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Unusually negative nitrogen isotopic compositions (δ^{15}N) of mangroves and lichens in an oligotrophic, microbially-influenced ecosystem

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Abstract. Extremes in δ^{15}N values in mangrove tissues and lichens (range =+4 to −22‰) were measured from a mangrove forest ecosystem located on Twin Cays, offshore islands in Belize, Central America. The N isotopic compositions and concentrations of NH_4^+/NH_3 in porewater, rainwater, and atmospheric ammonia, and the δ^{15}N of lichens, mangrove leaves, roots, stems, and wood were examined to study the biogeochemical processes important for establishing these unusual N isotopic ratios. Dwarfed *Rhizophora mangle* trees had the most negative δ^{15}N, whereas fringing *Rhizophora* trees, the most positive δ^{15}N values. Porewater ammonium concentrations had little relationship to N isotopic fractionation in mangrove tissues. In dwarfed mangroves, the δ^{15}N of fine and coarse roots were 6–9‰ more positive than leaf tissue from the same tree, indicating different sources of N for root and leaf tissues. When P was added to dwarfed mangrove trees without added N, δ^{15}N increased within one year from −12‰ to −2‰, approaching the δ^{15}N of porewater ammonium (δ^{15}N=+4‰). Isotopically depleted ammonia in the atmosphere (δ^{15}N=−19‰) and in rainwater (δ^{15}N=−10‰) were found on Twin Cays. We propose that foliar uptake of these atmospheric sources by P-stressed, dwarfed mangrove trees and lichens can explain their very negative δ^{15}N values. In environments where P is limiting for growth, uptake of atmospheric N by *Rhizophora mangle* may be an important adaptive strategy.

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1 Introduction

Mangrove trees, a wide range of species that can be characterized by their ability to grow in brackish or full salinity seawater along tropical and subtropical coasts, can be growth limited by N, P, or both (Feller, 1995; Feller et al., 1999). While it is reasonable to assume that N limitation will have some effect on the isotopic composition of plants (Evans, 2001), there are no clearly justifiable expectations about P limitation's effect on N isotopic compositions. P is taken up by plants through the roots, typically, assisted by the action of membrane bound phosphatases (Muchhal and Raghothama, 1999; Smith, 2001). Experiments on fertilized mangrove trees have shown that when a tree with the primary limitation of P is given this nutrient the tree responds rapidly and dramatically by adding shoots, roots, and new leaves (Feller, 1995). In this paper, we demonstrate how stable N isotopes might be useful in assessing an ecosystem's nutrient status with respect to N and P, without manipulation or long-term fertilization.

The δ^{15}N of plants reflects the net effect of many processes including the δ^{15}N of the source N, enzymatic fractionations within a plant, and plant-microbial interactions in soil (Dawson et al., 2002). The majority of terrestrial plants have δ^{15}N near 0‰ in temperate zones, however, different species growing in the same environment can vary by as much as 10‰ (Handley and Scrimgeour, 1997; Amundson et al., 2003). Mangrove trees, in general, have N isotopic compositions that reflect the overall nutrient status of the ecosystem (Fry et al., 2000; Muzuka and Shunula, 2006; Bouillon et al., 2008). For example, trees growing in regions associated with human populations had δ^{15}N similar to that of

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the N released from sewage treatment plants. Accordingly, we have previously reported that *Rhizophora mangle* L. (Red mangrove) found on a frigate bird rookery only a few kilometers from the site of the present study had δ^{15}N as positive as +17‰, which was identical to the N in the sediment and in the birds' droppings (Wooller et al., 2003b).

Plants can utilize N dissolved in soils or by absorption through their leaves (e.g., Garten et al., 1990; Leith et al., 2002) and volatile ammonia originating from animal colonies can influence the δ^{15}N of plant leaves (Erskine et al., 1998; Frank et al., 2004). For example, negative δ^{15}N (to −8‰) have been measured in grasses, vascular plants, and mosses, which were collected growing downwind of major bird rookeries. Plants collected in the immediate vicinity of the colonies incorporated the enriched δ^{15}N (up to +18‰) from the marine N at the base of the birds' food web. Either following a rainfall event or made available through dry deposition, ammonia and nitrate can be taken up by plants and incorporated into biomass.

The stable N and C isotopic compositions of mangrove trees fertilized with N or P have been studied previously (McKee et al., 2002). These authors found that leaves of *R. mangle* trees fertilized with P had positive δ^{15}N values compared with unfertilized or N-fertilized trees. The controls without fertilizer and the N-fertilized trees had negative δ^{15}N values to −8‰. In conditions where the concentration of ammonia or nitrate exceeds what a plant needs for immediate growth, isotopic fractionations of N increase during uptake or internal processing (Yonemaya et al., 1991; Fogel and Cifuentes, 1993). Clarkson et al. (2005) proposed a similar mechanism in their study of bog plants in New Zealand.

Based on previous work (Wooller et al., 2003a, b; Smallwood et al., 2003; Muzuka and Shunula, 2006), a number of lines of evidence suggested that the explanation for depleted δ^{15}N was incomplete. For example, Wooller et al. (2003b) noted that *R. mangle* leaves from unfertilized trees collected at random locations throughout the islands used by McKee and coworkers, had δ^{15}N considerably lower than the values they had found, making the hypothesis of fractionation during uptake from the soil problematic even though porewater dissolved nitrogen concentrations were much lower than those in fertilized plots. Subsequent work on the biochemical partitioning of the N isotopic signal in *R. mangle* leaves showed that the signal was not associated with any particular leaf component or biochemical fraction (Smallwood et al., 2003). Rather, differences between dwarf trees with low δ^{15}N and tall or fringing trees with δ^{15}N near 0‰ were similar in all chemical fractions. Muzuka and Shunula (2006) measured mangrove δ^{15}N as low as −3‰, values below those expected for nitrogen fixation only.

In this paper we further explored the causes of the wide variations in the stable N isotopic compositions in mangroves at Twin Cays, Belize, by sampling mangrove trees from two tree species, as well as organisms that rely primarily on atmospheric N sources (i.e., lichens), organisms that fix atmospheric N (e.g., microbial mats), and the isotopic compositions of potential soil, water, and atmospheric sources of this critical nutrient.

2 Materials and methods

2.1 Study site and collections

Twin Cays is a highly oligotrophic, peat-based archipelago, located 12 km off the coast of Belize and approximately 3 km inside the barrier reef. These islands are part of a Smithsonian research area with long-term studies on mangroves, sponges, seagrasses, and coral ecosystems (Woodruffe, 1995; Ruetzler and Feller 1996; Wooller et al., 2004). Twin Cays hosts a diverse array of microbial mats (Joye and Lee, 2004). On Twin Cays, microbial accumulations often occurred in thick (>1 m) banks forced to the edges of interior ponds by wind and waves, which we refer to as "floc".

Samples, including leaves, roots, bark, stems, and wood of mangrove trees (*R. mangle* and leaves only from *Avicennia germinans* (L.) Stearn. (black mangrove)), microbial mats, and lichens were sampled from October 2000 to April 2004 (Wooller et al., 2003a, b). Samples were dried at 50–70°C at the Smithsonian Caribbean Coral Reef Ecosystem laboratory at Carrie Bow Cay, approximately 3 km from Twin Cays.

2.2 Fertilization experiments

For the N and P fertilization studies, we sampled the three fertilization sites established in January 1998, extending from the fringe into the dwarf zone (Feller et al., 2003). I. C. Feller and coworkers of the Smithsonian Institution maintained these fertilization plots. In addition, 9 dwarf red mangrove trees were chosen for a one-time experiment. Three trees served as controls; three trees received 150 g of P_2O_5 (0:20:0) buried within 1 cm of a major prop root; three trees were fertilized with 150 g of P_2O_5 (0:20:0), 1 m away from the nearest prop root. This fertilizer aliquot was located so that no other mangrove tree was within 2 m. Leaves were sampled periodically over the next several years for isotopic and elemental compositions; trees were also assessed for internodal length, production of new prop roots, and overall size.

2.3 Bulk C and N stable isotope analyses

For leaf bulk stable C and N isotope analyses, a small aliquot of dried leaf tissue was sampled, taking care that no veins, invertebrate or fungal damage, or discolored portions of the leaf were used. Lichens were scraped off of bark with spatulas or razor blades taking care not to include bark tissue in the sample. An aliquot (∼400 to 1000μg) of each sample was analyzed using continuous-flow, stable isotope ratio mass spectrometry (Finnigan MAT, Delta^{plus}XL) (as described in Wooller et al., 2003b).

2.4 Ammonium concentration and isotopic measurements

Porewater ammonium concentrations were determined by collecting 10–50 ml at 10 cm depth using a sipper system. Water samples were filtered at the laboratory on Carrie Bow Cay with glass fiber filters (GF/F). Filtered water samples were stored in the refrigerator and analyzed typically within 2 days following collection. For samples that contained H_2S, 0.2 ml of HCl was added to the porewater prior to analysis following the method of Solarzano (1969).

To measure $\delta^{15}N$ of ammonium, we collected 120 ml of porewater from 5–10 cm depth using sippers, filtered and analyzed as above. Samples were collected from multiple locations around Twin Cays. Filtered porewater (110 ml) was decanted into a specimen cup with a Teflon sandwich containing filter paper dosed with 5–10 microliters of $H_2SO_4-KH_2SO_4$ solution following a modification of the method of Stark and Hart (1996). Ammonium sulfate with a known isotopic composition was used as a standard. Its starting $\delta^{15}N$ composition was 0.1‰, and after passive diffusion, $\delta^{15}N$ was 1.0±1.1‰. Values in Table 6 have been corrected accordingly.

2.5 Atmospheric ammonia emissions and isotopic measurements

To measure ammonia emissions into air, we deployed ammonium sensitive badges (K&M Environmental, Virginia, USA) that absorbed ammonia gas through a Teflon membrane, producing a color change by reacting with an ammonia sensitive chemical indicator (see Kring et al., 1981). The average ammonia emission over the period of exposure is estimated by dividing the dose by the time of exposure (i.e. the data reported by the badges are in units of ppm NH_3-hr). Badges were deployed in the field by tying them onto mangrove prop roots or stems at a height of 20–50 cm above the mean high tide. Readings were taken over time up to 24 h.

Badge readings were affected by several factors. Air turbulence is one of the major factors influencing NH_3 emission and can result in highly localized measurements with higher variability. Mangrove stand structure was also a factor, because in dense forests (e.g. fringe or transition zones), wind speed and air diffusion was minimal. Air and water temperature are well known additional factors that affect ammonia emissions in general (for review see Asman et al., 1998); higher soil and water temperatures, for example, result in higher NH_3 emissions. We acknowledge these limitations and deployed 50 badges in our 2003 study (Table 5) and 10 closely monitored badges in our 2004 study (Fig. 5).

To collect ammonia in the field for isotopic analyses, we deployed Teflon sandwiches (see above) contained in mesh bags, which were tied to mangrove branches or PVC pipes around the islands during February 2003 and March 2004. The tags were left out for a period of 6 days, which is considered adequate for absorbing atmospheric ammonia gas (Roadman et al., 2003). Within a localized dwarf mangrove pond, $\delta^{15}N$ for 6 similar samples was −15.7±5.8‰. Tags that showed evidence of leaking or were too low for an accurate measurement on the mass spectrometer were discarded.

2.6 Laboratory flux experiment

In March 2004, we conducted flux studies at the Carrie Bow Marine Laboratory using peat or mat cores (diameter = 5 cm) collected from the field the previous day. Experiments with duplicate cores were started before sunrise by hanging a K&M badge (see above) inside of the core barrel and incubated the cores in full daylight at ambient air temperatures (26 to 29°C). Ammonia readings were taken every hour for 6 h, or until the ammonia detecting badges reached saturation ($\gg$300 ppm NH_3).

2.7 P concentrations

Porewaters for P analysis were collected, filtered and frozen for subsequent measurement in Los Angeles on a Latchatt Automatic Analyzer at USC. Total P was determined on leaves by combusting approximately 100 mg of leaf material at 500°C for 2 h. The resulting ash was weighed, digested using methods modified from Jensen and Thamdrup (1998), and then analyzed for P with spectrophotometric methods (Presley, 1971).

3 Results

3.1 Stable isotopic signature of mangroves from Twin Cays, Belize

The dominant tree species on Twin Cays, Belize, *R. mangle* (red mangrove), had an unusually large range in bulk leaf $\delta^{15}N$ of −21.6 to 4.0‰ (Mean=−4.4±4.7; n=400) as well as $\delta^{13}C$ from −31.6 to −20.3‰ (Mean=−26.2±1.5‰). *R. mangle* from the fringes of the island had the lowest $\delta^{13}C$ values and the highest $\delta^{15}N$ values, whereas dwarf trees at interior locations had the highest $\delta^{13}C$ values and lowest $\delta^{15}N$ values (Fig. 1a and b; Table 1 and 2). *A. germinans* had $\delta^{15}N$ of −0.7±0.1‰ (range=−11.2 to +3.8‰) and $\delta^{13}C$ of −26.2±2.6‰ (range=−30.7 to −23.2‰) in leaves from 100 different trees (Fig. 2).

The greatest variations of $\delta^{15}N$ were found in the leaves of *R. mangle* located in the islands' interior. Very negative $\delta^{15}N$ (<-8‰) were measured only in dwarf (i.e. <1 m tall) or interior tall trees (Table 1). Many of these dwarf trees had very short internodal lengths (<0.2 cm) reflecting their slow growth. Isotopic heterogeneity was extreme over very short distances (e.g., 2 m or less). For example, dwarf trees within 1 m of interior tall trees often differed from each other in terms of $\delta^{15}N$ by up to 14‰.

The $\delta^{15}N$ of fringe *R. mangle* varied less than interior trees (Table 1). The $\delta^{13}C$ of fringe trees were more positive on the

Table 1. Isotopic compositions of *Rhizophora mangle* leaves collected from Twin Cays, Belize, 2000–2004. Recruits are recently rooted mangrove propagules collected in all zones.

	Mean δ^{15}N ±1 std. dev.	Range δ^{15}N	Mean δ^{13}C ±1 std. dev.	Range δ^{13}C
Dwarf (n=202)	−6.8±4.7	−21.6 to 1.4	−25.6±1.3	−22.7 to −30.7
Interior Tall (n=45)	−4.4±4.5	−12.8 to 2.8	−27.2±1.3	−24.9 to −30.1
Transition (n=28)	−1.9±3.0	− 11.1 to 0.9	−25.9±0.9	−24.2 to −27.9
Fringe (n=114)	−0.6±1.7	−7.4 to 2.5	−27.0±1.3	−23.4 to −30.6
Recruits (n= 8)	−4.5±2.3	−7.1 to −1.2	−27.0±2.3	−23.8 to −31.3

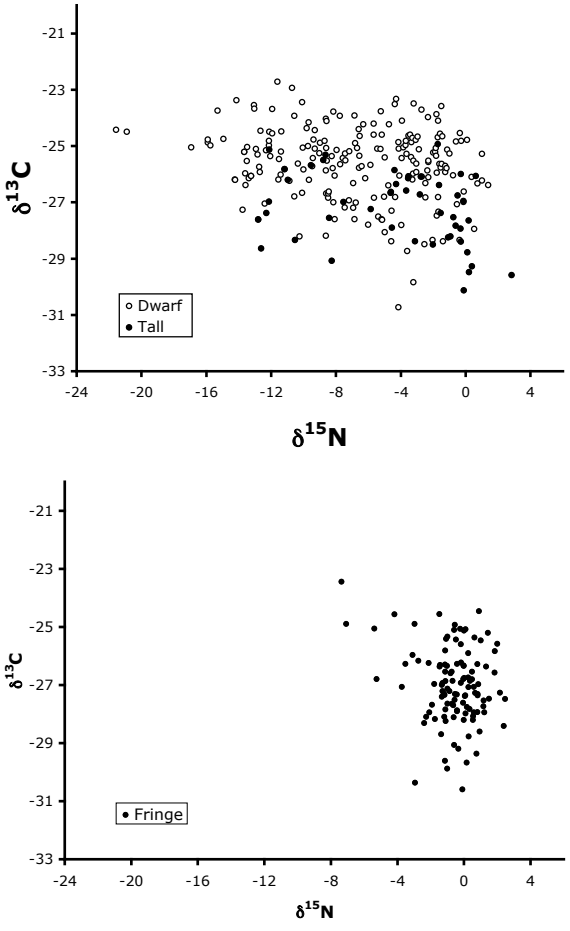

Fig. 1. The δ^{15}N and δ^{13}C of *Rhizophora mangle* leaves on Twin Cays, Belize. All data from these trees were collected from trees not included in specially fertilized experimental plots. A. Interior Tall and Dwarf Trees. B. Fringe Trees.

Table 2. Statistical analysis of *Rhizophora mangle* fresh leaf isotopic compositions as a function of stand structures. t Test: ***, highly significant; *, weakly significant.

Natural Trees			
δ^{15}N	Interior Tall	Transition	Fringe
Dwarf	0.001***	< 0.001***	< 0.001***
Transition	0.04*		
Fringe	< 0.001***	< 0.001***	
Fertilized Trees			
P vs. Control Fringe	0.71		
P vs. Control Trans	0.41		
P vs. Control Dwarf	< 0.001***		
P vs. N Fringe	< 0.001***		
P vs. N Trans	< 0.001***		
P vs. N Dwarf	< 0.001***		
Control vs. N Fringe	< 0.001***		
Control vs. N Trans	< 0.001***		
Control vs. N Dwarf	0.007		

exterior, wave-beaten portions of the island than in the inner channels (p<0.001).

The results of isotopic analysis of a single dwarf tree, sacrificed in its entirety, from the Batfish Pond site are summarized in Table 3. Although chosen at random, it represented one of the many examples of trees with extremely low δ^{15}N. The δ^{15}N of above ground tissues varied little among flowers, buds, leaves, stems, wood, and prop roots. Below ground tissues varied in δ^{15}N from −15.6 to −3.1‰, most noticeably within the roots which are actively involved in nutrient and water absorption. Striking in all cases was the absence of variation or gradients in δ^{15}N through the shoot, from the substrate surface to

Table 3. Isotopic compositions of tissues from a single dwarf tree from the Batfish Pond region. "Functioning prop root" indicates that it has penetrated the substrate and produced one or more classes of finer roots. "Smaller roots" were in contact with the substrate, ranging in diameter from <0.5 mm to >8 mm.

Tissue	Range δ^{15}N	Mean δ^{15}N ±1 st. dev.	Range δ^{13}C	Mean δ^{13}C ±1 st. dev.
Above Ground				
Bud and Flower (n=4)	−13.7 to −18.1	−16.8±2	−23.7 to −24.8	−24.3±0.5
Leaves (n=5)	−15.7 to −17.8	−16.9±0.8	−24.6 to −27.2	−25.9±1
Branches and Stems (n=6)	−15.1 to −17.4	−16.3±0.9	−24.9 to −26.8	−25.7±0.8
New Prop roots (n=6)	−16.7 to −20.2	−17.7±2	−23.3 to −25.5	−24.5±0.8
Functioning Prop roots (n=5)	−15.2 to −17.4	−16.2±1.1	−22.3 to −25.4	−23.9±1.4
Below Ground				
Functioning prop roots (n=3)	−12.4 to −13.6	−13.1±0.6	−24.6 to −25.1	−24.8±0.3
Roots in contact with sediment (n=10)	−3.1 to −11.1	−7.8±4	−24.2 to −26.2	−25.2±0.8

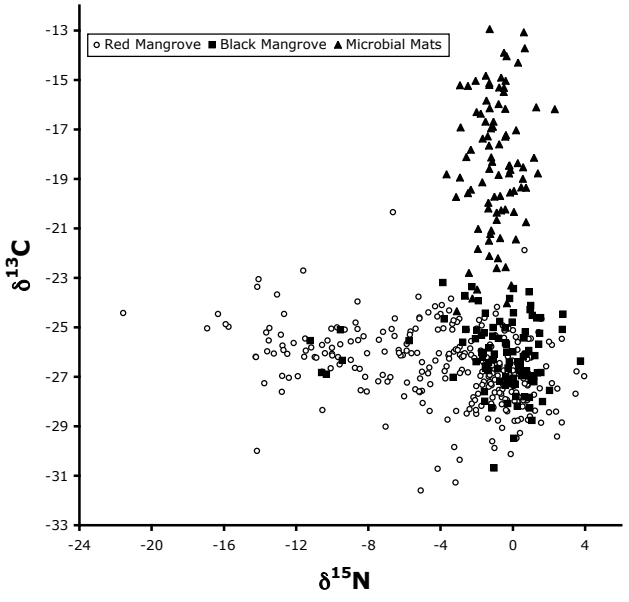

Fig. 2. The δ^{15}N and δ^{13}C relationships between *Rhizophora mangle*, *Avicennia germinans*, and microbial mats.

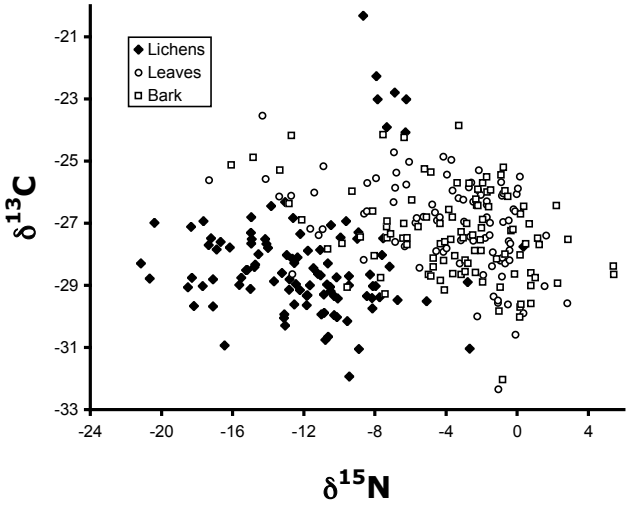

Fig. 3. The δ^{15}N of bark, leaves, and lichens growing on *R. mangle* trees at Twin Cays. The data set is for paired sets of leaf:bark:lichen collections sampled from fringe, transition, and dwarf regions around the islands.

the most distal leaves, or with leaf developmental stage. Equally noteworthy were the higher and variable values of δ^{15}N in the active roots. Similar, but not as dramatic, results were measured in six dwarf *Rhizophora* specimens from other Twin Cays locations (1 m height): leaves (δ^{15}N=−11.0±4‰), stems (δ^{15}N=−8.8±2‰), and below ground fine roots (δ^{15}N=−5.2±3‰).

3.2 Stable isotopic composition of microbial mats and lichens

The δ^{15}N of bacterial and algal mats distributed on the islands averaged −0.9±1.3‰ (range=−3.7 to +2.3; n=90) (Fig. 2). These δ^{15}N values indicate a source of N from N fixation (e.g., Macko et al., 1986). Lichens had a δ^{15}N range from 0.4‰ in the fringe zone to −21‰ in the floc and dwarf zones (Fig. 3). The δ^{15}N of the lichens was not related to the δ^{15}N of the bark that the lichens were found growing on (r^2=0.167) or leaves growing on nearby branches. Patches of lichens with very negative δ^{15}N values were found around floc zones (δ^{15}N=−13.1±5‰; n=13) and interior dwarf

zones (δ^{15}N=−12.3±4‰; n=45). Lichens on the fringe trees had slightly more positive values (δ^{15}N=−11.7‰; n=18), while the δ^{15}N of the fringing mangrove leaves was near 0‰. The δ^{15}N of lichens was always more negative than both the bark and a corresponding leaf that was collected at the same tree height from the sediment surface.

3.3 Fertilization experiments

In the long-term fertilization plots (Feller et al., 2003) at the Dock, Boa Flats, and Lair sites, trees fertilized with P had leaf δ^{15}N that were around 0‰ with no significant difference across the tree height gradient. Dwarf trees fertilized with urea (δ^{15}N=0‰), had δ^{15}N values as low as −12.4‰, whereas those from fringe and transition trees likewise fertilized, were more positive (Tables 2 and 4). At the Dock site, some of the N-fertilized trees as well as control trees measured in 2003 had more negative N isotopic compositions than the same trees measured in 1998 (McKee et al., 2002). The δ^{15}N in P-fertilized tree leaves was as positive as 2.6‰ at this time, similar to δ^{15}N in unfertilized fringe trees.

In 2002 a one-time, P fertilization experiment was started to test how quickly, and to what extent, a single dose of P can affect the growth and functioning of dwarf *R. mangle* trees. Trees that received P input directly next to a major prop root displayed stimulated growth within 7–8 months, as evidenced by increased internodal distances (from 0.1 cm to >5 cm) and more positive δ^{15}N (Fig. 4). Trees that were fertilized with P one meter away from major prop roots experienced a lag phase relative to those trees fertilized proximally. The δ^{15}N and δ^{13}C and the growth of the control trees, approximately 25 m away from the P fertilized areas, remained constant during the experimental period.

3.4 Ammonia concentrations, fluxes, and isotopic compositions of air and porewaters

In unfertilized regions hosting tall, interior *R. mangle* trees, porewaters from 5–10 cm depth averaged 37.1±21μM NH_4 (n=18). In the transition zone, concentrations were 5±2.8μM NH_4 (n=5), whereas in the fringe they were 13.2±10μM NH_4 (n=5). Interior zones dominated by dwarf *R. mangle* had porewaters ranging from 1.8 to 88μM NH_4 (mean=19.3±17; n=42). Within the floc regions, porewaters ranged from 5.8 to 413.8μM NH_4 (mean=98±140; n=7), while within a microbial mat directly, porewaters averaged 159±102μM NH_4 (n=8). Porewaters sampled in sediments directly fertilized with N had an average 52±63μM NH_4 (n=7), however, those in porewaters sampled where P was applied averaged 1.5±3μM NH_4 (n=6).

The relationship between ammonium concentrations in porewaters and the coexisting N isotopic compositions in mangrove leaves was significant in fertilized (R^2=0.29; P<0.0001; n=108) and natural samples (R^2=0.16; P=0.005; n=50), but indicates that while porewater NH_4 concentration is an important factor in determining δ^{15}N, it is not the major one. In specific examples, dwarf trees fertilized with urea had higher porewater ammonium concentrations (e.g., 200μM) with δ^{15}N values down to −10‰, compared to unfertilized dwarf trees growing in sediment with 20–30μM ammonium with δ^{15}N in their leaves as low as −18‰.

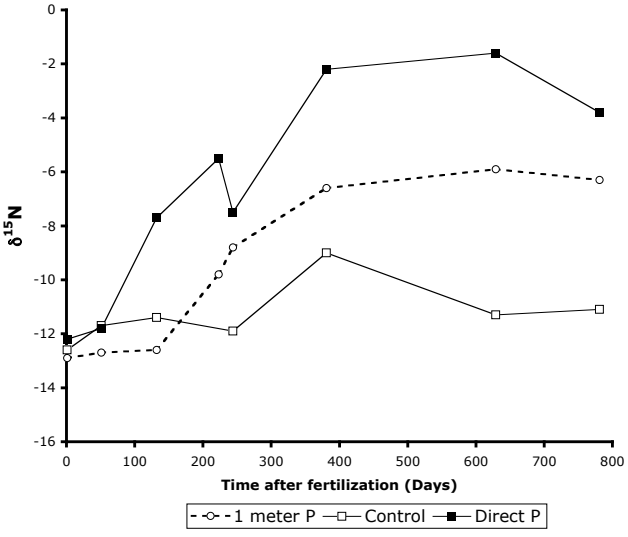

Fig. 4. Response of δ^{15}N to the addition of P to sediments over a period of approximately two years. No additional N source was added to these trees.

Ammonia emissions in situ (February 2003 and March 2004) were measured coincident with porewater concentrations. They were highest over mats and floc (Table 5; Fig. 5). In February 2003, we sampled the δ^{15}N of atmospheric NH_3, NH_4 from underlying porewaters, and rainwater (Table 6). Both atmospheric NH_3 and the NH_3/NH_4 in rainwater on Twin Cays had very negative isotopic compositions relative to those measured in porewaters and in rainwater collected on Carrie Bow Cay. The isotopic fractionation between the atmospheric ammonia (Mean δ^{15}N=−19.5‰) and the ammonium in porewaters (Mean δ^{15}N=4.3‰) is 23.8‰, which fits within the range of isotopic fractionations (19 to 30‰) between these two N species that have been measured during chemical isotope fractionation experiments in the laboratory (Thode et al., 1945; Hermes et al., 1985).

Flux experiments with small cores incubated on Carrie Bow Cay showed that ammonia fluxes related positively to porewater concentrations (r^2=0.7; P=0.02; n=14). From microbial mats and floc samples, rates of 2.0 to 3.0μmole NH_3/m^2/hr were measured in comparison to fringe cores, which had an average flux of 0.7μmole NH_3/m^2/hr.

3.5 P and N concentrations and ratios in red mangrove leaves and underlying sediments

P concentrations in freshly collected *R. mangle* leaves were extremely low all over Twin Cays (0.06±0.02% Total P (TP);

Table 4. N isotopic composition of fresh leaves from fertilized *R. mangle* experimental plots on Twin Cays, Belize. Samples were collected and measured in 2003. P (Phosphorus), N (Nitrogen) and C (Control) refer to the fertilization experimental treatments.

Treatment-Stand Structure	Mean(n=3 for each location and treatment)			
	Boa Flats δ^{15}N ‰	Dock δ^{15}N ‰	Lair δ^{15}N ‰	Range δ^{15}N ‰
P-Fringe	0.4	0.8	0.9	−0.2 to 1.7
P-Transition	0.5	1.0	0.6	−0.8 to 2.6
P-Dwarf	−0.5	−0.5	0.4	−1.6 to 1.1
C-Fringe	0.2	0.8	0.5	0.5 to 1.5
C-Transition	0.7	1.6	−3.6	−7.2 to 2.7
C-Dwarf	−3.2	−5.2	−7.3	−8.9 to 0.7
N-Fringe	−1.0	−4.1	−2.8	−5.6 to 0.7
N-Transition	−2.3	−4.4	−6.0	−8.0 to −0.7
N-Dwarf	−4.8	−9.7	−8.6	−12.4 to −3.1
	Boa Flats N/P$_{(at)}$	Dock N/P$_{(at)}$	Lair N/P$_{(at)}$	Average N/P$_{(at)}$
P-Fringe	27.6	33.2	26.2	29.0
P-Transition	29.5	33.4	24.1	29.0
P-Dwarf	31.5	38.3	28.9	32.9
C-Fringe	32.8	34.0	46.2	37.7
C-Transition	41.2	47.2	57.1	48.5
C-Dwarf	52.7	42.6	54.1	49.8
N-Fringe	26.2	40.5	61.0	42.6
N-Transition	35.1	78.2	48.8	54.0
N-Dwarf	36.7	42.8	35.4	38.3

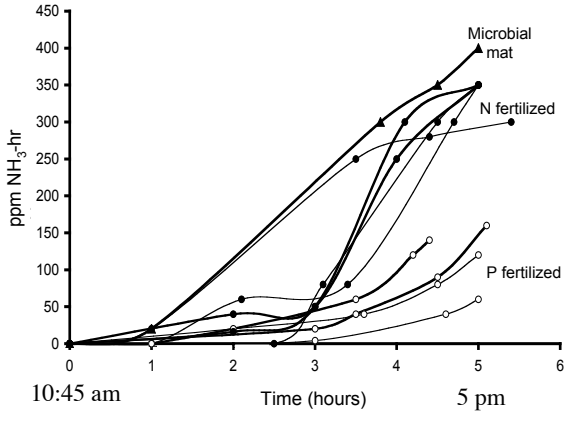

Fig. 5. Ammonia emissions from Batfish Pond, Twin Cays by ammonia-sensing badges. Experiments were initiated at 10:45 am when badges were first exposed to air. Readings were taken approximately every hour until 5 pm. The tide decreased during the course of the experiment; the water depth ranged from 0 cm (dry) to 50 cm. Badges were placed about 0.5–0.7 m from the sediment surface by suspending them from *Rhizophora mangle* branches on P-fertilized trees (open circles); N-fertilized trees (closed circles), and microbial mat (triangle).

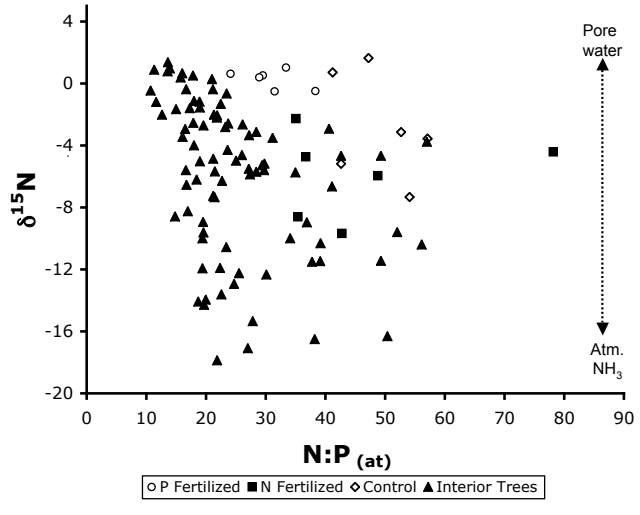

Fig. 6. The δ^{15}N of *Rhizophora mangle* leaves as a function of N:P (at) ratios. Interior mangrove trees include dwarf, transition, and larger trees growing on inland creeks. These trees were collected in regions not influenced by any fertilization experiments. Control trees were sampled adjacent to actively fertilized sites.

Table 5. Ammonia emissions from Twin Cays. Floc refers to unconsolidated microbial growth floating on surface peat sediments. Floc often occurred in thick (>1 m) banks forced to the edges of interior ponds by wind and waves. Microbial mats are consolidated sedimentary features with distinct layering (see Joye and Lee, 2004).

Zone/Treatment	Mean ppm NH_3-hr Mean	St dev.	Range	n
Dwarf	19.4	14.0	0–53.3	26
Floc	39.3	53.0	8.9–133	5
Microbial Mat	44.5	54.4	5.7–150	7
N-Fertilized	30.4	32.7	8.9–100	8
P-Fertilized	5.0		0–10	2
Transition	8.6		0–17.2	2

Table 6. Isotopic compositions of ammonium plus ammonia from porewaters, rain, and atmosphere from Twin and Carrie Bow Cays, Belize February 2003.

Sample	δ^{15}N	Range	Number
Air on Twin Cays	−19.5±7.1	−30 to −7.3	18
Rain on Twin Cays	−10.6±4.1	−14.3 to −5.9	4
Rain on Carrie Bow	1.0±3.6	−4.4 to 2.6	8
Porewaters	4.3±4.1	−3.8 to 11.8	11

n=71). Red mangrove leaves sampled on the East Island remote from direct coastal access and completely removed from fertilization plots, had 0.08±0.04% TP with ranges from 0.03 to 0.20% TP (n=35). These %TP values were elevated slightly from trees receiving chronic, episodic P fertilization (0.07±0.03% TP; n=27) (p=0.04) in the three fertilization experiments maintained on the islands (Feller et al., 2002). Tissue levels, thus, appear to be constant in fertilized trees, but total tree levels were much higher as many more leaves, branches, and stems were produced. Trees receiving a one-time input of P had significantly greater total P contents (0.10±0.05% TP; n=6) in growing leaves 2 years after the initial fertilization (p=0.05) than control trees (0.07±0.02% TP; n=6). Total P in sediments in dwarf and transition zones had a mean value of 0.051±0.016% (n=34).

In a representative subset of *Rhizophora*, N concentrations were 1.04±0.26% (n=74), and there was no relationship between %N in leaves and their δ^{15}N (P=0.73) or %TP in leaves (P=0.33; n=54). Conversely, %N in mangrove leaves was correlated with corresponding %TP in sediments (R^2=0.21; P=0.005; n=34). The relationship between N:P in this particular subset of leaves and δ^{15}N had a significant exponential relationship with δ^{15}N decreasing with increased N:P (R^2=0.34; $P<0.001$; n=52). Moreover, at two of the four individual fertilization sites an exponential relationship was also highly significant: single application P experiment (R^2=0.47; P=0.0004); n=22; and the long term Boa Flats experiment (R^2=0.25; P=0.01; n=26), but not in the other two long-term fertilization experiments (see Fig. 6 for pooled δ^{15}N vs. N/P).

4 Discussion

Nitrogen isotopic compositions of mangrove tissues were not simply related to the inorganic N concentrations in sediments. Higher sediment N concentrations should result in increased N isotope fraction during uptake and biosynthesis in roots (e.g., Yonemaya et al., 1991; Fogel and Cifuentes 1993, McKee et al., 2002). Based on the NH_4 concentrations in porewaters and the coexisting N isotopic compositions in mangrove leaves, other biogeochemical processes must be more important factors.

Trees fertilized with P and trees growing nearby with equivalent sedimentary and leaf total P concentrations had the most positive δ^{15}N (+2 to −1‰), whereas those trees with lower available P had more negative δ^{15}N (−5 to −21‰). The relative amount of P then seems to be a critical factor in determining δ^{15}N values. Lovelock et al. (2006) demonstrated that P limitation of mangroves results in water deficiency, lower stomatal conductance, and less conductive xylem. When mangroves were fertilized with P, root surface area increased per unit leaf area. Accordingly, we propose that with greater root biomass, P becomes more available to catalyze enzymes needed for active NH_4 transport. In addition, increased xylem conductance allows mangroves to efficiently assimilate and translocate porewater N from roots into leaves, resulting in dramatic shifts in δ^{15}N from negative values to more positive values similar to δ^{15}N in porewater NH_4. Differences in δ^{13}C of the two most extreme tree heights, fringe and dwarf, provide further support for lower stomatal conductance and photosynthetic rates (Cheeseman and Lovelock, 2004).

Clarkson et al. (2005) recently measured a correlation between % total P in foliar tissue and δ^{15}N in plants growing in peat bogs. They concluded that P limitation reduced mycorrhizal colonization of roots, which in turn potentially resulted in increased N isotope fractionation. Clarkson et al. (2005) did not, however, report δ^{15}N for root tissue or the δ^{15}N of potential N species in the environment. In our study, coarse and fine roots had more positive δ^{15}N by 4–13‰ than aboveground tissues: leaves, prop roots, stems, wood, and bark. Porewater ammonium δ^{15}N values from mats, floc zones, dwarf regions, and underneath some N-fertilized trees averaged 4‰ (n=12). This similarity between porewater and root values implies that evidently, these roots incorporate N from sediments.

Hobbie et al. (2000, 2005) have shown that a substantial N isotopic fractionation occurs during early colonization by ectomycorrhizal (ECM) and ericoid mycorrhizal (ERM) fungi associations with δ^{15}N in leaves of colonizing species averaging -9 ± 2‰. Mature plants infected with AM fungi had δ^{15}N has low as -4‰; our leaves with δ^{15}N less than -10‰ were collected from dwarf mangrove trees that were well established. Mangrove ecosystems at Twin Cays have not shown evidence of mycorrhizal associations with mangrove trees (C. Lovelock, personal communication), although arbuscular mycorrhizal (AM)-type mycorrhizal associations have been found in mangroves colonizing the Ganges River estuary (Sengupta and Chaudhuri, 2002). It is unlikely that AM fungal associations could explain the full extent of the δ^{15}N variation we determined at Twin Cays.

The intimate relationship between P availability and N isotopic fractionation was shown clearly and dramatically in the results of fertilization studies in as little as 3 months (Fig. 4). After 5 months, the δ^{15}N increased from -14‰ to -9‰ in leaves that had P applied directly at roots. After 8 months, P-fertilized trees, regardless of the application method, showed evidence of responding by having longer internodes, thinner leaves, more leaves, new prop roots, and reproductive tissues. Control trees were unchanged. At the same time, δ^{15}N in leaves increased in all P treatments: direct root application, δ^{15}N=-2‰ and 1 m distant root application δ^{15}N=-4‰. In conclusion, P availability can explain the continuum of δ^{15}N values from negative to positive values, but not the extremely negative δ^{15}N themselves.

Although the potential sources of N for mangroves could include dissolved nitrate or amino acids in the sediment, the negative δ^{15}N is likely explained by the incorporation of an atmospheric NH_3 pool. Nitrate levels in unamended surface sediments from Twin Cays were rarely above 25% of the total dissolved N pool, and there were no indications that any sediment pool had a δ^{15}N different from 0‰ (data not shown). Amino acids in porewaters are another potential source of N for mangroves, although their uptake by mangroves has not been specifically documented. In a review by Lipson and Nasholm (2001), they state that in ecosystems where microbial activity and biomass have large seasonal cycles, organic N uptake might be a significant source of N. Again, however, the total sediment δ^{15}N, together with the decreased ^{15}N depletion in the roots compared to the leaves suggests amino acid uptake was not a significant source.

On Twin Cays, extensive microbial mats and floc zones have high N_2 fixation rates (Joye and Lee, 2004) resulting in high concentrations of underlying porewater NH_4. Emissions of NH_3 were highest over microbial mats especially under high light and temperature conditions. Typically, dwarf *Rhiozphora* trees had the most depleted N isotopic compositions when they were growing in or adjacent to microbial mats or floc zones. In transition and fringe zones, microbial mats were found to be less abundant and competitive with mangrove trees for N (Lee and Joye, 2006). NH_3 emissions

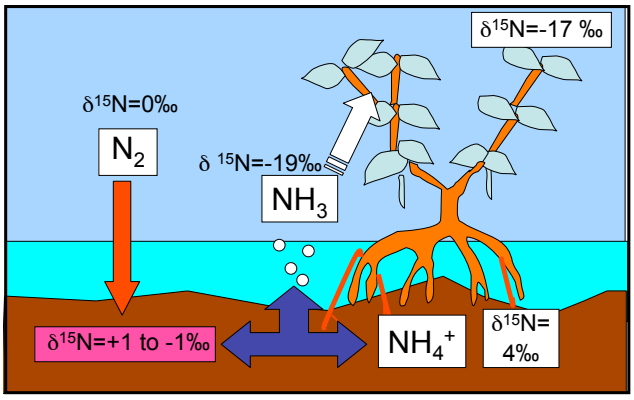

Fig. 7. Diagram for nitrogen isotope pathways in microbially-dominated mangrove ecosystems. Isotopically-depleted NH_3 is passively taken up by leaves, then incorporated into biomass.

in these tree-height zones were low or below detection. Mangrove trees growing in fringe zones had the most positive δ^{15}N. We conclude, therefore, that the microbial community influence on δ^{15}N in mangrove tissues could be the most important determinant in complex mangrove ecosystems.

Ammonia in the Twin Cays' atmosphere was always depleted in ^{15}N, with isotopic compositions averaging -19 ± 4‰ (n=20). Ammonium in rainwater (δ^{15}N=-10 ± 3‰; n=4) was also depleted in ^{15}N. Thus, uptake of N from atmospheric or rainwater pools is a potential explanation of the δ^{15}N of the most negative dwarf trees. This is further supported by the results of lichen analyses. The δ^{15}N of lichens collected from trees in the dwarf, transition, floc, and fringe zones of the island also have very negative values (as low as δ^{15}N of -22‰). Lichens, and other epiphytes, do not have roots, thus must use atmospheric sources of N for their nutrient requirements; therefore the possibility that an isotopically depleted N source is available to them for growth is strong (Hietz et al., 2002; Bouillon et al., 2004; Tozer et al., 2005). Additional fractionation may occur in the absorption of NH_3 via diffusive processes (Tozer et al., 2005). Because the extent and magnitude of this fractionation is not known, simple mass balance calculations for quantifying the proportions of atmospheric vs. root uptake in mangroves is speculative.

We propose that N isotope fractionation may be a passive process related to leaf proximity to volatilized ammonia and is proportional to the relative amount of uptake of N by the roots (Fig. 7). Vallano and Sparks (2007) proposed an equation for estimating the magnitude of foliar nitrogen incorporation:

$$\text{X foliar} = \frac{\delta^{15}\text{N leaf} - \delta^{15}\text{N soil N}}{\delta^{15}\text{N atmosphere} - \delta^{15}\text{N soil N}} \quad (1)$$

where in our mangrove ecosystem δ^{15}N soil N=4‰ and δ^{15}N atmosphere=-19.5‰. Admittedly, there are unknowns in

this simple mixing model because there is insufficient data to determine isotopic fractionations in foliar absorption and incorporation at this time. However, using this equation as stated, we calculated that dwarf mangrove trees on Twin Cays potentially derive 45±20% of their N through atmospheric input into leaf tissue. For taller fringing mangroves 20±7% of their N supply might originate from the air. Therefore, foliar uptake of ammonia is very likely an important, critical source of N for the Twin Cays mangrove ecosystem, as has been shown with terrestrial vegetation particularly in polluted areas and maybe increasing as atmospheric pollution increases (e.g. Krupa, 2003; Vallano and Sparks, 2007). Because P-limited mangrove trees put less energy into below ground biomass (e.g., McKee et al., 2007), foliar uptake may be especially important in trees that are P limited.

5 Conclusions

We conclude that P-limited dwarf mangrove trees, growing adjacent to ammonia sources, in addition to lichens on trees in floc and dwarf zones, obtain a portion of their N from atmospheric sources (i.e. ammonia) with isotopically distinct isotopic compositions from porewater N. The very negative δ^{15}N of mangroves and lichens on the oligotrophic islands of Twin Cays are key in estimating the primary and secondary nutrient limitations for this ecosystem. Twin Cays, located away from terrestrial runoff and with limited outside atmospheric influences, is an area where dwarf mangrove trees struggle to survive. We propose that their success depends in part on their taking advantage of the microbial community comprised of N-fixing cyanobacteria and other photosynthetic microbes. A small, but significant portion, of the ammonia fixed by microbes is released by physical processes into the atmosphere where it is available for uptake by leaves on trees growing in high salinity, low P, anoxic sedimentary porewaters. This strategy must also depend on leaf proximity to volatilized ammonia sources, such as exists at Twin Cays, and the resulting δ^{15}N reflects the relative amount of uptake of N by leaves and roots. Thus, foliar uptake of ammonia is a critical source of N for the Twin Cays mangrove ecosystem. Finally, we predict that in this and other highly oligotrophic ecosystems, δ^{15}N can be powerful indicators of the integrated P and N cycling.

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