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ISOTOPIC FRACTIONATION DURING CELLULOSE SYNTHESIS IN TWO MANGROVE SPECIES: SALINITY EFFECTS

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Key Word Index—*Avicennia germinans*; *Rhizophora mangle*; stable isotope ratio; mangroves; biological recorder; sea level rise; salinity; cellulose.

Abstract—Carbon, non-exchangeable hydrogen, and oxygen isotope ratios of cellulose of *Avicennia germinans* and *Rhizophora mangle* plants hydroponically grown under different salinities (0, 18, 45% sea water, but with irrigation waters having the same isotopic ratios) were measured to determine the possibility of using isotopic ratios of plant tissues as biological recorders of sea level rise. There was a large variability in the δD values of leaf nitrated cellulose between different treatments and even within a single treatment for both *A. germinans* and *R. mangle*. Thus, δD values of non-exchangeable hydrogens of cellulose cannot be used as a historical tracer for utilization of ocean water or freshwater by mangroves. In contrast, δ^{18}O values of cellulose were not significantly different between different salinity treatments for both mangroves, indicating that δ^{18}O of cellulose can be used as a sea water tracer. δ^{13}C values of cellulose did not vary directly with salinity as has been observed with other plants. δ^{13}C values of cellulose from *A. germinans* were the lowest for plants growing at 18% sea water, with cellulose from plants growing in 0 and 45% sea water having significantly higher δ^{13}C values. δ^{13}C values of cellulose from *R. mangle* were the highest for plants grown in 45% sea water, with plants grown in 0 and 18% sea water having equally lower δ^{13}C values.

INTRODUCTION

The mangrove swamp is an association of halophytic trees and shrubs growing in tidal waters of tropical and subtropical coastlines [1–3]. Mangroves can tolerate a large range of salinities, and previous studies have shown that water uptake by mangroves can range from highly saline to freshwater [4–7]. Because of their ability to use water with different salinities and their possession of a woody trunk with several years of growth increments, mangroves may be ideal biological recorders of sea level rise.

Potential climatic recorders are the carbon, hydrogen and oxygen stable isotopic composition of plant biomass. Several researchers have shown that salinity can affect the discrimination against ^{13}C in plants during photosynthesis [8–11]. However, with mangroves these changes have not been as clear cut [12]. Other studies have demonstrated that hydrogen and oxygen isotope ratios of plant cellulose can be used as climatic markers [13–15]. Sternberg and Swart [6] and Sternberg *et al.* [16] demonstrated that freshwater and ocean water have different hydrogen and oxygen isotopic composition. They proposed that this difference in isotopic composition, if incorporated into cellulose of mangroves, may be the basis of examining sea level rise, using mangroves as biological recorders. Before this technique can be used, however, physiological and biochemical effects on isotopic incorporation into leaf and stem cellulose, associated with saline conditions, must be understood. To determine these effects, we analysed the carbon, hydrogen and oxygen isotopic characteristics, as well as the growth for two commonly found neotropic mangrove species *A. germinans* and *R. mangle*. These plants were grown under different salinities with source waters having the same isotopic composition. Thus, any observed differences in isotopic composition in the biomass of these plants have to be attributed to the effect of their physiology and/or biochemistry on isotopic fractionation associated with different salinity treatments. If these differences are significant for different treatments, then it is questionable whether isotopic analysis of that particular element can be used as a tracer for sea water or freshwater utilization by mangroves.

RESULTS

δD and δ^{18}O values of the nutrition solution during the experimental period were not significantly different between different treatments. The mean δD values of the nutrition solutions of 0, 18 and 45% sea water were $+6.6 \pm 0.12$‰ and $+2.3 \pm 0.07$‰ for *A. germinans* and *R. mangle*, respectively. The mean δ^{18}O values of the nutrition solutions were $+0.2 \pm 0.2$‰ and $+0.3 \pm 0.12$‰ for *A. germinans* and *R. mangle*, respectively.

δD values of nitrated cellulose of stems and leaves of *A. germinans* grown in 0, 18 and 45% sea water are shown in Fig. 1A. δD values of nitrated cellulose of leaves decreased with increasing salinity. This pattern was not observed in stem nitrated cellulose. δD values of stem nitrated cellulose were significantly higher than those of the leaves. δD values of nitrated cellulose of leaves of *R. mangle* grown in different salinities also varied with different salinity treatments (Fig. 2A). Plants grown in 18% sea water solution had nitrated cellulose with δD values significantly lower than those grown in 0 and 45% sea water solutions.

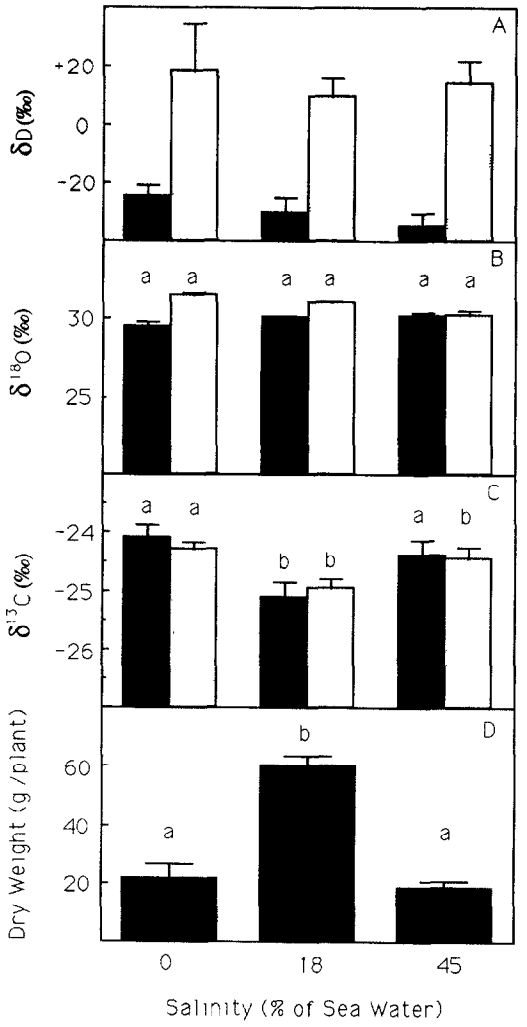

Fig. 1. Salinity effects on isotope ratios and biomass of *A. germinans*. (A) Hydrogen, (B) oxygen, (C) carbon and (D) biomass produced during the experimental period. Leaf values are represented by solid bars, stem values by stippled bars. Means of oxygen, carbon and biomass represent four, four and six replications, respectively. Bars for different treatments of the same tissue with different letters represent significant difference between treatments ($P < 0.05$). No statistical test was done for hydrogen since only two replicates of each treatment were analysed.

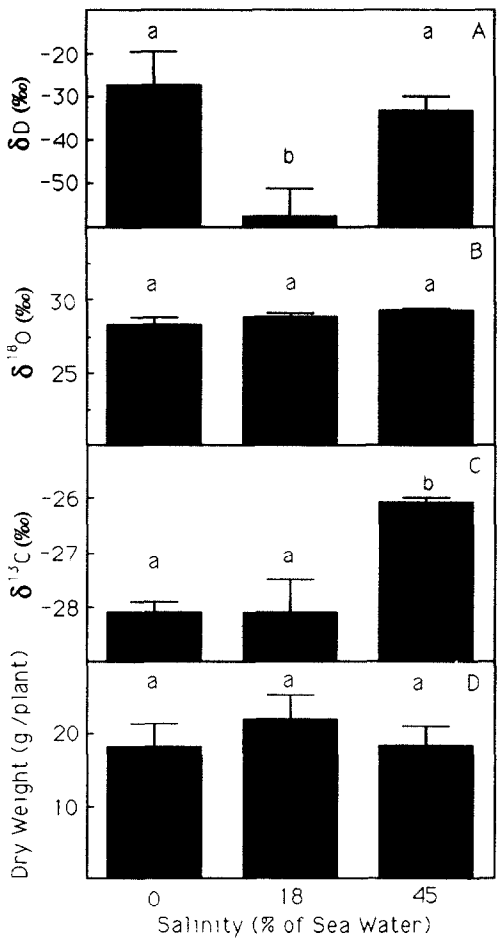

Fig. 2. Salinity effects on isotope ratios and biomass of *R. mangle*. (A) Hydrogen, (B) oxygen, (C) carbon and (D) biomass produced during the experimental period. Leaf values are represented by solid bars. Each mean represents six replicates, except for four and five replicates for oxygen of 0 and 45% sea water, respectively. Bars for different treatments of same tissue with different letters represent significant difference between treatments ($P < 0.05$).

$\delta^{18}O$ values of cellulose of stems and leaves of *A. germinans* are shown in Fig. 1B. There were no differences in $\delta^{18}O$ values of stem and leaves between the salinity treatments. For plants grown at 0 and 18% sea water, $\delta^{18}O$ values of stem were higher than those of leaves. Similarly, no significant differences were observed in $\delta^{18}O$ values of leaf cellulose between treatments for *R. mangle* (Fig. 2B).

However, there were differences in $\delta^{13}C$ values of stem and leaves between treatments (Fig. 1C), with $\delta^{13}C$ values of leaves and stem of plants grown at 18% sea water having significantly lower values relative to those grown at 0 and 45% sea water. This pattern is the inverse of the pattern of biomass produced under each treatment (Fig. 1D), with plants at 18% sea water having the highest productivity relative to those grown in 0 and 45% sea water solutions. There were no differences in $\delta^{13}C$ values between cellulose of leaves and stems of *A. germinans* grown at each treatment (Fig. 1C). $\delta^{13}C$ values of leaf cellulose from *R. mangle* grown at different salinities were the highest at 45% sea water relative to the values for *R. mangle* grown at 0 and 18% sea water (Fig. 2C). There was no clear correlation between $\delta^{13}C$ values of leaf cellulose and biomass productivity at different salinities for *R. mangle* (Fig. 2D).

DISCUSSION

The hydrogen isotopic composition of cellulose of plants is ultimately determined by the hydrogen isotopic composition of water available for its synthesis, which for terrestrial plants is leaf water. The hydrogen isotopic composition of cellulose also seems to vary in relation to the biochemistry of the plant [17]. This variability is most

marked in relation to the presence or absence of CAM in plants, but even within C_3 plants there seems to be a large amount of variation, probably due to metabolic peculiarities of the plant [17]. It has been previously observed that during cellulose synthesis there is a large percentage of carbon-bound hydrogens exchanging with water during enzyme-mediated reactions, which causes an overall enrichment in the carbon bound hydrogens relative to the water where synthesis occurred (about +150‰ relative to the δD of the water available during cellulose synthesis) [18, 19]. Accordingly, we expected that given certain environmental stresses which are sufficient to cause metabolic changes in the plant (such as high salinity), there should be an effect on the hydrogen isotopic composition of its metabolites, including cellulose. Our results are consistent with this expectation; there were differences in the δD values of nitrated cellulose of leaves of plants subjected to different salinity treatments (Figs 1A, 2B). Further, this difference in δD values was due to metabolic effects, since the oxygen isotopic composition did not differ to a great extent. Another expectation was that stem cellulose may be more enriched than leaf cellulose. Leaf cellulose may be synthesized directly from photosynthetic products whereas the pathway of synthesis of stem cellulose is more complex and involves synthesis of sucrose and translocation. Thus, exchange between carbon-bound hydrogens and water, with its concomitant enrichment during cellulose synthesis, may occur to a greater extent during synthesis of stem cellulose. Again, our expectation was fulfilled; *A. germinans* stem cellulose was significantly more enriched relative to leaf cellulose.

Like hydrogen, the oxygen isotopic composition of cellulose of terrestrial plants is determined by leaf water [20]. δ^{18}O values of plant cellulose seem to be insensitive to the photosynthetic mode used by the plant, and average about 27‰ higher than the water available for synthesis [21]. It is thought that water labels carbohydrates during cellulose synthesis when carbonyl oxygens undergo a reversible hydration [22, 23]. Cellulose synthesized from different carbohydrates will have a variable amount of oxygen exchanging with and labelled by water [18, 19, 22]. For example, cellulose synthesized from starch will have as much as 40% of its oxygen exchanging with water, and cellulose synthesized from glycerol may have as much as 70% of its oxygen exchanging with water [19, 22]. Equilibration of carbon-bound oxygen occurs rapidly, and thus it is thought that δ^{18}O values of cellulose reflect complete equilibration of metabolic oxygen with water. Accordingly, we expected that differences in salinity should not have any effect in the isotopic ratios of leaf cellulose. This expectation was fulfilled in that neither *A. germinans* nor *R. mangle* showed any significant difference in δ^{18}O values of cellulose between different salinity treatments. We also expected that δ^{18}O values of leaf cellulose should be higher than those of stem cellulose. The reason being that isotopically enriched leaf water will label all the carbohydrates synthesized, including those destined for cellulose synthesis. Sucrose transported to the stem from the leaves however may re-exchange 40% of its oxygen with stem water, which is not as enriched as leaf water, during cellulose synthesis. In this case our expectation was not fulfilled, and δ^{18}O values of stem cellulose of *A. germinans* were in some cases higher than those of leaf cellulose. Further studies are necessary to determine the reason for this discrepancy. One possible explanation is that leaf cellulose is synthesized during the night where leaf water which is not undergoing transpiration, shows no isotopic enrichment relative to stem water.

Carbon isotopic variation in plant matter is largely determined by the photosynthetic mode of the plant, with C_3 plants having lower δ^{13}C values, averaging −27‰, while C_4 and CAM plants have δ^{13}C values averaging about −13‰ [24]. There is also a variability of carbon isotope ratios in C_3 plants, and an important factor determining this variability is the CO_2 concentration of the internal leaf space (C_i) [25]. Lower C_i will decrease discrimination against ^{13}C and thus increase the δ^{13}C values of photosynthates. The internal CO_2 concentration in C_3 leaves is largely determined by the stomatal conductance to CO_2. Thus, environmental factors which decrease stomatal conductance will decrease the internal CO_2 concentration, and thus decrease discrimination against ^{13}C during photosynthesis. As a result, plants exposed to such environmental factors will have biomass with an overall higher δ^{13}C value. An environmental factor relevant to this study is the increase in salinity. Several workers have reported that increase in salinity decreases stomatal conductance and thus increasing the δ^{13}C value of photosynthates [8–11]. With mangroves, however, the results were somewhat unclear, δ^{13}C values of mangroves seemed to decrease with higher salinity [12]. Our results on carbon isotopic composition of *A. germinans* and *R. mangle* may explain this inconsistency. The relationship between salinity and carbon isotopic discrimination for *A. germinans* is not a simple linear relation, but rather curvilinear with plants having the lowest δ^{13}C value at 18% sea water (Fig. 1C). Closely related to stomatal conductance are the increase in productivity of plants and the lowering of water-use efficiency in plants with higher stomatal conductance. The carbon isotopic composition of plant tissue of *A. germinans* is consistent with these trends: plants having the highest productivity also had the lowest carbon isotopic composition (Fig. 1D). The pattern of δ^{13}C values for *R. mangle* was different, with plants grown at 0 and 18% sea water showing no difference in carbon isotopic composition, and those at 45% sea water showing a significant increase. Thus, for *R. mangle*, stomatal limitation to photosynthesis may increase only at salinities above 18% sea water.

CONCLUSIONS

Our isotopic analysis indicates that stable isotopes may have potential as indicators for sea level rise. However, the relationship between plant isotopic composition and salinity is complex, and thus careful analysis is necessary before proper interpretation is accomplished. There are species-specific physiological and biochemical effects on the δD values of nitrated cellulose, which may mask the input of deuterium-enriched saline water. On the other hand, δ^{18}O values of cellulose are not affected by salinity, and thus may faithfully record isotopic changes in source water associated with sea water intrusion. The relationship between δ^{13}C values of cellulose from mangroves and salinity is not a simple linear relation. For *A. germinans*, an increase in δ^{13}C values of leaf or stem tissue cannot necessarily be interpreted as an increase in the salinity of the available water, since it can also be caused by decrease in the salinity. For *R. mangle*,

an increase in $\delta^{13}C$ values of leaf appears only at 45% sea water, reflecting the low sensitivity of the species to salinities below 45%.

EXPERIMENTAL

Greenhouse studies. Seedlings of *A. germinans* were collected from trees growing at Matheson Hammock Park (Dade County), along the coast of the Atlantic Ocean, Florida, U.S.A. Propagules of *R. mangle* were collected at Manatee Bay (Monroe County), Florida, U.S.A. Seedlings and propagules were cultivated in vermiculite beds and irrigated with tap water for 6 months. Seedlings and propagules of similar dimensions and fr wts were then selected from the population, and divided into 5 groups of 6 The seedlings were placed in foam containers (27 × 43 × 30 cm) for hydroponic culture. Each box contained a gravel layer 8 cm deep, 15–18 l of nutrient soln, and 6 seedlings. Seedlings were held in spongy corks in holes which were made in the cover of the box, with their roots immersed in the nutrient soln. Air was pumped into the boxes, using aquarium pumps. Nutrient soln for mangrove was used as in Boto *et al.* [26], and supplemented with the appropriate concn of dry sea salt to maintain constant salinity. The salinities were adjusted at a rate of 9% of sea water/day to give the 5 final concentrations of 0, 18, 45, 90 and 135% sea water. Water level was maintained by adding tap water every other day, and solns were changed biweekly. The plants were grown for 9 months after transplanting, before isotopic ratios and biomass were measured, so that isotopic ratio was studied on tissues which had developed fully under the different salinity regimes. When grown at 90 and 135% sea water, plants of both species died or did not produce sufficient new biomass for isotope ratio analysis. Therefore, isotope ratios were analysed only for plants grown in 0, 18 and 45% sea water.

Isotopic analysis. The nutrient soln was sampled for hydrogen and oxygen isotopic ratio biweekly, just before soln replacement. At the end of the experiment, leaves, stems and roots were separated, oven-dried (80° for 48 hr), and weighed. Midribs were removed from the leaves, and tissues were ground using a Wiley mill. Cellulose was extracted and nitrated as described previously [17]. Oxygen isotope ratios of cellulose, and hydrogen and carbon isotope ratios of nitrated cellulose were determined as in previous studies [17] All isotopic analyses were done in a VG PRISM mass spectrometer. Isotope ratio is expressed in δ units using PDB standard for carbon, and SMOW for hydrogen and oxygen.

Statistical analysis. Statistical tests were conducted using the Statistical Analysis System [27]. Differences between treatments were tested with Duncan's multiple range test (ANOVA).

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