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Carbon and nitrogen stable isotopic variation in *Laguncularia racemosa* (L.) (white mangrove) from Florida and Belize: implications for trophic level studies

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Abstract

Carbon and nitrogen stable isotopic data from the primary producers in mangrove ecosystems are needed to investigate trophic links and biogeochemical cycling. Compared with other mangrove species (e.g. *Rhizophora mangle*) very few measurements have been conducted on the white mangrove, *Laguncularia racemosa*. The carbon and nitrogen stable isotopic and elemental compositions of *L. racemosa* were analyzed and compared from Florida and Belize. δ^{13}C values of *L. racemosa* from Florida (mean = –26.4‰) were slightly higher than those from Twin Cays, Belize (mean = -27.4‰), which may be due to higher salinity in some parts of the Florida site. There was no difference between the δ^{15}N values from *L. racemosa* from these two sites (Florida mean = 0.6‰; Belize mean = 0.3‰), which are indicative of nitrogen derived from nitrogen fixation in a planktonic marine system. However, higher δ^{15}N values from *L. racemosa* at Man of War Cay in Belize (11.4‰ and 12.3‰), which is fertilized by roosting marine birds (∼14.0‰), illustrate that *L. racemosa* can sensitively reflect alternative nitrogen sources. Although the isotopic data could not distinguish between *Avicennia germinans, R. mangle* and *L. racemosa* in Belize the *L. racemosa* had considerably higher C/N ratios (46.5 – 116.1) compared with the Florida samples (42.2 – 76.0) or the other mangrove species. Unlike some previous findings from *R. mangle*, substrate characteristics (e.g. salinity, NH_4^+, and H_2S) were not related to the isotopic or elemental composition of *L. racemosa*. δ^{13}C, δ^{15}N and C/N were analyzed for ecosystem components from *L. racemosa* habitats at Twin Cays, including other plants (e.g. *R. mangle*, *A. germinans* and seagrass), detritus, microbial mats and sediments. Results from mass-balance calculations show that mangrove detritus composes very little of the sediment, which is principally composed of microbial biomass (80 – 90%). Detritus at some sites is also influenced by sources other than that from *L. racemosa*, including seagrass leaves.

Introduction

Laguncularia racemosa (L.) (white mangrove[1]) occupies a narrow ecological niche in the mangrove ecosystems of North and South America and West Africa. With the exception of sites with relatively low salinity (Flores-Verdugo et al., 1987) *L. racemosa* is seldom the dominant tree species in mangrove ecosystems (Davis, 1940) and is generally found on elevated soils where tidal inundation is infrequent (Thom, 1967;

[1] Not the white mangrove *Avicennia marina* (Forrsk.) Vierh found in South Africa (Lambert et al., 1989)

Lugo & Snedaker, 1974). The species prefer salt concentrations averaging 15 – 20 parts per thousand (ppt) but can tolerate a broad range in salinity (0 to 90 ppt) (Jimenez, 1985). *L. racemosa* secretes salt and is often reduced in stature at high salinity (>50 ppt) (Jimenez, 1985). At the locations discussed in this paper, namely Belize and Florida, *L. racemosa* is not the dominant species and inhabits transition zones between different stand structures of *Rhizophora mangle* L. (red mangrove) in Belize (Feller & McKee, 1999) and contributes to a species gradient from *R. mangle* to *Avicennia germinans* (L.) Stearn (black mangrove) in some parts of Florida (Davis, 1940; Feller et al., in press).

Biomass from *L. racemosa* supports a variety of communities, including microbes, which are fueled by organic root exudates (Alongi et al., 1993), herbivorous invertebrates (Stowe, 1995) and marine zoosporic fungi (Fell et al., 1980; Newell et al., 1987). Nitrogen and phosphorus availability influences primary production and growth in mangroves (Clough, 1992). Nitrogen-fixing bacteria associated with the roots of *L. racemosa* such as the heterotroph *Bacillus licheniformis* and *Pseudomonas stutzeri*, are responsible for solubilizing insoluble forms of phosphate (such as calcium phosphate) in addition to nitrogen fixation (Krotzky & Werner, 1987; Alongi et al., 1992, 1993; Vazquez et al., 2000). Since *L. racemosa* bark and leaves are composed of between 10 and 24% soluble tannins (Walsh, 1977) and tannins may inhibit microbial activity, which in turn can decrease rates of herbivory (Bohlolli et al., 1977; Gonzalez-Farias & Mee, 1988; McKee, 1995), increased input of *L. racemosa* detritus may decrease detrital degradation (Gonzalez-Farias & Mee, 1988) with subsequent export of organic matter from *L. racemosa* into the ocean (Flores-Verdugo et al., 1987). *L. racemosa* with their associated micro-organisms can therefore alter their geochemical environment.

Analyses of the stable carbon and nitrogen isotopic composition of ecosystem components (i.e. leaves, detritus and sediments) can be used to asses the interplay of physical and chemical environmental parameters and their relationship to the growth of *L. racemosa*. Plants receiving nitrogen via atmospheric (N) fixation by microbes can be distinguished from those using combined nitrogen sources (e.g. NO_3 or NH_4^+). Unlike other mangrove species, such as *Rhizophora mangle* (Flemming et al., 1990; Lin et al, 1991; Lin & Sternberg, 1992b, 2c, 1994; Cifuentes et al., 1996; Fry et al., 2000), there have been very few isotopic measurements on *L. racemosa* (Lin & Sternberg, 1992a).

The aim of this study was to measure the carbon and nitrogen stable isotopic composition of *L. racemosa* from Florida and Belize. This study also aimed to investigate whether environmental parameters known to influence the carbon and nitrogen isotopic composition of other plants influenced the variation in *L. racemosa*, including salinity (Ish-Sholom-Gordon et al., 1992; Lin & Sternberg, 1992c) and soil nitrogen characteristics (e.g. ammonium concentration, nitrogen fixation and anthropogenic nitrogen) (Fry et al., 2000; McKee et al., in press). At sites with *L. racemosa* the degree to which mangrove detritus and sediment were influenced by contributions from white mangroves was examined. It was unknown whether detritus derived from *L. racemosa* is an important contributor to higher trophic levels. Our study in both tidal and salt water settings provides a base-line survey of isotopic variation in *L. racemosa*, supplying information for researchers concerned with the eco-physiology of this species.

Study sites

Florida

The study site in Florida is located at Mosquito Impoundment (MI) 23, in the Avalon State Recreation Area on the lagoonal side of Hutchinson Island, St. Lucie Co, Florida and has been described by Feller et al. (in press). Maintained between 1966 and 1974, MI 23 is now derelict and is overgrown with mangroves. As a MI the site was periodically flooded with seawater from the lagoon, but now the site receives water primarily from rainfall. MI 23 is characterized by a distinctive gradient in tree height. The interior of the site is dominated by dwarf (∼1 – 2 m) *Avicennia germinans*, which are interspersed with *L. racemosa*. *R. mangle*, which is restricted to the fringe zone, is often the tallest tree at the site (∼4 – 5 m). *L. racemosa* is located in the interior of the impoundment, primarily in the transition zone between the site's interior and the fringe. The sedimentary substrate here is composed mainly of sand and shell fragments.

Belize

Fieldwork in Belize, Central America was principally conducted at Twin Cays (16° 50′ N, 88° 06′ W), a peat based, 92-ha archipelago of mangrove islands inside

the crest of a barrier reef 12 km off the shore of Belize. The vegetation is dominated by *R. mangle*. A tree height gradient exists that includes a narrow seaward fringe of tall (5 – 6 m) *R. mangle* trees, which occur mainly in the low intertidal zone around the islands' periphery. Tree height decreases rapidly towards the island's interior through a transition zone (2 – 4 m tall). *L. racemosa* is primarily located at scattered locations in the transition zone where it is mixed with *R. mangle* and *A. germinans* (Koltes et al., 1998). The interior of the island is dominated by stands of dwarf (∼1 – 1.5 m) *R. mangle*. A wet season occurs from July to October, and average rainfall is 218 cm yr^{-1} (Rutzler & Ferraris, 1982). Further information and description of the island has been provided by Feller et al. (1999) and McKee et al. (in press). Samples were also collected from Man of War Cay, which is ∼200 m in diameter and ∼5 km NE from Twin Cays, in the same chain of barrier islands. Man of War Cay, a seabird colony for over 25 years, is inhabited by Frigate birds and Blue-Footed Boobies, which roost in the mangrove trees on the island. All three mangrove tree species are represented on the island and are considerably taller (up to ∼20 m high) than those at Twin Cays.

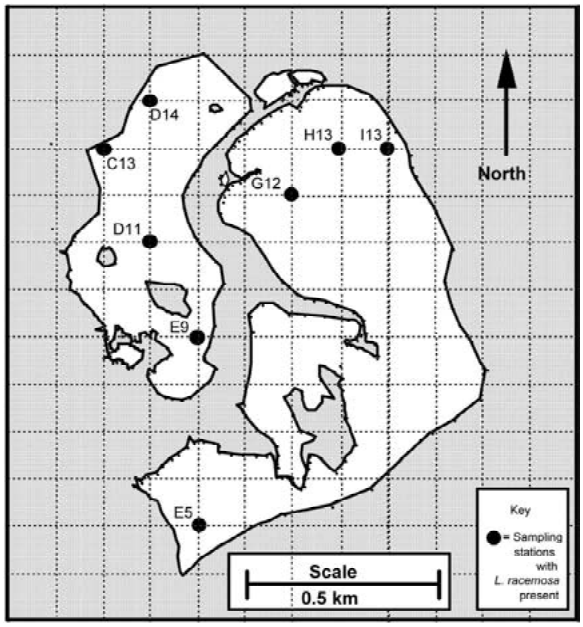

Figure 1. The location of sampling stations on Twin Cays, Belize.

Methods

Field collection of L. racemosa from Florida

A full-expanded, canopy leaf was removed from fourteen *L. racemosa* trees at MI 23, Florida, during January 2000. The leaves were kept refrigerated from the field to the Geophysical Laboratory, Carnegie Institution of Washington (CIW), where they were freeze dried. Pore water from a depth of 10 cm was removed from beneath 10 *L. racemosa* trees that had been sampled for stable isotope analyses. Salinity of the pore water samples was measured using a hand-held refractometer (Vista A366ATC).

Field collection of samples from Belize

A grid of ∼50 stations covering Twin Cays was established using a geographical positioning system (Garmin GPS 12 XL) (Fig. 1 and Table 1) during October and November 2000. *L. racemosa* was located at 8 of these stations, (Fig. 1 and Table 1). A fully expanded canopy leaf was taken from a *L. racemosa* and other primary producers (e.g. *R. mangle* and *A. germinans*) at each station, along with a sample of the surface sediment and the detritus. The results from the analyses of the other primary producers are being reported separately. Detritus was defined as the dead fraction of organic matter (with attached bacterial/microbial community). Detritus was collected from the sediment/water interface. Typically this fraction contained the finely divided remains of mangrove biomass, rotted seagrass, or decaying algal material depending on the location. This detritus fraction also included the microbial population responsible for its degradation.

Without landing at Man of War Cay, so as not to disturb the nesting seabirds, leaves were collected from two *L. racemosa*, four *R. mangle* and two *A. germinans* trees situated at the island's edge. A sample of *Ulva maritima* and a Frigate bird's feather were also collected. A surface sediment sample from the island was taken by pushing, from the boat, a 3 m-long piece of PVC pipe with a 3 cm internal diameter into the sediment.

Detritus and leaf samples were dried at 50 °C under N_2 at the Smithsonian Marine Station, Carrie Bow Cay, Belize (∼2 km from Twin Cays). Samples were then packaged and transported to the Geophysical Laboratory for stable isotope analyses. Sediment samples were stored on ice in Belize, transported, and stored at 4 °C prior to analysis.

Table 1. Descriptions of sites with *L. racemosa* present at Twin Cays, Belize

Site code	Latitude, Longitude	% composition *R. mangle*	*A. germinans L. racemosa*	Transition,	Fringe, notes Dwarf	Site
C13	16° 50′ 0.0, 88° 6′ 26.5	30%	10%	60%	Fringe (Marine)	*Thalassia* leaves present covering the substrate. Dead *R. mangle* also present.
D11	16 °49′ 51.9, 88° 6′ 22.4	40%	40%	<10%	Transition	Purselane present and *R. mangle* senescing in the area. Microbial mats present in the locality.
D14	16° 50′4.0, 88o 6′ 22.4	70%	30%	<10%	Transition	Purselane and dead *L. racemosa* present.
E5	16° 49′27.7, 88° 6′18.2	30%	40%	30%	Transition	Purselane and stumps of large *A. germinans* present.
E9	16° 49′43.8, 88° 6′ 18.2	80%	10%	<10%	Transition	Cyano-bacterial mats present
G12	16° 49′55.9, 88° 6′ 10.1	90%	0%	<10%	Transition	*Rhodopseudomonas. sphaeroidies* mat present.
H13	16° 50'0.0, 88° 6′5.9	80%	0%	10%	Dwarf (Pond)	*Rhodopseudomonas sphaeroidies* mat present.
I12	16° 49′55.9, 88° 6′1.8	30%	60%	<10%	Transition	*A. germinans* stumps present.

Measurements of pore water from Twin Cays, Belize

Water samples taken from the surface and from pore water at 5 cm and 10 cm depths in the substrate were collected at each of the stations where *L. racemosa* was present at Twin Cays. Each pore water sample was filtered through a pre-combusted GF/F 47 mm glass fiber filter. The filters were kept at ∼4 °C during transport to the CIW, where they were freeze-dried for stable isotope analyses. The salinity of each filtrate was measured at Carrie Bow Cay using a refractometer (Vista A366ATC), pH (Orion 250A pH meter), and alkalinity (Saarazin et al., 1999) were measured on site. The rest of the filtered and frozen or fixed (depending on the prescribed method) water samples were transported to the University of Southern California where concentrations of phosphate (after Presley, 1971), hydrogen sulfide (after Cline, 1969) and ammonium (after Solorzano, 1969) were determined.

Carbon and nitrogen stable isotope analyses

An aliquot (∼700 to 800 μg) of each freeze dried sample collected from Florida and Belize was weighed into a tin capsule, which was sealed and introduced via the EA carousel (Wooller et al., 2001) into the autosampler (A2100) of a CE Instruments, NA 2500 series, elemental analyzer (EA) at the Geophysical Laboratory, CIW. Within the EA, the sample was combusted with ultra pure oxygen at 1020 °C in a quartz oxidation column containing Chromium (III) Oxide and Silvered Cobalt (II,III) Oxide. The resulting gases, mixed with zero grade helium as the carrier gas, were passed through a quartz reduction column, containing copper reduced wire grains, held at 650 °C. Purified combustion gases (CO_2 and N_2) were separated in a molecular sieve gas chromatographic column prior to entering a Finnigan Conflo II interface. The Conflo II allowed the CO_2 to be diluted with helium gas and the subsequent analyses of the carbon and nitrogen isotopic composition from a single preparation. Isotope ratios of the combustion gases were analyzed using continuous-flow, stable isotope ratio mass spectrometry (Finnigan MAT, Delta^{plus}XL). The results are presented in standard notation:

$$\delta^h X = \left[\frac{\left(\frac{X^h}{X^l}\right) SAM}{\left(\frac{X^h}{X^l}\right) STD} - 1 \right] \times 1000$$

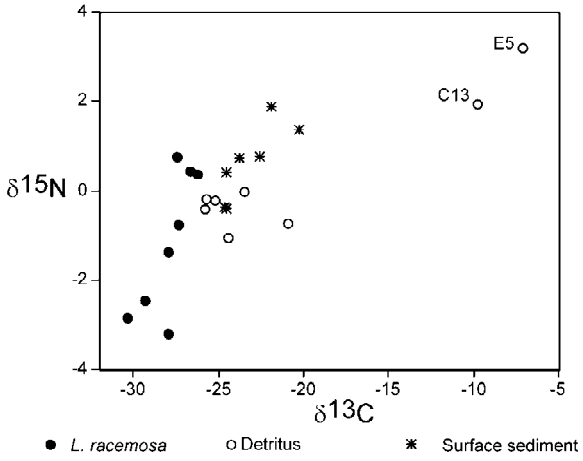

Figure 2. The δ^{13}C, δ^{15}N and C/N values of ecosystem components from *L. racemosa* habitats, Twin Cays, Belize.

where X is either carbon or nitrogen, h is the heavier isotope, l is the lighter isotope, SAM. is the sample, and STD is the standard. Both N_2 and CO_2 samples were analyzed relative to internal, working gas standards. Nitrogen stable isotope ratios (δ^{15}N) are expressed relative to air (δ^{15}N = 0.0‰); carbon isotope ratios (δ^{13}C) are expressed relative to Pee Dee Belemnite (δ^{13}C = 0.0‰). Acetanalide (C_8H_9NO) was analyzed as a check on the accuracy and precision of isotopic ratios and elemental compositions by the elemental analyzer. Precision for δ^{15}N was + 0.2‰(N% = ± 0.5) and for δ^{13}C was ± 0.27 (C% = ± 5.4).

Results

The stable isotopic (carbon and nitrogen) signature of L. racemosa

The δ^{13}C values of *L. racemosa* leaves from Florida (mean = –26.4) (Table 2) were higher than those from Belize (mean = –27.4) ($t\geq$ crit. at $p < 0.1$). There was also a significant difference in the C/N values between the Florida and Belize *L. racemosa* leaves ($t\geq$ crit. at $p < 0.01$), with the Belize samples having on average significantly higher C/N values than the Florida samples (Table 2). No significant difference between the two *L. racemosa* populations was found with respect to δ^{15}N. The δ^{13}C, δ^{15}N and C/N values of *L. racemosa* compared with the other two tree species, *R. mangle* and *A. germinans* were almost identical (Fig. 2). *L. racemosa,* however, shows by far the highest C/N values, compared with any component of the ecosystem (Fig. 2), reaching ∼116 at site D11.

The δ^{15}N values of leaves from Man of War Cay were elevated compared with those measured in leaves from Twin Cays or Florida. More positive δ^{15}N values were also seen in *R. mangle*, *A germinans*, *Ulva* sp., and surface sediment from Man of War Cay (Table 3) compared with those from Twin Cays (Fig. 2). The δ^{15}N values of *L. racemosa* leaves from Man of War Cay (11.4 and 12.3) were similar to the δ^{15}N values of a Frigate bird feather (δ^{15}N = 14.0) found at the site. The N% of *L. racemosa* leaves from Man of War Cay (Table 3) were also higher (∼ 0.5%) than those from either Florida or Twin Cays (Fig. 2 and Table 2).

Physical and chemical habitat characteristics and the stable isotopic signature of L. racemosa

Salinity of the pore water at 10 cm depth beneath *L. racemosa* at Florida ranged between 33 and 47 (Mean = 40, ± 4, n = 10), whereas that from Twin Cays ranged from 33 to 40 ppt (Table 2). A weak negative relationship ($t\geq$ crit. at $p < 0.05$, n = 10) was demonstrated between the salinity of pore water at 10 cm beneath *L. racemosa* trees and the δ^{15}N of the leaves from those trees at Florida. However, no significant relationships were demonstrated between any of the other variables derived from *L. racemosa* leaves (Table 2) and salinity. Moreover, no significant relationship was detected between any of the isotope and elemental data from *L. racemosa* and salinity from Twin Cays. Although the highest pore water salinity, at 10 cm, was measured in substrates from Florida (47 ppt), there was no significant difference between the Florida and Belize pore water salinity measured at 10 cm.

Alkalinity and pH at *L. racemosa* sites at Twin Cays ranged from a mean = 10.77 (micromoles), ± 4.7 and mean = 7.4, ± 0.3, respectively. Phosphate concentrations were often below detection levels at sites with *L. racemosa* present at Twin Cays. Phosphate was detected only at sites E5 (surface = 0.81 μM), E9 (10 cm depth = 0.24 μM) and G12 (surface = 0.38 μM). Hydrogen sulfide was generally below detection limits and only detected at three sites. At site D11 pore water H_2S was 42.8 μM at 10 cm depth; at station I12 pore water H_2S was 97.1 μM at 5 cm and rose to 103.0 μM at 10 cm. Hydrogen sulfide concentrations were highest at Site E5 in the surface water (420.72 μM). Ammonium concentrations (Fig. 3) ranged between 7.6 and 173.0 μM in pore water samples (5 and 10 cm depths) with the highest

Table 2. The stable isotope and elemental (C and N) data for *L. racemosa* sampled from Florida and Twin Cays, Belize (n.d. = data not determined)

Site	Site salinity (ppt)	δ^{15}N	δ^{13}C	%N	% C	C/N (atomic)	
FLORIDA MOSQUITO IMPOUNDMENT							
	n.d.	–2.1	–26.5	0.6	47.7	76.0	
	n.d.	–3.0	–25.7	0.7	47.0	67.9	
	n.d.	–2.4	–25.6	0.8	47.1	57.2	
	n.d.	–1.6	–26.2	0.7	45.3	69.0	
	n.d.	–3.1	–25.7	0.7	49.3	71.5	
	33	1.2	–25.0	0.8	41.6	52.7	
	35	1.0	–25.6	0.9	46.2	49.5	
	40	0.2	–28.9	1.7	49.9	29.4	
	40	0.3	–26.8	0.9	44.3	50.2	
	38	1.0	–25.5	0.8	41.6	45.3	
	41	–0.7	–27.2	1.1	44.6	42.2	
	42	–1.2	–26.1	0.7	47.8	67.7	
	44	0.5	–27.4	0.7	43.7	59.4	
	45	0.3	–29.0	1.0	48.5	46.4	
	47	–0.8	–25.3	1.0	49.0	51.1	
Mean (one sigma)	40 (±4)	–0.7 (±1.5)	–26.4 (±1.2)	0.9 (±0.3)	46.2 (±2.7)	55.7 (±12.9)	
TWIN CAYS, BELIZE							
C13	35	1.5 (0.5)	–27.0 (0.5)	1.2 (0.1)	46.8 (1.6)	46.5 (1.3)	(*n*=5)
D11	33	–3.0 (1.4)	–26.4 (0.6)	0.4 (0.0)	40.3 (0.9)	116.1 (7.3)	(*n*=5)
D14	40	–0.9 (0.6)	–28.6 (0.6)	1.0 (0.1)	44.6 (1.1)	51.2 (2.8)	(*n*=6)
E5	46	0.3 (1.9)	–27.5 (0.4)	0.4 (0.1)	36.0 (1.8)	112.6 (17.9)	(*n*=6)
E9	36	–1.3 (1.5)	–27.5 (1.1)	0.5 (0.1)	45.6 (0.9)	106.2 (17.6)	(*n*=6)
G12	33	–0.4 (1.7)	–27.4 (0.4)	0.8 (0.1)	43.8 (1.7)	62.8 (7.5)	(*n*=4)
H13	36	1.2 (1.1)	–25.8 (0.6)	0.8 (0.1)	45.7 (1.9)	68.1 (3.0)	(*n*=5)
I12	38	0.8 (1.0)	–26.8 (0.5)	0.8 (0.1)	46.6 (1.4)	71.2 (6.1)	(*n*=5)
Mean (one sigma)	37 (±4)	–0.2 (±1.5)	–27.4 (±1.2)	0.7 (±0.3)	43.7 (±3.7)	79.3 (±28.1)	

concentrations detected at sites E5 (107.38 μM at 10 cm) and G12 (173.03 μM at 5 cm). Sites C13 and D11 had no detectable ammonium in their pore water or surface water. In the surface water of all other sites ammonium concentrations ranged from 4.1 to 13.3 μM.

The stable isotopic (carbon and nitrogen) signature of ecosystem components in L. racemosa habitats

At Twin Cays the detritus and sediment at sites with *L. racemosa* were ^{13}C-enriched relative to fresh *L. racemosa* leaves from the same sites (Tables 2 and 4).

Table 3. The stable isotope and elemental (C and N) data for *L. racemosa* and samples taken from Man of War Cay, Belize

Sample type	δ^{15}N	δ^{13}C	%N	% C	C/N (atomic)
L. racemosa	11.4	–26.7	1.2	48.4	45.6
L. racemosa	12.3	–26.5	1.9	51.1	32.2
A. germinans (n = 2)	20.2	–27.3	2.0	52.3	31.7
	(±0.1)	(±0.7)	(±0.4)	(±0.1)	(±5.8)
R. mangle (n = 6)	9.1	–27.2	1.5	51.1	39.7
	(±1.3)	(±1.5)	(±0.2)	(±0.8)	(±5.2)
Frigate Bird feather	14.0	–13.0	17.0	52.3	3.6
Ulva	6.1	–12.2	1.6	36.3	25.8
Surface sediment	2.0	–23.0	0.8	34.7	51.9

Table 4. Carbon and nitrogen stable isotopic and elemental characteristics of components from sites at Twin Cays, (n.d. = data not determined)

Sample type	Site	δ^{15}N	δ^{13}C	%N	% C	C/N (atomic)
Surface sediment	C13	n.d.	n.d.	n.d.	n.d.	n.d.
	D11	0.4	–24.5	2.3	36.2	18.8
	D14	1.4	–20.2	1.7	29.4	20.3
	E9	–0.4	–24.6	1.6	30.9	23.0
	G12	–0.4	–24.6	1.3	33.3	30.6
	H13	0.7	–23.8	1.0	32.5	39.5
	E5	1.9	–21.9	1.9	32.7	20.3
	I12	0.8	–22.6	3.0	35.3	13.9
Mean		0.6	–23.2	1.8	32.9	23.8
(one sigma)		(±0.8)	(±1.7)	(±0.7)	(±2.4)	(±8.6)
Detritus	C13	3.2	–7.14	2.0	38.3	22.6
	D11	–0.2	–25.2	1.6	30.5	22.2
	D14	–1.1	–24.4	1.0	43.0	50.7
	E5	1.9	–9.8	1.1	20.2	22.0
	E9	–0.4	–25.9	1.7	27.8	18.6
	G12	–0.2	–25.8	1.5	28.2	21.8
	H13	–0.7	–21.0	1.7	17.8	13.1
	I12	–0.0	–23.5	2.3	28.0	14.4
Mean		0.3	–20.3	1.6	29.2	23.2
(one sigma)		(±1.5)	(±7.5)	(±0.4)	(±8.4)	(±11.7)
Purselane	D11	–0.2	–29.0	23.7	1.8	15.2
Purselane	E9	0.1	–27.6	21.9	2.0	13.1
Microbial mat	E9	–0.4	–22.6	32.7	3.1	12.2
Thalassia sp. (n = 3)		2.6	–7.3	38.2	2.9	15.4
		(±0.5)	(±1.8)	(±2.3)	(±0.5)	(±1.7)
Halimeda sp.		2.0	–13.1	23.8	1.9	14.6
Sargassum sp.		2.0	–14.1	31.7	1.5	25.5
Seagrass (n = 3)		1.13	–7.8	35.6	2.8	15.6
		(±2.5)	(±0.4)	(±1.8)	(±0.6)	(±4.1)

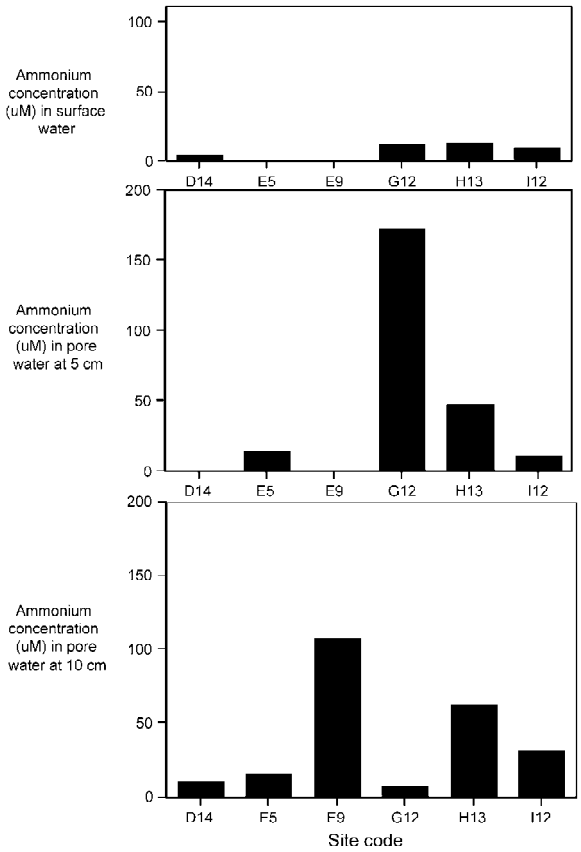

Figure 3. Ammonium concentration (μM) of peat pore water (5 cm and 10 cm depths) and surface water from *L. racemosa* habitats, Twin Cays, Belize.

With the exception of sites C13 and E5 the δ^{13}C of detritus was ~3‰greater than the *L. racemosa* leaves ($t\geq$ crit. at p <0.01) and the δ^{13}C of sediment was ~ 4‰ higher than *L. racemosa* leaves ($t\geq$ crit. at p <0.001). The δ^{13}C values of detritus at the sites C13 and E5 were considerably ^{13}C-enriched (δ^{13}C = –7.1‰and –9.8‰) relative to either the sediment or *L. racemosa* leaves and was isotopically similar to fresh *Thalassia* sp. sampled and measured from Twin Cays (Fig. 2). The remains of *Thalassia* sp. were observed at site C13 (Table 1).

Detritus from sites C13 and E5 was also enriched in ^{15}N (δ^{15}N = 3.2‰ and 1.9‰) relative to *L. racemosa* leaves (mean δ^{15}N = -0.2‰) and to the sediment (mean δ^{15}N = 0.6‰). Although these two stations show exceptionally different δ^{15}N values the surface sediment was generally more enriched in ^{15}N compared with fresh *L. racemosa* leaves at all stations with *L. racemosa* present. Detritus δ^{15}N values isotopically resembled a mixture of mangrove, Purselane, and microbial nitrogen. Given the δ^{15}N variability exhibited by seagrass the detritus nitrogen could also be from these sources. The sediment and detritus had significantly higher N% values (t>crit. at p <0.001) and significantly lower C% values (t>crit. at p <0.001) when compared with *L. racemosa* leaves.

The C/N values (Table 3 and Fig. 2) of the sediment and detritus are significantly lower when compared with fresh *L. racemosa* leaves (t>crit. at p <0.001). There was no significant difference between any of the isotopic or elemental data of sediment compared with detritus. With the exception of site D14 the detritus at the sites appeared similar to a number of other ecosystem components that were measured (Fig. 2), including the microbial mat, Purselane, seagrass, *Halimeda* sp. and *Thalassia* sp. D14 in contrast has a higher detrital C/N value compared with all other sites (Fig. 2).

Discussion

Carbon stable isotopic variation in plants is largely determined by the photosynthetic mode of plants. *L. racemosa* leaves measured from Twin Cays and Florida had δ^{13}C values indicative of a plant utilizing C_3 photosynthesis (averaging –27‰) (Lajtha & Marshall, 1994). The variability in δ^{13}C exhibited by C_3 plants is primarily determined by variations in the concentration of CO_2 of the internal leaf space (Farquar et al., 1982), primarily determined by the stomatal conductance to CO_2. A number of environmental factors can influence stomatal conductance, including salinity, humidity, soil moisture and temperature, which will subsequently influence stable isotope fractionation. The δ^{13}C values exhibited by *L. racemosa* from Florida and Belize are comparable to the average value for C_3 plants (–27‰) and would therefore seem to show that the *L. racemosa* studied are not experiencing physiological stress that would cause enrichment in ^{13}C. Unlike the findings from an investigation of *R. mangle* carried out by Lin & Sternberg (1992c), there was no correlation between the δ^{13}C of *L. racemosa* leaves and salinity values of pore water from either Florida or Belize (Table 2). Salinity values were relatively uniform across both sites and are less than 50 ppt; a level above which tends to stunt the growth of *L. racemosa* (Jimenez, 1985). The highest salinity values were recorded in Florida compared with Twin Cays, Belize, which may be responsible for the slightly higher δ^{13}C values in Florida, implying greater water

use efficiency needed to cope with higher salinity. At 2.5 – 3 m height in Florida and 3 – 4.5 m height in Belize, the δ^{13}C values fit the previously demonstrated pattern of variation for *L. racemosa* from Florida (Lin & Sternberg, 1992b), with a predicted value of ∼ –27.2‰ for a 4 m high tree. Compared with the values demonstrated for dwarf (scrub) (∼ –26.2‰) and fringe *L. racemosa* trees (∼ –27.2‰) (Lin & Sternberg, 1992b), the *L. racemosa* we examined from Florida (mean = –26.4‰) and Belize (–27.4‰) appear to be relatively water use efficient.

The δ^{15}N values from both Belize and Florida do not show the high values demonstrated for *R. mangle* from some sites in Florida (Fry et al., 2000), which are thought to be indicative of anthropogenic influence. Twin Cays is a remote island (∼12 km off shore) and the *L. racemosa* from this site did not show high δ^{15}N indicative of an anthropogenic nitrogen source. The *L. racemosa* were also isolated from direct tidal influence. In this respect they were similar to the *R. mangle* most distant from the shore line studied by Fry et al. (2000), which were found to have lower δ^{15}N values. The δ^{15}N values from *L. racemosa* are more indicative of nitrogen derived from nitrogen fixation in a planktonic marine system (Macko & Ostrum, 1994). Nitrogen fixation seems to supply the majority of combined nitrogen for the ecosystems at Twin Cays and Florida. Mangroves have, however, an ability to respond to other nitrogen sources, as evident from the relatively high δ^{15}N values from the leaves of mangroves from Man of War Cay, which are fertilized by guano and uric acid from roosting birds at the site. Dense microbial mats consisting of *Rhodopseudomonas sphaeroides* and *Rhodospirillum rubrum*, both nitrogen-fixing, photosynthetic microbes, were common constituents of surface sediments in Belize. It is also possible that microorganisms associated with *L. racemosa*, including *Pseudomonas stutzeri* (Vazquez et al., 2000), could also be responsible for the fixation of atmospheric nitrogen (Krotzky & Werner, 1987; Alongi et al., 1992, 1993).

The high C/N values in some *L. racemosa* leaves from Belize (such as E5, E9 and D11) do not appear to be related to limited nitrogen since at least two of these sites (E5 and E9) both have detectable levels of ammonium present in the surface and pore waters (Fig. 3).

Samples from Belize were used to examine the degree to which *L. racemosa* resembles other organic components of the ecosystem. Two of the sites with *L. racemosa* had detritus that was clearly derived from seagrasses. The most likely candidate is probably *Thalassia* sp., the presence of which was recorded at site C13 (Table 1) and resembles the δ^{13}C, δ^{15}N and C/N values of the detritus (Fig. 2). *L. racemosa* grows in transition zones, which often receive considerable quantities of marine detritus. It is obvious from our data and from observation that microbial and algal mats overprint a mangrove biogeochemical signature in surface sediments. The sediments may have been isotopically enriched in both ^{13}C and ^{15}N by receiving a slight proportion of algae or seagrass. *L. racemosa* does not have an isotopic or elemental composition completely distinct from *R. mangle* or *A. germinans*. One possible distinguishing trait, the C/N, was measured in the Belize leaves from Twin Cays. When fertilized by guano, however, C/N values in all three mangrove species declined. One of our goals was to determine the contribution of *L. racemosa* biomass to sedimentary organic matter. By mass balance using δ^{13}C and C/N data (from mangrove leaves, microbial mat, and sediments) mangrove derived carbon contributes only 10 – 20% to sedimentary organic C, whereas the microbial biomass is the overwhelming source (80 — 90%). The δ^{15}N of *L. racemosa*, microbes, sediments, and detritus were undistinguishable from one another, most likely because of a very tight biogeochemical cycle among these four pools of organic nitrogen.

In those parts of ecosystems where *L. racemosa* predominate (e.g. Flores-Verdugo et al., 1987; Chen & Twilley, 1999), fluxes of organic carbon to nearby estuaries should have terrestrial, photosynthetic isotopic signals. Nitrogen-exported from *L. racemosa* stands would be primarily microbial-fixed nitrogen from sedimentary bacteria. Food webs in offshore marine or estuarine environments that are influenced *L. racemosa* detritus should have δ^{13}C near the average value of –27‰. Because of the positive carbon isotopic shift in trophic transfer (Newman, 1991), herbivores supported completely by *L. racemosa* detritus should have δ^{13}C of up to –25.5‰. For nitrogen, the δ^{15}N values of *L. racemosa* from oligotrophic waters indicate incorporation of microbially fixed nitrogen. However, because the N content of some *L. racemosa* leaves is so low, animals would have to consume a dietary source with a higher N content. Therefore, influence of *L. racemosa* on nitrogen isotopic systematics in food webs adjacent to major growth stands seems unlikely.

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