

Fine-scale mapping of land-derived nitrogen in coral reefs by $\delta^{15}\text{N}$ in macroalgae

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Abstract

We measured the C/N ratio and $\delta^{15}\text{N}$ values of two brown macroalgae—*Padina* spp. and *Dictyota* sp., which are distributed over all the subtropical fringing reefs of the Ryukyu Islands, Japan—to evaluate the feasibility of these algae as indicators of the terrestrial nitrogen load to the reef. The correlations between the distance from the shoreline and algal C/N ratio and surrounding NO_3^- concentrations were not clear, although their average values among the reefs seemed to indicate differences in nitrogen loadings from the land. The $\delta^{15}\text{N}$ values of these algae, on the other hand, linearly or curvilinearly decreased from +8‰ to +2‰ with increasing distance from the shoreline, indicating the difference in nitrogen sources available to macroalgae. The slope of the decline among eight study areas had different characters, which seemed to depend on the residence time of reef seawater and the fluxes of terrestrial nitrogen. Using $\delta^{15}\text{N}$ values of brown algae as an indicator, we confirmed that primary producers, such as macroalgae on the reefs, assimilated land-derived nitrogen and successfully evaluated time-integrated effects of terrestrial nitrogen on coral reef algae, which had been missed by conventional monitoring of the water column nutrients.

Direct anthropogenic influences on coral reefs are major concerns because the global population is increasing, especially in tropical and subtropical countries with coral reefs. Furthermore, coral reefs are threatened by global climate

change (Hoegh-Guldberg 1999; Kleypas et al. 1999), which will exacerbate problems already weakened by local perturbations, such as pollution and sediment flow (Cuet et al. 1988; Arceo et al. 2001). Shifts from coral to macroalgae, which have been reported worldwide, may result from many factors: reduction in herbivory because of overfishing (Hughes et al. 1999), eutrophication due to land-derived nitrogen (Bell 1992; Lapointe 1997), and strong synergism between both (McCook et al. 2001).

In subtropical coral reef areas, terrestrial nitrogen coming from groundwater or rainfall often arrives in pulses and is spatially and temporally variable (Andrews and Müller 1983; Ayukai 1993). Therefore, the measured short-term distribution of nitrogen concentrations or salinity in the water column does not necessarily represent the long-term influence of terrestrially derived materials. Although nitrogen inputs from terrestrial areas have been estimated at many sites (e.g., Lewis 1985; Furnas et al. 1995; Umezawa et al. in press), finding clear evidence for a relationship between nitrogen inputs and biological reactions in natural environments has been difficult. Detailed mapping of the area affected by terrestrial nitrogen loading within reefs must be known if we

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are to understand the quantitative relationships linking eutrophication and biological reactions such as the abundance of benthic algae.

The chemical composition of macroalgae and coral tissues should reflect the influences of terrestrial nitrogen because the nitrogen content of tissues represents time-integrated nutrient availability (Weeler and Bjoernsaeter 1992; Fong et al. 1998). Many studies have demonstrated positive responses in algal nitrogen content or the C/N ratio to nutrient conditions in the laboratory or mesocosms (Fujita 1985; Schafelke and Klump 1998; Naldi and Wheeler 1999), but few studies have been done in natural marine environments (Horricks et al. 1995; Lapointe 1997; Fong et al. 2001).

Time-integrated sewage and other terrestrial inputs to coastal waters have been used to trace using the natural abundance of nitrogen isotopes in estuarine seaweed and seagrass (Fourqurean et al. 1997; McClelland and Valiela 1998; Costanzo et al. 2001) and coral tissues and stomatopods at coral reefs (Mendes et al. 1997; Sammarco et al. 1999; Heikoop et al. 2000a,b; Risk and Erdmann 2000). In the narrow coral reefs around Ishigaki Island (our study site), some species of macroalgae are ubiquitous, whereas seagrasses and corals are found only on the inner and outer sides of the reefs, respectively. Macroalgae directly take up nutrients only from the water column, whereas seagrass does so mainly from interstitial water, and corals are mixotrophs. This makes benthic macroalgae more appropriate indicators of the long-term availability of nitrogen in water columns than seagrass or coral tissues. Because the terrestrial inputs incorporated into the algal tissue are averaged over their active growing period (approximately several months), this reflects the land-derived nitrogen availability.

In this study, we surveyed the spatial distributions of macroalgal $\delta^{15}\text{N}$, the C/N ratio, and dissolved inorganic nitrogen (DIN) concentrations in surrounding seawater at eight coral reefs and bays that had different hydrographic and topographic features. The background nitrogen inputs (i.e., in the absence of anthropogenic nitrogen input) among these sites in this small area (about 20 by 30 km) should be similar. Our goal was to determine whether these chemical components in macroalgae could be used as an indicator of anthropogenic N loading (Cuet et al. 1988; Heikoop et al. 2000b). To understand nitrogen enrichment at these reefs and nitrogen sources utilized by macroalgae, we compared these distribution patterns with the corresponding characteristics of each area (e.g., nitrogen loading from each watershed, residence time of seawater), especially with reference to the differences in outflow (i.e., groundwater, rivers, and sewer drains).

Materials and methods

Study site—Ishigaki Island (Ishigaki-jima), southwest of the Ryukyu Islands, Japan ($24^{\circ}21'–31'N$, $124^{\circ}4'–16'E$) (Fig. 1), is surrounded by a well-developed 0.5–2.0-km-wide fringing reef. In contrast to the flat topography of the southern area, low mountains (maximum 526 m above mean sea elevation) range across the central region of the island from east to west, from which steep and gentle slopes extend north and south, respectively. The climate is subtropical, the an-

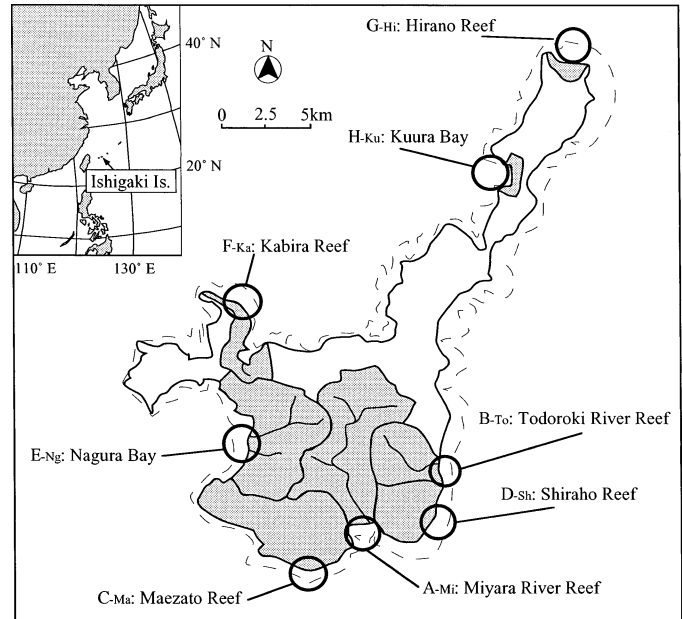


Fig. 1. Map of Ishigaki Island with location of each watershed (shaded areas) and reef (circles). The dotted line shows coral reefs around the island. Main rivers are shown by solid lines.

nual average temperature is 24.3°C , and the humidity is 78% (Ishigaki-jima Meteorological Observatory). Average annual precipitation is about 2,000 mm; rain is especially abundant during the monsoon season (March to May) and typhoon season (July to September).

Benthic algae and seawater were collected at eight coral reefs or bays (Miyara, Todoroki, Maezato, Shiraho, Nagura, Kabira, Hirano, Kuura) at Ishigaki Island (Fig. 1). The watersheds adjoining these reefs and their boundaries were determined from surface landform and tributary streams (Fig. 1). Nitrogen loadings to these watersheds were estimated by human and domestic animal population data and land use (Table 1). In the southern area, many people, fields, and pastures were concentrated on limestone-based flat land, whereas the northern area had small numbers of people and fields. Therefore, the estimated nitrogen loading was different in each watershed. In this paper, we use letters (A–H) for these locations, indicating the order of estimated values of nitrogen loading per unit length of shoreline (Table 1); we also use an abbreviation of the location name after the letter (e.g., Miyara area is A-Mi). The outflow pattern from each watershed depends on whether the ground is permeable (e.g., limestone, gravel) or impermeable (e.g., granite, andesite). On the beaches at the D-Sh and F-Ka watersheds, which are mainly limestone and gravel, many groundwater seepages occur, especially at low tide (Table 1). On the other hand, freshwater input through natural river and sewer drains was usually observed at A-Mi, B-To, and C-Ma watersheds. Table 2 gives the topographical and biological features of these reefs. At most study areas, we saw a diverse community with coral and algal zonation from the inner reef to the crest, whereas the topography (e.g., depth, width of moat, conditions of reef crest, and others) differed between the reefs (Nakamori et al. 1992; Tanaka 1999; Yamano et al. 2000). Dense patches of green macroal-

Table 1. The geological and geographical features of each watershed. The letters A–H are assigned to the watersheds in the order of estimated nitrogen loading per unit length of the shoreline.

Location	Total area† (km ²)	Shoreline length (km)	Estimated nitrogen loading‡			Note (major land use and out flow pattern)
			Total§ (Mg N yr ⁻¹)	Per unit area of watershed (Mg N km ⁻² yr ⁻¹)	Per unit length of shoreline (Mg N km ⁻¹ yr ⁻¹)	
A-Mi Miyara	34.2	1.3	128(48.6)	4	99	Sugarcane, woods, river
B-To Todoroki	12.8	1.8	122(73.1)	10	70	Pasture, sugarcane, river
C-Ma Maezato	31.0	16.0	848(92.2)	27	53	Town, sewer drains
D-Sh Shiraho	8.8	6.0	189(89.1)	21	31	Pasture, sugarcane, groundwater seepage
E-Ng Nagura	27.2	7.0	90(52.7)	3	13	Rice field, pineapple, river
F-Ka Kabira	8.6	9.3	66(80.1)	8	7	Rice field, woods, groundwater seepage
G-Hi Hirano	2.1	4.1	16(70.7)	8	4	Fields, woods, groundwater seepage
H-Ku Kuura	2.4	2.5	9(47.1)	4	4	Fields, woods, small streams

† The area was measured using a digitizer (HTG-3648S; Hitachi) from a 1:25,000 geomorphologic map.

‡ The estimation was conducted by the per-capita method, largely following Umezawa et al. (in press) and Umezawa (2000).

§ The values in parentheses are the percentage (%) of nitrogen loadings from feces of humans and domestic animals.

gae, such as *Ulva* spp., *Enteromorpha* spp., and *Monostroma* spp., occurred as a dense belt on reef rocks along the shoreline from winter to spring at A-Mi, B-To, C-Ma, and D-Sh. Mixed seagrass beds (e.g., *Thalassia hemprichii*, *Cymodocea* spp., *Halodule* spp., and *Halophila* spp.) covered the bottom near shore at B-To, D-Sh, E-Ng, and H-Ku year-round. Phaeophyta, such as *Dictyota* sp., *Turbinaria ornata*, and *Padina* spp., were widely distributed on the reef from inshore to further offshore, whereas *Sargassum* spp. canopies were dense on the reef crest in summer. *Porites* spp., *Montipora* spp., *Acropora* spp., and *Heliopora coerulea* were predominant among the branching corals around the well-developed reef crest at B-To, D-Sh, and F-Ka.

Sampling—Around Ishigaki island, >100 species of macroalgae have been reported (Ohba and Aruga 1982). We set one or three transects perpendicularly to the shoreline at every coral reef or bay (Fig. 2) and collected about 20 representative species (e.g., *Cladophora* spp., *Caulerpa* spp., *Halimeda* spp., *Enteromorpha* spp., *Ulva* spp., *Dictyota* spp.,

Turbinaria ornata, *Hormophysa cuneiformis*, *Sargassum* spp., *Padina* spp., *Acanthophora* sp.) at depths between 0.5 and 3.0 m (mainly 0.5–1.0 m) on the reef rock or patch corals. Detailed sampling point maps were created using a global positioning system (GPSII plus, Garmin). Among the species collected, we analyzed a total of 98 samples of *Padina* spp. and 47 samples of *Dictyota* sp. that were not well differentiated morphologically. These two species appeared at all the reefs and were distributed widely from near shoreline to reef crest. Therefore, they were appropriate indicator species to evaluate the distribution of land-derived nitrogen. These algae were kept cool during transport to the laboratory, where they were gently brushed with filtered seawater to remove obvious organic and inorganic contaminants (i.e., epiphytes and foraminifera). They were then dried at 60°C until the weight became constant, powdered, and homogenized with an agate mortar and pestle for the determination of nitrogen stable isotope ratio ($\delta^{15}\text{N}$) and the ratio of organic carbon to total nitrogen content (C/N) in the tissues.

At >120 stations, we took duplicate seawater samples for

Table 2. The topographical and biological features of each reef.

Location	Total area* (km ²)	Bed slope† (Δh)	Reef width (m)	Crest condition†	Major substrate†
A-Mi Miyara River Reef	0.75	0.1–0.2	700–800	Not developed	Reef rock, seaweed, seagrass
B-To Todoroki River Reef	0.95	0.3–0.4	600	Well developed	Reef rock, seaweed, seagrass, coral
C-Ma Maezato Reef	0.75	n.d.	300–400	Not developed	Reef rock, seaweed, coral
D-Sh Shiraho Reef	1.4	0.45–0.55	600–850	Well developed	Sand, seagrass, seaweed, coral
E-Ng Nagura Bay	—	0.15–0.25	—	—	Sand, seagrass
F-Ka Kabira Reef	1.3	0.50–0.65	800–1,000	Well developed	Sand, seaweed, coral
G-Hi Hirano Reef	2.0	0.9–1.1	1,200	Not developed, big channel	Sand, coral
H-Ku Kuura Bay	—	0.4–0.5	—	—	Sand, seagrass

* The boundary of each reef was mainly determined by reef geomorphology and circulation of seawater. We did not define the area of Nagura and Kuura Bay because there is no obvious boundary (reef crest) between outer and inner ocean in this area. The area and reef width was measured using a digitizer (HTG-3648S, Hitachi) from a 1:25,000 geomorphologic map.

† Slope of bed (Δh = depth (m)/distance (100 m)), crest condition, and major substrate were inferred from field observation, Tanaka (1999), and the *Distribution Map of Coral Reefs* (Ministry of the Environment, Government of Japan, 1989–1992). The average depth of each moat is 1.0–2.5 m, mostly corresponding with the slope of the bed. n.d., not determined.

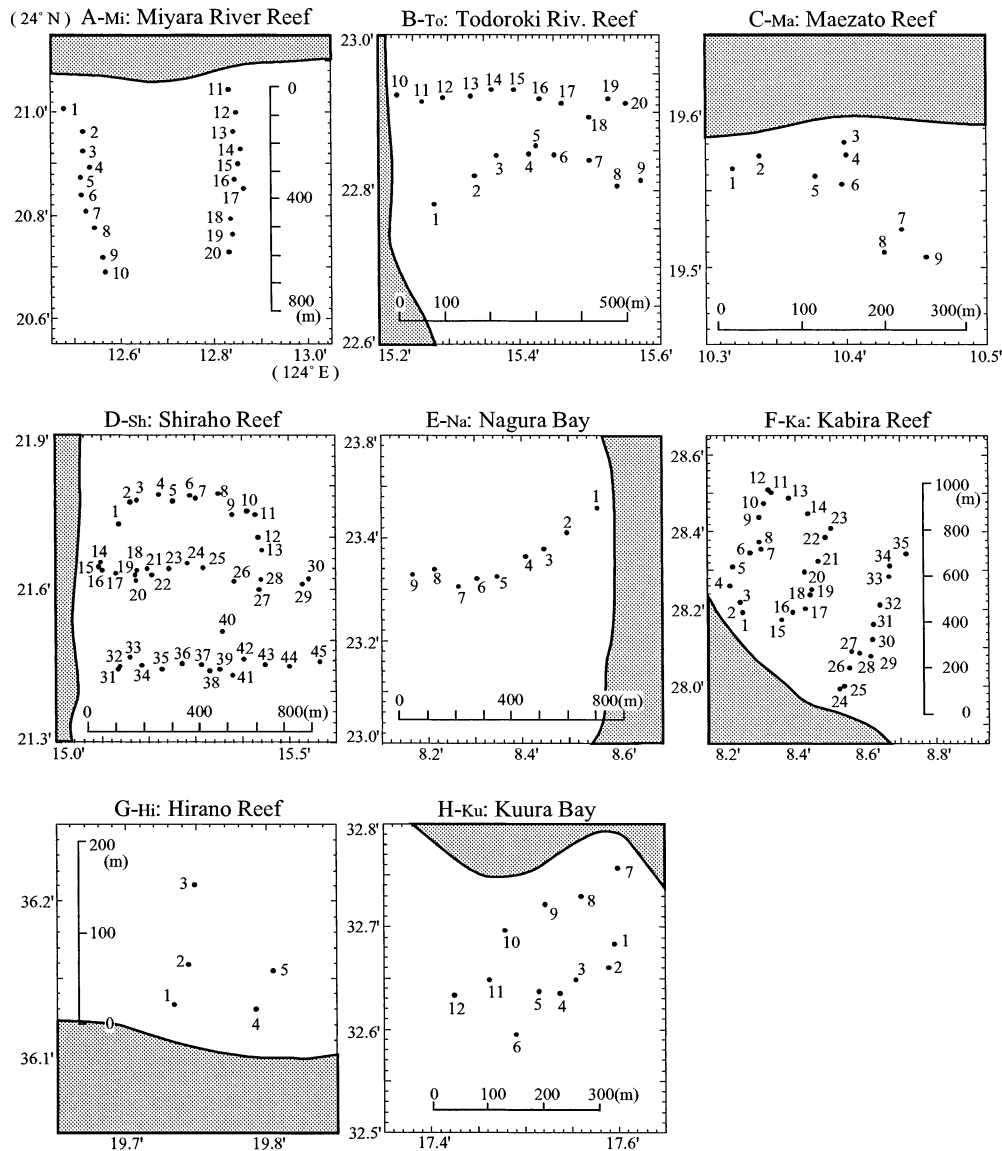


Fig. 2. Detailed distribution of sampling stations at each reef. Shaded areas indicate terrestrial area. From the viewpoint of main terrestrial outflow patterns, these areas were classified by groundwater (D-Sh Reef, F-Ka Reef, G-Hi Reef), by river (A-Mi. Reef, B-To Reef, E-Ng Bay), by small stream (H-Ku Bay), and by sewer drain (C-Ma reef).

nutrient analyses (NO_3^- , NO_2^- , NH_4^+ , and PO_4^{3-}). These samples were taken at low tide when nutrient concentrations were maximal. These samples were transferred to 10-ml acrylic tubes and stored in a freezer for later analysis. Analyses were performed within a few weeks.

The samples for the analysis of the $\delta^{15}\text{N}$ values of nitrate in freshwater were collected from wells or rivers near the coastline at each watershed. These samples were transferred to 1-liter plastic bottles and kept frozen until later analysis.

Analytical methods—Nutrient concentrations (NO_3^- , NO_2^- , NH_4^+ , and PO_4^{3-}) were colorimetrically measured with an autoanalyzer (AACS II, BRAN+RUEBBE).

Macroalgal samples (3.00–6.00 mg dry weight) were

transferred into a small silver container (5 × 8 mm, CE Instruments), which had been soaked in pure ethanol overnight to remove organic matter, and dried well. A drop of 1.0 N HCl was added to remove inorganic carbon, and then the containers were dried at 40°C to remove any remaining HCl. Carbon and nitrogen contents of these algae were measured using a CHN analyzer (NA-1500, Fisons Instruments). Algal samples for determining nitrogen stable isotope ratios were subjected to the same pretreatment, except that a tin container (5 × 8 mm, CE Instruments) was used and HCl was not added. The wrapped tin containers were combusted at 1050°C in an elemental analyzer (NA-1500, Fisons Instruments), and the combustion products (N_2) were introduced to an isotope-ratio mass spectrometer (DELTA^{plus}, Fi-

Table 3. Nitrate concentrations and $\delta^{15}\text{N}$ values of nitrate in groundwater and river water.

Group/watershed*	NO_3 conc. (μM)	$\delta^{15}\text{N}^\dagger$ (‰)	Recovery ‡ (%)	Note
Wastewater				
Maezato(C-Ma)	3.4	n.d.	—	
Groundwater in the well:				
Shiraho (D-Sh)	259.7	6.7	90.6	
Kabira (F-Ka) §	181.2	7.8		
Spring water around shoreline:				
Shiraho (D-Sh)	217.2	7.7	99.7	
Kabira (F-Ka)	40.5	3.2	88.9	
Hirano (G-Hi)	280.1	5.2	86.1	
River water:				
Todoroki River (B-To)	244.0	7.4	94.3	
Miyara River (A-Mi)	78.3	6.9	97.9	
Nagura River (E-Ng)	18.0	5.7	104.8	
Outer reef seawater				
Seawater 1	2.1	n.d.	—	
Seawater 2	1.5	n.d.	—	
Standard (KNO_3)				
Solution 1	30	0.5	109.1	
Solution 2	150	0.9	93.1	
Solution 3	250	0.6	95.6	
Crystalline (n=5)	—	0.57	100.2	± 0.09 SD

* All samples except for the standard sample were analyzed with duplicates (SD ≤ 0.13 max.).

† The values of wastewater at Maezato and offshore seawater were not analyzed. Liu et al. (1996) suggested that the estimated errors were within $\pm 0.3\%$ for samples with nitrate concentrations of $>20 \mu\text{M}$ and within $\pm 1.2\%$ for a concentration of $>5 \mu\text{M}$.

‡ Recovery (%) is the ratio of the amounts of nitrogen in the filter sample detected by the CHN analyzer relative to nitrate concentrations in the initial water sample analyzed by AACS II.

§ The groundwater data at Kabira (F-Ka) was from Miyajima (pers. comm.).

nigan Mat) in a continuous flow using a He carrier. Ratios of $^{15}\text{N} : ^{14}\text{N}$ were expressed relative to atmospheric nitrogen. Ratios of $^{15}\text{N} : ^{14}\text{N}$ were calculated as

$$\delta^{15}\text{N} = \left\{ \left(\frac{\text{R}(\text{sample})}{\text{R}(\text{standard})} \right) - 1 \right\} \times 1,000 \text{ (‰)}$$

(where R = $^{15}\text{N}/^{14}\text{N}$)

The reproducibility of the isotope analysis was checked with isotopically known L- α -alanine every five original samples (SD ≤ 0.19 , $n = 8\text{--}15$ for $\delta^{15}\text{N}$), and the values for the algal samples were corrected for the change in the sensitivity of the mass spectrometer. The $\delta^{15}\text{N}$ values of the algal samples were measured twice; the difference in the duplicate values was 0.13‰ on average and 0.28‰ maximum.

Nitrate in groundwater or river water was extracted in three steps, (1) sample concentration, (2) conversion of nitrate to ammonia using Devarda's alloy, and (3) gas-phase diffusion of ammonia onto an acidified glass fiber disk to analyze the $\delta^{15}\text{N}$ values in the nitrate (Sigman et al. 1997). We also analyzed sets of standard samples of distilled water amended with nitrate (KNO_3) to 30–250 μM by using the same preparation procedure, and crystalline KNO_3 (precombusted at 120°C for 2h) directly, to confirm the Devarda's alloy blank size and the fraction of recovery at the ammonia diffusion step. The glass fiber disks, on which extracted nitrate was trapped as ammonium, were wrapped in small tin containers (5 \times 8 mm; CE Instruments). The $\delta^{15}\text{N}$ values of the filter samples were measured twice following the same

procedure outlined for algal samples. The difference in duplicate values was 0.17‰ on average and 0.49‰ maximum.

Results

The sum of nitrate and nitrite concentrations in groundwater and river water, which flowed into each coral reef, ranged from 18.0 to 280 μM (Table 3). In most locations, ammonium was negligible compared with nitrate, as was also reported by Umezawa et al. (in press). The wastewater flowing into the C-Ma reef had a nitrate concentration of 3.4 μM , and ammonium concentrations were extremely high (over 500 μM). The nitrate values of spring water around the shoreline were lower compared to groundwater in the well at D-Sh and F-Ka watersheds, which was explained by mixing with seawater around the beach (Umezawa et al. in press). The values in offshore deep water 7 km from the reef, which we presumed to flow up to the reef, were 1.5–2.1 μM . The $\delta^{15}\text{N}$ values of nitrate in groundwater and river water ranged from 5.2 to 7.8‰ with 86.1 to 104.8% recovery of nitrate. A distinctly low value of 3.8‰ was observed in spring water at Kabira beach (Table 3). These $\delta^{15}\text{N}$ values of freshwater were nearly the same as those reported at Miyako Island (i.e., +4.3–9.7‰, stable at each area all year around), next to Ishigaki Island (Yamamoto et al. 1995). Several groundwater currents, each with different nitrogen sources, could account for the variation in $\delta^{15}\text{N}$ values of nitrate in the groundwater within the same watershed.

The low recovery rate may be attributed to incomplete

Table 4. The mean values with standard deviations (SD) of $\delta^{15}\text{N}$ (‰), the C/N ratio in macroalgal tissues, and nitrate concentrations (μM) in ambient water columns at each coral reef and bay.

Site	Species	<i>n</i>	$\delta^{15}\text{N}$		C/N		NO_3^-		
			Mean	SD	Mean	SD	Mean	SD	
A-Mi	Miyara	<i>Padina</i>	14	6.9	0.9	19.7	2.1	2.3	4.2
		<i>Dictyota</i>	0	—	—	—	—	—	—
B-To	Todoroki	<i>Padina</i>	15	4.6	2.0	17.0	2.9	13.7	28.9
		<i>Dictyota</i>	2	2.7	0.1	15.9	0.5	0.76	0.01
C-Ma	Maezato	<i>Padina</i>	7	5.9	1.1	18.7	2.2	15.4	24.5
		<i>Dictyota</i>	2	4.7	0.1	14.6	0.6	2.0	0.2
D-Sh	Shiraho	<i>Padina</i>	27	3.8	1.1	17.6	1.9	1.7	1.2
		<i>Dictyota</i>	14	3.9	0.5	17.1	2.4	1.6	0.8
E-Ng	Nagura	<i>Padina</i>	7	3.2	0.5	27.4	2.6	0.07	0.08
		<i>Dictyota</i>	3	2.8	0.4	21.5	2.5	0.04	0.01
F-Ka	Kabira	<i>Padina</i>	28	3.2	1.0	19.2	2.6	0.54	3.50
		<i>Dictyota</i>	16	3.1	0.7	16.4	1.7	0.54	0.36
G-Hi	Hirano	<i>Padina</i>	3	3.6	1.0	20.4	1.5	1.1	0.7
		<i>Dictyota</i>	3	1.9	0.3	20.2	1.6	0.82	0.20
H-Ku	Kuura	<i>Padina</i>	7	2.5	0.5	25.1	4.2	0.09	0.12
		<i>Dictyota</i>	6	2.4	0.4	15.3	5.8	0.07	0.05

ammonia uptake or a low efficiency of nitrate reduction. Sigman et al (1997) suggested that combustion of Devarda's alloy in air might decrease its nitrate reduction efficiency. In any case, the actual $\delta^{15}\text{N}$ values in terrestrial nitrate may be a little higher than the measured values because incomplete ammonia uptake out of aqueous solution strongly fractionates the nitrogen isotopes to lower values (Sigman et al. 1997).

In our study, the blank size of Devarda's alloy and $\delta^{15}\text{N}$ fractionation at the ammonia diffusion step were estimated to be negligible because the $\delta^{15}\text{N}$ values of KNO_3 solution with different concentrations were similar to the values of crystalline KNO_3 analyzed directly (Table 3).

The nitrate concentrations at each reef around Ishigaki Island ranged widely from 0.0 to 120 μM (full data are available in Web Appendix 1 at www.aslo.org/lo/toc/vol_47/issue_5/1405a1.pdf). At areas subject to point source nitrogen from rivers or sewer drains, the average nitrate concentration in reef water was relatively high. At B-To, high NO_3^- values (>30 μM) were observed only at the shallow area north of the river mouth and at a distance of <200 m from the shoreline, whereas <1.0 μM was observed at the areas >200 m distant. At A-Mi, NO_3^- concentrations over the reef were 0.1–2.4 μM with no gradients. At C-Ma, which is adjacent to residential areas, high NO_3^- was observed, especially at the surface, from 23.9 to 73.1 μM , despite low nitrate concentrations in flowing wastewater (Table 3). This indicates high temporal variability of nitrogen loading from residential areas. However, at areas subject to non-point source inputs of nitrogen from groundwater, the average nitrate concentration was low, despite input of high nutrient concentrations. At F-Ka and G-Hi, NO_3^- was distributed evenly all over the moat (from 0.2 to 0.9 μM), although some high values were also observed along the beach or at springs of groundwater (e.g., Sta. 29 at F-Ka and Sta.4 at G-Hi). At D-Sh, where the seawater residence time is relatively longer than at the other areas because of a well-developed reef crest (Nakamori et al. 1992; estimates from

Yamano et al. 1998), the NO_3^- concentration was slightly higher, from 0.2 to 4.9 μM . The NO_3^- concentration at E-Ng and H-Ku bays was near the limits of analytical detection. Concentrations of phosphate in reef water around Ishigaki Island were near the limits of analytical detection, except along the shoreline at C-Ma and B-To (0.3–0.5 μM).

We had hypothesized that nutrient concentrations at each area would gradually decrease with increasing distance from the land, which was considered to be the main nitrogen source to the reefs. However, this pattern was found only at B-To, D-Sh, and F-Ka ($p < 0.05$, Spearman's rank correlation). This may be because the mixing ratio varies spatially and temporally between freshwater with high nutrients and offshore seawater with low nutrients.

The $\delta^{15}\text{N}$ values of macroalgae ranged from +8.3 to +1.9‰ from shoreline to reef crest (i.e., +8.3 to +5.3‰ at A-Mi reef, +7.6 to +2.4‰ at B-To reef, +7.2 to +4.8‰ at C-Ma reef, +5.8 to +2.0‰ at D-Sh reef, +3.9 to +2.7‰ at E-Ng bay, +5.7 to +2.2‰ at F-Ka reef, +4.3 to +2.1‰ at G-Hi reef, and +3.6 to +1.9‰ at H-Ku bay, Web Appendix 1). There were highly significant differences in the mean $\delta^{15}\text{N}$ values between some reefs (i.e., A-Mi vs. B-To, D-Sh, E-Ng, F-Ka, G-Hi, and H-Ku; C-Ma vs. D-Sh, E-Ng, F-Ka, and H-Ku, $p < 0.001$, *t*-test, Table 4); significantly positive relationships were related to nitrogen loading per unit length of shoreline ($p < 0.05$, Spearman's rank correlation) and the mean nitrate concentrations ($p < 0.05$, Spearman's rank correlation).

The C/N ratio of *Padina* spp. ranged from 11.8 to 30.1 from shoreline to reef crest (15.8–23.3 at A-Mi, 11.8–21.0 at B-To, 15.6–21.3 at C-Ma, 12.5–21.1 at D-Sh, 24.4–30.0 at E-Ng, 12.0–25.4 at F-Ka, 19.3–22.2 at G-Hi, and 19.7–30.1 at H-Ku, Web Appendix 1). The mean C/N ratio differed significantly between some reef combinations (i.e., E-Ng vs. A-Mi, B-To, C-Ma, D-Sh, and F-Ka; H-Ku vs. B-To, D-Sh, and F-Ka, $p < 0.001$, *t*-test, Table 4); it showed no consistent relationship with nitrogen loading per unit length of shoreline, whereas a significant negative relationship with

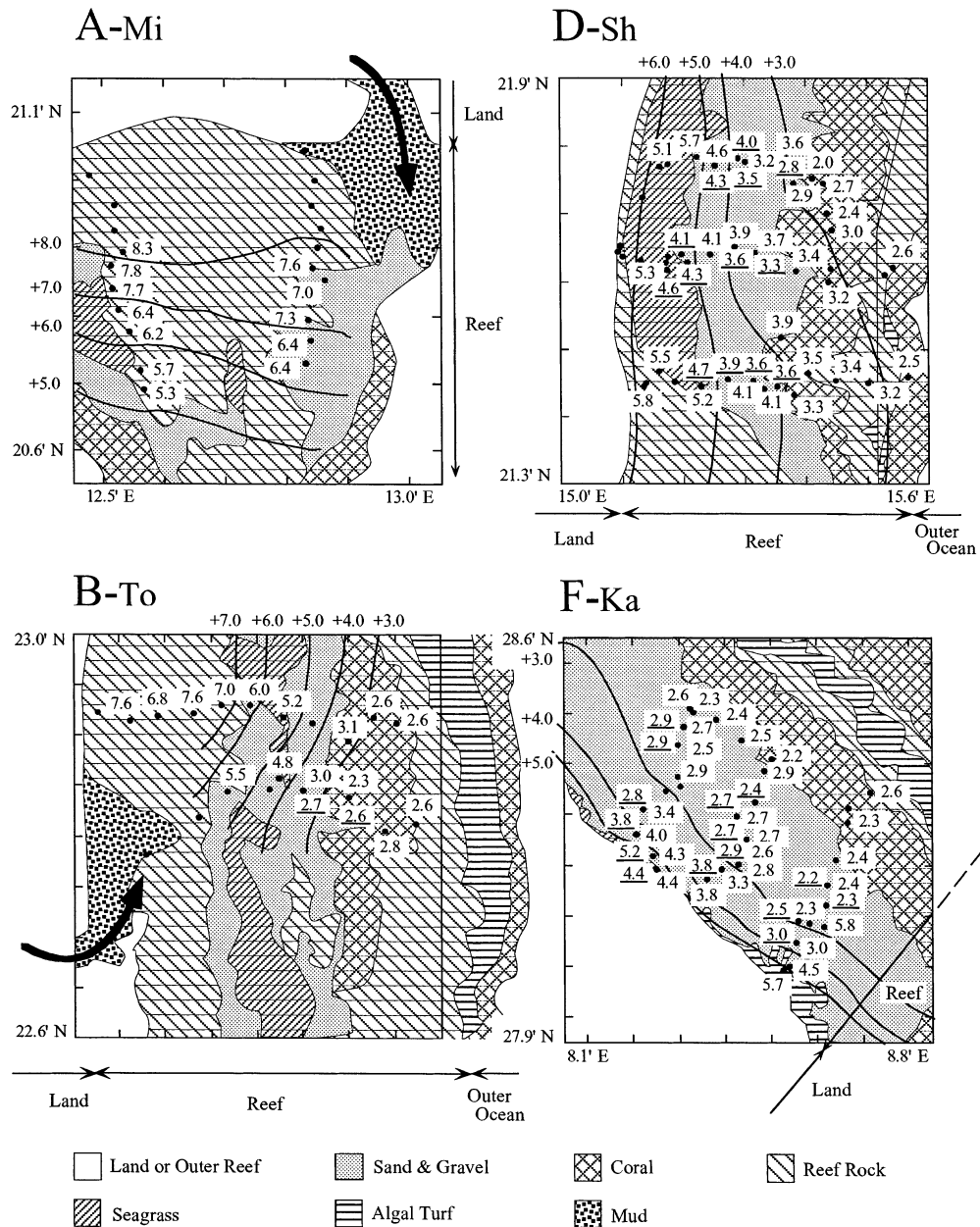


Fig. 3. Distribution of the $\delta^{15}\text{N}$ value of macroalgae (*Padina* spp., *Dictyota* sp.) on the map of organisms and substratum at A-Mi Reef, B-To Reef, D-Sh Reef, and F-Ka Reef. The maps of organism and substratum at each reef were made by analyzing aerial photographs (1995–1997) together with field observations and modifying Nakamori et al. (1992), Yamano et al. (2000), and the Distribution Map of Coral Reefs (1989–1992). The values for *Dictyota* sp. are underlined, and the others are for *Padina* spp. The arrows in panels A-Mi and B-To indicate the current of a flowing river.

the mean nitrate concentrations at each area was found ($p < 0.05$, Spearman's rank correlation).

The $\delta^{15}\text{N}$ values of *Padina* spp. and *Dictyota* sp. at A-Mi, B-To, D-Sh, and F-Ka using two or three sampling transects were plotted on a map of vegetation and substratum (*Distribution Map of Coral Reefs*, Marine Biotic Environment Survey (1989–1992), 4th National Survey on the Natural Environment, Ministry of the Environment, Japan). The values of $\delta^{15}\text{N}$ at these four areas (Fig. 3) clearly decreased

with distance from the shoreline. However, the C/N ratio of these algae showed no distinct gradients away from the shoreline.

Discussion

We observed a large variation (11.8–30.1) of the C/N ratio of *Padina* spp. The ratio was high at E-Ng and H-Ku, where the nitrate concentrations in seawater were nearly undetect-

able, and low at the other areas. The C/N ratio of *Padina* spp. showed significant negative relationships with the mean nitrate concentrations at each area. This suggests that tissue nutrient content might also be a reliable indicator of water quality at our study sites, when compared among reefs and bays. However, this trend was not present in *Dictyota* sp., which showed no distinct gradients away from the shoreline. This might indicate that tissue nutrient levels depend on growth rates, physiology, storage capacity, and morphological forms, as well as supply (Fujita 1985; Gomez and Wiencke 1998; Fong et al. 2001). Also, tissue nutrient content is reliable as a bioindicator only in a region where nutrients are limiting to growth (Horrocks et al. 1995). Therefore, the C/N ratio of seaweed in our study areas does not reflect a variation of terrestrial nitrogen input.

On the other hand, the algal $\delta^{15}\text{N}$ values showed distinct gradients from the shoreline and did not seem to be affected by a difference in algal species or physiology. For example, different species of green foliose algae, *Ulva fasciata* and *U. pertusa*, collected at the same place along the shoreline of D-Sh, had indistinguishable $\delta^{15}\text{N}$ values (i.e., 4.67 ± 0.18 ($n = 5$) and 4.58 ± 0.15 ($n = 4$), respectively (Umezawa unpubl. data). The $\delta^{15}\text{N}$ values of even the different genera of brown algae, *Padina* spp. and *Dictyota* sp., collected at the same places were within 0.3‰, showing similar decreasing trends with increasing distance from the shoreline. This suggests that macroalgae that grow within the same nutrient environments have similar $\delta^{15}\text{N}$ values, at least among related species.

Nitrogen isotopes are useful in tracing terrestrial input to coastal systems when the nitrogen sources are isotopically distinct from other sources (Macko and Ostrom 1994; Paerl and Fogel 1994). The $\delta^{15}\text{N}$ values in nitrate, which was the dominant land-derived nitrogen source at our study sites, were 5.2–7.8‰. To use it as an indicator of terrestrial input, the $\delta^{15}\text{N}$ values of the other nitrogen sources must be distinctly different from those values.

At oligotrophic coral reefs, nitrogen fixation by epiphytic cyanophytes can be an important nitrogen source (Larkum et al. 1988; Bell et al. 1999). At our study sites, previous studies showed high nitrogen fixation rates by cyanobacteria or bacteria at Kabira (F-Ka) and Shiraho (D-Sh) reefs (Kayanne et al. pers. comm.; Miyajima et al. 2001). Because N_2 dissolved in water has an isotopic composition very similar to that of atmospheric dinitrogen (Cline and Kaplan 1975), the small fractionation associated with nitrogen fixation implies that the $\delta^{15}\text{N}$ values of nitrogen in reef ecosystems supplied by nitrogen fixation should be approximately 0‰ (Wada and Hattori 1978).

The $\delta^{15}\text{N}$ values of nitrate or ammonia in rainwater vary with sources, season, and their location. For instance, $\delta^{15}\text{N}$ values of nitrate and ammonia in agricultural fields are -2.0 to $+4.7$ ‰ and -12.5 to $+3.6$ ‰, respectively, where chemical fertilizers of atmospheric nitrogen origin (0‰) are mainly used (Heaton 1986; Pearl and Fogel 1994). Moreover, the $\delta^{15}\text{N}$ values of ammonia in rainwater may further decrease because of ammonia volatilization, which has a strongly negative fractionation. We assumed $\delta^{15}\text{N}$ values of nitrate or ammonia in rainwater to have low values around 0‰, although they were not measured.

The $\delta^{15}\text{N}$ values in nitrate or particulate organic nitrogen (PON) of pelagic seawater range widely (from $+4$ to $+20$ ‰) depending on the area, depth, and season (Altabet and Deuser 1985; Liu and Kaplan 1989). However, the East China Sea near Ishigaki Island has abundant nitrogen-fixing cyanobacteria such as *Trichodesmium* (Marumo and Asaoka 1974) and low $\delta^{15}\text{N}$ values in diatoms (i.e., $+1$ to $+3$ ‰) and in PON (i.e., -1.5 to $+1.0$ ‰) were reported (Wada and Hattori 1978; Saino and Hattori 1987). Although the $\delta^{15}\text{N}$ values in diatoms and PON may not accurately reflect the $\delta^{15}\text{N}$ of nitrate because of fractionation between different trophic levels (Goericke et al. 1994), we assumed that the $\delta^{15}\text{N}$ values in nitrate flowing into coral reefs from the outer ocean were about $+1$ to $+3$ ‰, the lower end of the range of typical seawater DIN values (Owens 1987). Even if the seawater nitrate did have slightly heavier $\delta^{15}\text{N}$ than assumed, the assumption that the terrestrial source is heavier would still hold, because freshwater nitrate $\delta^{15}\text{N}$ values are probably underestimated because of incomplete recovery of nitrate during sample preparation (see Results). Considering the above information, the distinctly higher $\delta^{15}\text{N}$ values of terrigenous nitrate, by 3–6‰ relative to all other nitrogen sources, make it possible to detect the areas of terrestrial nitrogen influence by $\delta^{15}\text{N}$ analysis.

$\delta^{15}\text{N}$ values in macroalgae plotted as a function of the distance from the shoreline at each study site decreased, and the gradients fitted a curvilinear or linear regression (Fig. 4). Logarithmic transformation is appropriate on vertical coral flanks, where the illumination quickly decreases (Heikoop et al. 1998). Macroalgal uptake is also controlled by light availability (Mann 1982), and intracellular fractionation may occur during uptake of inorganic nitrogen by microalgae (Wada and Hattori 1978; Montoya and McCarthy 1995). However, light-related fractionation was not likely to occur because the light available to macroalgae collected in this study were uniformly high because of the low turbidity of the reef water. Therefore, the decreasing gradients of algal $\delta^{15}\text{N}$ should be due primarily to differences in the isotopic composition of nutrient sources.

Whether the data fit better with linear or curvilinear regression lines may be associated with other factors such as lateral transport by physical mixing and biological uptake in the vicinity of the shoreline (Mendes et al. 1997; Sammarco et al. 1999). Here, we discuss the different features of these regression lines, with special reference to local conditions (e.g., inflow pattern of freshwater, nitrogen loading per unit length of shoreline, and the exchange ratio between seawater and freshwater). The study reefs and bays were classified into three categories: (1) reefs having high nitrogen loading from rivers or sewer drains (point sources), (2) reefs with moderate nitrogen loading through groundwater (nonpoint sources), and (3) bays without direct input by rivers or streams.

In the first category (i.e., A-Mi, B-To, and C-Ma), the gradients of $\delta^{15}\text{N}$ values fitted a linear regression line better than a curvilinear one, judging from the correlation coefficient (r^2) and the average residual values (Fig. 4). At A-Mi, the $\delta^{15}\text{N}$ values decreased the least of the eight study sites, and $\delta^{15}\text{N}$ values were high. Although the Miyara River makes a channel cutting the reefs, the large body of river

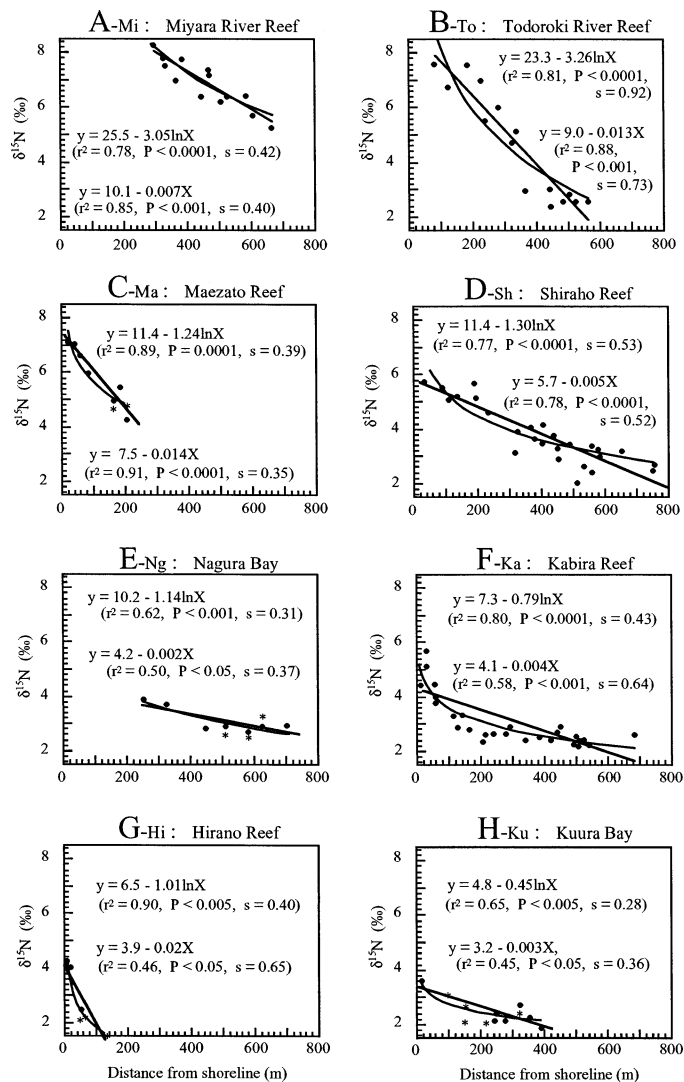


Fig. 4. The relationships between macroalgal $\delta^{15}\text{N}$ values and the distance of sampling stations from the shoreline at each reef or estuary (August 1999). Station is represented by the distance in meters from the shoreline, where terrestrial nitrogen flows in. Solid circles represent the $\delta^{15}\text{N}$ values of *Padina* spp. The $\delta^{15}\text{N}$ values of *Dictyota* sp. are represented by an asterisk only at the four areas (C-Ma, E-Ng, G-Hi, and H-Ku), where not enough *Padina* spp. was collected. Linear or logarithmic regression line, correlation coefficient (r^2), p -value, and average residual value(s) are shown on each graph.

water flowing to the bay spreads out over this shallow reef. The ^{15}N enrichment of the macroalgae (5.3‰) living 667 m from the shoreline suggests that this station is still influenced by the terrestrial nitrogen. At B-To, the macroalgae affected by ^{15}N -rich terrestrial nitrogen were farther from the beach in the northern area (Fig. 3). Moreover, the contour lines were sparse near the beach, but dense 200 m from the shoreline. This unevenness might be attributed to a lower dilution rate by offshore water in the northern part, because the river usually flushes out in a north direction where the developed reef rock has a shallow area (Watanabe 1999). At C-Ma, the algal $\delta^{15}\text{N}$ values rapidly decreased within 200 m of the

shoreline, despite the large estimated nitrogen loading per unit length of shoreline (Table 1). The sewage water from the drains apparently spreads over the water surface as a thin layer because of the moderate currents and greater depth of the inner reef. In fact, the nitrate concentration at the bottom was less than a tenth of that at the surface (Web Appendix 1). Therefore, the nutrients from the wastewater were not fully available to the benthic macroalgae, even in the vicinity of the shore. Except for C-Ma, which has specific geographical features, the land-derived nitrogen available for benthic organisms is conveyed far away (by advective flow) in a short time in areas affected by point sources. Therefore, the regression lines in these areas approach the one-dimensional mixing model. In watersheds drained by a river or sewer drain, the shoreline length is small relative to the size of the area because many branches or drains collect water from a vast area to produce a single larger flow. Therefore, the nitrogen loading per unit length of shoreline becomes large, despite small loading per unit area of the watershed (Table 1). The above situation occurred at the coastal area of eastern Australia, where freshwater runoff is mainly from rivers (Furnas and Mitchell 2000) and the effects of terrestrial inputs spread beyond 10 km (Sammarco et al. 1999; Costanzo et al. 2001).

The second category of reefs (i.e., D-Sh, F-Ka, and G-Hi), received moderate nitrogen loading through groundwater (nonpoint sources). The offshore gradients of $\delta^{15}\text{N}$ in these areas fitted curvilinear regression lines better than linear lines, although the validity of the two models were similar for D-Sh (Fig. 4). At F-Ka, the values of algal $\delta^{15}\text{N}$ decreased rapidly within 200 m of the shoreline, and the algal $\delta^{15}\text{N}$ contour lines were denser than at D-Sh, which has a similar outflow pattern through groundwater. The estimated nitrogen loading per unit length of the shoreline at F-Ka was a fourth or fifth the values at D-Sh (Table 1, Umezawa et al. in press). This might explain the differences in $\delta^{15}\text{N}$ distribution patterns between the two areas. At F-Ka, offshore water enters the reef across the northeastern reef edge, flows landward, and drains from the southeast channel. The high exchange between freshwater and seawater, which is strengthened by the northern dominant wind throughout the year (Yamano et al. 1998), could be another reason for this narrow area of terrestrial nitrogenous influence.

At D-Sh, the 1‰ contour lines were parallel to the shoreline with a small bulge in the southern area (Fig. 3). The general circulation pattern of water on this reef flat is offshore water that enters across the reef edge, especially in the northern part, and flows southward along the coastal line in the moat, finally draining offshore mainly through a depression in the southern part (Nakamori et al. 1992; Kawahata et al. 2000). The slightly extended area of enrichment in algal ^{15}N in the southern area could be explained by this current pattern, which entrains terrestrial nitrogen. At both D-Sh and F-Ka, however, the algal $\delta^{15}\text{N}$ contour lines were nearly parallel to the shoreline, and correspondence with the current direction was not as conspicuous as expected. This suggests that nitrogen uptake by macroalgae could occur mainly at low tide, when the circulation nearly stops because of isolation from the offshore water and the seepages of groundwater increase. The previous observation that the high

uptake rate was obtained at high N flux after N starvation (Fujita 1985) supports the idea that nutrient uptake occurs predominately at low tide.

At G-Hi, the $\delta^{15}\text{N}$ values decreased markedly within 100 m from the shore. This reef has a big channel and no well-developed reef crest, and the mean water depth is deeper than at D-Sh and F-Ka. Moreover, the estimated terrestrial nitrogen loading per unit length of the shoreline was half of that at F-Ka (Table 1). This potentially high dilution rate might be the reason why the area of influence of terrigenous nitrogen was narrow. The $\delta^{15}\text{N}$ value (1.6‰) 142 m from the shoreline was markedly lower than the minimum values (i.e., +2 to +3‰) of the other seven areas, suggesting that nitrogen fixation is active in the moat.

Because the groundwater discharge is irregular along the shoreline (Johannes 1980), the nitrogen loading per unit length of shoreline seems to be small, compared with the large nitrogen loading per unit area of the watershed (Table 1). At the reefs having significant nonpoint nitrogen sources, the nitrogen flowing through sediment seems to be easily taken up by benthic organisms near the shore (Lewis 1985; Miyajima et al. 2001), and at the same time, inflowing nitrogen is diluted with seawater primarily by diffusion rather than advection. Therefore, the regression lines decreased curvilinearly, and the area showing a terrestrial influence is restricted.

Finally, in the areas without direct inputs by river or streams (i.e., E-Ng and H-Ku), the gradients of $\delta^{15}\text{N}$ values were nearly flat and values were low, despite high $\delta^{15}\text{N}$ values of terrestrial nitrate (Fig. 4; Table 3). *Padina* spp. and *Dictyota* sp. at the E-Ng bay occurred further than 250 m from the shoreline, where the river flowing into the bay did not directly extend out. Similar gradients of algal $\delta^{15}\text{N}$ regression lines at E-Ng and at a part of D-Sh might be due to similar nitrogen loadings per unit length of shoreline (Table 1). The distribution of $\delta^{15}\text{N}$ values in macroalgae at the H-Ku area suggests that terrigenous nitrogen was not the main source. The watershed size and the estimation of total nitrogen loading at H-Ku were very small (Table 1), and we found few terrestrial nitrogen sources along the shoreline, except for small streams after heavy rainfall. The low nitrate concentrations in ambient seawater were consistent with the low $\delta^{15}\text{N}$ values (Web Appendix 1). At the open bays facing the outer ocean such as E-Ng and H-Ku, the freshwater was so rapidly diluted with seawater that the terrestrial nitrogen was hard to detect even near the shoreline, except around river mouths.

At all the areas, except A-Mi, the $\delta^{15}\text{N}$ values of macroalgae decreased to +3‰ or +2‰ at about 500 m from the shoreline, where corals become important. These values were similar to the assumed $\delta^{15}\text{N}$ values of nitrate in offshore water and from atmosphere-derived nitrogen. The effect of anthropogenic nitrogen on macroalgae was minor around the reef crest, although the effect was considerable for macroalgae distributed near the shoreline. Yamamuro et al. (1995) also suggested that the corals at Shiraho (D-Sh) used nitrogen originating from nonterrestrial sources. Near the reef crest at D-Sh, F-Ka, and G-Hi, corals such as *Porites* spp., *Montipora* spp., *Acropora* spp., and *Heliopora coerulea* flourish without being covered with macroalgae, whereas at

the inner reef of A-Mi, corals were smothered with terrestrial sediments and were covered with macroalgae.

Some features of algal $\delta^{15}\text{N}$ values were difficult to explain as the result of mixing ^{15}N -rich freshwater and ^{15}N -poor seawater. For example, the $\delta^{15}\text{N}$ values of nitrate in groundwater wells around D-Sh and F-Ka were 0.9–2.1‰ higher than those of macroalgae near the terrestrial nitrogen sources. The opposite was true at A-Mi and B-To; that is, macroalgal $\delta^{15}\text{N}$ values near terrestrial nitrogen were 0.2–1.4‰ higher than the values expected from the $\delta^{15}\text{N}$ values of nitrate in freshwater (Fig. 4; Table 3). We suggest that this is because of variation in terrestrial nitrogen $\delta^{15}\text{N}$ values that were not detected by our sampling (Wassenaar 1995). Also, $\delta^{15}\text{N}$ fractionation associated with algal uptake at high concentrations of external nitrogen (i.e., 3.3–230 μM at D-Sh and 1.5–55.3 μM at F-Ka [Umezawa et al. in press] and 122.8 μM at B-To; Web Appendix 1) could explain the lower algal $\delta^{15}\text{N}$ values than expected by the mixing pattern, if we assume that nitrogen uptake is similar to that of phytoplankton (Wada and Hattori 1978; Montoya and McCarthy 1995). The low $\delta^{15}\text{N}$ nitrogen resulting from nitrogen fixation could be another factor at the inner reefs of D-Sh and F-Ka (Kayanne et al. pers. comm.; Miyajima et al. 2001), where the rapid decrease in algal $\delta^{15}\text{N}$ with distance from shore resulted in a curvilinear regression line. If these processes are important, the terrestrial nitrogen effects inferred from algal $\delta^{15}\text{N}$ values would be underestimated.

Conversely, the enrichment of $\delta^{15}\text{N}$ in nitrate due to fractionation associated with denitrification (Liu and Kaplan 1989; Horrigan et al. 1990) could explain the higher algal $\delta^{15}\text{N}$ values than expected. Fourqurean et al. (1997) suggested that a gradient of increasing $\delta^{15}\text{N}$ in *Zostera marina* from shoreline at Tomales Bay, California, likely resulted from denitrification. The increasing $\delta^{15}\text{N}$ in DIN would also result from fractionation during nitrate uptake by primary producers (Cline and Kaplan 1975; Altabet et al. 1986). However, even if the $\delta^{15}\text{N}$ values in macroalgae were shifted upward by these effects, it would be minor.

Salinity and many chemical components (e.g., CH_4 , ^{222}Rn , $^{226}\text{Ra}/^{228}\text{Ra}$) have been used as indicators of the effects of terrestrial materials on coastal areas (Bunga et al. 1996; Hussain et al. 1999; Krest et al. 1999). However, these variables suggest only the input of freshwater and are not evidence of effects on benthic organisms. For example at B-To, Watanabe (1999) indicated the influence of terrestrial soils by measuring Al_2O_3 , Fe_2O_3 , TiO_2 , and some clay minerals, which are specifically contained in the land soils. The amount of Al_2O_3 in the surface sediments suggested that land soils spread farther than land-derived nutrients. We assumed that the distribution of some clay minerals indicates the maximum extent of terrestrial matter by floods, which occur several times a year, whereas the $\delta^{15}\text{N}$ in macroalgae indicates the extent of terrestrial nitrogen under normal conditions. Besides, in mass balance estimates of nitrogen sources to coral reefs (Furnas et al. 1995; Umezawa et al. in press), the estimated contribution ratio of terrestrial nitrogen simply represents the potential amount of available nitrogen for benthic primary producers. However, the contents of algal tissue represent actually assimilated nitrogen under the concentration and flux of nitrogen at their places of growth. Therefore,

$\delta^{15}\text{N}$ in macroalgae is a better indicator of anthropogenic nitrogen inputs, especially when we need to assess the cause of macroalgal blooms, which have been considered to be one of the reasons for accelerating coral reef degradation. The $\delta^{15}\text{N}$ of coral tissue is also a direct indicator of effects on coral health and metabolism (Heikoop et al. 2000b).

Sammarco et al. (1999) reported the relative importance of nutrient sources across the central Great Barrier Reefs on the 100-km scale, suggesting that the $\delta^{15}\text{N}$ signals derived from land would probably have been masked by mixing with open ocean nitrate on a narrower reef. Heikoop et al. (2000b) also reported that no clear $\delta^{15}\text{N}$ sewage signals were found about 1 km away from the city, Zanzibar, probably because of rapid dilution by seawater. Our data on the $\delta^{15}\text{N}$ in macroalgae on a fringing reef, however, showed clear gradients across the reefs, even at the very small (1 km) scale of coral reefs here.

Land-derived nitrogen loading to coral reefs is highly correlated with human activities in adjacent watersheds (Umezawa et al. in press). Our study suggests that the area influenced by anthropogenic nitrogen depends on the outflow pattern of terrestrial water and the hydrodynamic structure of coral reefs. The geographical and hydrological features both of the land and in the coastal waters are important determinants of the availability and influences of terrestrial nitrogen on benthic communities in these areas.

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