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Microbial nitrogen transformations in unconsolidated coral reef sediments

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ABSTRACT: Major nitrogen (N) pools and bacterial transformations of N were examined in carbonate sediments of 3 reefs in the central area of the Great Barrier Reef, Australia. Depth distributions of nitrate (NO_3^-) and ammonium (NH_4^+) and rates of NH_4^+ production, N_2 fixation (nitrogenase activity by C_2H_2 reduction) and denitrification were measured in muddy sediments of an inshore reef and in fine-, medium- and coarse-grained sediments at a midshelf and shelf edge reef. Ammonium efflux was estimated from pore water profiles. Estimates of potential rates of NH_4^+ and NO_3^- utilization were made in the upper 2 cm of sediments at the midshelf and shelf edge reefs. Highest concentrations of NH_4^+ (up to 70 μM at 8 cm) were observed in muddy carbonate sediments of inshore Pandora Reef, with somewhat lower concentrations (up to 20 μM) in fine-grained sands of the other 2 sites. Relatively small NH_4^+ pools, usually less than 10 μM, typified coarse-grained sediments. Nitrate was generally undetectable in these sediments. Rates of NH_4^+ efflux among sites ranged from 0 to 4 μmol N m^{-2} h^{-1}, with highest fluxes associated with muds and fine-grained sands. Ammonification rates in the upper 2 cm ranged from 6 to 26 μmol N m^{-2} h^{-1} among sites, generally increasing with depth. Nitrogenase activity was detected in all sediments examined, with highest rates near the surface. N_2 fixation could account for more than 50 % of NH_4^+ production in the upper 2 cm of sediment at 3 of 4 sites. The potential in the upper 2 cm for NH_4^+ consumption (nitrification and assimilation) ranged from 10 to 60 μmol N m^{-2} h^{-1}, while NO_3^- reduction potential ranged from 10 to 80 μmol N m^{-2} h^{-1} suggesting these may be quantitatively important pathways. Inhibitor experiments indicated that much of the NH_4^+ utilization might be by nitrification. Very high nitrification rates [up to 3.8 nmol N (g dry sed.)$^{-1}$ h^{-1} or 70 μmol N m^{-2} h^{-1}] were confirmed at 1 site by a ^{15}N isotope dilution method. Low denitrification rates were also detected in these environments, and in many cases under apparently oxic conditions. However, highest rates noted were less than 5 % of the rate of NO_3^- reduction. While shallow carbonate sands may be poor in organic material, they are active sites of bacterial N transformations. The NH_4^+ and NO_3^- pools in the upper few cm appear to be highly dynamic, with estimated turnover times of substantially less than 1 d. It is also noteworthy that bacterial N_2 fixation appears to account for a much larger fraction of NH_4^+ turnover than in shallow temperate zone sediments.

INTRODUCTION

Coral reefs are often likened to oases of biological productivity in a marine desert. The oligotrophic tropical waters which bathe reefs are often devoid of essential macronutrients (D'Elia 1988), and particularly nitrogen (N) which is considered by many (but not all, e.g. Smith 1984) to be the primary limiting nutrient for these systems (D'Elia 1988). The apparent enigma of high biological productivity despite low inputs of N has been essentially solved by research over the last 2 decades which has identified varied and intensive sites of biological N_2 fixation on and around reefs (Capone 1983, 1988, D'Elia 1988, D'Elia & Wiebe 1990).

Coral reefs are composed of consolidated areas of coral growth interspersed with areas of unconsolidated sediment containing coral and algal carbonate skeletons. Coral reef sediments have essentially been ignored until recently with regard to their importance in coral reef nutrient cycling, possibly because of their low organic content. Nonetheless, unconsolidated areas of reef sediment often greatly exceed the areal extent of the reef proper. Furthermore, the recent findings that O_2 can be depleted within centimeters of the sediment surface (King et al. 1990) suggests that

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anaerobic biogeochemical processes are operative around the reef and may be important in the various nutrient cycles (DiSalvo 1973, Nedwell & Blackburn 1987).

Microorganisms in the sediment are capable of various N transformations which can affect the forms and concentration of N. Many of the microbial N transformations, such as denitrification and ammonification, are predominantly anaerobic, and may therefore occur in the sediments.

We therefore concurrently examined the distribution of inorganic N species, sediment surface nitrification and the depth distribution of N_2 fixation, ammonification, and denitrification in sediments of the Great Barrier Reef at 3 sites ranging from an oligotrophic outer shelf reef to a more eutrophic reef closer to shore.

MATERIALS AND METHODS

Study sites. Microbial N transformations were investigated in shallow (1 to 25 m water depth) carbonate sediments at several sites along the central portion of the Great Barrier Reef, Australia (Fig. 1). Bowl Reef is located on the outer edge of the Great Barrier Reef. Hopkinson Reef is due west of Bowl, on the middle shelf. Pandora Reef is relatively close to the mainland in the inner reef track. Sampling location details for each site are given in Table 1. Sampling and experiments occurred during two 2 wk cruises aboard the RV 'Sirius' (Nov 1985) and RV 'Lady Basten' (Dec 1985) of the Australian Institute of Marine Sciences.

Sediment cores. Cores of up to the top 10 cm of sediment were manually collected by SCUBA or snorkeling using plastic core tubes (diameter 3.5 cm) or, for microbial assays, cut-off 30 or 50 cc plastic syringes, 10 cm length × 2 cm or 2.6 cm diameter, respectively. Barrel cores were gently inserted into the sediments to minimize disturbance. Syringe cores were used as piston cores, with the plunger in place and slowly withdrawn as the core was inserted. For each, black rubber stoppers were used to seal core ends. Samples were returned to the shipboard laboratory and processed as soon after sampling as possible, usually within 2 h.

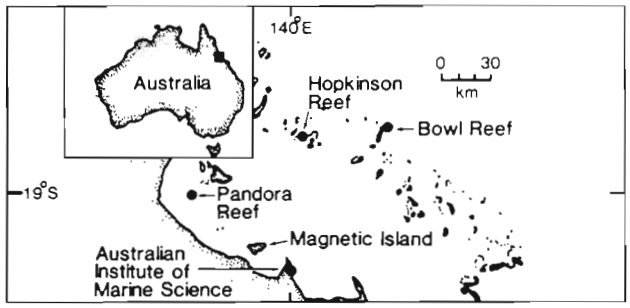

Fig. 1. Map of reef site locations

Pore water analysis. Sediments were analyzed for pore water NH_4^+, NO_3^- and NO_2^- using standard (manual) seawater methods (Strickland & Parsons 1972). Pore waters were collected by 2 methods. Cores returned to shipboard were segmented in 2 cm increments and the pore waters collected by vacuum filtration in a Hoefer 10-position vacuum filtration manifold. Alternately, in coarse- or medium-grained sediments, we used a modified teflon 'sipper' with low dead volume with a 1 cm porous frit near the tip which was manually inserted into the sediment. At 2 cm intervals

Table 1 Site characteristics and interstitial ammonium concentrations; filt: filtered; sip: sipped

Site	Depth (m)	Sediment type	Location	Date/method	NH_4^+ concentration (μM) 0–2 cm	6–8 cm
Pandora	10	Mud	Forereef	11 Nov/filt	15.0	67.0
				12 Nov/filt	6.1	57.0
Hopkinson 1	10	Fine sand	Backreef	5 Nov/sip	0.8	17.9
				5 Nov/filt	11.3	11.6
				6 Nov/filt	1.5	18.1
Hopkinson A	20	Fine sand	Backreef	9 Nov/filt	4.0	19.4
Hopkinson B	10	Medium sand	Backreef	9 Nov/filt	1.8	8.4
Hopkinson C	2	Coarse sand	Reef flat	20 Dec/filt	6.6	23.5
				20 Dec/sip	2.9	7.6
Hopkinson D	20	Medium sand	Forereef	8 Nov/sip	5.3	5.9
Bowl A	10	Medium sand	Backreef	13 Dec/sip	2.4	17.6
				14 Dec/filt	19.2	29.8
Bowl B	20	Fine sand	Backreef	12 Dec/sip	2.6	8.4
Bowl C	10	Coarse sand	Backreef	11 Dec/sip	1.2	1.2

down to about 10 cm, ca 10 ml was withdrawn by plastic syringe and returned to the surface for analysis.

Solid phase analysis. Known volumes of sediment in 2 cm intervals were dried at 105 °C. The weight loss per volume was used to estimate sediment porosity (Berner 1980). Dried sediments were analyzed for total CN on a Leco CHN600 and, after dissolution of carbonates, for organic carbon (Sandstrom et al. 1986).

Ammonium fluxes. Diffusive NH_4^+ fluxes were computed (Berner 1980) for sites with an NH_4^+ gradient in the upper sediment column, using a molecular diffusion coefficient (D) of 19.8×10^{-6} cm^2 s^{-1} (Li & Gregory 1972, Krom & Berner 1980) corrected for tortuosity using the measured porosity and assuming a formation factor (F) of 2.228 for the calculation of the sediment diffusion coefficient (D_s). No correction was made for adsorption (Rosenfeld 1979).

Ammonification. For the depth profiles of NH_4^+ production in carbonate sands, triplicate samples were collected in 60 cc Plastipak syringes with plungers in place but from which the end had been removed to allow for coring. Cores were taken to a depth of at least 8 cm. The open ends were capped in the field with a # 6 black rubber stopper. Upon return to the ship, the plunger was carefully removed by breaking the seal with a needle. Approximately 10 ml of ambient seawater was gently added to provide an overlying water phase. The plungers were then carefully replaced on all cores and kept sealed until harvested. Cores were sampled at zero time and at various time intervals to establish the rate of NH_4^+ production. At sampling times, the plunger was gently removed by breaking the seal with a needle. Overlying water was removed by syringe, and the samples were filtered through Whatman GF/C filters and refrigerated until analyzed for NH_4^+.

The plunger was then replaced and used to extrude and section the core from the bottom up, in 8 to 6, 6 to 4, 4 to 2, and 2 to 0 cm segments. Each segment was placed in a filtration tower of a Hoefer filtration apparatus, and the pore waters were passed through GF/C filters into scintillation vials in the manifold. For the initial samples (e.g. zero time and 6 h), replicates for each horizon had to be combined to obtain sufficient volume (2 to 3 ml) for analysis. At subsequent time points, individual replicates could be analyzed separately since they required dilution.

Acetylene reduction and acetylene blockage assays. Nitrogenase activity was measured on both cruises using the acetylene reduction assay (Stewart et al. 1967, Hardy et al. 1968) as adapted for marine sediments (Capone 1982, O'Neil & Capone 1989). Because of instrument problems, denitrification was estimated only on the second cruise by the acetylene blockage method (Payne 1984, Slater & Capone 1987). Acetylene (C_2H_2), generated from calcium carbide, was added to the experimental flasks to a final volume equal to 20 % of the gas head space volume (vol:vol). Flasks were incubated in the dark and experiments were carried out within 2 °C of ambient temperature (25 to 30 °C). Control experiments with sediment and no additions of C_2H_2, as well as flasks containing filtered seawater alone with additions of C_2H_2, were also conducted.

In general, samples of the top 4 cm of sediment from 30 cc syringe cores were extruded directly into 175 ml wide mouth Erlenmeyer flasks which were then sealed with black rubber stoppers. Alternately, for depth profiles, 2 cm increments were extruded into flasks. For most experiments, samples were not slurried by addition of seawater. For anaerobic experiments, stoppered flasks were gassed with N_2 for 2 to 3 min through incurrent and excurrent hypodermic needles inserted through the stopper. Flask headspace was occasionally sampled and checked for O_2 concentrations using electron capture gas chromatography.

Gas samples (100 µl) were withdrawn from the headspace of the experimental flasks with a gas tight syringe and analyzed immediately for ethylene (C_2H_4) production using flame ionization or nitrous oxide (N_2O) by electron capture detection on a Shimadzu Mini 2 gas chromatograph. Gases were separated on 2 m × 3 mm Porapak R columns, held at 70 °C. Injector temperature = 150 °C; N_2 carrier flow = ca 30 ml min^{-1}; H_2 = ca 30 ml min^{-1}; air flow = ca 300 ml min^{-1}.

Peak heights of C_2H_4 or N_2O were measured on a Hewlett-Packard 3390A integrator and converted to nmol of gas by comparison to peaks of C_2H_4 or N_2O standards of known concentrations. Sediment samples were dried to constant weight at 60 to 100 °C, and rates of C_2H_4 or N_2O production were normalized to dry weight of sediment. Rates of C_2H_2 reduction or N_2O production as nmol (dry wt sed.)$^{-1}$ h^{-1} were determined by performing linear regression analysis to determine the average slope of replicates for each parameter over the initial 12 to 24 h of incubation.

Ammonium utilization, nitrification and nitrate reduction. The potentials for NH_4^+ utilization and NO_3^- reduction in the upper 2 cm were assessed in aerobic slurry incubations with defined NO_3^- and NH_4^+ concentrations by noting changes in these pools over 24 h periods. On 2 occasions, nitrification rate was determined using the ^{15}N-NO_3^- isotope dilution method (Koike & Hattori 1978, Horrigan & Capone 1985). The top 2 cm from seven 50 cc syringe cores were combined and sieved through a 2 mm mesh. For each experiment, 5 cc portions of sediment were dispensed into six 125 ml Erlenmeyer flasks. Experiments were initiated by addition of 50 ml of a 10 µM NO_3^- (as $^{15}NO_3^-$) and 10 µM NH_4^+ solution. Flasks were placed

in a water bath in the dark for incubation. Two flasks were harvested at 0, 24 and 48 h. For harvesting, the contents of the flask were filtered onto a 4.25 cm Whatman GF/C filter previously rinsed with distilled water. After filtering, the sediment was collected and placed in a tared scintillation vial for dry weight analysis. The filtrate was collected, with about 20 ml used for nutrient analysis. For ^{15}N experiments, the remainder was frozen for subsequent solvent extraction of NO_3^- and determination of $^{15}N:^{14}N$ isotopic ratio by mass spectrometry (Horrigan & Capone 1985).

On one occasion (22 Dec, Hop C), parallel experiments also examined the use of chlorate (Belser & Mays 1982) and N-Serve (Webb & Wiebe 1975) as specific inhibitors of nitrification. Chlorate was added to a final concentration of 10 mM, while N-Serve dissolved in DMSO was added to a final concentration of 10 ppm (wt/vol). From these flasks, 10 ml subsamples of the liquid phase were removed over the time course of the experiment for inorganic N analysis.

RESULTS

Site characteristics

Among the 3 sites, highest NH_4^+ levels in bottom waters were found at Pandora Reef (Table 2). Bottom water NH_4^+ levels were lower at Bowl and Hopkinson Reefs (Table 2). Bottom NO_3^- levels at Pandora Reef were also greater than either Bowl or Hopkinson Reefs.

Ammonium concentrations in the upper 10 cm of sediments from Bowl and Hopkinson Reefs were generally similar for comparable sediment types. For fine-grained sediments, NH_4^+ concentrations increased rapidly over the upper few cm, leveling off at 10 to 30 µM by 7 cm (Fig. 2A, Table 1). Ammonium concentrations in coarse-grained sands were generally much lower, usually less than 10 µM by 10 cm depth (Fig. 2B). An exception to this was the reef flat sediment at Hopkinson Reef (Hop C; Fig. 2B) which had an increase in NH_4^+ with depth more typical of the finer grained sands. Fine muds were sampled only at Pandora Reef, and these sediments had the sharpest gradients and highest concentrations (Fig. 2C, Table 1). The lowest NH_4^+ concentrations were noted in sediments from Bowl Reef (Bowl C; Fig. 2B), a station which we observed to be densely populated with *Callianassa* shrimp.

Table 2. Bottom water ammonium and nitrate concentrations; means ± SE, with number of replicates in parentheses

Station/Month	Ammonium (µM)	Nitrate (µM)
Hopkinson/Nov	0.67 ± 0.30 (6)	0.70 ± 0.21 (5)
Hopkinson/Dec	0.61 ± 0.23 (7)	0.27 ± 0.03 (7)
Pandora/Nov	1.61 ± 0.68 (10)	1.18 ± 0.25 (10)
Bowl/Dec	0.55 ± 0.55 (11)	0.59 ± 0.17 (7)

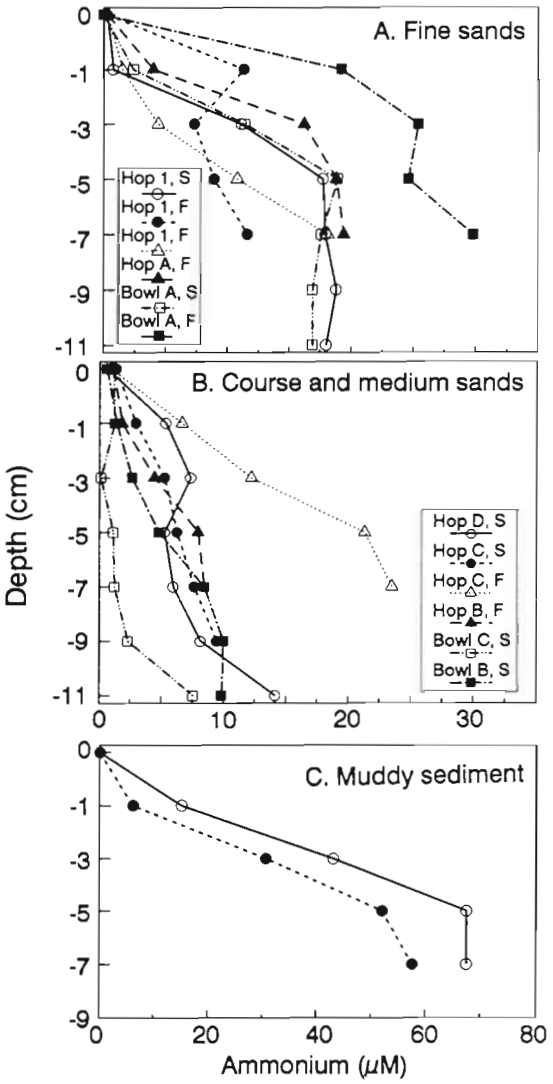

Fig. 2. Depth distributions of ammonium in (A) fine, (B) medium and coarse and (C) muddy carbonate sediments. See Table 1 for more detail on site designation and characterization. Hop: Hopkinson; S: sippers; F: filtered

With respect to the 2 procedures used for collecting pore waters, sipper values (S) were generally lower than values derived by vacuum filtration of sediment segments (F), particularly for the shallowest horizons (0 to 2 cm). Closer agreement between the 2 sampling protocols occurred for fine sands at deeper horizons (e.g. Hop 1 and Bowl A; Table 1). Sippers were incapable of obtaining samples in the muddy sediments of Pandora Reef. Subsequent analyses were restricted to data from filtration-derived samples.

Table 3. Solid phase analysis of study sites. Where indicated, values are means of triplicate determination (± SE)

Particulate N (% dry wt)	Organic C (% dry wt)	C/N	Porosity
Pandora Reef (mud)			
0–2 0.170 ± 0	0.550 ± 0.060	3.2	0.708
6–8 0.140	0.530	3.8	0.709
0–8 0.145	0.538	3.7	0.707
Hopkinson Reef A (fine-grained)			
0–2 0.083 ± 0.007	0.233 ± 0.003	2.8	0.526 ± 0.009
6–8 0.050	0.270	5.4	0.506 ± 0.008
0–8 0.064	0.232	3.6	0.506
Hopkinson Reef B (medium-grained)			
0–2 0.133 ± 0.013	0.240 ± 0.006	1.8	0.496 ± 0.006
6–8 0.070	0.190	2.7	0.476 ± 0.001
0–8 0.094	0.215	2.3	0.479
Hopkinson Reef C (coarse-grained)			
0–2 0.067 ± 0.009	0.263 ± 0.007	4.0	0.560 ± 0.007
6–8 0.043 ± 0.020	0.243 ± 0.035	5.6	0.512 ± 0.002
0–8 0.054	0.242	4.5	0.534

Bulk phase analyses were performed on sediments from several of the sites (Table 3). Particulate N was highest in the muddy Pandora Reef sediment, and lowest in the coarse-grained sands. The muddy sediment had about twice the organic C content of the sands (Table 3). Porosities were also highest in the muddy sediments, compared to the sands. Sediment diffusion coefficients ($D_s \times 10^6$ cm^2 s^{-1}) calculated for several of the sites were 12.6 (Pandora, mud), 16.9 (Hop B, fine), 17.9 (Hop A, medium) and 15.9 (Hop C, coarse).

Ammonification

Ammonium production in intact cores was examined at several of the stations (Fig. 3; Table 4). At Pandora Reef, NH_4^+ production rates were relatively constant with depth, ca 0.25 nmol (g dry sed.)$^{-1}$ h^{-1}. Similarly, at Hop A, relatively high rates of NH_4^+ production occurred through the upper 8 cm, with the lowest rates in the upper 2 cm. At Hop B, while little ammonification was noted in the upper 4 cm, high rates of NH_4^+ production occurred below 4 cm. In contrast, at 2 sites with medium-grained sands, substantial NH_4^+ production was found only in the upper 2 cm. At shallow, coarse-grained Hop C, NH_4^+ production, while highest at the surface, was substantial down to the deepest horizon sampled (6 to 8 cm) (Fig. 3).

Ammonium and nitrate utilization

Net changes in NH_4^+ and NO_3^- pools were also examined over 24 h periods in aerobic sediment slurries of the upper 2 cm from several of the sites (Table 4). Relatively high potential rates of NH_4^+ utilization, either through nitrification or assimilation, occurred in all cases examined. Values ranged from 0.54 to 2.9 nmol (g dry sed.)$^{-1}$ h^{-1}. Similarly, most sites also had appreciable rates of NO_3^- removal, ranging from 0.5 to 1.8 nmol (g dry sed.)$^{-1}$ h^{-1}. Medium-grained Hop B had lowest rates of both NH_4^+ and NO_3^- consumption while fine-grained Hop 1 had highest rates of each (Table 4). In the coarse-grained reef flat sediments of Hop C, very

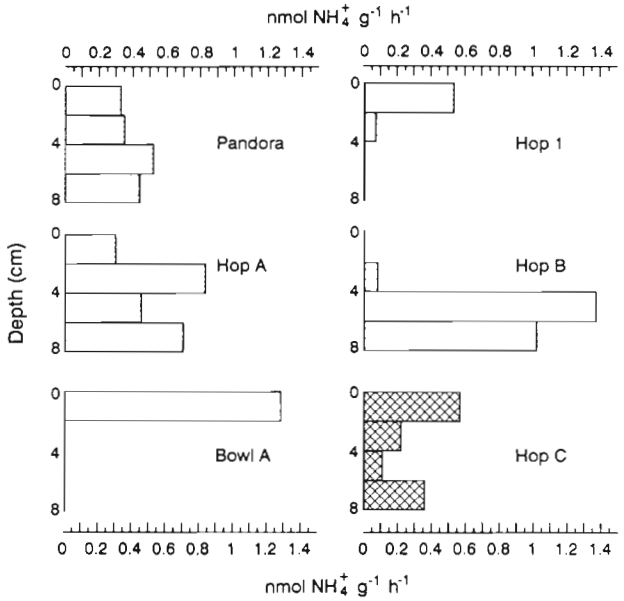

Fig. 3. Depth distributions of ammonium production at several sites. Fine stipple indicate muds and fine-grained sediments, medium stipple are medium-grained and cross hatch are coarse-grained

Table 4. Rates of net ammonium production, net ammonium and nitrate utilization, denitrification and nitrogen fixation. Nitrogen fixation data previously reported in O'Neil & Capone (1989). Where indicated, data are means ± SE with number of replicates in parentheses. Hop: Hopkinson; nd: not determined

Site	NH_4^+ production[a]	NH_4^+ utilization[b]	NO_3^- reduction[b]	Denitrification[c]		Nitrogen fixation[c]	
	[nmol (g dry sed.)$^{-1}$ h^{-1}]			$-O_2$	$+O_2$	$-O_2$	$+O_2$
				[pmol (g dry sed.)$^{-1}$ h^{-1}]		[nmol C_2H_4 (g dry sed.)$^{-1}$ h^{-1}]	
Pandora	0.34	nd	nd	nd	nd	0.44 + 0.05 (2)	0.34 ± 0.05 (2)
Hop 1	0.30	2.9	1.8	nd	nd	nd	nd
Hop A	0.57	2.2	1.8	0.0	6.0 ± 0 (0)	0.13 ± 0.013 (4)	0.27 ± 0.05 (4)
Hop B	0.04	4.1	0.65	3.2 ± 2.44 (3)	9.0 ± 2.4 (4)	0.08 ± 0.01 (3)	0.15 ± 0 (2)
Hop C	0.40	2.8	–2.7	4.6 ± 2.8 (2)	16.2 ± 3.7 (6)	0.04 (1)	0.46 ± 0.23 (3)
Hop D	nd	2.1	1.6	22.6 ± 12.8 (3)	3.0 ± 1.2 (3)	0.16 ± 0.09 (5)	0.22 ± 0.09 (5)
Bowl A	0.64	2.3	1.8	91.6 ± 18.5 (7)	10 ± 2.0 (3)	0.07 ± 0.006 (4)	0.77 ± 0.09 (3)
Bowl B	nd	0.54	0.46	7.0 ± 3.8 (6)	10.0 ± 2.0 (2)	0.14 ± 0.03 (6)	0.36 ± 0.05 (2)
Bowl C	nd	nd	nd	666 ± 212 (4)	22.2 ± 13.8 (3)	0.28 (1)	nd

[a] Averaged over top 4 cm from intact core incubations
[b] From aerobic slurry assays of top 2 cm
[c] From flask assays (not slurried) of top 4 cm of minimilly disturbed sediment

high rates of net NO_3^- production, rather than removal, occurred (Table 4).

Nitrification

For Hop C, the only site which exhibited net rates of NO_3^- production, nitrification was examined by several methods on 2 dates (Table 5). On 18 Dec, total nitrification rates based on $^{15}NO_3^-$ isotope dilution were about 7.6 fold greater than concurrent NO_3^- reduction rates [3.8 vs 0.5 nmol (g dry sed.)$^{-1}$ h^{-1}], based on ^{15}N. Net NH_4^+ depletion and NO_3^- accumulation rates were about 74% and 71% of the total nitrification rate, respectively, and 85% and 82% of the net nitrification rate.

Table 5. Comparison of nitrification estimates in coral reef sediments by several methods at Stn Hopkinson C

Date	Assay	Nitrification (nmol g^{-1} h^{-1})
18 Dec	$^{15}NO_3^-$ isotope dilution	3.8
		3.3 (net)[a]
	NO_3^- accumulation	2.7
	NH_4^+ depletion	2.8
22 Dec	$^{15}NO_3^-$ isotope dilution	0.88
		0.50 (net)
	NO_3^- accumulation	0.31
	NH_4^+ depletion	1.95
	Chlorate (ΔNO_3^-)	0.72
	N-Serve (ΔNO_3^-)	0.60
	(ΔNH_4^+)	2.21

[a] Less estimate of ^{15}N-nitrate reduction

On 22 Dec, ^{15}N-nitrification rates were only 23% of the rate on the previous sampling. Total nitrification was about 2.3 times the concurrent ^{15}N-NO_3^- reduction rate [0.88 vs 0.38 nmol N (g dry sed.)$^{-1}$ h^{-1}]. The ^{15}N-based estimate was 2.8 times the NO_3^- accumulation rate and 45% of the net NH_4^+ accumulation rate. Inhibitor assays of nitrification using chlorate, performed on 22 Dec, did not detect any NO_2^- accumulation; however, they did result in NO_3^- consumption, compared to accumulation in controls and thus yielded an estimate of nitrification about 82% of the gross rate of nitrification based on the ^{15}N method. Similarly, assays using N-Serve resulted in production of NH_4^+ and depletion of NO_3^- and yielded estimates of nitrification of 68% (based on decrease in NO_3^- production rate) and 251% (based on decrease in NH_4^+ consumption rate) of the ^{15}N-based estimate (Table 5).

Denitrification

Low denitrification rates occurred in most of the sediments examined (Table 4). Highest rates were found in medium- and coarse-grained sediments from Bowl Reef incubated under anaerobic conditions. Consistently higher denitrification rates were noted in aerobically, compared to anaerobically, incubated sediments from 2 of the sites at Hopkinson Reef (Hop C; Table 4). Depth profiles performed at Bowl A found similar rates of activity down to 8 cm while a depth profile at Hop C detected denitrification only in the upper 4 cm (data not shown).

Table 6. Areal rates of ammonification and nitrogen fixation in reef sediments. NH_4^+ turnover calculated from the NH_4^+ production rate / NH_4^+ inventory (from Table 2) for the indicated depth interval. nd: not determined

Site	NH_4^+ production		N_2 fixation		N_2 fix/NH_4 prod		NH_4^+ turnover	
	0–2 cm	0–8 cm	0–2 cm	0–8 cm	0–2 cm	0–8 cm	0–2 cm	0–8 cm
	(μmol NH_4^+ m^{-2} h^{-1})						(h^{-1})	
Pandora	6.6	32.9	3.6	5.3	0.55	0.16	0.04	0.009
Hopkinson 1	10.6	12.0	nd	nd	–	–	0.15	0.022
Hopkinson A	6.0	45.6	4.9	8.5	0.82	0.19	0.05	0.048
Hopkinson C	11.4	25.2	8.2	12.9	0.72	0.51	0.15	0.037
Bowl A	25.6	25.6	1.5	5.0	0.06	0.20	0.10	0.021

N_2 fixation

Nitrogenase activity was detected in all sediments examined (also reported in O'Neil & Capone 1989). At all stations, activity was highest in the upper 2 cm (Fig. 4). For the fine- through coarse-grained sediments, nitrogenase activity in the upper few cm was

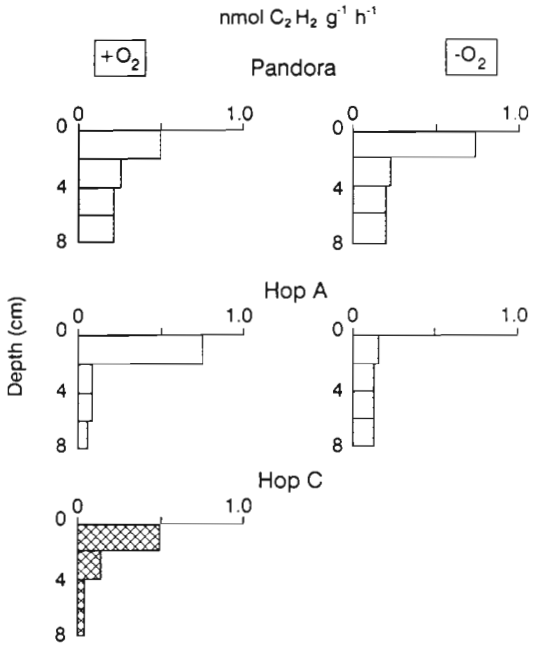

Fig. 4. Depth distribution of C_2H_2 reduction at several sites. Fine stipple are muddy sediments, medium stipple are fine-grained whereas cross hatch are coarse-grained

generally higher under aerobic conditions. In Pandora Reef muds, nitrogenase activity in surficial sediments was somewhat higher under anaerobic conditions. Activities ranged from a high of about 0.77 nmol C_2H_2 (g dry sed.)$^{-1}$ h^{-1} at Bowl A under aerobic conditions to rates of about 0.13 nmol C_2H_4 (g dry sed.)$^{-1}$ h^{-1} at Hop A under anaerobic conditions (Table 4).

Areal estimates

In order to compare rates of activity and pool sizes within and among sites, values were converted to a m^{-2} basis, integrated either over the top 2 cm, or from 0 to 8 cm (Tables 6 & 7). Ammonium efflux was computed from the concentration gradient over the top few cm according to Fick's First Law, and using a diffusion coefficient adjusted for porosity and tortuosity, but not adsorption. For ammonification, intact core, depth profile data were used (Fig. 3). Similarly, depth profiles of N_2 fixation (Fig. 4) and denitrification determined in minimally disturbed sediments were used. A 3:1 ratio of C_2H_2 reduced to N_2 fixed was assumed. Ammonium utilization and NO_3^- reduction in the upper 2 cm were

Table 7 Areals rates of denitrification, nitrate reduction, ammonium consumption, integrated to 2 cm, and ammonium efflux in reef sediments. Units: μmol N m^{-2} h^{-1}

Site	Denitrif.[a]	NO_3^- reduction[b]	NH_4^+ util.[c]	NH_4^+ efflux
Pandora	nd[d]	nd	nd	3.9
Hop 1	nd	36	58	2.2
Hop A	0.12[e]	37	45	1.7
Hop B	0.18[e]	78	12.5	0.44
Hop C	0.32[e]	9.4	48	1.1
Hop D	0.45[f]	33	43	0.66
Bowl A	1.8[f]	36	47	2.5
Bowl B	0.20[e]	9.3	11	nd
Bowl C	13.3[f]	nd	nd	0

[a] Denitrification, extrapolated to 2 cm sediment depth from results of Table 4, assuming no difference in rates over the upper 4 cm, and a bulk sediment density of 1 g cm^{-3}
[b] Extrapolated to 2 cm sediment depth from NO_3^- reduction data of aerobic slurries as presented in Table 4 using the same assumptions as for denitrification
[c] As for denitrification and NO_2^- reduction, using the NH_4^+ utilization estimates from aerobic slurries
[d] Not determined
[e] Aerobic assay results
[f] Anaerobic assay results

based on the NH_4^+ and NO_3^- utilization potentials observed in aerobic slurry assays (Table 4). Graphical summaries of pools and fluxes for the 4 stations with the most complete information are presented in Figs. 5 through 8.

At Pandora Reef, PON pools greatly exceeded NH_4^+ pools in the upper 2 cm, but were similar when integrated over 8 cm depth (Fig. 5). The calculated NH_4^+ efflux was 3.9 μmol N m^{-2} h^{-1} (Table 7). Ammonification rates were 6.6 μmol N m^{-2} h^{-1} for the upper 2 cm and 33 μmol N m^{-2} h^{-1} for 0 to 8 cm. Rates of N_2 fixation were 55 % of the NH_4^+ production rate and 92 % of the efflux estimate in the upper 2 cm, and accounted for 16 % of ammonification over the entire 8 cm depth interval.

At Hop A, PON increased from about 1200 μmol N m^{-2} in the upper 2 cm to about 4400 μmol N m^{-2} over 0 to 8 cm, while NH_4^+ increased from 115 μmol N m^{-2} to 942 μmol N m^{-2} over the same interval (Fig. 6). The diffusion-driven NH_4^+ flux rate was computed to be 1.7 μmol N m^{-2} h^{-1} (Table 7). While surficial (0 to 2 cm) ammonification rates were comparable to Pandora Reef, rates over the entire depth interval were somewhat higher (Table 6). N_2 fixation could account for 82 % of the NH_4^+ production in the upper 2 cm, and about 19 % of the NH_4^+ production over the upper 8 cm

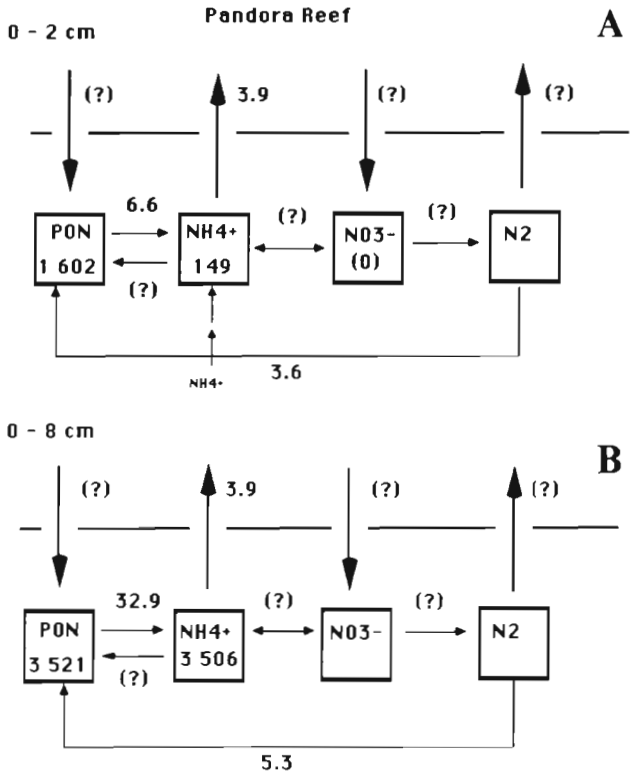

Fig. 5. Summary of pool sizes (μmol N m^{-2}) and transformations (μmol m^{-2} h^{-1}) of nitrogen in the (A) 0–2 cm and (B) 0–8 cm sediment horizons of Pandora Reef

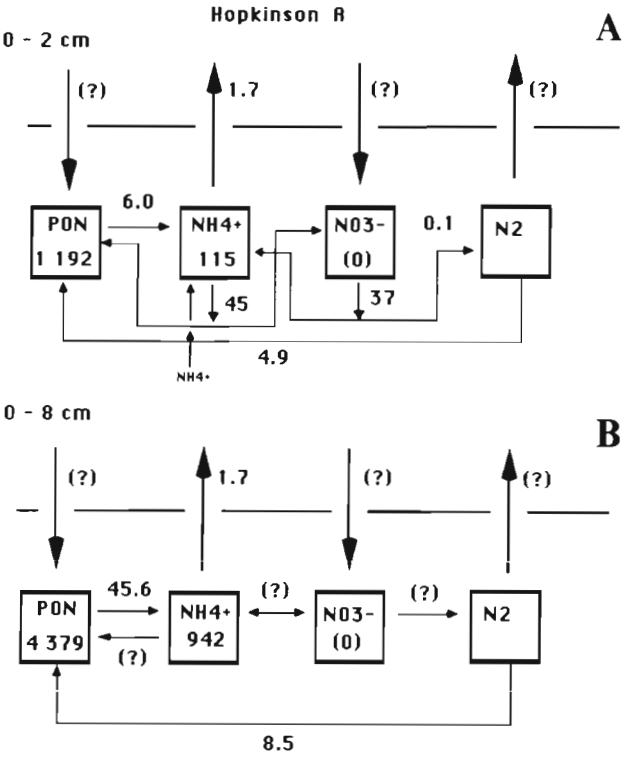

Fig. 6. Summary of pool sizes (μmol N m^{-2}) and transformations (μmol m^{-2} h^{-1}) of nitrogen in sediments of Hopkinson Reef, Site A, as for Fig. 5

(Table 6). In the upper 2 cm, NH_4^+ utilization potential in slurries was about 7.5 fold greater than NH_4^+ production (Table 7). Nitrate reduction potential in these same assays was about 82 % of the NH_4^+ removal rate. Only a small fraction of the NO_3^- reduction appeared to be denitrified.

At Hop C, PON pools increased from 976 μmol N m^{-2} in the upper 2 cm to about 3500 μmol N m^{-2} over the 8 cm interval, while NH_4^+ increased from 74 μmol N m^{-2} to 680 μmol N m^{-2} (Fig. 7). Ammonium efflux was estimated to be 1.1 μmol N m^{-2} h^{-1}. Ammonification rates increased from 11.4 μmol N m^{-2} h^{-1} at the surface to 25.2 μmol N m^{-2} h^{-1} over the top 8 cm. As for Pandora and Hop A, N_2 fixation could account for 72 % of the NH_4^+ production in the surface sediments, and 51 % of NH_4^+ production over the top 8 cm (Table 6). In the upper 2 cm, the potential for NH_4^+ utilization was also quite high and similar to Hop A (Table 7). Nitrate reduction potential was only about 20 % of the NH_4^+ consumption rate, and denitrification could account for 3.4 % of NO_3^- reduction.

Highest surface NH_4^+ production rates were found at Bowl A and this activity was essentially confined to the upper 2 cm (Figs. 3 & 8, Table 6). Ammonium efflux was estimated to be 2.5 μmol m^{-2} h^{-1}. In contrast to the other sites, N_2 fixation only accounted for about 6 % of the

NH_4^+ production in the upper 2 cm, but 20 % when integrated over the upper 8 cm. Ammonium and NO_3^- utilization potential and denitrification rates in the upper 2 cm at Bowl A were very similar to Hop A (Table 7).

DISCUSSION

While shallow carbonate sediments around coral reefs are often characterized as relatively poor in their content of organic and inorganic nutrients, particularly when compared to shallow temperate zone sediments, they nonetheless appear to be sites of active N transformations. Previous studies have found NH_4^+ production, N_2 fixation, nitrification, NO_3^- reduction and denitrification to occur in various strata of these sediments (D'Elia & Wiebe 1990). However, in the absence of concurrent assessment of each process along with N pools, it has been difficult to evaluate the relative importance of each process. Inherent methodological limitations of some of the procedures employed (see below), including the use of inhibitors and assays of potential rates, preclude a definitive assessment of the sedimentary N cycle of these systems at present. Nonetheless, within the limits of the methods used, we can now provide a more comprehensive understanding of several important N species and transformations.

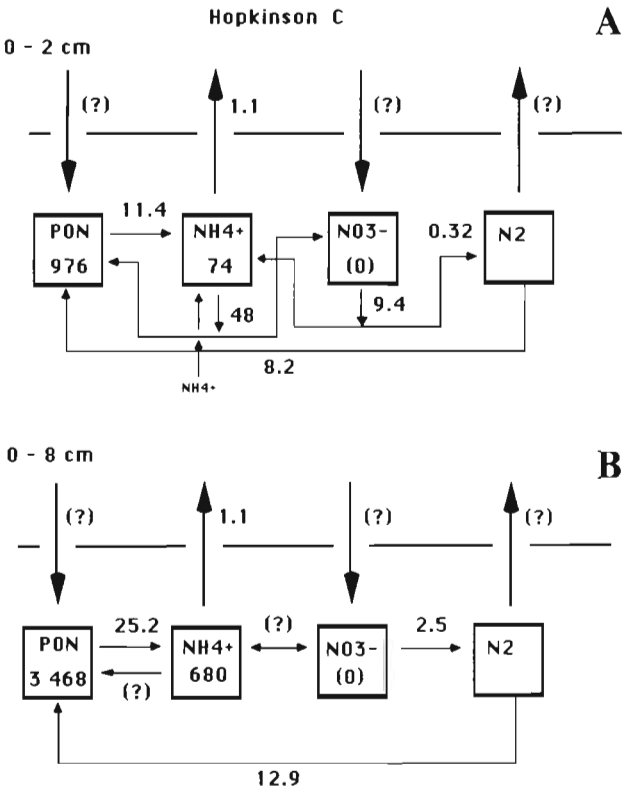

Fig. 7. Summary of pool sizes (μmol N m^{-2}) and transformations (μmol m^{-2} h^{-1}) of nitrogen in sediments of Hopkinson Reef, Site C, as for Fig. 5

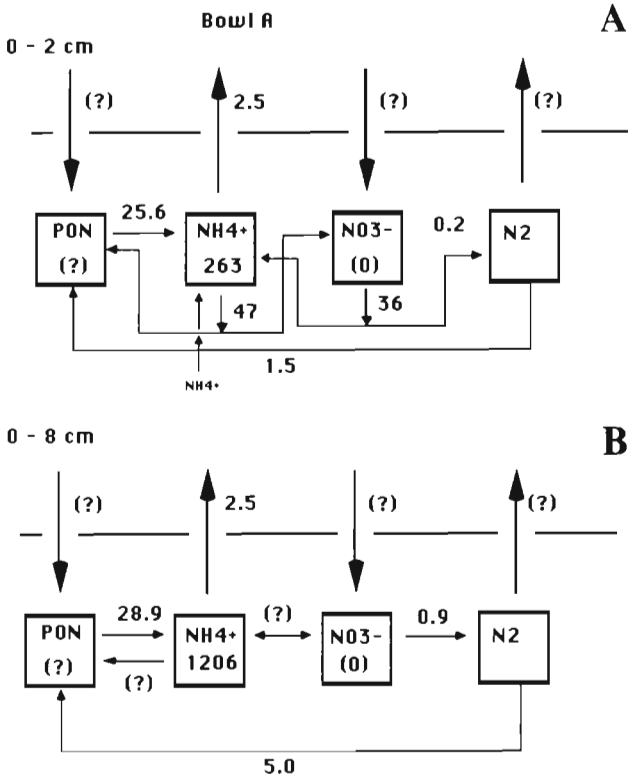

Fig. 8. Summary of pool sizes (μmol N m^{-2}) and transformations (μmol m^{-2} h^{-1}) of nitrogen in sediments of Bowl Reef, Site A, as for Fig. 5

Ammonium concentrations and fluxes

Relative to temperate zone areas, there is sparse information on pore water nutrients of reef environments, possibly because of the difficulty in obtaining samples from consolidated substrata (D'Elia & Wiebe 1990). Several of the available studies are summarized in Table 8.

We found NH_4^+ concentrations to vary substantially among sediment types. Relatively low NH_4^+ concentrations were noted in coarse- and medium-grained carbonate sediments, usually less than 10 μM at 10 cm depth (Fig. 2). Fine-grained sediments had steeper NH_4^+ gradients, reaching 15 to 30 μM by 8 cm. Highest NH_4^+ concentrations (Table 1) and inventories (Figs. 5 to 8) in our study were found in muddy sediments, reaching up to 60 μM at 8 cm depth.

Several previous studies have estimated NH_4^+ fluxes in tropical carbonate sediments based on pore water profiles or by use of benthic chambers (Table 9). Two studies reported relatively poor agreement between

Table 8. Reported maxima in ammonium concentrations in tropical carbonate sediments

NH_4^+ concentration (μM)	Comment	Source
40–300	Cores, centrifuge, by 14 cm; various sites nearshore Bermuda	Hines et al. (1982)
2.3–16.3	Sipper, upper 10 cm; Davies Reef, Great Barrier Reef	Entsch et al. (1983)
9.6–64	Extract, upper 10 cm, same site	Entsch et al. (1983)
20–40	Sipper, by 15 cm; fringing reefs, SW Puerto Rico	Corredor & Morrell (1985)
15–30	Core, by 8 cm, sands	This study
60	Core, by 8 cm, muds	This study

Table 9. Reported rates of nitrogen transformations

Transformation rate (μmol m^{-2} h^{-1})	Comment	Source
NH_4^+ efflux		
0.7–0.96	Modeled, diffusive; fringing reefs, SW Puerto Rico, Mona Is.	Corredor & Morrell (1985)
0–0.8	Modeled, diffusive; Tikehau Lagoon, French Polynesia	Charpy et al. (unpubl.)
50–704	Anoxic, chamber; same site	Charpy et al. (unpubl.)
0.39	Modeled, diffusive; Hydrolab., St. Croix	Fisher et al. (1990)
35	Dark chamber; same site	Fisher et al. (1990)
3.0	Chambers; backreef, Tague Bay, St. Croix	Williams et al. (1985)
0.4–4.0	Modeled, diffusive	This study
Ammonification		
4.5	Direct, tube pack; same site	Williams et al. (1985)
5–300	Modeled from SO_4^+ red.; same sites	Hines et al. (1982)
4.4–28	In core, 0–2 cm	This study
9.8–50	In core, 0–8 cm	This study
Deposition		
125–618	Sediment traps; same sites	Fisher et al. (1990)
109	Sedient traps; Tuamotu lagoon, French Polynesia	Charpy & Charpy-Robaud (1991)
357	Sediment traps, One Tree Is., Great Barrier Reef	Koop & Larkum (1987)
Denitrification		
19	C_2H_2 blockage; lagoonal seds., Bahamas	Seitzinger & D'Elia (1985)
50–100	C_2H_2 blockage; fringing reefs, SW Puerto Rico & Mona Is.	Corredor & Capone (1985)
0.12–13		This study
N_2 fixation		
2.7	C_2H_2 red.[a]; $^{15}N_2$; Barbados	Patriquin & Knowles (1972)
5.1	C_2H_2 red.; Kanehoe Bay, Hawaii	Hanson & Gunderson (1976)
1.7–12.7	C_2H_2 red.; Great Barrier Reef	Wilkinson et al. (1984)
6.5–12.8	C_2H_2 red.; muds, same sites	Corredor & Capone (1985)
0.4–3.6	C_2H_2 red.; sands, same sites	Corredor & Capone (1985)
3–12	C_2H_2 red.; muds, Bermuda	O'Neil & Capone (1989)
0.4–11.5	C_2H_2 red.; sands, Bermuda	O'Neil & Capone (1989)
6	C_2H_2 red.; Hydrolab., St. Croix	King et al. (1990)
4–10	C_2H_2 red.; $^{15}N_2$; Australia	O'Donohue et al. (1991)
5–13	C_2H_2 red.	This study

[a] Acetylene reduction method

modeled and empirically determined fluxes using benthic chambers. Charpy-Roubaud et al. (unpubl.) found NH_4^+ release in benthic chambers was often several-fold higher than modeled fluxes, particularly in chambers with artificially lowered O_2 concentrations. Similarly, modeled fluxes reported by Fisher et al. (1990) were about 0.4 μmol m^{-2} h^{-1} compared to directly measured averaged rates of about 35 μmol m^{-2} h^{-1} using benthic flux chambers (Table 9). In Fisher et al., rates varied greatly over the diel cycle, with net NH_4^+ influxes during the day which were attributed to photoautotrophic activity. Williams et al. (1985) reported NH_4^+ efflux rates in backreef sediments averaging 3 μmol m^{-2} h^{-1}; these rates compared favorably to

depth-integrated ammonification of 4.5 μmol m^{-2} h^{-1}. They noted rapid turnover of NH_4^+ pools, with average residence times of 2.2 h. Hines et al. (1982) predicted ammonium production rates from measured sulfate reduction for the top 14 cm of organically enriched carbonate sediments of Bermuda (Table 9) with residence times of 0.4 to 25 d.

Our estimates of NH_4^+ efflux, based strictly on the diffusion driven flux, ranged from 0.4 to 3.9 μmol m^{-2} h^{-1} (Table 7). Ammonification rates in the upper 2 cm ranged from 6 to 26 μmol m^{-2} h^{-1}, and from 12 to 46 μmol m^{-2} h^{-1} for the upper 8 cm. Except for Pandora Reef, where NH_4^+ efflux could account for 59% of surficial ammonification, NH_4^+ efflux was less than 30% of ammonification. Ammonium turnover in the upper 2 cm was rapid, ranging from 0.04 to 0.15 h^{-1} (6.6 to 25 h) (Table 6).

Surficial sediments at most sites incubated aerobically in the dark also exhibited very high potentials for NH_4^+ consumption (Table 7). At Hop C, in shallow water just behind the reef crest, parallel rapid production of NO_3^- indicated that nitrification could explain a large fraction of this flux. We confirmed this with ^{15}N and inhibitor studies (Table 5). Our areal rates are comparable to the observations of Webb & Wiebe (1975) detailing high nitrification rates associated with the reef flat. At most of the deeper sites, however, NO_3^- was consumed rather than produced, making it less obvious that nitrification was a primary fate for NH_4^+. Ammonium removal may also be associated with autotrophic or heterotrophic uptake. Sumi & Koike (1990), using a ^{15}N procedure, have recently reported that in surficial sediments, microbial assimilation of NH_4^+ can at times exceed ammonification. Rapid NO_3^- removal, as observed at most deeper sites, may also be attributed to assimilatory NO_3^- reduction (D'Elia & Wiebe 1990). However, denitrification occurred under apparently aerobic conditions (Table 4) and may also contribute to apparent NO_3^- removal (see below).

N_2 fixation

Several recent studies have observed nitrogenase activity in non-vegetated, shallow carbonate sediments from a diverse range of environments (O'Neil & Capone 1989, King et al. 1990, O'Donohue et al. 1991) (Table 9). Nitrogenase activities reported in these studies, ranging from 0.4 to 12 μmol m^{-2} h^{-1}, were very similar to our present results, which ranged from 5 to 13 μmol m^{-2} h^{-1}. Several of these authors (O'Neil & Capone 1989, King et al. 1990) speculated that N_2 fixation might be more important in the N cycle of these environments, compared to more N rich temperate sediments. However, the supporting data (NH_4^+ distributions, inventories and production rates) to assess the relative importance of N_2 fixation to the nitrogen cycle were lacking. In the present study, we have directly compared N_2 fixation to concurrent rates of NH_4^+ production and found that, at 3 out of 4 sites, N_2 fixation in surficial sediments (0 to 2 cm) could account for greater than 50% of the NH_4^+ production (Table 6). This is in sharp contrast to unvegetated shallow sediments in temperate zones where N_2 fixation may only account for a very small fraction of the nitrogen input to and turnover within the sediment (e.g. Nixon & Pilson 1983).

Some concern has been expressed about the validity of C_2H_2 reduction-based estimates of N_2 fixation in marine sediments (Capone 1988). One concern has been the supposition that N_2 fixation should be inhibited at the very high ambient NH_4^+ concentrations often found in shallow temperate zone sediments (Capone 1988). While the sensitive C_2H_2 reduction assay can detect nitrogenase activity in such systems, it has also been shown that C_2H_2 reducing activity can be far below potential levels and may indeed be inhibited (Capone 1988). With respect to the carbonate sediments examined herein, nitrogenase activity was maximal near the surface (Fig. 4) where NH_4^+ concentrations were often the lowest and generally less than 10 μM (Fig. 2, Table 1). This is well below concentrations known to inhibit nitrogenase synthesis or activity (e.g. Neilson & Nordlund 1975, Zumft & Castillo 1978, Kleinschmidt & Kleiner 1981). Thus, it is not surprising that N_2 fixation may be a more quantitatively important process in these systems.

With regard to converting C_2H_2 reduction to N_2 fixation, Patriquin & Knowles (1972) and more recently O'Donohue et al. (1991) directly compared C_2H_2 reduction with direct $^{15}N_2$ fixation in vegetated and non-vegetated carbonate sediments and found empirically derived ratios very close to the theoretical 3 to 1 value.

Denitrification

Denitrification has been reported in 2 previous studies of coral reef sediments. Seitzinger & D'Elia (1985), using the C_2H_2 blockage procedure, estimated denitrification rates of 19 μmol N m^{-2} h^{-1} (integrated to 5 cm) (Table 9). Corredor & Capone (1985), using the same method, found denitrification ranging from 50 to 100 μmol m^{-2} h^{-1}.

In general, our areal estimates presented here, restricted to 2 cm depth (Table 7), are on the low range of these previous estimates. We only performed limited studies on the depth distribution of denitrification. As mentioned, at 1 site (Bowl A), denitrification rates were relatively similar at each horizon down to 8 cm and

integration to greater depth would probably bring our estimates closer to these earlier observations.

However, there are potential limitations with the present (and previous) denitrification estimates. For one, the C_2H_2 block of N_2O reductase becomes ineffective at ambient levels of NO_3^- below 5 to 10 µM (see Slater & Capone 1989 and references therein). Also, limitation of denitrification by nitrification appears to occur in temperate sediments (Jenkins & Kemp 1984, Horrigan & Capone 1985) and has been suggested as an important controlling factor for denitrification in tropical sediments (Corredor & Capone 1985). Further confounding the quantitative estimation of denitrification rates by C_2H_2 blockage in low NO_3^- environments is the fact that nitrification, and hence NO_3^- production, is also inhibited by C_2H_2 (Payne 1984).

Sediment NO_3^- was generally undetectable and therefore well below 5 µM. Additions of NO_3^- over the range 10 µM to 500 µM produced an immediate, concentration-dependent stimulation of denitrification (Capone unpubl.) indicating a very high potential for denitrification. Thus, the present estimates should be considered as minima, particularly in light of high rates of NO_3^- production and removal as suggested by nitrification assays and potential NH_4^+ and NO_3^- utilization.

One interesting aspect of the denitrification data is that assays conducted under aerobic conditions often resulted in rates similar to and, at times, greater than those conducted in parallel under anoxic conditions (Table 4). A particularly noteworthy case was for samples obtained from the very shallow reef flat near the reef face at Hop C. This site is often exposed to high wave action and turbulence and the coarse-grained sands and high flow would suggest relatively well-aerated conditions near the sediment water interface. (No sediment O_2 data was collected in our study.) While denitrification is traditionally viewed as an anaerobic process with the induction of the denitrifying enzymes often dependent upon low O_2 (Payne 1981), organisms with a capacity for aerobic denitrification are known (Robertson & Kuenen 1984) and aerobic denitrification has been suggested in some natural environments (Dodds & Jones 1987). Alternatively, the coarse skeletal particles which comprise these sands may allow the development of anoxic microzones as has been previously shown by Patriquin & Knowles (1975).

Deposition and fate of particulate nitrogen

While we did not directly determine particulate N (PN) sedimentation rates in our study, several investigations have provided data on the rate of PN input to shallow carbonate environments (Table 9). Recent estimates range from about 100 to 600 µmol N m^{-2} h^{-1} (Table 9). If PN sedimentation was similar at our sites, only a small portion of such input can be accounted for by NH_4^+ efflux. Our downcore PN analysis (Table 3) indicated preservation of well over half the surficial PN in the 6 to 8 cm horizon. Nonetheless, other fates for deposited nitrogen are indicated and need to be quantified. In this regard, the observations of both Fisher et al. (1990) and Charpy-Roubaud et al. (unpubl.) that chamber-derived fluxes can greatly exceed diffusively modeled estimates deserve further examination. Similarly, underestimation of denitrification could account for further, unaccounted losses.

Conclusions

The NH_4^+ pool in the upper few cm of tropical carbonate sediments appears to be highly dynamic, with inferred internal turnover times of substantially less than a day. Nitrification appears to be a quantitatively important N-cycle component in shallow, reef flat areas. Denitrification can be detected, even in apparently oxic sediments, but its quantitative importance is unresolved. Our data indicate that bacterial N_2 fixation accounts for a large fraction of the NH_4^+ produced within or released from the upper layers of the sediments.

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