1 **Hydrogen isotope response to changing salinity and rainfall in Australian mangroves**
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3 *S. Nemiah Ladd^{a, 1, *} and Julian P. Sachs^a*
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9 ^a*School of Oceanography, University of Washington, Box 355315, Seattle, WA 98195, USA*
10 snladd@uw.edu, jsachs@uw.edu
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13
14 ¹*Present address: EAWAG, Surface Waters Research and Management, Seestrasse 79, 6047*
15 *Kastanienbaum, Switzerland; +41 79 869 4345; Nemiah.Ladd@eawag.ch*
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19 **Corresponding author*
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ABSTRACT

Hydrogen isotope ratios ($^2\text{H}/^1\text{H}$, $\delta^2\text{H}$) of leaf waxes covary with those in precipitation and are therefore a useful paleohydrologic proxy. Mangroves are an exception to this relationship because their $\delta^2\text{H}$ values are also influenced by salinity. The mechanisms underlying this response were investigated by measuring leaf lipid $\delta^2\text{H}$ and leaf and xylem water $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values from three mangrove species over 9.5 months in a subtropical Australian estuary. Net $^2\text{H}/^1\text{H}$ fractionation between surface water and leaf lipids decreased by 0.5-1.0‰ ppt⁻¹ for *n*-alkanes and 0.4-0.8‰ ppt⁻¹ for isoprenoids. Xylem water was ^2H depleted relative to surface water, reflecting ^2H discrimination of 4-10‰ during water uptake at all salinities and opportunistic uptake of freshwater at high salinity. However, leaf water ^2H enrichment relative to estuary water was insensitive to salinity and identical for all species. Therefore variations in leaf and xylem water $\delta^2\text{H}$ values cannot explain the salinity-dependent ^2H depletion in leaf lipids, nor the 30‰ range in leaf lipid $\delta^2\text{H}$ values among species. Biochemical changes in direct response to salt stress, such as increased compatible solute production or preferential use of stored carbohydrates, and/or the timing of lipid production and subsequent turnover rates, are more likely causes.

INTRODUCTION

Hydrogen isotopes of plant leaf waxes are a valuable source of information about the water cycle, and $\delta^2\text{H}_{\text{Wax}}$ ($\delta^2\text{H} = (^2\text{H}/^1\text{H}_{\text{Sample}})/(^2\text{H}/^1\text{H}_{\text{VSMOW}}) - 1$) values are increasingly used as a paleoclimate proxy (Sachse *et al.*, 2012; Gao *et al.*, 2014). Over large environmental gradients, the primary cause of variability in $\delta^2\text{H}_{\text{Wax}}$ values are the $\delta^2\text{H}$ values of local precipitation (Huang *et al.*, 2004; Sachse *et al.*, 2004; Hou *et al.*, 2008; Polissar & Freeman, 2010; Sachse *et al.*,

2012; Tipple & Pagani, 2013), which is directly related to environmental variables such as temperature, rainfall rate, and moisture source (Dansgaard, 1964; Craig & Gordon, 1965; Gat, 1996).

However, changes in $\delta^2\text{H}_{\text{precipitation}}$ are not the only cause of variability in $\delta^2\text{H}_{\text{Wax}}$ (Sachse et al., 2012; Gao et al., 2014). Other environmental variables have demonstrated influence on $\delta^2\text{H}_{\text{Wax}}$ in some settings. Factors such as plant type (Luo et al., 2006; Smith & Freeman, 2006; Hou et al., 2007; Feakins & Sessions, 2010a), relative humidity (Feakins & Sessions, 2010b; Douglas et al., 2012; Kahmen et al., 2013a; Kahmen et al., 2013b; Tipple et al., 2014), and light levels (Luo & Yang, 2008; Yang et al., 2009) can affect the offset between $\delta^2\text{H}_{\text{Wax}}$ and $\delta^2\text{H}_{\text{Water}}$, which is defined by the fractionation factor $\alpha_{\text{Lipid-Water}}$ ($\alpha_{\text{Lipid-Water}} = (\delta^2\text{H}_{\text{Lipid}} + 1000)/(\delta^2\text{H}_{\text{Water}} + 1000)$). On tropical and subtropical coastlines where mangroves are present, salinity is another important variable that can affect $\alpha_{\text{Lipid-Water}}$. Mangroves contribute ~30% of total organic material to coastal marine sediments in the tropics and subtropics (Alongi and Mukhopadhyay, 2014). It is therefore essential to understand this effect in order to accurately apply the $\delta^2\text{H}_{\text{Lipid}}$ proxy in low-latitude coastal sediments.

Increased salinity is correlated with apparent isotope fractionation in mangroves growing along spatial salinity gradients at a single point in time (Ladd & Sachs, 2012; Ladd & Sachs, in review). In the Brisbane River, $\alpha_{\text{Lipid-Water}}$ decreased by $1.5 \pm 0.3\text{‰}$ PSU^{-1} for both $n\text{C}_{31}$ - and $n\text{C}_{33}$ -alkanes produced by *Avicennia marina* (gray mangroves) growing along a salinity gradient of 6-35 PSU (Ladd & Sachs, 2012). For the pentacyclic tripterpenoid taraxerol produced by *Rhizophora* spp. (red mangroves) growing along three Micronesian estuaries and four marine lakes in Palau, $\alpha_{\text{Lipid-Water}}$ decreased by $0.9 \pm 0.2\text{‰}$ PSU^{-1} . For n -alkanes produced by the same plants, $\alpha_{\text{Lipid-Water}}$ decreased by $1.0 \pm 0.3\text{‰}$ PSU^{-1} (Ladd & Sachs, in review).

The main goal of the present study was to investigate how changes in apparent isotope fractionation manifest themselves throughout the year by studying mangroves at a single location with highly seasonal and episodic precipitation. Similar time series of temperate plants have produced conflicting results, with some indicating that leaf wax isotopes reflect the environmental conditions during leaf flush (*Sachse et al., 2010; Tipple et al., 2013*), and some suggesting leaf wax regeneration and isotopic shifts throughout the growth season (*Sachse et al., 2009; Gao & Huang, 2013*). More relevant for evergreen tropical plants, such as the mangroves studied here, was a recent study of evergreen broadleaf trees from California (*Quercus agrifolia*) (*Sachse et al., 2015*). The results of this study indicated that leaf waxes were formed and regenerated over the first three months of the leaf's existence, and subsequently were isotopically stable (*Sachse et al., 2015*). Leaf lipids from mangroves, which produce new leaves throughout the year (*Wium-Andersen & Christensen, 1978; Duke et al., 1984; Coupland et al., 2005*) may thus represent aggregate annual conditions.

Another major goal of the present study was to better understand the mechanism that results in greater net discrimination against ^2H during the synthesis of mangrove lipids at high salinity. Previous hypotheses for increased fractionation at high salinity are (1) increased discrimination against ^2H during water uptake by roots, (2) increased production of compatible solutes, resulting in changes in the NADPH pool used for lipid synthesis, and (3) salt on the leaf surface, which increases the relative humidity along the leaf boundary layer and decreases the vapor pressure difference between the interior and exterior of the leaf (*Ladd & Sachs, 2012*). This third mechanism should be more pronounced for mangroves that secrete salt, such as *Avicennia* and *Aegicerus*, than for mangroves that lack this function, such as *Rhizophora*.

Analyzing the isotopic composition of xylem water ($\delta^2\text{H}_{\text{XW}}$) and leaf water ($\delta^2\text{H}_{\text{LW}}$) is an effective method to investigate causes of net fractionation during lipid synthesis (Feakins & Sessions, 2010a; Romero & Feakins, 2011; Kahmen et al., 2013b; Tipple et al., 2013; Gao et al., 2014). Here we included weekly measurements of these values from adjacent salt-secreting and non-secreting trees over a 9.5-month period to test the relative likelihood of each of the three hypotheses summarized above.

METHODS

Study site and sample collection

Mobbs Bay (S 28°53.068', E 153°34.211') is located at the mouth of the Richmond River in South Ballina, New South Wales, Australia (**Figure 1**). South Ballina has a humid subtropical climate, with mean maximum temperatures ranging from 19.9 °C in July to 28.2 °C in January (Australian Bureau of Meteorology). Mean annual rainfall is 1768.6 mm/year, with a wet season from January to June and a dry season from July to December. On average, March is the wettest month, with an average of 214.3 mm/month, and September is the driest month with 61.3 mm/month (Australian Bureau of Meteorology).

The study period began on October 1, 2012 and lasted until July 17, 2013. During weekly site visits, surface water salinity (1 cm depth) was measured with a refractometer (VeeGee Scientific, Kirkland, WA) with accuracy of ± 1 ppt. Salinity varied from 36 ppt on October 24, 2012 to 1 ppt on March 6th, 2013. 4 mL of water was collected from 1 cm below the surface for isotopic analysis.

One *Avicennia marina* individual and one *Rhizophora stylosa* individual growing adjacent to one another on the fringe of the mangrove swamp were selected for study. In March

2013, one *Aegiceras corniculatum* individual was added to the study. The roots of all three trees were fully flooded at high tide. Each week, two sun-exposed branches were collected from each tree. One or two leaves from each branch were stored in an 11 mL glass screw cap vial for leaf water analysis. The remaining leaves from each branch were stored in Whirlpak plastic bags for lipid analysis. An ~5 cm length of well-suberized stem on each branch was stored in an 11 mL glass screw cap vial for xylem water analysis. All samples were stored in a cooler with ice and transferred to a -20 °C freezer, where they remained prior to analysis.

Rainwater samples were collected regularly throughout the study period in East Ballina, NSW (3.5 km NNE along the coast from Mobbs Bay at S 28°51.623', E 153°35.633'; **Figure 1**). 4 mL of rainwater falling on the rooftop of a single story dwelling (~3-4 m high) and flowing to ground level through a downspout was collected once or twice per rain event and stored in a glass screw cap vial sealed with electrical tape.

Leaf and xylem water extraction

Leaf and xylem water samples were cryogenically extracted on a vacuum line in the Agricultural Sciences Institute at ETH-Zurich, following methods modified from those of West et al. (2006). Samples were heated to 80 °C, while attached to a glass U-tube that was submerged in liquid Nitrogen. The sealed line was kept at a pressure below 60 mTorr for at least two hours. After this time, the U-tubes were removed from the liquid N₂, sealed, and the collected water was allowed to thaw. The water was then filtered through a 0.45 µm PTFE filter to remove any particulate material and transferred to 2mL screw cap vials, which were sealed with Parafilm until analysis.

Water isotope measurements

Leaf and xylem water samples were analyzed by a Thermal Conversion Elemental Analyzer (TC/EA) coupled to a Delta V Isotope Ratio Mass Spectrometer (IRMS; Thermo Scientific, Waltham, MA) in the Sustainable Land Use Group in the Botany Department of the University of Basel, Switzerland. Each sample and standard was injected six times in sequence. The first three injections of each sample were discarded in order to avoid memory effects. Thermo Isodat 3.0 software normalized raw isotopic ratios to the VSMOW (Vienna Standard Mean Ocean Water) scale using pulses of reference gas before and after the sample injection. Additionally, two in-house water standards of known isotopic composition were analyzed after every six samples throughout each sequence. The slope and intercept of the linear regression between the measured and known δ values of these standards was used to further normalize sample $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values and to correct for any instrumental drift throughout the course of the sequence. An additional laboratory standard was analyzed after every 12 samples. This standard was not used for any of the isotopic corrections, but was used to assess instrument performance over time and the accuracy of the isotope corrections. Average absolute offset from the known value of this standard was 1.0‰ for $\delta^2\text{H}$ measurements and 0.2‰ for $\delta^{18}\text{O}$ measurements. Average precision of triplicate sample injections was 0.9‰ for $\delta^2\text{H}$ measurements and 0.2‰ for $\delta^{18}\text{O}$ measurements.

Surface water and rainwater $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values were measured by a Picarro Cavity Ring Down Spectroscopy (CRDS) L2130i Water Isotope Analyzer at the University of Washington in Seattle. Measurements were normalized to the VSMOW scale using three laboratory standards of known isotopic composition. The three standards were analyzed between every six samples throughout the run. All samples and standards were injected six times, and the

first three measurements were discarded in order to avoid memory effects. Average precision of triplicate analyses was 0.2‰ for $\delta^2\text{H}$ measurements and 0.05‰ for $\delta^{18}\text{O}$ measurements.

Lipid extraction and purification

Four intact whole leaves from each week (2 from each branch sampled) were selected for analysis and treated as a single sample. Leaves were rinsed with DI water to remove debris, then cut into ~0.5 cm pieces using solvent-washed scissors prior to freeze-drying. Dry leaves were ground with a solvent-cleaned mortar and pestle, and lipids from ~0.5 g of dry leaf were extracted using accelerated solvent extraction (ASE-200, Dionex Corp., Sunnyvale, CA, USA) with 9:1 Dichloromethane:Methanol (DCM:MeOH) at 100 °C and 1500 psi for 3 x 5 min static cycles. The resulting total lipid extract (TLE) was evaporated to dryness under a stream of N_2 .

An aliquot of the TLE (~10 mg) was purified using silica gel column chromatography. The stationary phase was 1g of Si gel (5% deactivated with water). Hydrocarbons were eluted with 8 mL hexane (Hex), aldehydes and ketones with 6 mL 1:1 Hex:DCM, sterols, alcohols and triterpenols with 8 mL 4:1 Hexane:Ethyl Acetate, and remaining compounds with 4 mL MeOH.

The alcohol fraction was acetylated at 70 °C for 30 minutes with 25 μL of acetic anhydride of known $\delta^2\text{H}$ composition in 25 μL of pyridine. The alcohol fraction of *R. stylosa* leaves of all species was dominated by taraxerol, with relatively small amounts of β -sitosterol, β -amyrin, and *n*-alkanols. Lupeol was the most abundant compound in the alcohol fraction of *A. marina* leaves, which also contained significant amounts of α -amyrin, β -amyrin, β -sitosterol, and stigmasterol. Although they were not the most abundant alcohols, β -sitosterol and stigmasterol were the best resolved compounds by gas chromatography (GC), and were thus targeted for isotopic analyses. Stigmastanol was the most abundant compound in the alcohol fraction of *A.*

193 *corniculatum*. Lipids in the alcohol fraction were quantified by Gas Chromatography – Mass
194 Spectrometry (GC-MS) using an Agilent (Santa Clara, CA, USA) 6890N gas chromatograph
195 equipped with an Agilent 7683 autosampler, a split-splitless injector operated in splitless mode
196 and an Agilent VF-17ms capillary column (60 m X 0.32 mm X 0.25 μ m) interfaced to an Agilent
197 5975 quadrupole mass selective detector. The oven temperature was increased from 110 °C to
198 320 °C at 4 °C/min, then held at 320 °C for 10 minutes. Quantification of target compounds was
199 performed by comparing integrated peak areas with a known amount of 5 α -cholestane, added to
200 the sample just prior to GC-MS analysis.

201 The hydrocarbon fraction of leaves of all species was dominated by odd-numbered *n*-
202 alkanes, with chain lengths ranging from C₂₇-C₃₃. In *R. stylosa* and *A. corniculatum* leaves, *n*C₂₉
203 was the most abundant *n*-alkane, followed by *n*C₃₁. In *A. marina* leaves *n*C₃₁ and *n*C₃₃ were the
204 most abundant alkanes. The *n*-alkanes were quantified with a GC instrument equipped with
205 flame ionization detection (GC-FID). An Agilent 6890N gas chromatograph equipped with an
206 Agilent 7683 autosampler, programmable temperature vaporization inlet (PTV) operated in
207 splitless mode, and an Agilent DB-5ms column (60 m X 0.32 mm X 0.25 μ m) was used with He
208 as carrier gas (2.4 mL/min). The oven temperature was increased from 60 to 150 °C at 15
209 °C/min, then at 6 °C/min to 320 °C (held 28 min). Quantification of *n*-alkanes was performed by
210 comparing integrated peak areas with a known amount of *n*C₃₇-alkane, added to the sample just
211 prior to GC-FID analysis.

212 These quantifications were used to determine an appropriate amount of solvent in which
213 to dissolve the sample so that it would produce a peak of ~20 Volt*sec (V*s) when analyzed by
214 Gas Chromatography – Isotope Ratio Mass Spectrometry (GC-IRMS).

216 Lipid $\delta^2\text{H}$ analyses

217 The $\delta^2\text{H}$ values of individual lipids were measured by GC-IRMS on a Thermo DELTA V
218 PLUS system (Thermo Scientific, Waltham, MA, USA). The GC (Trace Ultra, Thermo) was
219 equipped with a split-splitless injector operated in splitless mode at 320 °C, a TRIPLUS
220 autosampler (Thermo Scientific), and a VF-17ms capillary column (60 m X 0.25 mm X 0.25 μm ,
221 Agilent). For taraxerol and stigmastanol analyses, the GC was heated from 120 °C to 260 °C at
222 20 °C/min, then at 1 °C/min to 300 °C, at 20 °C/min to 325 °C and then held at 325 °C for 20
223 min. For β -sitosterol and stigmansterol from *A. marina* the GC was heated from 120 °C to 260
224 °C at 20 °C/min, then at 1 °C/min to 300 °C, at 20 °C/min to 325 °C and then held at 325 °C for
225 40 min. For *n*-alkane analyses, the GC was heated from 120 °C to 250 °C at 20 °C/min, then at 6
226 °C/min to 325 °C and then held at 325 °C for 12 min. Helium was used as the carrier gas at a
227 constant flow of 1.1 mL/min. Compounds were pyrolyzed in a ceramic reactor at 1400 °C. 1 μL
228 of sample was injected along with 0.5 μL of a mix of *n*-alkanes of known isotopic composition
229 (A. Schimmelmann, Indiana University, Bloomington, Indiana). For isoprenoid analyses, this
230 mix included $n\text{C}_{26-}$, $n\text{C}_{28-}$, $n\text{C}_{32-}$, $n\text{C}_{34-}$ and $n\text{C}_{41}$ -alkanes. For *n*-alkane analyses, the co-injection
231 standards were $n\text{C}_{21}$ and $n\text{C}_{23}$. At the beginning and the end of the sequence, as well as after
232 every 4-6 sample injections, a mixture of additional *n*-alkane standards of known isotopic
233 composition was analyzed with the co-injection standards, in place of a sample. For isoprenoid
234 sequences, this external standard was $n\text{C}_{38}$ -alkane, which elutes within 1 minute of taraxerol
235 under these GC conditions. For *n*-alkane sequences, $n\text{C}_{28-}$, $n\text{C}_{32-}$ and $n\text{C}_{34}$ -alkanes were used as
236 external standards.

237 Thermo ISODAT software V.2.5 was used to control instrumentation and calculate $\delta^2\text{H}$
238 values. The slope and intercept of the relationship between measured and known $\delta^2\text{H}$ values of

the *n*-alkane standards was used to correct lipid $\delta^2\text{H}$ values for instrument bias. Offsets between corrected and known standard $\delta^2\text{H}$ values were used to further correct for drift, retention time, and peak area for each sequence. Each sample was measured in triplicate. Reported analytical uncertainties are the standard deviation of triplicate sample analyses. The H_3^+ factor was determined each day using pulses of a reference gas of varying heights and averaged 1.7‰.

RESULTS

Relationship between salinity and $\delta^2\text{H}/\delta^{18}\text{O}$ values of different water pools

There was an 84‰ range in $\delta^2\text{H}_{\text{Precipitation}}$ values throughout the study period, from $17.8 \pm 0.2\text{‰}$ to $-67.3 \pm 0.2\text{‰}$ (**Table S1**). Values of $\delta^{18}\text{O}_{\text{Precipitation}}$ varied by $\sim 11\text{‰}$ throughout the study period, from $0.21 \pm 0.05\text{‰}$ to $-10.97 \pm 0.05\text{‰}$ (**Table S1**). Lighter rainwater isotopes corresponded to periods of heavier rainfall in the summer and autumn, with heavier rainwater isotopes predominant in the drier spring. Surface water isotopes in Mobbs Bay varied between $-29 \pm 0.2\text{‰}$ and $6 \pm 0.2\text{‰}$ for $\delta^2\text{H}$ and $-5.15 \pm 0.05\text{‰}$ and $0.48 \pm 0.05\text{‰}$ for $\delta^{18}\text{O}$, changing in concert with rain events, with lower values during wet episodes and higher values during dry periods (**Figure 2a-c**).

Salinity and surface water isotopes ($\delta^2\text{H}_{\text{SW}}$ and $\delta^{18}\text{O}_{\text{SW}}$) at the study site were well correlated throughout the year (**Figure 3**; $R^2 = 0.76$ for $\delta^2\text{H}$; $R^2 = 0.85$ for $\delta^{18}\text{O}$). A 1 ppt increase in salinity resulted in increases of $0.77 \pm 0.07\text{‰}$ for $\delta^2\text{H}_{\text{SW}}$ and $0.14 \pm 0.01\text{‰}$ for $\delta^{18}\text{O}_{\text{SW}}$ (**Figure 3**).

Xylem water isotopes ($\delta^2\text{H}_{\text{XW}}$ and $\delta^{18}\text{O}_{\text{XW}}$) in *R. stylosa* and *A. marina* were positively correlated with salinity as well, but with significantly shallower slopes than the surface water (**Figure 3**). For *R. stylosa*, $\delta^2\text{H}_{\text{XW}}$ increased by $0.45 \pm 0.09\text{‰ ppt}^{-1}$ ($R^2 = 0.39$) (**Figure 3d**) and

262 $\delta^{18}\text{O}_{\text{XW}}$ increased by $0.08 \pm 0.02\text{‰ ppt}^{-1}$ ($R^2 = 0.40$) (**Figure 3a**). For *A. marina*, a 1 ppt increase
 263 in salinity was associated with a $0.43 \pm 0.08\text{‰}$ increase in $\delta^2\text{H}_{\text{XW}}$ ($R^2 = 0.44$) (**Figure 3e**) and
 264 with a $0.07 \pm 0.02\text{‰}$ increase in $\delta^{18}\text{O}_{\text{XW}}$ ($R^2 = 0.36$) (**Figure 3b**). Xylem water isotopes and
 265 salinity were not correlated in *A. corniculatum*, which was only sampled for the last 14 weeks of
 266 the study period ($p = 0.79$ for $\delta^2\text{H}_{\text{XW}}$ and 0.82 for $\delta^{18}\text{O}_{\text{XW}}$) (**Figures 3c and 3f**).

267 The apparent fractionation factors between surface water and xylem water ($\alpha^2_{\text{XW-SW}} =$
 268 $(\delta^2\text{H}_{\text{XW}} + 1000)/(\delta^2\text{H}_{\text{SW}} + 1000)$ and $\alpha^{18}_{\text{XW-SW}} = (\delta^{18}\text{O}_{\text{XW}} + 1000)/(\delta^{18}\text{O}_{\text{SW}} + 1000)$) were
 269 negatively correlated with salinity for *R. stylosa* and *A. marina* (**Figure 4**). For *R. stylosa*, $\alpha^2_{\text{XW-SW}}$
 270 decreased by $0.3 \pm 0.1\text{‰ ppt}^{-1}$ ($R^2 = 0.20$) (**Figure 4a**) and $\alpha^{18}_{\text{XW-SW}}$ decreased by $0.06 \pm$
 271 0.02‰ ppt^{-1} ($R^2 = 0.23$) (**Figure 4d**). For *A. marina*, $\alpha^2_{\text{XW-SW}}$ decreased by $0.3 \pm 0.1\text{‰ ppt}^{-1}$ (R^2
 272 $= 0.28$) (**Figure 4b**) and $\alpha^{18}_{\text{XW-SW}}$ decreased by $0.06 \pm 0.02\text{‰ ppt}^{-1}$ ($R^2 = 0.23$) (**Figure 4e**).
 273 Salinity was not significantly correlated with either $\alpha^2_{\text{XW-SW}}$ or $\alpha^{18}_{\text{XW-SW}}$ in *A. corniculatum* ($p =$
 274 0.14 for $\alpha^2_{\text{XW-SW}}$ and 0.12 for $\alpha^{18}_{\text{XW-SW}}$) (**Figures 4c and 4f**).

275 Leaf water isotopes ($\delta^2\text{H}_{\text{LW}}$ and $\delta^{18}\text{O}_{\text{LW}}$) were positively correlated with salinity in *R.*
 276 *stylosa* and *A. marina*, with slopes that were significantly steeper than the relationship between
 277 salinity and xylem water, but statistically indistinguishable from the relationship between salinity
 278 and surface water isotopes (**Figure 3**). For *R. stylosa*, a 1 ppt increase in salinity was associated
 279 with a $0.9 \pm 0.2\text{‰}$ increase in $\delta^2\text{H}_{\text{LW}}$ ($R^2 = 0.35$) (**Figure 3d**) and with a $0.22 \pm 0.07\text{‰}$ increase
 280 in $\delta^{18}\text{O}_{\text{LW}}$ ($R^2 = 0.25$) (**Figure 3a**). For *A. marina*, a 1 ppt increase in salinity was associated with
 281 a $0.7 \pm 0.2\text{‰}$ increase in $\delta^2\text{H}_{\text{LW}}$ ($R^2 = 0.28$) (**Figure 3e**) and with a $0.16 \pm 0.06\text{‰}$ increase in
 282 $\delta^{18}\text{O}_{\text{LW}}$ ($R^2 = 0.17$) (**Figure 3b**). Leaf water isotopes in *A. corniculatum* were not correlated with
 283 salinity ($p = 0.73$ for $\delta^2\text{H}_{\text{LW}}$ and 0.74 for $\delta^{18}\text{O}_{\text{LW}}$) (**Figures 3c and 3f**).

No significant correlations existed between salinity and the net hydrogen isotope fractionation between surface water and leaf water, $\alpha^2_{\text{LW-SW}}$, in any species, (**Figures 5a-c**) Likewise, no significant correlations existed between salinity and the evaporative enrichment of leaf water relative to xylem water, $\alpha^2_{\text{LW-XW}}$ (**Figures 5d-f**). Oxygen isotopic enrichment of leaf water relative to surface water and to xylem water was similarly uncorrelated with salinity and is not shown.

Temporal variability of lipid $\delta^2\text{H}$

Leaf lipid $\delta^2\text{H}$ values varied throughout the year, and differed significantly between *R. stylosa* and the other two species (**Figures 2d and 2e**). For *R. stylosa*, $n\text{C}_{29}$ -alkane $\delta^2\text{H}$ values ranged from -138‰ to -108‰, and averaged $-118 \pm 8\text{‰}$ (1σ) (**Figure 2d**). Insufficient lipid quantities precluded analysis in some samples, but from the 25 weeks where $\delta^2\text{H}$ values of the $n\text{C}_{31}$ -alkane were measured, they varied from -141‰ to -115‰ and averaged $-128 \pm 7\text{‰}$ (**Figure 2d**). The pentacyclic triterpenoid measured from this plant, taraxerol, had $\delta^2\text{H}$ values that varied from -192‰ to -154‰, with an average value of $-173 \pm 10\text{‰}$ (**Figure 2e**).

For *A. marina*, $n\text{C}_{31}$ -alkane $\delta^2\text{H}$ values varied from -169‰ to -143‰ and averaged $-154 \pm 6\text{‰}$ (**Figure 2d**). The $\delta^2\text{H}$ values of $n\text{C}_{33}$ -alkanes from this plant ranged from -180‰ to -142‰ with an average value of $-158 \pm 10\text{‰}$. β -sitosterol $\delta^2\text{H}$ values varied from -218‰ to -185‰, averaging $-200 \pm 7\text{‰}$ (**Figure 2e**). For stigmasterol, $\delta^2\text{H}$ values ranged from -224‰ to -195‰, with an average of $-207 \pm 7\text{‰}$ (**Figure 2e**).

Finally, $n\text{C}_{29}$ -alkane $\delta^2\text{H}$ values from *A. corniculatum*, which were only measured from March 28th onwards, varied from -169‰ to -146‰, and averaged $-163 \pm 8\text{‰}$ (**Figure 2d**). The $n\text{C}_{31}$ -alkane $\delta^2\text{H}$ values from this plant, which were measured for only 11 weeks, ranged from -

168‰ to -148‰, averaging -162 ± 5 ‰ (**Figure 2d**). Stigmastanol $\delta^2\text{H}$ values from *A. corniculatum* ranged from -242‰ to -218‰, with an average of -230 ± 7 ‰ (**Figure 2e**).

The apparent fractionation factor between surface water and leaf lipids ($\alpha_{\text{Lipid-SW}}$) was negatively correlated with salinity for all lipids from *A. marina* and *R. stylosa*, and for $n\text{C}_{29}$ -alkane from *A. corniculatum* (**Figure 6**). There was no significant correlation for *A. corniculatum* $n\text{C}_{31}$ -alkane ($p = 0.21$) or stigmastanol ($p = 0.39$) (**Figure 6**). Significant differences in the y-intercept of these linear relationships existed ($p < 0.0001$), but the slopes were not significantly different ($p = 0.10$).

DISCUSSION

Fractionation during water uptake and transpiration by mangroves

Mangroves and other woody halophytes discriminate against ^2H by as much as 11‰ during water uptake by roots (*Lin & Sternberg, 1993; Ellsworth & Williams, 2007*). Corresponding discrimination has not been observed against ^{18}O , permitting the use of $\delta^{18}\text{O}_{\text{XW}}$ to determine mangroves' environmental water source (*Lin & Sternberg, 1994; Ewe et al., 2007; Lambs et al., 2008; Wei et al., 2013; Santini et al., 2015*). Assuming the $\delta^{18}\text{O}$ values of xylem water from mangroves in Mobbs Bay represent the isotopic composition of their water source, surface (-1 cm) water was not the only water used by these trees, since $\delta^{18}\text{O}_{\text{XW}}$ values were usually 2-3‰ depleted in ^{18}O relative to surface water at salinity $> \sim 20$ ppt (**Figures 2b, 3a-3c**). Other potential water sources for the mangroves in Mobbs Bay include groundwater (*Ewe et al., 2007; Lambs et al., 2008*), freshwater runoff during rain events (*Wei et al., 2013; Santini et al., 2015; Reef et al., 2015*), and foliar uptake of rain or dew (reviewed in *Reef & Lovelock, 2015*). Foliar water uptake is an important water sources for some plants (*Burgess & Dawson,*

2004; Johnson & Smith, 2008; Eller et al., 2013). Thus far only indirect evidence, such as reverse xylem flow during rainfall events (De Groot, 2013) and at night (Rada et al., 1989; Hao et al., 2009) has suggested that foliar water uptake might occur in mangroves (Reef & Lovelock, 2015).

It seems more likely that mangroves are able to selectively take up sporadically available freshwater through their roots during rain events. When *A. marina* seedlings were grown in split plots where each half of the plant's roots were exposed to different water treatments, the mangroves used three times as much water from the low salinity (5 ppt) half than from the seawater salinity side (35 ppt) (Reef et al., 2015). This change in water uptake rate was observed within hours of decreasing salinity from one root chamber from 35 to 5 ppt, and demonstrates that mangroves may be able to increase water uptake through only one portion of their roots in response to freshwater runoff (Reef et al., 2015).

Salinity and $\alpha^{18}_{\text{XW-SW}}$ are negatively correlated, implying that the studied mangroves relied less on surface water as the salinity of that water increased (**Figures 4d and 4e**). The selective use of fresher water sources at high salinities has been documented previously (Ewe et al., 2007; Lambs et al., 2008; Wei et al., 2013; Ladd & Sachs, in review) and is consistent with increasingly negative $\delta^{18}\text{O}_{\text{XW}}$ values as salinity increased in Mobbs Bay mangroves.

^2H depletion of xylem water from *R. stylosa* and *A. marina* relative to surface water was observed at all salinities, even when $\delta^{18}\text{O}_{\text{XW}}$ values were not depleted relative to surface water (**Figures 2, 3d-3f**). Furthermore, $\delta^2\text{H}_{\text{XW}}$ values were lower than those predicted from $\delta^{18}\text{O}_{\text{XW}}$, assuming a constant relationship between the two isotopes in both xylem water and local surface water (**Figure 7**). Discrimination against ^2H during water uptake by halophyte roots has been reported previously and likely caused low $\delta^2\text{H}_{\text{XW}}$ values in Mobbs Bay mangroves relative to

$\delta^2\text{H}_{\text{SW}}$ (Lin & Sternberg, 1993; Ellsworth & Williams, 2007; Ladd & Sachs, in review). The magnitude of this effect can be determined from the relationships between $\alpha_{\text{XW-SW}}$ and salinity for each isotope. For both *A. marina* and *R. stylosa*, the y-intercept of the regression between $\alpha^{18}_{\text{XW-SW}}$ and salinity is 1.000 (**Figure 4d and 4e**), indicating that when surface water is fresh, it is the primary water source for the mangroves. The y-intercepts for the linear regression between $\alpha^2_{\text{XW-SW}}$ and salinity are 0.993 ± 0.003 for *R. stylosa* and 0.994 ± 0.002 for *A. marina* (**Figures 4a and 4b**). ^2H discrimination during water uptake is thus $7 \pm 3\text{‰}$ by *R. stylosa* and $6 \pm 2\text{‰}$ for *A. marina*. These values compare favorably with published reports of 2 - 11‰ ^2H depletion in the xylem water of mangroves and other woody halophytes (Lin & Sternberg, 1993; Ellsworth & Williams, 2007).

While the xylem water isotope values are informative about the water pools accessed by mangroves growing in Mobbs Bay, and suggest that mangroves discriminate against ^2H during water uptake, they cannot explain the inverse relationship between salinity and $\alpha_{\text{Lipid-SW}}$ observed here and elsewhere (**Figure 6**; Ladd & Sachs, 2012; Ladd & Sachs, in review). This is because the leaf water isotopes track surface water isotopes much better than xylem water isotopes (**Figures 3, 5**), and leaf water is the source of at least 96% of the hydrogen for leaf lipids synthesized by dicots (Kahmen et al., 2013b). There is no salinity dependence for either $\alpha^2_{\text{LW-SW}}$ or $\alpha^{18}_{\text{LW-SW}}$ in any of the three species studied (**Figures 5a-c**). This result suggests that environmental variables such as relative humidity and the isotopic composition of ambient water vapor have a more significant impact on leaf water isotopes in mangroves than the isotopic composition of xylem water.

Differences in biosynthetic fractionation among different species of mangroves

In contrast to the nearly identical xylem water and leaf water isotopes among the three species, there were significant differences in the $\delta^2\text{H}_{\text{Lipid}}$ values (**Figure 2**). In particular, the $\delta^2\text{H}$ values of $n\text{C}_{31}$ -alkane, the one compound that was produced in sufficient abundance by all three species, was consistently enriched in *R. stylosa* relative to the other two species by ~25-30‰ (**Figure 2d**). This interspecies variability highlights a potential complication of using n -alkane $\delta^2\text{H}$ values to reconstruct past hydroclimate, as a large shift in sedimentary $n\text{C}_{31}$ -alkane $\delta^2\text{H}$ values in a setting like Mobbs Bay could reflect a change in the dominant mangrove species rather than being driven solely by hydrological conditions.

Significant variation in interspecies $\delta^2\text{H}$ values was also observed in isoprenoidal lipids. Taraxerol from *R. stylosa* was enriched by ~30‰ relative to β -sitosterol and stigmasterol from *A. marina* and by ~60‰ relative to stigmastanol from *A. corniculatum* (**Figure 2**). These differences could be due to the different biosynthetic pathways from which pentacyclic triterpenols and sterols are produced. For example, pentacyclic triterpenols produced by *Daucus carota* are enriched by 30-60‰ relative to sterols produced by the same plant (Sessions *et al.*, 1999), and the pentacyclic triterpenol lupenol is enriched by 30-40‰ relative to β -sitosterol produced by *Spartina alterniflora* (Sessions, 2006). The observation that the most enriched isoprenoid is from the plant that also produced the most enriched n -alkane may therefore be a coincidence.

Previous studies observed differences in biosynthetic fractionation up to 70‰ among different plants growing at the same site (Feakins & Sessions, 2010a; Feakins & Sessions, 2010b; Romero & Feakins, 2011; Eley *et al.*, 2013). It is unlikely that this variability is due to changes in the isotopic fractionation associated with individual biosynthetic steps, which are typically consistent with temperature and reaction rate (Schmidt *et al.*, 2003; Wang *et al.*, 2009).

Rather, variability in $\alpha_{\text{Lipid-LW}}$ is more likely to be caused by different sources of NADPH, differences in the relative use of stored carbohydrates, or by different timing of lipid production. These possibilities are explored below.

The hydrogen in lipids is derived from three sources: intracellular water, carbohydrate molecular precursors, and NADPH (*Sessions et al., 1999; Schmidt et al., 2003; Zhang et al., 2009*). Of these, variations in the relative amounts of H from NADPH derived from photosynthesis or from the pentose phosphate cycle have the most potential to profoundly influence $\delta^2\text{H}_{\text{Lipid}}$, as $\delta^2\text{H}$ values of the two NADPH pools can differ by several hundred per mil (*Schmidt et al., 2003; Zhang et al., 2009*). The different compatible solutes employed by each species could cause variability in the NADPH sources for lipid synthesis. *A. marina* produces the amino acids asparagine and glycine betaine as its main compatible solutes (*Ashihara et al., 1997; Hibino et al., 2001*) while *A. corniculatum* produces aspartic acid and alanine (*Datta & Ghosh, 2003*). *R. stylosa*, on the other hand, primarily uses the soluble carbohydrates pinitol and manitol (*Hibino et al., 2001*). Because the primary NADPH source of H to amino acids is the relatively enriched pool from the pentose phosphate cycle (*Schmidt et al., 2003*), it is possible that the production of amino acid-based compatible solutes in *A. marina* and *A. corniculatum* results in relatively more use of depleted NADPH from photosynthesis for lipid synthesis in those two species as compared to *R. stylosa*.

Another possible explanation for variability in biosynthetic fractionation among these mangrove species could be different reliance on stored carbohydrates as lipid precursors. Stored carbohydrates are enriched in ^2H relative to current photosynthate, and biosynthetic products derived from stored carbohydrates are likewise enriched in ^2H (*Yakir & DeNiro, 1990; Yakir, 1992; Roden & Ehrlinger, 2000*). If lipid synthesis in *R. stylosa* is more reliant on stored

carbohydrates than in the other two species, that could account for the observed differences in *n*-alkane $\delta^2\text{H}$ values among species. *A. marina* is more salt tolerant than *R. stylosa* (Clough, 1984; Naidoo, 1985; Clough & Sim, 1989), and consistently has higher photosynthetic rates than *R. stylosa* growing at the same site (Clough & Sim, 1989). *A. marina* may therefore have more primary photosynthate available for leaf wax synthesis than *R. stylosa*, which may be more likely to rely on stored carbohydrates for lipid production during times of salt stress.

Another possibility is that differences in the timing of lipid production account for the differences in $\delta^2\text{H}$ values among species. This could include production of lipids at different times of day and during different seasons, as well as different lipid residence times within the leaf. All leaf water measurements made in this study were from leaves collected in the early-to-mid afternoon, in order to capture the maximum diurnal enrichment of leaf water. However, leaf water isotopes fluctuate throughout the day with changes in relative humidity, temperature and stomatal conductance (Kahmen *et al.*, 2008; Ferrio *et al.*, 2009). If *R. stylosa* fixes a larger proportion of carbon during the afternoon, when leaf water is most enriched, it would be expected to have more enriched lipids than *A. marina* or *A. corniculatum* if they fix a larger fraction of carbon in the morning and/or evening.

Daily water use patterns and photosynthetic activity differ for adjacent *R. stylosa* and *A. marina* growing on North Stradbroke Island, ~200 km north of Mobbs Bay (Vandegehuchte *et al.*, 2014). Stomatal resistance in *R. stylosa* is relatively constant throughout daylight hours, while *A. marina* has much higher stomatal resistance rates during the afternoon than in the morning or evening (Vandegehuchte *et al.*, 2014). This pattern is consistent with *A. marina* being more photosynthetically active during cooler parts of the day, when leaf water should be less enriched, which would result in relatively depleted leaf waxes.

Another possibility is that different species may produce lipids during different seasons. Temperate plants typically produce leaf waxes soon after leaf flush in the spring, and some studies have shown the isotopic signature of leaf waxes during this time of high production persists throughout the growing season (Sachse *et al.*, 2010; Tipple *et al.*, 2013), while other work indicates that leaf waxes are regenerated and isotopically variable over time (Sachse *et al.*, 2009; Gao *et al.*, 2012; Gao & Huang, 2013). Leaf production in mangroves, and other tropical evergreen plants, is not limited to one season and occurs throughout the year (Wium-Andersen & Christensen, 1978; Duke *et al.*, 1984; Coupland *et al.*, 2005). Seasonal variability in afternoon $\delta^2\text{H}_{\text{LW}}$ values in Mobbs Bay was more than 60‰ in both *A. marina* and *R. stylosa* (**Figures 2 and 3**). If *R. stylosa* produces relatively more leaf lipids during drier seasons, when $\delta^2\text{H}_{\text{LW}}$ values are high, it would have ^2H -enriched lipids compared to *A. marina* and *A. corniculatum*.

In studies of leaf longevity from Phuket, Thailand, and Darwin, Australia, *Rhizophora* leaves had greater average longevity than *A. marina* leaves (Wium-Andersen & Christensen, 1978; Coupland *et al.*, 2005). *Rhizophora* leaves persisted on average for 11.2 ± 0.2 months, as compared to *A. marina* leaves, which lasted an average of 6.3 ± 0.1 months. If *R. stylosa* has the longest leaf residence time among Mobbs Bay mangroves, its $\delta^2\text{H}_{\text{Lipid}}$ values might be more reflective of average conditions in Mobbs Bay throughout the year, while lipids in *A. marina* leaves would be more responsive to changing environmental conditions.

Likewise, lipid turnover in existing leaves could account for differences among species. While most leaf waxes are produced soon after leaf flush, new waxes can form on mature leaves that have been abraded by wind, rain, or fauna, changing the overall $\delta^2\text{H}_{\text{Wax}}$ values (Sachse *et al.*, 2009; Gao *et al.*, 2012; Gao & Huang, 2013). If *A. marina* and *A. corniculatum* leaf waxes are more susceptible to abrasion during rain events, and are subsequently regenerated using the

relatively fresher, isotopically depleted water associated with those storms, their lipids would be less enriched than those of *R. stylosa*. Lipid turnover rates have not been investigated for sterols and triterpenols, which are not part of the epicuticular wax, but are located in sub-epidermal parts of the leaf. These compounds are likely less susceptible to abrasion than waxes, and the fact that changes in their $\delta^2\text{H}$ values do not covary with those of *n*-alkanes (**Figure 2**) suggests that different factors control the residence times of different lipid classes.

Relationship between salinity and net isotopic fractionation in mangrove lipids

Net fractionation during mangrove lipid synthesis, $\alpha_{\text{Lipid-SW}}$, was inversely related to salinity (**Figure 6**), as observed previously for *A. marina* growing along the Brisbane River (Ladd & Sachs, 2012) and for *Rhizophora* spp. growing along estuaries in Micronesia and around marine lakes in Palau (Ladd & Sachs, *in review*). Part of this dependence was driven by changes in $\delta^2\text{H}_{\text{SW}}$ values, which were large and positively correlated with salinity (**Figure 3**), making it more appropriate to look at lipid $\delta^2\text{H}$ values in order to assess changes in lipid composition due to variable salinity (**Figure 2d and 2e**). Changes in $\delta^2\text{H}_{\text{SW}}$ alone do not explain the decreases in $\alpha_{\text{Lipid-SW}}$ at higher salinities, as $\delta^2\text{H}_{\text{Lipid}}$ values decreased for 7 of the 10 lipids studied here even as $\delta^2\text{H}_{\text{SW}}$ values increased (**Figure 2d and 2e**). These results contribute to the growing body of evidence that mangroves discriminate more strongly against ^2H during lipid synthesis as salinity increases, regardless of species or local climate (Ladd & Sachs, 2012; Ladd & Sachs, *in review*).

The results of this study make it possible to reevaluate the hypotheses initially proposed to explain the inverse relationship between salinity and net isotopic fractionation in mangroves by Ladd and Sachs (2012). One hypothesis was that discrimination against ^2H during water

uptake increased with increasing salinity. The data presented here do not support this hypothesis. While all three species discriminated against ^2H during water uptake, there was no systematic change in the magnitude of that fractionation as a function of salinity (**Figure 7**). The decrease in $\alpha^2_{\text{XW-SW}}$ as salinity increased was associated with a decrease in $\alpha^{18}_{\text{XW-SW}}$ (**Figure 4**), which has not been observed to be sensitive to water isotope fractionation by roots (*Lin & Sternberg, 1993; Ellsworth & Williams, 2007*), and is thus more plausibly explained by the opportunistic utilization of fresher, more depleted water.

The utilization of isotopically depleted, fresher water sources at higher salinity cannot explain the inverse correlation between salinity and $\alpha^2_{\text{Lipid-SW}}$ however, because the isotopic depletion in the xylem water is not observed in the leaf water from which leaf lipids are synthesized (**Figures 5**). Since there is no change in $\alpha^2_{\text{LW-SW}}$ with salinity (**Figures 5a-5c**), the decrease in $\delta^2\text{H}_{\text{XW}}$ values relative to $\delta^2\text{H}_{\text{SW}}$ values, while interesting from the perspective of salinity tolerance and water use by mangroves, is less relevant to understanding the mechanism responsible for the inverse relationship between salinity and $\alpha_{\text{Lipid-SW}}$.

Likewise, the fact that there are no systematic decreases in net leaf water enrichment with increasing salinity argues against the third hypothesis proposed by Ladd and Sachs (2012), which is based on increased salt secretion by *A. marina* at higher salinity. We hypothesized that hydration of salts on the surfaces of leaves could diminish leaf-water ^2H -enrichment by both lowering the vapor-pressure differential across the leaf surface, and by depleting the $\delta^2\text{H}$ value of water vapor near the leaf surface (since hydration water of salts is ^2H -depleted relative to water vapor (*Matsuo et al., 1972*)). Both of these hypotheses were based on increased salt on the leaf surface at high salinity. As such, they should be more pronounced for the salt secreting mangroves *A. marina* and *A. corniculatum*. Since there was no difference in $\alpha^2_{\text{LW-SW}}$ between the

salt secreting mangroves with salt-coated leaves and the non-secreting tree with no visible salt deposits on its leaves, and since there was no relationship between salinity and α_{LW-SW}^2 in any of the three species (**Figure 5**), it seems unlikely that the hypothesis relating salt on the leaf surface to reduced leaf water enrichment is correct.

Ultimately, the entire variability in apparent fractionation between leaf lipids and source water in Mobbs Bay mangroves must be explained either by variability in biosynthetic fractionation, $\alpha_{Lipid-LW}$, or by variable lipid production in response to fluctuating salinity. Salinity dependent changes in $\alpha_{Lipid-LW}$ can account for differences among species and for differences within an individual tree. The second hypothesis posed by Ladd and Sachs (2012) to explain the Brisbane River data, that increased compatible solute production at high salinity results in less H from NADPH derived via the pentose phosphate cycle being incorporated into lipids, is the only previously proposed hypothesis that would manifest itself as changes in $\alpha_{Lipid-LW}$. However, the data presented here cannot confirm or refute this hypothesis. Other processes described above that could result in differences in $\alpha_{Lipid-LW}$ among species, such as variability in the amount of stored carbohydrates used for lipid synthesis, could also explain differences in $\alpha_{Lipid-LW}$ within a species over time, as in this study, or over spatial salinity gradients (Ladd & Sachs, 2012; Ladd & Sachs, *in review*). While the data here does not definitively explain why there would be more biosynthetic discrimination against 2H at higher salinity, it does suggest that this is a promising avenue for further investigation of the mechanisms responsible for the inverse relationship between salinity and $\alpha_{Lipid-SW}$.

Alternatively, some or all of the observed salinity dependence of $\alpha_{Lipid-SW}$ for mangrove lipids (**Figure 6**), could be due to variable rates of leaf and leaf lipid production in response to salinity. If mangroves in seasonal locations such as Mobbs Bay preferentially produce leaves and

leaf lipids during rainy periods when salinity and $\delta^2\text{H}_{\text{SW}}$ are low, the apparent net fractionation and apparent biosynthetic fractionation would appear larger during times of high salinity, when $\delta^2\text{H}_{\text{SW}}$ and $\delta^2\text{H}_{\text{LW}}$ are relatively high (**Figure 2**). Since mangrove leaves are produced throughout the year (*Wium-Andersen & Christensen, 1978; Duke et al., 1984; Coupland et al., 2005*), and since leaf lipids can be regenerated over the existence of a leaf (*Sachse et al., 2009; Gao et al., 2012*), the influence of newly synthesized lipids would be muted by the continued presence of older compounds.

CONCLUSIONS

$\delta^2\text{H}$ values of leaf lipids and $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of surface, xylem, and leaf water were measured weekly in *R. stylosa*, *A. marina*, and *A. corniculatum* mangroves in Mobbs Bay, New South Wales, Australia over 9.5 months. When salinity exceeded 20 ppt, xylem water $\delta^{18}\text{O}$ values decreased relative to surface water, suggesting that the mangroves relied more on non-surface water sources, such as groundwater, episodic rain events and/or foliar water uptake. Even at times when there was no ^{18}O -depletion, xylem water $\delta^2\text{H}$ was depleted by 4-10‰ relative to estuary surface water in all three species, implying discrimination against ^2H during water uptake.

Although xylem water became more ^2H - and ^{18}O -depleted relative to surface water as salinity increased from 1-36 ppt, there were no systematic changes in leaf water $\delta^2\text{H}$ or $\delta^{18}\text{O}$ values relative to surface water. Leaf water $\delta^2\text{H}$ values were also similar among species. In all three species, the ^2H -depletion in leaf lipids relative to estuary and leaf water increased with salinity by 0.5-1.0‰ ppt⁻¹ in *n*-alkanes and by 0.4-0.8‰ ppt⁻¹ in isoprenoids. Since there were no systematic changes in leaf water enrichment with salinity, changes in leaf and xylem water $\delta^2\text{H}$

values cannot explain the salinity dependence of ^2H -depletion in leaf lipids. More likely causes are (i) increased compatible solute production at high salinity causing changes in the source of NADPH used in lipid synthesis, (ii) reduced utilization of stored carbohydrates for lipid synthesis as salinity increases, or (iii) variability in the timing of lipid production and turnover rates. An $\sim 30\text{‰}$ ^2H enrichment of *n*-alkanes and isoprenoids in *R. stylosa* leaves relative to the other two species can be explained by either different classes of compatible solutes, a different proportional reliance on stored carbohydrates in lipid synthesis, different timing of leaf lipid production, or different turnover rates of leaves and/or leaf lipids compared to *A. marina* and *A. corniculatum*.

Further investigation into the causes of biosynthetic fractionation in leaf lipids and into the timing of lipid production and replacement rates in mangroves will better constrain the use of $\delta^2\text{H}_{\text{lipid}}$ values from mangroves as a paleohydrologic proxy, and offer improved understanding of carbon and lipid metabolisms within trees.

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References

- Alongi D.M. & Mukhopadhyay S.K. (2014) Contribution of mangroves to coastal carbon cycling in low latitude seas. *Agricultural and Forest Meteorology* [doi:10.1016/j.agrformet.2014.10.005](https://doi.org/10.1016/j.agrformet.2014.10.005)
- Ashihara H., Adachi K., Otawa M., Yasumoto E., Fukushima Y., Kato M., Sano H., Sasamoto H. & Baba S. (1997) Compatible solutes and inorganic ions in the mangrove plant *Avicennia marina* and their effects on the activities of enzymes. *Zeitschrift für Naturforschung* 52, 433-440.
- Burgess S.S.O. & Dawson T.E. (2004) The contribution of fog to the water relations of *Sequoia sempervirens* (D. Don): foliar uptake and prevention of dehydration. *Plant, Cell and Environment* 27, 1023–1034.
- Clough B.F. (1984) Growth and salt balance of the mangroves. *Avicennia marina* (Forsk.) Vierh. and *Rhizophora stylosa* Griff. in relation to salinity. *Australian Journal of Plant Physiology* 11, 419–430
- Clough B.F. & Sim, R.G. (1989) Changes in gas exchange characteristics and water use efficiency of mangroves in response to salinity and vapour pressure deficit. *Oecologia* 79, 38–44.
- Coupland G.T., Paling E.I. & McGuinness K.A. (2005) Vegetative and reproductive phenologies of four mangrove species from northern Australia. *Australian Journal of Botany* 53, 109–117.
- Craig H. & Gordon L. (1965) Deuterium and oxygen 18 variations in the ocean and the marine atmosphere. In: Tongiorgi, E. (Ed.), *Proceedings of a Conference on Stable Isotopes in Oceanographic Studies and Paleotemperatures*. CNR-Laboratorio di Geologia Nucleare, Pisa, pp. 9-130.
- Dansgaard W. (1964) Stable isotopes in precipitation. *Tellus* 16, 436-468.
- Datta P.N. & Ghose M. (2003) Estimation of osmotic potential and free amino acids in some mangroves of the Sundarbans, India. *Acta Botanica Croatica* 62, 37-45.
- De Groote S. (2013) Impact of dew and rain on the water relations of the mangrove species *Avicennia marina* (Forssk.) Vierh. Master's thesis, University Ghent, Faculty of Bioscience Engineering.
- Douglas P.J.M., Pagani M., Brenner M., Hodell D.A. & Curtis J.H. (2012) Aridity and vegetation composition are important determinants of leaf-wax δD values in southeastern Mexico and Central America. *Geochimica et Cosmochimica Acta* 97, 24-45.

- Duke N.C., Bunt J.S. & Williams W.T. (1984) Observations of the floral and vegetative phenologies of north-eastern Australian mangroves. *Australian Journal of Botany* 32, 87–99.
- Eley Y., Dawson L., Black S., Andrews J. & Pedentchouk N. (2013) Understanding 2H/1H systematics of leaf wax n-alkanes in coastal plants at Stiffkey saltmarsh, Norfolk, UK. *Geochimica et Cosmochimica Acta* 128, 13–28.
- Eller C.B., Lima A.L. & Oliveira R.S. (2013) Foliar uptake of fogwater and transport belowground alleviates drought effects in the cloud forest tree species, *Drimys brasiliensis* (Winteraceae). *New Phytologist* 199, 151–162.
- Ellsworth P. & Williams D. (2007) Hydrogen isotope fractionation during water uptake by woody xerophytes. *Plant and Soil* 291, 93–107.
- Ewe S., Sternberg L. & Childers D. (2007) Seasonal plant water uptake patterns in the saline southeast Everglades ecotone. *Oecologia* 152, 607–616.
- Feakins S.J. & Sessions A.L. (2010a) Crassulacean acid metabolism influences D/H ratio of leaf wax in succulent plants. *Organic Geochemistry* 41, 1269–1276.
- Feakins S.J. & Sessions A.L. (2010b) Controls on the D/H ratios of plant leaf waxes in an arid ecosystem. *Geochimica Cosmochimica Acta* 74, 2128–2141.
- Ferrio J.P., Cuntz M., Offermann C., Siegwolf R., Saurer M. & Gessler A. (2009) Effect of water availability on leaf water isotopic enrichment in beech seedlings shows limitations of current fractionation models. *Plant, Cell and Environment*, 32, 1285–1296.
- Gao L., Burnier A. & Huang Y. (2012) Quantifying instantaneous regeneration rates of plant leaf waxes using stable hydrogen isotope labeling. *Rapid Communications in Mass Spectrometry*, 26, 115–122.
- Gao L., Edwards E.J., Zeng Y. & Huang Y. (2014) Major evolutionary trends in hydrogen isotope fractionation of vascular plant leaf waxes. *PLOS one*, DOI: 10.1371/journal.pone.0112610
- Gao L. & Huang Y. (2013) Inverse gradients in leaf wax δD and $\delta^{13}C$ values along the grass blades of *Micanthus sinensis*: implications for leaf wax reproduction and plant physiology. *Oecologia*, DOI: 10.1007/s00442-012-2506-6
- Gat J.R. (1996) Oxygen and hydrogen isotopes in the hydrologic cycle. *Annual Review in Earth and Planetary Sciences* 24, 225–262.
- Hao G.Y., Jones, T.J., Luton, C., Zhang, Y.J., Manzane, E., Scholz, F.G., Bucci, S.J., Cao, K.F. & Goldstein, G. (2009) Hydraulic redistribution in dwarf *Rhizophora mangle* trees driven by interstitial soil water salinity gradients: impacts on hydraulic architecture and gas exchange. *Tree Physiology* 29, 697–705.
- Hibino T., Meng Y.L., Kawamitsu Y., Uehara N., Matsuda N., Tanaka Y., ..., Takabe T., 2001. Molecular cloning and functional characterization of two kinds of betaine-aldehyde dehydrogenase in betaine-accumulating mangrove *Avicennia marina* (Forsk.) Vierh. *Plant Molecular Biology* 45, 353–363.
- Hou J., D'Andrea W. & Huang Y. (2008) Can sedimentary leaf waxes record D/H ratios of continental precipitation? Field, model and experimental assessments. *Geochimica et Cosmochimica Acta* 72, 3503–3517.
- Hou J., D'Andrea W., MacDonald D. & Huang Y.S. (2007) Hydrogen isotopic variability in leaf waxes among terrestrial and aquatic plants around Blood Pond, Massachusetts (USA). *Organic Geochemistry* 38, 977–984.

- Huang Y., Shuman B., Wang Y. & Webb T. (2004) Hydrogen isotope ratios of individual lipids in lake sediments as novel tracers of climatic and environmental change: a surface sediment test. *Journal of Paleolimnology* 31, 363-375.
- Johnson D.M. & Smith W.K. (2008) Cloud immersion alters microclimate, photo- synthesis and water relations in *Rhododendron catawbiense* and *Abies fraseri* seedlings in the southern Appalachian Mountains, USA. *Tree Physiology* 28, 385-392.
- Kahmen A., Hoffmann B., Schefuss E., Arndt S.K., Cernusak L.A., West J.B. & Sachse D. (2013a) Leaf water deuterium enrichment shapes leaf wax n-alkane dD values of angiosperm plants II: Observational evidence and global implications. *Geochimica et Cosmochimica Acta* 111, 50-63.
- Kahmen A., Schefuss E. & Sachse D. (2013b) Leaf water deuterium enrichment shapes leaf wax n-alkane dD values of angiosperm plants I: Experimental evidence and mechanistic insights. *Geochimica et Cosmochimica Acta* 111, 39-49.
- Kahmen A., Simonin K., Tu K.P., Merchant A. & Callister A. (2008) Effects of environmental parameters, leaf physiological properties and leaf water relations on leaf water delta ¹⁸O enrichment in different Eucalyptus species. *Plant, Cell & Environment* 31, 738-751.
- Ladd S.N. & Sachs J.P. (2012) Inverse relationship between salinity and n-alkane δD values in the mangrove *Avicennia marina*. *Organic Geochemistry* 48, 25-36.
- Ladd S.N. & Sachs J.P. (in review) Influence of salinity on hydrogen isotope fractionation in *Rhizophora* mangroves from Micronesia. *Geochimica et Cosmochimica Acta*.
- Lambs L., Muller E. & Fromard F. (2008) Mangrove trees growing in a very saline condition but not using seawater. *Rapid Communications in Mass Spectrometry* 22, 2835-2843.
- Lin G. & Sternberg L. (1993) Hydrogen isotopic fractionation by plant roots during water uptake in coastal wetland plants. In *Stable Isotopes and Plant Carbon/Water Relations* (eds Ehleringer, J., Hall, A. & Farquhar, G.), pp. 497-510. Academic Press, Inc., San Diego.
- Lin G. & Sternberg L. (1994) Utilization of surface water by red mangrove (*Rhizophora mangle* L.): An isotopic study. *Bulletin of Marine Science* 54, 94-102.
- Liu W.G. & Yang H. (2008) Multiple controls for the variability of hydrogen isotopic compositions in higher plant n-alkanes from modern ecosystems. *Global Change Biology* 14, 2166-2177.
- Liu W.G., Yang H. & Li L. (2006) Hydrogen isotopic composition of n-alkanes from terrestrial plants correlate with their ecological life form. *Oecologia* 150, 330-338.
- Matsuo S., Friedman I. & Smith G.I. (1972) Studies of quaternary saline lakes – I. Hydrogen isotope fractionation in saline minerals. *Geochimica et Cosmochimica Acta* 36, 427-435.
- Naidoo G. (1985) Effects of waterlogging and salinity on plant-water relations and on the accumulation of solutes in three mangrove species. *Aquatic Botany* 22, 133-143.
- Polissar P.J. & Freeman K.H. (2010) Effects of aridity and vegetation on plant-wax δD in modern lake sediments. *Geochimica et Cosmochimica Acta* 74, 5785-5797.
- Rada F., Goldstein G., Orozco A., Montilla M., Zabala O. & Azócar A. (1989) Osmotic and turgor relations of three mangrove ecosystem species. *Functional Plant Biology* 16, 477-486.
- Reef R. & Lovelock C.E. (2015) Regulation of water balance in mangroves. *Annals of Botany* 155, 385-395.
- Reef R., Markham H.L., Santini N.S. & Lovelock C.E. (2015) The response of the mangrove *Avicennia marina* to heterogeneous salinity measured using a split-root approach. *Plant and Soil*, doi 10.1007/s11104-015-2489-2.

- Roden J.S., Lin G. & Ehleringer J.R. (2000) A mechanistic model for interpretation of hydrogen and oxygen isotope ratios in tree-ring cellulose. *Geochimica et Cosmochimica Acta* 64, 21-35.
- Romero I.C. & Feakins S.J. (2011) Spatial gradients in plant leaf wax D/H across a coastal salt marsh in southern California. *Organic Geochemistry* 42, 618-629.
- Sachse D., Billault I., Bowen G.J., Chikaraishi Y., Dawson T.E., Feakins S.J., ... Kahmen A. (2012) Molecular paleohydrology: interpreting the hydrogen-isotopic composition of lipid biomarkers from photosynthesizing organisms. *Annual Review of Earth and Planetary Science* 40, 212-249.
- Sachse D., Dawson T. & Kahmen A. (2015) Seasonal variation of leaf wax *n*-alkane production and $\delta^2\text{H}$ values from the evergreen oak tree, *Quercus agrifolia*. *Isotopes in Environmental Health and Sciences* 51, 1-19.
- Sachse D., Gleixner G., Wilkes H. & Kahmen A. (2010) Leaf wax *n*-alkane δD values of field-grown barley reflect leaf water δD values at the time of leaf formation. *Geochimica et Cosmochimica Acta* 74, 6741-6750.
- Sachse D., Kahmen A. & Gleixner G. (2009) Significant seasonal variation in the hydrogen isotopic composition of leaf-wax lipids for two deciduous tree ecosystems (*Fagus sylvatica* and *Acer pseudoplatanus*). *Organic Geochemistry* 40, 732-742.
- Sachse D., Radke J. & Gleixner G. (2004) Hydrogen isotope ratios of recent lacustrine sedimentary *n*-alkanes record modern climate variability. *Geochimica et Cosmochimica Acta* 68, 4877-4889.
- Santini N.S., Reef R., Lockington D.A. & Lovelock C.E. (2015) The use of fresh and saline water sources by the mangrove *Avicennia marina*. *Hydrobiologia* 745, 59-68.
- Schmidt H.L., Werner R.A. & Eisenreich W. (2003) Systematics of ^2H patterns in natural compounds and its importance for the elucidation of biosynthetic pathways. *Phytochemistry Reviews* 2, 61-85.
- Sessions A. (2006) Seasonal changes in D/H fractionation accompanying lipid biosynthesis in *Spartina alterniflora*. *Geochimica et Cosmochimica Acta* 70, 2153-2162.
- Sessions A.L., Burgoyne T.W., Schimmelmann A. & Hayes J. M. (1999) Fractionation of hydrogen isotopes in lipid biosynthesis. *Organic Geochemistry* 30, 1193-1200.
- Smith F.A. & Freeman K.H. (2006) Influence of physiology and climate on δD of leaf wax *n*-alkanes from C_3 and C_4 grasses. *Geochimica et Cosmochimica Acta* 70, 1172-1187.
- Tipple B.J., Berke M.A., Doman C.E., Khachaturyan S. & Ehleringer J.R. (2013) Leaf *n*-alkane record the plant-water environment at leaf flush. *Proceedings of the National Academy of Science* 110, 2659-2664.
- Tipple B.J., Berke M., Hambach B., Roden J.S. & Ehleringer J.R. (In Press) Predicting leaf wax *n*-alkane (2) H/(1) H ratios: controlled water source and humidity experiments with hydroponically grown trees confirm predictions of Craig-Gordon model. *Plant, Cell and Environment*. doi: 10.1111/pce.12457.
- Tipple B.J. & Pagani M. (2013) Environmental control on eastern broadleaf forest species' leaf wax distributions and D/H ratios. *Geochimica et Cosmochimica Acta* 111, 64-77.
- Vandegehuchte M.W., Guyot A., Hubau M., DeGroote S.R.E., De Baerdemaeker N.J.F., Hayes M., ..., Steppe K. (2014) Long-term versus daily stem diameter variation in co-occurring mangrove species: environmental versus ecophysiological drivers. *Agricultural and Forest Meteorology* 192-193, 51-58.

- Wang Y, Sessions A.L., Nielsen R.J. & Goddard W.A. (2009) Equilibrium $^2\text{H}/^1\text{H}$ fractionations in organic molecules. II. Linear alkanes, alkenes, ketones, carboxylic acids, esters, alcohols and ethers. *Geochimica et Cosmochimica Acta* 73, 7076-7086.
- West A.G., Patrickson S.J. & Ehleringer J.R. (2006) Water extraction times for plant and soil materials used in stable isotope analysis. *Rapid Communications in Mass Spectrometry* 20, 1317-1321.
- Wei L., Lockington D.A., Poh S.C., Gasparon M. & Lovelock C.E. (2013) Water use patterns of estuarine vegetation in a tidal creek system. *Oecologia* 172: 485-494.
- Wium-Andersen S. & Christensen B. (1978) Seasonal growth of mangrove trees in southern Thailand. II. Phenology of *Bruguiera cylindrica*, *Ceriops tagal*, *Lumnitzera littoria* and *Avicennia marina*. *Aquatic Botany* 5, 383-390.
- Yakir D. (1992) Variations in the natural abundance of ^{18}O and deuterium in plant carbohydrates. *Plant, Cell and Environment* 15, 1005-1020.
- Yakir D. & DeNiro M.J. (1990) Oxygen and hydrogen isotope fractionation during cellulose metabolism in *Lemna gibba* L. *Plant Physiology* 93, 325-332.
- Yang H., Pagani M., Briggs D.E.G., Equiza M.A., Jagels R., Leng Q. & LePage B.A. (2009) Carbon and hydrogen isotope fractionation under continuous light: implications for paleoenvironmental interpretations of the High Arctic during Paleogene warming. 160, 461-470.
- Zhang X., Gillespie A. & Sessions A. (2009) Large D/H variations in bacterial lipids reflect central metabolic pathways. *Proceedings of the National Academy of Sciences* 106, 12580-12586.

Figure Captions

Figure 1 Study site in Ballina, New South Wales. Satellite image from Google Earth.

Figure 2: Rainfall amount (black bars, panel a), salinity (blue circles, panel a), $\delta^{18}\text{O}$ (panel b) and $\delta^2\text{H}$ (panel c) of surface (SW), xylem (XW), and leaf (LW) water, and $\delta^2\text{H}$ of *n*-alkanes (panel d) and isoprenoidal lipids (panel e) from October 1, 2012 to July 17, 2013. In all panels, Rs is *R. stylosa* (red symbols), Am is *A. marina* (gray symbols), and Ac is *A. corniculatum* (purple symbols). Error bars represent one standard deviation of triplicate measurements.

Figure 3 Surface (SW, blue), xylem (XW) and leaf (LW) water $\delta^{18}\text{O}$ values (panels a-c) and $\delta^2\text{H}$ values (panels d-f) for *R. stylosa* (red, panels a and d), *A. marina* (gray, panels b and e) and *A. corniculatum* (purple, panels c and f) growing in Mobbs Bay as a function of salinity. X-error bars indicate the precision of the refractometer used to measure salinity. Y-error bars represent the standard deviation of triplicate measurements. Regression lines and 95% confidence intervals are shown for relationships that are significantly correlated at the $p < 0.05$ level.

Figure 4 Fractionation factors between xylem water and surface water for hydrogen isotopes (panels a-c) and oxygen isotopes (panels d-f) for *R. stylosa* (white squares, panels a & d), *A. marina* (gray diamonds, panels b & e) and *A. corniculatum* (black circles, panels c & f) growing in Mobbs Bay as a function of salinity. X-error bars indicate the precision of the refractometer used to measure salinity. Y-error bars represent the standard deviation of triplicate measurements. Regression lines and 95% confidence intervals are shown for relationships that are significantly correlated at the $p < 0.05$ level.

Figure 5 Fractionation factors between leaf water and surface water for hydrogen isotopes ($\alpha^2_{\text{LW-SW}}$; panels a-c) and leaf water and xylem water for hydrogen isotopes ($\alpha^2_{\text{LW-XW}}$; panels d-f) for *R. stylosa* (white squares, panels a & d), *A. marina* (gray diamonds, panels b & e) and *A. corniculatum* (black circles, panels c & f) growing in Mobbs Bay as a function of salinity. X-error bars indicate the precision of the refractometer used to measure salinity. Y-error bars represent the standard deviation of triplicate measurements.

Figure 6 Net fractionation factors between leaf lipids and surface water for *n*-alkanes (panel a) and isoprenoids (panel b) for *R. stylosa* (Rs, red), *A. marina* (Am, gray) and *A. corniculatum* (Ac, purple) growing in Mobbs Bay as a function of salinity. X-error bars indicate the precision of the refractometer used to measure salinity. Y-error bars represent the standard deviation of triplicate measurements. Regression lines and 95% confidence intervals are shown for relationships that are significantly correlated at the $p < 0.05$ level.

Figure 7 Predicted and measured xylem water $\delta^2\text{H}$ values for (a) *R. stylosa* (Rs), (b) *A. marina* (Am) and (c) *A. corniculatum* (Ac) growing in Mobbs Bay from October 1, 2012 to July 17, 2013. Predicted xylem water $\delta^2\text{H}$ values are based on measured $\delta^{18}\text{O}$ values of xylem

848 water and the local water line. Error bars on measured lines represent one standard
849 deviation of triplicate measurements.
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Figure 1 Study site in Ballina, New South Wales and sampled trees

855 **Figure 2**

$\delta^{18}\text{O}$					$\delta^2\text{H}$					
m	b	R ²	p	n	Water	m	b	R ²	p	n
0.14 ± 0.01	4.8 ± 0.3	0.85	<0.0001	39	Surface (all panels)	0.77 ± 0.07	-23 ± 2	0.76	<0.0001	39
0.08 ± 0.02	4.5 ± 0.4	0.40	<0.0001	39	<i>R. stylosa</i> xylem (panels a & d)	0.45 ± 0.09	-30 ± 2	0.39	<0.0001	39
0.22 ± 0.07	2 ± 2	0.25	0.002	36	<i>R. stylosa</i> leaf (panels a & d)	0.9 ± 0.2	-9 ± 6	0.35	0.0001	36
0.08 ± 0.02	4.3 ± 0.4	0.36	<0.0001	37	<i>A. marina</i> xylem (panels b & e)	0.43 ± 0.08	-29 ± 2	0.44	<0.0001	37
0.16 ± 0.06	4 ± 2	0.17	0.01	37	<i>A. marina</i> leaf (panels b & e)	0.7 ± 0.2	-2 ± 5	0.28	0.0006	37
-0.01 ± 0.06	2 ± 1	0.01	0.82	14	<i>A. cornic.</i> xylem (panels c & f)	0.1 ± 0.2	-15 ± 5	0.01	0.79	14
0.1 ± 0.01	6 ± 4	0.01	0.74	14	<i>A. cornic.</i> leaf (panels c & f)	0.1 ± 0.4	9 ± 10	0.01	0.73	14

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$\alpha_{\text{Lipid-SW}}$	m (‰)	b	R ²	p	n
<i>R. stylosa</i> nC_{29} ($R_s \alpha_{nC29-SW}$)	-0.5 ± 0.1	0.898 ± 0.003	0.30	0.003	39
<i>R. stylosa</i> nC_{31} ($R_s \alpha_{nC31-SW}$)	-0.8 ± 0.2	0.895 ± 0.004	0.50	0.0001	24
<i>A. marina</i> nC_{31} ($Am \alpha_{nC31-SW}$)	-0.8 ± 0.1	0.868 ± 0.003	0.52	<0.0001	38
<i>A. marina</i> nC_{33} ($Am \alpha_{nC33-SW}$)	-1.1 ± 0.2	0.872 ± 0.005	0.50	<0.0001	38
<i>A. corniculatum</i> nC_{29} ($Ac \alpha_{nC29-SW}$)	-1.0 ± 0.4	0.867 ± 0.009	0.35	0.03	14
<i>A. corniculatum</i> nC_{31} ($Ac \alpha_{nC31-SW}$)	-0.9 ± 0.7	0.87 ± 0.02	0.17	0.21	11
<i>R. stylosa</i> taraxerol ($R_s \alpha_{\text{Taraxerol-SW}}$)	-0.4 ± 0.2	0.840 ± 0.005	0.12	0.03	36
<i>A. marina</i> stigmasterol ($Am \alpha_{\text{Stigmasterol-SW}}$)	-0.7 ± 0.1	0.811 ± 0.004	0.40	<0.0001	37
<i>A. marina</i> β -sitosterol ($Am \alpha_{\beta\text{-sitosterol-SW}}$)	-0.8 ± 0.1	0.824 ± 0.004	0.44	0.0001	36
<i>A. corniculatum</i> stigmastanol ($Ac \alpha_{\text{stigmastanol-SW}}$)	-0.3 ± 0.3	0.781 ± 0.007	0.07	0.39	13

876 **Figure 7**

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