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$\delta^{15}\text{N}$ of seagrass leaves for monitoring anthropogenic nutrient increases in coral reef ecosystems

M. Yamamuro ^{a,*}, H. Kayanne ^b, H. Yamano ^c^a Institute for Marine Resources and Environment, AIST Tsukuba Central 7, 1-1-1 Higashi, Tsukuba 305-8567, Japan^b Department of Earth and Planetary Science, Graduate School of Science, University of Tokyo, Bunkyo-ku 113-0033, Japan^c Social and Environmental Systems Division, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan

Abstract

In a coral reef environment, a slight increase in dissolved inorganic nitrogen (DIN; $\geq 1.0 \mu\text{M}$) can alter the ecosystem via macroalgal blooms. We collected seagrass leaves from the tropical and subtropical Pacific Ocean in five countries and examined the interactions between nutrient concentrations (C, N, P), molar ratios of nutrients, and $\delta^{15}\text{N}$ to find a possible indicator of the DIN conditions. Within most sites, the concentrations of nutrients and their molar ratios showed large variations owing to species-specific values. On the other hand, almost identical $\delta^{15}\text{N}$ values were found in seagrass leaves of several species at each site. The correlations between $\delta^{15}\text{N}$ and nutrient concentrations and between $\delta^{15}\text{N}$ and molar ratios of nutrients suggested that nutrient availability did not affect the $\delta^{15}\text{N}$ value of seagrass leaves by altering the physiological condition of the plants. Increases in $\delta^{15}\text{N}$ of seagrass leaves mostly matched increases in DIN concentrations in the bottom water. We suggest that $\delta^{15}\text{N}$ in seagrass leaves can be a good tool to monitor time-integrated decrease/increase of DIN concentrations at a site, both in the water column and the interstitial water.

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1. Introduction

Coral reefs are threatened by various anthropogenic stresses, including eutrophication (Pennisi, 1997). Even a slight increase in nutrients in the water may alter the ecosystem of a reef; for example, a slight increase in dissolved inorganic nitrogen (DIN; $\geq 1.0 \mu\text{M}$) in reef water can induce the dominance of macroalgae that sometimes cause coral death (Lapointe, 1997). The early detection and prevention of nutrient enrichment are the most desirable management options for coral reefs; however, the concentrations of DIN (ammonium, nitrate, and nitrite) in reef water are often below the limit of detection. Therefore, a sensitive indicator of nutrients would be a useful monitoring tool. Moreover, nutrient concentrations in the water column change seasonally or episodically; consequently, the integrated or average effect of raised nutrient concentrations is difficult to

detect by monitoring the concentrations in the water column itself.

Natural differences in the $^{15}\text{N}/^{14}\text{N}$ ratios (usually expressed as $\delta^{15}\text{N}$) of organisms have been related to elemental cycling rates and to nitrogen sources. One advantage of analyzing stable isotopes is that they reflect a time-integrated measure of assimilated nitrogen rather than an instantaneous value. This allows $\delta^{15}\text{N}$ to be used as an indicator to detect increases in nitrogen loads while they are still too low to cause any detectable change in flora (McClelland et al., 1997). Because sewage effluent elevates the $\delta^{15}\text{N}$ in marine plants (including seagrass), the $\delta^{15}\text{N}$ of these plants can also be used to detect and map the effect of sewage nitrogen under extremely eutrophic conditions (Costanzo et al., 2001). Anthropogenic influences in coral reef ecosystems have also been examined with $\delta^{15}\text{N}$ of coral tissue (Heikoop et al., 2000) and benthic macroalgae (Umezawa et al., 2002). Such an examination of $\delta^{15}\text{N}$ of seagrass leaves alone has not yet been reported.

Tropical seagrass beds mostly develop landward of coral reefs, where anthropogenic eutrophication would have the first influence. Since $\delta^{15}\text{N}$ is not affected by

* Corresponding author. Tel./fax: +81-298-613766.

E-mail address: m-yamamuro@aist.go.jp (M. Yamamuro).

drying or preservation in formalin or ethanol (Kaehler and Pakhomov, 2001), historical seagrass samples, which are usually preserved in the dried condition or in a formalin solution, can be also used to compare the nutrient conditions in the past. Unlike macroalgae, seagrass appears all year round, and because there are only 24 species in the Indo-Pacific region (Short et al., 2001), far fewer than tropical macroalgae or corals, identification of the samples requires less effort.

In oligotrophic water, the typically lighter $\delta^{15}\text{N}$ of reef macroalgae is attributed to the importance of nitrogen made available through nitrogen fixing organisms (Yamamuro, 1999). In an oligotrophic oceanic island, the nitrogen fixed by epiphytic cyanobacteria was of the same order as that required for seagrass production in a tropical seagrass bed (Iizumi, 1994). Rates of nitrogen fixation are reported to be higher on bare reef substrates than in the macroalgae zone (Shashar et al., 1994), although Williams and Carpenter (1998) pointed out the possibility of underestimating nitrogen fixation in the algal turf zone on reefs owing to the reduced water flow during measurement. These findings suggest that in oligotrophic reef environments nitrogen fixation is the major source of nitrogen and seagrass leaf $\delta^{15}\text{N}$ may be an indicator of the DIN conditions on reefs, with lower DIN concentrations reflected by $\delta^{15}\text{N}$ values closer to 0‰ to -2‰ (Wada and Hattori, 1991; Shearer and Kohl, 1993) due to the increased contribution from nitrogen fixation.

On the other hand, groundwater nitrate influenced only by atmospheric deposition typically has $\delta^{15}\text{N}$ values of 2–8‰ (McClelland et al., 1997), while the $\delta^{15}\text{N}$ signature of treated sewage is approximately 10‰ (Costanzo et al., 2001). Groundwater nitrate generated from human and animal wastes shows heavier values (10–20‰), owing to the volatilization of ^{14}N -rich ammonia during the early stages of wastewater degradation (McClelland et al., 1997). These findings suggest that the input of land-derived DIN would elevate the $\delta^{15}\text{N}$ in seagrasses, unless the land were oversupplied with synthetic fertilizers which show rather low $\delta^{15}\text{N}$ values (-8 to 7‰; Macko and Ostrom, 1994).

Although the tissue nutrient contents as well as the C:N:P ratio in seagrass leaves reflect the nutrient conditions of seagrasses (Erftemeijer et al., 1994; Udy and Dennison, 1997), sometimes the nutrient contents in seagrasses do not change in response to nutrient conditions but rather the growth rates change (Udy and Dennison, 1997). A correlation between tissue nitrogen concentration and $\delta^{15}\text{N}$ may occur because primary producers can utilize ^{14}N in preference to ^{15}N when the DIN pool is not limited (Pennock et al., 1996).

In this study, we examined the nutrient concentrations, molar ratio of nutrients, $\delta^{15}\text{N}$, and their interactions in seagrass leaves to seek an indicator of the DIN concentration in a reef environment.

2. Materials and methods

2.1. Study sites

The main sampling was carried out at Kabira reef, Ishigaki Island, Japan in August 1996 (lat 24°28'N, long 124°08'E; Fig. 1(A)), Green Island, Australia in October 1996 (lat 16°45'S, long 145°59'E; Fig. 1(B)), and Dravuni Island, Fiji in December 1995 (lat 18°45'S, long 178°30'E; Fig. 1(C)). Additional samples were collected at three sites (100 m offshore at Hirano, 300 m offshore at Kuura, and 400 m offshore at Miyara; Tanaka, 1999), Ishigaki Island, Japan in March 1999, Dravuni Island, Fiji in October 1989 and 1991, and the coast of Thailand (lat 7°22'N, long 99°18'E) in December 1998. We also analyzed the nutrient concentrations in samples reported in Yamamuro et al. (1995) from Palau.

The water depth is approximately -2 m from mean sea level (MSL) at K-1 and K-2. In the Green Island reef, station G-1 is 50 m and G-2 is 600 m off the island's east end. The water depth at these stations is approximately -1.5 m from MSL. The area is categorized as a sanded flat (Baxter, 1990). At Dravuni Island, D-1 is 60 m off the shoreline and is more exposed to wave action than D-2 (Nishihira and Suzuki, 1997). D-2 is near a village and within a *Syringodium isoetifolium*-dominated seagrass bed 50 m off the shoreline. The water depth at D-1 and D-2 is approximately -3 m from MSL.

2.2. Analytical methods

Seagrass samples were cleaned with brushes under a binocular microscope to remove all visible attached materials, rinsed with filtered seawater, and dried at 60 °C to a constant weight. The dried seagrasses were powdered and homogenized with an agate mortar and pestle before we determined the $\delta^{15}\text{N}$ and the concentrations of organic C, N, and P.

The concentrations of organic carbon and nitrogen in seagrass leaves were determined using a Yanaco MT-5 CHN analyzer following the method of Yamamuro and Kayanne (1995). Samples were measured 2–4 times with $\text{SD} \leq 1.1\%$ for C and $\leq 0.23\%$ for N. The phosphorus concentration was determined colorimetrically following the method described in Ohtsuki (1982). It was measured 2–3 times with $\text{SD} \leq 0.42 \text{ mg g}^{-1}$.

To determine the ratios of stable nitrogen isotopes, samples were combusted at 1020 °C in an elemental analyzer (Fisons Instruments EA1108), and the combustion products (N_2) were introduced to an isotope-ratio mass spectrometer (Finnigan Delta Plus) with a He carrier.

The $^{15}\text{N}:^{14}\text{N}$ ratio ($\delta^{15}\text{N}$) was expressed relative to N_2 in air. It was calculated as:

$$\delta^{15}\text{N} = \{R(\text{sample})/R(\text{standard}) - 1\} \times 1000 (\text{‰}),$$

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

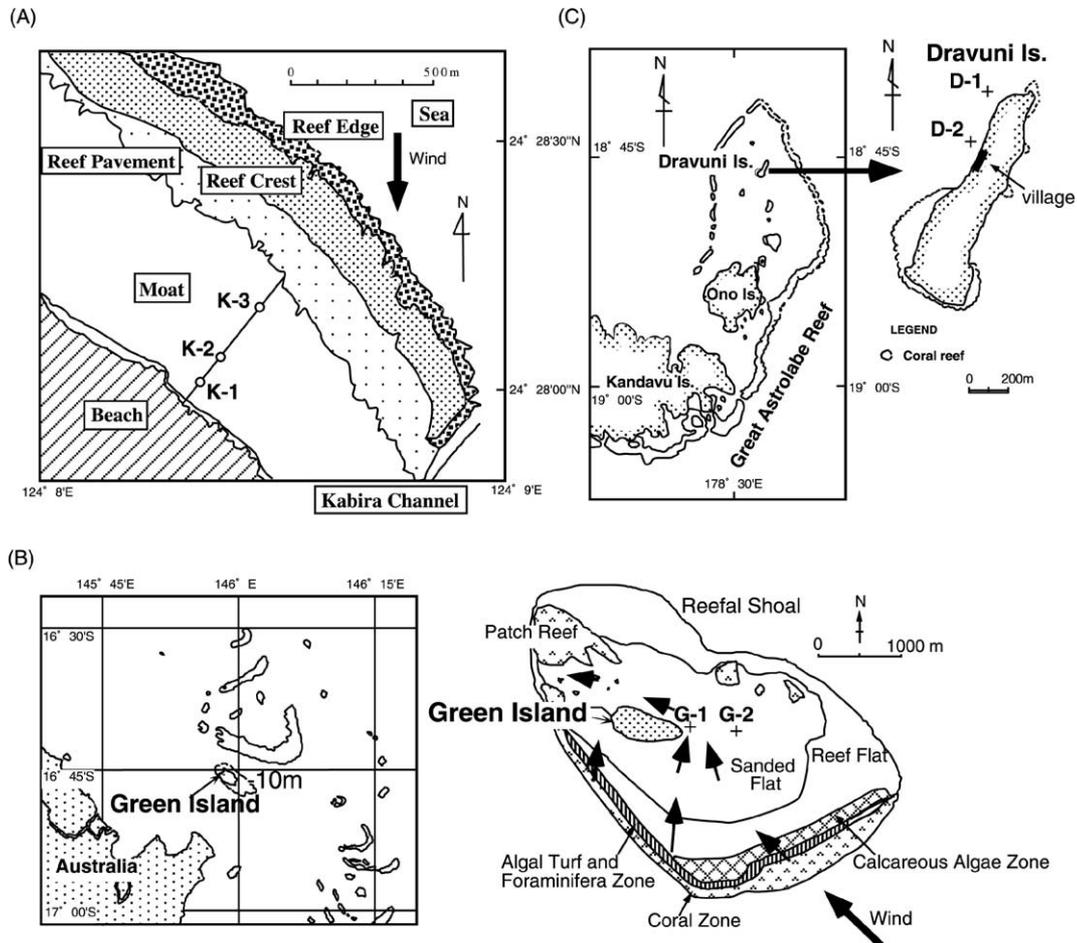


Fig. 1. Study sites. (A) Kabira reef, Japan. (B) Green Island, Australia. (C) Dravuni Island, Fiji. Bold arrows in (B) depict the dominant water circulation pattern (Yamano et al., 2000).

Machine drift during the analyses was checked with *L-a*-alanine ($\delta^{15}\text{N}$: 7.61‰) after every fifth sample. The accuracy of the absolute values was determined using interlaboratory-determined nitroarginine ($\delta^{15}\text{N}$: -4.64‰), glycoamine ($\delta^{15}\text{N}$: -11.00‰) and proline ($\delta^{15}\text{N}$: -8.52‰) following the method of Minagawa et al. (1984). Samples were measured 2–4 times with $\text{SD} < 0.6\text{‰}$.

3. Results and discussion

Tissue nitrogen concentrations in seagrass leaves of various species showed a large variation within a single location i.e. G-1 where nitrogen concentrations varied between 2.1% and 3.1% (Table 1). Most of the values measured in this study were within this range. The C:N ratio of seagrass leaves also showed a wide variation at G-1 (14–21) which covers most of the observed ratios in this study.

Phosphorus concentrations in the seagrass leaves were not identical in any location. Most of the observed

values fell within the range found at G-2 (1.5–4.6 mg g^{-1}). Consequently, N:P of the seagrass leaves observed in this study were within the range observed at G-2 (13–46).

On the other hand, $\delta^{15}\text{N}$ values were almost identical in seagrass leaves from various species at each site (e.g., G-1 in Table 1). Grice et al. (1996) found the same trend in Moreton Bay in Australia—the $\delta^{15}\text{N}$ values of seagrass leaves appeared to be affected more by the collection site than by the species of seagrass or by light intensity. They also found higher $\delta^{15}\text{N}$ values at a more eutrophic site (western Moreton Bay = 8.6–8.8‰) than at a site further from anthropogenic influences (eastern Moreton Bay = 2.6–4.5‰). These findings suggest that the $\delta^{15}\text{N}$ of seagrass leaves reflects the $\delta^{15}\text{N}$ of the DIN source at the site.

To examine if nutrient conditions (nitrogen and phosphorus) affected the $\delta^{15}\text{N}$ of seagrass, we calculated the correlations between $\delta^{15}\text{N}$ and nutrient concentrations and $\delta^{15}\text{N}$ and their molar ratios. Significant ($p < 0.05$) correlations were found only with nitrogen concentration ($r = 0.66$, $p = 0.0015$; Fig. 2) and C:N

Table 1

Mean, standard deviation (SD), and number of determinations (*n*) of organic carbon, nitrogen and phosphorus concentrations, their molar ratios, and the stable nitrogen isotope ratio of seagrass leaves sampled at several coral reefs

| Sample, species and location | Organic carbon (%) | | | Nitrogen (%) | | | Phosphorus (mg g ⁻¹) | | | C:N (Molar) | C:P | N:P | δ ¹⁵ N (‰) | | |
|--|--------------------|------|----------|--------------|------|----------|----------------------------------|------|----------|----------------|-----|------|-----------------------|------|----------|
| | Mean | SD | <i>n</i> | Mean | SD | <i>n</i> | Mean | SD | <i>n</i> | | | | Mean | SD | <i>n</i> |
| <i>Kabira, Japan</i> | | | | | | | | | | | | | | | |
| K-1 | | | | | | | | | | | | | | | |
| <i>Thalassia hemprichii</i> | 34.91 | 0.68 | 2 | 2.53 | 0.07 | 2 | 1.83 | 0.02 | 3 | 16.1 | 493 | 30.6 | 4.97 | 0.16 | 2 |
| K-2 | | | | | | | | | | | | | | | |
| <i>Thalassia hemprichii</i> | 32.20 | 0.47 | 2 | 2.94 | 0.03 | 2 | 2.39 | 0.23 | 3 | 12.8 | 348 | 27.2 | 4.30 | 0.14 | 2 |
| K-3 | | | | | | | | | | | | | | | |
| <i>Thalassia hemprichii</i> | 31.21 | 0.20 | 2 | 2.91 | 0.02 | 2 | 2.09 | 0.06 | 3 | 12.5 | 386 | 30.8 | 2.50 | 0.06 | 2 |
| <i>Green Island, Australia</i> | | | | | | | | | | | | | | | |
| G-1 | | | | | | | | | | | | | | | |
| <i>Halodule uninervis</i> | 36.86 | 0.35 | 2 | 2.09 | 0.23 | 3 | 2.96 | 0.13 | 3 | 20.6 | 322 | 15.6 | 2.68 | 0.01 | 2 |
| <i>Thalassia hemprichii</i> | 36.90 | 0.08 | 2 | 3.10 | 0.12 | 2 | 2.01 | 0.05 | 2 | 13.9 | 474 | 34.1 | 2.47 | 0.05 | 2 |
| <i>Halophila ovalis</i> | 33.30 | 0.18 | 2 | 2.50 | 0.03 | 2 | 4.30 | 0.12 | 2 | 15.5 | 200 | 12.9 | 2.00 | 0.23 | 2 |
| <i>Cymodocea rotundata</i> | 39.12 | 0.27 | 2 | 2.63 | 0.05 | 4 | 2.11 | 0.07 | 2 | 17.4 | 478 | 27.6 | 2.24 | 0.08 | 2 |
| G-2 | | | | | | | | | | | | | | | |
| <i>Thalassia hemprichii</i> | 37.60 | 0.21 | 2 | 3.23 | 0.05 | 2 | 1.57 | 0.01 | 3 | 13.6 | 619 | 45.5 | 1.62 | 0.08 | 2 |
| <i>Halophila ovalis</i> | 32.79 | 0.25 | 2 | 2.68 | 0.10 | 2 | 4.57 | 0.42 | 3 | 14.3 | 185 | 13.0 | 1.78 | 0.12 | 2 |
| <i>Dravuni Island, Fiji</i> | | | | | | | | | | | | | | | |
| D-1 | | | | | | | | | | | | | | | |
| <i>Halophila ovalis</i> | 30.81 | 0.17 | 2 | 2.51 | 0.10 | 2 | 4.40 | 0.05 | 2 | 14.3 | 181 | 12.7 | -1.41 | 0.52 | 3 |
| D-2 | | | | | | | | | | | | | | | |
| <i>Syringodium isoetifolium</i> | 24.51 | 1.10 | 3 | 0.81 | 0.06 | 3 | 0.72 | 0.01 | 3 | 35.1 | 880 | 25.1 | -0.47 | 0.29 | 3 |
| <i>Syringodium isoetifolium</i> ^a | 24.42 | 0.83 | 2 | 0.90 | 0.08 | 3 | 1.31 | 0.00 | 2 | 31.7 | 480 | 15.2 | -0.48 | 0.36 | 3 |
| <i>Syringodium isoetifolium</i> ^b | 25.42 | 0.38 | 2 | 1.04 | 0.03 | 3 | 1.08 | 0.00 | 2 | 28.5 | 608 | 21.3 | -0.72 | 0.20 | 3 |
| <i>Halodule uninervis</i> | 33.81 | 1.07 | 3 | 1.55 | 0.13 | 5 | 1.98 | 0.13 | 2 | 25.4 | 441 | 17.3 | -0.06 | 0.26 | 2 |
| <i>Hirano, Japan</i> | | | | | | | | | | | | | | | |
| <i>Thalassia hemprichii</i> | 36.16 | 0.14 | 2 | 2.78 | 0.10 | 2 | 1.65 | 0.01 | 3 | 15.2 | 566 | 37.3 | 5.17 | 0.10 | 2 |
| <i>Kuura, Japan</i> | | | | | | | | | | | | | | | |
| <i>Thalassia hemprichii</i> | 34.66 | 0.54 | 2 | 2.59 | 0.02 | 2 | 1.51 | 0.01 | 3 | 15.6 | 593 | 38.0 | 2.31 | 0.18 | 2 |
| <i>Miyara, Japan</i> | | | | | | | | | | | | | | | |
| <i>Thalassia hemprichii</i> | 35.82 | 0.68 | 2 | 2.82 | 0.07 | 2 | 1.54 | 0.02 | 3 | 14.8 | 601 | 40.5 | 3.88 | 0.17 | 2 |
| <i>Palau</i> ^c | | | | | | | | | | | | | | | |
| <i>Thalassia hemprichii</i> | 32.96 | 0.09 | 2 | 2.72 | 0.01 | 2 | 2.50 | 0.07 | 3 | 14.1 | 341 | 24.1 | 2.23 | — | 1 |
| <i>Thailand</i> | | | | | | | | | | | | | | | |
| <i>Thalassia hemprichii</i> | 37.54 | 0.00 | 2 | 2.89 | 0.02 | 2 | 2.84 | 0.02 | 2 | 15.2 | 341 | 22.5 | 2.88 | 0.18 | 2 |

^a October 1989.

^b October 1991.

^c δ¹⁵N for Palau is from Yamamuro et al. (1995).

ratio ($r = -0.62$, $p = 0.0036$). Since the latter likely reflects the strong negative correlation between nitrogen concentration and C:N ratio ($r = -0.97$, $r < 0.0001$), only the nitrogen concentration of seagrasses is likely to affect the δ¹⁵N.

The effect of nitrogen concentration on δ¹⁵N of seagrass leaves has two possible causes. One may be a physiological change whereby an increased availability of nitrogen changes the isotope fractionation. The other may be a physical change—the increased nitrogen concentration means an increased uptake of land-derived DIN which, at the same time, increases the δ¹⁵N owing to the high δ¹⁵N value of land-derived DIN.

Physiological interaction was reported by Udy et al. (1999) when they increased the amount of source nitrogen by inserting fertilizer into the sediment. Their results showed that δ¹⁵N of seagrass leaves decreased with nitrogen enrichment, and they assumed that the increased nitrogen availability increased the discrimination against the ¹⁵N isotope. In our case, however, increased concentrations of nitrogen in seagrass leaves—which most probably indicates an increased availability of nitrogen—increased the δ¹⁵N of the leaves, suggesting that the significant correlation between δ¹⁵N and nitrogen concentration in the leaf is due to an increased input of nitrogen with high δ¹⁵N value.

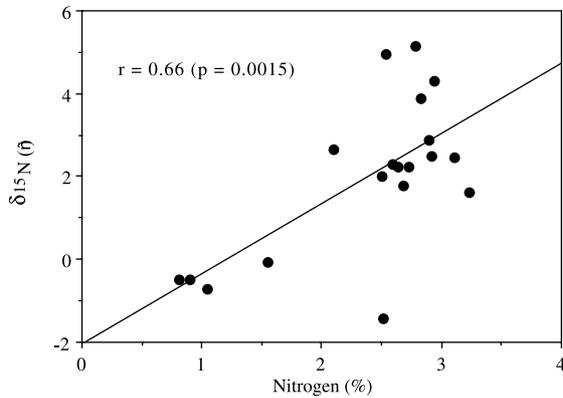


Fig. 2. Correlation between nitrogen concentration and $\delta^{15}\text{N}$ of seagrass leaves.

In Fig. 3, the sampling sites are ordered from those with low seagrass $\delta^{15}\text{N}$ at the top of the chart down to those with high $\delta^{15}\text{N}$ at the bottom. The concentration of DIN in the bottom water at G-2 is less than at G-1; we assumed this, because G-2 is more strongly affected by oligotrophic water from the outer reef (Fig. 1(B)). The concentration of DIN in the bottom water at D-1 is less than at D-2; we assumed this is because D-2 is closer to the village (Fig. 1(C)). If the increased concentration of DIN is mostly due to an increase in land-derived DIN, it would be reflected as an elevated value of $\delta^{15}\text{N}$, because groundwater influenced only by atmospheric deposition typically has $\delta^{15}\text{N}$ values of 2–8‰ (McClelland et al., 1997) and the $\delta^{15}\text{N}$ signature of treated sewage is approximately 10‰ (Costanzo et al., 2001), both of which are higher than the $\delta^{15}\text{N}$ of DIN originating from nitrogen fixing plants which show a $\delta^{15}\text{N}$ value closer to 0‰ to –2‰ (Wada and Hattori,

1991; Shearer and Kohl, 1993). Since nitrogen fixation is suppressed with increased concentrations of DIN, any increase in DIN would change the DIN source for seagrass from the low $\delta^{15}\text{N}$ originating from nitrogen fixing plants to the high $\delta^{15}\text{N}$ of anthropogenic origin.

However, if the site is under the influence of DIN of fertilizer origin—whose $\delta^{15}\text{N}$ values are rather low (–8‰ to 7‰; Macko and Ostrom, 1994)—an increase in DIN will lead to a decrease in seagrass $\delta^{15}\text{N}$. In fact, when Udy et al. (1999) added fertilizer ($\delta^{15}\text{N}$: 0‰) to the sediment of seagrass beds in Green Island, Australia, the $\delta^{15}\text{N}$ of *Halodule uninervis* decreased from 1.7‰ to –2.0‰, and the $\delta^{15}\text{N}$ of *S. isoetifolium* decreased from 1.3‰ to –3.6‰ when only nitrogen was added.

In our study, an increase in $\delta^{15}\text{N}$ of seagrass leaves mostly corresponded with an increase in the DIN concentration in bottom water. Effects of enriched DIN of anthropogenic origin have also been reported in estuaries (McClelland et al., 1997; McClelland and Valiela, 1998) and coastal environments (Hansson et al., 1997). We assumed that the effect of low- $\delta^{15}\text{N}$ fertilizer is negligible in coral reef regions, presumably because agriculture that requires such vast use of fertilizer may not be common. Sewage (treated and/or untreated) may dominate the DIN of anthropogenic origin in reef environments.

Seagrasses are considered to take up nutrients through their leaves (e.g., Hemminga and Stapel, 1999), and some tropical seagrasses assimilate ammonium and phosphate through both roots and leaves (Stapel et al., 1996). Therefore, the $\delta^{15}\text{N}$ of seagrass leaves may reflect the $\delta^{15}\text{N}$ of source DIN in both the bottom water and interstitial water. On the other hand, $\delta^{15}\text{N}$ of benthic macroalgae may reflect the DIN of only the water

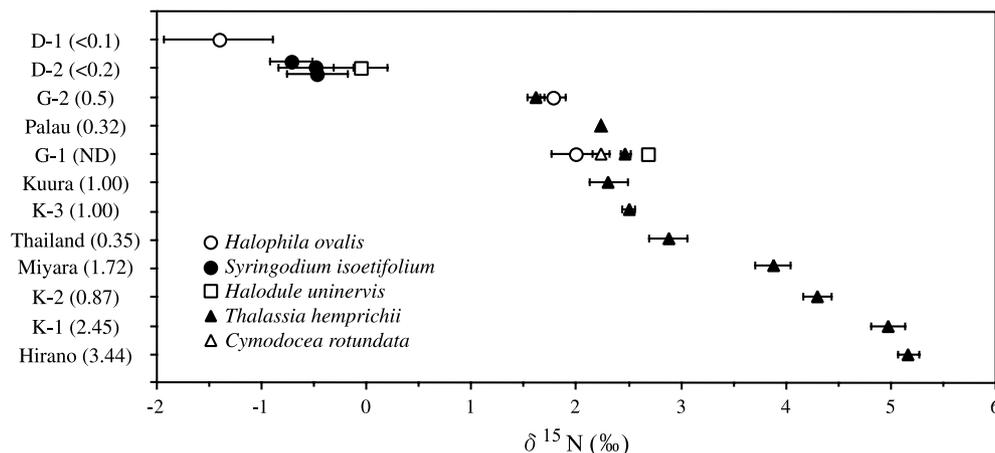


Fig. 3. Means (symbols) and standard error (bars) of $\delta^{15}\text{N}$ for tropical seagrass leaves. Concentrations (μM) of DIN in bottom water are in parentheses. They were measured simultaneously with the seagrass sampling at D-1, D-2, G-2, and Thailand. ND means no data was available. References for DIN concentrations are from Yamamuro (unpublished) for D-1 and D-2, Miyajima et al. (1998) for G-2, Hata (personal communication) for Palau, Umezawa et al. (1999) for Thailand. For other sites, we calculated the mean of the data reported in Tanaka (1999) without counting the exceptionally high concentration.

column. This may be the reason why Lapointe (1997) found that the elevation of $\delta^{15}\text{N}$ in macroalgae correlated significantly with rainfall, and that the variation of $\delta^{15}\text{N}$ in macroalgae was nearly 10‰ over four months. In the case of seagrass leaves, Yamamuro et al. (2001) observed monthly changes in the $\delta^{15}\text{N}$ of *Halophila ovalis* leaves growing in the tidal flat over six months, including both dry and monsoon seasons, and found only 1.2‰ change, while the change in $\delta^{13}\text{C}$ was 8‰. The seagrass $\delta^{13}\text{C}$ may have changed dramatically due to changes in the physiological condition of leaves, but the $\delta^{15}\text{N}$ did not change so dramatically because the leaf $\delta^{15}\text{N}$ reflects the $\delta^{15}\text{N}$ of the source nitrogen which is mostly from the interstitial environment in this case, and the interstitial DIN does not change as quickly as the DIN in the water column.

The threshold concentration of DIN in bottom water reported for macroalgal overgrowth of seagrass and coral reef communities is approximately 1.0 μM (Lapointe et al., 1993). Our results cannot show the level of $\delta^{15}\text{N}$ that may indicate a bottom water DIN concentration of 1.0 μM , because seagrass leaves may reflect the $\delta^{15}\text{N}$ of source DIN in both the bottom water and the interstitial water. Nevertheless, it can be a good indicator to detect time-integrated increases in DIN concentrations in the water and sediment in a coral reef environment providing we monitor the change of $\delta^{15}\text{N}$ in seagrass leaves within the same location. Since the $\delta^{15}\text{N}$ signature of seagrass leaves is not species-specific, it can be a more applicable indicator than macroalgae or coral tissues. Further work measuring seagrass $\delta^{15}\text{N}$ together with bottom and interstitial nutrient concentrations may present the possibility that seagrass $\delta^{15}\text{N}$ values can be used to determine the nutrient concentration itself.

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