

Thalassia testudinum phosphate uptake kinetics at low in situ concentrations using a ^{33}P radioisotope technique

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Abstract

A new ^{33}P -tracer technique was used to define low-level phosphate (P_i) uptake kinetics for a dominant tropical seagrass, *Thalassia testudinum*. We established seagrass P_i uptake kinetics at high (western Bay) and low (eastern Bay) nutrient sites in a fine-grained carbonate lagoon of south Florida (Florida Bay), where P limitation of seagrass has been documented. Sediment P_i adsorption kinetics were also investigated to test whether carbonate sediments could sequester P_i to the threshold levels we established for *T. testudinum*, defined as S_{\min} or the physiological threshold value where P_i uptake is positive. At P_i levels characteristic of Florida Bay ($\leq 0.26 \mu\text{mol L}^{-1}$), leaf and root P_i uptake was linear with increasing levels of P_i , and similar affinities (α) were found for leaves and roots ($0.12\text{--}0.30$ and $0.10\text{--}0.20 \mu\text{mol g dry weight}^{-1} \text{h}^{-1}$). S_{\min} for roots and leaves was extremely low ($0.004\text{--}0.009 \mu\text{mol L}^{-1}$) regardless of the P status of the site. While *T. testudinum* was able to take up P_i at nanomolar levels, the uptake rates were insufficient for plant requirements. Thus, sediment pools or transient fluxes of P to the water column must sustain high seagrass production rates in the Bay. Sediment adsorption–desorption equilibrium for P_i was 10-fold lower in the eastern versus western Bay sites, meeting $<10\%$ and $>87\%$ of the P demand for *T. testudinum*, respectively, a result that might account for the reported P limitation of seagrass biomass and production in eastern Florida Bay.

In the past, nitrogen (N) was considered to be the most important nutrient limiting primary production in marine environments (Barber 1992); however, it is now more widely recognized that other essential macronutrients, such as phosphate (P_i) and trace elements, may control primary productivity, particularly in tropical and subtropical marine ecosystems (e.g., Martin and Fitzwater 1988; Wu et al. 2000; Fourqurean and Ziemann 2002). Subtropical and tropical seagrass meadows characterized by fine-grained carbonate sediments with a high adsorptive capacity for P_i and low allochthonous sources of nutrients tend to have extremely low P_i concentrations in pore water and overlying surface water (Fourqurean et al. 1992a; Jensen et al. 1998; Koch et al. 2001). This low P_i availability has been shown to limit sea-

grass primary producers (Powell et al. 1989; Short et al. 1990; Fourqurean et al. 1992b) regardless of the fact that seagrass similar to other submerged aquatic plants can sequester nutrients from both roots and leaves (Patriquin 1972; Carignan and Kalff 1980; Rattray et al. 1991). While both above- and below-ground tissues participate in nutrient sequestration, it is commonly purported that seagrasses meet their nutritional demand for P by acquiring P_i through root uptake (Denny 1980; Brix and Lyngby 1985). However, this paradigm has been established based on studies from mesotrophic seagrass systems in both temperate and tropical locations, where concentrations of P_i in pore water tend to exceed those in the overlying surface waters, sometimes by orders of magnitude (Carignan and Kalff 1980; Bulthuis and Woelkerling 1981; Stapel et al. 1996). The relative importance of below-ground versus above-ground P_i uptake is still in question in oligotrophic carbonate-dominated seagrass ecosystems where pore-water P_i concentrations are similar to levels in the water column (Gras et al. 2003). Another factor to consider in these carbonate seagrass systems is that sediments may efficiently compete for P_i with roots; thus, the role of leaf sequestration may become more important. In order to address these issues, the P_i uptake kinetics of seagrass roots and leaves, as well as sediment adsorption kinetics, must be more clearly understood in tropical and subtropical carbonate-based seagrass systems.

Currently, only one study has quantified P_i uptake kinetics

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for the dominant western Atlantic tropical seagrass *Thalassia testudinum* (Gras et al. 2003), and very few studies have been conducted on other seagrass species (Thursby and Harlin 1984; Perez-Llorens and Niell 1995; Stapel et al. 1996). In general, nutrient-uptake experiments on seagrass have focused on defining the maximum P_i uptake rates (V_{\max}) and half saturation constants (K_m) for modeling purposes. The problem with applying these parameters for models in highly oligotrophic systems is that the nutrient uptake rates are being defined at concentrations far exceeding nutrient concentrations found in situ ($<0.1 \mu\text{mol L}^{-1} P_i$). For reasons pointed out by Healey (1980), when competition for a limiting nutrient becomes intense, V_{\max} and K_m no longer reflect the true competitive ability of a given organism. At low substrate availability, nutrient affinity (or α) and S_{\min} , defined as the physiological threshold value where P_i uptake is positive, are the two critical nutrient-uptake parameters. This results from the fact that species able to take up nutrients at the lowest concentrations (S_{\min}) and with the highest affinity (α) tend to have the competitive advantage. In seagrasses, these parameters have been elusive for two reasons: (1) uptake studies on seagrasses have been conducted at relatively high P_i levels that lack sensitivity to define low-level P_i uptake kinetics (Thursby and Harlin 1984; Perez-Llorens and Niell 1995; Stapel et al. 1996) and (2) because analytical detection limits have prevented an accurate estimate of P_i uptake in the nanomolar range, which is required to elucidate kinetics close to physiological thresholds for P_i uptake (Gras et al. 2003).

In order to overcome these limitations, we used a highly sensitive ^{33}P -radiotracer technique to examine uptake kinetics at extremely low P_i levels. We also determined P_i kinetics at saturation levels to compare P_i kinetics using ^{33}P with our earlier study using a traditional chemical approach observing nutrient loss over time (Gras et al. 2003). We focused on defining the P_i uptake kinetics of roots and leaves for the dominant western Atlantic tropical seagrass, *T. testudinum*, using plants collected from both high (western) and low (eastern) nutrient sites in a subtropical carbonate-dominated estuary, Florida Bay, where P limitation of seagrass has been documented. To compare *T. testudinum* S_{\min} for P_i with that of the sediment from western and eastern Bay sites, we estimated the sediment critical crossover concentration (Ce_0) (Froelich 1988), defined as the point where net adsorption-desorption of P_i is zero. We examined whether fine-grained carbonate sediments could sequester P_i down to the threshold levels observed for *T. testudinum*. Last, we explored the contribution of leaf versus root P_i uptake in meeting seagrass P demand based on the site-specific Ce_0 and in situ P_i concentrations using average *T. testudinum* nutrient content and productivity rates in Florida Bay.

Methods

Sampling site and harvesting—The study was conducted using seagrass from Florida Bay, a shallow semienclosed lagoon (<2 m) at the southern tip of the Florida peninsula (Fig. 1). The Bay supports extensive seagrass meadows dominated by the tropical seagrass *Thalassia testudinum*. *T.*

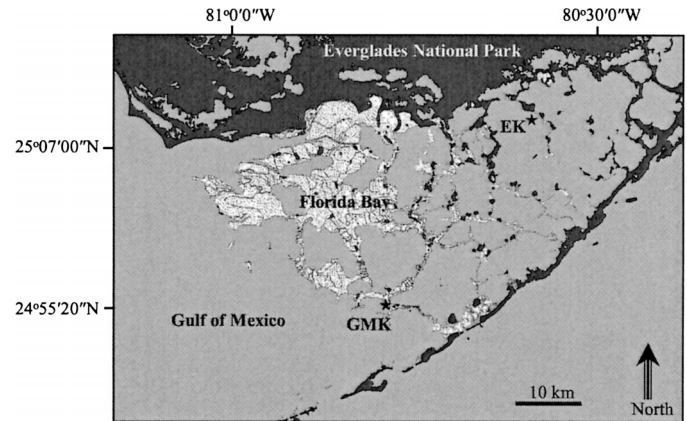


Fig. 1. Map illustrating the location of Florida Bay at the terminus of the South Florida peninsula. Two study sites are depicted: Eagle Key (EK), the eastern Bay site, and Green Mangrove Key (GMK), the western Bay site. Note shallow mud banks and numerous mangrove islands throughout the Bay.

testudinum biomass and primary production decreases from the western to the eastern Bay as a consequence of decreasing P availability evidenced by leaf C:P and N:P ratios (Fourqurean et al. 1992a,b). For this study, we collected *T. testudinum* from two sites in the Bay. Our first site, Green Mangrove Key ($24^{\circ}55'20''\text{N}$, $80^{\circ}47'33''\text{W}$) is located in the southwestern portion of the Bay and is influenced by nutrient inputs from the Gulf of Mexico (Fig. 1). Our second site, Eagle Key, is in the northeastern part of the Bay ($25^{\circ}09'30''\text{N}$, $80^{\circ}34'59''\text{W}$) and is dominated by nutrient input from the highly oligotrophic, particularly with respect to P, Everglades to the north (Fig. 1).

T. testudinum plants were collected intact using a 20-cm-diameter corer. Sediment was gently washed from the below-ground tissue and healthy shoots with intact apical rhizome tips and intact roots were collected. Plant shoot segments were kept moist and transported back to the laboratory in coolers. In the laboratory, they were placed in aquaria with aeration and held overnight in low-nutrient seawater from northeastern Florida Bay in close proximity to Eagle Key ($P_i < 0.01 \mu\text{mol L}^{-1}$). Prior to uptake experiments, roots and rhizomes were washed in seawater from the Eastern Bay and epiphytes gently removed by sliding the blade through two fingers; the epiphytes were easily dislodged using this technique.

^{33}P uptake—Following preliminary tests of incubation time (see *Time series*), two uptake experiments using ^{33}P were conducted: one during May–June 2004 and the other June 2005. In 2004, a series of triplicate incubation bottles (300 mL) were filled with glass-fiber filter (GF/F)-filtered low-nutrient seawater ($P_i < 10 \text{ nmol L}^{-1}$ measured by the MAGIC method; Karl and Tien 1992) spiked with carrier-free $^{33}\text{PO}_4^{3-}$ (65 kBq) and one of 10 P_i amendments (0.0, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 5.0, 10.0, and $20.0 \mu\text{mol L}^{-1}$). Approximately 10 mg dry weight of excised root ($0.012 \pm 0.003 \text{ g}$) or fresh green leaf ($0.015 \pm 0.002 \text{ g}$) were added to each incubation bottle and incubated at room temperature (26°C) for 60 min while gently being shaken on

a shaking platform. In June 2005, the experimental set-up was modified slightly to include six amendments (0.0, 0.025, 0.05, 0.1, 0.2, and 0.25 $\mu\text{mol L}^{-1}$) but in five replicates each. At the same time, in order to test for differences in anoxic versus oxic P-uptake rates, excised roots from Eagle Key and Green Mangrove Key were incubated in deoxygenated GF/F-filtered seawater amended to three different concentrations (0.0, 0.1, and 0.25 $\mu\text{mol L}^{-1}$). Prior to hypoxia experiments, a test was conducted to determine the length of time for degassing (helium). In a 10-min period, oxygen concentrations were reduced to 10% O_2 saturation; therefore, during the experiment, water was degassed for 30 min prior to ^{33}P amendments to ensure a low-level O_2 treatment.

In all incubations, the amount of tissue used per bottle was kept low so that the external phosphate concentration remained relatively unchanged during incubation (<2% taken up by the tissue). After incubation, the tissue was washed for 1 min in succession with 0.5 mol L^{-1} HCl, 0.5 mol L^{-1} H_3PO_4 , and distilled water to remove any ^{33}P attached to the leaf/root surface as either adsorbed PO_4^{3-} or bound to calcium-carbonate sediment/epiphyte remnants. Tests with tissues incubated in phosphate-free $^{33}\text{PO}_4^{3-}$ showed that virtually all radioactivity adsorbed to the surface was removed after washing. Counts on the washing medium likewise showed decreased radioactivity with each wash. Subsequently, the tissues were blotted with paper towels, weighed, and cut into small pieces, and the tissue ^{33}P activity counted using liquid scintillation techniques (Beckman LS 6500 Liquid Scintillation Counter). Counts were later corrected for background, decay, and quenching by using quenching standards with known activity. Phosphate-uptake rates were assumed linear over the incubation time (tested in preliminary studies, see *Time series*) and were calculated as the absolute ^{33}P incorporated per gram dry weight of tissue per incubation time and multiplied by the background corrected P_i concentration. In May/June 2004, ambient P_i concentrations were below detection limits and it was decided to use the detection limit of 0.01 $\mu\text{mol L}^{-1}$ as background concentration, although this would have slightly overestimated uptake rates if concentrations were lower than 0.01 $\mu\text{mol L}^{-1}$. In June 2005, ambient concentrations were 0.015 $\mu\text{mol L}^{-1}$.

Calculating uptake rates from initial $^{33}\text{PO}_4^{3-}$ incorporation into plant tissue are based on two assumptions: (1) the active uptake process must be unidirectional, with negligible leakage of phosphorus back into the incubation medium, and (2) the uptake does not include energy-independent phosphate-phosphate isotope exchange. No experimental data for either of the assumptions are, to our knowledge, available for *T. testudinum* or other higher plants and were treated as being insignificant in our calculations.

Time series—Time-series incubations were conducted in March (leaves) and May (roots) 2004 with plants from Eagle Key to test whether the ^{33}P uptake was independent of incubation time or was subject to feedback inhibition. The set-up for the leaves was as described above but with five amended concentrations (0.0, 0.05, 0.075, 0.1, and 0.25 $\mu\text{mol L}^{-1}$) and three incubation times, 15, 30, and 120 min. Two substrate concentrations (0.025 and 0.1 $\mu\text{mol L}^{-1}$

PO_4^{3-}) were used to validate linearity over time for roots. All incubations were run in triplicate.

Sediment adsorption-desorption experiment—Sediment adsorption-desorption parameters were determined on sediment from Eagle Key and Green Mangrove Key in March 2004. Approximately 0.5 g wet weight sediment was suspended in 50 mL artificial seawater (ultrapure grade chemicals) amended with PO_4^{3-} to one of eight initial concentrations (0.0, 0.0125, 0.024, 0.050, 0.075, 0.125, 0.250, and 0.500 $\mu\text{mol L}^{-1}$) and varying pH (7.0, 7.2, 7.4, 7.6, 7.8, 8.0). The latter experiment was conducted to test for the effect of pH on the sediment PO_4^{3-} adsorption capacity, as it is known to affect PO_4^{3-} adsorption to pure aragonite (Millero et al. 2001). The sediment was kept in suspension for 24 h, centrifuged, and the supernatant filtered through an acid-washed glass-fiber filter (Whatman GF/F). The final equilibrium P_i concentration was measured as molybdate-reactive P in a 5-cm light path. Changes in P_i concentrations were plotted as a function of final P_i concentration in a buffer diagram (δP_i vs. $P_{i\text{end}}$ plot; Froelich 1988) to estimate the critical crossover concentration (C_{e0}) defined as the P_i concentration where no net adsorption-desorption occurs. Normally, the data are fit to a Langmuir isotherm to describe saturation adsorption kinetics, but because ambient concentrations were far below saturation, linear regression was applied. Using this approach, the C_{e0} is defined as the point on the P_i -axis where δP_i is zero; the slope of the line is the linear adsorption capacity (LAC).

Plant nutrient analyses—Freeze-dried leaf tissue from both sites was digested for C:N:P elemental analyses. Total phosphorus in plant tissue was determined on dried samples (sample digested using COE 3-227; analyzed using EPA 365.2) and measured spectrophotometrically (Spectronic Genesys 5 Spectrophotometer). Total carbon and total nitrogen were analyzed by mass spectroscopy (Carlo-Erba NA 1500 Series 2 Elemental Analyzer) from dried samples.

Statistical analysis—Leaf and root V_{max} and K_m values were estimated by fitting the data to a nonlinear hyperbolic model in a V versus $[P_i]$ plot (Fig. 2) and analyzed for significant differences between sites and tissue type according to Bates and Watts (1988) using nested nonlinear regression analysis followed by an analysis of variance (ANOVA). Affinities (α) were calculated as the initial slope of the hyperbola fit using linear regression (Fig. 2) and were examined for significant differences between tissue type and sites using ANCOVA followed by the T' methods according to Sokal and Rohlf (1995) when significant interactions were observed. S_{min} was calculated as the intersection of the linear regression with the $[P_i]$ axis (Fig. 2). Significant differences between P_i uptake rates and incubation time were also examined using ANCOVA. The computer program “R” (www.r-project.org) and SigmaStat (SigmaStat) were used for all statistical analyses.

Results

Time series—For all three incubation times, ^{33}P incorporation into *T. testudinum* leaf tissue was a linear function of

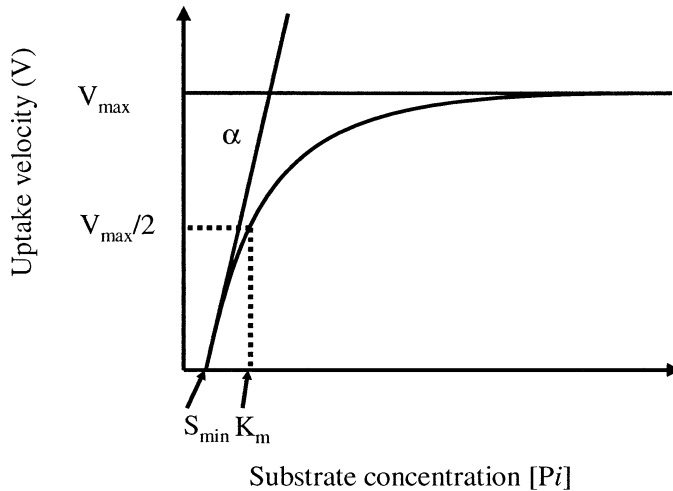


Fig. 2. Theoretical plot of uptake velocity (V) as a function of substrate concentration (Pi) as described by Michaelis–Menten kinetics. V_{\max} is the maximum velocity at substrate saturation, K_m is the half-saturation constant, S_{\min} is the physiological threshold value where Pi uptake is positive, and α is the affinity defined as the initial slope of the hyperbolic curve.

concentration below $0.26 \mu\text{mol L}^{-1}$ (Fig. 3a). Uptake rates increased slightly with incubation time, mainly between 15 and 30 min, with only a small increase between 30 and 120 min. These data indicate an initial lag time in the Pi uptake; however, no statistical differences among slopes were found. Based on these initial tests, it was concluded that leaf Pi uptake rates were not significantly affected by potential internal feedback inhibition or phosphate isotope exchange during the 15–120 min incubations, and a 60-min incubation period was set for the study. Root uptake rates were comparable with those for leaves at specific Pi concentrations measured (Fig. 3b).

Pi uptake kinetics at saturation—Across a broad range of Pi concentrations, rates of Pi uptake followed a nonlinear hyperbolic model (Fig. 4), from which Michaelis–Menten

kinetic parameters were estimated (Table 1). V_{\max} and K_m values were higher in leaves from Eagle Key compared with Green Mangrove Key (2004), but relatively high variance, especially at high Pi concentrations (Fig. 4), precluded establishing any statistical differences between tissue types or sites (Table 1).

Low-level Pi uptake kinetics—At Pi concentrations $\leq 0.26 \mu\text{mol L}^{-1}$, Pi uptake rates showed a strong linear relationship with concentration (Figs. 5, 6; and Table 1). In leaf and root tissues from both sites and years, affinities were quite similar, ranging from 0.10 to 0.30 (Table 1). Comparing between sites, leaf affinities at Green Mangrove Key were approximately 50% higher than at Eagle Key in 2004 and 2005; however, high natural variability within the system precluded discerning any significant differences between the two sites. Root affinities were either the same as the leaves, as was the case at Eagle Key, or they were significantly lower, as was found at Green Mangrove Key in 2005. Because root affinity may be influenced by oxygen in the media, we examined the uptake kinetics both with and without degassing the media before incubations. Based on these results, we could not differentiate a significant difference between root uptake in oxic and hypoxic conditions and root Pi -uptake rates appeared to be independent of media oxygen concentration in the short-term incubations (Fig. 7).

Linear kinetics allowed us to articulate the low levels at which *T. testudinum* was able to sequester Pi . It was interesting that Pi threshold values (S_{\min}) were in the nanomolar range for both *T. testudinum* leaves and roots at both sites (Table 1). This seemed to be the case regardless of the fact that the two sites where the plants were collected had different nutrient states with respect to P. The more P-enriched state at Green Mangrove Key was evidenced in the twofold higher leaf N:P ratios found at Eagle Key (39.0 ± 3.3) compared with Green Mangrove Key (22.2 ± 3.8).

Sediment P kinetics—In general, sediment net Pi adsorption–desorption was a linear function of Pi concentration in the slurries (measured as the postincubation concentration)

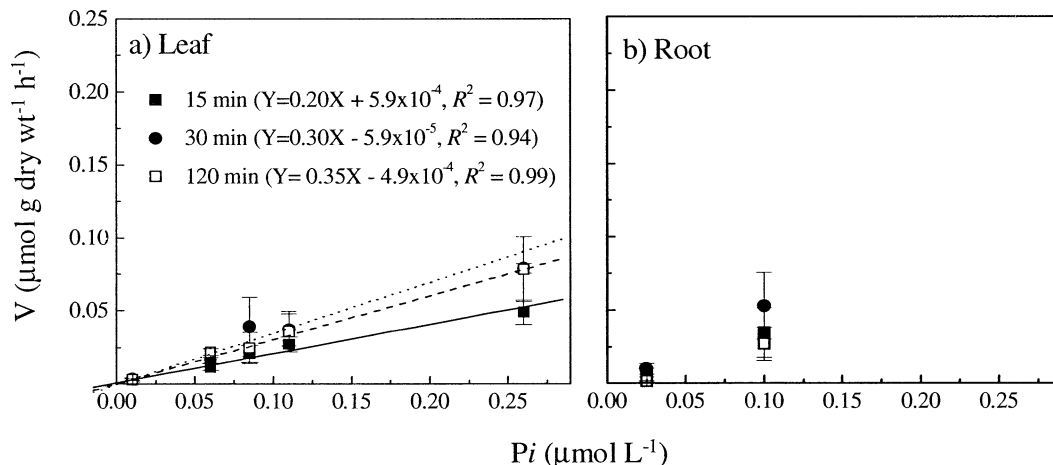


Fig. 3. The effect of incubation time (15, 30, and 120 min) on ^{33}P -uptake rates by (a) leaves and (b) roots of *Thalassia testudinum*. Lines represent best fit using linear regression. Error bars represent \pm SD ($n = 3$).

Table 1. *Thalassia testudinum* leaf (L) and root (R) Pi uptake saturation kinetic parameters V_{\max} ($\mu\text{mol g dry weight}^{-1} \text{h}^{-1}$) and K_m ($\mu\text{mol L}^{-1}$) and low in situ Pi linear kinetic parameters S_{\min} ($\mu\text{mol L}^{-1}$) and α ($\text{L g dry wt}^{-1} \text{h}^{-1}$) based on plants collected from Eagle Key and Green Mangrove Key in Florida Bay (2004 and 2005). All estimates are means \pm standard error.

	Saturation kinetics			Low-level Pi uptake kinetics				
	V_{\max}	K_m	R^2	S_{\min}	α	n	R^2	p
Eagle Key (eastern Bay)								
L (2004)	1.28 \pm 0.24	7.89 \pm 3.60	0.89	0.006	0.12 \pm 0.01	15	0.87	<0.01
L (2005)	nd*	nd	nd	0.008	0.15 \pm 0.01	30	0.83	<0.01
R (2004)	0.74 \pm 0.22	3.34 \pm 3.40	0.57	0.005	0.18 \pm 0.03	15	0.77	<0.01
R (2005)	nd	nd	nd	0.004	0.17 \pm 0.02	30	0.64	<0.01
Green Mangrove Key (western bay)								
L (2004)	0.58 \pm 0.05	2.18 \pm 0.69	0.93	0.009	0.23 \pm 0.03	15	0.83	<0.01
L (2005)	nd	nd	nd	0.008	0.30 \pm 0.02	30	0.93	<0.01
R (2004)	nd	nd	nd	nd	nd	nd	nd	nd
R (2005)	nd	nd	nd	0.008	0.10 \pm 0.01	30	0.84	<0.01

* nd = not determined.

as exemplified by the plots presented in Fig. 8. This linear relationship was significant at $p < 0.05$ using a one-sided t -test with a few exceptions (pH 7.6 at Eagle Key and pH 7.4 and 7.8 at Green Mangrove Key), probably due to sediment heterogeneity. The mean Ce0, based on the dataset with significant regression lines, was an order of magnitude lower at Eagle Key ($0.017 \pm 0.015 \mu\text{mol L}^{-1}$, $n = 5$) compared with Green Mangrove Key ($0.292 \pm 0.104 \mu\text{mol L}^{-1}$, $n = 4$). Further, the average LAC was six times higher at Eagle Key (19.5 ± 8.6) than Green Mangrove Key (3.0 ± 0.6), alluding to a greater role of sediment in sequestering Pi in the eastern region of Florida Bay. While site differences were found for Ce0 and LAC, estimated Ce0 and LAC values showed no significant pH effect at either site.

Discussion

Using a ^{33}P -tracer technique, previously used for evaluating Pi uptake kinetics in P limited cyanobacteria and eukaryotic algae in culture (Currie and Kalff 1984; Falkner et al. 1989; Istvanovics and Herodek 1995), we report herein for the first time that seagrasses have a high capacity to take up Pi at extremely low levels. In this study, S_{\min} for Pi uptake was in the nanomolar range (0.004 – $0.009 \mu\text{mol L}^{-1}$) for both roots and leaves of *T. testudinum*. These threshold values are comparable with those reported for P-limited cyanobacteria and eukaryotic algae (<0.001 – $0.015 \mu\text{mol L}^{-1}$) (Admiraal and Werner 1983; Button 1985; Istvanovics et al. 2000). These data lead us to suggest that *T. testudinum* can be a competitor for Pi at extremely low Pi levels, comparable with unicellular organisms considered superior to macroflora in nutrient scavenging due to their small size (Currie and Kalff 1984; Istvanovics and Herodek 1995). This potential to sequester Pi at nanomolar concentrations would be advantageous to tropical seagrasses from carbonate environments where Pi levels are frequently below detectable limits. In Florida Bay, Pi in surface waters has an average concentration of $0.03 \mu\text{mol L}^{-1}$ and ranges from below detection (at which time total phosphorus = $0.02 \mu\text{mol L}^{-1}$) to $0.2 \mu\text{mol L}^{-1}$ (long-term data 1989—from Boyer et al. 1997,

1999). While surface-water Pi concentrations in Florida Bay are consistent across the Bay, pore-water nutrients are greater in the western region of the Bay, where plant production and biomass is also highest (Fourqurean et al. 1992a). Even though plant biomass and productivity is greatest in the western regions of the Bay, S_{\min} remained extremely low from plants collected across this nutrient gradient. These data indicate that the Pi threshold level is species-, rather than site-specific, and noninducible.

We also observed very little difference in the leaf uptake kinetics of Pi at low in situ Pi concentrations ($\leq 0.26 \mu\text{mol L}^{-1}$) regardless of the trophic state of the site where *T. testudinum* was collected. Rates of Pi uptake using ^{33}P were also consistent with our results using more traditional chemical techniques (Gras et al. 2003). We found the α in the present study, ranging between 0.12 and 0.30 for leaves using ^{33}P and 60-min incubations, to be very close to those from our last study, where α was calculated to be 0.16–0.23 for *T. testudinum* leaves in 10-h incubations (Gras et al. 2003). These similarities in the kinetics were found even though, in our previous kinetic experiments, the lowest Pi concentration was an order of magnitude higher ($0.5 \mu\text{mol Pi L}^{-1}$; Gras et al. 2003) than in this study. The consistency of our *T. testudinum* Pi kinetic results, along with those found for *Thalassia hemprichii* from southeast Asia (α range 0.13–0.19; Stapel et al. 1996), have important consequences for modeling Pi uptake in tropical seagrass ecosystems dominated by *Thalassia*. Based on these studies, a linear relationship should be employed when describing the Pi uptake kinetics, and this relationship appears to hold across a wide range of low Pi concentrations and site-specific trophic states. Differences between leaf and root Pi uptake rates at low Pi concentrations ($\leq 0.26 \mu\text{mol L}^{-1}$) are relatively similar assuming the current variance in the system. The affinity for *T. testudinum* roots ranged between 0.09 and 0.24 in this study and Gras et al. (2003).

Based on current data, *T. testudinum* leaves and roots have relatively similar linear kinetics for Pi across a broad range of Pi concentrations. Consequently, the relative importance of leaf versus root uptake in meeting the plant requirements

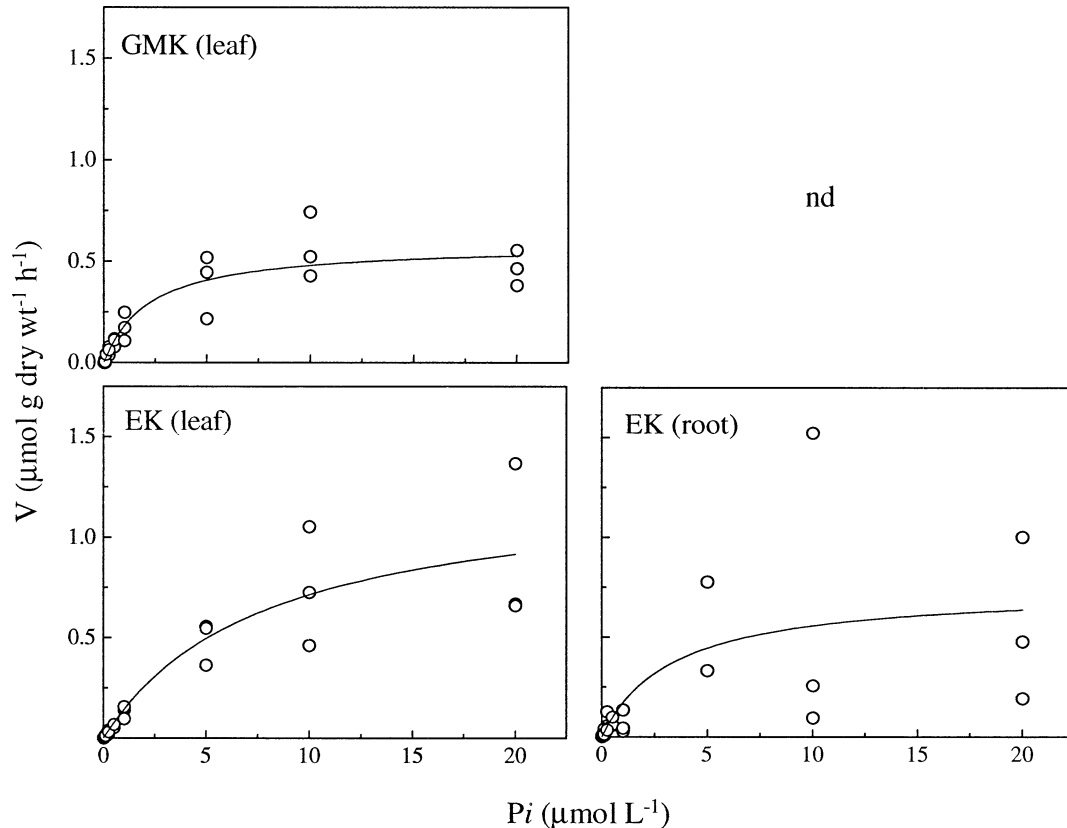


Fig. 4. P_i uptake rate for leaves (left panel) and roots (right panel) of *Thalassia testudinum* at Eagle Key (EK) and Green Mangrove Key (GMK) as a function of P_i concentration described by Michaelis–Menten saturation kinetics. Lines are best fit to the hyperbolic model. Not determined (nd).

for P will be defined by the availability of nutrients to these tissues. In the water column, resuspension of fine carbonate sediment particles and biotic processes maintain a consistently low P_i concentration in surface waters across Florida Bay (Boyer et al. 1997, 1999) and, on average, P_i to leaves from this source is low. Using the average surface-water P_i concentrations and previously estimated *T. testudinum* requirements for P in Florida Bay, $0.0216 \mu\text{mol P g dry weight}^{-1} \text{ h}^{-1}$ (Gras et al. 2003), we calculate that leaf uptake at Eagle Key and Green Mangrove Key can only satisfy approximately 13–15% and 23–30% of *T. testudinum* P nutritional requirements, respectively. These calculations lead us to suggest that the main P source for *T. testudinum* in Florida Bay is not the water column.

Another major source of P to seagrass is the large solid-phase P pool in the sediments (Jensen et al. 1998); however, the availability of this pool to the surrounding pore water is regulated by sediment adsorption/desorption reactions. It has been shown that adsorption to aragonite (a pure phase calcium carbonate) is affected by pH at high phosphate concentrations ($4 \mu\text{mol L}^{-1}$) (Millero et al. 2001). Burdige and Zimmerman (2002) further suggest that, in the biogeochemically dynamic seagrass rhizosphere, the oxygenated region around the roots might directly or indirectly modify P sorptive reactions by shifting pH in the sediment. In the present study, we found no significant effect of pH (7.0–8.0) on the P_i adsorptive kinetics in Florida Bay sediments; however,

pH adjustments of the seawater were prior to adding the sediment (in a 1 : 100 sediment : seawater ratio) and the sediment may have modified pH of the slurry such that differences in pH after 24 h were less pronounced. In our current research (data not presented), we are refining these experiments and so, at this time, we will not exclude a possible pH effect. We did observe, however, a strong east–west difference in the threshold adsorptive capacity of the sediment that would significantly influence the P_i available to seagrass roots.

At the eastern Bay site, P_i was adsorbed by the sediment down to extremely low levels ($0.017 \pm 0.015 \mu\text{mol L}^{-1} \text{P}_i$), approaching the root S_{min} for *T. testudinum* ($0.004\text{--}0.005 \mu\text{mol L}^{-1} \text{P}_i$), and within the range of overlying surface-water P_i concentrations (Boyer et al. 1999). At this sediment P_i equilibrium concentration, root uptake of P_i from pore water at Eagle Key would only satisfy 6.5% of the P requirements for *T. testudinum* based on a root : leaf biomass of 0.66 (Fourqurean and Zieman 1991). While this is only based on the P_i concentration where adsorption is in equilibrium with desorption at the Eagle Key site, these calculations suggest that there is a potential for competition between the sediment and roots for P_i in eastern Florida Bay. These dynamics are in contrast with the western Bay site, where the crossover threshold between sediment adsorption and desorption of P_i was calculated as $0.292 \pm 0.104 \mu\text{mol L}^{-1}$, 10 times higher than at Eagle Key. At the western Bay

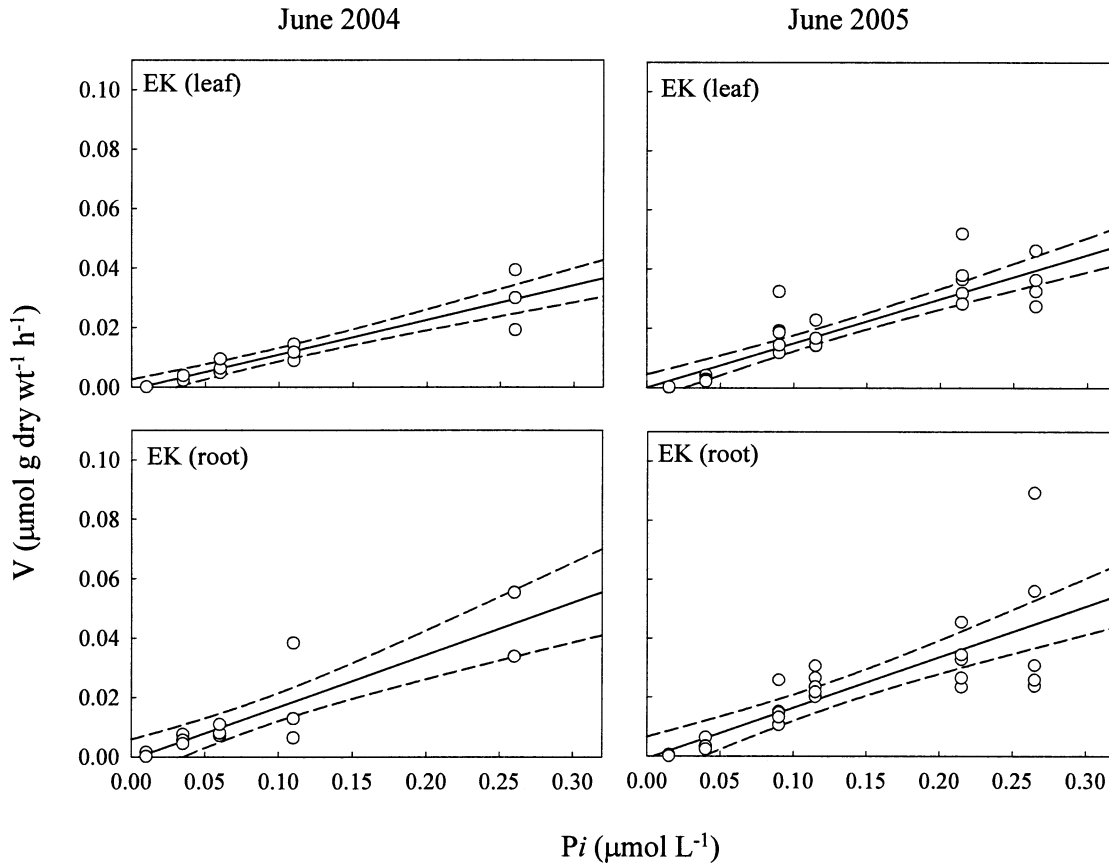


Fig. 5. Pi uptake rate for leaves and roots of *Thalassia testudinum* at Eagle Key (EK) at low Pi concentrations ($\leq 0.26 \mu\text{mol g dry weight}^{-1} \text{h}^{-1}$) in June 2004 (left panel) and 2005 (right panel). Solid lines represent best fit using linear regression and dotted lines represent 95% confidence intervals.

site, pore-water Pi at the $Ce0$ concentration could account for 87% of the P requirements for *T. testudinum*; thus, competition between sediments and plants for pore-water nutrients may not be as important in the western versus eastern Bay, also indicated by greater labile exchangeable P in western Bay sites (Zhang et al. 2004). The lower $Ce0$ and higher LAC at Eagle Key are probably a consequence of the finer grained sediment in the eastern Bay. Very fine carbonate particles ($< 10 \mu\text{m}$) have been shown to significantly influence the sediment P sorption potential (Suess 1973). Low P inputs to eastern Florida Bay may also influence sediment adsorptive potential, as was found in Bermuda by McGlathery et al. (1994). They reported higher adsorption capacity in sediments from offshore sites compared with inner island sites characterized by P enrichment. A survey of pore-water nutrients collected from 18 sites throughout the Bay by Fourqurean et al. (1992a) indicate a very broad Pi range from 0.05 to 33.5 $\mu\text{mol L}^{-1}$ Pi (median of 0.34 $\mu\text{mol L}^{-1}$). Based on these data ($n = 286$), $\sim 50\%$ of the samples had Pi levels below the concentration (0.2–0.34 $\mu\text{mol L}^{-1}$) where we calculated that root uptake would not meet *T. testudinum* P requirements (0.0216 $\mu\text{mol P g dry weight}^{-1} \text{h}^{-1}$; Gras et al. 2003). Thus, *T. testudinum* in the northeastern region of the Bay probably are Pi limited due to competition for Pi with carbonate sediments.

In contrast with the broad range of pore-water Pi concen-

trations in the Bay, surface-water Pi remains close to the limits of detection (Boyer et al. 1997, 1999). It is interesting that, even though surface-water Pi levels are consistently low, leaf uptake of Pi saturates at concentrations 10–100 times higher than ambient surface waters in the Bay (V_{max} 0.58–1.28 $\mu\text{mol L}^{-1}$). These data indicate *Thalassia's* ability to adapt to transient periods of increased Pi availability, perhaps during rapid remineralization events. Leaf V_{max} values determined in this study are in line with other studies of *Thalassia* (1.9–3.2 $\mu\text{mol g dry weight}^{-1} \text{h}^{-1}$; Stapel et al. 1996; Gras et al. 2003), as well as temperate seagrass species, albeit with considerable variance (0.9–43 $\mu\text{mol g dry weight}^{-1} \text{h}^{-1}$; Thursby and Harlin 1984; Perez-Llorens and Niell 1995). Thus, based on the limited available saturation kinetic data for seagrass, it appears that similar kinetics (V_{max}) are operating in tropical and temperate species regardless of the fact that temperate estuaries tend to be more eutrophic. Even more interesting is the lack of a clear site or species difference in the half-saturation constant, usually an indicator of a species' ability to compete under relatively low-nutrient conditions; low K_m indicates a high competitive ability. We estimated leaf K_m values to be lower at Green Mangrove Key (2.18) than Eagle Key (7.89), showing that low nutrient availability at Eagle Key did not lower leaf K_m . The K_m calculated for *T. testudinum* in this study was slightly lower than our earlier estimates (12; Gras et al. 2003) and

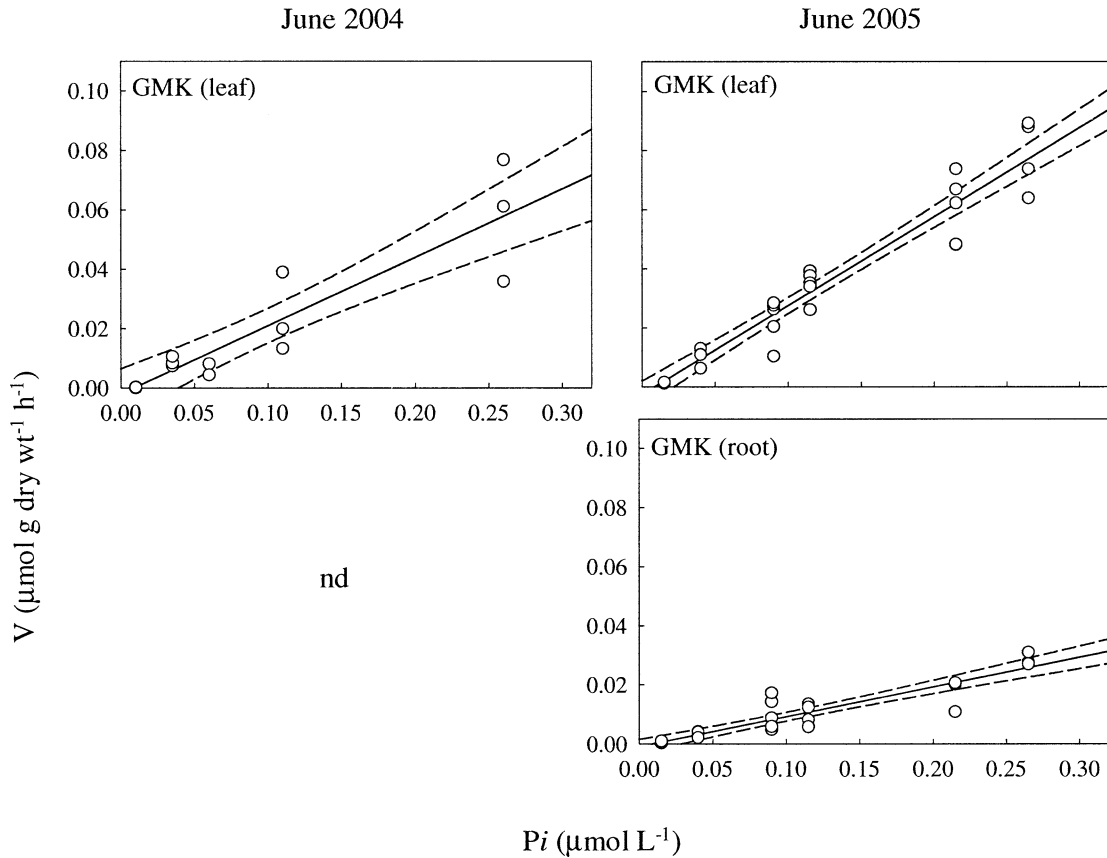


Fig. 6. P_i uptake rate for leaves and roots of *Thalassia testudinum* at Green Mangrove Key (GMK) at low P_i concentrations ($\leq 0.26 \mu\text{mol g dry weight}^{-1} \text{h}^{-1}$) in June 2004 (left panel) and 2005 (right panel). Solid lines represent best fit using linear regression and dotted lines represent 95% confidence interval. Not determined (nd).

those determined for *T. hemprichii* (7.7–15), but are within the range reported for temperate species (3.1–12.1; Thursby and Harlin 1984; Perez-Llorens and Niell 1995), again alluding to the fact that no major kinetic differences exist between temperate and tropical seagrass species in terms of leaf P_i uptake at saturation. Unfortunately, there is not enough data to contrast root kinetics among region; however, based on our results (Gras et al. 2003; this study) and data

for *Ruppia maritima* by Thursby and Harlin (1984), V_{\max} and K_m tend to be 25–50% lower for roots than leaves, perhaps indicating a higher affinity for P_i by roots than leaves at P_i levels approaching saturation.

In conclusion, seagrasses have a very high capacity to take up P_i at extremely low levels, on par with S_{\min} reported for unicellular organisms. This low S_{\min} would allow seagrass to take up P_i at low water-column concentrations; however,

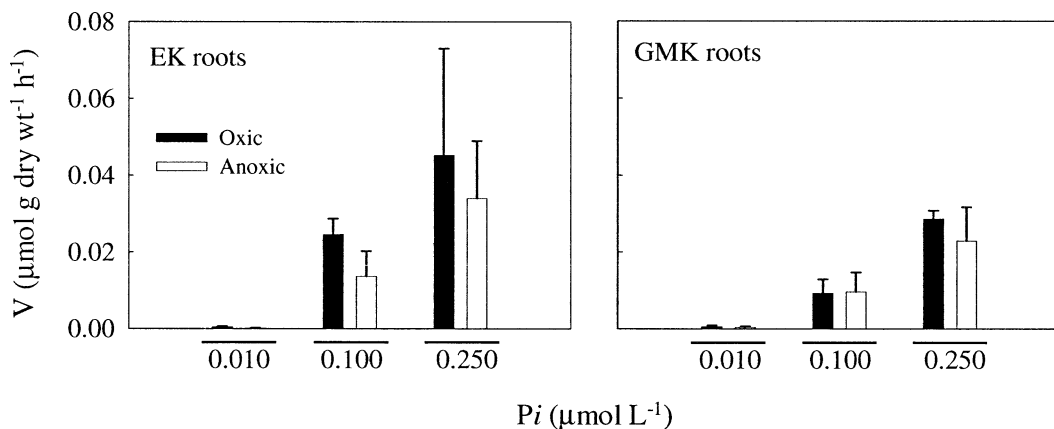


Fig. 7. P_i uptake rates for roots at Eagle Key (EK) and Green Mangrove Key (GMK) incubated in oxic versus hypoxic media at three different P_i concentrations (0.01, 0.10, and $0.25 \mu\text{mol L}^{-1}$; \pm standard deviations, $n = 5$).

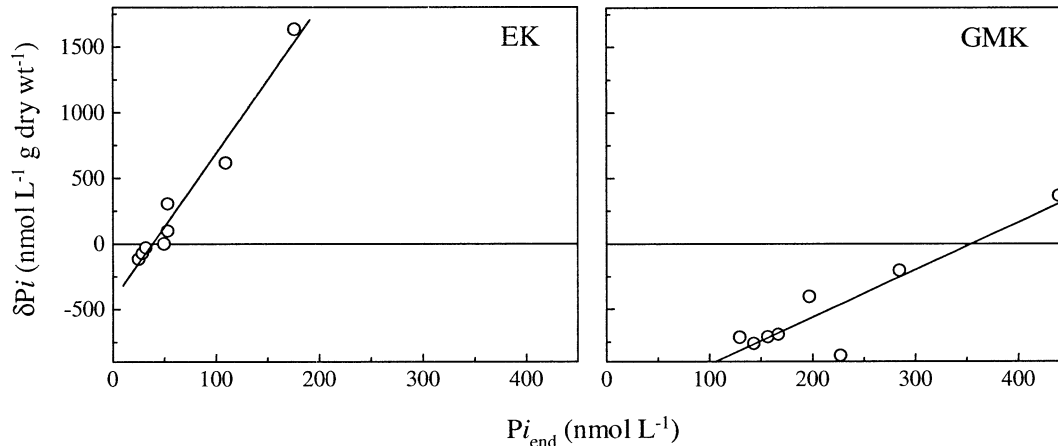


Fig. 8. Representative plots of net P_i adsorption-desorption onto sediment suspended in low P_i seawater from the two sites; Eagle Key (EK) at pH = 7.6 and Green Mangrove Key (GMK) at pH = 8.0. The point on the X-axis where the regression line crosses the Y-axis at zero is the critical crossover concentration (C_{e0}); the slope is the linear adsorption capacity (LAC).

based on the nutrient requirements of *T. testudinum* in the Bay, seagrass uptake rates at nanomolar concentrations do not represent a sufficient nutritional supply. Thus, sediment pools, or transient fluxes of P_i to the water column must sustain the high rates of seagrass production in the Bay. Pore-water P_i at the adsorption-desorption threshold for P_i at our eastern Bay site met $\leq 10\%$ of the P demand for *T. testudinum*, while at the western Bay site, accounted for 87% of the P requirements. These data lead us to suggest that sediment and root competition for pore-water P_i accounts for P limitation of seagrass production in eastern Florida Bay. While *T. testudinum* has the capacity to take up P_i at extremely low levels, it has relatively similar linear and saturation kinetics across nutrient gradients. Thus, based on our work and a limited number of other studies, there does not appear to be a unique adaptation of tropical seagrass species to oligotrophic environments based on kinetic parameters. Consequently, defining P pools in the water column and sediment, their turnover rates, and transient fluxes to the water column and pore water are important for modeling P dynamics in seagrass ecosystems.

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