JARQ 34, 69–73 (2000)

Nitrogen Fixation Activity by Periphytic Blue-Green Algae in a Seagrass Bed on the Great Barrier Reef

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Abstract

Acetylene reduction activity, an index of nitrogen fixation, by epiphytic and epibenthic algae and coral debris was measured in seagrass beds around Green Island, Australia. Epiphytic blue-green algae showed a high activity, ranging from 56.1 ± 31.9 to 729 ± 105 mmol (g chl a) $^{-1}$ d^{-1} on a chlorophyll basis, or 3.9 μmol N_2 m^{-2} d^{-1} to 16 mmol N_2 m^{-2} d^{-1} on an areal basis. The activities on an areal basis were comparable to those reported in a Fijian seagrass bed, whereas activities on a chlorophyll basis were higher on Green Island than on Fiji. A relatively high activity was observed on the leeward side of the island, partly due to the high seagrass biomass and partly due to the high epiphyte biomass. This trend of high activity on the leeward side of the island suggested that the biomass and/or activity of nitrogen fixing blue-green algae could be affected by unknown mechanism (s) related to the island environment. Algae on (or in) coral debris, which were abundant around live corals, were able to reduce acetylene.

Discipline: Fisheries / Environment
Additional key words: acetylene reduction, stable isotope ratio

Introduction

Seagrass beds in coastal waters in the tropics are among the most productive ecosystems[1], although they are often washed with nutrient poor waters[20]. From seagrass beds, there are inevitable losses of nutrients by export of detached leaves, exudates, leaching, grazing[14], etc. To compensate for those losses, nutrients should be supplied, and the supply sometimes limits the production of dominant producers in seagrass beds, e.i. seagrasses themselves for nitrogen[6] or for phosphorus[7,17].

In a Fijian seagrass bed, atomic ratio of nitrogen to phosphorus of nutrients in a water column ranged from 2.5 to 4.7, values which are lower than those of seagrasses (7.9~49) and epiphytes (87~102)[20], suggesting that nitrogen might be the limiting nutrient for the primary producers in tropical seagrass beds. As tropical seagrasses take up nutrients mainly from sediments where nutrient concentrations are relatively high, nitrogen supply to the sediments has been a main target of interest, and belowground nitrogen fixation has been investigated[4,13,15]. However, in tropical coral reefs and seagrass beds, nitrogen-fixing blue-green algae commonly occur on reef sediments or seagrass shoots[3,8,11,21,22]. In a Papuan seagrass bed, nitrogen fixation (acetylene reduction) activity was detected on the surface of almost every object at the bottom including seagrasses, shells, detrital leaves, etc.[9]. In a Fijian *Syringodium*-dominated bed, a nitrogen-fixing blue-green alga, *Hydrocoleum cantharidosmum* (Mont) Gom (=*Microcoleus lyngbyaceus* (Kutzing) Crouan, *sensu* Drouet), formed tuft-shape colonies on leaves, showing that the magnitude of nitrogen fixation was in the same order as that of nitrogen requirement for seagrass production[10]. Nitrogen fixed in the aboveground parts of seagrass beds are likely to be cycled through detrital microbial chains in water columns or sediments and utilized by primary producers.

In a seagrass bed on a lagoonal reef around Green Island on the Great Barrier Reef, Australia, spatial variability of nitrogen fixation (acetylene reduction) by blue-green algae was investigated to examine the significance of nitrogen fixation on a coral cay.

Materials and methods

1) Study site

Green Island (16°46'S., 145°58'E.) is a coral cay (ca. 4 km in long axis and ca. 2 km in short axis), ca. 27 km offshore from Cairns, Australia (Fig. 1). A lagoonal platform or planar reef has developed around the island[2],

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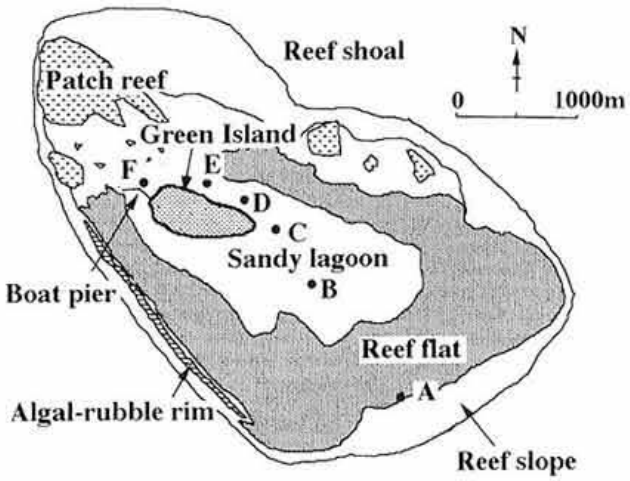

Fig. 1. Study site

with a wider area on the windward sides (eastern and southern) of the island than on leeward sides. Several species of seagrasses (*Thalassia hemprichii, Cymodocea serrulata, Cymodocea rotundata, Halodule uninervis, Syringodium isoetifolium* and *Halophila ovalis*) formed meadows in the lagoon (Lee Long, W., personal communication). Dense patches were observed on the leeward side of the island, while sparse seagrass patches were scattered all over in the sandy lagoon. Six stations (A, B, C, D, E, F) were set up in the lagoon along the water current over the reef (from SE to NW), namely Stn. A on the windward edge of the lagoon, ca. 1.7 km SSE of the island, and Stn. F on the leeward side, ca. 100 m NW of the island. Water depth at all the stations was shallow at low tide, less than 30 cm, except for that at Stn. F, which was ca. 3 m.

2) Acetylene reduction assay

Acetylene reduction activity was measured to estimate the nitrogen fixation activity[18]. Samples consisted of intact leaves with epiphytes, epiphytes that were scraped from leaves with a razor blade, epibenthic algal mats that were carefully peeled from the sediment and cut into rectangles, and coral debris. Samples were put in plastic containers (80 or 230 mL in volume), together with GF/F glassfiber-filtered seawater so as to leave 40 or 110 mL of headspace. Acetylene was injected into the headspace through a rubber septum on the lid of the container. Final concentration of acetylene was 10 to 15% (v/v). Containers were incubated in an outdoor tank for one day. Ethylene concentration in the headspace was analyzed with an ethylene analyzer (GC-2, Kiya Seisakusho Co.). *In situ* temperature and irradiance were monitored at 10 min intervals with temperature and irradiance recorders (MDS-T, MDS-L , Alec Electronic Co.). Temperature in the tank, which was 22~27°C, was ca. 1°C higher than that in the field. Irradiance in the tank was 60% of *in situ* irradiance at Stn. E.

3) Isotope ratio analysis of blue-green algae

The entangled visible materials other than blue-green algae were removed with a pair of pincettes under a binocular microscope. After being freeze-dried, the organisms were powdered and homogenized by using an agate mortar and pestle. To determine the stable isotope ratios, organisms were combusted at 1,050°C in a CN analyzer (Fisons Instruments EA1108), and the combustion products (N_2 gas) were introduced into an isotope-ratio mass spectrometer (Finigan Mat 252) in a continuous flow of He carrier. Isotope ratios were expressed as the deviation from a standard represented by the following equation:

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

where $R = {}^{15}N/{}^{14}N$, standard = N_2 in air.

4) Biomass

At each station, 3 quadrates (50×50 or 10×10 cm) were set up and seagrasses above the sediment were harvested. Samples were dried at ca. 60°C for dry weight measurement. Chlorophyll a content of epiphytes was analyzed fluorometrically after extraction with N,N-dimethylformamide[19].

Results and discussion

Seagrasses were widely observed on the reef (Fig. 2). At Stn. A, *T. hemprichii* formed sparse patches in sand pockets on reef flats. As the quadrates were placed on these patches, the biomass shown in the figure corresponded to that in the patches. Total aboveground biomass values were 3.5 ± 0.8, 63.4 ± 72.9, 98.9 ± 35.3, 42.6 ± 5.6, 96.7 ± 24.6, and 55.2 ± 16.0 g m^{-2} at Stns. A, B, C, D, E, and F, respectively. As shown by standard errors, the distribution of each seagrass was patchy, but seagrass beds, irrespective of their species composition, developed on the sandy lagoon around the island. This finding indicates that substrata to which surface epiphytes could become attached were commonly distributed on the reef, except on the windward edge of the reef.

Intact seagrass leaves showed a high acetylene reduction activity (Table 1). As young leaves, which accumulated few epiphytes, did not show any activity (data not shown), epiphytes were assumed to be responsible for the activity[9]. It was observed that a blue-green alga, *H. catharidosmum*, was the dominant alga, forming tufts on leaves of seagrasses, as observed in a Fijian seagrass bed[10].

One-way analysis of variance (ANOVA) of the

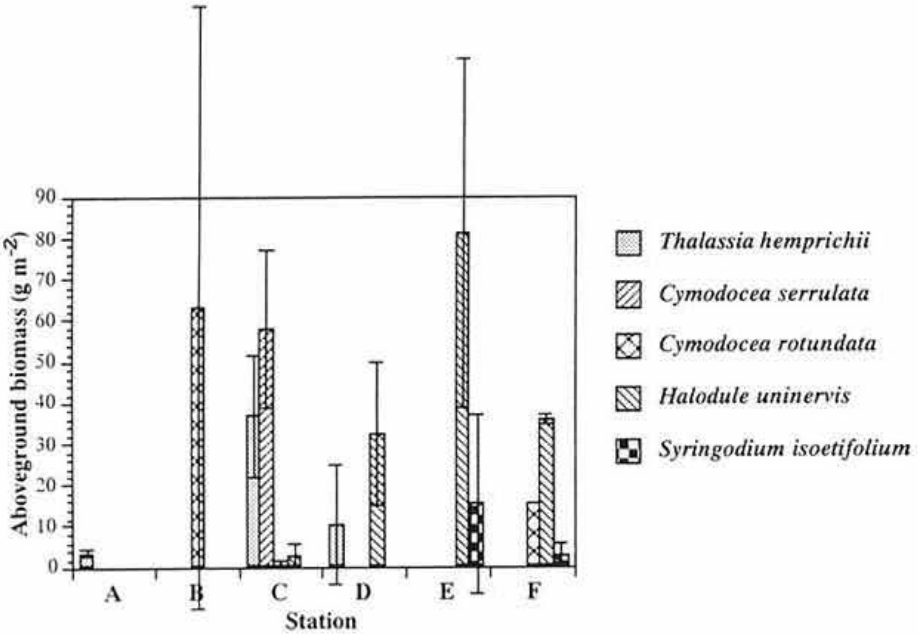

Fig. 2. Aboveground biomass of seagrasses around Green Island
Mean and standard errors are shown.

activity on a chlorophyll basis indicated that the activity of the epiphytes at Stn. C was significantly ($p<0.01$) higher compared with that of the epiphytes at other stations (Stns. B, E and F). It was observed that the concentrations of particulate organic carbon and nitrogen in seawater tended to increase when the seawater flowed around the island from Stns. A to F[12]. Concentrations of dissolved inorganic nitrogen and phosphorus in pore water of the sediments were higher in the seagrass beds than in unvegetated areas[12], suggesting that the supply of nutrients for epiphytic algae could be more abundant at Stns. C to F than at Stns. A and B (Fig. 2). Nitrogenous and phosphorous nutrients recycled from organic matter which were supplied from seagrasses or transported from other areas and trapped in the seagrass beds may be an important source of nutrients for epiphytic algal communities. However, the low isotopic ratios of algal nitrogen suggested that nitrogen fixation is the main source of

Table 1. Acetylene reduction activity by epiphytic algae and coral debris in seagrass beds around Green Island

	Station	Acetylene reduction rate (mean ± s.e.)	Unit
Intact leaves			$\mu mol\ m^{-2}d^{-1}$
	A	17.2 ± 9.54	
	B	73.7 ± 41.5	
Epiphytes			$mmol\ (g\ chl\ a)^{-1}\ d^{-1}$
	B	56.1 ± 31.9	
	C	729 ± 105	
	D	nd[a)]	
	E	143 ± 1.7	
	F	234 ± 42.4	
Epibenthic algal mat			
	E	325 ± 82.5	
	Southern reef	11.6[b)]	
Coral debris			$\mu mol\ g^{-1}d^{-1}$
	E	0.28 ± 0.08	

a) : Not determined. b) : Single determination.

Table 2. Stable isotope ratio of organic nitrogen in algae on Green Island

	$\delta\ ^{15}N$ (‰), mean ± s.e.
Epiphytes on *Thalassia hemprichii*	−0.73 ± 0.03
Filamentous algae on a dead macrophyte	−1.11 ± 0.02
Epibenthic algae	−0.79 ± 0.1

$\delta\ ^{15}N(‰) = \{R(sample)/R(standard) - 1\} \times 1000$, where $R = {}^{15}N/{}^{14}N$, standard = N_2 in air. All the samples were collected at Stns. E and F.

nitrogen for these algae (Table 2).

The same epiphytic algae with a tuft shape (*H. catharidosmum* was the dominant alga) were found to fix nitrogen in a Fijian seagrass bed[10]. Acetylene-reducing activity by the epiphytes was 25.2 ± 0.75 mmol reduced (g chl a)$^{-1}$ d^{-1}. Epiphytes in seagrass beds on Green Island showed a higher reduction activity than that in Fijian seagrass beds. The difference might be due to the grazing effect. A cage experiment on a coral reef on the Great Barrier Reef indicated that fish grazing reduces algal biomass and leads to the shift of the epiphytic community from red algae to rapidly colonizing and growing blue-green algae in a year[23]. Though data on fish grazing or fish biomass at our study site were not available, fish were apparently abundant on Green Island whose environment is protected for a marine park. Instead, local people are fishing in the Fijian seagrass beds. Even if the dominant alga is the same, fish grazing is likely to improve microenvironments of light and/or nutrients on the leaf surface and stimulate the turnover of algae, hence the enhancement of the nitrogen fixation ability.

Acetylene reduction activity on an areal basis was higher on the leeward side than on the windward side (Fig. 3), partly due to the larger seagrass biomass on the leeward side, or to the larger abundance of nitrogen-fixing algae on seagrass leaves. *Thalassia hemprichii* on the eastern (windward) edge of the reef accumulated fewer epiphytes. This distribution of the nitrogen fixation activity around the island suggests that the biomass of epiphytic and epibenthic blue-green algae is controlled by factor(s) other than nitrogen supply through nitrogen fixation. Supply of nutrients from the island or mechanisms other than nutrient supply could enhance the production of epiphytes on seagrass leaves, resulting in the increase of nitrogen fixation. After seagrasses started to thrive since the construction of a resort on the northwestern side of the island (Lee Long, W., personal communication), the biomass and nitrogen fixation activity of epiphytes may have increased. Further studies should be carried out to analyze the effect of the island environment on nitrogen fixation around the island.

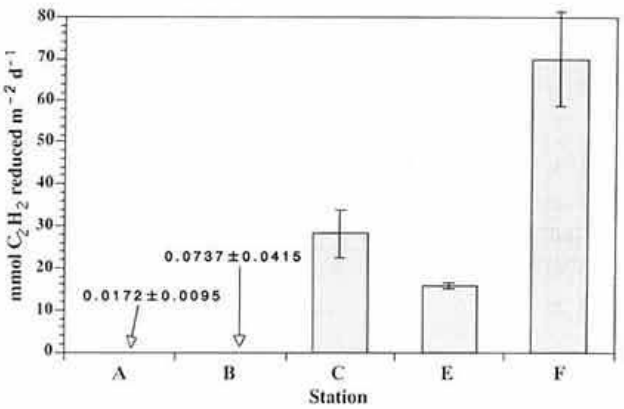

Fig. 3. Acetylene reduction activity by epiphytic algae in seagrass beds around Green Island
Acetylene reduction by epibenthic algae is not included.

Nitrogen fixation activity was estimated to be 3.9 μmol N_2 m^{-2} d^{-1} at Stn. A and 16 mmol N_2 m^{-2} d^{-1} at Stn. F, assuming that the C_2H_2/N_2 conversion factor is 4.4[16]. The average activity at all the stations (5.2 mmol N_2 m^{-2} d^{-1} or 145 mg N m^{-2} d^{-1}) was comparable to the rate estimated in a Fijian seagrass bed (113 mg N m^{-2} d^{-1})[10]. However, these activities were larger by 2 to 3 orders of magnitude than those reported in seagrass beds at Weipa and Groote Eylandt in northern Australia[14], for unknown reasons.

Coral debris displayed an acetylene reduction activity (Table 1). It remains to be determined whether the activity was related to algae on the surface of the samples or algae penetrating into the skeleton of corals[5]. Dead corals play an important role in supplying a support for nitrogen-fixing organisms and in enhancing nitrogen import to the ecosystem.

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(Received for publication, March 18, 1999)