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# Ecophysiology of Stromatolitic Microbial Mats, Stocking Island, Exuma Cays, Bahamas

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**Abstract.** Intertidal stromatolites, covered by cyanobacterial mats, were recently discovered at Stocking Island, Exuma Cays, Bahamas. Ecophysiological responses ($CO_2$ fixation, $N_2$ fixation, and photoacclimation) of these cyanobacterial mats to experimental manipulations were examined to identify potential environmental variables controlling community structure and function. The mats exhibit horizontal zonation that shifts from soft to crusty to hard in a seaward direction. Cluster analysis of chemotaxonomic photopigments (chlorophylls and carotenoids) revealed that visually distinct mat types are composed of distinct phototrophic assemblages. Under reduced irradiance, diatoms within the mats photoacclimated by increasing accessory photopigments (diadinoxanthin, fucoxanthin, and chlorophyll $c_1c_2$) and cyanobacteria reduced the photoprotective carotenoid echinenone. In a 4-day nutrient addition bioassay experiment, nitrate, phosphate, dissolved organic carbon, and trace metal enrichments did not enhance $CO_2$ fixation, but phosphate enrichments tripled $N_2$ fixation rates. The addition of DCMU increased $N_2$ fixation rates relative to nonamended light and dark rates, indicating light (photosystem I) enhanced nitrogenase activity. Soft mats appear to represent the early stages of colonization and stabilization of mat communities. Active growth following stabilization results in the formation of partially-lithified crusty mats, which eventually become highly-lithified and form hard mats. Collectively, our results suggest that Stocking Island stromatolitic mats have low growth rates and consequently exhibit slow responses to increased nutrient availability and changes in ambient irradiance. In general, intertidal stromatolitic mats at Stocking Island appear to exhibit low rates of $CO_2$ and $N_2$ fixation relative to nonlithifying temperate cyanobacterial mats. Although production is low, res-

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piration is likewise low, leading to the suggestion that high production to respiration ratios (P:R) may be necessary for lithification of intertidal stromatolitic mats.

## Introduction

Laminated sedimentary structures known as stromatolites, thought to be of cyanobacterial origin, are the earliest known macrofossils [36]. Dominating the early fossil record, stromatolites reached a peak in abundance and diversity in the Precambrian and declined from Middle Ordovician time onward [10, 31]. Paleoenvironmental interpretation of ancient stromatolites has been hampered by a scarcity of modern analogs. Recently, intertidal stromatolites were discovered on the eastern shore of Stocking Island, Bahamas [28]. These were the first modern stromatolites recognized in an intertidal environment of normal marine salinity (36–37‰). Preliminary investigations revealed that these stromatolites appeared to be actively growing, with growth believed to be associated with microbial mats covering the stromatolite surface [28]. The Stocking Island discovery provides an opportunity to characterize the ecophysiological properties of stromatolitic microbial mats and to investigate the role(s) of these mats in stromatolite formation.

Several factors may regulate the biotic component of stromatolites [4, 5, 13, 21, 24]. Primary production ($CO_2$ fixation) and the conversion of $N_2$ to $NH_3$ ($N_2$ fixation) by certain cyanobacteria and eubacteria are important metabolic indicators of the potential contribution of microbial mats to carbon and nitrogen budgets of intertidal communities (B. Bebout, Ph.D. Thesis, University of North Carolina, Chapel Hill, 1992 and [5, 22, 23, 32]). $N_2$ fixation helps circumvent chronic N-limitation in oligotrophic marine systems [21, 37, 39]. This process may meet mat and community N demands or serve as a supplementary (rather than exclusive) source of "new" nitrogen for mat growth [24, 37]. In either case, the environmental factors regulating $N_2$ fixation may subsequently control mat $CO_2$ fixation, primary production, and growth of stromatolites. Quantification of $CO_2$ and $N_2$ fixation, and the factors regulating these processes in stromatolites, may provide insight into the processes (or mechanisms) controlling production in these communities.

The primary goals of this study were to ascertain and quantify the variability in mat composition and ecophysiological processes ($CO_2$ fixation, $N_2$ fixation, and photoacclimation) and make a first attempt to discern the variables that control community structure and production of intertidal stromatolitic mats at Stocking Island, Bahamas.

## Materials and Methods

### *Study Site*

Stocking Island is located at the southern end of the Exuma Cays, ca. 2 km east of Georgetown, Great Exuma Island, Bahamas (Fig. 1). The eastern side of Stocking Island is fronted by wide, carbonate sand beaches exposed to the open ocean conditions of Exuma Sound. The seawater salinity in Exuma Sound is relatively constant (36–37‰). Ambient seawater is supersaturated with aragonite and calcite [3, 7, 38]. Tides in the Exuma Cays are diurnal with an average range of ca. 1.0 m [9].

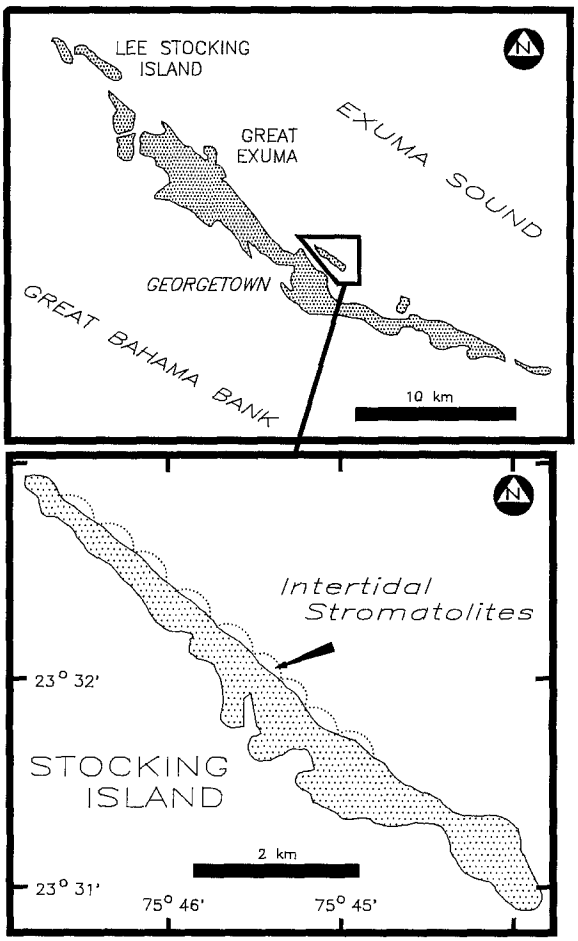


**Fig. 1.** Maps showing the location of the intertidal stromatolites on Stocking Island, Bahamas. *Arrow* indicates the location of the study site and horizontal transect (Fig. 2).

Intertidal stromatolites, forming tabular structures and isolated heads, are major components of a reef complex that stretches 3 km along the shore, extending 50 m seaward from the beach to shallow subtidal depths of 3 m. Stromatolites occur in the backreef intertidal zone of the reef complex. The upper 10–30 cm of the stromatolites are exposed for ca. 2 h at low tide; exposed surfaces are periodically washed by waves and do not completely dry out during low tide. Waves generally break seaward of the stromatolites on a shallow, subtidal algal ridge. The surfaces of the stromatolites are colonized by filamentous cyanobacterial mats, diatoms, and macroalgal turfs. The cyanobacterial mats are dominated by *Schizothrix gracilis,* with occasionally abundant patches of *Lyngbya* spp., *Oscillatoria* sp., *Phormidium* sp., *Calothrix* sp. and *Gloeothece* sp. (B. Bebout, Ph.D. Thesis). Macroalgal turfs are composed primarily of phaeophytes (*Dictyota* sp., *Lobophora variegata*), chlorophytes (*Acetabularia calyculus, Batophora oerstedii, Cladophoropsis macromeres, Dictyospheria cavernosa, Ernodesmis verticillata*), and rhodophytes (*Laurencia papillosa,* and *Neogoniolithon strictum*) (R. Steneck, pers. comm.). Gastropods, urchins, and chitons were not observed on the stromatolites, but are abundant on nearby Pleistocene bedrock outcrops and the subtidal algal ridge [28].

**Table 1.** Definitions of field terms used to describe surface features of intertidal stromatolites at Stocking Island, Bahamas

| | |
|---|---|
| Morphological appearance | |
| Flat | Surface of substrate is relatively smooth with a vertical relief < 1 cm. |
| Knobby | Surface of substrate composed of rounded protrusions with vertical relief > 1 cm. |
| Degree of hardness | |
| Soft | Surface has soft, smooth texture with a thin (0.1 mm) uniform cyanobacterial layer near surface. A sample from a "soft" surface will readily smear in the palm of your hand. |
| Crusty | Surface layer with hardened, thin crust (< 1 mm thick) and deeper layers of loosely-bound (poorly-lithified) aggregates. A sample from a "crusty" surface does not readily disintegrate when smeared in the palm of your hand, but will fall apart if moderate pressure is applied. |
| Hard | Surface layer with thick (> 1 mm), hardened crust and deeper layers composed of tightly-bound (well-lithified) aggregates. A sample from a hard surface will not disintegrate when smeared in the palm of your hand. |
| Epiphytic community | |
| Few epiphytes | Epiphytes occupy < 25% of substrate surface. |
| Microturf | Epiphytes (< 1 mm in height) cover > 25% of substrate surface. Microturf is composed of cyanobacteria and/or small macroalgae. |
| Macroturf | Epiphytes (> 1 mm in height) cover > 25% of substrate surface. Cyanobacteria may form a mat beneath the macroalgal canopy. |

## *Description of Mat Types*

The cyanobacterial mats and algal turfs associated with the intertidal stromatolites at Stocking Island exhibit structural and compositional variation across the intertidal zone [28]. For descriptive purposes, we devised a simple classification scheme based on easily-discernable field characteristics. The system was based on overall morphological appearance (flat or knobby), degree of hardness (soft, crusty, or hard), and epiphytic community characteristics (few epiphytes, microturf, or macroturf) of the mat and associated stromatolite (Table 1). Mats/stromatolitic surfaces were assigned one of each of the attributes (e.g., flat, hard, microturf) at the time of collection. We will use this terminology to describe the mat types examined in the following experiments. Our primary interest was in examining processes mediated by cyanobacteria; therefore we limited our collections to flat morphotypes with few epiphytes or microturf to minimize the influence of macroalgal species. The cyanobacteria in the flat morphotypes with few epiphytes seems to consist almost entirely of small trichomes of *Schizothrix* sp. (2–5 μm across), with 2–5 trichomes bundled within thick sheaths. The microturf contains a diverse species assemblage, but the most abundant cyanobacteria in this morphotype is *Calothrix* sp. *Calothrix* trichomes were twisted together in darkly pigmented sheaths. Knobby morphotypes were difficult to sample quantitatively and macroturfs were dominated by macroalgal species. All experiments were conducted during 9–14 May 1993.

## *Experimental Methods*

*Nutrient Addition Bioassay.* To examine the potential for nutrient limitation of mat production, we exposed sections of flat, crusty, microturf mats collected from the surface of stromatolites to nutrient enrichments, followed by measurements of community $CO_2$ and $N_2$ fixation responses. Large sections (50 $cm^2$) of this mat type were collected randomly from intertidal stromatolites at Stocking Island and transported to a nearby location that was protected from wind, but received full sunlight exposure. The 50 $cm^2$ mat squares were placed in 500-ml plastic containers cleaned with 0.1 N HCl and deionized

$H_2O$, and each container was filled with 250 ml of ambient seawater collected from the beach at Stocking Island. Nitrate ($NaNO_3$, 5 μM final concentration), phosphate ($H_2PO_4$, 2 μM), a trace metal mixture (1 μM Fe and 0.5 μM each Mn, Cu, Zn, Mo, Co, and B), and mannitol (10 mM) were each added to four containers, and an additional four containers (control) received no nutrient additions (total of 5 treatments in 20 containers). Previous studies of tropical cyanobacterial mats indicated that these nutrient levels should be sufficient to elicit a measurable response [24]. All containers were exposed to ambient temperature and irradiance for a period of 4 days. Salinity in the containers was measured daily with a refractometer and adjusted (by adding deionized water) to maintain the desired salinity (ca. 37‰). $CO_2$ and $N_2$ fixation ($^{14}CO_2$ and acetylene reduction, respectively) rates were measured on day 4 to determine the community response to the different treatments. After $NaH^{14}CO_3$ or acetylene was added (see below), samples were incubated for 5.5 h (0830 to 1400 local time) on day 4 under ambient irradiance and temperature conditions. Previous work [24] indicated that at least a 3–4 day period was needed to measure growth responses in mats inhabiting oligotrophic waters such as those surrounding Stocking Island.

*Photoacclimation Responses.* The effects of irradiance on the photopigment composition of the flat, crusty, microturf mat were evaluated to determine how light affects the relative concentrations of mat photopigments (i.e., chlorophylls and carotenoids) as well as to identify possible photoprotective pigments. Small sections (50 $cm^2$) of flat, crusty, microturf mat collected from intertidal stromatolites on Stocking Island beach were exposed to three different irradiance regimes during a 4-day period. Sections were placed in 500-ml acid-cleaned polyethylene containers and covered with 250 ml seawater. Four containers were assigned to three different treatments (total of 12 containers): 100%, 20%, and 0% (dark) of full irradiance. On days 2 and 4, three samples (1 $cm^2$) were collected from each container, placed in 20-ml scintillation vials, and frozen for later analysis. Salinity was checked daily and adjusted with deionized water to maintain a constant salinity of ca. 37‰.

*Effects of Inhibitors and DOC.* The effects of the photosynthetic (photosystem II) inhibitor DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) and dissolved organic carbon (DOC as mannitol) on $CO_2$ and $N_2$ fixation by soft and hard mats (both were flat morphotypes with few epiphytes) were evaluated in a series of parallel incubations. The rationale for using DCMU and DOC was based on prior research where both stimulated mat $N_2$ fixation potentials due to a reduction in localized $O_2$ concentrations [24, 25]. The experimental design consisted of six treatments [control, dark, mannitol (3 m*M* final concentration), formalin (2%), and DCMU (10 μM)] for each of the two mat types (hard and soft). The experiment was conducted in duplicate to measure both $CO_2$ and $N_2$ fixation rates, requiring a total of 36 samples of each mat type. Mat samples (1 $cm^2$) were added to the incubation vials (see below) and incubated for 6 h (1000–1600) under ambient irradiance and temperature conditions.

*Comparison with Another Mat.* $CO_2$ and $N_2$ fixation rates of hard and crusty mat types (both flat morphotypes with few epiphytes) were compared with a nonstromatolitic soft mat (dominated by *Schizothrix*) collected from the lagoonal side of Stocking Island. Light and dark rates of $N_2$ and $CO_2$ fixation were determined in triplicate for all three mat types in parallel 5.5 h (0900–1430) incubations under ambient temperature and irradiance.

*Vertical $O_2$ and pH Profiles.* The microscale vertical distributions of dissolved $O_2$ and pH gradients were determined for several different mat types. Oxygen (Diamond General model 768-20R) and pH (Microelectrodes, Inc. model MI-418) needle electrodes were attached to a micromanipulator, and measurements were obtained at 500-μm intervals in the mat. All profiles were obtained during mid-afternoon under full sunlight to coincide with $CO_2$ fixation measurements.

## *Analytical Methods*

*Nitogen ($N_2$) Fixation.* Rates of $N_2$ fixation were estimated using the acetylene reduction (AR) method to assess nitrogenase activity [34]. Samples were obtained by cutting 1-$cm^2$ mat sections with a scalpel.

These were placed in 37-ml glass serum vials with 20 ml of the ambient water and capped with rubber serum stoppers. Acetylene (5 ml), generated from calcium carbide, was injected into the water phase of each sample. Samples were oriented with the top of the mat facing upward and incubated in a flowing water bath covered with 1 layer of neutral density screen (ca. 70% of ambient irradiance). Samples for dark incubations were wrapped in two layers of aluminum foil and placed in the water bath. After incubation, vials were vigorously shaken for 30 s to equilibrate aqueous and gas phases, and 10 ml of the gas phase was displaced into evacuated 15-ml vials for later analysis. Ethylene concentrations were determined by injecting 300 μl of the headspace gas into a gas chromatograph (Shimadzu GC9A, flame ionization detector, 2 m Porapak-T column, 80°C).

*Carbon ($CO_2$) Fixation.* Samples of mat material were collected as described above, placed in 20-ml glass scintillation vials (with polyethylene caps) along with 20 ml of the ambient incubation water and 200 μl of $NaH^{14}CO_3$ (3.5 μCi; 58 μCi μmol$^{-1}$). Incubation procedures were the same as described above. After incubation, the mat sample was dried and fumed with concentrated HCl for 6 h in a covered container to remove unincorporated and abiotically-precipitated $^{14}C$. Dissolved inorganic carbon (DIC) in the incubation water was determined by infrared analysis (Beckman model 864 infrared Analyzer). Samples were stored in scintillation vials for ca. 5 days before counting. The scintillation cocktail (Cyto-Scint, ICN, Inc.; 5 ml) was added and samples stored in darkness 24 h prior to counting. Counts were obtained using a Beckman TD 5000 Liquid Scintillation Counter and converted to DPM using a quench curve (based on calibrated $^{14}C$ hexadecane; NEN) constructed using mat material from Stocking Island. Counting efficiencies for the mat samples ranged from 70 to 90%.

It should be noted that the methodologies used to measure $CO_2$ and $N_2$ fixation in mats have limitations. Utilization of the added substrates may be diffusion-limited and unevenly distributed within the mat. In addition, others have demosntrated that the $^{14}C$ method may underestimate primary production in microbial mats [29]. However, the control treatments in our experimental designs allowed us to evaluate the responses to manipulated variables.

*Photopigments.* Determination of the presence and relative concentrations of chemotaxonomic photopigments (i.e., chlorophylls and carotenoids characteristic of taxonomic groups) allowed an indirect way of comparing the phototroph composition among the different mat types. A total of 57 samples was collected from 9 mat types and the concentrations of 12 photopigments (chlorophyllide *a*, fucoxanthin, *cis*-neoxanthin, violaxanthin, lutein, zeaxanthin, chlorophyll $c_1c_2$, chlorophyll *b*, diadinoxanthin, antheraxanthin, echinenone, and β-carotene) were used to construct a "photopigment profile" for each sample. Photopigment concentrations were normalized to chlorophyll *a* (Chl *a*) and subjected to cluster analysis (Ein*Sight Software Package) to construct a cluster analysis dendrogram. Samples for photopigment analysis were obtained using a scalpel to cut 1 cm$^2$ sections. Mat sections were placed in 20-ml plastic scintillation vials and frozen until later analysis. At 72 h prior to analysis, 10 ml of extraction solvent (45% methanol, 45% acetone, 10% deionized $H_2O$) was added, samples were sonicated for 30 s, covered wih aluminum foil and returned to the freezer (−20°C) to allow slow extraction of photopigments. Photopigments were analyzed by high performance liquid chromatography (HPLC) using an in-line photodiode array spectrophotometer (PDAS) [40]. Photopigments were identified by comparing absorbance spectra and retention times with known standards. After extraction, the remaining mat material (and sediment) was dried for 24 h in a drying oven at 50°C. Dried material was removed from the vials and weighed. Pigment concentrations were normalized to sediment weight or Chl *a* for comparisons and statistical analyses.

*Statistical Analyses.* Statistical analyses for most experiments consisted of a mixed model (models I and II) 2-way analysis of variance (ANOVA) procedure. The assumptions of ANOVA were checked prior to analyses and data transformed when necessary. *A posteriori* multiple comparisons of means was achieved using the Bonferroni procedure with $\alpha = 0.05$.

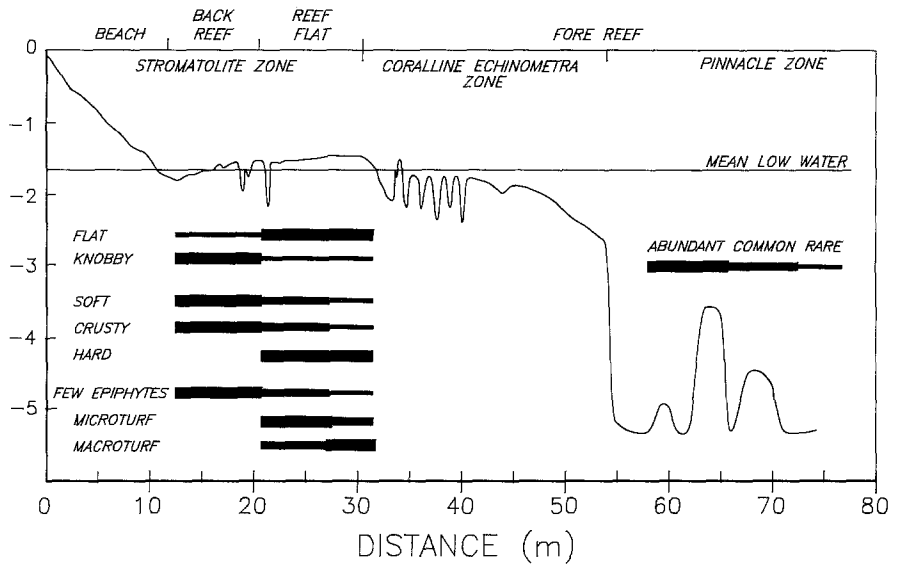


**Fig. 2.** A horizontal transect across the stromatolite zone at Stocking Island. The general distribution and abundance of different mat types along the transect are shown with *horizontal bars*. Terminology for mat types is explained in Table 1.

## Results

The distribution of mat types at Stocking Island along a transect perpendicular to the beach is shown in Fig. 2. Mat types progress from knobby, soft, with few epiphytes at the shoreward end of the transect to microturf and macroturf along the seaward end. Macroturf and (to some degree) microturf mats were generally located in the high-energy surf and splash zone. In the shoreward zone of lower surf energy, epiphyte colonization was rare. The microbial mats covering stromatolites are spatially heterogenous and exhibit distinct variations in community structure. The macroturf areas contain a variety of attached macroalgal species (chlorophytes, rhodophytes, phaeophytes, and coralline algae) in addition to cyanobacterial mat species. Because our purpose was to evaluate the responses of the cyanobacterial components of the mats, we more closely examined those areas that were not covered by macroturf.

The results of the cluster analysis of the concentrations of 12 photopigments in 9 mat types show that, in general, each mat type has a distinctive photopigment profile and suggests that the relative abundance and phototroph species composition differs among the mat types (Fig. 3). The first dichotomy (ca. 55% similarity) separated hard macroturf mats from the others. The second dichotomy (ca. 75% similarity) divides the crusty mats from the soft and nonstromatolitic soft mats. The clustering of mat types into groups with high similarity values in the dendrogram partially validates our field classification system (with respect to phototrophic functional groups). Samples collected from a particular mat type were more similar (in terms of photopigment composition) to one another than samples collected from different mat types. These results suggest that mat phototrophic community compo-

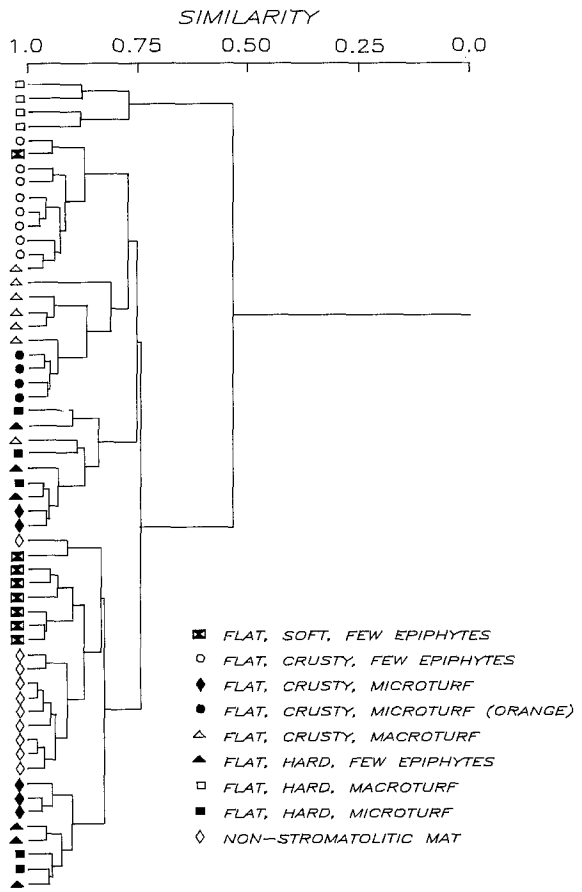


**Fig. 3.** Cluster analysis dendrogram based on the similarity of "photopigment profiles" for nine different mat types. Flat, crusty, microturf (orange) refers to a particular type of flat, crusty, microturf mat that had a distinctive orange coloration. Symbols that are clustered together in the dendrogram indicate that the photopigments for a particular mat type were more similar to one another than to different mat types.

sition differs among the different mat types identified by our field classification system (Table 1).

The three most abundant photopigments (other than Chl *a*) were chlorophyll *b* (Chl *b*), fucoxanthin, and zeaxanthin. Chl *b* is a chemotaxonomic photopigment for chlorophytes (green algae), fucoxanthin is characteristic of chrysophytes (primarily diatoms) and phaeophytes (brown algae), and zeaxanthin is indicative of cyanobacteria [33]. The relative abundances of Chl *b*, fucoxanthin, and zeaxanthin (normalized to Chl *a* concentration) were determined for each of the mat types used in the cluster analysis to further illustrate the variability in the relative abundance of major taxonomic groups in different mat types (Fig. 4). The flat, hard, macroturf mats contain higher concentrations (relative to Chl *a*) of Chl *b*, fucoxanthin, and zeaxanthin than soft and crusty mats, which reflects the low similarity (55%) between hard, macroturf, and other mat types shown in the cluster anlaysis. Crusty mats

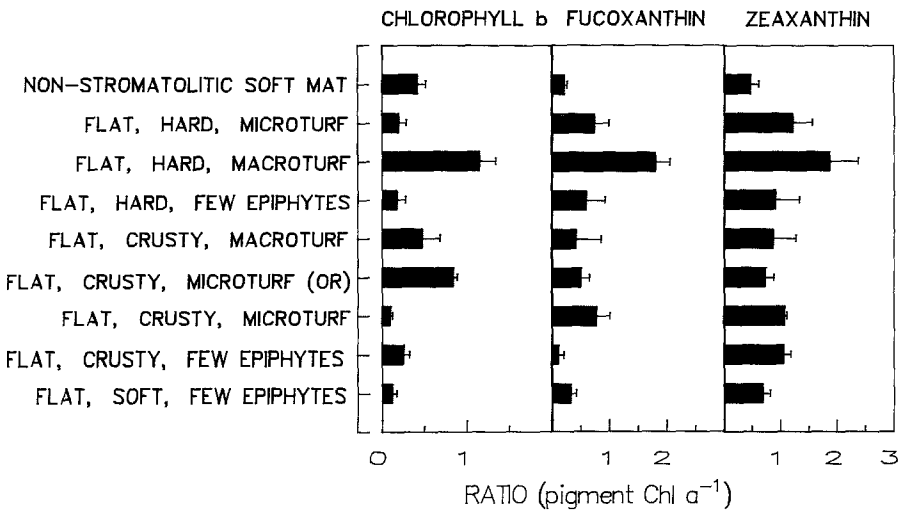


**Fig. 4.** Histograms of three most abundant photopigments (other than Chl *a*) in the different mat types. Photopigment concentrations were normalized to Chl *a*. Values are the mean ± 1 SD.

generally have a higher Chl *b* concentration than soft mats, indicating a higher abundance of chlorophytes.

## Nutrient Addition Bioassay

$CO_2$ fixation rates of flat, crusty, microturf mats were not enhanced by inorganic nutrient and DOC (mannitol) enrichments after a 4-day period (1-way ANOVA, $P < 0.05$) (Fig. 5). However, the phosphate treatment promoted higher rates of $N_2$ fixation than any of the other treatments (1-way ANOVA, $P < 0.01$; Bonferroni, $P < 0.05$).

### *Photoacclimation Responses*

Nine different photopigments were present in sufficient quantities to be identified and quantified (Fig. 6). Changes in the concentration of each photopigment were analyzed using a randomized complete-block ANOVA (block = day, treatment = light exposure level), and a significant light effect was detected for echinenone concentrations only ($P < 0.01$). Multiple comparisons revealed that the full irradiance (control) treatment had significantly higher concentations of echinenone than the screened (20% irradiance) treatment ($P < 0.05$). However, the dark treatment was not significantly different from either the control or the screened treatment. With the exception of echinenone, the flat crusty microturf mat did not reveal altered concentrations of photopigments under reduced light levels.

Photopigment concentrations were also normalized to Chl *a* to determine if ratios of accessory pigments to photosynthetic reaction centers changed over the 4-day

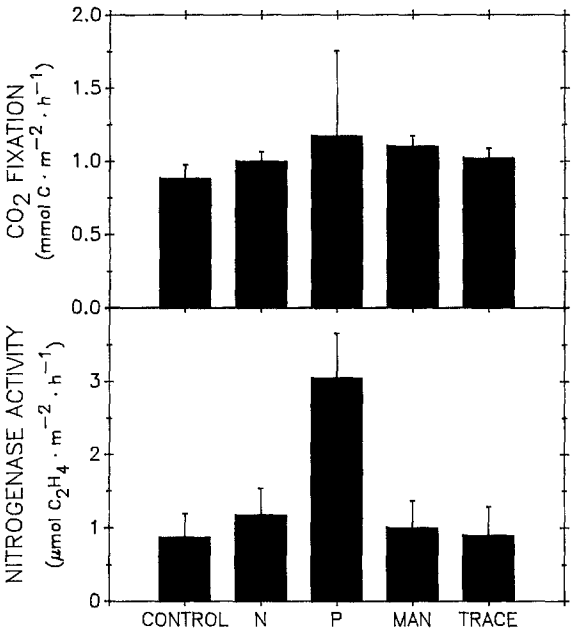


**Fig. 5.** Results of the nutrient addition bioassay using flat, crusty, microturf mats. Abbreviations are N ($NO_3$), P ($PO_4^-$), MAN (mannitol DOC), and TRACE (trace elements). Values are the mean ± 1 SD.

incubation period (Fig. 7). Diadinoxanthin, fucoxanthin, chlorophyll $c_1c_2$, and echinenone all showed significant treatment effects (RCB ANOVA on arcsine square-root transformed values, $P < 0.01$). With the exception of echinenone, screened treatments had significantly higher photopigment to Chl *a* ratios than full irradiance treatments but were not significantly different from dark treatments. Echinenone to Chl *a* ratios were significantly higher in the control than in the screened and dark treatments ($P < 0.05$). These results suggest that the flat, crusty, microturf mat increased the amount of diadinoxanthin, fucoxanthin, chlorophyll $c_1c_2$, and decreased echinenone per number of reaction centers in order to photoacclimate to lower light levels.

### *Inhibitor and DOC Effects on $N_2$ and $CO_2$ Fixation*

The effects of DCMU and DOC (mannitol) additions on $N_2$ and $CO_2$ fixation rates of *Schizothrix*-dominated soft and hard mat types (both flat morphotypes with few epiphytes) were determined (Fig. 8). The soft mat had a significantly higher rate of $CO_2$ fixation than the hard mat (2-way ANOVA, $P < 0.01$). For both mat types, there was a significant treatment effect on $CO_2$ fixation ($P < 0.01$), with the DCMU, formalin, and dark treatments being lower than the control and mannitol treatments. The similarity between the dark and DCMU-incubated samples indicated that there was no measurable bacterial anoxygenic photosynthesis (which is insensitive to DCMU) in either of the mat types. The addition of DOC did not

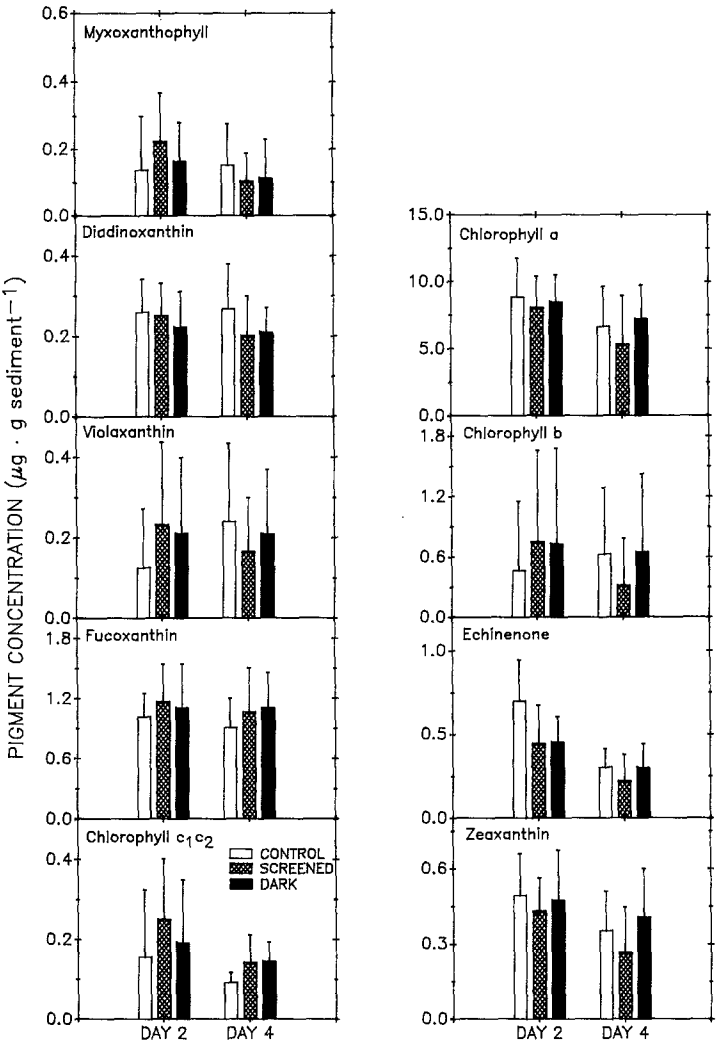


**Fig. 6.** Results of the photoacclimation experiment showing changes in photopigment concentrations in the three treatments (control, screened, dark) after 2 and 4 days. Values are the mean ± 1 SD.

enhance $CO_2$ fixation. The hard mats consistently exhibited higher $N_2$ fixation rates than the soft mats during the incubation period (2-way ANOVA, $P < 0.01$). There was also a significant treatment effect on $N_2$ fixation ($P < 0.01$), with DCMU amended rates being higher than the control and other additions. The dark treatment was lower than the control ($P < 0.05$). The results of this experiment suggest that, under identical incubation conditions, soft mats have higher $CO_2$ fixation rates than hard mats, but hard mats have higher rates of $N_2$ fixation than soft mats. The addition of DCMU to these mats resulted in a near doubling of rates of $N_2$ fixation for both mat types.

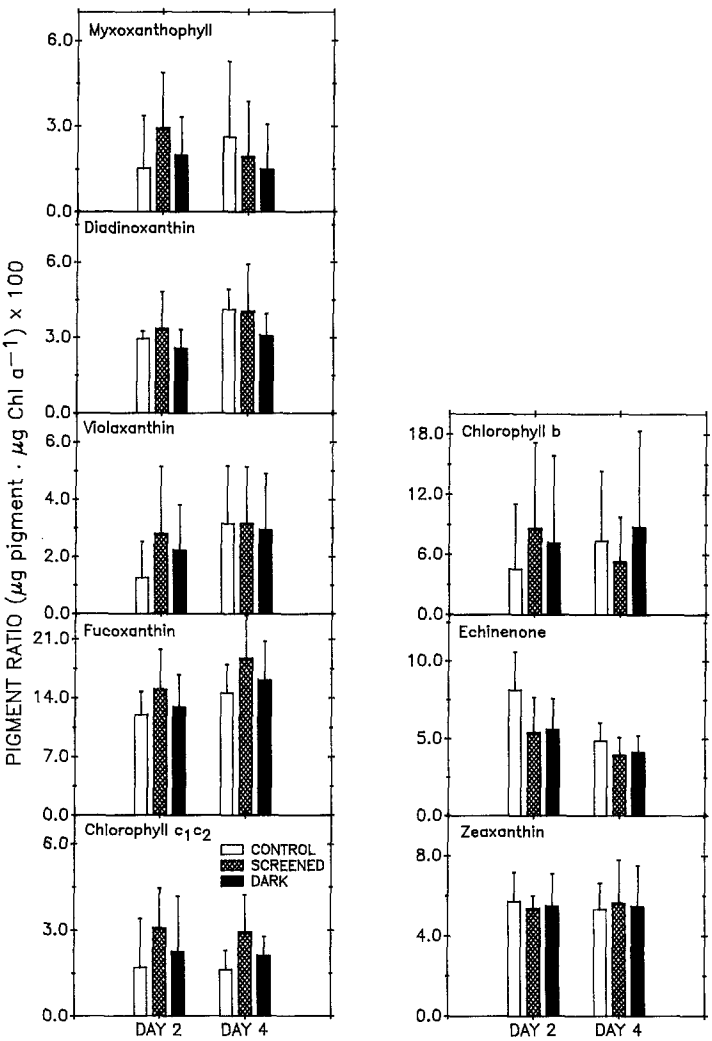


**Fig. 7.** Results of the photoacclimation experiment showing changes in the ratio of each photopigment to Chl *a* in three treatments (control, screened, dark) after 2 and 4 days. Values are the mean ± 1 SD.

### *Rates Comparisons for Different Mat Types*

For $CO_2$ fixation, overall rates were significantly different for each *Schizothrix*-dominated mat type (2-way ANOVA, $P < 0.01$) (Fig. 9). The crusty mats with few epiphytes exhibited the highest $CO_2$ fixation rates, followed by the non-stromatolitic soft mat and hard mats with few epiphytes. Similarly, light and dark $N_2$ fixation rates were significantly different (2-way ANOVA, $P < 0.01$). The non-stromatolitic soft mat had the highest rates of $N_2$ fixation, followed by crusty, and hard mats. Dark rates of $N_2$ fixation were very low relative to the light rates for all

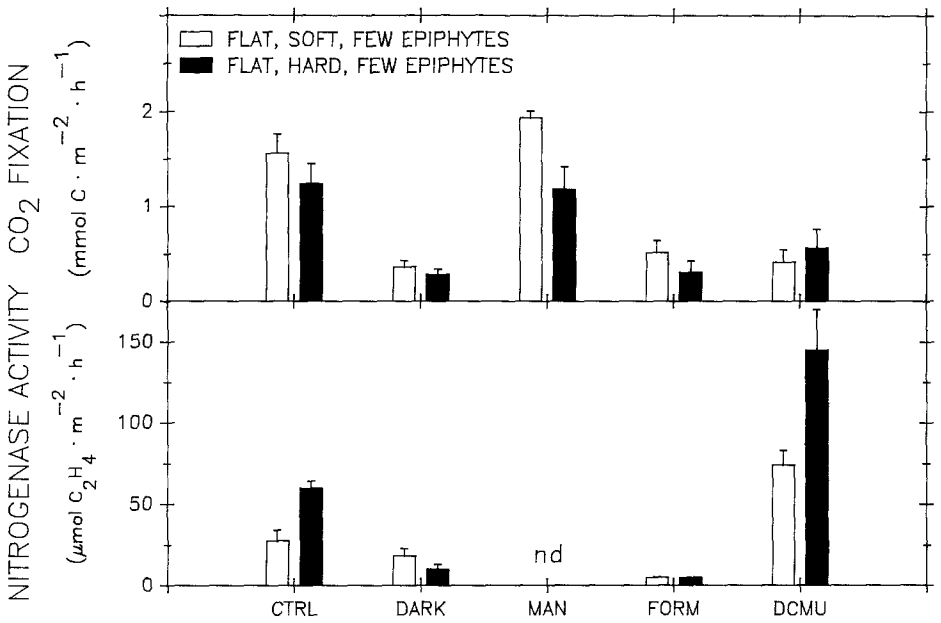


**Fig. 8.** Results of the addition of inhibitors and DOC on $CO_2$ and $N_2$ fixation by soft and hard mats with few epiphytes. Abbreviations are CTRL (control), DARK (dark incubation), MAN (mannitol DOC), and FORM (formalin). Values are the mean ± 1 SD.

three mat types. Rates of $CO_2$ and $N_2$ fixation for several mat types, based on control values from the above experiments, are compared in Table 2. Soft and hard mats with few epiphytes or microturfs have lower $CO_2$ and $N_2$ fixation rates than crusty and nonstromatolitic soft mats.

### *Vertical $O_2$ and pH Profiles*

For crusty, microturf, and nonstromatolitic soft mat types, the microscale vertical distribution of pH in the upper 5 mm of the mat exceeded 7.5 and generally increased (pH 8 to 9) with depth. The dissolved $O_2$ concentration ranged from 100 to 250% saturation and was at or above 100% saturation over the entire 5-mm depth interval in flat crusty and flat hard mats (with few epiphytes). The most notable features of these profiles are the absence of anoxic microzones or anoxic layers within the mat and the relatively high pH within the respective mats.

## Discussion

Our field classification system for intertidal microbial mats on stromatolites at Stocking Island (Table 1) was based primarily on physical characteristics. Chemotaxonomic photopigment analyses of each mat type demonstrated that the photopigments and relative quantities of photopigments vary among mat types. The variations in identities and quantities of photopigments may be attributed to differences

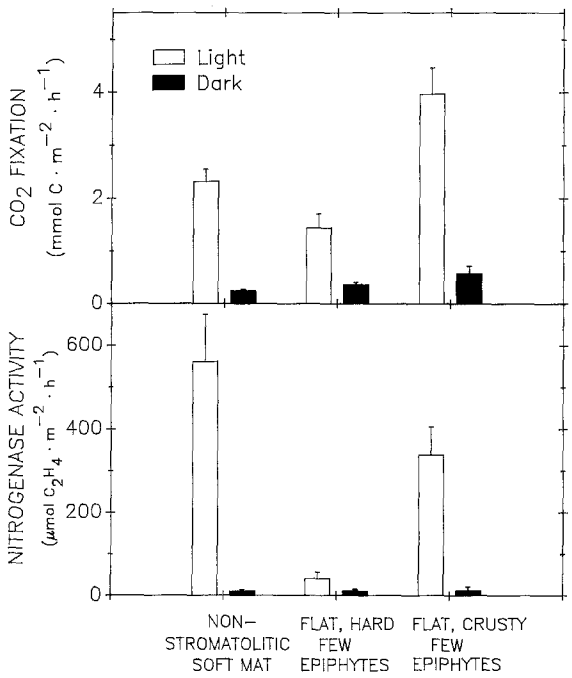


**Fig. 9.** $N_2$ and $CO_2$ fixation rates of three *Schizothrix*-dominated mat types. Values are the mean ± 1 SD.

**Table 2.** Comparison of production and nitrogenase activity rates for a variety of microbial mat types

| | $CO_2$ Fixation (mmol C $m^{-2}$ $h^{-1}$) | | Nitrogenase activity (μmol $C_2H_4 m^{-2}$ $h^{-1}$) | |
|---|---|---|---|---|
| Mat type | Light | Dark | Light | Dark |
| Flat, soft, few epiphytes | 1.56 ± 0.20 | 0.37 ± 0.07 | 27.7 ± 6.6 | 18.5 ± 4.5 |
| Flat, hard, few epiphytes | 1.26 ± 0.29 | 0.29 ± 0.06 | 49.5 ± 18.4 | 10.3 ± 3.0 |
| Flat, hard, few epiphytes | 1.42 ± 0.32 | 0.37 ± 0.04 | 40.8 ± 15.4 | 12.1 ± 3.4 |
| Flat, hard, microturf | 1.05 ± 0.37 | nd[a] | 0.9 ± 0.6 | nd |
| Flat, hard, microturf | 0.88 ± 0.18 | nd | nd | nd |
| Flat, crusty, few epiphytes | 3.96 ± 0.60 | 0.58 ± 0.14 | 338.4 ± 68.1 | 13.0 ± 8.6 |
| Flat, crusty, microturf | 2.28 ± 0.29 | 0.20 ± 0.09 | 312.5 ± 17.1 | 13.5 ± 2.6 |
| Nonstromatolitic soft | 2.32 ± 0.23 | 0.24 ± 0.03 | 561.2 ± 113.6 | 11.2 ± 3.5 |

[a] nd, Rates not determined

in the phototrophic community composition or possibly photoacclimation responses by the same assemblage [8, 20, 30, 33]. Differences in $N_2$ and $CO_2$ fixation rates for the various mat types provide further support for the hypothesis that mat types are physiologically distinct. Therefore our results indicate that the microbial mats in the stromatolite zone of Stocking Island are spatially diverse with respect to species composition and production-related processes.

Mat types exhibited a horizontal zonation that shifted from soft to crusty to hard in a seaward direction. Similarly, epiphyte growth progressed from total absence to

microturf to macroturf along the horizontal gradient. Although we cannot explain the mechanisms responsible for this gradient, it is likely that wave exposure, duration of subaerial exposure at low tide, and relative hardness of the substrate are influential factors [13, 28]. Grazing, especially in the intertidal, is not likely to be an important factor because herbivores are rare in the stromatolite zone (R. Steneck, pers. comm.).

Absolute concentrations of photopigments (except for echinenone) were not altered in flat, crusty, microturf mats when they were exposed to a lower irradiance (20% of ambient). However, when accessory pigment concentrations were normalized to the total number of photosynthetic reaction centers (Chl *a*), the ratios of diadinoxanthin, fucoxanthin, and chlorophyll $c_1c_2$ to Chl *a* increased in the shaded treatments but not in the dark treatments. Diadinoxanthin, fucoxanthin, and chlorophyll $c_1c_2$ are accessory photopigments diagnostic of diatoms (Chrysophyceae) which are commonly used to photoacclimate to low-light environments [8, 11, 12, 26, 33]. Echinenone, a cyanobacterial photopigment, plays a photoprotective role in high-light surface-dwelling planktonic cyanobacteria, and the reduction in echinenone at low irradiances may reflect a photoacclimation response by cyanobacteria [20]. In general, the dark treatment in this experiment did not differ from the controls (100% irradiance). The absence of a photoacclimation response in the dark treatments may be due to low community growth rates and hence an inability of phototrophs to rapidly synthesize additional photopigments *de novo*. One implication of these results is that as mat layers become buried by new growth, the diatoms trapped in the deeper layers will photoacclimate to the lower irradiance levels. By increasing carotenoid levels, diatoms may be able to capture enough light energy to sustain photosynthesis. The cyanobacterial component of these mats does not seem to readily photoacclimate to lower light levels and may exhibit a diminished capacity for photosynthesis at lower light levels following burial. Additional experiments, with a gradient of light values coupled with photophysiological measurements (i.e., PI-curves), should be undertaken to better characterize mat responses to changes in the ambient light environment.

Nitate, phosphate, DOC, and trace metal additions to flat, crusty, microturf mats did not enhance $CO_2$ fixation during the 4-day experimental period. This result suggests that factor(s) other than nutrients limit growth of this particular mat type. Similarly, Paerl et al. [24] showed that nutrient and DOC additions failed to increase $CO_2$ fixation in a wide variety of tropical, subtropical, and temperate microbial mat communities during 3- to 4-day bioassay incubations. One possible explanation for the absence of nutrient stimulation may be that mats require longer exposure times to elevated nutrient levels before exhibiting a measurable response [24]. Rates of $CO_2$ fixation by the Stocking Island stromatolitic mats were low (ca. 10–20 mg C $m^{-2}h^{-1}$) compared to microbial mats in North Carolina (ca. 50–100 mg C $m^{-2}$ $h^{-1}$) [1, 24] and Tomales Bay, California (ca. 60–120 mg C $m^{-2}$ $h^{-1}$) [24]. However, rates were comparable to stromatolitic mats in the hypersaline Storr's lake on San Salvador Island, Bahamas, where nutrient limitation was also undetectable (B. Bebout, Ph.D. thesis and [27]). The low rates of $CO_2$ fixation indicate low productivity and growth rates for the Stocking Island mats and may offer a possible explanation for our inability to detect nutrient limitation within a 4-day bioassay exposure period. Longer incubations may be necessary to document the effects of nutrient enhancement on $CO_2$ fixation.

The $N_2$ fixation rates measured in the nutrient bioassay were low compared to mats of the same type that were not preincubated for 4 days. However, the addition of phosphate tripled $N_2$ fixation rates. Phosphate availability has been shown to be limited in some carbonate environments [14], but it is unclear why the addition of this nutrient has such a profound effect on $N_2$ fixation by Stocking Island mats. DOC commonly stimulates $N_2$ fixation in temperate and subtropical mats, and Paerl et al. [24] suggested that the DOC is either used directly as an energy source or promotes the formation of anoxic microzones needed to support the $N_2$ fixing process. Perhaps DOC additions result in an instantaneous stimulation of $N_2$ fixation, and the effect is dissipated over long incubation periods such as the 4-day preincubation in our experiment. A similar nutrient bioassay experiment on microbial mats in a hypersaline lagoon on a nearby island (San Salvador, Bahamas) also showed phosphate stimulation and a lack of DOC enhancement of $N_2$ fixation in short-term (3-day) nutrient addition bioassays [27].

The addition of DCMU greatly reduced $CO_2$ fixation in soft and hard mats, indicating that $CO_2$ fixation was reliant on photosystem II (PSII) activity and that the contribution of anoxygenic photosynthesis (by bacteria) was negligible. In contrast, $N_2$ fixation was enhanced by the addition of DCMU. Although these results support earlier conclusions that DCMU-stimulated $N_2$ fixation is proof of in situ $O_2$ inhibition of nitrogenase (i.e., DCMU alleviates $O_2$ inhibition of nitrogenase), Bebout et al. [2] have suggested several alternative mechanisms by which DCMU may also enhance the role of photosystem I (PS I) in supporting $N_2$ fixation in microbial mats. In the bioassay experiment, DCMU-enhanced rates far exceeded the dark rates, indicating that light (exclusively utilized by PS I) plays a role in enhancing nitrogenase activity.

Because of possible differences in the rates at which radiolabeled bicarbonate becomes available to the photosynthetic members of each of the mat communities, photosynthetic rates determined by the incorporation of this tracer may not be directly compared across communities. Incorporation of radiolabeled bicarbonate in these communities probably does not reflect total carbon fixation but rather fixation of inorganic carbon derived from the water column (the pool traced by added bicarbonate). Similar problems may exist in cross-community comparisons using acetylene reduction as a measure of nitrogen fixation. However, this is less likely because the diffusion of acetylene into the mat communities is not likely to limit the reduction of this gas, and there is no production of acetylene within the community that would dilute the added acetylene.

Another important consideration is that these rates may exhibit longer term (daily to weekly) periodicities that were not measured in this study. For example, mat $N_2$ fixation rates are known to vary by as much as an order of magnitude over a diel cycle [1, 2, 35]. Long-term (i.e., $>2$ h) periodicities in fixation rates must be documented in order to determine the relative contributions of the different mat types examined in this study. The relative abundance of heterocystous vs. nonheterocystous cyanobacterial species within the different mat types may further complicate short term measurements. However, our results do provide an interesting "snapshot" of the relative $CO_2$ and $N_2$ fixation rates of the different mat types during the daytime and during a particular season.

Crusty mat (with few epiphytes) $CO_2$ fixation rates were nearly double those obtained for nonstromatolitic soft mats and hard mats. Although the nonstroma-

tolitic mat exhibited the highest rate of $N_2$ fixation, crusty mat $N_2$ fixation was nearly 6 times higher than that of the hard mat. In all cases, dark $N_2$ fixation was barely detectable and suggests a tight coupling between photosynthesis (oxygenic and/or anoxygenic) and nitrogenase activity. Other studies have shown that dark rates may, at times, exceed light rates due to photosynthetic $O_2$ inhibition of nitrogenase activity [2]. The incubations for this experiment were initiated in early morning (0900), when the mats may not have had sufficient light exposure to build up energy reserves necessary to support high $N_2$ fixation rates [2].

The relatively high pH (>7.5) within the matrix of all mat types is conducive to carbonate precipitation and lithification [6, 17, 19]. The absence of anoxic microzones and layers within these mats suggests that respiration rates and chemical oxygen demand are very low relative to diffusive and $O_2$ production (photosynthetic) processes. In contrast to nonlithifying mats, stromatolitic mats exhibit a much higher production-to-respiration ratio (P:R). Although production ($CO_2$ fixation) is low, respiration also appears to be low, suggesting that heterotrophic utilization of phototrophic biomass is small. High P:R seems to be a common characteristic of mats associated with lithified structures in a variety of tropical and subtropical environments (B. Bebout, Ph.D. thesis and [15, 18, 27]) and may play a key role in the lithification process in these stromatolites.

A comparison of $CO_2$ and $N_2$ fixation rates of the various stromatolitic mat types of Stocking Island indicates distinct differences and trends (Table 2). Soft and hard mats have similar $CO_2$ fixation rates, but crusty mats appear to fix $CO_2$ derived from the water column at more than twice the rate of other mat types under identical incubation conditions. In general, $N_2$ fixation rates for Stocking Island mats are comparable to other temperate and subtropical mats [2, 24] and almost double the rates measured in the hypersaline Storr's Lake [27]. Crusty mats seem to fix $N_2$ at nearly 6 times the rate of hard and soft mats. Although *Schizothrix* is the dominant cyanobacterial species in these mat types, the large rate disparity may be attributable to differences in the diel maxima of $N_2$ fixation by the other (less abundant) community components.

One hypothesis is that the soft mats represent an early stage of colonization and sediment stabilization; this stage is followed by active growth that results in the formation of partially-lithified crusty mats that eventually become highly lithified and form what we term hard mats. The high degree of lithification, which we suspect is related to high P:R ratios, slows diffusive properties and ultimately limits growth of microbial mats.

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