Dissolved organic nitrogen uptake by seagrasses

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Abstract

We examined the ability of seagrasses to take up dissolved organic nitrogen (DON) with leaves (in situ) and roots (laboratory) in an oligotrophic tropical offshore meadow in Indonesia using ^{15}N-labeled nitrogen (N) substrates. We compared the uptake of urea and amino acids with that of ammonium (NH_4^+) and nitrate (NO_3^-) and determined uptake kinetics of amino acids for the seagrasses *Thalassia hemprichii, Halodule uninervis*, and *Cymodocea rotundata* in comparison with the macroalgae *Sargassum* sp. and *Padina* sp. Uptake rates of small DON substrates for macroalgae were higher than those for seagrass leaves for all N substrates, but the seagrass roots also had a considerable uptake capacity. Seagrass leaves preferred urea, NH_4^+, and NO_3^- over amino acids, and there were differences between species. Seagrass roots, however, took up amino acids at rates comparable to NH_4^+, whereas uptake rates of urea and NO_3^- were much lower. The ability to take up DON enables seagrasses and macroalgae to shortcut N cycling and gives them access to additional N resources. In oligotrophic environments, uptake of amino acids by roots may provide seagrasses with a competitive advantage over macroalgae.

Seagrasses, like all other plants, need nitrogen (N) to maintain their high productivity. In contrast to many terrestrial plants, resorption of N from senescing leaves in seagrasses is low and loss due to leaf detachment is high (Stapel and Hemminga 1997; Romero et al. 2006). Because seagrasses flourish in oligotrophic areas, it seems paradoxical that they can collect sufficient N to survive. It is well established that seagrasses take up N by both leaves and roots in amounts depending on the relative availability in the sediment and in the water column (Stapel et al. 1996; Lee and Dunton 1999). The majority of the research on N uptake by seagrass leaves and roots has focused on uptake of ammonium (NH_4^+) and nitrate (NO_3^-; Stapel et al. 1996; Romero et al. 2006). As concentrations of these inorganic N sources are generally low in seagrass habitats (Lee and Dunton 1999; McGlathery et al. 2001), this raises the question if other important N sources exist.

Recent insights indicate that the dissolved organic nitrogen (DON) pool contains a "doughnut" fraction composed of relatively labile compounds such as amino acids and urea, with a relatively high turnover (Bronk et al. 2007). Dissolved amino acids and urea are released during decomposition of organic material. Besides, urea is also produced by animal excretion (Bronk 2002) and is often present in run-off from agricultural fields to coastal waters (Glibert et al. 2006). Particularly in oligotrophic environments, where most N is available in the dissolved organic form (Bronk 2002; Bronk et al. 2007), small DON molecules could serve as potentially important N substrates for seagrasses. Whereas it is clear that certain groups of organisms such as phytoplankton may obtain a substantial part of their N nutrition from this DON fraction (Bronk et al. 2007), it remains largely unknown whether seagrasses can do the same.

Recent studies demonstrated that N present in phytodetritus from plankton trapped within the sediment can be rapidly taken up by seagrass plants (Evrard et al. 2005; Barrón et al 2006). These in situ labeling experiments, however, did not reveal whether N was taken up in the dissolved inorganic form (DIN) after complete mineralization or as DON. Various terrestrial plants (Chapin et al. 1993; Persson and Nasholm 2001) and micro- and

macroalgae (Probyn and Chapman 1982; Linares and Sundback 2006) are able to utilize DON; one might therefore ask whether seagrasses may be able to take up small organic N molecules, like amino acids and urea. Until now, only *Halophila ovalis* has been shown to grow on amino acids, but not on urea, at 1.7 m mol L^{-1} in cultures (Bird et al. 1998). If seagrass species could utilize small DON molecules, this could be highly advantageous for their N supply, especially in oligotrophic environments. We hypothesize that seagrasses can take up small molecules of DON both by leaves and roots and used ^{15}N-labeled substrates to test this hypothesis.

Materials and methods

Site description—All in situ experiments and material collection was performed in a seagrass meadow on the island Bone Batang, Sulawesi, Indonesia. This uninhabited island (5°01′ S; 119°19′30″E) is located ~15 km offshore in the Spermonde Archipelago, 2 km north of Barang Lompo (*see* Stapel et al. 1996 for area details). The island has a large reef flat with coarse carbonate sediment, comparable to Barang Lompo (93–100%; Erftemeijer and Middelburg 1993) and is covered by meadows of the seagrasses *Thalassia hemprichii, Halodule uninervis*, and *Cymodocea rotundata* and the macroalgae *Sargassum* sp. and *Padina* sp.

Water sampling—Pore-water samplers (Rhizon 10-cm soil moisture sampler, Eijkelkamp Agrisearch Equipment) were placed in a frame at 6-cm-depth intervals. Tubing was added to extract pore water from the samplers without disturbing the sediment. The frame was inserted in the seagrass meadow with the depths of pore-water samplers at 1.5, 7.5, and 13.5 cm. A hard polyvinyl chloride (PVC) tube (5 cm length) was placed over the closed sampling tubes to prevent damage and algae growth. Five frames were placed in the meadow in May 2004, and sampling occurred in November 2005. Pore water was sampled by adjusting a syringe to the tube in which a vacuum was applied. A 40 mL sample was collected after discarding the first 5 mL. The water column was sampled at each pore-water location by deploying rhizon soil samplers at 10 cm above the sediment. The collected water samples were filtered, frozen, and transported to The Netherlands for automated colorimetric analysis of urea, NH_4^+, NO_3^-, and NO_2^- (Middelburg and Nieuwenhuize, 2000).

Uptake by above-ground tissue—In November 2005, uptake rates of NH_4^+, NO_3^-, urea, and amino acids were measured for the above-ground parts of three seagrass species, *T. hemprichii, H. uninervis*, and *C. rotundata*, and two macroalgae, *Sargassum* sp. and *Padina* sp., for comparison. Uptake of different dissolved N substrates by aboveground tissue was measured in situ at ~1 m depth during daytime (09:00 to 14:00 h). We used four ^{15}N labeled sources: NH_4^+, NO_3^-, urea (Cambridge Isotope Laboratories NLM-233), and a mixture of 16 algal amino acids (Cambridge Isotope Laboratories NLM-2161; *see* Middelburg and Nieuwenhuize 2000; Veuger et al. 2004). The initial concentration for all N substrates was

10 μmol L^{-1}, and in addition, initial concentrations of 1 μmol L^{-1}, 2 μmol L^{-1}, and 5 μmol L^{-1} were also examined for the amino acids. The ^{15}N label was added to filtered seawater (Whatman GF/C) collected offshore shortly before the incubations, which were performed in triplets.

We incubated *Sargassum* sp. and *Padina* sp. by placing 5-cm-long pieces of thalli in a small plastic bag and adding 60 mL seawater containing the ^{15}N-labeled substrate. The bags were closed and placed underwater. The seagrass leaf incubations were performed in 60-mL plastic bags placed over intact plants in the field. A notched rubber membrane (25 mm diameter, 4 mm thick) was attached around the base of seagrass shoots (one shoot for *T. hemprichii*, two for *C. rotundata*, and three for *H. uninervis* to have comparable amounts of leaf material). The notch was closed using plasticide, and a 12-cm-long plastic bag was sealed around the membrane with cable ties enclosing the shoots to create a leaf compartment. All water was removed from the leaf compartment through a small tube in the rubber membrane using a syringe. Directly afterward we injected 60-mL seawater with ^{15}N-labeled source through the tube into the compartment.

After 1 h incubation, the seawater with ^{15}N-label was removed. Seagrass leaves were directly separated from the shoots and washed in seawater to remove adsorbed label. Epiphytes were removed from the incubated material using a scraper blade, and above-ground material was rinsed with aquadest. Below-ground material of the incubated seagrasses was harvested using small cores (6 cm diameter, 15 cm depth). The material was sorted in the laboratory and rinsed with aquadest. All plant material was oven dried at 70°C for 48 h and dry weight (DW) was measured.

Uptake by below-ground tissue—Incubations with seagrass roots were performed in the laboratory. Seagrass cores (diameter 18 cm) up to 20-cm depth were collected in the field. Complete seagrass shoots were carefully washed out of the cores to minimize damage to the roots and to remove all detritus. Leaf epiphytes were removed using scraper blades. We divided the plants into single shoots with roots and about 3-cm horizontal rhizome. The plants were kept in seawater overnight. The same number of shoots per seagrass species and concentrations of N substrates were used as for the above-ground uptake experiments. The below-ground seagrass parts were placed in a small bag containing 80-mL filtered seawater with the ^{15}N-labeled substrate, and a small tube receiving bubbling air was placed in the bag for circulation. The seagrass leaves were wrapped in wet tissue without contact to the incubation medium to prevent drying out and uptake through the leaves. After 1 h incubation we cut the leaves from the below-ground parts, washed all material in seawater, and rinsed with aquadest. The below-ground parts were then divided into roots and rhizomes and dried.

Analyses—N contents and at.% ^{15}N of dried plants were determined with a Fisons NA 1500 elemental analyzer coupled to a Finnigan Delta S mass spectrometer via a Conflo II interface (Middelburg and Nieuwenhuize 2000).

Table 1.   Mean concentrations (μmol L^{-1}) of N sources measured in the water column and pore water at different depths (in cm) in the seagrass meadow (SD; $n = 5$).

	NH$_4^+$	NO$_3^-$	NO$_2^-$	Urea
Water column	0.67 (0.26)	0.51 (0.19)	0.03 (0.01)	0.99 (0.29)
Pore water				
1.5	0.55 (0.09)	0.33 (0.12)	0.00 (0.01)	0.80 (0.12)
7.5	0.44 (0.07)	0.31 (0.16)	0.00 (0.01)	0.84 (0.09)
10.5	0.66 (0.19)	0.32 (0.20)	0.01 (0.01)	1.01 (0.38)

Background δ^{15}N composition was measured in three replicate samples for all species, parts, and experiments. ^{15}N enrichment was calculated by subtracting the background from the incubated values. Uptake rates (in μmol N source g DW^{-1} h^{-1}) were estimated from ^{15}N enrichment, incubation time, and weight of the incubated material. Total ^{15}N enrichment in plant material was corrected for the amount of μmol N (μmol N source)$^{-1}$: 1 for NH$_4^+$ and NO$_3^-$, 2 for urea, and 1.39 for amino acids. For seagrasses, it was assumed that rhizome uptake was negligible relative to root uptake (Stapel et al. 1996). We therefore calculated the uptake rates normalized to root biomass. Transport of ^{15}N from the incubated plant part to the non-incubated plant parts, i.e., the roots and rhizomes during leaf incubations and vice versa, was estimated for seagrasses. For all incubations, the depletion of the substrate in the incubation medium was determined by comparing the total amount of ^{15}N added with the total amount of ^{15}N recovered in the plants after incubation.

Statistics—Differences between species and N sources were tested using two-way analysis of variance (ANOVA; Sokal and Rohlf 1995). Significant effects were followed by univariate analysis of variance and post-hoc Tukey's-b test. Before ANOVA, uptake rates (μmol N source g DW^{-1} h^{-1}) were square-root transformed to ensure quality of error variances (Levene's test). The uptake kinetics of amino acids was analyzed assuming Michaelis-Menten models using nonlinear regression to estimate the half saturation constant (K_m) and maximum uptake rates (V_{max}). All analyses were performed in SPSS 14.0.

Results

Concentrations of inorganic N and urea were low (≤ 1 μmol L^{-1}) in both the water column and pore waters in the seagrass meadow (Table 1). NO$_3^-$ concentrations were highest in the water column, whereas NO$_2^-$ concentrations were negligible. NH$_4^+$ and urea had comparable concentrations in the water column and pore water. Pore-water concentrations of urea were comparable to total inorganic N concentrations.

Our results clearly show that all macrophyte species studied were able to take up dissolved amino acids and urea with their aboveground tissues (Fig. 1A) as well with their roots (Fig. 1B; seagrasses only). For the above-ground tissues, the uptake rates for macrophyte species and N sources were significantly different (ANOVA $F_{12,40} = 12.03$, $p < 0.001$). Uptake rates were different per N

substrate and were highest overall for NH$_4^+$, followed by NO$_3^-$, urea, and amino acids (ANOVA $F_{3,40} = 222.8$, $p < 0.001$). Comparing uptake rates for the different macrophyte species revealed that *Sargassum* sp. had the highest uptake rates for all N substrates followed by *Padina* sp., *T. hemprichii* and *C. rotundata*, and *H. uninervis* (ANOVA $F_{4,40} = 116.4$, $p < 0.001$). Comparing N sources for each species showed that uptake rates of NH$_4^+$ and NO$_3^-$ were significantly higher compared to urea, whereas amino acids

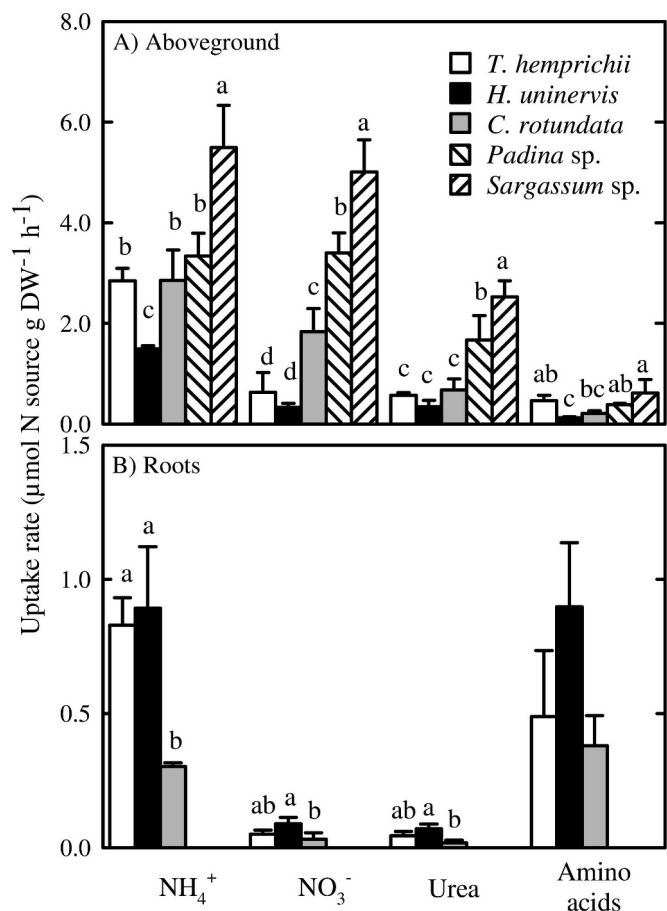Fig. 1.   Mean (A) above-ground and (B) root uptake rates (μmol g DW^{-1} h^{-1}) at 10 μmol L^{-1} of the different N sources for the macrophyte species (+SD). Significant differences between macrophyte species per N source are denoted (ANOVA and post-hoc Tukey's-b test; above-ground NH$_4^+$ $F_{4,10} = 27.97$, NO$_3^-$ $F_{4,10} = 54.47$, urea $F_{4,10} = 35.50$, and amino acids $F_{4,10} = 10.22$; root NH$_4^+$ $F_{2,6} = 23.25$, NO$_3^-$ $F_{2,6} = 5.348$, and urea $F_{2,6} = 11.15$, respectively; all $p < 0.05$; $n = 3$).

had the lowest uptake rates for *Sargassum* sp. (ANOVA $F_{3,8} = 66.10$, $p < 0.001$) and *Padina* sp. (ANOVA $F_{3,8} = 64.45$, $p < 0.001$). The leaf uptake rates for *C. rotundata* followed the same order but were significantly different for all N substrates (ANOVA $F_{3,8} = 43.69$, $p < 0.001$). The leaf uptake rates of *H. uninervis* were significantly lower for amino acids and higher for NH_4^+ (ANOVA $F_{3,8} = 101.3$, $p < 0.001$), whereas *T. hemprichii* only had significantly higher uptake rates for NH_4^+ compared to the other N substrates (ANOVA $F_{3,8} = 33.50$, $p < 0.001$).

Uptake rates of seagrass roots differed between species and N sources (ANOVA $F_{6,24} = 3.705$, $p = 0.010$) and showed a remarkably high uptake rate for amino acids (Fig. 1B). That is, the root uptake rates for amino acids were comparable to those of NH_4^+ and significantly higher than those for urea and NO_3^- (ANOVA $F_{3,24} = 135.5$, $p < 0.001$). This preference was strongest for *H. uninervis* (ANOVA $F_{3,8} = 51.96$, $p < 0.001$) and *C. rotundata* (ANOVA $F_{3,8} = 56.10$, $p < 0.001$), and relatively weak for *T. hemprichii* (ANOVA $F_{3,8} = 40.45$, $p < 0.001$). Overall, roots of *H. uninervis* had the highest uptake rates, followed by *T. hemprichii* and then *C. rotundata* (ANOVA $F_{2,24} = 26.05$, $p < 0.001$). Comparing the three different seagrass species per N source showed that for most N sources *H. uninervis* had the highest, *C. rotundata* the lowest, and *T. hemprichii* the intermediate root uptake rates (Fig. 1B).

Uptake kinetics of amino acids followed Michaelis-Menten kinetics in most cases, except for *T. hemprichii* leaves, where the uptake rates increased linearly in the concentration range used (1–10 μmol L^{-1}; Fig. 2; Table 2). Seagrass roots had the highest uptake affinity for amino acids (i.e., highest $V_{max} : K_m$ ratio), followed by macroalgal thalli. The amino acid uptake affinity of seagrass leaves was much lower than that of macroalgae. Amino acid uptake rates of seagrass roots were overall comparable or higher compared to leaves at all concentrations.

At the end of the leaf uptake experiment, a negligible amount (<1%) of the total ^{15}N-label in the seagrass was recovered from the below-ground parts. Conversely, there was substantial transfer of ^{15}N from roots to leaves. Nitrate taken up by roots was particularly transported from roots to leaves, with 15% of the total NO_3^- taken up by the roots allocated to the leaves for *T. hemprichii*, 8% for *H. uninervis*, and 20% for *C. rotundata*, respectively. In the last two species, urea (4% and 21%) and amino acids (2% and 8%, respectively) were also transferred from roots to leaves. The depletion of the N substrates during uptake experiments ranged from 0.3% to 51% (Table 3), with most depletion occurring for macroalgae because of their high uptake rates. Almost no depletion was calculated for NO_3^- and urea uptake by the seagrass roots.

Discussion

The experiments presented here demonstrate that small DON molecules have the potential to contribute significantly to the overall N demand of the studied tropical seagrasses and macroalgae. All three seagrasses, *T. hemprichii, H. uninervis*, and *C. rotundata*, could take up both urea and amino acids. The ability of macroalgae to
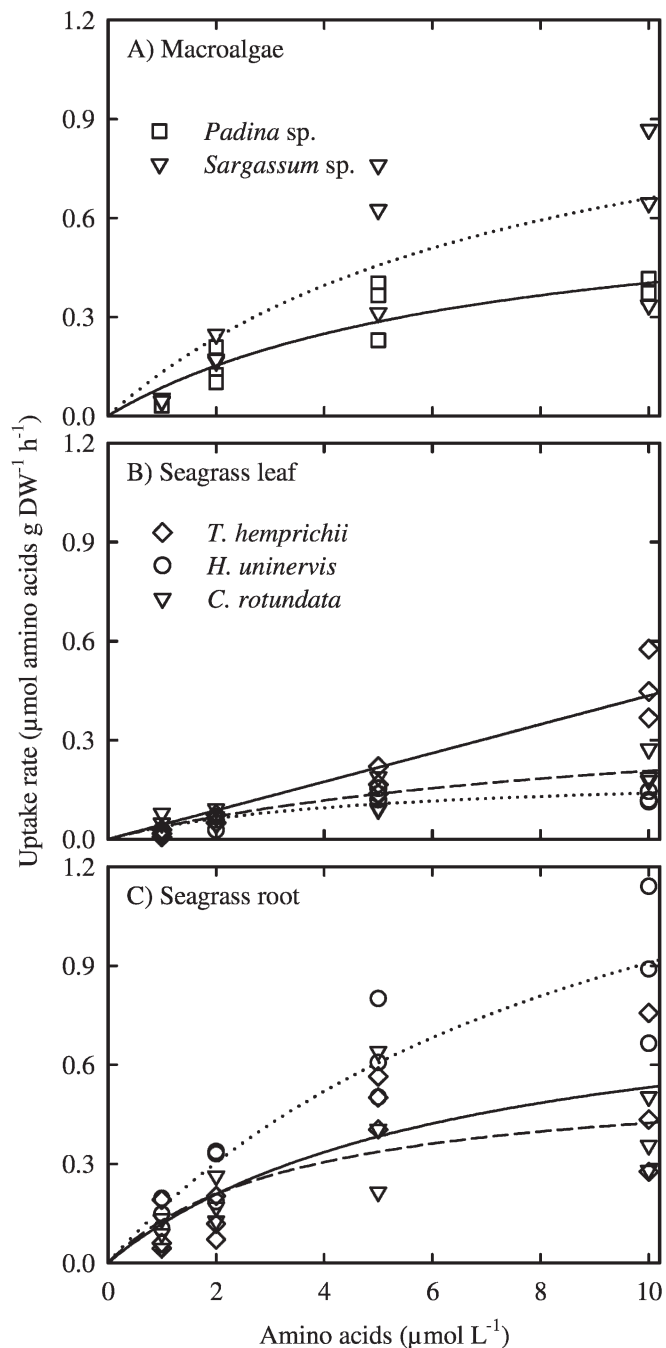

Fig. 2. Amino acid uptake rates for (A) macroalgae thalli (solid line, *Padina* sp.; dotted line, *Sargassum* sp.), (B) seagrass leaves, and (C) seagrass roots (solid line, *T. hemprichii*; dotted line, *H. uninervis*; dashed line *C. rotundata*, respectively).

take up DON has been shown before (Probyn and Chapman 1982; Phillips and Hurd 2004; Tyler et al. 2005), and we confirm this ability for tropical species of the genera *Sargassum* and *Padina*. To our knowledge, this is the first study showing the ability of seagrasses to take up organic N substrates in situ and at concentration levels approaching those in nature. Leaves preferred urea and inorganic N sources over amino acids. Roots took up

Table 2. Uptake kinetics parameters for amino acids. Maximum uptake rate V_{max} (μmol N source g DW^{-1} h^{-1}) and half saturation constant K_{m} (μmol L^{-1} N source) were derived from nonlinear regression of the data using the Michaelis-Menten equation. For data not displaying saturation kinetics, linear regression equations are given (V = amino acids uptake rate, S = substrate concentration in μmol L^{-1}). Coefficient of determination (r^2), SE of the estimates, and the affinity for amino acids uptake at low concentrations (V_{max}: Healey 1980) are given as well.

	K_{m}	V_{max}	V_{max}: K_{m}	r^2
Leaf				
T. hemprichii		$V = 0.043\ S$		0.90
H. uninervis	4.55 (2.39)	0.20 (0.05)	0.044	0.79
C. rotundata	10.5 (8.24)	0.42 (0.20)	0.040	0.74
Thalli				
Sargassum sp.	8.05 (7.94)	1.19 (0.64)	0.148	0.66
Padina sp.	7.01 (3.72)	0.69 (0.19)	0.098	0.85
Root				
T. hemprichii	6.41 (5.80)	0.87 (0.40)	0.136	0.63
H. uninervis	9.98 (6.00)	1.82 (0.64)	0.182	0.85
C. rotundata	3.50 (2.86)	0.57 (0.19)	0.163	0.54

amino acids at comparable rates to NH_4^+, whereas uptake rates for urea and NO_3^- were much lower.

The pool of natural dissolved free amino acids consists of many compounds (Middelburg and Nieuwenhuize 2000; Veuger et al. 2004) and therefore a mixture of 16 was used. Natural DON consists of an even more complex mixture of compounds, such as proteins, oligopeptides, purines, nucleic acids, and humic substances. Usually <20% of the DON pool can be identified, including urea and free amino acids, whereas up to 70% is potentially bioavailable (Seitzinger et al. 2002; Stepanauskas et al. 2002). The uptake kinetics of amino acids by the macrophytes showed saturation uptake kinetics most often, in agreement with earlier observations for macroalgae (Tyler et al. 2005).

Seagrass leaf uptake was measured using intact plants in the field. Translocation of ^{15}N out of the collected and analyzed materials may therefore lead to an underestimation of the uptake. However, we consider the export of ^{15}N from the incubated shoots outside the collected area due to translocation negligible for two reasons. First, the measured concentration of label in the below-ground parts of the seagrass plants after 1 h incubation was very low (1% of the total ^{15}N) compared to leaves. Second, Marbá et al. (2006) showed that the maximum translocation rate of

nutrients between seagrass ramets is less than 1 cm h^{-1} (80 cm over a time span of 4 days). Our incubations lasted only 1 h, and the sediment cores from which the root and rhizome material was collected had a radius of 3 cm, indicating translocation out of the collected area is highly unlikely.

A useful estimation of a species' competitive uptake ability for nutrients is the comparison of uptake at low concentrations (Healey 1980; Harrison et al. 1989). Comparing uptake rates of the different N substrates at 10 μmol L^{-1} showed that the macroalgae *Sargassum* had, overall, the highest uptake rates for all N sources studied (NH_4^+, NO_3^-, urea, and amino acids). Uptake rates of the different N sources for the macroalgae *Padina* were comparable or higher compared to leaf uptake rates of seagrass species. These findings correspond with the main body of studies showing that N uptake rates are generally higher for macroalgae than seagrasses (Phillips and Hurd 2004; Romero et al. 2006).

The relative importance of DIN and DON for the N demand of macrophytes depends on their availability and the preferences of the macrophytes. Both N pools are generally elevated in sediment relative to the water column (Burdige and Zheng 1998; McGlathery et al. 2001). In the

Table 3. Mean depletion (%) of N sources during 1-h uptake experiments (SD). Initial concentration of the N sources (in μmol L^{-1}) are given below each source ($n = 3$).

	NH_4^+ 10	NO_3^- 10	Urea 10	Amino acids			
				10	5	2	1
Leaf/Thalli							
T. hemprichii	36.2 (1.4)	7.4 (5.6)	9.7 (0.9)	6.0 (0.2)	4.4 (1.1)	4.7 (0.4)	2.6 (1.0)
H. uninervis	22.5 (4.5)	4.7 (1.0)	5.0 (2.0)	1.9 (0.1)	4.0 (1.1)	4.5 (1.8)	3.8 (0.3)
C. rotundata	29.3 (3.2)	21.5 (7.5)	11.5 (6.7)	2.9 (0.6)	2.2 (0.9)	5.7 (1.7)	7.9 (3.5)
Sargassum sp.	50.8 (2.7)	36.6 (9.5)	27.0 (7.4)	9.1 (4.5)	15.8 (5.0)	9.0 (1.9)	3.6 (0.1)
Padina sp.	45.0 (10.9)	42.6 (6.4)	19.9 (8.3)	4.1 (0.7)	11.2 (3.0)	6.9 (3.1)	4.3 (0.6)
Root							
T. hemprichii	6.4 (0.7)	0.5 (0.2)	0.4 (0.2)	3.0 (1.8)	5.8 (2.2)	4.4 (3.0)	6.2 (3.0)
H. uninervis	8.5 (1.6)	0.8 (0.2)	0.7 (0.2)	6.5 (2.2)	8.2 (3.5)	8.5 (3.4)	8.7 (3.1)
C. rotundata	4.2 (1.1)	0.5 (0.4)	0.3 (0.1)	2.5 (0.3)	9.0 (4.6)	4.8 (1.7)	5.9 (1.3)

Florida Keys, DIN concentrations of $NO_2^- + NO_3^-$ (0.33–0.94 μmol L^{-1}) and NH_4^+ (1.6–3.4 μmol L^{-1}) were comparable to our study, whereas total DON concentrations were much higher (31–81 μmol L^{-1}; Boyer et al. 1999). The same pattern was observed in pore-water concentrations in seagrass carbonate sediments, where DON comprised 50–75% of the total dissolved N pool (McGlathery et al. 2001). Despite higher concentrations in pore water, root and leaf uptake of DIN for seagrasses is often found to be about the same (Stapel et al. 1996; Lee and Dunton 1999). In the studied meadow, total DIN concentration was comparable to urea in the water column. Although the leaf uptake rate for urea is lower, the N content is double and overall the importance of DIN and urea as N sources for leaf uptake may be comparable for seagrasses. The comparable uptake rates of NH_4^+ and amino acids by roots, combined with the high DON pool in the sediment, indicates that organic N uptake may be more important than NH_4^+.

Pore-water NO_3^- concentrations are often much lower compared to NH_4^+ in seagrass carbonate sediments (Erftemeijer and Middelburg 1993; McGlathery et al. 2001), but only small differences were measured in this study. Urea concentrations in the sediment were comparable to total DIN concentrations and contain twice the amount of N. Urea is produced by bacteria or macrofauna in the sediment and can thus be a significant component (Burdige and Zheng 1998; Glibert et al. 2006). However, the uptake of urea and NO_3^- by roots was low. Because the low uptake rates of NO_3^- and urea are not caused by the low availability in sediments, processing these N sources may introduce problems for the roots. Processing urea and NO_3^- requires energy (Roth and Pregnall 1988; Tyler et al. 2005), which may restrict the use by roots compared to leaves. Our experimental setup for measuring root uptake limited photosynthesis by the leaves, which may have inflicted the low uptake capacity of the roots. The relative high translocation of these sources to the leaves may also indicate the lack of processing capacity in the belowground parts. The low uptake rates of urea and NO_3^- by roots suggest that these N sources are less important in the sediment of this meadow.

N uptake rates by the roots of seagrasses show that they prefer amino acids in addition to NH_4^+. Both N species are released through decomposition of organic material. The turnover of amino acids by microbial activity can vary greatly in soils, marine surface waters, and sediments with half lives varying from minutes to hours (Veuger et al. 2004; Jones et al. 2005). Seagrass roots therefore have to compete with microbes for uptake, making high affinities understandable (high $V_{max} : K_m$). Part of the DON uptake by roots in natural sediments can be facilitated by microbes in the rhizosphere (Jones et al. 2005). Sediment washing of the roots before the uptake experiments resulted in removal of a large part of the rhizosphere and minimized the effects of uptake through root-associated bacteria. Direct uptake of amino acids by seagrass roots may explain the fast uptake of N derived from particulate organic matter by seagrass roots found in previous studies (Evrard et al. 2005; Barrón et al. 2006). Seagrass roots had the highest competitive ability for amino acids, followed by macroalgae thalli, whereas seagrass leaves had the lowest values. This suggests that amino acid uptake by roots is more important compared to leaf uptake for seagrasses.

In oligotrophic environments where production is often limited by available N, DON can form the largest pool of fixed N (Bronk et al. 2007). The high concentration of DON compared to DIN indicates that if seagrass species are able to take up organic N sources, as shown here, DON may in fact present an important N source for such seagrass species. In the studied meadow, total DIN concentration was comparable to urea in the water column. Although the leaf uptake rate for urea is lower, the N content is double, and overall the importance of DIN and urea as N sources for leaf uptake may be comparable for seagrasses. The comparable uptake rates of NH_4^+ and amino acids by roots combined with the high DON pool in the sediment indicates that organic N uptake may be more important than NH_4^+. The ability of seagrasses to take up DON enables them to directly access DON, i.e., the N recycling pathway provides a shortcut because no mineralization step is involved. Therefore DON uptake provides seagrasses with an efficient mechanism to sustain high productivity in an environment depleted in inorganic nutrients.

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