PRIMARY PRODUCTION, NITROGEN CYCLING AND THE ECOSYSTEM ROLE OF MANGROVE MICROBIAL MATS ON TWIN CAYS, BELIZE

by

ROSALYNN Y. LEE

(Under the Direction of Samantha B. Joye)

ABSTRACT

The seasonal variability of porewater nutrient concentrations, metabolism, nitrogen cycling (denitrification and nitrogen fixation), and primary production (oxygenic and anoxygenic photosynthesis and chemoautotrophy) was examined in benthic mangrove environments on Twin Cays, Belize. Twin Cays mangroves exhibit a tree-height gradient from tall seaward fringe trees, through a transition of intermediate tree heights to short dwarf trees surrounding interior ponds and lagoons. Detailed investigations of steady state porewater profiles of nutrients and terminal metabolic products in dwarf mangrove soils revealed strong seasonal differences in salinity, organic carbon and nitrogen inventories, redox conditions and reduced manganese concentrations. Substantial rates of organic matter remineralization were coupled primarily to sulfate reduction. Redox conditions contributed to variability in mat nitrogen fixation and denitrification response to nutrient addition, while dissolved organic carbon did not. Nitrogen fixation was controlled primarily by the sensitivity of nitrogenase to oxygen inhibition, whereas denitrification was limited by nitrate availability.

Community composition of photosynthetic organisms appeared to be controlled by light fluctuations due to mangrove canopy light gaps and by differential tolerance to environmental stresses such as desiccation or nitrogen limitation. Dwarf mangrove cyanobacteria-dominated

microbial mats achieved a high biomass of photopigments in well-illuminated soils. Transition and fringe soils were more shaded and contained diatoms and green algae and less cyanobacteria and anoxygenic photosynthetic bacteria than in dwarf soils. Oxygenic photosynthesis was the primary mode of carbon fixation (56%) in all habitats under full sun, with a lesser contribution by anoxygenic photosynthesis (32%) and chemoautotrophy (12%). *In situ* light conditions underscore the gradient from highest rates of carbon fixation in dwarf mangrove habitat mats (0.21 g C m^{-2} d^{-1}) to diminished rates in shaded transition and fringe mangrove habitat mats (0.08 and 0.05 g C m^{-2} d^{-1}, respectively). Well-lit mats associated with dwarf mangrove habitats fix 18-20% of the net primary productivity of Twin Cays' dwarf mangrove trees and can supply 5-28% of the nitrogen requirement of Twin Cays' dwarf mangrove trees via nitrogen fixation. Light limitation restricts the fixation of carbon and nitrogen in transition and fringe mangrove habitat mats which account for only <0.3% of the net production and <2% of the nitrogen requirement of the respective mangrove trees.

INDEX WORDS: Mangrove, microbial mat, cyanobacteria, porewater nutrients, benthic metabolism, primary production, respiration, carbon fixation, nitrogen fixation, denitrification, desiccation, oxygenic photosynthesis, anoxygenic photosynthesis, chemoautotrophy, natural abundance isotopic signatures

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CHAPTER 1

INTRODUCTION

Mangrove ecosystems dominate tropical coastlines, covering 18 million hectares worldwide (Spalding et al. 1997), and serve important economic and ecological functions acting as nurseries for commercially important aquatic organisms that contribute to coastal, estuarine and deep-sea fisheries (Ronnback 1999, Mumby et al. 2004), as habitat for resident and migratory birds, as nutrient and particulate filters from upland sources, and as protection from physical damage of shoreline due to tidal waves, erosion, hurricanes, and tsunamis (Mitch & Gosselink 1993). More than half of the world's original mangrove habitats have been destroyed (Kelleher et al. 1995, Spalding et al. 1997), with about 70% of that loss occurring in the last 20 years (Valiela et al. 2001). Anthropogenic pressures leading to the destruction of mangrove habitat include over-harvesting for timber and fuel-wood (Hussein 1995), clearing for aquaculture and salt-pond construction (Terchunian et al. 1986, Primavera 1997), mining, and pollution and damming of rivers which alter salinity levels in the mangrove (Wolanski 1992).

Mangrove habitats in terrestrial-riverine coasts and oceanic islands vary from 10.5 m tall (on average, Lugo 1990), dense, seaward fringe trees adjacent to the shore, to sparser ≤1.5 m tall "stunted" trees located away from the shoreline (Lugo & Snedaker 1974, Koltes et al. 1998, Feller et al. 2003). In this dissertation, these "stunted" mangrove habitats will be referred to as "dwarf" mangrove habitats for consistency with the published manuscript from this dissertation (Lee & Joye 2006, Ch. 4). The perception of dwarf mangrove habitats as unproductive "stunted"

or “scrub” forests (Pool et al. 1977) has been used to justify the devaluation and subsequent destruction of these habitats for shrimp aquaculture; this type of habitat accounts for 20-50% of mangrove destruction worldwide (Primavera 1997).

Mangrove soils are typically nutrient-deficient (Alongi & Sasekumar 1992) despite being rich in organic matter, suggesting highly efficient recycling of nutrients which are regenerated by mangrove litter decomposition (Holguin et al. 2001). Mangroves must also adapt to the stresses of intertidal environments, including high salinity and reducing soil conditions (Kathiresan & Bingham 2001). Seasonal monsoonal rains affect porewater salinity, redox potential, pH, and soil biogeochemical processes (Alongi et al. 1999, Alongi et al. 2004). Numerous studies investigating benthic metabolism and nutrient transformations in fringe mangrove soils have demonstrated the relationship between organic matter availability and sulfate reduction with mangrove density and speciation (Nedwell et al. 1994, Sherman et al. 1998), however data from dwarf mangrove zones is lacking. In **CHAPTER 2**, I characterize the seasonal variability of porewater nutrients and metabolic end products in dwarf mangrove soils from Twin Cays, Belize (Fig. 1.1), to identify spatial and temporal patterns of benthic metabolism and nutrient dynamics.

Studies of benthic productivity in mangroves are limited and typically demonstrate low photosynthetic biomass and activity. The primary producers in mangrove soils and sediments include eukaryotic microalgae and cyanobacteria. Light intensity is commonly described as the primary factor limiting benthic primary production due to mangrove canopy shading (Alongi 1988, Kristensen et al. 1988, Alongi & Sasekumar 1992, Alongi 1994), although a variety of other controls have also been proposed. Nutrients (both nitrogen and phosphorus) can significantly limit mangrove benthic primary production and microalgal growth (Kristensen et al. 1988, Alongi et al. 1993). Inhibition by physical factors other than low light may include high

temperatures, wide salinity variations, and surficial erosion by hydrological processes such as rainstorms (Alongi 1990). The growth and density of benthic phototrophs may also be constrained by grazing or inhibition by soluble phenolic compounds such as mangrove-derived tannins (Potts & Whitton 1980, Alongi 1990, Alongi 1994). Most benthic mangrove studies have examined diatom-dominated microalgal sediments and soils. Mangrove microbial mat studies are few, but have documented diverse photosynthetic communities of significant biomass (Potts & Whitton 1980, Dor & Levy 1984). Mangrove canopy coverage, tree basal area and tree height controls light availability to the benthos. In **CHAPTER 3**, I examined the effect of habitat differences in light, elevation and inundation on the distribution and composition of microphytobenthic assemblages, including microbial and microalgal mats, and rates of oxygenic photosynthesis. I also examined the effect of light availability on photosynthetic oxygen production in relation to community composition of benthic microbial mats and microphytobenthos.

Benthic communities play an active role in the nutrient status of benthic environments. Nutrient limitation in temperate coastal ecosystems is due primarily to the relative lack of nitrogen (N) (Howarth 2006), while tropical and subtropical mangroves appear to be primarily phosophorus (P) limited (Boto & Wellington 1983, Feller et al. 1999). Within mangrove habitats, nutrient limitation patterns have been found to vary from P limited dwarf mangrove habitats to N limited fringe mangrove habitats (Boto & Wellington 1983, McKee 1993). On oligotrophic offshore mangrove islands, inputs of N depend upon atmospheric and oceanic inputs and nitrogen fixation, the microbial conversion of N_2 to NH_4^+, and are balanced by loss via denitrification, the facultative anaerobic microbial reduction of NO_3^- to gaseous end products including N_2O and N_2, and export or burial. High rates of N_2 fixation (up to 4.2 mg N m^{-2} d^{-1};

Zuberer 1976) in mangrove environments have been documented in association with leaf litter, pneumatophores, and soils (Holguin et al. 2001). In contrast, denitrification rates in mangrove habitats are considered a negligible part of the N budget (Rivera-Monroy & Twilley 1996, Kristensen et al. 1998). However, neither of these processes has been well studied in habitats occupied by benthic mats in mangrove forests. In **CHAPTER 4**, I investigated spatial and temporal dynamics of nitrogen fixation and denitrification with respect to daily- and seasonally-varying physical and chemical environmental forces.

Mangrove forests dominate tropical intertidal landscapes (Por 1984) and are often regarded as highly productive ecosystems (Clough 1992). Benthic microbial mats and microphytobenthos can also attain high rates of productivity, contributing up to 50% of estuarine primary production (Underwood & Kromkamp 1999). Mangrove primary production is commonly investigated in terrestrial-riverine forests consisting of tall (10.5 m average) trees (Lugo 1990). In these mangrove systems, canopy shading limits both the distribution and activity of benthic phototrophs (Kristensen et al. 1988, Alongi & Sasekumar 1992, Lee et al. in preparation, Ch. 3). In contrast, sparser "dwarf" mangrove trees (≤1.5 m tall) exist farther from the shoreline (Lugo & Snedaker 1974, Feller et al. 2003) and these habitats can support significantly greater quantities of benthic photosynthetic biomass and rates of primary productivity as well as nitrogen fixation relative to shaded forests (Lee et al. in preparation, Ch. 3, Lee & Joye 2006, Ch. 4). In **CHAPTER 5**, I investigated rates of benthic carbon fixation and patterns of natural abundance signatures of carbon and nitrogen in mangrove forests under a gradient of light levels from well-lit dwarf mangrove habitats to densely shaded fringe forests on Twin Cays, Belize. I hypothesized that an inverse relationship occurs across the tree-height gradient between the productivity of mangrove trees and the productivity of the benthic

communities and documented the significance of microbial mats in the contribution of carbon and nitrogen inputs to mangrove ecosystems.

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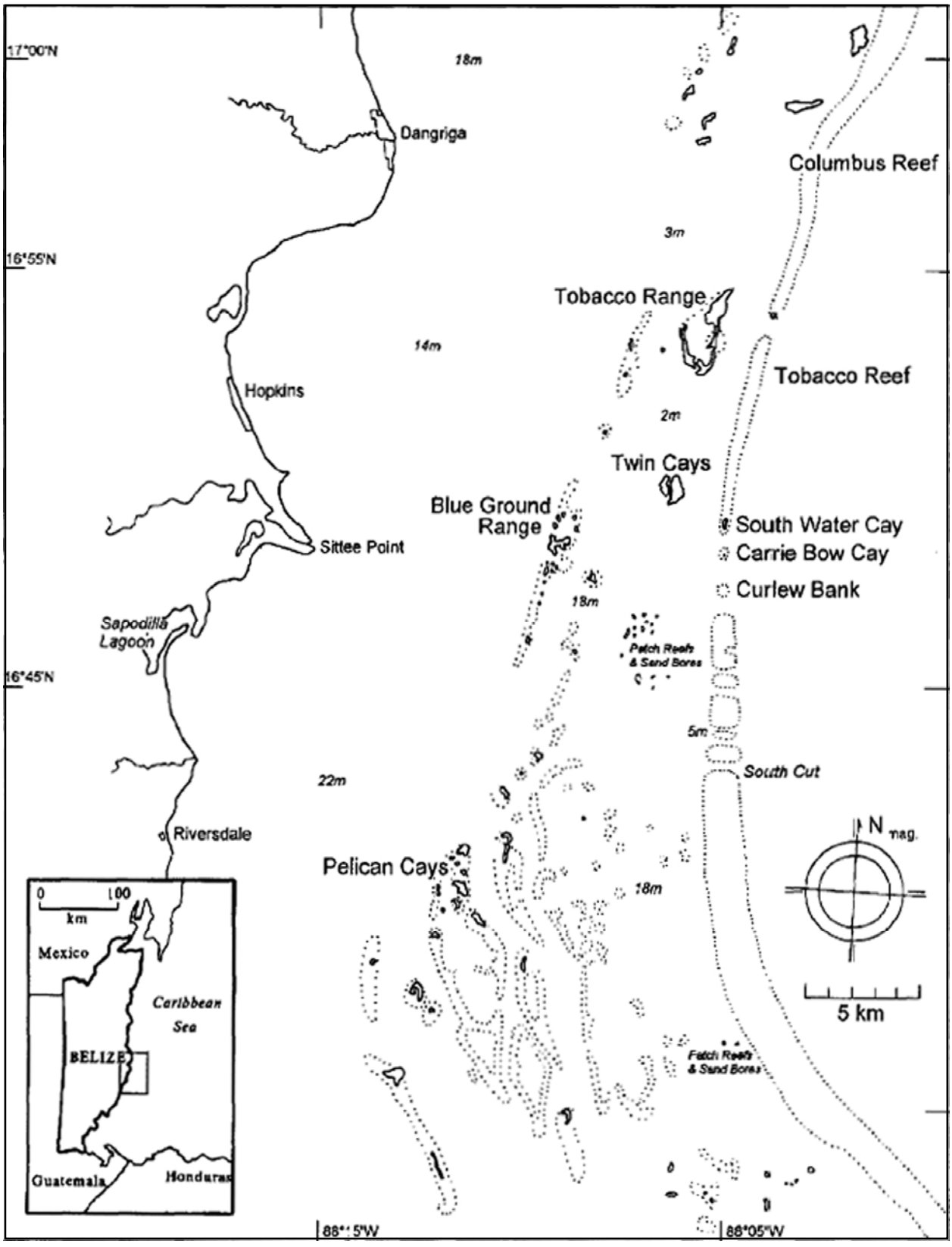
FIGURE CAPTIONS

Figure 1.1. Study area location relative to Belize (Koltes et al. 1998).

Figure 1.1


17°00'N
16°55'N
16°45'N
88°11'5"W
88°05'W
18m
3m
14m
2m
22m
5m
Dangriga
Hopkins
Sittee Point
Sapodilla Lagoon
Riversdale
Columbus Reef
Tobacco Range
Tobacco Reef
Twin Cays
Blue Ground Range
South Water Cay
Carrie Bow Cay
Curlew Bank
Patch Reefs & Sand Bores
South Cut
Pelican Cays
N mag.
5 km
0 100
km
Mexico
Caribbean Sea
BELIZE
Guatemala
Honduras

CHAPTER 2

POREWATER BIOGEOCHEMISTRY AND SOIL METABOLISM IN STUNTED RED MANGROVE HABITATS (TWIN CAYS, BELIZE)[1]

[1]Lee RY, Porubsky WP, Feller IC, McKee KL, Joye SB. Prepared for submission to *Biogeochemistry*.

ABSTRACT

Seasonal variability in mangrove soil metabolism was examined by comparing steady state porewater profiles of pH, chloride, sulfate, sulfide, ammonium, nitrate/nitrite, phosphate, dissolved organic carbon, nitrogen, and phosphorus, reduced iron and manganese, dissolved inorganic carbon, methane and nitrous oxide from three sites on Twin Cays, a pair of oceanic mangrove islands that lie offshore from Belize, during wet and dry periods of the year. The interior of the islands consisted of short, 'stunted' red mangrove trees surrounding shallow lagoons. During the wet season, the input of rainwater decreased the salinity of overlying pond and shallow pore waters. Increased infiltration of rainwater through soils combined with higher tidal heights led to increased organic carbon inventories and more reduced soil pore waters. During the dry season, evaporation increased both surface water and porewater salinities, while lower tidal heights led to less reduced soil pore waters. Rainfall strongly affected dissolved organic carbon and nitrogen inventories, possibly due to increased decay of mangrove litter during the wet season. During both times of year, high concentrations of reduced metabolites, such as ammonium and hydrogen sulfide, accumulated at depth, indicating substantial rates of organic matter mineralization coupled primarily to sulfate reduction. Nitrous oxide and methane concentrations were supersaturated significantly indicating considerable rates of nitrification and/or incomplete denitrification and methanogenesis, respectively. More reducing soil conditions during the wet season promoted the production of reduced manganese. Contemporaneous activity of sulfate reduction and methanogenesis was likely fueled by the presence of noncompetitive substrates, e.g., methylated amines.

INTRODUCTION

Microbial activity drives the mineralization of organic matter in intertidal soils and sediments thereby influencing porewater nutrient availability and the speciation of redox-active compounds (Paerl & Pinckney 1996). Terminal metabolism couples organic matter oxidation to the reduction of a terminal oxidant and produces a variety of end products ranging from dinitrogen and nitrous oxide gases, reduced iron and manganese, sulfide and methane, indicating denitrification, metal reduction, sulfate reduction and methanogenesis, respectively. Terminal metabolic processes recycle complex organic matter back to inorganic forms, such as bicarbonate, ammonium and phosphate. These inorganic nutrients can support additional ecosystem primary production.

Mangrove soils are typically nutrient-deficient (Alongi 1996, Alongi & Sasekumar 1992, Boto & Wellington 1984) despite being rich in organic matter, suggesting highly efficient recycling of the inorganic nutrients regenerated during mangrove litter decomposition (Holguin et al. 2001). In natural (i.e., non-anthropogenically influenced) mangrove systems, nitrogen fixation is an important source of new nitrogen (N) but this process is spatially and temporally limited (Lee & Joye 2006, Ch. 4). The high productivity of mangroves is thus largely driven by internal nutrient recycling, which is coupled to organic matter mineralization.

Mangrove soils can experience high salinity and reducing conditions (Kathiresan & Bingham 2001); however, in the tropics, increased rainfall during the wet season or monsoonal period can affect porewater salinity, redox potential and pH, as well as soil biogeochemical processes (Alongi et al. 1999, 2004). Numerous studies have investigated benthic metabolism and nutrient transformations in mangrove soils along the ocean or river edge, often referred to as the fringe zone, demonstrating a relationship between organic matter availability and sulfate

reduction and mangrove density and speciation (Nedwell et al. 1994, Sherman et al. 1998). Similar data from more landward or interior, stunted mangrove zones, is lacking.

The objective of this study was to characterize steady-state porewater biogeochemical signatures of mangrove soils and to use these data to evaluate pathways of benthic metabolism and nutrient regeneration. We quantified steady state porewater profiles of dissolved species during a wet and dry season using diffusion equilibration samplers. We hypothesized that wet-dry seasonality would strongly influence the flushing of soils, leading to changes in the inventories of metabolic constituents in porewater and the redox status of the soils. These changes were in turn hypothesized to affect the rates of carbon mineralization and nutrient regeneration.

METHODS

Twin Cays is a pair of oceanic mangrove islands located 15.5 km off the coast of Belize at 16˚ 50' N, 88˚ 06' W (Feller et al. 2003, Lee & Joye 2006, Ch. 4). Twin Cays is one of many "island mangroves" (Rützler & Feller 1996) that lie along the Caribbean coastline of Central America. Island mangroves differ from mainland mangroves in that they have limited terrestrial influence (i.e., they are not impacted by river runoff except following extreme climate events like hurricanes) and are constantly bathed by full salinity ocean water. The Twin Cays islands are forested primarily by *Rhizophora mangle* (red mangrove) but *Avicennia germinans* (black mangrove) and *Laguncularia racemosa* (white mangrove) are also present in some areas. The islands consist of ~9 m of mangrove peat that accumulated during the Holocene as the islands accreted to keep pace with rising sea level (Macintyre & Toscano 2004). The Holocene peat lies atop Pleistocene limestone deposits (Macintyre & Toscano 2004, Macintyre et al. 2004). *R.*

mangle exhibits a decreasing tree-height gradient from the seaward edges of the islands to the interior where stunted trees, ≤1.5 m tall, surround treeless ponds. Stunted mangrove habitats account for approximately forty percent of the surface cover on Twin Cays (Rodriguez & Feller 2004) making these habitats a dominant feature of this, and other similar, islands. Stunted mangrove zones are water logged except during extremely low tides, which occur in late spring and early summer.

We examined soil processes at three sites inhabited by stunted *R. mangle*: the Dock, the Lair and the Weather Station; at two times of year, between August and September 2002 and April and May 2003; to investigate the temporal and spatial variability of soil biogeochemical signatures. Seasonality at Twin Cays results from variations in precipitation and tidal height rather than from fluctuations in temperature (Lee & Joye 2006, Ch. 4). Water temperature at Carrie Bow Cay, a Smithsonian Institution field station located 3.5 km from Twin Cays, was similar in April-May and August-September, while tidal height (from -25 and +27 cm relative to mean sea level) and rainfall (1.3 mm d^{-1}) was significantly lower in April-May than in August-September (from -11 and +30 cm relative to mean sea level and 4.6 mm d^{-1}, respectively) (Opishinski 2002-2003; Lee & Joye 2006, Ch. 4).

Steady state profiles of dissolved constituents in stunted mangrove soils were obtained using porewater diffusion equilibration samplers (hereafter referred to as "peepers"; Hesslein 1976). Ultra high molecular weight polyethylene peepers with thirty 18 ml chambers were assembled with 0.2 μm Biotrans® nylon membranes and nylon screws while submerged in helium-purged deionized water (details provided in Weston et al. 2006). Peepers were transported to the field site in 0.15 mm thick polypropylene bags of helium-purged deionized water with no headspace. Peepers were inserted vertically in the peat soil approximately 6

months before collection due to the limited number of field visits permitted per year. The oxygen-free deionized water in the peeper chambers equilibrated with the porewater and at the time of collection reflected the soil dynamics over only the previous 6-8 weeks of equilibration due to continuous diffusion-mediated exchange. For simplicity, peepers collected in September 2002 will be referred to as from September (although they represent the biogeochemical signature of August and September), and peepers collected in May 2003 will be referred to as from May (although they represent the biogeochemical signature of April and May). Peepers were removed from the soil, placed in helium-purged 0.15 mm thick polypropylene bags, and transported to the Smithsonian Institution field station on Carrie Bow Cay for sampling; the time between retrieval and sampling was about 30 minutes. After transfer of the peepers into helium-purged glove bags, equilibrated porewater from each chamber was sampled through the nylon membrane using a gas-tight glass syringe fitted with an 18G needle.

Water from each peeper chamber was analyzed immediately for pH and sub-samples were collected for determining concentrations of ammonium (NH_4^+), nitrate + nitrite (NO_x), phosphate (PO_4^{3-}), dissolved organic carbon (DOC), nitrogen (DON), and phosphorus (DOP), dissolved inorganic carbon (DIC), hydrogen sulfide (H_2S), sulfate (SO_4^{2-}), chloride (Cl^-), reduced iron (Fe^{2+}) and manganese (Mn^{2+}), methane (CH_4) and nitrous oxide (N_2O) back at the University of Georgia (UGA) laboratory. The pH of the soil pore water was determined in one ml of unfiltered porewater (from each peeper) chamber using a Sensorex® low volume flow-through pH electrode assembly. The pH sensor was calibrated using NBS® pH 4 and 7 standards. Other sub-samples were stored in acid-washed, ultrapure deionized water rinsed, and 500 °C combusted glass vials. One ml of unfiltered porewater was injected into helium-purged, crimp-sealed 6 ml headspace vials and acidified with 0.1 ml of concentrated phosphoric acid

(after removal from the glove bag) for analysis of DIC, CH_4, and N_2O. The other vials were sealed with teflon-lined screw caps.

An unfiltered water sample (0.1-0.5 ml) was pipetted into a vial containing 0.5 ml zinc acetate (20 weight %), as a preservative, for subsequent H_2S analysis. The remaining water from each chamber was filtered through a 0.2 μm Target® cellulose filter and further aliquotted. NH_4^+ samples (0.1-0.5 ml) were pipetted into vials containing 0.2 ml phenol reagent (22 ml phenol, 198 ml ethanol, 8 ml deionized water) for preservation. Four milliliters (ml) of filtered water was placed in a 7 ml vial and preserved with 0.1 ml of concentrated nitric acid (after removal from the glove bag); this sample was stored at 4 ºC and used for subsequent DOC, PO_4^{3-}, DOP, Cl^-, SO_4^{2-}, Fe^{2+}, and Mn^{2+} analysis. The remaining filtered porewater was stored 4 ºC in vials for later analysis of NO_x and DON back at the UGA lab.

All samples were analyzed within 3 weeks of collection. Ammonium was analyzed colorimetrically via the phenol hypochlorite method (Solorzano 1969). Nitrate+nitrite was measured by vanadium reduction and nitric oxide detection on an Antek® Nitrate/Nitrite Reduction system inline with a chemiluminescent nitric oxide detector (Valderrama 1981, Garside 1982). Phosphate was analyzed colorimetrically using the molybdate blue method (Strickland & Parsons 1972). DOC was measured by high temperature combustion and infrared CO_2 detection on a Shimadzu® Total Organic Carbon (TOC) 5000 analyzer. DON was calculated as the difference between total dissolved nitrogen (TDN) and dissolved inorganic nitrogen (DIN = $NH_4^+ + NO_x$); TDN was measured by high temperature oxidation on a Shimadzu® TOC 5000 analyzer inline with an Antek® chemiluminescent nitric oxide detector (Álvarez-Salgado & Miller 1998). DOP was calculated as the difference between total dissolved phosphorus (TDP) and dissolved inorganic phosphorus (DIP = PO_4^{3-}); TDP was measured

colorimetrically as PO_4^{3-} after combustion and acid hydrolysis (Solorzano & Sharp 1980). H_2S was analyzed colorimetrically using the Cline method (Cline 1969). SO_4^{2-} and Cl^- were quantified using ion chromatography on a Dionex® system. Fe^{2+} and Mn^{2+} were analyzed colorimetrically using the ferrozine and formaldoxime methods, respectively (Stookey 1970, Armstrong et al. 1979).

DIC, CH_4 and N_2O were quantified by gas chromatography. CH_4 and DIC were measured on a Shimadzu® flame ionization detection gas chromatograph with an Alltech® Carbosphere column and Shimadzu® methanizer, which converted DIC to CH_4. N_2O was measured on a Shimadzu® electron capture detection gas chromatograph with a HayeSep® DB column. Concentrations of gases (DIC, CH_4 and N_2O) were determined by comparison of sample peak areas to the areas generated by certified gas standards from Scott Specialty Gases® (a mix of 10% DIC and 10% CH_4 in a balance of ultrapure He and a mix of 500 ppm N_2O in a balance of ultrapure He).

The pore water profiles from individual peepers incubated at the three different sites were averaged to evaluate spatial variability in pore water constituents at each sampling time. After averaging, total sediment inventories at four depth intervals (i.e., 0-5, 5-10, 10-20, 20-40 cm) and over the total measured depth range ('ALL', 0-40 cm) were calculated using a porosity-corrected trapezoidal integration of pore water profiles. The DIC produced by microbial respiration in soils, hereafter noted DIC_R, was calculated by correcting pore water DIC concentrations for the DIC originating from the overlying seawater (Eq. 1):

$$\text{Eq. (1)} \qquad \mathbf{DIC_R} = [DIC]_{PW} - [DIC]_{OLW}$$

where $[DIC]_{PW}$ is the pore water DIC concentration and $[DIC]_{OLW}$ is the overlying water DIC concentration. Mangrove peat is organic rich (65-95% organic matter, Feller et al. 2003) and is

comprised mainly of coarse and fine roots (~80%) plus leaves and wood (~20%) derived from mangrove trees; the carbonate content of surficial peat is insignificant (McKee & Faulkner 2000, Feller et al. 2003). Thus, it is unlikely that carbonate dissolution contributed to the observed DIC_R values. Estimates of the calcium carbonate saturation index (SI), calculated for *in situ* pH and bicarbonate concentration and assuming calcium concentrations equivalent to those in seawater (i.e., 10.53 mM), suggested that carbonate precipitation is unlikely ($SI \leq 1$; data not shown).

Net rates of SO_4^{2-} reduction were estimated from the observed depletion of SO_4^{2-} over the depth profile. Since the ratio of Cl^- to SO_4^{2-} in seawater does not vary (Pilson 1988), the expected concentration or inventory of SO_4^{2-} can be calculated from the measured concentration or inventory of Cl^-. Sulfate depletion was calculated using Eq. 2:

Eq. (2) $$\mathbf{SO_4^{2-}_{dep}} = [Cl^-_M * R_{SW}^{-1}] - SO_4^{2-}_M$$

where Cl^-_M and $SO_4^{2-}_M$ are the measured concentrations of Cl^- and SO_4^{2-}, respectively, and R_{SW} is the molar ratio of Cl^- to SO_4^{2-} in surface seawater ($R_{SW} = 19.33$; Weston et al. 2006). Sulfate depletion provides an estimate of microbial sulfate reduction, as this parameter reflects the difference between the expected ($=[Cl^-_M * R_{SW}^{-1}]$) and measured SO_4^{2-} ($SO_4^{2-}_M$) concentration, thus reflecting the net microbially-mediated conversion of SO_4^{2-} to H_2S (Weston et al. 2006). Inventories of DIC_R were compared to $SO_4^{2-}_{dep}$ to estimate soil respiration coupled to SO_4^{2-} reduction, assuming a stoichiometry of 2 moles of DIC produced per mole of SO_4^{2-} reduced (Canfield et al. 1993a). Ratios of inorganic nutrient inventories to $SO_4^{2-}_{dep}$ inventories were used to estimate the magnitude of nutrient regeneration coupled to anaerobic microbial metabolism.

Data for the total carbon (C), nitrogen (N), and phosphorus (P) content of microbial mats, and both leaves (live and senescent) and roots (fine and coarse) from stunted red mangrove trees

was either obtained from the literature or determined using a ThermoFinnigan FlashEA 1112 Elemental Analyzer (for C and N; Kristensen & Andersen 1987) or ashing/acid digestion (for P; Aspila et al. 1976). Samples were air-dried and ground using a mortar and pestle. For C and N analysis, both acidified (1 N HCl) and unacidified samples were analyzed on a ThermoFinnigan Flash EA 1112 Series NC analyzer to determine total C and N as well as carbonate-C content. The carbonate content was calculated as the total C (unacidified sample C content) minus the organic C (acidified sample C content). Total P content was determined using an adaptation of the Aspila et al. (1976) method. A known amount of sediment (ca. 15 mg) was baked for 2 hrs at 550°C and then transferred into 50 ml plastic centrifuge tube containing 25 ml of 2M HCl. Next, the sediment plus acid was heated (95 ºC) in a water bath for 2 hrs. An aliquot was then filtered (0.45 μm filter) and analyzed for phosphate using the molybdenum blue method (peat, mat, leaves; Strickland & Parsons 1972) or by ICP-MS (roots).

RESULTS

Triplicate peepers from three stunted mangrove habitats on Twin Cays demonstrated substantial variability in constituent distribution over depth within a single sampling period (Fig. 2.1). During either wet (September) or dry (May) periods, geochemical variability in the top 10 cm layer of soil was typically low (Fig. 2.2). The chlorinity below 13 cm (equivalent to a salinity of 36.8‰) was similar in September and May (Fig. 2.2). Above 13 cm, the chlorinity differed significantly between September, when chlorinity decreased towards the surface, and May, when chlorinity increased towards the surface. Although pH was slightly more acidic in May than in September, soils were generally circumneutral and the pH tended to decrease with depth (Fig. 2.2). SO_4^{2-} concentrations decreased with depth, paralleling increases in H_2S

concentrations. Reduced metabolites, including NH_4^+, Fe^{2+}, Mn^{2+}, and H_2S, increased to high concentrations (~200 μM, 10 μM, 20 μM, and 13 mM, respectively) with depth indicating reducing conditions, especially in September. Ortho-phosphate, DIC and CH_4 concentrations also increased with depth below the surface (Fig. 2.2). Dissolved inorganic carbon was the only constituent whose concentration was greater in May than in September. Dissolved organic carbon and DON concentrations increased significantly with depth in September, while DOP increased slightly with depth in May. NO_x concentrations were consistently low, but detectable, over the entire depth profile in both seasons. Below the upper flushed layer of sediments, concentrations of biologically produced trace gases were extremely high. Concentrations of N_2O (up to 375 nM) greatly exceeded the concentrations predicted from equilibrium with atmospheric N_2O (~6 nM). Similarly, pore water CH_4 concentrations (up to 80 μM) exceeded concentrations predicted from equilibrium with atmospheric CH_4 (~1.2 nM) (Fig. 2.2). Concentrations of N_2O and CH_4 did not exceed calculated saturation values (3 mM for N_2O or 1.9 mM for CH_4, estimated from solubility data presented in Weiss & Price (1980) and Yamamoto et al. (1976) respectively; data not shown) meaning that bubble formation and ebullition is not an important mechanism for trace gas release from this habitat.

Total dissolved nitrogen was composed primarily of NH_4^+ and secondarily of DON; the concentrations of both TDN and DON were higher in September (Fig. 2.2). Because of differences in the proportions of PO_4^{3-} and DOP present at depth at the different sampling times, TDP concentrations were comparable. We compared the observed porewater inorganic C:N:P molar ratios to the Redfield ratio (106:16:1; Redfield 1958) and to potential soil organic matter sources in dwarf mangrove zones, mainly microbial mats, green and senescent *R. mangle* leaves, and fine and coarse roots of *R. mangle* (Table 2.1). Porewater DIN:DIP ratios in the upper 10

cm did not differ significantly over time but ratios at depth (35-50 cm) were lower in September compared to May (Fig. 2.2 & 2.3; Table 2.1); DIN:DIP ratios consistently indicated P limitation with respect to the Redfield ratio. The DIC_R:DIN molar ratios in May (31:1 and 59:1 in surface and deep samples, respectively, Table 2.1) were greater than in September (6:1 and 37:1 in surface and deep samples, respectively, Table 2.1) and the DIC_R:DIN was lower in surficial porewater than at depth (Table 2.1). The DIC_R:DIP ratios followed a similar pattern with values being higher in May than in September; the ratios consistently increased with depth (Table 2.1).

Organic C, N and P species contributed significantly to the porewater C-N-P pools (Fig. 2.2 & 2.3). Similar to the DIN:DIP ratios, the TDN:TDP ratios indicated P limitation relative to the Redfield ratio (Fig. 2.3). The dissolved organic C:N:P molar ratios demonstrated little variability over season or depth and the average DOC:DON:DOP ratio was 4899:39:1. DOC:DON ratios were generally higher in May than September while DOC:DOP ratios were generally higher in September than in May (Fig. 2.3).

Porewater constituent inventories integrated over various depths (0-5, 5-10, 10-20, and 20-40 cm) as well as over 'ALL' depths (0-40 cm) were compared to examine seasonal variability (Fig. 2.4 & 2.5). Although Cl^-, SO_4^{2-}, H_2S, DIC, NH_4^+, PO_4^{3-} and DOP exhibited differences at specific depths, inventories over 'ALL' depths were not significantly different. Only DOC, DON, and Mn^{2+} inventories over 'ALL' depths differed significantly seasonally ($p < 0.05$, 0.10, and 0.09, respectively; 2-tailed t-test), being 5-10 times higher in September than in May (Fig. 2.4). Inventories of $SO_4^{2-}{}_{dep}$ and CH_4 were not significantly different between September and May (Fig. 2.5).

Molar ratios of H_2S, DIC_R, NH_4^+ and PO_4^{3-} to $SO_4^{2-}{}_{dep}$ were comparable in May and September over 'ALL' depths and at deep depths (10-40 cm; Fig. 2.6). In May, H_2S and $SO_4^{2-}{}_{dep}$

inventories were not significantly different, suggesting that H_2S accumulated proportionately as SO_4^{2-} was consumed by sulfate reduction. In September, the H_2S produced exceeded the expected inventories due to SO_4^{2-} depletion. DIC_R ratios indicated that SO_4^{2-} reduction dominated organic carbon mineralization, assuming a reaction stoichiometry of 2 moles of CO_2 produced per mole of SO_4^{2-} reduced. Redfield ratios of NH_4^+ and PO_4^{3-} to $SO_4^{2-}{}_{dep}$ would be 0.3 and ~0.019, respectively, but such ratios were rarely achieved in the porewater. If SO_4^{2-} reduction was coupled solely to remineralization of senescent *R. mangle* leaf organic matter, 0.018 mol NH_4^+ and 0.00013 mol PO_4^{3-} would be produced per mol of SO_4^{2-} reduced.

DISCUSSION

The lack of strong geochemical gradients in the upper 10 cm of mangrove soils can be attributed to porewater flushing by physical and biological processes, including precipitation, tidal drainage, or root activities (Alongi et al. 1999, 2004). Variation in precipitation and tidal height may drive significant exchange of pore fluids, either pore water on offshore mangrove islands or groundwater on terrestrial mangroves (Ridd & Sam 1996, Ridd et al. 1997, Sam & Ridd 1998). However, in most systems, such hydrological forcing functions are poorly constrained (Lee 1995, Twilley & Chen 1998). Wet-dry seasonality significantly affects soil water levels and salinities in tropical mangrove habitats (Alongi et al. 2004). Porewater advection through mangrove soils results from changes in hydraulic head, which varies daily and seasonally as a function of the tidal height (Ridd et al. 1997). Twilley and Chen (1998) showed that tides alleviated mangrove soil saturation deficits, and that the frequency of tidal inundation made higher elevation mangrove habitats more sensitive to changes in precipitation. In Rookery Bay, the cumulative tidally-driven water inputs and effluxes were of similar magnitude, around

12000 mm yr^{-1} (Twilley & Chen 1998) meaning that tidal fluctuations efficiently flushed the peat soils.

At Twin Cays, daily and seasonal variations in both precipitation and tidal height (Lee & Joye 2006, Ch. 4) likely generated the consistent 10-cm deep zone that was characterized by negligible gradients in porewater constituent concentrations (Fig. 2.2). Though the average tidal range at Twin Cays is ~20 cm (Rützler & Feller 1996), there is substantial variation in tidal forcing over the seasons. During the spring and early summer, tidal elevations below mean sea level tides are common and these low tides are coincident with reductions in precipitation, leading to extremely dry conditions in stunted mangrove zones (Lee & Joye 2006, Ch. 4). During this period, which can be considered the dry season, decreased precipitation combined with high evaporation rates increased shallow porewater salinity while lower tidal heights increased soil exposure to the atmosphere. Dry season soils were thus more oxidized and contained lower concentrations of reduced species such as H_2S and NH_4^+ (Fig. 2.2). The spring-early summer hydrological regime contrasts markedly with that of the fall-winter. During the wet fall-winter season, stunted mangrove zones were inundated continuously because of increased precipitation and higher tidal heights, which decreased shallow porewater salinity and likely increased pond water infiltration through the soils respectively. Wet season soils were characterized by higher inventories of dissolved organic matter (DOM) and elevated rates of anaerobic microbial metabolism (see below). Differences in hydrology thus contributed significantly to the observed differences in porewater geochemistry.

Dissolved organic matter (DOM) in the soil porewater exhibited high DOC:DON and DOC:DOP ratios; these organic constituents were likely derived from the decay of mangrove litter and roots (Alongi et al. 2005). Soluble, reactive mangrove leaf litter leachates (e.g., sugars,

carbohydrates and amino acids) are quickly consumed by microbes (Benner et al. 1986), while more refractory litter components, e.g., lignins, decay an order of magnitude more slowly (Robertson 1988). Soluble phenolic tannins represent a significant fraction of *R. mangle* leaf litter leachates, but tannins inhibit microbial degradation of DOM only at high concentrations (g l^{-1}; Benner et al. 1986). An additional source of organic matter to soils in the dwarf mangrove zone is surficial microbial mats. These cyanobacterial exhibited high rates of primary production (Joye & Lee 2004) and nitrogen fixation (Lee & Joye 2006, Ch. 4). Mineralization of and/or leakage of labile DOM derived from microbial mats likely contributed the observed sediment-water interface peaks in DON observed at both sampling times (Fig. 2.2). Similarly, autochthonous microbial N-inputs via nitrogen fixation likely contributed to the high DIN:DIP ratios observed in soils from both seasons (Fig. 2.2, Table 2.1; Lee & Joye 2006, Ch. 4). DON and DOC concentrations exhibited no gradient with depth in May, but in September concentrations increased 10- and 5-fold, respectively, at depth. Litter fall from Twin Cays stunted mangroves is twice as high in the fall relative to the spring (Koltes et al. 1998). We hypothesize that increased litterfall, combined with increased inundation during the wet season, resulted in greater DOC and DON inputs to the soils by leaching of mangrove and microbial mat derived DOM and microbial and/or invertebrate breakdown of mangrove leaves and detritus to DOM (Fig. 2.2 & 2.3).

Twin Cays soils were anoxic below the upper 1 to 2 cm (Joye & Lee 2004), so anaerobic metabolic processes, including denitrification, iron and manganese reduction, sulfate reduction, and methanogenesis, dominated organic carbon turnover over the depth profiles that we evaluated (~0-40 cm). High denitrification rates in mangrove soils can be fueled by *in situ* (e.g., nitrification) or tidal inputs of NO_x (Alongi et al. 1999). Though offshore oceanic waters are

usually characterized by low NO_x concentrations, NO_x concentrations in incoming tidal waters can be elevated because these waters flow past the dense communities of sponges living on the submerged roots of mangrove trees that line the island fringe (Rützler et al. 2000, 2004). These sponges are host to a variety of microbial symbionts, including nitrogen fixing and nitrifying bacteria (Diaz & Ward 1997, Diaz et al. 2004, Rützler et al. 2000, 2004). These porous sponges may be hot-spots of N cycling on coral reefs and in mangrove habitats but linkages between the sponge-supported microbial N cycling and the surrounding ecosystem are poorly understood. Sponges are an important N source to associated algae on coral reefs (Davy et al. 2002), and in mangrove environments, sponges may serve as a NO_x source for fringing mangrove trees and/or for the microbes inhabiting mangrove soils. The highest *in situ* denitrification rates observed in Twin Cays soils were measured in the fringing mangrove zone (Lee & Joye 2006, Ch. 4). *In situ* denitrification rates in soils from stunted mangrove sites were lower than rates in the fringing mangrove zone but potential rates, determined in the presence of added NO_3^-, were high (Lee & Joye 2006, Ch. 4), indicating that these soils possess a high potential for denitrification when NO_x is available. Rates of potential denitrification in deeper (<40 cm) soils were lower than rates observed in surficial mats, but rates were still substantial (~1 nmol cm^{-3} h or ~1 μM h^{-1}; Porubsky & Joye, unpublished data). Clearly, substantial dissimilatory sinks for NO_x exist in these soils.

Under natural conditions, *in situ* nitrification can also be an important source of NO_x for denitrification in mangrove soils. Nitrification in surface soils may be stimulated by benthic primary production, which significantly increases oxygen concentrations in the upper 1 to 2 cm of these soils (Joye & Lee 2004). Similarly, nitrification deeper in soils may be stimulated by oxygen translocated by plant roots or animal burrows (Kristensen et al. 1998, Kristensen &

Alongi 2006). Efficient consumption of NO_x via biological assimilation and/or denitrification in Twin Cays soils maintained the observed low NO_x concentrations in the soil porewater (Fig. 2.2).

The surprisingly high concentrations of N_2O observed in these mangrove soils (Fig. 2.2), between 15 and 400 nM, are from 2 to 65 times greater than the concentrations expected if the soil pore fluids were in equilibrium with atmospheric N_2O concentration, which would yield a porewater N_2O concentration of about 6 nM. N_2O can be produced during both nitrification and denitrification; it is unclear whether N_2O is produced as a by-product during dissimilatory nitrate reduction to ammonium or anammox. In previous studies of N_2O dynamics in mangrove habitats (Corredor et al. 1999, Bauza et al. 2002), a strong correlation was observed between N_2O flux and DIN, mainly NH_4^+, concentration. Because of this N_2O-NH_4^+ correlation, the high N_2O fluxes were attributed to nitrification (Corredor et al. 1999, Bauza et al. 2002). The high sulfide concentrations present in these soils (0.5 to 20 mM, Fig. 2.2) probably inhibit nitrification (Joye & Hollibaugh 1995) and may also block the terminal enzymatic step of denitrification, i.e., the reduction of N_2O to N_2 (Sørensen et al. 1978, Joye 2002), resulting in incomplete denitrification where NO_x is reduced only to N_2O (Brundet & Garcia-Gil 1996). It is tempting to speculate that H_2S short-circuited denitrification, thus generating the extremely supersaturated N_2O concentrations observed in these mangrove soils (Fig. 2.2). However, N_2O concentrations were elevated in aerobic surface sediments as well as deeper sulfidic sediments, suggesting that multiple processes were involved in N_2O production. Understanding the processes regulating N_2O dynamics in these habitats requires further study but given the observed extremely high porewater N_2O concentrations, we conclude these soils almost certainly support a substantial

flux of N_2O to the atmosphere, as suggested by previous studies (Corredor et al. 1990, Alongi et al. 2005).

Fe^{2+} and Mn^{2+} reduction rates are often low in mangrove soils (Alongi et al. 1999, 2005) but the reasons for this are not clear (Alongi et al. 1999). In island mangrove environments, iron and manganese oxide concentration in soils depend on allochthonous inputs, which come from the ocean (via seawater delivery), land (via terrestrial runoff) or atmosphere (volcanic or dust inputs). Concentrations of Fe and Mn in seawater are extremely low making it unlikely that seawater infiltration provides a substantial Fe or Mn source to such islands. Terrestrial runoff reaches Twin Cays only following anomalous weather events, such as hurricanes or tropical storms. For example, in October 1998, the flood-waters from Hurricane Mitch carried dissolved nutrients and particulates more than 40 km offshore from Belize (Muller-Karger et al. 2005). Strong storms occur regularly and periodic delivery of nutrient and particle laden waters to island mangrove systems could serve as an important source of bioactive materials to these habitats. Finally, atmospheric inputs of Saharan dust are known to be an important source of metals to offshore islands in the Atlantic and Caribbean (Muhs et al. 1990, Hayes et al. 2001).

Twin Cays soil porewaters contained concentrations of dissolved, reduced Fe^{2+} up to10 μM and Mn^{2+} up to 50 μM, suggesting active cycling of metal oxides in this habitat (Fig. 2.2). The peat soils contained considerable concentrations of total iron and manganese (7 to 100 μmol Fe (g dry weight)$^{-1}$ and 1 to 5 μmol Mn (g dry weight)$^{-1}$, respectively; K. L. McKee, unpublished data). The concentrations of total Fe in Twin Cays soils were similar to those reported for terrestrial mangrove environments, ~81 μmol Fe (g dry weight)$^{-1}$, but the concentrations of total Mn at Twin Cays (~3 μmol (g dry weight^{-1})) were lower than those reported for terrestrial mangroves, 36 μmol Mn (g dry weight)$^{-1}$ (Alongi et al. 2005). Despite the lower abundance of

manganese oxides, the high concentrations of porewater dissolved Mn^{2+} suggest that the available manganese oxides present are reactive and the change in dissolved Mn^{2+} concentrations over time suggest that the factors driving rates of metal reduction vary seasonally (Fig. 2.2 & 2.4). The high dissolved Mn^{2+} concentrations observed in the wet season suggest more active Mn cycling during this time by either direct biological Mn oxide reduction (Burdidge 1993) or by reductive dissolution of manganese oxides associated with the anaerobic oxidation of H_2S (Canfield et al. 1993b). In contrast, during the dry season, more oxidized soil conditions may have retained Mn^{2+} on the solid phase by adsorption to solid phase metal oxides (Canfield et al. 1993b).

Previous studies have suggested a link between mangrove root dynamics and iron and manganese reduction activity in mangrove soils (Alongi et al. 1999, 2001, 2005). Though Fe and Mn reduction typically account for only a small fraction of total organic carbon mineralization in mangrove soils, metal reduction is consistently documented in mangrove soils (Alongi et al. 1999). Alongi et al. (1999, 2001, 2005) concluded that iron and manganese cycling was stimulated by the presence and activity of mangrove roots. Belizian and other mangrove peats are comprised largely -- up to 80% -- of coarse and fine roots (McKee & Faulkner 2000). Alongi et al. observed that the production of dissolved Fe^{2+} and Mn^{2+} was correlated strongly with the density of live roots, leading the authors to speculate that root organic exudates stimulated microbial metal reduction. The important role of plant roots in metal cycling has been documented previously in salt marsh environments (Lacerda et al. 1993) where metal oxides precipitate as plaques on roots (Sundby et al. 1998). Alongi et al. (2001) observed that rates of anaerobic microbial metabolism correlated positively with root density but not with soil organic carbon content (Alongi et al. 2001), providing evidence that roots enhance

microbial metal cycling by providing carbon substrate. Thus roots may influence both concentration electron acceptors, iron and manganese oxides, as well as provide reductant, as organic carbon exudates, for soil microbial communities.

The presence of roots was not the only factor that appeared to influence metal cycling in Twin Cays soils: Mn^{2+} concentrations were substantially higher during the wet season than during the dry season (Fig. 2.2 & 2.4). Alongi and colleagues (Alongi et al. 2001) documented extremely high rates of both Fe and Mn reduction (about 1.5 mmol m^{-2} d^{-1}) in Thialand mangrove soils. Pore water Fe^{2+} concentrations were 10-30 μM (Alongi et al. 2001), which are comparable to the Fe^{2+} concentrations we documented at Twin Cays. Interestingly, rates of Fe and Mn reduction, as well as rates of sulfate reduction, were 2 to 4 times higher during the wet season than during the dry season (Alongi et al. 2001). At Twin Cays, litterfall is highest in the fall (Koltes et al. 1998) and we documented significantly higher porewater DOC concentrations at this time as well. We hypothesize that increased litterfall and subsequent degradation of detritus increases the porewater concentrations of labile DOC, fueling higher rates of metal reduction, especially manganese reduction, as well as other anaerobic processes (see below).

As in other coastal marine environments, the dominant pathway for organic matter oxidation in Twin Cays soils appeared to be SO_4^{2-} reduction (Kristensen et al. 1991, 1995, Canfield et al. 1993a, Alongi et al. 1999). SO_4^{2-} depletion profiles indicated fairly similar SO_4^{2-} reduction rates in the wet and dry seasons (~100 μmol cm^{-2} of SO_4^{2-} depletion; Fig. 2.2 & 2.5). During the wet season, however, H_2S inventories (300 μmol cm^{-2}; Fig. 2.4) exceeded net sulfate reduction, as estimated from $SO_4^{2-}{}_{dep}$ (100 μmol cm^{-2}; Fig. 2.4 & 2.5), suggesting an additional H_2S source. Green *R. mangle* leaves are about 0.31% sulfur by weight while senescent leaves are 0.67% sulfur by weight; senescent *R. mangle* leaves have a N:S ratio of about 1 (Fry & Smith

2002). The decomposition of sulfur rich mangrove detritus may offer a biogenic organic sulfur source, such as amino acids or fulvic and humic acids, whose mineralization could contribute to porewater H_2S pools. Significant contributions of organic sulfur to porewater H_2S pools have been documented in other mangrove soils and sediments (Altschuler et al. 1983, Holmer et al. 1994), where the mineralization of mangrove-derived organic sulfur compounds was postulated to lead to H_2S accumulation.

Root exudation of labile organic carbon may stimulate sulfate reduction as well as metal reduction (Alongi et al. 2001, 2005, Kristensen & Alongi 2006). We compared sulfate reduction rates, as estimated from SO_4^{2-} depletion ($SO_4^{2-}{}_{dep}$) values, to DOC concentrations and found no correlation between $SO_4^{2-}{}_{dep}$ and DOC (data not shown). This finding mirrors that of Alongi et al. (2001, 2005) and suggests that root organic carbon exudates may be closely linked to microbial sulfate reduction rates. Weston et al. (2006) found no correlation between $SO_4^{2-}{}_{dep}$–derived sulfate reduction rates and porewater DOC concentrations in temperate intertidal sediments from Georgia and South Carolina, concluding that the bulk DOC pool consisted of largely refractory organic matter. They also suggested that the observed refractory nature of the DOC pool could drive the sediment microbial community to be limited by the availability of labile dissolved organic carbon; this was particularly true during summer when high temperature supported high rates of microbial metabolism (Weston et al. 2006, Weston & Joye 2005). Integrated rates of sulfate reduction (also estimated from $SO_4^{2-}{}_{dep}$ and confirmed using $^{35}SO_4^{2-}$ radiotracer experiments; Weston et al. 2006) observed in temperate sediments (10-400 μmol cm^{-2}) were comparable to the integrated rates of sulfate reduction estimated for these mangrove sediments (~100 μmol cm^{-2}; Fig. 2.6). Unlike the situation at the sites studied by Weston et al. (2006), porewater SO_4^{2-} concentrations never reached zero in these mangrove soils (Fig. 2.2).

Thus, the estimates of integrated sulfate reduction rates obtained from our ~45 cm deep peeper deployments probably underestimate the total activity of the bioactive sediment column. Alongi et al. (2005) proposed that substantial microbial activity persists to at least the depth where live roots cease to exist, which is 1-2 m down the soil profile. Future studies should evaluate the depth distribution and metabolic controls on sulfate reduction in these and other mangrove soils.

Methane inventories (0.45 μmol cm^{-2}) were small relative to SO_4^{2-} depletion inventories (100 μmol cm^{-2}; Fig. 2.5), suggesting that methanogenesis was not a dominant pathway for organic matter oxidation in Twin Cays soils. However, CH_4 concentrations were high (up to 80 μM) during both seasons, suggesting that methanogenesis rates were substantial and consistent over time. Methane is not commonly detected in the porewater from mangrove soils (Alongi et al. 1999, 2001, 2004), indicating that methanogenesis either does not occur or that methane does not accumulate because it is consumed efficiently by aerobic and/or anaerobic oxidation (Giani et al. 1996). A few studies have reported methane efflux from mangrove soils (Hariss et al. 1988, Barber et al. 1988, Sotomayor et al. 1994, Lu et al. 1999, Alongi et al. 2005) but the fluxes were typically below 100 μmol m^{-2} d^{-1}. However, methane fluxes from a sewage impacted site in Puerto Rico were high, up to 5 mmol m^{-2} d^{-1} (Lu et al. 1999). The paucity of data on methane concentrations and fluxes from mangrove habitats makes it impossible to conclude whether these soils are an important source of methane to the atmosphere as are other wetland soils. Further work is needed to fully understand this topic.

One of the most intriguing aspects of porewater methane biogeochemistry at Twin Cays was accumulation of methane in the presence of sulfate (Fig. 2.2). Methanogenesis and sulfate reduction do not typically occur contemporaneously because SO_4^{2-} reduction is more energetically favorable than methanogenesis (Capone & Kiene 1988). Furthermore, the sulfate

dependent anaerobic oxidation of methane occurs in SO_4^{2-} containing environments (Valentine & Reeburgh 2000) so methane would not be expected to accumulate in sulfate-rich soil layers. Methanogens and sulfate reducers compete for some substrates, like acetate or hydrogen, and methanogens are typically outcompeted by SO_4^{2-} reducing bacteria. However, simultaneous activity of these functional microbial groups can occur in SO_4^{2-} rich sediments if methanogens use non-competitive substrates, like methylated amines, or if competitive substrates are abundant, relieving competition (Oremland & Polcin 1982). Noncompetitive substrates, including methanol, trimethylamines and dimethylsulfide, can fuel methanogenesis in SO_4^{2-} containing mangrove sediments (Purvaja & Ramesh 2001, Lyimo et al. 2000, Mohanraju et al. 1997). These substrates are produced through a variety of pathways; methanol by anaerobic bacterial metabolism, methylated amines from the decomposition of organic osmolytes, e.g., choline or glycine betaine, and dimethylsulfide by the catabolism of dimethylsulfoniopropionate or amino acids (Lyimo et al. 2002). The distribution of methanogenic microbes and patterns of methanogenesis in these and other stunted mangrove soils is a worthy subject for future investigations as such soils may prove to be an important source of atmospheric methane.

Nutrients released from the remineralization of organic matter, including NH_4^+, PO_4^{3-}, DON and DOP, increased with depth and were present at slightly greater concentrations during the wet season (Fig. 2.2 & 2.5). Reducing conditions in these soils increased slightly with more frequent submergence during the wet season, as has been documented in other mangrove soils (Alongi et al. 2004). The observed ratios of NH_4^+ and PO_4^{3-} suggest remineralization of organic matter composed of a combination of senescent leaf material, microbial mat-derived organic matter, mangrove roots and/or root exudates. PO_4^{3-} and DOP in these dwarf mangrove soils were low, but similar to concentrations of dissolved P in a variety of other mangroves, while

dissolved N concentrations were high compared to other mangroves (Sherman et al. 1998, Middelburg et al. 1996, Alongi et al. 1992). These greater N inventories may be the result of high rates of nitrogen fixation observed in soils from Twin Cays stunted mangrove habitats (Lee & Joye 2006, Ch. 4). The porewater DIN:DIP and TDN:TDP ratios are higher than the Redfield ratio (Fig. 2.3) but lower than expected from mineralization of mangrove leaf litter. DIC_R:DIN and DIC_R:DIP ratios varied substantially between May and September, being higher in May. These data suggest that nutrients, particularly phosphorus, are efficiently immobilized by soil microbial communities and that different organic matter sources fuel soil microbial activity during different times of year.

The same peeper designs and analytical methods used in this study were used to evaluate porewater stoichiometry in estuarine creek bank sediments from Georgia and South Carolina (Weston et al. 2006), which allows direct comparisons of these data sets. SO_4^{2-} was not completely consumed in Twin Cays soil while it was consumed at shallow depths (~15 cm in summer) in estuarine sediments from Sapelo Island (coastal Georgia) or the Okatee estuary (South Carolina). In temperate estuarine sediments, H_2S concentrations were generally related to SO_4^{2-} reduction rates but reoxidation and/or pyritization depleted H_2S inventories. In contrast, mangrove sediments had H_2S concentration inventories that exceeded SO_4^{2-} depletion inventories, potentially due to the diagenesis of sulfur-rich mangrove organic matter. Methane inventories in Twin Cays were similar to those observed in saline estuarine sediments. Dissolved organic carbon inventories from temperate estuarine and Twin Cays sites were similar and DOC pools at both sites appeared to be recalcitrant. Denitrification played a minor role in organic matter oxidation in both the mangrove soils and estuarine sediments discussed here and was limited by low NO_x inventories. Georgia salt marsh sediments also contained lower H_2S

inventories than in Twin Cays soils (Koretsky et al. 2003, Weston et al. 2006); while Fe^{2+} inventories in temperate salt marsh and creek bank sediments were both much greater, suggesting a more significant role of iron reduction in the temperate marsh sediments than in Twin Cays soils.

Summary

Benthic metabolism in the predominantly anoxic soils in stunted mangrove habitats on Twin Cays was dominated by SO_4^{2-} reduction, but terminal metabolic products of metal reduction, denitrification and methanogenesis suggested these processes also occurred. Nitrous oxide concentrations were consistently high over depth and time, suggesting that stunted mangrove soils are a source of N_2O to the atmosphere. Sulfate reduction was responsible for most organic matter remineralization, but the presence of SO_4^{2-} at depth (~40 cm) suggested carbon limitation of sulfate reducers, possibly due to the refractory nature of mangrove-derived DOM. High porewater CH_4 concentrations indicated significant rates of methanogenesis occurring at depths where SO_4^{2-} was abundant, suggesting that methanogens and sulfate reducers were active in the same depth horizons. Twin Cays stunted mangrove soils contained Cl^-, SO_4^{2-}, SO_4^{2-} depletion NH_4^+, NO_x, DOC, DON, DIC and CH_4 inventories similar to saline temperate estuarine sediments, but seasonality in mangrove litter input and hydrological regimes drove variations in DOM oxidation patterns and nutrient regeneration. During the wet season, leaching of mangrove litter in the overlying water may have resulted in high concentrations of DOM in the porewater, providing substrates for substantial H_2S production that exceeded sulfate reduction and also fueled simultaneous methanogenesis and metal reduction.

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Table 2.1. Porewater inorganic C:N:P ratios and C:N:P content and ratios of dominant organic matter (OM) sources in Twin Cays stunted mangrove habitats.

Porewater	**n**	**%C**	**(se)**	**%N**	**(se)**	**%P**	**(se)**	**C:N:P (molar)**	**Reference**
May									
porewater, 0-10	3							1524:49:1	This work
porewater, 35-50	3							3110:52:1	This work
September									
porewater, 0-10	3							310:52:1	This work
porewater, 35-50	3							1099:29:1	This work
OM Sources									
microbial mat	20	29.5	1.2	2.9	0.5	0.094	0.017	124:14:1	This work
green leaf	3	48.6	4.1	1.13	0.02	0.038	0.001	3300:66:1	Smallwood et al. 2003 Feller et al. 2003
senescent leaf	81	46.2	2.8	0.49	0.05	0.007	0.000	15567:155:1	Feller et al. 2003
fine root	10	41.8	0.5	0.75	0.04	__	__	48:1:__	This work
coarse root	10	38.1	0.3	0.43	0.02	__	__	76:1:__	This work

FIGURE CAPTIONS

Figure 2.1. Porewater steady state profiles of Cl^-, DIC, DOC, and SO_4^{2-} from individual peepers in September. Three peepers were incubated in replicate stunted mangrove zones on each sampling date. Symbols are consistent throughout the four panels and refer to peepers from Weather Station (○), Lair (◊) or Dock (▷) sites.

Figure 2.2. Average steady state porewater profiles of Cl^-, SO_4^{2-}, H_2S, $SO_4^{2-}{}_{dep}$, pH, NH_4^+, NO_x, DON, PO_4^{3-}, DOP, Fe^{2+}, Mn^{2+}, DIC, DOC, CH_4 and N_2O from September (filled circles) and May (open circles). CH_4 and N_2O are also expressed in units of % saturation relative to the atmosphere. Dotted lines indicate base of mixed surface soil layer at 10 cm. Error bars = standard deviations.

Figure 2.3. Molar ratios of porewater DIN:DIP, TDN:TDP, DIC_R:DIN, DIC_R:DIP, DOC:DON and DOC:DOP over 0-5, 5-10, 10-20, 20-40 cm and 'ALL' (0-40 cm) depths in September and May. Dotted lines indicate Redfield C:N:P ratios of 106:16:1; double solid lines indicate senescent *R. mangle* C:N:P ratios (Table 2.1). * = DOP below detection; error bars = standard deviations.

Figure 2.4. Porewater inventories of Cl^-, SO_4^{2-}, H_2S, DOC, DIC, NH_4^+, DON, DIP, DOP and Mn^{2+} over 0-5, 5-10, 10-20, 20-40 cm and 'ALL' (0-40 cm) depths in September and May. Error bars = standard deviations.

Figure 2.5. Porewater inventories of $SO_4^{2-}{}_{dep}$ and CH_4 over 0-5, 5-10, 10-20, 20-40 cm and 'ALL' (0-40 cm) depths in September and May. Error bars = standard deviations.

Figure 2.6. Molar ratios of porewater H_2S, DIC_R, NH_4^+ and PO_4^{3-} to $SO_4^{2-}{}_{dep}$ over 0-5, 5-10, 10-20, 20-40 cm and 'ALL' (0-40 cm) depths in September and May. Dotted lines indicate stoichiometric ratios of SO_4^{2-} reduction coupled to oxidation of Redfield organic matter; double solid lines indicate N:P:SO_4^{2-} reduction ratios of 1:0.018:0.00013 when coupled to oxidation of senescent *R. mangle* (see text for details). * = no $SO_4^{2-}{}_{dep}$; error bars = standard deviations.

Figure 2.1

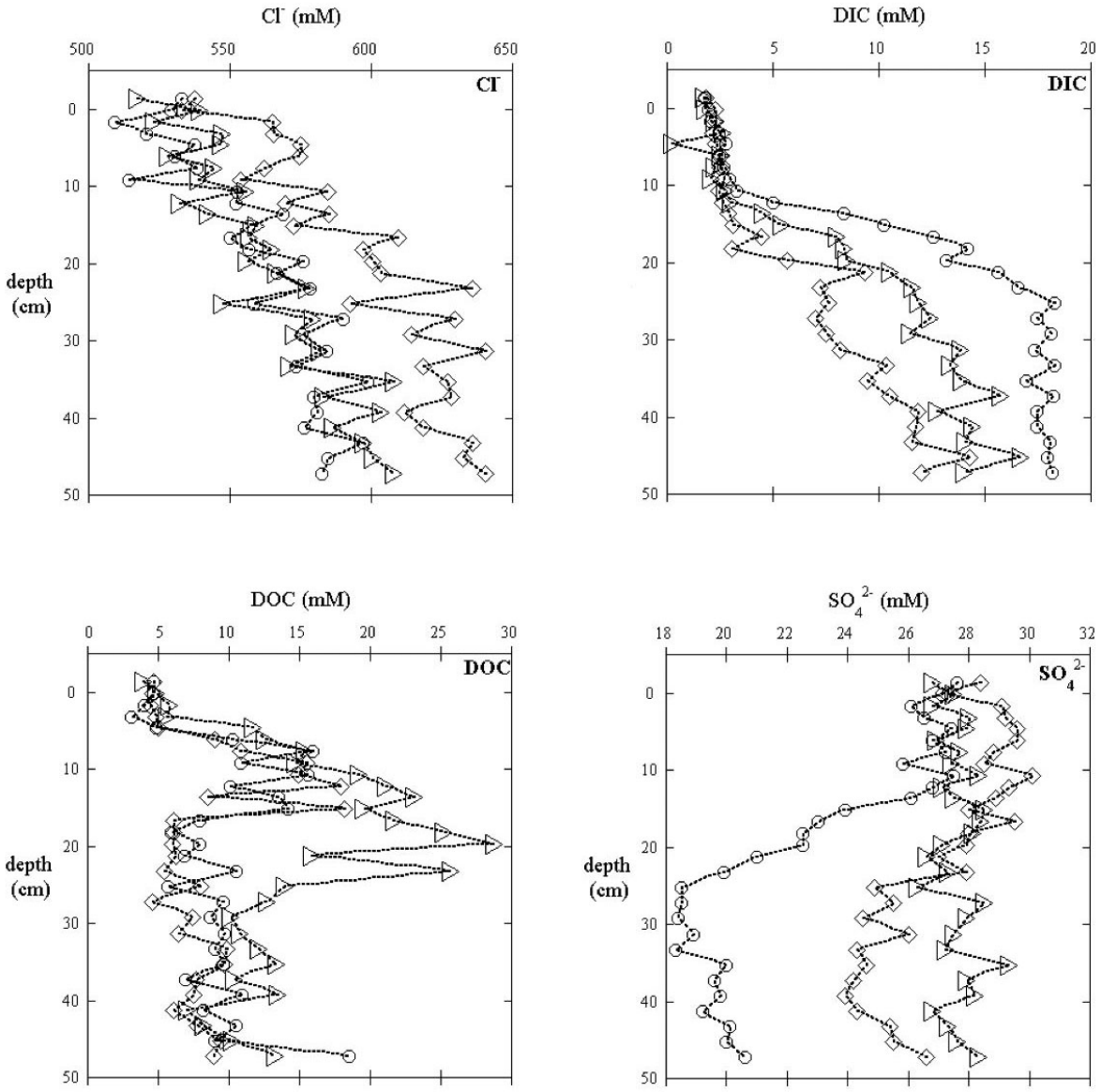

Cl⁻ (mM)
500
550
600
650
Cl⁻
DIC (mM)
0
5
10
15
20
DIC
depth (cm)
0
10
20
30
40
50
DOC (mM)
0
5
10
15
20
25
30
DOC
SO4 2- (mM)
18
20
22
24
26
28
30
32
SO4 2-

Figure 2.2

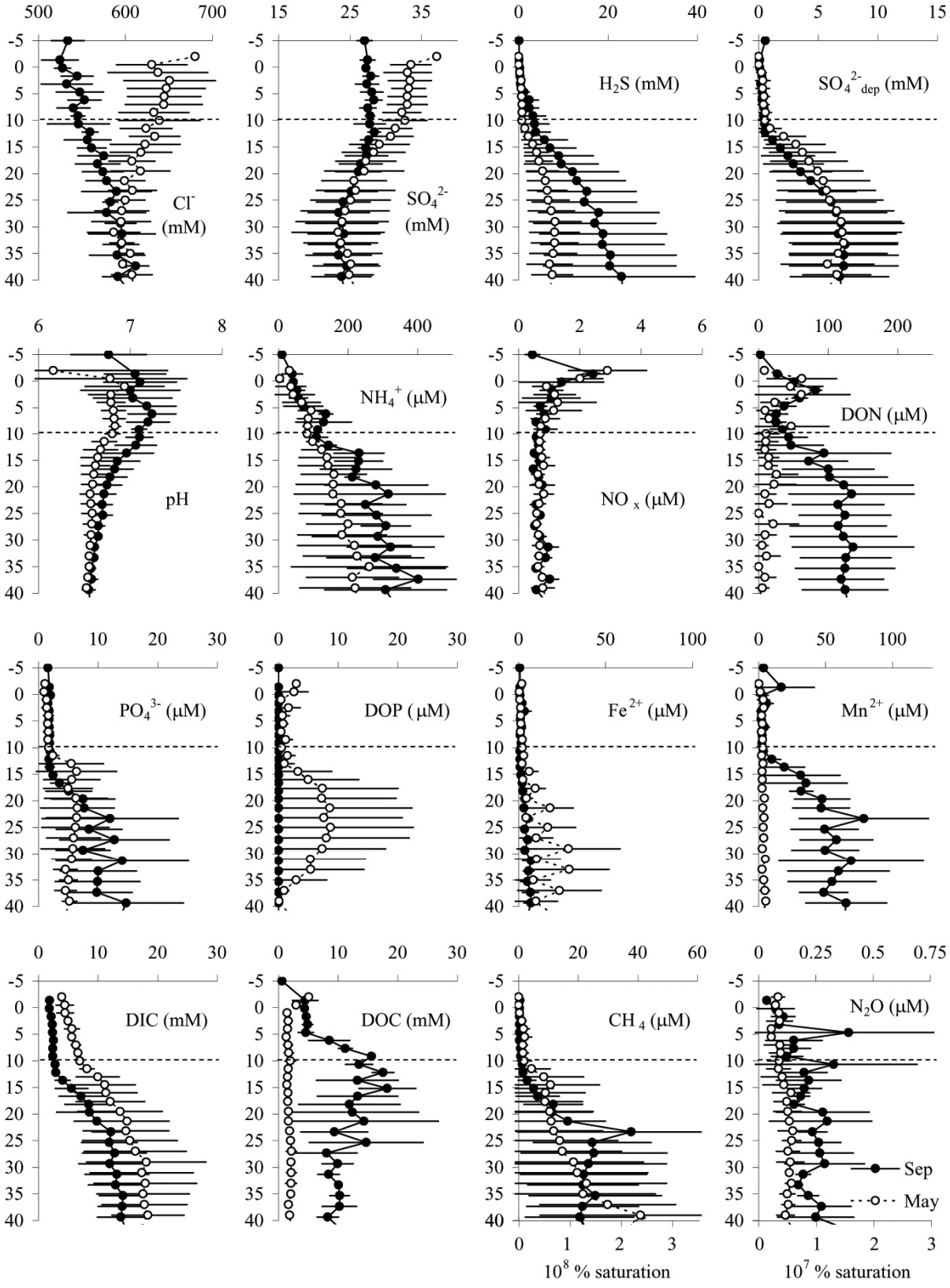

Cl- (mM)
SO4 2- (mM)
H2S (mM)
SO4 2- dep (mM)
pH
NH4+ (µM)
NO x (µM)
DON (µM)
PO4 3- (µM)
DOP (µM)
Fe2+ (µM)
Mn2+ (µM)
DIC (mM)
DOC (mM)
CH 4 (µM)
N2O (µM)
Sep
May
10^8 % saturation
10^7 % saturation

Figure 2.3

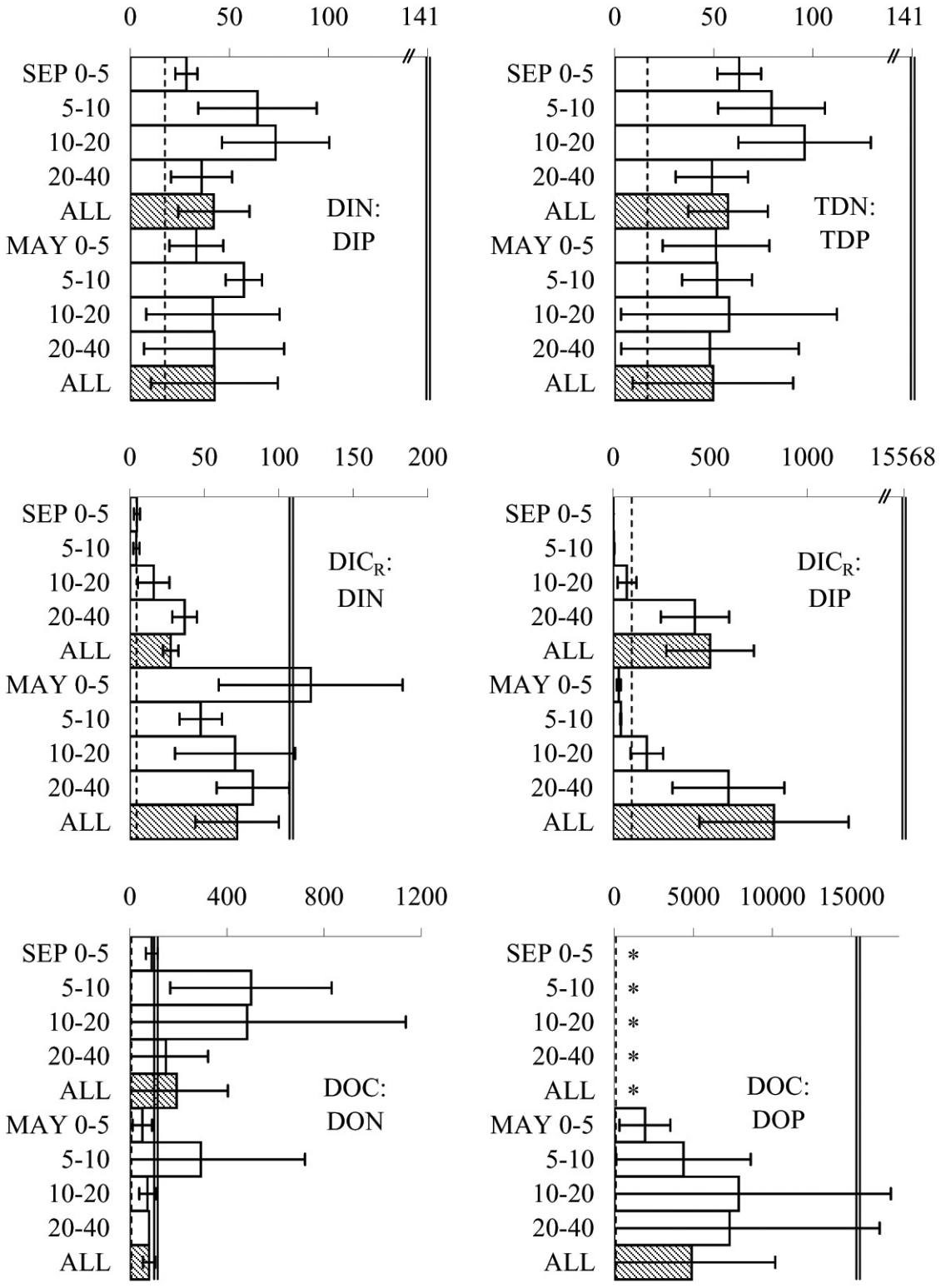

0
50
100
141
SEP 0-5
5-10
10-20
20-40
ALL
MAY 0-5
5-10
10-20
20-40
ALL
DIN:
DIP
TDN:
TDP
0
50
100
150
200
0
500
1000
15568
DIC$_R$:
DIN
DIC$_R$:
DIP
0
400
800
1200
0
5000
10000
15000
DOC:
DON
DOC:
DOP
*

Figure 2.4

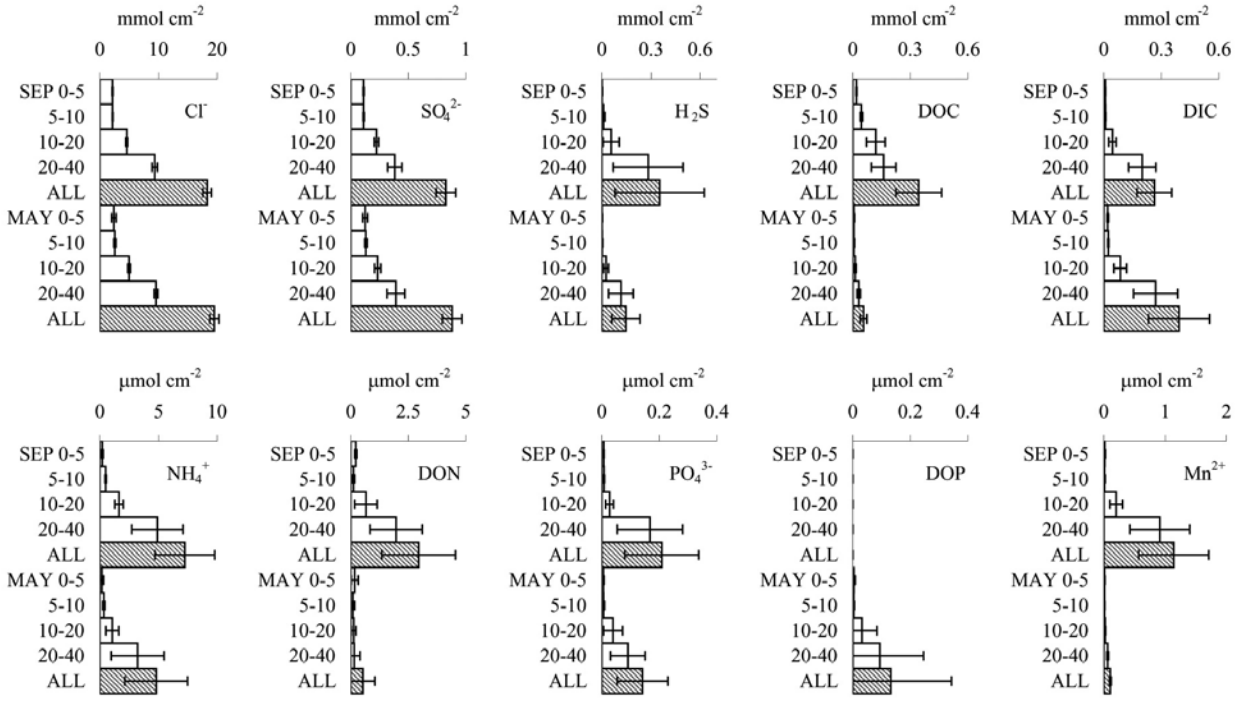

mmol cm-2
0 10 20
Cl-
mmol cm-2
0 0.5 1
SO4 2-
mmol cm-2
0 0.3 0.6
H2S
mmol cm-2
0 0.3 0.6
DOC
mmol cm-2
0 0.3 0.6
DIC
µmol cm-2
0 5 10
NH4+
µmol cm-2
0 2.5 5
DON
µmol cm-2
0 0.2 0.4
PO4 3-
µmol cm-2
0 0.2 0.4
DOP
µmol cm-2
0 1 2
Mn2+
SEP 0-5
5-10
10-20
20-40
ALL
MAY 0-5
5-10
10-20
20-40
ALL

Figure 2.5

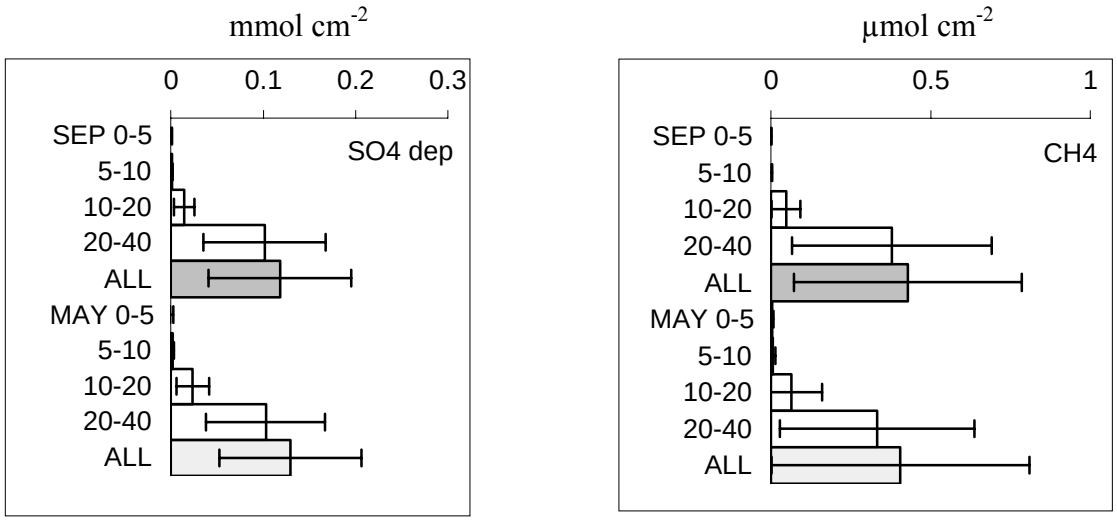
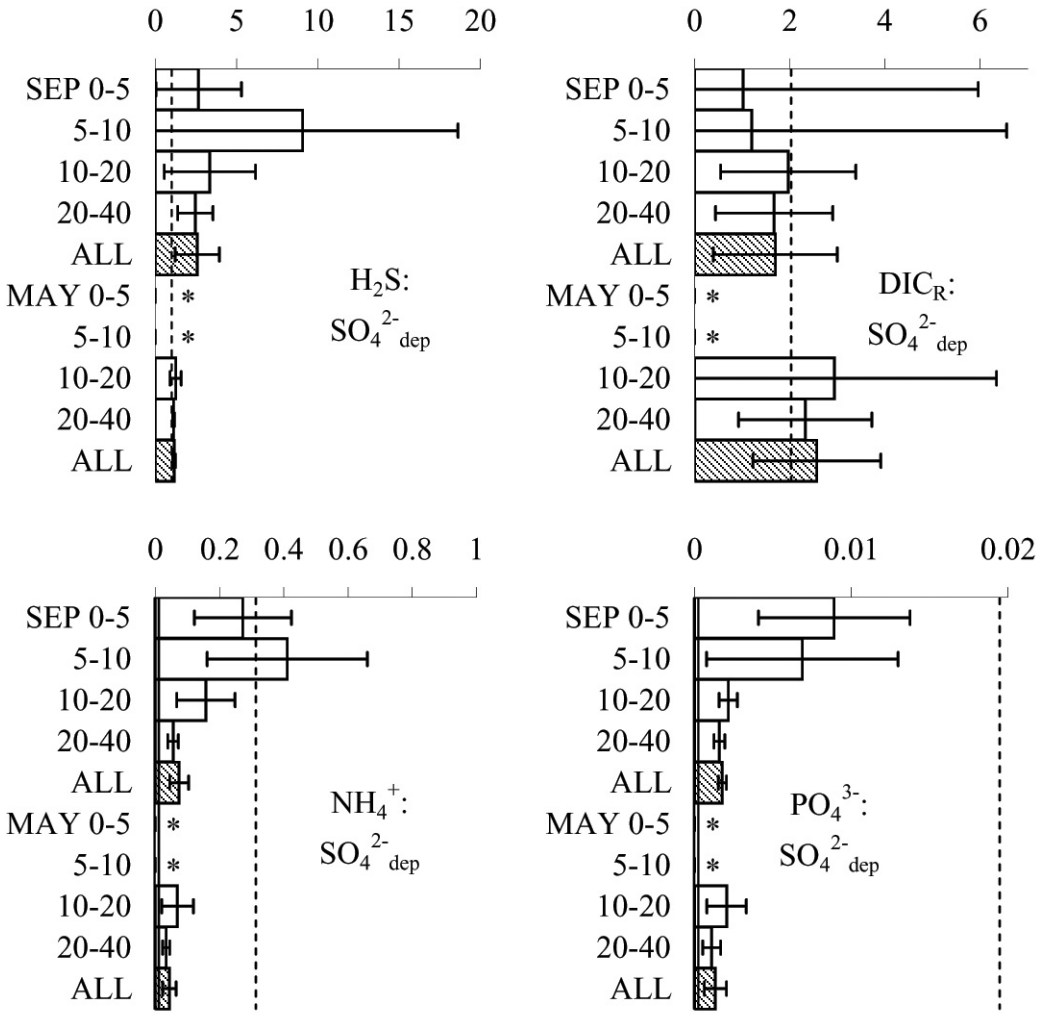

mmol cm-2
0
0.1
0.2
0.3
SEP 0-5
5-10
10-20
20-40
ALL
MAY 0-5
5-10
10-20
20-40
ALL
SO4 dep
µmol cm-2
0
0.5
1
SEP 0-5
5-10
10-20
20-40
ALL
MAY 0-5
5-10
10-20
20-40
ALL
CH4

Figure 2.6


0 5 10 15 20
SEP 0-5
5-10
10-20
20-40
ALL
MAY 0-5
5-10
10-20
20-40
ALL
H2S:
SO4 2- dep
0 2 4 6
DICR:
SO4 2- dep
0 0.2 0.4 0.6 0.8 1
NH4 +:
SO4 2- dep
0 0.01 0.02
PO4 3-:
SO4 2- dep

CHAPTER 3

PATTERNS OF NET AND GROSS PRIMARY PRODUCTION IN MANGROVE SOILS, TWIN CAYS, BELIZE: FIELD RESULTS AND MODELING [1]

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ABSTRACT

We investigated primary production and respiration in benthic microbial mats inhabiting mangrove soils at Twin Cays, Belize. Cyanobacteria-dominated microbial mats with high concentrations of chlorophyll *a* inhabited well-illuminated soils in interior regions of the island where dwarf mangroves lead to high light levels on the soil surface. Soils under taller mangrove trees in transition and fringe mangrove zones were poorly illuminated; diatoms and green algae dominated these soils. The composition of the benthic photosynthetic community was controlled by differences in irradiance due to mangrove canopy light gaps and by differential tolerance to environmental stresses such as desiccation or nitrogen limitation. Highest chlorophyll *a* concentrations were observed in dwarf mangrove soils, but even the much lower chlorophyll *a* concentrations in transition and fringe soils exceeded those reported in other mangrove forests. Seasonal variation in temperature, chlorophyll *a* concentration, and gross oxygenic photosynthesis (GPP) was insignificant. Community composition affected chlorophyll-specific gross photosynthetic efficiency, which varied between habitats. Respiration was proportional to GPP during the day, and calculated diel integrated rates of net O_2 production were proportional to chlorophyll *a* concentration. Dwarf mangrove stands supported the highest rates of benthic gross and net photosynthesis. Over a diel cycle, well-lit dwarf mangrove habitat mats were net autotrophic while mangrove shaded transition and fringe mats were net heterotrophic. Our analysis suggests that anthropogenic nutrient inputs can shift heterotrophic benthic systems to autotrophy.

INTRODUCTION

Microphytobenthic assemblages, including microbial (cyanobacteria and photosynthetic bacteria) and microalgal mats, proliferate in shallow marine environments such as intertidal flats, coastal embayments and lagoons (MacIntyre et al. 1996). Microbial mats are laminated associations of unicellular, filamentous or heterocystous cyanobacteria, often coexisting with various eukaryotes (e.g., diatoms) and photosynthetic and/or heterotrophic bacteria. Microalgal mats commonly include unicellular eukaryotic algae such as the bacillariophytes (diatoms), chlorophytes (green algae) and dinophytes (dinoflagellates). Phototrophic microphytobenthos can be a significant source of primary production, contributing as much as 50% of the total primary production in estuarine ecosystems (Underwood & Kromkamp 1999).

Studies of benthic productivity in mangroves are limited and typically demonstrate low photosynthetic biomass and activity. Light availability is commonly described as the primary limiting factor due to mangrove canopy shading (Alongi 1988, Kristensen et al. 1988, Alongi & Sasekumar 1992, Alongi 1994), although a variety of other controls have also been proposed. Nutrients (both nitrogen and phosphorus) can significantly limit mangrove benthic primary production and microalgal growth (Kristensen et al. 1988, Alongi et al. 1993). Inhibition by physical factors other than low light may include high temperatures, large salinity variations, and surficial erosion by hydrological processes such as rainstorms (Alongi 1990). Microalgal and cyanobacterial growth and density may also be constrained by grazing and inhibition by soluble phenolic compounds such as mangrove-derived tannins (Potts & Whitton 1980, Alongi 1990, Alongi 1994). Most mangrove benthos studies have examined microalgae in sediments and soils dominated by diatoms. Mangrove microphytobenthic studies are few, but some studies have

documented diverse photosynthetic communities accounting for significant biomass accumulation (Potts & Whitton 1980, Dor & Levy 1984).

In this study, we investigated the primary productivity of benthic microbial and microalgal mats from oceanic mangrove island habitats with differential light, elevation and inundation regimes. We examined the influence of seasonality and mangrove habitat on benthic O_2 dynamics and identify which controls affect the community composition and photosynthetic productivity of mangrove benthic environments.

METHODS

Study site

Twin Cays is a pair of oceanic mangrove islands located off the coast of Belize (16˚ 50' N, 88˚ 06' W; Feller et al. 2003, Lee & Joye 2006, Ch. 1). *Rhizophora mangle* dominates the islands' vegetation and creates three distinct habitats due to its decreasing tree-height gradient towards the center of the islands. The island-edge "fringe" habitat is comprised of tall (5-7 m) *R. mangle*. Tree height decreases through a "transition" habitat composed primarily of *R. mangle* mixed with stands of *Avicennia germinans* and *Laguncularia racemosa*. The island interiors are composed of treeless ponds surrounded by "dwarf" *R. mangle* less than 1.5 m tall. Sediment surface light levels are inversely related to canopy coverage (Woodroffe 1995, Feller & Mathis 1997) and affect the composition of surficial microbial populations. Fringe and transition mangrove habitat mats are inhabited by thin (<1 mm thick) patchy communities of diatoms, eukaryotic algae and non-heterocystous coccoidal and filamentous cyanobacteria (Lee & Joye 2006, Ch. 4). In contrast, dwarf mangrove habitat soils are densely populated by a thin surficial

layer of diatoms, mm-thick layers of coccoidal, non-heterocystous filamentous and heterocystous filamentous cyanobacteria, and μm- to mm-thick layers of purple sulfur bacteria.

Seasonality

We examined temporal and spatial variation in photosynthetic activity on Twin Cays over 7 field expeditions to 8 dwarf, 4 transition and 4 fringe mangrove sites. The climate at Twin Cays is driven by tropical wet-dry seasonality. Sampling trips in March 2002, May 2003, and June 2001 represent warmer, drier seasons, while the wet season was represented by September 2002, October 2001, November 2000, and February 2004 (Opishinski 2000-2004, Lee & Joye 2006, Ch. 4). Monthly average temperature and solar radiation at the Smithsonian Institution Field Station on Carrie Bow Cay (3.5 km from Twin Cays) were slightly higher (by 3 ˚C at 30 ˚C and 360 W m^{-2} at 1381 W m^{-2}) in early through late summer (May through October) compared to winter. Tidal ranges and rainfall were lowest (by 35 cm at 42 cm below mean sea level and 12.6 mm d^{-1} at 0.2 mm d^{-1}) in spring and summer (February through June). Combined water and temperature stress (i.e. lowest tides and rainfall and highest temperatures) was greatest in May and June and least in September, October and November.

Surficial mat characterization

Photopigment concentration (chlorophyll *a* and other pigments), density, and porosity were determined in surficial mats, here defined as samples containing microbial mat and underlying peat soil to a total depth of 1 cm. Triplicate surficial microbial mat cores were collected with a cut-off 5 cc syringe (1.03 cm^2 surface area). Pigment samples were immediately preserved with magnesium carbonate and frozen. Samples were later amended with a 45:45:10

acetone:methanol:deionized water mixture by volume and sonicated, and chlorophyll *a* concentrations were determined by spectrophotometry (Strickland & Parsons 1972). Mat samples from May 2003 were analyzed by HPLC for chlorophyll *a*, chlorophyll *b*, bacteriochlorophyll *a*, echinenone, myxoxanthophyll, zeaxanthin, fucoxanthin, and β-carotene (HPLC Photopigment Analysis Lab, Texas A&M University) to characterize photosynthetic community diversity. The bulk photosynthetic community was estimated from chlorophyll *a*, green algae from chlorophyll *b*, anoxygenic photosynthesizers from bacteriochlorophyll *a*, and cyanobacteria from echinenone, myxoxanthophyll, and zeaxanthin. Fucoxanthin indicates diatoms, brown algae and dinoflagellates, but the latter two organisms were not typically present under microscopic observation. β-carotene, a photoprotective carotenoid, can indicate UV/PAR stress when ratios relative to chlorophyll *a* are high (Palmisano et al. 1989).

Density was calculated as g wet soil (gws) per cm^3. Percent water (g per gws) was calculated as mass lost after 24 h at 60 °C and converted to porosity (pore volume per volume of wet sediment). Photosynthetically-active radiation (PAR) at the soil surface was measured simultaneously in all habitats using LI-COR® 2π quantum sensors and a LI-COR® datalogger.

O_2 and gross photosynthesis microprofiling

At each site, 6-10 microbial mat cores were collected using bevel-edged, 4 cm diameter, 7 cm deep PVC tubes. Cores were sealed with vinyl caps or o-ring fitted plugs, and seams were sealed with electrical tape. Cores were maintained in a large reservoir of site-specific overlying water under ambient light and temperature regimes.

O_2 microprofiles were measured within 1-3 days of core collection. Oxygen microprofiles and gross oxygenic photosynthesis (GPP) rates were measured simultaneously

using 20-30 μm outside-diameter Unisense® O_2 microsensors and a Unisense® microelectrode system (picoammeter, AD converter, computer-controlled micromanipulator, and Sloper data acquisition program). GPP was determined using the light-dark shift method (Revsbech et al. 1981). Profiles were obtained in 100 μm intervals from above the soil-water interface to depths of zero O_2. A variety of PAR levels (up to maximum sunlight or approximately 2000 μE m^{-2} s^{-1}) were simulated using a full-spectrum light source (Fostec® 8300). GPP profiles were obtained at a minimum of two light levels (200 and 2000 μE m^{-2} s^{-1}) at times during the day when equal to ambient solar radiation, while dark (no light) profiles were measured at night. To account for heterogeneity between core samples, triplicate profiles at each light level were measured in multiple cores. After profiling, chlorophyll *a*, density and percent water samples were collected as described above for core-specific soil characterization. Areal GPP was obtained from integration over active mat depths using a trapezoidal approximation and core-specific density and porosity measurements. Photosynthetic efficiency was evaluated on a per unit chlorophyll basis calculated from areal GPP rates using core-specific areal chlorophyll *a* concentrations.

Modeling

Net O_2 production/consumption rate profiles were quantified using an inverse approach. Assuming quasi-steady state conditions, the net rate profile (R_{net}) was determined by solving

$$0 = \frac{\partial}{\partial z}\left(\phi D_{sed}\frac{\partial C}{\partial z}\right) + R_{net}$$

and adjusting R_{net} to match the measured O_2 profiles. In this procedure, the rate profile is simplified sequentially, and realizations of different complexity are compared statistically. This procedure, described in Meile et al. 2001, is embedded in Monte Carlo simulations, and the maximum production rate was constrained by measured GPP. Effective sediment diffusion

coefficients (D_{sed}) were calculated from measured porosity, temperature and salinity data (Boudreau 1997). *In situ* respiration rates as a function of depth in the mat were calculated as the difference between measured GPP and modeled net rates (R = GPP - Net). Nighttime net rates were evaluated by setting GPP to 0 and fitting measured nighttime O_2 profiles to identify respiration ($R_{night} = Net_{night}$). Areal net O_2 production or consumption can be computed using Fick's law:

$$J = -D\frac{\partial C}{\partial x}$$

using the concentration gradient in the diffusive boundary layer, or the sediment-water interface, accounting for porosity variations (Jørgensen & Boudreau 2001).

GPP, respiration and net rate profiles were trapezoidally integrated over depth to obtain per area-values. Extrapolation of GPP, respiration and net rate per area-values to actual daily light availability was based on PAR data from the Carrie Bow Cay weather station (Opishinski 2000-2004) and estimates of shading in each habitat (Lee & Joye 2006, Ch. 4). Nighttime rates were integrated assuming constant rates over 12 hours of night. Light availability over 12 hours of daytime was estimated by first factoring in the light level reaching the mat surface relative to full sun in each habitat (0.34 fringe, 0.69 transition, 1.00 dwarf; Lee & Joye 2006, Ch. 4). Next, daytime saturated GPP was conservatively estimated to occur at PAR >1000 $\mu E\ m^{-2}\ s^{-1}$. For the remaining daytime hours, the unsaturated GPP rate was linearly interpolated from rates measured at unsaturated PAR levels. Respiration and net rates were integrated accordingly.

RESULTS

Chlorophyll *a* concentration varied primarily within and between habitat type and only slightly with season (Fig. 3.1). Dwarf mangrove habitat mats contained more chlorophyll *a* than

transition and fringe mangrove habitat mats, which contained similar concentrations (both below 100 mg m^{-2}). Seasonal differences in chlorophyll *a* were not apparent in transition and fringe mangrove habitat mats, while moderately lower concentrations were found in dwarf mangrove habitat mats during the dry season compared to the wet season.

Detailed photopigment analysis of May 2003 microbial mat samples from dwarf, transition and fringe mangrove habitats revealed a variety of photosynthetic pigments (Fig. 3.2). Dominant pigments in dwarf mangrove habitat mats included chlorophyll *a* (84.8 mg m^{-2}), bacteriochlorophyll *a* (36.7 mg m^{-2}), fucoxanthin (20.5 mg m^{-2}), myxoxanthophyll (9.5 mg m^{-2}), chlorophyll *b* (7.0 mg m^{-2}), and zeaxanthin (6.2 mg m^{-2}). The ratio of transition and fringe mangrove habitat mat pigments to dwarf mangrove habitat mat pigments was commonly less than 1, except for chlorophyll *b*, suggesting a larger green algal component in fringe mangrove habitat mats. Fringe and transition to dwarf ratios of bacteriochlorophyll a, echinenone, myxoxanthophyll, and zeaxanthin indicated 56-100% more anoxygenic photosynthetic bacterial and cyanobacterial pigments in dwarf habitats. β-carotene was also significantly greater in dwarf mangrove habitat mats (2.6 mg m^{-2}). Fucoxanthin concentrations indicated that diatoms were most abundant in dwarf and fringe mangrove habitat mats and present in lower concentrations in transition mangrove habitat mats.

O_2 microprofiles varied with PAR in terms of concentration and penetration depth (Fig. 3.3 and 3.6). An example of the variability within a habitat is shown in Fig. 3.3 for two dwarf mangrove habitat mats from May 2003. O_2 concentrations are lower at low light levels (e.g., 50 and 100 μE m^{-2} s^{-1}), but increased PAR did not result in an increasing pattern of increasing O_2 production. In dwarf mangrove habitat mats at site BFD, highest PAR levels (nearly equivalent to full sun) produced the highest concentrations of O_2 at shallow depths, while 1000 μE m^{-2} s^{-1}

PAR produced the highest concentrations of O_2 at depth. In dwarf mangrove habitat mats at site WSD, O_2 concentrations were highest at 250 and 500 μE m^{-2} s^{-1} PAR and decreased at high PAR of 1900 μE m^{-2} s^{-1}. Large error bars also indicate the variability in O_2 production within a site.

Mat variability within habitats from the same season, e.g., the same two dwarf mangrove sites in May 2003 (Fig. 3.4), was also evident in measured gross photosynthetic rates. GPP and chlorophyll specific GPP typically exhibited a Michaelis-Menten relationship versus PAR. Although GPP rates under high light conditions were comparable between sites WSD and BFD, different rates were measured at low and intermediate light conditions (Fig. 3.4a). Differences within dwarf mangrove habitat mats are further evident when comparing chlorophyll specific GPP (Fig. 3.4b).

GPP variability in dwarf habitats was high for any given month, and average monthly GPP varied between 5.8-13.5 mmol O_2 m^{-2} h^{-1} (9.5 mmol O_2 m^{-2} h^{-1} average, 2.7 mmol O_2 m^{-2} h^{-1} standard deviation) and 14.9-24.1 mmol O_2 m^{-2} h^{-1} (19.6 mmol O_2 m^{-2} h^{-1} average, 4.5 mmol O_2 m^{-2} h^{-1} standard deviation) at 10% and 100% full sunlight (2000 μE m^{-2} s^{-1}), respectively (Fig. 3.5a). GPP in dwarf and fringe mangrove habitat mats were comparable across the range of irradiances and generally exceeded transition mangrove habitat GPP. Seasonal differences in GPP were minimal in all habitats, with slightly (but not significantly) lower rates of GPP in the driest month of June than during the wet season (Fig. 3.5a).

Trends in chlorophyll specific GPP were similar to trends in areal GPP in mats from dwarf mangrove habitats (Fig. 3.5b). Chlorophyll *a* concentrations were highly variable within the habitats sampled each month and varied somewhat seasonally, but throughout the year, GPP efficiency per chlorophyll within each habitat was similar at any particular light level (200, 500

and 2000 μE m^{-2} s^{-1}). GPP normalization to chlorophyll concentration revealed greatest per chlorophyll *a* efficiencies in fringe mangrove habitat mats.

Maximum daylight oxygen profiles varied across dwarf, transition and fringe mangrove habitat mats (e.g., Fig. 3.6a from June 2001). All mangrove habitat mats exhibited subsurface maximum O_2 concentrations. Compared to dwarf habitats, fringe and transition habitats typically exhibited lower O_2 concentrations, profiles with less sharp peaks, and shallower O_2 penetration depths. GPP profiles paralleled O_2 profiles with respect to shape (subsurface maxima and depth of activity) and magnitude across habitat types (dwarf > fringe > transition) (Fig. 3.6b). In the shallow (upper) section of mats from all habitats, net O_2 production occurred as GPP exceeded O_2 respiration. In deeper layers, net O_2 consumption occurred as GPP ceased while respiration resumed. Integration of daytime activity over depth showed that respiration rates were tightly related to GPP (e.g., Fig. 3.6; respiration rate = 0.79 * GPP, $r^2 = 0.94$).

Seasonal differences in depth-integrated net rates were not exhibited in June and October 2001 measurements (data not shown). Therefore rates from both seasons were combined to evaluate depth-integrated rates in each mangrove habitat. In all habitats, net O_2 production occurred under full sun conditions (Fig. 3.7). Net O_2 consumption occurred at night at rates never exceeding full sun rates. When mangrove tree shading was accounted for in each habitat, integration of net O_2 production rates in the day and night revealed distinct differences between habitats (Fig. 3.8). Dwarf mangrove habitat mats, characterized by large O_2 subsurface maxima leading to diffusive loss of O_2 to the overlying water, were the only sites of net O_2 production during the day, and nighttime respiration rates were only slightly lower in magnitude than daytime rates. Transition and fringe mangrove habitat mats exhibited net respiration during both day and night. Daily integrated rates of GPP are balanced by respiration rates to maintain rates

of net O_2 production in dwarf mangrove habitat mats (3.8 ± 4.2 mmol m^{-2} d^{-1}) and net O_2 consumption in transition (7.4 ± 4.9 mmol m^{-2} d^{-1}) and fringe mangrove habitat mats (16.1 ± 0.8 mmol m^{-2} d^{-1}) (Fig. 3.9).

DISCUSSION

Microphytobenthic community composition

Photosynthetic biomass and community composition exhibited spatial patterns related to light availability in dwarf, transition and fringe mangrove habitats. Mats in mangrove-shaded fringe and transition habitats were never as developed in terms of thickness, density, biomass and phototroph diversity as in exposed dwarf soils. Laminated cyanobacterial mats in Twin Cays' dwarf habitats contained the highest photosynthetic biomass, in terms of chlorophyll *a*, which were in the same range as in other well-illuminated and developed mats from mangroves and other tidal flats (Potts 1980, Potts & Whitton 1980, Pinckney et al. 1995). The pattern of decreasing algal proportions and increasing cyanobacterial proportions from inside the mangrove forest to outside in the Aldabra lagoon (Western Indian Ocean; Potts & Whitton 1980) was similar to our observations on Twin Cays. Specifically, eukaryotic algae decreased by 1-2 orders of magnitude from within the mangrove forest to outside the forest in the intertidal Aldabra lagoon, while cyanobacterial abundance increased from 19% to 30%. Fringe and transition mangrove habitat mats contained fewer cyanobacterial and anoxygenic photosynthetic bacterial pigments compared to dwarf mangrove habitat mats, while eukaryotic photosynthetic pigments (indicating green algae and diatoms) were slightly more abundant in fringe habitats.

However, bulk photosynthetic biomass and community structure across Twin Cays' dwarf, transition and fringe habitats and other mangroves is not controlled only by light

availability. Although light gaps in the mangrove canopy of Twin Cays fringe habitats account for 22% of the fringe habitat area, the light provided by light gaps fringe mangrove habitat soils did not support the accumulation of high photosynthetic biomass (Feller & McKee 1999). Potts & Whitton (1980) also concluded that light availability is not the primary factor dictating the development of cyanobacteria mats inside the forest compared to the open lagoon, because illuminated sediments inside the Aldabran forest commonly lacked cyanobacterial mat coverage. Sediments intensely shaded (~90%) by trees in the Ao Nam Bor mangrove forest, Thailand, were populated by microalgae to the same degree as sediments exposed to direct sun (Kristensen et al. 1988).

Other factors such as light fluctuations, environmental stressors and nutrient availability may explain the observed spatial patterns in photosynthetic community composition and distribution. Light fluctuations can be caused by patchy cloud cover, but regularly occur throughout the day in transition and fringe habitats through dynamic gaps between mangrove leaves, branches and prop roots that shade the benthos. Such fluctuating light conditions depress growth rates least in diatoms, intermediately in green algae and most in cyanobacteria (Nicklisch 1998, Mitrovic et al. 2003), which can lead to the observed dominance of diatoms (Litchman 1998). Dwarf habitats are well lit and these constant, commonly high light conditions can favor the dominance of cyanobacteria and green algae over diatoms (Litchman 1998).

Cyanobacterial tolerance to environmental stresses such as extremes in salinity, desiccation, temperature and ultraviolet radiation (D'Antoni D'Amelio et al. 1989, Des Marais et al. 1992, Stal et al. 1996, Stal 2000) enable their proliferation in dwarf habitats where soil redox conditions are more reduced and benthic surface PAR is more intense than in fringe and transition soils (Lee & Joye 2006, Ch. 4). Additionally, in fringe and nearby transition soils,

prop root sponges can be a significant source of nitrate (Diaz & Ward 1997, Miller-Way & Twilley 1999), and cyanobacteria may be outcompeted by eukaryotic algae, especially diatoms, that respond to nitrate inputs (Berg et al. 2003). In dwarf mangrove habitat mats that lack external nitrate sources, such as from prop root sponges, nitrogen fixing cyanobacteria provide autochthonous new nitrogen to the system (Lee & Joye 2006, Ch. 4).

Photosynthetic physiology and the balance between oxygen consumption and production

Benthic GPP rates were greatest in habitats with the greatest chlorophyll *a* concentrations (Fig. 3.1), and exhibited the same decreasing spatial pattern from dwarf to fringe and transition habitats. When GPP rates were normalized to chlorophyll *a*, chlorophyll specific GPP rates decreased with increasing chlorophyll *a* concentrations as has been observed in other benthic microalgal assemblages due to greater light attenuation associated with increased biomass (Dodds et al. 1999), except at chlorophyll concentrations < 10 mg chl *a* m^{-2}. Maximum rates of chlorophyll specific GPP occurred in soils with chlorophyll *a* concentrations of 10-30 mg m^{-2}. Therefore although dwarf mangrove habitat mats had high concentrations of chlorophyll *a* contributing to high areal rates of photosynthesis, the photosynthetic efficiency of dwarf mangrove habitat mats was low compared to fringe and transition mangrove habitat mats. Insignificant seasonal variability in chlorophyll *a* concentrations and rates of benthic oxygenic photosynthesis was observed in mats from Twin Cays dwarf, transition and fringe mangrove habitats, which is consistent with patterns observed in soils and sediments from other mangroves with narrow seasonal temperature variations (Alongi 1988, Alongi 1994, Rajesh et al. 2001). In contrast, dry tropical fringe mangrove sediments from Chunda Bay, Australia, exhibited much

higher GPP during the warmer seasons due to greater seasonal temperature ranges (14-40 °C; Alongi 1994).

Depth-integrated net O_2 rates which rely on the interpretation of the entire measured O_2 profile compare reasonably well with diffusive O_2 exchange fluxes estimated from the top two measured O_2 values. In addition, depth profiles of respiration and net O_2 production rates estimated using the inverse model showed a strong correlation between daytime respiration and GPP over depth suggesting that microbial respiration was tightly coupled to oxygenic photosynthesis as observed in other microbial mats (Grötzschel & de Beer 2002).

Diel integration of net rates under natural light conditions indicated net O_2 production in dwarf mangrove habitat mats and net O_2 consumption in transition and fringe mangrove habitat mats. Twin Cays results are comparable to the benthic gross and net photosynthetic rates measured in other mangrove environments (Table 3.1). Benthic productivity was controlled by light availability and fluctuations and therefore greater in constantly well-lit mangrove lagoons, ponds, channel mudbanks, mudflats, and sandflats than in forested soils fluctuatingly shaded by well-developed mangrove canopies and prop roots. Well-lit sediments and soils tend towards net autotrophy while those shaded by mangrove forests tend towards net heterotrophy. Seasonal variations in benthic primary productivity are poorly represented in Table 3.1, but may only be important in mangrove environments with high temperature variations, as discussed above, or high nutrient inputs (see below).

Nutrient effects on soil trophic status

Light is the primary control on the functioning of soils and sediments receiving minimal anthropogenic nutrient inputs, hereafter termed “natural”, such as in Twin Cays, but significant

inputs of nutrients, e.g., from anthropogenic sources, alter the role of the benthos. Soils and sediments in shrimp farm mangroves exhibit greater benthic primary production rates than in natural forests (Table 3.1). In contrast to natural systems, the benthic environment associated with shrimp farms under both shaded and unshaded light regimes was net autotrophic (Alongi et al. 2000, Holmer et al. 2001). Additionally, in mangrove soils surrounded by shrimp farms, seasonality affects benthic productivity. Nutrients and organic matter discharge from shrimp farms were flushed through the mangroves and were more efficiently retained in the dry season than the wet season (Holmer et al. 2001). Dry season nutrient retention promoted high rates of GPP and net primary production (NPP) in intertidal zones compared to the wet season, when nutrients were flushed offshore. These relationships between nutrient inputs and benthic trophic status suggest ecosystem-level effects of anthropogenic development in mangrove environments under wet and dry seasonality.

Summary

Light availability, light fluctuations, environmental stresses and nutrient availability in Twin Cays' mangroves influenced benthic photosynthetic biomass and community composition, and therefore rates of photosynthetic activity across dwarf, transition and fringe mangrove habitats. Mangrove sediments and soils in environments with high light, such as Twin Cays dwarf soils, or high nutrient inputs, such as shrimp farm soils in Thailand and Vietnam, exhibit net autotrophy, while light limited systems under natural nutrient conditions, such as Twin Cays transition and fringe soils, exhibit net heterotrophy. Future studies on mangrove benthic primary production need to focus on the effects of *in situ* light regimes, including variability due to seasonal cloudiness and canopy gaps. Integration over dynamic light conditions is necessary to

capture *in situ* rates of benthic primary productivity in mangrove environments, where inverse modeling analysis suggests a tight spatial coupling between oxygen production and respiration.

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Table 3.1. Summary of mangrove benthic gross photosynthesis (GPP) and net primary production (NPP) rates. [1]*A.* = *Avicennia*, *B.* = *Bruguiera*, *C.* = *Ceriops*, *N.* = *Nypa*, *R.* = *Rhizophora*, *S.* = *Sonneratia* spp.; [2]mmol O_2 m^{-2} d^{-1}; [3]c = C units converted to O_2 assuming 1:1 stoichiometry, d = daytime light variability factored in integration, s = seasonal average.

Vegetation[1]	Location	GPP[2]	NPP[2]	Notes[3]	Source
NATURAL WELL-LIT FLATS/BANKS	***Average:***	***58.6***	***3.8***		
Dwarf *R. mangle* mangrove cyanobacterial mats	Twin Cays, Belize	125.3	3.8	s, d	This study
A. marina mangrove bay cyanobacterial mats	Shurat Arwashie, Sinai	50.0		d	Dor & Levy 1984
Mangrove fringed pond sediments	Nethravathi estuary, India		7.7	c	Rajesh et al. 2001
Mangrove lagoon sandflat	Sweeting's Cay, Bahamas	72.3	24.2	d	Koch & Madden 2001
Fringe mangrove sandflat	Chunda Bay, Australia	31.8	-2.4	s	Alongi 1994
Mangrove creek bank	Hinchinbrook Island, Australia	13.6	-14.4		Alongi 1994
NATURAL SHADED FOREST	***Average:***	***11.1***	***-13.3***		
Transition *R. mangle* mangrove (69% full sun)	Twin Cays, Belize	22.5	-7.4	s, d	This study
Fringe *R. mangle* mangrove (34% full sun)	Twin Cays, Belize	28.2	-16.1	s, d	This study
A. marina mangrove microalgal sediment	Shurat Arwashie, Sinai	11.3		d	Dor & Levy 1984
R. apiculata sunlight-exposed benthos mangrove	Ao Nam Bor, Thailand	24.7	-7.6		Kristensen et al. 1988
R. apiculata shaded benthos mangrove (11% full sun)	Ao Nam Bor, Thailand	14.6	-4.7		Kristensen et al. 1988
R. apiculata -B. parviflora mangrove	Fly River delta, Papua New Guinea	20.4	-2.4		Alongi et al. 1993
N. fruticans mangrove	Fly River delta, Papua New Guinea	3.6	-19.8		Alongi et al. 1993
A. marina -S. lanceolata mangrove	Fly River delta, Papua New Guinea	1.2	-17.4		Alongi et al. 1993
Low-intertidal mixed *R.* mangrove (36% full sun)	Hinchinbrook Island, Australia	1.5	-14.1	s	Alongi 1994
Mid-intertidal mixed *R.* mangrove (12% full sun)	Hinchinbrook Island, Australia	0.4	-16.2	s	Alongi 1994
High-intertidal mixed *R. -C.* mangrove (43% full sun)	Hinchinbrook Island, Australia	1.9	-21.6	s	Alongi 1994
Fringing *R. stylosa -A. marina* mangrove	Chunda Bay, Australia	2.8	-18.5	s	Alongi 1994
SHRIMP FARM WELL-LIT FLATS	***Average:***	***65.5***	***24.3***		
Shrimp farm mangrove pond benthos	Mekong delta, Vietnam		4.5	c	Alongi et al. 2000
Shrimp farm-surrounded mangrove channel mudflat	Bangrong Mangrove, Thailand	78.8	41.0	s	Holmer et al. 2001
Shrimp farm-surrounded low-intertidal mangrove mud/sandflat	Bangrong Mangrove, Thailand	52.2	27.5	s	Holmer et al. 2001
SHRIMP FARM SHADED FOREST	***Average:***	***40.6***	***9.5***		
Shrimp farm-surrounded mid-intertidal mixed *R.* mangrove	Bangrong Mangrove, Thailand	42.0	12.0	s	Holmer et al. 2001
Shrimp farm-surrounded high-intertidal mixed *R. -C.* mangrove	Bangrong Mangrove, Thailand	39.2	7.0	s	Holmer et al. 2001

FIGURE CAPTIONS

Figure 3.1. Seasonal chlorophyll *a* distribution. Error bars = standard deviations; n.m. = not measured; difference relative to dwarf mangrove habitat mats (2-tailed *t*-test) is significant at $p \leq 0.1$ (*) or $p \leq 0.05$ (**).

Figure 3.2. Pigment concentrations from May 2003 relative to dwarf concentrations. Chl a = chlorophyll *a*; Chl b = chlorophyll *b*; BChl a = bacteriochlorophyll *a*; Echin = echinenone; Myxo = myxoxanthophyll; Zeax = zeaxanthin; Fuco = fucoxanthin; B-Car = β-carotene. Error bars = standard deviations.

Figure 3.3. O_2 depth profiles across a range of PAR from 50 to 1900 μE m^{-2} s^{-1} at two dwarf sites (BFD, WSD) from May 2003. Error bars = standard deviations of triplicate profiles. 50 μE m^{-2} s^{-1} PAR error bars not shown for clarity.

Figure 3.4. Michaelis-Menten relationship of (a) gross oxygenic photosynthesis (GPP) and (b) chlorophyll *a* specific GPP with PAR at two dwarf sites (BFD, WSD) from May 2003. Error bars = standard deviations.

Figure 3.5. Seasonal variability of (a) gross oxygenic photosynthesis (GPP) and (b) chlorophyll *a* specific GPP in dwarf, transition and fringe mangrove habitat microbial mats at 200, 500 and 2000 μE m^{-2} s^{-1} PAR, respectively. Error bars = standard deviations of triplicate profiles; n.m. = not measured.

Figure 3.6. Example profiles of (a) measured net O_2 concentrations and (b) corresponding calculated rates from June 2001 at 2000 μE m^{-2} s^{-1} PAR. In (b), GPP and R are plotted in absolute terms; positive net rates indicate net production, and negative net rates indicate net consumption of O_2.

Figure 3.7. Benthic net oxygen production (+) and consumption (-) rates in dwarf (D), transition (T) and fringe (F) mangrove habitats under full sun (2000 μE m^{-2} s^{-1} PAR) and at night quantified by inverse modeling. Error bars = standard deviations.

Figure 3.8. Integrated daytime and nighttime rates of net O_2 production (+) and consumption (-) in dwarf (D), transition (T) and fringe (F) mangrove habitat mats. Error bars = standard deviations.

Figure 3.9. Benthic gross oxygenic photosynthesis (GPP), respiration (R), and net O_2 production (+) and consumption (-) rates in dwarf (D), transition (T) and fringe (F) habitats integrated over a 24 hr cycle. Error bars = standard deviations resulting from pooling all sites in a given habitat.

Figure 3.1

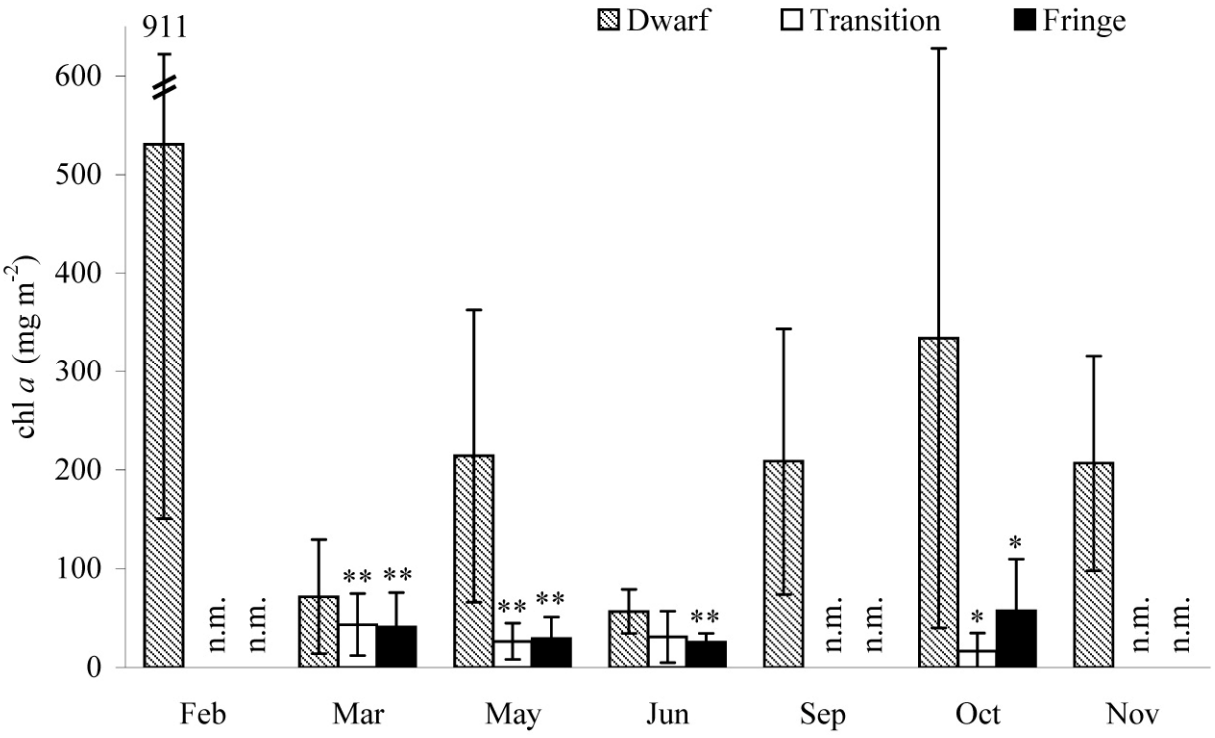

Dwarf
Transition
Fringe
911
600
500
400
300
200
100
0
chl a (mg m-2)
n.m.
n.m.
**
**
**
**
**
n.m.
n.m.
*
*
n.m.
n.m.
Feb
Mar
May
Jun
Sep
Oct
Nov

Figure 3.2

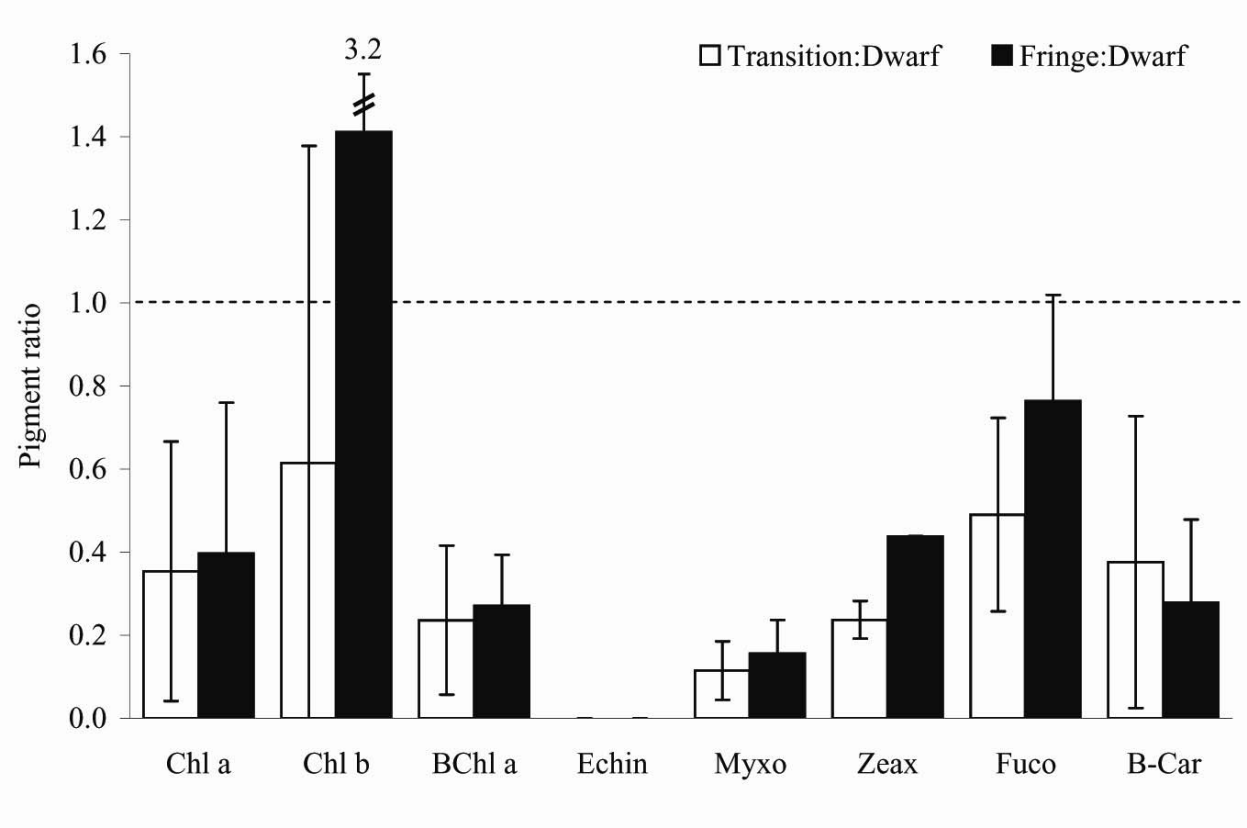

Transition:Dwarf
Fringe:Dwarf
Pigment ratio
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
3.2
Chl a
Chl b
BChl a
Echin
Myxo
Zeax
Fuco
B-Car

Figure 3.3

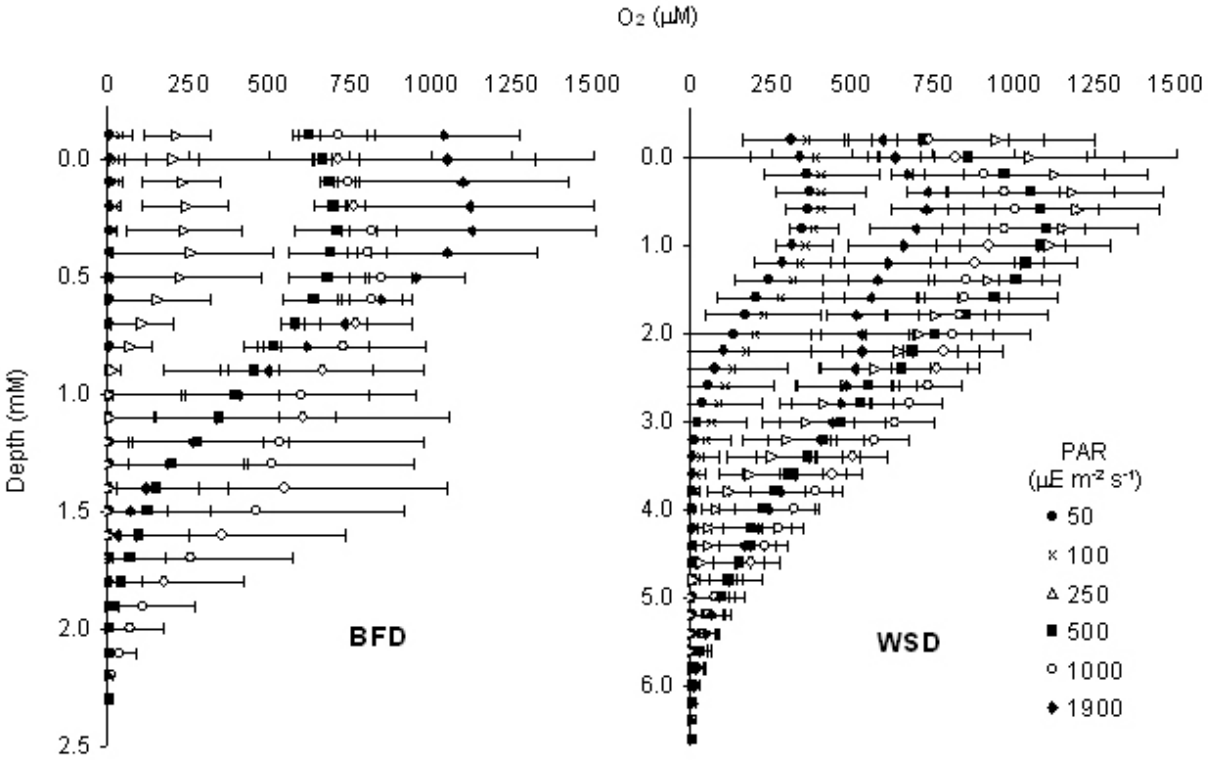

O2 (µM)
0 250 500 750 1000 1250 1500
0.0
0.5
1.0
1.5
2.0
2.5
Depth (mM)
BFD
0 250 500 750 1000 1250 1500
0.0
1.0
2.0
3.0
4.0
5.0
6.0
WSD
PAR
(µE m-2 s-1)
50
100
250
500
1000
1900

Figure 3.4

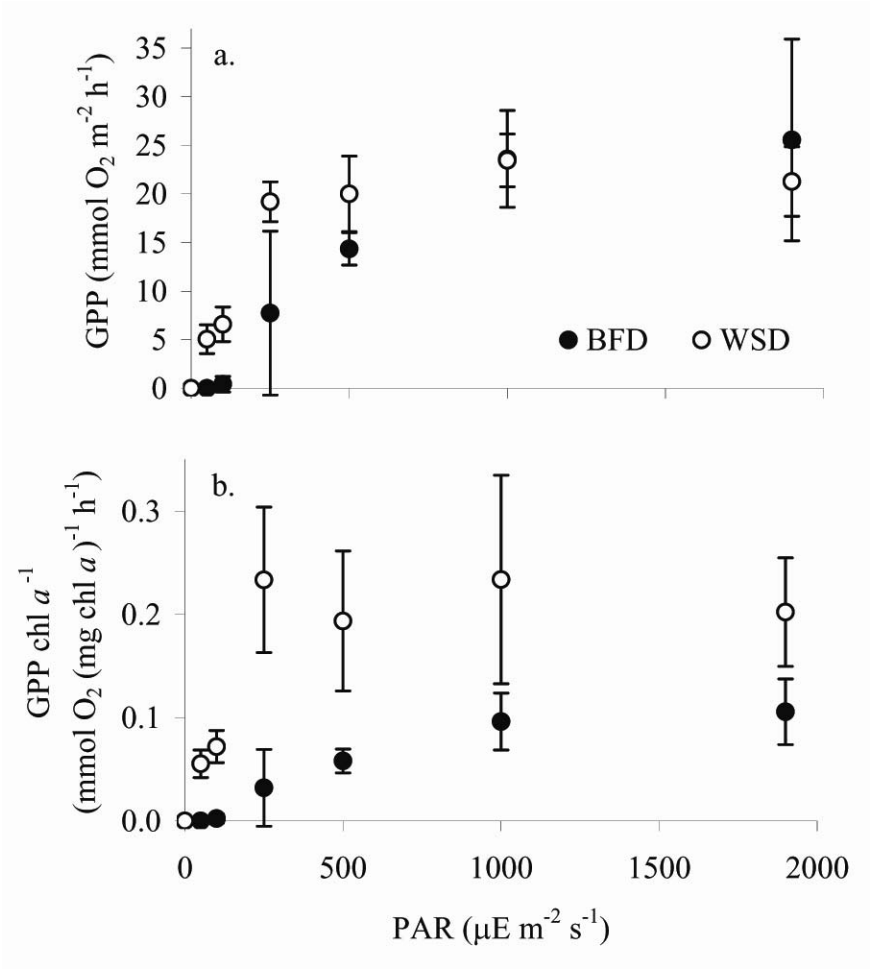

a.
GPP (mmol O_2 m^{-2} h^{-1})
35
30
25
20
15
10
5
0
BFD
WSD
b.
GPP chl a^{-1}
(mmol O_2 (mg chl a)$^{-1}$ h^{-1})
0.3
0.2
0.1
0.0
0
500
1000
1500
2000
PAR (μE m^{-2} s^{-1})

Figure 3.5

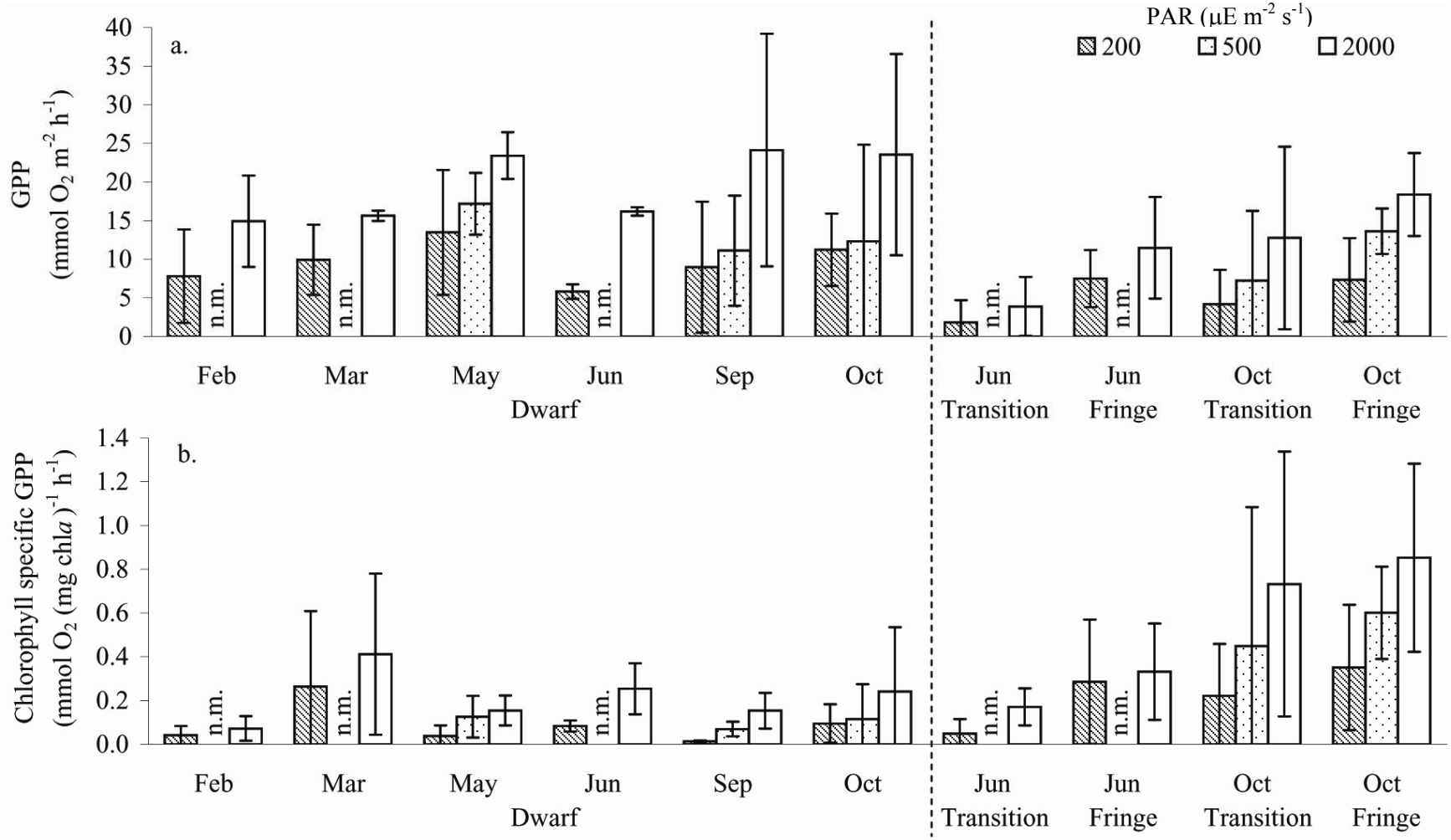

PAR (µE m-2 s-1)
200
500
2000
a.
GPP
(mmol O2 m-2 h-1)
40
35
30
25
20
15
10
5
0
n.m.
Feb
Mar
May
Jun
Sep
Oct
Dwarf
Jun
Transition
Jun
Fringe
Oct
Transition
Oct
Fringe
b.
Chlorophyll specific GPP
(mmol O2 (mg chla)-1 h-1)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0

Figure 3.6

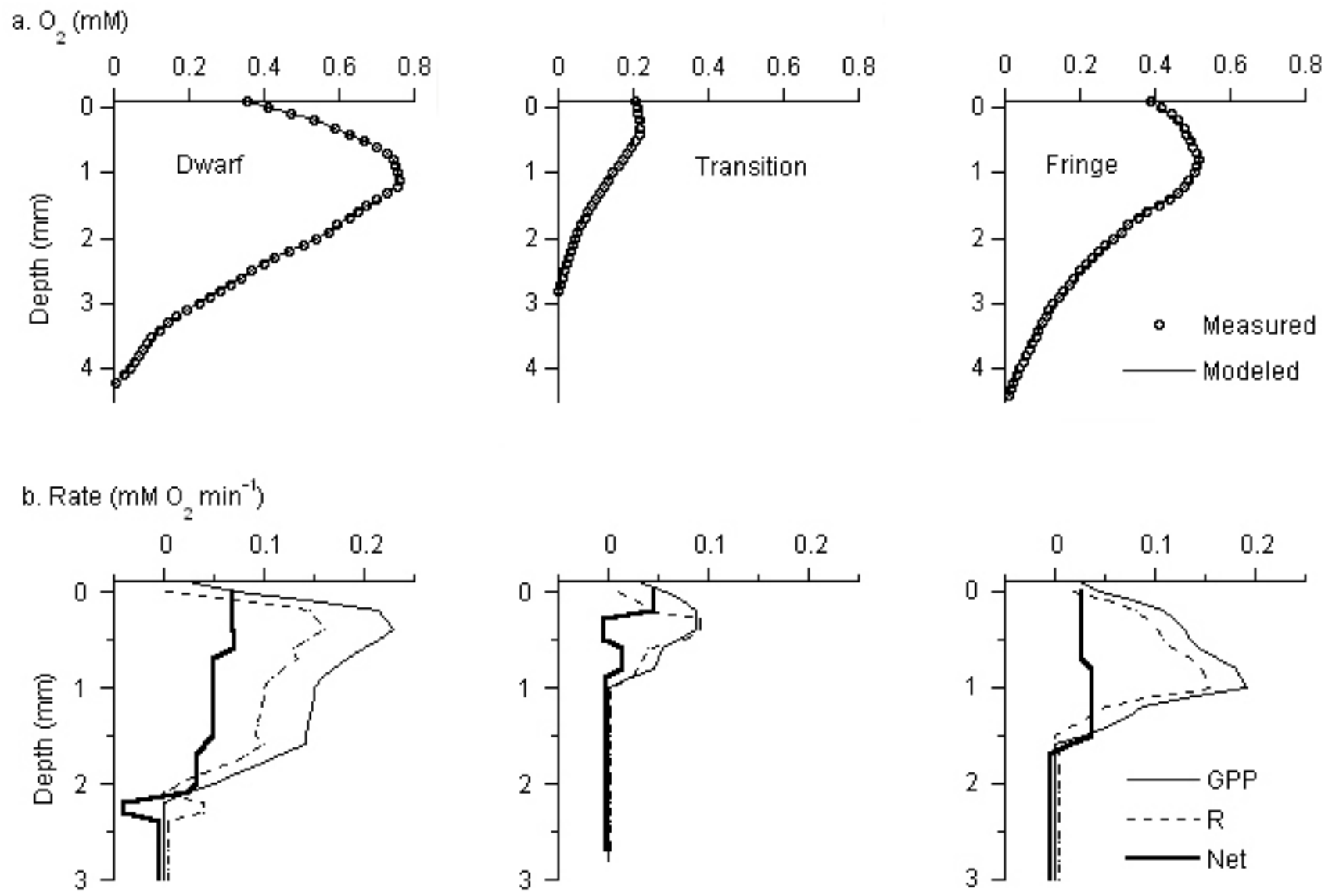

a. O_2 (mM)
0 0.2 0.4 0.6 0.8
Depth (mm)
0 1 2 3 4
Dwarf
Transition
Fringe
Measured
Modeled
b. Rate (mM O_2 min^{-1})
0 0.1 0.2
0 1 2 3
GPP
R
Net

Figure 3.7

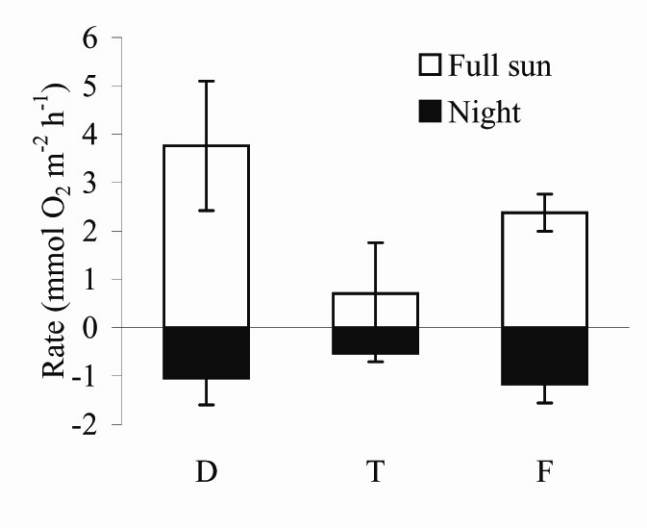


Figure 3.8

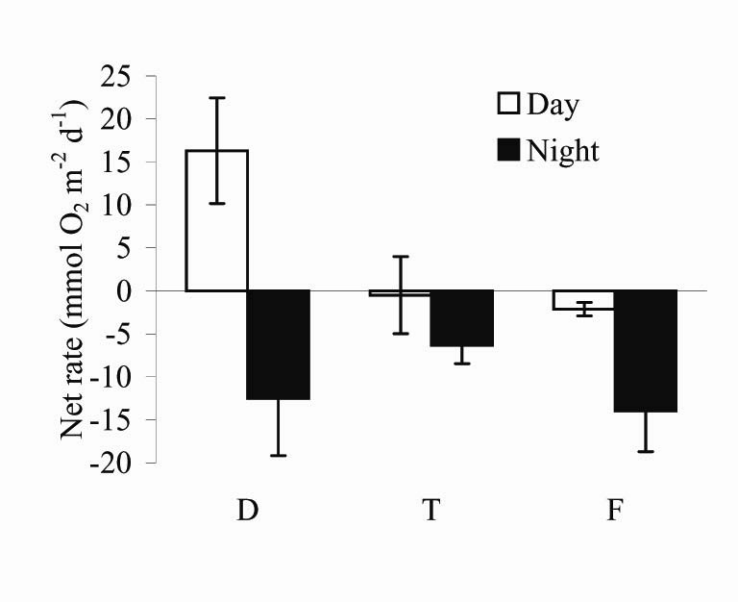
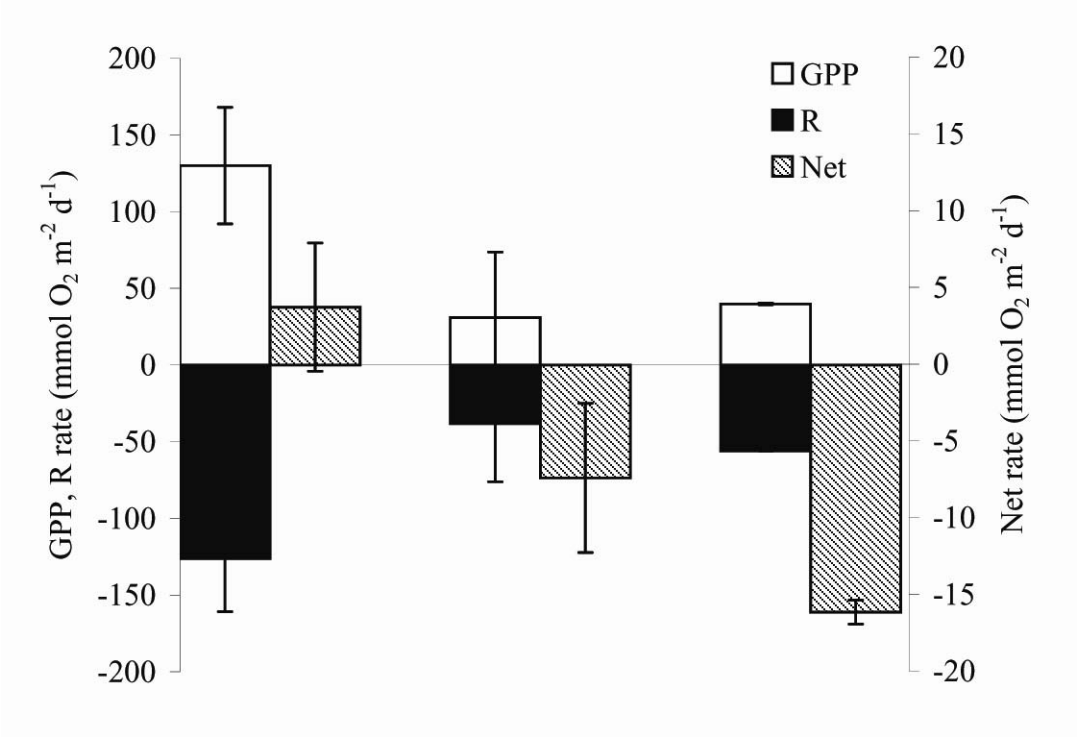


Figure 3.9


GPP
R
Net
GPP, R rate (mmol O_2 m^{-2} d^{-1})
Net rate (mmol O_2 m^{-2} d^{-1})
200
150
100
50
0
-50
-100
-150
-200
20
15
10
5
0
-5
-10
-15
-20

CHAPTER 4

SEASONAL PATTERNS OF NITROGEN FIXATION AND DENITRIFICATION IN OCEANIC MANGROVE HABITATS[1]

[1] Lee RY, Joye SB. 2006. *Marine Ecology Progress Series*. 307:127-141.
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ABSTRACT

Mangrove peat soils are home to a variety of microbial communities that may play a vital role in system-level elemental cycling. We examined rates of nitrogen fixation and denitrification in benthic microbial mats on Twin Cays, Belize, a pair of oceanic mangrove islands. A tree-height gradient across the islands created distinct habitats for benthic microbes. Seawater flushing of the benthos and tree height decreased landward from tall, dense trees on the island fringe through a transition zone of high elevation and intermediate tree heights. In the center of the islands, microbial mats with dense communities of cyanobacteria and purple sulfur bacteria covered the benthic surface of shallow ponds and around dwarf trees. Wet-dry seasonality, tidal cycles and elevation controlled the extent of mat exposure to desiccation and UV radiation. Nitrogen fixation was controlled primarily by the sensitivity of nitrogenase to oxygen inhibition, whereas denitrification was limited by oxidant (nitrate) availability. Diel patterns of nitrogen fixation varied with the type of cyanobacteria dominant in each mat. Dissolved inorganic nitrogen concentration influenced both nitrogen fixation and denitrification rates. Redox conditions contributed to variability in mat nitrogen fixation and denitrification response to nutrient addition, while dissolved organic carbon did not. Microbial mat nitrogen cycling likely contributes to the nutrient (nitrogen and phosphorus) limitation patterns observed in the mangrove trees; in dwarf habitats, mats serve as a source of nitrogen via nitrogen fixation, while in fringe and transition habitats, mats compete with the trees for nitrogen via denitrification.

INTRODUCTION

Microbial mats proliferate in shallow aquatic ecosystems, including tidal flats and coastal and hypersaline lagoons because of their ability to tolerate extremes in salinity, desiccation, temperature and ultraviolet radiation (Stal 2000). Benthic microbial mats are also found in intertidal mangrove environments (Potts 1980, Mann & Steinke 1993, Paling & McComb 1994). Mats may flourish especially in 'dwarf' mangrove forests, because the low stature and thin canopies of the trees allow abundant photosynthetically-active radiation (PAR) to reach the sediment surface (Lee et al. in preparation, Ch. 3).

Microbial mats play an active role in the nutrient status of benthic environments. Nutrient limitation in marine environments is due primarily to the lack of nitrogen (N) (Howarth 1988). At oligotrophic offshore mangrove islands, inputs of N depend upon atmospheric and oceanic inputs and dinitrogen (N_2) fixation which are balanced by loss via denitrification, export and burial. High rates of N_2 fixation in mangrove environments have been documented in association with leaf litter, pneumatophores, and soils (Holguin et al. 2001). In contrast, denitrification rates in mangrove habitats are considered a negligible part of the N budget (Rivera-Monroy & Twilley 1996, Kristensen et al. 1998). However, neither of these processes has been well studied in benthic mats in mangrove forests.

To quantify the role of benthic microbial mats in oceanic mangrove ecosystem N cycling, we investigated spatial and temporal dynamics of N_2 fixation and denitrification with respect to daily and seasonally varying physical and chemical environmental forces. Diel cycles of photosynthetically active radiation (PAR) influence O_2 concentration dynamics due to variations in O_2 production and consumption, and O_2 concentration may alter activity of the O_2-sensitive nitrogenase enzyme and influence facultative denitrifying microbes. Daily and seasonal changes

in environmental parameters such as temperature, tidal height, and desiccation also affect patterns of N cycling. Substrates such as organic carbon, nitrogen, phosphorus and trace metals may limit microbial activity in oligotrophic oceanic mangrove habitats, and additions of these nutrients may alter rates of N_2 fixation and denitrification. Our objectives were to document the primary effects of N availability and tidal hydration on daily and seasonal patterns of N cycling in oceanic mangrove microbial mats and to demonstrate the adaptation of these mats to a dynamic environment. We hypothesized that microbial mats play a key role in the productivity of oceanic mangrove islands, and that microbial mats may contribute to the previously documented system-scale patterns of nutrient limitation (Feller et al. 2003).

METHODS

Study site

Twin Cays is a well-described 92 ha pair of peat-based tropical oceanic mangrove islands located off the coast of Belize (McKee et al. 2002, Feller et al. 2003). The primary vegetation on the islands is *Rhizophora mangle*, and its tree height gradient generates distinct benthic habitats delineated by gradients in benthic-surface available PAR, tidal inundation, water table height, porewater salinity and porewater sulfide concentrations. The semidiurnally inundated 'fringe' habitat on the edge of the islands consists of tall (5 to 7 m) *R. mangle*. Benthic-surface light availability is low in fringe habitats due to high tree basal area and thick canopies. Landward vegetation shifts to a 'transition' habitat of intermediate-height (2 to 4 m) *R. mangle* mixed with *Avicennia germinans* and *Laguncularia racemosa* stands on higher elevation with infrequent flooding (<50 times yr^{-1}). The typically flooded interior 'dwarf' mangrove habitat is lowest in elevation and features less dense, shorter (<1.5 m) mangrove trees with more open canopies,

resulting in high benthic surface light availability. Dwarf mangrove habitats and associated treeless lagoons comprise approximately 44% of the island area (Rodriguez & Feller 2004) (Fig. 4.1). The dwarf zone is home to laminated, cyanobacteria-dominated microbial mats that vary from several mm up to cm in thickness. In contrast, the sparse benthic microbial community in the transition and fringe habitats, dominated by eukaryotic microalgae and cyanobacteria, was consistently less than 1 mm thick.

Like most tropical systems, Twin Cays exhibits wet-dry seasonality, with rainy and slightly cooler fall and winter seasons contrasting with dry and warmer spring and summer seasons. The semidiurnal tides also exhibit a seasonal cycle, with extreme low tides common in spring/summer and extreme high tides common in fall. These tidal variations affect the daily exposure/submergence regimes of soils and microbial mats.

Spatial variation in rates of N_2 fixation and denitrification was examined in fringe, transition and dwarf microbial mats from 8 sites on Twin Cays (Fig. 4.1). We conducted 6 field expeditions in November 2000, June and October 2001, March and September 2002, and May 2003 to examine spatiotemporal variability in N dynamics. The September, October, and November trips reflect cooler, wetter conditions. Monitoring data collected from the Smithsonian Institution's Field Station on Carrie Bow Cay (3.5 km from Twin Cays) indicated monthly solar radiation maxima of 1021 to 1168 W m^{-2} and average monthly rainfall of 6.7 to 12.8 mm d^{-1} (Opishinski 2000–2003). Low tides varied between 7 and 16 cm below mean sea level, and high tides varied between 28 and 30 cm above mean sea level. The March, May, and June trips reflect warmer, drier conditions. During these months, Carrie Bow Cay received monthly solar radiation maxima of 1149 to 1381 W m^{-2} and average monthly rainfall of 0.2 to 1.7 mm d^{-1}. Low tides varied between 20 and 42 below mean sea level and high tides varied

between 4 and 32 cm above mean sea level. June 2001 is notable among all the dates because minus tides and low rainfall resulted in severe desiccation of Twin Cays soils and microbial mats.

Environmental states

Physical and chemical parameters were investigated to characterize the benthic environment. Gradients in benthic PAR availability across fringe, transition and dwarf habitats were logged simultaneously over hours to days using a LICOR pyranometer. Surficial mat samples consisted of microbial mat and any adjacent underlying soil (peat) to a total depth of 1 cm. Surficial mat porosity (g water per g wet sample [gws]) and organic content (g per g dry sample [gds]) were calculated as mass lost after 24 h at 60°C and loss-on-ignition after 24 h at 500°C, respectively. Benthic chlorophyll *a* (chl *a*) was monitored seasonally to evaluate photosynthetic capacity. Surface mat sub-samples (1 cm deep with a 1.03 cm^2 surface area) were preserved immediately with $MgCO_3$ and frozen. Upon return to the laboratory, chl *a* samples were extracted and sonicated in a 45% acetone, 45% methanol and 10% deionized water mixture, then analyzed by spectrophotometry with a correction for phaeophytin (Strickland & Parsons 1972). Mat samples were also collected for microscopic examination and identification. Porewater and overlying water pH and dissolved chemical species were monitored to quantify the conditions of the benthic nutrient and redox environment.

Porewater was collected at 10 cm depth using a PVC piezometer. Dissolved organic carbon (DOC), inorganic and organic N (NH_4^+; $NO_3^- + NO_2^- = NO_x^-$; DON = total dissolved N [TDN] – dissolved inorganic N [DIN = $NH_4^+ + NO_x^-$]) and phosphorus (P) (PO_4^{3-}; DOP = total dissolved phosphorus [TDP] – dissolved inorganic phosphorus [DIP = PO_4^{3-}]), sulfur (SO_4^{2-},

H_2S), reduced iron (Fe^{2+}) and salinity (total salts and Cl^-) samples were immediately 0.2 μm filtered and preserved, then stored at 4°C. Filtered overlying water and pore water aliquots were fixed in sample to preservative ratios of 5:0.2 NH_4^+:phenol reagent (22 ml phenol, 198 ml ethanol, 8 ml deionized water), 4:0.1 DOC/PO_4^{3-}/TDP/Fe^{2+}/Cl^-:concentrated ultrex nitric acid, and 5:0.5 H_2S:20% weight/weight zinc acetate. NO_x^-/TDN samples were filter-sterilized.

All dissolved components were analyzed as soon as possible (within 3 weeks of collection). Colorimetric assays for NH_4^+ (phenol hypochlorite method; Solorzano 1969), PO_4^{3-} (molybdate antimony ascorbic method; Strickland & Parsons 1972), H_2S (Cline's method; Cline 1969), and Fe^{2+} (ferrozine method; Stookey 1970) were conducted with a Shimadzu® UV-1601 spectrophotometer. DOC was measured using high temperature combustion and infrared CO_2 detection in a Shimadzu® TOC-5000 Total Organic Carbon analyzer. NO_x^- was measured on an Antek® 745 Nitrate/Nitrite Reducer (vanadium reduction assembly) inline with an Antek® 7050 chemiluminescent nitric oxide detector (Álvarez-Salgado & Miller 1998). TDN was analyzed by high temperature combustion in a Shimadzu® TOC-5000 inline with an Antek® 7020 chemiluminescent nitric oxide detector. TDP was combusted and acid hydrolyzed (Solorzano & Sharp 1980) then analyzed spectrophotometrically as PO_4^{3-}. Cl^- was quantified using ion chromatography (Dionex® DX500).

Diel experiments

Diel experiments were conducted to examine fluctuations in rates of N_2 fixation and denitrification in relation to hourly changes in solar flux, which drive variations in rates of oxygenic photosynthesis and thus porewater O_2 concentration (Joye & Lee 2004, Lee et al. in preparation, Ch. 3). Rates of N_2 fixation and denitrification were measured contemporaneously

using the acetylene reduction and acetylene block techniques, respectively (Joye & Paerl 1994). To convert acetylene reduction rates to N_2 fixation rates, we assumed a conversion factor of 4:1 C_2H_2:N_2 reduced (Postgate 1982).

Individual incubations (time points) during diel experiments spanned 4 to 6 h intervals over 24 to 36 h. For each time point, sub-samples of the surface mat (1 cm deep with a 1.03 cm^2 surface area) were placed into 20 ml serum vials containing 10 ml of GF/F filtered site-specific overlying water (GF/F OLW). Triplicate samples were included for each treatment. Treatments included: light, dark, light plus NO_3^- (1 mM) and glucose (2 mM), dark plus NO_3^- (1 mM) and glucose (2 mM), and light plus 3-(3,4 dichlorophenyl)-1,1 dimethylurea (DCMU; 10 μM), an inhibitor which blocks photosystem-II (PS-II), the O_2 producing step of photosynthesis. Samples were incubated under natural light and temperature regimes.

Additional experiments were used to identify shortterm (hourly timescale) nutrient controls on N_2 fixation and denitrification, including day and night incubations with amendments of NH_4^+ (0.1, 0.5, 1 mM), NO_3^- (0.1, 0.5, 1 mM), glucose (0.5, 1, 2 mM), acetate (2 mM), and lactate (2 mM) under light and dark conditions.

Bioassay experiments

Longer-term (days-long timescale) controls on N_2 fixation and denitrification were examined in bioassay experiments. Triplicate 5 × 5 cm^2 by 1 cm deep mat sections were incubated in individual plastic tubs (Rubbermaid® 3870) under control (no addition) and treatment (nutrients added) conditions. Mat sections were submerged in 250 ml of GF/F OLW. Treatments included additions of the following nutrients to the GF/F OLW: NH_4^+ (0.1, 0.5, 1 mM), NO_3^- (0.1, 0.5, 1 mM), NH_4^+ plus NO_3^- (0.05 mM NH_4^+ and 0.1 mM NO_3^-), PO_4^{3-} (0.01

mM), glucose (0.5, 1, 2 mM), acetate (1 mM), lactate (1 mM), sequestrine-complexed iron (7.2 μM), SL-8 trace metal solution (Fe:Zn:Mn:Co:Cu:Ni:Mo = 7.5:0.5:0.5:0.8:0.1:0.1:0.1 μM; Atlas 1995), and a vitamin solution (0.1% Vitamix containing biotin, thiamine, B_{12}, nicotinamide, folic acid, Ca pantothenate, riboflavin; Lidstrom 1988). After nutrient incubation under natural light and temperature regimes for 72 h, N_2 fixation and denitrification rates under light and dark conditions were determined as described above.

Hydration experiments

Under the extremely dehydrated surface mat conditions of June 2001, experiments were conducted to elucidate the effects of desiccation and rehydration on daytime N_2 fixation and denitrification rates. To investigate short-term (hourly) effects of rehydration, desiccated microbial mats were incubated as described above (1 cm deep × 1.03 cm^2 surface area sub-samples in 20 ml vials), but under a suite of different conditions: dry (no water addition), moist (with 3 drops of GF/F OLW), wet (with 10 ml of GF/F OLW), wet/dry (dry incubation following 20 min of rehydration with GF/F OLW), and wet/moist (incubation with 3 drops of GF/F OLW after 20 min of rehydration with GF/F OLW). Longer-term effects of rehydration and desiccation were investigated in dehydrated and moist mats after 1 to 5 d of mat moisture content manipulation. Dehydrated (dry) mats were rehydrated (i.e. submerged in GF/F OLW) for 1, 2, or 5 d or alternately rehydrated and dried (i.e. submerged in GF/F OLW on the first day, removed from OLW on the second day, submerged on the third day, etc.) over 5 d. Likewise, moist (wet) microbial mats were desiccated (i.e. air exposed) for 1, 2, or 5 d or alternately dried and rehydrated (i.e. exposed on the first day, submerged on the second day, exposed on the third day, etc.) over 5 d. Longer-term rehydration incubations were conducted in wet (10 ml of GF/F

OLW) and dry (no water addition) incubations under helium as well as air headspaces to differentiate the effects of oxygenation from dehydration.

RESULTS

Each of the study sites contained diverse assemblages of cyanobacteria, including filamentous species (e.g. *Oscillatoria*, *Lyngbya*, *Microcoleus, Phormidium*, *Johannesbaptistia*, *Spirulina* and heterocystous *Nodularia* and *Scytonema* spp.) and unicellular species (e.g. *Aphanocapsa*, *Chroococcus*, *Gloeocapsa* spp.), and composition varied seasonally (Joye & Lee 2004). Heterocystous cyanobacteria (HC) communities were present at dwarf mangrove habitats in November 2000 (at site WS), June 2001 (WS), March 2002 (NWD and WS), September 2002 (L and WS), and May 2003 (WS), while only non-heterocystous cyanobacteria (NHC) were present in all other mats. The cyanobacterial layer in dwarf and pond habitats was usually overlain by a diffuse film of pennate diatoms and underlain by a multi-mm thick layer of purple sulfur bacteria. Photosynthetic biomass in fringe and transition microbial mats was similar, ranging from 7.4 to 68.2 mg chl *a* m^{-2}, and was much lower than that observed in dwarf and pond mats (20.9 to 499.9 mg chl *a* m^{-2}) (Tab. 4.1). Porosity and organic content of the surficial mat from all habitats was similar. PAR reaching the benthic surface was not strictly inversely-related to chl *a*, but decreased steadily from dwarf through transition to fringe habitats.

Overlying and porewater chemistry varied seasonally and spatially. Fringe habitats were consistently flushed semi-diurnally with oligotrophic ocean water, while transition habitats were typically exposed to air, preventing the accumulation of reduced chemical species on short (daily) time scales. During the wet season, dwarf habitats were flushed so that pond water composition was 35 to 37‰ salt, 7.98 pH, 3 µM NH_4^+, and less than 1 µM NO_x^-, PO_4^{3-}, Fe^{2+},

and H_2S (similar to transition and fringe habitat overlying waters) (data not shown). During the dry season and under the influence of extreme low tides, dwarf habitats were flooded less frequently, resulting in increased overlying water salinities (40‰) and an order of magnitude higher NH_4^+ concentrations (30 μM) (data not shown).

Throughout the year, average porewater salinities (10 cm beneath mats) were slightly hypersaline and reflected tidal inundation regimes, with maximal salinities (49.5 ‰) in elevated transition soils, similar to the salinities observed in poorly flushed dwarf soils. Fringe soil porewater salinities were similar to that of overlying ocean waters (Tab. 4.1). In all soils, pH was between 6.87 and 7.23, NO_3^-, PO_4^{3-}, Fe^{2+}, and DOP concentrations were low (<1.3 μM NO_3^-, 1.9 μM PO_4^{3-}, 3.2 μM Fe^{2+}, and 2 μM DOP), and DOC concentrations were high (1.10 to 1.56 mM). Porewaters were very reducing in dwarf soils, with elevated concentrations of NH_4^+ (54.1 to 458.7 μM) and H_2S (0.44 to 4.08 mM). Well-flushed fringe and rarely flooded transition soil porewaters were similarly less reducing with concentrations of NH_4^+ and H_2S consistently below 20 μM and 0.65 mM, respectively.

While DOP concentrations did not fluctuate across habitats, DON increased gradually with distance from the ocean at 39.8 μM in the fringe to 101.4 μM in dwarf soils, thus skewing the DON:DOP ratio. Similarly, DIP concentrations did not fluctuate across habitats, thus the DIN:DIP ratio was skewed with the same pattern as DIN concentration. In all habitats, dissolved inorganic, organic and total N:P ratios were above the Redfield ratio of 16:1 indicating excess nitrogen, especially in dwarf soils.

Diel patterns of N_2 fixation and denitrification in dwarf mangrove habitats varied seasonally and across sites (Fig. 4.2). Within sites, N_2 fixation rates varied as a function of community composition and PAR intensity. Mats containing HC (WS November 2000 and WS

March 2002) exhibited higher daytime N_2 fixation rates, while NHC mats (NWD and BF November 2000 and WS June 2001) exhibited higher nighttime rates. Daytime dark rates in HC-containing mats were lower than light rates while daytime dark rates in NHC-containing mats were equal to or greater than light rates. N_2 fixation rates increased by an order of magnitude in DCMU-amended daylight-incubated mats relative to rates observed in unamended daylight incubations. NO_3^- and glucose addition had a slight negative effect (if any) on dwarf mat N_2 fixation, which was most evident in dark treatments. In contrast, while unamended denitrification rates were negligible, NO_3^- plus glucose addition led to significant increases in activity. Potential (NO_3^- plus glucose amended) denitrification rates were higher during the dry season (June 2001 and March 2002) than in the wet season (November 2000). Dark potential rates were often higher than daytime light potential rates and DCMU-amended rates. Although diel patterns of potential denitrification did not mirror N_2 fixation activity, higher rates of N_2 fixation were often associated with higher rates of potential denitrification (note rate scales in Fig. 4.2).

Island-wide N_2 fixation and denitrification rates exhibited minor variation across season, but differences were observed between mangrove habitats (Fig. 4.3). NO_3^- plus glucose addition had no significant impact on averaged island-wide N_2 fixation rates in either daytime or nighttime incubations. The level of DCMU stimulation of daytime N_2 fixation rates varied across season and habitat. Daytime and nighttime denitrification rates were enhanced by NO_3^- plus glucose addition, especially in transition and fringe habitats. Unamended denitrification rates in all habitats were low and did not vary significantly throughout the year. Nighttime potential denitrification rates did not change with season, but March daytime potential rates were lower than those observed in June and October. Integrating daytime and nighttime rates of N_2

fixation and denitrification in unamended treatments shows that N_2 fixation always exceeded denitrification (Fig. 4.4), and rates of N_2 fixation varied throughout the year across the different habitats. Trapezoidal integration of N cycling rates revealed that annual N inputs via N_2 fixation were much higher than removal by denitrification, generating a net N input of 45.7 mmol N m^{-2} y^{-1} (Fig. 4.4).

Short-term (hours-long) nutrient amendments had both negative and positive effects on rates of N cycling (Tab. 4.2 & 4.3). In all mangrove habitats (fringe, transition, and dwarf) and under all light conditions, denitrification rates were unaffected by NH_4^+ or glucose additions. NO_3^- concentration (from 0.1 to 1 mM) was the main stimulus for denitrification, evidenced by low rates in glucose-only treatments and similarly high rates in NO_3^- only and NO_3^- plus glucose treatments. Fringe and transition habitat potential denitrification rates always exceeded those in the dwarf zone, and different carbon sources (glucose, acetate or lactate) yielded similar rates.

NH_4^+ and NO_3^- addition had inconsistent effects on N_2 fixation in short-term nutrient amendment experiments. NH_4^+, NO_3^-, and glucose stimulated N_2 fixation in WS-HC mats in November 2000, but except for glucose, inhibited N_2 fixation rates in May 2003. Unamended rates of N_2 fixation in WS-HC mats on these dates were significantly different. In NHC-containing mats, nutrient (NH_4^+, NO_3^- and organic carbon) amendments had a negative or no effect on N_2 fixation rates.

Over longer (days-length) time scales, the response of N_2 fixation and denitrification to nutrient enrichment was similar to short-term effects (Tab. 4.4 & 4.5). Long-term NH_4^+ enrichment was either inhibitory to N_2 fixation at concentrations above 0.1 mM or had no effect. Long-term NO_3^- enrichment was typically also inhibitory at concentrations above 0.1 mM, but stimulatory in one instance (BF-NHC mats in May 2003). Glucose alone stimulated N_2 fixation

rates, especially at night. Phosphate rarely stimulated N_2 fixation ($p < 0.05$ in only 1 of 7 bioassays), while acetate, lactate, vitamins and trace metals had no significant effect on N_2 fixation. As in short-term experiments, longer-term denitrification rates were controlled by NO_3^- concentration. NO_3^- addition increased denitrification rates at concentrations as low as 0.1 mM. Additions of NH_4^+, organic carbon (glucose, acetate, or lactate), phosphate, vitamins, or trace metals had no significant effect on denitrification.

Hydration of desiccated microbial mats from June 2001 generated immediate effects on rates of N cycling (Fig. 4.5). HC-containing mats contained greater bulk concentrations of cyanobacteria than NHC-containing mats. Both desiccated HC and NHC mats required moist incubations to fix N_2, with significantly higher rates under OLW-submerged incubations. Maximum rates of N_2 fixation after short-term rehydration (20 min to 4 h) were significantly lower than rates in non-desiccated mats. Both desiccated HC and NHC mats showed evidence of denitrification under all hydration regimes (dry, moist, wet, wet/dry, and wet/moist). Rates of denitrification were enhanced along an increasing moisture gradient with greatest rates after a 20 min wet pre-incubation. Unlike non-desiccated microbial mats (Figs. 2 to 4), rates of denitrification in dehydrated mats exceeded N_2 fixation rates under all degrees of rehydration.

Under longer-term rehydration regimes, N_2 fixation again dominated N cycling activity compared to denitrification (Fig. 4.6). Non-desiccated (wet) NHC microbial mats exhibited higher N_2 fixation rates than both desiccated (dry) NHC and desiccated (dry) HC mats. Negligible rates of N_2 fixation occurred in dry incubations compared to wet incubations in both wet and dry NHC mats. One day of wet mat dehydration decreased rates of N_2 fixation to the same degree as daily-alternating and 2 and 5 d of dehydration. After ≥1 d of dehydration and subsequent wet incubation, NHC mat N_2 fixation rates consistently equaled dry NHC mat

fixation after >1 d of rehydration and wet incubation. No difference in N_2 fixation was evident between any treatment of wet NHC and dry NHC mats incubated under air or helium.

N_2 fixation in desiccated HC mats increased after 1 d of rehydration to maximum rates after 2 d of rehydration. N_2 fixation after 5 d of rehydration was the same as after 1 d. Unlike wet and dry NHC mats, N_2 fixation in desiccated HC mats occurred under both wet and dry incubations following rehydration. N_2 fixation in dry HC mats was insignificantly enhanced by incubation under helium.

Denitrification rates were also affected by rehydration regimes. Both non-desiccated and desiccated NHC mats exhibited minimal rates of denitrification, and hydration had no impact on denitrification activity. Desiccated HC mats exhibited denitrification under dry conditions, and activity in dry incubations often exceeded activity in wet incubations. As rehydration durations increased from 1 to 2 to 5 d, rates of denitrification in desiccated HC mats decreased from maximal rates after 1 d of rehydration to minimal rates after 5 d of rehydration. In all mat types studied, denitrification rates in incubations under air were the same as in under helium-purged conditions.

DISCUSSION

Physiological controls

Diel patterns of N_2 fixation in Twin Cays fringe, transition and dwarf microbial mats were controlled primarily by strategies to decrease O_2 inhibition of the nitrogenase enzyme. Mats dominated by HC demonstrated their ability to photosynthesize and fix N_2 contemporaneously by greater daytime N_2 fixation rates, while NHC-containing mats fixed N_2 at low rates under daytime O_2-rich conditions and exhibited maximal rates during low O_2

conditions at night. HC can fix N_2 during the day in specialized heterocysts lacking O_2-generating PS-II and surrounded by thick cell walls of glycolipid and polysaccharide that serve as a barrier to O_2 diffusion into the cell. Temporal separation of daytime photosynthetic O_2 production from nighttime N_2 fixation occurs in unicellular and filamentous cyanobacteria lacking heterocysts (Stal 1995). Other organisms are able to support N_2 fixation by exploiting deeper anoxic layers or existing within surficial anaerobic microzones of the microbial mat (e.g. sulfate reducing bacteria), while phototrophic sulfur bacteria possess only PS-I, which does not produce O_2 (Paerl & Pinckney 1996).

The sizeable stimulation of N_2 fixation by DCMU did not simply reflect the release of inhibition of O_2-sensitive N_2 fixers, but underscored the importance of PS-I in supplying energy and reductant in support of N_2 fixation. Cyanobacteria and phototrophic sulfur bacteria may use H_2S as a source of electrons for CO2 fixation, and cyanobacteria may funnel electrons from H_2S or NADH/NADPH oxidation (generated from the catabolism of fixed carbon) through PS-I to fix N_2 (Bebout et al. 1993). Phototrophic release of fixed-carbon (e.g. DOC) may stimulate heterotrophic N_2 fixation (Paerl et al. 1987, Paerl 1990). Rates of N_2 fixation in dark incubations over a diel cycle were relatively constant, showing that a fraction of the community fixing N_2 was independent of light-driven stimulation. In contrast to diel patterns of N_2 fixation, denitrification rates were influenced by O_2 only when ample NO_3^- was available. The controls on denitrification in Twin Cays' habitats are discussed further in subsequent sections.

Physical environmental controls

N_2 fixation and denitrification by Twin Cays' mangrove microbial mats were affected by a variety of factors including the physical environment, redox conditions, and community

composition. Shaded and tidally-flushed or air-exposed fringe and transition soils were colonized by oxygenic phototrophs including primarily diatoms and eukaryotic algae, and a fraction of unicellular and non-heterocystous filamentous cyanobacteria. In contrast, dwarf habitat mats were dominated by cyanobacteria, including unicellular and non-heterocystous and heterocystous filamentous forms, purple sulfur bacteria, and other microbes more tolerant of heat, salt, sulfide, irradiation, and desiccation stresses.

N_2 fixing communities proliferated in all habitats during wet seasons, but during dry spring and early summer seasons, N_2 fixation was influenced strongly by inundation patterns. Under dry season low tides in March and June, transition habitat N_2 fixation rates were lowest because the combination of low tides and high elevation resulted in the greatest exposure of these mats. The extreme low tides, decreased rainfall and increased temperatures in June, decreased rates of dwarf mat N_2 fixation, while fringe mats maintained average rates of N_2 fixation due to continual flushing by dry season high tides.

During the dry season, extreme low tides exposed the mats for days at a time to direct solar irradiation and desiccation. Under desiccated conditions, rates of N_2 fixation were immeasurable in NHC-containing mats, and low, but measurable in HC-containing mats, which were encased in yellow-brown colored sheaths, indicative of the UV-absorbing pigment scytonemin. Wetting of dried mat restored cellular water content, which may have altered local O_2 concentrations by restoring metabolisms that generate anoxic conditions at depth, as well as restoring oxygenic photosynthesis at the surface. Metabolic functions in rehydrated *Nostoc* began with respiration, followed by photosynthesis, and finally N_2 fixation (Potts 1999). Denitrification was more resilient to dehydration than N_2 fixation. We suspect this resilience resulted from physiological factors because denitrification was often inhibited by photosynthetic

O_2 production, suggesting that denitrification and N_2 fixation occurred in similar depth horizons, yet distinct redox microzones.

In non-desiccated and dehydrated NHC-containing mats, exposure to atmospheric O_2 was not inhibitory under wet conditions, possibly because O_2 diffusion through water is slower than in air and respiration maintained O_2 concentrations at a level non-inhibitory to N_2 fixers. Denitrification in NHC-containing mats was insignificant. In desiccated HC-containing mats, recovery of N_2 fixing activity was rapid after rehydration. HC-containing mats are thus presumably more resilient to changes in hydration, which may explain their greater abundance in the high intertidal relative to NHC-containing mats (Potts 1980). Alternating changes in water content in the high intertidal may also contribute to nitrification-linked denitrification, which may explain the occurrence of higher denitrification rates in HC-containing mats.

Chemical environmental controls

Twin Cays' microbial mat redox conditions were controlled by seasonal changes in hydrology, autochthonous production of O_2 by phototrophs, O_2 consumption by biotic and abiotic processes, and anaerobic metabolism. Photosynthetic O_2 production had a substantial diel effect on the environment of N_2 fixing and denitrifying bacteria by directly altering local O_2 concentration and the redox states of metabolic reactants. Elevated concentrations of reduced chemical species, such as H_2S and NH_4^+, accumulated at depth, especially in the almost continually-submerged dwarf habitat mats, and diffused towards the microbial mats to be metabolized by a diverse array of mat microbes, e.g. chemoautotrophs, heterotrophs, or photoautotrophs, or to pass through the microbial mats and flux into the overlying water.

N_2 fixation incurs a large metabolic cost (16 ATP per N_2 reduced), and environmental NH_4^+ availability can repress nitrogenase synthesis (Postgate 1982). N_2 fixation in Twin Cays' dwarf mats was inhibited when NH_4^+ concentrations exceeded 0.5 mM (data not shown). This value falls within the inhibitory range of 50 to 500 μM observed in other studies (Capone 1988, Valiente et al. 1997). Transition and fringe fixation rates varied independently of porewater NH_4^+ concentrations which were consistently less than 20 μM, and thus not likely the primary factor controlling N_2 fixation in those habitats.

Negative effects of H_2S on N_2 fixation have been attributed to pH-dependent direct sulfide toxicity (Tam et al. 1982). But microbial mat N_2 fixers, including sulfur- oxidizing bacteria and cyanobacteria, can oxidize H_2S (Bebout et al. 1993). Cyanobacteria also demonstrate a differential tolerance to H_2S addition based on morphology. HC-dominated mats along the Mediterranean coast exhibited decreased N_2 fixation under 1 to 10 mM H_2S addition, while NHC-mats were stimulated by the same H_2S amendments (Villbrandt & Stal 1996). Most dwarf mats observed on Twin Cays were dominated by NHC and purple sulfur bacteria, and thus may have been capable of sustaining N_2 fixation rates across the broad ranges of *in situ* H_2S concentrations.

Substrate limitation of denitrification by NO_3^- and glucose was evident in all habitats, and transition and fringe mats exhibited greater rates of denitrification than dwarf mats. Caribbean coral reef and mangrove prop root sponges have been found to release large amounts of NO_3^- to the surrounding environment (Diaz & Ward 1997). Fringe and transition mats may experience significant and erratic inputs of NO_3^- from sponges on reefs and fringe prop roots, so that when NO_3^- is available, the existing denitrifying population is capable of rapid consumption.

Denitrifiers in fringe and transition habitats also had the advantage of living in less sulfidic conditions compared to dwarf habitats. Sulfide is inhibitory to denitrification (Sorensen et al. 1980) and also nitrification (Joye & Hollibaugh 1995), which may be coupled to denitrification in these fluctuating aerobic-anaerobic, NH_4^+-rich environments. Unfortunately, acetylene inhibits nitrification, while sulfide interferes with the acetylene block measurement of denitrification. Quantification of coupled nitrification-denitrification in these habitats is a topic for future study.

Short-term (hourly) and long-term (days-long) nutrient controls

NO_3^- was the primary control on denitrification in both short- and long-term nutrient incubations, while NH_4^+, DOC, P, vitamins, and trace metals had no effect. Denitrification was primarily NO_3^- limited, but when NO_3^- was available, nighttime potential denitrification rates exceeded daytime rates, suggesting that denitrifiers were inhibited by O_2 during the day. The large variability in diel activity may have been due to the heterogeneity of denitrifier populations or the presence of anaerobic microzones (Paerl & Pinckney 1996).

Labile DOC (e.g. glucose) has been observed to stimulate aerobic respiration, and by decreasing O_2 concentrations, stimulate N_2 fixation in NHC-containing mats more than in HC-containing mats (Paerl et al. 1987, Villbrandt & Stal 1996). In this system, DOC stimulation of N_2 fixation occurred only in HC-containing mats. DOC stimulation of O_2 respiration may have enhanced photosynthetic sulfur bacterial H_2S oxidation, thus decreasing local H_2S concentrations and relieving H_2S-inhibition of HC N_2 fixation. Since HC are more sensitive to sulfide (as noted above), DOC stimulation of H_2S oxidation would influence activity in HC-containing mats more than in NHC-containing mats. Longer-term DOC addition significantly increased N_2 fixation in

some dark treatments attesting that stimulation of N_2 fixation by DOC is not due to increased oxygen consumption alone, but also that DOC was used as a carbon and energy source for heterotrophic N_2 fixation (Paerl et al. 1993).

Environmental availability of fixed N (e.g. NH_4^+ and NO_3^-) can inhibit N_2 fixation by suppressing nitrogenase synthesis and 'switching-off' nitrogenase activity, but the majority of mats demonstrated no significant change in N_2 fixation with NH_4^+ or NO_3^- addition irrespective of habitat or season. Paerl et al. (1989) also noted the absence of DIN inhibition of N_2 fixation with additions of up to ~55 μM NH_4^+ in Shackleford Banks (NC, USA) microbial mats, while DIN inhibition of N_2 fixation has been documented at a variety of concentrations in aquatic environments (e.g. 4 to >70 μM DIN; Horne & Commins 1987, MacKay & Elser 1998). High porewater NH_4^+ concentrations in both Twin Cays dwarf habitats (257.4 μM NH_4^+ at 10 cm depth) and Shackleford Banks (~8.8 μM NH_3) may have repressed nitrogenase activity prior to experimental N amendment. We suspect that in dwarf mats stimulated by DIN additions, heterotrophic O_2 respiration was stimulated, which decreased O_2 inhibition of N_2 fixation.

Phosphorus, vitamins and trace metals did not limit activity of N_2 fixers. Similar results have been found in other environments, including Bahamian stromatolites and mats from Mexican lagoons, North Carolinian coastal islands, and California coastal marshes (Paerl et al. 1987, 1993). In contrast, some environments, including North Carolinian mats (Pinckney et al. 1995), have exhibited phosphate limitation of N_2 fixation. Clearly, nutrient controls on N_2 fixation limitation vary locally, and each site needs to be examined as an independent system.

Ecosystem-level importance of microbial mats

The adaptation of Twin Cays microbial mat communities to redox and nutrient conditions in each habitat influences their role as either a source or sink of N in the system. Fringe and transition mats demonstrated a significantly greater denitrification capacity than dwarf mats, while N_2 fixation dominated dwarf habitats. Integrated unamended denitrification rates across all sites (9.9 mmol N m^{-2} yr^{-1}) were much lower than those of N_2 fixation (55.7 mmol N m^{-2} yr^{-1}), clearly showing that benthic processes serve as an important net source of N to the oligotrophic Twin Cays mangrove ecosystem (Joye & Lee 2004).

Variability in benthic N dynamics helps explain nutrient limitation patterns of mangrove trees in each habitat. Twin Cays fringe mangrove trees are N-limited, while dwarf trees are P-limited, and transition trees are co-limited by N and P (Feller et al. 2003). Microbial mats serve as a significant N source to dwarf mangrove trees via N_2 fixation, thereby alleviating N-limitation and contributing to the observed P-limitation of trees in this zone. Fringe and transition mats have the potential to serve as sources of N to their respective habitats, but elevated rates of denitrification in fringe and transition microbial mats may limit DIN availability to fringe and transition mangrove trees by competing for available NO_3^-. Coupled nitrification-denitrification could further exacerbate N limitation in mats and trees from these habitats.

The rates of N_2 fixation and denitrification observed in Twin Cays microbial mats were comparable to rates of N cycling observed in other mangrove cyanobacterial mats and soils (Tab. 4.6). Denitrification rates in all mangrove environments were broadly related to NO_3^- inputs associated with land use, such as agriculture, industry, sewage and shrimp-farming (Corredor et al. 1999, Alongi et al. 2000, 2002), which suggests that mangrove mats, particularly those in fringe and transition habitats, may naturally mitigate anthropogenic DIN inputs. Efforts aimed at

conservation and restoration of mangrove forests should consider microbial processes such as those observed in cyanobacterial mats and soils (Holguin et al. 2001, Rejmánková et al. 2004), as these processes may influence the productivity and potential recovery of mangrove habitats.

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Table 4.1. Mat and soil characteristics of Twin Cays fringe, transition, and dwarf habitats averaged over all seasons. PAR ratio: PAR at benthic surface relative to dwarf habitat PAR between 08:00-15:00; standard deviations in parentheses.

Characteristic	Fringe habitat	Transition habitat	Dwarf habitat
PAR ratio	0.34 (0.17)	0.69 (0.69)	1.00 (0.00)
chl *a* (mg m^{-2})	28.7 (6.5)	30.1 (14.4)	114.1 (70.6)
Porosity (g gws^{-1})	0.86 (0.09)	0.84 (0.12)	0.84 (0.07)
Organic content (g gdw^{-1})	0.65 (0.04)	0.57 (0.07)	0.55 (0.06)
Porewater at 10 cm depth:			
pH	6.96 (0.44)	6.87 (0.24)	7.23 (0.26)
Salinity (ppt)	36.2 (3.7)	41.3 (7.4)	39.9 (4.3)
NH_4^+ (μM)	12.9 (11.2)	15.3 (5.6)	257.4 (136.6)
NO_x^- (μM)	0.9 (0.6)	1.0 (0.5)	1.3 (0.7)
DON (μM)	39.8 (5.9)	58.2 (14.9)	101.4 (35.0)
PO_4^{3-} (μM)	0.4 (0.2)	0.7 (0.4)	1.9 (2.8)
DOP (μM)	1.6 (0.9)	1.5 (0.8)	1.6 (0.4)
DIN:DIP	52.1 (48.9)	27.7 (18.1)	337.3 (290.8)
DON:DOP	35.3 (28.8)	45.9 (17.6)	66.1 (28.2)
TDN:TDP	27.6 (5.5)	36.1 (6.7)	133.3 (67.5)
Fe^{2+} (μM)	0.5 (0.4)	1.0 (0.6)	3.2 (3.7)
H_2S (mM)	0.43 (0.14)	0.48 (0.15)	1.32 (1.40)
DOC (mM)	1.10 (0.69)	1.24 (0.93)	1.56 (0.77)

Table 4.2. Rates of N_2 fixation (μmol N m^{-2} h^{-1}) in short-term nutrient amendment experiments. *Difference relative to control (2-tailed *t*-test) is significant at $p < 0.1$. Am: NH_4^+; Ni: NO_3^-; G: glucose. Subscript: mM concentration; NHC: nonheterocystous cyanobacterial mats; HC: heterocystous cyanobacterial mats. NWD: North West Dock; WS: Weather Station; BF: Boat Flats; D: Dock.

	Nov 00 NWD-NHC		**Nov 00 WS-HC**	
	Light	*Dark*	*Light*	*Dark*
Control	8.1	16.5	1.4	0.4
$Am_{0.5}$	6.7	1.2*	32.8*	6.8*
$Ni_{0.5}$	10.0	3.8*	23.0	10.3*
G_1	8.5	9.8	24.0*	19.6*
$G_1Ni_{0.5}$	7.6	3.9*	21.1*	0.7*

	May 03 BF-NHC		**May 03 WS-HC**	
	Light	*Dark*	*Light*	*Dark*
Control	0.5	0.2	11.8	3.8
$Am_{0.1}$	1.0*	0.3	9.5	2.6
$Ni_{0.1}$	0.6	0.3	8.4	0.8*
Am_1	0.7	0.3	1.1	1.2*
Ni_1	1.1	0.2	6.2	0.6*
$Am_{0.1}Ni_{0.1}$	0.9	0.3	5.8	0.7*
$G_2Am_{0.1}Ni_{0.1}$	1.7	0.3	6.6*	1.3*
$G_2Am_1Ni_1$	0.6	0.3	0.7	1.2*
$G_{0.5}$	0.6	0.2	7.8	10.0*
G_2	1.7	0.2	8.8	7.8

	Mar 02 D-NHC					
	Day Dwarf	*Night Dwarf*	*Day Transition*	*Night Transition*	*Day Fringe*	*Night Fringe*
Control	4.7	22.5	9.2	0.3	9.4	0.1
G_2Ni_1	1.8*	6.7*	1.2*	0.1*	1.2	0.0
ANi_1	1.8*	4.7*	2.0*	0.0*	1.5	0.2*
LNi_1	5.6	3.7	6.2	0.0*	1.7	0.0

Table 4.3. Rates of denitrification (μmol N m^{-2} h^{-1}) in short-term nutrient amendment experiments. *Difference relative to control (2-tailed *t*-test) is significant at $p < 0.1$. Am: NH_4^+; Ni: NO_3^-; G: glucose. Subscript: mM concentration; NHC: nonheterocystous cyanobacterial mats; HC: heterocystous cyanobacterial mats. NWD: North West Dock; WS: Weather Station; BF: Boa Flats; D: Dock.

	Nov 00 NWD-NHC		**Nov 00 WS-HC**	
	Light	*Dark*	*Light*	*Dark*
Control	0.3	0.2	0.0	0.1
$Am_{0.5}$	0.1	0.3	1.9	0.3
$Ni_{0.5}$	14.8*	41.0*	8.2*	41.4*
G_1	0.0*	0.0*	0.0	0.0*
$G_1Ni_{0.5}$	12.6*	54.0*	17.6	76.6*

	May 03 BF-NHC		**May 03 WS-HC**	
	Light	*Dark*	*Light*	*Dark*
Control	0.1	0.0	0.0	0.0
$Am_{0.1}$	0.0	0.0	0.0	0.0
$Ni_{0.1}$	3.0*	0.6	0.3	45.4*
Am_1	0.0*	0.0	0.1	0.0
Ni_1	23.1	1.4	2.7	103.6*
$Am_{0.1}Ni_{0.1}$	4.0*	0.2*	4.2	70.8*
$G_2Am_{0.1}Ni_{0.1}$	1.4*	0.2*	3.0*	75.0*
$G_2Am_1Ni_1$	11.1	0.0	5.4	113.8*
$G_{0.5}$	0.0*	0.0	0.0	0.5
G_2	0.0	0.0	0.0*	0.0*

	Mar 02 D-NHC					
	Day Dwarf	*Night Dwarf*	*Day Transition*	*Night Transition*	*Day Fringe*	*Night Fringe*
Control	0.4	0.0	0.7	0.1	2.0	1.1
G_2Ni_1	21.4	78.2*	130.6*	271.4*	103.0*	172.8.*
ANi_1	12.9	72.6*	107.8*	223.0*	100.5	170.8*
LNi_1	22.2*	92.2*	145.4*	142.4*	159.4*	273.4*

Table 4.4. Rates of N_2 fixation (μmol N m^{-2} h^{-1}) in nutrient bioassay experiments. *Difference relative to control (2-tailed *t*-test) is significant at $p < 0.1$. Am: NH_4^+; Ni: NO_3^-; P: PO_4^{3-}; G: glucose; see text for other treatment details. Subscript: mM concentration; HC: heterocystous cyanobacterial mat; NHC: non-heterocystous cyanobacterial mat. WS: Weather Station; BF: Boa Flats; NWD: North West Dock.

	Nov 00 WS-HC		**Nov 00 BF-NHC**		**Nov 00 NWD-NHC**		**Jun 01 WS-NHC**		**Mar 02 WS-NHC**	
	Light	*Dark*	*Light*	*Dark*	*Light*	*Dark*	*Light*	*Dark*	*Light*	*Dark*
Control	0.2	1.3	9.0	22.9	5.6	16.0	3.7	4.2	46.6	15.1
$Am_{0.05}Ni_{0.1}$	0.2	0.5	7.1	3.8	3.8	0.1*	3.4	2.3	39.5	17.0
P	1.4*	2.1	11.8	23.8	8.4	18.5	3.7	4.7	48.1	11.8
$PAm_{0.05}Ni_{0.1}$	0.5*	0.8	8.5	5.4	2.3*	0.2*	2.7	4.7	54.7	7.6
G_1	2.4	18.5*	23.0	112.1*	11.3	38.5*	4.0	10.9	58.0	25.9
$G_1Ni_{0.5}$	0.3	1.2	4.3*	3.2	4.5	0.8*	1.2*	0.9*	43.4	1.7*
A	--	--	--	--	--	--	--	--	57.9	13.9
L	--	--	--	--	--	--	--	--	52.7	22.7
Fe	0.3	4.7	7.6	18.5	9.2	13.2	2.5	3.8	69.8	14.7
TM	--	--	--	--	--	--	--	--	64.8	6.5
V	--	--	--	--	--	--	--	--	64.7	15.6

	May 03 BF-NHC		**May 03 WS-HC**	
	Light	*Dark*	*Light*	*Dark*
Control	1.0	1.6	2.6	0.5
$Am_{0.1}$	4.0	3.1	1.9	0.4
$Ni_{0.1}$	2.3*	1.5	1.7	0.4
$Am_{0.5}$	1.9	1.5	0.8*	0.3
$Ni_{0.5}$	1.3	1.3	0.7*	0.3
Am_1	18.5	1.4	0.4*	0.1*
Ni_1	1.9*	1.5	0.5*	0.1*
P	22.6	1.9	1.0*	0.3
$PAm_{0.1}Ni_{0.1}$	7.9	1.8	1.4*	0.3
$G_{0.5}$	14.9	2.5	0.5*	0.2
G_2	9.0	1.4	3.3	1.3
$G_2Am_{0.1}Ni_{0.1}$	8.2	5.8	0.5*	1.0

Table 4.5. Rates of denitrification (μmol N m^{-2} h^{-1}) in nutrient bioassay experiments. *Difference relative to control (2-tailed *t*-test) is significant at $p < 0.1$. Am: NH_4^+; Ni: NO_3^-; P: PO_4^{3-}; G: glucose; see text for other treatment details. Subscript: mM concentration; HC: heterocystous cyanobacterial mat; NHC: non-heterocystous cyanobacterial mat. WS: Weather Station; BF: Boa Flats; NWD: North West Dock.

	Nov 00 WS-HC		**Nov 00 BF-NHC**		**Nov 00 NWD-NHC**		**Jun 01 WS-NHC**		**Mar 02 WS-NHC**	
	Light	*Dark*	*Light*	*Dark*	*Light*	*Dark*	*Light*	*Dark*	*Light*	*Dark*
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	1.2
$Am_{0.05}Ni_{0.1}$	0.4*	2.6*	3.6*	9.6*	19.6*	106.0*	0.1	0.4	0.2*	1.0
P	0.0*	0.0	0.2	0.0	0.1	1.3	0.0	0.0*	0.2*	1.2
$PAm_{0.05}Ni_{0.1}$	0.1	3.3	4.5	6.2	16.6	83.1	0.2	0.1	1.3	2.7
G_1	0.0	0.0	0.1	0.0	0.0	0.2	0.0	0.2	2.3	1.2
$G_1Ni_{0.5}$	1.4*	2.6	5.3	91.2*	23.2*	93.2*	8.0*	37.5	3.0	4.4*
A	--	--	--	--	--	--	--	--	1.3	1.2
L	--	--	--	--	--	--	--	--	1.8	0.6
Fe	0.0	0.1	0.0	0.0	0.1	0.9	0.0	0.1	1.7	0.9
TM	--	--	--	--	--	--	--	--	1.1	0.9
V	--	--	--	--	--	--	--	--	2.2	0.9

	May 03 BF-NHC		**May 03 WS-HC**	
	Light	*Dark*	*Light*	*Dark*
Control	0.1	0.0	0.4	0.0
$Am_{0.1}$	0.1	0.0	0.5	0.0
$Ni_{0.1}$	0.0*	0.0	0.1	0.0*
$Am_{0.5}$	0.0*	0.0	0.0	0.0
$Ni_{0.5}$	0.0*	0.0	0.0	0.0*
Am_1	0.5	0.0	0.0	0.3
Ni_1	4.2	0.0	5.8	34.1
P	0.0	0.0	0.0	0.2
$PAm_{0.1}Ni_{0.1}$	0.0	0.0	0.0	0.0*
$G_{0.5}$	0.0	0.0	0.0	0.0*
G_2	0.0	0.1	4.2	2.2*
$G_2Am_{0.1}Ni_{0.1}$	0.0	0.0	2.6	2.0*

Table 4.6. Summary of mangrove N_2 fixation (NFIX) and denitrification (DNF) rates (mmol N m^{-2} d^{-1}). R: *Rhizophora*; A: *Avicennia*; L: *Laguncularia*; C: *Ceriops*; B: *Bruguiera* spp. HC: heterocystous cyanobacteria, NHC: non-heterocystous cyanobacteria.

Vegetation	NFIX	DNF	Benthos	Land use/impact	Location	Source
Planted and regenerated mangrove (R, C)	0–0.6[a]	0–3.8	Silt, sand, and/or clay	Mangrove forest	Sawi Bay, Thailand	Alongi et al. (2002)
Fringe, basin mangrove (R, A, L)		0.001–0.23	Clay	Inland of tidal creek	Terminos Lagoon, Mexico	Rivera-Monroy & Twilley (1996)
Riverine/fringe mangrove (R, A, L)	2.3		Silt/clay	Tidal creek	Caete Estuary, Brazil	Dittmar & Lara (2001)
Mangrove, salt flat (R)	0.28–0.39[a]	0.01–0.05	Silt, sand slurries	Mangrove forest	Makham Bay, Thailand	Kristensen et al. (1998)
Mangrove, salt flat (R, C)	0.14–0.30[a]		Saltpan sediments	Mangrove tidal channel	Missionary Bay, Australia	Boto & Robertson (1990)
Mangrove, salt flat (R, C)	0–0.28[a]		Cyanobacterial mat	Mangrove tidal channel	Missionary Bay Australia	Boto & Robertson (1990)
Mangrove (A, B)	0.46–0.65		Cyanobacterial mat	Mangrove research reserve	Mgeni Estuary, South Africa	Mann & Steinke (1993)
Fringing mangrove salt flats (A)	0.096–0.255[a]		NHC mat	Coastal salt flats	Dampier Archipelago, Australia	Paling et al. (1989)
Emerging mangrove (R, A, L)	0.09–0.63[a]		Sand, mud	Emerging mangrove	Tampa Bay, Florida	Zuberer & Silver (1978)
Fringe mangrove	0.53	0.12	Sediment	Agricultural discharge	Joyuda Lagoon, Puerto Rico	Morell & Corredor (1993)
Fringe, center, rear mangrove (R, A)	0–2.4	0.2–2	Sediment	East of Falmouth Town	Oyster Bay, Jamaica	Nedwell et al. (1994)
Mangrove, salt flat (R, C)	0–0.24[a]		Sediment	Mangrove tidal channel	Missionary Bay, Australia	Boto & Robertson (1990)
Mangrove (A)	0.0064–0.80[a]		Sediment	Mangrove forest	North Island, New Zealand	Hicks & Silvester (1985)
Fringe mangrove (R, A, L)		1.20–2.16[b]	sediment	Sewage effluent	Puerto Rico	Corredor et al. (1999)
Planted and regenerated mangrove (R)	0.49–2.85[a]	0–4.4	sediment	Shrimp farm	Mekong delta, Vietnam	Alongi et al. (2000)
Fringe through dwarf mangrove (R, A, L)	0–1.21	0–1.11	HC & NHC mat, peat	Mangrove forest	Twin Cays, Belize	This study

[a]Rate assumes 3:1 ratio of C_2H_4:N_2 reduction; [b]N_2O only

FIGURE CAPTIONS

Figure 4.1. Twin Cays, Belize. WS: Weather Station, L: Lair, HL: Hidden Lake, BF: Boa Flats, WP: West Pond, D: Dock, SCC: South of Clear Cut, NWD: North West Dock. Inset illustrates the tree-height gradient across a transect from the fringe through the transition to the dwarf habitat. Adapted from Feller (1996).

Figure 4.2. N_2 fixation and denitrification rates from dwarf habitat diel experiments with and without 3-(3,4 dichlorophenyl)-1,1 dimethylurea (DCMU) or nitrate (N) and glucose (G) amendments. Error bars are standard deviations; HC: heterocystous cyanobacterial mat; NHC: non-heterocystous cyanobacterial mat; horizontal axis bars indicate daytime (open) and nighttime (filled). WS: Weather Station; BF: Boa Flats; NWD: North West Dock.

Figure 4.3. Seasonal day and night (a) N_2 fixation rates and (b) denitrification rates with and without 3-(3,4 dichlorophenyl)-1,1 dimethylurea (DCMU) or nitrate (N) and glucose (G) amendments in dwarf (D), transition (T), and fringe (F) habitats. Error bars are standard deviations.

Figure 4.4. Seasonal N cycling rates in dwarf (D), transition (T), and fringe (F) habitats. Error bars are standard deviations; annual rates determined by trapezoidal integration.

Figure 4.5. Hourly hydration effects on N_2 fixation (NFIX) and denitrification (DNF) rates (μmol N m^{-2} h^{-1}) in a desiccated heterocystous cyanobacterial (HC) dwarf mat and a desiccated non-heterocystous cyanobacterial (NHC) dwarf mat. Daytime incubations included dry: control,

i.e. no overlying water (OLW); moist: 3 drops OLW; wet: 10 ml OLW; wet/dry: dry incubation following a 20 min submersion in OLW; wet/moist: 3 drops OLW incubation following a 20 min submersion in OLW. Error bars are standard deviations. *Difference relative to control (2-tailed *t*-test) is $p < 0.1$.

Figure 4.6. Longer-term (1 to 5 d) rehydration and/or desiccation effects on N_2 fixation (NFIX) and denitrification (DNF) rates (μmol N m^{-2} h^{-1}) in (a) a non-desiccated non-heterocystous cyanobacterial (NHC) dwarf mat, (b) a desiccated NHC dwarf mat, and (c) a desiccated heterocystous cyanobacterial (HC) mat. Daytime incubations occurred with (wet) or without (dry) 10 ml overlying water and under air or helium. 0: control, i.e. dry incubation of desiccated mat or wet incubation of non-desiccated mat; 1: 1 d opposite; 2: 2 d opposite; 5: 5 d opposite; 5': 5 d alternate. Opposite: hydration of desiccated mat or desiccation of non-desiccated mat; alternate: alternating days of hydration and desiccation. Error bars are standard deviations. *Difference relative to control (2-tailed *t*-test) is significant at $p < 0.1$; nm: not measured.

Figure 4.1

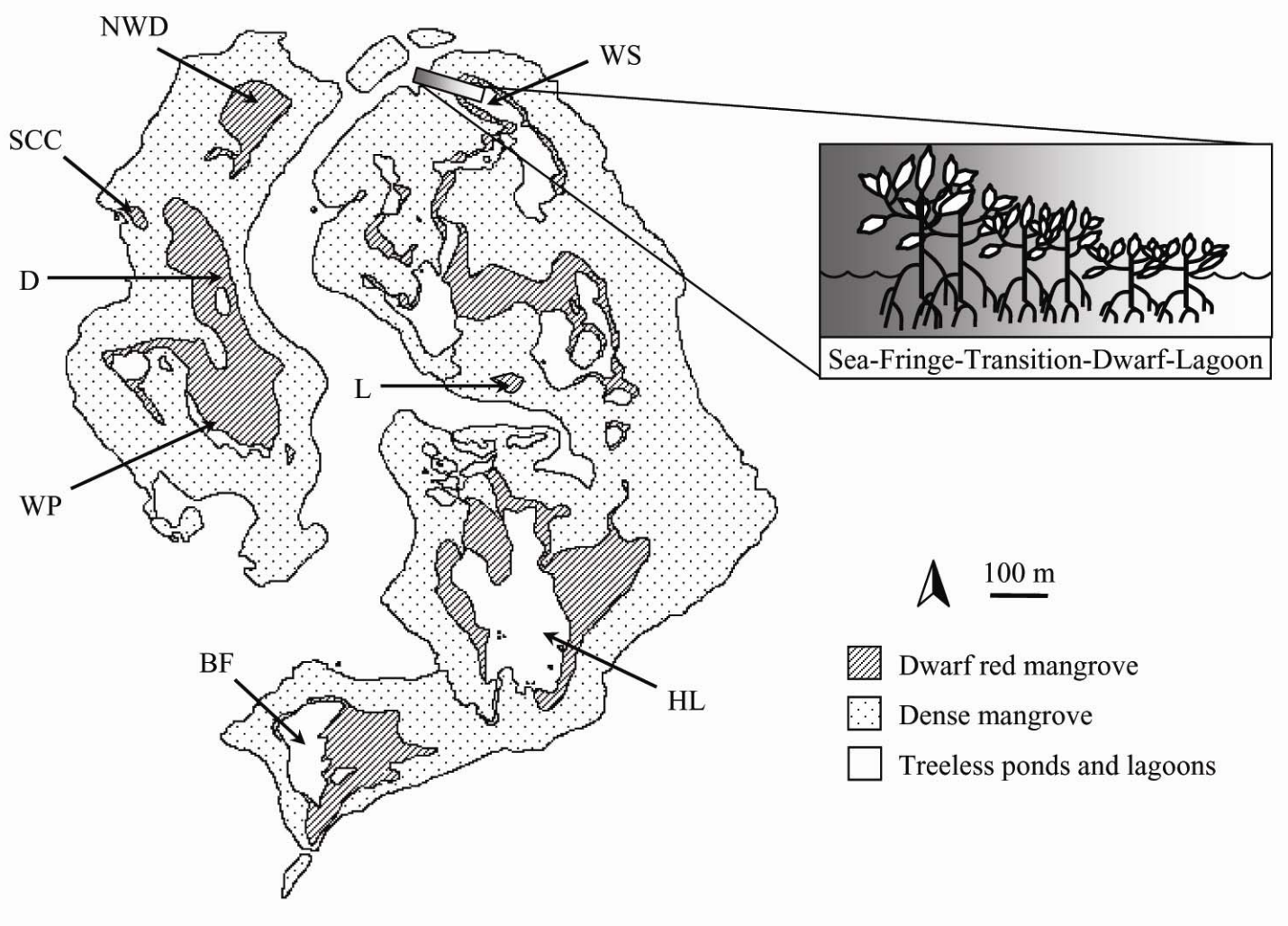

NWD
WS
SCC
D
L
WP
BF
HL
Sea-Fringe-Transition-Dwarf-Lagoon
100 m
Dwarf red mangrove
Dense mangrove
Treeless ponds and lagoons

Figure 4.2

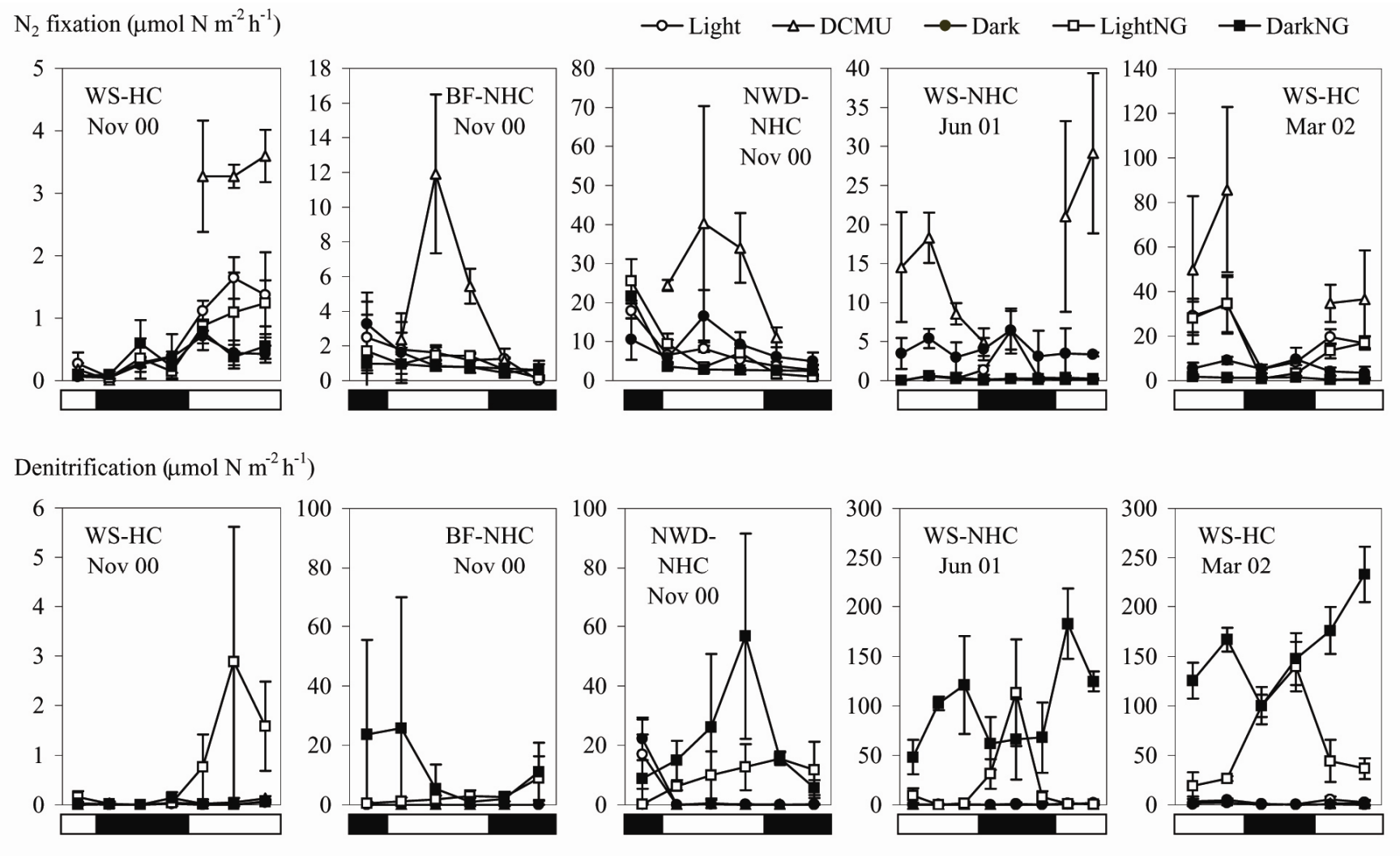

N2 fixation (µmol N m-2 h-1)
Light
DCMU
Dark
LightNG
DarkNG
WS-HC Nov 00
BF-NHC Nov 00
NWD-NHC Nov 00
WS-NHC Jun 01
WS-HC Mar 02
Denitrification (µmol N m-2 h-1)
WS-HC Nov 00
BF-NHC Nov 00
NWD-NHC Nov 00
WS-NHC Jun 01
WS-HC Mar 02

Figure 4.3

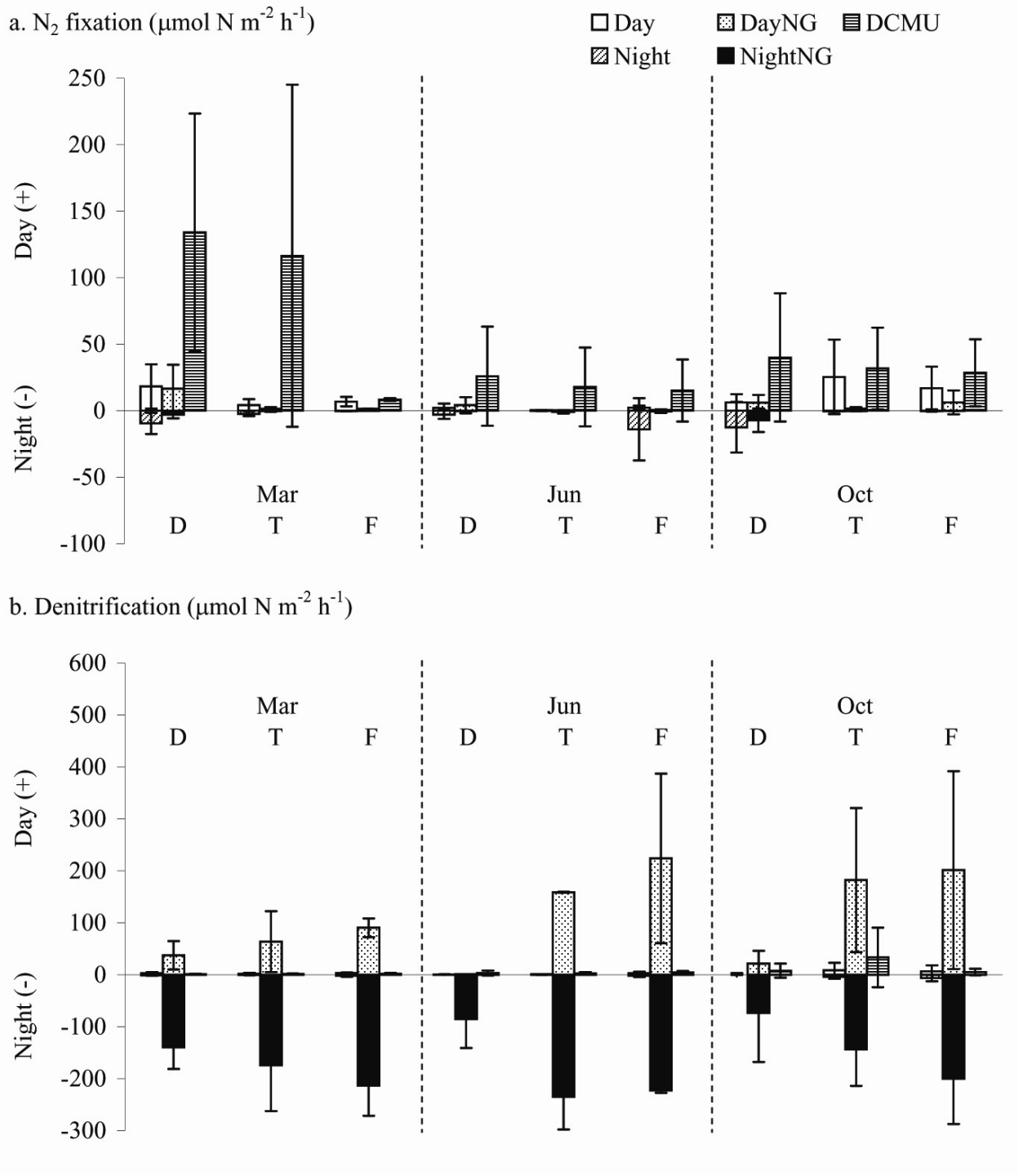

a. N_2 fixation (μmol N m^{-2} h^{-1})
Day
DayNG
DCMU
Night
NightNG
250
200
150
100
50
0
-50
-100
Day (+)
Night (-)
Mar
Jun
Oct
D
T
F
b. Denitrification (μmol N m^{-2} h^{-1})
600
500
400
300
200
100
0
-100
-200
-300

Figure 4.4

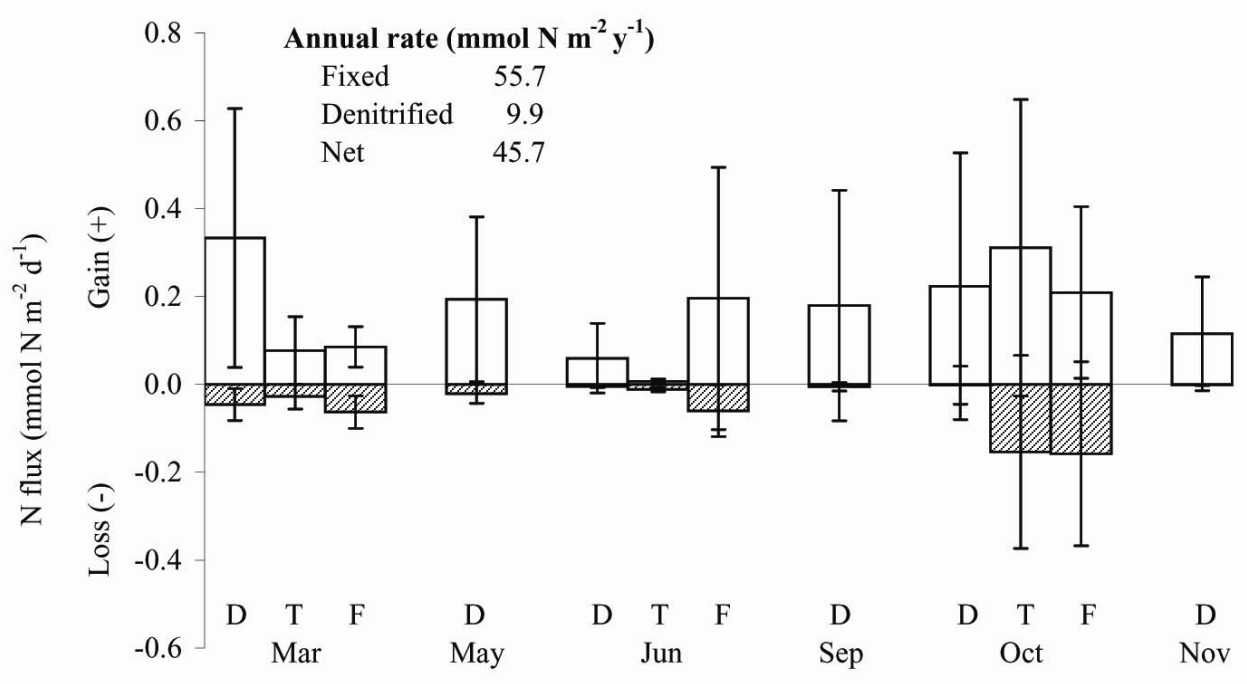

Annual rate (mmol N m-2 y-1)
Fixed 55.7
Denitrified 9.9
Net 45.7
N flux (mmol N m-2 d-1)
Gain (+)
Loss (-)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
D T F
Mar
D
May
D T F
Jun
D
Sep
D T F
Oct
D
Nov

Figure 4.5

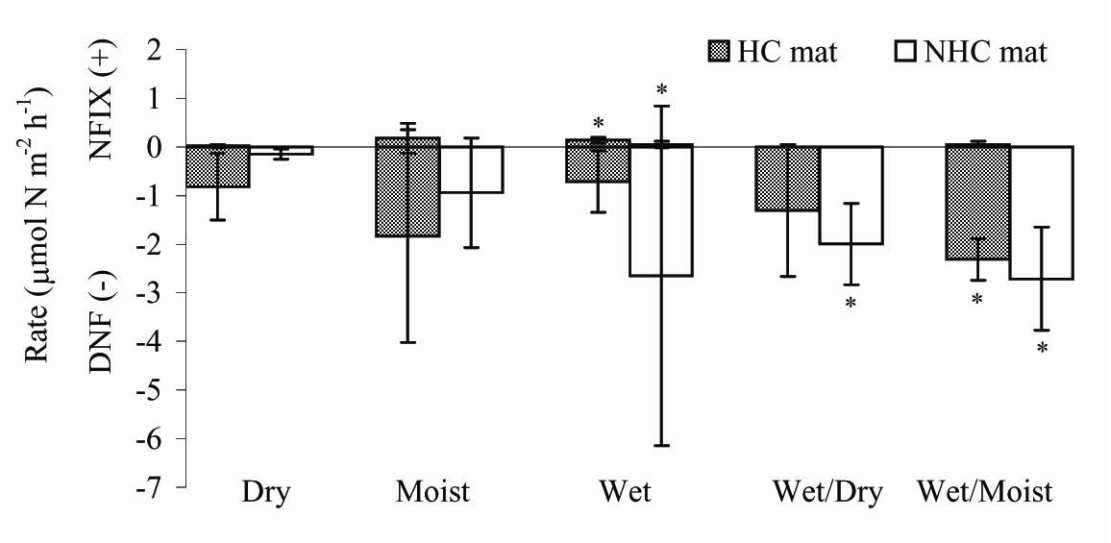
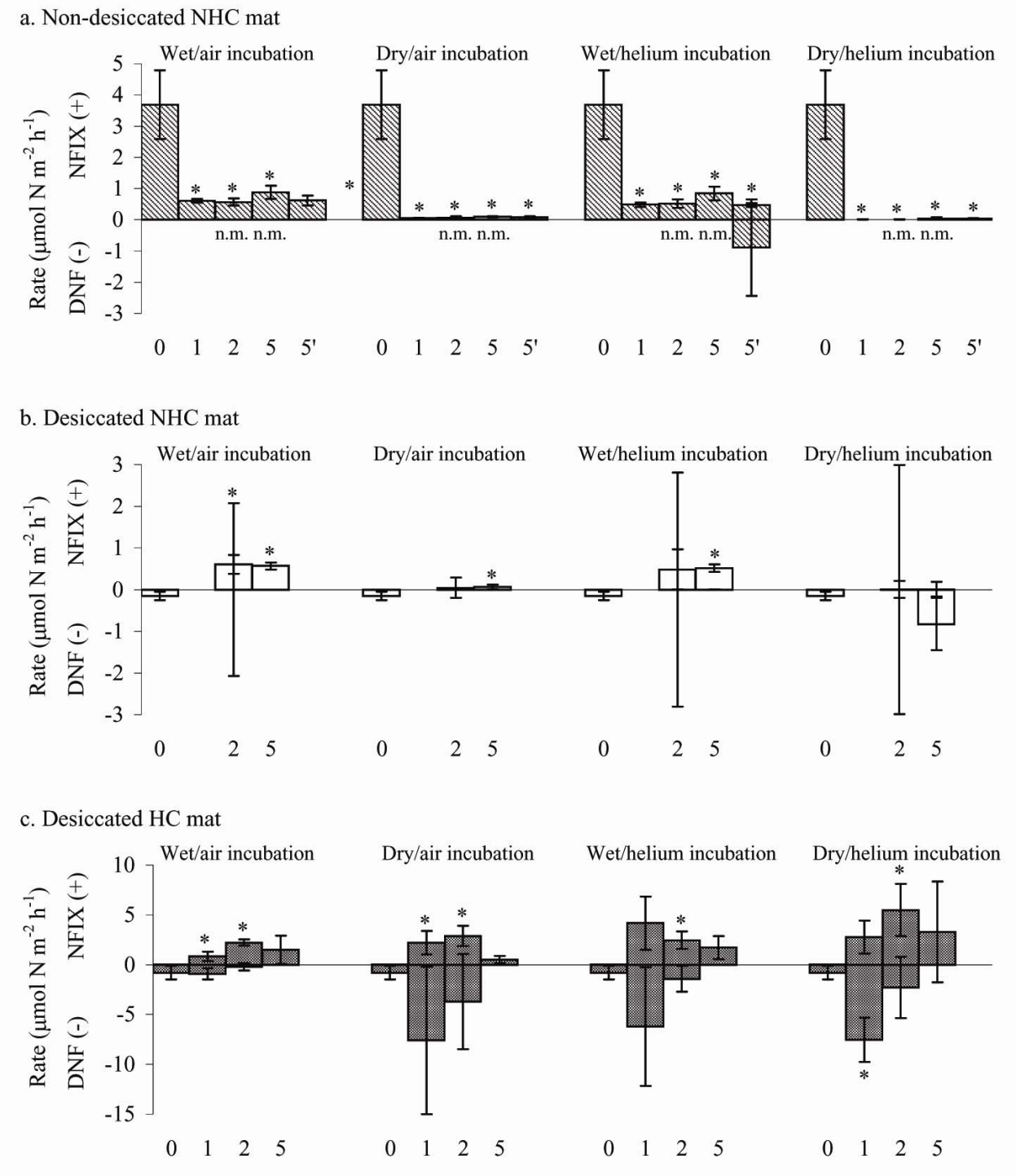

HC mat
NHC mat
Rate (µmol N m-2 h-1)
NFIX (+)
DNF (-)
2
1
0
-1
-2
-3
-4
-5
-6
-7
Dry
Moist
Wet
Wet/Dry
Wet/Moist
*

Figure 4.6


a. Non-desiccated NHC mat
Wet/air incubation
Dry/air incubation
Wet/helium incubation
Dry/helium incubation
Rate (µmol N m-2 h-1)
NFIX (+)
DNF (-)
n.m. n.m.
0 1 2 5 5'
b. Desiccated NHC mat
0 2 5
c. Desiccated HC mat
0 1 2 5

CHAPTER 5

ECOSYSTEM ROLE OF BENTHIC MICROBIAL MATS IN CARBON FIXATION AND NITROGEN DYNAMICS ON OCEANIC MANGROVE ISLANDS[1]

[1] Lee RY, Feller IC, Lovelock CE, Wooller MJ, Fogel ML, Montoya JP, Joye SB. Prepared for submission to *Ecosystems*.

ABSTRACT

Both mangrove forests and microbial mats are commonly considered highly productive components of coastal ecosystems. In the oceanic mangrove islands of Twin Cays, Belize, both photosynthetic communities exist in an opposing gradient of *Rhizophora mangle* to microbial mat coverage. Tall trees fringe the islands and light availability limits accumulation of benthic photosynthetic biomass in this zone. A transition mangrove habitat consists of intermediate mat and tree biomass, and dense microbial mats proliferate in the interior under and between "dwarf"-height trees. Benthic microbial mats were sampled from each habitat and analyzed for stable isotopic and elemental analysis of carbon and nitrogen. Rates of carbon fixation were determined by following the incorporation of ^{13}C bicarbonate into biomass. Benthic primary production in all habitats was dominated by algal, cyanobacterial and purple bacterial activity with highest rates of carbon fixation observed in well-lit dwarf mangrove habitat; lower rates were documented in shaded transition and fringe mangrove zones. Oxygenic photosynthesis was the primary mode of carbon fixation in all habitats under full sun while ~32% was attributed to anoxygenic photosynthesis and ~12% to chemoautotrophy. *In situ* light conditions emphasize the gradient from highest rates of carbon fixation in dwarf mangrove habitat mats (0.21 g C m^{-2} d^{-1}) to diminished rates in shaded transition and fringe mangrove habitat mats (0.08 and 0.05 g C m^{-2} d^{-1}, respectively). Mats associated with well-lit dwarf mangroves account for 18-20% of the net primary productivity of the habitat and may supply 5-28% of the nitrogen requirement of dwarf mangroves. Light limitation restricts the fixation of carbon and nitrogen in transition and fringe mangrove habitat mats so that

island-wide, microbial mats account for 2.3% of the net habitat production relative to the mangrove trees and the nitrogen requirement of Twin Cays mangrove trees.

INTRODUCTION

Mangrove ecosystems dominate tropical coastlines, covering 18 million hectares worldwide (Spalding et al. 1997), and serve important economic and ecological functions. For example, mangroves act as nurseries for commercially important aquatic organisms that contribute to coastal, estuarine and deep-sea fisheries (Ronnback 1999, Mumby et al. 2004), as habitat for resident and migratory birds, and as nutrient and particulate filters from upland sources (Mitch & Gosselink 1993). Mangroves also protect the shoreline from physical damage due to tidal waves, erosion, hurricanes, and tsunamis (Mitch & Gosselink 1993). More than half of the world's original mangrove habitats have been destroyed (Kelleher et al. 1995, Spalding et al. 1997), with about 70% of that loss occurring in the last 20 years (Valiela et al. 2001). Anthropogenic pressures leading to the destruction of mangrove habitat include over-harvesting for timber and fuel-wood (Hussein 1995), clearing for aquaculture and salt-pond construction (Terchunian et al. 1986, Primavera 1997), mining, and pollution and damming of rivers that alter salinity levels in mangrove habitats (Wolanski 1992).

Mangrove forests dominate tropical intertidal landscapes (Por 1984) and are often regarded as highly productive ecosystems (Clough 1992). Benthic microbial mats and microphytobenthos can also attain high rates of productivity, contributing up to 50% of estuarine primary production (Underwood & Kromkamp 1999). Mangrove primary production is commonly investigated in terrestrial-riverine forests consisting of tall (10.5 m average) trees (Lugo 1990). In these mangrove habitats, canopy shading limits both the distribution and

activity of benthic phototrophs (Kristensen et al. 1988, Alongi & Sasekumar 1992, Lee et al. in preparation, Ch. 3). In contrast, sparser "dwarf" mangrove trees (≤1.5 m tall) exist farther from the shoreline (Lugo & Snedaker 1974, Feller et al. 2003) and can support significantly greater accumulations of benthic photosynthetic biomass and rates of primary productivity as well as nitrogen fixation (Lee et al. in preparation, Ch. 3, Lee & Joye 2006, Ch. 4). The perception of dwarf mangrove habitats as unproductive "stunted" or "scrub" forests (Pool et al. 1977) has been used to justify the devaluation and subsequent destruction of these habitats for shrimp aquaculture, which accounts for 20-50% of mangrove destruction worldwide (Primavera 1997).

We investigated rates of benthic carbon fixation and natural abundance signatures of carbon and nitrogen in mangrove forests under a gradient of light levels from well-lit dwarf mangrove habitats to densely shaded fringe forests on Twin Cays, Belize. We hypothesized that an inverse relationship would characterize the productivity of mangrove trees compared to benthic microbial communities across the gradient from dwarf through transition to fringe mangrove habitats. We then evaluated the importance of microbial mats in the carbon and nitrogen budget in this mangrove ecosystem.

METHODS

Study Site

Twin Cays is a pair of oceanic mangrove islands located off the coast of Belize (16˚ 50' N, 88˚ 06' W; Feller et al. 2003). *Rhizophora mangle*, the dominant vegetation, exhibits a decreasing tree height gradient with distance from the shore. At the edges of the islands, fringe mangrove habitats contain 5-7 m tall densely distributed trees. The centers of the islands contain ponds and lagoons surrounded by more sparsely distributed "dwarf" height trees less than 1.5 m

tall. Intermediate height trees of *R. mangle* mixed with *Avicennia germinans* and *Laguncularia racemosa* populate the transition mangrove habitat between the fringe and dwarf mangrove habitats. Soil surface light availability is inversely related to tree density and canopy coverage resulting in differences in benthic community composition and activity across the mangrove tree-height gradient (Lee et al. in preparation, Ch. 3). Well-lit dwarf mangrove habitats support mm to dm thick cyanobacteria-dominated mats composed of a thin surficial layer of diatoms over a dense layer of coccoidal and heterocystous and non-heterocystous filamentous cyanobacteria which often overlie a visible layer of purple sulfur bacteria. Shaded fringe and transition mangrove habitat soils are colonized by a thin (<1 mm) layer of diatoms, eukaryotic algae and coccoidal and non-heterocystous cyanobacteria growing directly on the peaty soils.

Twin Cays tropical seasonality consists of increased precipitation and higher tidal heights during the wet fall-winter months and decreased precipitation and lower tidal heights during the dry spring-summer months. To capture seasonal and spatial variation, we conducted field surveys to 18 dwarf mangrove sites in August 2000, 8 dwarf mangrove sites in November 2000, and 3 dwarf, transition and fringe mangrove sites in June and October 2001. At the Smithsonian Institution Carrie Bow Cay field station, located 3.5 km southeast of Twin Cays, rainfall in June 2001 averaged 1.7 mm d^{-1} and extremely low tidal heights ranged from 42 cm below to 4 cm above mean sea level (Opishinski 2000-2002). August 2000 data was unavailable, but September 2000 data indicated slightly higher rainfall rates of 5.6 mm d^{-1} with higher tidal heights from 17 cm below to 19 cm above sea level. In October, average rainfall was considerably high at 11.7 mm d^{-1} and tidal heights were higher from 14 cm below to 35 cm above mean sea level (average of 2000 and 2002 data; 2001 data unavailable). November 2000

also exhibited wet seasonality with the highest rainfall rates of 12.8 mm d^{-1} and higher tidal ranges between 16 cm below and 28 cm above mean sea level.

Elemental and stable isotope analysis

Surficial mat samples consisting of the top layer of mat and associated soil to a total depth of 1 cm were collected in dwarf, transition and fringe mangrove habitats for natural abundance stable isotopic analyses of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) and for carbon and nitrogen elemental analysis. Samples from August, November and June were carefully sectioned to characterize bulk surficial mat compared to microbial mat only, peat only, upper mat (green surface layers) or lower mat (pink, red and/or brown deeper layers). Samples were preserved by freezing until return to UGA. Each sample was freeze-dried, ground, weighed into a tin capsule, and analyzed for ^{13}C, ^{15}N, %C and %N content on an elemental analyzer (EA) inline to an isotope ratio mass spectrometer (IRMS) (CE Instruments NA2500 EA with a Finnigan MAT, Delta^{plus}XL IRMS or with a Micromass Optima IRMS). Isotopic values are expressed in standard del notation:

$$\delta^{13}C \text{ or } \delta^{15}N = ([R_{sample}/R_{standard}] - 1) * 1000 [‰]$$

where R is the ratio of ^{13}C:^{12}C or ^{15}N:^{14}N, respectively, compared to the standards Pee Dee Belemnite or atmospheric N_2, respectively. Acetanilide (C_8H_9NO) was used for calibration.

Carbon fixation assays

Net carbon fixation rates were evaluated in dwarf, transition and fringe mangrove habitat mats. Surficial mats to a total depth of 1 cm were subsampled using a cut-off 5 cc syringe with a surface area of 1.03 cm^2 and placed in 20 ml serum vials containing 10 ml of GF/F filtered site-

specific overlying water. ^{13}C-$NaHCO_3$ was added to each vial to achieve a final concentration of 9 mM. Control treatments received no ^{13}C-$NaHCO_3$ additions. Carbon fixation by all autotrophs was represented in daytime (full sunlight) and shaded (10% full sunlight achieved by screening; June only) treatments. Chemoautotrophy was represented by nighttime treatments. Photoautotrophy (PS) was calculated by the difference between daytime and nighttime treatments. Anoxygenic PS was estimated in daytime treatments amended with 10 μM DCMU (3-(3,4 dichlorophenyl)-1,1 dimethylurea), an inhibitor which blocks photosystem-II (PS-II), the O_2 producing step of photosynthesis. Oxygenic PS was calculated by the difference between daytime and DCMU treatments. All treatments were carried out in triplicate over 6 hour incubations under natural temperature and light regimes at the Carrie Bow Cay field station. Supernatant liquid was carefully poured off to ensure all organic matter remained in the vials, and the vials were frozen until return to the laboratory at UGA. Samples were thawed and acidified in a concentrated HCl fume bath for 24 hours to volatilize unincorporated dissolved inorganic carbon (DIC). Samples were prepared and analyzed as described above for ^{13}C and %C. DIC concentrations in the overlying water was quantified by infrared detection of CO_2 on a Shimadzu® Total Carbon analyzer.

Diel integrated carbon fixation rates were corrected for diel light variability in each habitat by estimating daily PAR data from the Smithsonian Institution Carrie Bow Cay weather station (Opishinski 2000-2002) and factoring in differential shading in each habitat. Nighttime rates were assumed to be constant over 12 hours. Ratios of light availability relative to full sun in each mangrove habitat (0.34 fringe, 0.69 transition, 1.00 dwarf; Lee & Joye 2006, Ch. 4) were used to correct for natural daytime light availability over 12 hours. Photosynthetic production was conservatively estimated to saturate at 1000 μE m^{-2} s^{-1} (half the value of full sunlight; Lee &

Joye 2006, Ch. 4) and saturated PS was assumed over the duration of the day when photosynthetically active radiation was >1000 μE m^{-2} s^{-1}. Rates of carbon fixation over the unsaturated duration of the day were linearly interpolated from rates measured at light levels <1000 μE m^{-2} s^{-1}.

RESULTS

Carbon and nitrogen stable isotope natural abundance composition exhibited small variations across season and habitat (Fig. 5.1). ^{13}C analysis demonstrated increasing depletion with depth from green upper mat layers ("matA") to pink/red/brown deeper mat layers ("matB") or mat only layers (i.e., bulk mat layers but no peat) to the peat. Bulk surficial mat (including associated peat to 1 cm depth) $\delta^{13}C$ signatures were nearly identical to mat only signatures. Bulk dwarf mangrove habitat mats were slightly more enriched in ^{13}C compared to fringe and transition mangrove habitat mats. Temporal differences were not related to wet-dry seasonality. Natural abundance of ^{15}N in mats exhibited similar patterns with greater isotopic depletion in peats. The ^{15}N composition of bulk surficial mats (to 1 cm depth) was dominated by microbial mat signatures rather than those of the underlying peat.

Carbon and nitrogen content did not vary significantly between seasons across habitat (Fig. 5.2). Carbon concentrations exhibited an insignificantly higher trend in fringe mangrove habitat mats with no significant difference between peat and bulk mats from all habitats. In contrast, nitrogen contents were lower in peats than in mats. Concentrations of nitrogen were highest in dwarf mangrove habitat mats compared to fringe and transition mangrove habitat mats so that on a mole to mole basis, C:N ratios were lowest in dwarf mangrove habitat mats (~14:1). C:N ratios in transition and fringe mangrove habitat mats and peats were higher (~ 20:1) due to

lower %N content and higher %C in fringe mangrove habitat mats. No difference in C or N concentration was observed in upper versus deeper mat layers.

Daytime (full sun) rates of carbon fixation were represented primarily by photoautotrophy. Chemoautotrophy was relatively constant across season and habitat fixing on average 1.9 mg C m^{-2} h^{-1} or 12% of the total daytime rate (Fig. 5.3). Of the phototrophic production, anoxygenic PS was 3-5 times greater in dwarf mangrove habitat mats than in transition or fringe mangrove habitat mats. Rates of total daytime carbon fixation were smaller in October because of smaller rates of oxygenic PS, but differences were insignificant because of spatial variability.

Rates of phototrophic carbon fixation under full sunlight were on average almost two times the rates under 10% full sunlight (Fig. 5.4). Maximum rates of phototrophic carbon fixation may therefore occur at about 20-40% full sunlight. These values are low compared to our conservative estimate of oxygenic PS saturation at 50% sunlight because anoxygenic photosynthesizers may saturate at lower irradiances. We chose to integrate daytime rates using 50% sunlight as the saturation irradiance to conservatively represent oxygenic, as well as anoxygenic, photosynthetic kinetics.

Factoring differences in habitat shading on daytime carbon fixation rates accentuated the pattern of decreasing rates from dwarf to transition to fringe mangrove habitat mats (Fig. 5.5). Diel integrated rates of carbon fixation were dominated by photoautotrophy which was controlled by habitat light availability.

DISCUSSION

Modes of primary productivity in microbial mats

Twin Cays mangrove benthic microbial mats were productive communities containing microbial populations that varied between habitat as a function of the gradient in mangrove tree-height (Lee et al. in preparation, Ch. 3). All of our bulk surficial mat samples contained ^{13}C signatures representative of Calvin cycle fractionation (Goericke et al. 1994) indicating the dominance of algal, cyanobacterial and purple bacteria in the fixation of carbon in all habitats. Dwarf mangrove habitat mats were the most well-developed in terms of lamination and concentrations of chlorophyll *a* as well as other photopigments, including cyanobacterial pigments (i.e., echinenone, myxoxanthophyll, and zeaxanthin) and bacteriochlorophyll *a* (Lee et al. in preparation, Ch. 3). Mats in dwarf mangrove habitats also exhibited the highest total rates of primary production and the highest rates of anoxygenic photosynthesis compared to transition and fringe mangrove habitat mats. Full sun rates of primary production in all habitats were comparable to rates of daytime CO_2 fixation in microbial mats from a variety of other environments including Tomales Bay, California (Paerl et al. 1993), coastal North Carolina (Paerl et al. 1989, Paerl et al. 1993, Paerl et al. 1996), Belizean wetlands (Rejmánková & Komárková 2000), and Salt Pond, Bahamas (Pinckney & Paerl 1997). The significant contribution (12-57%) of anoxygenic photosynthetic organisms to CO_2 fixation in dwarf, transition and fringe mangrove habitat mats are also in the range of other microbial mats, e.g., 22-46% in Guerrero Negro, Mexico (Javor & Castenholz 1984), 10% in Bird Shoal, North Carolina (Paerl et al. 1996), 25% in Salt Pond, Bahamas (Pinckney & Paerl 1997), and 26% in Ebro Delta, Spain (Martinez-Alonso et al. 2004). Chemoautotrophic production was significant

in all mats varying from 8-15% of daytime photosynthetic rates. These rates are similar to upper range of other cyanobacteria-dominated mats, such as from Guerrero Negro (2-12%, Javor & Castenholz 1984), Stocking Island, the Bahamas (8-20%, Pinckney et al. 1995), and Bird Shoal (10%, Paerl et al. 1996), compared to minimal (≤1%) rates in Salt Pond (Pinckney & Paerl 1997) and Ebro Delta (Martinez-Alonso et al. 2004). These comparisons emphasize the high potential rates of all modes of primary production in Twin Cays mats from oxygenic photosynthesis to anoxygenic photosynthesis to chemoautotrophy.

Benthic productivity in mangrove environments

The relative contribution of each carbon fixing physiological group to total carbon fixation under full sun was comparable across mats from all habitats (i.e., dominance by oxygenic then anoxygenic photosynthesis and a small proportion of chemosynthesis), and photosynthetic activities were proportional under low (10%) and full sun (100%) irradiances, indicating similar community photosynthetic responses in all habitats to a range of light availabilities. Therefore *in situ* daytime light availability throughout the year supported a stronger gradient in CO_2 fixation from highest rates in well-lit dwarf mangrove habitat mats (1.21 ± 0.47 mmol C m^{-2} h^{-1}) to intermediate rates in partially-shaded transition mangrove habitat mats (0.44 ± 0.34 mmol C m^{-2} h^{-1}) and lowest rates in well-shaded fringe mangrove habitat mats (0.23 ± 0.14 mmol C m^{-2} h^{-1}). Light quality and quantity are major controls on benthic photosynthesis in mangrove systems (Lee et al. in preparation, Ch. 3). For example, high rates of daytime CO_2 assimilation (1.40 ± 0.15 mmol C m^{-2} h^{-1}) were measured in well-lit Ao Nam Bor, Thailand, mangrove sediments, while rates were significantly lower in sediments shaded by *R. apiculata* prop roots and canopies located only 5 m away (0.97 ± 0.10 mmol C m^{-2} h^{-1};

Kristensen et al. 1988). Benthic primary production in shrimp farms in Bangrong Mangrove, Thailand, also exhibited a gradient in daytime carbon fixation from 1.0-6.3 mmol C m^{-2} h^{-1} in shaded high- and mid-intertidal mangrove sediments to 3.4-9.2 mmol C m^{-2} h^{-1} in well-lit low-intertidal and mangrove channel mudflats (Holmer et al. 2001). Although nutrient inputs due to shrimp farming support significantly higher daytime rates of primary production than in natural mangrove sediments (Lee et al. in preparation, Ch. 3), over a diel cycle, net heterotrophy occurs in Bangrong Mangrove shrimp pond shaded sediments compared to net autotrophy in exposed sediments from the same area (Holmer et al. 2001). In contrast, all Twin Cays' mats sustained net autotrophy over a diel cycle across the differentially sun-exposed range of habitats.

These results contrast with our evaluations of benthic primary productivity using O_2 microelectrodes which revealed net heterotrophy in transition and fringe mangrove habitats and net autotrophy only in dwarf mangrove habitat mats (Lee et al. in preparation, Ch. 3). While many measurements of benthic productivity are estimated by O_2 production methods, in mats inhabited by anoxygenic photosynthetic and chemosynthetic organisms, such as those of Twin Cays, carbon uptake or flux assays are necessary to capture the sum of carbon fixed by these non-oxygenic phototrophic populations.

Ecosystem C and N input by mats and mangrove trees

To evaluate the importance of microbial mats to system-scale carbon fixation on Twin Cays, we compared our measurements of microbial mat net primary production (NPP) to Twin Cays specific measurements of leaf NPP (Tab. 5.1). Cheeseman & Lovelock estimated leaf area index and measured net maximal assimilation in dwarf and fringe *R. mangle* leaves in June and October 2002 (Cheeseman and Lovelock 2004). We roughly estimated leaf NPP in transition

(intermediate-height) mangrove habitats as the average of dwarf (short) and fringe (tall) mangrove data. We also made distinctions of where mats were found in the interior based on the classification of Rodriguez & Feller (2004): dwarf mangrove habitats included *R. mangle* stands ≤1.5 m tall in interior ponded areas, shallow open pond habitats contained sparse vegetation, and thick mat crusts were found alongside ponds on a deep flocculent layer. Although the coverage by microbial mats in these interior areas varied from a conservative estimate of 50% in dwarf mangrove and open pond habitats (Joye & Lee 2004) to 100% in pure microbial mat habitats, our measurements of NPP in dwarf mangrove habitats represented mats from all three interior zones because of proximity and our goal to assess the variability in the diversity of well-lit microbial mats. Mangrove leaf productivity was always an order of magnitude greater than that of microbial mats, and both fall in the median ranges of other mangrove and microphytobenthos observations (Cebrian 2002). Only <0.3% of the primary production was represented by microbial mats relative to the trees in transition and fringe mangrove zones. In dwarf mangrove habitats, microbial mats contributed ~5% of the mangrove production. When factoring in the other habitats related to the shallow interior ponds, the proportion increases to ~18-20%.

These values are similar to Ao Nam Bor, Thailand, benthic production rates which represented 4-20% of literature estimates for *R. apiculata* (Kristensen et al.1988). In contrast (though based on measurements of O_2 production), in Shurat Arwashie, Sinai, gross production by microalgae represented only 0.9% of *Avicennia marina* production (Dor & Levy 1984), while in Fly River Delta, Papua New Guinea *R. apiculata-Bruguiera parviflora*, *Nypa fruticans*, and *A. marina-Sonneratia lanceolata* forests, microalgal sediments were net heterotrophic (Alongi et al. 1993). But while mangrove trees fix relatively more carbon than microbial mats in mangroves, mangrove leaves contain difficult to degrade structural polymers such as lignins and soluble

phenolic tannins which can inhibit microbial degradation of leaf litter leachate and lignocellulose (Benner et al. 1986) or inhibit consumption of leaves by the mangrove crab *Neosarmatium smithi* (Neilsen et al. 1986). Mangrove leaves are also nutritionally poorer than mats with C:N ratios 8-9 times greater in senescent leaves (Feller et al. 2003) and 3-4 times greater in green leaves (Wooller et al. 2003). While benthic productivity in mangroves is highly variable, further investigation is clearly required for understanding its relative importance as a labile organic carbon source in mangroves. In addition to providing a carbon source for higher trophic levels (McIvor et al. in preparation), mats may also serve a number of other important functions in mangrove habitats, such as supplying a phosphorus source to trees by concentrating phosphorus (Joye et al. unpublished data), facilitating mangrove propagule settling and nutrition due to significant vertical accretion rates (McKee et al. unpublished data), and potentially affecting the net flux of CO_2 to the atmosphere.

N_2 fixation in Twin Cays dwarf, transition and fringe mangrove habitat surficial mats is a significant source of N to the system (Lee & Joye 2006, Ch. 4), and is confirmed by ^{15}N signatures indicative of N_2 fixation (i.e., close to 0‰ of atmospheric N_2; Goericke et al. 1994). Similar ^{15}N signatures were observed in deeper (20 cm) cores of Twin Cays mangrove peat and in leaves of Twin Cays *R. mangle* and *L. racemosa* indicating N_2 fixation as the primary source of N to the system (McKee et al. 2002, Wooller et al. 2003). Dwarf mangrove habitat and pond-associated mats support the highest inputs of N through N_2 fixation (Lee & Joye 2006, Ch. 4) and could supply 5-28% of the N required by mangrove leaves based on conversions of NPP by leaf C:N ratios (Wooller et al. 2003; Tab. 5.1). Benthic light availability limits transition and fringe mangrove habitat mat coverage, therefore N supplied by these mats consists of less than 2% of the mangrove leaf N requirement.

Across Twin Cays, benthic microbial mats fix 2.3% of the C relative to the *R. mangle* trees and supply 2.3% of the N required by the *R. mangle* trees. These proportions identify mats as important sources of fixed C and N to mangrove systems with differential importance across the different mangrove habitats. Of course these ratios may be considerably different when considering the net production of entire mangrove trees (i.e., including woody and below-ground biomass), thus further investigation is needed to understand the ecosystem contribution of benthic productivity in Twin Cays and other mangrove environments.

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Table 5.1. Rates of mangrove leaf and microbial mat net primary production (NPP) with respect to relative coverage in each habitat. [a]leaf area index (LAI) and maximum assimilation rates (A_{max}) from Cheeseman & Lovelock 2004; [b]transition mangrove leaf data estimated by averages of dwarf and fringe; [c]calculated using a maximum LAI of 1; [d]Wooller et al. 2003; [e]N required based on NPP; [f]N_2 fixation (NFIX) rates from Lee & Joye 2006, Ch. 4; see text for details.

	June					**October**				
	Mats	Open pond	Dwarf	Tran-sition	Fringe	Mats	Open pond	Dwarf	Tran-sition	Fringe
Leaf area index (LAI)[a]	0	0	0.7	1.5[b]	2.3	0	0	0.7	1.5[b]	2.3
A_{max} (µmol CO_2 m^{-2}_{leaf} s^{-1})[a]	0	0	7.3	7.0[b]	6.6	0	0	4.9	4.4[b]	3.9
Mangrove leaf NPP (g C m^{-2} d^{-1})	0	0	2.7	3.6[c]	3.4[c]	0	0	1.8	2.3[c]	2.0[c]
Mangrove leaf C:N (g g^{-1})[d]	-	-	42.4	47.8	51.3	-	-	42.4	47.8	51.3
Mangrove leaf N_{req} (mg N m^{-2} d^{-1})[e]	-	-	62.6	75.4	66.7	-	-	42.0	47.8	39.4
Mangrove coverage	0%	0%	100%	100%	100%	0%	0%	100%	100%	100%
Microbial mat NPP (g C m^{-2} d^{-1})	0.24	0.24	0.24	0.10	0.07	0.18	0.18	0.18	0.06	0.04
Microbial mat NFIX (mg N m^{-2} d^{-1})[f]	1.58	1.58	1.58	0.18	5.22	5.96	5.96	5.96	8.30	5.58
Microbial mat coverage	100%	50%	50%	10%	10%	100%	50%	50%	10%	10%
Coverage corrected:										
Mangrove leaf NPP (g C m^{-2} d^{-1})	0	0	2.7	3.6	3.4	0	0	1.8	2.3	2.0
Microbial mat NPP (g C m^{-2} d^{-1})	0.24	0.12	0.12	0.01	0.01	0.18	0.09	0.09	0.01	0.00
Microbial mat : mangrove leaf NPP (Mats, open pond & dwarf)	-	-	4.47% (17.88%)	0.28%	0.20%	-	-	4.92% (19.67%)	0.27%	0.18%
Mangrove leaf N_{req} (mg N m^{-2} d^{-1})	0	0	62.6	75.4	66.7	0	0	42.0	47.8	39.4
Microbial mat NFIX (mg N m^{-2} d^{-1})	1.58	0.79	0.79	0.02	0.52	5.96	2.98	2.98	0.83	0.56
Microbial mat : mangrove leaf N (Mats, open pond & dwarf)	-	-	1.26% (5.06%)	0.02%	0.78%	-	-	7.09% (28.37%)	1.74%	1.42%

FIGURE CAPTIONS

Figure 5.1. Stable isotopic carbon and nitrogen composition of dwarf (D), transition (T) and fringe (F) mangrove habitat mats from August, November, June and October. Mat only samples are indicated by striped bars, peat only samples by filled bars, and bulk surficial mats by open bars. "MatA" represents upper mat only layers while "matB" represents deeper mat only layers. Error bars = standard deviations.

Figure 5.2. Carbon and nitrogen content and C:N ratios of dwarf (D), transition (T) and fringe (F) mangrove habitat mats from August, November, June and October. Mat only samples are indicated by striped bars, peat only samples by filled bars, and bulk surficial mats by open bars. "MatA" represents upper mat only layers while "matB" represents deeper mat only layers. Error bars = standard deviations.

Figure 5.3. Full sun carbon fixation rates in dwarf (D), transition (T) and fringe (F) mangrove habitat mats from June and October. Anoxygenic PS, oxygenic PS and chemoautotrophy are represented in each column. Error bars = standard deviations of total full sun carbon fixation rates.

Figure 5.4. Full sun versus 10% sun net PS carbon fixation rates in mats from all habitats in June 2001. Error bars = standard deviations.

Figure 5.5. Integrated rates of carbon fixation over 12 h nighttimes, 12 h daytimes and full 24 h days in dwarf (D), transition (T) and fringe (F) mangrove habitat mats from June and October.

Figure 5.1

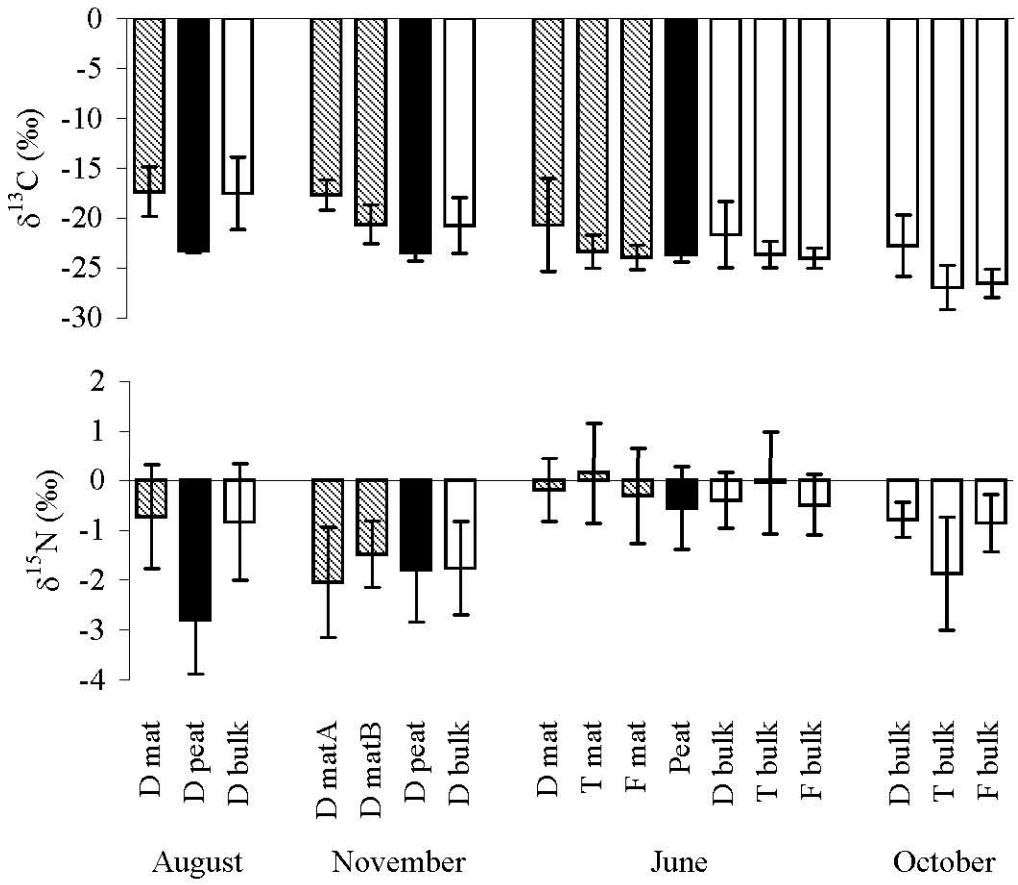

0
-5
-10
-15
-20
-25
-30
δ13C (‰)
2
1
0
-1
-2
-3
-4
δ15N (‰)
D mat
D peat
D bulk
D matA
D matB
D peat
D bulk
D mat
T mat
F mat
Peat
D bulk
T bulk
F bulk
D bulk
T bulk
F bulk
August
November
June
October

Figure 5.2

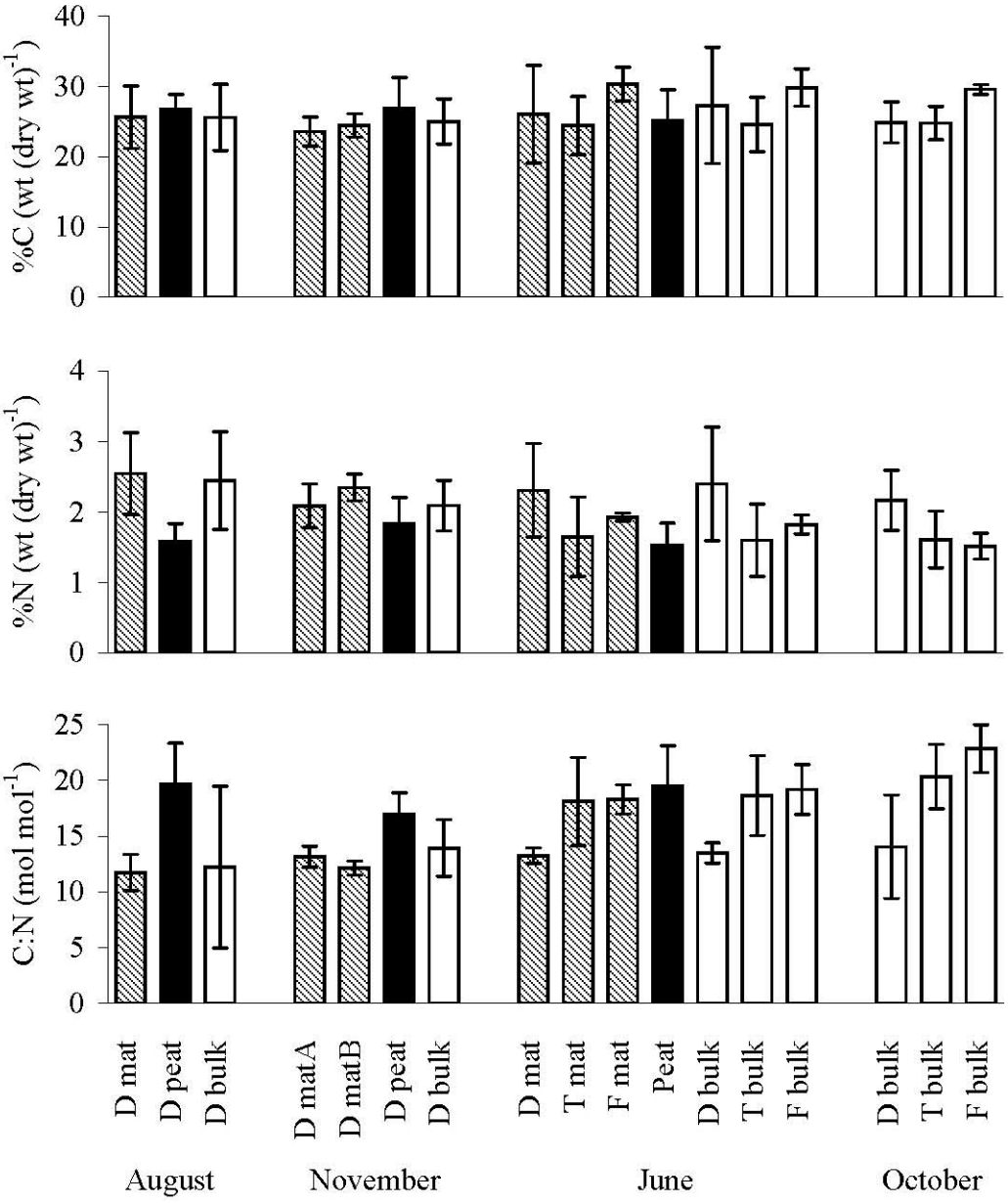

%C (wt (dry wt)-1)
40
30
20
10
0
%N (wt (dry wt)-1)
4
3
2
1
0
C:N (mol mol-1)
25
20
15
10
5
0
D mat
D peat
D bulk
D matA
D matB
D peat
D bulk
D mat
T mat
F mat
Peat
D bulk
T bulk
F bulk
D bulk
T bulk
F bulk
August
November
June
October

Figure 5.3

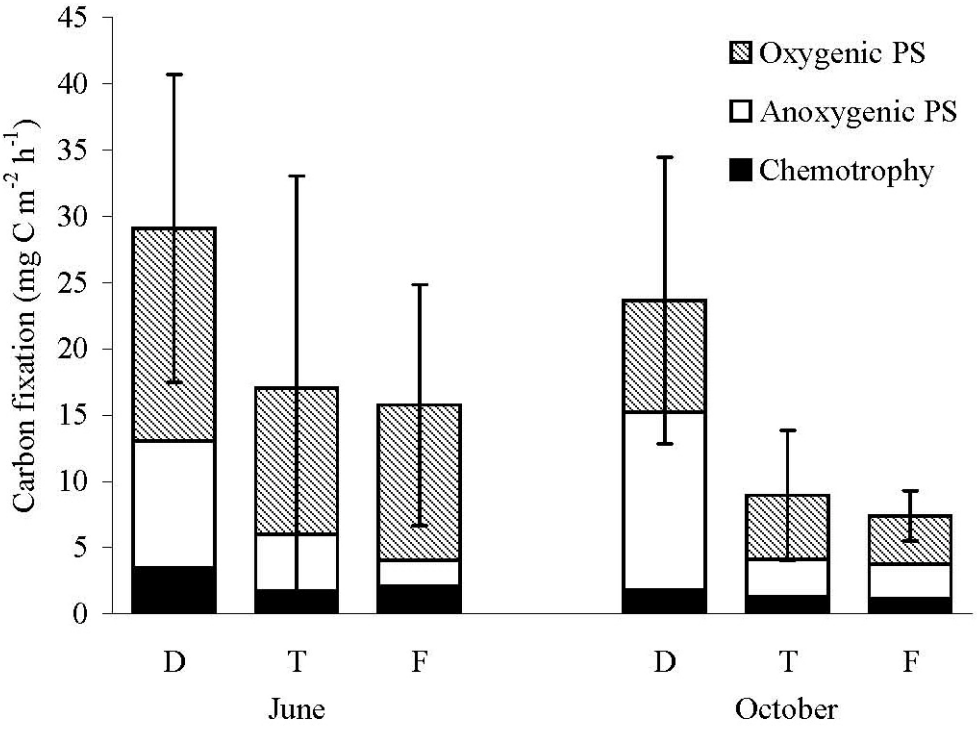

45
40
35
30
25
20
15
10
5
0
Carbon fixation (mg C m-2 h-1)
Oxygenic PS
Anoxygenic PS
Chemotrophy
D
T
F
D
T
F
June
October

Figure 5.4

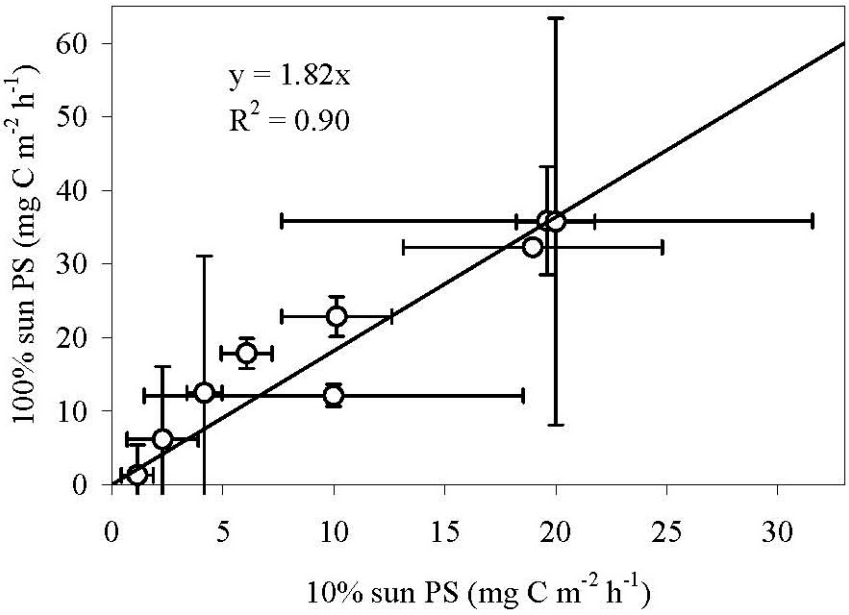
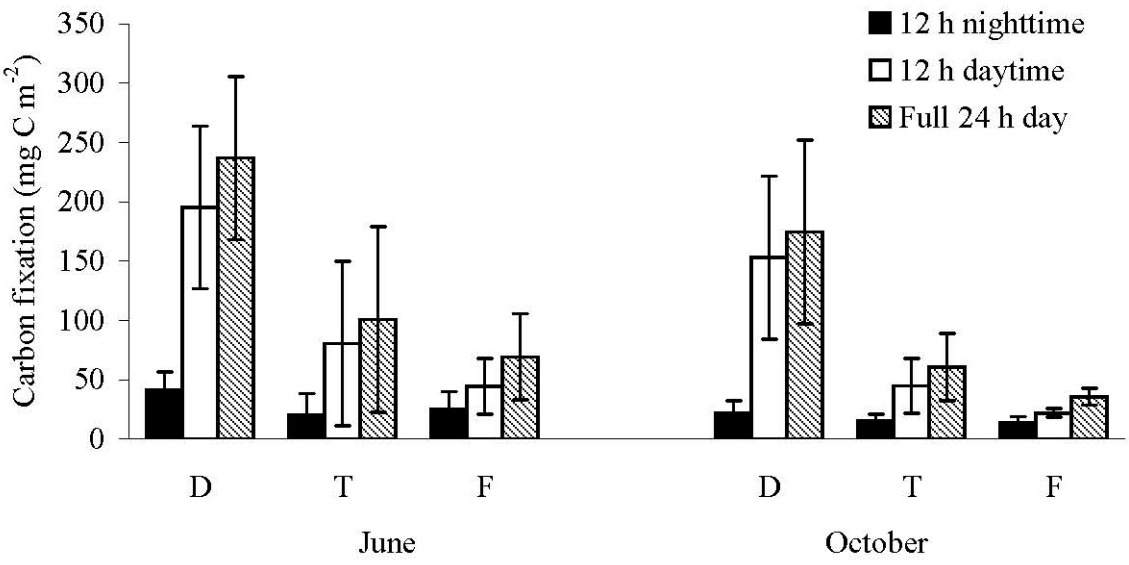

y = 1.82x
R2 = 0.90
100% sun PS (mg C m-2 h-1)
10% sun PS (mg C m-2 h-1)
0
10
20
30
40
50
60
0
5
10
15
20
25
30

Figure 5.5


Carbon fixation (mg C m-2)
350
300
250
200
150
100
50
0
12 h nighttime
12 h daytime
Full 24 h day
D
T
F
D
T
F
June
October

CHAPTER 6

CONCLUSIONS

The research presented here expands the knowledge base on biogeochemistry (**Chapter 2 & 4**) and primary production (**Chapter 3 & 5**) in benthic environments in general and specifically in mangrove systems which have been poorly documented. Benthic environments, from soils to estuarine and off-shore sediments, are characterized by various modes of benthic metabolism (**Chapter 2**). In the predominantly anoxic dwarf mangrove soils of Twin Cays, Belize, sulfate reduction dominated organic matter remineralization as observed in many other estuarine and saline environments, while concentrations of the products of other major terminal metabolic processes, including metal reduction, denitrification and methanogenesis, were low. Seasonality in mangrove litter input and hydrological regimes appeared to drive variations in nutrient regeneration.

While nutrient regeneration coupled to organic matter mineralization supplied a significant source of ammonium to mangrove sediments at depth, surficial microbial mat communities were active in the import and export of N from the system (**Chapter 4**). The adaptation of Twin Cays microbial mat communities to redox and nutrient conditions in each habitat influences their role as either a source or sink of N in the system. Nitrifying bacteria associated with fringe mangrove prop root sponges likely supply a source of nitrate to fringe and transition mangrove habitat mats which demonstrated a significantly greater denitrification capacity than dwarf mangrove habitat mats. N_2 fixation dominated dwarf mangrove habitat mats due to both the lack of available N as nitrate from fringe mangrove prop root sponges and the community composition more tolerant to the environmental stresses of exposed dwarf mangrove habitats. Integrated denitrification rates across all sites were much lower than those of N_2

fixation, clearly showing that benthic processes serve as an important net source of N to the oligotrophic Twin Cays mangrove ecosystem and may help explain nutrient limitation patterns of mangrove trees in each habitat.

Twin Cays benthic light availability, light fluctuations, environmental stresses and nutrient availability influenced benthic photosynthetic biomass and community composition, and therefore rates of photosynthetic activity across dwarf, transition and fringe mangrove habitats (**Chapter 3**). A number of factors can influence the balance between autotrophy and heterotrophy in mangrove soils. Mangrove sediments and soils in environments with high light, such as Twin Cays dwarf mangrove habitats, exhibit net autotrophy (in oxygen terms). Light limited soils, such as in Twin Cays transition and fringe mangrove forests, exhibit net heterotrophy (in oxygen terms). However, it appears that anthropogenic nutrient inputs (e.g., shrimp farming) can shift these net heterotrophic mangrove soils towards net autotrophy. Furthermore, net autotrophy was evident in all sediments when anoxygenic photosynthesis and chemoautotrophy (in carbon terms) were included (**Chapter 5**).

Although benthic microbial mats only fix up to 5% of the C compared to that of *R. mangle* forests of Twin Cays and supply up to 7% of the N demand, these proportions are significant and identify mats as important sources of fixed C and N to mangrove systems. Efforts aimed at conservation and restoration of mangrove forests should consider microbial processes such as these investigated in Twin Cays cyanobacterial mats and soils, as these processes may influence the productivity and potential recovery of mangrove habitats.