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Effects of simulated herbivory on nitrogen enzyme levels, assimilation and allocation in *Thalassia testudinum*

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Abstract

Recent estimates suggest that turtlegrass (*Thalassia testudinum*) can persist while being heavily grazed by smaller herbivores (e.g., sea urchins and bucktooth parrotfish) in many areas of the western North Atlantic. Based on studies conducted in terrestrial and aquatic ecosystems, we hypothesized that seagrasses compensate for grazer-induced losses of aboveground production by (1) increasing the uptake of growth-limiting nitrogen from the surrounding environment and (2) translocating this newly acquired nitrogen to grazed tissues. We tested our hypotheses by comparing the activity levels of nitrogen metabolizing enzymes (nitrate reductase (NR) and glutamine synthetase (GS/T)) and the distance of nitrogen translocation, in the roots and leaves of mechanically damaged and undamaged plants. These experiments were repeated along a seasonal productivity gradient (in mid summer, early fall, and late fall). Significantly greater enzyme activity (GS/T) was measured in the roots of clipped plants in all months. NR and GS/T activity was also significantly higher in partially clipped leaves than in unclipped outer leaves in two of three months. The degree of leaf damage had no impact on the enzyme activity of inner (i.e., younger) leaves. Nitrogen-15, as either ammonium or nitrate, was translocated up to 50 cm away from the application point within 48 h. Defoliation, however, did not lead to increased ¹⁵N accumulation in clipped segments, suggesting that turtlegrass does not preferentially translocate nitrogen toward grazed areas.

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Keywords: Glutamine synthetase; Glutamine transferase; Gulf of Mexico; ¹⁵N; Nitrate reductase; Seagrass

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1. Introduction

Herbivory on seagrasses, although reduced in a historical context (Thayer et al., 1984; Jackson, 1997), continues to play an important role in the transfer of energy from plants to animals in many coastal settings (e.g., Valentine and Heck, 1999; Kirsch et al., 2002; Alcoverro and Mariani, 2002). Even so, seagrasses can persist while being grazed (Ogden and Zieman, 1977; Zieman et al., 1984; Mitchell, 1987; Valentine et al., 1997, 2000; Cebrián et al., 1998), and the mechanisms by which seagrasses respond to grazer-induced leaf loss remain unknown (Valentine and Heck, 1999). Based on studies in other ecosystems, we know that plants can compensate for losses to grazers resulting in enhanced growth by either increasing nutrient uptake from the surrounding environment (Lehman and Scavia, 1982; Cargill and Jeffries, 1984; Bianchi, 1988; Williams and Carpenter, 1988; Day and Detling, 1990; Seagle et al., 1992; Sand-Jensen et al., 1994; McNaughton et al., 1996) or translocating nutrients among physiologically integrated ramets (Jónsdóttir and Callaghan, 1990). While seagrasses compensate for leaf loss via the rapid recycling of stored nitrogen (Dawes and Lawrence, 1979; Bjorndal, 1980; Iizumi and Hattori, 1980; Short and McRoy, 1984; Zieman et al., 1984; Pedersen and Borum, 1993) and the transport of carbohydrates along common rhizomes (Libes and Boudouresque, 1987; Tomasko and Dawes, 1989a,b), we do not know whether grazing also triggers increased nitrogen uptake from the surrounding environment by surviving tissues (Valentine and Heck, 2001), or for that matter, whether it is possible for nitrogen to be preferentially translocated along common rhizomes towards areas that have experienced recent leaf loss.

Since nitrogen is required for the synthesis of all enzymes involved in photosynthesis and metabolism, we expected to see increased nitrogen-metabolizing enzymatic activity (either nitrate reductase (NR) or glutamine synthetase (GS)) when turtlegrass leaves were damaged. Nitrate reductase [EC 1.6.6.1], one of the best-characterized, environmentally induced enzymes in plants (Oaks and Hirel, 1985; Kleinhofs et al., 1986; Campbell, 1999), is the enzyme responsible for converting nitrate into nitrite. Glutamine synthetase [EC 6.3.1.2], is common to the biochemical pathways that assimilate both nitrate and ammonium. Therefore, GS should be an indicator of ammonium and nitrate metabolism. It is also environmentally regulated, although not as strongly as NR (Pregnell et al., 1987; Schmidt and Mohr, 1989; Figueroa, 1996).

Here we report the findings of our study which was designed to assess the degree to which turtlegrass responds to varying levels of tissue damage by herbivores by (1) increasing nutrient uptake from the surrounding environment and (2) translocating freshly assimilated nitrogen in unclipped to clipped ramets. In addition, we sought to determine if this response might vary along a seasonal productivity gradient.

2. Materials and methods

2.1. Description of study site

The study site, St. Joseph Bay, Florida, is a sheltered, high salinity embayment in the northeastern Gulf of Mexico (Stewart and Gorsline, 1962; Folger, 1972; Valentine and

Heck, 1993). The bay is oligotrophic, with water column dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) values seldom exceeding 3 and 0.2 μM , respectively (Heck et al., 2000). Phytoplankton abundance is also low, with chlorophyll a (chl a) concentrations usually less than 5 $\mu\text{g chl a/L}$. Photosynthetically active radiation (PAR) is high, with up to 40% of surface irradiance (between 200 and 400 $\mu\text{E}/(\text{m}^2 \text{s})$) reaching the seagrass canopy at mid-day.

A seagrass habitat occupying approximately 25.6 km^2 of bottom occurs within St. Joseph Bay (McNulty et al., 1972). Seagrass production is seasonal, with maximum leaf biomass reaching approximately 150 g ash free dry weight/ m^2 during the summer (Iverson and Bittaker, 1986; Valentine and Heck, 1991, 1993). Large monospecific stands of *Thalassia testudinum*, intermixed with smaller patches of *Halodule wrightii*, *Syringodium filiforme*, and unvegetated sand flats, dominate the seagrass beds and have remained essentially unchanged since at least 1972 (Savastano et al., 1984; Iverson and Bittaker, 1986).

3. Experimental methods

We documented turtlegrass physiological responses to herbivore-induced nutrient losses along a seasonal productivity gradient by conducting three separate simulated herbivory experiments: (1) during the height of the growing season (August), (2) just after the peak of the growing season (September), and (3) when the growing season was largely over (November). The experiments were performed in shallow (<2 m deep) water. Experimental locations were haphazardly selected sites far away from marshes or tidal creeks. Surface run-off from these areas was a concern because it might contain substrate that could induce synthesis or activate nitrogen-metabolizing enzyme activity. This could mask synthesis or activation responses to simulated herbivory. We also selected sites far removed from areas where urchins were present, in an attempt to reduce the impacts of previous grazing events on our experiments.

While nutrients contained in sediment porewaters play a key role in controlling seagrass growth, leaf uptake of nutrients from the water column is also important (Stapel et al., 1996; Terrados et al., 1997). Therefore, each experiment consisted of three treatments designed to test whether seagrasses compensate for grazer-induced leaf loss by increasing nutrient uptake in surviving or partially damaged leaves and/or in the roots of grazed plants. These treatments were: (1) intact shoots with undamaged older (outer) leaves (a control), (2) partially removed older leaves (approximately the top 2.5 cm was removed from the leaves, which ranged in length from 10–12 cm, with scissors while leaving the youngest (inner) leaf intact), and (3) completely removed older leaves (the youngest leaf was left intact) (Fig. 1). Both categories of damage represent the range of leaf damage created by smaller seagrass herbivores such as sea urchins and bucktooth parrotfishes (e.g., Conacher et al., 1979; Greenway, 1976; Lobel and Ogden, 1981; Williams, 1990; Cebrián et al., 1996; Kirsch et al., 2002). Each treatment was replicated three times during each month of the study.

Leaf manipulations were performed on all ramets enclosed within haphazardly located plots (0.25 m^2 in size). Each plot represented a single treatment replicate. Twenty-four hours after manipulations were performed, single samples were collected from each treatment

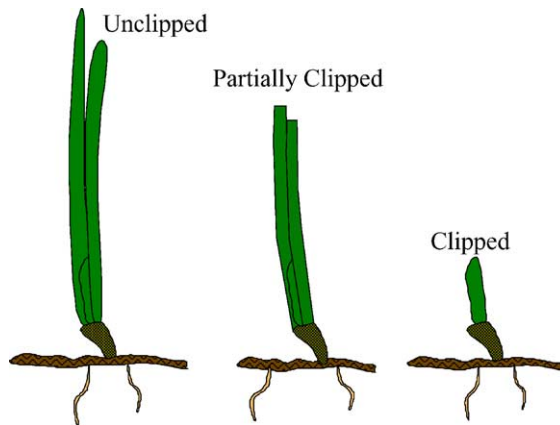


Fig. 1. Simulated herbivory treatments for enzyme activity comparisons in the leaves and roots of turtlegrass.

replicate with a 15-cm diameter PVC core. From each core sample, all similar tissues were pooled into groups (i.e., old undamaged leaves, partially clipped old leaves, young inner leaves, or roots) to obtain enough plant material for enzyme analyses. Tissue samples were placed in plastic pre-labeled tubes, which were placed in liquid nitrogen for transport to the laboratory, where they were stored at -80°C until analysis.

3.1. Enzyme assays

To measure nitrate reductase activity, turtlegrass tissue (100 mg fresh weight (FW) of leaf or root tissue per mL buffer) was homogenized with a mortar and pestle on ice in a buffer containing 50 mM potassium phosphate pH 7.8, 5 mM EDTA, 1 μM FAD (flavine adenine dinucleotide), 5 mM KNO_3 , 5 mM β -mercaptoethanol, polyvinylpyrrolidone polymer (10 mg/mL grinding buffer), and proteinase inhibitors (final concentrations of 10 μM pepstatin, 10 μM leupeptin, and 1 mM phenylmethyl sulfonyl fluoride). Extracts were clarified by centrifugation for 10 min at $12,000 \times g$ at 4°C .

Assays were conducted by combining 1.8 mL assay medium (15 mM potassium phosphate, 10 mM potassium nitrate, and 0.24 mM NADH) and 0.2 mL of clarified plant extracts, and incubating for 30 min at 30°C . The reactions were terminated at high temperature (80°C) for 10 min and phenazine methosulfate was added to a final concentration of 1.5 mM in order to reduce any remaining NADH that might interfere with the colorimetric determination of nitrite formed. Nitrite was quantified spectrophotometrically at 540 nm after the addition of 1 mL of 0.02% *N*-1-naphthylethylenediamine and 1 mL of 1% sulfanilamide in 1.5N HCl.

Glutamine synthetase catalyzes the formation of glutamine from ammonium ions and glutamate, and is coupled to the hydrolysis of ATP (Pregall et al., 1987). This enzyme also possesses glutamine transferase (GS/T) activity. However, the physiological relevance of this activity is unknown (Pregall et al., 1987). This activity catalyzes the exchange of hydroxylamine with the amide group of glutamine to produce γ -glutamyl hydroxamate,

which can be quantified spectrophotometrically. This GS/T activity allows for more sensitive assay of γ -glutamyl hydroxamate. Here we chose to measure GS/T activity instead of directly measuring GS. The procedure used was the same as that described by [Pregnall et al. \(1987\)](#) with only a slight modification of their tissue extraction method. Extracts were prepared as described above for the NR assays with the omission of KNO_3 and FAD from the solution.

Acid phosphatase (AP) was also assayed as a control to determine the general metabolic state of the harvested tissue, and to assure that the enzymes were not being inactivated during tissue disruption and extract preparation. Acid phosphatase is a common name for orthophosphate monoester phosphohydrolase [EC 3.1.3.2]. This enzyme is found in the central vacuole of plants and is involved in the recovery of inorganic phosphate from molecules that are being recycled in that cellular compartment ([Duff et al., 1994](#)). It is commonly assayed through the hydrolysis of *p*-nitrophenyl phosphate (colorless) to form *p*-nitrophenol (yellow in color) and inorganic phosphate. Assays were prepared by combining: 0.5 mL of assay medium (4 mM *p*-nitrophenyl phosphate in 40 mM acetate buffer pH 4.8) with 0.1 mL of clarified tissue extract. Assay tubes were incubated for 30 min at 30 °C. Reactions were terminated with addition of 3.4 mL of 0.1N NaOH. Product was measured spectrophotometrically at 405 nm. Enzymatic activities are reported as μmol s of product per gram of tissue fresh weight per hour (h) for both leaf and root tissue. Corn (leaf and root) and spinach (leaf) tissues were assayed as well to obtain enzyme activity from well-characterized species for comparison with those from the seagrass.

3.2. Stable isotope experiments

To assess the potential for grazing to trigger increased nitrogen sharing among ramets along a common rhizome, we conducted a second set of experiments using ^{15}N as a marker to measure nitrogen transport. To conduct this assessment, we exposed the leaves or roots found along 12 haphazardly selected, 1 m long, rhizomes to either ($^{15}\text{NH}_4$) $_2\text{SO}_4$ or K^{15}NO_3 in sealed labeling chambers that were clamped around the plant. Selected rhizomes were exposed from the sediments to identify the growing tips. Our experimental design consisted of four treatments: (1) labeled shoots on rhizomes where leaves had been removed, (2) labeled roots on rhizomes where leaves had been removed, (3) labeled shoots on rhizomes where leaves were left intact and (4) labeled roots on rhizomes where leaves were left intact. Treatments were equally divided among the 12 rhizomes. The clipping treatments consisted of dividing the selected rhizomes into 2–50 cm lengths. All leaves along the 50-cm section extending from the growing tip inward into the meadow was left intact, while all leaves were removed from the next 50-cm section of rhizome ([Fig. 2](#)). The leaves on the remaining rhizomes were left intact and the labeled chambers were attached 50 cm from the rhizome tip. Label chambers were attached either to the roots or shoots located 50 cm from the growing tip.

To insure a sufficiently high ^{15}N signal for later analysis, the concentration used in the bathing chamber for each nutrient was 5 mM. In the bay, ambient pore water nutrient concentrations were 2.22–15.92 μM NH_4^+ and 0.69–2.41 μM $\text{NO}_3^- + \text{NO}_2^-$; ambient water column nutrient concentrations were 1.27–9.33 μM NH_4^+ and 0.40–1.82 μM $\text{NO}_3^- + \text{NO}_2^-$. Due to differences in volume of the leaf and root chambers, the molar ratio of

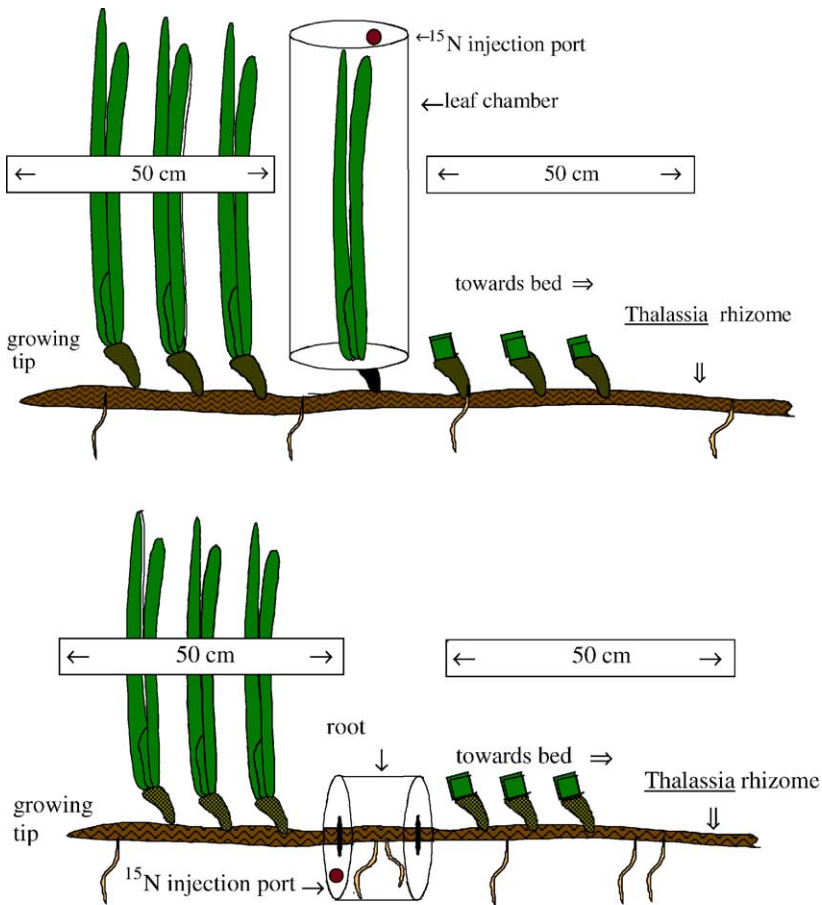


Fig. 2. Experimental design for nitrogen isotope translocation experiment.

nitrogen in the leaf chambers to the root chambers was standardized to 6:1. The significance of this ratio may not be great, however, due to the larger biomass of the leaf tissue as compared to the root tissue. This experiment was also repeated in August, September, and November.

We used labeling chambers constructed of 32 mm thick acrylic to isolate either leaf (a chamber size of 5 cm × 15 cm) or root tissue leaf (a chamber size of 2.5 cm × 5 cm) (Fig. 2). The isotope label was then injected into the acrylic chambers through a rubber septum and left to incubate with the plant tissue for approximately 48 h. After incubation, tissue from the origin of tracer application, along with 1 cm sections of rhizome harvested at 20 and 50 cm in either direction from the point of labeling were collected. Field samples were placed in plastic tubes and kept on ice during transport to the laboratory. The tissue was dried in an oven at 60 °C for 4 days then ground to a fine powder using a mortar and pestle. To prevent cross-contamination of samples between grindings, the mortars and

pestles were washed with soap and water, thoroughly rinsed with distilled water, and rinsed again with ethanol before grinding the next sample. A subsample of this tissue powder was processed through an on-line elemental analyzer (Heraeus, CHN–O Rapid) interfaced with a Finnigan Delta-S isotope ratio mass spectrometer through a trapping box system. Isotope ratio mass spectrometric analysis of labeled material was performed following published procedures (e.g., Nichols and Keeney, 1976; Boon et al., 1986; Martinelli et al., 1992). The ^{15}N contents of the samples are given by $\delta^{15}\text{N} = \{[R(\text{sample})/R(\text{air})] - 1\} \times 1000$, where $R = ^{15}\text{N}/^{14}\text{N}$. The precision of analysis was $\pm 0.3\%$. We did not determine the nature of transported metabolites.

3.3. Statistical analyses

We used two separate statistical analyses to detect the impact of the simulated herbivory treatments on plant enzyme activity. Analysis of the effect of the complete loss of outer leaves on nitrate reductase, glutamine transferase, and acid phosphatase activity in both undamaged young leaves (i.e., inner leaves) and roots was conducted using a two-way analysis of variance (ANOVA) with the time of the experiment (month) and the degree of cropping as treatments. A second two-way ANOVA was used to compare enzyme activity (e.g., nitrate reductase and glutamine transferase) in partially clipped and unclipped outer leaves with month and clipping as treatments. When significant differences were limited to treatments effects (i.e., no interaction as detected), the data were reanalyzed with the interaction term removed from the model. *A posteriori* comparisons of significant treatment effects were made using the Scheffe's multiple comparison test (Day and Quinn, 1989). When significant interactions between treatments were detected, comparisons between factors were not analyzed further statistically, but instead patterns were explained in terms of the interaction.

All the tests above were performed after verification of normality and equal variance (using Levene's test). When the assumption of equality of variance was not met, the data were log transformed then analyzed as discussed above. All test statistics were considered to be significant if $P < 0.05$.

Data from the ^{15}N uptake and translocation experiments were not normally distributed. This led us to use a multivariate nonparametric location test to detect the impacts of clipping on the translocation of ^{15}N (Hettmanesperger, 1984). Because of the small sample size, we had to pool monthly replicates of each treatment to conduct this test. Treatment effects were then analyzed to reveal the predominant directional movement of the label by comparing label in similarly located defoliated or undefoliated rhizome segments. Again, the results of this test were considered significant if $P < 0.05$.

4. Results

4.1. Enzyme activity

The responses of the nitrogen metabolizing enzymes to outer leaf damage from clipping (regardless of whether the whole leaf was completely or partially removed) varied significantly with season (Tables 1 and 2; $P < 0.05$). While treatment effects were not seen in

Table 1

Two-way analysis of variance on NR activity in the young leaves, outer leaves and roots of shoots experiencing varying levels of herbivory in August, September, and November in St. Joseph Bay, Florida

Treatments	d.f.	SS (%)	<i>P</i>
Outer leaves			
Intercept	1	47.6	0.000
Date	1	27.3	0.000
Treatment	1	8.1	0.000
Date × treatment	1	5.2	0.001
Error	7	11.8	
Total	11	100	
Inner leaves			
Intercept	1	34.3	0.000
Date	1	28.7	0.000
Treatment	2	8.9	0.000
Date × treatment	2	18.5	0.000
Error	10	9.6	
Total	16	100	
Roots			
Intercept	1	26.5	0.000
Date	2	47.5	0.000
Treatment	2	3.8	0.178
Error	22	22.2	
Total	27		

Sum of squares are presented as a proportion of the total sum of squares. Differences were considered significant when $P < 0.05$. Bolding indicates statistically significant differences.

August or September, strong treatment effects were apparent for both nitrogen metabolizing enzymes in November. In August, GS/T activity in the leaves and roots of clipped shoots was similar or slightly lower than in the leaves and roots of the control treatments (Fig. 3). Similarly, strong treatment effects on leaf GS/T activity were not detected in September. GS/T activity was greater in roots in September than in August, with the only apparent treatment effect being a reduction in GS/T activity in the roots of plants with partially clipped leaves. In contrast, in November GS/T activity was consistently higher in both the leaves and roots of clipped plants, regardless of treatment, than in the unclipped plants. These findings indicate that the impacts of herbivory on GS/T activity are strongly mediated by the season in which leaf damage occur.

Significant month and treatment effects and significant month × treatment interactions were detected for NR activity in young leaves and remaining outer leaves of partially damaged plants, respectively. As with GS/T activity, NR activity was highest in November (Fig. 4) and leaf clipping, regardless of treatment, or leaf age was found to lead to greater activity than measured in the leaves and roots of unclipped shoots. Leaf NR activity was below detection limits in August. NR activity in the roots of clipped and unclipped plants also varied significantly with time. While clipping was also observed to trigger noticeably higher NR activity in roots in August and November (Fig. 4), the treatment variance was large and differences were not found to be significant in our experiments.

Table 2

Two-way analysis of variance on GS/T activity in the young leaves, outer leaves and roots of shoots experiencing different levels of herbivory in August, September, and November in St. Joseph Bay, Florida

Treatments	d.f.	SS (%)	P
Outer leaves			
Intercept	1	85.5	0.000
Date	2	2.1	0.021
Treatment	1	2.6	0.003
Date × treatment	2	5.5	0.001
Error	11	4.3	
Total	17		
Inner leaves			
Intercept	1	74.4	0.000
Date	2	0.8	0.282
Treatment	2	2.2	0.048
Date × treatment	4	10.4	0.001
Error	16	12.2	
Total	25		
Roots			
Intercept	1	71.9	0.000
Date	2	8.7	0.003
Treatment	2	1.9	0.201
Date × treatment	4	7.7	0.028
Error	18	9.8	
Total	27		

Sum of squares are presented as a proportion of the total sum of squares. Differences were considered significant when $P < 0.05$. Bolding indicates statistically significant differences.

We found little evidence of treatment effects (either for month the experiment was conducted or the degree of outer leaf damage; Table 3) on acid phosphatase activity (Fig. 5). However, clipping impacts on the AP activity of outer leaves were found to change with time for outer leaves (month × treatment interaction, $P < 0.05$). Inspection of the data suggests that AP activity was higher in unclipped outer leaves in August. No other differences were noted. This trend was expected, as AP is a housekeeping metabolic enzyme that is involved in recycling of intracellular phosphate.

4.2. ¹⁵Nitrogen uptake and translocation

¹⁵N-nitrate and ¹⁵N-ammonium were taken up by the leaves and rhizome/roots. ¹⁵N enrichment was always greatest in and around the labeling site, regardless of where the isotope was introduced. Enrichment for ¹⁵N was found at least 50 cm from the labeling site within 48 h of the experiments initiation indicating that turtlegrass can translocate nitrogen a substantial distance in a short period of time. This was true for both ¹⁵NH₄ and ¹⁵NO₃ labels and in treatments with and without leaf removal (Figs. 6–9). Although the tracer experiments revealed some increases in label accumulation in the defoliated segments of clipped turtlegrass rhizomes, the responses were not statistically significant regardless if

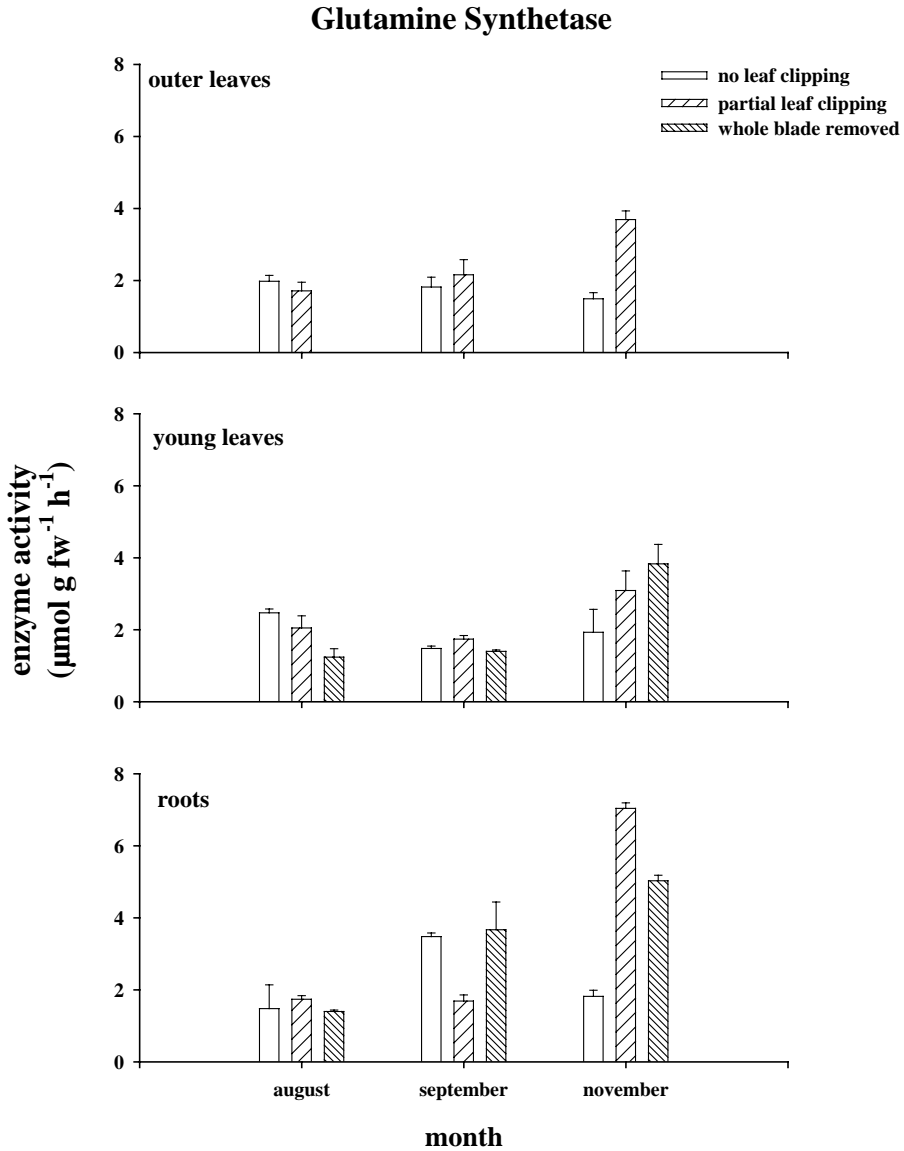


Fig. 3. Glutamine transferase activity (mean \pm S.D., $n = 3$) in the (a) young leaves, (b) roots and (c) outer leaves of shoots whose outer leaves were untouched, partially clipped or wholly removed in August, September, and November.

treatments were labeled at the roots ($\chi^2 = 8.389$, d.f. = 4, $P > 0.07$ and $\chi^2 = 4.36$, d.f. = 4, $P > 0.36$ for treatments where roots were labeled with $^{15}\text{NH}_4$ and $^{15}\text{NO}_3$, respectively) or the leaves ($\chi^2 = 4.36$, d.f. = 4, $P > 0.36$ and $\chi^2 = 4.23$, d.f. = 4, $P > 0.37$ for treatments where leaves were labeled with $^{15}\text{NH}_4$ and $^{15}\text{NO}_3$, respectively). These results suggest that

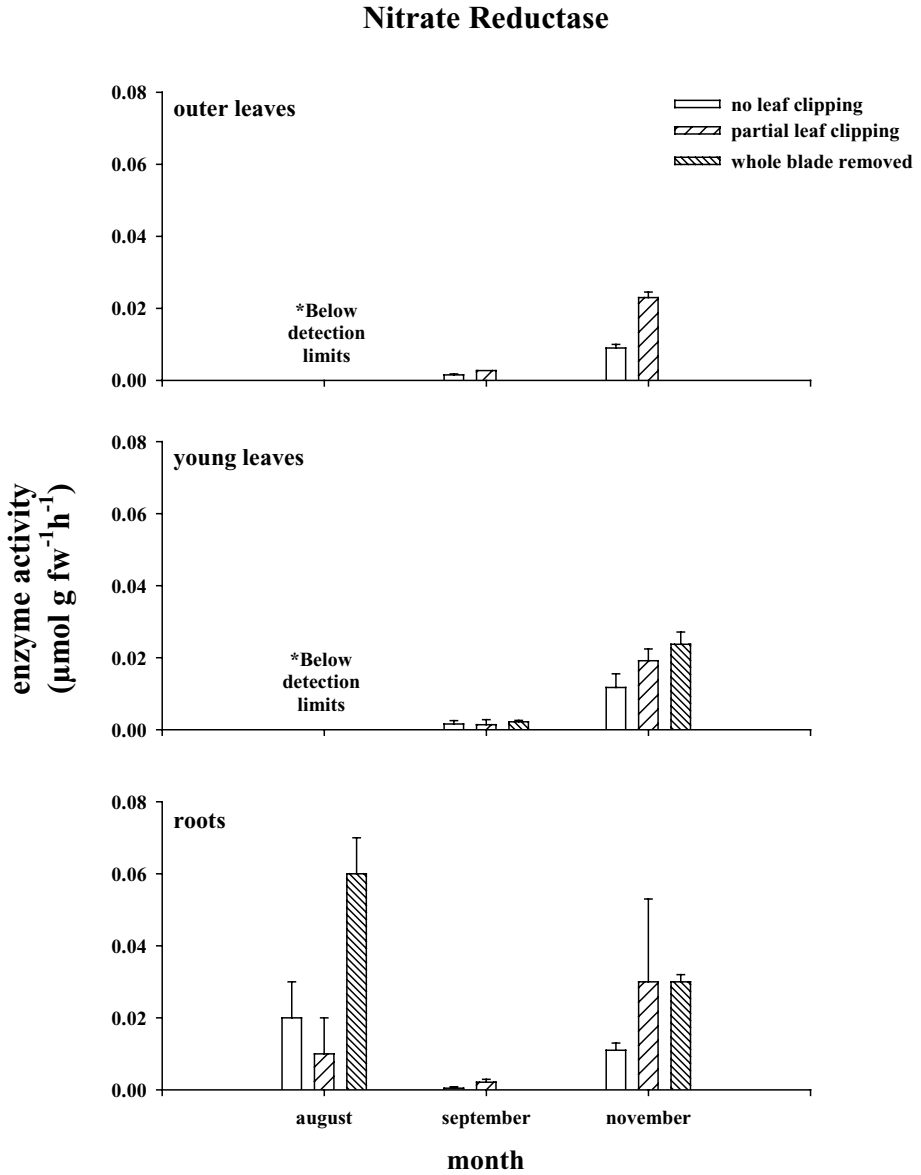


Fig. 4. Nitrate reductase activity (mean \pm S.D., $n = 3$) in the (a) young leaves, (b) roots and (c) outer leaves of shoots whose outer leaves were untouched, partially clipped or wholly removed in August, September, and November.

Table 3

Two-way analysis of variance on AP activity in the young leaves, outer leaves and roots of shoots experiencing different levels of herbivory in August, September, and November in St. Joseph Bay, Florida

Treatments	d.f.	SS (%)	<i>P</i>
Outer leaves			
Intercept	1	94.5	0.000
Date	2	0.4	0.008
Treatment	1	0.2	0.024
Date × treatment	2	0.6	0.002
Error	11	4.3	
Total	17		
Inner leaves			
Intercept	1	92.3	0.000
Date	2	0.5	0.378
Treatment	2	0.3	0.515
Date × treatment	4	1.5	0.218
Error	17	5.4	
Total	26		
Roots			
Intercept	1	68.1	0.000
Date	2	3.0	0.236
Treatment	2	2.6	0.285
Date × treatment	4	9.4	0.081
Error	18	16.9	
Total	27		

Sum of squares are presented as a proportion of the total sum of squares. Differences were considered significant when $P < 0.05$. Bolding indicates statistically significant differences.

turtlegrass does not preferentially translocate nitrogen to compensate for nitrogen lost in clipped leaves, counter to our expectations.

In contrast to the low nitrogen metabolizing enzyme activity measured in the seagrass tissues, terrestrial crop plants that were used as assay controls had relatively high activity (Table 4).

Table 4

Comparison of enzyme activity assayed from turtlegrass and two well-characterized crop plants

	NR activity (μmol $\text{NO}_2/(\text{h g FW})$)	GS/T activity (μmol $\gamma\text{-glutamyl}$ $\text{hydroxymate}/(\text{h g FW})$)	AP activity (μmol $p\text{-nitrophenol}/(\text{h g FW})$)
<i>Thalassia</i>			
Leaf	0.02	1.2–3.7	0.4–1
Roots and rhizomes	0.6	1.2–7.5	0.1–0.2
Corn			
Leaf	7.7	795	
Roots and rhizomes	1.4	555	
Spinach			
Leaf	110	2500	300–380

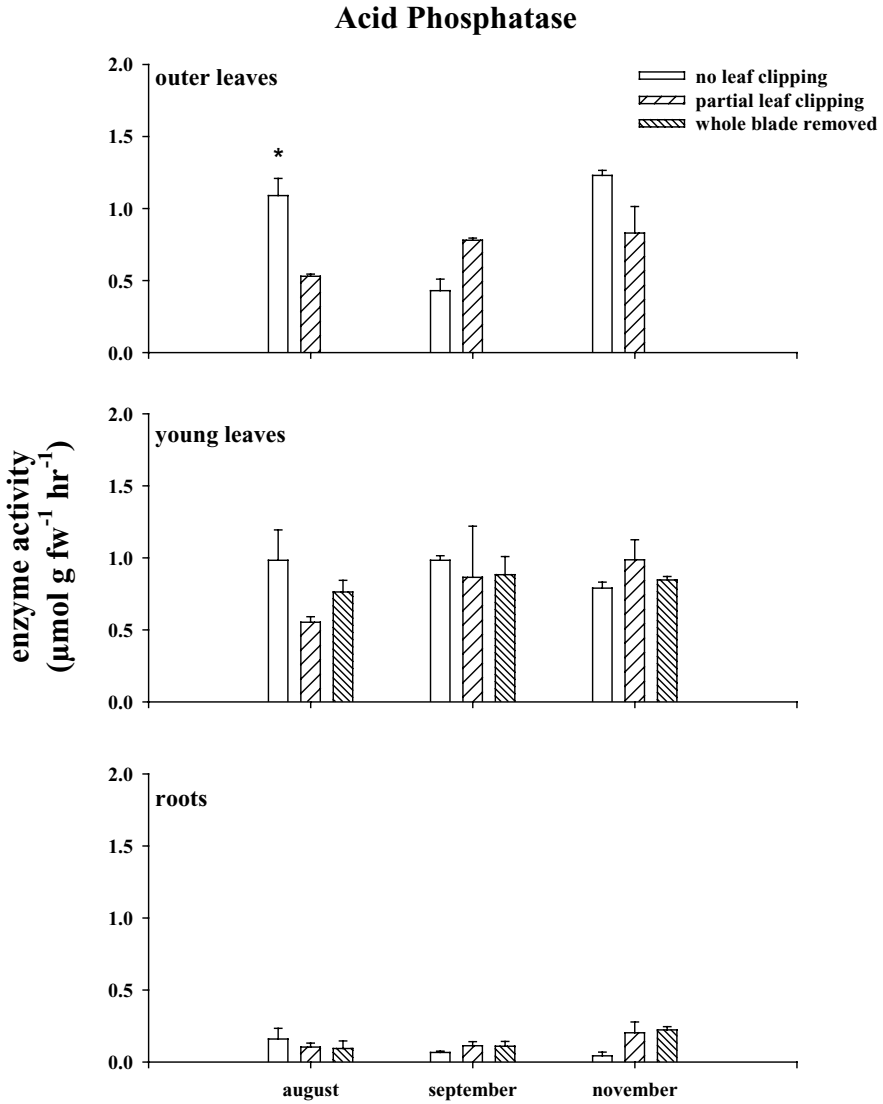


Fig. 5. Acid phosphatase activity (mean \pm S.D., $n = 3$) in the (a) young leaves, (b) roots and (c) outer leaves of shoots whose outer leaves were untouched, partially clipped or wholly removed in August, September, and November.

5. Discussion

Physiological effects of grazer-induced leaf loss are not well understood (Dawes and Lawrence, 1979; Valentine et al., 1997, 2000; Cebrián et al., 1998; Valentine and Heck, 1999, 2001). Because grazing damages photosynthetic tissues, it may be critical that seagrasses

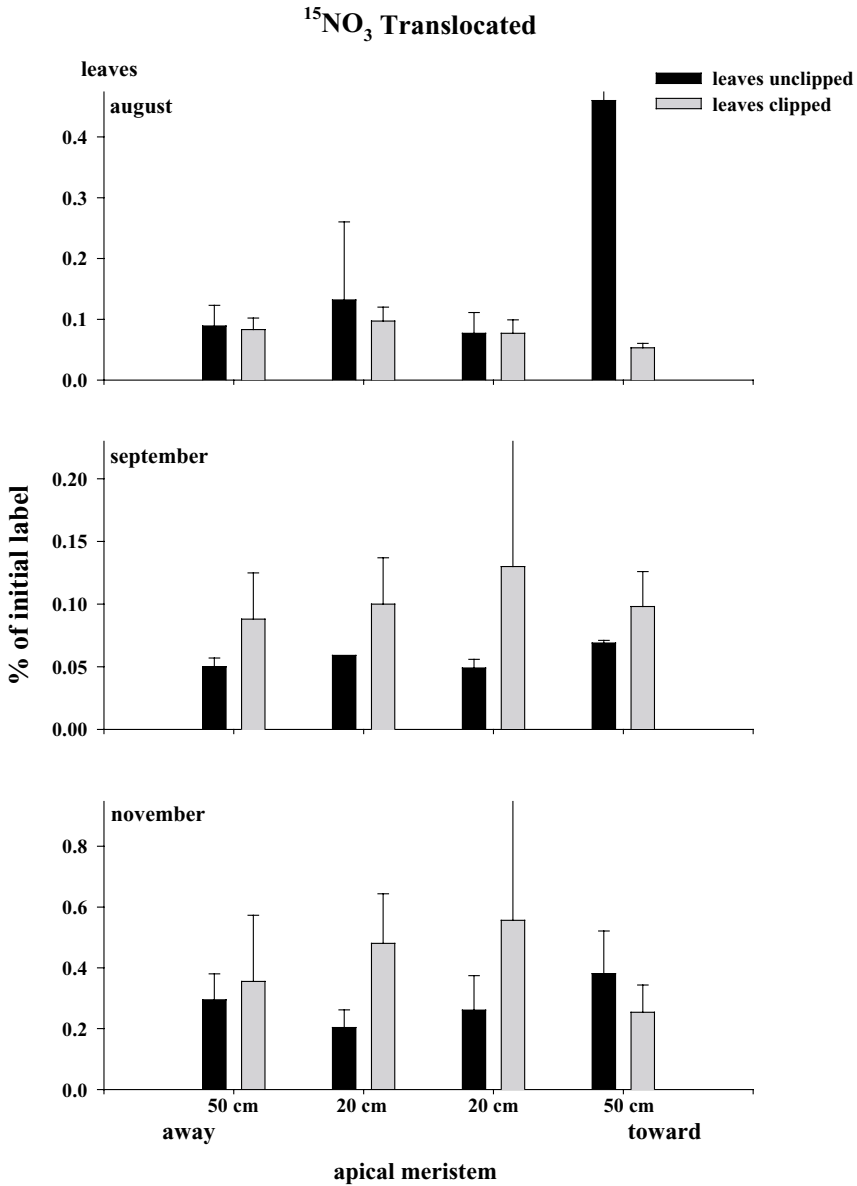


Fig. 6. Translocation of $^{15}\text{NO}_3$ along a rhizome in August, September, and November. The label was introduced to the plant through the leaves of a single shoot.

rebuild these tissues to sustain growth (Kraemer et al., 1997; Hemminga et al., 1999; Stapel and Hemminga, 1997). To do this, the synthesis of large quantities of proteins as well as chlorophyll should be needed. Since both of these compounds contain large amounts of nitrogen, we hypothesized that the enzymes involved in nitrogen assimilation would be

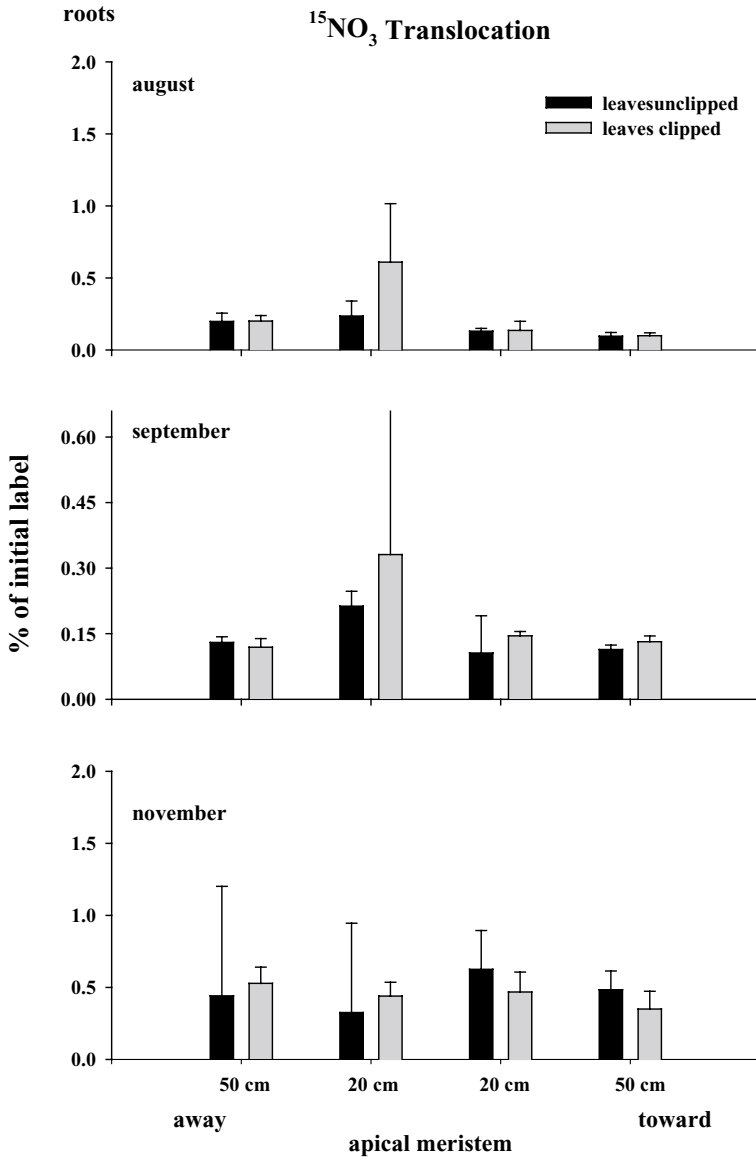


Fig. 7. Translocation of ¹⁵NO₃ along a rhizome in August, September, and November. The label was introduced to the plant through the roots of a single shoot.

among the first to be used in the recovery effort, as has been shown in plant-herbivore studies conducted in other ecosystems (Lehman and Scavia, 1982; Cargill and Jeffries, 1984; Bianchi, 1988; Williams and Carpenter, 1988; Sand-Jensen et al., 1994). Seagrasses are opportunistic plants that take nutrients up both from interstitial sediment porewater (e.g.,

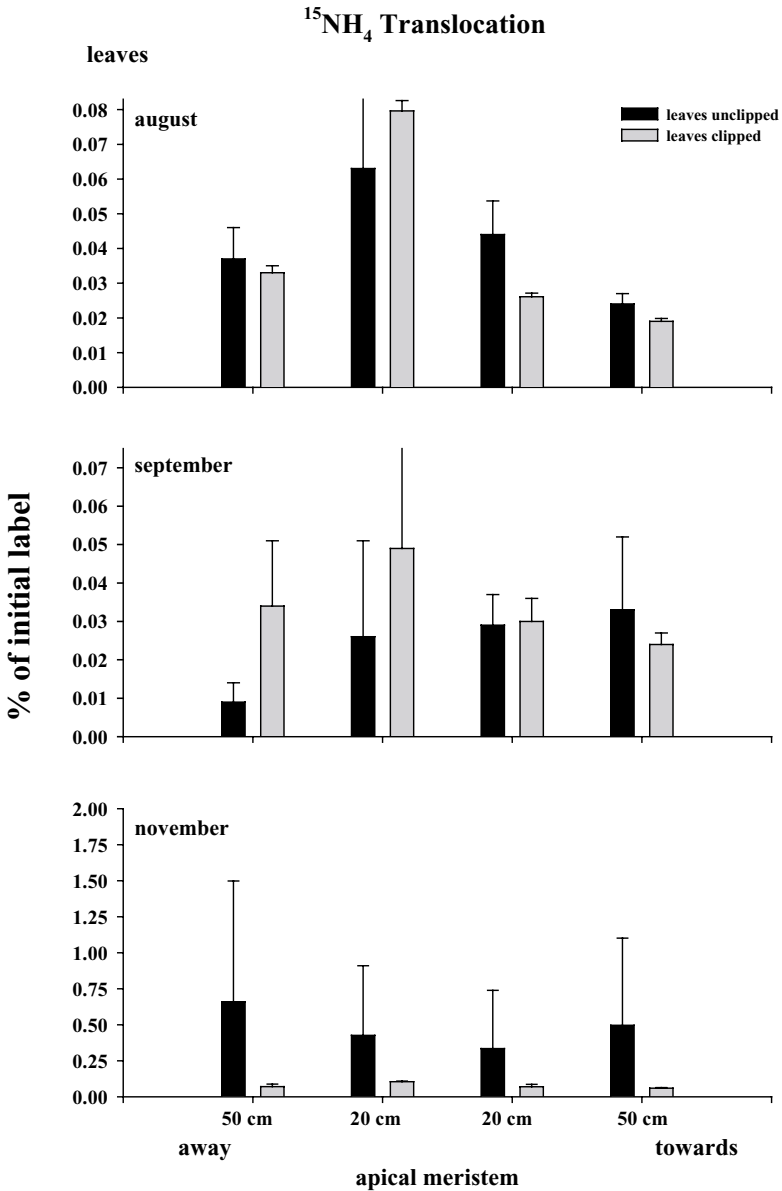


Fig. 8. Translocation of $^{15}\text{NH}_4$ along a rhizome in August, September, and November. The label was introduced to the plant through the leaves of a single shoot.

McRoy and McMillan, 1977; Penhale and Thayer, 1980; Short and McRoy, 1984; Pregnell et al., 1987), and the water column (Patriquin, 1972; Thursby and Harlin, 1982; Short and McRoy, 1984; Brix and Lyngby, 1985; Roth and Pregnell, 1988; Pedersen and Borum, 1992, 1993; Stapel et al., 1996; Terrados and Williams, 1997). As a result, we monitored

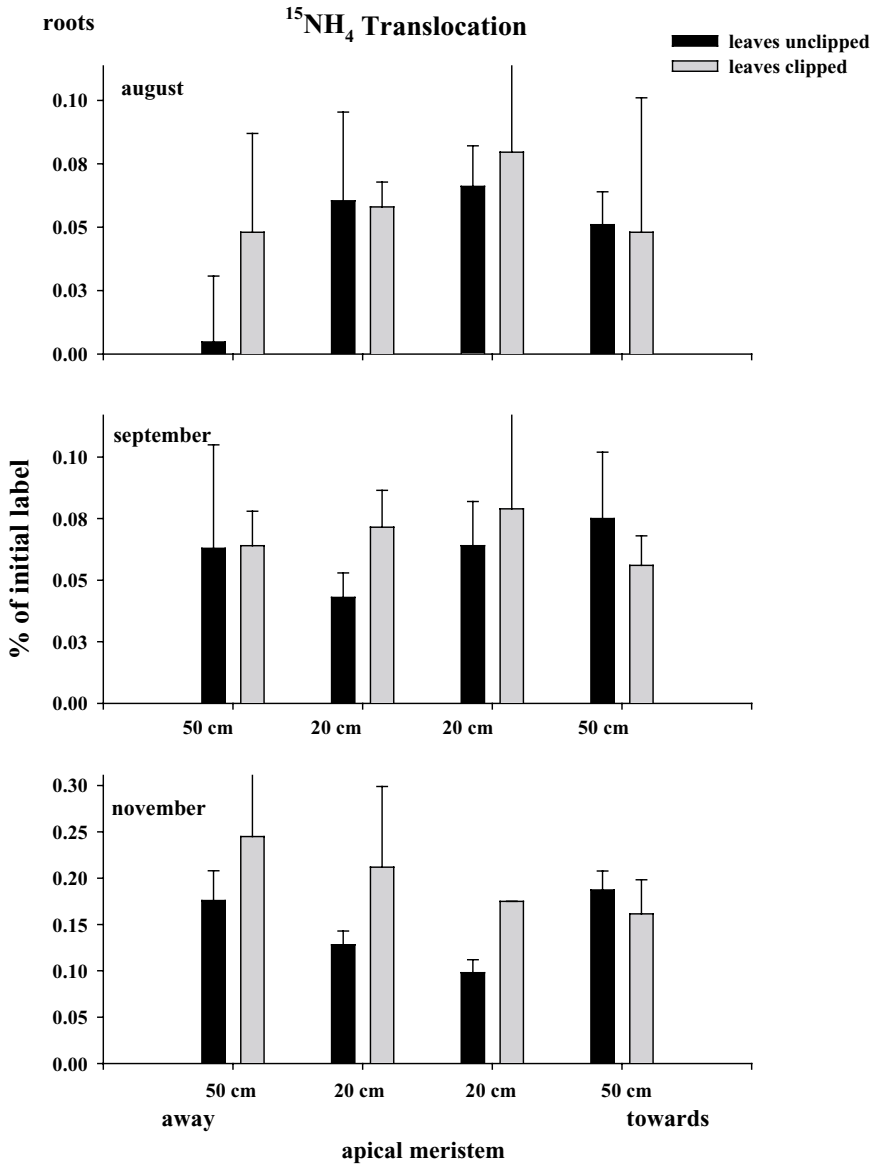


Fig. 9. Translocation of $^{15}\text{NH}_4$ along a rhizome in August, September, and November. The label was introduced to the plant through the roots of a single shoot.

the activities of two enzymes (NR and GS/T), both in the leaves and in the roots, in hopes of detecting grazer-induced increases in nitrogen uptake from the surrounding environment.

In our study, we did find that the impacts of simulated herbivory on nitrogen uptake by turtlegrass varied significantly with season. As a result, clear interpretation of turtlegrass

responses (in terms of GS/T and NR activity) to clipping were difficult. While the partial and the complete removal of outer leaves led to consistently higher GS/T and NR activity in the clipped plants than measured in unclipped plants in November, few differences were noted in the other months of this experiment. We also found no evidence that the clipping of leaves along common rhizomes led to increased levels of nitrogen translocation towards grazed areas.

The reasons for these modest responses to simulated grazing by smaller herbivores remain uncertain. It is possible that the length of our experiments was too short to adequately test our hypothesis, but it is of note that previous investigators have found significant changes in carbohydrate translocation along common seagrass rhizomes in as little as 24 h after leaf clippings (Tomasko and Dawes, 1989b; Libes and Boudouresque, 1987) and that 24-h incubation periods are standard for terrestrial plants (Taiz and Zeiger, 2002).

It is also possible that turtlegrass growth is not controlled by nitrogen availability in St. Joseph Bay, Florida. Recent studies conducted in this bay have found that nitrogen enrichment of either the water column or sediment porewaters can significantly increase nitrogen content in turtlegrass leaves yet have no impact on net aboveground turtlegrass production (Heck et al., 2000; Valentine and Heck, 2001). In addition, a preliminary analysis of the C:N:P ratios of turtlegrass leaves collected from the study area yielded values that were similar to those reported by Fourqurean et al. (1992) from phosphorus limited turtlegrass in Florida Bay (Fourqurean pers. comm.).

Previous studies have shown that turtlegrass can compensate for the losses of leaf tissue to herbivores by either increasing the production of surviving aboveground tissues or by increasing the production of young shoots. This increased growth can rapidly replace local biomass thus allowing seagrasses to persist even under chronic grazing (Zieman et al., 1984; Mitchell, 1987; Valentine et al., 1997, 2000; Valentine and Heck, 2001). We believe that this ability to increase leaf renewal has led to a significant underestimate of the amount seagrass production being consumed by marine herbivores in some areas. To date, few studies have considered the rate at which seagrasses replace damaged tissues or the processes that aid in this replacement, as has been shown to be important in other aquatic plant–herbivore interactions (Lehman and Scavia, 1982; Bianchi, 1988; Williams and Carpenter, 1988; Sand-Jensen et al., 1994; Valentine and Heck, 1999). How these plants respond physiologically to this form of damage remains uncertain, but increased uptake of nitrogen from sediment porewaters and the overlying water column were not found to be the probable pathways for this compensation in St. Joseph Bay. As such, we believe that there is a need to develop a better understanding of the mechanisms by which seagrasses compensate for losses to grazers before we will be able to accurately estimate energy flow from seagrasses to nearshore food webs.

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