

Inorganic nitrogen acquisition in the seagrass *Thalassia testudinum*: Development of a whole-plant nitrogen budget

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Abstract

Whole-plant nitrogen (N) uptake experiments were used to quantify the N budget of *Thalassia testudinum* growing under different sediment nutrient regimes at two locations in the western Gulf of Mexico. At both sites, Corpus Christi Bay (CCB) and lower Laguna Madre (LLM), Texas, concurrent measurements of plant biomass and levels of dissolved inorganic nitrogen (DIN) in the water column and sediments were made over a 12-month period (October 1996–October 1997). Water-column NH_4^+ and $\text{NO}_3^- + \text{NO}_2^-$ concentrations were not significantly different between study sites (ca. $1.2 \mu\text{M}$ [NH_4^+] and $0.7 \mu\text{M}$ [$\text{NO}_3^- + \text{NO}_2^-$] at both sites), but sediment NH_4^+ concentrations in CCB ($87 \mu\text{M}$) were significantly higher than in LLM ($26 \mu\text{M}$). The higher sediment NH_4^+ levels at CCB correlated with significantly higher leaf biomass at CCB, but there was no difference in root biomass between study sites. Leaf NH_4^+ uptake showed clear seasonal variation: V_{max} was highest in summer and fall, but K_m was highest in winter. V_{max} of leaf NO_3^- uptake did not change with season, but K_m decreased with increasing incubation temperature. There were no clear differences in leaf NH_4^+ and NO_3^- uptake rates between study sites, although leaf NH_4^+ uptake affinity was higher than that of NO_3^- . Root NH_4^+ uptake was variable with season and did not saturate at the experimental NH_4^+ concentrations at either site (0 – $300 \mu\text{M}$). Based on these measurements, N acquisition was highest during summer and fall and lowest during winter and spring. Roots and leaves contributed nearly equally to total plant N acquisition (root $\text{NH}_4^+ = 52\%$; leaf $\text{NH}_4^+ = 38\%$; and leaf $\text{NO}_3^- = 10\%$) at both sites. Annual N acquisition in CCB was double that of LLM (97.03 and $53.49 \text{ g N m}^{-2} \text{ yr}^{-1}$, respectively), but $>50\%$ of N uptake was not incorporated into biomass at either site. DIN turnover time ranged from 0.21 to 0.91 d in the water column and from 0.95 to 1.75 d in sediment pore water, indicating the importance of DIN regeneration processes for supporting seagrass production. The similarity in the relative tissue contributions between plants at both sites, despite a significant difference in sediment NH_4^+ pool sizes, results from the higher fraction of biomass allocated to below-ground tissues in plants living under low-sediment N conditions (LLM). In N-sufficient sediments, overall plant productivity is greater as *T. testudinum* is able to allocate a greater proportion of its biomass into photosynthetic aboveground tissues.

Throughout coastal ecosystems of the world, seagrasses are known to achieve high levels of production (McRoy and McMillan 1977; Zieman and Wetzel 1980). Because of their rapid seasonal growth, seagrasses require high N incorporation and play an important role in the cycling of N in shallow estuarine ecosystems, which likely contributes to the high productivity of these regions (Kenworthy et al. 1982; Hemminga et al. 1991; Blackburn et al. 1994). Dissolved organic matter (DOM) released through exudation, leaching, and decomposition of seagrass tissues is an important source of nutrients in the water column and sediments (Wood and Hayasaka 1981; Moriarty and Pollard 1982; Smith et al. 1984; Boon et al. 1986; Moriarty et al. 1986; Chin-Leo and Benner 1991). This organic matter also can control abun-

dance and production of heterotrophic bacteria, which transfer seagrass production to the microbial food webs and higher trophic levels (Wood and Hayasaka 1981; Moriarty et al. 1986; Blum and Mills 1991). Since seagrasses are able to utilize either the sediments or the water column for an N source, N cycles in seagrass beds are complex. In addition, little is known about the roles of leaves vs. roots in N acquisition and whole-plant N budgets.

In contrast to terrestrial plants, aquatic vascular plants, including seagrasses, can take up inorganic N through both leaf and root tissues (Iizumi and Hattori 1982; Thursby and Harlin 1982, 1984; Short and McRoy 1984; Stapel et al. 1996; Pedersen et al. 1997; Terrados and Williams 1997). The major N sources for seagrasses are NH_4^+ and NO_3^- in the water column for leaves and NH_4^+ in sediment pore waters for roots. With the exception of areas characterized by high river inflow, water-column NH_4^+ and NO_3^- concentrations in seagrass beds are usually $<3 \mu\text{M}$ (Tomasko and Lapointe 1991; Dunton 1996; Stapel et al. 1996; Terrados and Williams 1997). By contrast, pore-water NH_4^+ concentrations range from $<20 \mu\text{M}$ to well over $200 \mu\text{M}$, significantly higher than that of the water column (Bulthuis and Woelkerling 1981; Iizumi et al. 1982; Fourqurean et al. 1992; Dunton 1996). Thus, sediment pore waters are often considered the main source of N for seagrass growth (Iizumi and Hattori 1982; Short and McRoy 1984; Zimmerman et al. 1987).

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Nevertheless, there is evidence that leaf tissues have higher N uptake affinities than root tissues at low DIN concentrations (Pedersen et al. 1997), and several studies have indicated that uptake by leaves can contribute considerably to the total N acquisition of seagrasses (Short and McRoy 1984; Pedersen and Borum 1992; Stapel et al. 1996; Terrados and Williams 1997). In addition, Zimmerman et al. (1987) developed a numerical model of N uptake for *Zostera marina*, and predicted that N acquisition by root tissues would not exceed 80% of the total plant N demands, even when pore-water NH_4^+ concentrations were $>500 \mu\text{M}$. Work by Iizumi and Hattori (1982) indicated that 55% of the N required for growth in *Z. marina* was supplied by the NH_4^+ in the sediment pore water. However, no in situ quantitative work has directly addressed or confirmed the relative importance of water-column vs. sediment pore-water DIN in the annual N budgets of seagrasses.

Tissue N uptake rates are dependent on photosynthesis because the energy for tissue N uptake is supplied by photosynthesis (Turpin et al. 1990; Huppe and Turpin 1994). Assimilation of N also requires carbon (C) skeletons produced by photosynthesis for NH_4^+ incorporation into amino acids. Since seagrass photosynthetic performance is closely correlated with water temperature and underwater irradiance, both of which exhibit seasonal trends (Herzka and Dunton 1997), N uptake rates should also vary seasonally. Leaf and root N uptake kinetics have been quantified for several seagrass species (Iizumi and Hattori 1982; Thursby and Harlin 1982, 1984; Short and McRoy 1984; Stapel et al. 1996; Pedersen et al. 1997; Terrados and Williams 1997), but there have been no studies of seasonal N uptake kinetics, which are required for calculation of whole-plant N budgets.

Two monotypic meadows of *T. testudinum* characterized by significantly different pore-water NH_4^+ concentrations but similar water-column DIN levels, were chosen as study sites in CCB and LLM, Texas. The present study addresses seasonal differences in NH_4^+ and NO_3^- uptake by leaves and NH_4^+ uptake by roots to generate annual whole-plant N budgets based on field measurements of DIN levels and plant biomass collected within two seagrass beds in the southwestern Gulf of Mexico. Because previous work has demonstrated that seagrass growth in the LLM is strongly N regulated (Lee and Dunton 1999), we hypothesized that (1) the relative contributions by leaf and root tissues to total N acquisition varied between the two study areas, and (2) these differences would influence rates of DIN turnover in the water column and sediment pore waters.

Materials and methods

Study sites—Experimental plants were collected from monotypic meadows of *T. testudinum* in CCB (27°49'N, 97°07'W) and LLM (26° 09'N, 97°12'W). These study sites, about 200 km apart, are located at a similar water depth (1.2 m) and have been the focus of several recent investigations on south Texas seagrasses (Dunton 1990, 1994; Quammen and Onuf 1993; Czerny and Dunton 1995; Lee and Dunton 1996, 1997; Herzka and Dunton 1997, 1998; Kaldy 1997). A greater percentage of the plant biomass in LLM is allo-

cated to belowground tissues, as reflected in below/above-ground biomass ratios at LLM (4.66) that are significantly higher than at CCB (1.63; Lee 1998).

Plant collection—Plants were collected seasonally (February, May, July, and October 1997) using a 15-cm-diameter core driven about 20 cm into the sediments. Intact cores ($n = 8-12$) were placed in 20-liter buckets for transport to the laboratory. Upon arrival, plants were gently cleaned of sediment using filtered seawater. The plants were separated into individual shoot units that included healthy leaves, roots, and a 3-cm horizontal rhizome. Epiphytes were removed by gently scraping. The plants were maintained in aerated filtered seawater at an ambient field temperature in preparation for incubations the following day.

Biomass samples ($n = 4-5$) were collected monthly with a 9-cm-diameter core as described above. Plants were thoroughly cleaned of epiphytes and sediments; separated into leaf, rhizome, and root tissues; and dried at 60°C to a constant weight. Biomass was expressed as areal estimates (grams dry weight per square meter).

Water-column and sediment DIN—To determine water-column NH_4^+ and $\text{NO}_2^- + \text{NO}_3^-$ concentrations, four replicate surface-water samples were collected monthly at the two study sites around midday from October 1996 to November 1997 and were frozen pending chemical analysis. In addition, sediment pore-water NH_4^+ concentrations were determined from four replicate sediment samples collected with a 60-ml syringe corer to a depth of 13 cm. Pore water from sediment samples was obtained by centrifugation ($5,000 \times g$ for 15 min) and then diluted (1:5, v/v) with low NH_4^+ seawater ($<0.1 \mu\text{M}$) collected offshore in the Gulf of Mexico. Concentrations of NH_4^+ and $\text{NO}_2^- + \text{NO}_3^-$ were measured using standard colorimetric techniques following the methods of Parsons et al. (1984).

Incubation chamber—Four plants were placed in a specially constructed 11.5-cm-diameter cylindrical Plexiglass, two-compartment, incubation chamber. The chamber spatially separated above- and belowground tissues (hereafter leaf and root compartments, respectively). To prevent leakage, shoots were fitted through a rubber stopper covered in thin rubber tubing. The rubber tubing was sealed tightly to the short shoot by a rubber band. The root compartment was screened from light and was fitted with two 1-cm-diameter ports to inject nutrients and collect water samples. In addition, a ventilation tube was placed in the root compartment to prevent a vacuum from forming when water samples were withdrawn. During the experiments, leakage was monitored by checking the water level in the ventilation tubing; an increase in water level indicated leakage between the two compartments.

Seawater used during incubations was collected from the study sites. Phytoplankton and bacteria were removed by filtering through a glass-fiber filter followed by a 0.2- μm pore-size polycarbonate filter. Circulation in the leaf compartment was provided by gentle aeration; water in the root compartment was not continuously mixed but was magnetically stirred prior to sample collection.

N uptake experiment—The experiments were conducted in a controlled environmental room with constant temperature and light intensity. Light was supplied on a 12:12 light:dark (LD) cycle; fluorescent lights provided about 300 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ to shoot tissues as measured by an LI-193SA spherical quantum sensor in conjunction with an LI-1000 datalogger (LI-COR). DIN uptake rates were determined for leaves and roots collected from both study sites in February, May, July, and October 1997 at ambient temperatures of 16, 25, 30, and 25°C, respectively. Incubations were also completed at night (2000–0500 h) in February and May to compare N uptake under dark and light conditions.

For leaf N uptake experiments, NH_4^+ concentrations in the root compartment were maintained at ambient levels, 30 and 120 μM for LLM and CCB plants, respectively. Similarly, NH_4^+ and $\text{NO}_3^- + \text{NO}_2^-$ concentrations in the leaf compartment were $<2 \mu\text{M}$ during measurement of root NH_4^+ uptake rates.

To obtain uptake rates at a variety of DIN concentrations for leaf or root tissues, experimental runs lasted approximately 10 h. At the start of a run, the target compartment in each of nine chambers was spiked with concentrated NaNO_3 or NH_4Cl solutions to yield a representative range of NO_3^- or NH_4^+ concentrations. For example, for leaf uptake determinations, leaf compartments were spiked to achieve NH_4^+ concentrations of 3–200 μM . One chamber without plants was incubated during each run as a control.

To eliminate a high initial uptake rate by adsorption, sampling for DIN measurements was started 1 h after addition of NH_4^+ and NO_3^- (Short and McRoy 1984). Water samples were collected from the chambers at consistent time intervals (1–2 h) and analyzed immediately to determine NH_4^+ and NO_3^- concentrations following the colorimetric techniques of Parsons et al. (1984).

The NH_4^+ and NO_3^- uptake rates by leaf tissues and NH_4^+ uptake rates by roots were assumed to be represented by decreases in the nutrient concentrations in target compartments over time. The NO_3^- or NH_4^+ concentrations were corrected for volume to determine the total N species content within a chamber. Total N content per compartment was then normalized to per gram dry weight of root or leaf tissue within a chamber and plotted against time. Dry weight of rhizome tissues was not included in the uptake rate calculation because N uptake by rhizome tissues is assumed to be negligible (Barnabas 1991, 1994; Stapel et al. 1996). The slope of the linear regression represents the uptake rate (micromoles per gram dry weight per hour). Because uptake rates vary as a function of nutrient concentrations, two–five measurements from a given chamber were used to calculate an uptake rate.

Uptake rates were plotted as a function of nutrient concentration. The NH_4^+ and NO_3^- uptake kinetics were derived using the Michaelis–Menten equation

$$V = V_{\max} \cdot S / (K_m + S)$$

where V (micromoles per gram dry weight per hour) represents actual uptake rate, V_{\max} is the maximum uptake rate, S (μM) is the nutrient concentration, and K_m (μM) is the half-saturation constant, numerically equal to S at $1/2 V_{\max}$. Uptake affinity is equivalent to V_{\max}/K_m .

Contribution of leaves and roots to total N acquisition—Daily N acquisition (N_D ; millimoles DIN per square meter per day) by leaves and roots was calculated using the following equations:

$$V_{\text{ambient}} = V_{\max} \cdot S_{\text{ambient}} / (K_m + S_{\text{ambient}})$$

$$N_D = V_{\text{ambient}} \cdot M_{\text{leaf or root}} \cdot 24/1000$$

where V_{ambient} is the N uptake rate at ambient N concentration, S_{ambient} is the ambient water-column or pore-water DIN concentration, and $M_{\text{leaf or root}}$ is leaf or root biomass (grams dry weight per square meter). Based on observed in situ water temperature, V_{\max} and K_m values calculated for February, May, July, and October experiments were applied to daily N acquisition calculations for the periods December–February, March–May, June–August, and September–November, respectively.

Statistics—Statistical analyses were performed on a microcomputer using SAS (1989). Significant differences in water-column and sediment pore-water DIN and leaf and root biomass among sampling times and study sites were tested using a two-way analysis of variance (ANOVA). To test significant differences between light and dark N uptake rates, the Michaelis–Menten equation was rearranged to yield the linear equation for Hanes–Woolf plot (Segel 1976): $S/V = (1/V_{\max})S + (K_m/V_{\max})$. N uptake rates were replotted using the linear equation, and significant differences between the two linear relationships for light and dark N uptake were tested using an analysis of covariance (ANCOVA). V_{\max} and K_m were compared among sampling times and study sites using 95% confidence intervals for these parameters.

Results

DIN and biomass—Water-column NH_4^+ concentrations ranged from 0.79 to 1.53 μM in CCB and from 0.70 to 1.43 μM in LLM. Levels of NH_4^+ varied significantly as a function of sampling date ($P < 0.001$), but no significant differences were observed between study sites ($P = 0.28$; Fig. 1A). Water-column $\text{NO}_3^- + \text{NO}_2^-$ concentrations at both sites ranged from 0.26 to 1.19 μM ; no significant difference was found between sites ($P = 0.16$), although significant seasonal variations were evident ($P < 0.001$; Fig. 1B). Sediment pore-water NH_4^+ concentrations ranged from 37 to 180 μM in CCB and from 8 to 45 μM in LLM. Average pore-water NH_4^+ concentrations in CCB (87 μM) were significantly higher than in LLM (26 μM ; $P < 0.001$; Fig. 1C).

Leaf biomass in CCB ranged from 128.5 g dry wt m^{-2} in January to 394.2 g dry wt m^{-2} in August, while root biomass ranged from 59.9 g dry wt m^{-2} in August to 126.0 g dry wt m^{-2} in October (Fig. 2A). Leaf and root biomass in LLM were also seasonally variable; leaf biomass ranged from 65.1 g dry wt m^{-2} in January to 204.9 g dry wt m^{-2} in September, and root biomass ranged from 46.0 g dry wt m^{-2} in October to 178.0 g dry wt m^{-2} in February (Fig. 2B). Leaf biomass in CCB was significantly higher than in LLM ($P < 0.001$), while root biomass was not significantly different between study sites ($P = 0.21$).

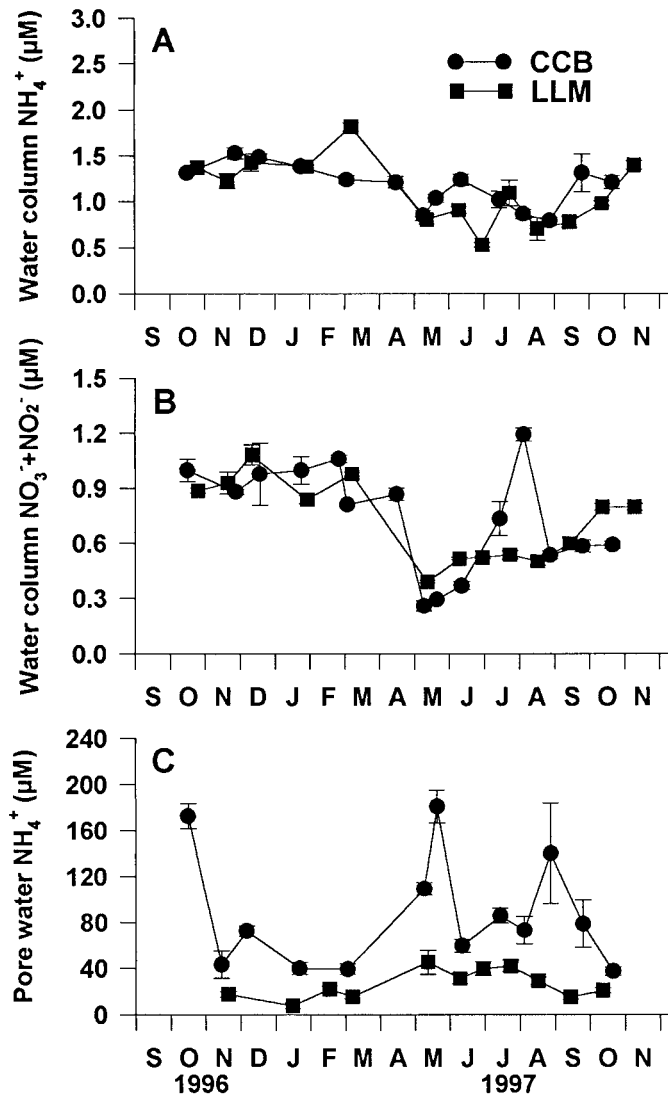


Fig. 1. Water-column NH_4^+ (A), $\text{NO}_3^- + \text{NO}_2^-$ (B), and sediment NH_4^+ (C) concentrations in CCB and LLM from October 1996 to November 1997. Values are mean \pm SE ($n = 4$). When no error bars appear, SE is less than the size of the symbol.

Leaf NH_4^+ and NO_3^- uptake—Leaf NH_4^+ uptake showed clear seasonal variation (Fig. 3). For CCB plants, the maximum uptake rate (V_{\max}) was highest in October ($16.4 \mu\text{mol g}^{-1} \text{ dry wt h}^{-1}$) and lowest in May ($8.3 \mu\text{mol g}^{-1} \text{ dry wt h}^{-1}$), while the half-saturation constant (K_m) was highest in February ($15.0 \mu\text{M}$) and lowest in October ($7.6 \mu\text{M}$) (Table 1). V_{\max} and K_m of leaf NH_4^+ uptake in LLM showed a similar trend to CCB, with high V_{\max} and low K_m values in October and lowest V_{\max} and high K_m values in February. Uptake affinity (V_{\max}/K_m) was lowest in winter (February) and highest in early fall at both sites (Table 1).

Leaf NO_3^- uptake also exhibited seasonal variation (Figs. 3, 4; Table 1). V_{\max} did not change significantly with season, but K_m decreased with increasing incubation temperature at both sites. Uptake affinity was lowest in February at both sites and highest during July in LLM (1.10) and during October in CCB (1.68). There were no obvious differences in

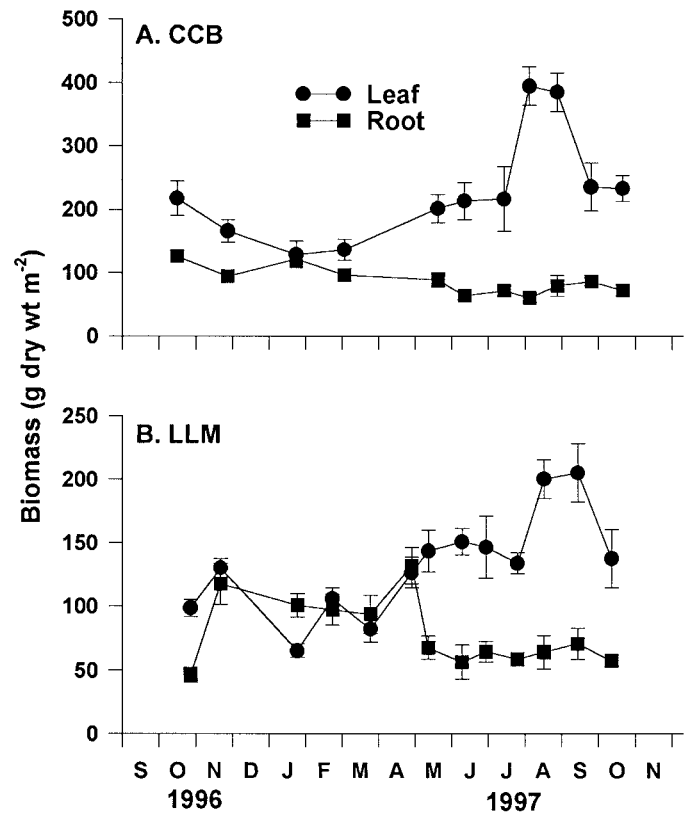


Fig. 2. *T. testudinum*. Seasonal changes in leaf and root biomass in CCB (A), and LLM (B). Values are mean \pm SE ($n = 4$). When no error bars appear, SE is less than the size of the symbol.

V_{\max} and K_m of leaf NO_3^- uptake between study sites. Leaf NH_4^+ uptake had higher V_{\max} values and similar or lower K_m values than leaf NO_3^- uptake; consequently, leaf NH_4^+ uptake resulted in higher uptake affinity than leaf NO_3^- uptake.

Root NH_4^+ uptake—In most cases, root NH_4^+ uptake did not saturate at the highest experimental NH_4^+ concentrations utilized (ca. $300 \mu\text{M}$). Therefore, V_{\max} and K_m of root NH_4^+ uptake estimated by the Michaelis–Menten model were much higher than those estimates for leaf uptake (Table 1). The lack of saturation in root NH_4^+ uptake may cause uptake kinetic parameters to be variable. Root NH_4^+ uptake was highly variable with season (Fig. 5; Table 1). For CCB plants, V_{\max} and K_m values were highest in October but lowest in May for V_{\max} and in February for K_m . For LLM plants, V_{\max} and K_m values were highest in May and lowest in February. There was no significant difference in root NH_4^+ uptake rates between sites. Root NH_4^+ uptake generally showed much lower uptake affinity than leaf NH_4^+ and NO_3^- uptake.

Light and dark N uptake—There were no differences between light and dark N uptake, as reflected by V_{\max} and K_m by leaf and root tissues (Fig. 6; Table 2). The uptake lines of the Hanes–Woolf plot corresponding to light and dark were also not significantly different ($P = 0.67, 0.15$, and 0.38 for leaf NH_4^+ , leaf NO_3^- , and root NH_4^+ uptake, respectively; Fig. 6).

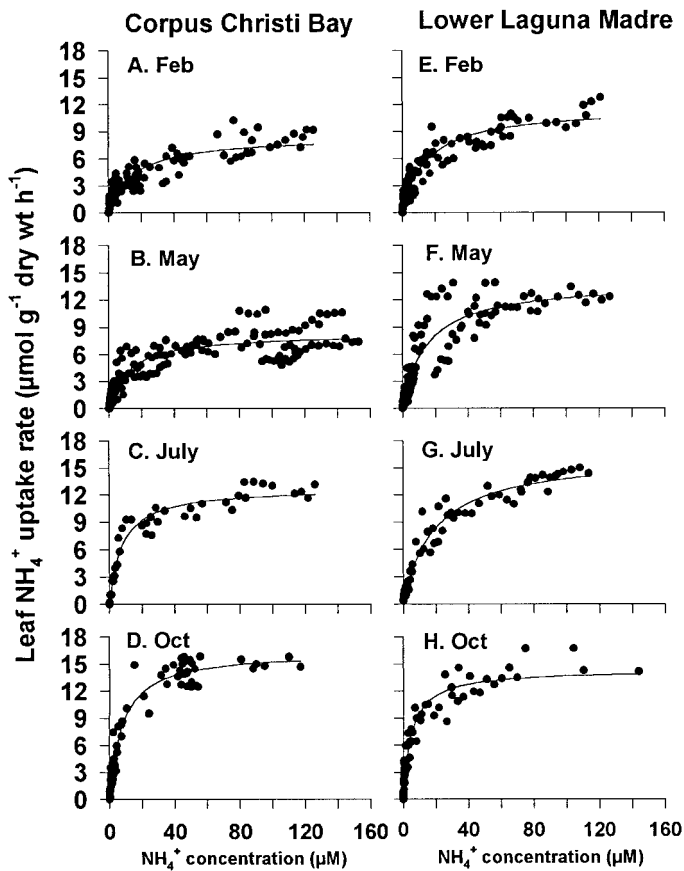


Fig. 3. *T. testudinum*. Leaf NH_4^+ uptake rates of plants from CCB and LLM in February, May, July, and October 1997 as a function of NH_4^+ concentration. The curves represent the best fits of the Michaelis–Menten equation.

N acquisition—Daily N acquisition from water-column NH_4^+ and NO_3^- by leaf tissues was highest during summer and fall and lowest during winter and spring, compared to sediment pore-water NH_4^+ acquisition by root tissues, which was variable and exhibited no clear seasonal trend (Fig. 7). In summer, leaf N uptake accounted for about 80% (CCB) and 65% (LLM) of total N acquisition, but N acquisition in winter was dominated by root uptake, which accounted for about 80% (CCB) and 70% (LLM) (Fig. 8). N acquisition was much higher in CCB than in LLM; the calculated annual N acquisition was 97.0 and 53.5 $\text{g N m}^{-2} \text{yr}^{-1}$ in CCB and LLM, respectively (Fig. 8). At both sites, NH_4^+ uptake by root tissues accounted for about 52% of total N acquisition, while leaf tissues assimilated about 38% of total N as NH_4^+ and about 10% as NO_3^- .

Discussion

Uptake kinetics—The results presented here demonstrate that both leaf and root tissues of *T. testudinum* are capable of significant N uptake as shown in previous studies on other seagrass species (Iizumi and Hattori 1982; Thursby and Harlin 1982, 1984; Short and McRoy 1984; Stapel et al. 1996; Pedersen et al. 1997; Terrados and Williams 1997). However,

Table 1. Parameters (V_{\max} and K_m) of the Michaelis–Menten model and uptake affinity (V_{\max}/K_m) for leaf and root N uptake for plants from CCB and LLM in February, May, July, and October 1997. Values in parentheses are 95% confidence intervals for V_{\max} and K_m .

	V_{\max} ($\mu\text{mol g}^{-1}$ dry wt h^{-1})	K_m (μM)	r^2	Affinity (V_{\max}/K_m)
Leaf NH_4^+ uptake				
CCB				
Feb	8.5 (7.8–9.2)	15.0 (11.1–18.9)	0.88	0.57
May	8.3 (7.9–8.8)	11.7 (9.1–14.2)	0.89	0.71
July	12.8 (12.1–13.5)	7.7 (5.6–9.7)	0.94	1.67
Oct	16.4 (15.7–17.1)	7.6 (6.3–8.9)	0.96	2.16
LLM				
Feb	11.7 (10.9–12.5)	15.2 (12.2–18.2)	0.92	0.77
May	14.0 (13.0–15.0)	14.7 (11.7–17.7)	0.88	0.95
July	16.5 (15.4–17.5)	19.4 (15.5–23.3)	0.96	0.85
Oct	14.4 (13.5–15.3)	5.1 (3.9–6.3)	0.93	2.82
Leaf NO_3^- uptake				
CCB				
Feb	5.9 (5.1–6.6)	38.5 (26.5–50.4)	0.91	0.15
May	3.8 (3.5–4.1)	5.3 (3.5–7.1)	0.72	0.72
July	3.7 (3.4–4.1)	6.3 (3.0–9.7)	0.72	0.59
Oct	3.7 (3.4–4.0)	2.2 (0.9–3.5)	0.66	1.68
LLM				
Feb	5.3 (4.7–6.0)	33.9 (24.3–43.4)	0.90	0.16
May	4.4 (4.0–4.8)	21.3 (14.9–27.8)	0.90	0.21
July	3.4 (3.1–3.7)	3.1 (1.7–4.5)	0.78	1.10
Oct	6.5 (6.0–7.0)	12.7 (8.9–16.4)	0.87	0.51
Root NH_4^+ uptake				
CCB				
Feb	14.0 (10.8–17.2)	89.2 (42.4–135.9)	0.78	0.16
May	10.9 (9.8–10.2)	172.1 (136.8–207.3)	0.97	0.06
July	25.6 (8.9–42.2)	757.1 (128.9–1385.3)	0.89	0.03
Oct	73.3 (35.7–111.0)	765.5 (296.1–1234.9)	0.88	0.10
LLM				
Feb	7.9 (6.4–9.3)	34.4 (15.0–53.7)	0.74	0.23
May	52.6 (32.5–72.7)	649.5 (351.5–947.5)	0.92	0.08
July	27.8 (11.5–44.1)	399.5 (233.8–565.2)	0.92	0.07
Oct	18.4 (15.2–21.2)	60.8 (40.9–80.7)	0.74	0.30

uptake kinetics can be highly variable among seagrass species (Table 3). Values of V_{\max} and K_m for NH_4^+ and NO_3^- uptake by *T. testudinum* leaf tissues were much lower than reported values for most other seagrass species (Thursby and Harlin 1982, 1984; Stapel et al. 1996; Pedersen et al. 1997). These results suggest that *T. testudinum* has a lower capacity for leaf N uptake but a higher uptake affinity than other seagrass species at low water-column DIN concentrations. The V_{\max} of root NH_4^+ uptake was lower than that of *Z. marina* (Thursby and Harlin 1982) but is similar to that of *Ruppia maritima* (Thursby and Harlin 1984) and *Amphibolis antarctica* (Pedersen et al. 1997). The K_m of root NH_4^+ uptake was higher than that of *R. maritima* but was within the range reported for *Z. marina* and *A. antarctica* (Iizumi and Hattori 1982; Thursby and Harlin 1982; Pedersen et al. 1997).

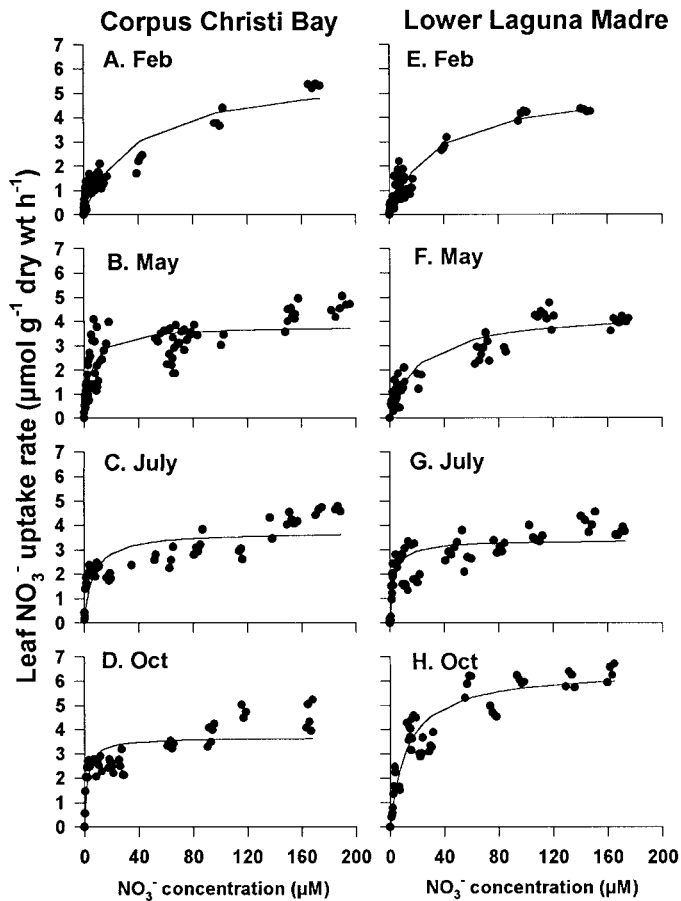


Fig. 4. *T. testudinum*. Leaf NO_3^- uptake rates of plants from CCB and LLM in February, May, July, and October 1997 as a function of NO_3^- concentration. The curves represent the best fits of the Michaelis–Menten equation.

Higher leaf uptake rates have been reported for NH_4^+ than for NO_3^- in *Phyllospadix torreyi* and *Z. marina* (Short and McRoy 1984; Terrados and Williams 1997). In the present study, leaf NH_4^+ uptake kinetics indicated higher V_{\max} and uptake affinity than those for NO_3^- uptake. Assimilation of NO_3^- by plants is influenced by the availability of photosynthate or stored carbohydrate and is energetically expensive (Thacker and Syrett 1972; Lara et al. 1987; Turpin 1991). Burkholder et al. (1992, 1994) demonstrated that NO_3^- utilization is an energetically costly process in *Z. marina* as chronic water-column NO_3^- enrichment leads to plant decline. Therefore, seagrass leaf tissues probably prefer the reduced N source (NH_4^+) to NO_3^- and take up NH_4^+ with higher affinity.

Pedersen et al. (1997) reported that leaf NH_4^+ uptake was much faster than root NH_4^+ uptake in *A. antarctica*. Similarly, in the present study, both leaf NH_4^+ and NO_3^- uptake affinities for *T. testudinum* were much higher than for root NH_4^+ . Seagrass leaf tissues are usually exposed to considerably lower NH_4^+ or NO_3^- concentrations than root tissues, which are surrounded by high NH_4^+ concentrations in pore water. Therefore, seagrass leaf tissues may have the ability to assimilate N under low DIN conditions, and the uptake may saturate

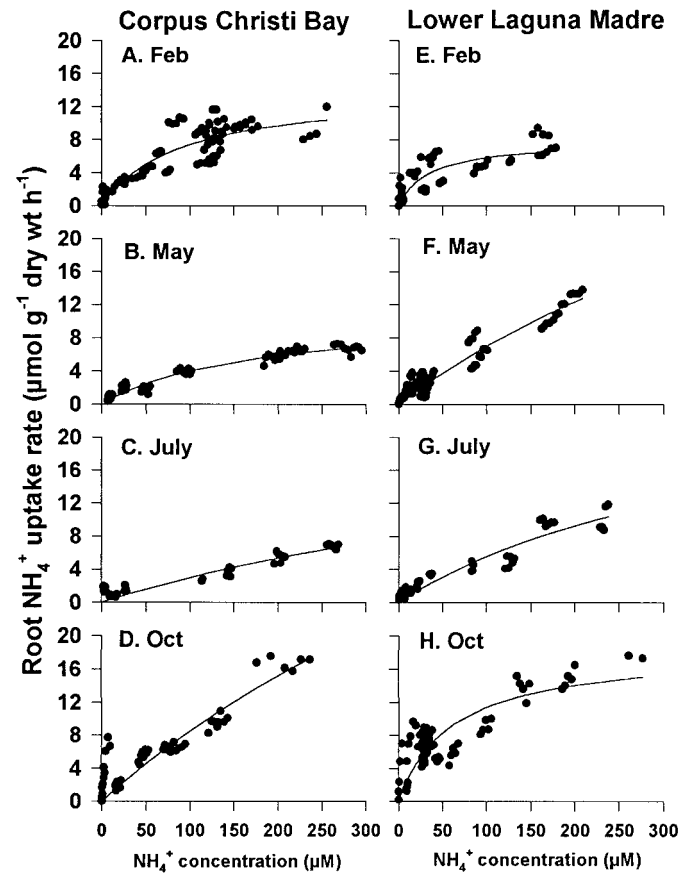


Fig. 5. *T. testudinum*. Root NH_4^+ uptake rates of plants from CCB and LLM in February, May, July, and October 1997 as a function of NH_4^+ concentration. The curves represent the best fits of the Michaelis–Menten equation.

at lower concentrations. Conversely, root NH_4^+ uptake rates increased continuously as a function of NH_4^+ concentration and exhibited saturation at much higher NH_4^+ concentrations than leaves. These N uptake patterns of leaf and root tissues likely reflect plant adaptations to life in oligotrophic-like waters of low DIN and relatively higher levels of NH_4^+ in sediment pore waters.

N acquisition—In the present study, *T. testudinum* root NH_4^+ uptake from the sediment accounted for about 50% of total plant N acquisition, with leaf NH_4^+ and NO_3^- uptake from the water column comprising the remaining 50% on an annual basis (Fig. 9). Iizumi and Hattori (1982) calculated N acquisition in *Z. marina* using average values of DIN concentrations, root/shoot ratio indices, and N uptake rates measured during spring, and they demonstrated that about 55% of the total N requirement for *Z. marina* growth was supplied by sediment NH_4^+ . From Zimmerman's model study (1987), contribution of root NH_4^+ uptake varied from 0 to 70% as a function of day length, and N uptake by roots accounted for 60% of total N acquisition for a photoperiod of 12 h.

This work demonstrates, however, that the contributions of leaves and roots to the total N budget of the plant change seasonally. In summer, leaf N uptake accounted for about

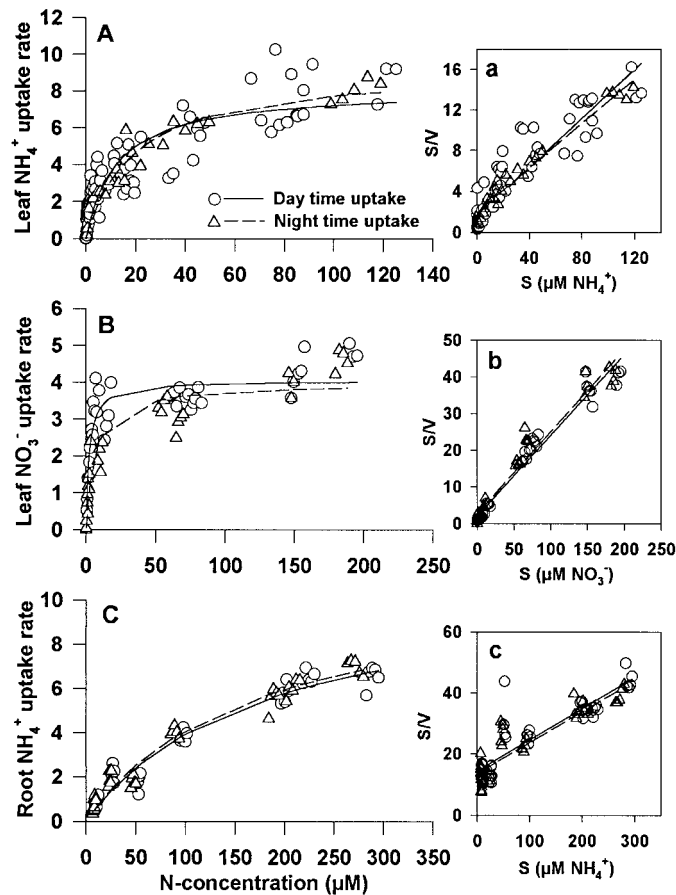


Fig. 6. *T. testudinum*. Comparisons of light and dark N uptake rates for leaf NH₄⁺ (A), leaf NO₃⁻ (B), and root NH₄⁺ (C). The curves represent the best fits of the Michaelis–Menten equation. All results were rearranged to yield the linear equation for the Hanes–Woolf plot (right inset) to test significant differences between light and dark N uptake using an ANCOVA. The experiments were conducted in February for leaf NH₄⁺ uptake and for leaf NO₃⁻ uptake and root NH₄⁺ uptake in May.

80% (CCB) and 65% (LLM) of total N acquisition. In contrast, N acquisition in winter was dominated by root uptake, which accounted for about 80% (CCB) and 70% (LLM). The distinct seasonal difference was driven by changes in leaf tissue acquisition, which was highest during summer and fall and lowest during winter and spring. N acquisition by root tissues was relatively constant with season. The distinct seasonal variations in leaf N acquisition were caused by higher leaf uptake rates and leaf biomass during summer and fall.

Total N acquisition by leaf and root tissues was high during fall and low during spring. This N acquisition pattern is consistent with previous reports of seasonal trends in tissue N content, protein, and amino acid levels (Harrison and Mann 1975; Pirc 1985; Dawes 1986; Pellikaan and Nienhuis 1988; Pirc and Wollenweber 1988; Dawes and Guiry 1992; Pérez-Lloéns and Niell 1993; Short et al. 1993; Lee and Dunton 1999). This suggests that during periods of high N uptake, uptake may exceed the N requirements, and the excess N will be stored as amino acids or proteins to meet high

Table 2. Parameters (V_{\max} and K_m) of the Michaelis–Menten model for light and dark N uptake by leaf and root tissues. Values in parentheses are 95% confidence intervals for V_{\max} and K_m .

	V_{\max} ($\mu\text{mol g}^{-1}$ dry wt h^{-1})	K_m (μM)	r^2
Leaf NH ₄ ⁺ uptake			
Day	8.1 (7.3–8.9)	12.6 (8.5–16.6)	0.85
Night	9.3 (8.3–10.3)	19.9 (13.8–26.0)	0.94
Leaf NO ₃ ⁻ uptake			
Day	4.0 (3.8–4.3)	2.3 (1.4–3.3)	0.75
Night	4.0 (3.6–4.3)	6.6 (3.3–9.8)	0.89
Root NH ₄ ⁺ uptake			
Day	10.9 (9.3–12.6)	178.1 (124.5–231.8)	0.96
Night	10.9 (9.4–12.5)	167.6 (119.2–215.9)	0.97

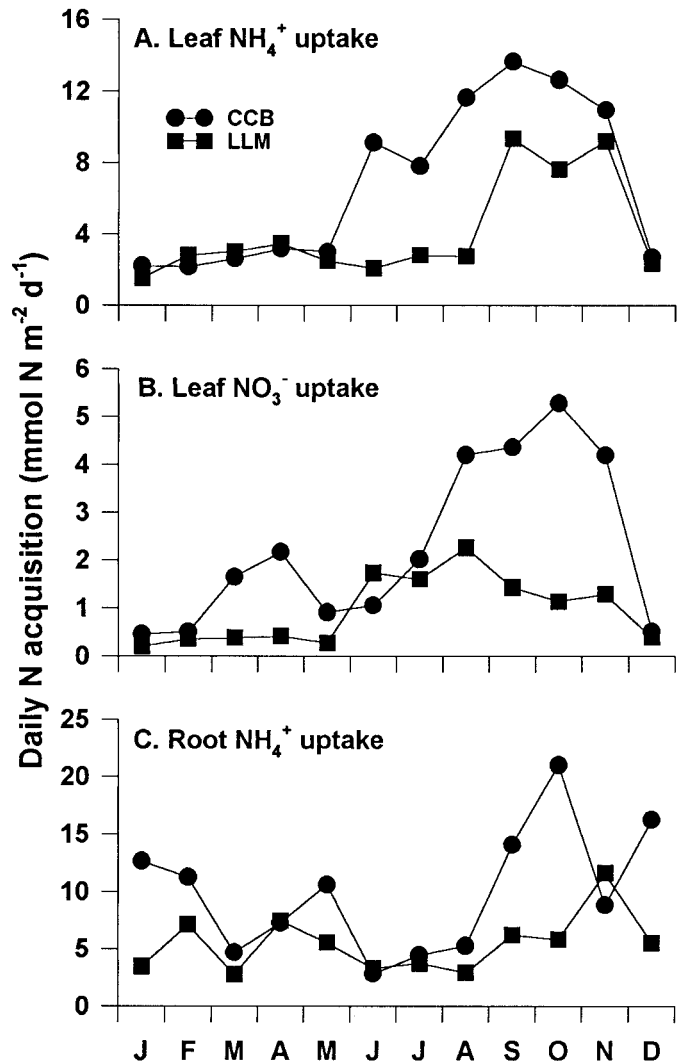


Fig. 7. Seasonal changes in daily N acquisition by leaves from water-column NH₄⁺ (A), NO₃⁻ (B), and by roots from sediment NH₄⁺ (C).

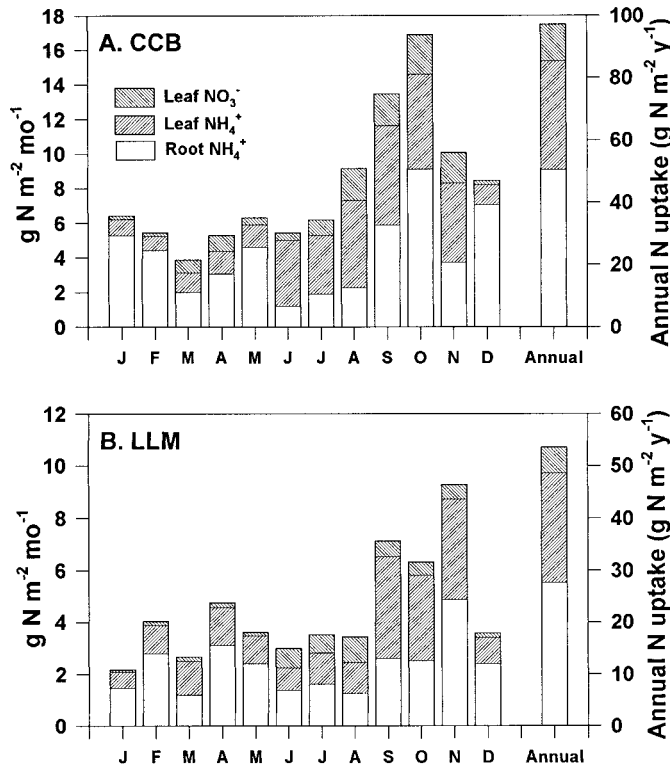


Fig. 8. Monthly and annual N acquisition by leaf and root tissue from the water column and sediment in CCB (A), and LLM (B).

N demands during periods of high production (Pirc 1985; Dawes 1986; Dawes and Guiry 1992).

Surprisingly, significant differences in pore-water NH_4^+ concentrations between the two sites (CCB vs. LLM) did not affect the contributions of leaves and roots to total N budget in *T. testudinum*. In contrast, Zimmerman et al. (1987) showed the effects of water-column and sediment DIN concentrations on patterns of N assimilation with a numerical model and noted that the contributions of leaf and root N uptake to total N acquisition varied as a function of

DIN concentrations. However, in the present study, there was no difference in the relative contributions of leaf and root uptake to total N acquisition between the two study sites, even though the sites differed significantly with respect to sediment NH_4^+ concentrations. This disagreement is probably a product of biomass allocation. Zimmerman et al. used a single root:shoot biomass ratio (0.20) for all calculations. However, terrestrial plant species adjust biomass allocation between roots and shoots in response to N supply (Tilman and Wedin 1991; Wilson and Tilman 1991). Root:shoot ratios increase with decreasing N supply, which suggests that greater root allocation is required for N uptake at low resource supply.

Seagrasses also allocate more biomass into belowground tissues under low-sediment N availability to increase the root surface area for N uptake; conversely, more biomass occurs in aboveground tissues under high-sediment N availability to increase C fixation (Lee 1998). Rhizome biomass at the low-sediment NH_4^+ site was about twofold higher than that at the high-sediment NH_4^+ site (Fig. 9). Even though N uptake by rhizome tissues is assumed to be negligible (Barnabas 1991, 1994; Stapel et al. 1996), higher rhizome allocation at low-sediment N condition would stimulate seagrass root tissues to explore larger areas for N uptake. The average below/aboveground biomass ratio (Lee 1998) at the low-sediment NH_4^+ site (LLM, 4.66) was significantly higher than that at the high-sediment NH_4^+ site (CCB, 1.63). Since tissue N uptake rates on a dry weight basis were not different for *T. testudinum* from the two study sites, the leaf N acquisition calculated for CCB was higher than for LLM due to the larger leaf biomass, which produced higher surface area for N uptake (Fig. 9). Root N acquisition was also higher in CCB than in LLM (51 and 28 $g\ N\ m^{-2}\ yr^{-1}$, respectively) due to higher sediment NH_4^+ concentrations at CCB (Fig. 9). Consequently, contributions of leaf and root N uptake to total N acquisition did not change in *T. testudinum* with sediment NH_4^+ concentrations. However, total annual N acquisition in CCB was significantly higher than in LLM (97.0 and 53.5 $g\ N\ m^{-2}\ yr^{-1}$, respectively).

C and N comprise about 35 and 2%, respectively, of *T.*

Table 3. Parameters (V_{max} and K_m) of the Michaelis–Menten model for tissue N uptake of various seagrass species.

Species	Tissue	DIN	V_{max} ($\mu mol\ g^{-1}\ dw\ h^{-1}$)	K_m (μM)	Area	Time	Source
<i>Thalassia hemprichii</i>	Leaf	NH_4^+	32–37	21–60	Indonesia	April–June	Stapel et al. (1996)
<i>Zostera marina</i>	Leaf	NO_3^-	—	23	Japan	March	Iizumi and Hattori (1982)
	Root	NH_4^+	—	30		May	
<i>Zostera marina</i>	Leaf	NH_4^+	20.5	9.2	Rhode Island, U.S.A.	Late summer	Thursby and Harlin (1982)
	Root	NH_4^+	211	104			
<i>Amphibolis antarctica</i>	Leaf	NH_4^+	82–604	133–1,041	Australia	January	Pedersen et al. (1997)
	Root	NH_4^+	16	66			
<i>Phyllospadix torreyi</i>	Leaf	NH_4^+	9.30–33.97	95.69–204.32	California, U.S.A.	July–August	Terrados and Williams (1997)
	Leaf	NO_3^-	4.39–16.98	24.97–75.47			
<i>Ruppia maritima</i>	Leaf	NH_4^+	243–270	9.0–17.7	—	—	Thursby and Harlin (1984)
	Root	NH_4^+	48–56	2.8–12.6			
<i>Thalassia testudinum</i>	Leaf	NH_4^+	8.3–16.5	5.1–19.4	Texas, U.S.A.	Seasonal	Present study
	Leaf	NO_3^-	3.4–6.5	2.2–38.5			
	Root	NH_4^+	7.9–73.3	34.4–765.5			

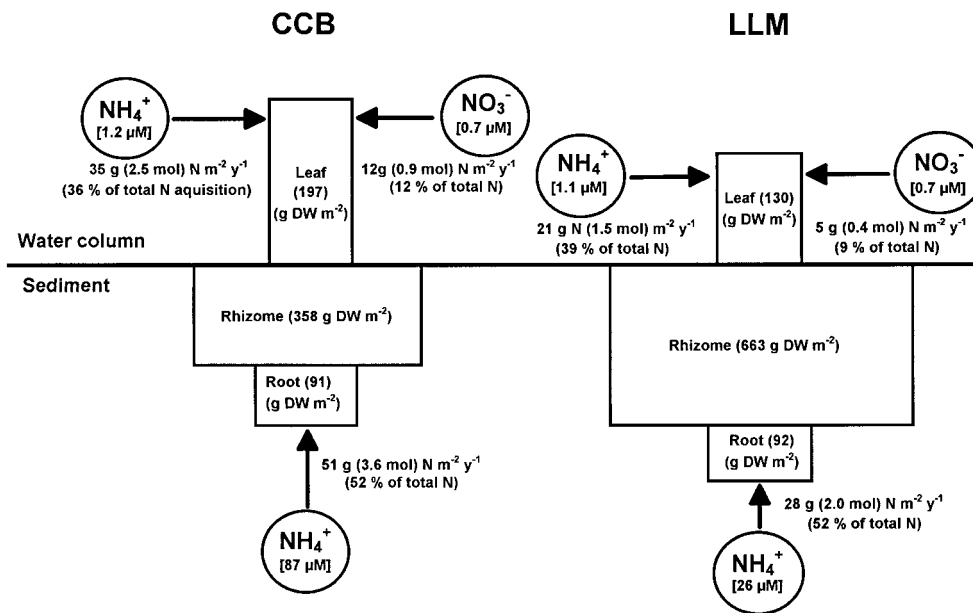


Fig. 9. N budget for *T. testudinum* in CCB and LLM. Values represent annual means; N budget calculations were derived using seasonal uptake rates, biomass, and ambient N availability. Size of boxes for plants corresponds to the average biomass of each plant part.

testudinum on a dry weight basis (Lee and Dunton 1999). Thus, calculated annual seagrass biomass production rates based on the annual N acquisition are 4,851 g dry wt $\text{m}^{-2} \text{yr}^{-1}$ (1,698 g C $\text{m}^{-2} \text{yr}^{-1}$) in CCB and 2,674 g dry wt $\text{m}^{-2} \text{yr}^{-1}$ (936 g C $\text{m}^{-2} \text{yr}^{-1}$) in LLM. Lee and Dunton (1996) estimated an annual blade production of 792 g dry wt $\text{m}^{-2} \text{yr}^{-1}$ (253 g C $\text{m}^{-2} \text{yr}^{-1}$) in CCB, while Kaldy (1997) estimated an annual total production of 953 g dry wt $\text{m}^{-2} \text{yr}^{-1}$ (340 g C $\text{m}^{-2} \text{yr}^{-1}$) in LLM. These results show that calculated biomass production based on the N acquisition is significantly higher than in situ measurements of production, suggesting that the N acquisition by leaf and root tissues is much higher than N utilized for biomass production at both sites. The difference between N acquisition and utilization suggests that not all N acquired by plant tissues is incorporated into biomass.

Consequently, although *T. testudinum* growth in LLM has been demonstrated to be N limited (Lee and Dunton 1999), >50% of the absorbed DIN was not incorporated into biomass. The difference between N acquisition and utilization may be explained by low efficiency of N assimilation into biomass. Excess N uptake over N utilization for biomass production has been reported for bacteria (Keil and Kirchman 1991; Jorgensen et al. 1993; Kirchman 1994). Kirchman (1994) calculated that bacterial production estimated from DIN uptake was about 10-fold higher than the microscopic-based estimate, suggesting that about 10% of the acquired DIN was incorporated in bacterial biomass. The imbalance between DIN uptake and bacterial production has been explained by excretion of dissolved organic nitrogen (DON; Kirchman 1994). Excretion of amino acids (Wood and Hayasaka 1981) and dissolved organic carbon (DOC; Penhale and Smith 1977; Wetzel and Penhale 1979; Moriarty et al. 1986; Ziegler 1998) by seagrass tissues has been re-

ported, and it is possible that some inorganic N acquired by seagrass tissues is also released as DON. It has been demonstrated that pore-water NH_4^+ concentrations in seagrass beds were maintained by the continual deamination of amino acids by bacteria in the rhizosphere of seagrasses (Smith et al. 1984; Boon et al. 1986). The amino acids to maintain this NH_4^+ regeneration are provided as exudates from seagrass roots (Wood and Hayasaka 1981; Smith et al. 1984; Boon et al. 1986).

Because root tissues were incubated in pure seawater instead of sediments, there is a possibility that we overestimated root DIN uptake rates due to increases in surface area contact with pore water and in rates of DIN diffusion from pore water to the root surface (Stapel et al. 1996). In addition, epiphytes on leaf tissues were removed to exclude N uptake by these organisms; their removal may cause increases in leaf surface area for N uptake and consequently, may cause an overestimate of leaf DIN uptake.

DIN pool and turnover rates—Root N uptake rates may be affected by the rates of N diffusion in the sediment. By comparing root uptake capacity with nutrient diffusion rates, Stapel et al. (1996) demonstrated that root uptake depends primarily on nutrient diffusion rates from pore water to the root surface. However, N for root uptake can be supplied by remineralization in the sediments in addition to diffusion (Hines and Lyons 1982; Holmer and Nielsen 1997). Thus, in situ N acquisition by roots probably depends on the combination of the uptake capacity of the roots, N diffusion, and remineralization rates in sediment. Rapid sediment DIN turnover rates have been reported in various seagrass beds (Capone 1982; Moriarty et al. 1985; Boon et al. 1986). In the present study, the DIN pool size in the water column and sediment was calculated from mean DIN concentrations and

Table 4. Estimated DIN pool size in water column and sediment, annual mean N uptake rate by seagrass, and DIN turnover time in water column and sediment in CCB and LLM.

	Pool size (mmol N m ⁻²)	Uptake rate (mmol m ⁻² d ⁻¹)	Turnover time (d)
CCB			
Water-column NH ₄ ⁺	1.42	6.80	0.21
Water-column NO ₃ ⁻ + NO ₂ ⁻	0.88	2.27	0.39
Sediment NH ₄ ⁺	17.40	9.93	1.75
LLM			
Water-column NH ₄ ⁺	1.33	4.12	0.32
Water-column NO ₃ ⁻ + NO ₂ ⁻	0.86	0.95	0.91
Sediment NH ₄ ⁺	5.20	5.45	0.95

volume of water (Table 4). It was assumed that DIN in the entire water column (1.2-m depth) was available for leaf uptake. Because most *T. testudinum* roots in CCB and LLM penetrate to a depth of only 20 cm in the sediment (Lee unpubl. data), pore-water DIN in the top 20 cm of sediment was assumed to be available for root uptake. Sediment porosity at both sites is about 0.5 (Lee unpubl. data). Assuming seagrass dominates DIN uptake in this system, DIN turnover time ranged from 0.21 to 0.91 d in the water column and from 0.95 to 1.75 d in sediment pore water (Table 4). The sediment DIN turnover time in this study is within the range found in other seagrass beds (0.4–6 d; Capone 1982; Moriarty et al. 1985; Boon et al. 1986). The water-column DIN turnover time calculated in this study is similar to other estimates for LLM (0.21–1.49 d) that were calculated using DIN remineralization rates (Ziegler 1998). However, bacteria can account for a large portion of total DIN uptake in marine environments (Kirchman 1994). Thus, actual DIN turnover rates at the study sites are probably faster than estimates calculated by only seagrass uptake. These rapid DIN turnover rates in seagrass beds indicate the importance of DIN regeneration processes for supporting seagrass production (Moriarty et al. 1985; Boon et al. 1986).

In conclusion, root and leaf tissues contributed equally to the N budget of *T. testudinum*. There were no differences in the contributions of leaf and root uptake to total N acquisition between the two study sites, despite a significant difference in sediment pore-water NH₄⁺ levels. The similar contribution patterns in tissue N acquisition at both sites were caused by biomass allocation patterns: high leaf biomass at high-sediment N conditions and high belowground biomass at low-sediment N conditions. Total N acquisition by leaf and root tissues was highest during fall and lowest during spring. Annual N acquisition in CCB was double that of LLM because of the significantly higher leaf biomass and higher sediment pore-water NH₄⁺ concentrations in CCB. N acquisition by leaf and root tissues did not match the amount incorporated into plant tissues; even at LLM, where the low DIN levels in the water column and sediments are believed to limit seagrass production, >50% of the taken-up N was not incorporated into biomass. The reason for the low assim-

ilation efficiency must be addressed in future research to further understand the N budget in seagrass beds.

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