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# Anoxygenic Photosynthesis and Nitrogen Fixation by a Microbial Mat Community in a Bahamian Hypersaline Lagoon

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**Simultaneous measurements of photosynthesis (both oxygenic and anoxygenic) and $N_2$ fixation were conducted to discern the relationships between photosynthesis, $N_2$ fixation, and environmental factors potentially regulating these processes in microbial mats in a tropical hypersaline lagoon (Salt Pond, San Salvador Island, Bahamas). Major photoautotrophs included cyanobacteria, purple phototrophic bacteria, and diatoms. Chemosystematic photopigments were used as indicators of the relative abundance of mat phototrophs. Experimental manipulations consisted of light and dark incubations of intact mat samples exposed to the photosystem II inhibitor DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea], a dissolved organic carbon source (D-glucose), and normal seawater (37‰). Photosynthetic rates were measured by both $O_2$ and $^{14}C$ methods, and nitrogenase activity (NA) was estimated by the acetylene reduction assay. Moderate reductions in salinity (from 74 to 37‰) had no measurable effect on photosynthesis, $O_2$ consumption, or NA. $CO_2$ fixation in DCMU-amended samples was ~25% of that in the control (nonamended) samples and demonstrated photosynthetic activity by anoxygenic phototrophs. NA in DCMU-amended samples, which was consistently higher (by a factor of 2 to 3) than the other (light and dark) treatments, was also attributed to purple phototrophic bacteria. The ecological implication is that $N_2$ fixation by anoxygenic phototrophs (purple phototrophic bacteria and possibly cyanobacteria) may be regulated by the activity of oxygenic phototrophs (cyanobacteria and diatoms). Consortial interactions that enhance the physiological plasticity of the mat community may be a key for optimizing production, $N_2$ fixation, and persistence in these extreme environments.**

Microbial mats are laminated communities that form interdependent layers of vertically stratified heterotrophs, chemoautotrophs, and photoautotrophs in aquatic habitats (35). The cohesive, turf-like matrix is constructed of interwoven cyanobacterial filaments (trichomes) covering the sediment surface. Mat microbes are aligned in discrete vertical layers that conform to chemical and light gradients within the mat (6, 8, 10, 29). These microbial communities are highly productive and significant contributors to carbon (C) and nitrogen (N) cycling in some shallow-water habitats (12, 17, 28). Primary productivity ($CO_2$ fixation) and the biological conversion of $N_2$ to $NH_3$ ($N_2$ fixation) are two major ecophysiological processes that determine the potential for phototrophic community growth (2, 17, 29). Nitrogen fixation, used by some prokaryotes (diazotrophs) to avoid chronic N limitation in oligotrophic systems, may meet N demands or provide supplementary "new" N for growth by N-requiring phototrophs (2, 17, 19, 34). The chemical and ecological interactions of $N_2$ fixation, $CO_2$ fixation, and related C and N cycling are of fundamental importance in controlling mat production. However, the contributions of individual mat components to the emergent properties (productivity, $N_2$ fixation, etc.) of the community are poorly understood.

Mat production is regulated by the complex interactions between C, N, and other nutrient (biogeochemical) cycles within the mat matrix and external exchange with adjacent environments (i.e., sediments, overlying water, and the atmosphere) (12). Although nitrogen dynamics in mats have been studied (2, 19, 23, 29), simultaneous measurements of $CO_2$ fixation, oxygenic-anoxygenic photosynthesis, and $N_2$ fixation are needed to understand the primary factors and mechanisms controlling community production. The contribution of each of the major phototrophic mat consortia (cyanobacteria, purple phototrophic bacteria, and eukaryotes) must be considered to understand the processes involved in structuring and maintaining these complex communities (8, 18).

The Bahamian Islands have many small hypersaline lagoons supporting benthic microbial mat communities (4, 16, 23). The calm, protective conditions and shallow waters of these lagoons provide an ideal habitat for the prolific growth of both structurally and functionally well-developed mats. Microbial mats in these lagoons are appropriate models because the stability of these systems promotes close coupling of biogeochemical gradients and community structure (6, 18). In addition, this stability leads to the development of distinct layers (community stratification) of physiologically diverse microbiota engaging in consortial interactions. The purpose of this study was to examine the relationship between oxygenic and anoxygenic mat photosynthesis, nitrogen fixation, and factors potentially controlling these processes in a typical Bahamian hypersaline lagoon habitat.

## MATERIALS AND METHODS

San Salvador Island (24°N, 72°W), in the eastern Bahamas, contains several shallow ponds and hypersaline lagoons (Fig. 1). Lagoon salinities vary from 45 to >100‰ seasonally, depending on rainfall during the wet season (summer and fall). Salt Pond is a small (0.05-$km^2$) enclosed, shallow (<0.5-m) hypersaline (74‰) lagoon carpeted with a thick (0.5- to 1.0-cm) mat dominated by cyanobacteria. The mat community is composed primarily of the filamentous cyanobacteria *Microcoleus chthonoplastes*, with a small amount (<5%) of *Lyngbya* spp. underlain by a layer of purple sulfur bacteria (*Chromatium* spp.) and covered by a thin film of benthic diatoms. Deep cores reveal alternating periods of prolific mat growth, sedimentation events, and a layer of pure gypsum at ~1.0 m in the sediment. The pH of the hypersaline water in Salt Pond is 7.95 to 8.00.

Large sections (500 $cm^2$ by 5 cm) of mat were collected on 23 to 29 March 1996 from Salt Pond and transported to the field station, and cores were obtained for individual incubations and photopigment analyses. Experimental manipulations consisted of light and dark incubations of mat samples exposed to the photosys-

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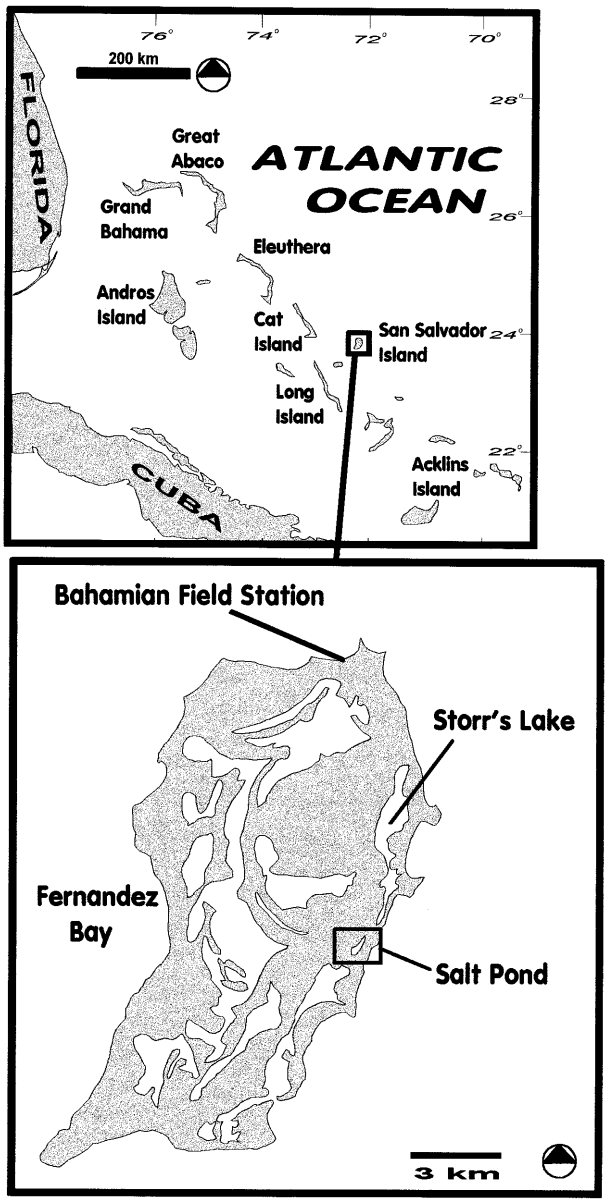


FIG. 1. Maps of San Salvador Island, Bahamas, and Salt Pond, a hypersaline lagoon containing the microbial mats examined in this study.

tem II inhibitor DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea; 10 μM final concentration], a dissolved organic carbon (DOC) source (DOC as D-glucose; 5.0 mM), and normal seawater (37‰). The final concentration of ethanol, associated with DCMU additions, was less than 0.25 μM and should not enhance heterotrophic activities over the short (<3-h) incubation periods. Incubations were conducted outside under ambient irradiance in small pools continuously flushed with flowing seawater for temperature control. For dark incubations, containers were wrapped in three layers of aluminum foil and submerged in the incubation pools. Air temperatures during the daytime period ranged from 24.1 to 32.9°C, and water temperatures varied from 27.1 to 34.6°C. All incubations were conducted between 10:00 a.m. and 2:00 p.m.

Photosynthetic rates of intact mat cores were measured by both $O_2$ and $^{14}C$ methods. For $O_2$ incubations, mat cores (18.5 $cm^2$ by 0.5 cm) were placed in clear plastic bags (1-pt Ziploc bags) and filled with water (100 to 300 ml) collected from Salt Pond. Bags containing the mat cores were submerged in a water bath, slowly filled with water, and then sealed underwater to minimize aeration and exclude air bubbles. The flexible plastic bags also facilitated gentle mixing of the water during incubations. The dissolved $O_2$ concentration and pH were measured simultaneously at fixed intervals during incubations. Incubation water (500 μl) was withdrawn from the sealed plastic bags with a gas-tight syringe and injected into a small-volume sample chamber (200 μl; Cameron Instruments BC202 gas cell and BGM 200 analyzer). The partial pressure of dissolved oxygen ($pO_2$) was determined with a Clarke-style oxygen electrode, and the pH was measured with a glass pH electrode. For these experiments, $O_2$ production was defined as the net increase in $O_2$ concentration per unit time and was equivalent to oxygenic net primary productivity. $O_2$ consumption was defined as the net reduction in $O_2$ concentration per unit time and represents the sum of aerobic respiration and abiotic oxygen consumption. Preliminary incubations were undertaken to determine optimal incubation times, assess the effects of elevated $pO_2$ on photosynthetic rates, and compare $O_2$ and $^{14}C$ methods.

For carbon ($CO_2$) fixation measurements, cores (1.15 $cm^2$ by 0.5 cm) were placed in 20-ml glass scintillation vials (with polypropylene caps) along with 20 ml of the incubation water and 0.2 ml of $NaH^{14}CO_3$ (3.5 μCi; 58 μCi $\mu mol^{-1}$). Following incubations, mat plugs were placed on drying paper, dried, and fumed with concentrated HCl for 6 h in a covered container to remove unincorporated and abiotically precipitated $^{14}C$. Preliminary experiments indicated that 6 h was sufficient to remove all inorganic $^{14}C$ (19). Scintillation cocktail (5 ml of Cyto-Scint [ICN Inc.]) was added, and each sample was stored in the dark for 24 h before being counted. Counts (Beckman TD 5000 liquid scintillation counter) were converted to disintegrations per minute by using a quench curve based on calibrated $^{14}C$ hexadecane (NEN, Inc.). The amount of dissolved inorganic carbon in the incubation water was measured by infrared gas analysis (Beckman model 864 infrared analyzer). $CO_2$ uptake rates for light-incubated samples were corrected for dark $CO_2$ uptake (i.e., $CO_2$ fixation = light uptake − dark uptake).

$N_2$ fixation was estimated by the activity of the $N_2$-fixing enzyme complex, nitrogenase. Nitrogenase activity (NA) was determined by the acetylene reduction assay (1, 31). Cores (1.15 $cm^2$ by 0.5 cm) were placed in 15-ml glass serum vials, 6 ml of incubation water was added, and the vials were capped with rubber serum stoppers. Acetylene (2 ml), generated from calcium carbide, was injected into each sample. After incubation, the vials were shaken vigorously for 30 s to equilibrate acetylene and ethylene, and 2 ml of the gas phase was transferred into evacuated 2-ml vials. Ethylene concentrations were quantified by injecting 300 μl of the headspace gas into a gas chromatograph (Shimadzu model GC9A with a flame ionization detector and a 2-m Porapak-T column at 80°C).

Core samples (0.50 $cm^2$ by 0.5 cm) for photopigment analysis were collected, stored in 2-ml microcentrifuge tubes, wrapped in foil, and frozen. Photopigment samples were sonicated (30 s) and extracted in 1.5 ml of solvent (45% methanol, 45% acetone, 10% deionized $H_2O$) (5, 22). Chemosystematic carotenoids and chlorophylls were used as indicators of the relative abundance of major microalgal groups (i.e., cyanobacteria, purple phototrophic bacteria, diatoms) (25). Photopigment quantification was achieved by high-performance liquid chromatography (14, 26, 32). Three reverse-phase $C_{18}$ columns were connected in series to optimize photopigment separations. The column order was a single monomeric reverse-phase $C_{18}$ column (Rainin Microsorb-MV; 0.46 by 10 cm, 3-μm packing) followed by two polymeric reverse-phase $C_{18}$ columns (Vydac 201TP; 0.46 by 25 cm, 5-μm packing). The nonlinear, variable-flow, binary gradient consisted of solvent A (80% methanol, 20% ammonium acetate [0.5 M adjusted to pH 7.2]) and B (80% methanol, 20% acetone) (26, 33). Absorbance spectra (380 to 670 nm; Shimadzu SPDm10av photodiode array detector) were obtained at 2-s intervals. Pigment peaks were identified and quantified at 440 nm (605 nm for bacteriochlorophyll *a*) by comparison of retention times and absorption spectra with authentic crystalline standards, including chlorophylls *a* and *b*, bacteriochlorophyll *a*, β-carotene (Sigma Chemical Co.), fucoxanthin, and zeaxanthin (Hoffman-LaRoche & Co.). All other pigments were identified by comparison to pigments extracted from phytoplankton cultures (36) and quantified by using the appropriate extinction coefficients (27).

Nonparametric statistical procedures (Wilcoxon rank sum W test, Kruskal-Wallis one-way analysis of variance) were used for comparisons of experimental treatment groups. A posteriori comparisons of means for multiple treatments were performed by a Bonferroni-type nonparametric procedure ($P < 0.05$) (15).

## RESULTS

The most abundant photopigments in the upper 5 mm of the Salt Pond microbial mat were chlorophyll *a* (29.36 ± 5.89 nmol $cm^{-2}$, mean ± 1 standard deviation [SD]), bacteriochlorophyll *a* (19.23 ± 5.48 nmol $cm^{-2}$), and zeaxanthin (18.68 ± 2.06 nmol $cm^{-2}$) (Fig. 2). These pigments are characteristic of oxygenic phototrophs, purple phototrophic bacteria, and cyanobacteria, respectively. Lower concentrations of diatoxanthin and fucoxanthin (diatoms) and of echinenone and myxoxanthophyll (cyanobacteria) were also detected. These data, combined with qualitative microscopic observations, indicate a mat phototroph community composed primarily of filamentous cyanobacteria and purple sulfur bacteria. Benthic diatoms, although present, were a minor component of the microalgal assemblage.

Although the response was highly variable, oxygenic photo-

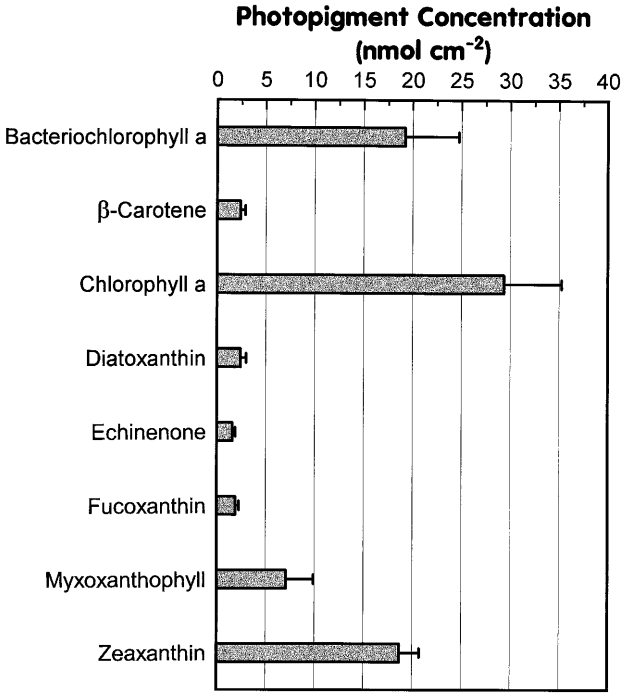


FIG. 2. Photopigment concentrations in the Salt Pond microbial mat. Values are the mean ± 1 SD for 10 samples.

synthesis (in nanomoles of $O_2$ per square centimeter per hour) was significantly reduced at higher $pO_2$ levels ($r^2 = 0.25$, $P < 0.01$, $n = 39$) (Fig. 3). The most severe inhibition and consistent response occurred at $pO_2$ greater than 23.33 kPa (125% $O_2$ saturation) and corresponded to incubation times of >2 h with our experimental setup. The $pO_2$ changes associated with oxygenic photosynthesis and $O_2$ consumption were also highly correlated with pH ($r^2 = 0.74$, $P < 0.001$, $n = 181$) (Fig. 4). A doubling in $pO_2$ translated into an increase of 0.4 pH unit in the overlying incubation water within the enclosed containers. Short incubation times had minimal impacts on mat oxygenic production, and all subsequent measurements of oxygenic photosynthesis were limited to <1 h.

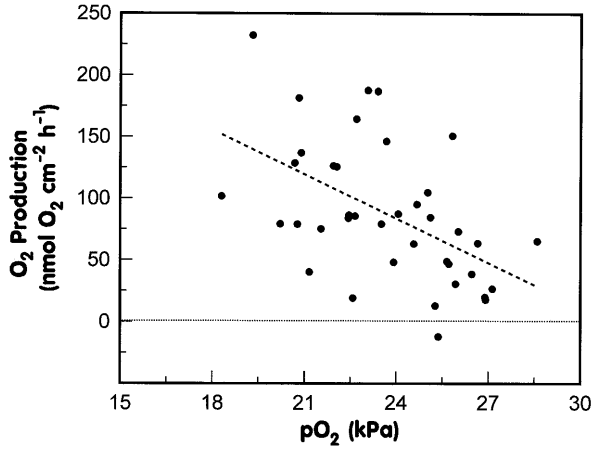


FIG. 3. Rate of oxygen ($O_2$) production plotted against the partial pressure of oxygen ($pO_2$) in the enclosed sample containers. The dashed line indicates the results of linear regression analysis ($r^2 = 0.252$, $P < 0.01$, $n = 39$).

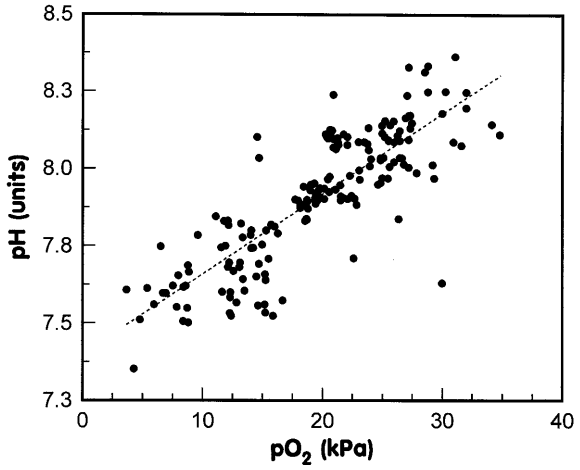


FIG. 4. pH plotted against the partial pressure of oxygen ($pO_2$) in the enclosed sample containers. The dashed line indicates the results of linear regression analysis ($r^2 = 0.744$, $P < 0.01$, $n = 181$).

The $O_2$ and $^{14}C$ methods for measuring photosynthetic rates were compared by using samples exposed to identical incubation conditions (Fig. 5). The $^{14}C$ method measures C assimilation associated with photosynthesis, while the $O_2$ method evaluates net oxygenic photosynthesis. The $O_2$ method gave rates that were more than double those of the $^{14}C$ method and suggests that the $^{14}C$ method underestimated oxygenic photosynthesis for this mat community. The relationship between the two methods was fairly constant within sample groups but highly variable between different sample groups and incubations. Calculations based on the water and mat volumes used for the $^{14}C$ incubations indicated that $pO_2$ levels were below the threshold (i.e., 23.33 kPa or 125% $O_2$ saturation) for oxygenic inhibition of photosynthesis. Dark uptake rates of $^{14}C$ were <1% of the light uptake rates, clearly indicating that the HCl fuming procedure effectively removed inorganic $^{14}C$ from the samples and that the dark uptake of $^{14}C$ was very low. Although the photosynthetic rates determined by the $O_2$ and $^{14}C$ methods were not equivalent, the relative rates (i.e., treat-

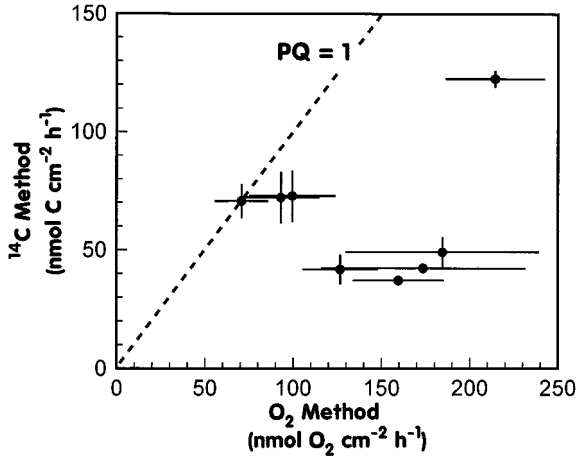


FIG. 5. Comparison of mat productivities by the $^{14}C$ and $O_2$ methods. The dashed line indicates a photosynthetic quotient (PQ) of 1 and illustrates a hypothetical 1 mol of $O_2$ evolved per mol of C fixed. Values are the mean ± 1 SD for five samples.

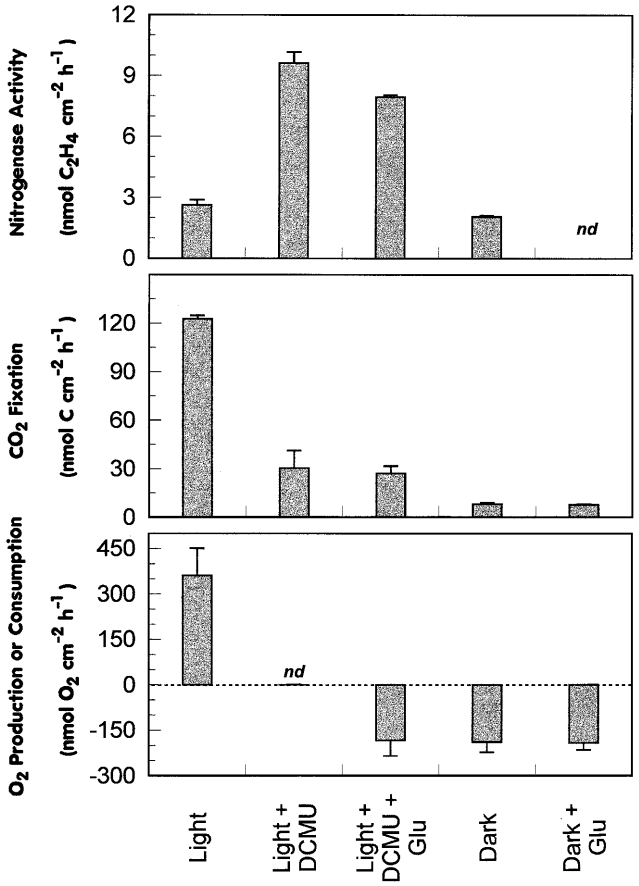


FIG. 6. Results of simultaneous measurements of $O_2$ production (positive values) or consumption (negative values), $CO_2$ fixation, and NA following experimental manipulations. Values are the mean ± 1 SD for five samples. The notation *nd* indicates that rates were not determined, and Glu is an abbreviation for the DOC addition (i.e., D-glucose).

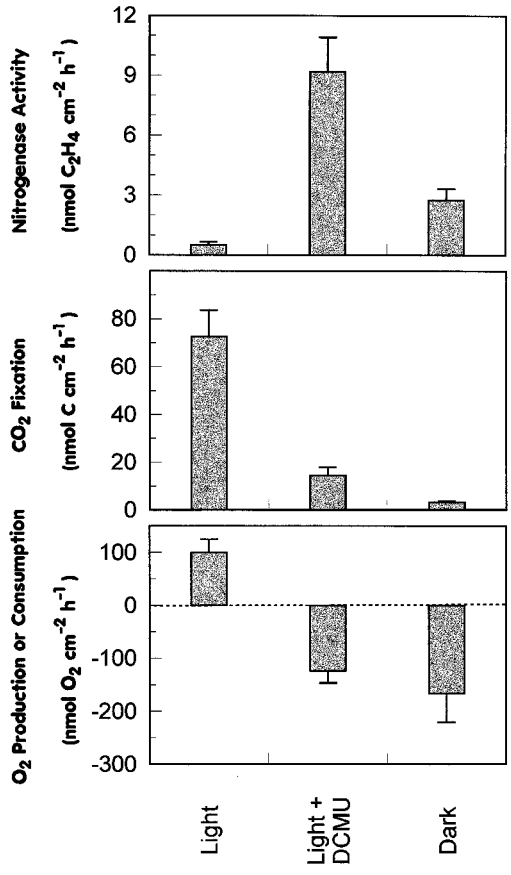


FIG. 7. Results of simultaneous measurements of $O_2$ production (positive values) or consumption (negative values), $CO_2$ fixation, and nitrogenase activity following experimental manipulations. Values are the mean ± 1 SD for five samples. The notation *nd* indicates that rates were not determined.

ments versus controls) provided useful insights into mat responses to the manipulated variables.

The effects of DCMU and a DOC source (DOC as D-glucose) on oxygenic photosynthesis, $O_2$ consumption, $CO_2$ fixation, and NA were evaluated in two incubations (Fig. 6 and 7). The DCMU additions effectively inhibited oxygenic photosynthesis completely, and D-glucose additions did not change light or dark $O_2$ consumption rates (Wilcoxon, $P = 0.14$) (Fig. 6). Although $O_2$ production rates differed for the two incubations, $O_2$ consumption rates were similar in the dark and DCMU treatments. $CO_2$ fixation continued after the addition of DCMU but at a lower rate than in the nonamended control (Light) (Kruskal-Wallis, $P < 0.05$; Bonferroni). D-Glucose additions did not enhance $CO_2$ fixation in DCMU-amended samples. Collectively, the $O_2$ and $CO_2$ fixation data suggest that nearly 25% of the total $CO_2$ fixed by these microbial mats can be attributed to anoxygenic photosynthesis. The addition of D-glucose had no measurable impact on respiration or photosynthetic rates, showing that the exogenous organic carbon provided was not readily assimilated by mat phototrophs.

NA was higher in the DCMU-amended samples, while the light and dark rates of NA in nonamended samples were not significantly different (Kruskal-Wallis, $P < 0.01$; Bonferroni) (Fig. 6 and 7). D-Glucose additions resulted in a small but significant reduction in NA relative to the light-plus-DCMU treatment ($P < 0.05$; Bonferroni). In combination with the productivity incubations, these results suggest that without oxygenic photosynthesis and exposure to light, NA is much higher (>300%) than dark rates.

The lower-salinity (37‰) incubation water had no effect on $O_2$ photosynthesis, $O_2$ consumption, $CO_2$ fixation, or NA (both light and dark) relative to Salt Pond water (74‰) (Wilcoxon, $P > 0.05$) (Fig. 8). However, the NA of the DCMU-amended samples was higher (by a factor of 3 to 4) than that of nonamended treatments (Kruskal-Wallis, $P < 0.01$; Bonferroni).

## DISCUSSION

The phototrophic community composition and total biomass (as chlorophyll *a*) of hypersaline microbial mats in Salt Pond were similar to those of temperate and tropical intertidal microbial mats (23–25). In addition, the molar ratio of carbon to nitrogen for the Salt Pond mat (9.2 ± 1.3; mean ± 1 SD) was close to values for coastal Atlantic intertidal mats in North Carolina (10.4 ± 1.8) (25). However, the Salt Pond mat had twice the concentration of zeaxanthin (cyanobacteria), more than five times the concentration of bacteriochlorophyll *a* (purple phototrophic bacteria), but only one-fourth the concentration of fucoxanthin (diatoms) compared to the North Carolina mats (25). The dominant phototrophs in the Salt Pond mat, as determined by photopigment analyses and qualitative microscopy, were filamentous cyanobacteria (*M. chthonoplastes*) and purple sulfur bacteria (*Chromatium* spp.). The implication for

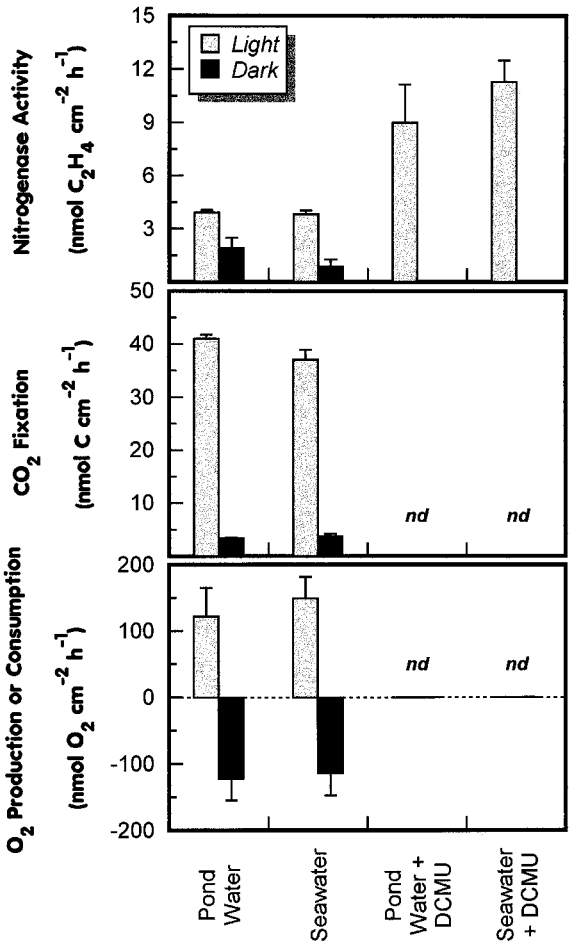


FIG. 8. Microbial mat responses to reduced salinity and DCMU additions under light and dark conditions. Pond water salinity was 74‰, while the seawater salinity was 37‰. Values are the mean ± 1 SD for five samples. The notation *nd* indicates that rates were not determined.

productivity measurements is that both oxygenic photosynthesis and anoxygenic photosynthesis are potentially important pathways for $CO_2$ fixation in this microbial mat community. An assessment of the relative contributions of mat community constituents required a combination of $O_2$ and $^{14}C$ methods to measure photosynthesis.

Dissolved $O_2$ levels above 125% saturation reduced the rate of oxygenic photosynthesis greatly in the Salt Pond microbial mats. At 150% saturation, oxygenic photosynthesis was almost completely inhibited. In shallow lagoons, the high photosynthetic rates of the mat community could easily lead to dissolved $O_2$ supersaturation. However, because these lagoons are shallow, the relatively high surface-area-to-water-volume ratio promotes rapid gas exchange and equilibration at the water-air interface. In deeper lagoons, where the surface-area-to-water-volume ratio is lower, the likelihood of $O_2$ supersaturation is greater and may limit mat development and production. This may help explain the more prolific mat growth around the margins of the lagoon. Subaerially exposed (but periodically wetted) mats at the water's edge are exposed to optimal conditions because diffusive exchange of photosynthetic $O_2$ with the atmosphere minimizes supersaturation. In addition, photoprotective pigments (myxoxanthophyll, β-carotene, diatoxanthin, and echinenone) and sheath pigments provide protective sunscreens in these high-light environments (9).

The positive (linear) relationship between $pO_2$ and pH corresponded to the measured high oxygenic photosynthetic activity of the microbial mat. $CO_2$ consumed during C fixation shifted the carbonate equilibrium and subsequently increased the pH of the incubation water. However, in the incubations, $pO_2$ levels that were inhibitory for oxygenic photosynthesis were achieved much sooner than high pH levels were. High aerobic respiration rates, which result in $CO_2$ release, caused a decrease in pH when oxygenic photosynthesis ceased. The range of pH exposure, especially in the well-buffered hypersaline lagoon water, represents a small change in the dissolved inorganic C concentration. The dissolved $CO_2$ concentration in the lagoon water should be sufficient to meet photosynthetic demands. Although slow diffusion could promote $CO_2$ limitation of phototrophs within the mat matrix, it is more likely that phototrophs experience $O_2$ inhibition before $CO_2$ becomes limiting. Based on these results, it is unlikely that the Salt Pond microbial mat experiences in situ $CO_2$ limitation of photosynthesis.

For the Salt Pond mat, photosynthetic rates based on the $O_2$ method were two to three times higher than those obtained by the $^{14}C$ method. Direct comparisons of the $O_2$ and $^{14}C$ methods for microalgal productivity measurements have been the subject of many studies. The reasons for the discrepancy between the two methods have been discussed in detail (21) and will not be addressed here. However, one important distinction is that the $O_2$ method measures photosynthesis only by oxygenic phototrophs. In contrast, the $^{14}CO_2$ method measures $CO_2$ fixation by both oxygenic and anoxygenic phototrophs but cannot be used to quantify aerobic respiration ($O_2$ consumption). Experiments incorporating both methods provided insights into the relative contributions of oxygenic and anoxygenic photosynthesis in these mat communities.

The addition of DCMU, which inhibits oxygenic (photosystem II, [PSII] i.e., noncyclic) photosynthesis, followed by light incubation provided an indirect measure of anoxygenic (PSI, i.e., cyclic) photosynthesis. This addition probably led to overestimates of anoxygenic photosynthesis because hypoxic and anoxic conditions are both conducive to and stimulatory for anoxygenic photosynthesis. The $O_2$ data (Fig. 6 and 7) show that DCMU effectively halted oxygenic photosynthesis and that $O_2$ consumption rates were the same in light and dark incubations. Rapid $O_2$ consumption resulted in hypoxic-anoxic conditions in the overlying incubation water and, presumably, within the mat matrix. Although photoheterotrophy has been observed in similar microbial mat communities (20), D-glucose additions to the Salt Pond mat did not affect $O_2$ consumption or photosynthetic rates during the incubations.

Moderate reductions in salinity (from 74 to 37‰) had no measurable effect on photosynthesis, $O_2$ consumption, or NA in these mats. In a similar study, Pinckney et al. (23) found that the NA and productivity of hypersaline mats could be increased at lower salinities. However, in these experiments, mats were preincubated for 48 h at the lower salinities before rates were determined. The short exposure times (<1 h) used here for the Salt Pond mat may not have been sufficient to allow acclimation to the lower (37‰) salinity.

A possible explanation for the higher NA in the DCMU-amended samples is that a reduction in ambient $O_2$ enhanced the NA. Nitrogenase, which is $O_2$ sensitive, exhibits its highest activity under anoxic conditions for nonheterocystous cyanobacteria as long as reductant (i.e., respirable organics) is available (2, 17). If anoxic conditions promoted a higher NA, the rates of NA should have been higher in the dark incuba-

tions than in the light-exposed incubations. However, the $O_2$ consumption rate was the same in light, dark, and DCMU treatments, demonstrating that the dark and DCMU treatments were exposed to nearly identical ambient $O_2$ concentrations. Therefore, it is unlikely that reduced $O_2$ levels alone promoted the observed increases in NA.

Under anoxic conditions in the light, purple phototrophic bacteria are capable of anoxygenic photosynthesis with $H_2S$ (rather than $H_2O$) as an electron donor (7, 11). Electrons generated by anoxygenic photosynthesis can be used for either $CO_2$ or $N_2$ fixation (3). High sulfide levels, which are characteristic of microbial mats under anoxic conditions, induce anoxygenic photosynthesis in some cyanobacterial species (8, 11, 28). Both *M. chthonoplastes* and *Lyngbya* spp., which normally conduct oxygenic photosynthesis, switch to anoxygenic photosynthesis when exposed to high levels of $H_2S$ (11, 28). The initiation of anoxygenic photosynthesis involves de novo synthesis of proteins and may require an induction period of several hours (11, 28). Therefore, it is conceivable that the enhanced NA rates in the DCMU treatments in our experiments could be attributed to electrons supplied by cyanobacterial anoxygenic photosynthesis. However, recent evidence suggests that *M. chthonoplastes*, the dominant cyanobacterium in the Salt Pond mat, lacks the dinitrogenase reductase gene (*nifH*) and is therefore unable to fix nitrogen (30). *Lyngbya*, which is a diazotroph capable of anoxygenic photosynthesis, was a minor constituent in this mat and could have made only a small contribution to the measured rates of NA and anoxygenic photosynthesis. Photosynthetic purple sulfur bacteria, which were a major component of the Salt Pond mat, can fix $N_2$ with energy supplied from either photosynthesis or anaerobic respiration (13). Collectively, the experimental results support the hypothesis that noncyanobacterial phototrophs were responsible for the enhanced NA in the DCMU-amended incubations.

The NA in the DCMU-amended samples exposed to light was consistently higher (by a factor of 2 to 3) than in the other treatments. However, the NA rates for the light and dark treatments were never significantly different. One possible mechanism for this response is that following DCMU addition and subsequent inhibition of PSII activity, high $O_2$ consumption rates shifted the oxic/anoxic interface in the mat. This shift allowed the upward migration of purple phototrophic bacteria (e.g., *Chromatium* spp.) into higher ambient irradiance. Higher light exposure (i.e., in situ irradiance) translated into higher rates of anoxygenic photosynthesis, which provided more energy for NA and $N_2$ fixation.

This proposed mechanism is supported by the experimental results. The photopigment data showed that purple phototrophic bacteria were a major component of this mat community. Oxygen consumption rates were high in both the DCMU and dark treatments, resulting in a reduction of the ambient DOC in the overlying water. D-Glucose (DOC) additions did not enhance NA, suggesting that $N_2$ fixation was not limited by the availability of exogenous organic carbon. High $O_2$ consumption, low ambient $O_2$, and no oxygenic photosynthesis produced conditions favorable for anoxygenic photosynthesis. $CO_2$ fixation rates in the DCMU-amended samples were ~25% of the control (nonamended) samples, further demonstrating the occurrence of photosynthetic activity by anoxygenic phototrophs. The fact that the rates of NA in the dark were always lower than those for DCMU treatment implies that in the absence of oxygenic photosynthesis, $N_2$ fixation was coupled closely to irradiance.

The ecological implication of these findings is that $N_2$ fixation by anoxygenic phototrophs (purple phototrophic bacteria and possibly cyanobacteria) appears to be regulated by the activity (or inactivity) of oxygenic phototrophs (cyanobacteria and diatoms). Conditions that promote high $O_2$ consumption and low oxygenic photosynthesis, such as stress induced by extreme hypersalinity or high $H_2S$ concentrations, should enhance $N_2$ fixation rates in these mats. Therefore, these microbial communities can obtain N for growth and maintenance over a wide range of environmental conditions. Cyanobacterial-bacterial consortium interactions that enhance the overall physiological plasticity of the mat community may be a key for survival and persistence in extreme environments.

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