

Effects of nitrogen enrichment on biomass allocation, growth, and leaf morphology of the seagrass *Thalassia testudinum*

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ABSTRACT: The effects of sediment ammonium (NH_4^+) enrichment on biomass allocation, growth, and leaf morphology of the seagrass *Thalassia testudinum* in Corpus Christi Bay (CCB) and lower Laguna Madre (LLM), Texas, were examined from May to October 1997. Prior studies had shown that shoot height and leaf biomass at CCB were significantly higher than those at LLM, and ambient sediment NH_4^+ concentrations in CCB (ca 100 μM) were significantly higher than those in LLM (ca 30 μM). It was hypothesized that the differences in plant morphology and biomass between the 2 areas could be related to differences in sediment nitrogen levels between 2 sites. To test this hypothesis, we conducted an *in situ* fertilization experiment at both sites over a 6 mo period. Results of this experiment revealed that seagrass growth, biomass and leaf size significantly increased as a result of sediment NH_4^+ enrichment at LLM, but had little effect on plant density, biomass and leaf morphology at CCB. In unfertilized plots, average leaf production rate (7.4 g dry wt $\text{m}^{-2} \text{d}^{-1}$) and shoot height (43.3 cm) at CCB were significantly higher than those at LLM (2.5 g dry wt $\text{m}^{-2} \text{d}^{-1}$ and 18.8 cm, respectively). After fertilization, leaf production rates and leaf size at LLM increased to reach equivalent levels of the CCB site. Leaf biomass at LLM increased significantly as a result of sediment NH_4^+ enrichment, but there was little change in below-ground biomass. The below-to above-ground biomass ratio at LLM (4.7) was about 3-fold higher than that at CCB (1.6) in unfertilized plots, but decreased significantly at LLM with sediment NH_4^+ enrichment, while the ratio at CCB remained unchanged. We conclude, based on seagrass growth responses to increases in sediment NH_4^+ , that sediment nitrogen availability at LLM limits seagrass productivity. *T. testudinum* responded to limited nitrogen conditions by increasing below- to above-ground biomass ratios. An ambient sediment NH_4^+ level of about 100 μM was considered to be the threshold concentration for nitrogen limitation of seagrass growth.

KEY WORDS: Seagrass · Sediment ammonium · *Thalassia testudinum* · Nitrogen enrichment · Nitrogen limitation · Growth · Biomass allocation

INTRODUCTION

Seagrasses are important primary producers in coastal and estuarine ecosystems. As autotrophs, seagrasses require light, inorganic carbon sources, and inorganic nutrients (e.g. nitrogen and phosphate). Because of their high production rates, e.g. 1000 g dry wt $\text{m}^{-2} \text{yr}^{-1}$ (McRoy & McMillan 1977), seagrass beds may assimilate and sequester large amounts of inorganic nitrogen (N) and phosphorus (P), dominating estuarine nutrient cycling (Kenworthy et al. 1982,

Romero et al. 1994). Thus, nutrient availability may play a significant role in regulating seagrass production in shallow and clear areas where light is plentiful. Fertilization studies have shown that the addition of N or P can stimulate seagrass growth resulting in increased biomass (Orth 1977, Harlin & Thorne-Miller 1981, Iizumi et al. 1982, Dennison et al. 1987, Short et al. 1990, Pérez et al. 1991, Murray et al. 1992, Williams & Ruckelshaus 1993), suggesting nutrient limitation for plant growth.

Seagrasses have access to inorganic-N sources in both the sediment and the water column (Iizumi & Hattori 1982, Thursby & Harlin 1982, Short & McRoy 1984, Stapel et al. 1996, Stapel & Hemminga 1997, Terrados

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& Williams 1997, Lee & Dunton 1999b). N enrichment of the water column can lead to decreased underwater light availability as a result of epiphyte growth and phytoplankton blooms that can lead to seagrass decline (Orth & Moore 1983, Silberstein et al. 1986, Giesen et al. 1990, Tomasko & Lapointe 1991). In addition, Burkholder et al. (1992, 1994) demonstrated that water column nitrate (NO_3^-) enrichment caused death of *Zostera marina* through a direct physiological effect unrelated to algal light attenuation. In most cases, however, sediment N enrichment enhanced seagrass growth, and no harmful effects have been reported (Bulthuis & Woelkerling 1981, Agawin et al. 1996, Alcoverro et al. 1997, Udy & Dennison 1997). Therefore, although a high N requirement has been demonstrated for seagrass growth, the plant responses to increased N availability may vary depending on the N sources.

Due to the high concentration of NH_4^+ in pore waters relative to the overlying water column, seagrasses obtain a large fraction of their N from the sediment via root tissues (Iizumi & Hattori 1982, Short & McRoy 1984, Zimmerman et al. 1987, Lee & Dunton 1999b). Sediment NH_4^+ is the most abundant source of N in seagrass beds (Agawin et al. 1996, Dunton 1996, Terados & Williams 1997) and consequently plays an important role in regulation of seagrass growth. The concentration of sediment NH_4^+ is highly variable, ranging from less than 20 μM (Bulthuis et al. 1992, Agawin et al. 1996, Udy & Dennison 1997) to well over 100 μM (Bulthuis & Woelkerling 1981, Iizumi et al. 1982, Fourqurean et al. 1992, Czerny & Dunton 1995, Dunton 1996). Most sediment NH_4^+ is supplied by remineralization processes in sediments (Jørgensen 1977, 1982, Hines & Lyons 1982, Boon et al. 1986, Holmer & Nielsen 1997). *In situ* sediment NH_4^+ concentrations represent a balance between NH_4^+ production and consumption. Thus, in most cases, existence of N limitation in each seagrass bed probably depends on *in situ* sediment NH_4^+ level, and the sediment NH_4^+ concentration may be used as an indicator of the N status for seagrass growth.

Seagrasses living under varying conditions of N availability may respond differently to sediment N enrichment, and N limitation can be predicted from plant responses. The present study focused on plant responses to sediment N fertilization in 2 distinct *Thalassia testudinum* populations in Texas: Corpus Christi Bay (CCB) and lower Laguna Madre (LLM). Previous studies demonstrated that plant height and leaf biomass in CCB were significantly higher than in the LLM (Lee & Dunton 1996, Herzka & Dunton 1997, Kaldy 1997). These differences were hypothesized to result from measured differences in the availability of pore water NH_4^+ in the 2 areas. The present study tested the hypothesis that while N availability at LLM is limiting for plant growth, the N requirements of *T. testudinum* at CCB are adequately met. Changes in productivity, biomass allocation, and leaf morphology were evaluated in response to *in situ* sediment NH_4^+ enrichment at the 2 sites. Pore water NH_4^+ , nitrate + nitrite ($\text{NO}_3^- + \text{NO}_2^-$) and phosphate (PO_4^{3-}) levels were monitored to assess sediment nutrient availability for *T. testudinum* growth.

MATERIALS AND METHODS

Study site. The study was conducted in monotypic meadows of *Thalassia testudinum* in CCB (27° 49' N, 97° 07' W) and LLM (26° 09' N, 97° 12' W), Texas, USA. The study sites are located at similar water depth (1.2 m; Fig. 1), and have been the focus of several

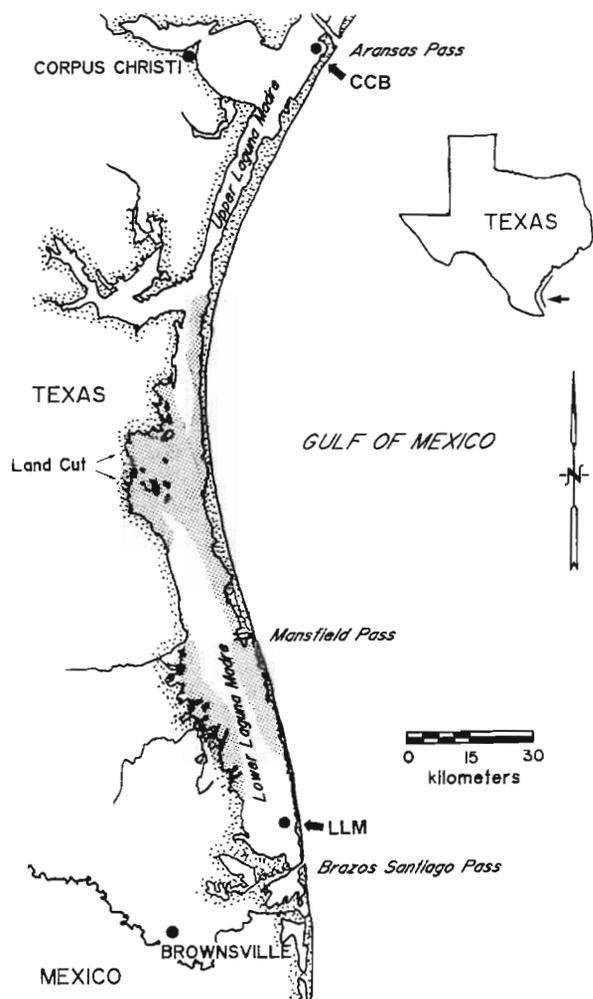


Fig. 1. Study sites in Corpus Christi Bay (CCB) and lower Laguna Madre (LLM), Texas, USA. Stippled areas represent wind-tidal flats, which are periodically flooded

recent studies on south Texas seagrasses (Dunton 1990, 1994, Quammen & Onuf 1993, Czerny & Dunton 1995, Lee & Dunton 1996, 1997, Herzka & Dunton 1997, 1998, Kaldy 1997). The CCB population is located on the eastern side of CCB and is characterized by high pore water NH_4^+ concentration (ca 100 μM) and high sand content in the sediment (Table 1). In contrast, the sediments at the LLM site, located 200 km to the south, have lower pore water NH_4^+ concentration (ca 30 μM) and sand content. *T. testudinum* plants growing at CCB are much taller and have higher leaf biomass. Water column NH_4^+ and $\text{NO}_3^- + \text{NO}_2^-$ at both sites were about 1 μM and were not significantly different. Underwater photosynthetically active radiation (PAR) was significantly higher at LLM than CCB (Table 1). The *T. testudinum* bed in LLM represents a relatively young population which replaced *Syringodium filiforme* in the 1970s and 1980s (Quammen & Onuf 1993).

Sediment NH_4^+ enrichment. At each site, 6 experimental plots (1.0 \times 1.0 m) were established in a homogeneous *Thalassia testudinum* meadow. Three served as controls and 3 were enriched with ammonium sulfate (21-0-0, Esco Corp., Dallas, TX). The fertilizer was wrapped in several layers of cheesecloth and in each plot, 25 packets consisting of 20 g of fertilizer were buried at 20 cm intervals in a horizontal grid and at about 10 cm sediment depth. The amount of N applied to each enriched plot was 105 g N m^{-2} based on manufacturer's nominal values. Agawin et al. (1996) compared sediment disturbed and undisturbed sites to

assess the effects of experimental sediment disturbance on seagrass. No significant sediment disturbance effects were found, and we presume the same for our study sites. The rhizome connections at the perimeter of each plot were cut to a sediment depth of about 30 cm to physiologically separate plants located within and outside the plots. The effects of physical damages as a result of rhizome cutting are very limited on plants within plots of this size and restricted to plants growing at the edge of the plots. In addition, rhizome cutting effects are also offset by cutting the rhizome connections at control plots. Ammonium fertilizer was added in early May 1997; plant and sediment samples were collected monthly from May to October from both control and treatment plots.

Sediment nutrient analyses. On each sampling date, 3 replicate sediment samples were collected randomly from each plot to a depth of ca 13 cm with a 60 ml syringe corer. Samples were placed on ice and frozen pending lab analyses. Sediment pore water was obtained by centrifugation (5000 $\times g$ for 15 min) and used for determination of pore water NH_4^+ , $\text{NO}_3^- + \text{NO}_2^-$ and PO_4^{3-} concentrations. Concentrations of NH_4^+ and PO_4^{3-} were determined using standard colorimetric techniques following the methods of Parsons et al. (1984) after dilution (1:5; v/v) with low nutrient sea water collected offshore in the Gulf of Mexico. Concentrations of $\text{NO}_3^- + \text{NO}_2^-$ were determined colorimetrically after running through a column containing copper coated cadmium which reduces NO_3^- to NO_2^- (Parsons et al. 1984).

Biological measurements. Measurements of shoot density, biomass, shoot height and blade width were completed monthly from May to October 1997. At each sampling date, 1 core sample from each plot was collected with a 9 cm diameter corer driven about 20 cm into the sediment. To assess the core size effect on shoot density and biomass, we compared shoot density and biomass on a m^2 basis estimated from samples collected by 15 cm diameter and 9 cm diameter corers. There were no significant differences in estimated shoot density and biomass between big and small core samples. Therefore, a 9 cm diameter corer was used to minimize destruction in experimental plots. Samples were thoroughly cleaned of epiphytes and sediments, separated into leaf (blade and sheath), rhizome (vertical and horizontal) and root tissues, and dried at 60°C to a constant

Table 1. Biological, physical and chemical characteristics of study sites in Corpus Christi Bay (CCB) and lower Laguna Madre (LLM), Texas. Values are from a long-term data set collected since 1990 (Herzka & Dunton 1997, 1998, Lee & Dunton 1997, Kaldy 1998, Dunton unpubl. data). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$: significant differences between the 2 study sites based on t -tests. Mean \pm SE (sample size)

| Parameter | Site | |
|--|-------------------------|---------------------------|
| | CCB | LLM |
| Sediment | | |
| Pore water ammonium (μM) | 115.08 \pm 11.12 (51) | 24.27 \pm 2.42 (19)*** |
| Sand content (%) | 91.19 \pm 0.47 (58) | 71.44 \pm 1.27 (35)*** |
| Clay + silt (%) | 8.81 \pm 0.47 (58) | 28.60 \pm 1.26 (35)*** |
| Water column | | |
| Ammonium | 1.16 \pm 0.06 (58) | 1.22 \pm 0.08 (38) |
| Nitrate + nitrite | 0.84 \pm 0.05 (58) | 0.84 \pm 0.06 (38) |
| <i>Thalassia testudinum</i> | | |
| Height (cm) | 41.42 \pm 0.63 (149) | 18.64 \pm 0.28 (196)*** |
| Leaf biomass (g dry wt m^{-2}) | 227.76 \pm 24.85 (15) | 159.42 \pm 11.32 (7)* |
| Root/shoot ratio | 1.35 \pm 0.10 (15) | 4.73 \pm 0.27 (7)*** |
| Underwater PAR | | |
| (mol photons $\text{m}^{-2} \text{d}^{-1}$) | 12.07 \pm 0.42 (362) | 15.44 \pm 0.50 (362)*** |

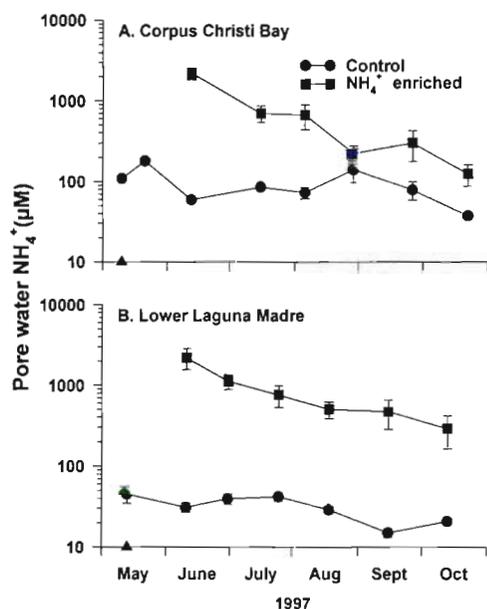


Fig. 2. Sediment pore water NH_4^+ concentrations in control and NH_4^+ enriched seagrass plots in (A) Corpus Christi Bay and (B) Lower Laguna Madre between May and October 1997. Triangle on the x-axis denotes the date of fertilizer additions. Values are means \pm SE ($n = 3$)

weight. Samples were weighed and biomass was converted to an areal estimate (g dry wt m^{-2}). Shoot density was estimated by counting the number of shoots inside the core. Shoot height was estimated by measuring the longest leaf length. Blade width was measured to the nearest 0.5 mm using the mature green leaves in cores collected for biomass.

Leaf production rates were measured using a modified blade marking technique (Zieman 1974, Kentula & McIntire 1986). Five randomly chosen shoots from each plot were marked just above the bundle sheath with a hypodermic needle and then harvested after a period of 3 to 4 wk. Leaf material was separated into leaf tissue produced before and after marking and dried at 60°C to a constant weight. The leaf production rate per shoot was determined by dividing the dry weight of new leaf tissue produced after marking by the number of days since marking. Areal production rates were obtained by multiplying the average leaf production per shoot in a given plot by its corresponding shoot density.

Statistics. All values are reported as means \pm 1 standard error (SE). Statistical analyses were performed on a microcomputer using a general linear procedure (SAS Institute 1989). Data were tested for normality and homo-

geneity of variance to meet the assumptions of parametric statistics, and assumptions were satisfied for all data tested. Differences in sediment nutrients, shoot density, shoot height, blade width and biomass among sampling time and between treatments were tested for significance using a 2-way ANOVA, with time as a block. When a significant difference among variables was observed, the means were analyzed by a Tukey multiple comparison test to determine where the significant differences occurred among variables.

RESULTS

Sediment nutrients

Pore water NH_4^+ concentrations in control plots at CCB (ca $100 \mu\text{M NH}_4^+$) were significantly higher than at LLM (ca $30 \mu\text{M NH}_4^+$) over the 6 mo experimental period ($p < 0.001$). During the first month following NH_4^+ enrichment, the concentrations of sediment pore water NH_4^+ increased to $2000 \mu\text{M NH}_4^+$ at both study sites. By the end of the experiment, 5 mo after NH_4^+ addition, it declined to about $200 \mu\text{M}$, which was still significantly higher than control plots (Fig. 2).

Since pore water $\text{NO}_3^- + \text{NO}_2^-$ and PO_4^{3-} concentrations did not exhibit significant seasonal trends, average values throughout the experiment are presented in Table 2. There were no significant differences in pore water $\text{NO}_3^- + \text{NO}_2^-$ concentrations between study sites ($p = 0.66$) and between NH_4^+ enriched and control plots ($p = 0.48$ and $p = 0.91$ at CCB and LLM, respectively). Pore water PO_4^{3-} concentration in control plots at CCB (ca $24 \mu\text{M PO}_4^{3-}$) was significantly higher than at LLM (ca $15 \mu\text{M PO}_4^{3-}$; $p < 0.001$). However, the pore water PO_4^{3-} concentrations were not significantly different between NH_4^+ enriched and control plots at both CCB ($p = 0.94$) and LLM ($p = 0.79$; Table 2).

Shoot density and leaf morphology

The average shoot density in control plots at LLM (ca 2000 m^{-2}) was significantly higher than at CCB (ca 1700 m^{-2} ; $p < 0.001$). Shoot densities at CCB ranged

Table 2. Average sediment pore water $\text{NO}_3^- + \text{NO}_2^-$ and PO_4^{3-} concentrations in control and NH_4^+ enriched seagrass plots in Corpus Christi Bay (CCB) and lower Laguna Madre (LLM). Values are means \pm SE ($n = 6$)

| | CCB | | LLM | |
|---|------------------|------------------|------------------|------------------|
| | Control | Fertilized | Control | Fertilized |
| $\text{NO}_3^- + \text{NO}_2^-$ (μM) | 3.42 ± 0.38 | 3.05 ± 0.24 | 3.07 ± 0.23 | 3.01 ± 0.24 |
| PO_4^{3-} (μM) | 23.80 ± 3.71 | 24.20 ± 3.54 | 15.08 ± 2.25 | 15.43 ± 1.86 |

from 1200 m⁻² in July to 2100 m⁻² in August and did not change as a result of sediment NH₄⁺ enrichment (Fig. 3A). In LLM, shoot densities in the enriched plots also did not increase relative to controls during the first 4 mo following NH₄⁺ enrichment, but were significantly higher in the enriched plots by October, 5 mo after fertilization ($p = 0.022$; Fig. 3B).

In control plots, shoot height was significantly higher at CCB than at LLM ($p < 0.001$). Sediment NH₄⁺ enrichment had no significant effect on shoot height at CCB ($p = 0.226$). Shoot height at CCB showed a significant seasonal trend ($p < 0.001$); values ranged from 30.3 cm in May to 49.5 cm in August (Fig. 3C). In contrast, shoot height at LLM significantly increased as a result of sediment NH₄⁺ enrichment ($p < 0.001$), increasing continuously in enriched plots from 17.7 cm in May to a maximum of 34.3 cm in September (Fig. 3D). Shoot height in control plots at LLM remained at ca 18 cm throughout the experiment.

Average blade widths in control plots at CCB (6.3 mm) were significantly higher than at LLM (5.2 mm; $p < 0.001$). At CCB, blade width in control plots ranged from 5.9 mm in June to 6.6 mm in October. There was no significant difference in blade width in enriched plots relative to controls (Fig. 3E). In contrast, blade width at LLM increased significantly relative to controls as a result of NH₄⁺ enrichment ($p < 0.001$; Fig. 3F).

Leaf production and plant biomass

Leaf production rates in control plots at CCB (7.35 g dry wt m⁻² d⁻¹) were significantly higher than those at LLM (2.50 g dry wt m⁻² d⁻¹; $p < 0.001$). The rate of leaf production at CCB was about 2-fold higher in fertilized plots relative to controls during the first month following NH₄⁺ enrichment, although there were no significant differences between fertilized and control plots during subsequent months ($p = 0.109$; Fig. 4). Leaf production rates in control plots at CCB showed a strong seasonal trend ranging from 4.86 g dry wt m⁻² d⁻¹ in June to 9.37 g dry wt m⁻² d⁻¹ in August. Sediment NH₄⁺ enrichment significantly stimulated leaf production at LLM ($p < 0.001$; Fig. 4). The average leaf production rate in control plots at LLM was 2.50 g dry wt m⁻² d⁻¹ and 8.21 g dry wt m⁻² d⁻¹ in fertilized plots.

Average leaf biomass in control plots at CCB (281 g dry wt m⁻²) was significantly higher than that at LLM (163 g dry wt m⁻²; $p < 0.001$; Fig. 5). In contrast, average total and rhizome biomass at LLM (889 and 663 g

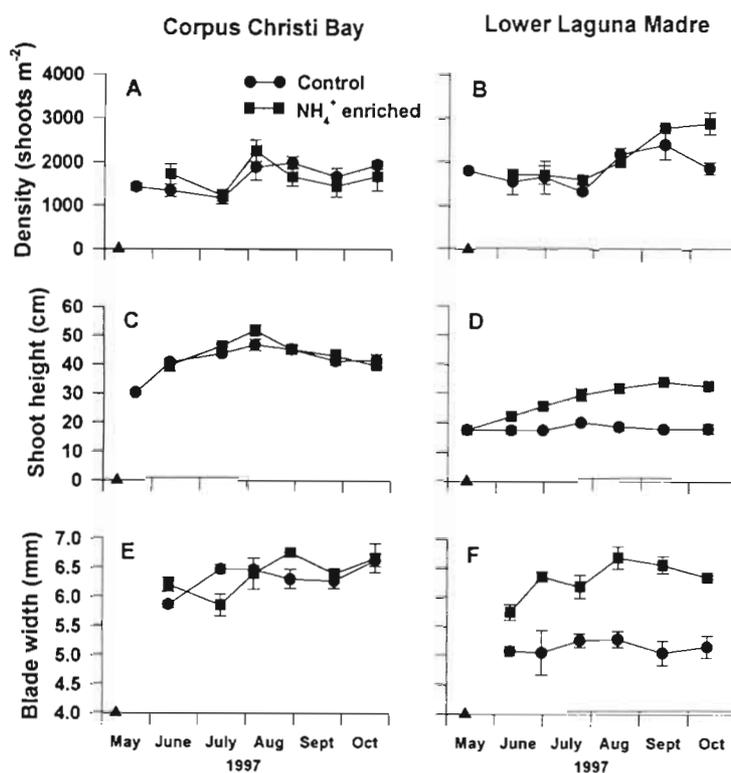


Fig. 3. *Thalassia testudinum*. (A, B) Shoot density, (C, D) shoot height and (E, F) blade width in control and sediment NH₄⁺ enriched plots at Corpus Christi Bay and lower Laguna Madre between May and October 1997. Triangle on the x-axis denotes the date of fertilizer additions. Values are means \pm SE ($n = 3$)

dry wt m⁻², respectively) were significantly higher than those at CCB (712 and 358 g dry wt m⁻², respectively; $p < 0.001$). Root biomass did not differ significantly between study sites ($p = 0.092$).

There was no significant effect of NH₄⁺ enrichment on total biomass as well as the biomass of different tissues at CCB ($p > 0.05$; Fig. 5). However, leaf biomass at the LLM site significantly increased as a result of sediment NH₄⁺ enrichment ($p < 0.001$). Leaf biomass in control plots remained at a weight of about 150 g dry wt m⁻² throughout the experiment, while continuously increasing from 143 g dry wt m⁻² in May to 468 g dry wt m⁻² in September in fertilized plots (Fig. 5). Root and total biomass at LLM did not change during the first 3 mo following NH₄⁺ enrichment, although a significant increase was observed during subsequent months ($p = 0.013$, root and $p < 0.001$, total biomass; Fig. 5). Sediment NH₄⁺ enrichment had no significant effect on rhizome biomass at LLM ($p = 0.54$).

Average below-above-ground biomass ratio in control plots at LLM (4.66) was significantly higher than that at CCB (1.63; $p < 0.001$). About 60% of total biomass was apportioned below ground in control plots at CCB. At LLM, about 82% of total biomass corre-

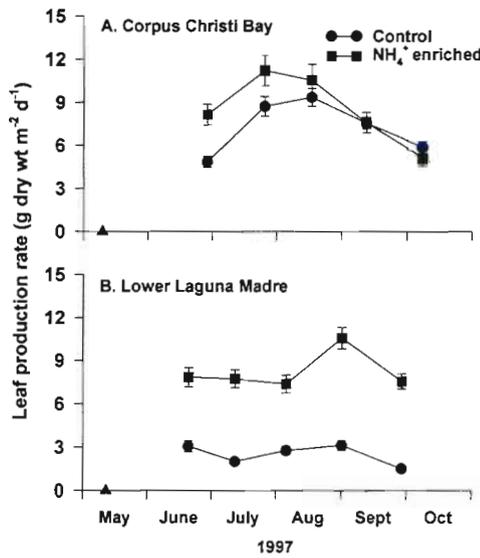


Fig. 4. *Thalassia testudinum*. Leaf production rate ($\text{g dry wt m}^{-2} \text{d}^{-1}$) in control and sediment NH_4^+ enriched plots at (A) Corpus Christi Bay and (B) lower Laguna Madre between May and October 1997. Triangle on the x-axis denotes the date of fertilizer additions. Values are means \pm SE ($n = 3$)

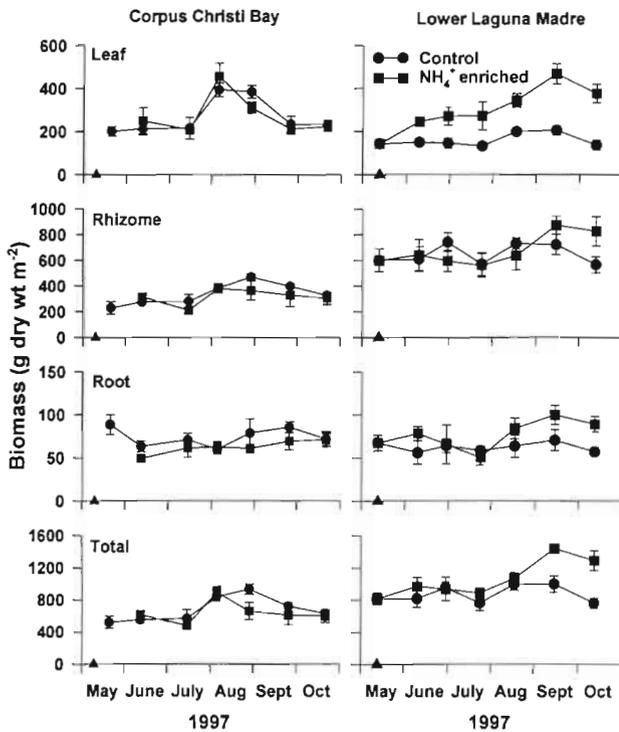


Fig. 5. *Thalassia testudinum*. Biomass of total and different plant components in control and sediment NH_4^+ enriched plots at Corpus Christi Bay and lower Laguna Madre between May and October 1997. Triangle on the x-axis denotes the date of fertilizer additions. Values are means \pm SE ($n = 3$)

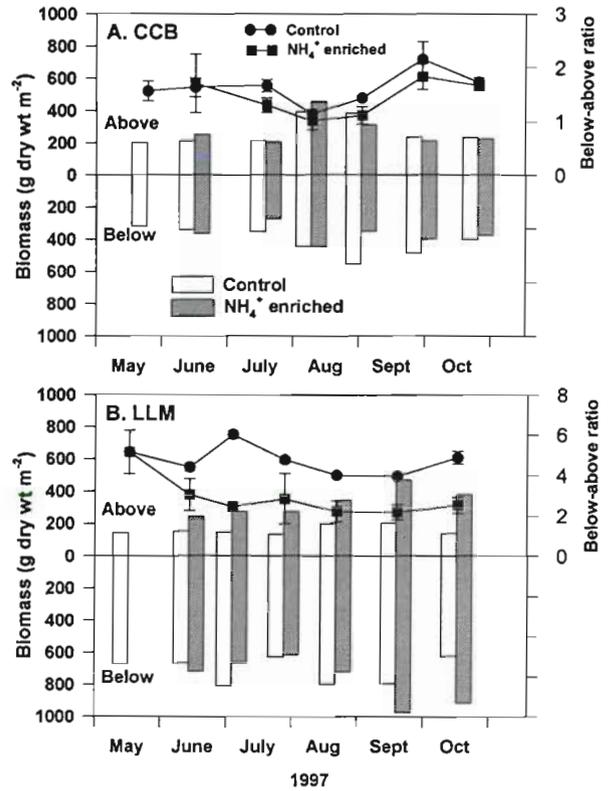


Fig. 6. *Thalassia testudinum*. Above- and below-ground biomass and below-above-ground biomass ratios in control and sediment NH_4^+ enriched plots at (A) Corpus Christi Bay (CCB) and (B) lower Laguna Madre (LLM) between May and October 1997

sponded to below-ground biomass in control plots and 72% of total biomass in fertilized plots. At CCB, there was no significant effect of NH_4^+ enrichment on below-above-ground biomass ratio ($p = 0.133$; Fig. 6). However, below-above-ground biomass ratio in enriched plots (2.53) at LLM was significantly lower than that in control plots (4.66; $p < 0.001$; Fig. 6).

DISCUSSION

N availability for seagrass growth

Increased N availability as a result of NH_4^+ fertilization had little effect on seagrass growth and leaf morphology at site CCB, which is characterized by having a higher ambient sediment pore water NH_4^+ level (ca $100 \mu\text{M}$). Despite a 20-fold increase in N availability as a result of the fertilization, no differences in shoot density, shoot height, blade width and biomass were observed between control and fertilized plots. A similar lack of change in leaf biomass and shoot density in response to enrichment was reported by Bulthuis &

Woelkerling (1981) in a *Heterozostera tasmanica* bed. They hypothesized that the high ambient pore water NH_4^+ level in their study area (200 to 1700 μM NH_4^+) provided an adequate reserve of N for seagrass growth. *Zostera marina* in Great Harbor, Massachusetts, showed little response in leaf production and biomass to sediment NH_4^+ manipulation, which resulted in concentrations of 100 to 1000 μM NH_4^+ (Dennison et al. 1987). Therefore, sediment NH_4^+ availability was considered to be in excess of seagrass N demand.

Short (1983) and Dennison et al. (1987) reported significantly smaller eelgrass leaf size and lower leaf production rates when sediment pore water NH_4^+ concentrations dropped below ca 100 μM , the suggested threshold concentration for N limitation for *Zostera marina*. The proposed threshold value of 100 μM is similar to saturation concentrations reported for *Z. marina* NH_4^+ uptake by root tissues (Iizumi & Hattori 1982, Thursby & Harlin 1982). In the present study, changes in sediment N availability from 100 to 2000 μM NH_4^+ had no significant effect on shoot density, leaf morphology and biomass of *Thalassia testudinum*. The lack of response to sediment NH_4^+ enrichment by *T. testudinum* plants at CCB suggests that ambient pore water NH_4^+ concentrations of 100 μM may provide an adequate pool of inorganic N for *T. testudinum* growth. This suggestion is consistent with reports for various seagrass species (Table 3). Seagrass growth has been limited at the range of ambient pore water NH_4^+ concentration from 7.4 to 137 μM , while seagrasses growing in the range of 100 to

1000 μM have been demonstrated to be provided with sufficient N for growth.

Although sediment NH_4^+ concentrations in seagrass beds are indicative of N limitation of seagrass growth, turnover rates of the sediment NH_4^+ pool and the regeneration of sediment NH_4^+ are also important factors that determine the degree of N limitation. Sediment NH_4^+ pools in seagrass beds have been reported to have rapid turnover rates ranging from 0.3 to 6 d (Capone 1982, Moriarty et al. 1985, Boon et al. 1986, Lee & Dunton 1999b). Bacterial N_2 fixation provides substantial inputs of N in seagrass beds (Capone & Taylor 1980, Capone 1982, Moriarty & O'Donohue 1993). Additionally, bacterial sulfate reduction in sediments is important in terms of the regeneration of sediment nutrients (Hines & Lyons 1982, Holmer & Nielsen 1997). Since NH_4^+ regeneration rates vary spatially and temporally (Jørgensen 1977, Moriarty & O'Donohue 1993), the sediment NH_4^+ pool size for N limitation of seagrass growth should also vary spatially and temporally.

Nitrogen availability in muddy sediments is usually higher than that in sandy sediments (Short 1983, 1987). In the present study, however, the sandy sediments at the CCB site were characterized by higher N availability. Processes of N input into sediments of seagrass meadows are N fixation in the rhizosphere and phyllosphere (Capone et al. 1979, Capone & Taylor 1980, Capone 1982, Moriarty & O'Donohue 1993), settlement of particulate organic matter (Kenworthy & Thayer 1984, Cooper 1989) and N uptake from the water col-

Table 3. Species-specific response to nitrogen fertilization and nitrogen limitation reported in *in situ* seagrass beds during growing season. (+) Increases from controls due to sediment nitrogen fertilization, (ns) no significant change, (L) nitrogen limitation for seagrass growth and (N) no nitrogen limitation

| Species | Ambient pore water NH_4^+ (μM) | Responses to N fertilization | | | | | N limitation | Location | Source |
|--------------------------------|--|------------------------------|--------------|---------------|-------------------|------------------|--------------|-----------------------------|-------------------------------|
| | | Leaf biomass | Shoot height | Shoot density | Shoot Growth rate | Tissue N content | | | |
| <i>Heterozostera tasmanica</i> | 11–19 | + | + | + | + | + | L | Port Phillip Bay, Australia | Bulthuis et al. (1992) |
| <i>Halodule uninervis</i> | 7.4 | + | + | + | + | + | L | Moreton Bay, Australia | Udy & Dennison (1997) |
| <i>Zostera capricorni</i> | 7.4 | ns | ns | ns | ns | + | L | Moreton Bay, Australia | Udy & Dennison (1997) |
| <i>Zostera marina</i> | 30–137 | | | | + | | L | Puget Sound, WA, USA | Williams & Ruckelshaus (1993) |
| <i>Thalassia hemprichii</i> | ca 100 | ns | | ns | ns | + | N | Indonesia | Erfemeijer et al. (1994) |
| <i>Zostera marina</i> | ca 400 | ns | | | ns | ns | N | Chesapeake Bay, USA | Murray et al. (1992) |
| <i>Zostera marina</i> | 100–1000 | ns | | | ns | | N | Great Harbor, MA, USA | Dennison et al. (1987) |
| <i>Thalassia testudinum</i> | 100 | ns | ns | ns | ns | + | N | CCB, TX, USA | Present |
| | 30 | + | + | ns | + | + | L | LLM, TX, USA | study |

umn (Hemminga et al. 1991). There are no available values on N fixation and sedimentation rates in these study areas, but limited riverine input into LLM (Texas Department of Water Resources 1983) is presumed to be one of the factors responsible for low sediment N availability in the LLM.

Short (1987) demonstrated that the sediment geochemistry in seagrass beds is important in determining the limiting nutrient to seagrass growth. Seagrasses growing in terrigenous sediments are N-limited, while seagrass growing in biogenic carbonate sediments are often P-limited due to binding of phosphate in the sediments (Short et al. 1990, Pérez et al. 1991, Fourqurean et al. 1992). In the present study, both CCB and LLM sites have terrigenous sediment, and sediment pore water phosphate concentrations at the study sites (24 and 15 μM at CCB and LLM, respectively) were much higher than those reported in P-limited seagrass beds (0.2 to 2.7 μM ; Short et al. 1990, Fourqurean et al. 1992, Agawin et al. 1996). If *Thalassia testudinum* at LLM is limited by P or both N and P, the growth at LLM should not increase as a result of N fertilization. However, in this study, *T. testudinum* growth and plant size at LLM increased significantly after sediment NH_4^+ enrichment. Therefore, low growth rates and short-narrow leaf morphology at LLM must be the result of low N availability at this site. Lower tissue N content in LLM than CCB also suggested N limitation in LLM (Lee & Dunton 1999a). *T. testudinum* growing in tropical environments and carbonate sediments, however, are likely to be P-limited rather than N-limited.

Plant responses to sediment NH_4^+ enrichment

Ambient sediment pore water NH_4^+ concentration at LLM (ca 30 μM) was approximately one-third that at CCB (ca 100 μM), and NH_4^+ fertilization significantly increased N availability. Leaf production rate, leaf morphology and biomass significantly responded to sediment NH_4^+ enrichment at LLM. Short (1983) reported a strong correlation between sediment NH_4^+ availability and leaf morphology for *Zostera marina*. Plants characterized by short and narrow leaves grew in low sediment N areas, while plants exhibiting long and wide leaves were found in high sediment N areas. In the present study, *Thalassia testudinum* plants in LLM (low sediment NH_4^+) had significantly shorter and narrower leaves than those at CCB (high sediment NH_4^+). Shoot length and blade width at LLM increased significantly as a result of sediment NH_4^+ enrichment. The increased leaf size in enriched plots at LLM was equivalent to that at CCB 4 mo after the NH_4^+ enrichment.

Shoot density was significantly higher at the low sediment NH_4^+ site (LLM) than at the high sediment

NH_4^+ site (CCB). Fertilization experiments contradict this relationship, showing the increase of shoot density as a result of sediment N fertilization at the low sediment NH_4^+ site. Short (1983) noted this contradiction in *Zostera marina* beds. With an increase in N supply by the N fertilization, plants initially respond by producing new shoots. However, plants become larger and self shading occurs after long-term N enrichment. Higher shoot densities become disadvantageous as shoots compete for light. Therefore, plants at LLM will produce fewer, but larger shoots after long-term N fertilization.

Increased leaf production rate as a result of N enrichment has been shown to be the most pronounced response of seagrass in a N-limited environment (Bulthuis & Woelkerling 1981, Agawin et al. 1996, Alcoverro et al. 1997, Udy & Dennison 1997). At ambient sediment NH_4^+ concentrations, leaf production rates at CCB were about 3-fold higher than those at LLM. In fertilized plots at LLM, however, leaf production rates increased significantly to about 3 times that of the control plots. Hence, leaf production rates at LLM after sediment NH_4^+ enrichment were similar to those at CCB. In LLM, increases in leaf production rate, shoot length and blade width in enriched plots to values similar to those observed at CCB suggest that *Thalassia testudinum* growth in the LLM site is limited by low sediment N availability, while plants at CCB are exposed to N-sufficient conditions.

Significant increases in leaf biomass in response to nutrient additions have been reported from various seagrass species (Orth 1977, Bulthuis et al. 1992, Agawin et al. 1996, Udy & Dennison 1997). In the present study, leaf biomass at LLM increased significantly as a result of sediment NH_4^+ enrichment, although there was little change in below-ground biomass. Similar biomass responses have been reported for *Zostera marina* (Orth 1977), *Halodule uninervis* and *Zostera capricorni* (Udy & Dennison 1997). These responses are indicative of a stimulating effect of sediment NH_4^+ enrichment on above-ground biomass. Increased NH_4^+ assimilation due to sediment NH_4^+ enrichment would require more carbon to incorporate assimilated NH_4^+ into amino acids (Turpin et al. 1990, Huppe & Turpin 1994). The increased carbon demand must be supplied by photosynthetic carbon fixation that is conducted in leaf tissues. Consequently, increases in leaf surface area are often associated with enhancement of leaf production rates and biomass to meet the increased carbon demand resulting from increased inorganic N assimilation during NH_4^+ enrichment.

In contrast, plants under sediment nutrient deficient conditions increase biomass allocation to below-ground tissues to expand surface area for nutrient uptake (Gleeson 1993, Vogt et al. 1993). In the present

study, the below/above-ground biomass ratio at LLM was about 3-fold higher than that at CCB. This biomass allocation trend agrees with general plant response to sediment nutrient availability. Since below-ground biomass did not respond to short-term NH_4^+ enrichment, the significant decrease in below-above-ground biomass ratios at LLM due to sediment NH_4^+ enrichment occurred as a result of enhanced leaf biomass. Increased carbon allocation to the shoots as a result of N fertilization and enhancement of below-ground carbon allocation as a result of elevated CO_2 concentrations have been reported in terrestrial grass species (Cotrufo & Gorissen 1997). Therefore, C and N availability may have a reverse effect on biomass allocation to above-or below-ground tissues: increasing root biomass at high C and/or low N availability and increasing leaf biomass at low C and/or high N availability. Changes in plant biomass allocation appear to reflect differences in N availability as the plant responds to imbalances in the ratio of nutrient versus carbon supplies.

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