Metabolic changes and compositional shifts in nutrient-enriched tropical reef sediment communities*

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SUMMARY: Four sets of nutrient enrichment experiments were carried out in 1994 and 1995 using sieved sand from a coral reef flat in the northwestern Philippines. The samples, prepared by packing the sediment in glass petri dishes, were maintained for 7 days in outdoor microcosms with varying nitrogen-phosphorus (N:P) ratios in the overlying water. The objective of the study was to detect changes in the composition and metabolism of sediment communities over short-term nutrient exposures. Net primary production and respiration rates of the microalgae increased within a few days in treatments having elevated levels of N, P and N+P. The addition of N and N+P significantly stimulated microalgal proliferation, even within a few days, while the addition of P alone did not elicit a significant response. Diatoms and cyanobacteria were the major taxonomic groups in the sediment samples, with the latter accounting for much of the community growth. The associated infaunal community in the sediment plates, dominated by crustaceans, nematodes and foraminifera, showed a high degree of variability between experiments, but this did not confound the effects of nutrient addition as there was no distinct trend with respect to either time or nutrient treatment. This study demonstrates that, in tropical shallow-water benthic systems, the direct effects of an episodic nutrient loading event can be observed within short time scales using microcosms derived from natural communities.

Key words: coral reef sediments, microcosm experiments, microphytobenthos, nutrient loading, infauna.

RESUMEN: CAMBIOS METABÓLICOS Y DE COMPOSICIÓN EN COMUNIDADES DE SEDIMENTOS DE ARRECIFE TROPICAL ENRIQUECIDOS CON NUTRIENTES. – Entre 1994 y 1995 se llevaron a cabo cuatro series de experimentos de enriquecimiento en nutrientes con arena tamizada que procedía de un arrecife de coral situado en el noroeste de las Filipinas. Las muestras, preparadas para su enema en platos de Petri de vidrio, se mantuvieron durante 7 días en microcosmos al aire libre con proporciones variables de nitrógeno:fósforo en el agua. El objetivo del estudio era detectar cambios en la composición y el metabolismo de las comunidades del sedimento cuando se sometían a episodios cortos de enriquecimiento con nutrientes. La producción primaria neta y las tasas de respiración de las microalgas aumentaban en poco tiempo en los tratamientos de un N, P y N+P significativamente. La adición de N y N+P estimulaba significativamente la proliferación de las microalgas en poco tiempo, mientras que la adición de P no provocaba una respuesta significativa. Las diatomeas y cianobacterias eran los grupos taxonómicos más abundantes y éstas últimas contribuían a la mayor parte del crecimiento de la comunidad. La comunidad de fauna estaba dominada por crustáceos, nemátodos y foraminíferos y mostraba una gran variabilidad entre los experimentos. Esta variabilidad no invalidaba los efectos de la adición de nutrientes, ya que la tendencia de las respuestas a los tratamientos de nutrientes no fue distinta. Este estudio demuestra que, en los sistemas bentónicos tropicales de poca profundidad, los efectos directos de una carga episódica de nutrientes pueden ser detectados en poco tiempo, mediante la utilización de microcosmos derivados de la comunidad natural.

Palabras clave: sedimentos de arrecife de coral, experimentos con microcosmos, microfitobentos, carga de nutrientes, infauna.

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INTRODUCTION

Characteristic populations of benthic microalgae inhabit sandy substrates in shallow marine environments, either occupying interstitial spaces or adhering to sediment particles. They account for much of the primary production in unconsolidated reef substrates and form an important trophic base for the benthic community, providing food for both meio- and macrofaunal herbivores that live on or within the sediment (Alongi, 1989; Blanchard, 1991).

Although the availability of light is probably the major environmental factor that influences microalgal production, it is apparently not the limiting factor in tropical clear-water sediment systems. For these systems, nutrient supply is probably the most critical factor in regulating benthic primary productivity (Sorokin, 1981), particularly in oceanic reefs. Aside from the autochthonous nutrient supply provided by biologically mediated nutrient regeneration and nitrogen fixation, these systems experience either chronic or episodic nutrient inputs from terrigenous groundwater (Johannes, 1980; D’Elia et al., 1981), river runoff (deAngelis and Gordon, 1985), rainfall (Paerl et al., 1990), and anthropogenic sources (e.g. Smith et al. 1981; Lapointe et al., 1990). Nitrogen and phosphorus from these sources have been measured to reach several orders of magnitude higher than ambient reef concentrations (see Lapointe et al., 1990).

Enrichment studies carried out in temperate benthic and pelagic systems (both experimental and natural) show that nutrient availability and concentrations influence the algal community structure (e.g. Sommer, 1994a; b; Jacobsen et al., 1995; Escaravage et al., 1996), shifting the algal dominance with respect to which nutrient is in stoichiometric excess. Some studies have shown that cyanobacteria dominate in systems with low N availability because of the ability of some species to fix elemental N into biologically usable forms (e.g. Pinckney et al., 1995). Escaravage et al. (1996) reported that in planktonic communities, flagellates eventually outnumber diatoms in high N and P environments because the latter experience a secondary limitation brought about by the depletion of Si from the water column. Sommer (1994a) noted that diatoms become dominant competitors at Si:N ratios >25:1.

Nutrient effects on aquatic communities are commonly assessed by means of growth bioassays carried out in controlled systems (e.g. Sullivan and Ritacco, 1988; Sundbäck and Snoeijts, 1991; Jacobsen et al., 1995). These experiments are conducted over a span of days to several weeks to detect and quantify consequent responses of the community to enrichment. Although such enrichment experiments are designed to detect Liebig limitation (Beardall et al., 2001), i.e. the maximum amount of biomass that can be supported by the system, and not the limitation of instantaneous growth, they are nevertheless important in determining the degree and speed at which communities respond to either episodic or chronic nutrient loading events.

Benthic microalgal studies, carried out in situ or in the laboratory, involve the use of intact sediment (e.g. Yap et al., 1994; MacIntyre and Cullen, 1995), artificial substrates (e.g. Carpenter, 1985) or processed sediment samples (e.g. Nilsson and Sundbäck, 1991). Although the use of intact samples is ideal, the use of manipulated or processed sediment (as in this study) enables organisms of interest to be isolated and increases the degree of control over experimental variables.

This paper describes several sets of nutrient enrichment experiments carried out in 1994 and 1995 in the northwestern Philippines to test the responses of coral reef flat sediment communities to inorganic nitrogen and/or phosphorus loading over 7 d. Significant changes in the Photosynthesis-Irradiance response curves and increases in the concentration of sediment chlorophyll a in nutrient-enriched treatments have been observed and these results are reported elsewhere (Dizon and Yap, 1999). Responses of interest for this aspect of the study include changes in net primary production and respiration rates over short periods of time, changes in microalgal densities, and possible shifts in sediment community structure. The experiments make use of sediment plates prepared from sieved sediments that contain representative natural microalgal communities and their associated infauna.

MATERIALS AND METHODS

Ten to fifteen sediment samples (pooled volume ca. 15L) were collected in a haphazard fashion from a shallow reef flat in Lucero, Bolinao and Pangasinan in the northwestern Philippines (approx. 16°24′41″N, 119°54′25″E). The collection site is about 500m wide with seagrass beds dominating the shoreward edge, coral communities proliferating oceanwards, and a distinct sand-rubble belt separating these two zones. Only the upper 1 cm of the oxic
layer was sampled with a flat, stainless steel scoop and brought to the field station (approximately 4 km south of the collection site), where it was sieved through a 1 mm mesh to exclude the macrofaunal fraction. Filtered seawater was added during this step to facilitate sieving and was later collected as it contained resuspended microalgae. The sediment was then carefully mixed in a large basin and packed into glass petri dishes (7 cm dia) for use as culture plates (= “sediment plates”).

Microalgae remaining in the larger sediment fraction were extracted by ultrasonication (Nilsson and Sundbäck, 1991; Voltolina, 1991). The supernatant was then decanted, poured through a 1 mm sieve and added to the algal suspension collected previously. This pooled suspension was partitioned between two glass tanks (up to 10 cm water depth) after which the sediment plates were placed on the bottom of these tanks (50 per tank) to allow the resuspended microalgae to reestablish in the sediment over a period of 3 d (Fig. 1). Throughout the settlement period, the tanks were left outdoors and were covered with a double layer of netting to reduce incident light and subsequent heat build up in the overlying water.

At the end of the settlement period, 6 treatment tanks were set up using glass aquaria (90 x 45 x 45 cm) laid across a table and alternately designated as control, nitrogen-enriched (N) and phosphorus-enriched (P) tanks (n = 2 per treatment; Fig. 1). These were filled with natural seawater (serially filtered to 0.2 μm and UV-treated) up to a depth of about 25 cm (volume = 104 L). Stirring was generated to homogenise the nutrient concentration in each tank by means of a motor-driven paddle (Montebon and Yap, 1995), while no aeration was provided during the experiment.

After the microalgae had been allowed to resettle on the sediment plates, the latter were carefully transferred from the settling tanks to the treatment tanks. Equal numbers of plates were taken from each settling tank for each treatment tank (n = 14 per treatment tank). After all the sediment plates were in place, stock solutions of analytical grade NaNO₃ and/or KH₂PO₄ were added to the appropriate treatment tanks to raise or decrease the ambient N:P ratio of the water column. Table 1 shows the resultant N:P values.

The entire set-up was covered with 2 layers of netting to reduce solar irradiance levels and to keep water temperatures within 2°C of ambient (typically below 32°C). Irradiance values measured in the covered tanks were lower than ambient, but well within the range of annual light intensities measured at the
collection site (500 to >2000 µE m^{-2} s^{-1}, Yap et al. 1994). Framed sheets of clear plastic were also used during the rainy months (August and September) to prevent a salinity decrease in the tanks.

Batch experiments following the protocol outlined above were conducted in April, September and December 1994 and will be referred to as Experiments 1, 2, and 3 respectively. Experiment 4, carried out in August 1995, was modified to investigate the effects of combined N and P enrichment. Three untreated (controls) and 3 treated (N+P-enriched) tanks were similarly laid out in an interspersed fashion in this particular experiment (Fig. 1).

Second-order effects of nutrient input, such as intensified trophic interactions, are known to set in after the direct effects, such as increased productivity and biomass accumulation, become detectable (Birkeland, 1988). Thus the study was intentionally kept short (7 d) for the specific purpose of minimising the effects of factors other than that of nutrient addition on microalgal metabolism and growth. With short nutrient incubation periods, the increasing effects of confounding factors such as nutrient regeneration and herbivory with time can theoretically be kept to levels much smaller than that of the variable of interest.

Three sediment plates were randomly collected from each settling tank prior to their transfer to the treatment tanks for purposes of determining initial community parameters for each set of experiments. Likewise, 3 plates were also sampled from each of the control and treatment tanks several days after nutrient addition (Days 1, 3 and 7) for primary production and respiration measurements. A different set of 3 plates was collected from each replicate tank for each day of respirometry experiments (i.e. sampling without replacement, so a total of 9 plates were taken from each tank during the 7-day experiment). The flowthrough respirometry set-up and experimental protocol used in these experiments is described in detail in Montebon and Yap (1995). Respiration rates were measured in the dark (“dark measurements”) by covering the acrylic respiration chambers (StrathKelvin RC400) with a black canvas material. The cover was then removed after ~ 0.5 h to allow the sediment plates to acclimate under 820 µE m^{-2} s^{-1} (910 µE m^{-2} s^{-1} in Experiment 1) for 15 min prior to the photosynthetic (“light”) measurements. The light intensities used were above the I_{10\%} (light saturation constant) values estimated for these communities (Dizon and Yap, 1999). Respiration and photosynthetic rates were derived using regression analysis and were expressed as area-specific (mg O₂ m⁻² h⁻¹) and biomass-specific (mg O₂ [mg Chl a]⁻¹ h⁻¹) rates.

After each respirometry experiment, the sediment samples were transferred to labelled glass jars and fixed in borax-buffered 10% formalin solution with Rose Bengal stain. From the 3 sediment plates taken from each tank per sampling day, one was randomly selected for microalgal analysis while another was selected for faunal analysis. Since there were 2 tanks per treatment, this resulted in a sample size of 2 plates (1 from each tank) each for faunal and algal analyses each sampling day for each treatment. The remaining plates in the tanks were used in Photosynthesis-Irradiance experiments (Dizon and Yap, 1999) and in other experiments using antibiotics to inhibit bacterial metabolism.

For the microalgal analyses, a 3 ml subsample was taken from each well-mixed sediment sample using a syringe corer. This subsample was washed 6 times with 20 ml filtered fresh water to resuspend unattached algal forms. To dislodge the remaining epipsammic algae from the sediment fraction, the sediment subsamples were immersed in an ultrasonic bath for 10 min followed by six 20-ml washings. Both algal suspensions (from the washings made before and after ultrasonication) were pooled and poured through 125 and 20 µm mesh sieves. The fraction retained in the 20 µm sieve was collected, transferred to a glass vial and resuspended in a 20 ml 5% formalin solution.

After vigorous shaking of each vial to mix its contents, a 1 ml aliquot was drawn and dispensed on a gridded Sedgwick-Rafter counting chamber (20 x 50 mm). Microalgal cells were counted using a Leitz inverted microscope (magnification = 320x) following a counting technique modified from Leupold (1988). Counts were made along 4 randomly selected 50 mm² transects. Algae were grouped into coarse taxonomic categories, namely: centric diatoms, pennate diatoms, coccoid/filamentous cyanobacteria, flagellates and unidentified algal cells.

Sediment for faunal analysis was sieved through a 250 µm mesh. The sediment fraction retained in the sieve was extracted from fauna by means of flotation and decantation through repeated washings (Riddle, 1988). Organisms that were extracted from the sediment were preserved in 70% ethanol and stored in glass vials. Using a stereomicroscope, the fauna were counted and sorted into major taxonomic groups, namely: crustaceans, foraminiferans,
nematodes, polychaetes, and “others” (i.e. organisms belonging to less common taxa).

Statistical analyses were carried out using SPSS for Windows (release 6.0, SPSS Corporation 1993). The effects of nutrient treatment and exposure time (i.e. number of days after nutrient addition) on net community photosynthetic ($P_n$) and respiration ($R$) rates were tested using 2-Way ANOVA. Appropriate transformations were applied on data sets that did not meet the parametric assumptions of normality and homoscedasticity (Sokal and Rohlf, 1981).

Microalgal counts, normalised per unit sediment volume, were tested for differences between treatments (controls, N, P, N+P) and through time (i.e. days) using Multivariate Analysis of Variance (MANOVA; Morrison, 1978). Data that did not satisfy the normality and homoscedasticity assumptions were transformed (usually log$_{10}$ [x]) until these were met. Faunal counts were similarly assessed using 2-way fixed effects MANOVA. As results of the different multivariate tests of significance generally agreed with each other in all the analyses, only the significance of the Pillai’s trace statistic will be reported under the MANOVA results.

RESULTS

A multivariate comparison of the algal abundances in samples taken from the settling tanks in the 4 experiments (representing different times of the year) revealed high natural variability among the batches (Pillai’s trace = 1.43, $p<0.05$). Differences were seen for all taxa, so they are most likely due to seasonal fluctuations. A more detailed profile of the seasonal variations in biotic and abiotic aspects of the sampling area is described in Yap et al. (1994) and Nacorda and Yap (1996). These between-batch differences would have obscured any significant between-treatment effects, so no further inter-batch comparisons were carried out. The separate experiments were primarily designed to elucidate nutrient effects rather than seasonal differences.

Nutrient levels

Ambient N:P values of the overlying water at the start of the experiment ranged from 4 to 28 in the control tanks. Initial ratios in the N tanks ranged from 302 to 452 while those in the P tanks were between 0.2 and 6. The N+P treatments had an initial mean ratio of about 17 (Table 1). In spite of the wide range in the N:P levels between same-treatment tanks and through time (e.g., 4–49 for the Controls, 198–452 for the N tanks, and 0.2–6 for the P tanks), differences between treatments were consistently maintained at one to two orders of magnitude. Total inorganic nitrogen levels achieved in the treatment tanks ranged from 50 to 88 $\mu$M while phosphate levels were between 3 and 9 $\mu$M. A more detailed profile of the nutrient concentrations in the tanks is presented in Dizon and Yap (1999).

Primary production

Area- and Chl $a$-specific $P_n$ (net photosynthesis) and $R$ (respiration) are presented in Figure 2. Sediment net production exhibited variations through time and between treatments. Photosynthetic rates of the treated plates (N- and P-enriched treatments) were higher than those of the controls in almost all of the samplings with a general tendency to increase.

### Table 1. – N:P values measured in the control and treatment tanks before nutrient addition and several days after addition; [n.d.], no data.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>N:P days after nutrient addition</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 Tank 1</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>13</td>
</tr>
<tr>
<td>Experiment 2 (Sep 1994)</td>
<td>Controls</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>N</td>
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</tr>
<tr>
<td></td>
<td>P</td>
<td>17</td>
</tr>
<tr>
<td>Experiment 3 (Dec 1994)</td>
<td>Controls</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>14</td>
</tr>
<tr>
<td>Experiment 4 (Aug 1995)</td>
<td>Controls</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>N+P</td>
<td>8</td>
</tr>
</tbody>
</table>
with time (at least up to Day 3 in all experiments). Photosynthetic rates (area- and Chl \( a \)-specific) of plates from both nutrient treatments exhibited noticeably higher values than those of the controls at the seventh day of the experiments (significant treatment effects in Experiments 2 and 3, Table 2), although differences were already apparent for the N-treated sediments as early as Day 3 (Fig. 2). Photosynthetic rates in the N+P sediment plates (Experiment 4) showed an increase at Day 3 but, at Day 7, dropped to levels similar to those measured from the INITIAL (Day 0) plates.

Results from ANOVA (Table 2) reveal that area-specific \( P_n \) differed significantly with exposure time in all experiments and varied with respect to nutrient treatment (interacting with exposure time) in 2 experiments. Chl \( a \)-specific production also differed significantly with exposure time in all experiments, and with respect to nutrient treatment in Experiments 2 and 3. Differences in community \( R \) due to exposure time were generally significant. With these results, the major factors influencing change in sediment metabolic rates were the duration of exposure to the nutrients (in days), and its interaction with the type of nutrient used (N or P, Table 2).

**Microphytobenthos**

Figure 3 depicts changes in the microalgal composition of the sediment plates through time. Major constituents of the samples were the pennate diatoms and cyanobacteria, with the latter (both colony and filamentous types) accounting for much of the change in algal densities. Flagellates and other unidentified algal cells comprised only 0–3% of the total microalgal counts.

Among the centric diatoms encountered in the samples, the families Thalassiosiraceae and Coscinodiscaceae were well represented. Pennate diatoms were represented by the families Naviculaceae,
Table 2. Summary of results from 2-factor ANOVA assessing the main and interaction effects of nutrient treatment (N) and nutrient exposure time (D) on microalgal production and respiration rates. * p < 0.05.

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
<th>Experiment 4</th>
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<tr>
<td></td>
<td>df F-ratio</td>
<td>df F-ratio</td>
<td>df F-ratio</td>
<td>df F-ratio</td>
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<tr>
<td>Area-specific net production</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutrient (N)</td>
<td>2, 9 3.00</td>
<td>2, 9 18.22*</td>
<td>2, 9 15.04*</td>
<td>1, 6 3.04</td>
</tr>
<tr>
<td>Exposure time (D)</td>
<td>2, 9 21.27*</td>
<td>2, 9 97.78*</td>
<td>2, 9 14.76*</td>
<td>2, 6 56.69*</td>
</tr>
<tr>
<td>N*D</td>
<td>4, 9 1.18</td>
<td>4, 9 11.45*</td>
<td>4, 9 5.26*</td>
<td>2, 6 1.71</td>
</tr>
<tr>
<td>Area-specific respiration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutrient (N)</td>
<td>2, 9 1.86</td>
<td>2, 9 0.83</td>
<td>2, 9 0.30</td>
<td>1, 6 9.47*</td>
</tr>
<tr>
<td>Exposure time (D)</td>
<td>2, 9 7.20*</td>
<td>2, 9 33.99*</td>
<td>2, 9 3.13</td>
<td>2, 6 11.81*</td>
</tr>
<tr>
<td>N*D</td>
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<td>4, 9 2.26</td>
<td>4, 9 0.27</td>
<td>2, 6 1.53</td>
</tr>
<tr>
<td>Chl a-specific net production</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutrient (N)</td>
<td>2, 9 0.34</td>
<td>2, 9 12.43*</td>
<td>2, 9 6.31*</td>
<td>1, 6 0.71</td>
</tr>
<tr>
<td>Exposure time (D)</td>
<td>2, 9 19.09*</td>
<td>2, 9 78.55*</td>
<td>2, 9 12.10*</td>
<td>2, 6 63.15*</td>
</tr>
<tr>
<td>N*D</td>
<td>4, 9 0.91</td>
<td>4, 9 9.96*</td>
<td>4, 9 0.78</td>
<td>2, 6 1.23</td>
</tr>
<tr>
<td>Chl a-specific respiration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutrient (N)</td>
<td>2, 9 2.78</td>
<td>2, 9 1.92</td>
<td>2, 9 2.14</td>
<td>1, 6 3.35</td>
</tr>
<tr>
<td>Exposure time (D)</td>
<td>2, 9 5.39*</td>
<td>2, 9 15.13*</td>
<td>2, 9 1.18</td>
<td>2, 6 5.30*</td>
</tr>
<tr>
<td>N*D</td>
<td>4, 9 0.44</td>
<td>4, 9 0.62</td>
<td>4, 9 0.50</td>
<td>2, 6 0.46</td>
</tr>
</tbody>
</table>

Fig. 3. Microalgal composition of sediment plates in Experiments 1 to 4. Note that the graphs are arranged according to treatment (columns) and experiment sets (rows). Flagellates and unidentified cells are not included as they comprise only 0-3% of the total counts. X-axis is not drawn to scale. Values are means of 2 samples per treatment.
Fragilariaceae, Phaeodactylaceae and Bacillariaceae. Commonly encountered coccoid microcolonies mostly belonged to the genera *Agmenellum* and *Anacystis*. Filamentous cyanobacteria were represented by *Spirulina* and *Oscillatoria*, as well as other heterocystous and non-heterocystous forms. Flagellates were represented by dinoflagellates (typically by the genus *Prorocentrum* and some thecate forms).

MANOVA results (Table 3) show significant density differences in the major algal groups due to nutrient treatment in 3 out of 4 experiments. Differences in algal abundances through time were significant in Experiments 1 and 4, while interaction effects were occasionally registered in all experiments.

The greatest responses (in terms of cell numbers) to nutrient addition were observed in the N and N+P treatments (Fig. 3). Algal densities in the control tanks were relatively stable through time (except in Experiment 1), while purely phosphorus-enriched tanks displayed relatively small temporal fluctuations. Growth responses in terms of biomass change of each taxon were not determined in this study as no record was made of algal cell sizes that would have allowed conversions of algal counts to biomass. The range in cell sizes within each taxon was large, particularly among the diatoms and the colonial cyanobacteria.

**Infauna**

The infauna showed large inter-batch order-of-magnitude differences (Fig. 4). The lowest counts were obtained from Experiment 2 (23 organisms cm⁻²). Three taxonomic groups were observed to be numerically dominant in almost all the sediment samples, namely, crustaceans, nematodes and foraminiferans. Other organisms recorded in the sediment samples were representatives of the following taxa: Cnidaria, Mollusca, Nemertea, Polychaeta, Pycnogonida, Sipunculida, and Turbellaria. Foraminiferans accounted for a substantial portion of the total counts in Experiment 1, while nematodes dominated the sediment in terms of abundance in Experiment 4. Crustaceans figured prominently in Experiment 3, where the other two taxa were also present in significant densities. No distinctive trend could be discerned, however, with respect to either nutrient treatment or time (2-way fixed effects MANOVA, *p*<0.05).

**DISCUSSION**

Pore water nutrient concentrations (50-100 µM DIN) in shallow-water tropical marine sediments are at least two orders of magnitude higher than those in the overlying water (Stimson and Larned, 2000). Ambient porewater nutrient concentrations from carbonate and terrigenous sediments (seagrass beds in South Sulawesi, Indonesia) have been reported to reach 100 µM NH₄ and 10 µM PO₄ (Erftmeijer *et al.*, 1994). However, access to this within-sediment nutrient supply by actively photosynthesising algae in the top sediment layers is apparently limited. The results of the experiments reported here indicate that nutrient regeneration from within the sediment plates was probably not significant. Concentrations
of NH$_3$ (a typical form of regenerated N) did not exceed 3 µM in all the experimental tanks. However, by enriching the overlying water column (i.e. providing easy access to nutrients) with nutrient concentrations that are of the same order of magnitude as porewater concentrations measured in the field, observable and measurable responses in the microphytobenthos are elicited.

The most significant responses, within a time scale of days, were obtained from the microalgal communities with the addition of nitrogen, and of nitrogen combined with phosphorus. Significant increases in cell number, particularly of the cyanobacteria, were detected along with significant increases in net community production. Community respiration also changed significantly over the relatively short duration of the experiment.

Nilsson and Sundbäck (1991), in their experiments on microalgae on sand-agar substrates, similarly noted an initial increase in the numbers of non-diatom microalgae following nutrient addition. Their results indicate that non-siliceous microalgae such as cyanobacteria may have an intrinsically faster initial growth response to N and/or P enrichment than diatoms. In the present study, significant interaction effects were noted, indicating that the microalgae responded variably, depending on both the time of sampling and the nutrient treatment that was applied. Variability was evident in both the diatom and cyanobacterial densities although much of the community growth is attributed to the latter.

With respect to community metabolism, the significant interaction of nutrient treatment and time in some of the experiments (Table 2) is an indication of the early effect of nutrient addition. The photosynthetic performance of these communities measured over a range of light intensities and over the same period of exposure has been shown to be nutrient-limited in a related study by Dizon and Yap (1999). In their study, the addition of inorganic nitrogen elicited an increase in the area-normalised metabolic rates of the microalgae (as well as significant increases in chlorophyll $a$ concentrations) while the addition of inorganic phosphorus brought about an enhancement of chlorophyll $a$-normalised photosynthetic rates of the sediment communities. Other studies using longer-term nutrient treatments (e.g., Nilsson and Sundhök, 1991; Pinckney et al., 1995) have also shown significant nutrient effects on benthic metabolic and growth rates.

Major infaunal groups encountered in the sediment samples were the nematodes, foraminiferans and crustaceans (mostly copepods), taxa that were similarly reported by Guzmán et al. (1987) to be the dominant meiofauna in two Costa Rican reef habitats. The orders-of-magnitude differences in the

![Fig. 4. – Mean infaunal abundances (organisms cm$^{-2}$) determined from the sediment plates in Experiments 1-4. Note differences in the scale of the vertical axis. Bars represent mean of 2 samples per treatment per day. Error bars are standard deviations of mean total counts.](image-url)
total faunal counts among the four batch experiments might be due to the seasonal fluctuations in the abundances of these organisms in situ. The findings of Nacorda and Yap (1996) on the seasonal fluctuations in macrofaunal abundances in the same reef flat is indicative of this. Yap et al. (1994), in their 2-yr study of the area, reported three distinct “seasons”, namely, a dry-cool season (Dec-Feb), a dry-warm season (Mar-Apr) and a wet season (May-Nov). These seasons were based on the monthly variations of several physico-chemical parameters such as salinity, temperature and light intensity.

A lack of significant temporal variation in faunal abundance was consistently observed in each of the four separate experiments within the 7-day observation period. This is typical in the early stages of long-term incubation experiments. Nutrient inputs lead to the enhancement of algal productivity and biomass accumulation. Prolonged exposure to elevated nutrient levels, however, brings about a subsequent response of other components. In nutrient enriched benthic mesocosms, Nilsson et al. (1991) reported the proliferation of oligochaete and copepod populations after 4 weeks. Sullivan and Rittaco (1988) noted increased rates of copepod egg production in enriched mesocosms in spite of the addition of copper, while Jacobsen et al. (1995) observed increased populations of choanoflagellates and microzooplankton in their fertilized enclosures. Such responses are most likely attributable to increased food availability. Consequently, these initial responses may elicit other “second-order” nutrient effects (Birkeland, 1988) such as subsequent community shifts toward r-selected organisms and intensified biological interactions (i.e. grazing and competition). Unless the experiment is designed to take these into account, community parameters of interest may be seriously confounded by these variables. In this study, the short incubation time was intended to prevent organic matter accumulation and to keep the experiment well within the generation times of most infaunal organisms, thus minimising both nutrient regeneration and the increase in herbivory rates that could potentially obscure the effects of nutrient enrichment on the microalgal fraction of the sediment.

Comparing our rates with those measured in situ, only the upper limits of the area-specific $P_n$ were within the range of rates measured at the Lucero reef flat, while values of $R$ were way below the in situ mean annual respiration value of $213 \pm 90 \text{mg O}_2 \text{m}^{-2} \text{h}^{-1}$ measured between 1989 and 1991 in the same vicinity as the collection site (Yap et al., 1994). These discrepancies are presumably due to the absence of components (e.g. larger algae and macroinfauna) that were excluded from the samples during the preparation of the sediment plates. This is evident in the autotrophic responses ($P/R > 1$) observed in the sediment plate communities. In situ metabolic measurements of the sand substrate in the Lucero reef flat showed a general tendency of the benthic community towards heterotrophy ($P/R < 1$; Yap et al., 1994; Nacorda and Yap, 1996) which implies that the macroinfaunal component of the sediment may significantly account for most of the energy consumption in the benthos. However, no significant correlation was established between macroinfaunal biomass and benthic respiration rates (Nacorda and Yap, 1996).

The use of sediment-filled glass petri dishes as experimental substrates has been demonstrated in this study to be suitable for short-term manipulative experiments on microalgal communities (also Dizon and Yap, 1999). The uniformity of the grain sizes and the exclusion of larger infaunal organisms (which might have contributed to significant loss of algal biomass through grazing and to nutrient input via excretion) enable a greater degree of control over variables and minimise the inclusion of experimental artifacts. Inoculation of the substrates by settlement from an algal suspension has the advantage of a shorter colonisation time and a reduction of the “selective effect of species-specific colonisation” (Nilsson and Sundbäck, 1991). The duration of the settlement period also allows enough time for the system to recover from the disruptive effects of sieving (Findlay et al., 1990). The shallow depth of the sediment plates (1 cm) greatly reduces the chances of forming anoxic lower layers that may have served as a source of regenerated nutrients.

This study demonstrates that tropical microphytobenthic assemblages are adapted to responding quickly to episodic nutrient inputs, indicating that this fraction of the benthos is basically opportunistic, and are thus able to proliferate as soon as access to nutrients is provided. Results from this study and from an earlier paper (Dizon and Yap, 1999) also indicate that these communities are nutrient-limited (both N and P). Nutrient loading on reef flat systems can stimulate high microalgal biomass production, though this may not be readily observed in nature due to the interplay of other factors involving other components of the system. Longer-term enrichment experiments (e.g. experiments simulating chronic...
nutrient loading events) and more holistic experiments (i.e. incorporating all benthic components) will be of interest in studying other related processes such as community structure shifts, nutrient uptake rates and capacities, macro- and meiofaunal responses, and nutrient regeneration processes.

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