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A taphonomic study of $\delta^{13}C$ and $\delta^{15}N$ values in *Rhizophora mangle* leaves for a multi-proxy approach to mangrove palaeoecology

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Abstract

The response of mangrove ecosystems to environmental change can be examined with stable isotopic tracers of C and N. The $\delta^{13}C$ and $\delta^{15}N$ of a taphonomic series of *Rhizophora mangle* L. (Red mangrove) leaves were analyzed from Twin Cays, Belize, to facilitate reconstruction of past mangrove ecosystems. On Twin Cays, fresh leaves of dwarf *R. mangle* trees (~0.5 m high) were found to have more negative $\delta^{15}N$ values (mean = −10‰) and more positive $\delta^{13}C$ values (mean = −25.3‰) compared to tall *R. mangle* trees (mean $\delta^{15}N = 0$‰, $\delta^{13}C = -28.3$‰). These isotopic differences can be related to nitrogen and phosphorus availability [Ecology 83 (2002) 1065]. We investigated three taphonomic stages in the fossilization of *R. mangle* leaves into peat with the following: (1) senescent leaves; (2) fallen leaves on the surface of the peat; and (3) sub-fossil leaves found within mangrove peat. In addition, by examining natural leaf assemblages we established that $\delta^{13}C$ and $\delta^{15}N$ of *R. mangle* leaves were not altered during senescence, despite a significant (50%) decrease in the N%. Modern dwarf and tall trees could still be identified from $\delta^{13}C$ and $\delta^{15}N$ analyses of the leaf assemblages found directly below a tree. Dwarf and tall trees could also be identified from $\delta^{13}C$ analyses of leaves that had decomposed for four months. Although dwarf and tall trees could not be statistically separated after four months according to $\delta^{15}N$ analyses, leaves with very negative $\delta^{15}N$ (−7‰) were only collected below dwarf trees. Leaf fragments were present in ~50 cm long cores of peat from four sites on the island, and their isotopic compositions were determined. The ranges of $\delta^{13}C$ (−29 to −22‰) and $\delta^{15}N$ (−11 to +2‰) values from sub-fossil leaves were similar to the ranges from modern leaves ($\delta^{13}C = -29$ to −23‰, $\delta^{15}N = -11$ to +1‰). The sub-fossil leaf isotopic compositions were independent values, in comparison to the uniform values of the surrounding peat. Because of the stability and persistence of the stable isotopic signals, they could contribute significantly to a multi-proxy approach to mangrove palaeoenvironmental reconstruction.

1. Introduction

Situated at the junction between terrestrial and marine ecosystems, mangrove habitats are particularly sensitive to alterations of sea-level (e.g. Blasco et al., 1996). The impact of future sea-level scenarios, related to anthropogenic global warming, on mangrove ecosystems continues to be debated (Woodroffe, 1988, 1990;

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Ellison and Stoddart, 1991; Parkinson et al., 1994; Swarzbach, 1999). Simulated sea-level changes profoundly influence the anatomy and physiology of mangroves (Ellison and Farnsworth, 1997). One way to understand future outcomes has been to examine how past mangrove ecosystems have reacted to environmental change (e.g. Suguio et al., 1988; Woodroffe, 1990; Martin and Suguio, 1992; Parkinson et al., 1994; Angulo and Suguio, 1995; Blasco et al., 1996; Angulo et al., 1999). Despite concerns over the turbation of pollen assemblages by tidal fluctuations, past mangrove dynamics can be reconstructed using palynological techniques (e.g. Spackman et al., 1966; Grindrod, 1985; Behling et al., 2001; Behling and Costa, 2001).

In many palaeoecological investigations, stable isotope data have successfully supplemented pollen data to resolve the reconstruction of past ecosystems (e.g. Cerling et al., 1997; Ficken et al., 2002; Wooller et al., 2003). We investigate the potential of using the stable isotopes of carbon and nitrogen in sub-fossil mangrove leaves as proxies of past mangrove eco-physiology. Stable isotope data derived from the measurement of sub-fossil mangrove leaves in addition to pollen data could provide a multi-proxy approach towards past mangrove environmental reconstruction. Sub-fossil leaves are preserved as a small fraction of mangrove peat (McKee and Faulkner, 2000). The leaves of modern tall and dwarf *Rhizophora mangle* L. (Red mangrove) often have distinctly different carbon and nitrogen stable isotopic characteristics, which are related to the nutrient status and salinity of the environment (e.g. Alongi et al., 1992, 1993; Lin and Sternberg, 1992a,b; McKee et al., 2002). Dwarf *R. mangle* trees have been noted as having more negative $\delta^{15}N$ values (−5.38‰) and more positive $\delta^{13}C$ values (−26.5‰) compared with tall *R. mangle* trees ($\delta^{15}N = 0.1$‰ and $\delta^{13}C = -28.4$‰) (see McKee et al., 2002, for a comprehensive review of the range of isotopic variation demonstrated for *R. mangle*). Dwarf trees are associated with sites that are often in the interior of mangrove islands in Central America, while tall trees are more often, but not exclusively, found around the edge of islands. The morphological differences are generally associated with phosphorus and nitrogen limitation, where the dwarf trees are phosphorus limited and the tall trees are nitrogen limited (McKee et al., 2002).

Compared with stable carbon isotopes, $\delta^{15}N$ measurements are not as commonly used as palaeoecological proxies, because nitrogen in leaves can be turned over by microbes and invertebrates during the degradation of primary biomass. The $\delta^{13}C$ values, on the other hand, alter very little during decomposition (Fogel et al., 1989; Fogel and Tuross, 1999). The direction of $\delta^{15}N$ changes can also vary during decomposition (Fogel and Tuross, 1999), although generally $\delta^{15}N$ values become more positive due to the preferential loss of ^{15}N-depleted ammonia by microbial deamination of proteins (Wada, 1980; Fogel et al., 1989; Benner et al., 1991). $\delta^{15}N$ values in plant remains can also be affected by nitrogen immobilization, which results from microbial incorporation of nitrogen from the pore water or by the reaction of nitrogen with humic substances (Rice, 1982; Benner et al., 1991; Hoch et al., 1992; Fogel and Tuross, 1999). Fogel and Tuross (1999) suggested that the significant alteration of $\delta^{15}N$ in organic material during decomposition preclude the use of $\delta^{15}N$ values when reconstructing palaeoenvironments. Nitrogen immobilization can reflect the sources of nitrogen that were immobilized during decomposition (Benner et al., 1991). However, in anoxic or tannin rich depositional environments, such as mangrove peat, decomposition rates are diminished (Gonzalez-Farias and Mee, 1988) and nitrogen can remain preserved (Bohlolli et al., 1977). The $\delta^{15}N$ of sub-fossil material can therefore be used to illustrate variations in the source of nitrogen into sedimentary environments (e.g. Finney, 1998). Decomposition rates of organic material are highly dependent on the type of plant species, the plant organ (e.g. roots or leaves) and chemical composition (Benner et al., 1987, 1991; Fogel and Tuross, 1999).

We investigated the potential of using carbon and nitrogen stable isotopic signatures in sub-fossil leaves of *R. mangle* as indicators of past mangrove stand structure and nutrient dynamics. Our approach was to study several stages during the taphonomy of *R. mangle* leaves in a natural setting (Fig. 1) and the influence of these stages on the stable isotopic composition of leaves. Three requirements were investigated:

1. The senescence of mangrove leaves on a tree. If the stable isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of *R. mangle* leaves are to be used in palaeoecological investigations, then the signatures should remain unaltered by senescence or fractionation should be systematic.
2. Fallen leaves on the surface of mangrove peat. If *R. mangle* leaves preserved in sediments are to be used to illustrate the past stand structure of mangrove ecosystems, then assemblages of leaves directly below modern dwarf and tall trees should possess dwarf and tall stable isotopic signatures, respectively.
3. Preservation of leaf tissue and chemistry within mangrove peat. If mangrove leaves are present in peat they need to retain sufficient carbon and nitrogen to measure both $\delta^{13}C$ and $\delta^{15}N$ respectively. Variations in both $\delta^{13}C$ and $\delta^{15}N$ from mangrove leaves from older sediments should be in accordance with the range of variation seen in modern mangrove leaves, rather than the surrounding peat that is composed primarily of fine roots and not likely to be contemporaneous with the leaves.

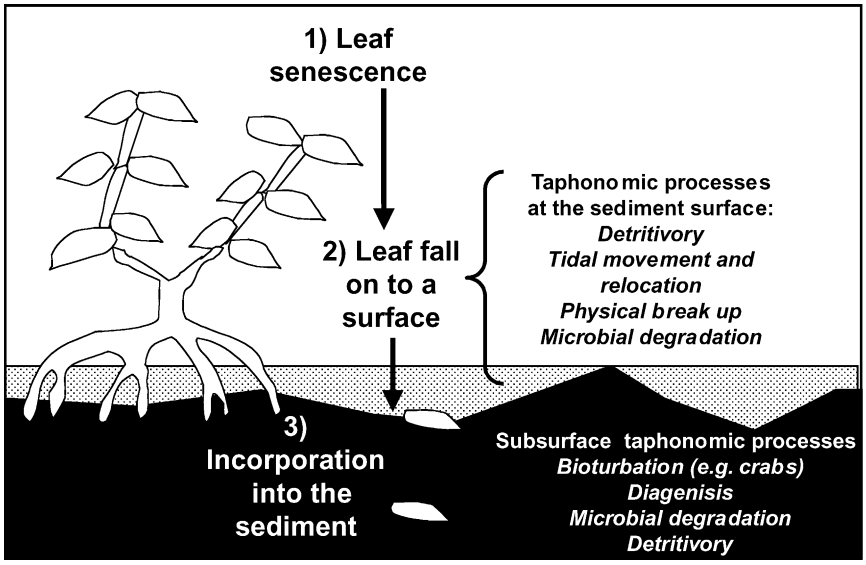

Fig. 1. A model of the taphonomic stages in the production of sub-fossil *R. mangle* leaves.

2. Study site

Fieldwork was conducted in Belize, Central America at Twin Cays (16°50′N, 88°06′W) (Fig. 2), a peat based, 92-ha archipelago of mangrove islands inside the crest of a barrier reef 12 km off the shore of Belize. These mangrove islands receive no direct terrigenous input, the only input being aolian dust transported to the island. Twin Cays is inundated with sea water and the vegetation is mostly dominated by *R. mangle*. A tree height gradient exists that includes a narrow seaward fringe of tall (5–6 m) *R. mangle* trees, which occurs 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) (Koltes et al., 1998). The interior of the island is dominated by stands of dwarf (~0.5–1.0 m) *R. mangle* with some discrete patches of tall trees (up to ~5 m). In some areas of the island mixed stands of *Avicennia germinans* (L.) Stearn. (black mangrove), *Laguncularia racemosa* (L.) Gaertn. f. (white mangrove) and *R. mangle* are found. Stands of *A. germinans* also occur on the island. The wet season occurs from July to October, with average rainfall of 218 cm year^{-1} (Rutzler and Ferraris, 1982).

3. Methods

3.1. Collection of samples from Belize

Fully expanded green ($n=5$) and senescent ($n=5$) leaves were collected from each of three tall, three dwarf, and two transition *R. mangle* trees from Twin Cays during January 2001. Directly under each tree, five fallen leaves were collected from the peat surface. These specimens were dried at 50 °C under N_2 at the Smithsonian Marine Station, Carrie Bow Cay, Belize. Samples were then packaged and transported to the Geophysical Laboratory, Carnegie Institution of Washington (GL, CIW) for stable isotope analyses.

3.2. Decomposition experiment

An experiment at Twin Cays to investigate the processes involved in leaf decomposition was established in April 2001. Senescent leaves were sampled from three dwarf trees and three tall trees at a site on the island. A senescent leaf from each one of the trees was freeze-dried for stable isotope analyses (T_0). Additional leaves were tethered using fishing line below the tree from which they were sampled and were in contact with the peat surface. A time series of tethered, decomposing leaves was collected at 2 weeks (T_1), 4 months (T_2) and 7 months (T_3) after the experiment was initiated. These samples were washed with de-ionized water, freeze-dried and then ground for stable isotope analyses.

3.3. Core sampling

Four sediment cores, between 35 and 50 cm length and 3 cm diameter, were taken from below stands of *R. mangle* on the island. The four coring sites were taken along a transect through a line of tall *R. mangle*. The four coring sites were spaced at approximately 4 m along this transect. The first core (Core 1) was taken from below a stand of dwarf (0.5–1 m high) trees that had very closely spaced internodal distances on their branches (approximately 0.25–0.5 cm) and were standing in

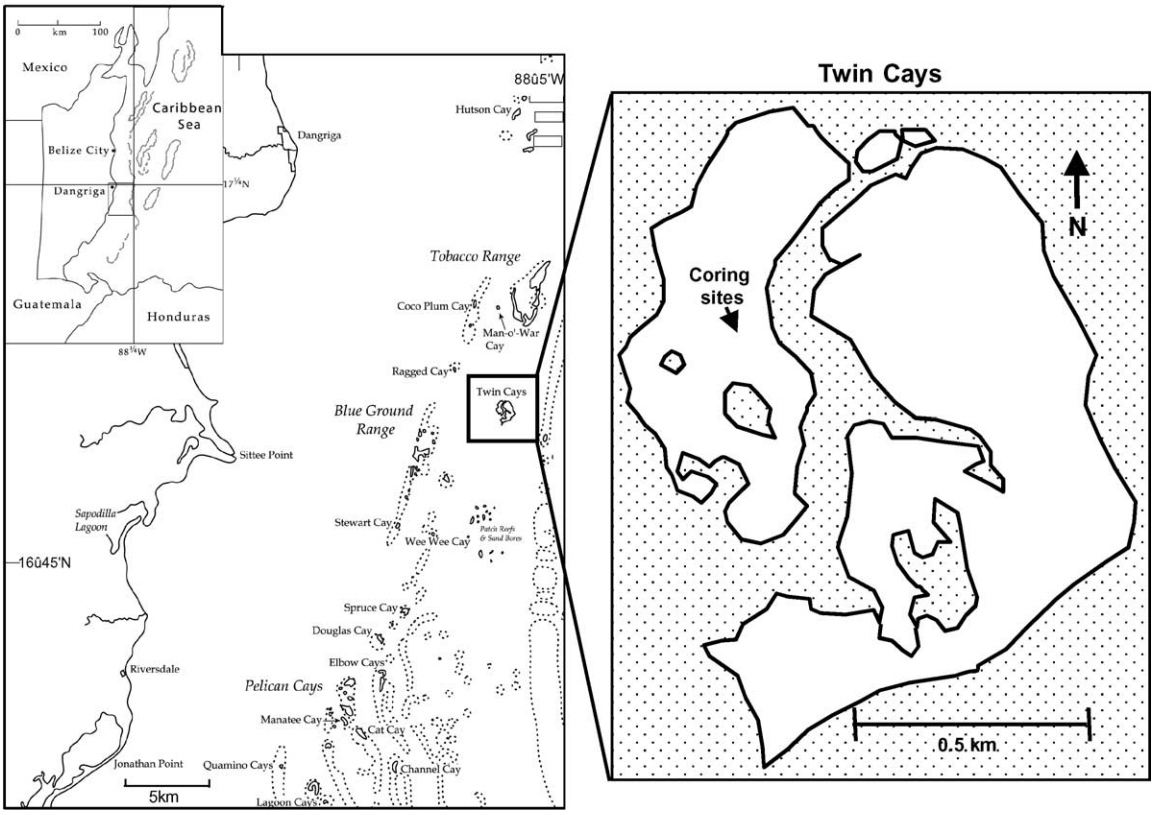

Fig. 2. Location of coring sites at Twin Cays, Belize (large scale map adapted from Smithsonian Caribean Coral Reef Ecosystems map).

approximately 0.5 m of sea water. The second core (Core 2) was taken from below a pure stand of *R. mangle* that was situated in a ~20 cm thick microbial mat. The trees at this second site were ~3–4 m high and had widely (~7–10 cm) spaced internodal distances on the most recent branches. Core 3 was taken from below a relatively tall (~4 m), pure stand of *R. mangle* trees that did not have widely spaced internodal distances. The final core (Core 4) was taken within a mixed stand of shorter (~1–2 m) *R. mangle* and *A. germinans*. The sediment cores were wrapped in cling film and foil and were stored on ice in Belize before transport to the Carnegie Institution of Washington. They were stored at 4 °C prior to analyses.

3.4. Sample preparation and carbon and nitrogen stable isotope analyses

The four sediment cores taken from Twin Cays were cut into 1 cm long sections, which were stored and picked for leaf fragments. Sub-fossil leaf fragments from each core section were rinsed with distilled water to remove seawater and were then freeze dried. Samples of the peat were taken at 2 cm intervals from the four cores. The sediment samples were rinsed with distilled water, to remove salt and freeze dried. Between 700 and 800 μg of green or fallen leaves or sediment, and ~900 μg of senescent leaves of dried sample collected from Belize was weighed into a tin capsule, which was then sealed. Samples were introduced via the EA carousel (Wooller et al., 2001) into the autosampler (A2100) of a CE Instruments, NA 2500 series, elemental analyzer (EA). 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:

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

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 ($\delta^{15}N$) are expressed relative to air ($\delta^{15}N$ designated 0.0‰); carbon isotope ratios ($\delta^{13}C$) are expressed relative to Pee Dee Belemnite ($\delta^{13}C$ designated 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 $\delta^{15}N$ was ±0.5‰ standard deviation (N% = ±0.8 S.D.) and for $\delta^{13}C$ was ±0.1 standard deviation (C% = ±2.9 S.D.).

4. Results

4.1. Green, senescent and fallen R. mangle leaves

Significant differences in the stable isotope compositions of leaves were measured from dwarf and tall tree types (Fig. 3a and b, Table 1). Little alteration of the carbon and nitrogen stable isotopic composition of *R. mangle* leaves occurred with senescence. The tall and dwarf trees were found to be statistically different for both the green and senescent leaves ($\delta^{13}C$ tall green vs. dwarf green, t stat. = 9.24, $n = 15$, $P = < 0.001$; $\delta^{13}C$ tall senescent leaves vs. dwarf senescent leaves, t stat. = 12.21, $n = 15$, $P = < 0.001$). One of the transition-zone trees showed a wide range of $\delta^{13}C$ values in fresh leaves, however. The greatest magnitude of difference between $\delta^{13}C$ values of senescent and fallen leaves was determined from dwarf trees rather than tall trees. The difference in $\delta^{13}C$ from fallen leaves sampled below tall and dwarf trees is smaller than that between fresh leaves; nonetheless these two populations are statistically different (Fig. 3c) ($\delta^{13}C$ tall fallen leaves vs. dwarf fallen leaves, t stat. = 2.07, $n = 15$, $P = < 0.05$). The lowest $\delta^{13}C$ values from the set of fallen leaves were measured on those leaves from below tall trees (Fig. 3c).

Table 1
The $\delta^{13}C$ and $\delta^{15}N$ of green, senescent and fallen *R. mangle* leaves (parentheses = 1 standard deviation)

	$\delta^{13}C$			$\delta^{15}N$		
	Green	Senescent	Fallen	Green	Senescent	Fallen
Dwarf	−24.2 (0.7)	−25.0 (1.1)	−25.7 (0.1)	−10.1 (0.6)	−10.3 (0.3)	−3.3 (0.9)
Tall	−27.6 (0.7)	−27.8 (0.3)	−26.3 (0.1)	0.0 (0.5)	0.2 (0.2)	0.1 (0.3)
Transition	−25.0 (1.4)	−26.1 (0.7)	−25.6 (0.1)	−2.2 (0.7)	−2.2 (1.3)	−1.0 (0.0)

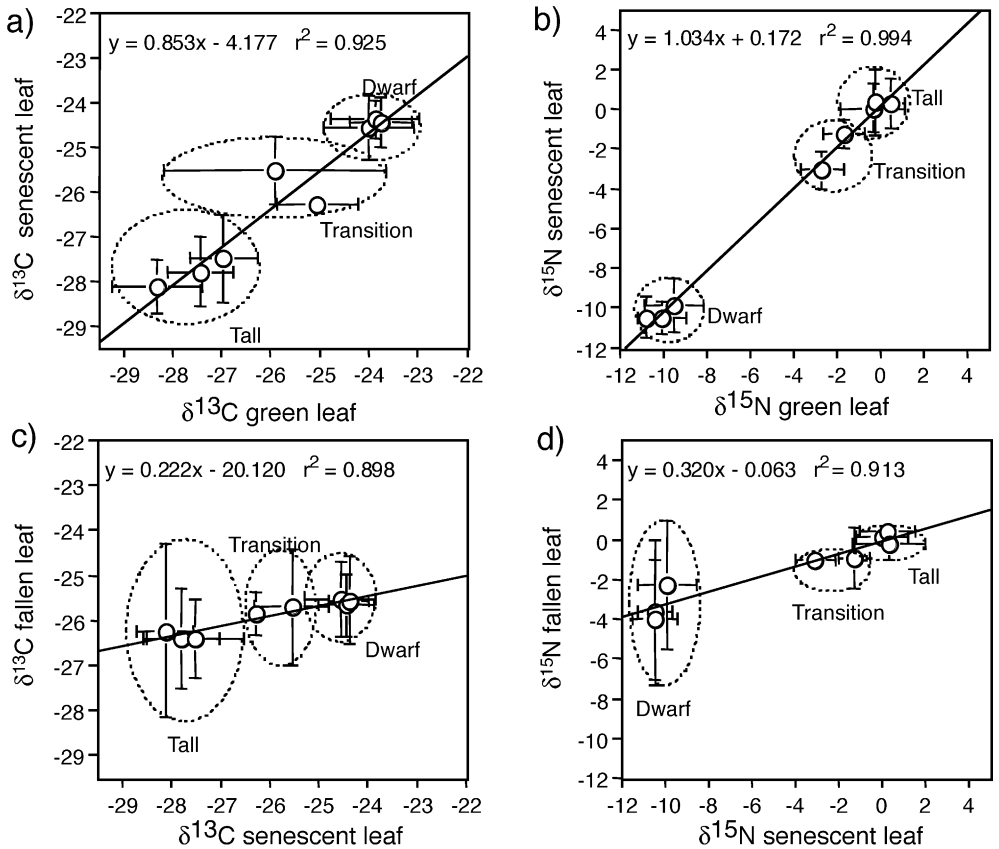

Fig. 3. $\delta^{13}C$ values of green vs. senescent (a) and senescent vs. fallen (c) and $\delta^{15}N$ values of green vs. senescent (b) and senescent vs. fallen (d) leaves from *R. mangle*.

The dwarf and tall populations of leaves could also be separated in terms of the $\delta^{15}N$ values of green, senescent and fallen leaves (Fig. 3b and d). Relationships among classes were statistically significant for all types: (1) $\delta^{15}N$ tall green leaves vs. dwarf green leaves, t stat. = −24.84, $n = 15$, $P = < 0.001$; (2) $\delta^{15}N$ tall senescent leaves vs. dwarf senescent leaves, t stat. = −6.39, $n = 15$, $P = < 0.001$; (3) $\delta^{15}N$ tall fallen leaves vs. dwarf fallen leaves, t stat. = 3.91, $n = 15$, $P = < 0.005$. The most negative $\delta^{15}N$ values in the set of fallen leaves occurred in leaves below dwarf trees. The $\delta^{15}N$ values of the fallen leaves below a dwarf tree were more positive by ~6‰ than the senescent leaves on the tree above them (Fig. 3d), whereas the $\delta^{15}N$ values from tall and transition sites were ~0–3‰ for both fallen and senescent leaves. The $\delta^{15}N$ values derived from fallen leaves below dwarf trees were more variable than those below tall trees (Fig. 3d).

The C% values of fallen leaves were not significantly different from senescent or green leaves for dwarf, tall and transition trees (Table 2). However, unlike carbon the N% of leaves altered significantly with senescence, decreasing by ~50% (Table 2). This change in the leaf composition explains the increasing C/N values with senescence.

There were also changes in the morphology and appearance of the *R. mangle* leaves in addition to the chemical changes that occurred with senescence and decomposition at the peat surface. Senescent *R. mangle* leaves were observed as bright yellow and were slightly curled relative to green leaves. *R. mangle* leaves that had fallen to the ground and had been on the peat surface for some time were generally dark brown to almost black. The external surface (cuticle) of these leaves often appeared shiny and remained relatively intact, although rubbing them could fragment these leaves. A microscopic examination of these dark leaves showed that the stomata remained relatively undamaged on the leaves. The contents of these blackened leaves were observed as soft, dark amorphous material.

4.2. *Decomposition of* R. mangle *leaves*

Leaves from dwarf and tall trees that were tethered and left exposed to the environment for a total of 4 months remained isotopically ($\delta^{13}C$) distinct from each other (Fig. 4a). A complete data set was not available for the four time points as only one leaf remained attached to one dwarf tree after 7 months. Loss of leaves may have been due to either physical breakage or biological activity, e.g. detritivory or microbial degradation. The $\delta^{13}C$ values of dwarf leaves were consistently more positive than those from tall trees, even after four months of decomposition at the peat surface (Fig. 4a). The $\delta^{13}C$ values from the leaves below the tall trees became slightly more positive (t stat. = −2.32, $P = < 0.05$) after 4 months, whereas there was no difference between those leaves below dwarf trees for this time period.

Dwarf and tall populations were distinctly different at time zero (senescent), with the dwarf leaves having more negative $\delta^{15}N$ values than the tall leaves. The leaves after 2 weeks of decomposition had a wider range of $\delta^{15}N$ values, which was most noticeable in the dwarf population of leaves (Fig. 4b). The wider range of values from both tall and dwarf leaves after 2 weeks meant that the two populations were not statistically distinct. The $\delta^{15}N$ values of tall and dwarf leaves converged after four months and were not significantly different (Fig. 4b).

The dwarf and tall populations could be statistically separated in terms of their C% from senescent leaves at time zero (t stat. = 3.7, $n = 3$, $P = < 0.05$), but not beyond this time point. The highest C% values were measured in leaves that had decomposed for at least four months (Fig. 5a). The N% content of leaves from tall trees decomposed for 4 months was greater than the senescent leaves and from those leaves taken from tall trees and decomposed for 2 weeks ($P = < 0.005$) (Fig. 5b). Although the largest N% values from the dwarf population of leaves were seen in those leaves decomposed between four and seven months there was no significant difference between the populations of senescent leaves, and those decomposed for 2 weeks and four months. Dwarf and tall populations of leaves could not be statistically separated in terms of their C/N for a time period of up to 4 months (Fig. 5c). The

Table 2
The C%, N% and C/N ratios of green, senescent and fallen *R. mangle* leaves (parentheses = 1 standard deviation)

	C%			N%			C/N		
	Green	Senescent	Fallen	Green	Senescent	Fallen	Green	Senescent	Fallen
Dwarf	46.6 (2.0)	44.7 (2.5)	44.2 (3.3)	1.1 (0.4)	0.5 (0.1)	1.1 (0.3)	45.9 (19.1)	88.5 (17.7)	42.9 (12.0)
Tall	46.2 (1.5)	45.9 (1.4)	43.6 (2.9	0.9 (0.1)	0.3 (0.1)	1.1 (0.3)	52.4 (5.9)	140.6 (21.2)	44.0 (18.1)
Transition	47.8 (1.7)	47.0 (2.6)	49.0 (3.6)	1.0 (0.2)	0.6 (0.2)	1.0 (0.3)	47.8 (8.5)	88.5 (27.3)	51.4 (18.2)

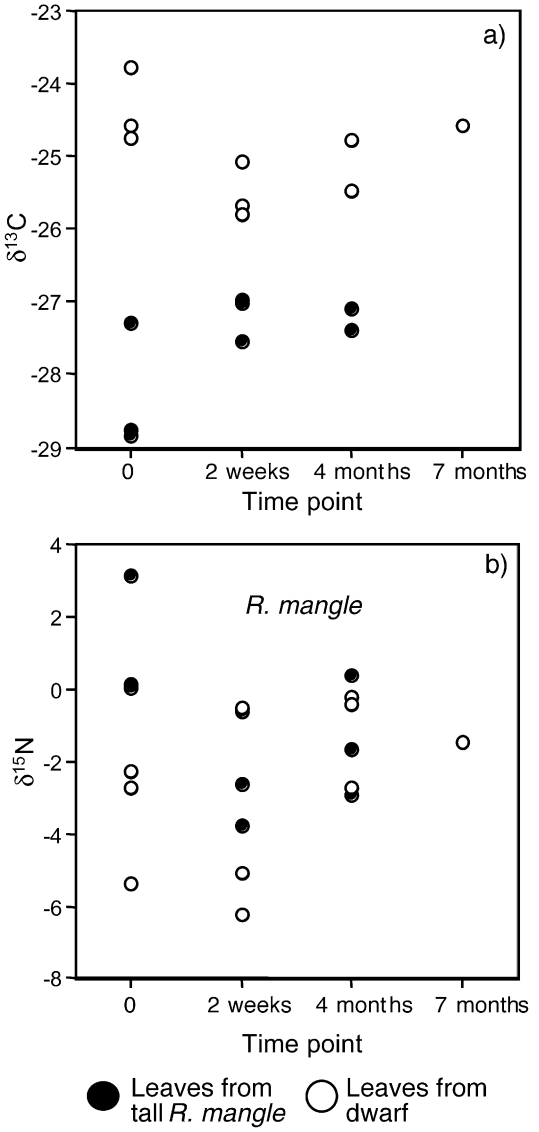

Fig. 4. $\delta^{13}C$ (a) and $\delta^{15}N$ (b) values of senescent (time point 0) *R. mangle* leaves from dwarf and tall trees along with leaves decomposed for 2 weeks (time point 1), 4 months (time point 2) and 7 months (time point 3).

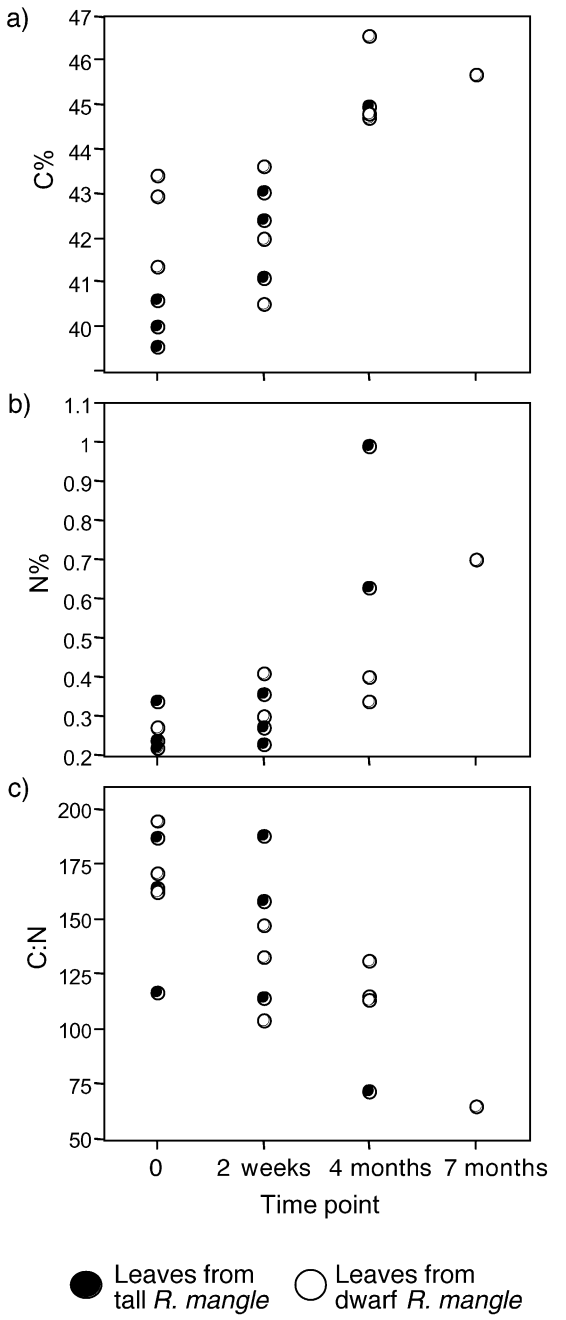

Fig. 5. C% (a), N% (b) and C/N (c) values of senescent (time point 0) *R. mangle* leaves from dwarf and tall trees along with leaves decomposed for 2 weeks (time point 1), 4 months (time point 2) and 7 months (time point 3).

highest C/N values were measured in senescent leaves whilst the lowest were measured in the leaves decomposed for four and seven months (Fig. 5c). The N% of fallen leaves was greater than senescent leaves. Initially green leaves decreased in N% as they became senescent, and then once fallen subsequently increased (Table 2). The larger N% values of fallen leaves correspond with a lower C/N of the same. The greatest C/N values were seen in senescent leaves from tall trees (Table 2).

4.3. R. mangle *leaves from mangrove peat*

4.3.1. *The peat*

The cores taken from the peat at Twin Cays were primarily composed of a matrix of fine roots and fine

organic matter containing leaf fragments, with the exception of Core 2 that had a substantial microbial mat composing the top 20 cm. The most apparent feature of the $\delta^{13}C$ values from the peat (Fig. 6) from the four cores was the similarity in the trends shown in cores 1 and 3, which showed relatively uniform values down core (mean peat $\delta^{13}C$ value for peat = −23.6‰, S.D. = 0.5, n = 79). Cores 2 and 4 showed more positive values (up to −16‰ in Core 2 and −20‰ in Core 4). The more positive $\delta^{13}C$ values from the peat in Core 2 between 20 cm and the surface could be accounted for by the fact that the top 20 cm were composed primarily of microbial mat rather than peat. Microbial mat was not readily observed in Core 4, which could otherwise have been used to explain the more positive $\delta^{13}C$ values of the peat compared with values from Cores 1 and 3.

The $\delta^{15}N$ values measured on the peat varied between −2‰ to 1.75‰ (mean = −0.3, S.D. = 0.6, n = 79) in all four cores. The $\delta^{15}N$ values of the microbial mat derived from Core 2 between 7 and 20 cm averaged 0‰ and varied very little throughout this depth range. The C% of the peat was between 12.5 and 50.2 (mean = 40.5, S.D. = 6.4, n = 79) and the N% was between 0.9 and 6.4 (mean = 2.1%, S.D. = 0.8, n = 79). The C/N values of the peat were generally lower than the sub-fossil leaf fragments, ranging from between 6.5 and 41.5 (mean = 21.9, S.D. = 6.7, n = 79). The C/N values of the microbial mat from Core 2 were between 10.6 and 15.9 (mean = 13.4, S.D. = 2.7, n = 10). The C% and N% values of the microbial mat taken from Core 2 were between 28.0–38.6 and 1.9–3.6 respectively.

4.3.2. Sub-fossil leaves

- Core 1: the $\delta^{13}C$ values from leaf fragments picked from Core 1, taken from a dwarf *R. mangle* site, ranged between ∼−29‰ and −23‰ (mean = 26.0‰, S.D. = 1.2‰, n = 36) (Fig. 8a). The $\delta^{13}C$ values lower than −27‰ were comparable to those measured from senescent and fallen leaves from tall *R. mangle* trees (Fig. 3c). The $\delta^{15}N$ values lower than −3.3‰ were comparable to those measured from the leaves from dwarf *R. mangle* trees (Table 1, Fig. 3d). Core 1 showed $\delta^{15}N$ values from sub-fossil leaves that were between ∼0‰ and 2‰ from 40 to 20 cm. From 20 cm to the surface there was generally a decrease in the $\delta^{15}N$ values to approximately −6‰ at just below the surface. The C% and N% of the leaves from Core 1 averaged at 46.4% (S.D. = 5.0%, n = 40) and 1% (S.D. = 0.4%, n = 40) respectively, showing relatively minor variation down core. The N% of leaves generally decreased with depth. The C/N also seemed to decrease with depth, from 60 at the base of the core to 30 at the top. A peak in the C/N values occurred at ∼20 cm.
- Core 2: the $\delta^{13}C$ values of sub-fossil leaves from Core 2 (Fig. 8b), sampled below rapidly growing *R. mangle* in a microbial mat, ranged between ∼−28‰ and −22‰ (mean = −25.8‰, S.D. = 1.3‰, n = 43). Core 2, showed $\delta^{15}N$ values of between −6‰ and 0‰ from the

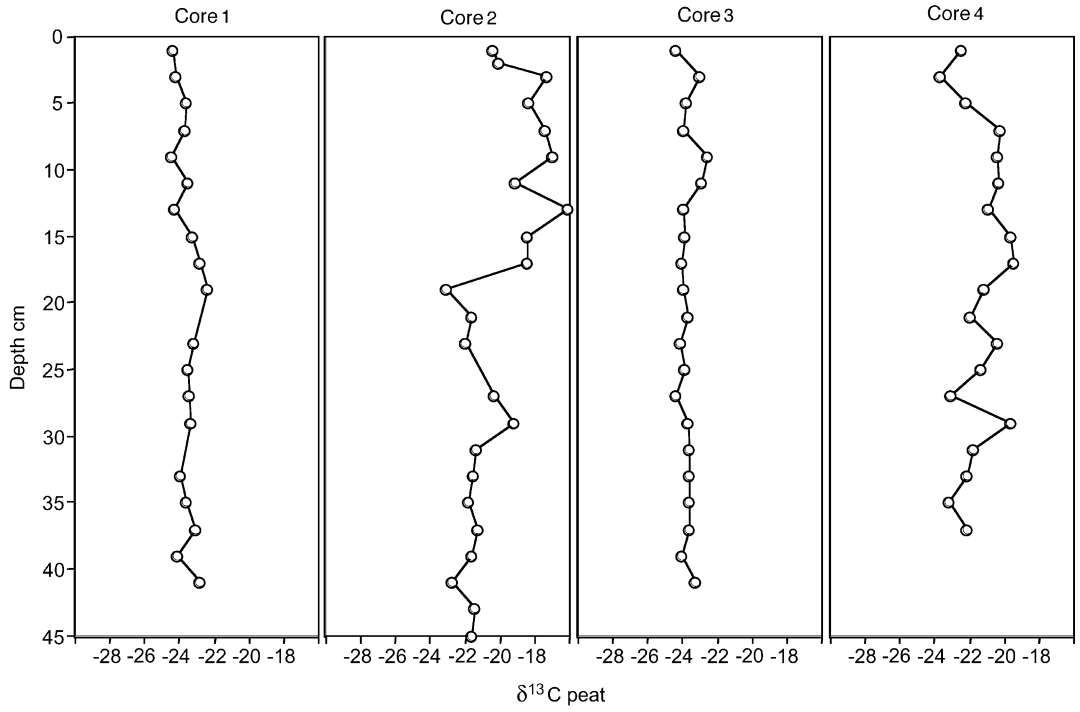

Fig. 6. The $\delta^{13}C$ values of sediment from four cores (Cores 1–4) taken from Twin Cays, Belize.

base of the core (~45 cm) to ~20 cm. Between 20 and 7 cm a number of the $\delta^{15}N$ values of the sub-fossil leaves analyzed were considerably more negative (down to −11‰), which were as negative as some $\delta^{15}N$ values seen in green and senescent leaves from dwarf *R. mangle* (Fig. 3b). The $\delta^{15}N$ values from the sub-fossil leaves in this core then increased from 7 cm to the surface, reaching ~1.5‰. The C% ranged between 35 and 55% and the N% ranged between 0.75 and 2.75%. The N% generally remained constant at ~1% from the base of the core to ~15 cm and then generally increased to the surface of the core. Like Core 1, the C/N values were higher at the base of the core (~60) and lower at the top of the core (~20).

- Core 3: leaf remains were absent from a large proportion (11–27 cm) of Core 3 (Fig. 8c), contrasting with Cores 1, 2 and 4. $\delta^{13}C$ values from Core 3 ranged between approximately −27‰ and −23‰ (mean = −25.0‰, S.D. = 1.2‰, $n = 25$), with the lowest values in the base section of the core. Some relatively negative $\delta^{15}N$ values (−6‰) were observed at the base of the core (42–30 cm) and at 6 cm. The rest of the $\delta^{15}N$ values ranged between −3‰ and 0‰. C% values ranged between 33 and 50%. N% values were highest (~2.8%) towards the top of the core (11–0 cm), while the lowest N% values (0.75%) were in the base of the core (27–41 cm). The C/N values generally decreased from the base of the core (45) to the top of the core (35).
- Core 4: the $\delta^{13}C$ values from the leaves in Core 4 (Fig. 8d) ranged between −29‰ and −24‰ (mean = −25.7‰, S.D. = 1.0‰, $n = 38$), with the lowest values measured at the surface. Core 4, taken from below a mixed species site of tall *R. mangle* with *A. germinans* present, showed $\delta^{15}N$ values of ~0‰ at the base (37 cm) of the core, which generally decreased to −6‰ at 20 cm. The $\delta^{15}N$ values then increased to −2‰ between 20 and 15 cm and then decreased to ~−9‰ at 13 cm. From 13 cm the $\delta^{15}N$ values generally increased to −1‰ at the surface. The C% values of the sub-fossil leaves ranged between 20 and 60. The N% value, which ranged between 2.25 and 0.25%, generally decreased down core.

Although the four cores demonstrated different trends in $\delta^{13}C$ and $\delta^{15}N$ derived from sub-fossil leaves there were some similarities between the cores. All of the cores generally showed a decrease in the N% of the sub-fossil leaves with depth and the C/N ratio generally increased with depth.

5. Discussion

5.1. *Taphonomy of* R. mangle *leaves*

Senescence of *R. mangle* leaves did not significantly alter the $\delta^{15}N$ and $\delta^{13}C$ values of the leaves investigated, despite a significant decrease (~50%) in the N% content of the leaves during senescence. Lack of a significant isotopic change suggests that the trees reabsorb an isotopically representative fraction of the biochemicals within the leaves. The N% decrease during senescence is the result of nutrient resorption (Feller et al., in press). The difference in $\delta^{15}N$ values between dwarf and tall *R. mangle* leaves are believed to be the result of processes related to the uptake of relatively small nitrogen compounds (e.g. ammonium) (McKee et al., 2002), which are ultimately synthesized into macro-molecules (e.g. amino acids) and set the isotopic composition of these compounds and leaves. The resorption of the limited class of N-containing compounds (i.e. amino acids and proteins) from the leaves may prevent fractionation of the nitrogen returning from the leaves and account for the lack of change in $\delta^{15}N$ with senescence despite an alteration of the N% of the leaves. Fallen leaves were collected from directly below each of the sampled trees. However, it was impossible to determine exactly which tree produced the leaves in an uncontrolled natural environment. The trees that were sampled were not in areas of vastly open space, hence leaf movement due to wind or currents was believed to be minimal. In spite of this uncertainty, tall and dwarf trees could still be identified using the $\delta^{15}N$ and $\delta^{13}C$ values of the fallen leaves below a tree (Fig. 3c and d). From the set of fallen leaves very negative (−28‰) $\delta^{13}C$ values were only ever determined for leaves below tall trees while very negative (−7‰) $\delta^{15}N$ values were only ever produced from the analyses of leaves below dwarf trees (Fig. 3c and d). A change in the $\delta^{13}C$ values between the senescent and fallen leaves is only significant (t stat. = 7.6, $n = 3$, $P = < 0.01$) between leaves from tall trees ($\delta^{13}C$ of senescent = ‰ and fallen = ‰) (Fig. 2c). However, a change in the $\delta^{15}N$ values between the senescent and fallen leaves was most noticeable, although not statistically different (t stat. = 2.1 $n = 3$, $P = > 0.1$), between leaves from dwarf trees ($\delta^{15}N$ of senescent = −10.3‰ and fallen = −3.3‰) (Fig. 2d). Comparatively speaking the $\delta^{13}C$ values of the senescent leaves from tall trees (~−27.8‰) and the $\delta^{15}N$ values of senescent leaves from dwarf trees (mean = −10.3‰) (Table 1) have the greatest difference compared with values of the peat on to which they ultimately fell ($\delta^{13}C = \sim -22.5$‰, $\delta^{15}N = \sim 0$‰) (Fig. 5). On the basis of mass balance calculations microbial biomass appears to compose the greatest proportion of the peat (Wooller et al., in press). It is reasonable to suspect that the $\delta^{13}C$ values of leaves from tall trees and the $\delta^{15}N$ values of leaves from dwarf trees are coming into 'equilibrium' with the isotopic composition of the peat through active infiltration of the leaves with both C and N from microbial biomass during leaf degradation and passive infiltration of the fragmenting leaves with particulate C and N from the peat matrix.

The decomposition experiment provided a number of controls that the "fallen leaf" investigation could not

allow for. The leaves all came from particular trees. Leaf assemblages were not mixed, and the time of leaf exposure at the peat surface was known. Ideally the decomposition of a single leaf, subsampled over time, would have allowed the measure of change in a specific leaf's chemistry over time. The increase in variability of $\delta^{15}N$ values of mangrove leaves after two weeks (Fig. 4b) can not be definitively separated from the possibility that the data may represent the broad range of $\delta^{15}N$ values that can exist in a single tree (Figs. 3a and 4b). However, the decomposition experiment is a closer simulation of the decomposition of an assemblage of leaves. The data from the decomposition experiment is consistent with the findings from the fallen leaf measurements and illustrate that dwarf and tall trees can be distinguished even after 4 months of decomposition, in terms of their $\delta^{13}C$ values. Establishing how much of the variation was caused by decomposition vs. how much of the variation existed in the initial collection of leaves at time zero remains difficult. Nonetheless, the most negative $\delta^{13}C$ values in all types of leaves were measured below tall trees and the most negative $\delta^{15}N$ values were seen in association with dwarf trees.

5.2. The incorporation of leaves into mangrove peat

At all four of the sites where cores were taken, leaf fragments from depths close to the surface had isotopic values consistent with the stand structure (dwarf vs. tall) present at each site. No systematic trends in either the $\delta^{15}N$ and $\delta^{13}C$ values from sub-fossil leaves with depth (increasing or decreasing) in all of the four peat cores taken (Fig. 8a–d) were detected. We conclude, therefore, that the sub-fossil leaves record an aspect of past environmental conditions, plant physiology and stand structure, rather than simply a steady-state diagenetic overprint. This conclusion is supported by the fact that the range of isotopic values from the cores (Fig. 8a–d) ($\delta^{13}C = -29‰$ to $-22‰$ and $\delta^{15}N = -11‰$ to $+2‰$) fall within the range of variation seen in the modern leaves ($\delta^{13}C = -29‰$ to $-22‰$ and $\delta^{15}N = -11‰$ to $+1‰$) (Fig. 3). Moreover, the sub-fossil leaves (Fig. 8) are clearly very different from the surrounding sediment (Fig. 7), which is relatively uniform in comparison to the leaf fragments. Fogel et al. (1989) found that while decomposed plant remains had $\delta^{13}C$ and $\delta^{15}N$ values similar to those of modern plant specimens, N% values of plant remains decreased over time and once in the sediments the chemical composition of plant fragments no longer resembled that of modern plant tissue. Early diagenetic alteration in $\delta^{13}C$ values of sedimentary and sub-fossil organic matter is complex (Macko et al., 1993; Meyers and Ishiwatari, 1993). Previous studies have found that loss of non-lignin organic matter results in depletion of ^{13}C by approximately $-4‰$ (Macko et al., 1993; Meyers and Ishiwatari, 1993, and references therein). Fogel et al. (1989) also suggested that diagenesis can result in the contamination of original material with organic matter from sedimentary sources. On the other hand $\delta^{15}N$ can often be shifted to more positive values as demonstrated by Benner et al. (1987) and Fogel et al. (1989), who showed that $\delta^{15}N$ values of plant remains, that were decomposed for 3 months, were shifted by up to $+7.8‰$. This finding is consistent with the $\delta^{15}N$ values of fallen leaves below dwarf mangrove trees, which were more positive (by $\sim +6‰$) than the senescent leaves on the trees directly above them (Fig. 3d). The $\delta^{15}N$ values, however, from tall and transition sites were within the range of $\sim$0–1‰ for fallen and senescent leaves.

Downcore C/N values of sub-fossil leaf organic matter generally increase, which is consistent with early diagenesis of a more labile nitrogen-containing fraction of organic matter degrading more rapidly than carbon (Bordovskiy, 1965; Patience et al., 1990). The accumulation of mangrove peat shown at the Tobacco range off of the coast of Belize shows a deposition rate of between $\sim$0.4 and $\sim$4 m per 1000 years (MacIntyre et al., 1995). We are therefore confident that the upper 50 cm of peat at the surface should represent between 100 and 1000 years. We investigated the potential of using carbon and nitrogen stable isotopic signatures in sub-fossil leaves of *R. mangle* as an indicator of past mangrove stand structure and nutrient dynamics. The examination of these four cores from Twin Cays show that leaf fragments do indeed persist to a depth of $\sim$50 cm. From our observations using stable isotope measurements of sub-fossil leaves we can consider the $\delta^{15}N$ values as a maximum, conservative estimate of the leaves' original $\delta^{15}N$ values, since diagenesis and 'dilution' of the leaf assemblage from dwarf trees will tend toward more positive $\delta^{15}N$ values (up to $\sim$0‰).

The taphonomic processing of leaf nitrogen does not simply result in a loss of nitrogen over time. Senescence does appear to result in a decrease of total nitrogen (Table 2), which demonstrates the extent of nutrient resorption from the leaves to the tree as a mechanism of nutrient conservation (Feller et al., in press). Once a leaf falls to the peat surface there seems to be an initial increase ($\sim$50%) in nitrogen (Table 2). This increase in nitrogen content of leaves likely represents the processes of 'nitrogen immobilization' that have been discussed elsewhere (Rice, 1982; Benner et al., 1991; Fogel and Tuross, 1999). Processes of nitrogen immobilization could be incorporating the signature of a dissolved nitrogen pool within the surface water or in the pore water, as previously observed by Benner et al. (1991). Dissolved NH_4+ can be rapidly taken up by bacteria during decomposition of plant material (Benner et al., 1991). Benner et al. (1991) suggest that nitrogen immobilization can result from the exogenous sources of nitrogen by the population of microorganisms attached to a decomposing leaf. The $\delta^{15}N$ values of some organic

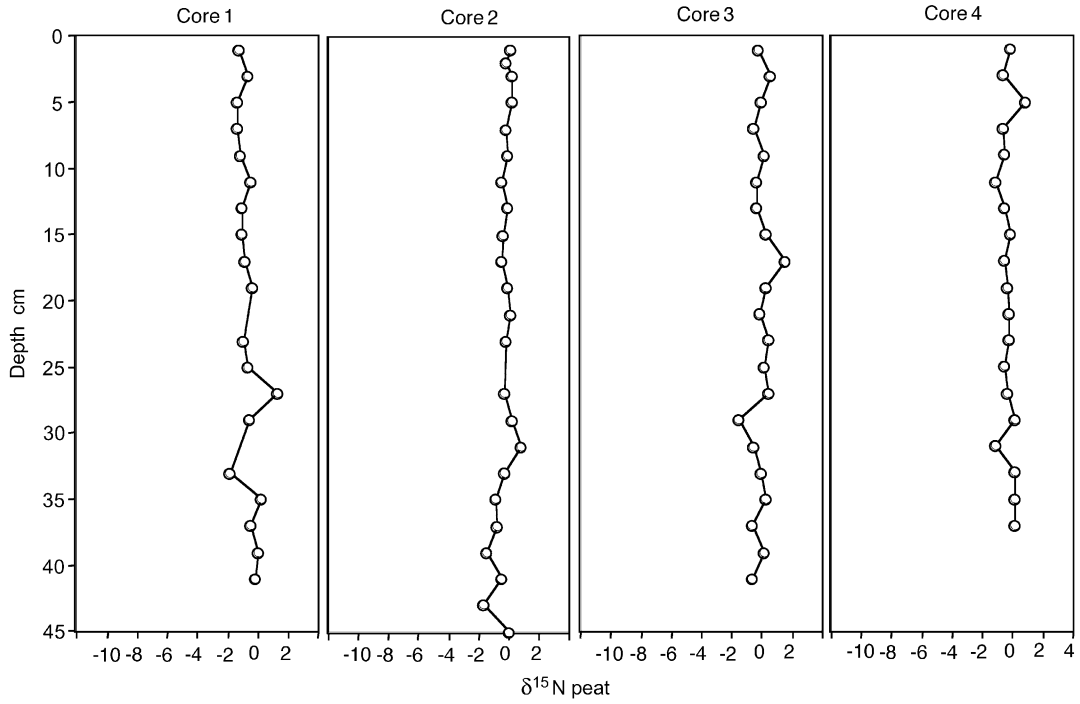

Fig. 7. The δ^{15}N values of sediment from four cores (Cores 1–4) taken from Twin Cays, Belize.

components from the Twin Cays ecosystem are influenced by nitrogen derived from atmospheric nitrogen fixation, yielding δ^{15}N values of ~0‰ (Wooller et al., in press). Ammonium is present in the sediments at Twin Cays at μM concentration (Wooller et al., in press) compared with the mM concentration observed by Benner et al. (1991) and Fogel and Tuross (1999). Once in the sediment there seems to be a gradual decrease in the N% values of the sub-fossil leaves from below ~10 cm in all of the cores examined.

There is a possibility that shifts in the δ^{13}C of the peat could be used to mark transitions towards microbial mats (e.g. Core 2) being present at a site in the past. The two sites dominated by *R. mangle* (Cores 1 and 3) showed very little variation in δ^{13}C values of the peat down core (Fig. 6). It is unclear why the peat below the stand of mangroves containing *A. germinans* should have relatively high δ^{13}C values (Core 4, Fig. 6). However, pollen present in the peat could be used to mark whether an abundance of *A. germinans* was present at a site, thus distinguishing microbial vs. *A. germinans* δ^{13}C shifts. *A. germinans* can posses organic matter with higher δ^{13}C values (Wooller and Fogel, unpublished data), which is probably a result of this species existing in sites with higher salinity, which would demand an elevated state of water use efficiency. In the cores with higher δ^{13}C values and lower C/N values (Cores 2 and 4) the proportions of microbial material composing the peat are even higher (80–90%), which are consistent with the findings of Wooller et al. (in press).

We acknowledge that the stages of taphonomy are exceedingly complex (Clark, 1988; Spicer, 1989) and have highlighted some of the main processes in Fig. 1. Relocation of leaves due to physical processes (tidal and wind) could be established by tagging and tracking an assemblage of fallen leaves over time. Marking numerous leaves below two close trees could also be used to examine the mixing of assemblages over time. The influences of crabs and herbivory on the leaf assemblage below a tree and the bioturbation involved during the creation of burrows by crabs also need to be investigated. McKee and Faulkner (2000) noted that surface litter is subject to tidal export, while Robertson (1991) noted the consumption of leaves by crabs and snails.

5.3. Towards a multi-proxy approach to mangrove palaeoecology

The ultimate control over mangrove development and succession at some of the cays in Belize is liable to be sea-level change (McKee and Faulkner, 2000). Twin Cays has formed on up to ~10 m of mangrove peat, which is one of the thickest deposits of mangrove peat in the world (Macintyre et al., 1995). The significance of these deposits, the base of which are dated to ~7000 years before present, is that they demonstrate that the

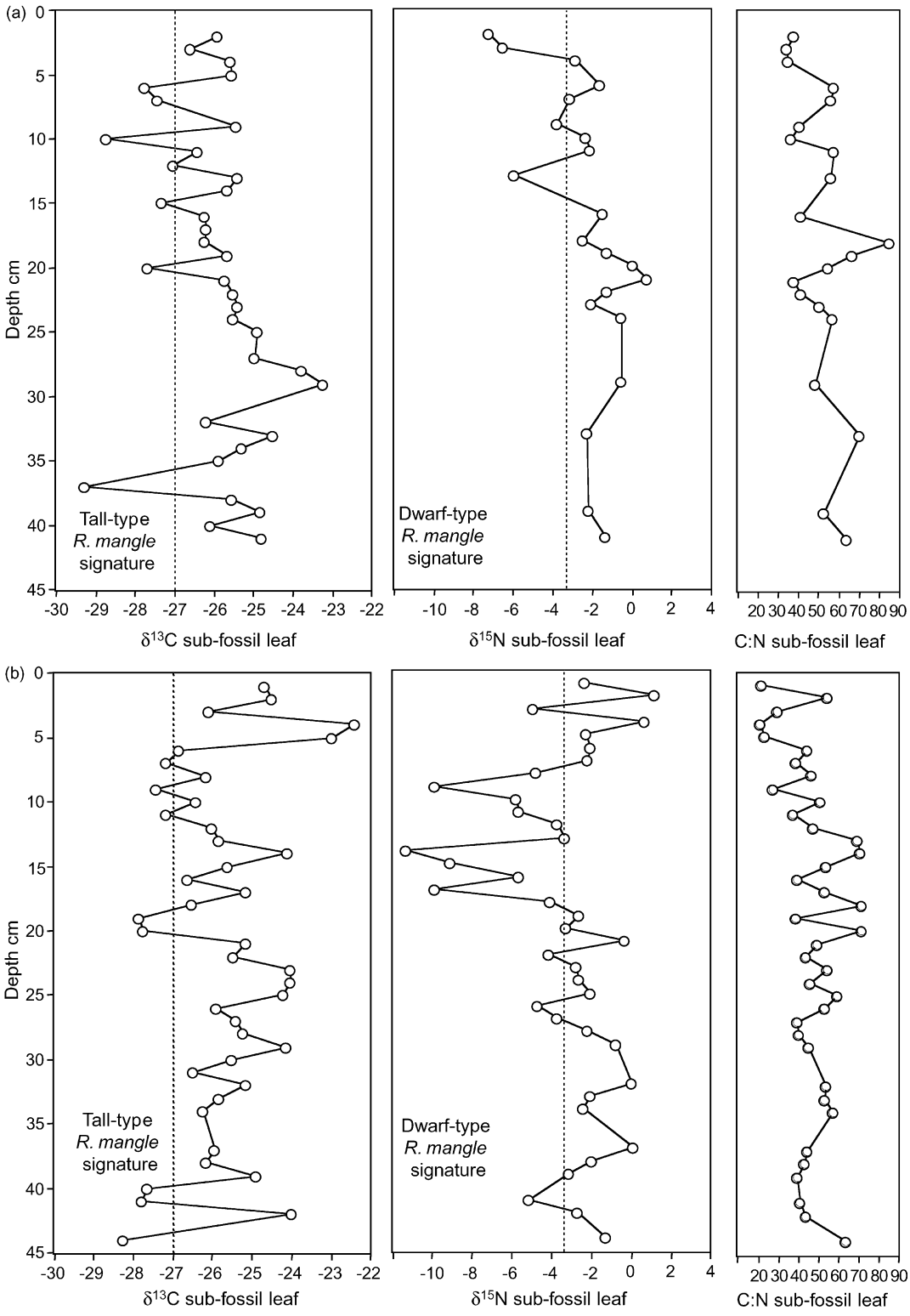

Fig. 8. The $\delta^{13}C$, $\delta^{15}N$ and C/N values of sub-fossil leaf fragments of *R. mangle* from four cores: (a) Core 1; (b) Core 2; (c) Core 3; (d) Core 4, taken from Twin Cays, Belize.

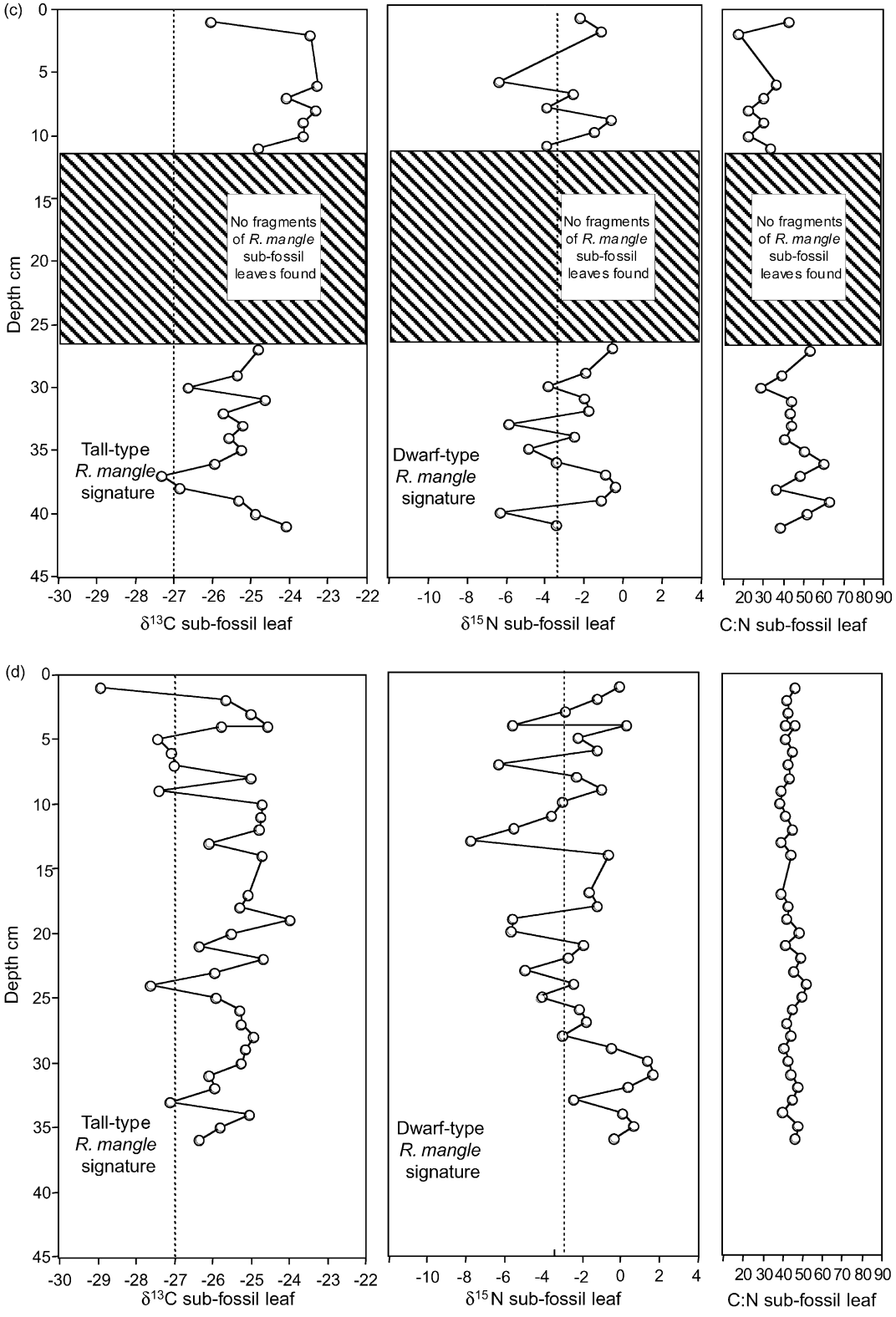

Fig. 8. (*continued*)

mangroves in this area were able to keep up with the rise in sea-level since the start of the Holocene (Macintyre et al., 1995). Other mangrove deposits to the south of Belize demonstrate that mangroves were unable to keep up with sea-level, became totally submerged and were then over lain with marine deposits (Macintyre et al., 1995).

McKee and Faulkner (2000) suggested that changes in vegetation composition associated with changes in hydroedaphic conditions imply that zonation of mangrove forests is strongly tied to processes controlling surface elevation in relation to mean sea level. Shifts in conditions, e.g. sea-level rise or fall, which are outside the optimum niche of *R. mangle* would impose physiological stress on the species that could potentially be tracked over time using the isotopic compositions of sub-fossil mangrove leaves.

Two main sea-level phases occurred during the Holocene: (1) an early-Holocene phase of rapid sea-level rise, the postglacial marine transgression; and (2) a mid- to late-Holocene phase of sea-level stability (Woodroffe, 1988, 1990). Researchers have debated whether there were fluctuations in the rate at which sea-level rose during the last ~7000 years, with some areas of the earth said to have experienced a sea-level 1–2 m higher than present between 6000 and 3000 years ago (Woodroffe, 1990). Two marked decreases in the rate, or even decreases in sea-level, are said to have occurred during the last 7000 years in Brazil at ~3500 and 5000 years before present (Suguio et al., 1988; Martin and Suguio, 1992). Western Samoa also appeared to experience a sea-level as much as 6 m below present about 5000 years ago. Evidence from Tonga supports a sea-level below 1.5 m lower than present 5600 years ago (reviewed by Woodroffe, 1990). Some anomalies also exist in the records of sea-level change from Central America, with some data implying an abrupt decrease in the rate of sea-level rise at ~5500 years ago (evidence reviewed by Macintyre et al., 1995). In fact, Woodroffe (1990) suggested that it is widely accepted that there has not been a single global sea-level pattern over the Holocene. Tropical coastlines have responded to more subtle hydro-isostatic adjustments compared with the polar regions, which have responded to glacial-isostatic responses (Woodroffe, 1990).

Multiple proxies of past mangrove vegetation preserved in continuous cores of peat could potentially be used to examine the response of mangrove ecosystems to Holocene environmental change. Fluctuations in $\delta^{13}C$ values from mangrove leaves could be used to illustrate changes in stand structure (dwarf and tall *R. mangle*) and infer changes in water use efficiency (e.g. Ish-Sholom-Gordon et al., 1992; Ellison and Farnsworth, 1997), possibly responding to fluctuations in sea-level thus affecting the local water table. Changes in the $\delta^{15}N$ values of leaves could be used to illustrate changes in mangrove stand structure and nutrient limitation, while pollen analyses could illustrate shifts in the composition of vegetation (e.g. Behling and Costa, 2001). A constant rise in sea-level over the last 7000 years would have imposed a constant physiological pressure on mangroves to respond. Fluctuation in the rate of sea-level rise, or indeed slight falls in sea-level, over the last 7000 years would, more likely, have produced fluctuations in the physiological response of mangroves.

Stable isotope analyses of sub-fossil mangrove leaves will probably be more useful as a relational (directional) sea-level indicator than as a fixed indicator. A number of hypotheses, regarding the physiological response of past mangroves, could potentially be tested using $\delta^{13}C$ of sub-fossil leaves of mangroves preserved in the mangrove peat. A constant physiological response may be manifest as constant $\delta^{13}C$ values over time. The response of mangroves to a fall in sea-level may impose comparatively 'drier' conditions and may be manifest as increasing physiological stress, in addition to the need for increased water use efficiency and a subsequent decrease in stable carbon isotope fractionation. In the long term this is more likely to prompt an ecological change and a shift to *A. germinans* dominated vegetation rather than a physiological response manifest as a shift towards relatively higher $\delta^{13}C$ values from the analysis of preserved *R. mangle* leaves. *A. germinans* is able to tolerate relatively higher salinities compared with *R. mangle*. Indeed flooding (rapid sea-level rise and an increase in the depth of standing sea water) seems to impose physiological stress, causing submergence of pneumatophors and a decrease in root respiration. As a modern analogue, areas with deeper standing water are generally those areas with dwarf *R. mangle* present, which have the highest $\delta^{13}C$ values.

Correlation between proposed sea-level changes (Suguio et al., 1988) and stable carbon and nitrogen isotopes of sub-fossil leaves from well-dated sediments could test the response of mangroves to past sea-level changes. McKee and Faulkner (2000) noted that, from analyses of cores from other cays in Belize, the changes in stand structure of mangroves progressed in cycles between *A. germinans* dominance and *R. mangle* dominance. Perhaps $\delta^{13}C$ analyses of sub-fossil leaves could be used to track the physiological response of *R. mangle* to encroaching *A. germinans* inferred from pollen analyses. From the short cores taken from Twin Cays it is clear that there are significant shifts in the stable isotopic composition of sub-fossil leaves (Fig. 8a–d). These data do seem to imply that shifts in stand structure have occurred during the past at the sites investigated.

Multiple proxies that can demonstrate changes in vegetation composition such as pollen, (e.g. Behling and Costa, 2001) or root identification (e.g. McKee and Faulkner, 2000) coupled with plant physiological proxies (C, N and O isotopes) or stomatal indices (e.g. Wooller and Agnew, 2002) could significantly advance

investigations of the response of mangroves to past environmental changes, including sea-level. Increases in the relative abundance of leaf fragments (fragment area and number) could also be used to infer the presence of die back zones in the sub-fossil record, which are known to have occurred in the recent past at a number of Belizian mangrove Cays (McKee and Faulkner, 2000). Bulk $\delta^{13}C$ analyses of sub-fossil leaves could be coupled with the judicious application of compound specific $\delta^{13}C$ analyses (e.g. sterols or lipids) to support 'dwarf' and 'tall' inferences down cores. Our preliminary findings (Smallwood, Wooller, Fogel, unpublished data) show that sterols, which are resistant to degradation (Volkman, 1986; Volkman et al., 1993), from *R. mangle* retain dwarf and tall stable carbon isotope biosignatures. Bulk analyses of sub-fossil *R. mangle* leaves could be used to generate initial profiles down cores of mangrove peat, indicating areas of dwarf vs. tall *R. mangle*. These analyses could be followed by the application of $\delta^{13}C$ analyses of sterols or lipids extracted from leaf fragments to test the bulk isotope data from sub-fossil leaves.

Records of past sea-levels have been proposed for the Atlantic coast of Central and South America (e.g. Angulo et al., 1999; Suguio et al., 1988, Angulo and Suguio, 1995) covering the last ~8000 years before present. Substantial debates have occurred over suggestions of dramatic changes in sea-level over this time (1–4 m sea-levels higher than current mean sea-level) (Suguio et al., 1988; Martin and Suguio, 1992; Angulo and Suguio, 1995; Angulo et al., 1999). Sea-level fluctuations during the Holocene are said to account for many of the marked vegetation changes in coastal ecosystems in eastern Amazonia (Behling and Costa, 2001). An integrated, multi-proxy approach will provide a holistic picture of mangrove ecosystem change covering most of the Holocene; an entirely novel insight into mangrove palaeoecology. Accurately dated low and high sea-level stands and transitional periods could allow precise timing of environmental change (i.e. sea-level change). A suite of radiocarbon dates from mangrove peat cores could provide a critical chronological framework to allow correlation with independent records of environmental change for the Atlantic coast of Central and South America (Suguio et al., 1988; Martin and Suguio, 1992; Angulo and Suguio, 1995; Angulo et al., 1999). Our data on the taphonomy of sub-fossil *R. mangle* leaves suggest that meaningful palaeoecological information can be derived from stable isotope measurements of the sub-fossil leaves in mangrove peat.

6. Conclusion

The aim of this investigation was to address the feasibility of utilizing the stable organic carbon and nitrogen isotopic signature of *R. mangle* leaves within a multi-proxy palaeoecological method. The method would be used to reconstruct past mangrove stand structures, more specifically to address the response of mangroves to sea-level fluctuations over the past 7000 years. Three taphonomic stages of mangrove leaves were examined and the following conclusions were drawn.

1. The stable isotopes of carbon were shown to fractionate systematically during leaf senescence. The nitrogen stable isotopic signature was unaltered during senescence, even after ~50% loss of the total nitrogen from the leaf during this process.
2. The stable isotopic signatures of carbon and nitrogen from fallen leaves collected directly below *R. mangle* trees can be used to identify dwarf and tall 'parent' trees.
3. Sub-fossil mangrove leaves were present in ~50 cm long cores of mangrove peat. These sub-fossil leaves contained sufficient organic carbon and nitrogen to accurately measure $\delta^{13}C$ and $\delta^{15}N$ values. A wide range of variation in $\delta^{13}C$ and $\delta^{15}N$ values measured in sub-fossil *R. mangle* leaves corresponded with the range shown in modern leaves. The wide range of $\delta^{13}C$ and $\delta^{15}N$ values in sub-fossil leaves contrasted with, and appeared to be independent of, the surrounding mangrove peat.

Stable organic carbon and nitrogen isotopes from sub-fossil *R. mangle* leaves holds promise for contributing to a multi-proxy method to investigate mangrove palaeoecological changes.

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