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Lignin methoxyl hydrogen isotope ratios in a coastal ecosystem

Sarah J. Feakins [a,*], Patricia V. Ellsworth [b], Leonel da Silveira Lobo Sternberg [b]

[a] University of Southern California, Department of Earth Sciences, Los Angeles, CA 90089, USA
[b] University of Miami, Department of Biology, Coral Gables, FL 33124, USA

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Abstract

Stable hydrogen isotope ratios of plant lignin methoxyl groups have recently been shown to record the hydrogen isotopic composition of meteoric water. Here we extend this technique towards tracing water source variations across a saltwater to freshwater gradient in a coastal, subtropical forest ecosystem. We measure the hydrogen isotopic composition of xylem water (δD_{xw}) and methoxyl hydrogen ($\delta D_{methoxyl}$) to calculate fractionations for coastal mangrove, buttonwood and hammock tree species in Sugarloaf Key, as well as buttonwoods from Miami, both in Florida, USA. Prior studies of the isotopic composition of cellulose and plant leaf waxes in coastal ecosystems have yielded only a weak correlation to source waters, attributed to leaf water effects. Here we find $\delta D_{methoxyl}$ values range from −230‰ to −130‰, across a 40‰ range in δD_{xw} with a regression equation of $\delta D_{methoxyl}$ ‰ = 1.8 * δD_{xw} − 178‰ ($R^2 = 0.48$, $p < 0.0001$, $n = 74$). This is comparable within error to the earlier published relationship for terrestrial trees which was defined across a much larger 125‰ isotopic range in precipitation. Analytical precision for measurements of δD values of pure CH_3I by gas chromatography–pyrolysis–isotope ratio mass spectrometry (GC–P–IRMS) is $\sigma = 6‰$ ($n = 31$), which is considerably better than for CH_3I liberated through cleavage with HI from lignin with $\sigma = 18‰$ ($n = 26$). Our results establish that $\delta D_{methoxyl}$ can record water sources and salinity incursion in coastal ecosystems, where variations sufficiently exceed method uncertainties (i.e., applications with δD excursions >50‰). For the first time, we also report yields of propyl iodide, which may indicate lignin synthesis of propoxyl groups under salt-stress.

1. INTRODUCTION

The hydrogen isotopic composition of methoxyl hydrogen on lignin polymers in plant tissues have recently been shown to record the hydrogen isotopic composition of precipitation (Keppler et al., 2007). The advantages of this technique are that only C bound H from methoxyl groups are sampled avoiding problems of H exchange in hydroxyl H. Furthermore the technique is rapid providing analytical advantages over other techniques including position-specific analysis of both δD (Epstein et al., 1976) and oxygen isotopes ($\delta^{18}O$) in cellulose (Sternberg et al., 2007a), as well as organic geochemical methods for isolation and analysis of plant leaf wax biomarkers in plants (Sessions et al., 1999) and sediments (Sachse et al., 2004). Each of these techniques finds useful application to paleoclimate reconstructions; however in applications to coastal saline environments each has faced challenges. Prior studies have found that source water signals are not uniquely recorded in saline environments: isotopic effects in the leaf lead to overprinting with a leaf water signal resulting in confounded signals recorded in both the oxygen isotopic composition of cellulose (Ellsworth et al., 2013) and in the hydrogen isotopic composition of plant leaf waxes (Romero and Feakins, 2011; Ladd and Sachs, 2012). The new lignin methoxyl δD approach has not yet been tested in a saline environment.

We have previously considered whether plant leaf waxes record spatial variations in source waters across a saltwater to freshwater gradient (Romero and Feakins, 2011). In that study we found that while the δD_{xw} decreased inland as expected, leaf water (δD_{lw}) displayed an inverse relationship. Leaf water was the dominant influence on δD_{wax}, but was

* Corresponding author. Tel.: +1 213 740 7168.
E-mail address: feakins@usc.edu (S.J. Feakins).

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attenuated by the initial xylem water gradient. In that study, it was suggested that δD_{lw} varied with inundation, recording a larger enrichment at the most inland sites, counteracting the source water effects (hypothesis a). Another study found a similar pattern in mangrove δD_{wax} across a salinity gradient in an Australian estuary, where δD_{wax} does not follow river water δD, but instead displays an inverse relationship with salinity (Ladd and Sachs, 2012). The authors give several hypotheses including (b) increased fractionation against D in the roots during water uptake, (c) increased relative humidity at the leaf surface due to secretion of salty brines, (d) water of hydration of leaf salts being incorporated in leaf water, or (e) increased contributions of isotopically depleted hydrogen from NADPH at high salinity (Ladd and Sachs, 2012). While xylem water was not directly measured in that study, elsewhere plant waters in mangroves, provide evidence that hypothesis (b), fractionation in the roots, is unlikely to be of sufficient magnitude, being <10‰ in a study of varied halophytes and xerophytes (Lin and Sternberg, 1994; Ellsworth and Williams, 2007). Another study proposed hypothesis (f) that salt alters the pathlength of leaf water flow and may lead to ^{18}O-depletion in saline environments relative to freshwater plants (Ellsworth et al., 2013). Although the effect of pathlength was observed in the oxygen isotope ratios of stem alpha cellulose ($\delta^{18}O_{cell}$) and phenylglucosazone ($\delta^{18}O_{pg}$), the same processes could apply to other leaf-based isotope proxies. Thus there are multiple competing hypotheses for the common problems affecting leaf-based isotope proxies and we cannot distinguish further between them here. Whatever the mechanism, the salinity-induced complications in the leaf water isotopic enrichment would ideally be circumvented in order to resolve source water signals with a plant-based proxy.

1.1. Lignin methoxyl hydrogen isotope approach

The lignin methoxyl hydrogen isotope approach has been tested on terrestrial tree wood sampled including species from Scandinavia to Yemen across a wide (125‰) isotopic range (Keppler et al., 2007). Like the nitration method for cellulose (Epstein et al., 1976), the methoxyl approach samples only non-exchangeable hydrogen on lignin. Lignin is formed in the xylem tissues and as such may avoid the complications that affect leaf-based isotopic proxies in saline environments (see Section 1). Analytical methods for measuring methoxyl hydrogen include cleavage of the methoxyl group with hydroiodic acid (HI) to yield methyl iodide (Keppler et al., 2007). The methyl iodide hydrogen isotopic ratio is determined by gas chromatography isotope ratio mass spectrometry (Greule et al., 2008). More recently, the method was refined with the addition of a cold trap before the IRMS to exclude HI generated during pyrolysis (Feakins et al., 2013) similar to an approach used for the analysis of chlorinated hydrocarbons (Chartrand et al., 2007). $\delta D_{methoxyl}$ values have been shown to do better than bulk wood δD in recording the δD of source waters in terrestrial trees in a global survey (Keppler et al., 2007) and tested against conventional techniques in an alpine ecosystem (Gori et al., 2013). Here we assess the potential of the $\delta D_{methoxyl}$ technique in application to a saline ecosystem for the first time.

Specifically we examine the prospects for the new lignin methoxyl hydrogen isotope technique to serve as a proxy of source water in a coastal ecosystem in South Florida including mangroves, and coastal hardwood hammocks. These two communities, which are characteristic of South Florida and Caribbean coastal regions, span a source water salinity gradient ranging from that of seawater (mangrove communities) to freshwater (hardwood hammocks). This study will allow us to test the potential of the new technique in saline environments.

1.2. Natural and anthropogenic influences on saltwater–freshwater gradients in coastal ecosystems

The coastal ecosystems in the Florida Keys have been affected by salt water intrusion associated with continued sea level rise exacerbated by diversion of freshwater inflows (Saha et al., 2011). In addition, periodic influxes of ocean water occur during tropical storms (Ross et al., 1994). As a result, rising salinity is pushing the boundary between saltwater tolerant mangroves and saltwater intolerant hammocks inland (Ross et al., 1994). The effects of sea level rise, however, are complex. Plant physiological behavior can affect the salinity of the soil and lead towards progression towards one of two stable states characterized by either freshwater or mangrove communities in close proximity, with hammock species depending on lenses of freshwater perched above the saline groundwater (Sternberg et al., 2007b). Satellites (Niedzielski and Kosek, 2011), tide gauges (Church and White, 2006) and proxy reconstructions (Gehrels et al., 2006) are all valued means of recording and reconstructing sea level rise (IPCC, 2007). However, mean sea level does not fully describe the impacts on an ecosystem. Coastal plants can modulate the flow of seawater and are prone to storm surges, leading to a variable freshwater to saltwater boundary. Furthermore freshwater diversion can cause a rise in salinity through processes that accelerate the local impacts of sea level rise (Saha et al., 2011). Water isotopic evidence can be helpful to trace actual water use by plants in ecosystems suffering the effects of sea level rise, freshwater inflow reductions, and perhaps increasing hurricane intensity (Emanuel, 2007).

The difference in the isotopic composition of ocean and freshwater (Sternberg et al., 1991) controls the isotopic composition of water available to plants. If that source water isotopic signal is not overprinted by leaf water processes such as transpiration and the pathway of water movement from the xylem to the leaf stomatal cavity (Barbour and Farquhar, 2003; as described in Section 1), then plant biochemicals may record the integrated signal of water accessed by the plant. Water sources may vary between adjacent trees of different species and during seasonal

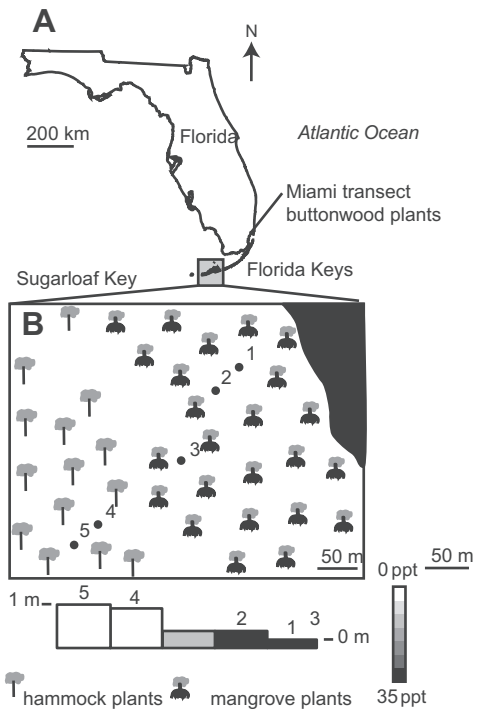

Fig. 1. Map of sample locations (after Ellsworth et al., 2013) (A) showing Sugarloaf Key and Miami study locations within Florida. (B) Sugarloaf Key showing transect Sites 1–5, mangrove and hammock plant coverage, elevations and salinities.

variations in freshwater inputs and water table levels there may be complex patterns of water uptake. We seek to trace this by the isotopic composition of plant photosynthetic products.

2. STUDY LOCATIONS AND SAMPLING STRATEGY

2.1. The coastal plant communities

The main study site was a transect in Sugarloaf Key located in the lower Florida Keys approximately 200 km southwest of Miami, Florida, USA (Fig. 1, Table 1). The coastal site comprises a flat area with a clear boundary between saltwater tolerant and saltwater intolerant plants. In the mangrove community sea water is the main water source, whereas hardwood hammocks are located inland where there is greater freshwater availability (Ross et al., 1994). We sampled the dominant species across the freshwater-saltwater gradient. The mangrove species sampled were *Avicennia germinans* (black mangrove) and *Rhizophora mangle* (red mangrove). These species are salt tolerant and have similar stature, but they differ in their method of avoiding excess salt uptake. While the red mangrove is primarily a salt excluder (i.e., it ultra filtrates salt during water uptake at the root level); the black mangrove secretes salt through specialized glands at the leaf epidermis. In the hardwood hammock community we sampled *Coccoloba diversifolia* (pigeonplum), *Pithecellobium guadalupensis* (blackbead) and *Bursera simaruba* (Gumbo-limbo). These species have a similar stature to the mangrove species, but they are intolerant of salinity.

One species, *Conocarpus erectus* (or buttonwood), tolerates brackish water salinities and grows in freshwater. This single species was sampled across a salinity gradient as a comparison for the multi-species transect at Sugarloaf Key. Samples were collected on and near the campus of the University of Miami, Coral Gables, Florida between Lake Osceola and the Atlantic Ocean (Fig. 1, Table 1). In addition *C. erectus* was sampled from Site 3 at the Sugarloaf Key transect where they are present in low abundances, they are not present in the flooded zones at Sites 1 and 2, nor in the sites further inland at Sites 4 and 5.

At each location, the soil, derived from the Miami Oolite bedrock, is shallow with low organic matter. Groundwater varies in depth and salinity, however hammock plants, utilizing freshwater from precipitation, can be found even in locations overlying saline groundwater (Ishshalom et al., 1992). Precipitation averages 1000 mm a^{-1} in the lower Florida Keys and 1500 mm a^{-1} in Miami with 60% falling in the summer months of May–October. Diurnal and mean monthly intra-annual temperature variations are modest (<10 °C) and temperatures have not been recorded below freezing.

2.2. Field sampling methods

A 300 m transect was delineated in Sugarloaf Key with the purpose of sampling different plant communities across a salinity gradient. Moving from the ocean inland we sampled 5 sites: 3 sites sampled mangroves and 2 sites sampled hammocks (Table 1). Five replicates were collected for each

Table 1
Site locations.

Site	Plant community	Long. (W)	Lat. (N)	Distance Inland (m)	Salinity (ppt)	Elevation (m)
Sugarloaf Key transect						
1	Mangrove	81.5443	24.6828	100	30.60	0.055
2	Mangrove	81.5446	24.6826	150	30.05	0.184
3	Mangrove	81.5449	24.6823	200	4.04	0.120
4	Hammock	81.5451	24.6821	300	0.79	0.901
5	Hammock	81.5454	24.6818	350	0.31	0.991
Miami transect						
From	Buttonwood	80.2628	25.6768	5		
To	Buttonwood	80.2781	25.7174	5000		

species at each sampling site. In Miami, we sampled *C. erectus* (buttonwood) across environments that range from freshwater to ocean water. For each sample, stems were collected approximately 30 cm basipetal to the leaves to prevent contamination by enriched leaf water. The well suberized stems were debarked, placed in a glass tube, sealed and maintained in a cooler.

3. ANALYTICAL METHODS

3.1. Water isotope analysis

Water was extracted from the stem and analyzed isotopically (as described in Vendramini and Sternberg, 2007). After water extraction, the same stem was ground and homogenized. One aliquot was used for cellulose extraction followed by oxygen isotope analysis (as detailed in Ellsworth et al., 2013), another aliquot of the ground stem was used for hydrogen isotope analysis of the methoxyl hydrogen reported here. Extracted water was analyzed at the Laboratory of Stable Isotope Ecology in Tropical Ecosystems at the University of Miami for oxygen and hydrogen isotope ratios by equilibration with an Isoprime© isotope ratio mass spectrometry via a Multiflow© system (Elementar, Germany). Water isotope ratios are reported relative to Vienna Standard Mean Ocean Water (VSMOW) and the precision of analysis is ±0.1‰ for oxygen and ±3‰ for hydrogen isotopes (1σ).

3.2. Methoxyl hydrogen isotopic analyses

Wood samples were ground with a ball mill. 10 mg of ground wood was placed in a 2 mL headspace GC vial. 0.5 mL of hydroiodic acid (55%, purchased from Spectrum Chemicals, Gardena, California) was added and capped with Al crimp top caps with PTFE/rubber TF2 septum gas tight septa (#50613370, Agilent Technologies, Lake Forest, California). Samples were placed in a heating block at 110 °C for 30 min (following methods established in Greule et al., 2008). Samples were then removed and kept at ambient conditions ~22 °C for at least 30 min to allow the CH_3I product to equilibrate in the headspace, prior to analysis. Samples were neutralized via the injection of 0.5 mL 5M KOH through the septum, analogous to the method of neutralization (using NaOH) reported in methoxyl (Li et al., 2012a) and ethoxyl quantification experiments (Li et al., 2012b).

Methyl iodide was analyzed by gas chromatography–mass spectrometry (GC–MS) at the University of Southern California (USC). The identity of compounds present were determined by analysis by Agilent GC–MS fitted with a ZB-5 ms column (30 m × 0.25 mm × 0.25 μm) a 1 mL/min constant column flow, an isothermal temperature program held at 32 °C for 5 min, with 1 μl injected via a split/splitless inlet (S/SL) with a split ratio of 5.

The δD values of methyl iodide were determined at USC using a Trace GC connected via a GC-Isolink pyrolysis furnace (at 1420 °C), passing through a cold trap (liquid nitrogen) for HI entrapment, and a Conflo IV interface to Delta V^{Plus} IRMS (all supplied by Thermo Scientific, Bremen, Germany). The cold trap consisted of a fused silica capillary (1 m × 0.32 mm ID) with 30 cm of a u-shaped bend in the capillary immersed in a dewar of liquid nitrogen (Chartrand et al., 2007; Feakins et al., 2013). The trapped HI was periodically purged by removing the capillary from the cold trap and venting. 10–60 μL of headspace gas from sample vials was injected using a gas tight syringe via a Triplus autosampler into a Programmable Temperature Vaporizing (PTV) inlet operated at constant temperature (200 °C) and with a split ratio of 5 needed to generate narrow peak chromatography of the gas phase analyte. For example we are able to achieve 4 V amplitude peaks with a half height width of 1.7 s; resolution is not a relevant concept here as only a single compound is present in most runs. We have previously shown an example chromatogram in Feakins et al. (2013). The GC was fitted with a ZB-5 ms column (30 m × 0.25 mm × 1 μm) and conditions included a 2 mL/min constant column flow, and an isothermal temperature program held at 32 °C for 7 min.

Peaks of hydrogen reference gas bracket the methyl iodide analyte peak during the course of the GC–P–IRMS run. One of the initial peaks was used for standardization of the isotopic analyses, while the other three bracketing peaks were treated as unknowns (precision averaged 1.1‰, 1σ, $n = 76$). Data were then normalized to the VSMOW/SLAP isotopic scale by comparison with an external standard of 99.7% purity CH_3I analyzed by offline combustion and analysis by dual inlet IRMS with δD values -95.6 ± 1.6‰ $n = 6$ (the δD value was analyzed and supplied by A. Schimmelmann, Indiana University,

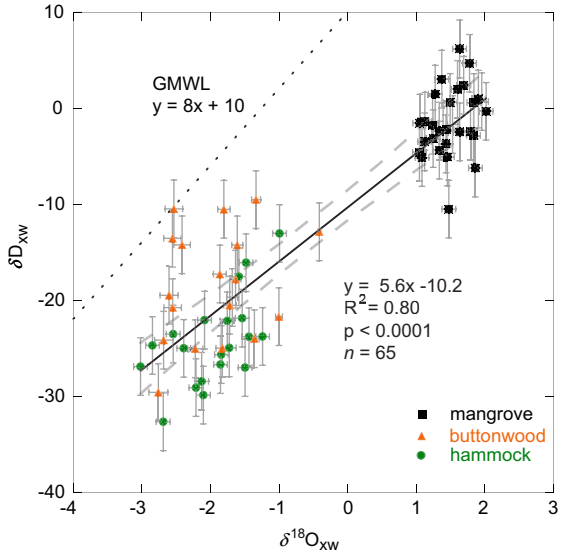

Fig. 2. Comparison of the oxygen and hydrogen isotopic composition of xylem water. Showing mangrove (black square), hammock (green circle) and buttonwood (orange triangle), regression (solid line), 95% confidence intervals (dashed lines). Error bars represent mean instrumental precision of 0.1‰ and 3‰ for δD and δ^{18}O respectively. Also shown, the Global Meteoric Water Line (GMWL, dotted line). (For interpretation of colors in this figure legend, the reader is referred to the web version of this article.)

Bloomington). The results are reported using conventional delta notation (δD ‰).

4. RESULTS AND DISCUSSION

4.1. Hydrogen isotopic gradient in plant xylem water across a salinity gradient

Across a gradient from coastal mangrove at Site 1 to more freshwater influenced, hammock forest at Site 5, measured δD_{xw} ranged from −33 to + 6‰ (Fig. 2, Table 2). This pattern reflects a gradient between meteoric water (more negative values) and sea water >0‰ (Sternberg et al., 1991). δD_{xw} values were not directly measured for 9 samples, including 4 isolated samples and the 5 buttonwoods sampled at Site 3 of the Sugarloaf Key transect. These missing δD_{xw} values are predicted (marked with a * in Table 2) based on the regression of the δD_{xw} to $\delta^{18}O_{xw}$ values measured directly in all other samples (Fig. 2).

The slope of the xylem water line ($\delta^{18}O_{xw}$ v. δD_{xw}, Fig. 2) is thought to reflect the slope of surface environmental waters in the region, which through evaporation fall on a shallower slope than the Global Meteoric Water Line (GMWL; Swart et al., 1989; Ewe et al., 2007). In most plants δD_{xw} has been shown to be a direct proxy for source water where water uptake by roots does not involve significant fractionation (Ehleringer et al., 1991, 1998; Williams and Ehleringer, 2000). In saltwater tolerant species, including mangroves, ultrafiltration in the roots may lead to fractionation relative to source water by up to 10‰ (Lin and Sternberg, 1994; Ellsworth and Williams, 2007). This can result in an offset in the xylem water slope from environmental waters (Ladd and Sachs, pers. comm., 5/9/2013). However environmental waters were not directly measured in this study as plant waters are considered to be the best way to determine source waters where environmental waters are heterogeneous, although this presents a problematic assumption in the case of fractionation during uptake. Thus we infer that δD_{xw} values likely reflect uptake of environmental waters with the possibility of minor fractionations at the saline end of the spectrum.

4.2. Methoxyl hydrogen isotopic composition

$\delta D_{methoxyl}$ values range from −230 to −130‰, and we report data for individual samples (Table 2, Fig. 3) as well as sorted by site (Table 3) and species (Table 4). The observed trend is from more negative $\delta D_{methoxyl}$ values at the most inland sites to more positive values at the more seawater influenced sites (Fig. 3). We find $\delta D_{methoxyl} = 1.8 \times \delta D_{xw} - 178$, $R^2 = 0.48$, $p < 0.0001$, $n = 74$, defined across a 40‰ range in δD_{xw}.

4.2.1. Quantification of uncertainty in methoxyl hydrogen isotopic determinations

The precision of replicate analyses of the external CH_3I standard was $\sigma = 6$‰, $n = 31$, s.e.m. = 1‰ across 5 days of analyses, indicating the analytical precision for analysis of pure CH_3I headspace injections, similar to previously reported precision (Feakins et al., 2013). A lignin in-house standard (alkali, low sulfonate content, purchased from Sigma–Aldrich), and a wood sample (*Piscidia piscipula*, a freshwater species) provides a control on reproducibility of the reaction of pure lignin and wood samples liberating CH_3I gas upon subsequent analysis by GC–P–IRMS. In this way we provide a check on not only instrument precision but also preparative chemistry, which is particularly important for the establishment of this relatively new method and volatile analyte. For lignin, 8 replicate vials had $\sigma = 16$‰ indicating a large reaction uncertainty, whereas the instrument precision of triplicate analyses averaged $\sigma = 6$‰, s.e.m. = 3‰. For the wood standard, 4 replicate vials had $\sigma = 19$‰ reaction uncertainty, and the instrument precision was $\sigma = 3$‰, s.e.m = 2‰. These results indicate that the instrument precision is considerably better than the total analytical uncertainty, when measuring CH_3I evolved from lignin and wood. The mean δD value of the USC lignin standard was found to be −248‰ ($\sigma = 18$‰, s.e.m = 3‰, $n = 26$), combining all vials and replicates to provide an overall measure of reproducibility) and the wood standard was −179‰ ($\sigma = 17$‰, s.e.m = 6‰, $n = 9$).

Replicate reactions and analyses of standards indicate a large increase in uncertainty between the CH_3I standard and CH_3I derived from reactions with lignin or wood. First, the need to puncture the septum during neutralization may compromise headspace integrity and isotopic composition; however similar headspace concentrations in experiments with and without neutralization indicate no problems with headspace integrity. Secondly, there may be differences in reaction efficiency between vials or heterogeneity in lignin polymers sampled. Thirdly, the presence of additional reactive compounds in headspace such as $HI_{(g)}$ may alter GC or pyrolysis conditions during isotopic analysis. The difference in precision between the CH_3I standard and CH_3I derived from reactions with lignin or wood, and between vials rather than between replicate injections from the same vial, implies that a larger uncertainty derives from differences in reaction chemistry rather than instrument precision. Complex biopolymers can be expected to introduce variability into reaction chemistry. However we are not completely able to exclude the possibility that the presence of additional reactive compounds in headspace such as $HI_{(g)}$ or additional reaction products may alter GC or pyrolysis conditions during isotopic analysis.

4.3. Identification of ethyl and propyl groups

In some samples we detected the presence of additional analyte peaks; whose identities were determined by GC–MS. We found a later eluting peak to be CH_3CH_2I (ethyl iodide) and $CH_3(CH_2)_2I$ (propyl iodide) through comparison to library spectra. The most prominent occurrences of these compounds were in the saltwater-influenced *C. erectus* (buttonwood). Trace amounts of ethyl and propyl iodide are also seen in the hammock and mangrove species, notably in a sample of *R. mangle* with the most isotopically enriched xylem waters. Propyl iodide was present in detectable quantities in all 5 of the samples of saltwater-influenced *C. erectus* samples; ethyl iodide was detectable but only quantified in two of the 5 samples. The amplitude

Table 2
Isotopic results by sample.

Species	Site	F or M[a]	Oxygen isotopes (‰)					Hydrogen isotopes (‰)					
			$\delta^{18}O_{xw}$	$\delta^{18}O_{cell}$	$\delta^{18}O_{pg}$	$\varepsilon_{cell/xw}$	$\varepsilon_{pg/xw}$	δD_{xw}	$\delta D_{methoxyl}$	σ	n	$\varepsilon_{methoxyl/xw}$	c.σ[b]
Sugarloaf Key transect													
Avicennia germinans	1	M	1.1	31.4	23.2	30	22	−1	−166	1	2	−165	3
Avicennia germinans	1	M	1.2	31.6	22.6	30	21	−2	−186	2	2	−184	4
Avicennia germinans	1	M	1.0	31.2	22.5	30	21	−5	−179	5	2	−175	6
Avicennia germinans	1	M	1.5	31.5	21.3	30	20	−10	−178	0	2	−169	3
Avicennia germinans	1	M	1.3	31.5	22.4	30	21	−2	−192	1	2	−190	3
Avicennia germinans	2	M	1.1	31.8	23.9	31	23	−5	−174		1	−170	3
Avicennia germinans	2	M	1.4	31.2	23.1	30	22	−2*	−168	1	2	−165	3
Avicennia germinans	2	M	1.8	31.5	23.7	30	22	5	−171	1	2	−175	3
Avicennia germinans	2	M	1.5	32.1	23.7	31	22	−5	−182	2	4	−178	4
Avicennia germinans	2	M	1.4	31.1	23.4	30	22	−2	−183	1	2	−181	3
Avicennia germinans	3	M	1.4	31.8	23.7	30	22	−4	−180	1	2	−177	3
Avicennia germinans	3	M	1.2	30.6	24.0	29	23	−3	−157	1	2	−155	3
Avicennia germinans	3	M	1.1	30.4	24.6	29	23	−3	−179	3	2	−177	4
Avicennia germinans	3	M	1.5	31.6	24.6	30	23	1	−177	1	2	−178	3
Avicennia germinans	3	M	1.1	32.1	24.0	31	23	−2	−163	2	4	−161	4
Conocarpus erectus	3	M	0.7	31.1		30		−6*	−214	2	2	−209	4
Conocarpus erectus	3	M	0.6	30.4		30		−7*	−223	6	2	−218	7
Conocarpus erectus	3	M	0.0	31.2		31		−10*	−203	2	2	−195	3
Conocarpus erectus	3	M	0.1	31.0		31		−10*	−215	0	2	−207	3
Conocarpus erectus	3	M	0.4	30.3		30		−8*	−197	2	2	−191	4
Rhizophora mangle	1	M	1.6	32.4	23.1	31	21	−2	−165	0	2	−163	3
Rhizophora mangle	1	M	1.5	32.3	24.3	31	23	−2*	−162	5	2	−160	6
Rhizophora mangle	1	M	1.6	32.2	23.6	31	22	2	−171	1	2	−172	3
Rhizophora mangle	1	M	1.9	32.3	23.3	30	21	−6	−174	3	2	−169	4
Rhizophora mangle	1	M	2.0	32.8	23.4	31	21	0	−162	3	2	−162	4
Rhizophora mangle	2	M	1.8	31.8	22.2	30	20	−3	−165	1	2	−163	3
Rhizophora mangle	2	M	1.8	31.8	23.3	30	21	1	−172	2	2	−172	4
Rhizophora mangle	2	M	2.0	31.9	22.6	30	21	1*	−173	1	2	−173	3
Rhizophora mangle	2	M	1.6	31.7	22.9	30	21	6	−165	1	2	−170	3
Rhizophora mangle	2	M	1.8	31.9	22.7	30	21	−2	−172	1	2	−170	3
Rhizophora mangle	3	M	1.7	32.2	23.5	30	22	2	−171	1	2	−173	3
Rhizophora mangle	3	M	1.3	31.8	22.5	30	21	−4	−181	3	2	−178	5
Rhizophora mangle	3	M	1.9	32.4	23.5	30	22	1	−179	1	2	−180	3
Rhizophora mangle	3	M	1.3	31.8	23.5	31	22	2	−190		1	−191	3
Rhizophora mangle	3	M	1.4	32.0	23.1	31	22	3	−185	3	2	−188	4
Coccoloba diversifolia	4	F	−2.1	30.5	23.9	33	26	−22	−219	2	2	−201	4
Coccoloba diversifolia	4	F	−1.5	29.5	23.6	31	25	−22	−207	0	2	−189	3
Coccoloba diversifolia	4	F	−1.8	29.7	24.0	32	26	−26	−205	0	2	−184	3
Coccoloba diversifolia	4	F	−1.2	30.2	23.8	31	25	−24	−242		1	−224	3
Coccoloba diversifolia	4	F	−2.5	30.8	23.3	33	26	−23	−180		1	−160	3
Coccoloba diversifolia	5	F	−1.6	29.4	22.2	31	24	−18					
Coccoloba diversifolia	5	F	−1.0	29.7	23.8	31	25	−13	−172	5	2	−161	6
Coccoloba diversifolia	5	F	−2.1	30.4	24.4	33	27	−30	−185	2	2	−160	3
Coccoloba diversifolia	5	F	−1.7	29.8	23.2	32	25	−25	−197	0	2	−176	3
Coccoloba diversifolia	5	F	−1.5	30.2	23.8	32	25	−16	−191	3	4	−178	4
Pithecellobium guadalupensis	4	F	−1.8	30.4	22.5	32	24	−22	−249	1	3	−232	3
Pithecellobium guadalupensis	4	F	−2.4	31.3	23.7	34	26	−25	−248	2	2	−229	4
Pithecellobium guadalupensis	4	F	−1.4	31.3	24.2	33	26	−24	−240	1	2	−222	3
Pithecellobium guadalupensis	4	F	−2.7	31.0	23.4	34	26	−33	−232	3	2	−206	4
Pithecellobium guadalupensis	4	F	−2.2	32.1	24.0	34	26	−29	−170	1	2	−146	3
Bursera simaruba	5	F	−3.0	29.3	21.7	32	25	−27	−194	9	2	−172	9
Bursera simaruba	5	F	−1.5	28.7	21.4	30	23	−27	−224	4	2	−203	5
Bursera simaruba	5	F	−2.1	28.5	21.8	31	24	−28	−214	1	2	−191	3

Bursera simaruba	5	F	−2.8	28.0	19.7	31	23	−25	−201		1	−180	3
Bursera simaruba	5	F	−1.9	28.7	21.4	31	23	−27	−235	3	2	−214	4
Miami transect													
Conocarpus erectus		F	−1.8	31.3		33		−25	−239	4	2	−220	5
Conocarpus erectus		F	−2.4	31.2		34		−14	−206		1	−195	3
Conocarpus erectus		F	−2.6	30.5		33		−21	−229	5	2	−213	6
Conocarpus erectus		F	−2.6	30.6		33		−14	−230	2	2	−219	3
Conocarpus erectus		F	−1.8	31.0		33		−10	−238	1	2	−230	3
Conocarpus erectus		F	−2.5	30.5		33		−10	−238	1	2	−230	3
Conocarpus erectus		F	−0.4	30.8		31		−13	−217	2	2	−207	4
Conocarpus erectus		F	−1.4	30.7		32		−24	−237	0	2	−218	3
Conocarpus erectus		F	−1.6	30.8		32		−18	−232	1	2	−218	3
Conocarpus erectus		F	−1.9	30.2		32		−17	−246	0	2	−232	3
Conocarpus erectus		F	−1.7	30.2		32		−20*	−218	2	2	−202	4
Conocarpus erectus		F	−1.3	30.0		31		−9	−228	1	2	−220	3
Conocarpus erectus		F	−1.6	30.5		32		−14	−198	0	2	−186	3
Conocarpus erectus		F	−1.7	31.0		33		−20	−215	2	2	−199	3
Conocarpus erectus		F	−1.0	30.7		32		−22	−224	2	2	−207	4
Conocarpus erectus		F	−2.8	29.1		32		−30	−239	2	2	−216	4
Conocarpus erectus		F		31.6				−22	−235	0	2	−217	3
Conocarpus erectus		F	−2.7	31.8		35		−24	−227	0	2	−208	3
Conocarpus erectus		F	−2.2	33.8		36		−25	−239	0	2	−220	3
Conocarpus erectus		F	−2.6	30.1		33		−19	−216	1	2	−201	3

Note, full analytical uncertainty includes the reaction chemistry uncertainty reported in the text for standards, also assessed by replicate samples above. Typical precision (σ) on various isotopic measurements are: $\delta^{18}O_{xw}$ 0.1‰, δD_{xw} 3‰, $\delta^{18}O_{cell}$ 0.3‰, $\delta^{18}O_{pg}$ 0.4‰. c. σ for $\varepsilon_{cell/xw}$ 0.3‰ and $\varepsilon_{pg/xw}$ 0.4‰.

[a] F = freshwater, M = marine.

[b] c. = compounded instrument precision.

* δD_{xw} assigned based on $\delta^{18}O_{xw}$ value and δD_{xw}–$\delta^{18}O_{xw}$ relationship (Fig. 2).

of the signal for these compounds was too low (<0.5 V) for rigorous assessment of isotopic composition as denoted by poor reproducibility of replicate isotopic determinations, however the isotopic composition appears to be similar to methyl iodide. This observation is consistent with methoxyl and propoxyl groups deriving from the lignin molecule in the wood samples. If the ethyl and propyl groups are indeed derived from the lignin molecule, their presence suggests plasticity and salinity stress-induced changes in the molecular structure of lignin. We speculate that the production of longer ether branches may be related to the osmolytes (e.g., mannitol) produced during salt stress which may provide propanol or ethanol for the polymerization reaction yielding lignin with propoxyl and ethoxyl as well as common methoxyl groups.

We quantified the relative and absolute abundance of the homologous series in the saltwater-influenced *C. erectus* samples using a GC–MS (Table 5). From samples weighing 10 mg, we measured CH_3I (methyl iodide, MI) yields of 50–100 μg mg dw^{-1} of wood, in comparison to 100 μg mg dw^{-1} from the lignin standard. In contrast detectable propyl iodide (PI) was only noted in a few samples and ethyl iodide (EI) in fewer still, with yields <1 μg mg dw^{-1}. We suspect that isotopic fractionation may be linked to variations in the lignin molecular structure associated with these longer chain ether branches. Overall we find that in samples with detectable PI, $\delta D_{methoxyl}$ values have a mean offset from predicted values of -18 ± 12‰ ($n = 5$; Table 5). However we did not observe a significant relationship between the relative abundance of PI and $\delta D_{methoxyl}$ here. While the presence of PI may indicate that $\delta D_{methoxyl}$ values will be more depleted than expected for a given source water, given the analytical uncertainties we do not find that we can attempt a correction for PI at this time. Exploring the production of propoxyl groups and the isotopic effects on both methoxyl groups may add to the power of this proxy to resolve biosynthetic and environmental signals.

4.4. Isotopic fractionations between source water and methoxyl hydrogen

The net fractionation between xylem water and methoxyl hydrogen ($\varepsilon_{methoxyl/water}$) is defined by Eq. (1):

$$\varepsilon_{methoxyl/w} = \alpha_{methoxyl/w} - 1 = \frac{\delta_{methoxyl} + 1}{\delta_w + 1} - 1 \quad (1)$$

Enrichment factors are reported in permil notation, which implies a factor of 1000 (Cohen et al., 2007). Note that we measured δD_{xw} to directly assess water sources and the net fractionation by individual plants in this study (Table 2). We calculate an average $\varepsilon_{methoxyl/water}$ of −190‰, $\sigma = 23$‰, $n = 74$ in this study. We find a regression relationship of $\delta D_{methoxyl} = 1.8 \times \delta D_{xw} - 178$, $R^2 = 0.48$, $p < 0.0001$, $n = 74$ (Fig. 3B), suggesting that the primary control on $\delta D_{methoxyl}$ results from source waters, however additional confounding effects lower the degree of correlation. Reaction chemistry variability has been demonstrated to produce significant scatter (see Section 4.2.1; $\sigma = 16$‰ on $n = 26$ replicate lignin standards). Exceeding

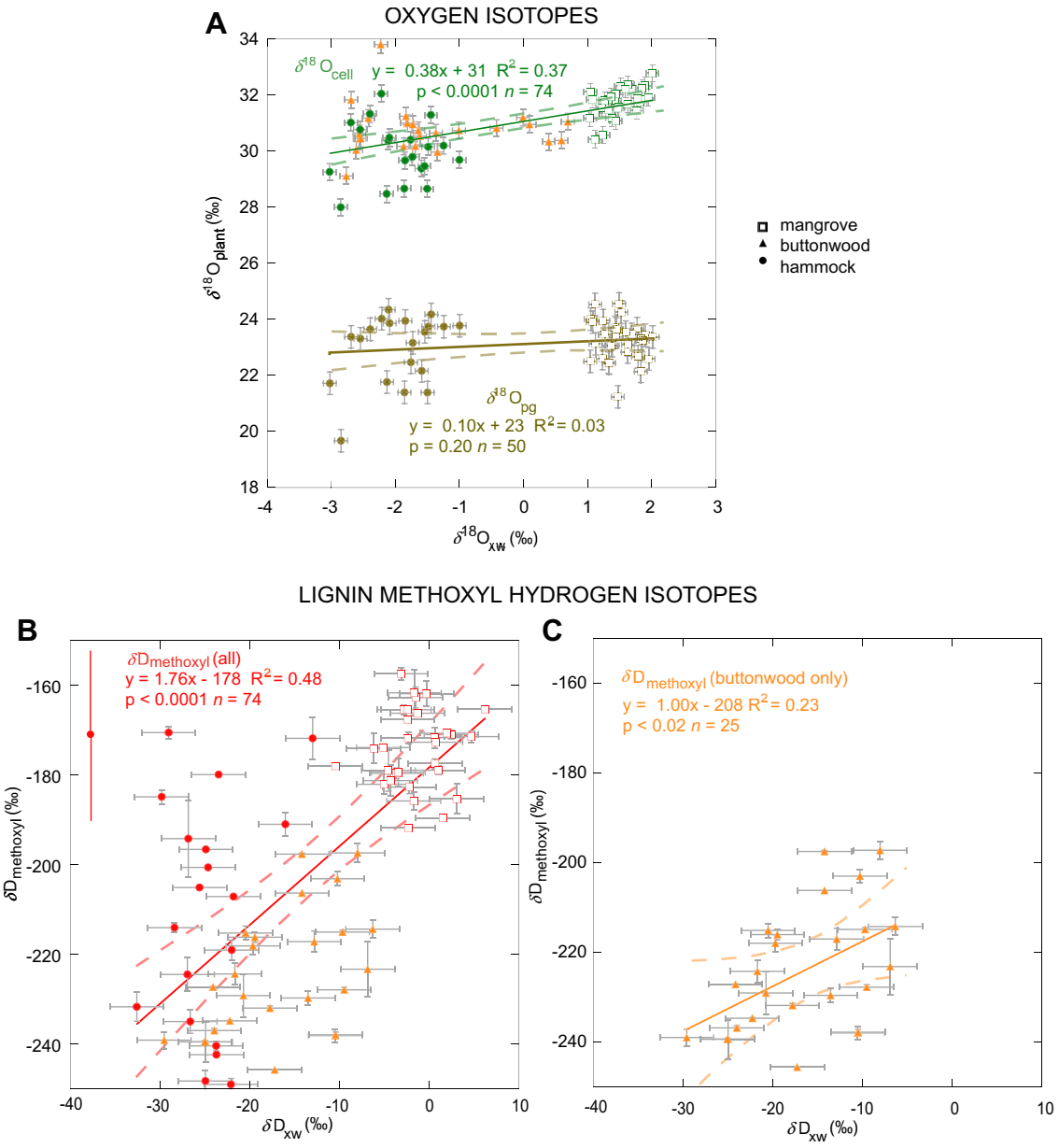

Fig. 3. Intercomparison of lignin and cellulose based-proxies for source water isotopic composition. A, $\delta^{18}O_{xw}$ versus $\delta^{18}O$ values of alpha cellulose (green symbols – Ellsworth et al., 2013; orange symbols – this study) and cellulose phenylglucosazone (brown symbols – Ellsworth et al., 2013). Regression (solid line), 95% confidence intervals (dashed lines). B, δD_{xw} versus $\delta D_{methoxyl}$ (this study). Mangrove (red open square), buttonwood (orange triangle), hammock (red circle). Overall regression (solid line) and, 95% confidence intervals (dashed lines). *X* error bars represent mean instrument uncertainty on δD_{xw}; *y* error bars represent σ of $\delta D_{methoxyl}$ instrument precision. Also shown *y* error bar (red) for replicate reactions of a lignin standard. C, As for B showing buttonwoods only (orange). (For interpretation of colors in this figure legend, the reader is referred to the web version of this article.)

measurement uncertainty, plant-to-plant variability contributes substantially, with the largest degree of spread observed in *C. diversifolia* ($\sigma = 23‰$, $n = 5$) and *P. guadalupensis* at Site 5 ($\sigma = 36‰$, $n = 5$; Table 3).

We also considered whether there are variations in fractionations between species and between plant communities. We found that the fractionations in the two mangrove species are identical within error (overall mangrove mean −173‰, $\sigma = 9‰$, $n = 30$, Table 4). The fractionations in the buttonwoods are apparently larger (overall buttonwood mean −207‰, $\sigma = 36‰$, $n = 25$, Table 4). The fractionations within the hammock species are intermediate (−193‰, $\sigma = 26‰$, $n = 19$, Table 4). However given the uncertainty in replicate chemistry, plant-to-plant variability and the numbers of plants surveyed, these offsets are not significantly different.

Variations in fractionations may also exist across the salinity gradient. The mean $\varepsilon_{methoxyl/water}$ values for marine

Table 3
Isotopic results across the environmental gradient.

Species	n	Oxygen isotopes (‰)												Hydrogen isotopes (‰)								
		$\delta^{18}O_{xw}$	σ[a]	n	$\delta^{18}O_{cell}$	σ	n	$\varepsilon_{cell/xw}$	σ	n	$\varepsilon_{pg/xw}$	σ	n	δD_{xw}	σ	n	$\delta D_{methoxyl}$	σ	n	$\varepsilon_{methoxyl/xw}$	σ	n
Sugarloaf Key transect																						
Site 1 – Marine (Mangrove)																						
Avicennia germinans	5	1.2	0.2	5	31.4	0.2	5	30.1	0.1	5	21.1	0.8	5	−4	4	5	−180	10	5	−177	10	5
Rhizophora mangle	5	1.7	0.2	5	32.4	0.2	5	30.6	0.2	5	21.8	0.6	5	−2	3	5	−167	5	5	−165	5	5
Site 2 – Marine (Mangrove)																						
Avicennia germinans	5	1.4	0.2	5	31.5	0.4	5	30.1	0.5	5	22.1	0.4	5	−2	4	5	−175	7	5	−174	6	5
Rhizophora mangle	5	1.8	0.1	5	31.8	0.1	5	30.0	0.1	5	20.9	0.5	5	0	4	5	−169	4	5	−170	4	5
Site 3 – Marine (Mangrove)																						
Avicennia germinans	5	1.3	0.2	5	31.3	0.8	5	30.0	0.7	5	22.9	0.4	5	−2	2	5	−171	10	5	−169	11	5
Rhizophora mangle	5	1.5	0.3	5	32.0	0.2	5	30.5	0.1	5	21.6	0.4	5	1	3	5	−181	7	5	−182	7	5
Conocarpus erectus	5	0.4	0.3	5	30.8	0.4	5	30.4	0.6	5	n.a.	n.a.		−8	2	5	−211	10	5	−204	11	5
Site 4 – Freshwater (Hammock)																						
Coccoloba diversifolia	5	−1.9	0.5	5	30.1	0.5	5	32.0	1.0	5	25.6	0.5	5	−23	2	5	−211	23	5	−192	23	5
Pithecellobium guadalupensis	5	−2.1	0.5	5	31.2	0.6	5	33.4	0.9	5	25.7	0.8	5	−27	4	5	−228	33	5	−207	36	5
Site 5 – Freshwater (Hammock)																						
Coccoloba diversifolia	5	−1.6	0.4	5	29.9	0.4	5	31.5	0.7	5	25.1	1.0	5	−20	7	5	−186	11	4	−169	10	4
Bursera simaruba	5	−2.3	0.6	5	28.6	0.5	5	31.0	0.8	5	23.5	0.9	5	−27	1	5	−214	17	5	−192	17	5
Miami transect																						
Freshwater (buttonwood)																						
Conocarpus erectus	19	−1.9	0.6	19	30.8	0.9	20	32.8	1.2	19	n.a.	n.a.		−19	6	20	−228	13	20	−213	12	20
Marine mean		1.3	0.2	5	31.6	0.3	5	30.2	0.3	5	21.7	0.5	5	−2	3	5	−179	8	5	−177	8	5
Freshwater mean		−1.0	0.5	7	30.6	0.6	7	31.6	0.8	7	24.1	0.7	5	−17	3	7	−203	16	7	−189	17	7

Instrument precisions are as reported in the footnote for Table 2. Analytical uncertainty as well as plant-to-plant variability accounts for the large σ in the methoxyl values reported here.

[a] σ represents the distribution of values for a species and site.

Table 4
Isotopic results by plant type.

Species	n	Oxygen isotopes (‰)												Hydrogen isotopes (‰)								
		$\delta^{18}O_{xw}$	σ	n	$\delta^{18}O_{cell}$	σ	n	$\delta^{18}O_{pg}$	σ	n	$\varepsilon_{cell/xw}$	σ	n	$\varepsilon_{pg/xw}$	σ	n	δD_{xw}	σ	n	$\delta D_{methoxyl}$	σ	n	$\varepsilon_{methoxyl/xw}$	σ	n
Mangrove species																									
A. germinans	15	1.3	0.2	15	31.4	0.5	15	23	0.9	15	30.1	0.5	15	22.0	0.9	15	−3	3.2	15	−176	9	15	−173	9	15
R. mangle	15	1.7	0.2	15	32.1	0.3	15	23	0.5	15	30.1	0.3	15	21.4	0.6	15	0	3.2	15	−172	8	15	−172	9	15
Mean	30	1.5	0.3	30	31.8	0.5	30	23.3	0.7	30	30.1	0.4	30	21.7	0.8	30	−1	3.4	30	−174	9	30	−173	9	30
Buttonwood																									
C. erectus	25	−1.4	1.1	24	30.8	0.8	25				32	1.4	24				−17	7	25	−224	14	25	−211	12	25
Hammock species																									
C. diversifolia	10	−1.7	0.5	10	30.0	0.5	10				31.8	0.8	10	25.3	0.8	10	−22	5.0	10	−200	22	9	−181	21	9
P. guadalupensis	5	−2.1	0.5	5	31.2	0.6	5				33.4	0.9	5	25.7	0.8	5	−27	0.9	5	−228	33	5	−207	36	5
B. simaruba	5	−2.3	0.6	5	28.6	0.5	5				33.4	0.8	5	23.5	0.9	5	−27	1.3	5	−214	17	5	−192	17	5
Mean	20	−2.0	0.5	20	30.0	1.1	20				32.9	1.2	20	24.9	1.2	20	−25	4.7	20	−214	26	19	−193	26	19

σ values represents the distribution of values by species. Instrument precisions are reported in the footnote for Table 2. Analytical uncertainty as well as plant-to-plant variability accounts for the large σ in the methoxyl values reported here.

influenced plants are −177‰, compounded $\sigma = 8$‰, $n = 35$, whereas the mean $\varepsilon_{methoxyl/water}$ values for freshwater influenced plants are −189‰, compounded $\sigma = 17$‰, $n = 39$. However given the uncertainty in replicate chemistry, plant-to-plant variability and the numbers of plants surveyed these offsets are not significantly different. We note that the buttonwoods surveyed include freshwater and saltwater influence yet also do not show a significant offset in fractionations between these environments (Table 3).

4.4.1. Greater heterogeneity in saltwater-intolerant taxa

We observe a greater scatter in the δD_{xw} versus $\delta D_{methoxyl}$ relationship in the hammock species versus the

Table 5
Abundances of alkyl iodides in seawater-influenced buttonwoods at Site 3 Sugarloaf Key.

Species	Oxygen isotopes (‰)			Hydrogen isotopes (‰)						Alkyl iodide Rel. Abund.			Abundance (μg mg dw^{-1})			Hydrogen isotope offsets (‰)	
	$\delta^{18}O_{xw}$	$\delta^{18}O_{cell}$	$\varepsilon_{cell/xw}$	δD_{xw}	$\delta D_{methoxyl}$	σ	n	$\varepsilon_{methoxyl/xw}$	c. σ	MI	EI	PI	MI	EI	PI	δD_{pred} [a]	$\Delta_{meas-pred}$ [b]
Conocarpus erectus	0.7	31.1	30	−6*	−214	2	2	−209	4	0.990	0.002	0.008	101	0.19	0.81	−189	−25
Conocarpus erectus	0.6	30.4	30	−7*	−223	6	2	−218	4	0.995	0.000	0.005	97		0.47	−190	−33
Conocarpus erectus	0.0	31.2	31	−10*	−203	2	2	−195	4	0.992	0.001	0.007	83	0.12	0.55	−196	−7
Conocarpus erectus	0.1	31.0	31	−10*	−215	0	2	−207	4	0.995	0.000	0.005	57		0.31	−195	−20
Conocarpus erectus	0.4	30.3	30	−8*	−197	2	2	−191	4	0.993	0.000	0.007	53		0.38	−192	−5

[a] δD_{pred} = predicted value of methoxyl from the measured δD_{xw} based on the regression in Fig. 3, $y = 1.8x - 178$.
[b] $\Delta_{meas-pred}$ is the residual of the measured $\delta D_{methoxyl-dDpred}$.
* c. σ abbreviations as defined in Table 2.

mangrove species (Fig. 3B, Table 4). The spread in *C. diversifolia* and *P. guadalupensis* is particularly great as noted above. This observation may reflect greater heterogeneity in the micro-topography, and seasonal water availability for the hammocks, versus greater homogeneity in water sources for seawater influenced mangroves. The hammocks use meteoric water from a superficial layer of densely packed litter and do not access the underlying saline groundwater (Ishshalom et al., 1992). In order to eliminate interspecies effects we examine evidence from the buttonwoods, which span a wider salinity gradient. We also find a large degree of scatter in the buttonwoods in the δD_{xw} versus $\delta D_{methoxyl}$ relationship (buttonwood, $R^2 = 0.23$, overall $R^2 = 0.48$, $p < 0.02$, $n = 25$; Fig. 3B). While scatter is inherent to all samples associated with reaction chemistry, the scatter is greatest in the hammocks and buttonwoods. This suggests that the issue is inherent to plants growing at, or near to, the limit of their salinity tolerance range rather than interspecies offsets *per se*. This scatter presents an obstacle to the application of the $\delta D_{methoxyl}$ proxy to saltwater-intolerant plants in saline environments to resolve all but very large changes in source water.

4.5. Comparison to oxygen isotope values in cellulose derivatives

Comparison of the new $\delta D_{methoxyl}$ proxy with the oxygen isotope proxies from $\delta^{18}O$ values of alpha cellulose ($\delta^{18}O_{cell}$) and cellulose phenylglucosazone ($\delta^{18}O_{pg}$) indicate that the new proxy is more sensitive to source water variations across the sampled salinity gradient (Fig. 3). The oxygen isotope proxies both suffer from low sensitivity of the proxy to source water variations. In cellulose, the constancy of fractionation across a salinity gradient may result from opposing leaf-based isotope effects (Section 1). Although the salinity would cause a lower leaf water ^{18}O enrichment as observed in other saltwater tolerant species, accompanying decrease in transpiration (via the Peclét effect) leads to leaf waters that are not as ^{18}O enriched. The effect is particularly marked on $\delta^{18}O_{pg}$ (slope of 0.10, $p < 0.0001$, $n = 74$) but also markedly influences $\delta^{18}O_{cell}$ (slope of 0.38, $p = 0.20$, $n = 50$) whereas no such attenuation is noted in the methoxyl proxy (slope of 1.8, $p < 0.0001$, $n = 74$). Attenuation of the source water signal in cellulose-based plant proxies in coastal environments hampers our ability to fully reconstruct source water changes, yielding scale compression in those archives.

It is not known whether leaf processes can affect the $\delta D_{methoxyl}$ values, however the proxy response indicates that a source water signal is recorded by $\delta D_{methoxyl}$ values ($\delta D_{methoxyl} = 1.76 * \delta D_{xw} - 178$, $R^2 = 0.48$, $p < 0.0001$, $n = 74$). The slope is greater than 1 because we find a smaller than expected fractionation in mangroves (mean fractionation $\varepsilon_{methoxyl/xw}$ -173 ± 1‰, $n = 30$; Table 4) and a larger than expected fractionation in freshwater hammocks (mean fractionation $\varepsilon_{methoxyl/xw}$ -193 ± 13‰, $n = 30$; Table 4). It remains possible that the salinity induced isotope effects may also affect the methoxyl hydrogen on lignin, purported to be only influenced by stem water (Keppler et al., 2007), however any isotope effects appear to increase the slope. The $\delta D_{methoxyl}$ proxy is therefore a more sensitive proxy of source water than $\delta^{18}O_{cell}$ or $\delta^{18}O_{pg}$, however the new technique suffers from large uncertainties at present.

4.6. Comparison to other methoxyl hydrogen calibration efforts

Previous studies of the lignin methoxyl hydrogen isotope approach have analyzed terrestrial trees of 11 species, with most data from *Picea abies* (spruce) and *Betula pendula* (birch) from Scandinavia as well as several other species from around the northern hemisphere. That study found $\varepsilon_{methoxyl/water}$ of −216‰, $\sigma = 19$‰, $n = 32$, which is not significantly different from the fractionations identified here. The authors reported a regression of $\delta D_{methoxyl} = 1.2 \times \delta D_{MAP} - 182$, $R^2 = 0.91$, $p < 0.0001$, $n = 32$ (Keppler et al., 2007), where source waters were approximated by estimates of mean annual precipitation (MAP) derived from the Online Isotopes in Precipitation Calculator (OIPC). We calculate an average $\varepsilon_{methoxyl/water}$ of −190‰, $\sigma = 23$‰, $n = 74$ in this study (Table 2), where source waters to the plants were directly determined from measurements of xylem water. While our study spans a more limited 40‰ range in source waters, the regression is not significantly different by Student's t test from the regression of the $\delta D_{methoxyl}$ data spanning a 125‰ range in δD_{MAP}

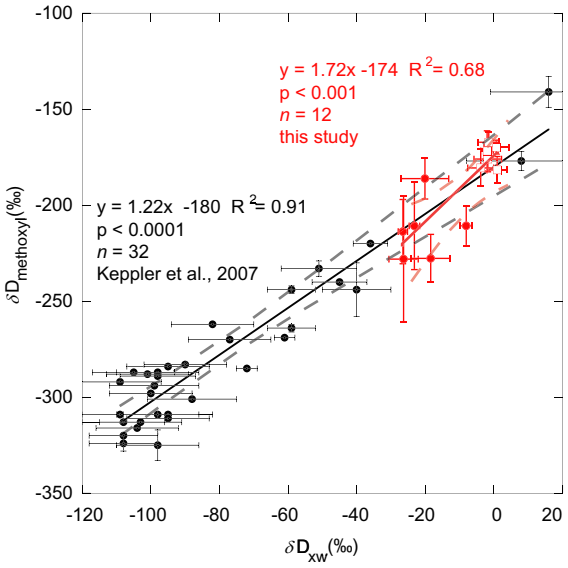

Fig. 4. Global comparison of the $\delta D_{methoxyl}$ proxy. This study, a subtropical, coastal ecosystem (red), plotting $\delta D_{methoxyl}$ versus δD_{xw} showing mean values for species and site averages; x error bars represent instrument precision on δD_{xw}; y error bars represent σ distribution of the site and species mean values; mangrove species, using saline water sources, are differentiated (open square). For comparison, a global collection of terrestrial plants (black), plotting δD_{MAP} (derived from the OIPC) versus $\delta D_{methoxyl}$ (black) showing mean of 2 samples; x error bars represent 95% CI on MAP values (derived from the OIPC), y error bars represent the 95% CI on samples where >2 replicates (Keppler et al., 2007). Local regression (red line), global regression (black line), 95% confidence intervals (dashed lines). Regression equations shown are not significantly different with a Student's t test. (For interpretation of colors in this figure legend, the reader is referred to the web version of this article.)

(Fig. 4). Even the saltwater-tolerant mangroves fall closely on the global calibration previously defined only for terrestrial species (Fig. 4).

4.7. Prospects for the lignin methoxyl proxy

Future directions for the lignin methoxyl hydrogen isotope technique in coastal applications could include applying the observed spatial response towards monitoring the changing pressures on freshwater hammock and buttonwood species in low lying areas of south Florida. $\delta D_{methoxyl}$ values, provide a means to track the annually integrated water uptake by these marginal freshwater-dependent ecosystems, however there is a large amount of scatter in the data from the plant-to-plant variability in the hammock species, as well as analytical uncertainties. This approach could monitor the influence of sea level rise and freshwater diversion that may be placing these biodiverse ecosystems at risk, so long as the magnitude of change exceeds measurement uncertainties. The technique is amenable to sampling a large number of trees in a single field visit and to capture the annually integrated water uptake – a pragmatic solution to monitoring conditions in challenging field conditions.

More broadly, this technique has now been demonstrated to work in terrestrial and coastal trees and so the potential for source water reconstructions using this approach appears to be widespread (Fig. 4). Reduction of sample size below 10 mg would facilitate application to sample limited studies, e.g. tree rings. Reduction of uncertainties would facilitate applications to reconstructing small isotopic excursions. Current uncertainties were ca. 18‰ (1σ) in replicate reactions, thus the $\delta D_{methoxyl}$ technique will be best applied where the magnitude of environmental changes exceeds analytical uncertainties.

Lignin is also liberated during plant decomposition and transported fluvially to depositional basins (Goni et al., 1997). Lignin can therefore also be extracted and isolated from aqueous and sedimentary samples. Lignin phenols provide diagnostic information about vegetation sources (e.g., conifers versus angiosperms) and if extracted in sufficient quantities, and isolated and purified by HPLC methods they can be prepared for radiocarbon analysis (Feng et al., 2013). Although the preparative chemistry for lignin monomer purification is extensive, these same purified extracts have great prospects for analysis for methoxyl hydrogen isotopic ratios, however at present sample sizes are commensurate with radiocarbon requirements. Sample sizes would ideally be reduced to facilitate environmental applications.

5. CONCLUSIONS

In this study we report the first measurements of the hydrogen isotopic composition of lignin methoxyl groups in mangrove, hammock and buttonwood species. We report a strong correlation between δD_{xw} and $\delta D_{methoxyl}$ data across an environmental gradient from freshwater to seawater influence ($R^2 = 0.5$, $p < 0.0001$) indicating that source waters explain about half the variability in methoxyl hydrogen isotopic composition of stem lignin. We find a lower correlation coefficient than reported for the global terrestrial dataset which spans a wider isotopic range (Keppler et al., 2007). Empirically we observe the greatest scatter in the hammocks which may reflect greater heterogeneity of source waters than measured by point sampling of δD_{xw} or other confounding factors in that environment. In contrast to the $\delta^{18}O_{cell}$, $\delta^{18}O_{pg}$ and δD_{wax} analyses, this technique offers advantages of speed of preparation and avoidance of leaf-based isotope effects. However, the large uncertainties associated with the method (e.g., ±18‰ for replicate analyses of the same sample) limit application of this method at present. The present level of uncertainty (±18‰) represents approximately one-third of the effect of source water on the hydrogen isotopic composition of lignin methoxyl groups from freshwater to saltwater (40‰) and thus presents a challenge to applying this method to reconstructions of changes in source water over time. Our findings demonstrate the potential of methoxyl hydrogen to record source waters in coastal mangrove and hammock ecosystems that are important tropical and subtropical coastal habitats. However the $\delta D_{methoxyl}$ technique will be best applied where the magnitude of environmental changes exceeds analytical uncertainties.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.gca.2013.07.012. These data include Google maps of the most important areas described in this article.

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