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PRIMARY RESEARCH PAPER

Comparison of the role of the foliar sheath in nutrient (ammonium and phosphate) acquisition by the seagrass *Thalassia hemprichii* (Ehrenb.) Aschers. at two different sites on tropical Hainan Island, China

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Abstract Generally, the foliar sheaths of seagrass contribute a large biomass to the dry weight of plants, and are found to be above-sediment biomass or, sometimes, below-sediment biomass. However, the role of foliar sheaths of seagrass in nutrient uptake has not yet been established. Thus, this study was performed to test whether the growth form of foliar sheaths affects the nutrient uptake properties of the seagrass. Two separate sets of morphotypes of the seagrass *Thalassia hemprichii* were collected from two different tropical meadows in coastal Hainan Island, China in the South China Sea. Ammonium (NH_4^+) and phosphate (P_i) uptake by solely blades and roots (experiment I), and above and below-sediment tissues (experiment II) of the two sets of specimens were examined in partitioned chambers using laboratory incubations. Curve profiles of the blade and root saturation uptake kinetics were shown to be similar for the two morphotypes of *T. hemprichii*. However, the above and below-sediment tissues uptake kinetics had different characteristics between the two morphotypes. For plants with above-sediment foliar sheaths, uptake by the above-sediment tissues contributed an important part of the whole plants' nutrient acquisition. In contrast, for plants with below-sediment foliar sheaths, the contribution of nutrient uptake by above-sediment foliar blade tissues seemed almost negligible. Therefore, the results demonstrated that foliar sheaths of the tropical seagrass *T. hemprichii* were able to absorb NH_4^+ and P_i. Especially interesting is that the capacity for uptake by robust foliar sheaths growing beneath the sediment was remarkable (we termed this the Zhang–Huang–Thorhaug effect). The role of sheaths in nutrient acquisition found in this study is critical in elucidating seagrass nutrient uptake strategies.

Keywords *Thalassia hemprichii* · Seagrass · Phosphate · Ammonium · Sheaths · Blades · Roots · Nutrient uptake kinetics · Hainan Island · South China Sea

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Introduction

Nitrogen (N) and Phosphorus (P) have been regarded by a series of investigators to be the two most

important nutrients limiting primary production in seagrass ecosystems (reviewed by Touchette & Burkholder, 2000; Romero et al., 2006). However, seagrasses can flourish in oligotrophic areas especially in tropical carbonate-dominated meadows (Fourqurean et al., 2001). It seems paradoxical that they can acquire sufficient nutrients to thrive. Thus, tropical seagrasses apparently have evolved efficient nutrient uptake strategies to maintain adequate nutrients in support of their high rates of primary productivity. A central question in the nutritional physiology of these submerged aquatic vascular plants has been the source of their nutrients. Seagrasses derive N and P, mostly as inorganic forms (N_i and P_i), from sediment pore water (especially NH_4^+), and the water column (mostly NO_3^- and PO_4^{3-}; reviewed by Touchette & Burkholder, 2000; Romero et al., 2006; Burkholder et al., 2007). The sediment and the water column, the two potential sources for uptake, were the bases for a historical controversy about the relative importance of leaf versus root uptake in meeting the nutrient requirements of submerged aquatic plants (Thorhaug and Schroeder, 1979a, b; Denny, 1980; Schroeder & Thorhaug, 1980; Brix & Lyngby, 1985). It is now well established that seagrasses take up N and P via both leaves and roots in amounts depending on the relative availability of nutrients in the sediment versus amounts in the water column (Stapel et al., 1996; Lee & Dunton, 1999), and nutrient uptake by leaves is regarded as a process which contributes a considerable part of nutrient demands (reviewed by Hemminga et al., 1991). Currently, there is a paucity of literature on nutrient uptake strategy in tropical seagrass ecosystems, with the exception studies focusing on quantification of the kinetics of N and P uptake by leaves and roots (Schroeder & Thorhaug, 1980; Stapel et al., 1996; Lee & Dunton, 1999; Gras et al., 2003; Cornelisen & Thomas, 2004; Nielsen et al., 2006; Vonk et al., 2008).

The sheaths contribute a large biomass to the dry weight of plants (Manzanera et al., 1998) and have been established as sinks of C, N, and P (Mateo & Romero, 1997). It is believed that their length can be measured in situ to calculate leaf growth accurately (Gaeckle et al., 2006). It has been hypothesized that the sheaths may be important in the respiratory budget of the ecosystem as sites of significant oxygen uptake (Manzanera et al., 1998). Foliar sheath and foliar blade morphology and anatomy are clearly very similar in cell and organ structure, except that the foliar blade contains pigmented material (Tomlinson 1969, 1972; Kuo 1978). Nonetheless, the role of foliar sheaths in nutrient uptake has not yet been established. Moreover, the flexible growth forms of the foliar sheath morphology seem to be species-specific and/or environmental-condition-specific in various species of seagrasses. Some studies have regarded it as an important portion of the below-sediment biomass (Durako, 1994; Manzanera et al., 1998; Jensen & Bell, 2001), whereas other studies have shown it to be above-sediment biomass (Lin & Shao, 1998; Kuo & Lin, 2010). Clearly, elucidating the role of living foliar sheaths in nutrient acquisition is necessary to improve our understanding of nutrient uptake strategy in seagrass.

Seagrass nutrient uptake strategy remains somewhat elusive for three reasons:

1. Most uptake studies on seagrasses have been conducted with different nutrient concentrations in blade and root chambers for one seagrass species from one study site only (Thursby & Harlin, 1982, 1984; Short & McRoy, 1984; Paling & McComb, 1994; Pérez-Lloréns & Niell, 1995; Terrados & Williams, 1997; Gras et al., 2003; Rubio et al., 2007; Vonk et al., 2008). Some studies also paid attention to uptake kinetics of one seagrass from several sites with different nutrient concentrations (Stapel et al., 1996; Lee & Dunton, 1999). However, no nutrient uptake data are available in the literature for one species of seagrass with considerable differences in morphology from two separate sites.
2. The important organ, the foliar sheath, was not taken into account when discussing the relative importance of above-sediment versus below-sediment nutrient uptake. In fact, the sheath was overlooked in some of the above studies.
3. Leakage from the upper chamber to the lower chamber could not be prevented in the methodology used in previous experiments (Stapel et al., 1996), thus there was scarce experimental data on saturation kinetics for seagrass roots especially in the tropical specimens tested.

In order to overcome the three limitations mentioned above, we used a newly-designed split acrylic container which enabled separation of the

above-sediment tissues from the below-sediment portions of intact shoots and in which a hydraulic pressure-balance technique was used to prevent leakage from the upper chamber to the lower chamber. Specifically, we tested the following hypotheses using laboratory experiments:

1. uptake kinetic data for solely blades or solely roots of *Thalassia hemprichii* (Ehrenb.) Aschers. would not vary significantly despite substantial differences between morphology at different study sites;
2. foliar sheaths growing as below-sediment biomass in carbonate-dominated seagrass meadows can play a measurable role in nutrient acquisition; and
3. the relative contributions by above and below-sediment tissues to total nutrient acquisition of the *Thalassia* specimens would vary between the two study areas because of the different sheath allocation.

Materials and methods

Field sampling

Sampling locations

Xincun Bay (referred to as XCB), an almost enclosed bay, is located on the southeast coast of Hainan Island, China, in the South China Sea (Fig. 1; 18°24′N,

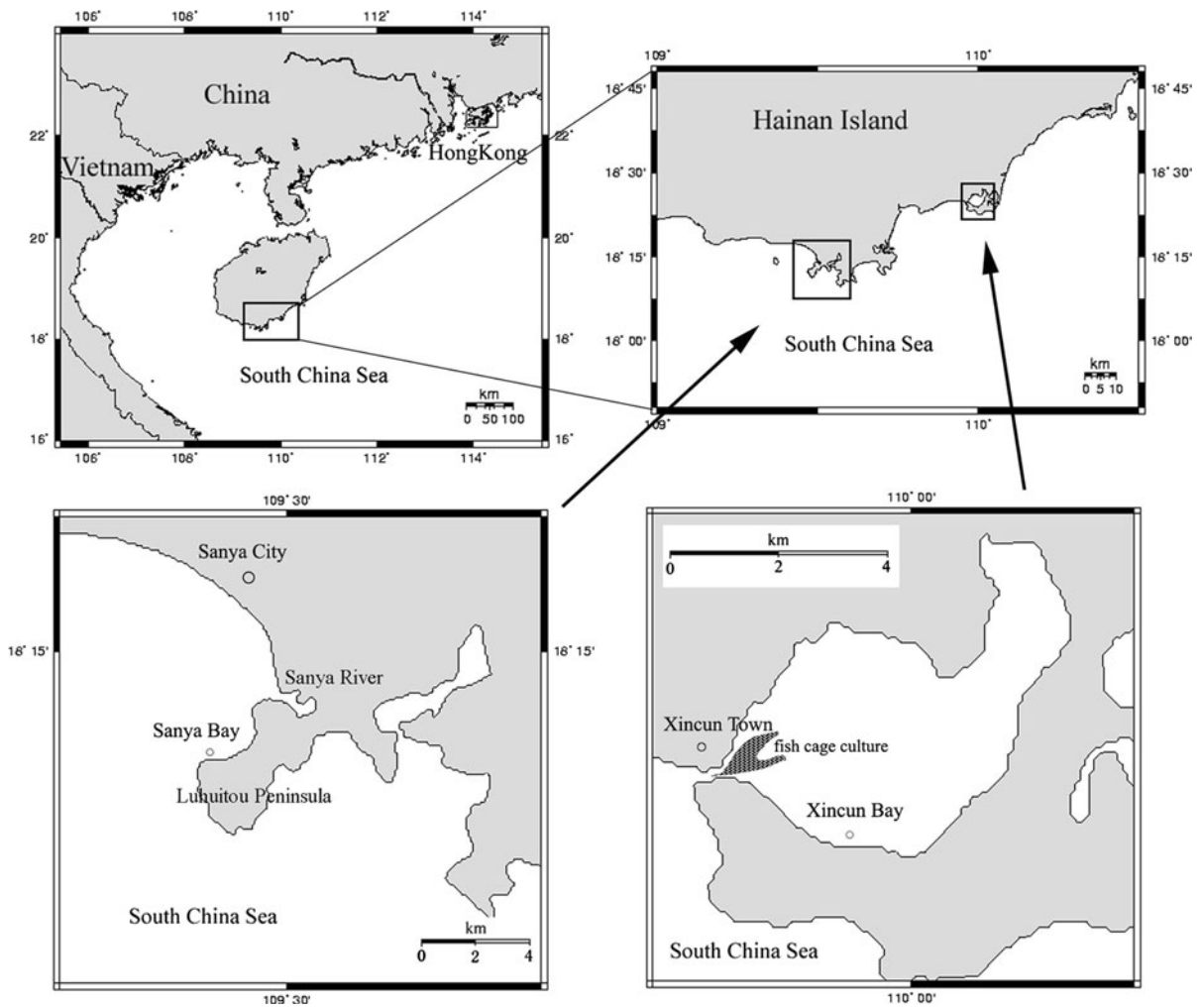

Fig. 1 Location of the two sampling sites

110°E). In this bay the average water temperature is 22.5°C in winter. *T. hemprichii* grows there generally within a mixed seagrass bed with *Enhalus acoroides*, *Cymodocea rotundata*, and *Halophila* species in an area of approximately 200 ha (Huang et al., 2006) wherein the sediment consists chiefly of mixed sandy terrigenous mud. This seagrass segment of the bay is dominated by nutrient inputs from a series of fish-cage mariculture systems that are located near the bay's entrance. This has led to a deterioration in water quality in recent years.

Sanya Bay (referred to as SYB), approximately 60 km away from XCB, is located at the southern tip of Hainan Island, on the west of Luhuitou Peninsula and in the central part of the Coral Reef National Park (Fig. 1; 18°12′46″N, 109°28′38″E). It is affected by nutrient input from Sanya River, but not from caged-fish mariculture. *T. hemprichii* mostly grows on intertidal reef flats, as a community of monospecies seagrass in a sediment which consists of relatively coarse carbonate sand plus coral rubble, in a total area of about 5 ha.

The sediment was judged similar to that in previous studies at these two sites by two scientific groups. SYB site was described as "Lots of broken coral rubble and medium size sand particles of calcium particles, bits of fine particles, all whitish in color in Sanya Bay" (Huang et al., 2007). The description of XCB site was "Fine sandy particles brown in color with grains of medium size sand particles in Xincun Bay" (Huang & Huang, 2008).

T. hemprichii collection and preparation

For the purpose of background investigation, *T. hemprichii* was collected during three seasons (July, October, and December 2009) in XCB. Plants were separated into foliar blades, rhizomes, foliar sheaths, and root tissues. The foliar sheaths were classified as above-sediment biomass, with the blades. In SYB, field sampling was conducted in two seasons, July and December, 2009, and the foliar sheaths were found to form a part of the below-sediment biomass. Sheath length was determined for each of the largest leaves in a shoot and measured from the meristem on the bottom of the leaf bases to the top of the unpigmented tissues. The sheath measured in these experiments was the relatively recent living sheath tissue joining the bottom of the blades to the rhizome and sometimes enwrapping emergent new foliar blades, not the broken older sheath cells clinging to the rhizome (Tomlinson, 1969, 1972; Kuo, 1978).

For cultivation experiments, plants were collected in December 2009 in XCB and SYB. Clumps were very carefully uprooted with intact roots, and the rhizomes washed while in place, then continuously immersed in seawater for transport to the laboratory. In the laboratory, sediment was gently washed from the below-sediment tissues, and then plants were separated into individual shoot units that included healthy leaves, roots, and a 3-cm horizontal rhizome. Epiphytes were gently removed by gently scraping with the thumbnail, initially at the time of collection and then during cultivation. Scraping was also performed before each experiment. Plants of approximately the same size (comparable leaf and root dimensions) were selected and kept in natural seawater at 25°C and at a photon flux ratio (PFR) of 200 μmol m^{-2} s^{-1}, using six strip lights (Philips TLD 18 W/33), with a photoperiod of 14/10 h light/dark. Before uptake experiments, decomposing sheaths which were not connected to actively photosynthetic blades were carefully removed, to prevent influx into the basal portion of the remaining foliar sheaths.

Water column and sediment porewater sampling and analysis

Water column samples were collected at the two study sites and were transported rapidly, on ice, to the laboratory for analysis. Sediment samples were collected with an aseptically-cleaned 8-cm-diameter corer driven approximately 13 cm into the sediments. Pore water from sediment samples was obtained by centrifugation (4,000 × *g* for 15 min) and then diluted (1: 5, *v*/*v*) with low nutrient seawater (measured at <0.1 μM) collected far offshore from SYB. After filtration (Whatman GF/C), the dissolved inorganic NH_4^+ and P_i concentrations of the samples were measured by use of standard colorimetric techniques following the methods of Parsons et al. (1984) using a CANY 722s spectrophotometer.

Laboratory analysis

Chamber deployment

A cylindrical hydroponic chamber (2 l volume of filtered seawater in the upper compartment, 1.5 l

volume of filtered seawater in lower compartment) which enabled the separation of the above-sediment tissues from below-sediment portions of intact shoots was used for nutrient uptake experiments. This chamber was similar to the split-chambers design of Thorhaug and Schroeder (1979b) and Gras et al. (2003), with the following changes: a branch pipe was added to achieve hydraulic pressure balance between upper and lower compartments so that leakage from the upper to the lower chamber could be entirely prevented. On the basis of this container, the plants were held in position (pre-enwrapped by PTFE thread-seal tape, see the section "Plant partition", below) and sealed with low-melting and quick-crystallizing polyester hot-melt adhesive (solid after the adhesive cools, also waterproof, and detachable). In addition, a ventilation tube was placed in the lower compartment to prevent a vacuum from forming when water samples were withdrawn.

Plant partition

In the uptake experiments for solely blades and solely roots, the roots or the blades were isolated independently. Specifically, for the "solely roots" uptake kinetics measurements, thread-seal tape was wrapped around the erect stem so that the lower compartment consisted only of rhizomes and roots, and, simultaneously, the upper compartment consisted of blade and sheath in this set of experiments. For the "solely blades" uptake kinetics measurements, the thread seal tape was wrapped around the top of the sheaths. Thus, solely blades were isolated in the upper compartment while foliar sheaths, rhizomes and roots were incubated in the lower chamber.

Because sheaths had been found to be growing beneath the sediment in SYB but above the sediment in XCB, different partitioning methods were used in the uptake experiments for above and below-sediment tissues, depending on the below or above-sediment placement of sheath at each site. Specifically, for the plants from SYB, the partitioning method was consistent with that used for "solely blades" uptake experiments, simulating the foliar sheaths and roots growing beneath the sediment in situ. Whereas for the plants from XCB, the partitioning method was in line with that for "solely roots" uptake experiments, leaving the foliar sheaths to be incubated with the foliar blades as the above-sediment biomass.

Biomass estimation and nutrient content determination

For field investigations and laboratory experiments, the blades, rhizomes, foliar sheaths, and roots were separated, dried to a constant weight (60°C), and then weighed. Nutrient uptake rates were normalized to dry weights. The dry weight of rhizome material was not included in the calculation of uptake rates, because nutrient uptake by seagrass rhizomes is limited (Short & McRoy, 1984; Brix & Lyngby, 1985; Barnabas, 1991; Stapel et al., 1996). Powdered samples were digested in duplicate for N and P content determination by a sulfuric acid–hydrogen peroxide method (Jiang, 2000). After that, nitrogen content was determined by use of an N analyzer (Buchi Auto Kjeldahl Unit K-300) and phosphate content was analyzed by use of a colorimetric method (Fourqurean & Zieman, 1992).

Experimental design

Blade and root nutrients uptake kinetics (experiment I)

Blade and root nutrients uptake experiments were carried out from December 2009 to January 2010. For the blade uptake experiments, NH_4^+ and P_i concentrations in the root compartment were maintained at ambient levels in situ, 10 and 20 μM NH_4^+ for SYB and XCB plants, 1.0 and 3.0 μM P_i for SYB and XCB plants, respectively. Similarly, concentrations in the blade compartment were 0.9 μM NH_4^+ for SYB and 2.0 μM NH_4^+ for XCB and 0.2 μM P_i for plants from both sites during the measurements of root NH_4^+ and P_i uptake rates. Five replicates of similar size plants from each treatment were preincubated for 48 h in a 4 l volume seawater glass aquarium with NH_4^+–P_i-free growth medium for NH_4^+ and P_i uptake. After preincubation, a series of nutrient concentrations for the target compartment were introduced into each chamber. Five of 6 chambers were spiked with concentrated $(NH_4)_2SO_4$ and KH_2PO_4 solutions to yield a representative range of NH_4^+ and P_i concentrations. For example, for blade uptake determinations, blade compartments were spiked to achieve

NH_4^+ concentrations of 1–180 μM for the 5 chambers. The sixth chamber was the control without plants but all other variables including handling and sampling were similar to those for the test chambers. Water samples were collected from the chambers at consistent time intervals (2 h) and analyzed immediately to determine NH_4^+ and P_i concentrations by use of the colorimetric techniques as described above. NH_4^+ and P_i uptake by *T. hemprichii* was estimated from the nutrient depletion in the solution in the target compartments over time. After each sampling, volume reduction (25 ml) for every chamber was taken into account and then total nutrient depletion was modified. During the period of incubation, all chambers were kept at 25°C and a PFR of 200 μmol m^{-2} s^{-1}. Uptake rates were plotted as a function of nutrient concentration. The NH_4^+ and P_i uptake kinetics were derived using the Michaelis–Menten equation

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

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 $\frac{1}{2}V_{\max}$. Uptake affinity (α) is equivalent to $V_{\max}/K_m$.

The nutrient contribution ratio of blade versus root was calculated by using the following equation:

$$R = V_{\text{ambient}} \times M_{\text{blade}}/(V_{\text{ambient}} \times M_{\text{root}})$$

where V_{ambient} is the nutrient uptake rate at ambient concentration, M_{blade} or M_{root} is blade or root biomass (grams dry weight per chamber), and R is the ratio of nutrient contribution by blade versus by root. Similarly, the contribution ratio of above versus below-sediment tissues was calculated by use of the method described above.

Above and below-sediment tissues nutrient uptake measurements (experiment II)

In order to test the role of the foliar sheath, the above and below-sediment tissues uptake experiments were carried out from December 2009 to January 2010 with plants collected from both sites. Partitioning methods used for the two study sites were as described above (in the section "Plant partition"). Experimental settings were consistent with the experiments described in the section "Blade and root nutrients uptake kinetics", except for the concentrations in the target compartment. Because environmental nutrient concentrations in the water column and the sediment porewater in situ were below the K_m, NH_4^+, and P_i concentrations added to the target compartment were far below saturation, and this did not enable modeling of the data to the Michaelis–Menten equation. Nevertheless, a linear regression was applied. Based on this method, we computed the affinities differently in measuring the uptake kinetics of the above and below-sediment tissues. Using this approach, the slope of the line represented the uptake affinity.

Data analysis

Two-way analysis of variance (ANOVA; 3 seasons × 2 sites) by use of the statistical software SPSS Ver. 16 (SPSS, Illinois, USA) was used to test for significant differences between the sites and the seasons for the dependent variables. The assumption of homogeneity of variances was tested with a Levene's test. Whenever significant differences were observed from ANOVA, a suitable post-hoc test was run to identify dependent variables that were significantly different. Either a Tukey HSD or Games-Howell post-hoc test was used to identify significantly different components. The Games–Howell nonparametric test was used when Levene's test failed.

PROC NLIN of SAS System 9.1 was used to fit nonlinear regression models (Michaelis–Menten equation), and the sum-of-squares reduction test (SSRT) was performed to compare values among models (Schabenberger & Pierce, 2002). Linear regression analysis (stepwise) was used to obtain the regression equation with high Pearson's correlation coefficients. Differences among slopes were tested by analysis of covariance in SPSS (Cohen et al., 2003). All statistical tests were assessed at $\alpha = 0.05$.

Results

Nutrient background and plant variables

Mean values of ambient environment nutrient concentrations measured for the three sampling periods

are summarized in Table 1 (data for SYB in October 2009 was unavoidably missing). At both locations, the water column concentrations of NH_4^+ and P_i ranged between NH_4^+ at 0.18 to 2.76 μM and P_i at 0.01 to 0.67 μM (Table 1). The variation of nutrient concentrations in the water column at sites XCB and SYB was dependent upon tide and wastewater discharge. Note that the average NH_4^+ concentration in XCB (1.98 μM) was double the average in SYB (0.88 μM), whereas P_i concentrations in the water column of XCB and SYB were almost the same. For both NH_4^+ and P_i concentrations, statistically significant differences were shown between sampling periods ($P < 0.001$ and $P = 0.03$, respectively).

NH_4^+ and P_i concentrations in the sediment of both sites were far higher and significantly different from in the water column (both $P < 0.001$) and were of far higher variability (from 5.97 to 34.69 μM for NH_4^+ and from 0.20 to 10.56 μM for P_i) (Table 1). There was a large average concentration difference between sites: at XCB, the average concentration of P_i in porewater was 3.41 μM whereas in SYB the average concentration was 1.23 μM. Similarly, average NH_4^+ concentration of porewater nutrients in XCB was consistently double of that at site SYB (XCB: 20.46 μM and SYB: 10.88 μM) (Table 1). Statistically significant differences were found between the two sites for both NH_4^+ and P_i concentrations in the sediment ($P = 0.002$ for NH_4^+ and $P = 0.011$ for P_i). Meanwhile, P_i concentrations in the porewater varied statistically significantly between sampling periods ($P = 0.039$).

Details of the seasonal variations in biological data for plants from XCB and SYB meadows monitored during the study are summarized in Table 1. Differences between the two sites for most biological data were pronounced, with the exception of N content and P content for both blade and sheath. A greater percentage of the plant biomass in SYB was allocated to below-sediment tissues, as reflected in above/below-sediment biomass ratios at SYB (0.27) that were statistically significantly lower than at XCB (0.46; $P < 0.001$) (Table 1). Plants from SYB had much longer averages for blades (13.5 cm) and longer sheaths (9.6 cm for average) than averages for plants from XCB (9.6 cm blades and 5.2 cm, sheaths, both $P < 0.001$). However, at SYB blades were statistically much narrower ($P < 0.001$) (Table 1) and sheaths grew beneath the sediments. Root/shoot biomass ratio was much higher at site XCB than at SYB ($P < 0.001$) whereas blade/shoot biomass ratio and sheath/shoot biomass ratio were significantly higher at SYB ($P = 0.019$ and $P < 0.001$, respectively). Comparison of the N and P content of blades and sheaths revealed no significant differences between the two locations, but the N and P content of the roots were statistically significantly higher at site XCB ($P = 0.010$) than at SYB ($P < 0.001$) (Table 1).

Nutrient uptake kinetics for blades and roots

Because for control cylinders there were no significant changes in either NH_4^+ or P_i concentrations in the water during the time course of the incubations (data measured but not shown, because there was no statistically significant difference), we were led to believe that the changes in nutrients' concentrations to a decreased level in the upper or lower compartments in the experiment was because of uptake by the target materials. Therefore, blank measurements were not taken into account in the calculations of kinetic data.

NH_4^+ and P_i uptake by blades and roots in this set of measurements were dependent on the concentration of dissolved nutrients in the external medium. An increase in nutrient concentration of the seawater medium resulted in increased uptake rates which apparently fit Michaelis–Menten kinetics.

NH_4^+ uptake kinetics by solely blades and roots of *T. hemprichii* from the two locations are presented in Fig. 2. The resulting kinetics and statistical data are listed in Table 2. All data were combined for curve fitting according to the Michaelis–Menten equation. It was shown that curve profiles of uptake kinetics for the plants from the two study sites were similar (Fig. 2). However, the estimates of the model data tested by covariance analysis revealed that there were significant differences of V_{max} of roots between the two sites (Table 2).

P_i uptake kinetics by solely blades and solely roots of *T. hemprichii* from the two locations are presented in Fig. 3. It was shown that the curve profiles of uptake kinetics for both blades and roots were similar between plants from sites SYB and XCB. The resulting kinetics and statistical data are also listed in Table 2. Covariance analysis revealed that there were significant differences between V_{max}, K_m, and α

Table 1 Summary of background data during sampling (±SD in parentheses)

Variables	July 2009		October 2009		December 2009		Average	
	XCB	SYB	XCB	SYB	XCB	SYB	XCB	SYB
Nutrient								
NH_4^+ concentration in water column (μM)	2.13 (±0.89), $n = 7$	1.80 (±0.1), $n = 3$	1.95 (±0.57), $n = 7$	–	1.71 (±0.44), $n = 6$	0.36 (±0.15), $n = 6$	1.98 (0.29–2.76)	0.88 (0.18–1.87)
NH_4^+ concentration in porewater (μM)	20.78 (±6.17), $n = 7$	9.46 (±2.3), $n = 3$	20.18 (±10.67), $n = 7$	–	20.41 (±5.44), $n = 6$	11.33 (±3.34), $n = 6$	20.46 (8.63–34.69)	10.88 (5.97–15.81)
P_i concentration in water column (μM)	0.11 (±0.06), $n = 7$	0.17 (±0.03), $n = 3$	0.17 (±0.09), $n = 7$	–	0.22 (±0.08), $n = 6$	0.19 (±0.04), $n = 6$	0.17 (0.01–0.67)	0.17 (0.09–0.25)
P_i concentration in porewater (μM)	3.77 (±2.41), $n = 7$	1.25 (±0.22), $n = 3$	5.66 (±3.03), $n = 7$	–	0.39 (±0.15), $n = 6$	1.35 (±0.55), $n = 6$	3.41 (0.20–10.56)	1.23 (0.69–2.13)
Seagrass								
Blade length (cm)	8.8 (±1.2), $n = 18$	13.6 (±2.1), $n = 7$	10.1 (±2.2), $n = 8$	–	10.2 (±2.1), $n = 24$	13.5 (±2.5), $n = 26$	9.6 (6.5–14.5)	13.5 (7.8–18.0)
Blade width (cm)	1.17 (±0.08), $n = 18$	0.98 (±0.11), $n = 7$	1.12 (±0.08), $n = 8$	–	1.20 (±0.08), $n = 24$	0.97 (±0.10), $n = 26$	1.17 (1.00–1.32)	0.97 (0.79–1.18)
Sheath length (cm)	5.02 (±0.67), $n = 6$	9.94 (±1.65), $n = 7$	5.14 (±0.77), $n = 5$	–	5.25 (±0.8), $n = 24$	9.57 (±1.41), $n = 23$	5.2 (4.0–7.0)	9.6 (6.8–13.1)
Above/below biomass ratio	0.48 (±0.05), $n = 3$	0.30 (±0.03), $n = 3$	0.43 (±0.06), $n = 3$	–	0.48 (±0.05), $n = 3$	0.26 (±0.05), $n = 3$	0.46 (0.39–0.54)	0.27 (0.21–0.32)
Blade/shoot biomass ratio	0.17 (±0.02), $n = 3$	0.19 (±0.03), $n = 3$	0.16 (±0.02), $n = 3$	–	0.16 (±0.01), $n = 3$	0.20 (±0.03), $n = 3$	0.16 (0.15–0.19)	0.19 (0.16–0.23)
Root/shoot biomass ratio	0.10 (±0.05), $n = 3$	0.03 (±0.01), $n = 3$	0.11 (±0.04), $n = 3$	–	0.10 (±0.01), $n = 3$	0.04 (±0.01), $n = 3$	0.10 (0.06–0.15)	0.03 (0.02–0.05)
Sheath/shoot biomass ratio	0.20 (±0.04), $n = 3$	0.30 (±0.02), $n = 3$	0.18 (±0.05), $n = 3$	–	0.18 (±0.04), $n = 3$	0.31 (±0.01), $n = 3$	0.19 (0.13–0.24)	0.30 (0.28–0.35)
Blade N content (%)	2.11 (±0.03), $n = 3$	2.35 (±0.20), $n = 3$	2.54 (±0.13), $n = 3$	–	2.57 (±0.08), $n = 3$	2.37 (±0.16), $n = 3$	2.41 (2.09–2.66)	2.36 (2.16–2.55)
Sheath N content (%)	1.22 (±0.04), $n = 3$	1.32 (±0.20), $n = 3$	1.25 (±0.09), $n = 3$	–	1.24 (±0.07), $n = 3$	1.30 (±0.18), $n = 3$	1.24 (1.15–1.32)	1.29 (1.11–1.54)
Root N content (%)	1.16 (±0.02), $n = 3$	1.06 (±0.06), $n = 3$	1.26 (±0.21), $n = 3$	–	1.45 (±0.17), $n = 3$	1.14 (±0.16), $n = 3$	1.29 (1.09–1.57)	1.10 (1.00–1.32)
Blade P content (%)	0.23 (±0.02), $n = 3$	0.24 (±0.01), $n = 3$	0.26 (±0.01), $n = 3$	–	0.25 (±0.02), $n = 3$	0.24 (±0.01), $n = 3$	0.25 (0.22–0.27)	0.24 (0.23–0.25)
Sheath P content (%)	0.14 (±0.01), $n = 3$	0.13 (±0.01), $n = 3$	0.14 (±0.01), $n = 3$	–	0.12 (±0.01), $n = 3$	0.15 (±0.02), $n = 3$	0.13 (0.12–0.16)	0.14 (0.12–0.17)
Root P content (%)	0.12 (±0.01), $n = 3$	0.10 (±0.01), $n = 3$	0.13 (±0.01), $n = 3$	–	0.15 (±0.01), $n = 3$	0.11 (±0.01), $n = 3$	0.13 (0.12–0.15)	0.11 (0.10–0.12)

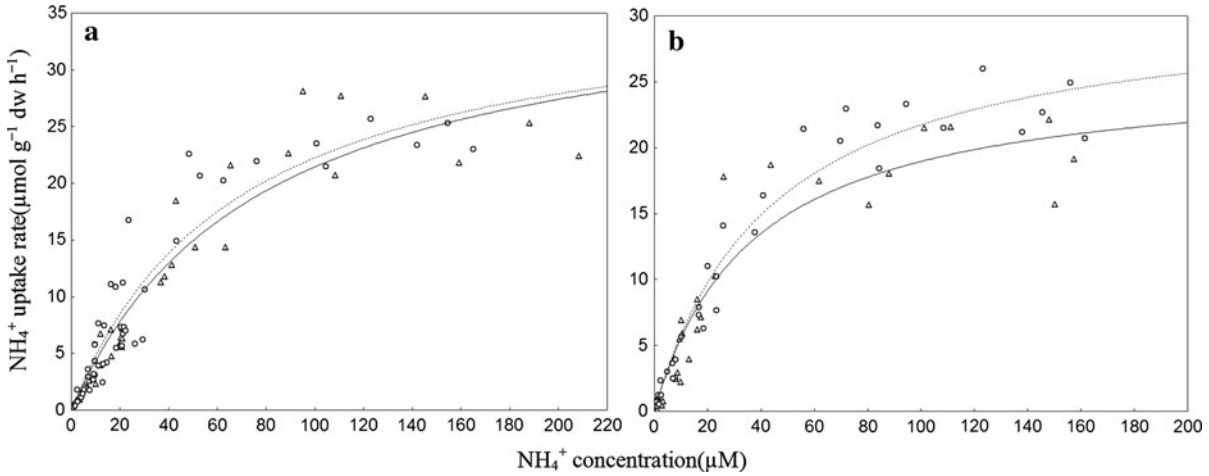

Fig. 2 *Thalassia hemprichii*. NH_4^+ uptake rate (μmol g^{-1} dw h^{-1}) by blades (**a**) and roots (**b**) of plants from sites XCB (*circles and dotted line*) and SYB (*triangles and solid line*) as a function of NH_4^+ concentration (μM) in the target organ compartment. The curves represent the best fits according to the Michaelis–Menten model (see Table 2 and text for explanation)

Table 2 NH_4^+ and P_i Michaelis–Menten kinetic data for blades and roots from each of the two sites SYB and XCB: V_{max} and K_m, with asymptotic standard errors of the data in parentheses (95% confidence limits)

Experiment	V_{max} (μmol g^{-1} dw h^{-1})	K_m (μM)	α	R^2	*P*-level
NH_4^+					
XCB blade	37.36 (30.64–44.08)	67.89 (44.33–91.48)	0.550	0.900	<0.001
XCB root	**31.27 (27.83–34.71)**	43.89 (31.38–56.40)	0.712	0.962	<0.001
SYB blade	37.93 (30.58–45.28)	76.89 (45.86–107.91)	0.493	0.919	<0.001
SYB root	**25.94 (21.48–30.40)**	36.86 (20.55–53.17)	0.704	0.903	<0.001
P_i					
XCB blade	**4.70 (3.86–5.54)**	**13.17 (8.77–17.57)**	**0.357**	0.932	<0.001
XCB root	5.59 (4.92–6.27)	4.33 (2.83–5.82)	1.291	0.927	<0.001
SYB blade	**3.63 (3.06–4.19)**	**7.40 (4.59–10.21)**	**0.491**	0.942	<0.001
SYB root	5.18 (4.39–5.98)	3.74 (2.21–5.27)	1.385	0.852	<0.001

The affinity (α) was calculated as V_{max}/K_m. The level of significance (*P*) and the R^2 of the regression are also included. Values in bold are statistically significantly different between sties ($P < 0.05$)

for blades between plants from the two sites SYB and XCB.

Above and below-sediment tissues uptake of nutrients and relative tissue contributions

NH_4^+ and P_i taken up by the above and below-sediment tissues increased linearly with ambient nutrient concentrations (Fig. 4). The slope of the linear regression represents the uptake affinity. For plants from both sites, much higher affinities were found in below-sediment tissues than above-sediment tissues for both NH_4^+ and P_i, which is a similar relationship as the α found for solely roots and solely blades in saturation kinetics. However, unlike the α relationship between sites found for solely roots in saturation kinetics, the below-sediment tissues of plants from SYB (including foliar sheaths and roots) had significantly higher affinities than those from site XCB (solely roots with no foliar sheaths) ($P < 0.05$). Moreover, uptake affinities of above-sediment tissues from XCB (including foliar sheaths and foliar blades) were slightly higher than those of plants from SYB (NH_4^+: 0.26 for XCB and 0.23 for SYB; P_i: 0.30 for XCB and 0.23 for SYB) (Fig. 4).

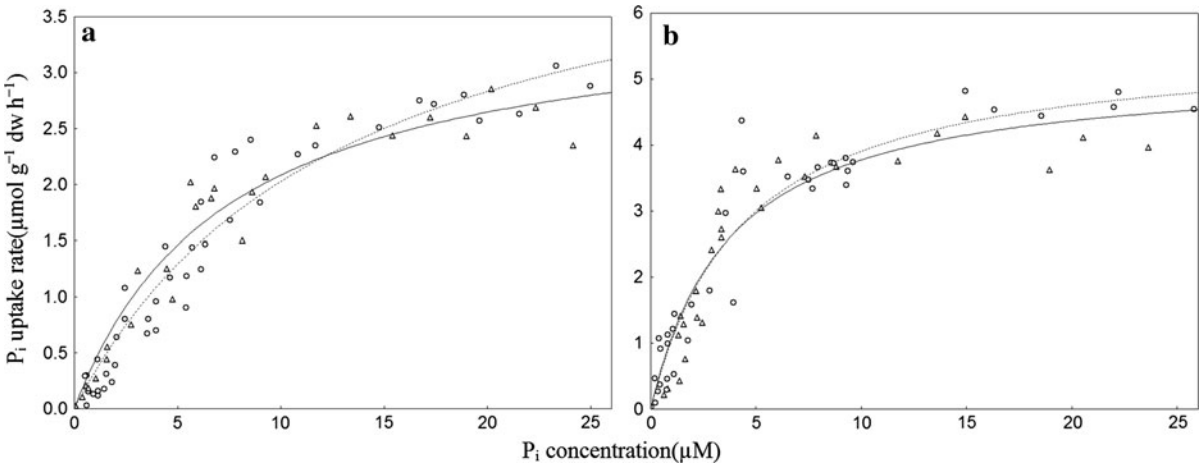

Fig. 3 *Thalassia hemprichii*. P_i uptake rate (μmol g^{-1} dw h^{-1}) by blades (**a**) and roots (**b**) of plants from sites XCB (*circles and dotted line*) and SYB (*triangles and solid line*) as a function of the P_i concentration (μM) in the target organ compartment. The *curves* represent the best fits according to the Michaelis–Menten model (see Table 2 and text for explanation)

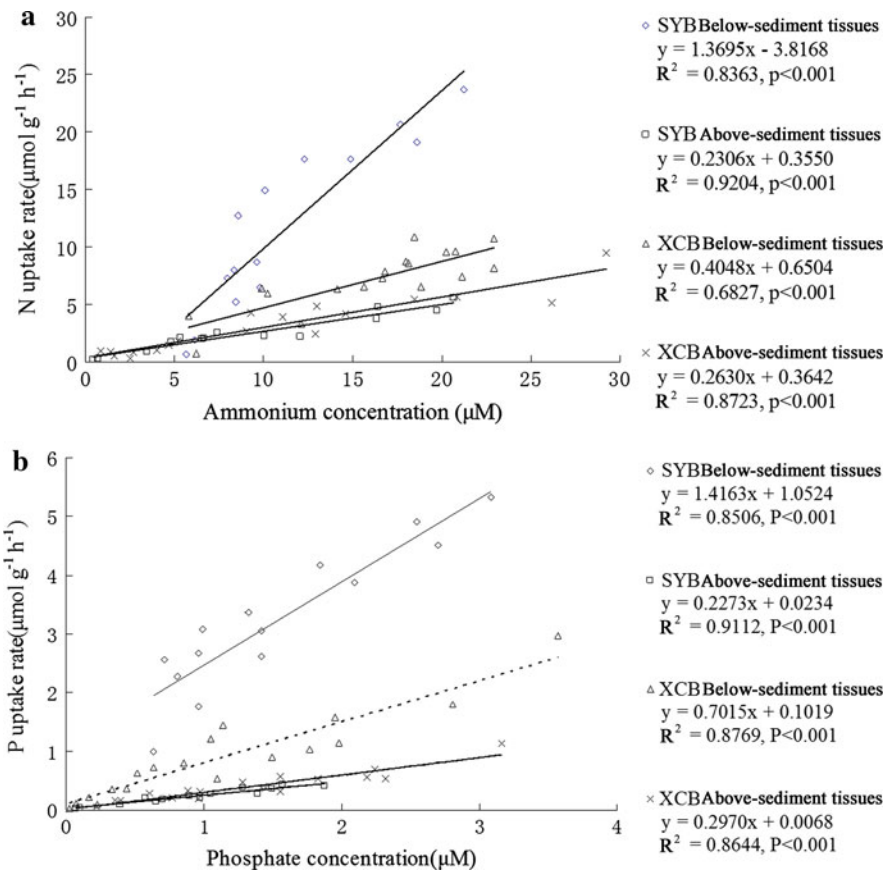

Fig. 4 *Thalassia hemprichii*. The NH_4^+ uptake rate (μmol g^{-1} dw h^{-1}) (**a**) and the P_i uptake rate (μmol g^{-1} dw h^{-1}) (**b**) by above and below-sediment tissues of plants from both sites as a function of the concentration (μM) in the target organ compartment

Accordingly, the uptake rates for different tissues, the nutrient contribution ratio of blade/root, and the contribution ratio of above/below-sediment tissues are found in Table 3. Table 3 reveals that blade uptake rates calculated by use of the Michaelis–Menten equation derived by saturation kinetics and

Table 3 Uptake rates for different tissues and nutrient contribution ratios of blade/root and above/below-sediment tissues of plants from two sites at natural ambient concentrations

Locations	XCB		SYB	
	Uptake rate (μmol g^{-1} dw h^{-1})	Contribution ratio	Uptake rate (μmol g^{-1} dw h^{-1})	Contribution ratio
NH_4^+				
Blade	1.06	0.39	0.43	0.61
Root	9.94		5.91	
Above-sediment tissues	0.88	0.50	0.56	0.05
Below-sediment tissues	8.93		11.08	
P_i				
Blade	0.06	0.09	0.08	0.53
Root	2.46		1.28	
Above-sediment tissues	0.06	0.12	0.06	0.02
Below-sediment tissues	2.49		2.79	

Uptake rates of blade and root were calculated by use of the Michaelis–Menten equation derived by saturation kinetics (Figs. 2, 3). Uptake rates of above and below-sediment tissues were calculated by use of the linear regression equations (Fig. 4). Biomass of the plants from each site held in laboratory experiments were used in the calculation of the contribution of each tissue to nutrient acquisition (data not shown, but the mean biomass ratio of above to below-sediment tissues was 5.03 for XCB and 0.98 for SYB; the biomass ratio of blade/root was 3.65 for XCB and 8.40 for SYB)

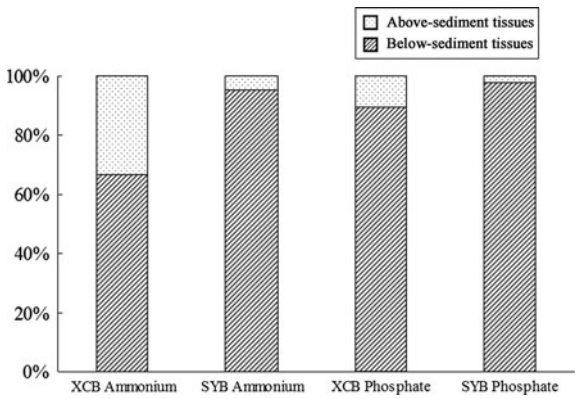

Fig. 5 NH_4^+ and P_i attributed to above and below-sediment tissues of plants from both sites at the ambient concentrations in situ

the above-sediment tissues uptake rates calculated by use of linear regression equations were similar for plants from the SYB site where foliar sheaths are below the sediment. Likewise, the root uptake rates calculated by saturation kinetics and the below-sediment tissues uptake rates calculated by use of linear regression equations were also close in value for plants from site XCB where foliar sheaths are above the sediment. However, the uptake rates by the below-sediment tissues (11.1 μmol g^{-1} dw h^{-1} for NH_4^+ and 2.8 μmol g^{-1} dw h^{-1} for P_i) were much higher than solely for roots (5.9 μmol g^{-1} dw h^{-1} for NH_4^+ and 1.3 μmol g^{-1} dw h^{-1} for P_i), and the above/below-sediment contribution ratio was much lower (0.05 for NH_4^+ and 0.02 for P_i) than blades versus roots (0.61 for NH_4^+ and 0.53 for P_i) for the specimens from SYB where robust and long sheaths grew beneath the sediment and were measured as "below sediment". In contrast, the above/below-sediment contribution ratio was slightly higher (0.50 for NH_4^+ and 0.12 for P_i) than blades versus solely roots (0.39 for NH_4^+ and 0.09 for P_i) for XCB where short sheaths occurred above the sediment. NH_4^+ and P_i contributions by the above and below-sediment tissues at the natural ambient concentrations are presented in Fig. 5.

Discussion

Environment heterogeneity and its effect on uptake kinetics

Environmental physical and chemical differences of the habitat at the two study sites seem to have created morphological differences in foliar sheaths, foliar blades, roots, and growth compartment of foliar sheaths for the *T. hemprichii* community at the two

study sites. The heterogeneity in sediment composition, sandy terrigenous mud for XCB and coarse carbonate sand and coral rubble for SYB, are the two differing dominant physical conditions. Additionally, low nutrient sediment concentration at site SYB (which is basically an open ocean site) versus the site XCB (a lower-energy embayment with additions of man-mediated fertilizer from fish culture), created nutrient concentration differences at the two sites. We theorize that the coarse carbonate sand and hard coral rubble in SYB may have limited the root growth and lateral prolongation of the rhizomes, thus causing the relatively simple root/rhizome architecture and a lesser biomass of roots with growth of the foliar sheaths below sediment than at site XCB wherein the roots/rhizomes easily could expand through the muddy sand. Generally, the sheath of this species *T. hemprichii* has been considered to be above-sediment tissues (Lin & Shao, 1998; Kuo & Lin, 2010). However, a central physiological difference of *Thalassia* specimens between the two sites seems to be the placement of the foliar sheath at the high energy site SYB below the sediment surface where sheaths have access to sediment porewater (sheaths are completely buried in the sediment) versus at the low energy site XCB where sheaths grow above the sediment so that sheaths have direct access to nutrients from the water column (with shoots bearing foliar sheaths and foliar blades thrust into the water column). An ecophysiological response to the formation of the well-developed below-sediment sheaths may be a reaction to heavy sedimentation movement plus high energy above sediment, whereas the above-sediment sheaths may be a reaction to continual input or erosion of sediment plus lower energy. One hypothesis is that relatively simple root architecture in the high energy SYB site requires more biomass growing beneath sediment to sustain itself and to avoid broken newly-produced blades in the high energy environment of the open ocean surf. On the other hand, Lee & Dunton (1999) working in the Laguna Madre, Texas, in the western Gulf of Mexico, also pointed out that *Thalassia testudinum* Banks & Sol. ex König had a higher fraction of biomass allocated to below-sediment tissues in plants when living under low nutrient sediment conditions. This plasticity of *Thalassia* morphological architecture, with greater relative production of below-sediment tissues in areas where below-sediment resources are scarce, is a basic tenet of plant physiological ecology. Thus, we hypothesized here that sedimentary environment (including chemistry and the physical regime) determines whether the sheaths are found below or above the sediment.

In general, nutrient uptake experiments on seagrass have focused on defining the maximum uptake rates (V_{max}) and half saturation constants (K_m) for modeling purposes (Pérez-Lloréns & Niell, 1995; Terrados & Williams, 1997; Gras et al., 2003; Rubio et al., 2007; Vonk et al., 2008). Some of the studies also focused on another characteristic, α (or nutrient affinity; Lee & Dunton, 1999; Nielsen et al., 2006).

Blade NH_4^+ V_{max} values determined in this study are consistent with those of Stapel et al. (1996) for *T. hemprichii* (32–37 μmol g dry weight^{-1} h^{-1}). The blade P_i V_{max} values determined in our study are slightly higher than those in two other studies of tropical *Thalassia* (1.9–3.2 μmol g dry weight^{-1} h^{-1}; Stapel et al., 1996; Gras et al., 2003). Thus, on the basis of the limited saturation kinetic data available for seagrass, the lack of a clear site or species difference suggests that similar kinetics (V_{max}) are operating in tropical and subtropical species of *Thalassia*.

K_m is an indicator of the ability of a species to compete for nutrients under relatively low-nutrient conditions—low K_m indicates a high competitive ability of the seagrass. For P_i, we estimated root K_m values to be lower for plants from site SYB (3.74 μM) than from XCB (4.33 μM), showing that lower nutrient availability in SYB sediment led to a lower root K_m. Similarly for NH_4^+, root K_m value was a little lower for plants from SYB (36.86) than from XCB (43.89). As stated above, the K_m for NH_4^+ of *T. hemprichii* in this study was in line with the study by Stapel et al. (1996) of *T. hemprichii* (21–60). The K_m for P_i of *T. hemprichii* in this study was almost the same as earlier estimates for *T. hemprichii* (7.7–15 for blade by Stapel et al., 1996) and for *T. testudinum* (12 for blade and 4 for root by Gras et al., 2003, 7.89 for blade and 3.34 for root by Nielsen et al., 2006), again suggesting that no major kinetic differences exist between sites or even species in terms of nutrient uptake at saturation. The consistency of our *T. hemprichii* nutrient kinetic results have important consequences for modeling nutrient uptake in tropical seagrass ecosystems dominated by *Thalassia*.

At low substrate availability, α is a critical nutrient uptake property (Nielsen et al., 2006). We found the α in this study, ranging between 0.36 and 1.39 for P_i using traditional chemical techniques, pre-starved, and 10-h incubations, to be much higher than those from southeast Asia, where α was calculated to be 0.13–0.19 for *T. hemprichii* blades in 10-h incubations (Stapel et al., 1996). These dissimilarities were found also in Nielsen's study, kinetic experiments of *T. testudinum* using a ^{33}P-tracer technique (Nielsen et al., 2006). Nevertheless, it has to be considered that in this study *T. hemprichii* plants were nutrient-starved for 2 days, and then it has to be taken into account that a thirst for nutrients was generated after starvation, which led to high-affinity nutrient uptake. In the NH_4^+ uptake experiment, we estimated α of *T. hemprichii* in the range 0.49 to 0.71. We noticed that the root affinity was estimated to be higher than the blade affinity in our study and differed to Lee and Dunton's α for *T. testudinum* which ranged between 0.03 and 0.30 for roots and 0.57 and 2.82 for leaves (Lee & Dunton, 1999). These reported data implies α can vary substantially and we wonder whether α is regulated by the previous history of plants' nutrient status.

The relative importance of above and below-sediment tissues and the role of the sheath

Despite much higher nutrient concentrations in the porewater, the blade uptake of NH_4^+ for the seagrass *T. hemprichii* was found to be a considerable part of the whole plant nutrient acquisition for plants from XCB (Table 3). This finding is consistent with the conclusion that even in the tropics, where water column nutrient concentrations are often very low, leaves clearly have a significant ability for NH_4^+ or P_i uptake (Stapel et al., 1996). Although it is commonly purported that seagrasses meet their nutritional demand for P by acquiring P_i through root uptake (Brix & Lyngby, 1985), it has become increasingly clear and generally accepted for an array of seagrass species, environmental conditions, and climatic zones (since the original studies of Schroeder and Thorhaug in 1980) that both above and below-sediment tissues of seagrasses participate in nutrient uptake. This study demonstrates that blade tissues have high uptake affinities for both NH_4^+ and P_i. Previous studies by Pedersen et al. (1997) and Lee & Dunton (1999) also demonstrated blade tissues have high N uptake affinities. Several other previous studies have indicated that uptake by blades can contribute substantially to total N acquisition by seagrasses, and in some situations nutrient uptake by the leaves may even be essential in meeting plant nutrient demands (Pedersen & Borum, 1992; Stapel et al., 1996; Terrados & Williams, 1997; Lee & Dunton, 1999; Lepoint et al., 2002).

In this study, average NH_4^+ and P_i concentrations found in the two study sites were consistent with prior reports that concentrations of nutrients in porewater can exceed those in column water (Carignan & Kalff, 1980; Stapel et al., 1996).Consequently, although above-sediment tissues did contribute to uptake for a specific proportion of nutrients for the whole plants, sediment pore water was found to be the main source of uptake for both NH_4^+ and P_i for seagrass growth in our study (Fig. 5; Table 3). Sediment pore water has been stated by some workers to be the main source of NH_4^+ and P_i for seagrass growth (Carignan & Kalff, 1980; Short & McRoy, 1984; Zimmerman et al., 1987). Some previous studies have suggested that the relative concentrations of nutrients in water or sediments determine the main site for uptake (Penhale & Thayer, 1980; Thursby & Harlin, 1984; Brix & Lyngby, 1985). Based on this hypothesis, at NH_4^+ concentrations approximately 6 μM and P_i concentrations less than 2 μM, which were still lower but close to the upper range experienced by *T. hemprichii* in XCB (2.76 μM for NH_4^+ and 0.67 for P_i) (Table 1), the above-sediment tissues of these plants would take up NH_4^+ and P_i to a similar amount as would the roots. However, in the much more extreme situation for *T. hemprichii* from SYB, sediment nutrients are almost the principal source of NH_4^+ and P_i, although nutrients in the sediment were not as high as at XCB. Apparently, this was because of substantial nutrient acquisition by the sheaths which was measured along with the contribution of the below-sediment tissues.

The nutrient contribution ratio of above to below-sediment tissues was far lower than blade to root ratio of plants from site SYB, and the contribution ratio of above to below-sediment tissues was slightly higher than blade to root ratio of plants from XCB. These data are our evidence that sheaths can contribute part of the nutrients. Evidence was also found that for both NH_4^+ and P_i, uptake affinity of below-sediment

tissues from SYB (which included the foliar sheaths) was substantially higher than for below-sediment tissues of plants from XCB, and uptake affinity of above-sediment tissues from XCB (which included foliar sheaths) was slightly higher than for above-sediment tissues of plants from SYB, again contributing information to our hypothesis that the foliar sheaths' uptake contributes toward nutrient acquisition of the whole plant. From these data we assert that the sheaths of the tropical seagrass *T. hemprichii* do have the ability to absorb NH_4^+ and P_i. This uptake capacity relates to Kuo's insightful remarks on the potential response capabilities of various cell types within the seagrass sheaths (Kuo, 1978). We note that the anatomy and morphology of both the foliar sheath and foliar blade are found to be similar to one another (Tomlinson, 1972), except that the foliar blade contains pigmented material. However, pigmented material is not absent in all seagrass species with sheaths, for instance *Syringodium filiforme* foliar sheaths frequently contain pigment (these are related to *Thalassia* evolutionarily). With a similar cellular structure in foliar sheaths and blades, one might expect to find similarities in the capacity for physiological responses such as uptake of nutrients. We feel this is the first time this participation of foliar sheaths in uptake has been demonstrated, especially in measuring active uptake.

It is noticed that below-sediment tissue-uptake rates for plants from site SYB were significantly higher than solely root tissue uptake, whereas above-sediment tissue-uptake rates for plants from XCB were, surprisingly, slightly lower than solely blade tissue uptake, indicating that the sheath tissue had different uptake affinities dependent on the growth form and allocation above or below sediment of the sheaths. Obviously, longer, more robust foliar sheaths (in some cases enwrapping green blades) growing beneath the sediment had a higher nutrient uptake affinity than shorter foliar sheaths growing above the sediment. Thus, higher uptake capacity was found for the robust sheaths growing beneath the sediment from which plants were subjected to a higher concentration gradient of these nutrient substances than for the concentration gradients acting on the blades in the water column (and which we termed the Zhang–Huang–Thorhaug effect). These data we interpret as a coupling process during which the nutrient uptake responses among blade, root, and foliar sheath are dependent on the morphology, location, and size of the sheath, and on the above and below-sediment nutrient concentration gradients.

Nutrient budget for *T. hemprichii*

Plant nutrient demands can be estimated from blade growth rates and plant N and P content because the N and P demand for blade growth generally constitutes ~95% of seagrass nutrient requirements (Erftemeijer et al., 1993; Stapel et al., 1996). Average blade production for *T. hemprichii* in XCB is 19.2 ± 6.7 mg g^{-1} per day (Xu et al. 2009), which is within the range of *T. hemprichii* in Indonesia of 12–56 mg g^{-1} per day (Stapel et al., 1996) and similar to *T. testudinum* in the Florida region (18.3 mg g^{-1} per day, Fourqurean et al., 2001). Using the yearly mean percent N (2.41%) and P (0.25%) for *T. hemprichii* blades collected from across XCB (Table 1), and assuming no translocation between plant parts, the yearly average plant demand for the XCB site is 1.37 μmol N g^{-1} dw h^{-1} and 0.061 μmol P g^{-1} dw h^{-1}. (Unfortunately, average blade production for *T. hemprichii* at the SYB site was not obtained. Considering that the N and P content of the blades were very close in the two sites, we can assume as a first estimate that the P and N requirements might be the same in the two study sites). The P requirement is consistent with that reported for *T. hemprichii* (0.047–0.075 μmol P g^{-1} dw h^{-1}; Stapel et al., 1996) in Indonesia. However, this P requirement is 2 times higher than that reported for *T. testudinum* (0.0216 μmol P g^{-1} dw h^{-1}; Gras et al., 2003) in NE Florida Bay, where blade tissues of *T. testudinum* are much lower (0.095 ± 0.039%) than those from XCB. The N requirement is slightly lower than that reported for *T. hemprichii* (1.6–2.3 μmol g^{-1} dw h^{-1}; Erftemeijer & Herman, 1994; Stapel et al., 1996). Thus, assuming a 1.37 μmol g^{-1} dw h^{-1} demand for N and a 0.061 μmol g^{-1} dw h^{-1} demand for P by *T. hemprichii*, we calculated whether above or below-sediment nutrient uptake or both uptake locations combined could meet the plant nutrient requirements at the ambient levels of NH_4^+ and P_i. Because the foliar sheaths can be important in nutrient acquisition, the uptake linear equation for above and below-sediment tissues was used in calculation rather than Michaelis–Menten non-linear kinetic equations for solely roots

or solely blades. On the basis of this exercise, uptake by above-sediment tissues at sites SYB and XCB seems to satisfy approximately 65 and 41% of *T. hemprichii* N nutritional requirements, respectively. At both SYB and XCB P_i equilibrium concentration, solely above-sediment tissue uptake of P_i from column water would seem to satisfy the P requirements for *T. hemprichii*. For roots at site XCB and below-sediment tissues in site SYB, seagrasses can obtain several times their N requirements and many times their P requirements from the porewater. Thus, *T. hemprichii* in the southeastern region of Hainan Island seems not to be P_i limited, which is different from other tropical and subtropical seagrass beds that were reported as P_i limited (Fourqurean & Zieman, 1992; Jensen et al., 1998; Koch et al., 2001; Nielsen et al., 2006, 2007).

Conclusion

The tropical and subtropical seagrass *Thalassia hemprichii* (Ehrenb.) Aschers. apparently has evolved efficient nutrient uptake strategies over the variety of environmental conditions they inhabit to maintain adequate nutrients in support of their high rates of primary productivity. We examined two sites wherein both nutrient concentrations and the morphology of *Thalassia hemprichii* differed markedly. In conclusion, *T. hemprichii* in the first experiment took up NH_4^+ and P_i via both blade and root according to Michaelis–Menten kinetics. Uptake kinetic data measured solely for blades or solely for roots of *T. hemprichii* were within a specific range, indicating that uptake characteristics are species-specific. We hypothesized that uptake characteristics of NH_4^+ and P_i may be similar for this species found over a large array of environmental conditions. In our second set of studies, in which we measured sheaths of below-sediment tissues for plants from site SYB and of above-sediment tissues for plants from XCB, both sediment placements of foliar sheaths of the seagrass *T. hemprichii* were found to be able to absorb NH_4^+ and P_i. Moreover, especially interesting in these results in this paper, was the much more remarkable uptake capacity for the well developed subsediment foliar sheaths, which acquired nutrients from the porewater with much higher concentrations than in the water column.

The role of sheaths in nutrient acquisition found in this study is critical in elucidating seagrass nutrient uptake strategies. Consequently, the expression of "above-sediment tissue versus below-sediment tissue" is more accurate than "leaf versus root" when we are discussing the historical controversy surrounding the relative importance of column water versus sediment porewater in defining the nutrient sources of submerged aquatic vascular plants. In short, when dealing with mature *Thalassia* plants, sheaths should be included in the experimentation. Furthermore, researchers should examine the definition of below-sediment-sheath and above-sediment-sheath when describing acquisition of materials and also when describing the variety of ecological morphotypes of *Thalassia*. The mechanism of formation of below-sediment-sheath needs more investigation. Further detailed elucidation of functional coupling among blades, roots, and sheaths (The Zhang–Huang–Thorhaug effect) for *Thalassia* during nutrient-uptake processes must also be sought by experimenting with plants with "no sheath" conditions (young seedlings), plants with sheaths covered with non-permeable material for potentially "no sheath uptake", versus whole plants with full sheaths of under-sediment and above-sediment types in comparative uptake studies potentially including use of radiotracers (Schroeder and Thorhaug, 1979b). This is also suggested for other species with major sheath development, for example *Posidonia*, *Enhalus*, *Syringodium*, and *Cymodocea*. Reciprocal transplants from one site to another and then repeating the same experiments with the plants once morphologically adjusted to the different environmentally conditions could explain much about the adaptability of the plasticity of the plant morphology versus its physiological uptake functions. Also, radiotracer experiments to locate exact intake sites of these nutrients are recommended.

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