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BENTHIC MICROBIAL MATS: IMPORTANT SOURCES OF FIXED NITROGEN AND CARBON TO THE TWIN CAYS, BELIZE ECOSYSTEM

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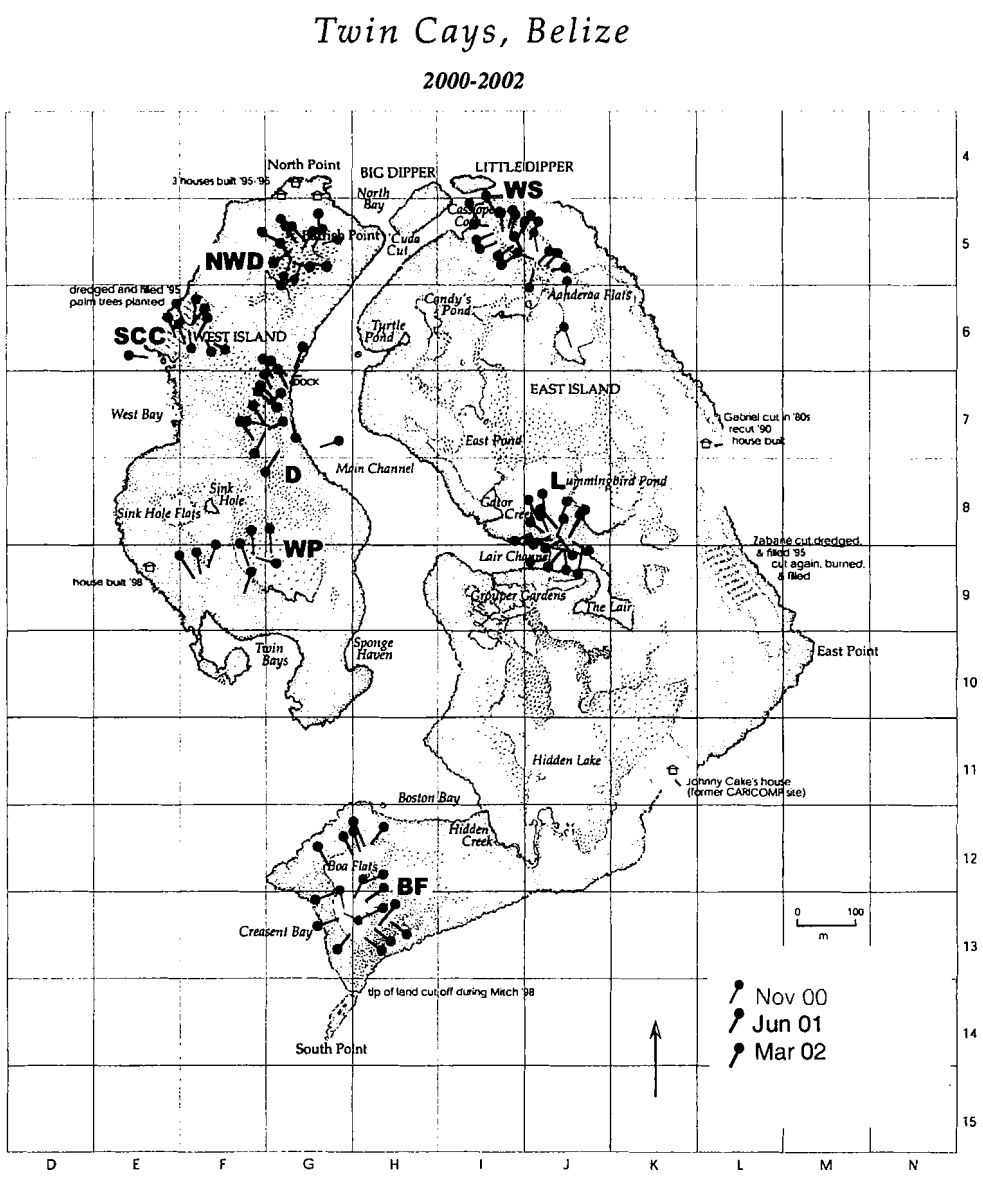

Figure 1. Site map of Twin Cays, Belize, noting the sampling locations and dates. Site abbreviations are: BF=Boa Flats; D=Dock; L=Lair; NWD=Northwest Dock; SCC=South Clear Cut; WP=West Pond; and, WS=Weather Station. See text for details.

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ABSTRACT

We collected surface microbial mats at sites on Twin Cays, Belize to determine rates of primary production and nitrogen transformations. A diverse array of cyanobacteria including filamentous, coccoidal and heterocystous cyanobacteria, as well as purple sulfur bacteria and heterotrophic bacteria, were important components of microbial mat communities. Sediment chlorophyll *a* concentrations illustrated a high photosynthetic biomass in surface sediments. Rates of primary carbon fixation, measured as gross oxygenic photosynthesis, and nitrogen fixation and denitrification, measured using specific metabolic inhibitors, were determined during day-and-night incubations. Primary production rates were similarly high across different mat types. Nitrogen fixation rates were substantial under *in situ* conditions and nighttime activity frequently exceeded daytime activity. *In situ* denitrification rates were very low in all incubations. In the presence of added nitrate, however, denitrification rates increased significantly during daytime and nighttime incubations so that they equaled or exceeded nitrogen fixation rates. Collectively, our data show that microbial mats are a significant source of fixed carbon and nitrogen to the Twin Cays ecosystem and suggest that mats may serve as an important component of the ecosystem's food web.

INTRODUCTION

Microbial mats are light-driven, laminated microbial ecosystems that are modern descendants of ancient microbial communities responsible for the initial oxygenation of the earth system (des Marias, 2003; Krumbein et al., 2003). Today, microbial mats may play a similarly important role in a variety of environments (Golubic, 1994) ranging from polar deserts (de los Rios et al., 2004) to hypersaline environments (Jørgensen et al., 1983; Campbell and Golubic, 1985; Paerl et al., 2003) to coral reefs (Wiebe et al., 1975), mangroves (Karsten et al., 1998) and intertidal marshes and mud flats (Bebout et al., 1987 and 1983; Skyring and Bauld, 1990; Joye and Paerl, 1994). Microbial mats are comprised of a diverse suite of autotrophic and heterotrophic microorganisms that carry out rapid rates of elemental cycling (Paerl et al., 2000). Despite incredible functional and phylogenetic diversity at the population level, microbial mats are highly structured communities with organization apparent at micrometer and centimeter scales through geochemical profiles (e.g., changes in oxygen or sulfide concentration over depth),

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differing colors (e.g., reflecting dominant pigments of the microbes in each layer) or varying textures (e.g., from the binding, trapping, or accumulation of sediments or organic byproducts such as empty sheaths).

Microbial mats are often referred to as "self-sustaining" ecosystems (Des Marais, 2003) where primary production (oxygenic and anoxygenic photosynthesis and, to a lesser extent, chemosynthesis) and organic matter recycling (fermentation and terminal metabolism) processes are tightly coupled. Even though mats are characterized by tight internal coupling, export of microbial mat-derived material to the larger ecosystem has been demonstrated in many settings. For example, mats support secondary production of associated benthic invertebrates (Pulich and Scalan, 1987; Bouillon et al., 2004), which then serve as food items for fish and birds and mats may provide fixed nitrogen to associated higher plants (Bashan et al., 1998).

We determined rates of primary production (i.e., oxygenic photosynthesis) and nitrogen cycling (i.e., nitrogen fixation and denitrification) in microbial mats collected from several sites on Twin Cays, Belize. We determined rates during both daytime and nighttime incubations to evaluate the impact of oxygen production via photosynthesis on nitrogen cycling. We also identified the dominant cyanobacteria in microbial mat samples using microscopic techniques to help explain the observed patterns of nitrogen cycling.

PREVIOUS WORK

Among the bacteria, many cyanobacteria are unique in their ability both to use dinitrogen (N_2) as a primary nitrogen (N) source through N_2 fixation and to evolve oxygen (O_2) via oxygenic photosynthesis, making them important in carbon (C), oxygen (O) and nitrogen (N) cycles. Cyanobacteria are some of the most conspicuous components of microbial mats. Diverse types of cyanobacteria inhabit microbial mats, including filamentous nonheterocystous (e.g. *Microcoleous spp.* and *Lyngbya spp.)*, coccoidal (e.g., *Chroococcus spp.* and *Aphanocapsa spp.)* and heterocystous (e.g., *Calothrix spp.* and *Nodularia spp.)* forms. In addition to playing important roles in C, N and O cycling, dense accumulation of filaments and excreted mucous help bind and stabilize sediments (Golubic et al., 2000), promoting establishment and eventual expansion of the mat.

Microbial mat communities are based ultimately on light availability because oxygenic and anoxygenic photosynthetic autotrophs produce the organic carbon that is later consumed by fermenting and heterotrophic bacteria. Oxygenic photosynthesis accounts for a large fraction (about 90% on average) of primary production in microbial mats and a large fraction of this activity is attributable to cyanobacteria (Kuhl et al., 2003). Nitrogen-fixing bacteria in mats provide the mat microbial community with an internal source of fixed N via N_2 fixation (Stal et al., 1984; Stal and Krumbein, 1985a; Stal and Heyer, 1987). The ability to provide primary sources of both fixed C and fixed N makes mats particularly important components of oligotrophic ecosystems.

One of the most interesting features of microbial mats is the simultaneous occurrence of processes that are metabolically incompatible, such as oxygenic

photosynthesis and anaerobic processes like N_2 fixation or sulfate reduction (Cohen and Rosenberg, 1989; Canfield and des Marias, 1991; Paerl et al., 1989 and 1994). The coexistence of aerobic and anaerobic processes in microbial mats results from tight coupling between production and consumption processes, redox heterogeneity and microzonation, and possibly novel metabolic adaptations (Paerl et al., 2000). Understanding how N cycling processes are regulated by O_2 concentrations in microbial mats is of particular interest because fixed N availability often limits primary production in marine environments (Howarth, 1991). In fact, N_2 fixation can be an extremely important N source in N-limited marine environments (Howarth et al., 1988a and 1988b).

Oxygen is a potent inhibitor of nitrogenase, the enzyme responsible for N_2 fixation (Bautista et al., 1985; Paerl et al., 1989; Fay, 1992; Stal, 1995). Maintaining high rates of primary production requires sufficient N supplies and N_2 fixation can alleviate N limitation. Consortial interactions between different microbes, such as tight coupling between O_2 production and consumption processes to maintain *in situ* O_2 concentrations at optimal (low) levels, may help optimize conditions for N_2 fixation in microbial mats (Steppe et al., 1996; Paerl et al., 2000). Alternatively, oxygenic photosynthesis and N_2 fixation may be separated temporally, e.g., high rates of oxygenic photosynthesis may occur during the day while N_2 fixation occurs at night when O_2 concentrations are lower (Bautista et al., 1985; Stal and Krumbein, 1987). Another possibility is that N_2 fixing microbes may spatially separate nitrogenase from potential O_2 toxicity by either isolating the enzyme within O_2-resistant heterocysts (Yates, 1977; Haselkorn, 1978) or by partitioning the cellular enzymatic machinery used in carbon fixation (O_2 evolution) from that of N_2 fixation (e.g., *Lyngbya aestuarii*, Paerl et al., 1991).

Oxygen concentration also influences other mat biogeochemical processes that are O_2-sensitive, such as denitrification, sulfate reduction and methanogenesis. Denitrification, or the reduction of nitrate to N_2, is mediated by denitrifying bacteria. Denitrifiers are facultative anaerobic heterotrophs, meaning that these microbes couple organic carbon oxidation to the respiration of either O_2 (when O_2 concentrations exceed about 20 μM) or nitrate (Joye, 2002). Denitrification is typically controlled by the availability of organic carbon, O_2 and nitrate (Joye, 2002). In many systems, nitrate availability limits denitrification rates but few studies have examined denitrification in microbial mats (Joye and Paerl, 1993 and 1994; Lee and Joye, in preparation).

MATERIALS AND METHODS

Study Site

This work was conducted at Twin Cays, Belize, a pair of oceanic mangrove islands that lie approximately 12 miles offshore (Feller et al., 2002). The islands are peat-based and receive no terrestrial (i.e., river) inputs of freshwater or sediment. The islands support distinct habitats, including mangrove forests, tidal creeks, and shallow ponds. Macroscopic forest vegetation is dominated by the red mangrove (*Rhizophora mangle*) but black (*Avicennia germinans*) and white (*Laguncularia racemosa*) mangroves are also present. Generally speaking, the mangrove forests can be divided into three zones based

on the height of the trees in that zone: fringe, transition and dwarf (Feller et al., 2002). The fringe zone, characterized by tall (about 5 m) trees, lies at the interface between the ocean or tidal creek and the edge of the mangrove forest. The transition zone, characterized by shorter trees (between 2-4 m), lies between the fringe and dwarf zones. The dwarf zone, characterized by short (about 1 to 1.5 m) trees, occupies a large fraction (about 37%, Rodriguez and Feller, this volume) of the island area. Surficial microbial mats are found throughout the different ecotones on Twin Cays but laminated microbial mats occurred mainly in the dwarf zone. The work described here was carried out in dwarf mangrove forests, which are ubiquitous in the interior of the islands.

Microbial mats were collected from seven dwarf mangrove sites, three on the east island (**BF**=Boa Flats, **L**=Lair and **WS**=Weather Station) and four from the west island (**D**=Dock, **NWD**=Northwest Dock, **SCC**=South Clear Cut and **WP**=West Pond) (Fig. 1). Two distinct areas were sampled at the WP site: one along the pond edge and one within the dwarf zone. The BF, WS and WP sites supported well-laminated microbial mats that grew atop thick (10's of cm) accumulations of organic debris (including old mat layers) along the edge of shallow ponds (Fig. 2a). The D and L sites supported localized accumulations of thin microbial mats that grew on top of a thin (<cm) organic layer that overlaid peat (Fig. 2a). At the NWD and WP dwarf sites, (noted WP-tab on Figs. 5 and 6), thin (<1 cm) laminated mats were found growing directly on top of peat. At the SCC site, thick (> cm) accumulations of mat grew on top of carbonate marl that was laid down when the region was clear cut.

Microscopy and Geochemical Characterization

The major cyanobacteria present in microbial mats were identified using epifluorescent microscopy. Individual layers of the microbial mats, delineated by changes in color, were carefully sampled using a sterile scalpel or dissecting pin. The sample was transferred to a microscope slide and examined under oil immersion. Multiple slides were examined for each depth horizon. Samples for chlorophyll *a* determination were collected in the field (n=3 to 5 for each mat type) and from samples used for microelectrode profiling (n=3 per core) by subcoring the mat with a cut-off 5 cc syringe. After collecting the subcore, excess organic material and/or peat were removed from the bottom of the core with a scalpel so that the sample contained only viable microbial mat. Each sample was transferred to a 15-ml centrifuge tube and 1-to-2 drops of a saturated magnesium carbonate solution were added to the mat to stabilize chlorophyll. Samples were stored frozen prior to analysis back at the University of Georgia laboratory. Chlorophyll *a* was determined by sonicating samples in a mixture of acetone, methanol, and deionized water at a ratio 45:45:10 to burst the cells. Next, pigments were extracted for 12-to-24 hours at 0°C. Following extraction, samples were centrifuged and chlorophyll concentrations were determined using a spectrophotometer (Lee and Joye, in preparation).

Samples for quantification of dissolved nutrient (dissolved inorganic nitrogen (DIN) = nitrate (NO_3^-) + ammonium (NH_4^+) and dissolved inorganic phosphate (DIP) = orthophosphate (PO_4^{3-})), concentrations and environmental data (hydrogen sulfide (H_2S) concentration, salinity and pH) were collected in the field from a depth of 5-to-10 cm

below the mat using a PVC piezometer and a hand-actuated vacuum pump. Samples were filtered through a 0.2 μm filter. Salinity was determined using a hand-held refractometer. pH was measured using a field electrometer calibrated with certified (National Bureau of Standards) buffers at pH 4 and 7. Other samples were either stored at 4°C (nutrients) or preserved (H_2S samples preserved by fixation with 500 μL of 20%-weight-per-volume zinc acetate solution) and then stored at 4°C. Nutrient and H_2S analyses were conducted using standard methods (Joye et al., 1996; Lee and Joye, in preparation).

Mats for use in experiments to determine N-cycling rates were collected the day before the experiment by cutting a 400-cm^2 section with a scalpel and sliding a flexible plastic sheet beneath the mat. Several (~3 to 4) sections were collected with care taken to maintain the integrity of the mat and placed into clean (10% HCl washed, deionized water-rinsed) plastic pans. Samples were transported to the laboratory and moistened with filtered (GF/F) ambient creek water to prevent desiccation. Incubations were performed using subsamples obtained by inserting minicorers (cut-off syringes with 1.15 cm^2 surface area) vertically into the large mat sections. From each core, the upper 0.5 cm was transferred to a 38-ml serum bottle for incubations as described below. Incubations were run at six-hour intervals during the day (about 0900 to 1500) or at night (2200 to 0400). At the same time as samples for N cycling rates were collected, four to five cores (5 cm diameter and 6 cm deep) were collected for determining rates of oxygenic photosynthesis. Water from adjacent tidal creeks was collected for use in the incubations described below.

Microbial Mat Primary Production

Depth profiles of O_2 concentrations and rates of oxygenic photosynthesis (via the light-dark shift method) were determined using O_2-specific microlectrodes (Revsbech et al., 1980; Revsbech et al., 1983a and 1983b; Joye et al., 1996). Microelectrode profiles were conducted in the laboratory using UNISENSE® microelectrodes, a UNISENSE® picoammeter, and a computer-controlled micromanipulator. In laboratory experiments, photosynthetically active radiation (PAR) flux was provided by a Fostec® full-spectrum light source. Oxygen concentrations and the rate of photosynthesis were determined at 100 μm intervals from about 1000 μm above the sediment water interface to the depth where oxygen concentration was zero. To evaluate heterogeneity in primary production rates, at least three profiles were obtained from each core and three-to-four cores were profiled from each site. PAR in the field and in the laboratory was measured using a LiCor model 2π quantum sensor coupled to a LiCor datalogger.

Microbial Mat Nitrogen Transformations

The combined acetylene (C_2H_2) reduction–acetylene block assay was used to estimate N_2 fixation and denitrification rates, respectively (Hardy et al., 1968; Joye and Paerl, 1994). We examined the effects of oxygenic photosynthesis on N cycling and coupling between photosynthetic CO_2 and N_2 fixation by comparing rates in different treatments during daytime and nighttime incubations. During daytime incubations, we

treatment allowed us to assess the effect of photosynthetic O_2 evolution on N transforming processes, i.e., light-supported N transformations in the absence of O_2 evolution. This treatment is noted "DCMU" on figures. Incubating samples in the dark (bottles wrapped in aluminum foil) eliminated activity coupled directly to light-generated (PSI or PSII) reductant and/or energy; this treatment provided estimates of *dark* N_2 fixation. To determine the impact of NO_3^- availability on rates of N_2 fixation and denitrification, we included a set of light-and-dark incubations amended with 1 mM nitrate and 2 mM glucose. These treatments provided *potential denitrification* rates and helped evaluate whether dissolved inorganic N concentrations regulate N_2 fixation. Rates in these treatments are referred to as *light NG* and *dark NG* later in the text and on the figures.

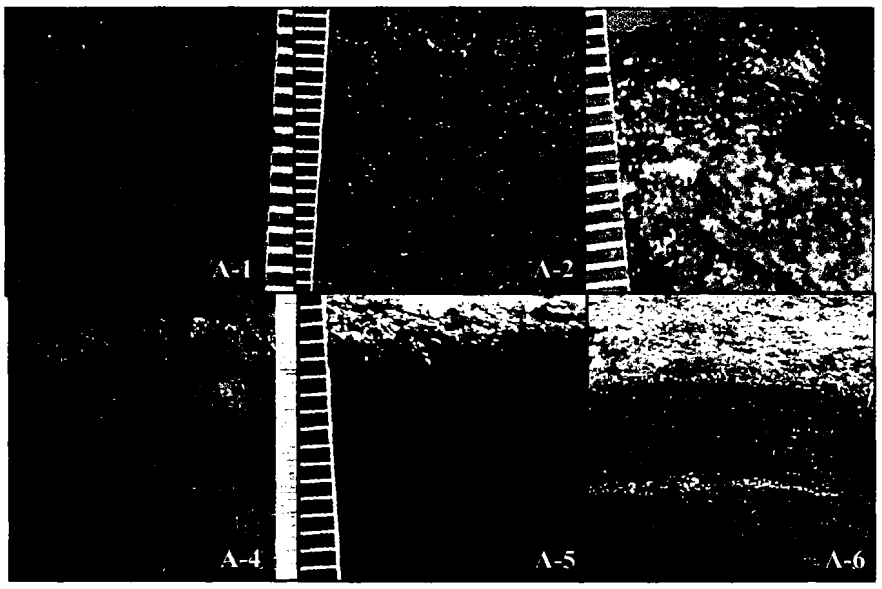
The details of the protocol have been described previously (Joye and Paerl, 1994). Briefly, samples were incubated for six hours during daytime or nighttime periods. DCMU-amended samples were run during the day only. Blanks (incubations of filtered creek water + C_2H_2 without any mat) were used to correct experimental treatments for trace ethylene (C_2H_4) contamination of C_2H_2. All treatments and blanks were run in triplicate. Ethylene production was quantified using flame ionization gas chromatography (Joye and Paerl, 1994). Production rates were transformed to N_2 fixation rates using an experimentally determined conversion factor (Lee and Joye, in preparation). Acetylene also blocks the transformation of N_2O to N_2 in denitrifying bacteria and denitrification rates were estimated by quantifying N_2O production rates during the incubation (Joye and Paerl, 1994). Nitrous oxide concentration was quantified using electron capture gas chromatography (Joye and Paerl, 1994; Lee and Joye, in preparation).

RESULTS

Microscopy and Geochemical Characterization

Twin Cays microbial mats are diverse in both overall morphology (Fig. 2A) and with respect to the cyanobacterial species present (Fig. 2B). The morphology of the mat varied as a function of the underlying substrate. Mats growing on top of peat had irregular (bumpy) surface topography (Fig. 2, A-3, A-4) while mats growing at the edges of ponds or atop organic layers had smoother surfaces. These different mats contained a variety of cyanobacteria. Mats growing directly on top of peat generally contained more heterocystous (e.g., *Nodularia spp.*) species whereas mats growing along the edges of ponds and those growing on organic layers overlaying peat were dominated by coccoidal (e.g., *Chroococcus spp.* and *Gloeocapsa spp.*) and filamentous, non-heterocystous species (e.g., *Microcoleus spp., Lyngbya spp., and Phormidium spp.*). Numerous heterotrophic bacteria and photosynthetic bacteria (e.g., *Chromatium*, *Thiocapsa*, and *Rhodopseudomonas*) were also observed in the microbial mats.

A.



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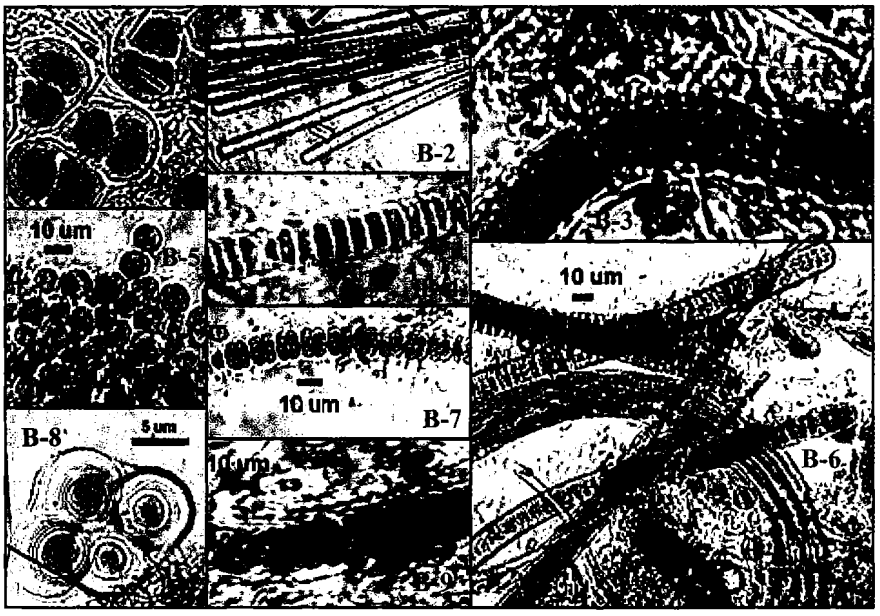

Figure 2. (A) Microbial mat morphologies observed on Twin Cays. Panels A-1 and A-2 show mats growing atop thin organic layers that overlay peat. Panels A-4 and A-5 show thin mats growing directly on top of peat. Panels A-3 and A-6 show mats from the SCC (3) and WP (6) sites. (B) Examples of the resident cyanobacteria in Twin Cays microbial mats: 1-Chroococcus sp.; 2-Microcoleus sp.; 3-Nodularia sp. (with opaque sheath); 4- Johannesbaptista sp.; 5- Aphanocapsa sp.; 6- Lyngbya sp. (thicker single filaments) and Microcoleus sp. (thin, multiple filaments within a sheath); 7-Johannesbaptista sp.; 8-Gloeocapsa sp.; and 9-Nodularia sp. (with clear sheath).

Average temperatures did not vary significantly between the three sampling dates. The maximum daily PAR flux was highest in June but day-to-day variation in PAR due to patchy cloud cover obscured significant differences between sampling dates. Variation in the concentration of chlorophyll *a* and pore water nutrients was noted (Table 1). Pore water at 10 cm was generally hypersaline. Chlorophyll *a* concentrations were highest in November 2000 but no significant differences were observed between the sites sampled at this time. In June 2001, chlorophyll *a* concentrations were significantly lower than those observed in November 2000. In March 2002, chlorophyll *a* concentrations were similar to those observed in June 2001 except at the WP site where high concentrations were observed. Pore water pH at 10 cm was usually between six and seven (data not shown). Pore water dissolved inorganic nitrogen (DIN = NH_4^++NO_3^-) concentrations varied substantially (~40 μM to >1.5 mM) beneath microbial mats. Dissolved inorganic phosphorus concentrations were very low (0.02 to 6 μM). The porewater DIN:DIP ratio varied but usually exceeded the Redfield Ratio of 15 (>100 to >1000). Pore water H_2S concentrations also varied, ranging from 0.19 to 3.5 mM.

Microbial Mat Primary Production

Pore water O_2 concentration was a function of PAR intensity and responded quickly to changes in the PAR availability (Fig. 3). Oxygen was rapidly consumed in sediments in the dark, limiting the oxygen penetration depth to 0.1 to 0.4 mm depth. As the PAR flux increased, oxygen concentrations gradually increased and the depth of oxygen penetration increased. A surface PAR flux greater than about 350 μE led to a subsurface peak in O_2 concentration (400 to 700 μM or >300% saturation) and an O_2 penetration depth between 1.1 and 2 mm (Fig. 3A). Integrating the gross oxygen production (GOP) rate over depth across increasing PAR availability showed that rates were similar at PAR fluxes between 100 and 2200 μE in the microbial mats from the edge of the Weather Station pond (values given represent the mean and error bars are standard deviation of the mean; n=3 profiles at each PAR flux). Even though the mean integrated GOP rates at ≥2000 μE were higher, the standard deviations were also higher so the rates were not significantly different from those observed at lower PAR fluxes.

GOP rates were similar across time and across sites. In June 2001, integrated GOP rates were comparable at the four sites examined (6 and 17 mmol O_2 m^{-2} hr^{-1} at 200 and 2000 μE, respectively) (Fig. 4A). Rates at the two light levels were only significantly different at the Dock site; however, when all data were pooled, rates at the higher PAR flux (2000 μE) significantly exceeded those observed at the lower PAR flux (200 μE).

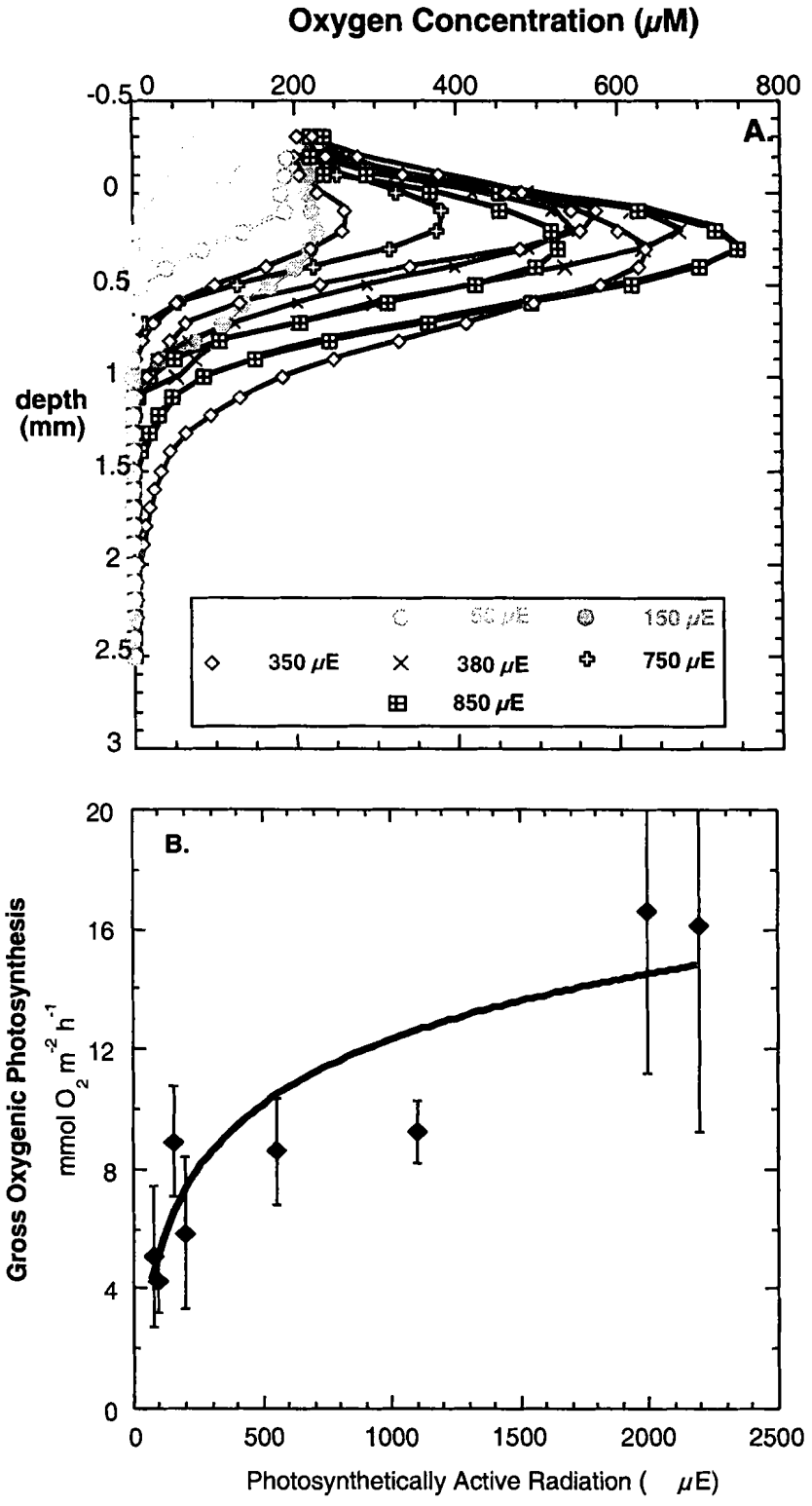

Figure 3. A. Pore water oxygen concentration over depth at different light levels (BF, Nov 2000). B. Gross oxygenic photosynthesis versus light availability. Values shown represent the mean and error bars are standard deviation of the mean of n=3 profiles at each PAR flux (WS, June 2001).

Table 1. Physical data and chlorophyll *a* and nutrient concentrations for the three sampling dates (average and (standard deviation) are shown).

Parameter:	**Nov-00** Salinity[3]	Avg. Temp.[1] Chlorophyll *a*[4]	27.6 °C DIN[5]	Avg. PAR[2] DIP[5]	1800 µE H_2S[6]
Site: **BF**	36	157.5 (57)	288 to 1200	0.2 to 0.38	0.01
D	n.d.	n.d.	n.d.	n.d.	n.d.
L	n.d.	n.d.	n.d.	n.d.	n.d.
NWD	41 (3)	129 (80)	62 (49)	1 (0.1)	0.19 (0.1)
SCC	n.d.	n.d.	n.d.	n.d.	n.d.
WP	n.d.	n.d.	n.d.	n.d.	n.d.
WS	41.5 (5)	280.8 (127)	704 (1254)	2 (3)	0.32 (0.35)
Parameter:	**Jun-01** Salinity[3]	Avg. Temp.[1] Chlorophyll *a*[4]	28.8 °C DIN[5]	Avg. PAR[2] DIP[5]	2400 µE H_2S[6]
Site: **BF**	49	48.9 (29)	274 to 310	0.2 to 1.5	0.4 to 0.5
D	48	50 (13)	313	0.37	0.44
L	51	84 (33)	86	0.27	0.31
NWD	40	28 (8)	59	0.17	0.56
SCC	n.d.	n.d.	n.d.	n.d.	n.d.
WP	n.d.	n.d.	n.d.	n.d.	n.d.
WS	n.d.	88.5 (11)	n.d.	n.d.	n.d.
Parameter:	**Mar-02** Salinity[3]	Avg. Temp.[1] Chlorophyll *a*[4]	27.2 °C DIN[5]	Avg. PAR[2] DIP[5]	2000 µE H_2S[6]
Site: **BF**	40	66 (24)	1607	6.5	3.5
D	33.5	63 (29)	43.3	0.4	0.47
L	34	74 (34)	45.5	0.2	0.42
NWD	42	58 (29)	78	0.8	0.32
SCC	42	77 (9)	156	0.5	0.44
WP	42	244 (12)	1.2	0.02	n.d.
WS	50	101 (69)	121	3	1.1
[1]average temperature	[2]photosynthetically active radiation	[3]part per thousand	[4]mg m^{-2}	[5]µM [6]mM	n.d.=no data

Adjusting the GOP rates to account for differences in chlorophyll *a* concentration (i.e., dividing the GOP rate, mmol O_2 m^{-2} h^{-1}, by the sediment chlorophyll *a* concentration, mg Chl *a* m^{-2}) yields a biomass specific production rate in units of mmol O_2 (mg Chl *a*)$^{-1}$ h^{-1} and permits a more robust comparison of activity across sites (Fig. 4B). Chlorophyll *a* specific GOP rates at the Dock site were significantly different between PAR fluxes but at the other sites, as well as for the pooled data, rates were not significantly different between PAR fluxes. In March 2002, GOP rates were similar to those observed in June 2001 but chlorophyll *a*-specific GOP rates were 2-to-10 times higher.

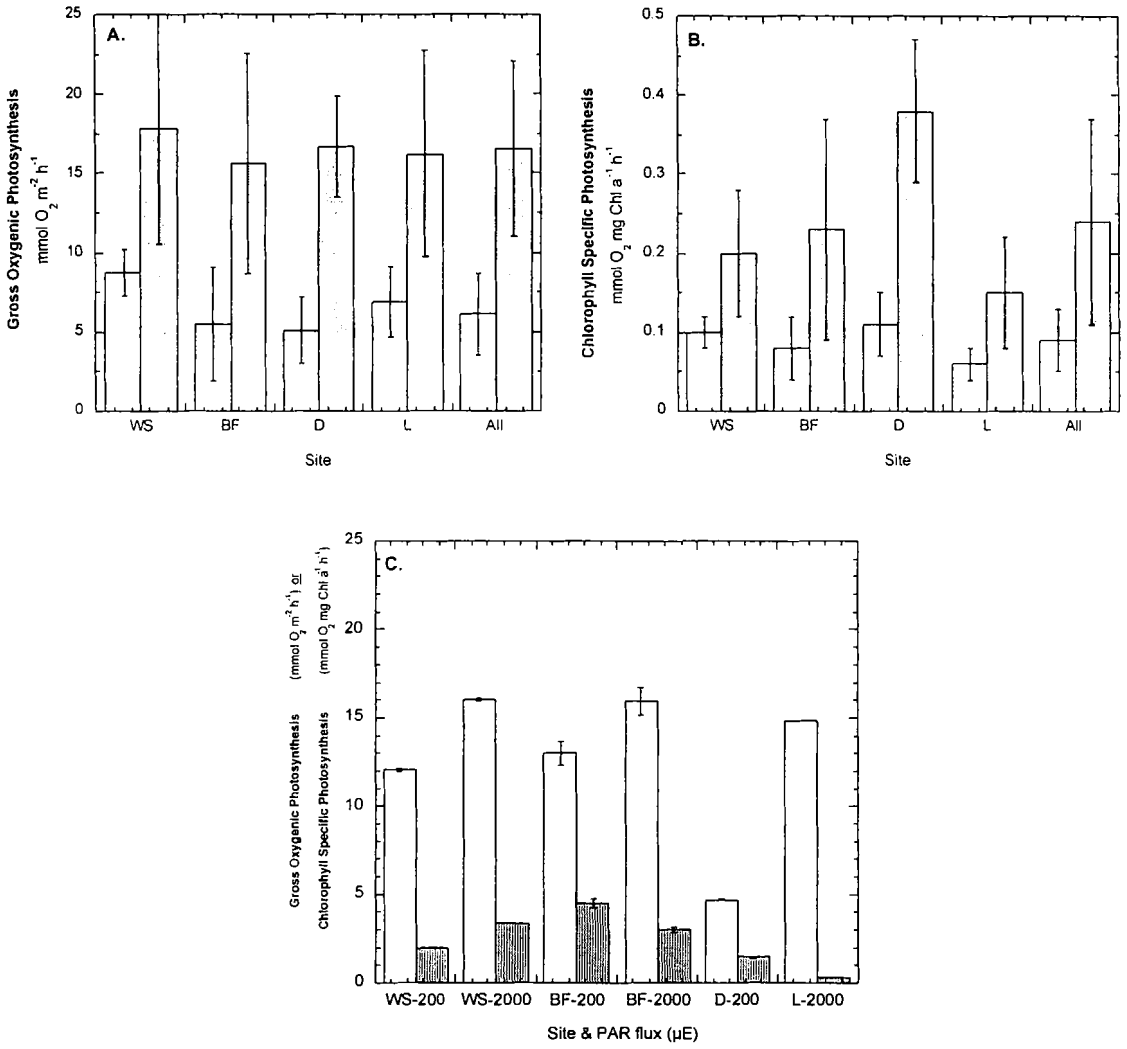

Figure 4. Rates of (A) GOP and (B) chlorophyll *a* specific GOP across sites in June 2001 (open=200 µE; hatched=2000 µE) and (C) GOP (open) and chlorophyll *a* specific GOP (speckled) across sites in March 2002 (only one PAR flux level available for D and L).

Microbial Mat Nitrogen Cycling

Nitrogen-fixation rates were variable but generally were higher in March compared to June (Fig. 5). Nighttime activity (15 to 20 µmol N m^{-2} h^{-1}) was generally equal to, or higher than, rates observed during daytime light (0 to 15 µmol N m^{-2} h^{-1}) or dark (2 to 20 µmol N m^{-2} h^{-1}) incubations. Samples amended with DCMU exhibited significantly higher N_2 fixation rates (30 to 500 µmol N m^{-2} h^{-1}) during the day relative to light samples (no DCMU), and DCMU-amended rates in March 2002 were significantly higher than rates observed in June 2001 (<100 to >250 µmol N m^{-2} h^{-1}). Addition of inorganic nitrogen such as NO_3^- (in +NG treatments) reduced N_2 fixation rates during daytime and nighttime incubations, although differences were not always significant. *In situ* denitrification rates were low (<2 µmol N_2O m^{-2} h^{-1}) but potential denitrification

rates rivaled (20 to 200 μmol N m^{-2} h^{-1}), and in some cases exceeded, rates of N_2 fixation (Fig. 6). Denitrification rates were generally higher in dark-incubated treatments during daytime incubations. Daytime-dark rates and nighttime rates in +NG treatments were not significantly different.

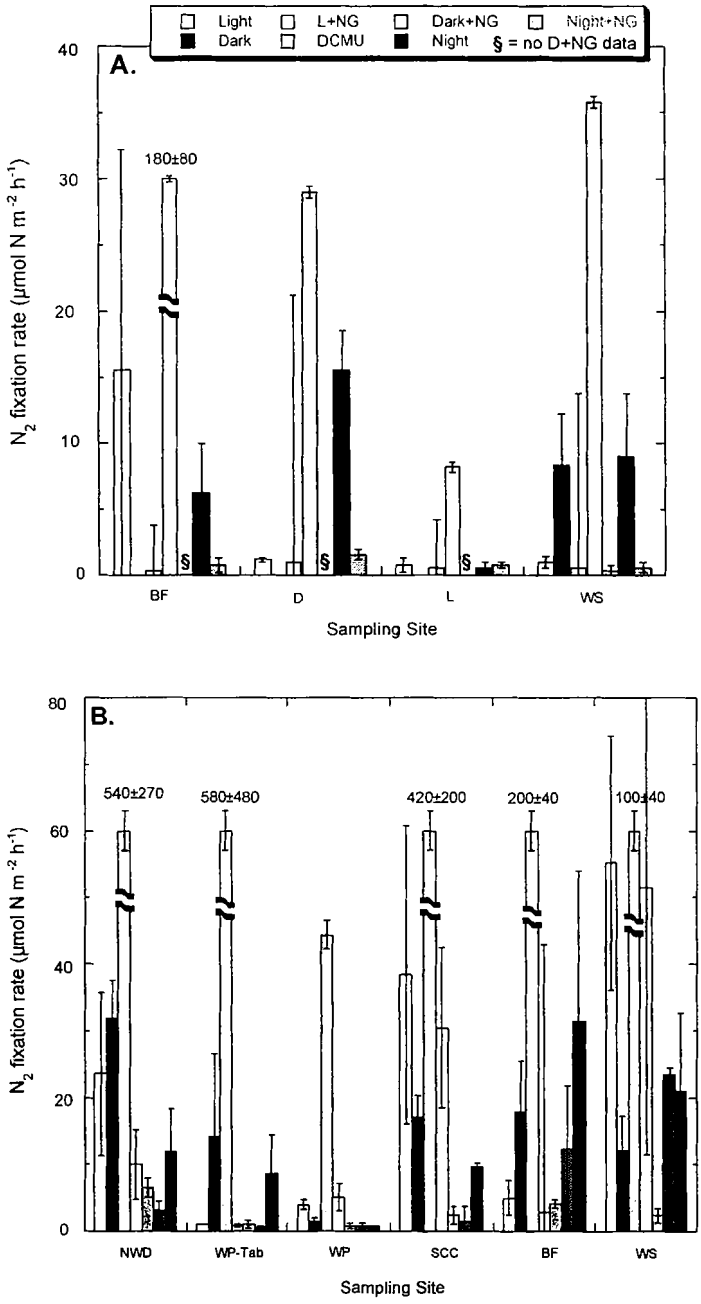

Figure 5. Day-night variability in N_2 fixation in microbial mats (**A**: 6/01, **B**: 3/02). Columns represent mean of n=3 samples; error bars=standard deviation of the mean. Maximum values and standard deviation for split columns are noted at the top of each column.

DISCUSSION

This is the first study to document the distribution of microbial mats and to evaluate their potential impact on biogeochemical cycling in oceanic mangrove ecosystems. Laminated microbial mats were abundant on surface sediments throughout the dwarf mangrove zone of Twin Cays. These microbial mats exhibited high rates of primary production, nitrogen fixation and potential denitrification suggesting they are important ecological and biogeochemical players in oceanic mangrove island systems.

A diverse suite of phototrophic microbes, including eukaryotes (diatoms) and prokaryotes (both cyanobacteria and photosynthetic bacteria), were observed in microbial mats. The photosynthetic bacteria observed in Twin Cays microbial mats (Fig. 2) are similar to those observed in other environments, including microbial mats from a hypersaline lagoon on San Salvador, Bahamas (Pinckney et al., 1995a and 1997), mats in evaporative salt ponds on Baja California Sur, Mexico (Lopez-Cortes, 1990; Bebout et al., 2002), saline lakes (Jørgensen et al., 1983) or mats occupying temperate intertidal flats (Joye and Paerl, 1994; Pinckney et al., 1995b). The dominant cyanobacteria in microbial mats varied between mats lying along the edges of ponds (e.g., WS, BF and WP) versus those in the dwarf zone occurring directly over the peat (e.g., NWD, WP-tab, L and D) versus those in an area where the mangrove had been removed via clear cutting (SCC). Mats along the pond edge were dominated by filamentous non-heterocystous cyanobacteria, including *Microcoleus* and *Lyngbya*. Coccoidal cyanobacteria and photosynthetic bacteria were also present. Mats overlying peat were unique in that they often contained heterocystous cyanobacteria, e.g., *Nodularia* (at NWD), in addition to coccoidal cyanobacteria, e.g., *Gloeocapsa* and *Chroococcus*, and some filamentous non-heterocystous forms (e.g., *Lyngbya*, at D, L, and WP-tab sites). Mats at the SCC site were dominated by *Spirulina*, a filamentous non-heterocystous cyanobacterium, and by *Aphanocapsa*, a coccoidal cyanobacterium. Photosynthetic bacteria, visible as a brown or pink layer beneath the cyanobacterial layer, were present in mats lying at the pond edge or on top of peat (see Fig. 2 A-1, A-2, A-4, and A-6). The dominant cyanobacteria in a particular mat sample helped explain, to some extent, the day-night patterns of N_2 fixation activity we observed (see below).

Microbial Mat Primary Production

The importance of oxygenic photosynthesis in Twin Cays microbial mats is evident from the depth distribution of dissolved O_2 in the pore water (Fig. 3A). In November 2000, dissolved O_2 penetrated only 0.1 to 0.5 mm into the microbial mat in the dark (0 μE). As the PAR flux increased, the shape-and-depth distribution of O_2 changed. The maximum pore water O_2 concentration increased from <100 μM at 0 μE to 800 μM O_2 at a PAR flux of 850 μE. The depth of the maximum O_2 concentration deepened from 0.2 to 0.7 mm and the O_2 penetration depth deepened by 1 mm, increasing from 0.5 mm in the dark to >1.5 mm at 850 μE. Though pore water O_2 concentrations increased with increasing PAR flux, the integrated rate of gross oxygenic photosynthesis (GOP) varied between 6 and 15 mmol O_2 m^{-2} h^{-1}across a range of PAR flux (Fig. 3B). We observed high variability between profiles because O_2 concentrations at a given depth often varied

by ±50%. This variability made it impossible to distinguish GOP at low PAR flux from that at high PAR flux. The relatively constant GOP rate at low (50 μE) and high (>1000 μE) PAR flux suggests that the phototrophic microbes in Twin Cays microbial mats are efficient (i.e., they photosaturate) at low PAR flux and that they are able to sustain high GOP activity even at high PAR fluxes. We did not observe any evidence of photoinhibition at PAR fluxes exceeding 2000 μE. Thus, Twin Cays microbial mats appear poised to photosynthesize at near maximum rates even at low PAR flux meaning that microbial mat light-driven primary production occurs during most of the daylight hours.

We did not quantify bacteriochlorophyll concentrations so the discussion of photosynthetic biomass below is based on chlorophyll *a* data. We stress, however, that photosynthetic bacteria are abundant in Twin Cays microbial mats and note that the importance of photosynthetic bacteria in benthic primary production is discussed elsewhere (Lee and Joye, in preparation). Photosynthetic biomass, as chl *a*, in Twin Cays microbial mats varied between about 30 and 300 mg chl *a* m^{-2} (Table 1) and biomass-specific primary production rates varied between 0.1 and 4 mmol O_2 (mg chl *a*)$^{-1}$ h^{-1} (Fig. 4). The chl *a* concentrations observed in Twin Cays microbial mats are comparable to those observed in other temperate and tropical sediments (e.g., Sournia et al., 1977; Potts, 1980; Joye et al., 1996; Pinckney and Paerl, 1997; Karsten et al., 1998; Camacho and de Wit, 2003).

Biomass-specific GOP rates in Twin Cays microbial mats in June 2001 were about an order of magnitude lower than those observed in tropical (Pinckney and Paerl, 1997) and temperate (Joye et al., 1996) microbial mats. In contrast, the biomass-specific GOP rates in Twin Cays microbial mats in March 2002 were comparable to those documented in tropical systems but were still about 3-times lower than those observed in high biomass temperate estuarine microbial mats. Seasonal variability in the biomass-specific GOP may reflect changes in microbial mat microbes' abilities to channel light-derived reducing equivalents into primary production. Lower tides which result in more severe exposure to UV in June may have stressed microbial mat microbes and led to lower photosynthetic efficiencies at that time compared with March which is in the 'wet' season (Lee and Joye, in preparation). UV-exposure has been shown to influence community structure and function in other microbial mats (Karsten et al., 1998; Sheridan, 2001) and future studies are planned to evaluate the impact of UV-exposure on the photophysiology of Twin Cays microbial mats.

Microbial mats are ubiquitous in dwarf mangrove zones along pond edges and along the bottom of shallow ponds. These habitats (dwarf red-and-black mangrove, open pond dwarf, floc zones and mixed dwarf stands; Rodriguez and Feller, this volume) account for about 44% of the land cover on Twin Cays (Rodriguez and Feller, this volume) covering about 332,854 m^2. We can derive a rough estimate of the annual primary production rate of microbial mats by assuming that half of this area (166,427 m^2) is covered by microbial mats (based on numerous surveys we have conducted in these areas, we consider this a conservative estimate) and assuming that, on an average day, a microbial mat receives ample PAR for 6 hours to support a GOP rate of 12 mmol O_2 m^{-2} h^{-1} (the average rate at low and high light, see Fig. 4). Using these values and assuming a photosynthetic quotient (O_2 evolved per CH_2O fixed) of one yields a value of 4.4×10^6

mol carbon or 5.3×10^7 g carbon fixed per year (gross) by microbial mats. Microbial mats are obviously an important source of labile organic carbon for the Twin Cays ecosystem.

Microbial Mat Nitrogen Transformations

This is the first study of microbial mats reporting contemporaneous data for biomass and rates of primary production, nitrogen fixation and denitrification. Nitrogen fixation rates in Twin Cays microbial mats were often comparable during the day and at night with rates ranging between 15-to-20 μmol N m^{-2} h^{-1} (Fig. 5). Nitrogen fixation activity measured in Twin Cays microbial mats is similar to that observed in other tropical and temperate microbial mats (Joye and Paerl, 1994; Pinckney and Paerl, 1997; Steppe et al., 2001). The day-versus-night patterns of N_2 fixation activity probably resulted from the various strategies used by the mat microbes to fix N_2. In mats dominated by heterocystous cyanobacteria (e.g., Fig. 5B, NWD), N_2 fixation activity during the day exceeded that at night. However, daytime activity was comparable in light and dark treatments suggesting that N_2 fixers had ample reductant to support activity in the dark. The source of this reductant appeared to be linked to photosynthesis because nighttime activity was much lower (one-half of daytime activity).

Oxygen production during photosynthesis was a potent regulator of N_2 fixation year round. Nitrogenase is inhibited by O_2 and the addition of DCMU, a photosystem-II (i.e., O_2 production) inhibitor, led to significant increases in nitrogen-fixation rates. At times, nitrogen-fixation rates in DCMU-amended treatments were an order of magnitude higher than those observed in light or dark treatments (Fig. 5B). As shown in Fig. 3, pore water O_2 concentrations increase rapidly as GOP rates increase. Similarly, blocking O_2 production with DCMU decreases pore water O_2 concentration at depths within the photic zone. The increase of N_2 fixation rates in DCMU treatments suggests that a substantial fraction of the nitrogenase present in these microbial mats is only active when freed from O_2 inhibition. This fraction of the population may be active during certain parts of the day (early morning or late afternoon) when ample light is available but pore water O_2 concentrations are below the threshold for inhibition of nitrogenase.

In mats dominated by filamentous nonheterocystous cyanobacteria (Fig. 6, A: WS and B: BF, WP-tab), daytime dark and DCMU-amended N_2 fixation rates exceeded light rates suggesting oxygen-regulation of N_2 fixation. However, in mats where both filamentous non-heterocystous cyanobacteria and coccoidal cyanobacteria were common, daytime light activity was comparable to that noted in dark treatments. Many cyanobacteria excrete copious amount of mucous which can relieve oxygen inhibition of nitrogenase by either stimulating heterotrophic bacterial oxygen consumption (i.e., respiration) or by serving as a diffusive shield against oxygen exposure. The existence of anoxic microzones may facilitate N_2 fixation in microbial mats (Tankere et al., 2002).

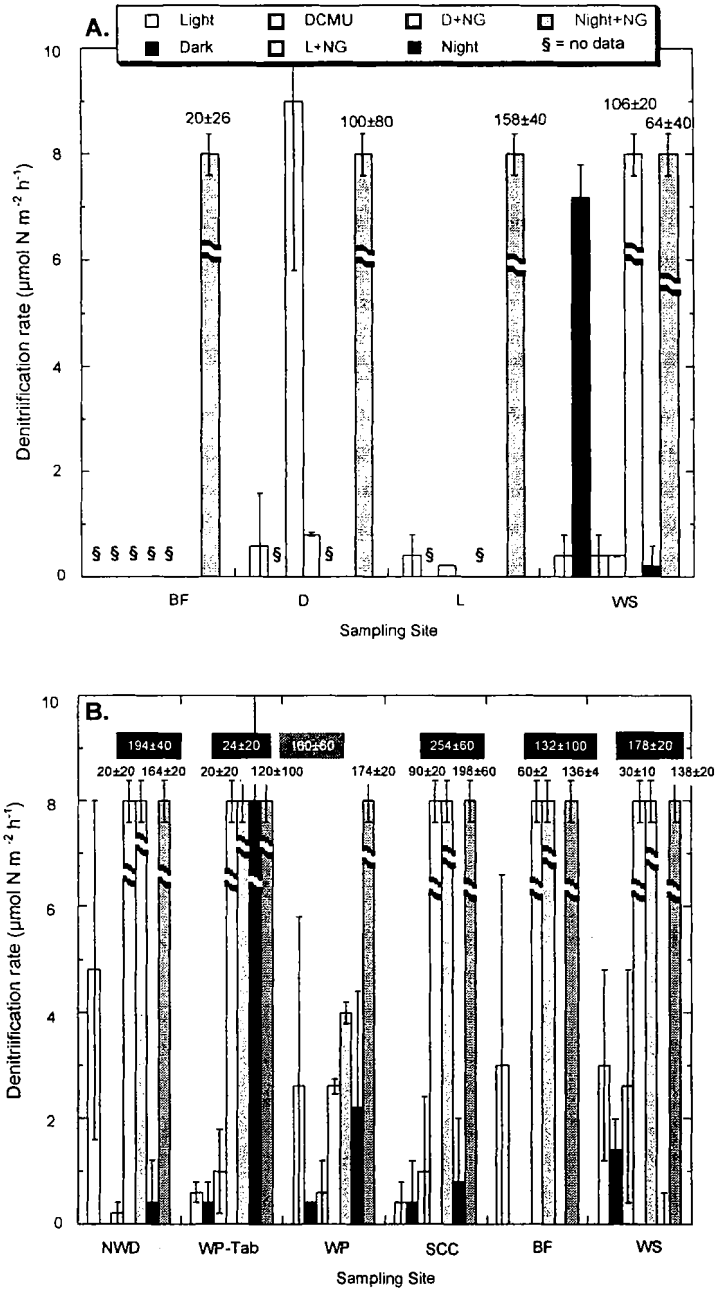

Figure 6. Day-night variability in microbial mat denitrification (**A**: 6/01, **B**: 3/02). Columns represent mean (n=3 samples) and error bars=standard deviation of the mean. Maximum rates and standard deviation for split columns are noted at the top of each column. Black or grey boxes refer to daytime-dark (black) or nighttime (gray) +NG treatments.

The variable pattern of N_2 fixation activity observed in Twin Cays microbial mats results from the different strategies used by N_2 fixing cyanobacteria to support the contemporaneous activity of the energy-demanding process of N_2 fixation with the energy-producing process of photosynthesis (Stal and Krumbein, 1985; Stal et al., 1994;

Steppe et al., 1996; Paerl et al., 2000). Part of the variability in N_2 fixation patterns likely results from the contribution of other microbes, e.g., sulfate-reducing bacteria or photosynthetic bacteria, to N_2 fixation. Cyanobacteria are certainly not the only microbes capable of N_2 fixation in microbial mats. A phylogenetically diverse suite of nitrogenase (*nif*H) genes has been observed in samples from similar microbial mats (Paerl et al., 1996 and 2000; Steppe et al., 1996; Omoregie et al., 2004). The experiments detailed here did not permit us to link directly a particular microbial group with N_2 fixation activity but ongoing and planned studies of *nif*H diversity and additional experiments aimed at determining the source of reductant for nitrogenase in these microbial mats may permit us to make such connections in the future.

Not surprisingly, the availability of inorganic nitrogen influenced N_2 fixation rates. The addition of inorganic nitrogen, such as nitrate (2 mM), in +NG treatments often resulted in substantial (80%) and instantaneous reduction in N_2 fixation rates (Fig. 5). However, in some cases, the addition of inorganic nitrogen had no effect on N_2 fixation (e.g., Fig. 5B, WS). Contrary to results of the +NG experiments, variability in pore-water nutrient and sulfide concentrations could not explain the patterns of N_2 fixation we observed. Nitrogen-fixation rates were not correlated with pore-water DIN concentrations (field DIN was usually < 500μM and was rarely >1000 μM, Table 1). Dissolved inorganic nitrogen concentrations (mainly ammonium; nitrate concentrations were usually <2 μM; data not shown) in the microbial mats were quite variable with the highest concentrations observed beneath mats along the pond edges (Table 1). Pore-water dissolved inorganic-phosphorus concentrations were extremely low (DIN:DIP ratios were always >16, suggestive of P limitation) and variability in DIP also did not correlate to N_2 fixation rates. Patterns in pore-water hydrogen-sulfide concentrations were also variable (highest beneath pond-edge mats) and did not help explain N_2 fixation rates. The available data suggest that the primary factors controlling N_2 fixation at these sites are pore-water oxygen concentrations and the availability of reductant (Lee and Joye, in preparation).

In situ denitrification rates in microbial mats were low (<4 μmol N m^{-2} h^{-1}). However, a tremendous potential for denitrification exists in these mats (up to 240 μmol N m^{-2} h^{-1}). Upon addition of nitrate and glucose, denitrification rates increased immediately and rates in light-and-dark treatments were sometimes comparable. At other times, rates in dark treatments exceeded those in light treatments, suggesting oxygen inhibition of denitrification. Nitrate availability clearly limited denitrification in these microbial mats and oxygen concentration was only a limiting factor when nitrate was available. Similar results were reported for a temperate microbial mat where denitrification rates were controlled by nitrate availability. Once nitrate became available, oxygen concentration also influenced (via inhibition) denitrification rates (Joye and Paerl, 1994).

Denitrification rates were generally higher in March 2002 compared to June 2001. Higher rates of denitrification during March may have resulted from wetter conditions which would generate anoxic conditions in the mat (as opposed to more aerobic conditions when the mat is dry and exposed during the day-time low-tide conditions that existed in June). Potential denitrification rates at night were remarkably similar between the different sites sampled in either June or March. Daytime *in situ* denitrification rates

were variable but the highest daytime denitrification activity was observed in mats with shallow O_2 penetration depths (Fig. 6B, WS, WP-E, BF). Shallow oxygen penetration depths may have permitted nitrate to flux through the aerobic zone into the anaerobic zone where it was accessible to denitrifying bacteria.

Overall, high rates of N_2 fixation and low denitrification rates under *in situ* conditions suggest that Twin Cays microbial mats are a net source of fixed N to the environment. Using the information presented above for primary production we can also extrapolate our N-fixation data to the Twin Cays system-scale. Based on the data presented in Fig. 5, a conservative average N_2 fixation is 10 μmol N m^{-2} h^{-1}. If we assume activity throughout the day-night cycle (the rate above averages day-and-night-time activity), then the average N input to the Twin Cays system is about 1.5 x 10^4 mol N or 2 x 10^5 g N per year. This is a conservative estimate that averages data from the different sites. Nonetheless, the magnitude of N_2 fixation activity underscores the importance of microbial mats as a significant N source for the Twin Cays ecosystem.

CONCLUSIONS

Microbial mat microorganisms are dynamic participants in biogeochemical cycling in Twin Cays sedimentary environments. Microbial mats are widespread occupying the sediment surface in dwarf mangrove zones, along pond edges and bottoms, and in clear-cut zones. A diverse assemblage of cyanobacteria was documented in Twin Cays microbial mats. In addition to cyanobacteria, eukaryotes (diatoms) and photosynthetic, chemosynthetic, and heterotrophic bacteria are also active in these microbial mats. Primary production and N_2 fixation by microbial mats represents a previously unrecognized source of fixed carbon and nitrogen to oceanic mangrove ecosystems. The absolute distribution of these mats on Twin Cays is unknown at present but remote sensing data suggests that up to 44% of the sediment could be occupied by microbial mats. Using conservative values for microbial mat distributions and activity, we estimate that these mats fix at least 5.3 x 10^7 g of carbon (gross) per year and 2 x 10^5 g N per year. Much of this labile organic carbon and nitrogen is probably transferred to other compartments in the Twin Cays food web. C-and-N cycling in microbial mats may also contribute to previously observed system-level patterns of nutrient limitation. Feller et al. (2002) reported that dwarf red mangrove trees on Twin Cays are phosphorus-limited. High rates of N_2 fixation by microbial mats within dwarf zones may enrich this zone with organic nitrogen, driving phosphorus limitation in the dwarf mangrove trees. Definitively linking microbial mats to the observed system-level patterns of nutrient limitation requires further study.

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