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 $\mu$M) inhibited nitrogenase activity, while glucose (1 mM) accelerated rates of C$_2$H$_2$ reduction. Much of the nitrogenase activity appears to be associated with sulfate-respiring bacteria. During the summer, rates of C$_2$H$_2$ reduction to 10 cm averaged about 1.5 to 2.5 nmol C$_2$H$_2$ X cm$^{-2}$h$^{-1}$ (0.1 to 0.2 nmol X 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...
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 N₂. In an attempt to identify factors controlling sediment nitrogenase activity, several experiments examined the effect of glucose, NO₃⁻ or NH₄⁺ additions. In these cases 200 ml of filtered seawater were included in each flask.

Nitrogenase activity was assayed by the C₂H₂ 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 C₂H₂ and C₂H₄ concentrations. Controls were periodically run without C₂H₂. 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 C₂H₄ 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 C₂H₂ reducing activity and the effect of Na₂MoO₄, a specific inhibitor of SO₄⁻² resiping bacteria (Taylor and Oremland, 1979), on sediment N₂ 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, C₂H₄ production was monitored periodically. Fig. 2 presents a typical example of the time course of C₂H₄ 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 C₂H₄ 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
The results from the seasonal survey of rhizosphere N₂ fixation are presented in Fig. 4. During similar periods, some spatial variation was apparent among the stations. However, during the summer (June–September), rates were comparable at all stations, ranging between 0.1 to 0.2 nmol C₂H₄ X g dry sed⁻¹ h⁻¹ (1.5 to 2.5 nmol C₂H₄ X 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 X cm⁻³, with macroorganic content of from 0.01 to 0.04 g X 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;
Comparison of the rates of C$_2$H$_4$ 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 1 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 x) greater than those measured in the rhizosphere samples. Nitrogenase activity in the sand bar was about 1/3 rhizosphere values.

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$_2$H$_4$ 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$_2$H$_4$ 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).

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$_2$H$_4$ 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$_2$H$_4$ 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$_2$H$_4$ production compared to controls (Fig. 5).

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

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

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

### Table 2. Nitrogen fixation (C$_2$H$_4$ reduction) by rhizosphere sediments from *Zostera marina*, *Ruppia maritima*, and mixed stands.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Site Date</th>
<th>Zostera stand</th>
<th>Sand patch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(nmol C$_2$H$_4$ cm$^{-3}$ h$^{-1}$)</td>
<td>(nmol C$_2$H$_4$ cm$^{-3}$ h$^{-1}$)</td>
</tr>
<tr>
<td><em>Zostera</em> bed</td>
<td>1 23 May 79</td>
<td>1.6 ± 0.4 (3)</td>
<td>1.1 ± 0.1 (3)</td>
</tr>
<tr>
<td>Mixed bed</td>
<td>2 26 Jun 79</td>
<td>2.4 ± 0.2 (5)</td>
<td>1.9 ± 0.6 (3)</td>
</tr>
<tr>
<td><em>Ruppia</em> bed</td>
<td>3 3 Jul 79</td>
<td>1.6 ± 0.2 (4)</td>
<td>1.6 ± 0.3 (4)</td>
</tr>
<tr>
<td></td>
<td>3 10 Jul 79</td>
<td>1.8 ± 0.2 (3)</td>
<td>1.0 ± 0.04 (3)</td>
</tr>
<tr>
<td></td>
<td>3 30 Jul 79</td>
<td>1.8 ± 0.2 (3)</td>
<td>2.4 ± 0.50 (2)</td>
</tr>
</tbody>
</table>
Table 4. Zostera marina. Effect of NH₄⁺ and NO₃⁻ on rhizosphere and non-rhizosphere N₂ fixation (C₂H₂ 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.

<table>
<thead>
<tr>
<th>Experiment Site</th>
<th>Addition (at 20h)</th>
<th>Rate (20-40h as % of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Zostera stand</td>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>100 µM NO₃⁻</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>100 µM NH₄⁺</td>
<td>68</td>
</tr>
<tr>
<td>Sand patch</td>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>100 µM NO₃⁻</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>100 µM NH₄⁺</td>
<td>61</td>
</tr>
<tr>
<td>II. Zostera stand</td>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>200 µM NO₃⁻</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>200 µM NH₄⁺</td>
<td>83</td>
</tr>
<tr>
<td>Sand patch</td>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>200 µM NO₃⁻</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>200 µM NH₄⁺</td>
<td>42</td>
</tr>
</tbody>
</table>

Additions of glucose and NO₃⁻ 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 C₂H₂ production. Nitrate (200 µM) negated this stimulatory effect.

The effect of Na₂MoO₄, a specific inhibitor of SO₄⁻² 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 C₂H₂ production while effecting an apparent stimulation of methano- 

Fig. 5. Zostera marina. Effect of NO₃⁻ on C₂H₂ reduction by rhizosphere sediments. Samples were collected on 26 June 1979 from Station 2. Glucose was added to a final concentration of 1 mM in o, ▲, □. Nitrate was added to a final concentration of 200 µM in □, □. Controls (i.e., no NO₃⁻ or glucose) were o, ▲. Additions were made at 7 h in situ equilibration devices. The concentration of NH₄⁺ 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, NH₄⁺ 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 NH₄⁺ concentration over the 0 to 10 cm depth averaged 116 µM. Ammonium was also measured using

Table 5. Effect of NaMoO₄ (20 mM) on methanogenesis and N₂ fixation (C₂H₂ reduction) by rhizosphere sediments from a mixed stand of Zostera marina and Ruppia maritima. Samples were collected at a site near Vaucharl Shores, Virginia, on 16 July 1980. Methanogenesis was monitored in flasks without C₂H₂ additions. All samples were incubated for 27 h.

<table>
<thead>
<tr>
<th>Depth interval (cm)</th>
<th>CH₄ production (nmoles g dry sed⁻¹)</th>
<th>C₂H₂ production (nmoles g dry sed⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-3</td>
<td>-Mo 6.8</td>
<td>+Mo 57.6</td>
</tr>
<tr>
<td>6-9</td>
<td>-Mo 1.2</td>
<td>+Mo 20.8</td>
</tr>
<tr>
<td>12-15</td>
<td>-Mo 0.44</td>
<td>+Mo 4.2</td>
</tr>
</tbody>
</table>
rately detect low levels of C$_2$H$_2$ production. During summer, area1 rates of C$_2$H$_2$ reduction have used it for the purpose of further discussion. Patriquin and Knowles (1972) for similar samples, I previous studies might also be the simple result of fixation. The apparent lag period of some of these in good agreement with determinations undertaken by extended assay (Patriquin and Knowles, 1972; Dicker et al., 1972; Day et al., 1975). This phenomenon has been shown to produce anomalous results because of the inhibitory nature of C$_2$H$_2$ (Brouzes and Knowles, 1971; Barber et al., 1976; David and Fay, 1977). Nonetheless, a number of investigators have resorted to extrapolation of experimentally derived rates of C$_2$H$_2$ production to in situ N$_2$ fixation. Prolonged exposure of samples to C$_2$H$_2$ has been shown to produce anomalous results because of the inhibitory nature of C$_2$H$_2$ (Brouzes and Knowles, 1971; Barber et al., 1976; David and Fay, 1977). 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$_2$H$_2$ 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 $1/4$ to $1/5$ 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$_2$H$_2$ production to in situ N$_2$ fixation. Prolonged exposure of samples to C$_2$H$_2$ has been shown to produce anomalous results because of the inhibitory nature of C$_2$H$_2$ (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$_2$H$_4$ 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$_2$H$_2$ reduction was detectable within minutes in assays of Zostera marina rhizosphere sediments during active periods. Also, rates of C$_2$H$_2$ 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$_2$H$_2$ 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; Wetz 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$_2$H$_2$ reducing conditions is indicative of differing efficiencies of reduction of the two substrates. While reduction by nitrogenase of C$_2$H$_2$ 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$_2$H$_2$ reduction assays of rinsed roots and rhizomes yielded an C$_2$H$_2$: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$_2$H$_2$ reduction...
ranged from about 1.5 to 2.5 nmol C₂H₄ × cm⁻²h⁻¹ (Fig. 3). Assuming a constant daily rate and a conversion ratio of 2.6:1, the calculated input of nitrogen by N₂ fixation equals 3.9 to 6.5 mg × m⁻²d⁻¹ (to 10 cm). The porosity of Great South Bay sediments is about 50 % (D. Hirschberg, pers. comm.), and with interstitial concentrations of NH₄⁺ ranging from 100 to 200 µM, this amounts to 70 to 140 mg N × m⁻² to 10 cm. If N₂ fixation were the only input, interstitial pools of NH₄⁺ 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 × m⁻²d⁻¹ for several sites during the summer (Capone and Taylor, 1980a). During a series of experiments, rhizosphere N₂ fixation was estimated to account for up to 47 % of the nitrogen demand of the plant. Patrquin and Knowles (1972) speculated that N₂ 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 × m⁻² (200 g dry wt × m⁻² above ground and 155 g dry wt × m⁻² below ground biomass) at our sites and estimates productivity to be between 3 to 4 g dry wt × m⁻²d⁻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 % (Patrquin, 1972; Harrison and Mann, 1975; Thayer et al., 1977; Aoi 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 × m⁻²d⁻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 (Patrquin, 1972). While this remains to be documented, any degree of recycling would increase the relative contributions of N₂ 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 N₂ fixation would supply 3 % and 28 %, respectively, of the calculated demand.

The high productivity of *Zostera marina*, relative to the measured concentrations of NH₄⁺, indicates a more rapid turnover of interstitial NH₄⁺ (0.6 to 6 d) than that calculated from N₂ fixation alone and, hence, other sources of NH₄⁺ 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 × cm⁻³ and an organic nitrogen content of 0.03 % amounts to about 40 g N × m⁻².

In this regard, Aller and Yingst (1980) have found a good correspondence between bacterial SO₄⁻² reduction and the rate of NH₄⁺ 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 NH₄⁺ regeneration, SO₄⁻² reducing bacteria have also been implicated in this (Table 5) and other studies (Dicker and Smith, 1980a; Nedwell and Aziz, 1986; Capone and Taylor, in prep.) as an important component of the N₂ fixing flora.

Ammonia might also be formed through an anaerobic dissimilatory reduction of NO₃⁻ (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 NO₃⁻. The generation of NO₃⁻ through nitrification would only represent a closed loop with respect to NH₄⁺ supply, but may be important in providing substrate for denitrification, a sink for combined nitrogen (Capone and Taylor, 1980b).

The inhibitory effect of NH₄⁺ and NO₃⁻ on *Zostera marina* rhizosphere N₂ 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 NH₄⁺ 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 NH₄⁺ (Table 5), as well as by NO₃⁻ (given the probable capacity for its dissimilatory reduction to NH₄⁺) are explicable in terms of probable synthetic regulation, while the reason for the apparent short term inhibition by NO₃⁻ (Figs. 5 and 6) is less clear. Dicker and Smith (1980c) have provided evidence to suggest that NO₃⁻ 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 NH₄⁺ 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


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