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Nitrogen Fixation (Acetylene Reduction) by Rhizosphere Sediments of the Eelgrass *Zostera marina*

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ABSTRACT: Nitrogen fixation (acetylene reduction) was consistently and immediately detectable in rhizosphere sediments of the eelgrass *Zostera marina L.* collected from several stations and at various times of the year. Nitrogenase activity was detected down to 12 cm with the major fraction occurring in the 0 to 6 cm segment. Nitrate and NH_4^+ (100 to 200 μM) inhibited nitrogenase activity, while glucose (1 mM) accelerated rates of C_2H_2 reduction. Much of the nitrogenase activity appears to be associated with sulfate-respiring bacteria. During the summer, rates of C_2H_2 reduction to 10 cm averaged about 1.5 to 2.5 nmol $C_2H_4 \times cm^{-2}h^{-1}$ (0.1 to 0.2 nmol × g dry $sed^{-1}h^{-1}$). This could account for from 3 to 28 % of the net nitrogen demand of the plant. While supplying a substantial fraction of the nitrogen required by eelgrass, rhizosphere N_2 fixation in *Z. marina* communities may represent a lesser input when compared to the tropical seagrass *Thalassia testudinum*. Information on the magnitude of other nitrogen transformations is needed to evaluate fully the importance of N_2 fixation in these systems.

INTRODUCTION

Since the conflicting and limited observations of Patriquin and Knowles (1972) and McRoy et al. (1973), there has been no further evidence to confirm or dispel the notion that microbial N_2 fixation is, in general, an important source of combined nitrogen in *Zostera marina* communities. Patriquin and Knowles (1972), working with samples collected from New Brunswick, Canada, had reported that substantial nitrogenase activity occurred in the root zone of *Zostera marina*. However, McRoy et al. (1973) soon questioned the quantitative validity of Patriquin and Knowles (1972) estimates on the basis of both the extended delay (2 d) and duration (2 to 3 d with glucose enrichment) of assay. Furthermore, McRoy et al. could find no measurable activity associated with the leaves, roots or sediments of *Z. marina* collected from sites in North Carolina and Alaska.

Similar observations were made in each of these investigations for the tropical counterpart of *Zostera marina, Thalassia testudinum*. In the case of *T testudinum*, a series of recent reports by Capone and Taylor (1977; 1980a, b; Capone et al., 1979) provide substantial new evidence for the common occurrence of nitrogenase activity at measurable levels in the rhizosphere of this seagrass. They also found that nitrogenase activity in the phyllosphere was considerably more variable both spatially and temporally (Capone and Taylor, 1977). The present communication examines the magnitude and significance of nitrogenase activity in *Z. marina* communities in Great South Bay on the south shore of Long Island, New York.

MATERIALS AND METHODS

Three stations were established along the east-west axis of the Great South Bay (Fig. 1). Station 1 is closest to Fire Island Inlet, located about 100 m north of Sand Island in the west central bay. Station 2 is 150 m west of Bird Island in the eastern reach of the bay (Bellport Bay). Station 3 is located in the central bay, about 100 m north of West Fire Island. The 3 stations represented a range of sediment types from a coarse sand substrate at Station 1, to a fine sand at Station 2. Experiments were also conducted with samples from a site near Vaucluse Shores, Virginia.

Rhizosphere sediments were manually collected in 3.4 cm diameter aluminium core tubes and sealed with

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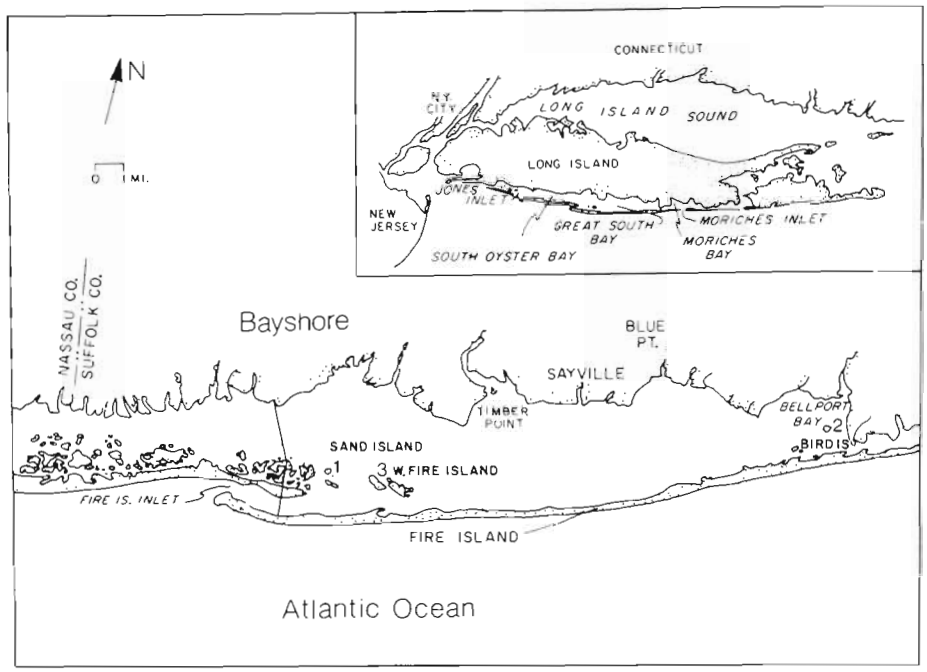

Fig. 1. Location of stations in Great South Bay, New York

rubber stoppers. Samples were returned to the laboratory and assays were set up within 3 h of collection. Assay procedures were essentially those developed for use in assaying *Thalassia testudinum* sediments (Capone and Taylor, 1980a). In general, 10 cm segments of sediments were incubated in 500 cc Erlenmeyer flask at *in situ* temperatures. Samples were extruded from the cores directly into the flasks while gassing with N_2. In an attempt to identify factors controlling sediment nitrogenase activity, several experiments examined the effect of glucose, NO_3^- or NH_4^+ additions. In these cases 200 ml of filtered seawater were included in each flask.

Nitrogenase activity was assayed by the C_2H_2 reduction method (Hardy et al., 1968). Acetylene was added to each flask to a final volume of about 12 % (v/v) and the gas phase of each flask was sampled at regular intervals for the determination of C_2H_2 and C_2H_4 concentrations. Controls were periodically run without C_2H_2. The gases were determined by flame ionization detection after separation on Porapak R (6' x 1/8'', 80 to 100 mesh). Peak height responses for unknowns were compared to a calibration standard. Ethylene production was expressed on both a dry weight of sediment and areal basis. Rates of nitrogenase activity were estimated from linear periods of C_2H_4 production by linear regression analysis.

Seasonal samplings were made, when possible, at all 3 stations with emphasis on the site near Bird Island (Station 2). On several occasions, whole foliar portions of the plant were assayed for nitrogenase activity in 900 Roux bottles *in situ*. Experiments with rhizosphere sediments were also run to determine the depth distribution of C_2H_2 reducing activity and the effect of Na_2MoO_4, a specific inhibitor of SO_4^{-2} respiring bacteria (Taylor and Oremland, 1979), on sediment N_2 fixation.

After termination of each assay, the sediments were passed through a 1 mm sieve to separate root material. Both sediments and root material were dried to constant weight at 105 °C. Selected samples from each site were analyzed for percent organic matter (loss on ignition) and total particulate carbon and nitrogen.

Samples of interstitial waters were obtained using *in situ* equilibrators (Hesslein, 1976) and also by centrifugation of core slices. Nutrients were determined by standard methods (Strickland and Parsons, 1972).

RESULTS

In all assays, C_2H_4 production was monitored periodically. Fig. 2 presents a typical example of the time course of C_2H_4 production by *Zostera marina* rhizosphere sediments. Samples of sediments extruded directly into deoxygenated seawater (slurries) exhibited short (½ to 2 h) lag periods, after which rates remained linear, often for longer than 24 h. Comparable samples assayed in flasks without seawater (static) showed immediately linear rates. The rates of C_2H_4 productions (i. e. slope of the linear portions of these lines) by the 2 methods were not significantly different ($p < 0.05$) in this, and in several similar experiments. During the course of the study, cores were assayed by

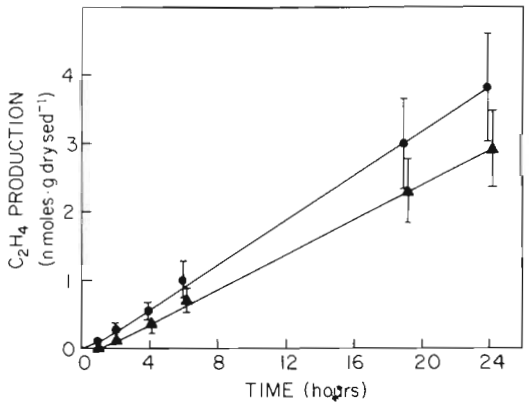

Fig. 2. *Zostera marina*. Time course of C_2H_4 production by rhizosphere sediments. Samples were incubated either with (▲) or without (●) deoxygenated seawater. Samples were collected from Station 2 on 15 May 1980 and incubated at 17 °C. Each point represents the mean of 3 replicates ± S. E. Slopes calculated from average values were not significantly different at the $P < 0.05$ level

both methods. Slurries were used particularly for investigations of the effect of combined nitrogen and glucose on nitrogenase activity. In all cases, reported rates were calculated from linear regression analysis after any apparent lag period. No C_2H_4 production was noted in assays lacking C_2H_2.

The depth distribution of nitrogenase activity was studied in several experiments. In one series of assays to 12 cm, the majority of the activity was found to occur in the upper 6 cm (Fig. 3). Cores longer than 12 to 15 cm were difficult to obtain by manual methods because of a dense shell layer commonly encountered at this depth.

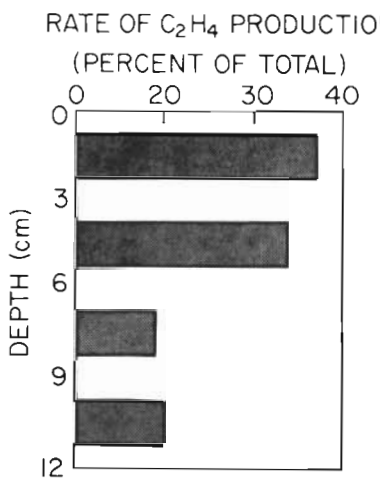

Fig. 3. *Zostera marina*. Depth distribution of C_2H_2 reduction in rhizosphere sediments Samples were collected on 14 August 1979 from Station 2. Integrated values of C_2H_2 reduction to 12 cm on this date equaled 3.4 nmol C_2H_4 $cm^{-2}h^{-1}$. Results are mean of replicate determinations

The results from the seasonal survey of rhizosphere N_2 fixation are presented in Fig 4. During similar periods, some spatial variation was apparent among

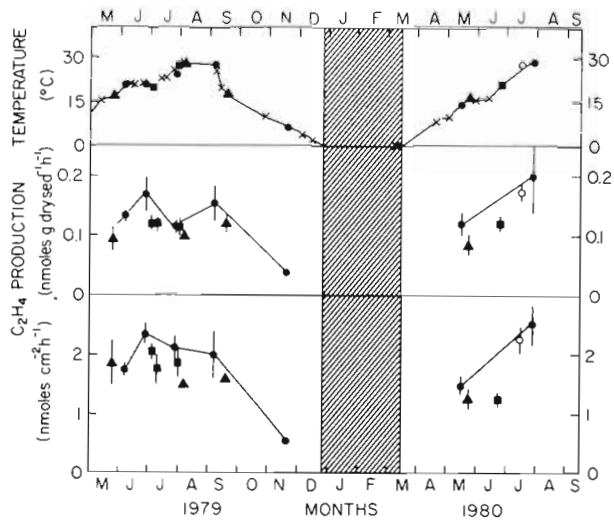

Fig. 4. *Zostera marina*. Seasonal and spatial variation in C_2H_2 reduction by rhizosphere sediments. The upper 10 cm of rhizosphere sediment were assayed as described in the text. Results are means ± S. E. for from 3 to 6 replicates. Closed triangles, samples from Station 1; closed circles, from Station 2; closed squares, from Station 3; open circles from a sampling at Vaucluse Shores, Virginia. Shaded area indicates periods of extensive ice cover on Great South Bay. Water temperatures are presented in upper portion of graph

the stations. However, during the summer (June–September), rates were comparable at all stations, ranging between 0.1 to 0.2 nmol C_2H_4 × g dry $sed^{-1}h^{-1}$ (1.5 to 2.5 nmol C_2H_4 × $cm^{-2}h^{-1}$). For Station 2, from which samples were obtained most often, some degree of seasonality in nitrogenase activity was noticed. Rates increased during the late spring/early summer and showed substantial decrease during the late fall. Sampling was curtailed from December through March because of ice cover on the bay. Water temperatures warmed more slowly during the spring of 1980, compared to 1979, and may partially account for the lower activities measured at two stations in May 1980.

A number of sediment parameters were periodically determined at Station 2, and the results are presented in Table 1. For Station 2, sediment densities over the 0 to 10 cm segment were between 1.3 to 1.9 g × cm^{-3}, with macroorganic content of from 0.01 to 0.04 g × cm^{-3}. Organic content, total carbon and total nitrogen all decreased with depth. Combusted samples were essentially devoid of measurable particulate carbon or nitrogen.

On several occasions, foliar portions of *Zostera marina* plants were also assayed for associated nitrogenase activity. On 4 dates in 1979 (9 May, Station 1;

Table 1. Sediment and macroorganic weight, and organic, carbon and nitrogen content of oven dried sediments from Station 2 on various occasions during 1979

Date	Depth interval	Sed Wt	MOM*	LOI**	C	N
		g cm^{-3}			%	
6 Jun	0–10	1.29	0.018	–	–	–
26 Jun	0–10	1.53	0.017	–	–	–
26 Jul	0–10	1.90	0.011	–	–	–
14 Aug	0– 3	1.27	0.017	0.71	0.38	0.027
14 Aug	3– 6	0.95	0.021	0.49	0.18	0.022
14 Aug	6– 9	1.58	0.007	0.24	0.08	0.017
14 Aug	9–12	1.28	0	0.18	0.06	0.011
14 Aug	0–12 ($\bar{x}$)	1.27	0.013	0.41	0.18	0.019
5 Sep	0–10	1.33	0.036	0.49	0.25	0.045
19 Nov	0–10	1.34	0.033	0.54	0.25	0.026

* Macroorganic matter retained on a 1-mm sieve
** Loss on ignition at 450 °C

26 June, Station 2; 3 July and 10 July, Station 3) no C_2H_4 production was noted for samples assayed *in situ* for up to 6 h. One experiment (30 July 1979, Station 3) did indicate low levels of nitrogenase activity (20 nmol C_2H_4 × g dry wt^{-1}h^{-1}) which, when translated into an areal basis (<0.4 nmol C_2H_4 × cm^{-2}h^{-1}), were far less than the activity in the rhizosphere.

A comparison of the rates of C_2H_4 production by rhizosphere sediments of *Zostera marina*, *Ruppia maritima* and from an area inhabited by both species, was made during a field trip to Vaucluse Shores on the eastern shore of Virginia. The results are presented in Table 2 and rates were very similar for all 3 sample types. Non-rhizosphere samples from a small sand patch within a seagrass bed and from a sand bar beyond the bed were also assayed. Rates of activity for the sand patch were comparable, and in fact slightly (1.5×) greater than those measured in the rhizosphere samples. Nitrogenase activity in the sand bar was about 1/3 rhizosphere values.

Table 2. Nitrogen fixation (C_2H_2 reduction) by rhizosphere sediments from *Zostera marina*, *Ruppia maritima*, and mixed stands. Samples were collected at a site near Vaucluse Shores, Virginia, USA, on 14 July 1980. Results are the mean of the indicated number of replicates ± S.E.

Sample	C_2H_4 Production	
	nmoles · g^{-1}h^{-1}	nmoles cm^{-2}h^{-1}
Zostera bed	0.18 ± 0.02 (4)	2.3 ± 0.3 (4)
Mixed bed	0.18 ± 0.07 (3)	2.4 ± 0.9 (3)
Ruppia bed	0.14 ± 0.03 (4)	2.3 ± 0.5 (4)

Nitrogenase activity in small bare patches within seagrass beds at the Great South Bay sites was determined on several occasions (Table 3). In general, rates were lower than adjacent rhizosphere sediments. On one date (20 Sept. 1979), C_2H_2 reduction was measured in sediments collected off Blue Point in mid bay, at a depth of about 3 m and devoid of any *Zostera marina*. Areal rates of C_2H_4 production equaled 0.69 ± 0.05 nmol × cm^{-2}h^{-1} (± S.E., n = 4). This was substantially lower than activities in the seagrass beds (Fig. 4).

Table 3. *Zostera marina*. Comparison of C_2H_2 reduction in rhizosphere sediments and adjacent sand patches. Samples all at 0 to 10 cm depth interval. Mean ± S.E. with number of replicates in parentheses

Site	Date	*Zostera* stand	Sand patch
		(nmoles C_2H_4 · cm^{-2}h^{-1}	
1	23 May 79	1.8 ± 0.4 (3)	1.1 ± 0.1 (3)
2	26 Jun 79	2.4 ± 0.2 (5)	1.9 ± 0.6 (3)
3	3 Jul 79	1.6 ± 0.2 (4)	1.6 ± 0.3 (4)
3	10 Jul 79	1.8 ± 0.2 (3)	1.0 ± 0.04 (3)
3	30 Jul 79	1.8 ± 0.2 (3)	2.4 ± 0.50 (2)

The effect of NO_3^- and NH_4^+ on sediment nitrogenase activity was tested in several experiments. In 2 of these, endogenous rates of C_2H_4 production were measured for 20 h, at which time the additions were made (Table 4). Ethylene production was then measured over the next 20 h. Nitrate and NH_4^+ at 100 or 200 µM were both effective in reducing nitrogenase activity in both *Zostera marina* and sand patch sediments, although the degree of inhibition was variable (Table 4). The higher concentrations (200 µM vs 100 µM) of either were, in general, more effective in reducing C_2H_4 production. For one experiment in which additions of 50 and 200 µM NO_3^- were made after 2 h, the lower concentrations of NO_3^- appeared to cause only a transient inhibition in the rate of C_2H_4 production compared to controls (Fig. 5).

Table 4. *Zostera marina*. Effect of NH_4^+ and NO_3^- on rhizosphere and non-rhizosphere N_2 fixation (C_2H_2 reduction). Experiments were conducted on 26 June 1979 (I) and 3 July 1979 (II) at Stations 2 and 3, respectively. In each experiment the indicated additions were made at 20 h and the rates over the subsequent 20 h period compared

Experiment	Site	Addition (at 20 h)	Rate (20–40 h as % of control)
I.	*Zostera* stand	None	100
		100 μM NO_3^-	74
		100 μM NH_4^+	68
	Sand patch	None	100
		100 μM NO_3^-	91
		100 μM NH_4^+	61
II.	*Zostera* stand	None	100
		200 μM NO_3^-	64
		200 μM NH_4^+	83
	Sand patch	None	100
		200 μM NO_3^-	62
		200 μM NH_4^+	42

Additions of glucose and NO_3^- were made in 2 experiments, with approximately similar results on both occasions. Fig. 6 presents the results from one of these experiments. Glucose supplements (1 mM) stimulated exponential increases in the rate of C_2H_4 production. Nitrate (200 μM) negated this stimulatory effect.

The effect of Na_2MoO_4, a specific inhibitor of SO_4^{-2} respiring bacteria (Taylor and Oremland, 1979), was investigated during one experiment. Both methanogenesis and nitrogenase activity were measured at three depths in sediment samples from a mixed bed of *Zostera marina* and *Ruppia maritima* (Table 5). Molybdate (20 mM) severely inhibited C_2H_4 production while effecting an apparent stimulation of methanogenic activity. Additionally, SO_4^{-2} respiration, as measured by $H_2{}^{35}S$ production, was completely inhibited by Na_2MoO_4 (Capone, unpubl.).

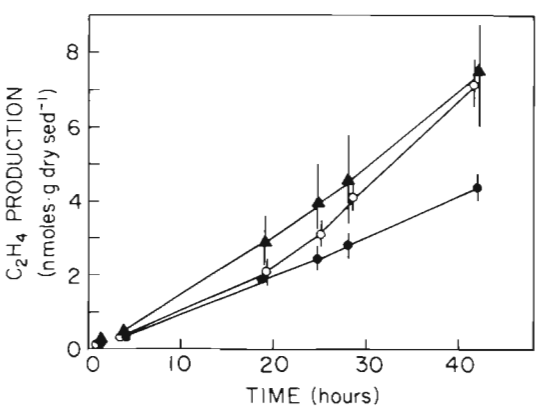

Fig. 5. *Zostera marina*. Effect of NO_3^- on C_2H_2 reduction by rhizosphere sediments. Samples were collected on 25 June 1980 from Station 3 and assayed with O (▲), 50 (○) or 200 (●) μM NO_3^-. Additions were made at 2 h. Means of replicate determinations with range indicated by bars

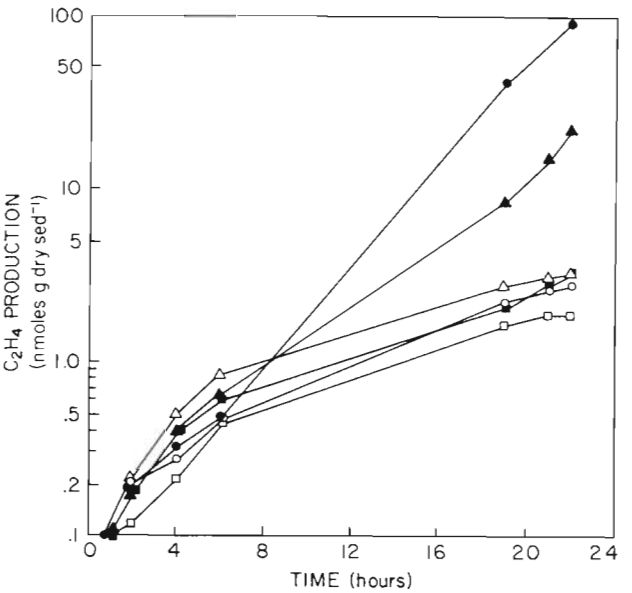

Fig. 6. *Zostera marina*. Effect of glucose and NO_3^- on C_2H_2 reduction by rhizosphere sediments. Samples were collected on 26 July 1979 from Station 2. Glucose was added to a final concentration of 1 mM in ●, ▲, ○. Nitrate was added to a final concentration of 200 μM in ○, □. Controls (i. e. no NO_3^- or glucose) were ■, △. Additions were made at 7 h

The concentration of NH_4^+ in the pore waters of *Zostera marina* stands at Station 3 was determined by 2 methods (Dietz and Capone, Abst, 45th Annual Meeting Am. Soc. Limnol. Oceanogr.). For 2 sets of sectioned cores from which the pore waters were separated by centrifugation, NH_4^+ levels averaged about 24 μM in the top 0.5 cm, increased to an average value of 160–180 μM by 3.0 cm and remained constant up to about 10 cm. The NH_4^+ concentration over the 0 to 10 cm depth averaged 116 μM. Ammonium was also measured using *in situ* equilibration devices. The concentration of NH_4^+ in 3 equilibrators (3 ports/equilibrator at each depth) was considerably more variable

Table 5. Effect of $NaMoO_4$ (20 mM) on methanogenesis and N_2 fixation (C_2H_2 reduction) by rhizosphere sediments from a mixed stand of *Zostera marina* and *Ruppia maritima*. Samples were collected at a site near Vaucluse Shores, Virginia, on 16 July 1980. Methanogenesis was monitored in flasks without C_2H_2 additions. All samples were incubated for 27 h

Depth interval (cm)	CH_4 production (nmoles g dry sed^{-1})		C_2H_4 production (nmoles · g dry sed^{-1})	
	− Mo	+ Mo	− Mo	+ Mo
0– 3	6.8	57.6	29.6	1.8
6– 9	1.2	20.8	19.1	0.8
12–15	0.44	4.2	12.7	0.5

ranging from 6 to 415 μM in the 6 to 7 cm depth segment and from 50 to 475 μM in the 9 to 11 cm depth segment.

DISCUSSION

Mutualistic associations of macrophytes and heterotrophic bacteria confer obvious ecological advantages. Given the energetic demands and oxygen sensitivity of nitrogenase, the rhizosphere environment provides a suitable habitat for the development of such associations. Nitrogen fixation has been reported to provide substantial inputs of nitrogen in the root zones of a variety of marine macrophyte communities including tropical seagrass (Patriquin and Knowles, 1972; Capone and Taylor, 1980b), salt marsh (Patriquin and McClung, 1978; Teal et al., 1979) and mangrove (Zuberer and Silver, 1978) systems.

Nitrogenase activity was consistently detectable in the rhizosphere of *Zostera marina* stands at several sites in Great South Bay, New York. Over the growing season (May through September), rates of C_2H_2 reduction in the 0 to 10 cm depth interval were quite comparable at several sites (Fig. 2). Similar activities were also detected at a site in Virginia (Table 2). From preliminary calculations, about ¼ to ½ of the activity appears to be associated with the roots and rhizomes (Capone and Budin, in press).

Several constraints limit the direct extrapolation of experimentally derived rates of C_2H_4 production to *in situ* N_2 fixation. Prolonged exposure of samples to C_2H_2 has been shown to produce anomalous results because of the inhibitory nature of C_2H_2 (Brouzes and Knowles, 1971; Barber et al., 1976; David and Fay, 1977). Nonetheless, a number of investigators have resorted to long term (days) assays in order to demonstrate N_2 fixation. Extended lag periods before the detection of nitrogenase activity have been reported for intact rhizosphere sediments and excised roots in seagrass (Patriquin and Knowles, 1972), *Spartina alterniflora* (Patriquin and Denicke, 1978; Dicker and Smith, 1980a), and mangrove (Zuberer and Silver, 1978) communities, as well as in terrestrial systems (Dobereiner et al., 1972; Day et al., 1975). This phenomenon has variously been attributed to substrate limitation (Day et al., 1975) and O_2 inactivation (Patriquin, 1978) of nitrogenase. Remedies have therefore included overnight preincubation (Day et al., 1975; Teal et al., 1979), extended assay (Patriquin and Knowles, 1972; Dicker and Smith, 1980a) and substrate additions in experiments intended to evaluate *in situ* rates of nitrogen fixation. The apparent lag period of some of these previous studies might also be the simple result of insufficient sensitivity in the assay system to accurately detect low levels of C_2H_4 production.

Recent studies by van Berkum and Sloger (1981) on *Spartina alterniflora* and several terrestrial grasses, and by Capone and Taylor (1980) on *Thalassia testudinum* have shown that O_2 exposure may indeed produce a lag period, which can be shortened or avoided by precautions minimizing sample exposure to O_2 during assay setup.

In this regard, C_2H_2 reduction was detectable within minutes in assays of *Zostera marina* rhizosphere sediments during active periods. Also, rates of C_2H_4 production were generally linear after minimal lag periods. The absence of a lag period in assays conducted without a liquid (seawater) phase may be indicative of either more rapid diffusion and equilibration of C_2H_2 to sites of activity or, alternately, more efficient purging of O_2 from these flasks.

The use of anaerobic assays to assess *in situ* rates of activity may underestimate total activity if obligately aerobic or microaerophilic N_2 fixing bacteria are a substantial component of the diazotrophic flora of the sediments. Since seagrasses are thought to facilitate the transport of gases to the sediments through their roots and rhizomes (Oremland and Taylor, 1977; Wetzel and Penhale, 1979), bacteria adapted to aerobic or microaerobic conditions might be expected on or near the roots and rhizomes. In fact, nitrogenase activity of excised roots and rhizomes, while optimal at reduced O_2 concentrations (maximum at 0.01 atm), was only slightly reduced under an anaerobic atmosphere but was substantially less under fully aerobic conditions (Capone and Budin, in press).

Another consideration concerns the validity of using the theoretical conversion factor (3 : 1) to extrapolate to N_2 fixation. The differential production of H_2 by nitrogenase under N_2 fixing and C_2H_2 reducing conditions is indicative of differing efficiencies of reduction of the two substrates. While reduction by nitrogenase of C_2H_2 at higher relative efficiencies than N_2 should increase the actual conversion ratio (Saito et al., 1980), the presence of uptake hydrogenase capable of recycling evolved H_2 has been shown to mitigate this effect (Lespinat and Berlier, 1981). Burris (1974) recommends direct calibration of each system with $^{15}N_2$ and where this has not been done, a ratio greater than 3 : 1 is more appropriate.

Although direct calibration of rhizosphere sediments was not performed, parallel $^{15}N_2$ fixation and C_2H_2 reduction assays of rinsed roots and rhizomes yielded an $C_2H_2 : N_2$ ratio of 2.6 : 1 (Capone and Budin, in press). Since this falls within the range of observed values (see Hardy et al. 1968; Saito et al., 1980) and is in good agreement with determinations undertaken by Patriquin and Knowles (1972) for similar samples, I have used it for the purpose of further discussion.

During summer, areal rates of C_2H_2 reduction

ranged from about 1.5 to 2.5 nmol $C_2H_4 \times cm^{-2}h^{-1}$ (Fig. 3). Assuming a constant daily rate and a conversion ratio of 2.6:1, the calculated input of nitrogen by N_2 fixation equals 3.9 to 6.5 mg $\times m^{-2}d^{-1}$ (to 10 cm). The porosity of Great South Bay sediments is about 50 % (D. Hirschberg, pers. comm.), and with interstitial concentrations of NH_4^+ ranging from 100 to 200 µM, this amounts to 70 to 140 mg N $\times m^{-2}$ to 10 cm. If N_2 fixation were the only input, interstitial pools of NH_4^+ would be replaced every 11 to 36 d.

Nitrogen fixation in the rhizosphere of the tropical seagrass *Thalassia testudinum* was recently determined to range between 5 and 38 mg N $\times m^{-2}d^{-1}$ for several sites during the summer (Capone and Taylor, 1980a). During a series of experiments, rhizosphere N_2 fixation was estimated to account for up to 47 % of the nitrogen demand of the plant. Patriquin and Knowles (1972) speculated that N_2 fixation might be a more important activity in *T. testudinum* communities compared to *Zostera marina* in light of the lower levels of available nitrogen in tropical waters and sediments, and the higher photosynthetic rates sustainable in these areas. Our results appear to confirm their conjecture.

The productivity of *Zostera marina* has been estimated in several studies (see McRoy and McMillan, 1977). Brinkhuis (unpubl.) has found standing crops of about 355 g dry wt $\times m^{-2}$ (200 g dry wt $\times m^{-2}$ above ground and 155 g dry wt $\times m^{-2}$ below ground biomass) at our sites and estimates productivity to be between 3 to 4 g dry wt $\times m^{-2}d^{-1}$ during the growing season. These values are consistent with previously published results (McRoy and McMillan, 1977). The nitrogen content of green leaves averages about 2.5 % (Patriquin, 1972; Harrison and Mann, 1975; Thayer et al., 1977; Aioi and Mukai, 1980). Assuming that nitrogen is required at this percentage by photosynthesis, one can calculate a total nitrogen demand of from 75 to 100 mg $\times m^{-2}d^{-1}$. It has been suggested that up to 70 % of the total nitrogen demand of *Z. marina* may be obtained by the plant from pools of labile nitrogen recycled from senescing plant tissue (Patriquin, 1972). While this remains to be documented, any degree of recycling would increase the relative contributions of N_2 fixation to the net or actual nitrogen demand. In the extreme cases of 0 % and 70 % of the nitrogen demand satisfied by internal plant recycling, our measured rates of N_2 fixation would supply 3 % and 28 %, respectively, of the calculated demand.

The high productivity of *Zostera marina,* relative to the measured concentrations of NH_4^+, indicates a more rapid turnover of interstitial NH_4^+ (0.6 to 6 d) than that calculated from N_2 fixation alone and, hence, other sources of NH_4^+ resupply to these sediments. The most likely source would be its release through degradation of the larger and presumably more refractory pool of organic nitrogen. A rough calculation using the data of Table 1, assuming a sediment dry weight of 1.3 g $\times cm^{-3}$ and an organic nitrogen content of 0.03 % amounts to about 40 g N $\times m^{-2}$

In this regard, Aller and Yingst (1980) have found a good correspondence between bacterial SO_4^{-2} reduction and the rate of NH_4^+ mineralization in anoxic muds. Sulfate reduction may account for the bulk of organic oxidation in marine sediments (Sorensen et al., 1978), including organically rich eelgrass systems (T. Wilson, unpubl.). Besides representing a probable agent of NH_4^+ regeneration, SO_4^{-2} reducing bacteria have also been implicated in this (Table 5) and other studies (Dicker and Smith, 1980b; Nedwell and Aziz, 1980; Capone and Taylor, in prep.) as an important component of the N_2 fixing flora.

Ammonia might also be formed through an anaerobic dissimilatory reduction of NO_3^- (Koike and Hattori, 1978; Sorensen, 1978). However, this could only be of minor impact in light of the low ambient levels and absence of obvious external sources of NO_3^-. The generation of NO_3^- through nitrification would only represent a closed loop with respect to NH_4^+ supply, but may be important in providing substrate for denitrification, a sink for combined nitrogen (Capone and Taylor, 1980b).

The inhibitory effect of NH_4^+ and NO_3^- on *Zostera marina* rhizosphere N_2 fixation may be a result of a variety of factors. The activity of glutamine synthetase, which appears to directly regulate nitrogenase synthesis, is itself controlled by intracellular NH_4^+ concentrations (Streicher et al., 1974; Barber and Evans, 1977). The evidence for direct allosteric regulation of nitrogenase by combined forms of nitrogen is scanty. In this study, the observed effects over the longer term by NH_4^+ (Table 5), as well as by NO_3^- (given the probable capacity for its dissimilatory reduction to NH_4^+) are explicable in terms of probable synthetic regulation, while the reason for the apparent short term inhibition by NO_3^- (Figs. 5 and 6) is less clear. Dicker and Smith (1980c) have provided evidence to suggest that NO_3^- inhibition of salt marsh sediment nitrogenase activity may be a result of competition for reducing power. Similarly, Capone and Carpenter (1982) recently noted that flushing of interstitial NH_4^+ from rhizosphere cores of *Z. marina*, as well as *Spartina alterniflora* and *Thalassia testudinum,* produced a rapid and substantial stimulation of nitrogenase activity.

Numerous questions remain to be answered in order to further unravel the complexities of the nitrogen cycle of seagrass communities and, indeed, other marine systems. The spatial and chemical relationship between the nitrogen fixing flora and the plant are the

focus of our present endeavors. The complementary activity of denitrifying bacteria and the role of nitrification in supplying substrate (i.e. NO_3^-) to this biological sink also require further elucidation.

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LITERATURE CITED

Aioi, K., Mukai, H. (1980). On the distribution of organic contents of eelgrass (*Zostera marina* L.) Jap. J. Ecol. 30: 189–192

Aller, R. C., Yingst, J. Y (1980). Relationship between microbial distributions and the anaerobic decomposition of organic matter in surface sediments of Long Island Sound, USA. Mar. Biol. 56: 29–42

Barber, L. E., Tjepkema, J. D., Russell, S. A., Evans, H. J. (1976). Acetylene reduction (nitrogen fixation) associated with corn inoculated with *Spirillum*. Appl. environ. Microbiol. 32: 108–113

Brouzes, R., Knowles, R. (1971). Inhibition of growth of *Clostridium pasteurianum* by acetylene: implication for nitrogen fixing assay. Can. J. Microbiol. 17: 1483–1489

Burris, R. H. (1974). Methodology. In: Quispel, A. (ed.) The biology of nitrogen fixation. North-Holland, Amsterdam, Oxford, pp. 9–36

Capone, D. G., Carpenter, E. J. (1982). Perfusion method for assaying microbial activities in sediments: applicability to studies of N_2 fixation by C_2H_2 reduction. Appl. environ. Microbiol. 43: 1400–1405

Capone, D. G., Taylor, B. F. (1977). Nitrogen fixation (acetylene reduction) in the phyllosphere of *Thalassia testudinum*. Mar. Biol. 40: 19–28

Capone, D. G., Taylor, B. F. (1980a). N_2 fixation in the rhizosphere of *Thalassia testudinum*. Can. J. Microbiol. 26: 998–1005

Capone, D. G., Taylor, B. F. (1980b). Microbial nitrogen cycling in a seagrass community. In: Kennedy, V (ed.) Estuarine perspectives. Academic Press, New York, pp. 153–161

Capone, D. G., Penhale, P. A., Oremland, R. S., Taylor, B. F. (1979). Relationship between productivity and N_2 (C_2H_2) fixation in *Thalassia testudinum* community. Limnol. Oceanogr. 24: 117–125

David, K., Fay, P. (1977). Effects of long-term treatment with acetylene on nitrogen fixing microorganisms. Appl. environ. Microbiol. 34: 640–646

Day, J. M., Neves, M. C. P., Dobereiner, J. (1975). Nitrogenase activity on roots of tropical grasses. Soil Biol. Biochem. 7: 107–112

Dicker, H. J., Smith, D. W. (1980a). Acetylene reduction (nitrogen fixation) in a Delaware salt marsh. Mar. Biol. 57: 241–250

Dicker, H. J., Smith, D. W (1980b). Enumeration and relative importance of acetylene-reducing (nitrogen-fixing) bacterial in a Delaware salt marsh. Appl. environ. Microbiol. 39: 1019–1025

Dicker, H. J., Smith, D. W. (1980c). Physiological ecology of acetylene reduction (nitrogen fixation) in a Delaware salt marsh. Microb. Ecol. 6: 161–171

Dobereiner, J., Day, J. M., Dart, P. J. (1972). Nitrogenase activity and oxygen sensitivity of the *Paspalum notatum – Azotobacter paspel.* association. J. gen. Microb. 71: 103–116

Evans, H., Barber, L. (1977). Biological nitrogen fixation for food and fiber production. Science, N. Y 197: 332–339

Hardy, R. W F., Holsten, R. D., Jackson, E. K., Burns, R. C. (1968). The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. Plant Physiol. 43: 1158–1207

Harrison, P. G., Mann, K. H. (1975). Chemical changes during the seasonal cycle of growth and decay in eelgrass *(Zostera marina)* on the Atlantic Coast of Canada. J. Fish. Res. Bd Can. 32: 615–621

Hesslein, R. H. (1976). An *in situ* sampler for close interval pore water studies. Limnol. Oceanogr. 21: 912–914

Koike, I., Hattori, A. (1978). Denitrification and ammonia formation in anaerobic coastal sediments. Appl. environ. Microbiol. 35: 278–282

Lespinat, P. A., Berlier, Y. M. (1981). The dependence of hydrogen recycling upon nitrogenase activity in *Azospirillum brasilense* Sp. 7. FEMS Microbiol. Lets. 10: 127–132

McRoy, C. P., Goering, J J., Chaney, B. (1973). Nitrogen fixation associated with sea grasses. Limnol. Oceanogr. 18: 998–1002

McRoy, C. P., McMillan, C. (1977). Production ecology and physiology of sea grasses. In: McRoy, C. P., Hefferich, C. (eds.) Seagrass ecosystems. Dekker, New York, pp. 53–87

Oremland, R. S., Taylor, B. F. (1977). Diurnal fluctuation of O_2, N_2 and CH_4 in the rhizosphere of *Thalassia testudinum*. Limnol. Oceanogr. 22: 566–570

Patriquin, D. G. (1972). The origin of nitrogen and phosphorus for growth of the marine angiosperm *Thalassia testudinum*. Mar. Biol. 15: 3546

Patriquin, D. G. (1978). Factors affecting nitrogenase activity (acetylene reducing activity) associated with excised roots of the emergent halophyte *Spartina alterniflora* Loisel. Aquat. Bot. 4: 193–210

Patriquin, D. G., Denike, D. (1978). *In situ* acetylene reduction assays of nitrogenase activity associated with the emergent halophyte *Spartina alterniflora* Loisel: methodological problems. Aquat. Bot. 4: 271–226

Patriquin, D. G., Knowles, R. (1972). Nitrogen fixation in the rhizosphere of marine angiosperms. Mar. Biol. 16: 49–58

Patriquin, D. G., McClung, C. (1978). Nitrogen accretion and the nature and possible significance of N_2 fixation (acetylene reduction) in a Nova Scotian *Spartina alterniflora* stand. Mar. Biol. 47: 227–242

Saito, S. M., Matsui, E., Salati, E. (1980). $^{15}N_2$ fixation, H_2 evolution and C_2H_2 reduction relationships in *Phaseolus vulgaris*. Physiol. Plant. 49: 37–42

Sorensen, J. (1978). Capacitiy for denitrification and reduction of nitrate to ammonia in a coastal marine sediment. Appl. envion. Microbiol. 35: 301–305

Sorensen, J., Jorgensen, B. B., Revsbech, N. P. (1979). A comparison of oxygen, nitrate, and sulfate repiration in coastal marine sediments. Microb. Ecol. 5: 105–115

Streicher, S. L., Shanmugan, K. T., Ausubel, F., Morandi, C., Goldberg, R. B. (1974). Regulation of nitrogen fixation in *Klebsiella pneumoniae:* evidence for a role of glutamine synthesis as a regulator of nitrogenase synthesis. J. Bacteriol. 120: 815–821

Strickland, J. D. H., Parsons, T. R. (1972). A practical handbook of seawater analysis, 2nd ed., Fisheries Research Board of Canada, Bulletin 167, Ottawa, Canada

Taylor, B. F., Oremland, R. S. (1979). Depletion of ATP in *Desulfovibrio* by oxyanions of Group VI elements. Current Microbiol. 3: 101–103

Teal, J. M., Valiela, I., Berlo, D. (1979). Nitrogen fixation by rhizosphere and free-living bacteria in salt marsh sediments. Limnol. Oceanogr. 24: 126–132

Thayer, G. W., Engel, D. W., LaCroix, M. W. (1977). Seasonal distribution and changes in the nutritive quality of living, dead and detrital fractions of *Zostera marina* L. J. exp. mar. Biol. Ecol. 30 (2): 109–127

van Berkum, P., Sloger, C. (1981). Comparing time course profiles of immediate acetylene reduction by grasses and legumes. Appl. environ. Microbiol. 41: 184–189

Wetzel, R. G., Penhale, P. A. (1979). Transport of carbon and excretion of dissolved organic carbon by leaves and root/rhizomes in seagrass and their epiphytes. Aquat. Bot. 6: 149–158

Zuberer, D. A., Silber, W. S. (1978). Biological dinitrogen fixation (acetylene reduction) associated with Florida mangroves. Appl. environ. Microbiol. 35: 567–575

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