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Aquatic Botany 76 (2003) 299–315

Aquatic botany

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Phosphorus uptake kinetics of a dominant tropical seagrass *Thalassia testudinum*

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Received 17 October 2002; received in revised form 23 April 2003; accepted 23 April 2003

Abstract

Although nitrogen is primarily the dominant nutrient limiting seagrass production in temperate estuaries, phosphorus (P) limitation can be important in tropical carbonate-dominated seagrass systems. While nitrogen uptake kinetics of seagrasses are moderately well established, very limited data exist on the dynamics of P-uptake. In this study, we determined the kinetics of dissolved inorganic phosphorus (P_i) uptake for a dominant tropical seagrass *Thalassia testudinum* across a range of P_i levels (0.5–25 μM). Under this broad range, leaf P_i-uptake (μmol g^{-1} dw h^{-1}) rates were similar under light (V_{max} = 1.90) and dark (V_{max} = 2.10) conditions, while root P_i-uptake rates declined 30% in the dark, and were significantly lower than leaves under both light (V_{max} = 0.57) and dark (V_{max} = 0.38) conditions. At lower P_i concentrations (0.5–5.0 μM), leaf V_{max} was 2–3-fold lower (0.50–0.77), while root V_{max} was the same at high and low P_i ranges. Based on linear and non-linear models of P_i-uptake kinetics for *T. testudinum*, leaves can contribute a majority of the P sequestered by the plant when surface and porewater P_i levels are equally low (0.05–0.5 μM). Based on the calculated P-demand of *T. testudinum* in South Florida, solely root or leaf uptake can account for the P requirements of *T. testudinum* when porewater or surface water P_i levels are 0.5 μM. However, when surface and porewater P_i levels are extremely low (<0.10 μM), such as in Florida Bay and other carbonate seagrass systems where P_i sequestration by the sediment is highly efficient, even root + leaf P_i-uptake rates do not meet the P requirements for growth and P-limitation may occur.

Keywords: Phosphorus; Nutrient uptake; Seagrass; Nutrient cycling; Kinetics

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doi:10.1016/S0304-3770(03)00069-X

1. Introduction

Seagrass ecosystems, including tropical and subtropical seagrasses found in oligotrophic environments, rank amongst the most productive coastal marine ecosystems worldwide (Odum, 1959; Fourqurean et al., 2001). In order for seagrasses to maintain these high rates of productivity, adequate nutrients must be continually supplied and/or recycled within the beds (Hemminga et al., 1991). Low allochthonous nutrient inputs and a high phosphorus (P) adsorptive capacity of carbonate sediments characterize tropical seagrass beds. This results in extremely low dissolved inorganic phosphorus (P_i) concentrations in the water column and sediment porewaters of seagrass ecosystems of the Caribbean, Bahamas, South Florida, and other tropical/subtropical regions (<0.03–0.6 μM; Patriquin, 1972; Morse et al., 1987; Fourqurean et al., 1993; Jensen et al., 1998; Koch et al., 2001), with some notable exceptions (Erftemeijer et al., 1994). Low surface and porewater P_i levels have been implicated in the observed P-limited growth of some tropical seagrass species (Short et al., 1985; Short, 1987; Powell et al., 1989; Fourqurean et al., 1992a,b).

In view of the efficiency of P sequestration by carbonate sediments and low P_i availability, tropical seagrasses should have evolved efficient P_i-uptake strategies to maintain adequate nutrients in support of high rates of primary productivity. This is particularly true in light of the fact that seagrasses reside in a dynamic environment with rapid leaf turnover rates and accompanied nutrient losses. Seagrasses have also been found to posses a limited capacity to resorb and retain limiting nutrients, an important plant strategy for nutrient conservation in oligotrophic environments (Hemminga et al., 1999). While many studies have focused on nutrient cycling in seagrasses at the ecosystem scale (see Alongi, 1998), a better understanding of P_i-uptake kinetics in seagrasses is required to accurately model growth and production in tropical environments that are P-limited. To date, few data are available on seagrass P_i-uptake kinetics for either temperate or tropical seagrass species (for review see Touchette and Burkholder, 2000). Also, controversy surrounds the relative importance of above-ground versus below-ground nutrient uptake in meeting the nutrient requirements of aquatic plants (Denny, 1980; Brix and Lyngby, 1985).

Although most seagrasses and other submerged aquatic vegetation (SAV) are physiologically capable of absorbing nutrients from both sediment and water column sources (Patriquin, 1972; McRoy and Barsdate, 1970; Carignan and Kalff, 1980; Thursby and Harlin, 1982, 1984; Short and McRoy, 1984; Brix and Lyngby, 1985; Rattray et al., 1991; Pedersen et al., 1997; Terrados and Williams, 1997), it has been assumed that nutrient uptake by SAV roots dominate over leaf uptake in estuaries where porewater nutrient concentrations are high relative to surface waters (Bole and Allan, 1978; Carignan and Kalff, 1980; Bulthuis and Woelkerling, 1981). While this paradigm is long standing, it is primarily based on evidence from temperate systems, where sediment fertility is generally high. We hypothesize that leaf P-uptake may be more important for seagrasses in tropical carbonate-dominated meadows where porewater P_i levels can be as low as surface waters. The fact that SAV leaves can take up P_i at rates that are equal to or greater than roots across a broad range of trophic states (McRoy et al., 1972; Penhale and Thayer, 1980; Thursby and Harlin, 1984; Stapel et al., 1996), supports the idea that leaf uptake could be important to the nutrient budgets of tropical seagrass communities.

Currently, no data are available on the P_i-uptake kinetics of the dominant tropical western Atlantic seagrass *Thalassia testudinum*. The objective of this study was to quantify P_i-uptake kinetics for *T. testudinum* roots and leaves under light and dark conditions across a range of P_i concentrations. We determined P_i-uptake kinetics at levels approaching saturation (up to 25 μM), as well as upper levels found in situ in oligotrophic carbonate-dominated seagrass beds (0.5 μM). In addition, we tested the root-dominance paradigm established in temperate systems that if root P_i supply rates are high, leaf P_i-uptake rates decline and vise versa (Thursby and Harlin, 1982, 1984; McRoy and Barsdate, 1970). We also determined if leaf and/or root P_i-uptake rates could meet plant P demand based on *T. testudinum* productivity rates in South Florida at low (0.05 μM), medium (0.10 μM), and high (0.50 μM) P_i concentrations.

2. Experimental methods

2.1. Thalassia testudinum collection and preparation

T. testudinum plants were collected in November 1999 and May 2000 from Buttonwood Sound in eastern Florida Bay (25°07′N, 80°27′W; approximate water depth 1 m). Plants were uprooted in sods to prevent root and rhizome damage and washed free of sediments. The plants were transported in a dark cooler to the Gumbo Limbo Marine Laboratory in Boca Raton, FL.

When plants were collected, 200 l of Florida Bay water was also taken from the middle of the northeastern portion of Florida Bay (adjacent to Duck Key) for use as experimental medium. At the lab, plants were gently separated into individual short shoots, each with at least 4 leaves, a section of rhizome (5–6 cm), and intact roots. Plants were acclimated for 5 days prior to experimentation in aerated low-nutrient Florida Bay water (35 PSU or ppt) at 25 °C on a 12 h photoperiod under light-saturated conditions (~500–600 μmol PAR m^{-2} s^{-1}). Following acclimation, epiphytes were gently scraped from the leaves, senescent tissue removed, and roots were carefully washed in low-nutrient seawater to remove carbonate sediments.

2.2. Partitioned chambers

A cylindrical hydroponic chamber (50 cm height × 15 cm diameter; 4 l upper compartment, 2 l lower compartment, Fig. 1) was used for P-uptake experiments. Chambers were constructed of clear acrylic plastic with two sampling ports in both upper and lower chambers. A dark acrylic disc in the center of the chamber held five short shoots and separated above- and below-ground plant material into two isolated compartments. All lower compartments were covered with foil during experiments to simulate dark sediment conditions. Individual *T. testudinum* shoots were threaded through rubber stoppers and sealed around the non-photosynthetic tissue of the short shoot with a non-toxic sealant (plumber's putty). The rubber stoppers were subsequently placed into the holes of the acrylic disc. To assure that water was not mixing between compartments, a red dye was added to the upper chambers of all experimental units at the end of the incubation. A sample of the lower

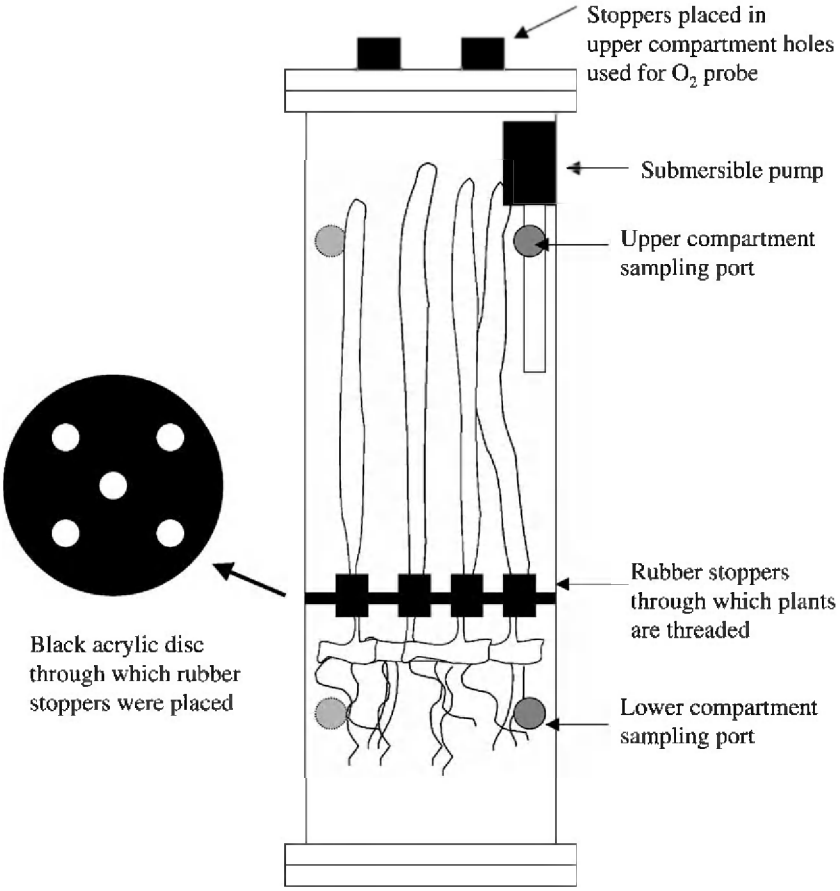

Fig. 1. Cylindrical hydroponic partitioned chamber (upper compartment: 4 l, lower compartment: 2 l) used for P_i-uptake experiments. Lower compartment was maintained under dark conditions during incubations.

chamber water was taken after approximately 1 h and examined spectrophotometrically at 520 nm (Shizumatzu UV 620). No dye was detected in the lower compartments, thus we felt confident that no leakage had occurred during incubations. The same chambers and methodology for plant insertion have been used subsequently to isolate different hypersalinity levels in upper and lower chambers with success (Koch, unpublished data), further validating the split chamber design.

2.3. Experimental setup and sampling

Prior to the experiment, Florida Bay water (~2001, 35 PSU) was filtered using 200 μm mesh filter bags (40 cm × 30 cm) and autoclaved to remove phytoplankton and bacteria. Although below-ground chamber experimental media was not purged with N_2 to create

anoxic conditions, water was left standing in large tanks (100 l) without light for 5 days before experiments commenced. During the incubations, the lower chamber was non-stirred to simulate low sediment porosity and boundary gradients under field conditions. However, during sampling, the chamber was placed on a stir plate and mixed briefly to ensure a homogenous sample. Medium in the leaf compartment was continuously circulated with a small submersible pump (Rio90) with a flow rate of ~4 l min^{-1}.

Water samples were extracted from either the upper or lower chamber by inserting syringe needles through two sampling ports with serum stoppers located on the sides of the hydroponic apparatus (Fig. 1). A 10 ml sample was extracted as an equal amount of P-free medium was replaced through the second port by slow injection. Each 10 ml sample for nutrient analysis was immediately frozen. P_i as soluble reactive P was analyzed colorimetrically with ammonium molybdate and antimony potassium tartrate (catalyst) under acidic conditions (Technicon Autoanalyzer II; APHA, 1995). Above-ground photosynthetic tissue and below-ground root tissue were separated, dried to a constant weight (60 °C), and weighed. Nutrient uptake rates were normalized to leaf and root dry weights. The dry weight of rhizome tissue was not included in the calculation of uptake rates, because nutrient uptake by seagrass rhizomes is limited (Short and McRoy, 1984; Brix and Lyngby, 1985; Barnabas, 1991, 1994; Stapel et al., 1996).

2.4. Fall experiment (November 1999)

2.4.1. Experiment #1 (low phosphorus levels)

To investigate leaf and root P_i-uptake rates under low levels, five P_i concentrations (0.5, 1.0, 1.5, 2.0, and 5.0 μM) were introduced into chamber compartments with five plants in each replicate chamber. When leaves were incubated with the five P_i concentrations, the lower root compartment received 1.0 μM P_i. When roots were incubated with the five P_i treatment levels, the leaf compartment received 0.5 μM P_i. Root compartments received a higher P_i concentration to represent upper P_i levels in Florida Bay (~1.0 μM porewaters and ~0.5 μM water column; Fourqurean et al., 1992b; Koch, unpublished data).

2.4.2. Experiment #2 (interaction experiment)

A second experiment was conducted in November 1999 to examine P-uptake rates when opposing compartments had a 10-fold higher P_i concentration. In this experiment, the same range of P_i concentrations (0.5, 1.0, 1.5, 2.0, and 5.0 μM) was added to leaf or root chambers. However, in leaf incubations, root compartments received 10.0 μM P_i, while during root incubations, leaf compartments received 5.0 μM P_i.

2.5. Spring experiment (May 2000)

2.5.1. Saturation kinetics

Results from the fall (November 1999) experiments (Experiments #1 and #2) indicated that saturation was approached, but perhaps not reached. Therefore, May experiments were conducted using a greater range of P_i levels (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 5.0, 7.0, 10.0, 15.0, and 25.0 μM). As in the November experiments, plants were incubated in replicate split chambers. Because high P_i levels in the opposite chambers had had

no effect on P_i-uptake in roots or leaves, opposite non-treatment compartments received 2.0 μM P_i.

2.6. Nutrient uptake rates and kinetics

Nutrient uptake rates in the light were calculated from P_i concentration changes in the chamber every hour over a 10 h period. Subsequent to light incubations, lights were extinguished to determine dark uptake rates. The next morning, after an approximate 14 h dark incubation, chambers were sampled for P_i. Phosphorus uptake rates (μmol P g^{-1} dw h^{-1}) in the light were calculated based on the linear slopes of P_i-uptake over the 10 h incubation. Dark uptake rates were based on P_i concentrations at the end of the light incubation (17:00 h) minus the concentration after the 14 h dark interval.

2.6.1. Michaelis–Menten kinetics

P_i-uptake rates as a function of P_i concentration was calculated using the Michaelis–Menten (MM) non-linear hyperbolic model. Plots of P_i-uptake versus substrate concentration (V versus [S]) were linearized using the Lineweaver–Burke double reciprocal transformation ($[S]^{-1}$ versus V^{-1}). In the Lineweaver–Burke transformation, the *y*-intercept is equal to $1/V_{max}$, while the *x*-intercept is equal to $-1/K_m$. The parameters V_{max} and K_m from the Lineweaver–Burke transformation were then used as initial starting parameters for the non-linear hyperbolic run (Sigma Plot, Jandel Inc., 1999). Standard errors of the model were generated from the last iteration of the model run. These error terms represent the asymptotic standard errors of the parameters and define the uncertainty in the estimate of regression coefficients. In general, the coefficients are within approximately two standard errors of the reported coefficient. Michaelis–Menten P_i-uptake parameters were defined at saturating (0–25 μM) and low P_i ranges (0–5 μM).

2.6.2. P_i-affinity

Leaf and root P_i-affinities were calculated using the initial linear slope of the hyperbolic curve at 0–2 μM P_i (Short and McRoy, 1984; Stapel et al., 1996). *t*-tests were performed to examine significant differences between slopes (V/[S]) for light versus dark incubations and root versus leaf P_i-affinities during May experiments. The P_i-affinities for November 1999 interaction Experiments #1 and #2 were also compared to determine if increases in ambient P_i concentrations affected leaf or root P_i-uptake rates in opposing chambers. All significant differences are reported at the $P < 0.05$ level.

3. Results

3.1. Phosphorus saturation kinetics (0–25 μM)

Leaves consistently showed a higher V_{max} than roots in both the light and dark across the 0–25 μM P_i range (Fig. 2, Table 1). Under light and dark conditions, leaf V_{max} was approximately 70–80% higher than root V_{max} (Fig. 2, Table 1). V_{max} in the leaf was similar under both light and dark conditions, while root V_{max} declined approximately 30% in the

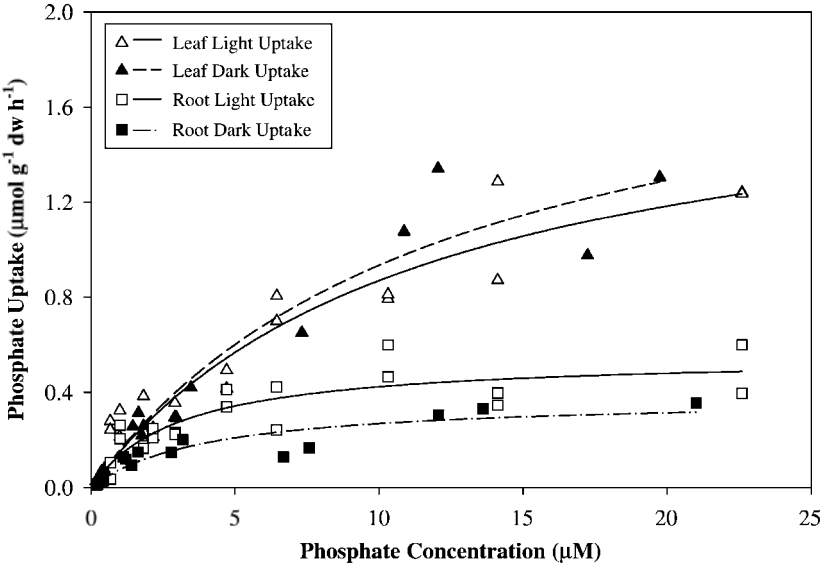

Fig. 2. P_i-uptake rates (μmol g^{-1} dw h^{-1}) in May by *Thalassia testudinum* leaves and roots in the light and dark as a function of P_i concentration (0–25 μM). Curves represent best fit using Michaelis–Menten kinetics.

Table 1
Michaelis–Menten kinetic parameters: V_{max} and K_m, with asymptotic standard errors of the parameters in parentheses

Experiment	P_i range (μM)	V_{max} (μmol g^{-1} dw h^{-1})	K_m (μM)	R^2	P-level
May					
Leaf light	0–25	1.90 (0.25)	11.3 (2.96)	0.92	<0.01
	0–5	0.50 (0.10)	1.20 (0.63)	0.66	<0.01
Leaf dark	0–25	2.10 (0.39)	12.4 (4.48)	0.94	<0.01
	0–5	0.77 (0.25)	3.48 (1.86)	0.93	<0.01
Root light	0–25	0.55 (0.06)	3.09 (0.95)	0.82	<0.01
	0–5	0.63 (0.22)	3.90 (2.33)	0.77	<0.01
Root dark	0–25	0.38 (0.05)	4.05 (1.48)	0.85	<0.01
	0–5	0.38 (0.13)	3.14 (1.71)	0.92	<0.01
November					
(1) Leaf light	0–5	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]
(2) Leaf light	0–5	0.93 (0.13)	1.05 (0.41)	0.85	<0.01
(1) Root light	0–5	1.12 (0.39)	2.69 (1.84)	0.79	<0.01
(2) Root light	0–5	1.27 (0.61)	3.01 (2.46)	0.77	<0.01

Parameters presented for high (0–25 μM) and low P_i range (0–5 μM) in May. November data presented for Experiments #1 (1) and #2 (2). The level of significance (P) and the R^2 of the regression are given.

[a] n.d.: not determined because of linearity.

dark (Fig. 2, Table 1). Half saturation constants (K_m) for the leaves in the 0–25 μM P_i range were higher than the roots in both the light and dark, indicating a lower P affinity in the leaf than the root at high P_i concentrations (Table 1). Plant exposure to light versus dark did not affect the half saturation constant (K_m) in leaves or roots of *T. testudinum*.

3.2. Phosphorus saturation kinetics (0–5 μM)

P-uptake kinetics within a lower range of P_i concentrations (0–5 μM) also fit a non-linear hyperbolic model (Fig. 3a–c; $R^2 = 0.66$–0.93; Table 1), with the exception of November leaf light data from Experiment #1 which was linear. These data indicate that P_i-uptake may be approaching saturation even at relatively low P_i concentrations (<5 μM). Leaf V_{max} was estimated to be ~3-fold lower at P_i concentrations <5 μM compared to the <25 μM range (Fig. 3a, Table 1). This decline in V_{max} resulted in leaf K_m estimates of an order of magnitude lower, while root K_m remained comparatively similar at high versus low P_i ranges (Table 1).

Based on the May and November experiments, seasonality shifts in V_{max} and K_m occurred. Root and leaf V_{max} were 2–3-fold higher in November than May (Table 1), and root and leaf K_m values were slightly lower in November. However, more seasonal data is required to draw firm conclusions about seasonal variability of P_i-uptake kinetics in *T. testudinum*.

3.3. Affinity for P at low P concentrations (0–2 μM)

The linear P_i-uptake kinetics observed at P_i concentrations <2.0 μM has been defined as P affinity or alpha, and corresponds more closely with upper levels of P_i concentrations found in Florida Bay and other tropical estuaries and lagoons. At these P_i levels, the affinity for P_i by the leaves was two-fold higher than roots under both light and in dark conditions during the growing season in May (Fig. 4a, Table 2). Contrary to findings in May, however, November leaf and root affinity in Experiments #1 and #2 were not significantly different (Fig. 4b, Table 2). Also, differences in light versus dark root uptake observed at higher P_i concentrations were not observed at <2 μM P_i (Table 2).

Table 2
Leaf and root P_i-affinity, defined as the slope of the linear equation at low P_i concentrations (0–2 μM)

Experiment	Equation	R^2	P-level
May			
Leaf light	$y = 0.2310x + 0.0315$	0.79	<0.001
Leaf dark	$y = 0.1916x - 0.0111$	0.63	<0.001
Root light	$y = 0.1022x + 0.0074$	0.99	<0.001
Root dark	$y = 0.0940x - 0.0006$	0.92	<0.001
November			
(1) Leaf light	$y = 0.1627x + 0.0407$	0.69	<0.001
(2) Leaf light	$y = 0.1939x + 0.1910$	0.64	<0.001
(1) Root light	$y = 0.2322x + 0.0117$	0.53	<0.001
(2) Root light	$y = 0.2360x - 0.0007$	0.90	<0.001

May results are for leaf and root in the light and dark. November results are for leaf and root in the light for Experiments #1 (1) and #2 (2). The level of significance (P) and R^2 of the linear model are given.

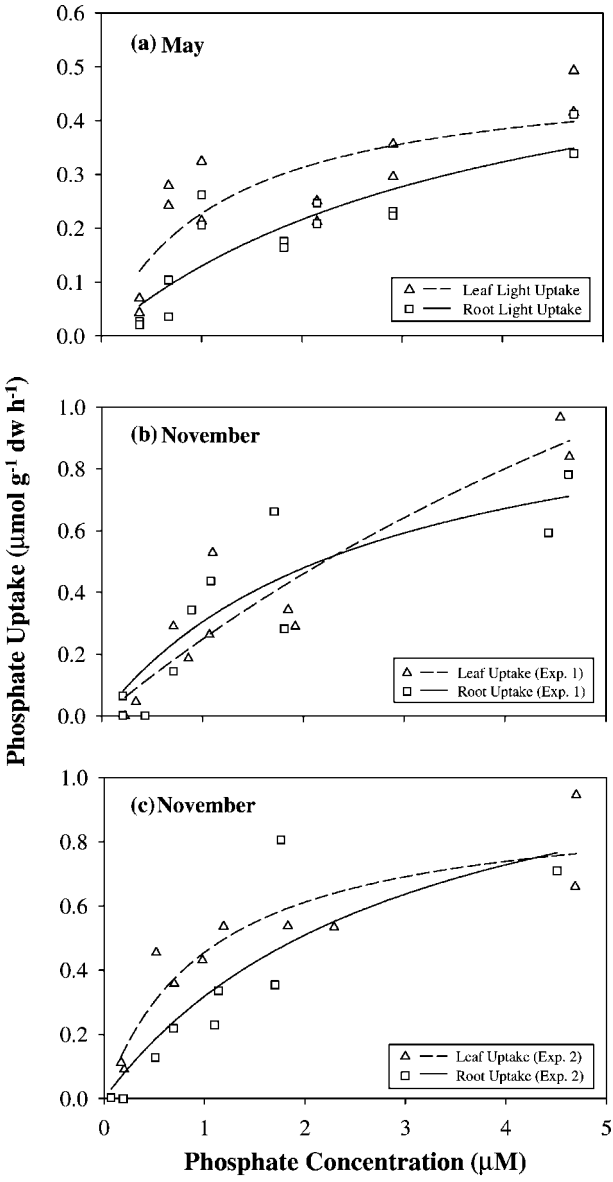

Fig. 3. P_i-uptake rates ($\mu mol\, g^{-1}\, dw\, h^{-1}$) by *Thalassia testudinum* leaves and roots in the light as a function of P_i concentration (0–5 μM) in (a) May 2000, (b) November 1999 (Experiment #1), and (c) November 1999 (Experiment #2). Curves represent best fit using Michaelis–Menten kinetics.

Leaf and root uptake rates from May were compared to November Experiment #1, due to the relatively low P_i concentrations used in the May experiments (2 μM, leaf and root) and November Experiment #1 (0.5 μM leaf, 1 μM root). The affinity for P_i by roots was significantly higher in November than in May, while the affinity for P_i in the leaves was not

significantly different from leaves in May and November (Table 2), suggesting that seasonal differences in leaf uptake may be minimal at P_i levels of 0.5–2.0 μM.

3.4. Interaction results

Ten-fold higher P concentrations in the opposite chamber compartment during November Experiments #1 and #2 did not result in significant changes in leaf or root P_i-uptake rates. No significant differences were found in the affinity of leaves for P_i between Experiments #1 and #2 (Fig. 4, Table 2). The affinity of roots for P was also similar when exposed to high versus low P_i levels in the leaf chamber (Table 2).

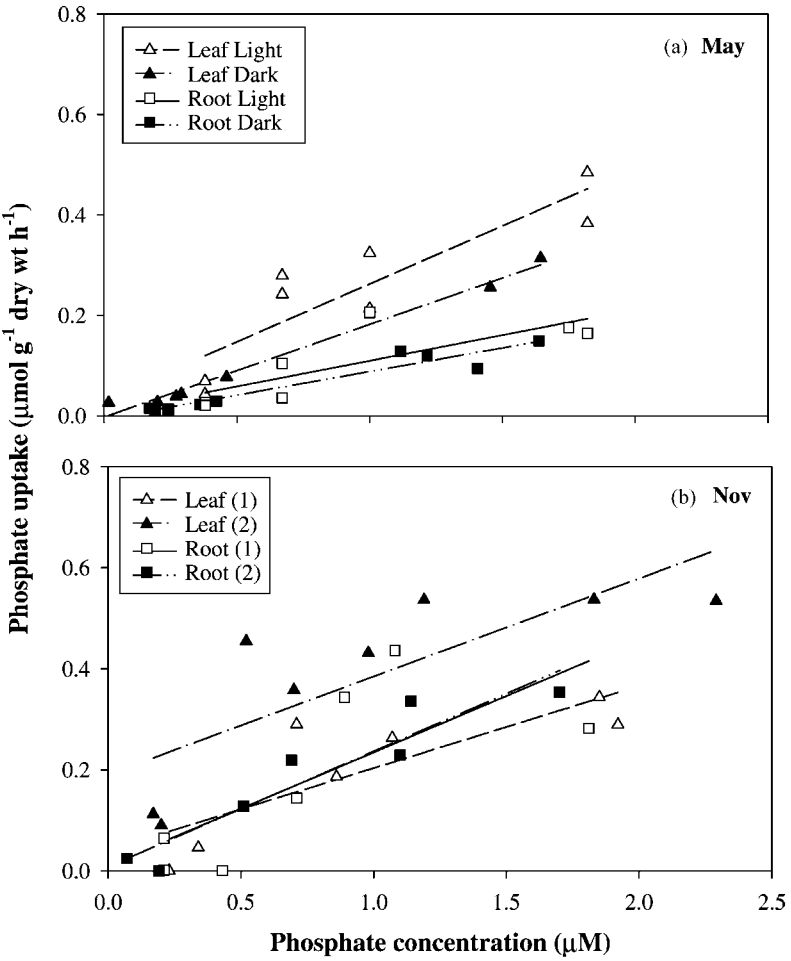

Fig. 4. Phosphorus affinity (V/[S]), defined as P_i-uptake rates (μmol g^{-1} dw h^{-1}) within the linear portion of the non-linear hyperbolic curve from 0 to 2 μM. Data are shown for (a) May 2000, and (b) November 1999 experiments for *Thalassia testudinum* leaves and roots.

4. Discussion

One of the dominant tropical Atlantic–Caribbean seagrasses, *T. testudinum*, is shown here to sequester P_i into both leaves and roots according to Michaelis–Menten kinetics. We ascribe these kinetics solely to absorption, because P-uptake rates were determined over a 10 h period, without determination of the initial adsorption of ions onto the plant surface, a rapid 5–20 min response (Short and McRoy, 1984; Perez-Llorens and Niell, 1995; Stapel et al., 1996). Many other tropical and temperate seagrass studies have shown Michaelis–Menten saturation kinetics at high P_i and inorganic nitrogen concentrations (McRoy and Barsdate, 1970; McRoy et al., 1972; Penhale and Thayer, 1980; Thursby and Harlin, 1984; Brix and Lyngby, 1985; Perez-Llorens and Niell, 1995; Stapel et al., 1996), with few exceptions (Paling and McComb, 1994).

Although inorganic nitrogen uptake kinetics have been described for several seagrass species (Touchette and Burkholder, 2000), *T. testudinum* P_i-uptake parameters can only be contrasted with three seagrass species for which P_i-uptake kinetics have been determined (Table 3). In the 25 μM P_i range V_{max} and K_m for *T. testudinum* leaves are remarkably similar to those of *Thalassia hemprichii*, a tropical seagrass of the Spermonde Archipelago in Indonesia (Table 3). This is the case regardless of the fact that *T. testudinum* plants from Florida Bay are exposed to surface water P_i concentrations over an order of magnitude lower (median = 0.04 μM, n = 2575, Boyer et al., 1997) than those experienced by *T. hemprichii* in Indonesia (0.8–1.4 μM; Erftemeijer and Herman, 1994). While P_i-uptake kinetics of the two *Thalassia* species is similar at high P_i concentrations, *T. testudinum* appears to approach saturation at P_i levels 50% lower (5 μM) than *T. hemprichii* (10 μM). However, the linear uptake affinity (α) for the two species were similar, ranging between 0.13 and 0.23 (Table 3). Thus, at P_i concentrations less than 2 μM, closer to the upper range experienced by *T. testudinum* in Florida Bay (1.1 μM, Boyer et al., 1997) and *T. hemprichii* in Indonesia (0.8–1.4 μM), the leaves of both *Thalassia* species take up P_i at similar rates.

Zostera noltii and *Ruppia maritima*, the only other two seagrass species for which P_i kinetics have been determined, exhibit slightly different P_i-uptake kinetics compared to *Thalassia*. Some of these differences may be attributable to experimental design. For example, the length of incubation for P_i-uptake and pretreatment of plants prior to uptake experiments. Over short 5 min incubations, *Z. noltii* excised leaves and leaves of intact plants show a V_{max} 10-fold greater than *Thalassia* (Table 3). These maximum P_i-uptake rates include both rapid adsorption onto the leaf surface and absorption of P_i by leaves. Further, these plants had been P-starved 24 h prior to uptake experiments. If maximum uptake rates are calculated from the longer 180 min incubation in the same study, *Z. noltii* P_i-uptake rates (0.9–3.0 μmol g^{-1} dw h^{-1}) at high concentrations of P_i (25–30 μM) align more closely with those of *Thalassia* (Table 3). In addition, linear affinities of *Z. noltii* leaves for P_i more closely follow those of *Thalassia* when utilizing data from the 180 min incubation (Table 3). Although *Thalassia* and *Z. noltii* show similar rate constants for P_i-uptake, an exception is found in the submerged macrophyte *R. maritima*, which exhibits a high V_{max} even during long incubations (12–15 h). These data, albeit limited, indicate consistent leaf P_i-uptake kinetic parameters among seagrass species when adjusted for experimental design with moderate inter-specific differences.

Table 3
A review of inorganic phosphorus (P_i), kinetic parameters, the maximum uptake rate at saturation V_{max} ($\mu mol\ g^{-1}\ dw\ h^{-1}$), the half saturation constant K_m (μM), and linear affinity (α) defined as the initial linear portion of uptake for seagrass and other SAVs across various P_i concentrations

Species experiment/site	Michaelis–Menten kinetics			Linear affinity kinetics		
	V_{max}	K_m	P_i (μM)	α	P_i (M)	Reference
Thalassia testudinum						
Leaf light (600 min)	1.9	11.93	0.5–25	0.23	0–2	This study
Leaf dark (720 min)	2.1	12.43	0.5–25	0.19	0–2	This study
Root light (600 min)	0.57	3.75	0.5–25	0.10	0–2	This study
Root dark (720 min)	0.38	4.05	0.5–25	0.09	0–2	This study
Thalassia hemprichii						
Leaf–mudflat (600 min)[a]	2.2	7.7	5–50	0.19	0–10	Stapel et al. (1996)
Leaf—reef coast (600 min)[a]	2.5	11	5–70	0.13	0–10	Stapel et al. (1996)
Leaf—reef shelf (600 min)[a]	3.2	15	5–50	0.13	0–10	Stapel et al. (1996)
Zostera noltii						
Excised leaf (5 min)	7.0	10.0	2.5–25	0.52[b]	2.5–5.4	Perez-Llorens and Niell (1995)
Leaf whole plant (5 min)	43.0	12.1	1.9–30	1.54[b]	1.9–5.8	Perez-Llorens and Niell (1995)
Excised leaf (180 min)[c]	0.9	7.0	2.5–25	0.14[b]	2.5–5.4	Perez-Llorens and Niell (1995)
Leaf whole plant (180 min)[c]	3.0	7.1	1.9–30	0.15[b]	1.9–5.8	Perez-Llorens and Niell (1995)
Ruppia maritima						
Leaf $-P_i$ root (900–1080 min)	14.1	9.2	2.5–20	–	–	Thursby and Harlin (1984)
Leaf $+P_i$ root (900–1080 min)	9.7	8.1	2.5–20	–	–	Thursby and Harlin (1984)
Root $-/+$ P_i leaf (900–1080 min)	4.6	3.1	2.5–20	–	–	Thursby and Harlin (1984)

[a] Maximum incubation time.
[b] Values are the linear slopes calculated by using initial $2P_i$ concentrations and associated uptake rates.
[c] Kinetic parameters generated by Edwards and Walker model (Fig. 4, Perez-Llorens and Niell, 1995).

Dependence on light for maximum nutrient uptake rates varies among seagrass species. Leaf P_i-uptake was light-independent in this study. We found no differences in leaf V_{max}, K_m, or α in light and dark conditions. These results refute the supposition that *T. testudinum* leaf P_i-uptake is promoted by or coupled to photophosphorylation. Our observations are consistent with results by Lee and Dunton (1999), who measured similar light and dark leaf uptake rates of NH_4^+ and NO_3^- for *T. testudinum*. The light-independent pattern is also consistent with shorter-term (2 h) kinetic experiments of P_i-uptake by *T. testudinum* leaf segments with and without epiphytes under both light and dark conditions (0–25 μM; Donovan and Koch, in preparation). By contrast, *Zostera marina* and *Z. notii* leaf P_i-uptake rates decline during dark periods (McRoy and Barsdate, 1970; McRoy et al., 1972; Brix and Lyngby, 1985), and therefore P_i-uptake may be linked to photophosphorylation in these species.

While *T. testudinum* leaf P_i-uptake rates were similar under light and dark conditions, root V_{max} declined by 33 and 40% in the dark across the 0–25 and 0–5 μM P_i range, respectively. This decline in root V_{max} implies that there may be a relationship between photosynthesis and root P_i-uptake in *T. testudinum*. Patriquin (1972) suggested that maximum P_i-uptake rates in *T. testudinum* roots occur during periods of active photosynthesis. However, Patriquin (1972) based this supposition on a weak correlation between rhizome water-soluble P_i (μmol g^{-1} wet wt.) and leaf growth rates (mg per shoot per day), without direct measurements of diel changes of soluble P_i in plant tissues. Our data indicate that root P_i-uptake is light dependent in *T. testudinum*.

Root P_i-uptake was significantly lower than leaves under both light and dark conditions in *T. testudinum*. Apoplastic barriers such as suberin lamellae, suberized hypodermal cell walls, and casparian bands are reported in seagrass species including *T. testudinum*, and may limit root uptake (Tomlinson, 1969; Barnabas, 1991). These restrictions, and the fact that seagrass leaves possess efficient apoplastic nutrient transfer routes to photosynthetic cells (Barnabas, 1988), could explain higher leaf V_{max} at saturating P_i concentrations. These results are consistent with *R. maritima*'s 2–3-fold greater V_{max} in leaves versus roots at high P_i concentrations (Table 3).

Root P_i-uptake kinetic parameters have not been reported for any other seagrass species that we could compare to our *T. testudinum* data. However, root P_i-uptake rates have been determined for *T. hemprichii* (Stapel et al., 1996) and *Z. marina* (Brix and Lyngby, 1985) at P_i levels within the range investigated in this study, and can be used to contrast seagrass leaf to root P_i-uptake ratios. At 6.4 μM P_i, *T. hemprichii* had similar leaf and root (1 μmol g^{-1} dw h^{-1}) P_i-uptake rates. In contrast, we found 2–3-fold higher P_i-uptake rates for *T. testudinum* leaves versus roots at 6.4 μM, based on the Michaelis–Menten model kinetics and the broad P_i range of 0–25 μM. When we applied kinetic parameters generated using lower P_i concentrations (0–5 μM) however, leaf:root P_i-uptake ratios (0.42:0.55 μmol g^{-1} dw h^{-1}) approach unity (0.76) under light conditions. At the low P_i range of 0–2 μM, linear affinities exhibited a leaf:root ratio of one during the November experiments and a ratio of two during the May growing season. The temperate counterpart to *T. testudinum*, *Z. marina* had a leaf to root P_i-uptake ratio of 1 in a 48 h ^{32}P translocation study, also treated with P_i concentrations of 6.45 μM (Brix and Lyngby, 1985). However, in the Brix and Lyngby (1985) study, rates of P_i-uptake (leaf range 0.03 to 0.10 and roots 0.06 μmol g^{-1} dw h^{-1}) were 10 times lower than those reported for *Thalassia* by Stapel et al. (1996) and in this study. The above synthesis suggests that seagrass leaves have the capacity to contribute as much as, or more, to the total acquisition of P_i by seagrass plants when exposed to similar P_i concentrations. The importance of leaf uptake was also evident when roots were exposed to higher P_i levels than leaves. These results are consistent with those found for *T. hemprichii* during short-term nutrient pulse experiments (Stapel et al., 1996). The potential importance of leaf uptake in oligotrophic environments is highlighted when we calculate the percent contribution of leaf versus root P_i to the overall P demand of *T. testudinum* from Florida Bay.

Plant nutrient demands can be estimated from leaf growth rates and plant P content because the N and P demand for leaf growth generally constitutes ~95% of seagrass nutrient requirements (Erftemeijer et al., 1993; Stapel et al., 1996). Average leaf production for *T. testudinum* in Florida Bay is 16.9 ± 0.7 mg g^{-1} per day (Zieman et al., 1999), averaging

Table 4
Leaf (L) and root (R) P_i-uptake rates ($\mu mol\ g^{-1}\ dw\ h^{-1}$) calculated based on a range of P_i concentrations in Florida Bay

Experiment	Range of P_i concentrations								
	Low (0.05 μM)			Mid (0.10 μM)			High (0.50 μM)		
May L: light									
MM (0–25 μM)	0.0079	n	51%	0.0158	n	52%	0.0764	y	53%
MM (0–5 μM)	0.0200	n	38%	0.0385	y	38%	0.1471	y	40%
LA (0–2 μM)	0.0431	y	78%	0.0546	y	76%	0.1470	y	72%
May L: dark									
MM (0–25 μM)	0.0084	n	65%	0.0168	n	65%	0.0812	y	66%
MM (0–5 μM)	0.0108	n	64%	0.0213	n	65%	0.0960	y	65%
LA (0–2 μM)	−0.0015	n	0%	0.0081	n	48%	0.8470	y	95%
May R: light									
MM (0–25 μM)	0.0075	n	49%	0.0148	n	48%	0.0671	y	47%
MM (0–5 μM)	0.0332	y	62%	0.0630	y	62%	0.2250	y	61%
LA (0–2 μM)	0.0125	n	23%	0.0176	n	24%	0.0585	y	29%
May R: dark									
MM (0–25 μM)	0.0046	n	35%	0.0092	n	35%	0.0418	y	34%
MM (0–5 μM)	0.0060	n	36%	0.0117	n	36%	0.0522	y	35%
LA (0–2 μM)	0.0041	n	100%	0.0088	n	52%	0.0464	y	5%
May L + R: light									
MM (0–25 μM)	0.01540	n	100%	0.03060	y	100%	0.14350	y	100%
MM (0–5 μM)	0.05320	y	100%	0.10150	y	100%	0.37210	y	100%
LA (0–2 μM)	0.05560	y	100%	0.07220	y	100%	0.20550	y	100%
May L + R: dark									
MM (0–25 μM)	0.01300	n	100%	0.02600	y	100%	0.12300	y	100%
MM (0–5 μM)	0.01680	n	100%	0.03300	y	100%	0.14820	y	100%
LA (0–2 μM)	0.00130	n	100%	0.01150	n	100%	0.88800	y	100%

P_i-uptake rates are calculated using Michaelis–Menten (MM) kinetics at high and low P_i concentrations as well as the linear affinity index (LA). It is noted whether the uptake rates satisfy (y: yes, n: no) leaf nutrient requirements, based on the calculated P demand of *Thalassia testudinum* in Florida Bay: 0.0216 $\mu mol\ g^{-1}\ dw\ h^{-1}$, and percent contributed by leaf vs. root tissue. The total uptake by the leaf and root is also calculated.

18.3 $mg\ g^{-1}$ per day for the South Florida region (Fourqurean et al., 2001). Using the mean percent P (0.095 ± 0.039%) for *T. testudinum* leaf tissues collected from 50 sites across Florida Bay (Fourqurean et al., 1992a), and assuming no translocation between plant parts, the yearly average plant demand for NE Florida Bay is 0.0216 $\mu mol\ P\ g^{-1}\ dw\ h^{-1}$. This P requirement is lower than that reported for *T. hemprichii* (0.047–0.075 $\mu mol\ P\ g^{-1}\ dw\ h^{-1}$; Stapel et al., 1996) in Indonesia, where *Thalassia* relative growth rates are higher (range: 12–56 $mg\ g^{-1}$ per day) than those in South Florida (3.2 to 34.2 $mg\ g^{-1}$ per day).

Assuming a 0.0216 $\mu mol\ P\ g^{-1}\ dw\ h^{-1}$ demand for P by *T. testudinum*, we calculated whether or not leaf or root P_i-uptake or both could meet the plant P requirements during the growing season (May) at 3 levels of P_i (0.05, 0.1, and 0.5 μM; Table 4). To assess model sensitivity to calculating P requirements, three modeling approaches were used to calculate

P_i-uptake rates: Michaelis–Menten non-linear kinetics at high and low ranges, and the linear affinity (α) (Table 4). Based on this exercise, some consistent patterns emerge. For example, leaf uptake accounts for >50% of the P_i taken up 72% of the time using all models, and 100% of the time using MM kinetics up to 25 μM P_i and α, with the exception of leaf-dark (0.05 and 1.0 μM). Secondly, at fairly low P_i levels of 0.5 μM, solely leaf or root uptake could satisfy the average requirements of P for *T. testudinum*. Finally, at the extremely low level of 0.05 μM P_i, greater light-dependency emerged as a factor in satisfying the P requirements of *T. testudinum*, even in the leaves that showed light independence for V_{max} and α. These results indicate that at very low ambient P_i levels, satisfying P requirements of the plants becomes extremely sensitive to the *y*-intercept where P_i-uptake could either be positive or negative. Several authors have tried to calculate S_{min} (the P_i concentration at which uptake is zero), but primarily using uptake kinetics based on experiments with P_i concentrations orders of magnitude greater than S_{min}. No studies to date have focused on seagrass nutrient uptake kinetics at levels found in highly oligotrophic tropical carbonate systems such as Florida Bay. At this time, it may be prudent not to over interpret calculated P_i-uptake rates at 0.05 μM based on experiments with the lowest uptake rates determined at 10-times higher P_i levels (0.5 μM). We are currently investigating the kinetics of P_i-uptake in *T. testudinum* at the extreme low range (0.02–1.0 μM), to determine if these P_i-uptake kinetics vary from those discernable for seagrass at higher ranges tested in this and other studies.

In conclusion, *T. testudinum* exhibited Michaelis–Menten kinetics across the broad range of P_i concentrations examined in this study (0–25 μM). Leaf P_i-uptake rates were light-independent. In contrast, root P_i-uptake rates declined 30% in the dark. At saturation, leaf V_{max} was 3–6 times greater than roots; while at lower P_i concentrations, uptake rates were more similar between roots and leaves. Leaf V_{max} declined 2–3-fold at lower P_i concentrations (0–5 μM). Root V_{max} remained the same at high and low P_i ranges. Applying linear and non-linear models of P_i-uptake kinetics, *T. testudinum* leaves contributed a majority of the P_i sequestered by the plant where surface and porewater P_i levels were equally low (0.05–0.5 μM). Based on the calculated P-demand of *T. testudinum* in South Florida, solely root or leaf uptake could account for the P requirements of *T. testudinum* at 0.5 μM P_i; while under highly oligotrophic conditions (0.05 μM), the sum of root and leaf P_i-uptake could not provide the P necessary for growth, particularly under dark conditions. These data suggest that P-limitation may occur in *T. testudinum* when surface and porewater P_i levels are extremely low (<0.10 μM), such as in carbonate seagrass systems where P_i sequestration by the sediment and associated biota are highly efficient, but is probably not a factor at higher P_i concentrations. Further research is required on P_i-uptake kinetics in the <0.50 μM P_i range.

Acknowledgements

This research was supported by the South Florida Water Management District (SFWMD) as a part of their ongoing Everglades research in Florida Bay. Everglades National Park is recognized for field support through the use of the Key Largo Ranger Station boats and facilities. Laboratory assistance was provided by several members of the Aquatic Plant

Ecology Lab. We would also like to thank Chelsea Donovan, Samantha Evans, Steve Kelly, and Karl Picard for their excellent advice and dedicated assistance in the field and laboratory. We are grateful for the time of two anonymous reviewers who significantly improved this manuscript.

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