Time (min)

Fig. 3:



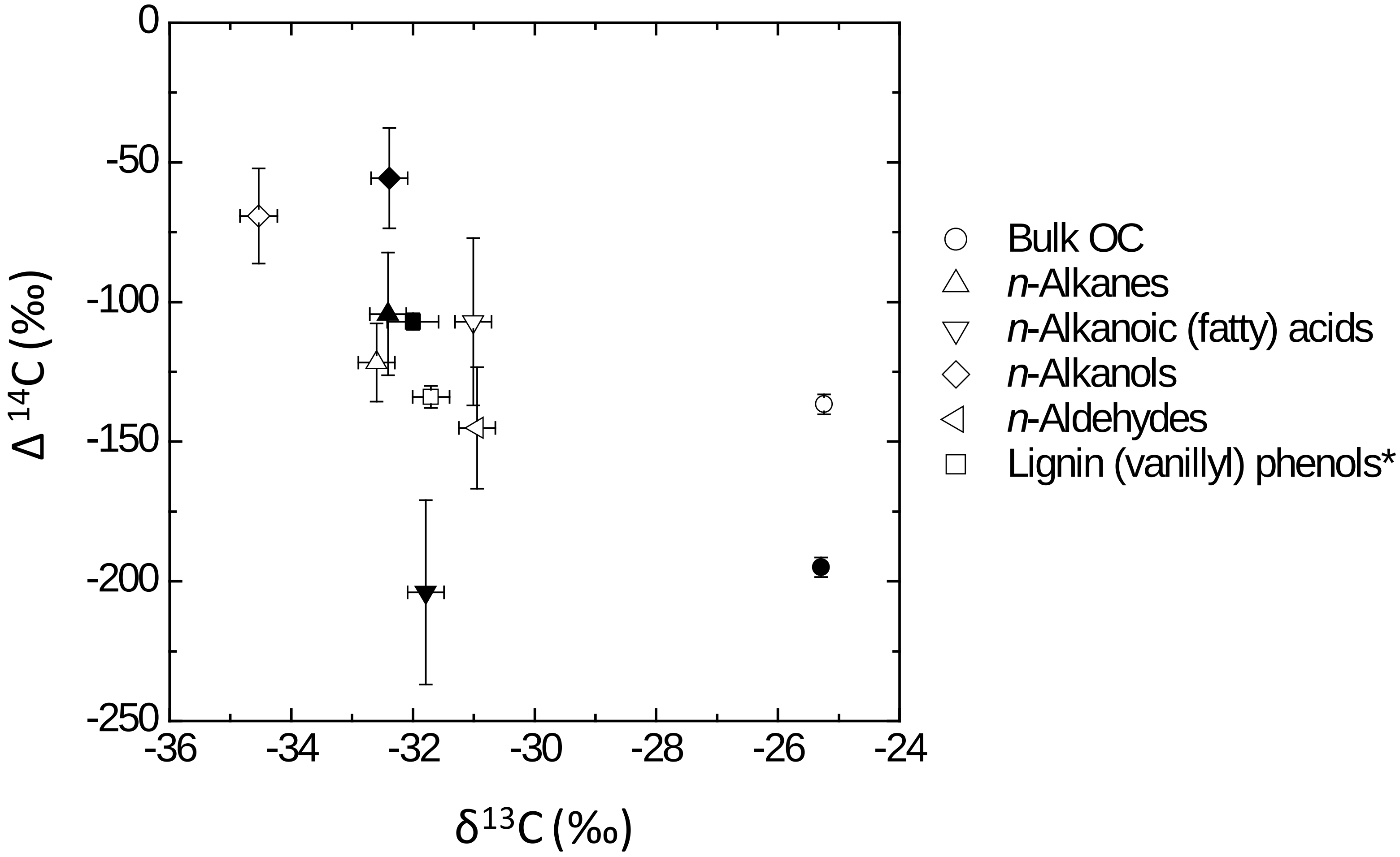
(a) Lignin phenols
PCGC
HPLC
δ13C (‰)
Δ14C (‰)
vanillin
acetovanillone
vanillic acid
syringealdehyde
+ acetosyringone
syringic acid
p-coumaric acid
ferulic acid
5-vanillovanillin
5-vanilloacetovanillone
dehydrovanillinvanillic acid
p-hydroxybenzaldehyde
+ di-hydroxybenzoic acid
(b) n-Alkanes
Carbon number
(c) n-Alkanoic (fatty) acids
Carbon number
(d) n-Alkanols
Carbon number
(e) n-Aldehydes
Carbon number

Fig. 4:



0.04
0.02
0.00
-0.02
-0.04
Average ΔF_m
of phenol standards
0.48
-0.05
0.15
0.35
0.55
0.75
0.95
F_m of procedural blank_HPLC

Fig. 5:



0
-50
-100
-150
-200
-250
-36
-34
-32
-30
-28
-26
-24
Δ ¹⁴C (‰)
δ¹³C (‰)
Bulk OC
n-Alkanes
n-Alkanoic (fatty) acids
n-Alkanols
n-Aldehydes
Lignin (vanillyl) phenols*

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

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Supplementary Information

Table S.1: Concentration, isolated mass, and corrected $\Delta^{14}C$ values of individual lignin phenols and lipids in the Washington margin sediments.

Compound	Concentration in sediments[1]		PCGC-based measurement						HPLC-based measurement					
			St 1			St 2			St 1			St 2		
	St 1	St 2	µg C	$\Delta^{14}C$ (‰)	Error (‰)	µg C	$\Delta^{14}C$ (‰)	Error (‰)	µg C	$\Delta^{14}C$ (‰)	Error (‰)	µg C	$\Delta^{14}C$ (‰)	Error (‰)
Lignin phenols														
Vanillin	3.3	2.5	150	-105	9	353	-116	24	235	-103	3	74	-150	6
Acetovanillone	0.8	0.6	36	-54	22	143	-87	16	43	-97	11	90	-99	5
Vanillic acid	0.8	0.7	66	-38	14	65	-83	14	122	-132	5	134	-106	5
Syringaldehyde	0.6	0.7				117	-23	14	132	-66	4	80	-77	7
Acetosyringone	0.2	0.2	27	-19	26	59	-57	22						
Syringic acid	0.1	0.2	20	-13	35	65	-40	14	22	-64	17			
p-Coumaric acid	0.1	0.1										46	-45	11
Ferulic acid	0.1	0.1							28	-67	19			
***n*-Alkanes**														
$C_{21, 23, 25}$			24	-588	15	29	-506	14						
$C_{22, 24, 26}$			17	-969	40	20	-747	17						
C_{27}	19	15	20	-100	34	28	-125	22						
C_{29}	23	26	23	-108	28	37	-117	23						
C_{31}	13	21				23	-125	27						
***n*-Alkanoic (fatty) acids**														

C_{16}	184	146	44	4	28	85	60	8						
C_{18}	58	50	18	179	46									
C_{22}	53	33	19	74	38	52	18	20						
C_{24}	84	58	29	-73	28	70	-28	18						
C_{26}	45	30	19	-204	33	32	-107	30						
***n*-Alkanols**														
C_{22}	10	7	15	-76	49									
C_{24}	15	14	20	-106	31									
C_{26}[2]	21	30	17	-56	41	87	-69	16						
C_{28}	18	21	22	2	35									
C_{30}	11	18	26	-62	34									
***n*-Aldehydes**														
$C_{22, 24, 26, 28, 30}$						66	-145	22						

[1] Concentration in sediments in the units of mg/100 mg OC for lignin phenols and µg/g OC for lipids; concentration is not provided for combined compounds.

[2] $C_{22, 24, 26, 28, 30}$ *n*-alkanols from St 2 were combined.

Table S.2: Recovery of phenol standards from two-SPE cleanup procedures (concentration assessed before and after SPE procedures on HPLC respectively; compounds sorted in elution order from HPLC). F1: aldehyde/ketone fraction; F2: acid fraction from LC-NH_2 SPE. nd: not detected.

Phenol	**1st assessment**		**2nd assessment**	
	F1	**F2**	**F1**	**F2**
pBd	nd	110%	nd	91%
Vd	nd	105%	nd	69%
Sd	nd	107%	nd	69%
pBn	102%	nd	90%	2%
Vl	78%	1%	65%	1%
pCd	nd	102%	nd	80%
Sl	75%	2%	70%	1%
Vn	80%	nd	78%	nd
Sn	103%	nd	89%	nd
Fd	nd	98%	nd	90%

Fig. S.1: GC-MS total ion chromatogram of lignin phenols isolated by HPLC from the Washington margin surface sediment, St 1 (analyzed as TMS derivatives).