²H/¹H fractionation in lipids of the mangrove *Bruguiera gymnorhiza* increases with salinity in
marine lakes of Palau

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Abstract

Hydrogen isotope ratios ($^2\text{H}/^1\text{H}$ or D/H) of sedimentary mangrove lipid biomarkers can be exploited as a quantitative proxy of past salinity and water isotopes. This approach is based on the observation that apparent $^2\text{H}/^1\text{H}$ fractionation between surface water and mangrove leaf lipids increases with surface water salinity. In order to better understand the mechanisms responsible for this empirical relationship, we analyzed the isotopic composition of surface water, xylem water, leaf water and leaf lipids from *Bruguiera gymnorhiza* mangroves growing around eleven marine lakes and a lagoon on the rock islands of Palau, spanning a salinity range of 5 – 32 parts per thousand (ppt). Net fractionation increased with increasing salinity for both $n\text{C}_{31}$ -alkane ($0.7 \pm 0.1\text{‰ ppt}^{-1}$) and for the pentacyclic triterpenoid lupeol ($0.5 \pm 0.2\text{‰ ppt}^{-1}$). These trends could not be attributed to changes in biosynthetic fractionation with salinity, but seem more likely to be due to increasing disequilibrium between xylem water and water vapor as salinity increases. In Palau's humid climate, this most likely causes leaf water to become less ^2H -enriched relative to surface water and to xylem water as salinity increases. This supposition is supported not only by measurements of leaf water ^2H enrichment, but also by the correlation ($R^2 = 0.66$) between leaf water isotopes and those of rain water, which are assumed to be in equilibrium with water vapor isotopes, and by the dependence of leaf water isotopes on water vapor isotopes in a Péclet modified Craig-Gordon model. These results should inform the application of sedimentary mangrove lipid hydrogen isotope ratios to infer past hydroclimatic changes.

Keywords: Hydrogen isotopes, leaf water, leaf waxes, lipid biomarkers, mangroves, salinity

1. Introduction

The hydrogen isotope composition of sedimentary lipid biomarkers is increasingly utilized to infer past hydrological conditions (Sachse *et al.*, 2012, and sources therein). The hydrogen in leaf waxes and other lipids synthesized by photoautotrophs is ultimately derived from environmental water, and lipids thus record changes in the isotopic composition of water ($^2\text{H}/^1\text{H}$ or $\delta^2\text{H}_{\text{Water}}$, where $\delta^2\text{H} = ((^2\text{H}/^1\text{H})_{\text{Sample}}/(^2\text{H}/^1\text{H})_{\text{VSMOW}} - 1)$ (Sessions *et al.*, 1999; Sauer *et al.*, 2001; Sachse *et al.*, 2012). The isotopic composition of environmental water is primarily determined by air temperature, precipitation amount, long-term average precipitation rate, and the evaporative source of moisture (Dansgaard, 1964; Craig and Gordon, 1965; Gat, 1996), and lipid based reconstructions of the changing composition of source water isotopes have provided important climatic information from diverse regions on a myriad of time scales (e.g. Schefuss *et al.*, 2005; Tierney *et al.*, 2008; van der Meer *et al.*, 2007; Sachs *et al.*, 2009; Feakins *et al.*, 2012; Konecky *et al.*, 2013; Vasiliev *et al.*, 2013; Niedermeyer *et al.*, 2014; Rach *et al.*, 2014; Nelson and Sachs, 2016).

The $\delta^2\text{H}$ values of lipids are not identical to those of the water from which the hydrogen is derived, and the lipids are almost always depleted in ^2H by 100-300‰ (Sessions *et al.*, 1999; Sauer *et al.*, 2001; Chikaraishi and Naraoka, 2003; Sachse *et al.*, 2012). The offset between the two terms is described by the net fractionation factor, $\alpha_{\text{Lipid-Water}} = (^2\text{H}/^1\text{H}_{\text{Lipid}})/(^2\text{H}/^1\text{H}_{\text{Water}})$. The net fractionation factor represents the cumulative effect of several intermediate fractionations, such as evaporative enrichment of precipitation in soil (Riley *et al.*, 2002), discrimination against water molecules containing ^2H during symplastic water uptake by halophytes (Lin & Sternberg, 1993; Ellsworth and Williams, 2007; Ladd and Sachs, 2015a, 2015b), evaporative enrichment of leaf water during transpiration (Dawson *et al.*, 2002; Kahmen *et al.*, 2008) and biosynthetic ^2H -depletion during lipid synthesis (Sessions *et al.*, 1999; Sauer *et al.*, 2001; Chikaraishi and Naraoka, 2003).

The magnitude of $\alpha_{\text{Lipid-Water}}$ can be sensitive to a number of environmental parameters, such as relative humidity (Kahmen *et al.*, 2013a, 2013b; Tipple *et al.*, 2015), plant type (Liu *et al.*, 2006; Smith and Freeman, 2006; Hou *et al.*, 2007), and salinity (Romero and Feakins, 2011; Ladd and Sachs, 2012, 2015a, 2015b; He *et al.*, in review). The salinity effect on hydrogen isotope fractionation is particularly important in tropical and sub-tropical coastal regions, where

salt-tolerant mangrove plants contribute up to 30% of the total organic matter preserved in sediments (Alongi and Mukhopadhyay, 2014).

Estuarine calibrations of *Avicennia marina* and *Rhizophora* spp. have demonstrated that $\alpha_{\text{Lipid-Water}}$ decreases by 0.7 - 1.8‰ per unit (ppt) increase in mean salinity in leaf wax *n*-alkanes. (Ladd and Sachs, 2012, 2015b; He *et al.*, in review). Similar decreases in $\alpha_{\text{Lipid-Water}}$ of $0.9 \pm 0.2\text{‰ ppt}^{-1}$ were observed in *Rhizophora* spp. growing along three estuaries in Micronesia for Δ -friedoolean-14-en-3 β -ol (taraxerol) (Ladd and Sachs, 2015b), a pentacyclic triterpenoid produced in high abundance by *Rhizophora* (Killops and Frewin, 1994; Koch *et al.*, 2003; Versteegh *et al.*, 2004).

The relationship between salinity and net fractionation in mangrove lipids permits quantitative reconstructions of past salinity and $\delta^2\text{H}_{\text{Water}}$ when coupled with measurements of $\delta^2\text{H}$ values of an algal lipid from the same sedimentary interval. Hydrogen isotope fractionation in algae has the opposite response to salinity as in mangroves, with less fractionation at high salinity (Schouten *et al.*, 2006; Sachse and Sachs, 2008; Sachs and Schwab, 2011; Chivall *et al.*, 2014; Nelson and Sachs, 2014; Sachs *et al.*, 2016). If mangroves and algae grow in the same water body, such as a brackish lake, and experience the same salinity and $\delta^2\text{H}_{\text{Water}}$ values, it is possible to use the empirical linear relationships between salinity and H isotope fractionation in each organism to solve for the salinity and $\delta^2\text{H}_{\text{Water}}$ values of the water body they lived in (Ladd and Sachs, 2012; Nelson and Sachs, 2016).

Determining the mechanism responsible for the inverse relationship between salinity and $\alpha^2_{\text{Lipid-Water}}$ in mangroves is important for robust hydroclimate reconstructions based on mangrove $\delta^2\text{H}_{\text{Lipid}}$ values. Past studies of mangroves growing in estuaries have constrained possible drivers of the increase in net fractionation with salinity by measuring xylem water and leaf water isotopes in addition to surface water and lipid isotopes (Ladd and Sachs, 2015a, 2015b). For *Rhizophora* mangroves growing in estuaries on the Micronesian island of Pohnpei, increased discrimination against ^2H during water uptake can account for 30% of the total change in $\alpha_{\text{Lipid-Water}}$ over a salinity range of 1-33 ppt (Ladd and Sachs, 2015b). However, when three species of mangroves at the same site in coastal Australia were monitored for almost one year, no changes in fractionation during water uptake were detected, and increased depletion of xylem water $\delta^2\text{H}$ values relative to those of surface water at high salinity was better explained by opportunistic use of alternative water sources as salinity increased (Ladd and Sachs, 2015a). Leaf water

enrichment relative to xylem water and surface water did not vary significantly with salinity or among taxa in either the Micronesian or Australian sample sets, implying that the majority of the increase in net fractionation with salinity was due either to increased biosynthetic fractionation at high salinity, or to variability in the timing of leaf and/or leaf lipid production (Ladd and Sachs, 2015a, 2015b).

The goal of the present study was to better constrain possible mechanisms for increased hydrogen isotope fractionation in mangroves growing at high salinity. We measured $\delta^2\text{H}$ values of surface water, xylem water, leaf water, and leaf lipids from *Bruguiera gymnorhiza* mangroves growing around brackish lakes on the rock islands of Palau. For calibration and process studies such as this, brackish lakes provide some significant advantages over estuaries where trees can experience large ranges of salinity over tidal and seasonal cycles. Additionally, such lakes can yield excellent sediment sequences for mangrove $\delta^2\text{H}$ -based climate reconstructions, motivating the need for lacustrine calibrations of this response.

2. Materials and Methods

2.1 Site description and sample collection

The Republic of Palau (7 °N, 134 °E) lies on the western edge of the Caroline Islands archipelago in the northwestern tropical Pacific Ocean (Fig. 1). Dozens of brackish lakes are located on the uninhabited rock islands south of Koror, and have been described in detail previously (Hamner *et al.*, 1982; Hamner and Hamner, 1998). These marine meromictic lakes are connected to the ocean at depth through fissures in the karst terrain and are typically highly stratified owing to the high rates of precipitation that exceed 3700mm yr⁻¹. The salinity of the surface water is constant through tidal cycles, but can vary by a few ppt due to frequency and intensity of rain events (Hamner *et al.*, 1982; Hamner and Hamner, 1998). Monitoring by the Coral Reef Research Foundation in Koror, Palau from 2007 – 2013 indicates that surface water salinity in the lakes was relatively stable in the years preceding our study, with standard deviations averaging 2 ppt.

During a month-long sampling campaign in Sept/Oct 2013, eleven lakes were selected to represent the widest possible range of surface water salinity (5-32 ppt) (Fig. 1). One of these lakes, Long Lake, is connected to the ocean at the surface and is more similar to an estuary than

to the other lakes. Fringing mangroves on the lagoon near Koror were also sampled to serve as a high salinity end member. Surface water salinity (1 cm below lake surface) was measured using a refractometer (Veegee Scientific, Kirkland, WA) with accuracy of 1 ppt. 4 mL of water from 1 cm below the lake surface was collected at each site for water isotope analyses.

At each site, three mature *Bruguiera gymnorhiza* trees were sampled. All sampled trees were south facing trees growing on the north shore of the lake. All trees were sampled in the early afternoon, between 12:00 and 15:00 local time. Two sun-exposed, south facing branches, ~1 m above the lowest leaves on the tree were collected. An ~4 cm section of woody stem was cut from 30 cm below the lowest leaves on the branch. The bark from this stem sample was removed with a pocketknife, and the sample was placed in an 11 mL glass screw-cap vial for subsequent vacuum-extraction of xylem water. The second most distal mature leaf from each branch was placed in an 11 mL glass screw-cap vial for vacuum-extraction of leaf water.

Four leaves were collected from each tree for a composite leaf lipid sample. One leaf was collected from each sun-exposed side of the tree (east, south, and west) and one leaf was collected from an interior, shaded branch (in most cases trees did not produce any leaves on the shaded north side). All leaves for lipid analyses were growing on branches ~1 m above the lowest leaves on the tree and were located at the second most distal position on the branch. All leaf and stem samples collected in the field were immediately placed in an insulated box containing ice, then transferred to a freezer at -20 °C upon return to the lab (typically 3-6 hours after collection).

During the one-month field campaign, a rainwater sampler was set up on the dock of the Coral Reef Research Foundation in Koror (Fig. 1) and sampled after every rain event. The sampler consisted of a 16 cm diameter funnel on top of a 1 L graduated cylinder. ~50 mL of mineral oil was poured in the bottom of the sampler before each deployment to prevent evaporation. 4 mL of rainwater was collected with a pipet from below the mineral oil – water interface, and any remaining traces of oil were removed by filtering the sample through a coffee filter. Rain samples were stored in screw-cap glass vials and sealed with electrical tape until analysis.

2.2 Leaf and xylem water extraction

Leaf and xylem water samples were cryogenically extracted on a vacuum line, following methods modified from West *et al.* (2006). Sample vials were screwed directly into a Swagelok fitting on the line and connected to a glass u-bend tube. The sample vials were lowered into water at 80 °C while the collection tubes were submerged in liquid nitrogen. After two hours, during which time pressure in the system remained below 60 mTorr, the collection tubes were removed and sealed. After the extracted ice in the collection tube had melted, it was filtered through a 0.45 µm PTFE filter to remove particulate material and transferred to a 2 mL screw-cap vial for isotope analysis.

2.3 Water $\delta^2\text{H}$ and $\delta^{18}\text{O}$ measurements

A Picarro Cavity Ring Down Spectroscopy (CRDS) L2130i Water Isotope Analyzer at the University of Washington measured surface water and precipitation $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values. Three laboratory standards of known isotopic composition were analyzed after every six samples and were used to normalize results to the Vienna Standard Mean Ocean Water (VSMOW) scale. All samples and standards were injected six times, and the first three measurements were discarded in order to avoid any memory effects from the previous analysis. Average precision of triplicate analyses was 0.15‰ for $\delta^2\text{H}$ and 0.05‰ for $\delta^{18}\text{O}$.

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; ThermoFisher Scientific, Waltham, MA) at the University of Basel, Switzerland. Each sample and standard was injected six times in sequence. In order to avoid any memory effects from the previous analysis, the first three injections of each sample were discarded. Thermo Isodat 3.0 software was used to calculate raw isotopic ratios on the VSMOW 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 (which ranged from 1.02 to 1.07) 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 isotopic value of this standard was 1.0‰

for $\delta^2\text{H}$ and 0.3‰ for $\delta^{18}\text{O}$. Average precision of triplicate sample injections was 1.3‰ for $\delta^2\text{H}$ and 0.2‰ for $\delta^{18}\text{O}$.

2.4 Leaf water model

In order to examine biosynthetic fractionation relative to longer-term average leaf water $\delta^2\text{H}$ values, expected leaf water $\delta^2\text{H}$ ($\delta^2\text{H}_{\text{LW}}$) values were determined using a Péclet-modified Craig-Gordon model of leaf water isotopic enrichment modified from that used by Kahmen *et al.* (2011) and described previously by Ladd and Sachs (2015b). The Craig-Gordon model predicts leaf water enrichment above xylem water (Δ^2H_e) using the equilibrium and kinetic fractionation factors (ϵ^+ and ϵ_k , respectively), and the isotopic difference between xylem water and water vapor (Δ^2H_{WV}), such that:

$$\Delta^2H_e = \epsilon^+ + \epsilon_k + (\Delta^2H_{\text{WV}} - \epsilon_k) * (e_a/e_i) \quad (1)$$

Temperature, relative humidity, and atmospheric pressure were estimated from climatological means for September and October (<http://www.ncdc.noaa.gov/cdo-web/>). Internal leaf temperature was assumed to equal air temperature. Stomatal conductance was estimated at $0.45 \pm 0.15 \text{ mol m}^{-2} \text{ s}^{-1}$ for salinity ≤ 15 ppt, and decreased by $0.015 \text{ mol m}^{-2} \text{ s}^{-1}$ for each unit increase in salinity above 15 ppt (based on observations of cultured *Rhizophora mangle* a mangrove of the same family as *B. gymnorhiza*, with similar leaf morphology; Lin & Sternberg, 1992). The $\delta^2\text{H}$ values of water vapor were calculated assuming equilibrium with precipitation during the three rain events closest to the date when the leaves were sampled. The model was also run with water vapor $\delta^2\text{H}$ values in equilibrium with average precipitation for the month-long study period.

Back diffusion of isotopically enriched water away from the site of evaporation was estimated using a Péclet effect ($\rho = L_M E / CD$, where L_M is the mixing path length, E is the transpiration stream, C is the molar concentration of water, and D is the diffusivity of $^1\text{H}^2\text{HO}$). L_M was informed by observations of *R. mangle* in Florida (Ellsworth *et al.*, 2013) and set at 60 ± 20.0 mm for all trees. Bulk enrichment of leaf water, Δ^2H_{LW} , was calculated using the Péclet effect:

$$\Delta^2 H_{LW} = \frac{\Delta^2 H_e(1-e^{-\rho})}{\rho} \quad (2)$$

Finally, $\delta^2 H_{LW}$ was calculated as:

$$\delta^2 H_{LW} = (\Delta^2 H_{LW} + \delta^2 H_{XW}) + (\Delta^2 H_{LW} * \delta^2 H_{XW})/1000 \quad (3)$$

Given the large number of variables in the model, and the significant uncertainties associated with many of them, a Monte Carlo simulation was used to determine the uncertainties associated with modeled leaf water values. The 1000-iteration Monte Carlo simulation was run in Matlab (MathWorks, Natick, MA, USA) and incorporated the uncertainties associated with all measured values and all estimates of environmental conditions or physiological parameters. The Matlab script used to run the leaf water model and associated Monte Carlo analysis has been previously published (Ladd and Sachs, 2015b). Average uncertainties associated with modeled leaf water were 4%. Additionally, sensitivity to each variable was assessed by changing each estimated input from its maximum to minimum value, while holding all other variables constant at their average value. A summary of the results of this sensitivity analysis is included in Table 1.

2.5 Leaf lipid extraction and purification

The composite sample of four leaves from each tree was freeze-dried and ground into a homogenous powder. ~0.5 g of dry leaf material was extracted with 9:1 Dichloromethane:Methanol (DCM:MeOH) by Accelerated Solvent Extraction (ASE, Dionex Corp., Sunnyvale, CA, USA). Samples were preheated at 100 °C and 1500 psi for 5 minutes, and extracted during three 5-minute stationary phases. The extracted total lipid extract (TLE) was evaporated to dryness under a stream of N₂. A 10 mg aliquot of the TLE was saponified overnight at 60 °C in 3 mL 1N KOH in MeOH and 2 mL of deionized water. After 12 hours, 1 mL of water saturated with NaCl was added as well as 4N HCl to acidify the sample to pH < 3. A phase separation was then performed by adding 2 mL of hexane, mixing, and pipetting off the supernatant. This step was repeated until two subsequent hexane rinses were colorless.

The resulting organic phase was evaporated under a stream of N₂, redissolved in 2 mL of hexane, and separated into compound classes using silica gel column chromatography. The sample was loaded onto 1 g of Si gel (deactivated with H₂O to 5% w/w). Alkanes were eluted with 10 mL of hexane. Ketones, aldehydes and esters were eluted with 6 mL of 1:1 Hexane/DCM. Sterols, *n*-alkanols and triterpenols were eluted with 10 mL 4:1 Hexane/Ethyl Acetate, and the remaining polar material was eluted with 4 mL MeOH. The alcohol fraction was acetylated with 25 µL of acetic anhydride of known isotopic composition and 25 µL of pyridine at 70 °C for 30 minutes. (3β,13ξ)-Lup-20(29)-en-3-ol (Lupeol), was the most abundant compound in the alcohol fraction, followed by Olean-12-en-3β-ol (β-amyrin). Alcohols were quantified by Gas Chromatography – Mass Spectrometry (GC-MS), as described previously (Ladd and Sachs, 2015a, 2015b). The alkane fraction was dominated by *n*C₃₁-alkane, and was quantified by Gas Chromatography – Flame Ionization Detection (GC-FID) as described previously (Ladd and Sachs, 2015a, 2015b). These quantifications were used to determine the volume of solvent needed to obtain optimal concentrations for isotopic analysis by Gas Chromatography – Isotope Ratio Mass Spectrometry (GC-IRMS).

2.6 Leaf lipid δ²H measurements

The δ²H values of individual lipids were measured by GC-IRMS on a Thermo DELTA V PLUS system (ThermoFisher Scientific, Waltham, MA, USA). The GC (Trace Ultra, ThermoFisher Scientific) was equipped with a split-splitless injector operated in splitless mode at 320 °C, a TRIPLUS autosampler (ThermoFisher Scientific), and a VF-17ms capillary column (60 m X 0.25 mm X 0.25 µm, Agilent). For lupeol analyses, the GC was heated from 120 °C to 260 °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 20 min. For *n*-alkane analyses, the GC was heated from 120 °C to 250 °C at 20 °C/min, then at 6 °C/min to 325 °C and then held at 325 °C for 12 min. Helium was used as the carrier gas at a constant flow of 1.1 mL/min. Compounds were pyrolyzed in a ceramic reactor at 1400 °C. 1 µL of sample was injected along with 0.5 µL of a mix of *n*-alkanes of known isotopic composition (A. Schimmelmann, Indiana University, Bloomington, Indiana). For lupeol analyses, this mix included *n*C₂₆-, *n*C₂₈-, *n*C₃₂-, *n*C₃₄- and *n*C₄₁-alkanes. For *n*-alkane analyses, the co-injection standards were *n*C₂₁ and *n*C₂₃. At the beginning and the end of the sequence, as well as after

every 4-6 sample injections, a mixture of additional *n*-alkane standards of known isotopic composition was analyzed with the co-injection standards, in place of a sample. For isoprenoid sequences, this external standard was *n*C₃₈-alkane. For *n*-alkane sequences, *n*C₂₈-, *n*C₃₂- and *n*C₃₄-alkanes were used as external standards.

Thermo ISODAT software V.2.5 was used to control instrumentation and calculate $\delta^2\text{H}$ 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 normalize for drift, retention time, and peak area for each sequence. Lupeol $\delta^2\text{H}$ values were corrected for the hydrogen added by acetylation using an isotopic mass balance, and the uncertainty in the acetic anhydride $\delta^2\text{H}$ values was propagated into the uncertainty in lupeol $\delta^2\text{H}$ values. 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‰.

2.7 Statistics

Statistical analyses were conducted using PRISM software (Graphpad Software Inc., La Jolla, CA, USA). Ordinary least squares regression was used to determine relationships between fractionation factors and salinity, modeled leaf water to measured leaf water, and leaf water isotopes to precipitation isotopes. Regression lines and 95% confidence intervals are only shown on data sets where the slope of the regression was significantly different from 0 at the $p < 0.05$ level. Differences between the slopes of various regressions were assessed using a two-tailed test of the null hypothesis that both slopes are equal. If slopes were not significantly different ($p > 0.05$), a pooled slope for all data was used to determine whether there was a significant difference in the y-intercepts. The results of all linear regression analyses are presented in Table 2.

3. Results

3.1 Water $\delta^2\text{H}$ and $\delta^{18}\text{O}$

Surface water isotopes ($\delta^2\text{H}_{\text{SW}}$ and $\delta^{18}\text{O}_{\text{SW}}$) were highly correlated with salinity for both hydrogen ($R^2 = 0.95$) and oxygen ($R^2 = 0.93$) (Fig. 2a; Table 2a). Xylem water isotopes ($\delta^2\text{H}_{\text{XW}}$ and $\delta^{18}\text{O}_{\text{XW}}$) were also positively correlated with salinity ($R^2 = 0.80$ for $\delta^2\text{H}_{\text{XW}}$; $R^2 = 0.76$ for $\delta^{18}\text{O}_{\text{XW}}$) (Fig. 2b; Table 2a). Xylem water H and O isotope ratios were always depleted relative to surface water. Measured $\delta^2\text{H}_{\text{XW}}$ values were 19 ± 6 ‰ lighter than corresponding $\delta^2\text{H}_{\text{SW}}$ values, while $\delta^{18}\text{O}_{\text{XW}}$ values were 2 ± 1 ‰ lighter than $\delta^{18}\text{O}_{\text{SW}}$ (Fig. 2; Table 2a). Although xylem water isotopes were depleted relative to lake surface water, the magnitude of that depletion was not sensitive to salinity and the fractionation factor expressing the offset between surface water and xylem water, $\alpha_{\text{XW-SW}}$, was not significantly correlated with salinity for either isotope (Fig. 3; Table 2a).

Leaf water isotopes ($\delta^2\text{H}_{\text{LW}}$ and $\delta^{18}\text{O}_{\text{LW}}$) were not significantly correlated with salinity (Fig. 2c; Table 2a). Leaf water was typically enriched relative to xylem water, but the magnitude of this enrichment decreased as salinity increased (Fig. 4), indicated by decreasing fractionation factors between leaf and xylem water ($\alpha^2_{\text{LW-XW}}$ and $\alpha^{18}_{\text{LW-XW}}$). This correlation was not significant for $\alpha^{18}_{\text{LW-XW}}$ (Fig. 4b; Table 2a). Net leaf water enrichment relative to surface water, $\alpha_{\text{LW-SW}}$, had weak positive correlations with salinity for both isotopes ($R^2 = 0.27$ for $\alpha^2_{\text{LW-SW}}$; $R^2 = 0.16$ for $\alpha^{18}_{\text{LW-SW}}$) (Fig. 4; Table 2a). $\delta^2\text{H}_{\text{LW}}$ values were better correlated ($R^2 = 0.68$) with average $\delta^2\text{H}_{\text{Rain}}$ during the precipitation events that occurred closest to the time when the leaves were sampled (Fig. 5a; Table 2b) than they were with $\delta^2\text{H}_{\text{SW}}$ ($R^2 = 0.16$) (Fig. 5b; Table 2b).

When the leaf water model was run using $\delta^2\text{H}$ values of the three most recent rain events to estimate water vapor $\delta^2\text{H}$ values, it captured approximately half of the variability in measured leaf water (the R^2 between measured and predicted values is 0.47, and the linear regression is nearly a 1:1 line) (Table 2b). When the water vapor $\delta^2\text{H}$ value was held constant and in equilibrium with average rainwater from the one-month study period, the model did not reproduce variability in $\delta^2\text{H}_{\text{LW}}$ ($R^2 = 0.002$) (Table 2b). Leaf water was more sensitive to changes in rainwater isotopes than to any other variable, although no variable could independently explain the full 45‰ range in measured leaf water (Table 1).

3.2 Lipid $\delta^2\text{H}$ values

Lupeol $\delta^2\text{H}$ values had a weak positive correlation with salinity ($R^2 = 0.14$; $p = 0.03$), as did $n\text{C}_{31}$ -alkane $\delta^2\text{H}$ values ($R^2 = 0.18$; $p = 0.01$) (Fig. 6; Table 2a). The slope of the linear regression was $0.4 \pm 0.2\text{‰ ppt}^{-1}$ for lupeol and $0.3 \pm 0.1\text{‰ ppt}^{-1}$ for $n\text{C}_{31}$ -alkane (Fig. 6; Table 2a). These slopes were significantly shallower than those of lake surface water $\delta^2\text{H}$ values relative to salinity (Figure 2a) ($p = 0.0004$ for lupeol and < 0.0001 for $n\text{C}_{31}$ -alkane). As such, the net fractionation factor, $\alpha_{\text{Lipid-SW}}$, decreased by $0.5 \pm 0.2\text{‰ ppt}^{-1}$ for lupeol and $0.7 \pm 0.1\text{‰ ppt}^{-1}$ for $n\text{C}_{31}$ -alkane (Fig. 7; Table 2). On the other hand, apparent biosynthetic fractionation between measured leaf water and leaf lipids, $\alpha_{\text{Lipid-LW}}$, displayed no trend with salinity ($R^2 = 0.003$ for $\alpha_{\text{Lup-LW}}$; $R^2 = 0.001$ for $\alpha_{n\text{C}_{31}\text{-LW}}$) (Fig. 8; Table 2a). Similarly, biosynthetic fractionation between lupeol and modeled leaf water was not correlated with salinity for either lipid ($R^2 = 0.002$ for lupeol and $R^2 = 0.08$ for $n\text{C}_{31}$ -alkane) (Table 2a). Calculating biosynthetic fractionation relative to modeled leaf water using monthly average values for water vapor $\delta^2\text{H}$ did not yield significant relationships with salinity ($R^2 = 0.01$ for lupeol and $R^2 = 0.006$ for $n\text{C}_{31}$ -alkane) (Table 2a).

4. Discussion

4.1 Water uptake by *B. gymnorhiza*

In all instances, xylem water ($\delta^2\text{H}_{\text{XW}}$ and $\delta^{18}\text{O}_{\text{XW}}$ values) was isotopically depleted in *B. gymnorhiza* relative to surface water ($\delta^2\text{H}_{\text{SW}}$ and $\delta^{18}\text{O}_{\text{SW}}$ values) (Fig. 2, 3). Since $\delta^{18}\text{O}_{\text{XW}}$ values are a reliable proxy for source water isotopes in mangroves (Lin and Sternberg, 1993; Ellsworth and Williams, 2007), the relatively low $\delta^{18}\text{O}_{\text{XW}}$ values indicate that *B. gymnorhiza* trees growing in marine lakes of Palau use other water sources in addition to lake surface water.

Other water sources that might have been available to these trees are deeper lake water, groundwater, freshwater runoff during storm events, or rain and/or dew deposited directly on leaves. Of these, deep lake water can be confidently excluded, since it is more saline than surface water and has correspondingly heavier water isotopes (Smittenburg *et al.*, 2011). Mangroves are able to make use of fresher groundwater in some cases (Ewe *et al.*, 2007; Lambs *et al.*, 2008). Because groundwater was not collected or analyzed it remains a possible water source for the studied trees.

Several recent studies have suggested that mangroves may be able to opportunistically take up freshwater runoff during and after storm events (Wei *et al.*, 2013; Reef and Lovelock, 2015; Reef *et al.*, 2015; Santini *et al.*, 2015), which would result in $\delta^{18}\text{O}_{\text{XW}}$ values that are depleted relative to lake surface water. Likewise, direct foliar uptake of rainwater or dew could also result in depleted $\delta^{18}\text{O}_{\text{XW}}$ values. Foliar uptake can be an important water source for some trees (Dawson, 1998; Burgess and Dawson, 2004; Johnson and Smith, 2008; Eller *et al.*, 2013). This process has not been directly observed in mangroves, but is consistent with observations of reverse xylem flow in these trees (Rada *et al.*, 1989; Hao *et al.*, 2008; Hoste, 2011).

Our previous studies of xylem water isotopes in *Rhizophora* spp. and *Avicennia marina* from estuaries and Palau's marine lakes are consistent with a mixed surface water and meteoric source, which could be either rainwater runoff taken up by roots, or foliar uptake (Ladd and Sachs, 2015a, 2015b). However, for *Rhizophora* spp. and *A. marina*, apparent fractionation between surface water and xylem water increased with salinity, indicating greater reliance on alternate freshwater sources at high salinity (Ladd and Sachs, 2015a, 2015b). For *B. gymnorhiza* in Palau's marine lakes, on the other hand, $\alpha_{\text{XW-SW}}$ did not change with salinity for either isotope (Fig. 3; Table 2a). This suggests that unlike in *A. marina* and *Rhizophora* mangroves, increased use of alternative freshwater sources as salinity increased did not occur in *B. gymnorhiza* from Palau's marine lakes. The one exception might be from the highest salinity (lagoon) sites, where $\delta^2\text{H}_{\text{XW}}$ and $\delta^{18}\text{O}_{\text{XW}}$ were relatively more depleted compared to surface water than at other sites (Fig. 3). As these sites experienced changing salinity over the course of the tidal cycle, the trees may have been able to selectively take up water during parts of the cycle where salinity was relatively low.

Although lake surface water could not be the only water source for *B. gymnorhiza* in the marine lakes of Palau, it seems likely that it was a significant water source. As salinity increased, $\delta^2\text{H}_{\text{XW}}$ and $\delta^{18}\text{O}_{\text{XW}}$ values increased with a nearly identical slope to the $\delta^2\text{H}_{\text{SW}}$ and $\delta^{18}\text{O}_{\text{SW}}$ values. This implies a relatively constant mixing ratio between lake surface water and the other water source(s) used by *B. gymnorhiza*. Additionally, while $\delta^{18}\text{O}_{\text{XW}}$ values always fell between surface water and rainwater $\delta^{18}\text{O}$ values, they were more highly correlated with $\delta^{18}\text{O}_{\text{SW}}$ ($R^2 = 0.65$) than with $\delta^{18}\text{O}_{\text{Rainwater}}$ ($R^2 = 0.20$), which was collected up to 10 km away from each lake (Figure 1), and may not be a perfect analog for rain at each site.

4.2 Controls on leaf water in *B. gymnorhiza* in Palau

For the mangroves in this study and elsewhere (Ladd and Sachs, 2015a, 2015b), xylem water was isotopically enriched relative to local precipitation, as expected for a mix of precipitation and seawater (section 4.1). For mangroves, $\delta^2\text{H}_{\text{XW}}$ was therefore out of equilibrium with local water vapor, and the magnitude of this disequilibrium must have increased with salinity, since a greater fraction of xylem water was derived from seawater.

At sites with high relative humidity, as in the marine lakes in Palau, the disequilibrium between xylem water and water vapor isotopes can have a profound effect on $\delta^2\text{H}_{\text{LW}}$ values. For the range of conditions expected in Palau, variability in rainwater $\delta^2\text{H}$ values can cause more variability in modeled $\delta^2\text{H}_{\text{LW}}$ values than any other parameter (Table 1). The $\delta^2\text{H}$ value of rainwater was highly variable during the one-month study period on Palau, as evidenced by the 45‰ range in precipitation $\delta^2\text{H}$ values (Table 3). This variability in precipitation $\delta^2\text{H}$ values can explain up to 24‰ of the observed 45‰ range in $\delta^2\text{H}_{\text{LW}}$ values. No other model variable had such a large effect on modeled $\delta^2\text{H}_{\text{LW}}$ values (Table 1). The observed range in temperature, relative humidity, and $\delta^2\text{H}_{\text{XW}}$ each can cause a 12 – 13‰ change in modeled $\delta^2\text{H}_{\text{LW}}$ values (Table 1). Further evidence for the importance of water vapor isotopes on leaf water isotopes comes from the relatively strong correlation between $\delta^2\text{H}_{\text{LW}}$ values and $\delta^2\text{H}_{\text{Rainwater}}$ (which is assumed to be in equilibrium with water vapor), while $\delta^2\text{H}_{\text{LW}}$ and $\delta^2\text{H}_{\text{SW}}$ values were poorly correlated (Fig. 5).

The observed decreases in $\alpha_{\text{LW-SW}}$ and $\alpha_{\text{LW-XW}}$ with salinity for both H and O isotopes (Fig. 4) can therefore be attributed to isotopic disequilibrium between xylem water and external water vapor. As salinity increased, the disequilibrium between surface (xylem) water and water vapor increased, and the leaf water became relatively more depleted; this explains why $\delta^2\text{H}_{\text{LW}}$ and $\delta^{18}\text{O}_{\text{LW}}$ values were mostly lower than those of xylem and surface water (Fig. 2, 4). Much of the scatter in leaf water values with salinity (Fig. 2c) is likely the result of temporal variations in water vapor isotopes during the one-month study, which we approximated here by the H and O isotopic composition of rainwater collected several kilometers from the site of each lake, at the CRRF dock, and averaged over 3 days (Table 3). Nevertheless, this variability is expected to be independent of lake water salinity at the height of the tree canopy, and was random with respect to the order in which samples were collected.

4.3 Causes of increased apparent fractionation in *B. gymnorhiza* lipids at increased salinity

Apparent biosynthetic fractionation ($\alpha_{\text{Lipid-LW}}$) by *B. gymnorhiza* mangroves was not correlated with salinity for either lupeol or $n\text{C}_{31}$ -alkane (Fig. 8; Table 2a). The lack of correlation could, in part, be driven by the sensitivity of leaf water isotopes to those of water vapor, assumed to have co-varied with the highly variable rainwater isotopes (Table 3). Measured leaf water represents a single point in time, while leaf lipid $\delta^2\text{H}$ values either reflect conditions when the leaf formed (Sachse *et al.*, 2010; Tipple *et al.*, 2013), or represent an accumulated signal over time (Gao *et al.*, 2012; Gao and Huang, 2013). Since $\delta^2\text{H}_{\text{LW}}$ at the time of lipid synthesis is unknown, it is impossible to determine if there is salinity-driven variability in $\alpha_{\text{Lipid-LW}}$ in *B. gymnorhiza* growing around marine lakes in Palau. However, given the poor correlations between salinity and biosynthetic fractionation relative to both measured and modeled leaf water (Fig. 8; Table 2a), it seems unlikely that changes in biosynthetic fractionation are responsible for the overall decrease in the net fractionation factor ($\alpha_{\text{Lipid-SW}}$) and salinity.

Rather, much of the increased offset between lipid and surface water $\delta^2\text{H}$ values with increasing salinity can be best explained by the sensitivity of leaf water isotopes to water vapor isotopes in humid environments such as Palau (Table 1). Leaf water is the direct source of hydrogen for leaf lipids in angiosperms (Kahmen *et al.*, 2013a). In the case of *B. gymnorhiza* studied here, the influence of water vapor $\delta^2\text{H}$ values, as approximated by $\delta^2\text{H}_{\text{Rain}}$ values, on $\delta^2\text{H}_{\text{LW}}$ was twice as large as that of $\delta^2\text{H}_{\text{XW}}$ (Table 1). As salinity increased, $\delta^2\text{H}_{\text{XW}}$ increased by $0.98 \pm 0.09\text{‰ ppt}^{-1}$ (Fig. 2b; Table 2a), reflecting the strong contribution of surface water to xylem water (Section 4.1). The influence of surface water isotopes, through the xylem water's contribution to leaf water, can be seen in the slight increase in $\delta^2\text{H}$ values of both lupeol and $n\text{C}_{31}$ -alkane with salinity (Fig. 6; Table 2a). However, the influence of $\delta^2\text{H}_{\text{SW}}$ on $\delta^2\text{H}_{\text{Lipid}}$ was diluted by the significant contribution of water vapor to leaf water. Although water vapor $\delta^2\text{H}$ values were highly variable, they were relatively more depleted compared to surface water at high salinity sites than at low salinity sites, where a larger proportion of the surface water was derived from rain (Fig. 2). The decrease in $\alpha_{\text{LW-SW}}$ was greater than or equal to the decrease in $\alpha_{\text{Lipid-SW}}$ for both lipids (Table 2a), and can therefore account for the change in $\alpha_{\text{Lipid-SW}}$ with salinity observed in *B. gymnorhiza* in Palau (Fig. 7).

The results from Palau contrast with those of *R. stylosa* and *A. marina* mangroves in a subtropical Australian estuary (Ladd and Sachs, 2015a). In a nine-month time series through large seasonal variations in rainfall rate and salinity, there was no trend in leaf water enrichment relative to surface water as salinity increased in either species (unlike in Palau, where leaf water ^2H -enrichment decreased with increasing salinity (Fig. 4)). Variability in leaf lipid $\delta^2\text{H}$ values between the two Australian species, and within each species over time, were best explained by either salinity-driven changes in biosynthetic fractionation, or by variability in the timing of lipid and leaf production (Ladd and Sachs, 2015a). In part, the lack of correlation between leaf water enrichment and salinity at the Australian site might have been due to its lower relative humidity, averaging 63% (*Australian Bureau of Meteorology*), in contrast to the 82% average humidity during the study period on Palau (<http://www.ncdc.noaa.gov/cdo-web/>). It is notable that during periods of intense rainfall in the Australian study, $\delta^2\text{H}_{\text{LW}}$ values were depleted relative to surface water and equal to $\delta^2\text{H}_{\text{XW}}$ values (Ladd and Sachs, 2015a), suggesting that at high relative humidity these trees also experienced a reduction in leaf water isotopic enrichment. As relative humidity decreases, the influence of water vapor isotopes on leaf water isotopes decreases, and leaf water enrichment relative to source water increases (Kahmen et al., 2013b). The results from Palau may therefore only be applicable to other sites with high relative humidity throughout the year.

5. Conclusions

The mechanisms responsible for increased net fractionation between surface water and leaf lipids in mangroves growing at high salinity were assessed by measuring the isotopic composition of surface water, xylem water, leaf water and leaf lipids from *Bruguiera gymnorhiza* mangroves growing around eleven brackish lakes and a lagoon, encompassing a salinity range of 5 – 32 ppt. The net fractionation factor, $\alpha_{\text{Lipid-SW}}$, decreased by $0.7 \pm 0.1\text{‰ ppt}^{-1}$ for $n\text{C}_{31}$ -alkane ($p < 0.0001$) and $0.5 \pm 0.2\text{‰ ppt}^{-1}$ for the pentacyclic triterpenoid lupeol ($p = 0.03$). These results contribute to a growing number of studies that demonstrate increasing net fractionation with increasing salinity for a variety of mangrove species, lipids, and environments (Ladd and Sachs, 2012, 2015a, 2015b; He et al., in review). However, unlike in previous studies

from estuarine sites in Australia and Pohnpei, Micronesia, none of this signal could be attributed to variations in biosynthetic fractionation.

Leaf water isotopic enrichment relative to surface water decreased with increasing salinity in *B. gymnorhiza*. As leaf water is the direct source of hydrogen for leaf lipids, this relationship could account for the increase in apparent fractionation between leaf lipids and surface water with increasing salinity. The decrease in leaf water isotopic enrichment with salinity was most likely due to the strong influence of water vapor isotopes on leaf water isotopes at high relative humidities. Modeled leaf water isotopes in Palau *B. gymnorhiza* mangroves were more sensitive to the isotopic composition of water vapor than to any other model variable (e.g., temperature, relative humidity, xylem water $\delta^2\text{H}$ values). Consistent with this result, leaf water isotopes were much better correlated with rainwater isotopes (a proxy for water vapor isotopes) than with lake surface water isotopes.

While the inverse correlation between salinity and $^2\text{H}/^1\text{H}$ fractionation factors in mangrove lipids is confirmed in *B. gymnorhiza* from brackish lakes in Palau, the mechanisms responsible for this trend are not necessarily consistent among mangrove species, hydroclimate, or hydrologic regimes. Consideration of these differences would be prudent when using hydrogen isotopes of sedimentary mangrove lipids to infer climate and environmental changes in past.

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Figure Captions

Figure 1 Map of study site in Palau. Rain samples and lagoon samples were collected on Koror. Lakes were sampled on the islands of Koror, Ongael, Ngeruktabel, and Mercherchar. The image of Mecherchar Island is indicative of the terrain around the sampled lakes. Satellite image from google earth.

Figure 2 Water $\delta^2\text{H}$ (open circles, left y-axis) and $\delta^{18}\text{O}$ (closed triangles, right y-axis) values plotted relative to surface water salinity. Panel a shows surface water isotopes relative to salinity. Panel b shows xylem water isotopes relative to salinity. Panel c shows leaf water isotopes relative to salinity. X-error bars represent the ± 1 ppt uncertainty associated with refractometer salinity measurements. Y-error bars represent one standard deviation of triplicate measurements and are frequently smaller than the marker size.

Figure 3 Fractionation factors between xylem water and surface water for hydrogen isotopes ($\alpha^2_{\text{xw-sw}}$, open circles, left y-axis) and oxygen isotopes ($\alpha^{18}_{\text{xw-sw}}$, filled triangles, right y-axis) relative to salinity. X-error bars represent the ± 1 ppt uncertainty associated with refractometer salinity measurements. Y-error bars represent propagated errors from triplicate analyses of each type of water isotopes (one standard deviation).

Figure 4 Fractionation factors between leaf water and xylem water ($\alpha_{\text{LW-XW}}^2$ and $\alpha_{\text{LW-XW}}^{18}$, filled triangles) and between leaf water and surface water ($\alpha_{\text{LW-SW}}^2$ and $\alpha_{\text{LW-SW}}^{18}$, open circles) plotted relative to salinity. Hydrogen isotopes are shown in panel a, and oxygen isotopes in panel b. X-error bars represent the ± 1 ppt uncertainty associated with refractometer salinity measurements. Y-error bars represent propagated errors from triplicate analyses of each type of water isotopes (one standard deviation), and are frequently smaller than the marker size.

Figure 5 Leaf water hydrogen isotopes ($\delta^2\text{H}_{\text{LW}}$) plotted relative to those of rainwater from the three most recent rain events (panel a) and of lake surface water (panel b). X-error bars in panel a represent one standard deviation of the most recent rain events. In panel b, x-error bars represent one standard deviation of triplicate water isotopes, and are typically smaller than the marker size. Y-error bars on both panels represent one standard deviation of triplicate measurements, and are frequently smaller than the marker size.

Figure 6 Lipid $\delta^2\text{H}$ values for $n\text{C}_{31}$ -alkane (gray squares) and lupeol (open circles) relative to salinity. X-error bars represent the ± 1 ppt uncertainty associated with refractometer salinity measurements. Y-error bars represent one standard deviation of triplicate analyses.

Figure 7 Net hydrogen isotope fractionation factors between leaf lipids and surface water for $n\text{C}_{31}$ -alkane ($\alpha_{\text{nC31-SW}}$, gray squares) and lupeol ($\alpha_{\text{LUP-SW}}$, open circles) relative to salinity. X-error bars represent the ± 1 ppt uncertainty associated with refractometer salinity measurements. Y-error bars represent propagated errors from triplicate analyses of water and lipid isotopes (one standard deviation).

Figure 8 Apparent biosynthetic fractionation factors between leaf lipids and measured leaf water for $n\text{C}_{31}$ -alkane ($\alpha_{\text{nC31-LW}}$, gray squares) and lupeol ($\alpha_{\text{LUP-LW}}$, open circles) relative to salinity. X-error bars represent the ± 1 ppt uncertainty associated with refractometer salinity measurements. Y-error bars represent propagated errors from triplicate analyses of water and lipid isotopes (one standard deviation).

Table 1: Changes in modeled leaf water ($\delta^2\text{H}_{\text{LW}}$) when changing one variable by indicated range and holding all other variables constant

Variable	Range	Change in $\delta^2\text{H}_{\text{LW}}$
Pressure	1000 – 1020 hPA	0‰
Relative humidity	60 – 100%	12‰
Temperature	10 - 44°C	12‰
Stomatal conductance	0.1 – 0.45 mol/m ² s	5‰
Effective path length	10 – 100 mm	6‰
$\delta^2\text{H}_{\text{Rainwater}}$	-19 – -52‰	13‰
$\delta^2\text{H}_{\text{Xylem Water}}$	-20 – -61‰	24‰

Table 2

Table 2a Summary of linear regression statistics for all water isotopes and various fractionation factors with salinity; bolded relationships are significant at the $p < 0.05$ level.

Variable (relative to salinity)	Slope	y-intercept	R ²	p	n
Surface water $\delta^2\text{H}$ (VSMOW, ‰)	1.25 ± 0.05	-42 ± 1	0.95	< 0.0001	34
Surface water $\delta^{18}\text{O}$ (VSMOW, ‰)	0.20 ± 0.01	-6.8 ± 0.3	0.93	< 0.0001	34
Xylem water $\delta^2\text{H}$ (VSMOW, ‰)	0.98 ± 0.08	-55 ± 2	0.80	< 0.0001	33
Xylem water $\delta^{18}\text{O}$ (VSMOW, ‰)	0.16 ± 0.02	-7.6 ± 0.4	0.76	< 0.0001	33
Leaf water $\delta^2\text{H}$ (VSMOW, ‰)	0.4 ± 0.3	-36 ± 6	0.07	0.14	33
Leaf water $\delta^{18}\text{O}$ (VSMOW, ‰)	0.04 ± 0.07	-3 ± 2	0.01	0.54	33
Lupeol $\delta^2\text{H}$ (VSMOW, ‰)	0.4 ± 0.2	-267 ± 4	0.14	0.03	35
$n\text{C}_{31}$ -alkane $\delta^2\text{H}$ (VSMOW, ‰)	0.3 ± 0.1	-182 ± 3	0.18	0.01	33
$\alpha^2_{\text{XW-SW}}$	-0.0002 ± 0.0001	0.985 ± 0.003	0.09	0.10	33
$\alpha^{18}_{\text{XW-SW}}$	$-3 \times 10^{-5} \pm 2 \times 10^{-5}$	0.9990 ± 0.0004	0.08	0.11	33
$\alpha^2_{\text{LW-XW}}$	-0.0006 ± 0.0003	1.020 ± 0.007	0.12	0.05	33
$\alpha^{18}_{\text{LW-XW}}$	$-0.00012 \pm 7 \times 10^{-5}$	1.004 ± 0.002	0.09	0.10	33
$\alpha^2_{\text{LW-SW}}$	-0.0008 ± 0.0002	1.005 ± 0.006	0.27	0.002	33
$\alpha^{18}_{\text{LW-SW}}$	-0.00015 ± 6 × 10⁻⁵	1.003 ± 0.001	0.16	0.02	33
$\alpha_{\text{LUP-LW}}$ (measured LW)	0.0001 ± 0.0003	0.769 ± 0.008	< 0.01	0.75	33
$\alpha_{\text{C31-LW}}$ (measured LW)	-0.0001 ± 0.0002	0.850 ± 0.006	< 0.01	0.86	31
$\alpha_{\text{LUP-LW}}$ (modeled LW, ind. water vapor)	$-7 \times 10^{-5} \pm 0.0003$	0.770 ± 0.006	< 0.01	0.78	34
$\alpha_{\text{C31-LW}}$ (modeled LW, ind. water vapor)	-0.0002 ± 0.0001	0.851 ± 0.003	0.08	0.11	32
$\alpha_{\text{LUP-LW}}$ (modeled LW, avg. water vapor)	0.0001 ± 0.0002	0.766 ± 0.004	< 0.01	0.57	34
$\alpha_{\text{C31-LW}}$ (modeled LW, avg. water vapor)	0.0001 ± 0.0001	0.846 ± 0.006	< 0.01	0.66	32
$\alpha_{\text{LUP-SW}}$	-0.0005 ± 0.0002	0.764 ± 0.005	0.14	0.03	35
$\alpha_{\text{C31-SW}}$	-0.0007 ± 0.0001	0.853 ± 0.003	0.55	< 0.0001	33

Table 2b Summary of linear regression statistics for leaf water $\delta^2\text{H}$ values relative to potential source water pools. Bolded relationships are significant at the $p < 0.05$ level.

Variable (relative to measured leaf water $\delta^2\text{H}$ values)	Slope	y-intercept	R ²	p	n
Rain water $\delta^2\text{H}$ (VSMOW, ‰)	1.0 ± 0.1	9 ± 5	0.68	< 0.0001	33
Surface water $\delta^2\text{H}$ (VSMOW, ‰)	0.5 ± 0.2	-20 ± 4	0.16	0.02	33
Modeled leaf water $\delta^2\text{H}$, individual water vapor (VSMOW, ‰)	1.2 ± 0.2	0 ± 6	0.47	< 0.0001	33
Modeled leaf water $\delta^2\text{H}$, average water vapor (VSMOW, ‰)	0 ± 0.6	-30 ± 13	< 0.01	0.99	33

Table 3: Rainwater isotopes from Koror, Palau

Rain date	Rain $\delta^2\text{H}$	Rain $\delta^{18}\text{O}$
September 17, 2013	-19.8 ± 0.4	-4.52 ± 0.09
September 18, 2013	-30.5 ± 0.1	-5.24 ± 0.04
September 21, 2013	-33.7 ± 0.4	-4.24 ± 0.06
September 23, 2013	-21.5 ± 0.1	-4.02 ± 0.03
September 25, 2013	-16.4 ± 0.4	-3.3 ± 0.2
September 27, 2013	-35.1 ± 0.1	-5.85 ± 0.08
September 30, 2013	-53.7 ± 0.1	-8.31 ± 0.02
October 5, 2013	-36.3 ± 0.2	-5.91 ± 0.06
October 5, 2013	-44.5 ± 0.1	-6.93 ± 0.01
October 6, 2013	-52.1 ± 0.1	-7.59 ± 0.03
October 7, 2013	-61.6 ± 0.1	-9.18 ± 0.09
October 8, 2013	-36.1 ± 0.1	-6.07 ± 0.05
October 9, 2013	-27.5 ± 0.4	-5.13 ± 0.05

Figure 2

Figure 4

Figure 5

Figure 7