Modern *n*-Alkane Abundances and Isotopic Composition of Vegetation in a
Gymnosperm-Dominated Ecosystem of the Southeastern U.S. Coastal Plain

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Abstract

Prior analyses of *n*-alkyl lipids in vegetation indicate low concentrations of *n*-alkanes in gymnosperm leaves and significant offsets in lipid carbon isotope values ($\delta^{13}\text{C}$) of angiosperms and gymnosperms. This finding has implications for paleoenvironmental studies utilizing *n*-alkyl lipid proxies in gymnosperm-dominated ecosystems, such as the pine ecosystems of the U.S. southeastern coastal plain. I provide here the *n*-alkane abundances and distributions for 19 of the most dominant taxa in the longleaf pine ecosystem along with their $\delta^{13}\text{C}$ values. Additionally, I assess modern relationships between stable hydrogen isotope values of *n*-alkanes ($\delta^2\text{H}_{\text{alkane}}$) and modern precipitation to constrain apparent fractionations (ϵ) necessary to conduct regional paleoclimate reconstructions. Gymnosperms of the coastal plain have extremely low *n*-alkane abundances relative to angiosperms and are unlikely to contribute significantly to sedimentary archives. Lake surface sediment *n*-alkane distributions, $\delta^2\text{H}$, and $\delta^{13}\text{C}$ values indicate that they are primarily sourced from the less-dominant angiosperms. Apparent $\delta^2\text{H}$ fractionations between *n*-C₂₉ alkanes and mean annual precipitation are $\sim 10.0\%$ VSMOW more positive for gymnosperms relative to angiosperms. Apparent $\delta^{13}\text{C}$

fractionations between *n*-C₂₉ alkanes and bulk leaf tissue are ~1.5‰ VPDB more positive for gymnosperms relative to angiosperms, but with overlap.

1. Introduction

Compound-specific hydrogen ($\delta^2\text{H}$) and carbon ($\delta^{13}\text{C}$) isotope measurements of *n*-alkyl lipids (e.g. *n*-alkanes) preserved in sedimentary archives combined with analyses of absolute and relative homologue abundances hold enormous potential as paleohydrological and paleoecological proxies (Sachse et al., 2012). However, applying such proxies requires an understanding of species-specific variations in homologue abundances and isotopic composition, which can vary widely between genera and species. The $\delta^2\text{H}$ value of *n*-alkanes ($\delta^2\text{H}_{\text{alkane}}$) has proven particularly variable between species, even within the same environment and using the same sourcewater, leading to enrichment factors (ϵ), or apparent fractionations, between the $\delta^2\text{H}_{\text{alkane}}$ value and that of the sourcewater that can vary by as much as 100‰ or more (Hou et al., 2007; Sachse et al., 2012). Thus, developing a more in-depth understanding of species-specific and site-specific lipid distributions and isotopic values is an essential first step to using these proxies to reconstruct paleoenvironments and allows for taxonomic correction of fossil $\delta^2\text{H}_{\text{alkane}}$ data using other vegetation proxies such as paired fossil pollen data (Feakins, 2013).

Most studies of modern *n*-alkane distributions and isotopic composition have focused on temperate and tropical angiosperm-dominated ecosystems (e.g. Bi et al., 2005; Sachse et al., 2006; Hou et al., 2007) with less attention given to gymnosperm-dominated ecosystems such as those of the U.S. southeastern coastal plain.

Diefendorf et al. (2015) documented remarkably low abundances of *n*-alkanes in gymnosperms of the western U.S. along with significant variations in the $\epsilon_{\delta^{13}\text{C}}$ values between bulk leaf tissues and *n*-alkanes, which has implications for paleoecological reconstructions based on these proxies as angiosperms would theoretically be overrepresented in the biogeochemical record. Additionally, Polissar and Freeman (2010) documented relatively small $\epsilon_{\delta^2\text{H}}$ values ($\sim$ -90‰) between the $\delta^2\text{H}_{\text{alkane}}$ value of lacustrine surface sediments and the $\delta^2\text{H}$ of lakewater in temperate alpine forest ecosystems, but systematic analyses of species-specific variations in $\delta^2\text{H}_{\text{alkane}}$ values of gymnosperms are rare. In this study I document *n*-alkane abundances, distributions, $\delta^{13}\text{C}_{\text{alkane}}$, and $\delta^2\text{H}_{\text{alkane}}$ values for 19 of the most common species in the native *Pinus palustris* Mill. (longleaf pine) ecosystem of the southeastern U.S. coastal plain. Additionally, I establish $\epsilon_{\delta^2\text{H}}$ and $\epsilon_{\delta^{13}\text{C}}$ values via comparison to local precipitation $\delta^2\text{H}$ and bulk organic matter $\delta^{13}\text{C}$, respectively.

2. Study Site

To minimize variations in sourcewater and microclimate, I sampled all vegetation along the eastern shore of Jones Lake located in Bladen County, North Carolina (34°40'58"N, 78°35'50"W, 21 m). Jones Lake lies within Jones Lake State Park and has a surface area of 0.9 km². The climate of Jones Lake is humid, subtropical with mean annual precipitation of 125 cm. Precipitation is seasonal with maximum precipitation in June, July and August (41.6 cm). According to the Online Isotopes in Precipitation Calculator (Bowen and Revenaugh, 2003; Bowen, 2016), the mean annual $\delta^2\text{H}$ of precipitation at Jones Lake is -35‰ VSMOW and the

mean $\delta^2\text{H}$ of growing season (MAM) precipitation is -28‰ VSMOW. Riparian forest is primarily comprised of *Pinus serotina* Michx. (pond pine), *Chamaecyparis thyoides* (L.) Brtton, Sterns & Poggenb. (white cedar), and *Taxodium ascendens* Brongn. (pond cypress), with an understory that includes *Magnolia virginiana* L. (sweet bay). Upland native vegetation in the region is predominantly *Pinus palustris* Mill. (longleaf pine) forest with *Quercus laevis* Walter (turkey oak) and *Aristida stricta* Michx. (wire grass) as dominant understory taxa (Ross, 2003).

3. Materials and Methods

I sampled roughly equivalent amounts of living leaf tissue of two or more adult individuals, depending on availability, of each species in May 2016. Additionally, I sampled *P. palustris* litter and sapling (grass-stage) needles from three different locations in the study area. Leaf material was lyophilized and total lipids extracted three times in a 2:1 (v/v) mixture of dichloromethane and methanol in Teflon bottles overnight on a shaker table. The total lipid extract (TLE) was base saponified with 1.0 N KOH in 4:1 (v/v) MEOH:H₂O for 2 h at 80 °C. After the addition of 3.0 ml of distilled H₂O, neutral lipids were extracted using hexane. The aliphatic fraction was isolated from the saponified lipid extract by solid phase extraction through a silica-gel column with hexane. *n*-Alkanes were further isolated using urea adduction.

Undisturbed surface sediments were collected from Jones Lake in 2012 using a clear PVC pipe fitted with a rubber piston. The TLE was extracted from lyophilized and powdered sediments using an accelerated solvent extractor (Dionex

ASE 350) with 2:1 (v/v) dichloromethane/methanol with three extraction cycles at 10.34 MPa and 100 °C. Subsequently, *n*-alkanes were purified from the TLE using the same methods applied to plant samples.

I identified and quantified *n*-alkane abundances via comparison with a *n*-C₇–C₄₀ alkane mixture (Sigma Aldrich) on a Thermo 1310 gas chromatograph equipped with an ISQ quadrupole mass spectrometer and flame ionization detector. Samples were injected using a Thermo TriPlus autosampler into dual PTV inlets operating in splitless mode at 300 °C. Compounds were separated 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. Average chain length (ACL) and carbon preference index (CPI) were calculated using the equations provided by Diefendorf et al. (2015).

Apparent fractionations between *n*-alkanes and mean annual precipitation $\delta^2\text{H}$ ($\epsilon_{\text{C29}} \delta^2\text{H}/\text{MAP}$) and bulk organic matter $\delta^{13}\text{C}$ ($\epsilon_{\text{C29}} \delta^{13}\text{C}$) were calculated according to the equations provided by Sachse et al. (2012) and Diefendorf et al. (2015), respectively.

Compound-specific $\delta^{13}\text{C}$ and $\delta^2\text{H}$ analyses were performed on a Thermo Delta V plus mass spectrometer interfaced with a Thermo 1310 gas chromatograph (GC) via an Isolink II device using a Thermo TG-5 SILMS silica column (60 m, 0.25 mm i.d., 0.25 µm film thickness). Injection temperatures were 300 °C and samples were injected by a Thermo AI 1310 autosampler in splitless mode. The GC oven temperature program was identical to that for quantification. Alkane standards

(Indiana University mixture B4) were injected following every third sample to monitor precision. Data correction to the VSMOW and VPDB scale and standard error propagation calculations were conducted using the methods of Polissar and D'Andrea (2014). Bulk leaf tissues and sediments were lyophilized, ground to a fine powder, and analyzed for $\delta^{13}\text{C}$ composition and C/N ratios using a Costech 4010 elemental analyzer interfaced with a Delta V Plus stable isotope mass spectrometer.

4. Results and Discussion

The $n\text{-C}_{29}$ alkane has become the most utilized biomarker of terrestrial vegetation in paleoenvironmental studies (Sachse et al., 2012), so for brevity, I focus my discussion on the single homologue. As documented by Diefendorf et al. (2011; 2015) for a variety of western U.S. gymnosperms, dominant gymnosperms of the southeastern U.S. have n -alkane abundances, ACL, and CPI values that are orders of magnitude lower than co-occurring angiosperms with the exception of *Taxodium ascendens* (Table 1; Figure 1). The $\delta^2\text{H}_{\text{alkane}}$ values of gymnosperms are, on average, $\sim 10\text{‰}$ more enriched in ^2H than angiosperms, but overlap between the two groups does exist (Table 1; Figure 1). Polissar and Freeman (2010) documented more positive $\varepsilon_{\delta^2\text{H}}$ values between sedimentary n -alkanes and precipitation in lakes surrounded by gymnosperm-dominated ecosystems than those of angiosperm-dominated ecosystems around the globe. Combined with the direct analysis of individual gymnosperm $\delta^2\text{H}_{\text{alkane}}$ values presented here, these data indicate the potential need for minor differential corrections of sedimentary $\delta^2\text{H}_{\text{alkane}}$ records from gymnosperm vs. angiosperm-dominated communities and in situations where

temporal shifts in the relative dominance or sedimentary organic matter (SOM) contributions of the two groups occur. The $\delta^2\text{H}_{\text{alkane}}$ value and $\epsilon_{\delta^2\text{H}/\text{MAP}}$ of the grass *Aristida stricta* is more negative than any of the tree taxa and should also be considered when quantitatively correcting or interpreting sedimentary *n*-alkane $\delta^2\text{H}$ records with evidence of significant changes in arboreal cover as previously highlighted by Nelson et al. (2013). The mean $\delta^{13}\text{C}_{\text{alkane}}$ values of woody gymnosperms and angiosperms are nearly identical, but mean $\epsilon_{\text{C}_{29} \delta^{13}\text{C}}$ values are $\sim 1.5\text{‰}$ higher for gymnosperms than co-occurring angiosperms, except *Quercus* spp., which overlap considerably. The *n*-C₂₉ $\delta^{13}\text{C}$ value of the monocot grass *Aristida stricta* is more positive than any of the woody taxa as it utilizes the C₄ photosynthetic pathway (Davis et al., 2002; Figure 1).

Jones Lake surface sediment *n*-alkane abundances and ACL are more similar to that of surrounding angiosperms than gymnosperms or monocots (Table 1, Figure 1), indicating that despite gymnosperm abundance, the majority of allochthonous *n*-alkanes are originating from the angiosperms due to higher production of *n*-alkyl lipids. The low CPI of the Jones Lake sediments indicates a significant contribution of even-chained *n*-alkanes to the SOM pool, perhaps from algae or other autochthonous sources. Low C/N ratios in the surface sediments of Jones Lake (~ 18) indicate relatively evenly-mixed terrestrial and aquatic contributions to SOM.

The underrepresentation of gymnosperm *n*-alkyl lipids in the SOM pool must be considered when reconstructing environmental change using compound-specific proxies in modern or ancient gymnosperm-dominated ecosystems, further

highlighting the importance of alternate proxies of vegetation when using this approach. While gymnosperms appear to contribute minimally to the total SOM pool of Jones Lake, past decreases in gymnosperm abundance (Frey, 1953; Taylor et al., 2011) could have theoretically decreased mean sedimentary $\delta^2\text{H}_{\text{alkane}}$ values without any change in hydrology, but the amplitude of this change is likely to be small (<10‰) due to the minimal contribution of gymnosperm lipids to SOM. Considering the overlap in mean $\delta^{13}\text{C}_{\text{alkane}}$ values, the sedimentary $\delta^{13}\text{C}_{\text{alkane}}$ record is likely insensitive to changes in relative gymnosperm vs. angiosperm dominance.

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Figure Caption

Figure 1. Carbon preference indices (CPI), average chain lengths (ACL), *n*-C₂₉ alkane δ^2H and $\delta^{13}C$ values, along with apparent fractionations between *n*-C₂₉ alkanes and mean annual precipitation δ^2H ($\epsilon_{C29 \delta^2H/MAF}$) and bulk organic matter $\delta^{13}C$ ($\epsilon_{C29 \delta^{13}C}$), for 19 of the most dominant taxa in the longleaf pine ecosystem of southeastern North Carolina and Jones Lake surface sediments. Error bars represent 1 S.E. and vertical bars represent mean values for each plant group.

Table 1. Mean δ^2H and $\delta^{13}C$ values of *n*-C₂₉ alkanes, carbon preference indices (CPI), and average chain lengths (ACL) for Jones Lake, NC modern vegetation groupings and surface sediments.

	Mean δ^2H_{C29} Alkane (‰ VSMOW)	Mean $\delta^{13}C_{C29}$ Alkane (‰ VPDB)	CPI	ACL
Woody Gymnosperms	-123.3±7.2	-32.4±0.3	9.5±14.8	24.9±3.0
Woody Angiosperms	-133.2±14.2	-32.8±2.0	42.1±29.3	28.7±1.2
Monocot	-158.1	-24.2	10.0	29.5
Lake Sediment	-138.2±2.0	-33.8±1.0	5.2	27.2