

## Short-term responses of coral reef microphytobenthic communities to inorganic nutrient loading

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### Abstract

The responses of coral reef flat microphytobenthos to short-term exposure to elevated levels of inorganic nitrogen (N) and phosphorus (P) were investigated in 1994 and 1995. Sand samples collected from the reef flat were maintained over 7 d in triplicate cultures with N-enriched (100  $\mu\text{M}$   $\text{NO}_3^-$ ), P-enriched (10  $\mu\text{M}$   $\text{PO}_4^{3-}$ ), and ambient seawater. A fourth experiment used a treatment of combined N and P enrichment. The sediment samples were assessed for chlorophyll *a* (Chl *a*) content and photosynthesis–irradiance (P-I) responses. P-I curves, constructed from area- and Chl *a*-specific metabolic rates, showed consistently higher maximal rates in the nutrient-enriched samples. Sediments exposed to enhanced levels of N exhibited the highest Chl *a* content while both N- and P-enriched samples showed increased photosynthetic yield. Very little depletion of nutrients in the water column was detected over time in the batch cultures except in the N+P-enriched treatments where nutrient values dropped to near-ambient levels. Results from these experiments point to N and P colimitation in tropical carbonate sediments.

Unconsolidated carbonate substrates, although apparently barren, are acknowledged to be biologically active components of coral reef systems (Kinsey 1977). In a tropical sandy reef flat studied by Yap et al. (1994), the same area investigated in this paper, gross primary production by the sand substrate constitutes about 10% of the aggregate per unit area production of the autotrophic components of the system. Yet this seemingly small contribution becomes substantial when the extent of the areal coverage of the substrate is considered (~85% of total reef flat area). The microphytobenthic communities responsible for this production have been studied extensively in various systems such as estuaries (Pinckney and Zingmark 1993a; MacIntyre et al. 1996), temperate shallow-water sediment systems (Sundbäck and Snoeijs 1991; MacIntyre and Cullen 1995), and coral reef lagoons (Charpy and Charpy-Roubaud 1990; Johnstone et al. 1990). Environmental factors influencing the production of these communities include irradiance (Daehnick et al. 1992; Pinckney and Zingmark 1993b), tidal stage and sun angles (Pinckney and Zingmark 1991), the abrasive effect of sand movement (Delgado et al. 1991), and nutrient input (Nilsson and Sundbäck 1991; Pinckney et al. 1995).

In tropical coral reef environments, dissolved nutrient concentrations in the overlying water column are generally lower than in temperate regions (Crossland 1983) except in cases where nutrients are supplied externally by groundwater (Johannes 1980; D'Elia et al. 1981), river runoff (Matson 1990), or direct anthropogenic input (Smith et al. 1981). Nutrient-limited responses have been confirmed in various coral

reef autotrophs such as coral zooxanthellae (e.g., Stambler et al. 1991), clam zooxanthellae (e.g., Belda et al. 1993), and seagrasses (e.g., Agawin et al. 1996). Small-scale experiments carried out on microphytobenthic communities in temperate systems (e.g., Sundbäck and Snoeijs 1991; Pinckney et al. 1995) have likewise confirmed nutrient limitation in sediment communities. On the other hand, large-scale in situ N and P enrichments, carried out in microatolls in One Tree Island, Great Barrier Reef (e.g., Kinsey and Domm 1974; Larkum and Koop 1997), have so far yielded ambiguous or conflicting results.

This study uses manipulative microcosm experiments to determine the short-term responses of tropical microphytobenthic communities to the increased availability of nutrients, particularly inorganic nitrogen and phosphorus. Results from these experiments should indicate the existence of a limiting role of one or both of these nutrients in terms of biomass formation and/or photosynthetic activity.

### Materials and methods

*Sediment collection*—Sediment samples were collected from a shallow reef flat on the western side of Santiago Island, Bolinao, Pangasinan in the northwestern Philippines (approximately 16°24'41"N, 119°54'25"E). The reef flat is ~500 m wide with seagrass beds dominating the shoreward edge, coral communities proliferating oceanward and a distinct sand-rubble belt lying in between these zones. The substrate is characterized by coarse, poorly sorted (0–1.00  $\phi$ ) coralline sand (Nacorda and Yap 1996). A pooled volume of ~15 liters of sediment samples was collected from about 1 m depth with a flat, stainless steel scoop, sampling only from the top 1 cm to avoid the dark, anoxic deeper layers.

*Preparation of sediment plates*—Immediately after sampling, the pooled sediment sample was sieved through a 1-mm mesh to exclude the macroinfaunal component (>1 mm) and to achieve grain size uniformity. Sediment plates were then prepared by carefully packing the sieved sediment in

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glass petri dishes (7 cm dia) up to a depth of 1 cm. Filtered (0.2  $\mu\text{m}$ ) sea water was added to the sediment to facilitate sieving and was subsequently collected to recover the resuspended microalgal fraction. Additionally, microalgae left attached to the larger sediment particles retained in the sieve were recovered by sonication (Nilsson and Sundbäck 1991) after which the overlying water was stirred, poured through a 1-mm sieve to exclude macrofauna, and mixed with the previously collected water used in sieving. This pooled algal suspension was equally partitioned between two 90-  $\times$  45-  $\times$  45-cm glass tanks (10 cm water depth). The sediment plates were then placed on the bottom of these tanks, arranged in compact rows to minimize the exposed area of the tank bottom and to maximize the sediment surface onto which the algae would settle. These tanks were left undisturbed and without aeration in an outdoor setup for 3 d to allow the microalgal community to reestablish in the sediment plates. Incident light intensity was kept below 2,000  $\mu\text{E m}^{-2} \text{s}^{-1}$  and the overlying water was maintained within 2°C of ambient sea water temperature by covering the tanks with several layers of netting.

*Nutrient enrichment experiments*—Randomly selected plates from the two settling tanks were transferred to six glass tanks (14 plates per tank) filled with filtered (0.2  $\mu\text{m}$ ) and UV-sterilized natural sea water (vol = 104 liters). These tanks were maintained outdoors over 7 d. Replicate tanks ( $n = 2$ ) were designated as the control, nitrogen-enriched (N), and phosphorus-enriched (P) tanks and were arranged in a single row following an interspersed design. Instead of providing aeration, a motor-driven paddle system was used to keep nutrient concentrations in the tanks homogeneous and to minimize the thickness of the diffusive boundary layers at the sediment–water interface that could limit the downward flux of nutrients into the sediment (cf. Jorgensen and Des Marais 1990). The action of the paddles was regulated to avoid resuspending the sediment in the petri dishes. Nutrient concentrations in the treatment tanks were increased by adding  $\text{NaNO}_3$  in the N tanks (calculated concentration = 100  $\mu\text{M NO}_3$ ) and  $\text{KH}_2\text{PO}_4$  in the P tanks (10  $\mu\text{M PO}_4$ ). Sea water in the control tanks was left untreated. Intense light, heat build up, and the introduction of allochthonous materials were minimized with the use of a double layer of netting and a roofing of clear plastic sheets over the entire outdoor setup.

*Water sampling and analysis*—Water samples were drawn from each tank with a 50-ml syringe before and after nutrient addition at predetermined periods. Five samples from each tank were pooled, filtered through 0.45- $\mu\text{m}$  acetate filters, and stored frozen in 250-ml high density polyethylene (HDPE) bottles until subsequent analysis for dissolved inorganic nitrogen (DIN,  $\text{NH}_3 + \text{NO}_3 + \text{NO}_2$ ) and dissolved inorganic phosphorus (DIP, measured as orthophosphate) using automated (Skalar Instruments) and spectrophotometric analytical procedures following Strickland and Parsons (1972).

*Photosynthesis–irradiance (P-I) experiments*—P-I responses of the sediment communities were determined using

a setup described in Montebon and Yap (1995b). Four respiration chambers (StrathKelvin Instruments RC 400), three of which contained a sediment plate collected from the treatment tanks, were kept immersed in a temperature-controlled water bath and incorporated in a flow-through system (flow range: 8–50  $\text{ml min}^{-1}$ ). The fourth chamber was used as a blank to measure dissolved oxygen concentrations of the inflowing water. Outflowing water from all the chambers was measured quasisynchronously by a polarographic oxygen electrode (StrathKelvin Instruments model 781b) fitted into a four-way stopcock (Montebon and Yap 1995a). Irradiance was provided by a 1,000-W halogen lamp and a series of black screens was placed in combination underneath the lamp to achieve different light levels for the experiments. The chambers were flushed for 5 min with aerated and filtered seawater between light levels. The microalgal communities in the plates were allowed to acclimate for 15 min to each light level prior to measurement.

Five light levels (plus an initial measurement of respiration in darkness) were used in the P-I experiments. This was to allow the measurement of more replicates (18 plates were measured over 24 h, with each batch of 3 plates requiring  $\sim 4$  h to construct a P-I profile). Because MANOVAR (see *Statistical analyses* below) is sensitive to the ratio of  $p$  (number of levels of the independent variable) to  $n$  (number of observations), it was necessary to opt for more replicates over irradiance levels to enable a more powerful analysis of treatment effects (Potvin et al. 1990).

*Estimation of P-I parameters and curve fitting*—A single P-I curve, along with its associated parameters, was generated for each sediment plate with the hyperbolic tangent model by Jassby and Platt (1976) using the curve-fitting function of SigmaPlot version 4.1 (Jandell Corporation). The numerical (iterative) model is defined as

$$P^n = P_{\text{max}} \tanh(I\alpha/P_{\text{max}}) + R_d$$

which estimates the maximal gross photosynthetic rate ( $P_{\text{max}}$ ), dark respiration rate ( $R_d$ ), and the initial slope of the curve ( $\alpha$ ) from inputs of net photosynthetic rates ( $P^n$ ) measured at specified irradiance levels ( $I$ ). The light saturation constant,  $I_k$ , was derived by dividing  $P_{\text{max}}$  by  $\alpha$ .

*Sediment chlorophyll a (Chl a)*—From a core subsample ( $\sim 7$  ml) taken from each plate at the end of each P-I experiment, Chl *a* was cold-extracted with 90% acetone over 24 h. Extraction was preceded by disrupting the algal cells with an ultrasonic homogenizer for 5 min. The supernatant was later decanted, centrifuged for 10 min at 3,000  $\times g$ , and analyzed spectrophotometrically (Lorenzen 1967).

*Batch experiments*—Sediment collection and batch culture experiments were carried out in April, September, and December 1994 and will be referred to as Experiments 1, 2, and 3, respectively. A fourth set, Experiment 4, was conducted in August 1995 to investigate the combined effect of elevated levels of inorganic N and P using three replicate tanks for controls and three for the N+P treatments (100  $\mu\text{M NO}_3 + 10 \mu\text{M PO}_4$ ). Experiments 1–3 were meant to serve as temporal replicates of each other. However, no at-

tempt was made to compare results of the different experiments statistically because between-experiment differences were too large to enable the detection of between-treatment differences. The primary reason for the temporal replication was to test the reproducibility of results.

*Statistical analyses*—Because several measurements were made on one sample over a range of light levels, the analysis of variance with repeated measures (ANOVAR) was the test of choice in comparing the P-I profiles generated in each experiment (Potvin et al. 1990). However, standard ANOVAR and ANOVAR with relaxed assumptions could not be carried out because the necessary assumptions of the tests were not met, thus, the multivariate counterpart, MANOVAR (using Pillai's trace statistic) was resorted to (Potvin et al. 1990). Homoscedasticity was verified using the univariate test (Cochran's test) instead of the multivariate Box-M test due to the singular matrix of the data set.

Chlorophyll *a* concentrations were compared among treatments (C, N, P [and N+P]) using a mixed model (two orthogonal factors and one nested factor) analysis of variance (Underwood 1981). Data from Experiment 1 were inverse-transformed to satisfy the assumptions of normality and homogeneity of variances while no transformations were necessary for the Chl *a* data from the other experiments.

P-I parameter estimates were compared between treatments using one-way ANOVA. In tests where significant differences were detected, a posteriori comparisons (Tukey's honestly significant difference [HSD],  $\alpha = 0.05$ ) were carried out to identify statistically distinct groups.

Curves fitted to the light-saturation equation were compared using a distribution-free statistical procedure that compares the overall shape of the response curves in detecting significant group (treatment) effects (Potvin et al. 1990). Pairwise comparisons were subsequently carried out when significant treatment effects were detected at the 0.05 level of significance.

## Results

*Nutrient levels*—Although falling short of the calculated final values of 100  $\mu\text{M}$  for nitrogen and 10  $\mu\text{M}$  for phosphorus, nutrient concentrations in the treatment tanks were several times greater than ambient seawater (i.e., control) concentrations (Fig. 1). In Experiments 1–3, relatively high nutrient concentrations were maintained in the treatment tanks over the 7-d experimental period. In Experiment 4, a drop to near-ambient levels was detected at day 7 for both DIN and DIP in all three treatment tanks. The apparent drop in DIP at day 3 in Experiment 3 may have been due to measurement error.

*Sediment Chl a*—Responses of the microalgal communities to elevated nutrient levels were evident in the tanks a few days after nutrient addition. A conspicuous algal film was observed to develop on the surface of the sediment plates. At the end of the exposure period, the nitrogen-enriched (N, N+P) plates exhibited the darkest colored algal mats in all four experiments. Chlorophyll *a* analyses confirmed these visual observations. Highest mean values of Chl

*a* were measured in the N-enriched samples in Experiments 1, 2, and 3 at day 7 (108.23–140.04 mg Chl *a*  $\text{m}^{-2}$ ) while in Experiment 4, highest concentrations ( $67.47 \pm 3.98$  mg Chl *a*  $\text{m}^{-2}$ ) were measured in the N+P-treated plates at day 3 (Fig. 2). Concentrations measured in this study lie within the range obtained from measurements previously made on sediment samples taken from the same collection site (66.50–209.40 mg Chl *a*  $\text{m}^{-2}$ ; Dizon et al. 1994) and are typical values for marine benthic communities (Parsons et al. 1984; MacIntyre et al. 1996). Using a mixed-model ANOVA, significant differences due to nutrient treatment and to sampling time were detected in all the experiments ( $P < 0.05$ ). Differences accounted for by the interaction of nutrient treatment and sampling time were likewise significant in Experiments 2, 3, and 4. In all experiments, Chl *a* concentrations measured from plates collected from replicate treatment tanks were not significantly different from each other. Likewise the interaction effects of sampling time and replicate treatment tanks were not statistically significant.

*Comparison of P-I responses*—The mean area-normalized photosynthetic rates of plates measured over a series of light levels are presented as scatterplots in Fig. 3. MANOVA results revealed significant between-treatment effects only in Experiment 2 ( $F = 17.90$ ,  $P = 0.001$ ). A pairwise comparison of the fitted curves further showed that the curves of the control, N and P plates of Experiment 2 were distinct from each other. Similar comparisons carried out for Experiments 3 and 4 also showed that the treatment groups (N, P, and N+P) exhibited higher photosynthetic rates than the controls (Exp. 3:  $F_{14,87} = 2.56$  and Exp. 4:  $F_{7,58} = 2.42$ ,  $P < 0.05$ ).

Net photosynthetic rates, when expressed in terms of the Chl *a* content of the sediment (Fig. 4), showed greater responses of the P-enriched plates than the control plates in Experiment 2 using MANOVA ( $F = 9.98$ ,  $P = 0.005$ ) and in both Experiments 2 and 3 using comparison of fitted curves ( $F_{10,57} = 11.90$  and  $F_{14,87} = 1.88$ , respectively). Nutrient enrichment had no significant effect on the photosynthetic responses of microphytobenthic communities in Experiments 1 and 4 (MANOVA and comparisons of fitted curves).

MANOVA results also showed that increasing irradiance levels exerted a significant effect on the photosynthetic performance of the microalgal communities in all the experiments ( $P < 0.001$ , see Figs. 3 and 4). The interaction of light and nutrient enrichment was significant for the area-specific rates of all the experiments (Fig. 3).

*P-I parameters*—P-I parameters generated from area- and chlorophyll-specific rates using the hyperbolic tangent function are presented as graphs in Fig. 5. Results of one-way ANOVA and post hoc comparisons are given in Table 1. Treatment effects were evident in the  $P_{\text{max}}$  and  $R_d$  parameters in most of the experiments and in the  $\alpha$  values in Experiments 2 and 4. Note that using the chlorophyll-specific rates, phosphorus appeared to elicit a significantly higher  $P_{\text{max}}$  in Experiments 2 and 3 and a significantly higher  $\alpha$  in Experiment 2.

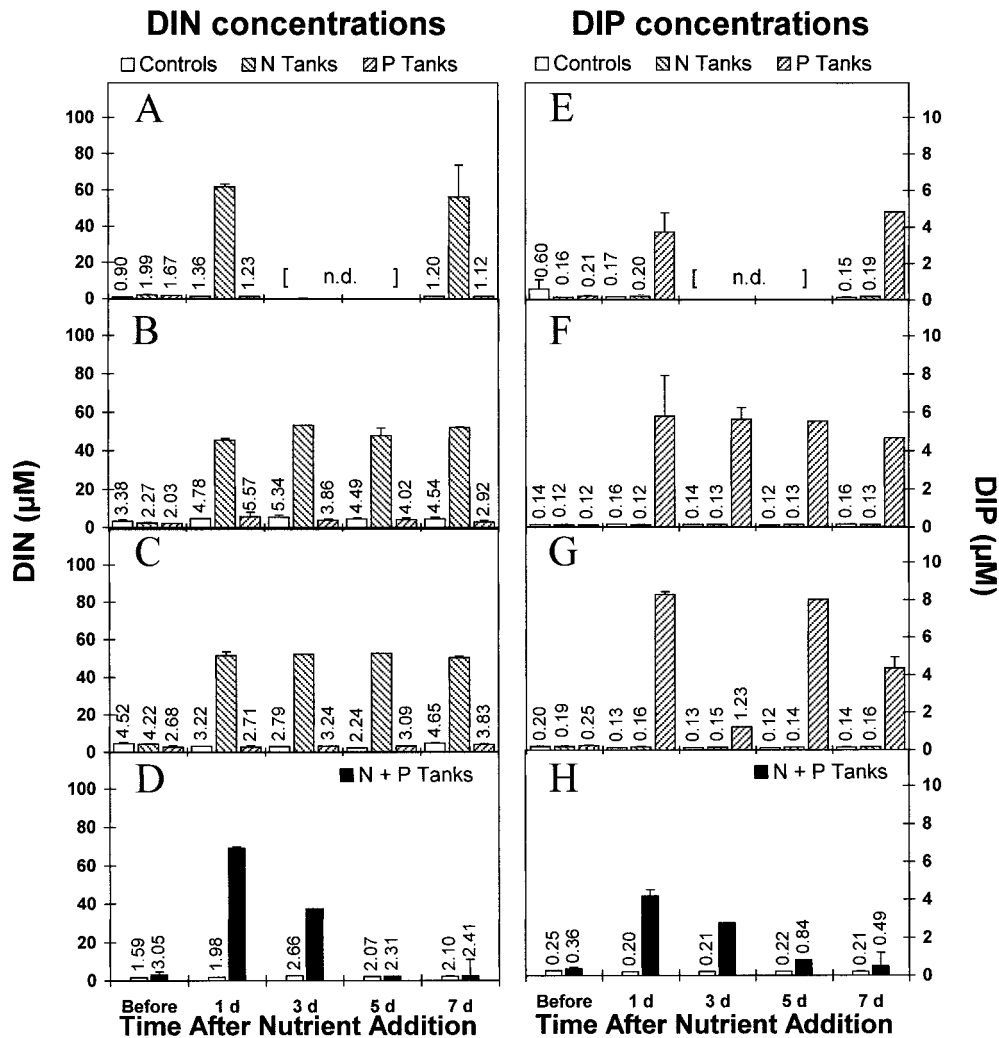


Fig. 1. Dissolved inorganic nitrogen (DIN, graphs A–D) and phosphorus (DIP, graphs E–H) levels in the treatment tanks over a period of 7 d. (A and E, Exp. 1 [APR 94]; B and F, Exp. 2 [SEP 94]; C and G, Exp. 3 [DEC 94]; D and H, Exp. 4 [AUG 95]). Bars are mean concentrations ( $n = 2$ , except in Exp. 4 where  $n = 3$ ). Error bars are standard deviations. n.d. = no data.

## Discussion

The use of sediment plates in this study was primarily to isolate the microalgal communities from other sources of nutrients, particularly regenerated nutrients from deeper sediment layers, besides the overlying water. Low concentrations of  $\text{NH}_3$ , not exceeding  $3.0 \mu\text{M}$  in all the experimental tanks, indicated that nitrogen regeneration was relatively minimal. This value was within the range of concentrations in the control tanks and is in the lower range of pore-water levels in seagrass beds proximal to the collection site (Agawin et al. 1996). Although the nutrient supply of microphytobenthic communities is mainly from interstitial water, nutrients from the overlying water can be just as efficiently taken up (Nilsson et al. 1991; Fong et al. 1993), provided that boundary layers at the sediment–water interface are thin enough to allow the vertical flux of nutrients.

The short duration of the experiments (7 d) was intended to minimize possible effects of increased meiofaunal bio-

mass and grazing rates that could have confounded the measurement of algal metabolic rates and biomass. However, the response of the microalgal communities within this short period was relatively rapid, involving observable changes in the sediment plates within days of nutrient addition. This is in contrast to the response lags ( $>7$  d) observed in similar enrichment studies (e.g., Nilsson and Sundbäck 1991; Nilsson et al. 1991). Results from our study indicate that secondary nutrient limitation (e.g., silicate depletion [Jacobsen et al. 1995; Escaravage et al. 1996]) does not always exert a significant effect on microalgal communities, particularly in benthic systems that have, aside from diatoms, significant populations of cyanobacteria.

Despite the observed responses of the benthos to elevated levels of nutrients, no appreciable decline in nutrient levels over time was noted for the experiments involving separate N and P enrichments (Experiments 1, 2, and 3; Fig. 1). This was largely due to the high concentrations used in the experimental enrichments that were designed to ensure that the

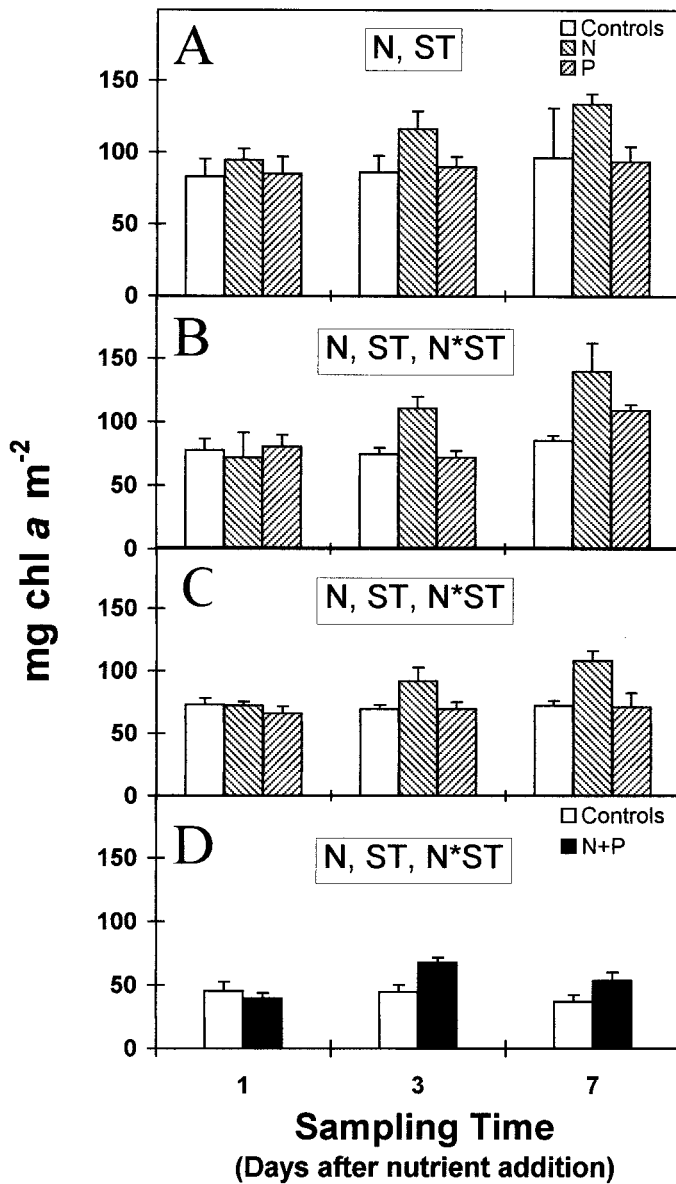


Fig. 2. Mean Chl *a* concentrations ( $\text{mg Chl } a \text{ m}^{-2}$ ) measured from the sediment plate samples of Experiment 1–4 (graphs A–D, respectively). Error bars are standard deviations;  $n = 6$  (Exp. 2,  $n = 4$ ). Factors that are significant ( $P < 0.05$ , mixed-model ANOVA) are enclosed in boxes (N = nutrient treatment, ST = sampling time, N\*ST = interaction).

nutrients (N or P) were consistently in excess throughout the duration of the experiments. These concentrations are comparable to in situ levels reported in other nearshore systems receiving terrigenous nutrient input (e.g., Joye and Pael 1993). Pinckney et al. (1995) used nutrient levels of the same order of magnitude in their study of seasonal fluctuations in the growth and  $\text{N}_2$  fixation rates of intertidal cyanobacterial mats in North Carolina. The effective nutrient uptake rates of the microphytobenthic communities in the treatment tanks were evidently orders of magnitude smaller than the available nutrient pool to allow an accurate interconversion of nutrient uptake with biomass gain, as was attempted through

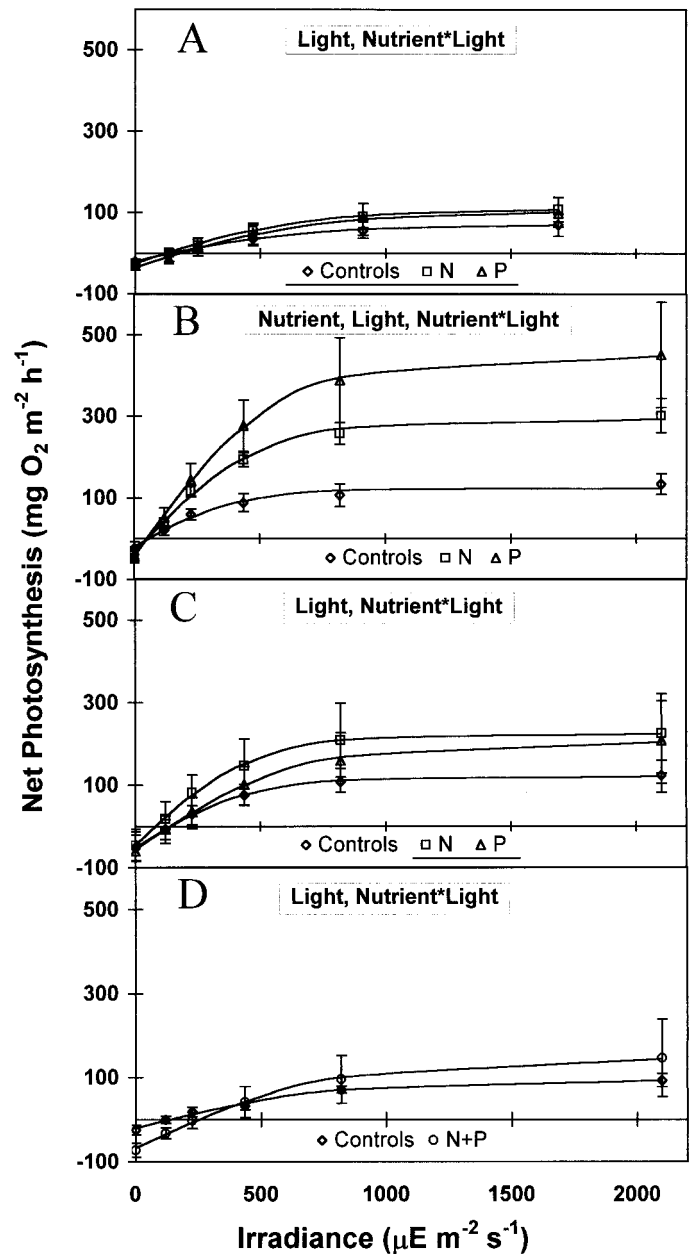


Fig. 3. Light saturation curves generated from area-specific photosynthetic rates. Graphs A–D refer to Experiments 1–4, respectively ( $n = 3$ , except in Exp. 2 where  $n = 2$ ). Error bars are standard deviations. Factors with significant effects on photosynthesis are enclosed in boxes (MANOVA,  $P < 0.05$ ). Treatments connected by a common underline are not significantly different (pairwise comparison of fitted curves,  $P < 0.05$ ).

mass-balancing calculations on the increase in Chl *a* concentrations in the N-enriched sediment plates and the observed N lost from the water column.

In contrast to the first three experiments, both N and P concentrations in all the replicate treatment tanks in Experiment 4 dropped to near ambient levels at the end of the experiment. To determine if instantaneous nonbiological uptake could account for this decrease, we carried out an en-

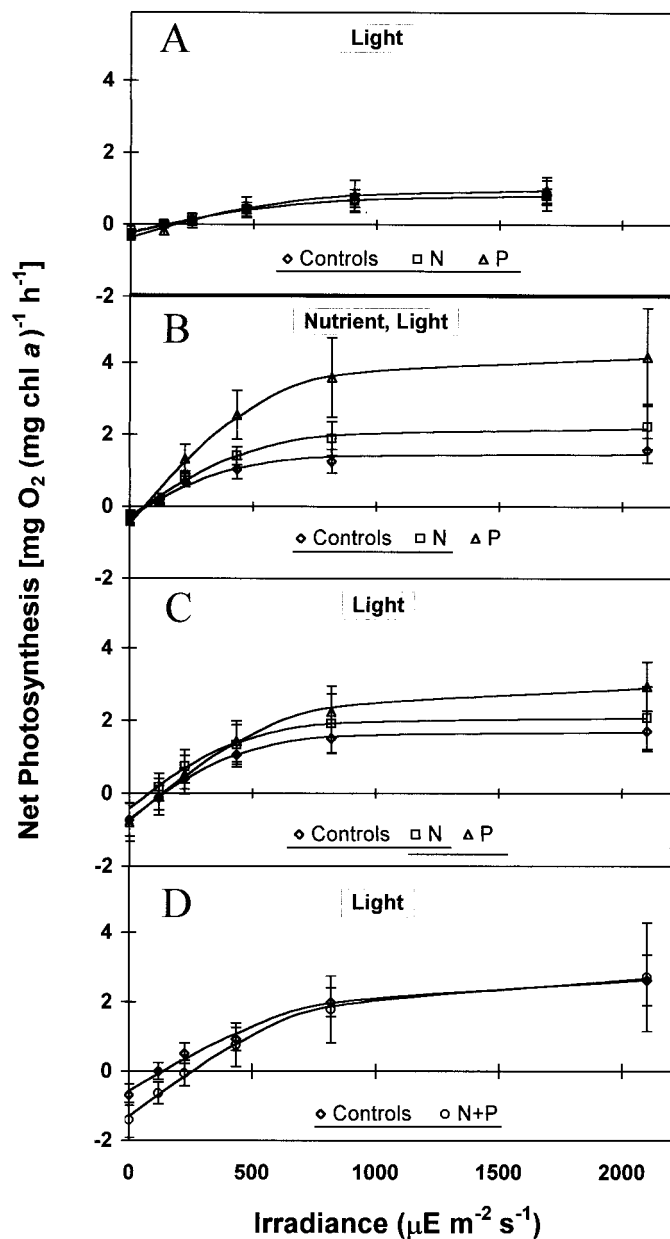


Fig. 4. Light saturation curves generated from biomass-specific photosynthetic rates. Graphs A–D refer to Experiments 1–4, respectively ( $n = 3$ , except in Exp. 2 where  $n = 2$ ). Error bars are standard deviations. Factors with significant effects on photosynthesis are enclosed in boxes (MANOVAR,  $P < 0.05$ ). Treatments connected by a common underline are not significantly different (pairwise comparison of fitted curves,  $P < 0.05$ ).

richment experiment identical to Experiment 4 except for the use of sterile (i.e., autoclaved) sediment plates. No changes in both N and P levels were detected in this experiment even after 24 h of nutrient addition, indicating that the observed nutrient decrease in Experiment 4 was more likely due to biological uptake.

Because the photosynthetic and growth response of the microalgal sediment communities showed only small increases relative to the controls, it is very likely that algal

communities may have developed over time in the overlying water or on the walls of the N+P tanks. However, no noticeable algal growth in the water column or on the glass walls was observed throughout the duration of the experiment. If these extrasedimentary communities were indeed present at significant densities, they could have accounted for part of the observed nutrient depletion. Translating the average amount of nitrogen lost from the water column of the N+P tanks ( $66.61 \mu\text{M}$ ) to Chl *a* concentrations yields a value of  $0.023 \text{ mg liter}^{-1}$  (assuming  $\text{N} = 40\%$  of biomass and  $\text{biomass} = 100 \times \text{mg Chl } a$ ). At such concentrations, the presence of microalgae in the water column would not have been visibly detectable (I. Flaming pers. comm.).

Nitrogen enrichment clearly effected an increase in microalgal biomass as estimated by sediment Chl *a* concentrations. This finding is consistent with those of other studies testing N influence on rates of increase in microalgal biomass (e.g., Nilsson et al. 1991; Pinckney et al. 1995). Both N and N+P treatments stimulated the proliferation of diatoms and cyanobacteria that formed the major microphytobenthic components in the sediment plates in all experiments (unpubl. data).

The role of nitrogen is well recognized in the synthesis of proteins and other nitrogenous compounds such as chlorophylls and phycobilins needed in the photosynthetic machinery. In turn, photosynthesis supplies the energy for the active uptake of N and its subsequent assimilation into algal cells in the presence of light (Turpin 1991). The photosynthetic process also provides the carbon framework (from CO<sub>2</sub> fixation) in which N is incorporated to form amino acids (Syrett 1981).

Phosphorus addition, on the other hand, did not elicit the same increase in microalgal biomass because this macronutrient, aside from being utilized in the synthesis of structural components of the cell, is also cycled in cellular energetic processes as adenosine di- and triphosphate (Parsons et al. 1984). In their nutrient loading study, Pinckney et al. (1995) likewise did not observe any significant P enrichment effects on biomass accretion of intertidal microbial mat communities despite the high concentrations used.

The consistently significant interaction effects between nutrient enrichment and light indicate that physiological responses of microphytobenthos to nutrient availability are strongly light dependent, as previously confirmed in phytoplankton studies dealing with specific uptake rates for N and P (Davis 1976 in Dugdale et al. 1981).

MANOVAR and the comparison of fitted curves showed that nutrient enrichment (N, P, and N+P addition) exerts a statistically significant effect on the photosynthetic performance of the sediment communities in all the experiments except in Experiment 1. This relatively consistent behavior is indicative of N and P colimitation in these tropical sediment communities.

Further evidence for nutrient-limited metabolism in microphytobenthos can be seen from results of the statistical comparison of P-I parameters, particularly  $P_{\text{max}}$  and  $R_d$ , that were consistently higher in sediment samples exposed to increased levels of inorganic nutrients (Fig. 5). In conditions where environmental factors (e.g., nutrients, temperature) play a key role in algal metabolism,  $P_{\text{max}}$  becomes a function

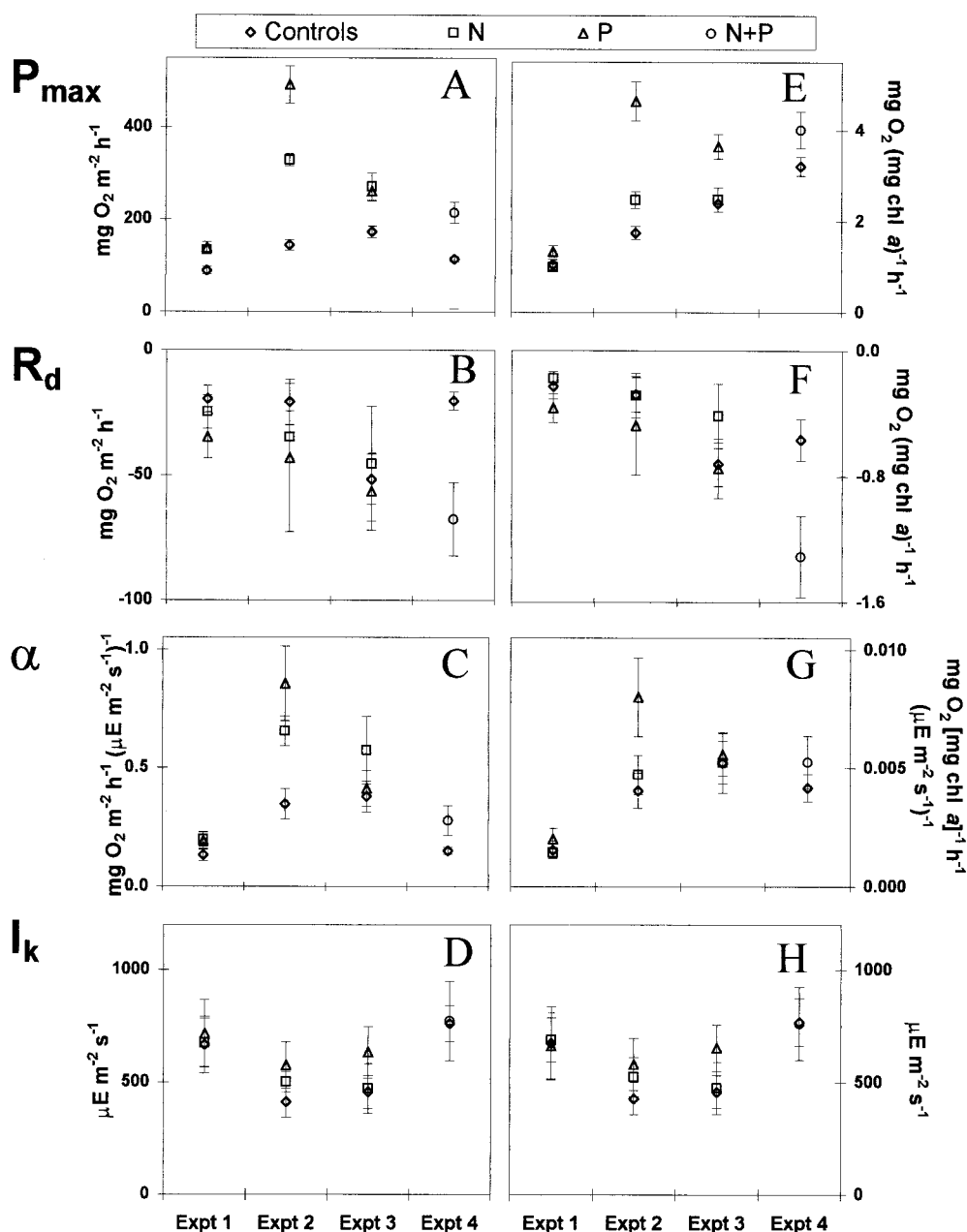


Fig. 5. Photosynthesis-irradiance parameters ( $P_{max}$ ,  $R_d$ ,  $\alpha$  and  $I_k$ ) generated from the fitted light saturation equations (Jassby and Platt 1976). Note that graphs are partitioned between parameters generated from area-specific photosynthetic rates (A–D) and those generated from biomass-specific rates (E–H). Error bars are standard errors.

of the limiting factor (Parsons et al. 1984) which, in this study, is the availability of N and P.

In this study the use of two parameters, namely, physiological performance and growth responses, defined a priori in order to detect significant responses of microphytobenthic communities to nitrogen and phosphorus loading, yielded several interesting results: (1) tropical microphytobenthic communities responded rapidly (on the order of days) to nutrient loading although their specific uptake rates for inorganic nutrients remain to be quantified, (2) the addition of

nitrogen in the water overlying the sediment communities elicited a significant increase in microphytobenthic biomass and a consequent enhancement in photosynthetic performance, while (3) the addition of phosphorus, although not eliciting the same quantitative response (increase in biomass), effected a consistent enhancement in the photosynthetic rates of the microalgal communities. Results from the experiment involving N+P loading indicate a need to investigate further the combined effects of N and P addition on phytoplankton-phytobenthos interactions.

Table 1. Results of between-treatment comparisons of P-I parameters.  $F$ -ratios are results obtained from one-way ANOVA while underscores indicate which treatments are not significantly different (Tukey HSD,  $P < 0.05$ ).

	Area specific				Biomass specific			
	$P_{\max}$	$\alpha$	$I_k$	$R_d$	$P_{\max}$	$\alpha$	$I_k$	$R_d$
Experiment 1	<u>C</u> <u>N</u> <u>P</u>	<u>C</u> <u>N</u> <u>P</u>	<u>C</u> <u>N</u> <u>P</u>	<u>C</u> <u>N</u> <u>P</u>	<u>C</u> <u>N</u> <u>P</u>	<u>C</u> <u>N</u> <u>P</u>	<u>C</u> <u>N</u> <u>P</u>	<u>C</u> <u>N</u> <u>P</u>
(Apr 1994)	$F_{2,15} = 4.04$	$F_{2,15} = 1.83$	$F_{2,15} = 0.44$	$F_{2,15} = 5.91$	$F_{2,15} = 1.49$	$F_{2,15} = 2.41$	$F_{2,15} = 2.08$	$F_{2,15} = 10.72$
Experiment 2	<u>C</u> <u>N</u> <u>P</u>	<u>C</u> <u>N</u> <u>P</u>	<u>C</u> <u>N</u> <u>P</u>	<u>C</u> <u>N</u> <u>P</u>	<u>C</u> <u>N</u> <u>P</u>	<u>C</u> <u>N</u> <u>P</u>	<u>C</u> <u>N</u> <u>P</u>	<u>C</u> <u>N</u> <u>P</u>
(Sep 1994)	$F_{2,9} = 20.67$	$F_{2,9} = 13.91$	$F_{2,9} = 0.91$	$F_{2,9} = 1.73$	$F_{2,9} = 11.13$	$F_{2,9} = 11.46$	$F_{2,9} = 0.57$	$F_{2,9} = 3.13$
Experiment 3	<u>C</u> <u>N</u> <u>P</u>	<u>C</u> <u>N</u> <u>P</u>	<u>C</u> <u>N</u> <u>P</u> *	<u>C</u> <u>N</u> <u>P</u>	<u>C</u> <u>N</u> <u>P</u>	<u>C</u> <u>N</u> <u>P</u>	<u>C</u> <u>N</u> <u>P</u> *	<u>C</u> <u>N</u> <u>P</u>
(Dec 1994)	$F_{2,15} = 3.14$	$F_{2,15} = 1.44$	$F_{2,15} = 3.80$	$F_{2,15} = 0.16$	$F_{2,15} = 6.77$	$F_{2,15} = 0.09$	$F_{2,15} = 3.80$	$F_{2,15} = 1.21$
Experiment 4	<u>C</u> <u>N</u> + <u>P</u>	<u>C</u> <u>N</u> + <u>P</u>	<u>C</u> <u>N</u> + <u>P</u>	<u>C</u> <u>N</u> + <u>P</u>	<u>C</u> <u>N</u> + <u>P</u>	<u>C</u> <u>N</u> + <u>P</u>	<u>C</u> <u>N</u> + <u>P</u>	<u>C</u> <u>N</u> + <u>P</u>
(Aug 1995)	$F_{1,10} = 9.91$	$F_{1,10} = 48.98$	$F_{1,10} = 0.24$	$F_{1,10} = 50.85$	$F_{1,10} = 1.31$	$F_{1,10} = 4.10$	$F_{1,10} = 0.24$	$F_{1,10} = 12.47$

\* Significant between group differences detected in ANOVA but not in the *a posteriori* tests.

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