Micronesica 37(2):271–285, 2005

A Nitrogen Budget, Including the Occurrence and Activity of nitrogen fixers in nitrogen-rich and nitrogen-poor habitats of Guam, Mariana Islands.

ERNEST A. MATSON

ANDREW S. E. QUENGA

Division of Natural Sciences and
Marine Laboratory
University of Guam, Mangilao, GU 96923
Email: eamatson@uog9.uog.edu

Abstract—In order to augment earlier studies on external nitrogen flux to Guam's reefs and to identify internal sources of fixed nitrogen for the nearshore marine communities, a broad survey of the occurrence and amount of nitrogen fixation (ethylene production) was done on two contrasting reef moats. Nitrogen fixation was ubiquitous; it was detected in all samples at both sites, including coarse and fine sand, epiphytes of macroalgae and common corals, rocks, rhizoids, fecal pellets, and sessile invertebrates, and was undetectable in dark controls. Paradoxically, while the leeward reef site is heavily enriched both with nitrate (up to ca. 20 μM final conc) it had nitrogen fixation rates 10–15 times greater than in the relatively nitrogen-poor windward reef moat. This windward site is dominated by oceanic tidal water (90% tidal prism), and, in contrast to the leeward site, its waters are enriched with aquifer nitrate only up to ca. 2 μM before they flood over the reef crest. Rates of fixation were a minor part of the nitrogen budget, were somewhat lower than at other tropical sites, and ranged from 0.02 to 60 ηmol N g^{-1} d^{-1}, with an average of 150 μmol N m^{-2} d^{-1} at the N-rich site and 11 μmol N m^{-2} d^{-1} at the nitrogen-poor site. Nitrogen fixation, which costs half that of assimilatory nitrate reduction, may be energetically favored in highly productive, relatively nitrogen-rich, euryoxic habitats.

Introduction

Tropical waters are considered to be nutrient poor, but gross primary production in coral reef communities proceeds at rates that are among the highest anywhere measured. Earlier discussions of this apparent paradox recognize the ability of these communities to efficiently recycle stored nutrients, and to remove nanomolar levels of nutrients from large volumes of tidal water that flow over them (Adey and Steneck 1985, Wiebe 1985, D'Elia 1988, Atkinson 1988, Szmant et al. 1990, Eyre and McKee 2002). However, until recently, only partial or

incomplete nitrogen budgets for coastal tropical reefs have been reported (Dufour et al. 2001, Bell et al. 1999, Matson 1993, Eyre and McKee 2002, Umezawa et al 2002a), in contrast to temperate coastal systems (McClelland and Valiela 1998, Boynton et al. 1995, Nixon et al 1995). In fact, Dufour et al. (2001) illustrate well the over-riding importance of hydrology and morphometry on nutrient regimes in atolls. Umezawa et al. (2002a,b) demonstrate the complex nature of nitrogen cycling, especially $\delta^{15}N$ enrichments and alterations within nearshore communities. Their data complement earlier work on Guam regarding the importance of the extreme patchiness of isotopically light (i.e., recently fixed) nitrogen in coastal carbonates (Matson 2004).

On Guam, substantial progress has been made towards identifying the important external nitrogen sources (Marsh 1977, Matson 1993). For example, as a result of the flux to the coastal zone of NO_3^{2-}-rich aquifer water (up to 8 μM NO_3^{2-} at 34‰ salinity, Matson 1993), the nitrate content of upwind North Equatorial surface current waters (0.12±0.05 μM; n~ 40) is enriched by up to ~2 μM. The effect is greater to leeward, where greater aquifer flux combined with upwelling increases the nitrate content by 15 fold. Elsewhere, parts of the nitrogen budget have been identified and include aquifer flow to the coastal zone (Umezawa et al. 2002b, Bell et al. 1999) that produces observable salinity gradients in which NO_3^{2-} and reactive P (RP) are highly enriched (Bokuniewicz 1980, Johannes 1980, D'Elia et al 1981, Entsch et al. 1983, Capone and Bautista 1985, Eyre and McKee 2002).

In view of the diversity of nitrogen supplies, and conspicuous lack of nitrogen fixation data, we did a broad survey of the distribution and activity of nitrogen fixers at a windward (N-poor) and leeward (N-rich) site to determine whether nitrogen fixation was (a) important in the nitrogen flux budget and (b) affected by differences in coastal nitrate flux upwind and downwind of Guam. At both sites, regardless of significant rates of in-situ nitrogen fixation, the nitrogen supply was dominated by N_2 fixed on land, oxidized to NO_3^{2-} and exported to the coast via aquifer water. In addition, nitrogen fixation was detected in all samples tested, with high rates in sediments and among the epiphytic community of highly branched macroalgae. These nitrogen fixation data supplement the existing knowledge of N flux and cycling on a high tropical island.

Materials and Methods

Study site

The northern carbonate plateau of Guam (Fig. 1) is composed nearly entirely of upraised limestone that overlays an extensive aquifer system (a so-called Ghyben-Herzberg lens), from which NO_3^{2-} -rich groundwater leaks through the shoreline transition zone around the entire periphery of northern Guam, largely to leeward of the prevailing NE trade winds (Matson 1993). In the southern, largely lateritic province of the island, total river discharge volume is equivalent to discharge from the northern aquifer, although almost all of river discharge occurs on

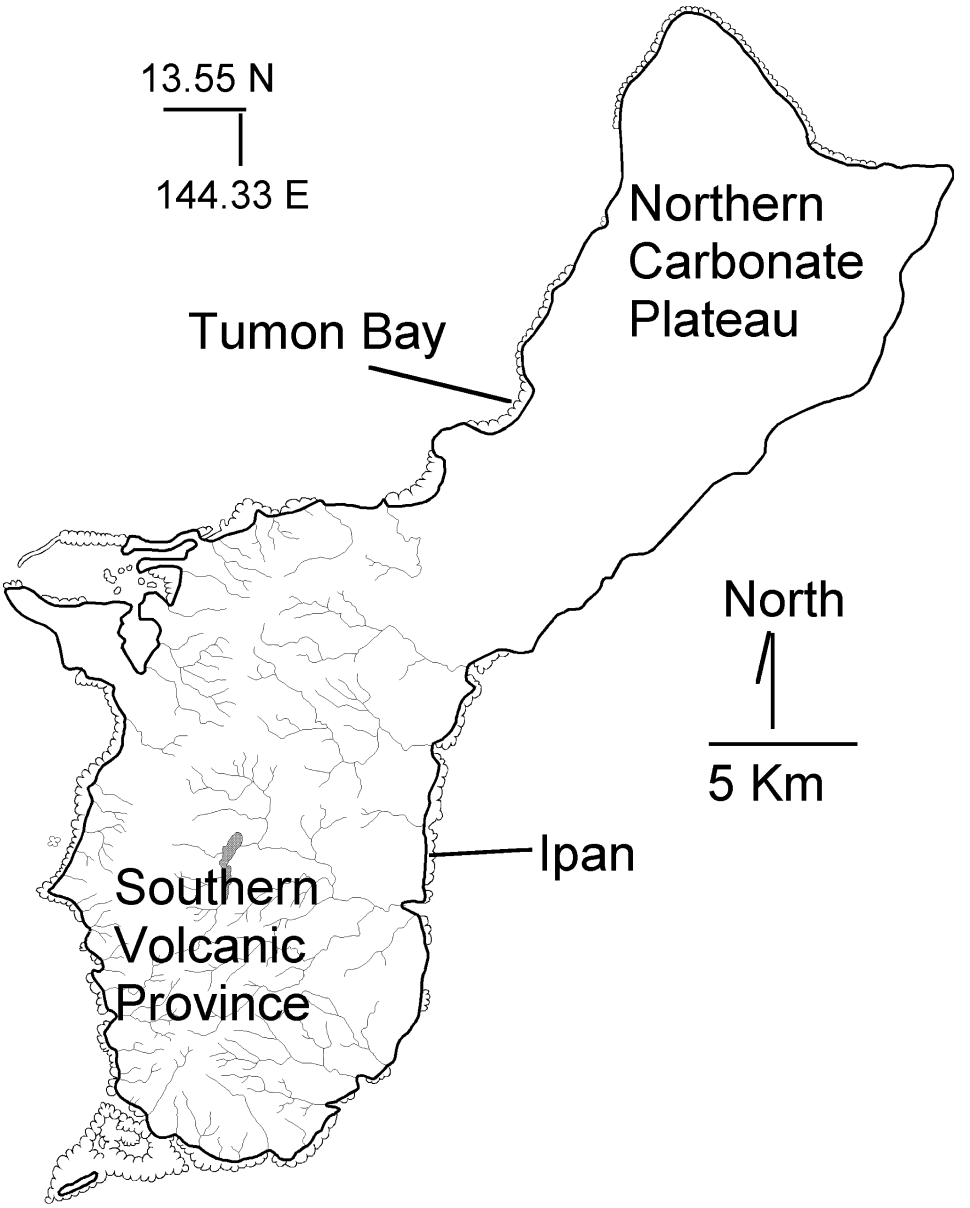

Figure 1. Map of Guam and the Tumon Bay and Ipan study areas.

the windward side during the June to December rainy season. Along the windward side of the island, wind-driven, longshore transport of surface waters carries nitrate enriched water from the northern aquifer lens year-round to the south along the extensive fringing reef of the east coast of Guam. In the rainy season, this is supplemented by both nitrate and (mostly) ammonium from the southern rivers (Matson 1991a).

Tumon Bay, on the leeward side of the island, is a semi-enclosed barrier reef moat, 3 km long, 300m wide and ca. 1 m deep. The tidal prism (ca. 70% of mean high water [MHW] volume) renews the water within 2–4 days, but tidal exchange is restricted to 2 or 3 narrow cuts through the reef crest, which is submerged under ca. 0.2 m of water at MHW. The moat is formed by a relic Pleistocene platform that is covered by a 2–40 cm layer of carbonate sands and rubble. Aquifer seepage into the moat accounts for 2.5% of the moat volume at MHW and average salinity in the moat ranges from ca. 30–34‰ (Matson 1993).

On the eastern windward shore, the Ipan fringing reef and reef flat is also ca. 300 m wide, averages only ca. 0.8 m deep, and is largely exposed at mean low water [MLW] during spring tides. In contrast with Tumon Bay, there is very little residual moat water at MLW: the tidal prism is ~ 90% of MHW. Usually, winds blow onshore ca. 10–20º north of east (perpendicular to the reef crest) and seas of 2–3 m are common.

This flat, scoured, platform has common patches of *Pocillopora*, algal turfs, beds of angiosperms *(Enhalus),* and rhodoliths. Sediments do not accumulate except in cryptic patches around corals and in a few sink holes. In these holes, diatom mats, cyanobacteria, and beds of *Enhalus* are common. Extensive patches of *Padina* and cyanobacteria are conspicuous among the nearshore macroflora along the shoreline. Holothurians (especially *Holothuria atra*) are common at both sites.

Nitrogen fixation in Tumon Bay sediments

For nitrogen fixation studies of the sediments, three cores were collected at each of 5 sites (0, 25, 50, 200, and 300 m) on three different occasions along a 300 m transect from the low water mark to the inner reef flat in NW Tumon Bay. Sediments were collected with core samplers made from 50 mL plastic syringes that were pushed about 5 cm into the sediment and then capped with rubber stoppers. Also, surface (top ca. 1 cm) sediment was collected in plastic bags. The cores and bags were kept submerged in a cooler with ambient sea water and returned to the University of Guam Marine Laboratory within ca. 1 h.

Nitrogen fixation by epiphytes, sediments, and rhizoids

A survey of nitrogen fixation (specifically ethylene production; Postgate 1972; Van Raalte et al 1974) by the microflora in sediments, and those associated with benthic algae, corals, sponges, and other flora and fauna was conducted at Tumon Bay and the Ipan reef flat. These samples were randomly chosen based on their frequent occurrence as members of the community so as to obtain a diverse

inventory of substrata for estimates of nitrogen fixation. No significant accumulation of sediment occurs on the windward Ipan reef flat (except among *Caulerpa* and *Enhalus* roots), so only samples of algae, corals and other sessile invertebrates were taken (a total of 27 samples), except for the 5 samples of surface sediment for chemical analyses described below.

Slurries of cores and surface sediments were incubated in stoppered, 50 mL plastic syringe tubes under a 10–15 mL headspace of acetylene (generated from commercial carbide in water). The tubes were shaken for 30 s, and incubated for 48 h in an outdoor flow-through seawater tank to maintain natural temperature and light conditions. Otherwise, whole corals, algae, sponges, etc. were suspended in ambient seawater in gas-tight, 1 liter ZipLoc® bags that were fitted with serum stoppers for gas sampling.

Ethylene concentration was measured with a flame ionization detector on a Perkin-Elmer Sigma 300 capillary gas chromatograph with a 2 m Spherocarb column (100 to 120 mesh), at 175ºC. The theoretical conversion ratio of 3 mol C_2H_4 produced to 2 mol N fixed (i.e., 1 mol N_2 reduced; Howarth et al. 1988) was used. Data were expressed as mol C_2H_4 produced g^{-1} dry $sediment^{-1}$ d^{-1} and mol N m^{-2} d^{-1} based on 48 h incubations and appropriate sediment conversion factors (below). Controls consisted of ambient water only, parallel samples or sediment cores without acetylene (to correct for natural production of ethylene), and, for sediments, cores wrapped in black electrical tape were used to estimate non-photosynthetic nitrogen fixation. No control sample showed any significant ethylene production and all recorded ethylene peaks were greater than 2 S.D. of the blank. The Welch equality of means test (BMDP® statistical analysis software) was used to determine significance at $p < 0.05$. Rates per day were calculated from least squares regressions of C_2H_4 content per gram dry material over time, all of which (except controls) had positive slopes that were significantly different from zero (Quenga 1993).

Total and Exchangeable Nitrogen Content

Total organic nitrogen (TON) content was measured in oven dried and ground samples of sediment (n=5) from both Tumon Bay and Ipan and in common algae *(Liagora* spp., *Halimeda opuntia, H. macrolobus, Hydroclathrus* spp., *Padina* spp., *Dictyota* spp., *Schizothrix* spp., *Gelidiopsis* spp., *Caulerpa racemosa),* fecal pellets of epifauna *(Holothuria atra)* and infauna (several polychaetes and a callianassid shrimp), surface sands, mats of cyanobacteria, rhodoliths, sponges (*Cinachyra* spp.) and small colonies of *Pocillopora damicornis.* Samples were analyzed in duplicate with a Carlo-Erba Model 1500 NCS elemental analyzer at 1000ºC (after Froelich 1980; but not at 1600ºC) against *p*-amino benzoic acid standards. The tin sample boats for the CNS analyzer, which are supplementary combustion catalysts, had been rinsed in acetone, oven dried, and then filled to their capacity (ca. 180 mg dry carbonate sediment). At greatest instrument sensitivity, this sets the lower detection limit of a 180 mg sample at about 1.5 µmol TN (total nitrogen) or 8.3 µmol TON g^{-1}.

Exchangeable NH_4^+ and NO_x (i.e., NO_2 + NO_3) were measured on 2 to 5 g subsamples of dried, homogenized sediment that were suspended in 20 mL 2.0 M KCl and shaken on a rotary shaker in plastic scintillation vials for at least 2 h (Rosenfeld 1979, Matson 1989). Ammonium in both the KCl extract and blanks was measured with an Orion ammonia probe, standardized each time in KCl. As the lower limit of detection ranged from 2 to 5 µM NH_4^+, the volumes of sediment were adjusted to keep the signal to noise ratio >5. NO_x in the KCl extract (and in water samples) was quantified with the spongy cadmium shaking method of Jones (1984) and read in a 5 or 10 cm spectrophotometer cell. These two KCl-exchange-able fractions are referred to as E-NH_4^+ and E-NO_x.

Tidal Water Flux

Water flow rate between the reef crest and the beach was measured at the Ipan site to determine nutrient flux in tidal waters. Six one gallon, plastic milk cartons were filled 7/8ths with water and launched into the surf on the reef crest at hourly intervals to measure water travel time to the beach, while the direction of travel was measured hourly on 2 occasions over 12 hr tidal cycles. Water flow times averaged 80–100 min from the fore reef crest to the beach (a distance of ca. 300 m) and depth varied from 0.2 to 0.6 m during the studies. For Tumon Bay, tidal flux was calculated from an extensive set of salinity data described elsewhere (Matson 1993).

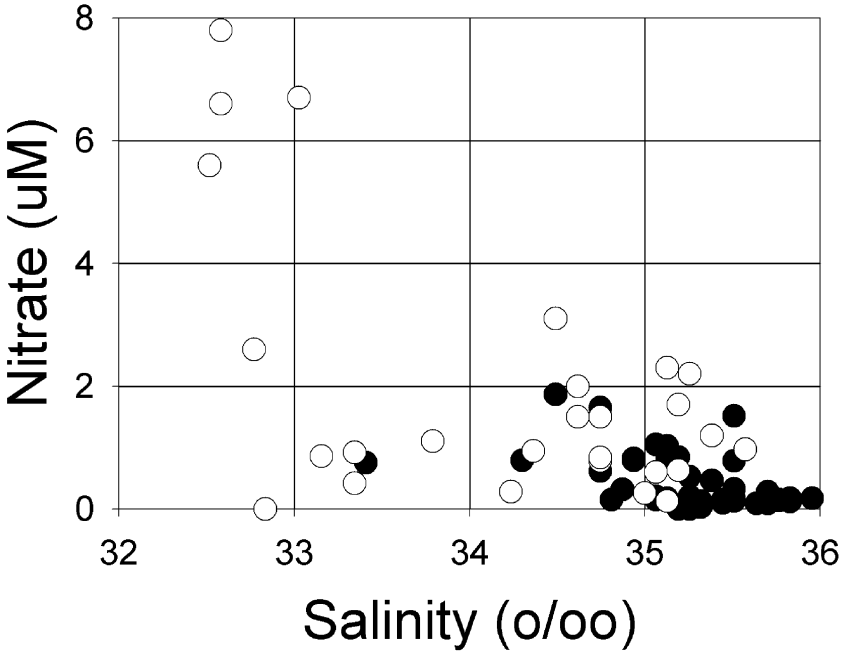

Figure 2. Nitrate in northern offshore (≥5 km) surface waters of Guam to windward (•) and leeward (º).

Results

Nitrogen in sediments and surface waters

Both sites had remarkably similar sedimentary nitrogen contents (Table 1), with TON averaging 24–28 μmol g^{-1}, although very little sediment occurs at the Ipan site in comparison with Tumon Bay. Exchangeable NH_4^+ at Ipan was approximately double that of Tumon Bay and this is attributed to the impact of NH_4^+ bound to particulates infrequently transported by the rivers during storms. In contrast, the substantial infiltration of nearshore sediments by the aquifer in Tumon Bay is obvious from the amounts of exchangeable nitrate, which is orders of magnitude higher than at Ipan. The nitrogen stocks, however, are dominated by the TON fraction, which are equivalent at both sites.

Nitrate levels in ocean waters are enriched by nearshore aquifer flux (Matson 1993). But at salinities greater than 33‰, the waters on the leeward coast of northern Guam are generally higher in nitrate than those to windward (Fig. 2). However, Tumon Bay waters are also internally enriched in nitrate derived from the constant flow of aquifer waters within the bay from inter- and sub-tidal seeps and springs (Fig. 3). Above 32‰ inside the bay, nitrate has been observed to be as high as 20 μM. The lowest nitrate concentration observed in Tumon Bay at 35‰ salinity was 0.68 μM. At Ipan, the incoming seawater on the fore reef slope averaged 0.27±0.37 μM (Table 1), which is ca. 0.15 μM higher than in the North Equatorial waters as little as 2 km upwind (Matson 1991a). Because this

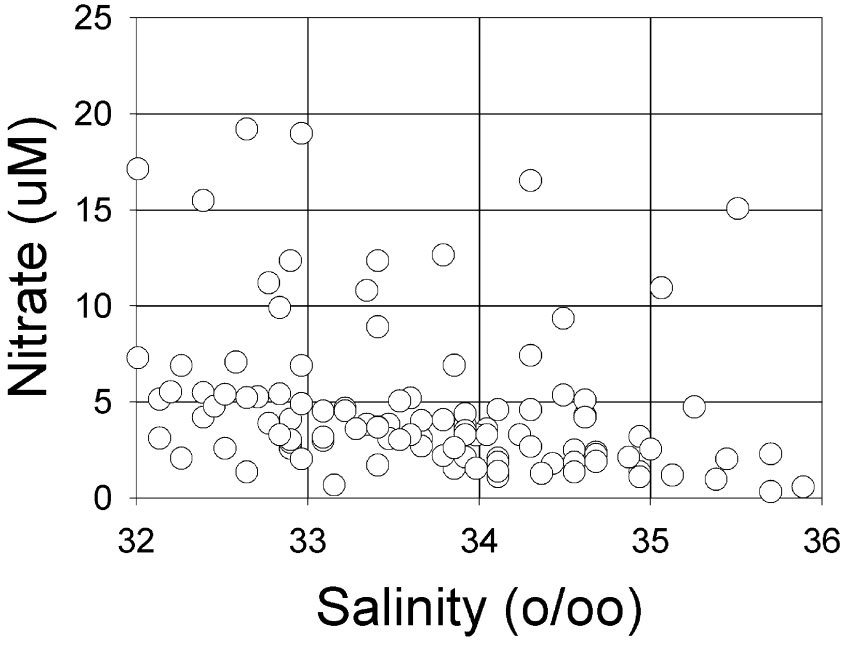

Figure 3. Nitrate in Tumon Bay.

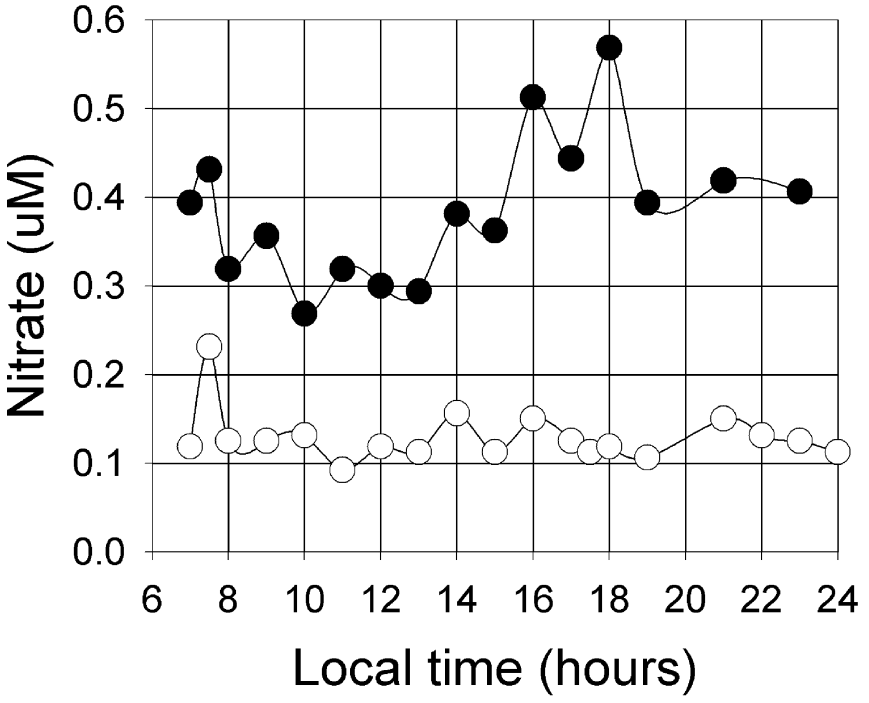

Figure 4. Net uptake of nitrate between the ocean waters at the reef crest (•) and the beach (º).

nearshore enrichment at the windward site persists in the dry season, it is attributed to longshore transport of aquifer water derived from upwind seawater transported along the coast. During flood tide, these waters then flow over the reef crest, into the moat through channels and over the reef flat where they are substantially depleted of nitrate by the time they reach the beach (Fig. 4).

Table 1. Average contents of three sediment N fractions and ambient floodwater nitrate for the Tumon Bay (leeward) and Ipan (windward) sites.

	TON	E-NH_4^+	E-NOx	Nitrate in flood water (n)
		µmol g^{-1} dry sediment (n=5)		µM
Tumon Bay	24±9.5	0.14±0.06	1.6±1.0	1.9±2.6 (24)
Ipan reef crest	28±5.3	0.30±0.08	0.0018±0.00042	0.27±0.37(33)

TON OF THE N FIXATION SURVEY SAMPLES

The total nitrogen content (Table 2) of the samples obtained for nitrogen fixation work ranged from 28 μmol N g^{-1} in coarse surface sands and rhodoliths to more than twice that in fresh polychaete feces, shrimp excavated mound sand (*Callianassa* spp.), sponges (*Cinachyra* spp.) and small, whole *Pocillopora* heads. Calcified algae had ca. 10 fold higher TON (from 330–460 μmol N g^{-1}), and noncalcified algae had the highest nitrogen content.

DISTRIBUTION OF N FIXERS

Every sample we incubated (except the dark and seawater-only controls) showed detectable nitrogen fixation. (Table 2). The primary data are expressed both per gram dry weight of the whole sample and a standard extrapolation to area

Table 2. Individual rates of ethylene production in single samples, calculated areal rates of NH_4^+ production, total nitrogen content, and N fixation per day as a % of TN.

Site and Sample	ηmol C_2H_4 g^{-1} d^{-1}	μmol N m^{-2} d^{-1}	μmol TON g^{-1} dry	N fixed d^{-1} as % of TN
Tumon Bay:				
surface sediment	29	140	31	0.06
Liagora	100	490	1400	0.005
Liagora	43	210	1400	0.002
polychaete feces 1	35	170	55	0.04
polychaete feces 2	14	68	98	0.01
lagoon sand 1	9.3	45	31	0.02
Callianassa mound sand	3.8	19	45	0.006
lagoon sand 2	2.5	12	31	0.005
sediment cores	0.61	4.5	31	0.002
Mean±1S.D.		150±140	350±600	
Ipan moat and reef flat:				
cyanobacterial mat	15	73	540	0.002
Caulerpa racemosa 1	5.8	28	2100	0.0002
Hydroclathrus	5.1	25	760	0.0004
Padina spp.	4	19	460	0.0006
Dictyota spp.	4	19	2100	0.0001
fine silt	1.7	8.3	100	0.001
Halimeda opuntia	1.4	6.8	330	0.0003
Halimeda macrolobus	1.3	6.3	330	0.0003
brown mat of algae	1.1	5.4	150	0.0005
Cinachyra	1.1	5.4	40	0.002
Schizothrix	0.80	3.9	720	0.0001
Gelidiopsis	0.38	1.9	650	0.00003
Pocillopora	0.31	1.5	56	0.0004
sediment in *Caulerpa* rhizoids	0.29	1.4	650	0.00002
rhodolith 1	0.13	0.63	28	0.0003
Caulerpa racemosa 2	0.11	0.54	2100	0.000003
rocks	0.064	0.31	28	0.0002
coarse sand	0.040	0.19	28	0.0001
rhodolith 2	0.029	0.14	28	0.0001
	Mean±1S.D.	11±28	590±720	

units using carbonate sediment density (0.73 g dry sediment cm^{-3}, Matson 1989). This is somewhat misleading because, for example, a relatively heavily textured 5 cm diameter rock encrusted with calcareous algae has substantial surface area but also weighs much more than the wrinkled surface of a sponge. Nonetheless, these serve as good first-order approximations for comparison with other nitrogen flux estimates.

In Tumon Bay, nitrogen fixation in sediment slurries was low in comparison with other samples and ranged between 0.46 ηmol C_2H_4 g^{-1} d^{-1} (50 m from shore) and 0.76 ηmol C_2H_4 g^{-1} d^{-1}. These rates extrapolate to areal rates of 3.3 to 5.5 µmol m^{-2} d^{-1}. In the surface sands and polychaete feces, rates ranged from 12 to 140 µmol N m^{-2} d^{-1} (Table 2). The highest rates observed in Tumon Bay (210 to 490 µmol N m^{-2} d^{-1}) were associated with an epiphyte-laden 500 g wet sample of *Liagora* spp. (Chlorophyceae, a seasonally common epibenthic alga), and in fresh polychaete feces.

As in Tumon Bay, nitrogen fixation in the nitrogen-poor Ipan site varied widely from 0.14 to 73 µmol N m^{-2} d^{-1} in the diverse suite of samples analyzed (Table 2), but the overall average of the sample suite was much lower (11 µmol N m^{-2} d^{-1}). The highest rates occurred in common cyanobacterial mats, and were also associated with epiphytes of algae such as *Caluerpa, Hydroclathrus, Padina,* and *Dictyota* spp. Nitrogen fixation rates in fine silts obtained from sediments among *Caulerpa* rhizoids ranged between 1–10 µmol N m^{-2} d^{-1}, similar to those in Tumon Bay lagoon sands.

Discussion

Distribution and Activity of N Fixers

We deliberately incubated fresh, whole samples of common individuals of "the sampled community" in sufficient water and in normal light regimes so as to not disturb (with the exception of sediment slurries) the intact nature of the associations. That N fixation was found in every sample is indeed interesting, but it would be additionally valuable to be able to adequately extrapolate the data to meaningful units comparable, for example, to reef surface area. Such an approach is problematical for several reasons, not the least of which is the complex 3 dimensional texture of nearly everything on the reef. For example, our extrapolation of, e.g., "rhodolith" rates per gram (of dense rock) to m^{-2} is misleading, because the surface area of these structures is rather great relative to their volume and/or weight. Worse, the surface area of a non-calcareous alga, such as *Gracillaria* (or even a partially calcified *Padina*, with less carbonate than *Halimeda)* is much greater than that of an equally-sized and heavily textured rock in comparison with its dry weight. Otherwise, that nitrogen fixers are ubiquitous even outside the common patches of cyanobacteria (Matson 2004) is certainly significant, but their relative importance to the nitrogen budget of Guam's reef communities appears to be minimal in contrast with the nitrogen from terrestrial fixation and nitrification that is exported to the reef via the aquifer. This also

appears to be true at Ishigaki Island, Japan, where, although common macroalgae have low $\delta^{15}N$ values indicative of nitrogen fixation by their epiphytes, the major nitrogen sources are by far due to tidal and aquifer fluxes (Umezawa et al. 2002a).

Nitrogen supplies to N-rich and N-poor habitats of Guam

At both the nitrogen-rich and nitrogen-poor sites, nitrogen is mostly supplied by the aquifer, but, at the nitrogen-poor Ipan site, the larger tidal prism increases the importance of ocean waters regardless of the upwind enrichment by the aquifer. (Table 3). The Guam values are compared with reef data from heavily agricultural watersheds in the Ryukyu Islands, Japan (Umezawa et al. 2002b), and, except for the much larger tidal flux (due to larger tidal prisms), both Nitrogen fixation and aquifer flux are similar. At Guam, at an average concentration of 1.9 µM NO_x in flood waters of Tumon Bay and a mean tidal prism volume of ca. 70%, calculated nitrate import from the ocean to the reef moat is on the order of 2700 µmol m^{-2} d^{-1} (1900µmol m^{-3} * 0.7 m $tide^{-1}$ * 2 tides d^{-1}= 2660 µmol m^{-2} d^{-1}). In addition, through direct leakage into Tumon Bay, the aquifer directly provides 4500 µmol NO_x m^{-2} d^{-1} (Matson 1993). In contrast, at the Ipan site, aquifer flow directly into the moat is nearly undetectable, so that tidal import dominates the supply and amounts to 380 µmol m^{-2} d^{-1} (270 µmol m^{-3} * 0.7 * 2 tides d^{-1}). However, as shown above, about half (55%) of the tidal nitrate at Ipan is derived from aquifer flow into the northeast coastal waters which is continuously enriched during longshore transport southward. These differences may also be due to morphological site differences, as in the Tuamotu Archipelago of the South Pacific, where the "aperture" of the atolls (i.e., the size of the tidal opening) appears to regulate nutrient limitation (Dufour et al., 2001)

Nitrogen fixation is much higher in the nitrate-rich milieu of Tumon Bay (a "small aperture" system): Nitrogen fixation rates extrapolated to unit areas averaged 150 µmol m^{-2} d^{-1} at Tumon Bay and 11 at Ipan (15 fold lower), where tidal

Table 3. Comparison of inorganic N sources to the proximal 300 m of coastal Guam and of Ishigaki Island, Ryukyu Islands, Japan.

Source	Leeward	Windward	Ryukyu[a]
		µmol N m^{-2} d^{-1}	
Tidal flux			
Oceanic N	170	170	3300
Aquifer N	2380	210	
(% from aquifer[b]	93	55	5–35)
Total Tidal flux	2550	380	3300
Direct Aquifer flux[c]	4500	"0"	790
N fixation	150	11	190
Total flux	7200	390	4280

[a]Umezawa et al. 2002b

[b]See text for calculated fraction of aquifer nitrate in tidal waters

[c]Directly into Tumon Bay from beach seeps, "none" occurs at Ipan.

water floods the entire reef flat (i.e., a "large aperture" system). These average rates are within the ranges observed in tropical sediments elsewhere (e.g., Wiebe 1985; Eyre and McKee 2002, Umezawa et al. 2002a) but, in comparison with other supplies, the contention that N is not a "limiting nutrient" on Guam's reefs is supported (Thacker et al. 2001): nitrogen fixation constitutes only about 2 percent of the total nitrate supplied to each site and a much lower percentage of the TON stocks, as in the Ryukyu Islands of Japan (Umezawa et al. 2002b, Table 3). Nonetheless, nitrogen fixation is sufficiently intense in patches so as to decrease the $\delta^{15}N$ ratio of Tumon Bay sediment nitrogen by ~6 ‰ (Matson 1991b).

Why then is nitrogen fixation so high in the presence of excess nitrate? Or is it more relevant to ask "why is it lower at the nitrogen-poor site? Or what is the purpose of nitrogen fixation here?" For example, Rudek et al. (1991) found high rates of nitrogen fixation in the presence of μM levels of NH_4^+. Below we speculate on an alternate role for marine nitrogen fixation on Guam.

Energetic Considerations

That a nitrogen-rich moat with lower tidal exchange than an nitrogen-poor, well-oxidized reef flat would have much higher rates of nitrogen fixation is paradoxical. Complete removal of nitrate from the waters of either site has never been observed, which implies that nitrogen does not limit activity, so, if nitrogen fixation is so metabolically expensive, why fix nitrogen at all?

Actually, nitrogen fixation in a euryoxic system such as Tumon Bay may merely be a method to acquire and assimilate nitrogen in a manner that is more efficient than the assimilatory reduction of nitrate. The saturation level of N_2 in seawater is of the order of 900 μM, which is ca. 10 fold higher than pore water nitrate levels in Tumon Bay (up to 70 μM). Thus, concentration differences may favor nitrogen fixation. From a metabolic standpoint, it "costs" ~12 mol ATP plus 3 pairs of electrons to reduce N_2 (oxidation state 0) to 2 NH_3 (, oxidation state -3; actually NH_4^+ at physiological pH), plus an extra 2e- (a total then of 8e- from catabolism) for H_2 disposal (Fay 1992). This is half the net cost of assimilatory reduction of NO_3^{2-} (N^{+5}) to NH_4^+ (N^{-3}), which also requires 8 (4 pairs) electrons, but which only generates one reduced N atom, not 2 as in N fixation. In addition to a net lower cost of N fixation, N fixation also allows for the simultaneous excretion of H_2 (Fay 1992, Madigan et al. 1997) which might be helpful, especially in euryoxic microzones (Paerl 1985) where the presence of O_2 might have a negative feedback on nitrogen fixation. Further, "dumping" of electrons in a euryoxic environment helps to lower the redox potential of the sediments and thus favor the oxygen-sensitive process of nitrogen fixation over assimilatory nitrate reduction. In this scenario, nitrogen fixation does not, in fact, mitigate nitrogen limitation in so-called oligotrophic tropical waters, but is merely a physiological adaptation (Paerl 1990) to acquire nitrogen relatively cheaply in a fluctuating O_2 regime.

Acknowledgments

We thank Cathy Crawford, Sharon Britos, Tina Ennis, Rick Wood, Kazu Sonoda, Kim Nadeau, Butch Irish and the late Kuni Sakamoto for valuable assistance in the field and laboratory. Steve Nelson, Dick Randall, Chuck Birkeland, Chris Lobban, Rob Rowan helped improve the manuscript. Research was supported by the U.S. Geological Survey, the National Science Foundation, and, of The University of Guam, The Water and Energy Research Institute, Marine Laboratory, and Research Council. Contribution No. 567 of the University of Guam Marine Laboratory.

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Received 25 Sep. 2002, revised 24 Aug. 2004