ABSTRACT

Title of Document:	NUTRIENT ENRICHMENT EFFECTS ON MANGROVE SEDIMENTS IN DIFFERING TREE HEIGHT ZONES IN BOCAS DEL TORO, PANAMA
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Red mangrove (*Rhizophora mangle* L.) forests have distinct tree-height zones, with tall trees fringing the ocean and shorter trees in interior stands. A long-term nitrogen (N) and phosphorus (P) fertilization experiment in Almirante Bay, Bocas del Toro Province, Panama has shown that tree-height zonation is primarily related to nutrient limitation. This experiment was used to test the effects of *in-situ* nutrient additions and tree zonation on mangrove sediments. The sediments underlying the experimental *R. mangle* trees were sampled and N_2 fixation, $\delta^{15}N$, chlorophyll *a*, percent N and P, and percent organic biomass were quantified. Both N and P additions significantly affected almost every parameter measured in both zones within this experiment. These results are likely to have implications for management since N and P inputs are predicted to increase throughout the tropics and subtropics worldwide.

NUTRIENT ENRICHMENT EFFECTS ON MANGROVE SEDIMENTS
FROM DIFFERING TREE HEIGHT ZONES IN BOCAS DEL TORO,
PANAMA

By

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Introduction

Land-use change is driving ecosystem change worldwide, especially in the coastal zone (Lotze *et al.* 2006). Urban and agricultural development and overexploitation of natural resources have led to increased nutrients and sediments entering marine ecosystems and counteracted the ability of the coastal ecosystems, such as wetlands, to absorb and process these pollutants before they reach sensitive offshore ecosystems. Due to ecological and human factors, the impacts of ecosystem change in the coastal zone are proceeding more rapidly in the tropics than the temperate zone (Downing *et al.* 1999). For example, 40% of global applications of industrial nitrogen (N) fertilizer take place in the tropics and subtropics, and over 60% is expected to occur in developing regions by 2020 (Matthews 1994). This increased N use may lead to excess runoff into coastal ecosystems, threatening sensitive habitats such as seagrass beds and coral reefs, unless these nutrients are intercepted and processed in wetlands such as mangrove forests. Mangrove forests are major wetland ecosystems in the tropics and subtropics, but at least 35% of mangrove forests have been destroyed in the last two decades worldwide (Valiela *et al.* 2001). The combination of coastal ecosystem destruction and increasing nutrient inputs contributes to coastal eutrophication, one of the greatest threats to the marine environment throughout the world (U.S. Commission on Ocean Policy 2004).

Mangrove forest wetlands dominate the intertidal zone in many tropical and subtropical areas. They are essential for protecting shorelines from erosion and storm events (Danielson *et al*. 2005), providing habitat for animals including commercially important fish species (Mumby *et al.*

2003), and protecting adjacent habitats from sedimentation (Thampanya *et al.* 2006). Mangrove ecosystems have been cited to protect seagrasses (Valiela and Cole 2002) and coral reefs (Alongi and McKinnon 2004) from land-derived N loading (Fig. 1). Fishing activities within and adjacent to mangrove ecosystems provide employment and sustenance to coastal communities (Ngoile and Shunula 1992). Despite the potential benefits they provide for human populations, mangrove forests are being cleared at an accelerating rate for aquaculture and urban development and are considered among the most threatened environments in the world (Valiela *et al.* 2001).

Research on the effects of increased nutrient inputs is ongoing in mangrove ecosystems (Feller 1995; Feller *et al.* 2003; Lovelock *et al.*
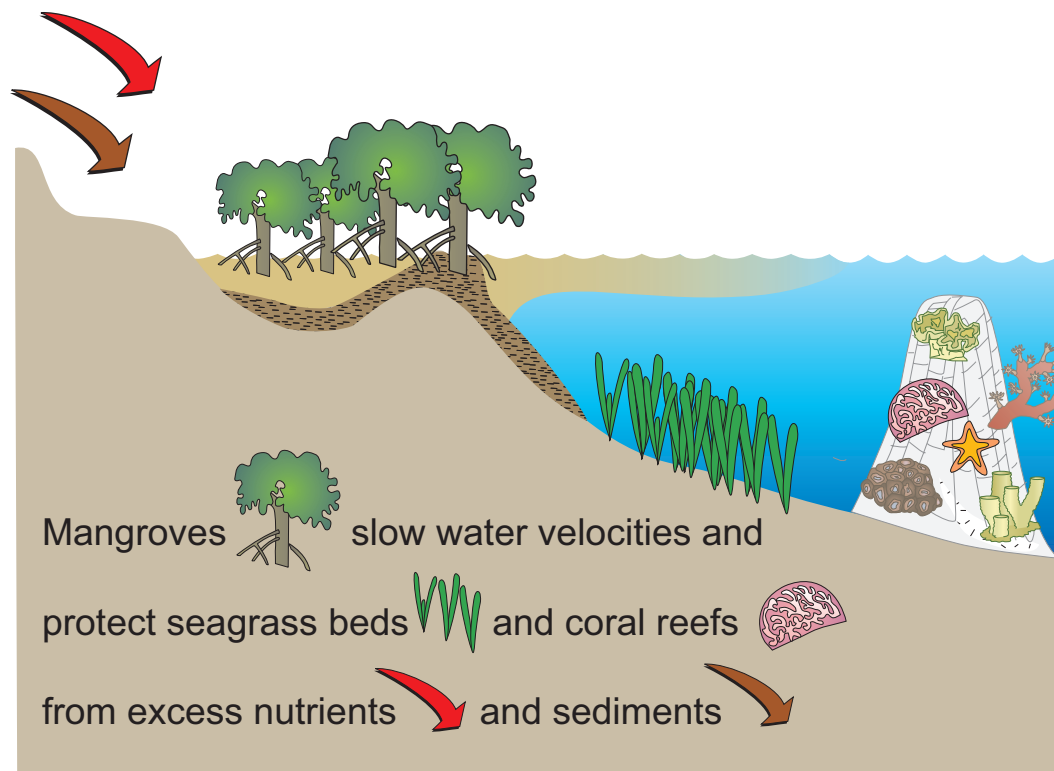

Figure 1. Mangrove ecosystems buffer seagrasses and coral reefs from nutrients and sediments. The benthic microbial community is essential for retention and processing of these pollutants (Symbols courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science).

2005). Most mangrove research has focused on large-scale aspects of the mangrove ecosystem, such as the effects of increased nutrients on the trees, while very few studies address impact of these nutrients on the sediment microbial community. This ubiquitous, but inconspicuous community is likely important for ecosystem functioning and nutrient cycling because microbes interact with the sediments and nutrients that are deposited, retained and processed within this system. Mangrove roots slow water velocities (Furukawa *et al.* 1997), limiting sediment transport. The shallow and warm benthic environment may provide an ideal environment for sediment microbes to assimilate, process and remineralize organic matter and nutrients (Alongi 1994).

Functioning of mangrove ecosystems is dependent on the sediment microbial activities that transform and recycle nutrients. The effects of eutrophication on the sediment microbial community are still poorly understood. Thus, this thesis seeks to elucidate some of the connections between the sediment microbial community and nutrient cycling within the mangrove ecosystem and seeks to answer the following questions:

Do long-term nutrient additions of nitrogen and phosphorus affect the characteristics and functioning of the sediment microbial community?

Do patterns of nutrient-limitation observed in the mangroves correspond to patterns in the sediment community?

Methods

Site description

The Bocas del Toro archipelago is located in Bahía Almirante on the northern Caribbean side of the Panama isthmus (9º 21'N, 82º 15'W) (Fig. 2). Carbonate sediments and a relatively small watershed characterize this lagoon, with channels allowing flushing to the Caribbean Sea (Carruthers *et al.* 2005). Small islands throughout the bay consist of mangrove trees perched on top of 3 - 4 m of peat overlying ancient coral reefs (Coates *et al.* 2005). Adjacent to these mangrove forests are vast seagrass beds and diverse coral reefs (Guzman *et al.* 2005). Since Bahía Almirante is outside the hurricane belt, the main natural disturbances are flooding and earthquakes, which may uplift and change the topography (Philips *et al.* 1997). Although mangrove forests are legally protected from destruction in Panama, humans clear mangroves for access to terrestrial forests in the centers of large islands and to build homes and docks at the water's edge, both directly and indirectly impacting mangrove and associated ecosystems.

The Bocas del Toro region has a high mean annual rainfall (3277 mm) (Kaufmann and Thompson 2005). The annual rainfall pattern does not have a pronounced dry season during the winter, but does have two distinct periods with lesser amounts of rain instead of just one (Kaufmann and Thompson 2005). High annual rainfall combined with a mountainous mainland watershed leads to large inputs of freshwater, nutrients and sediments into the bay. Total rainfall during January 2005 during this study was 691 mm which was greater than the 15-year average for the

Figure 2. A satellite image of the isthmus of Panama with the area of study, the Bocas del Toro archipelago, circled. The map in the right corner illustrates where Panama is located in context to the Caribbean and the United States (Credit: Jeff Schmaltz, MODIS Rapid Response Team, NASA/GSFC).

month of January (~180 mm) (Kaufmann *unpublished data*; Kaufmann and Thompson 2005).

Bordered by the Caribbean Sea, mangrove islands and reef barriers reduce the influence of winds and tides, creating a highly variable semi-lagoonal ecosystem. The tidal range of the Bocas del Toro archipelago is small (0.25 m) with complex patterns of changing diurnal tides (Cubit *et al.* 1989).

Three species of mangrove tree are found within the Bocas del Toro region, *Avicennia germinans, Laguncularia racemosa,* and *Rhizophora mangle*, but *R. mangle* dominates the mangrove islands of Bahía Almirante (Guzman *et al.* 2005). The forest selected for this study is typical for this region and throughout the Caribbean, consisting of a monoculture
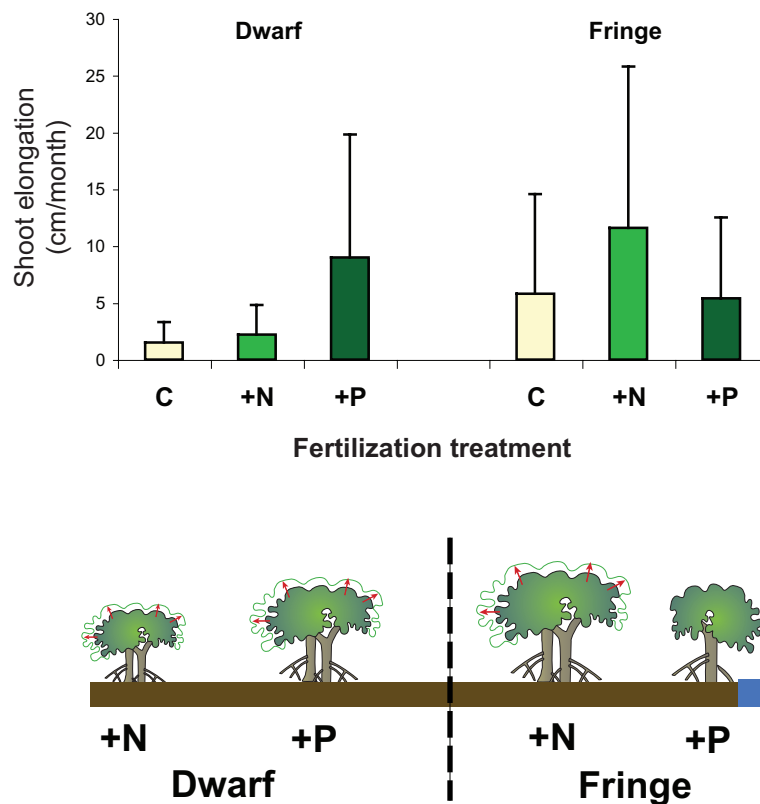
Figure 3. Long-term fertilization of N and P resulted in a small increase in growth in the N addition dwarf, an almost 10-fold increase of the P addition dwarf, and a 2-fold increase in the N addition fringe trees (Feller unpublished data).

of *R. mangle* with a tree-height zonation where the trees fringing the water are of taller stature (> 4 m), decreasing with distance from the coast, into large stands of scrub or dwarf forests ($\leq$ 1 m), which occupy over half the area of the study sites (Lovelock *et al.* 2005). This variation in tree-height in this area has been linked to nutrient limitation (Lovelock *et al.* 2004), although taller trees towards the interior of the islands have similar nutrient availability (such as available P) to the dwarf trees, so tree-height must be affected by several interconnected factors (Lovelock *et al.* 2005) (Fig. 3).

Experimental design

In 1999, a fertilization experiment was established to investigate the impacts of long-term nutrient inputs of nitrogen (N) and phosphorus (P) on the mangrove island ecosystems in Bahía Almirante (Feller *unpublished data*). Sites were selected on three different islands, Isla San Cristóbal, Cayo Solarte and Isla Popa, due to accessibility and to coincide with permanent seagrass plots set up by Guzman and Guevera (1998a; 1998b; and 1999). Although the sites were selected to be replicates, variability does exist amongst sites, such as distance from the mainland, hence freshwater discharge and nutrient inputs may also vary amongst sites (Fig. 4).

On each island, nine replicate trees were selected in the two zones, dwarf and fringe, for yearly fertilization for a total of five years. Trees were fertilized with either 300 g of urea as N fertilizer or triple super phosphate fertilizer as P fertilizer or cored and not fertilized for control. Intervals of 10 m were left between fertilization transects as buffer zones against possible lateral migration of fertilizer treatments. The fertilizer was delivered to the sediments by coring about 30 cm deep into the substrate, placing a 150 g dose enclosed within dialysis tubing and sealing the hole with a peat plug. Each tree selected for this study received two doses of 150 g for a total of 300 g per experimental tree. This coring method was used to ensure that fertilizer was not lost to tidal flushing. For the controls, the substrate was cored and sealed but not fertilized. These methods were based on previous studies, which resulted in dramatic growth increases in response to addition of the limiting nutrient and created small patches of fertilized trees immediately around the point of fertilization (Feller 1995, Feller *et al.* 2003).
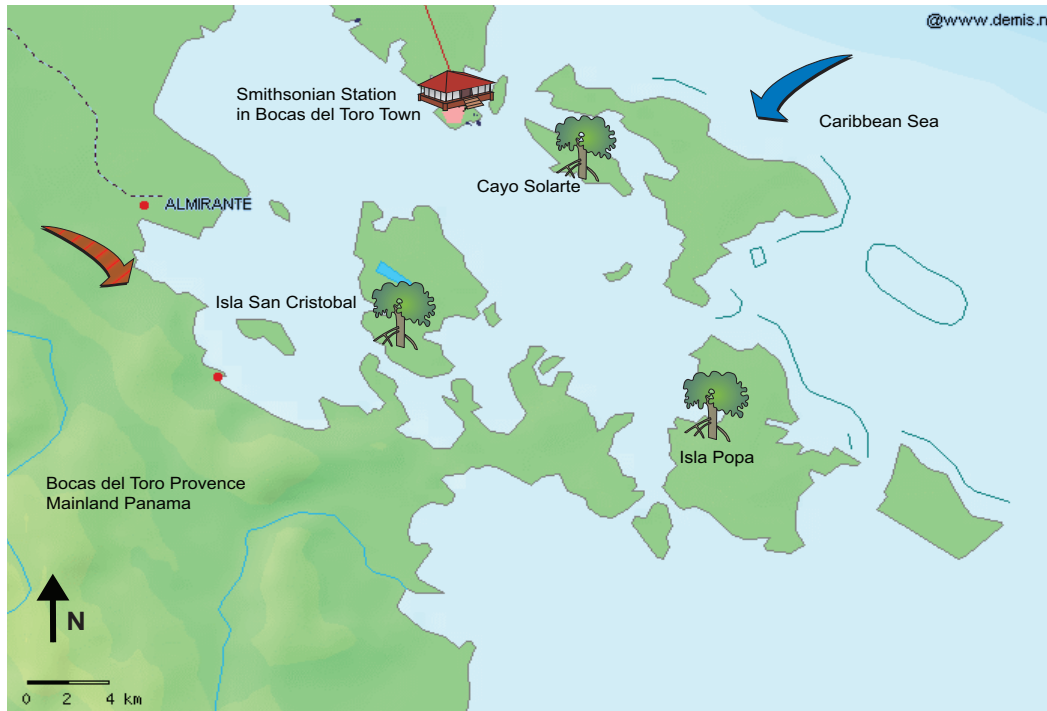
Figure 4. A map of Bocas del Toro archipelago with the study sites marked with mangrove trees. The bay receives oceanic flushing (blue arrow) from the Caribbean Sea, while sediment and nutrients (red/brown arrow) enter the bay from mainland Panama (image courtesy of www.demis.nl).

In an experiment in Belize, with the same experimental design, fertilization with N or P caused a significant increase in the bioavailability of NH_4-N or PO_4-P in the sediments, respectively, which may indicate that nutrients were available for uptake by the sediment microbial community (McKee *et al.* 2002).

Sample collection

Prior to sampling, mangrove sediments were last fertilized in April 2004. At the time of fertilization, the area under the roots within 10 cm of the fertilization was marked off with flagging to prevent trampling. All samples were collected in January 2005 from the flagged area. Small sediment cores (2 cm deep) were collected for $\delta^{15}N$, nutrient and pigment analysis using a 30 cc syringe corer.

Nitrogen fixation

Nitrogen fixation was measured on sediment cores using the acetylene reduction assay (Capone 1988). This assay is a substrate analogue assay that exploits the non-specificity of nitrogenase, the enzyme used to fix nitrogen, for its natural substrate and its capacity to reduce other triply bonded small molecules. Nitrogenase reduces acetylene (C_2H_2) to ethylene (C_2H_4), and the change in concentration of ethylene is quantified to calculate enzyme activity (see below).

A 2 cm deep peat core was collected under each of the experimental trees within 10 cm of the point of fertilization using a 5 cc syringe. Any obvious leaves or foreign matter were removed, but mangrove roots were not separated from cores. Whole cores were used for the assay to keep the vertical zonation and structural integrity of the sediment microbial community intact.

Samples were immediately transferred to the Smithsonian Marine Station in Bocas del Toro for processing. Whole sediment cores were placed in 15 mL flasks. Flasks were then sealed with rubber 'subaseals,' through which 0.6 mL of acetylene gas (collected from an acetylene tank after being passed through water to remove impurities) was added (10% of the headspace). The flasks were incubated outside under full natural light and ambient temperature (25 - 27°C). Ethylene production was quantified using a flame ionization gas detector (FID) on a Shimadzu Gas Chromatograph 8, using ultra-pure carrier grade N as the carrier gas.

The molar amount of ethylene produced per gram sediment per time is referred to as the 'nitrogenase activity rate'. To calculate nitrogen fixation rates, total ethylene volumes were converted to total volumes of

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fixed N by applying the theoretical 3:1 ratio of moles acetylene reduced: moles of dinitrogen fixed (Postgate 1982). Total micromoles of N were calculated and plotted against incubation time. Rates of N_2 fixation were calculated with linear regressions and rates among samples were compared.

Since there was considerable lag between the beginning of the incubation and the initial production of ethylene, rates were calculated for the first 24 h after an initial lag of 10 h. The analysis was conducted twice for each site.

Organic content

Following the final measurement of acetylene reduction, the sediment cores were dried at 70°C, weighed and combusted in a muffle furnace at 500°C for 24 h to remove organics and reweighed. Organic matter was expressed as the percent difference between ashed and dry weights (Gaudette *et al.* 1947).

$\delta^{15}N$ *and nutrient content*

Sediment and senescent leaf samples for nutrient and $\delta^{15}N$ analysis were dried at 70° C and ground for 3 min with a stainless steel ball grinder. $\delta^{15}N$ signature and N concentration were determined on a continuous flow isotope ratio mass spectrometer at UC Davis Stable Isotope Facility. Stable isotope ratios are reported with reference to a standard. The difference between the material under consideration (in this case the sediments or leaves) and the standard is expressed in parts

per thousand according to the following formula: $\delta^{15}N = [(\delta^{15}N/\delta^{14}N_{sample}/\delta^{15}N/\delta^{14}N_{standard}) - 1] \times 10^3$. The standard for $\delta^{15}N$ is atmospheric N. Phosphorus concentration was determined using an inductively coupled plasma spectrophotometer (ICP) (Analytical Services, Pennsylvania State University, State College, PA).

Pigments

Small sediment cores (2 cm deep) were collected for benthic chlorophyll *a* (chl *a*) analyses using a 30 cc syringe corer. Cores were processed within the same day of collection, using methods adapted from Parsons *et al.* 1984. Cores were extracted into a mortar and pestle and ground with 90% acetone. The mixture was placed in a centrifuge tube, which was filled to the 30 mL mark, and refrigerated in the dark for at least 5 h before extraction. Samples were shaken to resuspend the sediment and centrifuged. Chl *a* was measured using absorbance at 665 nm on a Spectronic spectrophotometer. Chlorophyll concentrations were calculated using the equations of Jeffries and Humphrey (1975).

Statistics

Two-way analysis of variance (ANOVA) testing analyzed for the significance of tree zone and site for the controls of all sites. Tukey's test was used as a post-hoc test for the controls and individual sites with a significance of $P < 0.05$.

All sites and treatments together were analyzed with a three-way mixed model ANOVA for the significance of zone, treatment and site. Within each individual site, two-way ANOVA testing for zone and

treatment was performed. When ANOVA found a significant interaction between nutrient treatment and zone, Least Square Means with the Tukey adjustment was used to examine pairwise differences within and among the treatment levels.

All data were analyzed for normality using the Shapiro-Wilkes test and homogeneity using Levene's Test for Homogeneity. Analysis on the controls was performed with SPSS 11.0 (SPSS, Inc. Chicago, IL, USA). All other data was analyzed using SAS (SAS Institute Inc, Cary, NC).

Results

Comparison of site controls

Patterns of nutrient limitation observed in the macrophyte community, with reduced growth in the dwarf zone and increased growth in the fringe zone, were similarly seen in the sediment microbial community. However, unlike the macrophyte community, this pattern varied among sites largely following a gradient from land to sea. Rates varied with distance from San Cristóbal, the island closest to land, to Isla Popa, the island farthest distance from land and more impacted by oceanic inputs.

Nitrogenase activity rates in the control sediments, as measured by the acetylene reduction assay, were greatest in the fringe zone at Isla Popa and in the dwarf zone at Cayo Solarte (Fig. 5A). Nitrogenase rates were lowest in the Isla San Cristóbal controls in both zones. These results varied significantly among sites but not zones and did not correlate with the other parameters measured (Fig. 5A).

Percent N in the control sediments in both zones increased with
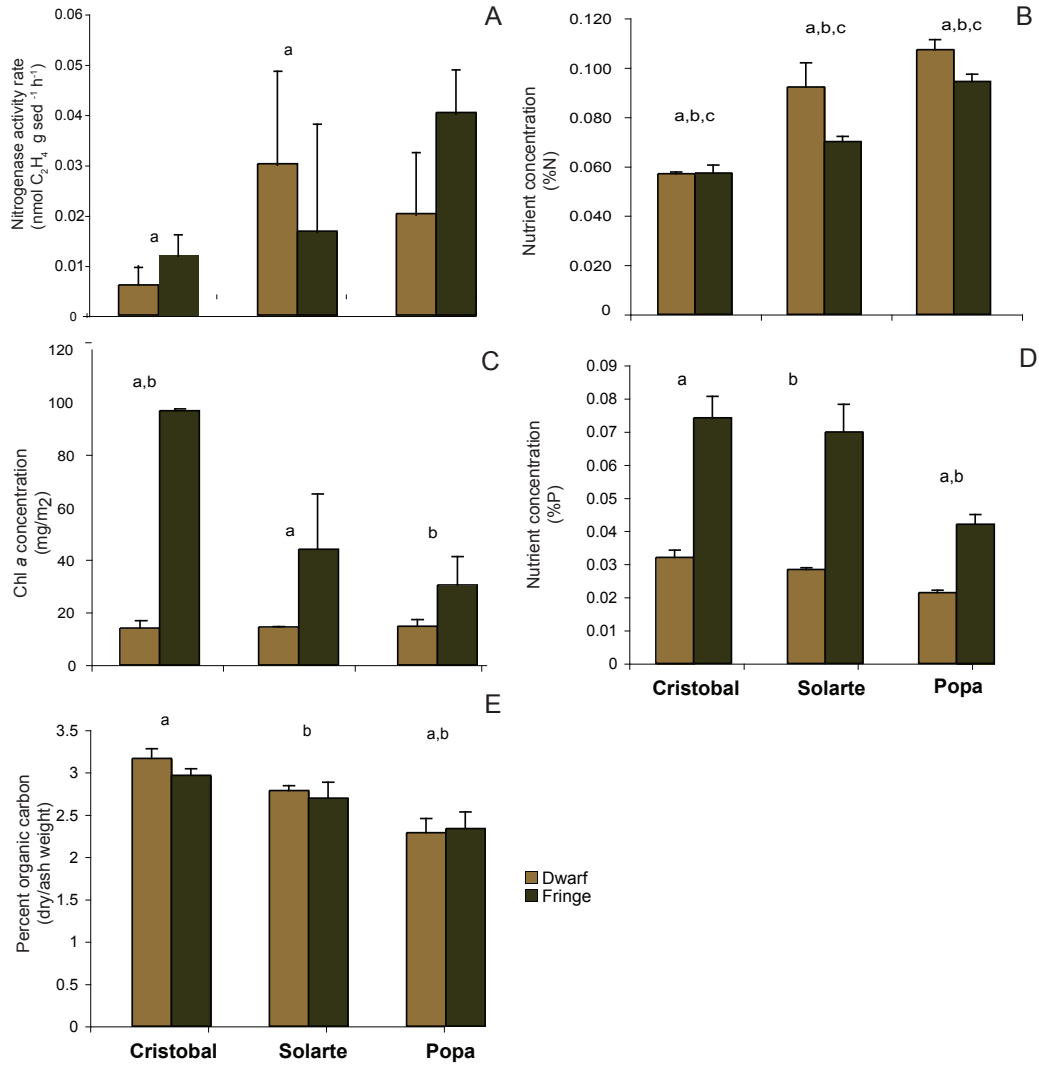
Figure 5. Comparison of site controls of sediments. Different lower case letters represent significant differences ($p < 0.05$) amongst sites.

distance from land, (greatest at Isla Popa and least at Isla San Cristóbal) (Fig. 5B), while percent P decreased (Fig. 5D). Both organic content of sediments and photosynthetic biomass, as measured by chl *a*, in the fringe zone decreased with distance from the mainland (Figs. 5C & E).

Dwarf/fringe comparison

Nitrogenase activity rates did not vary significantly between the dwarf and fringe zones, although the dwarf zone had relatively high rates at Cayo Solarte, while the fringe zone had high rates at Popa (Fig. 6a).

The lack of significant difference may indicate that N_2 fixation is not an important source of N to the macrophyte community, since the patterns of nitrogenase activity rate did not follow the patterns of macrophyte growth. However, the low nitrogenase rates during the sampling period may be attributable to seasonal effects, such as high rainfall seen during the sampling period. More static parameters measured seem to indicate that N_2 fixation could be an important source of N in the the dwarf zone. For example, $\delta^{15}N$ values of the sediments, which are often indicative of N_2 fixation, did vary significantly among zones and were much lower in the dwarf than the fringe zone, which may indicate greater N_2 fixation in this zone (Fig. 7a). Also, greater N content was found in the sediments in the dwarf zone (Fig. 6b). However, the $\delta^{15}N$ values of the senescent mangrove leaves, with greater values in the dwarf zone than the fringe,
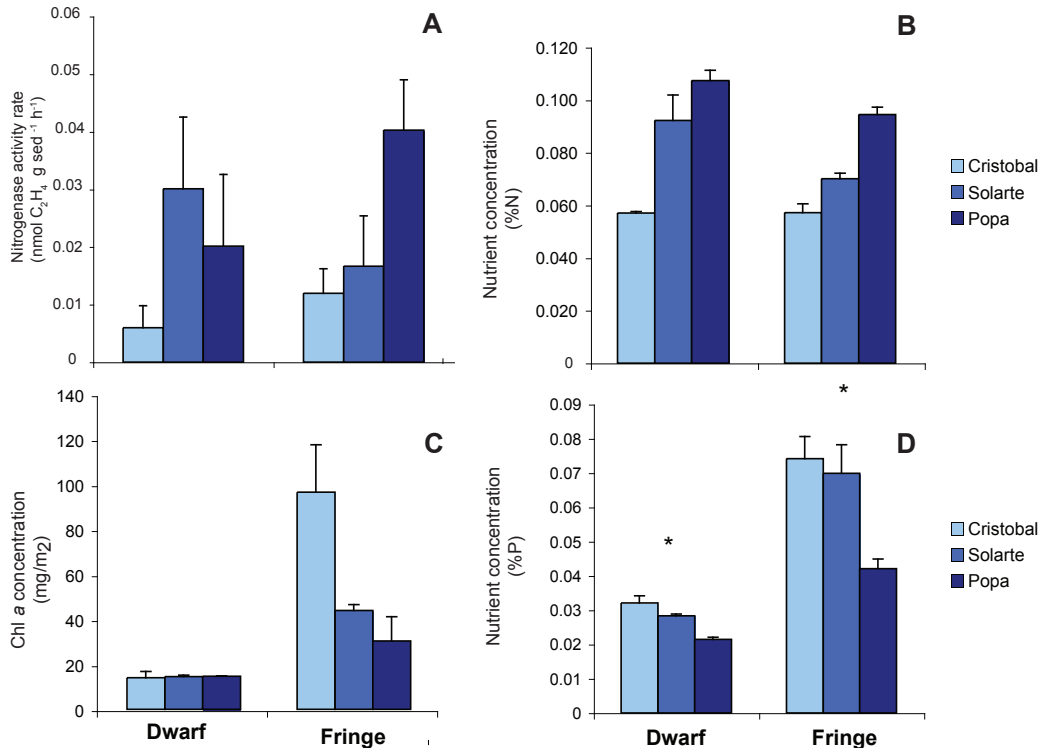
Figure 6. Comparison of dwarf and fringe zone control sediments. Astericks represent a significant difference ($p < 0.05$) between the dwarf and fringe zones.

did not reflect the same values as the sediments, perhaps indicating that the red mangrove trees are either not utilizing the N fixed by the sediment microbial community or are not selecting for one isotope of N over another (Fig. 7B).

Chl *a* and P content was greater in the fringe zone than the dwarf, possibly from greater external inputs from increased tidal flushing (Fig. 6C and D).

Fertilization response

The sediment microbial response was similar to the macrophyte response with nutrient enrichment. Similar to the trees, which increased in size with P fertilization, nitrogenase activity rates in the dwarf zone had a strong response to P fertilization at all sites (Fig. 8). Within the dwarf zone, the control and the P additions were significantly different from each other (p = 0.0001). With N fertilization, nitrogenase rates did not vary significantly from the control. When all sites were included, there was a

Figure 7. A comparison of sediment and mangrove leaf $\delta^{15}N$ values in the control zones.

significant treatment effect ($p < 0.0001$), but not a significant site or zone effect, although there was a significant treatment X zone ($p = 0.0003$) and site X treatment effect ($p < 0.0001$). Zone X treatment effects were: N X fringe, P X dwarf, and P X fringe (all $p = 0.0001$).

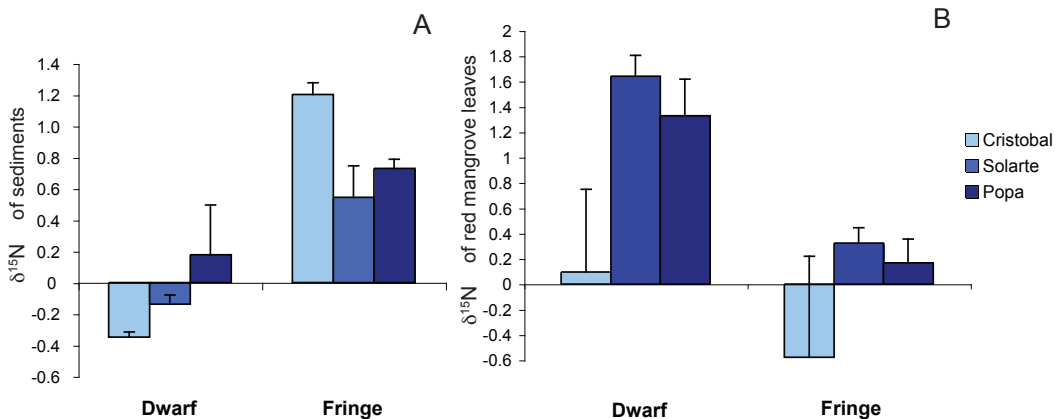The response of the sediment microbial community to fertilization varied within sites. At Isla San Cristóbal, P fertilization resulted in the greatest nitrogenase activity rates in both the fringe and dwarf zone among all sites. N_2 fixation was not detected in the controls at this site (Fig. 8a). In both Cayo Solarte and Isla Popa's fringe zones, nitrogenase rate was greatest at N fertilized plots, similar to the growth patterns seen in the N-fertilized fringe trees.

$\delta^{15}N$ is often used as both an indicator of N_2 fixation, with lower numbers indicating atmosphere N or an addition of N fertilizer created from atmospheric N. Within the dwarf zone, N addition significantly depleted $\delta^{15}N$ ($p < 0.0001$) (Fig. 9). This result suggests a signal from the N fertilizer used in the fertilization experiment, since this result is contrary to the results from the acetylene reduction assay, which detected no difference in nitrogenase activity rates in the dwarf control and N-fertilized treatments. P additions also significantly decreased the $\delta^{15}N$ values in the dwarf zone from the control ($p < 0.0001$), reflecting the results of greater nitrogenase activity rates in the dwarf zone (Fig. 9). In the fringe zone, $\delta^{15}N$ values were reduced with both N and P fertilization, although not significantly, suggesting that N_2 fixation or the contribution of external N may not be as an important in this zone.

Percent N of the sediments did not vary significantly amongst the controls and the fertilized treatments at any of the sites (Fig. 10). Chl *a* of the sediment microbial community in the dwarf zone followed a similar
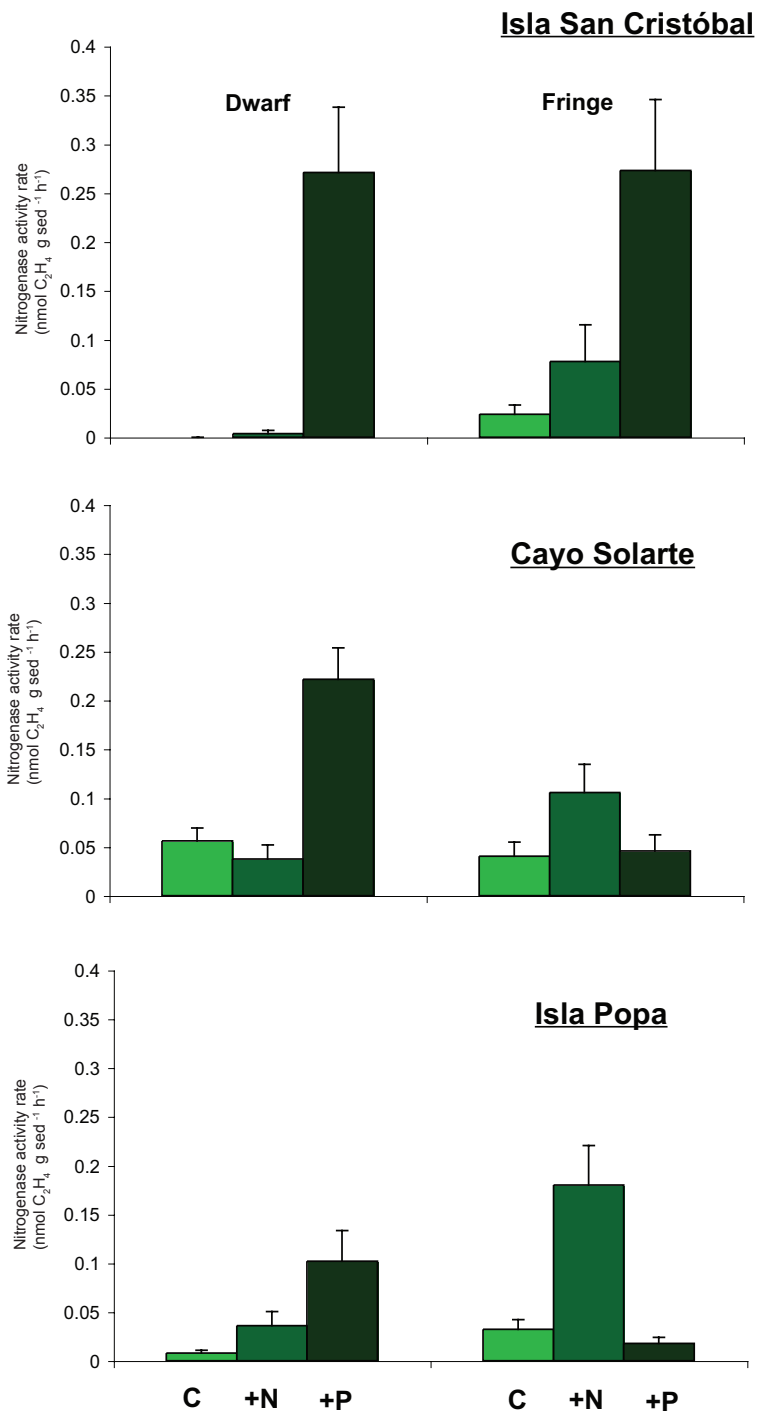
Figure 8. Rates of N$_2$ fixation as measured by the acetylene reduction assay for the three sites. Treatment codes are C = control, +N = nitrogen addition, and +P = phosphorus addition.

Figure 9. $\delta^{15}N$ values for the three sites. Treatment codes are C = control, +N = nitrogen addition, and +P = phosphorus addition.
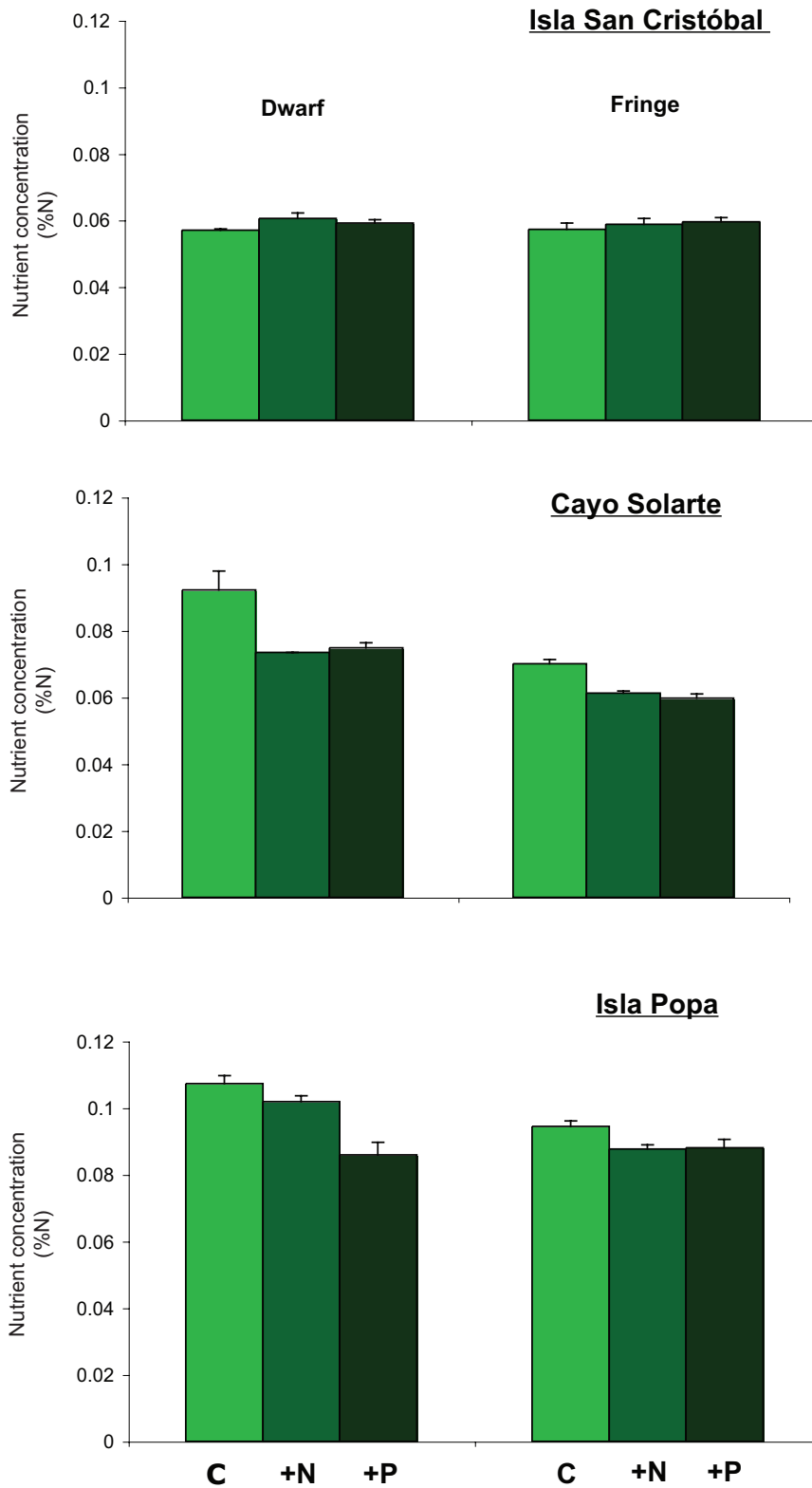
Figure 10. %N for the three sites. Treatment codes are C = control, +N = nitrogen addition, and +P = phosphorus addition.
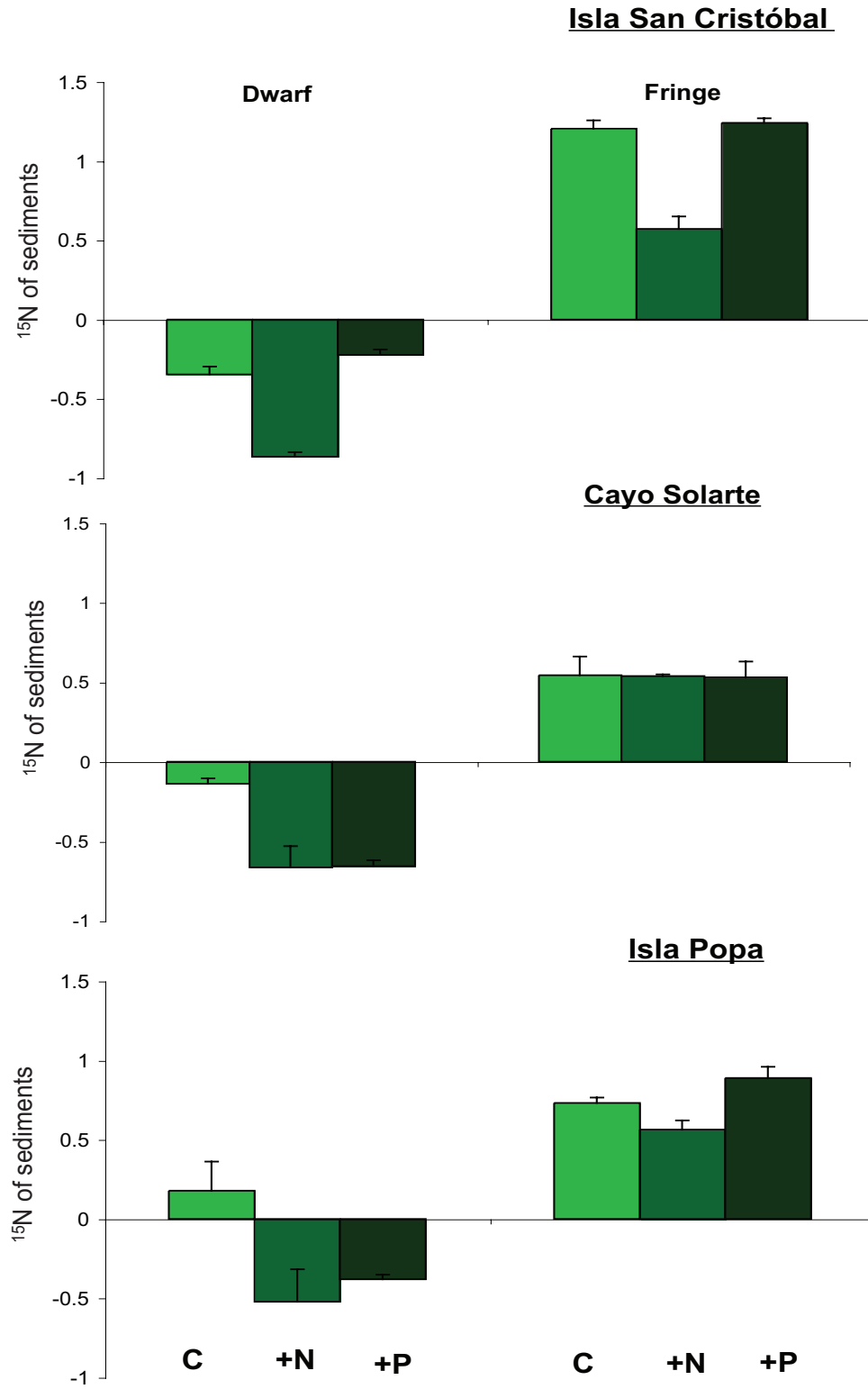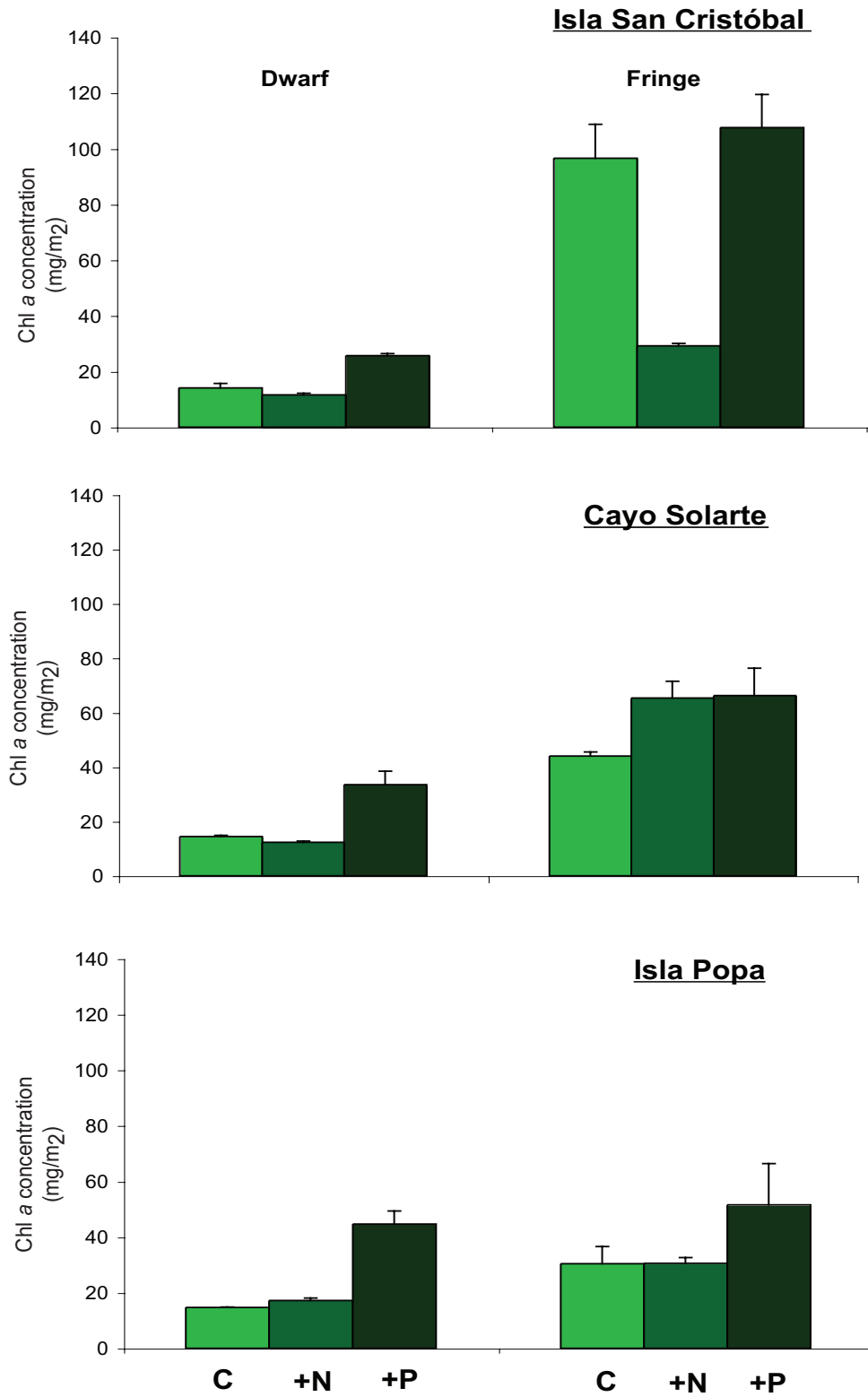
Figure 11. Chlorophyll *a* for the three sites. Treatment codes are C = control, +N = nitrogen addition, and +P = phosphorus addition.

pattern to that found in the macrophyte community (Fig. 11). The patterns

of chl *a* results were also similar to nitrogenase activity rates results in

the dwarf zone, with no significant difference between the control and N

addition treatment but an increase in chl *a* in the P fertilized zone. In the

fringe, N addition reduced the amount of chl *a* compared to the control,

while P addition increased the amount of chl *a*. There was a significant

treatment effect (p = 0.0016) and a significant zone effect (p < 0.0001), but

not a significant zone X treatment effect.

Discussion

The most significant pattern of nutrient limitation in the macrophyte

community is the difference in growth seen between the fringe and the

dwarf zones. This difference has been partially attributed to nutrient

limitation, which may be related to the sediment microbial community,

which creates 'new' nitrogen through N_2 fixation. In turn, zone-specific

differences in tree-height may create microhabitats in the environment

for the sediment microbial community. Differences in solar radiation,

temperature and flow created by the physical differences between the

taller trees on the fringe and the shorter dwarf trees within the interior of

the islands may create diverse microhabitats. Thus, tree-height variability

on the scale of meters may greatly influence microenvironment variability

on the scale of micro-meters to millimeters.

Site comparison

Although the mangrove sites were selected as replicates,

environmental and geographical differences exist among the sites. The

experimental sites were on islands of differing sizes and distances from the mainland, with Isla San Cristóbal being the closest to the mainland inputs of nutrients and sediments. Isla Popa is classified in many studies as more affected by oceanic inputs (Carruthers *et al.* 2005). Cayo Solarte is in the middle of the gradient, closer to Isla San Cristóbal and closest to Isla Colon and Isla Bastimentos, where the two major settlements in Bahía Almirante are located (Lovelock *et al.* 2005).

Environmental factors

While salinity was not significantly affected by zone when the sites were grouped, within sites significant differences in salinity were observed, which may have influenced the differing characteristics of mangrove sediments in response to nutrient fertilization (Lovelock *et al.* 2005). Cayo Solarte had significant differences in salinity between the dwarf and fringe zone, with fresh water inputs in the dwarf zone. Isla San Cristóbal also had significant differences between the zones, with freshwater inputs in the fringe zone. Isla Popa had no significant differences in salinity between the zones (Lovelock *et al.* 2005). Nitrogenase activity rates followed similar within-island patterns of salinity, with greater rates in the Cayo Solarte dwarf compared to that of the fringe zone and significantly greater rates in the Isla San Cristóbal fringe than the dwarf zones ($p > 0.00$) and no significant difference between the Isla Popa zones. However, salinity did not significantly correlate with nitrogenase activity rates at any of the sites, which may be an artifact of the relatively low N compared to the high natural variability and patchiness of the sediments. Differences in salinity may support a variety of microhabitats for the sediment microbial community, which may increase diversity and allow quick adaptation to

variations in climate, such as the high rainfall and low temperatures found during this study (Rejmánková *et al.* 2004).

Distance of the dwarf zone from the fringe zone and the coastline may also affect the characteristics of the sediments. Isla Popa for instance has a more narrow fringe/transition zone (i.e. < 25 m wide), with the dwarf zone closer to the ocean than either Isla San Cristóbal or Cayo Solarte where the dwarf zones are ~50 m from the coast (Lovelock *et al.* 2005). This could be a reflection and a cause of some of the differences seen among the sites since Isla Popa's dwarf zone may be more exposed to oceanic flushing. Although the entire Bahía Almirante is microtidal (0.25 m), it is uncertain how much tidal flushing reaches the dwarf zone. Inundation and desiccation on a tidal basis have been shown to affect community composition and N_2 fixation patterns in mangrove microbial mats in Belize (Lee and Joye 2006).

Nutrient gradient from land to sea

Bahía Almirante is a small bay almost entirely surrounded by land with a limited watershed, yet the bay is influenced by oceanic circulation from the adjacent Caribbean Sea. Whether the water quality of the bay is more lagoonal and impacted by high nutrients or more influenced by the Caribbean Sea and oligotrophic is uncertain. A previous study (Carruthers *et al*. 2005) reported that high values of nutrients in the Bay were correlated with high abundances of *Thalassia testudinum*, suggesting the Bay was largely influenced by lagoon inputs and received relatively little oceanic flushing. However, a study of plankton by D'Croz *et al.* (2005) found that Bahía Almirante's water quality, especially when compared to the nearby Laguna Chiriquí, was closer to oceanic.

Comparing the fringe zone controls, many of the measured mangrove sediment parameters showed a gradient from the major mainland rivers toward the Caribbean Sea. Percent N in sediments in both zones increased with distance from mainland while %P decreased. This may reflect terrigenous sources of P, while N has a variety of sources, including N_2 fixation. Nitrogenase activity rates also increased in the fringe controls with distance from mainland, possibly also accounting for some of the increased percent N in the sediments. Both organic biomass and chl *a* in the fringe decreased with distance from the mainland, also possibly reflecting an increased uptake of nutrients and corresponding increases in productivity.

Due to the short distance from the mountains to the coast and high rainfall throughout the area, water, sediments and nutrients enter the bay in pulses from mainland. The water from these outflows may form a superficial lens of water rich in organic matter available for the microbial community. Water temperature data showed very little vertical mixing during much of the year; this is consistent with previous observations that waters at 2 m and 4 m depth were much colder than at 20 m depth (Kaufmann and Thompson 2005). Although these data may only apply to the local geographic area near Isla Colon where the water temperature measurements were made, Isla San Cristóbal and perhaps Cayo Solarte may also experience this pulse of freshwater coming off the mainland. Unfortunately, these data only exist for Isla Colon. Isla Popa, situated closer to the Caribbean Sea, may be more mixed and have more oceanic influences than Isla San Cristóbal or Cayo Solarte, which are more influenced by the mainland. This lack of mixing may also explain why, despite seeing a nutrient gradient in the mangrove tree response, this

gradient is not seen in the seagrass bed which experienced uniformly high nutrients throughout the bay. Using *T. testudinium* as a bioindicator of water quality and nutrient status, N and P content of leaf tissues were found to be high relative to the Caribbean (Carruthers *et al*. 2005). It was hypothesized that high nutrient availability was partially a consequence of high, pulsing runoff from the mainland (Carruthers *et al*. 2005). Such pulses may be influenced by continued change of land use, principally the conversion of forested land to more cropland, resulting in more nutrients and sediments. This hypothesis may be supported by a shift in coral communities seen in the Bahía Almirante, from *Porites* spp. to *Agaricia* spp., which is suspected to be related to shifts in water quality over the last few decades (Aronson *et al.* 2004). However, coral cover and diversity are relatively high in Bahía Almirante, with 33 species and coral to a depth of 40 m, compared to the nearby Laguna Chiriquí, which has seven species and coral only to a depth of 3 m (Guzman and Guevara 1998b).

$\delta^{15}N$ values are often used as an indicator of sources of N, but in this study the sediments did not show a gradient and did not vary significantly among the sites. $\delta^{15}N$ values of the sediments in the fringe were relatively low (0.83‰) compared to the seagrasses (3.19‰) but still higher than the dwarf zone and may indicate a variety of local sources of N for the fringe trees, including N_2 fixation and external inputs, such as seagrass and macroalgal wrack. The $\delta^{15}N$ of the fringe trees was very low (-0.26‰) and more depleted closer to land (-0.52‰ at Isla San Cristóbal compared to 0.36‰ at Isla Popa), indicating possible uptake of N from N_2 fixation. The island sediments were very patchy, with large (~8 - 20 cm diameter) laminated cyanobacteria mats (Joye *pers. comm.*) occurring at Isla Popa in the dwarf zone and at Isla San Cristóbal the transition

between dwarf and fringe. Cayo Solarte has small (~1 cm) patchy mats occurring on leaf and wood fragments within the dwarf. This heterogeneity of sediments may indicate sources of N not sampled in this study that may be having an impact on nitrogen cycling and the $\delta^{15}N$ values.

Differences in tree-height zones

Within all sites there were distinct differences between the sediments of dwarf and fringe zones. Dwarf zone sediments had significantly less P and chl *a* than the fringe zone, but significantly more N. The sediment microbial community may serve as a significant source of N to the dwarf mangrove trees via N_2 fixation, which may alleviate N limitation and contribute to the P limitation of this zone. This may be reflected by the lower $\delta^{15}N$ values in the dwarf sediments compared to the fringe zone.

Fringe zone $\delta^{15}N$ values were also low but slightly higher values than the dwarf. This may reflect nutrients from the water column, as well as seagrass and macroalgal wrack. Sources of N for the fringe could include epibiont sponges, macroalgae and cyanobacteria that often occur on the submerged roots in the fringe zone (Diaz 2005; Bashan *et al.* 1998, France *et al.* 1998).

Although leaf area index, one measurement of light availability and canopy cover over the sediments, did not correlate with any parameters included in this study, it was significantly different between the fringe and dwarf zones ($p < 0.0001$) (Lovelock *et al.* 2005). Within the monotypic forest of *R. mangle*, light availability provides a variety of environments for microbes to exist. Increased canopy shading in the fringe could prevent desiccation and provide protection from heat, while the dwarf zone's lack

of shading could provide more sunlight for photosynthesis and create hypersalinization from increased evaporation.

Light limitation has also been cited as a factor for low chl *a* biomass in mangroves (Alongi 2005). However, the amount of chl *a* in the dwarf zone was much less than that in the fringe zone, indicating nutrient limitation or another stressor on the microalgal community. Light limitation was also not correlated with N_2 fixation as illustrated by the higher rates of acetylene reduction in the P fertilized dwarf sediments, which were increased in size and hence shading of the sediments.

Nutrient availability and immobilization also varied across zones. Increased tidal flushing in the fringe delivers external nutrients, such as P, and removes potential stressors, such as sulfide. The root structure of the fringe zone retards the flow of water allowing the sediments to drop out before reaching the dwarf zone, even when it is inundated. Phosphorus content of the sediments was significantly greater in the fringe than in the dwarf zone. A study at Isla San Cristóbal found that extractable P decreased with distance from the water but sulfide increased (Lovelock *et al*. 2004).

Mangrove sediments as bioindicators

Coral reefs and seagrass beds are often seen as indicators of water quality of coastal areas in tropical areas (Udy and Dennison 1997; Spurgeon 1992), but they do not always exist in close proximity with coastal areas in question. Water quality and phytoplankton may be used as indicators but vary quickly with rainfall events and seasonal fluctuations. In addition, SCUBA equipment, boats and other expensive expenditures are often necessary to monitor offshore habitats. In contrast,

mangrove forests are often accessible by walking. Due to pulses of fresh water and rates of mixing, mangrove sediments may be a better indicator than coral reefs and seagrass beds, since the water column can be stratified and upper nutrients are mixed first, before mixing with the rest of the water column.

Nitrogen fixation has been suggested for use as an indicator of ecosystem health (Holguín *et al.* 2006) since some have reported a correlation of N pollution and the cessation of N_2 fixation (Holguín *et al.* 2001). Nitrogen fixation incurs a large metabolic cost for the diazotrophic organism (16 ATP per N_2 reduced) and can be inhibited by NH_4^+ availability (Postgate 1982; Capone 1988). However, in the present study nitrogenase activity rates increased in the fringe zone sediments with N additions. Other studies have noted the increase or lack of suppression of N_2 fixation with the additions of N (Lee and Joye 2006; Paerl *et al.* 1998). Nitrogen additions may not have been large enough to cause the supression of N_2 fixation.

$\delta^{15}N$ is also often used as an indicator of N_2 fixation (Rejmánková *et al.* 2004) and of increased anthropogenic N inputs such as sewage (Udy and Dennison 1997; Cole *et al.* 2005). Low $\delta^{15}N$ ratios (~0‰) reflect N_2 fixation or N fertilizer which is derived from the atmospheric N whereas high $\delta^{15}N$ ratios (~10‰) reflect anthropogenic sources of N such as sewage (Grice *et al.* 1996; Voss and Struck 1997). However, there was no correlation between nitrogenase activity rates or the percent N in the sediments and $\delta^{15}N$ values. Different sources of N coming from both within and outside the ecosystem may have confounded any clear indication of N_2 fixation or external N. The $\delta^{15}N$ values may indicate that colder water temperatures and high rainfall changed normal N_2 fixation patterns during the time

period of this study. Further investigation of year-round rates and larger spatial sampling may clarify the relationship between $\delta^{15}N$ measurements, N_2 fixation and external N.

The differences seen in the seagrass, mangrove, and phytoplankton nutrients and sediment nutrients suggest that perhaps no one aspect of the Bocas del Toro ecosystem should be used as an indicator. Several parameters need to be studied in order to more comprehensively understand how nutrients are transported and retained among components of this ecosystem. For example, the mangrove sediments and the *R. mangle* fringe tree leaves showed a N gradient from the mainland to the ocean (Fig. 6B), similar to D'Croz et al. (2005) findings in water quality. However, Carruthers *et al.,* (2005) did not find a significant difference in %N in the seagrass leaf tissue. To get a larger view of nutrient distribution within the larger ecosystem, multiple measures of ecosystem function may need to be sampled in conjunction with each other.

Response to phosphorus fertilization

Phosphorus is an important nutrient, known to be limiting or co-limiting with N, especially in carbonate mangrove ecosystems (Feller 1995). Phosphorus has a strong binding affinity for sediments rich in calcium carbonate and may be less available to plants or the microbial community when these types of sediments are present, commonly in tropical and semi-tropical waters (Lapointe *et al.* 1992). Sediments in Bahía Almirante are predominantly coarse carbonate sands and may be a sink for P (Carruthers *et al.* 2005).

The fertilization of the dwarf zone sediments with P resulted in a clear boost in nitrogen fixation rates at all sites. The dwarf trees also

responded to P fertilization with a 10-fold increase in size (Lovelock *et al.* 2004, Feller *unpublished data*). This may be partially the result of feedbacks between the trees and the sediment microbial community. When initially fertilized, the dwarf zone trees responded to both N and P fertilization, with a 2-fold increase in size with N fertilization, indicating that the trees are possibly co-limited by both N and P. With the fertilization of P, the diazotrophs may increase the production of N, possibly explaining the dramatic change in the P fertilized dwarf trees. In turn, the dwarf trees produced more P-rich leaves (Feller *unpublished data*), giving the microbial community a rich substrate for increased N_2 fixation.

The sediments under the fringe trees did not share the same patterns of nutrient-limitation as the sediments under the dwarf trees. While Isla San Cristóbal's fringe P treatment showed an increase in nitrogenase activity rate over the control, neither Isla Popa nor Cayo Solarte had a significant change from the control. However, at all sites, nitrogenase activity rates increased in the fringe with N fertilization, suggesting the sediments were similarly N limited as the fringe trees (Feller *unpublished data*). One theory put forth to explain increases in N_2 fixation with N fertilization is when sediments are stimulated by N additions; heterotropic O_2 respiration becomes stimulated, which decreases O_2 inhibition of N_2 fixation (Lee and Joye 2006). When N_2 fixation rates from all sites were combined and averaged, both N and P were found to increase N_2 fixation in the fringe trees, suggesting co-limitation within the mangrove environment.

Lack of external inputs of P in the dwarf zone may help explain the dramatic differences in response of both the trees and the sediments to P fertilization. Extractable P declined from the fringe to the dwarf stands

at Isla San Cristóbal (Lovelock *et al.* 2005). Within the controls, there was a significant difference in the amount of P found in the sediments between the dwarf and the fringe zones. Increased P concentration in the P-fertilized dwarf zone suggests that the fertilizer P is being distributed through the sediments and retained within the ecosystem. Alternatively, external P may be flowing into this area coincidently.

Several studies have found little to no effect of short-term (< 1 month) nutrient additions on rates of N_2 fixation in sediments (Lee and Joye 2006, Rejmánková and Komárková 2005). A study in Belizian marshes did find that N_2 fixation increased with longer term P fertilization and decreased with additions of N, somewhat associated with microbial community composition changes (Rejmánková and Komárková 2005). Previous studies along with the present study, suggest that microbial sediment communities may adapt to and retain additional nutrients over a longer time frame, but over the short term are unable to adapt to nutrient regime shifts.

Nitrogen contributions from the sediment community

In many environments, N_2 fixation, the reduction of atmospheric dinitrogen to ammonium catalyzed by the nitrogenase enzyme, can be a significant component of the local N cycle (Capone 2001). The nitrogenase enzyme is extremely sensitive to oxygen, making the anoxic sediments in the mangrove ecosystem an ideal environment for this process. In coastal estuaries, sediments are anoxic for much of the year and may become anoxic within millimeters or centimeters of the sediment water interface (Revsbech *et al.* 1980). Low levels of nitrogenase activity have been detected in a variety of benthic marine environments, including

near shore, salt marsh, and estuarine sediments and mangroves (Capone 1993).

When all sites were viewed together, highest rates of nitrogenase activity rates were seen in the dwarf P zone, followed by the fertilized fringe, for which there was no significant difference between N and P addition treatments. $\delta^{15}N$ did not clearly reflect the patterns of nitrogenase activity rates, as the $\delta^{15}N$ values were low suggesting that N_2 fixation was occurring somewhere in the ecosystem.

Many factors can control rates of N_2 fixation, such as species composition, light, tidal frequency and flushing, and herbivore grazing (Burns 2003; Paerl 1985; Pickney *et al.* 2003). However, these factors interact, often making it difficult to pinpoint the exact factor controlling N_2 fixation.

Rates of nitrogenase activity were lower than may be expected from the depleted $\delta^{15}N$ values of the sediments, possibly due to environmental factors. Sea surface temperatures were very low in January, with an average of 26.8 C, compared to the January-February mean of 27.5 ºC, and a minimum temperature of 26.6 ºC. N_2 fixation has been shown to decrease with low temperatures (Toledo *et al.* 1995). Rainfall was very high (691 mm), much greater than the monthly average (average ~180 mm) for the area (Kaufmann and Thompson 2005). Turbidity due to rainfall and reduced temperatures may suppress nitrogen fixation.

Differences in microbial community composition can change rates of N_2 fixation and has been shown to change with long-term nutrient addition (Burns 2003; Rejmánková and Komárková 2005). While cyanobacteria are assumed to be the most important fixers of N within microbial mats (Bebout *et al.* 1993), there are a variety of diazotrophs

found within the sediment layer in the mangrove ecosystems. Associated with the photosynthetic members of the sediment microbial community are diverse heterotrophic, chemolithotrophic, and chemoorganotrophic bacteria, many of which can fix N (Burns 2003; Paerl and Pinckney 1996). While studies specific to Panama do not exist, a study with similar monospecific stands of *Rhizophora mangle* in Belize found within the mangrove sediment mats a diverse assemblage of cyanobacteria, pennate diatoms and purple sulfur bacteria (Lee and Joye 2006, Joye and Lee 2004). Most studies of benthic microbial community in mangrove ecosystems use microscopy as the identification method and may underestimate the diversity of the microbial community that may be captured with newer molecular methods (Pedrós-Alió 2007). Even if sediment biotic diversity is low compared to other ecosystems, often the functional significance of each component of estuarine ecosystems is great, so shifts in diversity may be especially important (Levin *et al.* 2001).

Mangrove tree and sediment interactions

Mangrove trees and the sediment microbial community are interconnected in several ways, providing feedback to each other. Mangrove trees are the primary producers of organic matter into the ecosystem, through both leaf fall and wood decay, providing both substrate and nutrients from leaves to the microbial community. The sediment microbial community contributes N through N_2 fixation to mangroves (Bashan *et al.* 1998). Up to 6% of the total N supplied to Australian mangroves may be supplied via N_2 fixation from sediment microbial communities (Boto and Robertson 1990).

Mangrove trees and the sediment community may have feedbacks that

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increase the overall productivity of the ecosystem. Bacteria have been found to preferentially decompose N-rich leaves (Mfilinge *et al.* 2003). Holguín *et al.* (2001) found that N incorporation doubled in *Avicennia* (black mangrove) leaves when incubated with a bacterial co-culture containing a nitrogen-fixing strain and a phosphate-solubilizing strain.

Mangrove trees and the diazotrophs in the sediments were found to be similarly nutrient limited in the current study. Nitrogenase activity rates and tree growth increased with P fertilization in the dwarf zone and with N fertilization in the fringe zone. $\delta^{15}N$ values of the sediments in the dwarf zone were the most depleted for the N addition treatment, which may indicate that the N fertilizer is being retained in the ecosystem. However, the $\delta^{15}N$ values of the dwarf trees were the most depleted in the P treatment. This may indicate that the N_2 fixed by the sediment microbial community is available for uptake by the dwarf trees and with increased tree growth, the nitrogen is being utilized. Within the fringe zone, N additions resulted in both the sediments and the trees becoming depleted in $\delta^{15}N$, following the nitrogenase activity rates and the tree growth response.

The sediment response to nutrients varied among parameters measured in the sediments. Chlorophyll *a* was greatest with P fertilization in the dwarf zone, similar to the nitrogenase activity rates and tree growth, but was also greatest with P addition in the fringe zone. The percent organic matter was reduced with nutrient additions in the dwarf zone but was increased with P addition in the fringe. However, these patterns did not follow for all parameters measured and may suggest co-limitation or limitation by other nutrients. One study of the saltmarsh ecosystem of

North Carolina found that plants were limited by N but microorganisms were limited by P (Sundareshwar and Morris 1999).

As shown in this study, the microbial community may be limited by similar or different nutrients as other organisms in the mangrove ecosystem and can vary even within the sites. Future studies should include simultaneous N and P additions to investigate the interactive effects of these nutrients.

Implications for Restoration

Increased knowledge of N_2 fixation and other sediment microbial properties are important for successful mangrove restoration projects. Mangrove ecosystem restoration is often recommended both after natural events, such as hurricanes and tsunamis, and as remediation for past destruction of the ecosystem by humans (Danielsen 2005, Alongi 2002). However, success rates of mangrove replanting have been limited and sediment restoration may be more essential than conventional methods of restoration, such as planting trees (Holguín *et al.* 2001, Lewis 2005).

Success of restoration efforts may be more easily measured by sediment biogeochemistry as a proxy for properly functioning, in lieu of waiting for trees to grow (McKee and Faukner 2000). N_2 fixation has been suggested both as a proxy for measuring mangrove ecosystem health (Sjöling *et al.* 2005) and as a tool to increase success of restoration projects (Bashan and Holguín 2002). Also, since microbial processes of sediments influence the productivity of tropical wetland ecosystems, they should be considered in conservation and restoration projects (Rejmánková *et al.* 2004).

Trophic connections

Mangrove trees are part of a coastal mosaic of ecosystems, including seagrass beds and coral reefs, which all share and provide habitat, nutrients and shore protection services (Harborne *et al.* 2006). While mangrove tree leaves and detritus are essential inputs of biomass into the coastal ecosystem (Davis *et al.* 2003, Holmer and Olsen 2002), the sediment microbial community may be an even more important pathway for nutrients (Paerl *et al.* 2000). Sediment cyanobacteria have been shown to be the preferred food source over vascular leaf tissue for crabs in mangrove forests in Puerto Rico (France 1998). Cyanobacteria, diatoms, bacteria and other microbial constituents provide a source of food for sediment and detritus feeding benthic fauna, both those that live within the sediment and those that come in with the tide to feed (Abed-Navandi and Dworshck 2005). These grazers have been cited as important in reducing and maintaining microalgal and microbial biomass, which may, in the case of snails and more stationary fauna, keep biomass and nutrients within the ecosystem and, in the case of fish and more mobile fauna, may be an important source of export of nutrients and biomass (Pinckney *et al.* 2003).

Fertilization with P resulted in an increased photosynthetic biomass (chl *a*) in both zones, which may provide a larger food source for organisms. Fertilization with N has been shown to increase both benthic microalgal abundance and herbivory (Hillebrand *et al.* 2000). However, chl *a* results in this study varied with N fertilization, and on the whole decreased with fertilization both zones. The result of co-fertilization with N and P, as may be the case for mainland runoff, was not tested in this

study but the combination has been shown in other estuaries to increase biomass but not grazing (Lever and Valiela 2005). Also, this experiment enriched the sediments with relatively little nutrients and it is still uncertain what is the upper limit of nutrient absorption for this sediment microbial community.

Bocas del Toro in a worldwide context

The basal area of the mangrove trees in Bocas del Toro are comparable to sites in Belize, Bonaire and Curacao, and the Dominican Republic, possibly reflecting similar environments and making this study a useful comparison for these ecosystemss (Guzman *et al.* 2005). The rates of N_2 fixation observed in Bocas del Toro sediments were low compared to the rates observed elsewhere in the Caribbean, even with P or N fertilization (Table 1). These low rates may be due to heavy rainfall and low sea surface temperatures (Kaufmann *unpublished data*) and seem contrary to the $\delta^{15}N$ measurements, which were low compared to worldwide values for sediments and would seem to indicate high N_2 fixation within both the dwarf and the fringe zones (Table 1). $\delta^{15}N$ measurements varied greatly even within Bahía Almirante, but all sites were lower than the worldwide average of 1.34‰ for mangrove sediments (Table 2). Percent N of the sediments were comparable to mangrove sediments in other parts of the world (Table 3).

Global trends of nutrient increases

Nitrogen is a major limiting nutrient in aquatic ecosystems (Ryther and Dunstan 1971). It is found in many biological molecules, including DNA and proteins, making it an essential element for all organisms. The supply

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Table 1. Summary of N_2 fixation rates (mmol N_2 m^{-2} d^{-1}) in *Rhizophora* spp. mangrove forest sediments (n.d. = not detected).

N_2 fixation	Benthos	Location	Source
n.d.	Peat/sediments	C dwarf zone	This study
n.d.	Peat/sediments	C fringe zone	This study
n.d.	Peat/sediments	+N dwarf zone	This study
0.01	Peat/sediments	+N fringe zone	This study
0.01	Peat/sediments	+P dwarf zone	This study
n.d.	Peat/sediments	+P fringe zone	This study
0-1.21	Cyanobacteria mat, peat	Twin Cays, Belize	Lee and Joye, 2006
0.53	Sediment	Joyunda Lagoon, Puerto Rica	Morell & Corredor (1993)
0-2.4	Sediment	Oyster Bay, Jamaica	Nedwell et al. (1994)
0.09-0.63	Sand, mud	Tampa Bay, Florida	Zuberer & Silver (1978)
0-0.6	Silt, sand, and/or clay	Sawi Bay, Thailand	Alongi et al. (2002)
2.3	Silt/clay	Caete Estuary, Brazil	Dittmar & Lara (2001)
0.28-0.39	Silt, sand slurries	Makham Bay, Thailand	Kristensen et al. (1998)
0-0.24	Sediment	Missionary Bay, Australia	Boto & Robertson (1990)
0.49-2.85	Sediment	Mekong delta, Vietnam	Alongi et al. (2000)
0.50	Sediment	Kisakasaka, Tanzania	Sjoling et al. (2005)
0.04	Sediment	S. Florida	Pelegrí et al (1997)

of fixed N demonstrably limits the productivity, composition, dynamics and diversity of many ecosystems, in that all these change when N is added. Nitrogen inputs into ecosystems are limited to external sources and the generation of N from internal processes, such as N_2 fixation from the microbial community.

Human activities are introducing an unprecedented amount of N into

Table 2. Summary of worldwide δ^{15}N data for mangrove sediments.

^{15}N sediment value	Location	Source
-0.4 $\pm$ 0.20	Cristobal, Dwarf zone	This study
1.2 $\pm$ 0.15	Cristobal, Fringe zone	This study
-0.1 $\pm$ 0.15	Solarte, Dwarf zone	This study
0.6 $\pm$ 0.30	Solarte, Fringe zone	This study
0.2 $\pm$ 0.46	Isla Popa, Dwarf zone	This study
0.7 $\pm$ 0.12	Isla Popa, Fringe zone	This study
-0.1 $\pm$ 0.50	All sites, dwarf zone	This study
0.8 $\pm$ 0.47	All sites, fringe zone	This study
-0.5 $\pm$ 0.05	Twin Cays, Belize, Dwarf zone	McKee et al. (2002)
0.1 $\pm$ 0.25	Twin Cays, Belize Fringe zone	McKee et al. (2002)
-3.2 $\pm$ 0.14	South Water Cay, Belize	Abed-Navandi & Dworschak (2005)
-1.5 $\pm$ 3.7	Laguna Joyunda, Puerto Rico	France (1998)
3.5 $\pm$ 1.5	Tanzania Coast	Muzaka & Shumula (2006)
5.2 $\pm$ 1.1	Andhra Pradesh, India	Boullion, et al. (2002)
2.1 $\pm$ 1.2	Gazi Bay, Kenya	Boullion, et al. (2004)
5.6	Andhra Pradesh, India	Boullion, et al. (2004)
-0.1 $\pm$ 0.3	Pambala, Sri Lanka	Boullion, et al. (2004)
1.8 $\pm$ 1.7	Galle, Sri Lanka	Boullion, et al. (2004)
-1	Pearl River estuary, China	Lee (2000)
4.11	Penisula Malaysia	Newell et al. (1995)

Table 3. Summary of %N data for mangrove sediments worldwide

%N	Substrate	Location	Source
0.09 ± 0.03	Peat/sediments	C dwarf zone	This study
0.07 ± 0.02	Peat/sediments	C fringe zone	This study
1.10 ± 0.1	Sand, silt or clay	Sawi Bay, Thailand	Alongi et al. (2002)
0.15 ± 0.2	Clay loam	Pará, Brazil	Schwendenmann et al. (2006)
0.10 ± .02	Sediment, silt or clay	Jiulong Jiang, China	Alongi (2005)
0.07 ± 0.11	Sediment	Vellar-Coleroon Complex, India	Alongi et al. (2005)
1.10	Sediment	French Guiana	Marchand et al. (2003)
0.09 ± 0.01	Sediment	Thailand	Kamp-Nielsen et al. (2002)
0.22 ± 0.2	Sediment	Gazi Bay, Kenya	Middleburg et al. (1996)
0.03 ± 0.02	Sediment	Tanzania Coast	Muzaka & Shumula (2006)

ecosystems worldwide. While non-human sources of N including lightning and N_2 fixing bacteria and algae introduce approximately 90 - 140 Tg of N per year (Galloway *et al.* 1996), human activities such as application of fertilizers, growing N_2 fixing crops and the burning of fossil fuels at least doubles this amount with the introduction of approximately 140 Tg of N per year to the terrestrial environment (Vitousek *et al.* 1997). In addition to the increases of N exported to ecosystems by humans, direct destruction of forested ecosystem releases 60 Tg of N per year from plant biomass and soils into the environment (Vitousek *et al.* 1997).

Land use change in tropical regions is expected to alter N fluxes as

countries develop (Matson *et al.* 1999). Already 40% of global applications of N take place in the tropics and subtropics and this is expected to increase by 60% by 2020 (Matthews 1994). Ecosystems in the Caribbean are adapted to oligotrophic conditions and therefore are sensitive to shifts to eutrophic conditions. Disturbed forested ecosystems export more N per unit area than undisturbed (Ortiz-Zayas *et al.* 2006) and mangrove ecosystems continue to be threatened by both direct destruction and land use change (Valiela *et al.* 1997).

In this study, long-term nutrient additions changed almost every parameter measured. Nitrogen additions affected the dwarf and fringe zones similarly. N additions increased nitrogenase activity rates for the dwarf N additions in both zones compared to the control, even if not significantly. N additions depleted $\delta^{15}N$ and decreased the %P, %N and percent organic matter compared to the controls. P additions also had similar effects on both the dwarf and fringe zone sediments. Nitrogenase activity rates, chl *a*, %P, and percent organic matter increased compared to the controls with P additions, while %N decreased. While these results varied by sites, these averages could be useful for computer models and coastal zone managers when predicting the effects of increased N and P separately on the mangrove ecosystem.

The N and P addition treatments in this experiment could be viewed as the future of the Bocas del Toro archipelago if development continues within the Bahía Almirante watershed without proper attention to water pollution controls. N and P additions significantly affected almost every parameter measured within this experiment. Within this experiment, there were significant differences in the effects of nutrient additions on

the different sites and different zones, which may indicate that effects will vary throughout the bay as the system becomes more eutrophic. Since nitrogenase activity rate was shown to increase with both N and P additions, future research is needed to elucidate if the mangrove environment will substantially shift from a net retainer of nutrients to an exporter of nutrients. Already within the Bahía Almirante, shifts in coral community data suggest that water quality has declined over the last few decades (Aronson *et al.* 2004).

The Bocas del Toro mangrove ecosystem has the potential for absorbing and immobilizing nutrients, especially P with the binding capacity of carbonate sediments. However, the further accumulation of organic matter in nearshore ecosystems can impair the capacity of the sediments for P immobilization (Corredor *et al.* 1999).

The economy of the Bocas del Toro archipelago is linked to tourism (Moody 2005). While degradation and destruction of coral reefs are caused by a variety of factors including sedimentation, destructive fishing, anthropogenic nutrient inputs, these issues will only be exacerbated with the loss of mangrove ecosystems (Szmant 2002).

These findings indicate that anthropogenic over-enrichment of sediments has the potential to alter ecological processes in the mangroves at Bocas del Toro. Scientists are only starting to have sufficient information to predict ecosystem responses to human-induced stresses within this system. Further research is necessary to predict the impact of increased nutrients and to find ways to prevent or compensate for any potential damage on the ecosystem. An additional challenge will be integrating microbial processes into management decisions and in the creation of predictive models (Azam and Worden 2004). However, while research is

ongoing throughout the world on mangrove ecosystems, efforts to include this research into management decisions need to be improved (Kairo *et al.* 2001).

Future research

Future experiments should include further investigations into the nitrogen cycle in the sediments of Bocas del Toro. Inputs of N from N_2 fixation and lagoonal inputs may be balanced by losses such as denitrification, export and burial need to be quantified, although denitrification rates are considered a relatively small part of the nitrogen cycle in mangrove habitats (Rivera-Monroy & Twilley 1996, Kristensen *et al.* 1998).

Depleted $\delta^{15}N$ values in both the sediments and the trees suggest that N_2 fixation is either much greater during other parts of the year or that it is taking place in another part of the ecosystem, such as laminate microbial mats or filamentous cyanobacteria also found within the ecosystem but not measured in this study. Greater temporal and spatial sampling could help explain the depleted $\delta^{15}N$ values and estimate year-round contribution of the sediment microbial community.

Future studies should also include in depth microbial studies, including molecular methods. For example, determination of *nifH* mRNA would address the question of which diazotrophs are actively fixing in the samples and if different organisms are responsible for nitrogenase activity within the different zones and treatments. Recent studies have discovered novel bacteria within the mangrove sediments, with a variety of functions (Liang *et al.* 2007). Therefore the sediment microbial community of the

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mangrove environment, both in terms of diversity and function, merits
future study.

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