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Seasonal patterns of nitrogen fixation and denitrification in oceanic mangrove habitats

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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.

KEY WORDS: Microbial mat · Cyanobacteria · Mangrove · Nitrogen fixation · Denitrification · Desiccation

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 (R. Y. Lee & S. B. Joye unpubl.).

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

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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).

MATERIALS AND 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. 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 ex-

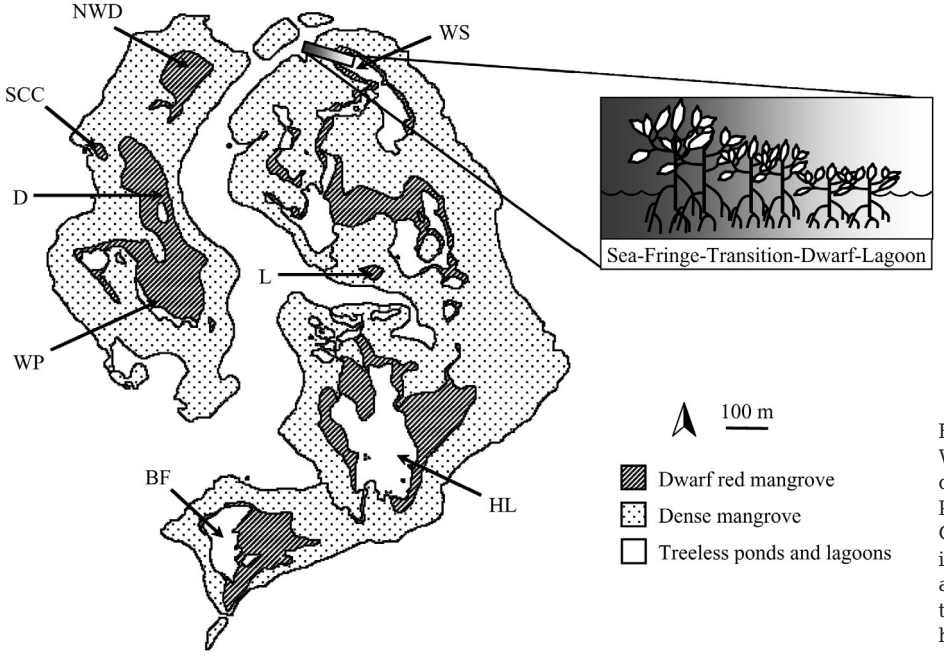

Fig. 1. Twin Cays, Belize. WS: Weather Station, L: Lair, HL: Hidden Lake, BF: Boa Flats, WP: West Pond, D: Dock, SCC: South of Clear Cut, NWD: North West Dock. Inset illustrates the tree-height gradient across a transect from the fringe through the transition to the dwarf habitat. Adapted from Feller (1996)

hibit 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. 1). We conducted 6 field expeditions in November 2000, June and October 2001, March and September 2002, and May 2003 to examine spatio-temporal 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; available at: http://web8.si.edu/belize). 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 LI-COR 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 & Joye unpubl.). 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 short-term (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}) (Table 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

Table 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 and 15:00 h; 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)

soil porewater salinities were similar to that of overlying ocean waters (Table 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. 2). Within sites, N_2 fixation rates varied as a

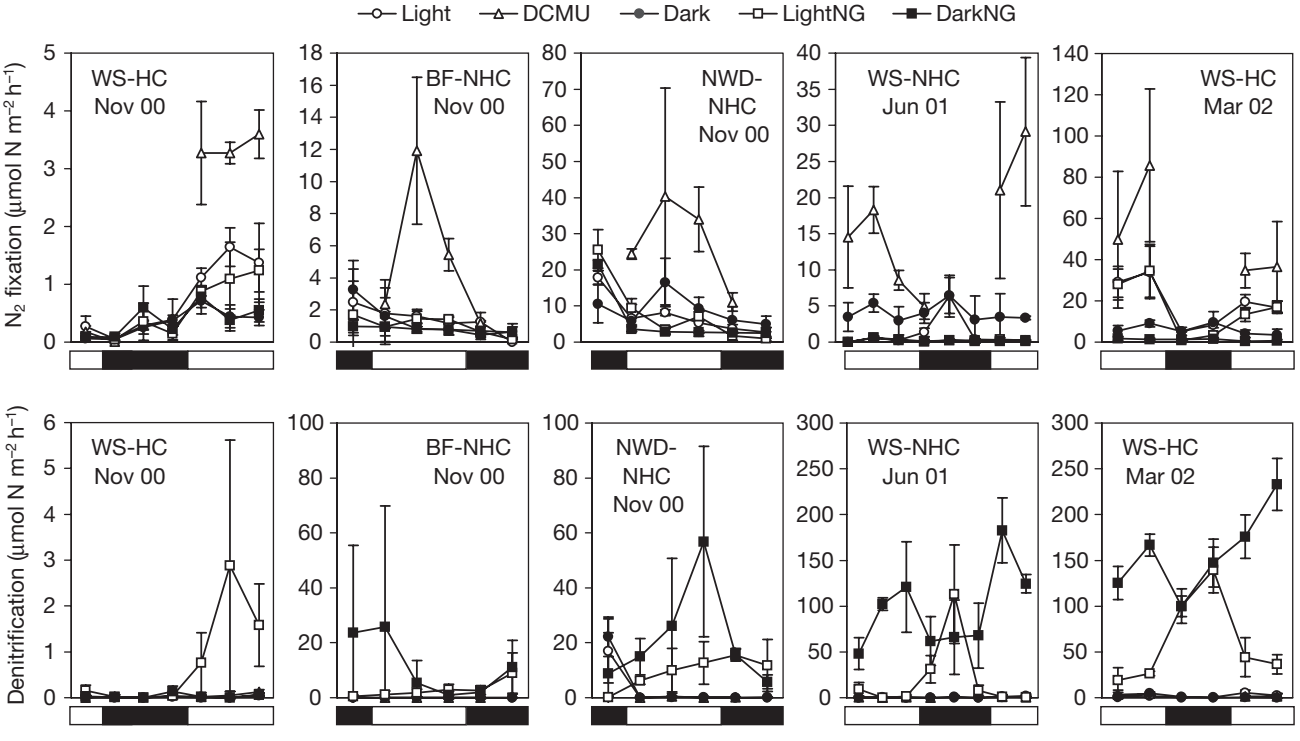

Fig. 2. N_2 fixation and denitrification rates from dwarf habitat diel experiments with and without 3-(3,4 dichlorophenyl)-1,1 dimethylurea (DCMU) or nitrate (N) and glucose (G) amendments. Error bars are standard deviations; HC: heterocystous cyanobacterial mat; NHC: non-heterocystous cyanobacterial mat; horizontal axis bars indicate daytime (□) and nighttime (■). WS: Weather Station; BF: Boa Flats; NWD: North West Dock

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. 2).

Island-wide N_2 fixation and denitrification rates exhibited minor variation across season, but differences were observed between mangrove habitats (Fig. 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

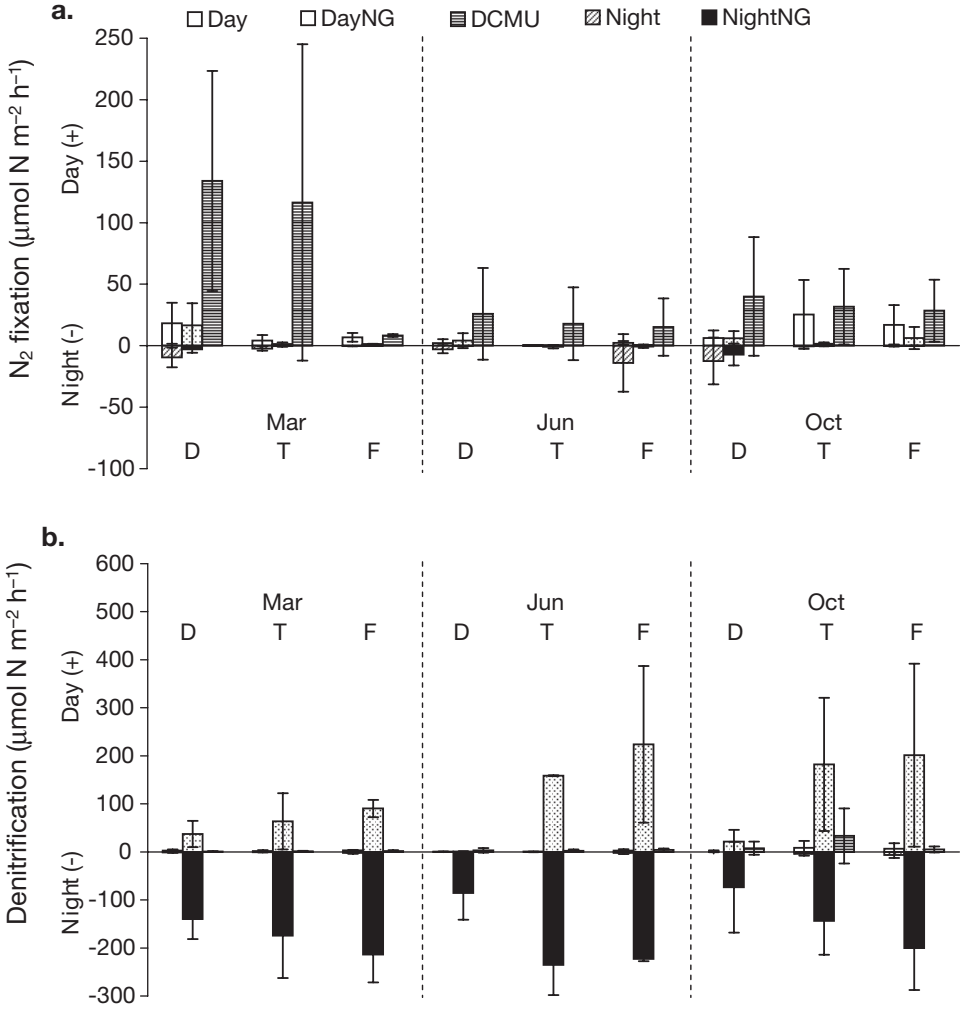

Fig. 3. Seasonal day and night (a) N_2 fixation rates and (b) denitrification rates with and without 3-(3,4 dichlorophenyl)-1,1 dimethylurea (DCMU) or nitrate (N) and glucose (G) amendments in dwarf (D), transition (T), and fringe (F) habitats. Error bars are standard deviations

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), 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).

Short-term (hours-long) nutrient amendments had both negative and positive effects on rates of N cycling (Tables 2 & 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 (Tables 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. 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

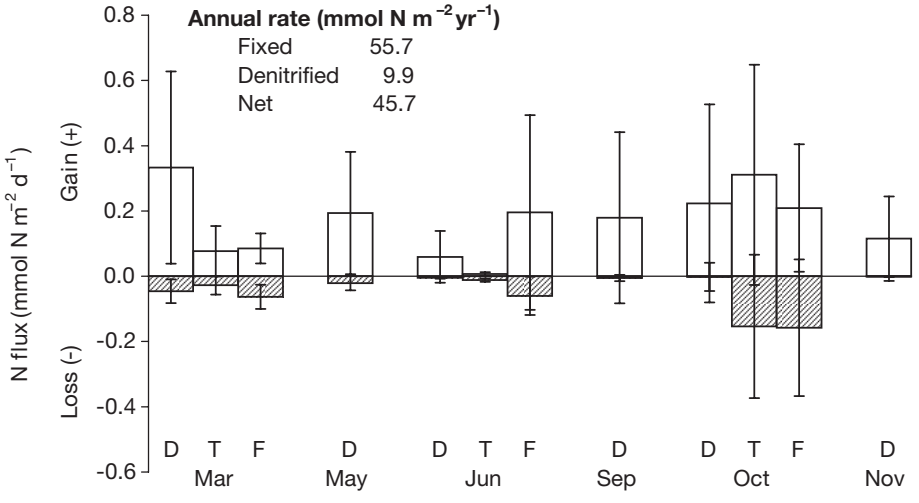

Fig. 4. Seasonal N cycling rates in dwarf (D), transition (T), and fringe (F) habitats. Error bars are standard deviations; annual rates determined by trapezoidal integration

Table 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: non-heterocystous 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
$AcetateNi_1$	1.8*	4.7*	2.0*	0.0*	1.5	0.2*
$LactateNi_1$	5.6	3.7	6.2	0.0*	1.7	0.0

Table 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: non-heterocystous 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*
$AcetateNi_1$	12.9	72.6*	107.8*	223.0*	100.5	170.8*
$LactateNi_1$	22.2*	92.2*	145.4*	142.4*	159.4*	273.4*

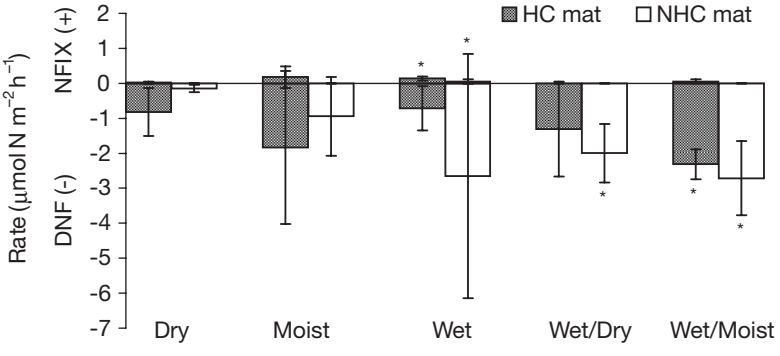

Fig. 5. Hourly hydration effects on N_2 fixation (NFIX) and denitrification (DNF) rates (µmol N m^{-2} h^{-1}) in a desiccated heterocystous cyanobacterial (HC) dwarf mat and a dessicated non-heterocystous cyanobacterial (NHC) dwarf mat. Daytime incubations included dry: control, i.e. no overlying water (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 $p < 0.1$

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. 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

Table 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			May 03 BF-NHC		May 03 WS-HC	
	Light	Dark	Light	Dark	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	Control	1.0	1.6	2.6	0.5
$Am_{0.05}Ni_{0.1}$	0.2	0.5	7.1	3.8	3.8	0.1*	3.4	2.3	39.5	17.0	$Am_{0.1}$	4.0	3.1	1.9	0.4
P	1.4*	2.1	11.8	23.8	8.4	18.5	3.7	4.7	48.1	11.8	$Ni_{0.1}$	2.3*	1.5	1.7	0.4
$PAm_{0.05}Ni_{0.1}$	0.5*	0.8	8.5	5.4	2.3*	0.2*	2.7	4.7	54.7	7.6	$Am_{0.5}$	1.9	1.5	0.8*	0.3
G_1	2.4	18.5*	23.0	112.1*	11.3	38.5*	4.0	10.9	58.0	25.9	$Ni_{0.5}$	1.3	1.3	0.7*	0.3
$G_1Ni_{0.5}$	0.3	1.2	4.3*	3.2	4.5	0.8*	1.2*	0.9*	43.4	1.7*	Am_1	18.5	1.4	0.4*	0.1*
Acetate	–	–	–	–	–	–	–	–	57.9	13.9	Ni_1	1.9*	1.5	0.5*	0.1*
Lactate	–	–	–	–	–	–	–	–	52.7	22.7	P	22.6	1.9	1.0*	0.3
Iron	0.3	4.7	7.6	18.5	9.2	13.2	2.5	3.8	69.8	14.7	$PAm_{0.1}Ni_{0.1}$	7.9	1.8	1.4*	0.3
Trace metals	–	–	–	–	–	–	–	–	64.8	6.5	$G_{0.5}$	14.9	2.5	0.5*	0.2
Vitamins	–	–	–	–	–	–	–	–	64.7	15.6	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 5. Rates of denitrification (μmol N m^{-2} h^{-1}) in nutrient bioassay experiments. See Table 4 for details

	Nov 00 WS-HC		Nov 00 BF-NHC		Nov 00 NWD-NHC		Jun 01 WS-NHC		Mar 02 WS-NHC			May 03 BF-NHC		May 03 WS-HC	
	Light	Dark	Light	Dark	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	Control	0.1	0.0	0.4	0.0
$Am_{0.05}Ni_{0.1}$	0.4*	2.6*	3.6*	9.6*	19.6*	106.0*	0.1	0.4	0.2*	1.0	$Am_{0.1}$	0.1	0.0	0.5	0.0
P	0.0*	0.0	0.2	0.0	0.1	1.3	0.0	0.0*	0.2*	1.2	$Ni_{0.1}$	0.0*	0.0	0.1	0.0*
$PAm_{0.05}Ni_{0.1}$	0.1	3.3	4.5	6.2	16.6	83.1	0.2	0.1	1.3	2.7	$Am_{0.5}$	0.0*	0.0	0.0	0.0
G_1	0.0	0.0	0.1	0.0	0.0	0.2	0.0	0.2	2.3	1.2	$Ni_{0.5}$	0.0*	0.0	0.0	0.0*
$G_1Ni_{0.5}$	1.4*	2.6	5.3	91.2*	23.2*	93.2*	8.0*	37.5	3.0	4.4*	Am_1	0.5	0.0	0.0	0.3
Acetate	–	–	–	–	–	–	–	–	1.3	1.2	Ni_1	4.2	0.0	5.8	34.1
Lactate	–	–	–	–	–	–	–	–	1.8	0.6	P	0.0	0.0	0.0	0.2
Iron	0.0	0.1	0.0	0.0	0.1	0.9	0.0	0.1	1.7	0.9	$PAm_{0.1}Ni_{0.1}$	0.0	0.0	0.0	0.0*
Trace metals	–	–	–	–	–	–	–	–	1.1	0.9	$G_{0.5}$	0.0	0.0	0.0	0.0*
Vitamins	–	–	–	–	–	–	–	–	2.2	0.9	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*

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 demon-

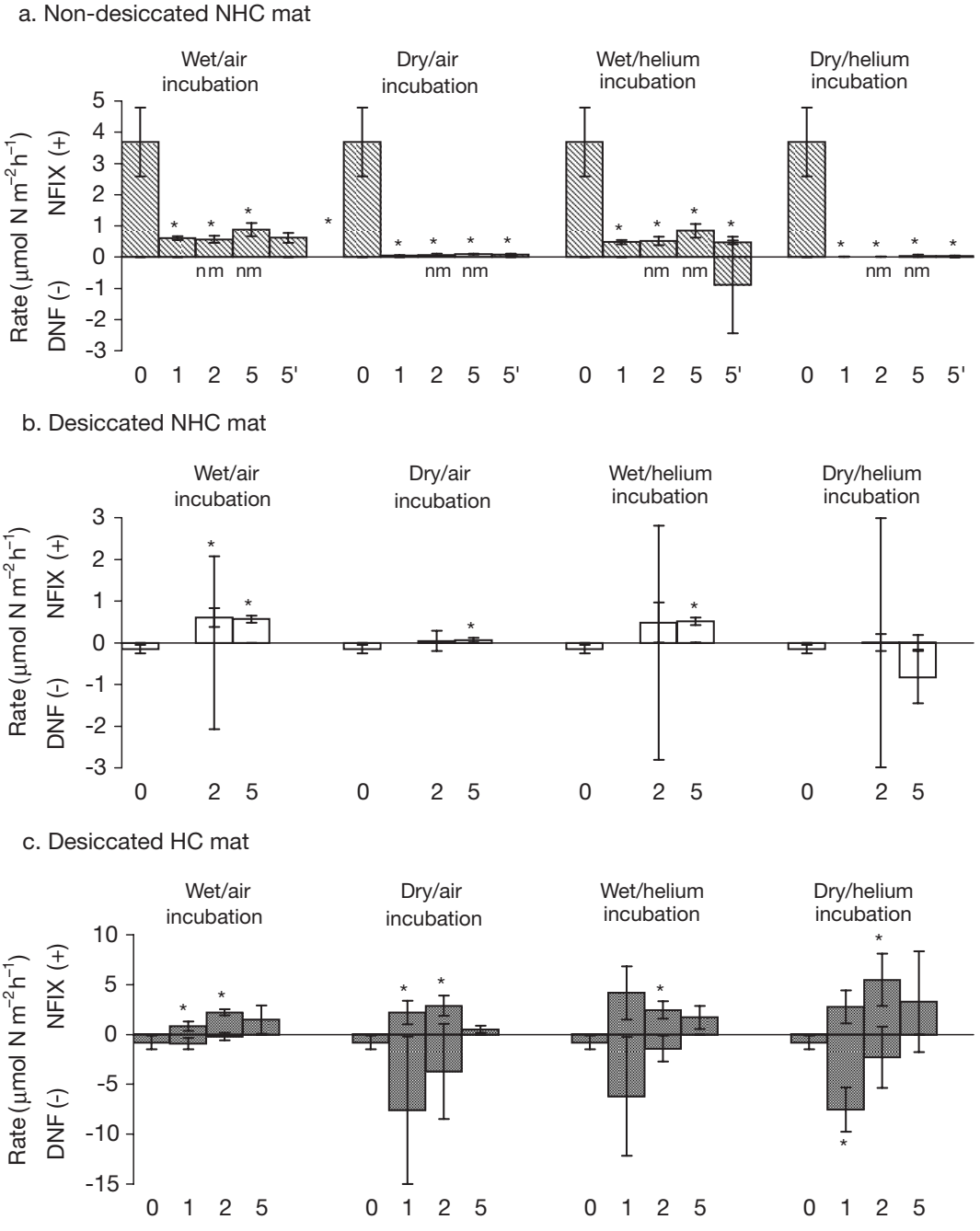

Fig. 6. Longer-term (1 to 5 d) rehydration and/or desiccation effects on N_2 fixation (NFIX) and denitrification (DNF) rates (µmol N m^{-2} h^{-1}) in (a) a non-desiccated non-heterocystous cyanobacterial (NHC) dwarf mat, (b) a desiccated NHC dwarf mat, and (c) a desiccated heterocystous cyanobacterial (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

strated 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 CO_2 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 (Table 6). Denitrification rates in all

Table 6. Summary of mangrove N_2 fixation (NFIX) and denitrification (DNF) rates (mmol $N^{-2}d^{-1}$). R: *Rhizophora*; A: *Avicennia;* L: *Laguncularia*; C: *Ceriops*; B: *Bruguiera* spp. ^{3}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

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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