

# Isotopic signatures of stem water reveal differences in water sources accessed by mangrove tree species

Catherine E. Lovelock · Ruth Reef · Marilyn C. Ball

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**Abstract** Mangrove trees are rooted in saline soils which can limit their growth. Access to alternative, less saline water sources may provide important water subsidies. We assessed the hydrogen and oxygen isotopic signatures of soil porewater, groundwater and atmospheric water sources (dew and rainfall) and tree stem water from three mangroves species over two sites that varied in elevation. Although stem water isotopic signatures were most similar to porewater, variation in isotopic values indicated trees also

accessed alternative water sources, the degree to which varied over sites and among species. *Rhizophora stylosa* had lowest values of stable isotopes among the species indicating significant groundwater utilization. In a long-term fertilization experiment we found that growth of *Ceriops australis* and *Lumnitzera racemosa* was nitrogen limited, while growth of *R. stylosa* was nitrogen limited to a lesser extent, suggesting groundwater may also provide nutritional benefits for *R. stylosa*. The uptake of alternative water sources in addition to saline porewater may improve metabolic function, differentially altering the performance of different species and over sites.

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C. E. Lovelock (✉) · R. Reef  
School of Biological Sciences, The University of Queensland, St Lucia, QLD 4072, Australia  
e-mail: c.lovelock@uq.edu.au

R. Reef  
School of Earth, Atmosphere and Environment, Monash University, Clayton, VIC 3800, Australia  
e-mail: ruth.reef@monash.edu

M. C. Ball  
Research School of Biology, The Australian National University, Acton, ACT 0200, Australia  
e-mail: Marilyn.Ball@anu.edu.au

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## Introduction

Mangrove forests grow along sheltered coasts and are strongly influenced by tidal inundation of soils with saline water. Mangrove species distributions and growth rates are influenced by a wide range of environmental factors, including salinity of soil porewater, nutrient availability and the frequency and intensity of inundation (Ball, 1988). Highly saline soils result in reduced photosynthetic carbon gain and growth (Ball, 1988; Ball et al., 1988; Reef & Lovelock, 2014). Thus access of species to alternative, less saline water sources, for example water from

rainfall, dew and groundwater, is likely to enhance tree growth and perhaps influence the diversity of mangrove communities through amelioration of highly saline conditions that can develop in surface soils due to limited tidal inundation and high evaporative demand.

The uptake of groundwater by mangrove trees has been observed in Florida, French Guiana, islands in the Indian Ocean and Australia (Semeniuk, 1983; Sternberg & Swart, 1987; Greaver & Sternberg, 2006; Ewe et al., 2007; Lambs et al., 2008; Wei et al., 2012; Lambs et al., 2016) and may be widely important in sustaining metabolic function of mangrove trees. Access to groundwater resources may also influence mangrove growth through access to the nutrients dissolved in groundwater. Growth of mangrove trees is often nutrient limited (Reef et al., 2010) and groundwater can contain high nitrogen concentrations (Valiela et al., 1990). Therefore, groundwater may provide an alternative water source to highly saline porewater and could also be an important source of nitrogen where nitrogen limits plant growth.

In plants, access to groundwater is influenced by both the depth of the groundwater and the rooting depth of the plants (Schulze et al., 1996; Casper & Jackson, 1997; Groom, 2004; February et al., 2011). In mangrove forests, the distribution of groundwater in soils is not well described, but freshwater lenses can occur on top of denser saltier water, groundwater can be held within porous sediments underlying mangrove forests and groundwater can often emerge at sea level and within mangrove habitats (Stieglitz, 2005; Santos et al., 2012; Maher et al., 2013). There have been few studies of the distribution of mangrove roots in the soil profile. However, most roots of mangrove trees in Kenya were within the top 20 cm of soil (Tamooh et al., 2008), although the presence of roots deeper (to 90 cm) in the sediment were observed in Florida (Castaneda-Moya et al., 2011), indicating a potential for some species to access resources deeper in the soil profile. Mangrove species differ in their tolerance of anoxic conditions, because of their different root characteristics (McKee, 1996), which may influence their maximum rooting depth. In a study of tolerance of root zone anoxia among species, McKee (1996) found that *Rhizophora mangle* L. had more highly developed aerenchyma, enabling the maintenance of root growth when exposed to anoxic conditions compared to *Avicennia germinans* (L.) L. or

*Laguncularia racemosa* (L.) Gaertn. f. which suffered reduced O<sub>2</sub> concentrations within roots and reduced root growth. Differences in tolerance of soil anoxia among mangrove taxa may limit the use of groundwater resources by some species, thereby differentially influencing growth rates and distributions of species (Yakir & Yechielie, 1995).

In addition to groundwater, plants may also utilize water vapour that condenses on leaf and stem surfaces. Some plant species can take up water that condenses on their leaves (Slayter, 1956; Munné-Bosch & Alegre, 1999; Martin & von Willert, 2000), most likely through stomatal pores (Eichert et al., 2008). This is commonly observed in cloud forests (Johnson & Smith, 2008; Eller et al., 2013) and in coastal areas where fog is common (Burgess & Dawson, 2004). Fog uptake by leaf surfaces of the cloud forest tree *Drimys brasiliensis* Miers contributed more than 40% of the leaves' water content leading to the reverse flow of sap from the leaves to the roots (Burgess & Dawson, 2004). Although not yet assessed in mangroves, uptake of atmospheric water from fog, dew or rainfall may be an important process that augments the saline water sources in soils.

At our study site in the South Arm of the Daintree River system, extensive mangrove scrub forests occur isolated from the main river channels and behind a series of low coastal dunes. These forests comprise a high diversity of species that occupy (1) permanently flooded soils that have 10–20 cm of brackish water overlying the soil surface and are underlain by hypersaline soil porewater; and (2) elevated areas where hypersaline soils are flooded intermittently by very high tides. *Rhizophora stylosa* Griff. and *Ceriops australis* (C.T.White) Ballment, T.J.Sm. & J.A.Stoddart are found in both habitats. In the permanently flooded sites, plant diversity is higher and *R. stylosa* and *C. australis* co-occur with two species of *Lumnitzera* (*L. racemosa* Willd. and *L. littorea* (Jack) Voigt) and other less abundant species, including *Xylocarpus granatum* J.König and *Bruguiera gymnorhiza* (L.) Lam.. *Rhizophora stylosa*, *C. australis* and *L. racemosa* have different aboveground root structures (Tomlinson, 1987). *Rhizophora stylosa* has “prop” roots, *C. australis* has fluted buttress roots while *L. racemosa* has no aboveground roots under most conditions but develops small rounded aboveground roots when growing in permanently inundated sites. Shallow fresh groundwater is available at our

site. The water table is within 2 m of soil surface in the sandy soils adjacent to the mangroves (visible in constructed ponds) and is pumped from bores for irrigation by local landholders that have developed the higher elevation sand dunes. Humidity in the region is high, and night time dew formation is common in the dry season. Based on differences in root morphologies of the species at our site, we used natural gradients in the potential availability of water sources in conjunction with a long-term nutrient fertilization experiment to test the following hypotheses:

1. Tree species access different water sources, due to variation in the characteristics and distribution of their roots. *R. stylosa*, with greater capacity to withstand anoxic soils compared to other species has greater access to groundwater sources compared to the other two species;
2. Species with access to groundwater would show reduced responsiveness to experimental nutrient additions;
3. At lower elevations plants access greater amounts of groundwater than those at higher elevations where the depth of the groundwater may exceed the rooting depth of trees and
4. Mangroves can access atmospheric water sources.

We used a stable isotope approach to assess both the characteristics of the water sources available at our field site and the water contained within the plants (Ehleringer & Dawson, 1992; Dawson & Ehleringer, 1993; Lin & Sternberg, 1993). We solved the isotope mixing equations to establish the most probable sources of the water held within the stems of plants (Phillips & Gregg, 2001). We assessed our hypotheses by contrasting the stable isotope composition of stem water among species and between two sites: one site at low elevation where we anticipated groundwater would be closer to the soil surface versus a higher elevation site where depth to the groundwater is greater. We expected stem water isotopic composition to reflect groundwater uptake at the lower elevation site. We assessed the potential uptake of atmospheric sources (dew) through assessing stem water before daybreak (predawn) on nights when dew was abundant, comparing to stem water at midday after approximately 6 h of evapotranspiration, when we anticipated water sources within the stems would

resemble water available at the roots (porewater and/or groundwater).

## Materials and methods

### Site description

Our experimental site was situated in extensive mangrove scrub forests within the South Arm of the Daintree River in northern Queensland (Lat. 16°22'S; Long. 145°25'E) (Fig. S1). The South Arm is a tidal tributary of the Daintree River which has tall and diverse mangrove forests (Duke et al., 1998). Mean annual rainfall in the region is 2,037 mm year<sup>-1</sup> (Australian Bureau of Meteorology, Port Douglas station). Although rainfall is highest in the wet season (November–February), rain usually occurs in all months, even throughout the dry season (July–October). Mean maximum annual air temperature is 27.9°C, with mean minimum temperatures of 16.8°C in the coolest month (July) and mean maximum temperatures of 30.3°C in December and January. The diversity of mangrove tree species is high (28 species, Duke et al., 1998) and they range from tall trees (~30 m) on the river's edge to extensive scrub forests (~2 m tall).

Two scrub forest sites within the tidally dominated South Arm of the river were selected for the study (Fig. S1). The Wet Scrub site is permanently inundated while the Dry Scrub site is rarely inundated. Mean porewater salinity (at 30 cm depth) was 39.7 ppt in the Wet Scrub, with 47.7 ppt in the Dry Scrub. Additionally, in the permanently inundated Wet Scrub the overlying layer of surface water was 7.7 ppt, but this overlying water did not occur in the Dry Scrub. Redox potential of the soils reflected differences in inundation with Eh of 120 ± 16 mV in the Wet Scrub and 241 ± 16 mV in the Dry Scrub (values and mean ± standard error, *N* = 24). Tree height was less than 2.5 m (mean tree height in Wet Scrub was 1.9 m and in the Dry Scrub 1.6 m) and the communities mainly comprised *R. stylosa*, *C. australis*, *L. racemosa* and *L. littorea*. In the Wet Scrub, leaf area index (LAI, m<sup>2</sup> leaf area per m<sup>2</sup> of soil surface area) was 0.88, while in the Dry Scrub, LAI was 0.38.

## Water sampling and isotopic analyses

All water sources and plant stem water were sampled between 20 and 22 June, 2011. To obtain the isotopic signatures of potential water sources during the field campaign, we sampled dew, groundwater, interstitial soil porewater and the overlying surface water that occurred in the Wet Scrub. We also sampled rainwater, river water and seawater on incoming tides in order to assess the variation in isotopic composition of all water at the site to create a local isotopic “water line” (after Liu et al., 2010) and to assess the contribution of tidal waters (river and seawater) to soil porewater and the overlying water in the Wet Scrub. There was heavy dew during the field campaign, from which we collected three samples on different nights of dew from the plastic-coated metal roof of a building that was within 300 m of the field site. Dew was collected on three successive evenings in clean plastic buckets attached below the roof and transferred to 10 ml plastic screw cap test-tubes with no headspace. These samples may have undergone some evaporation as the dew flowed over the metal roof. No rain occurred during the sampling period, although there were small rainfall events 9 days prior to the field campaign (on 9 and 11 June rainfall was 0.6 and 1.6 mm, respectively); thus three rainwater samples were collected from a covered rainwater tank (where evaporation is reduced) that collects rain from the roof of a building that was within 300 m of the field site. These samples represent a temporal composite of rainfall that may have undergone some evaporation.

The isotopic composition of the local fresh groundwater, which in the coastal sands of the region occurs as an unconfined aquifer that typically flows in the porous sediments along hydrological gradients from the terrestrial to the marine environment (Stieglitz, 2005; Santos et al., 2012), was collected three times over the sampling campaign from a 4-m-deep bore on private property that was 300 m from the study site. The water table of the groundwater was visible in excavated ponds in the terrestrial environment adjacent to the mangrove and was close to the soil surface (within 2 m).

River water was sampled from a boat at 30 cm below the water surface at three approximately equidistant locations along the Daintree River between the study site and the river mouth (over 10 km of the river). Seawater was sampled at three

locations at the river mouth, which is approximately 10 km distant from the study site on an incoming tide. The overlying surface water in the Wet Scrub site was sampled with a syringe at three locations. Three interstitial soil porewater samples were collected from both the Wet and Dry Scrub sites from 30 cm depth within the soils using a suction device (McKee et al., 1988). All water samples were stored at 5°C until analysis. Salinity of the different water sources was measured using a Refractometer W/ATC 300011, SPER Scientific (Scottsdale, AZ, USA).

In order to assess the isotopic composition of the stem water of trees, we collected terminal, leaf-bearing stems of three replicate trees (approximately 8 mm in diameter and 100 mm in length) of each species in each habitat at midday and predawn between 20 and 22 June, 2011. We sampled predawn when dew was observed on the plants and before transpiration commenced, thereby assessing the hypothesis that plants take up dew into their tissues via leaves or bark which is incorporated into stem water. Midday sampling was used to assess the water sources within the plant after it had been actively transpiring for ~6 h, thereby assessing the stem water when transpiration rates are high and testing the hypothesis that groundwater sources are available to some species. Stems were harvested and immediately sealed within vials and stored frozen (−20°C) until analysis. For predawn samples, stem segments were quickly wiped (5 s) with paper towel to remove external moisture.

To establish the  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  isotopic composition of water in plants, stem water was extracted from each sample by cryogenic distillation using a vacuum system at the Stable Isotope Laboratory at the University of Western Australia following the procedures of West et al. (2006). Extractions were performed for 2–3 h which is sufficient to avoid fractionation during extraction (West et al., 2006). The water extracted from the stems and the source water was analysed for  $\delta^{18}\text{O}$  and deuterium using an Isotopic Liquid Water and Continuous Water Vapor Analyzer (Picarro L1102-I, Santa Clara, California, USA). Results were normalized with the international standards provided by the International Atomic Energy Agency (IAEA): Vienna Standard Mean Ocean Water (VSMOW), Standard Light Antarctic Precipitation 2 (SLAP2) and Greenland Ice Sheet Precipitation (GISP) (Paul et al., 2007). Elemental

concentrations in groundwater were assessed in three samples from the same bore using persulfate digestion and simultaneous colorimetric analysis (Johnes & Heathwaite, 1992) at the Queensland Government Forensic analytical laboratories (QLD Government, Australia).

Differential nutrient limitations to tree growth among sites and species can provide insights into the availability of sources of nutrients (Feller et al., 2003). Because groundwater is found as a lens within porous sands at our site, and has been observed to provide a source of nitrogen to wetland plants in other similar ecosystems (Valiela et al., 1990), we used an established long-term fertilization experiment at the sites (directly adjacent to where trees were sampled for stem water described above) to assess differences in nutrient limitations among sites and species, thereby providing a complimentary assessment of possible plant uptake of groundwater. The long-term fertilization experiment, where trees were dosed with nitrogen (N, as urea) or phosphorus (P, as triple superphosphate) or left as unfertilized controls (C), was established in 2009. Eighteen pairs of *R. stylosa* and *C. australis* trees in the Dry Scrub and 18 triplets of *R. stylosa*, *C. australis* and *L. racemosa* in the Wet Scrub were selected and randomly assigned to treatments. Fertiliser was applied annually as two 250 g doses of fertiliser encased in dialysis tubing and inserted in holes cored between the target trees (after Feller, 1995). Assuming that we influenced approximately 4 m<sup>2</sup> of the forest floor then application rates are approximately equivalent to 5.8 kg N ha<sup>-1</sup> year<sup>-1</sup> and 3.4 kg P ha<sup>-1</sup> year<sup>-1</sup>. Control trees were cored but not fertilized. Core holes were capped with extracted soil. Thus the final design comprised 6 replicate N- and P-fertilized trees and 6 replicate control plots at each site. On each tree, 5 terminal unbranched twigs were tagged and linear extension was monitored annually for 3 years to provide a measure of tree growth (Feller, 1995). The mean extension rates of five twigs for each tree were calculated to provide a mean value for each tree which was used to assess the differences in nutrient limitation among species and sites.

#### Data analysis

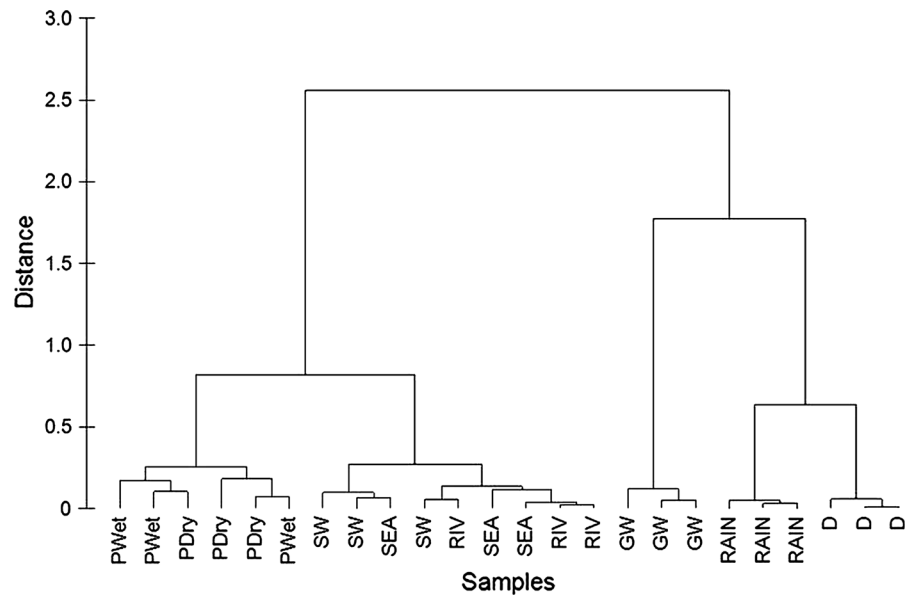
We used regression analysis to assess the relationship among  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  for both water sources and plant stem water. We tested the difference between water

and plant regression lines using analysis of covariance (ANCOVA). We assessed the influence of site, species and time of day on stable isotope composition in stem water using repeated measures ANOVA where Site and Species were fixed effects in the model and time of day was the repeat. To assess the influence of fertilization treatments on the growth of trees (stem extension), we used a generalized linear model where Site, Species and Fertiliser treatments were fixed effects in the model. *Lumnitzera racemosa* was excluded from the cross-site comparisons to avoid an unbalanced design but was included in two separate models for analysis of stable isotopes and growth in the Wet Scrub community. Stem extension rates were log-transformed prior to analysis. All statistical analyses were done using the DataDesk v6.3.1 (Data Descriptions Inc., Ithaca, New York, USA).

For modelling the contribution of different water sources to plant stem water, we used source partitioning models (Phillips & Gregg, 2001). We constructed a range of models using different inputs (i.e. different water sources; midday or predawn samples and a combined dataset of both midday and predawn samples). (1) We used a combined predawn and midday stem water dataset and water sources that were immediately available to the plants, using the mean isotopic values for groundwater, dew and porewater at each site; (2) We used a combined predawn and midday stem water dataset and water sources grouped into categories (Surface Water, Groundwater, Atmospheric) based on their similarity from a cluster analysis done using Euclidian distance within the Cluster procedure of Primer 7 (Primer-E, Ivybridge, UK) (Fig. 1). Water sources were grouped as Atmospheric (rain and dew), Groundwater and a combined Surface Water group. The Surface Water category encompassed tidal waters (river and seawater), water overlying the sediment surface from the Wet Scrub and interstitial soil porewater from both Wet Scrub and Dry Scrub (Table S1); (3) We considered the midday samples only, where we removed atmospheric sources (no dew or rain) and used only the oxygen isotope data, as in this case we had only two potential water sources; (4) We considered the predawn samples with three sources (porewater, dew and groundwater). In all models a 9 ‰ adjustment was used as the “trophic enrichment” factor for  $\delta^2\text{H}$  values as this is the value recommended for the most salinity-tolerant trees studied by Ellsworth & Williams (2007) and



**Fig. 1** Cluster analyses of water samples from different sources. Data were clustered based on Euclidean distance. *PWet* Porewater from Wet Scrub; *PDry* porewater from Dry Scrub; *SW* surface water in the Wet Scrub; *SEA* seawater from river mouth; *RIV* river water; *GW* groundwater from house bore; *RAIN* rainwater; *D* Dew



because mangroves have little apoplastic water uptake (Moon et al., 1986). Source partitioning was solved unambiguously (as there were three sources and two isotopes) using a determined linear mixing model formulated in R v3.0 (R Development Core Team, 2008) as described in Phillips & Gregg (2001). The mass balance equations for dual isotope, three end-member linear mixing models, using the mean isotopic signatures for two elements (i.e. O and H) with different sources (here A, B, C) and where the mixture in the plant is M, are as follows:

$$\overline{\delta^{18}\text{O}_M} = f_A \overline{\delta^{18}\text{O}_A} + f_B \overline{\delta^{18}\text{O}_B} + f_C \overline{\delta^{18}\text{O}_C}$$

$$\overline{\delta^2\text{H}_M} = f_A \overline{\delta^2\text{H}_A} + f_B \overline{\delta^2\text{H}_B} + f_C \overline{\delta^2\text{H}_C}$$

$$1 = f_A + f_B + f_C.$$

The calculation of the variation around the mean contribution of each source follows the methodology of Phillips & Gregg (2001).

## Results

### Characterizing water sources

Abundance of  $\delta^{18}\text{O}$  varied from approximately zero in soil porewater to  $-8\text{‰}$  in groundwater while,  $\delta^2\text{H}$

varied over a wider range, from approximately zero in soil porewater to  $-52\text{‰}$  in groundwater (Table 1). Rainwater and dew had similar  $\delta^{18}\text{O}$  signatures, but dew had less negative values of  $\delta^2\text{H}$  than rainwater ( $\delta^2\text{H}$  of dew was  $-22.12 \pm 0.15\text{‰}$ , while rainwater was  $-32.06 \pm 0.23\text{‰}$ ). Soil porewater from the Wet and Dry Scrub was similar (Fig. 1). The overlying surface water in the Wet Scrub was similar to water sampled from river and seawater sampled on incoming tide, leading them to be grouped together in the cluster analysis (Fig. 1).

Plotting  $\delta^2\text{H}$  against  $\delta^{18}\text{O}$  to obtain a site water line gave an intercept of 1.42 ‰ and slope of 6.06 (Fig. 2). The intercept was lower than the Australian water line (intercept 8.21 ‰), but had a similar slope (slope of 7.10, Liu et al., 2010) (Fig. 2). The plant water line had an intercept that was approximately 9 ‰  $\delta^2\text{H}$  lower than the relationship between the  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  of the water sources, as can be expected due to root fractionation (Ellsworth & Williams, 2007), but had a similar slope ( $\sim 6.1$ ) and was not statistically different from the site water line. The isotopic signature of water sources varied linearly with their salinity, although groundwater had a lower  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  than other “fresh” samples and fell below the regression line (Fig. 2B, C). Analysis of the elemental composition of the groundwater indicated it had high levels of total N ( $79 \pm 0.1\text{ }\mu\text{M}$ ) and levels of total P that were at the detection limits of the analyses

**Table 1** Mean isotopic signatures of water sources and collection location (in parentheses) in the Daintree River

Potential source	$\delta^{18}\text{O}$ ‰	$\delta^2\text{H}$ ‰
Groundwater (house bore)	$-7.93 \pm 0.05$	$-51.46 \pm 0.60$
Rainwater (water tank)	$-5.64 \pm 0.03$	$-32.06 \pm 0.23$
Dew (roof collected)	$-5.12 \pm 0.05$	$-22.12 \pm 0.15$
River water (main channel)	$-2.11 \pm 0.09$	$-11.83 \pm 0.41$
Seawater (river mouth)	$-1.87 \pm 0.14$	$-11.29 \pm 1.09$
Water overlying the sediment surface (wet scrub)	$-1.62 \pm 0.14$	$-9.08 \pm 0.70$
Porewater (dry scrub)	$-0.51 \pm 0.11$	$-2.51 \pm 0.98$
Porewater (wet scrub)	$-0.20 \pm 0.18$	$0.10 \pm 0.83$

Values are means  $\pm$  standard errors for three replicate samples

(0.09  $\mu\text{M}$ ). The N:P molar ratio of the groundwater was 851.

### Plant isotopic signatures

The isotopic composition of water in stems varied over sites, with  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  of plants being significantly lower in the Wet Scrub compared to the Dry Scrub (for  $\delta^{18}\text{O}$ ,  $F_{1,16} = 24.2$ ,  $P = 0.0002$  and  $\delta^2\text{H}$ ,  $F_{1,25} = 22.2$ ,  $P = 0.0002$ ). Within each site, plant species varied in the isotopic composition of their stem water (Fig. 3). *Rhizophora stylosa* tended to have more negative  $\delta^{18}\text{O}$  than *C. australis* and *L. racemosa* (Main effect of Species:  $F_{2,12} = 10.3$ ,  $P = 0.0025$ ). At the predawn sampling, stem water values for *R. stylosa* were 1 ‰ for  $\delta^{18}\text{O}$  and 9 ‰ for  $\delta^2\text{H}$  lower, while at midday the isotopic composition of species tended to be more similar (Fig. 3, Site  $\times$  Time interaction  $F_{1,16} = 6.38$ ,  $P = 0.0224$ ).

### Nutrient limitations to plant growth

Stem growth rates were enhanced with fertilization (Fig. 4; main effect Fertilization,  $F_{2,60} = 13.9$ ,  $P < 0.0001$ ) and differed among species (main effect Species,  $F_{2,60} = 14.59$ ,  $P = 0.0003$ ). In the Dry Scrub, both species responded similarly to N and P fertilization. In the Wet Scrub, both N and P increased stem growth of *C. australis* and *L. racemosa*, but N fertilization did not enhance stem growth of *R. stylosa* at this site, although stem growth of *R. stylosa* was significantly enhanced with P fertilization.

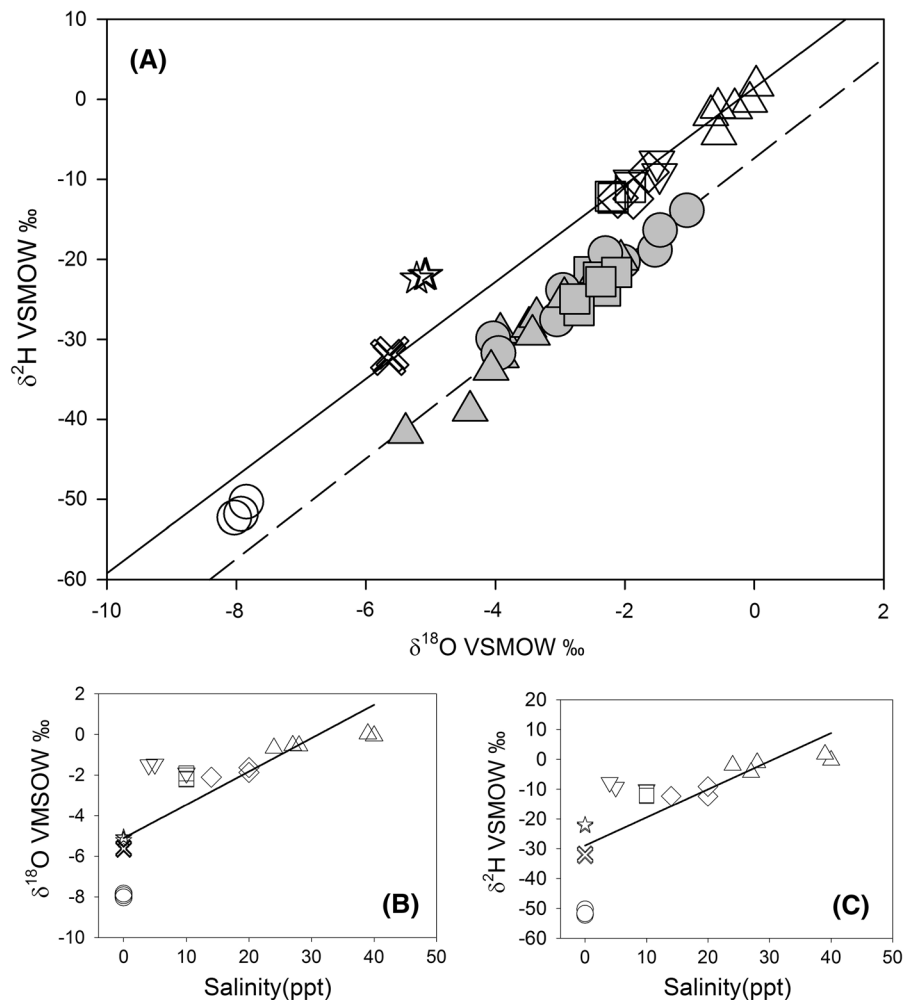
### Modelled contributions to stem water

Differences in isotopic composition among species and sites gave rise to models of differences in the

proportional contribution of differing water sources. Our modelling indicated that in both sites, stem water mainly (45–78%) comprised soil porewater (or overlying water in the Wet Scrub) (Table 2), which is derived from tidal water (Fig. 1). Groundwater provided the next largest contribution (8–41%), while dew contributed least (8–20%) and errors about the contribution of dew were very high and similar to the magnitude of the estimated contribution, such that the contribution of dew could be zero (Table 2). Limiting the model to predawn samples (removing noon samples) increased the estimated proportional contribution of dew to stem water to 10–27% (data not shown), but errors were very large and thus our initial hypothesis of uptake of dew in these species was not supported by the data. These modelled results were broadly similar whether we used site-specific isotopic values of porewater and the isotopic value of dew (Table 1) or the more general Surface water and Atmospheric water categories (Table S1), although using the grouped Surface water and Atmospheric water categories gave higher standard errors (Table S2).

Comparing sites, porewater contributions to stem water were higher in the Dry Scrub, while groundwater contributions were higher in the Wet Scrub, consistent with our hypothesis (Table 2). When we considered the contributions of only two water sources at noon (porewater and groundwater, omitting dew), and using only oxygen isotopes, results were similar to our three source model, with porewater contributing 53–83% of stem water and groundwater 17–47% (Table 3). Errors about the contributions in this two source model were much lower (in the order of 3–6%) than the models that considered three water sources.

Comparison of water source contributions among species indicated that stem water of *R. stylosa* tended to comprise greater proportion of alternative sources,



**Fig. 2** The relationship among  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in water and plant samples (A). Water samples are from groundwater (circles), rainwater (obtained from a rainwater tank, crosses), dew (stars), river water (squares), seawater (diamonds), surface water from the Wet Scrub (downward triangles) and porewater (upward triangles). Plant stem water is from *Rhizophora stylosa* (grey triangles), *Ceriops australis* (grey circles) and *Lumnitzera racemosa* (grey squares). The regression lines are Local water line (solid),  $Y = 1.42 (\pm\text{SE } 0.27) + 6.06 (\pm\text{SE } 1.1) \times X$ ,

mostly groundwater (up to 41% in the Wet Scrub) compared to *C. australis* (28%) and *L. racemosa* (24%) (Table 2). In the two water source model, using only the noon samples, *R. stylosa* had higher contributions of groundwater to stem water in both sites than the other species, but in the Wet Scrub groundwater contributions to stem water of *C. australis* was similar to *R. stylosa* (45 and 47% respectively), while contributions to stem water of *L. racemosa* were lower (30%).

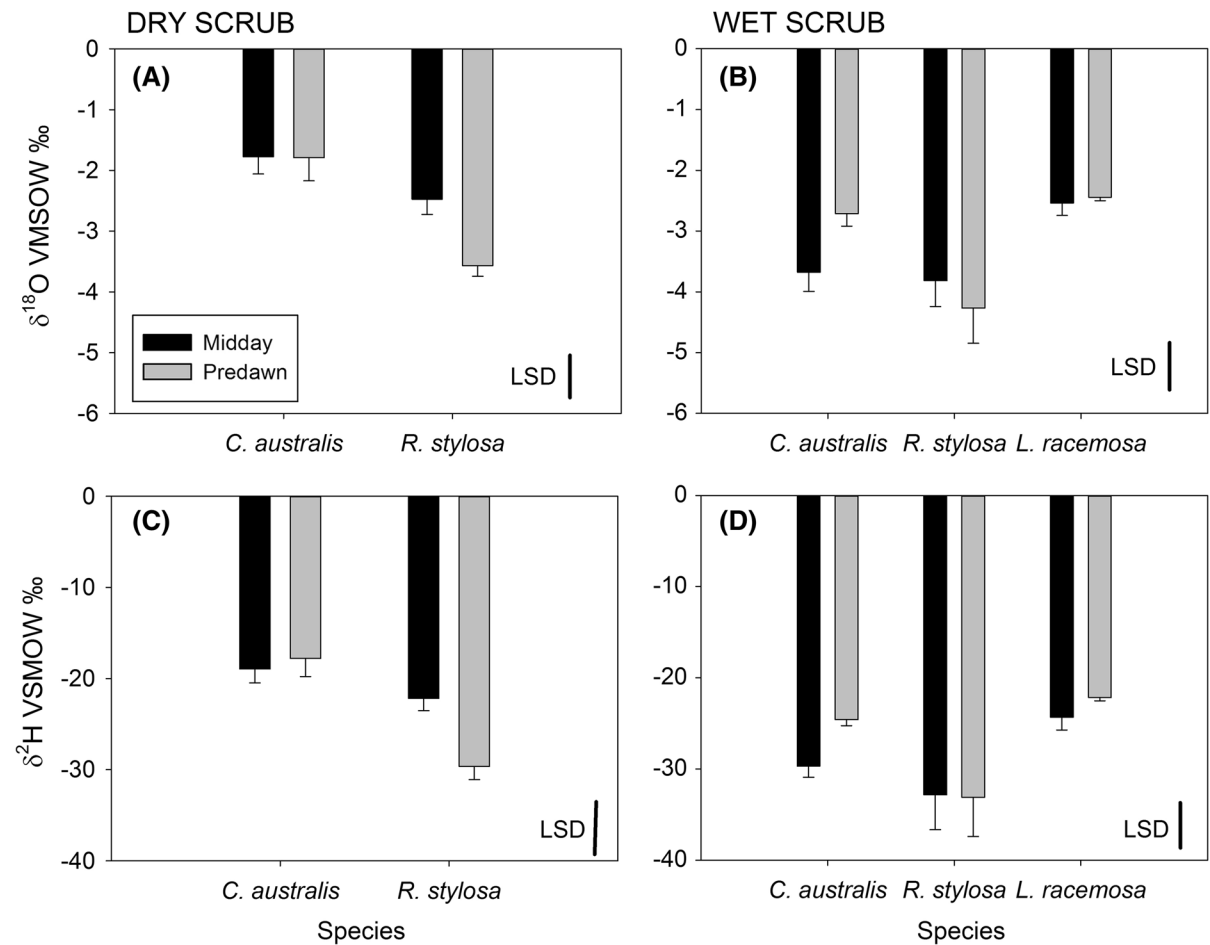
$R^2 = 0.96$ ,  $P < 0.0001$ . Plant water line (dashed),  $Y = -7.36 (\pm\text{SE } 0.93) + 6.26 (\pm\text{SE } 0.30) \times X$ ,  $R^2 = 0.94$ ,  $P < 0.0001$ . **B** The relationship between  $\delta^{18}\text{O}$  and salinity of water sources,  $Y = -4.86 (\pm\text{SE } 0.44) + 0.15 (\pm\text{SE } 0.03) \times X$ ,  $R^2 = 0.64$ ,  $P < 0.0001$  and **C** The relationship between  $\delta^2\text{H}$  and the salinity of water sources at the study site,  $Y = -27.0 (\pm\text{SE } 2.9) + 0.87 (\pm\text{SE } 0.17) \times X$ ,  $R^2 = 0.54$ ,  $P < 0.0001$ . Symbols are the same for **A**

## Discussion

### Variation in isotopic composition of water sources

Variation in isotopic composition of different water sources and their relationship to salinity of the water sources were similar to that observed in other estuarine systems where mixing of seawater and freshwater sources occurs (e.g. Bratton et al., 2009; Wei et al., 2012). The values  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  of soil porewater were





**Fig. 3** Isotopic signatures of stem water of three mangrove species: *Ceriops australis* (*C. australis*), *Rhizophora stylosa* (*R. stylosa*) and *Lumnitzera racemosa* (*L. racemosa*) between midday (*black bars*) and predawn during a heavy dew (*grey*

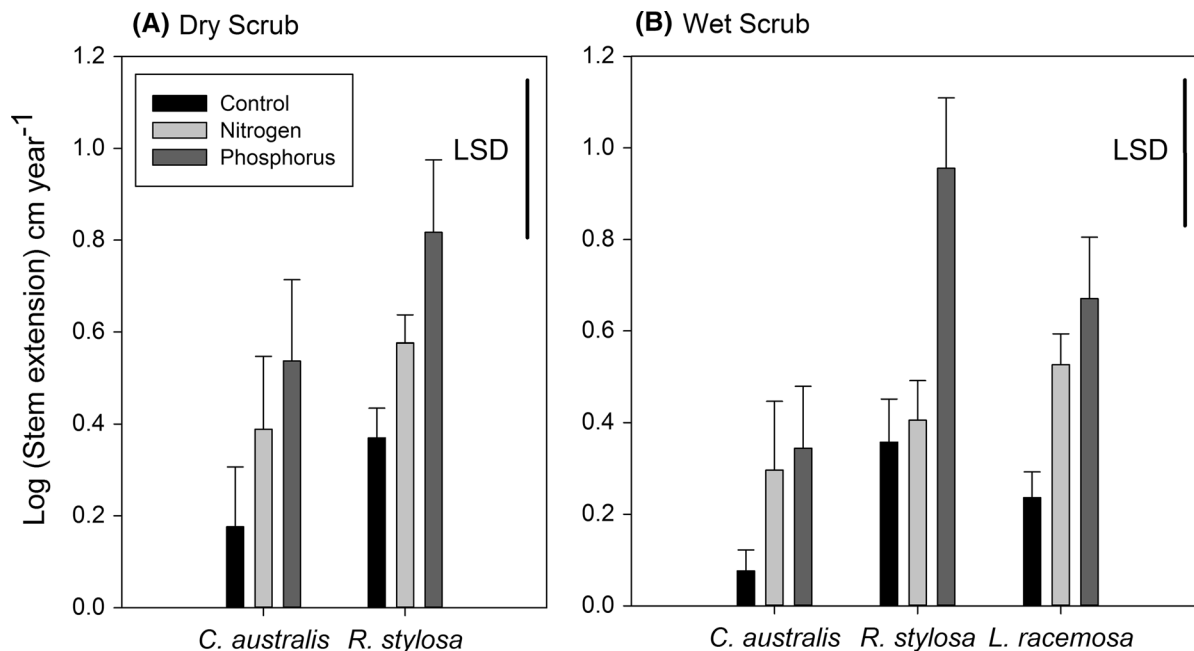
*bars*) at two sites; the Dry Scrub (**A** and **C**) and Wet Scrub (**B** and **D**). The least significant difference (LSD) bar within the plots indicates differences among means that are significant ( $P < 0.05$ )

consistent with porewater being derived from tidal water that had been evaporated with loss of lighter isotopes (Fass et al., 2007). The isotopic signature of the brackish surface water in the Wet Scrub was not significantly different from the river water and seawater samples and thus is likely refreshed with tidal flow. Fresh groundwater in this system, flowing within the porous soils, typical of other unconsolidated aquifers on the east coast of Australia and elsewhere (Stieglitz, 2005; Larsen & Cox, 2011; Santos et al., 2012), had an isotopic signature that was similar to other unconsolidated coastal aquifers in Queensland (Larsen et al., 2011). The isotopic composition of the groundwater was indicative of water derived from heavy rainfall indicating that this aquifer is likely

replenished during intense rainfall events (Liu et al., 2010).

#### Variation in isotopic composition of stem water

The isotopic composition of stem water of mangroves species in our study varied between sites and among species, indicating the incorporation of different water sources into their stem tissues. Seasonal and spatial variation in water sources comprising stem water of mangroves has been observed in the neo-tropics and islands in the Indian Ocean (Sternberg & Swart, 1987; Greaver & Sternberg, 2006; Ewe et al., 2007; Lambs et al., 2009; Lambs et al., 2016), and differing patterns in stem water source composition have been observed



**Fig. 4** Growth rates of mangrove trees, measured as stem extension of terminal shoots, in the Dry Scrub (A) and Wet Scrub (B) in three species: *Ceriops australis* (*C. australis*), *Rhizophora stylosa* (*R. stylosa*) and *Lumnitzera racemosa* (*L. racemosa*). Only *C. australis* and *R. stylosa* occur in the Dry

Scrub. Trees were unfertilized control trees (black), fertilized with nitrogen (grey) or fertilized with phosphorus (dark grey). Values are means of six trees  $\pm$  standard errors. The Least Significant Difference (LSD) bar indicates differences among means that are significant ( $P < 0.05$ )

**Table 2** Percentage use of the three water sources by different mangrove species at each site based on analysis of two isotopes (Phillips & Gregg, 2001)

	Groundwater	Porewater	Dew
Dry scrub			
<i>C. australis</i>	8 $\pm$ 10	78 $\pm$ 10	14 $\pm$ 19
<i>R. stylosa</i>	21 $\pm$ 13	59 $\pm$ 13	20 $\pm$ 25
Wet scrub			
<i>C. australis</i>	28 $\pm$ 12	55 $\pm$ 11	16 $\pm$ 23
<i>R. stylosa</i>	41 $\pm$ 18	45 $\pm$ 15	14 $\pm$ 33
<i>L. racemosa</i>	24 $\pm$ 7	67 $\pm$ 7	9 $\pm$ 14

Values are means  $\pm$  standard errors of 6 samples per cell where samples from predawn and noon were pooled

**Table 3** Percentage use of two water sources by different mangrove species at each site based on analysis of oxygen isotopes (Phillips & Gregg, 2001)

	Groundwater	Porewater
Dry scrub		
<i>C. australis</i>	17 $\pm$ 4	83 $\pm$ 4
<i>R. stylosa</i>	26 $\pm$ 4	74 $\pm$ 4
Wet scrub		
<i>C. australis</i>	45 $\pm$ 4	55 $\pm$ 4
<i>R. stylosa</i>	47 $\pm$ 6	53 $\pm$ 6
<i>L. racemosa</i>	30 $\pm$ 3	67 $\pm$ 3

Values are means  $\pm$  standard errors of 3 samples per cell collected at noon

in co-occurring tree species in estuarine vegetation (Wei et al., 2012; Lambs et al., 2016). Additionally, stem water composition has been shown to vary with experimental manipulation of water sources (Reef et al., 2015). Here we show that *R. stylosa* had different stem water isotopic compositions than two other co-occurring species, which was consistent with

hypothesized uptake of groundwater, possibly facilitated by the morphology of the root system of *R. stylosa* and its capacity to tolerate anoxic conditions and therefore deploy roots deep in the soil profile. Our isotopic data (Fig. 3) and the isotope mixing model results (Tables 2, 3) indicated that *R. stylosa* stem water comprised a greater proportion of groundwater

than other species, although variability in proportional sources estimated by the mixing models was high, particularly when three sources were used in the models, which reduced confidence in the mixing model analyses.

Access to groundwater in this species was also consistent with its growth responses to fertilization, where growth of *R. stylosa* was enhanced with fertilization with P but not N, which is abundant in the groundwater (79  $\mu\text{M}$  total N; N:P ratio of 851) but low in the tidal water (total N is  $\sim 15 \mu\text{M}$ , Davies & Eyre, 2005). While stem water of *C. australis* and *L. racemosa* generally comprised a lower proportion of groundwater than *R. stylosa*, and they were N limited in both sites, there was evidence that they also accessed groundwater in the Wet Scrub site, particularly in the analysis of noon samples where only groundwater and porewater were considered as sources. Our results suggest that groundwater may have a role in the nutrition of trees, but alternative hypotheses, that *R. stylosa* has access to alternative sources of N (other than groundwater), or that the anoxic soil conditions limits root nutrient uptake (McKee et al., 2002) in *C. australis* and *L. racemosa* more than in *R. stylosa*, require further investigation.

Elevation in the intertidal influences the frequency and intensity of tidal inundation and also depth of the groundwater table. We anticipated that at the more elevated Dry Scrub site, plants would have comparatively reduced access to groundwater due to increased depth of the groundwater compared to the lower elevation Wet Scrub site, although depth to groundwater varies with tidal cycles and seasons (Michael et al., 2005; Santos et al., 2012). The significant difference in isotopic composition of stem water between the two sites, with the Wet Scrub having more negative values (Fig. 3), and the high sensitivity of *R. stylosa* to P fertilization, but not N fertilization in the Wet Scrub compared to responses in the Dry Scrub (Fig. 4), indicate that groundwater was less available to plants at the elevated Dry Scrub site. Variation in elevation and rooting depth has been linked to differing access to water sources in freshwater wetland ecosystems (Saha et al., 2010), other Australian vegetation growing on sandy soils (Groom, 2004) and with salt water intrusion in coastal vegetation (Zhai et al., 2016). Thus the proximity of groundwater to the soil surface may be an additional

factor contributing to variation in the patterns of growth in some mangrove species over the intertidal zone.

Uptake of atmospheric water occurs in many plant species, and can be important for sustaining photosynthetic carbon gain in water-limited environments (Slayter, 1956; Munné-Bosch & Alegre, 1999; Martin & von Willert, 2000). In our study, there were high levels of uncertainty about the modelled proportion of atmospheric water in plant stems and thus we found no clear evidence to support the hypothesis that mangroves utilize dew. However, low levels of replication, high levels of spatial variation among sampled branches within trees and equilibrium of stem water with other water pools within the plant, reduced our ability to detect uptake of dew in this experiment. More detailed anatomical studies with water tracers will aid in investigating potential pathways of water uptake in the canopy and for establishing the importance of atmospheric sources of water to mangroves.

## Conclusion

Partitioning of resources is one of the most important processes giving rise to the stable co-existence of species within plant communities (Silvertown, 2004; Fargione & Tilman, 2005). Our study indicates that although mostly soil porewater was accessed by mangrove trees, differential access to groundwater by species could contribute to the maintenance of productivity and diversity in some mangrove habitats. Reductions in groundwater availability, e.g. due to human extraction or altered hydrology, sea level rise and salt water intrusion, or changes in the frequency of cyclones and atmospheric moisture levels with climate change are likely to influence the productivity and diversity of mangrove forests.

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**Author contribution** All authors conceived, designed and performed the experiments. CEL and RR analysed the data. CEL wrote the manuscript; RR and MCB edited the manuscript.

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