Anomalously low radiocarbon content of modern *n*-alkanes

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Abstract

Compound-specific radiocarbon (^{14}C) ages of lipid biomarkers commonly pre-date co-occurring macrofossils or sediment matrices in lacustrine and marine sedimentary sequences. Hypothetically, this age offset is the result of long residence and transport times of organic matter in the terrestrial realm and is referred to as 'pre-aging'. Here we measure the ^{14}C content of *n*-alkanes extracted from living leaf tissue of six different plant species. In all samples, the modern *n*-alkanes contained anomalously low ^{14}C content resulting in apparent radiocarbon ages ranging from ~530 to 1580 cal yr B.P. while bulk tissue samples yielded the expected modern ^{14}C ages. These results indicate a potentially strong fractionation against ^{14}C during lipid biosynthesis also documented for ^{13}C and ^{2}H stable isotope systems. This fractionation could, at least partially, explain observed age offsets between lipid biomarkers and co-occurring sediments.

1. Introduction

Numerous studies have documented large offsets in the radiocarbon (^{14}C) ages of terrestrially derived (long-chain) lipid biomarkers and co-occurring macrofossils or sediment matrices in lacustrine and marine sedimentary sequences. These age offsets have been used to estimate mean residence times of carbon in the terrestrial realm in carbon cycling budgets (e.g., Pearson and Eglinton, 2000; Pearson et al., 2001; Blair et al., 2003; Smittenberg et al.,

2006; Drenzek et al., 2007; Kusch et al., 2010) and as indicators of remobilization of sequestered carbon due to changing climate or land use (e.g., Feng et al., 2013; Gierga et al., 2016; Douglas et al., 2018). Increasingly, lipid biomarker ^{14}C ages are also used to establish chronologies for sediment records used in proxy-based analyses of paleoenvironmental change, particularly when the environmental proxies of interest include lipid biomarkers (Uchikawa et al., 2008; Douglas et al., 2014; Haggi et al., 2014). In these applications, terrestrially derived lipid biomarkers typically pre-date co-occurring macrofossils or sediment matrices by anywhere from a few centuries to several millennia.

The use of compound-specific ^{14}C ages to establish sedimentary chronologies poses a unique theoretical complication for proxy-based environmental reconstructions. Leaf-derived lipids, particularly long-chain *n*-alkanes, undergo processes in their transport to sedimentary basins (wind abrasion, aeolian transport, overland flow) that are similar to those of many other commonly employed proxies of environmental change, such as fossil pollen, charcoal, macrofossils, and bulk terrestrial organic matter (Simoneit et al., 1977; Schefuß et al., 2003), but the ^{14}C ages of terrestrially derived lipids routinely pre-date the inferred ages of these other proxies. There is nothing particularly unique about the mobilization, deposition, or chemistry of lipid biomarkers relative to many other terrestrial proxies, so the commonly observed age offset between these sedimentary components is perplexing. Based on strong temporal relationships between bulk sedimentary $\delta^{13}C$ and long-chain *n*-alkane $\delta^{13}C$ values, Lane et al. (2016) proposed that such age offsets between biomarkers and other proxies may be rare in small watersheds, yet they are found in some small watersheds and remain insufficiently explained in some larger watersheds.

Here we offer an alternative to invoking differential residence and transport for lipid biomarkers as compared to other terrestrially derived materials as the sole explanation for offsets in apparent age. We hypothesize that terrestrial plants strongly fractionate against ^{14}C

during lipid biosynthesis, a process that would result in artificially 'old' ^{14}C ages at the time of formation. Large apparent isotopic offsets (ε values) of >10‰ VPDB for $\delta^{13}C$ and >50‰ VSMOW for $\delta^{2}H$ between lipid biomarkers and bulk leaf tissues illustrate the potential for strong biosynthetic fractionations (Sachse et al., 2012; Diefendorf and Freimuth, 2017). We tested our hypothesis by measuring the ^{14}C content of *n*-alkanes isolated from six modern plant species and comparing results with the ^{14}C content of the modern plant tissue.

2. Study Site

We selected our study site to minimize potential variations in atmospheric carbon dioxide concentrations and isotopic composition. All plants were sampled along the eastern shore of Jones Lake in Bladen County, North Carolina (34°40'58"N, 78°35'50"W, 21 m), within a radius of 300 m. Jones Lake is located ~80 km from the nearest medium-sized city of Wilmington, NC and is surrounded by low-density traffic conditions, meaning localized fossil-fuel contributions to atmospheric CO_2 should be minimal.

3. Materials and Methods

Living leaf tissues from individuals of *Liquidambar styraciflua* L. (C_3 angiosperm tree) and *Quercus nigra* L. (C_3 angiosperm tree) were sampled in May 2016 and from *Magnolia virginiana* L. (C_3 angiosperm tree), *Quercus virginiana* Mill. (C_3 angiosperm tree), *Aristida stricta* Michx. (C_4 graminoid), and *Digitaria sanguinalis* (L.) Scop. (C_3 graminoid) in May 2019. All leaf tissues were collected at or below 3 m height. Only newly developed leaves were sampled. Leaf material was rinsed gently with distilled water and oven dried at 50 °C for a week. Lipids were extracted three times with hexane in Teflon bottles overnight on a shaker table. The aliphatic fraction of the lipid extract was purified using solid phase extraction through a silica-gel column with hexane. *n*-Alkanes were further isolated via

repeated (2x) urea adduction. A hexane blank was processed identically to assess potential carbon contamination during extraction and purification. We avoided use of freeze driers, accelerated solvent extractors, or additional solvents to minimize potential contamination of samples with fossil fuel-derived carbon. All glassware was pre-combusted prior to use.

Sample *n*-alkane purity and abundance were assessed via comparison with a *n*-C_7–C_{40} alkane mixture (Sigma Aldrich) on a Thermo 1310 gas chromatograph equipped with an ISQ quadrupole mass spectrometer and flame ionization detector. Homologue mass was determined via comparison to a five-point calibration curve derived from the *n*-C_7–C_{40} alkane mixture (Sigma Aldrich). Samples were injected (splitless) using a Thermo TriPlus autosampler into dual PTV inlets operating at 300 °C. Homologue separation was accomplished using Thermo TG-5 SILMS silica columns (30 m, 0.32 mm i.d., 0.32 μm film thickness). The oven temperature program for the GC was 70 °C isothermal for 1 minute, 20 °C/min to 180 °C, 4 °C/min to 320 °C, 320 °C isothermal for 5 minutes, 30 °C/min to 350 °C, and 350 °C isothermal for 1 minute.

The $\Delta^{14}C$ and $\delta^{13}C$ measurements were conducted on mixtures of all *n*-alkane homologues extracted from each species. This approach assured abundant carbon for the $\Delta^{14}C$ measurement, thereby increasing measurement precision in comparison to $\Delta^{14}C$ determinations on individual homologues. The blank extract and *n*-alkane homologue mixtures extracted from leaf samples collected in 2016 and 2019 and bulk leaf tissues from samples collected in 2019 were submitted to the National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) facility at the Woods Hole Oceanographic Institution for ^{14}C measurement. Pre-modern radiocarbon ages were calibrated using Calib 8.2 (Stuiver et al., 2020) and the IntCal 20 calibration dataset (Reimer et al., 2020). Weighted mean calibrated ages were used for single age estimates (Telford et al., 2004). The $\delta^{13}C$ values for alkane mixtures are those reported by NOSAMS measured on a dual-inlet stable isotope mass

spectrometer. The $\delta^{13}C$ composition of bulk leaf tissues were measured using a Costech 4010 elemental analyzer interfaced with a Delta V Plus stable isotope mass spectrometer at the University of North Carolina Wilmington.

4. Results and Discussion

The GC-FID quantification of all sample mixtures indicated >90% purity of *n*-alkanes by mass in all samples except *Magnolia virginiana*, which was 76.6% alkane by mass, but in which a series of late-eluting compounds comprised ~20% of the sample mass (Table 1). The late-eluting compounds are not urea contamination as urea elutes much earlier in the run using our analytical setup (Figure 1). Instead, we suspect these compounds are wax-derived fatty-acids based on characteristic mass 313, 341, and 369 fragments (m/z), poor chromatography typical of underivitized wax components, and the late elution. Given the EI source a molecular ion was not definitive. Considering long-chain ($>C_{27}$) alkanes are ~85% carbon by mass, the vast majority of the carbon from all mixtures measured for $\Delta^{14}C$ was *n*-alkane derived. The $\delta^{13}C$-corrected radiocarbon measurements of the *n*-alkanes yielded weighted mean calibrated radiocarbon ages spanning from 533 to 1580 cal yr B.P., while the four bulk leaf tissue samples analyzed contained modern abundances of ^{14}C (Table 2). These results indicate that biosynthetic fractionation during *n*-alkane synthesis discriminates strongly against ^{14}C and the variability in ^{14}C content between samples indicates that this discrimination is potentially species-specific.

Further, there is no significant linear relationship between $\Delta^{13}C_{\text{bulk leaf tissue-alkane}}$ and $\Delta^{14}C$ ($r = 0.28$, $F = 0.35$, $p = 0.59$), indicating that a key assumption of $\delta^{13}C$ corrections of radiocarbon ages may not be applicable to *n*-alkane radiocarbon age estimates. This basic assumption is that ^{14}C is assimilated into biological tissues at roughly half the concentration

of ^{13}C relative to ^{12}C (Stuiver and Robinson, 1974; Stuiver and Polach, 1977). Based on our results, it appears this ratio may be larger for *n*-alkanes and potentially other lipids.

The only potential explanation for the low radiocarbon content of the modern *n*-alkanes other than biosynthetic fractionation is that they were contaminated by ^{14}C-deplete compounds, which would most likely need to be fossil fuel-derived. We find this explanation unlikely for several reasons. First, we intentionally designed our *n*-alkane extractions to eliminate steps thought to be most prone to introducing 'old carbon' to biomarker samples, such as contamination by pump oil during lyophilization, introduction of stationary phase material from a GC column (i.e. column bleed) when using a preparative fraction collector, cross contamination between samples on an accelerated solvent extraction system, and use of high boiling point solvents for extractions (e.g., methanol). We attempted *n*-alkane purifications avoiding urea adduction by using the molecular sieve approach of Grice et al. (2008), but could not replicate their high *n*-alkane recoveries. Second, based on mass balance calculations, a radiocarbon age that is artificially 1500 years older than modern would require roughly 20% of the measured carbon to be fossil-fuel derived (Olsson and Osadebe, 1974). With the exception of the *M. virginiana* sample, our GC-FID data indicate that no more than 10% of our sample carbon could be derived from radiocarbon 'dead' compounds. It is possible that very low or very high boiling point compounds other than *n*-alkanes were present in the samples, but eluted outside of our GC-MS and GC-FID analytical windows or did not elute from our non-polar column. However, any low boiling point compounds would have likely been removed from the sample during vacuum line purification procedures prior to radiocarbon measurements at NOSAMS and any high boiling point compounds would still have likely originated from the plant sample, hence they would not explain the anomalously low radiocarbon content of the sample. Finally, the blank hexane sample put through all of the same extraction steps as the plant samples did not yield adequate CO_2 for radiocarbon

152 determination, indicating little or no radiocarbon contamination during the extraction process.
153 Thus, while minor contamination of our samples by old carbon is certainly possible, such
154 contamination is unlikely to wholly explain such large age offsets between the *n*-alkane
155 extracts and bulk leaf tissues.

156 No systematic assessments of ^{14}C incorporation into terrestrially derived lipids have
157 been conducted to date. Further comparisons of lipid and leaf ages, and assessments of
158 potential carbon contamination during biomarker extraction and isolation, are both needed.
159 Assessment of potential ^{14}C contamination using a variety of extraction and purification
160 protocols is currently underway by our research group. We present these pilot data to
161 stimulate further research into potential species-specific fractionation of ^{14}C by plants during
162 lipid biosynthesis, and as a cautionary note to researchers using compound-specific
163 radiocarbon ages to temporally constrain carbon cycle dynamics in modern systems, or to
164 construct sediment age-depth models.

165

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Figure Caption

Figure 1. Example flame ionization detector chromatograms of *n*-alkane extracts from a modern leaf of *Aristida stricta* (A), *Magnolia virginiana* (B), and sediments from a lake in Haiti. Note the lack of any non-alkane compounds in the *A. stricta* sample (A), the late eluting compounds in the *M. virginiana* sample (B), and the urea contamination in the lake sediment sample from Haiti (C).

Table 1. Percent *n*-alkane composition (by mass) of purified plant leaf extracts for six modern plant specimens from Jones Lake, NC.

Species	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}	C_{35}	Total *n*-Alkane	Other Compounds
Liquidambar styraciflua L.	0.0	0.0	0.2	0.0	2.5	0.6	34.5	2.0	41.9	2.2	14.5	0.2	1.2	98.1	1.9
Magnolia virginiana L.	6.1	1.5	5.2	3.9	25.8	4.8	25.8	0.7	1.4	0.2	1.0	0.1	0.1	76.6	23.4
Quercus nigra L.	0.1	0.1	0.5	0.1	2.4	1.4	81.9	0.9	12.1	0.1	0.1	0.0	0.0	99.2	0.8
Quercus virginiana Mill.	0.3	0.5	3.3	1.2	7.7	2.6	55.0	2.5	15.2	0.5	0.2	0.1	2.0	91.0	9.0
Aristida stricta Michx.	1.4	2.2	7.0	5.2	16.3	6.3	19.1	3.8	24.4	1.5	10.9	0.3	1.0	99.4	0.6
Digitaria sanguinalis (L.) Scop.	0.9	0.3	0.9	0.7	1.6	0.6	3.1	1.6	32.3	2.5	42.8	1.2	7.2	95.6	4.4

Table 2. Bulk leaf tissue and *n*-alkane stable carbon isotope ($\delta^{13}C$), radiocarbon ($\Delta^{14}C$), and calibrated radiocarbon age data for six modern plant specimens from Jones Lake, NC.

Species	Plant Type	Material	$\delta^{13}C$	Lab Number	$\Delta^{14}C$	Age (^{14}C yr B.P.)	1σ	Calibrated Age Range +/– 2σ (cal yr B.P.)	Area Under Curve	Weighted Mean Calibrated Age (cal yr B.P.)
Liquidambar styraciflua L.	Angiosperm Tree (C_3)	Bulk Leaf Tissue	–31.4	—	—	—				—
		n-Alkanes	–34.7	OS-137262	–138.80	1,140	15	1070–973	0.98	1027
								1174–1169	0.02	
Magnolia virginiana L.	Angiosperm Tree (C_3)	Bulk Leaf Tissue	–27.5	OS-152473	4.71	Modern				Modern
		n-Alkanes	–31.3	OS-151479	–191.15	1,640	15	1452–1417	0.17	1511
								1487–1474	0.07	
								1547–1513	0.77	
Quercus nigra L.	Angiosperm Tree (C_3)	Bulk Leaf Tissue	–28.7	—	—	—				—
		n-Alkanes	–30.4	OS-137265	–195.95	1,690	65	1462–1413	0.10	1580
								1711–1468	0.90	
Quercus virginiana Mill.	Angiosperm Tree (C_3)	Bulk Leaf Tissue	–29.3	OS-152472	5.34	Modern				Modern
		n-Alkanes	–32.7	OS-151478	–84.23	640	20	597–556	0.57	605
								660–625	0.43	
Aristida stricta Michx.	Graminoid (C_4)	Bulk Leaf Tissue	–14.1	OS-152470	3.64	Modern				Modern
		n-Alkanes	–25.2	OS-151476	–141.41	1,160	15	1031–993	0.30	1073
								1124–1052	0.54	
								1175–1163	0.16	
Digitaria sanguinalis (L.) Scop.	Graminoid (C_3)	Bulk Leaf Tissue	–29.8	OS-152471	–0.39	Modern				Modern
		n-Alkanes	–36.3	OS-151477	–70.49	520	15	545–518	1.00	533

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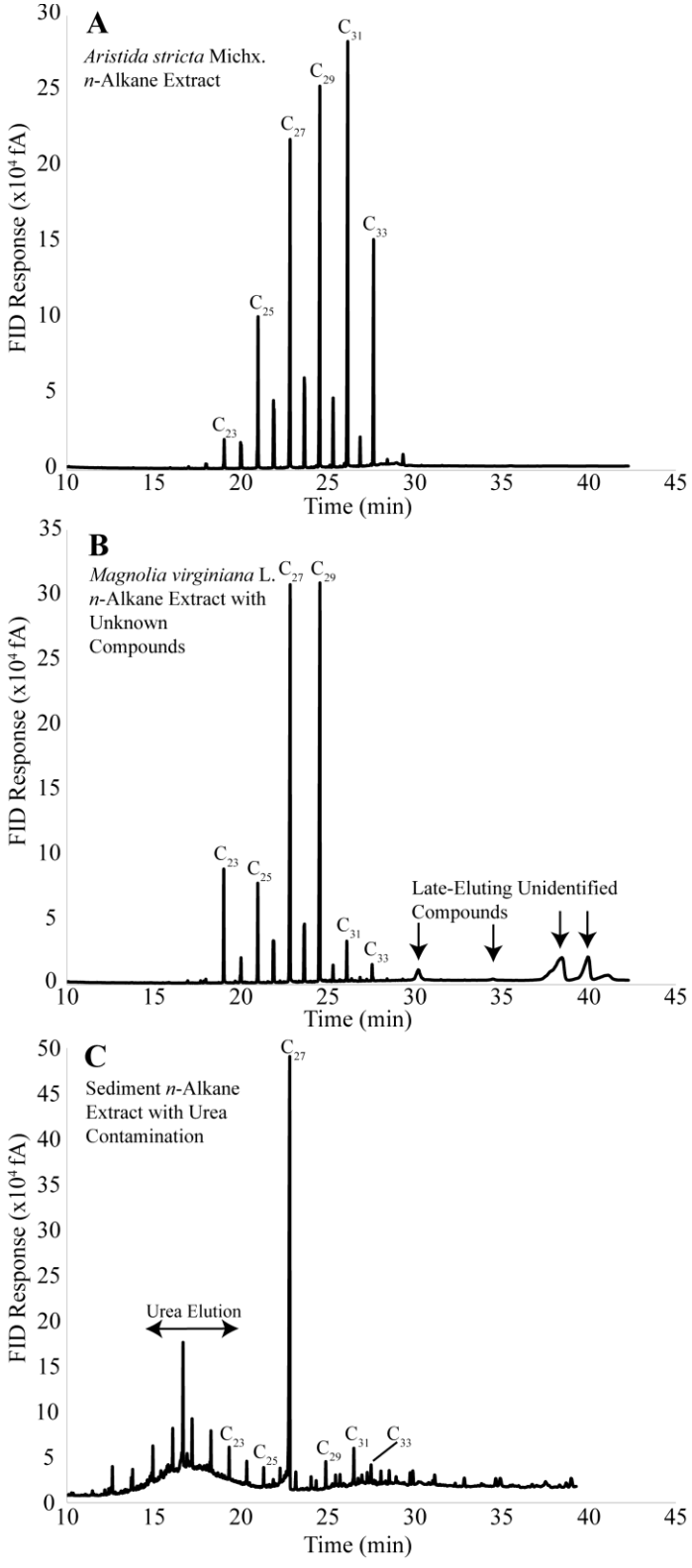
A
Aristida stricta Michx.
n-Alkane Extract
C23
C25
C27
C29
C31
C33
FID Response (x10⁴ fA)
Time (min)
B
Magnolia virginiana L.
n-Alkane Extract with
Unknown
Compounds
Late-Eluting Unidentified
Compounds
C
Sediment *n*-Alkane
Extract with Urea
Contamination
Urea Elution

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