

The role of organic acid exudates in liberating phosphorus from seagrass-vegetated carbonate sediments

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Abstract

Sediment-bound phosphorus (P) is a potential nutrient source for P-limited seagrasses inhabiting carbonate sediments. We explored the role of organic acid (OA) exudation by seagrasses in liberating mineral P from carbonate sediments. Organic acids can act to increase available P by dissolving carbonate sediment, competing with P for binding sites and complexing dissolution end products, and also by fueling microbial processes that change pore-water pH. We used dialysis tubing placed around individual roots in situ to quantify dissolved species immediately adjacent to roots (root zone) and compared these to bulk pore-water concentrations in vegetated and nonvegetated sediments. Total OA concentrations were highest in the root zone ($29.8 \pm 1.8 \mu\text{mol L}^{-1}$) compared to bulk measures of 15.5 ± 1.9 and $7.5 \pm 0.6 \mu\text{mol L}^{-1}$ in vegetated and nonvegetated sediments, respectively. Phosphate concentrations were also highest in the root zone and were linearly related to OA concentrations ($R^2 = 0.63$). Organic acid concentrations increased along a seagrass productivity gradient, and ratios of OA concentrations to productivity showed a significant response to a gradient in P-limitation of seagrasses. Organic acid concentrations found in and around roots, compared to those found in bulk sediment measures, indicate that seagrasses are a significant source of OA. Sampling at small spatial scales (mm) immediately adjacent to the roots is critical, because bulk sediment pore-water measures did not capture the observed fluctuations caused by the rapid reaction and consumption of OA in the sediment. Root-zone processes can liberate considerable quantities of P, and OA exudates likely contribute significantly to the success of *T. testudinum* in P-limited environments.

Organic acid exudation from seagrass roots has the potential to liberate phosphorus (P) from carbonate marine sediments that are characteristic of many subtropical and tropical environments. Since organic acid (OA) exudates from terrestrial plant roots have been shown to increase the dissolution of phosphate (P_i) rocks (Ryan et al. 2001; Strom et al. 2002; Jones et al. 2003) it is hypothesized that seagrasses may exhibit an analogous mechanism. However, there have been no attempts to address the dissolution of carbonate sediments by OA exudates in marine environments and the potential of this process to make mineral P_i available to P-limited seagrasses.

Organic acids are here defined as low-molecular-weight organic compounds that contain at least one carboxyl group and are not amino acids. The conceptual diagram in Fig. 1 illustrates the simplified biotic and abiotic mechanisms by which OA exudates, along with O_2 release, may influence P_i availability in marine carbonate sediments surrounding seagrass root tips. Organic acids can react abiotically with carbonate sediment minerals and solubilize P_i through a number of mechanisms. First, the organic anion may displace P_i on adsorption sites on minerals and organic matter or decrease the total amount of adsorption sites available (Jones 1998; Ryan et al. 2001; Strom et al. 2001). Second, the organic anion may chelate metal and carbonate dissolution end products, such as Ca^{2+} ions, that

would otherwise immobilize P_i (Knight et al. 1992; Kirk 1999; Strom et al. 2001). Third, if the excretion of the organic anion is accompanied by the excretion of protons (H^+) or if it is released as an acid, a localized decrease in pH will be observed around the root surface and the dissolution of carbonate minerals will release P_i (Bolan et al. 1997; Kirk 1999; Jones et al. 2003). The consumption of OA by microbes also likely contributes to carbonate sediment dissolution through the production of aqueous CO_2 and H^+ . The aerobic respiration of sediment organic matter (which may include OA) results in the production of aqueous CO_2 , which lowers the saturation state of carbonate minerals in sediment pore waters (Burdige and Zimmerman 2002; Hu and Burdige 2007). Organic acids may also be consumed through fermentation and sulfate reduction, by which CO_2 and end products such as ethanol, acetic, and lactic acids are produced. Sulfate reduction results in the net production of acidity due to the tight coupling between sulfide oxidation and sulfate reduction, which is balanced by diel fluctuations in seagrass O_2 release (Ku et al. 1999; Burdige and Zimmerman 2002; Hu and Burdige 2007).

The mechanisms of OA exudation are largely unknown and their contribution to sediment dissolution depends on how and in what magnitude they are metabolized by microbes as well as whether they are released as anions or acids (Strom et al. 2002; Jones et al. 2003). In terrestrial sediments, the reaction of organic anions with carbonate minerals has been shown to increase P_i concentrations and sediment dissolution, even though the anions do not directly increase acidity (Kirk 1999; Ryan et al. 2001). This is due to multivalent OA, which have the most significant effect on P_i availability because they compete

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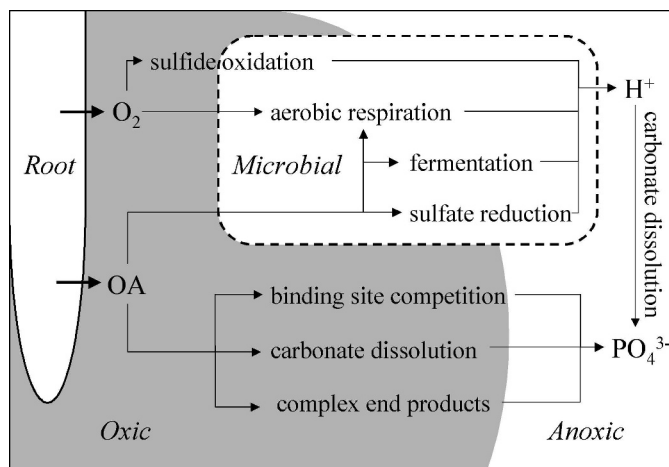


Fig. 1. Conceptual diagram of the simplified mechanisms of seagrass-mediated carbonate sediment dissolution. The shaded areas represent the oxic zone due to O_2 release from seagrass roots. Mechanisms inside the dash-outlined box represent mechanisms mediated by microbial activity. The stoichiometry is not balanced and the arrows do not represent magnitude, because these values are unknown and require further investigation.

with P_i for binding sites and shift the dissolution equilibria by complexing its end products. Multivalent OA also represent the best substrates for microbial degradation, which results in the net production of acidity. Although the contribution of sediment acidity provided directly by OA and that provided by microbial processes are difficult to separate, both of these processes are largely mediated by OA exudation in the root zone (Ryan et al. 2001; Jones et al. 2003). The multivalent OA we examine here are succinic, malic, maleic, fumaric, oxalic and citric and the monovalent OA we examine are acetic, lactic, propionic and formic acids.

The high affinity of marine carbonate sediments for P_i is generally thought to be the main reason why primary production in many tropical and subtropical environments is P-limited (Bosence 1989; Fourqurean and Zieman 1992). A number of studies have investigated total sediment dissolution rates and seagrass uptake rates and found that P_i concentrations provided by rates of bulk sediment dissolution are not sufficient to relieve P-limitation (Jensen et al. 1998; Gras et al. 2003; Nielsen et al. 2006). Recent evidence suggests that there are increased rates of sediment dissolution in marine rhizosphere sediments, and that high rates of oxidation caused by seagrass O_2 release may provide the required amount of acid to explain enhanced rates of dissolution (Ku et al. 1999; Burdige and Zimmerman 2002; Burdige et al. 2008). Organic acid exudation by seagrasses may also help to explain increases in sediment dissolution in the rhizosphere, either directly by reacting with carbonate minerals, or indirectly by providing the necessary substrates for the bacterially mediated oxidative processes. The mechanisms and dynamics of OA reactions in the sediment are of particular importance for P_i liberation, because they have the potential to prevent the adsorption of P_i onto carbonates.

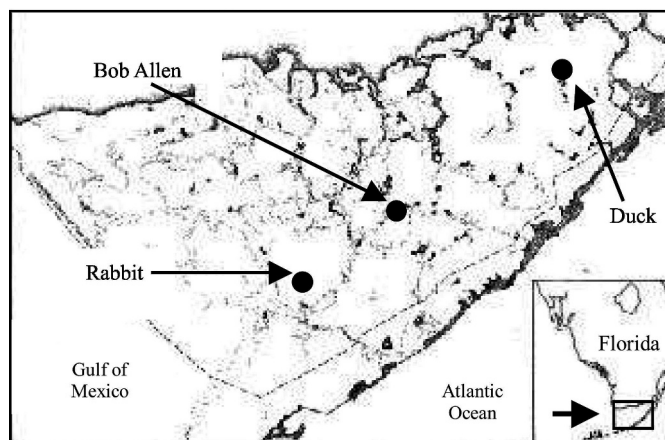


Fig. 2. Map of Florida Bay with sites of Duck, Bob Allen, and Rabbit from northeast to southwest across the Bay (adapted from Fourqurean and Zieman 1992).

The seagrass *Thalassia testudinum* has as much as 85% of its biomass in below-ground structures (Fourqurean and Zieman 1991) and has been shown to modify sediment characteristics such as O_2 concentrations and pH (Burdige and Zimmerman 2002). Recently, Burdige et al. (2008) modeled carbonate dissolution rates in seagrass sediments based on oxygen release from seagrass roots and showed that rates of dissolution were positively correlated with seagrass density. Seagrasses are known release O_2 from root tips (Pedersen et al. 1998; Frederiksen and Glud 2006) and the root tips are expected to be the dominant region of OA exudation, which may cause pockets of high rates of dissolution and reduced P_i adsorption to occur around root tips. It is likely that most exudate activity occurs in this microenvironment, because the processes that consume O_2 and OA in the sediments are rapid.

Although there is evidence that increased rates of carbonate sediment dissolution occur in the rhizosphere, little evidence has been presented for its importance on P_i liberation. This may be due to the more recalcitrant nature of P_i -containing minerals, for which OA may have a much greater potential for releasing P_i than do simple changes in acidity. We therefore examined the potential role of organic acid exudation by seagrass roots in liberating sediment-bound P_i in *T. testudinum* meadows in Florida Bay. Because Florida Bay has a well-established P availability and seagrass productivity gradient (Fourqurean et al. 1992; Fourqurean and Zieman 2002), it provides an ideal system to investigate the link between seagrass productivity, OA exudates, microbial processes, sediment dissolution and P dynamics. We report here the first evidence of the importance of organic acid exudation from marine seagrass roots in liberating mineral-bound P_i in the rhizosphere.

Methods

Site description—Florida Bay is a shallow (<3 m) estuary with extensive seagrass communities dominated by *T. testudinum* (Zieman 1989; Fourqurean and Zieman

1992), located at the southern tip of Florida, USA (Fig. 2). The sediments of Florida Bay are comprised primarily of accumulating biogenic carbonate mud and are overlaid by waters with long residence times, which produce a clear and P-depleted water column (Bosence 1989). The bay is bounded on the east and south by the emergent Pleistocene reef ridge of the Florida Keys: the western portion of the bay is open to flushing from the Gulf of Mexico, and the northern regions receive freshwater inputs from the Everglades of mainland Florida (Fourqurean and Zieman 1992). Three sites were chosen along a northeast to southwest transect across the bay and include Duck Key, Bob Allen Keys, and Rabbit Key Basin (Fig. 2). These sites represent gradients in P availability (lowest in northeast) and productivity (highest in southwest; Fourqurean and Zieman 2002), and are permanent monitoring sites of the Florida Coastal Everglades Long Term Ecological Research Program. At each site, four sampling locations were chosen randomly in monotypic beds of *T. testudinum* and in nonvegetated areas located nearby. Sampling was done in June 2005 and January, June, and August 2006.

Sediment and seagrass characteristics—Small 4-cm inside diameter (ID) \times 60-cm-length polyvinyl chloride (PVC) cores were used to sample bulk sediments with four cores taken in each of the vegetated and nonvegetated sediments at each site. Cores were sectioned in 10-cm intervals, homogenized, and frozen for later analysis. Small 4-cm ID \times 30-cm-length PVC cores were used to sample vegetated sediments for use as intact sediment cores for dissolution experiments with 24 samples taken at each site. The organic content of sediments was determined by weight loss after combustion at 450°C for 5 h. Total P was measured using the persulfate digestion method. Four large 15-cm ID \times 60-cm PVC sediment cores were used to sample seagrass biomass at each site. Live root material was separated from dead plant matter based on its buoyancy after removal of attached sediment (Bricker 2003). Samples of leaf, apical meristem, root, and rhizome material were freeze-dried, ground, and extracted in triplicate with 80% methanol, followed by centrifugation at 5000 g and filtering, with an aliquot run on the DIONEX ion chromatograph (IC) to determine OA concentrations, as described below. Internal-root concentrations of OA (by volume) were calculated for comparison to root-zone concentrations, by using a root of known length, diameter, and weight.

Pore-water analysis—Sediment pore-water samples were taken at 0-, 5-, 15-, 25-, and 35-cm depths using a small-diameter stainless steel probe and the method described by Berg and McGlathery (2001) at each sampling location. Four replicates were obtained at each depth in each of the vegetated and nonvegetated sediments at each site. Sampling was done by self-contained underwater breathing apparatus (SCUBA) divers, and pore-water samples were filtered through a sterile 0.45- μ m filter and put on ice immediately after being brought to the surface. pH and fluoride (F^-) concentrations were measured immediately using an Orion advanced pH and mV field meter (Orion

model 290A+). pH was determined using a low-maintenance triode pH electrode with automatic temperature compensation and an internal Ag-AgCl reference (Orion model 91-09). The electrode was calibrated using standard pH buffers (pH 4.01, 7.00, and 10.01). F^- concentrations were measured using an Orion 290A+ field meter and a combination F^- electrode (Orion model 96-09), after adjusting the ionic strength of each sample with total ionic strength adjuster buffer III (TISABIII). Sulfide (S^-) samples were fixed with equal parts of 0.2 mol L^{-1} zinc acetate and analyzed with the standard spectrometric technique described in Cline (1969) using the methylene blue method. Nutrient samples (P_i and ammonium) were frozen and analyzed on a Lachat Auto-Analyzer (Quick-Chem 8500 Automated Ion Analyzer).

Root-zone sampling—We used standard dialysis tubing to sample the microenvironment at the root tip (Fisherbrand Micropore, 3000–6000 kDa pore size, diameter of 0.8 cm). Individual root tips were located at five 15-cm depths by digging carefully into the sediment by hand and allowing the fine sediment to clear. Ten-centimeter portions of hydrated dialysis tubing were slipped over the exposed root tip. The dialysis tubing was closed with a dialysis clip 5 mm beyond the root tip, with flagging tape attached to the clip for locating later. The dialysis tubing was then filled with ambient-water-column seawater using a small syringe inserted into the open end of the tubing; the tube was sealed around the root with a zip-tie immediately upon filling, carefully reburied, and left to equilibrate for two weeks. Two types of controls were set up by placing dialysis tubes containing cut segments of roots (simulating root mortality), and dialysis bags containing ambient seawater (to examine sampling artifacts due to dialysis tubing). Both were buried at a depth of 10 cm in the vegetated sediments and handled and analyzed as dialysis samples above. Pore-water sampling was done at the time when the dialysis tubing was deployed. Root-zone samples were recovered by cutting the root above the dialysis tube and immediately bringing each sample above water, where the fluid in the dialysis tubing was removed using a syringe. Samples were filtered and processed for F^- , S^- , and OA concentrations. Sample recovery was low and many replicates should be used for this type of sampling. The low sample volume (<2.0 mL) did not allow for the measurement of pH, or nutrient data by standard colorimetric techniques (as was done for bulk pore-water measurements). However, the OA analysis method (see ion-chromatography description below) allowed for the analysis of F^- and P_i concentrations.

Organic acid analysis—OA samples were initially filtered and frozen and then concentrations were determined using a DIONEX ICS-3000 ion chromatograph using suppressed ion conductivity detection. The ICS-3000 was fitted with a gradient pump, pressurized eluent delivery system, internal de-gasser, a DIONEX AS11 column, a 4-mm Anion Self Regenerating Suppressor (ASRS), and a DIONEX temperature-controlled autosampler. A gradient method was developed with a variable gradient of 0.5 mmol L^{-1} NaOH, 100 mmol L^{-1} NaOH, and 100% methanol, with

Table 1. Site characteristics of vegetated sediments, averaged across all sampling dates (June 2005 and January, June, and August 2006). Standard errors are represented by \pm values. Duck is in the northeast of Florida Bay and Rabbit is in the southwest of Florida Bay, Florida, USA. Productivity measures are from data sets provided by J. C. Zieman (unpubl.) and J. W. Fourqurean (unpubl.) and are in $\text{mg short-shoot}^{-1} \text{d}^{-1}$ (seagrass short-shoot specific) and $\text{g m}^2 \text{d}^{-1}$ (areal). All data are from the vegetated bulk sediments.

Site	Productivity		Organic matter	Sed. total P	PO_4^{3-}	NH_4^+	Roots	Rhizomes
	($\text{mg ss}^{-1} \text{d}^{-1}$)	($\text{g m}^2 \text{d}^{-1}$)	(% dry wt)	($\mu\text{mol g}^{-1}$)	($\mu\text{mol L}^{-1}$)	($\mu\text{mol L}^{-1}$)	(g dry wt m^{-2})	(g dry wt m^{-2})
Duck	0.67 ± 0.06	0.41 ± 0.04	5.4 ± 0.11	1.4 ± 0.06	0.5 ± 0.03	11.1 ± 2.1	93.0 ± 13.9	161.6 ± 59.7
Bob Allen	0.86 ± 0.13	0.28 ± 0.04	8.1 ± 0.31	2.1 ± 0.09	0.7 ± 0.03	13.7 ± 2.7	225.0 ± 29.8	281.5 ± 84.1
Rabbit	1.58 ± 0.11	1.65 ± 0.11	15.9 ± 0.57	4.8 ± 0.16	0.9 ± 0.11	5.5 ± 1.2	1083.8 ± 115.5	1039.0 ± 330.1
F_2 -value	26.95	25.45	90.59	221.47	3.35	3.99	41.66	10.18
p -value	<0.0001	<0.0001	<0.0001	<0.0001	0.040	0.022	<0.0001	0.0001

a total time of 58 min. The pump flow rate was 1 mL min^{-1} , and the suppressor was set to 125 mV with an external water flow of 15 mL min^{-1} through the suppressor. We used On Guard II combination Ba^{2+} , Ag^+ , and H^+ cartridges to remove large portions of anions (specifically chloride and sulfate) in the seawater solution that would normally mask the detection of other anions. Recoveries of each analyte were calculated based on the retention efficiencies of each when run through the salt-removal cartridges in a seawater matrix. Standards containing known quantities of 10 different OA and two anions were made for: lactic acid, acetic acid, propionic acid, formic acid, succinic acid, malic acid, maleic acid, fumaric acid, oxalic acid, citric acid, fluoride, and P_i . Tartaric and malonic acid were found to co-elute with carbonate, which was very high and variable in concentration among samples and, thus, could not be determined.

Dissolution experiments—Intact sediment cores were used to examine the release of F^- and P_i from Florida Bay sediments and how this varied with acid type and acid concentration. Sediment dissolution was investigated by injecting OA samples into 10-cm intact sediment cores using a pore-water probe. The cores were incubated at room temperature in an Instant Ocean bath at a salinity of 35 with only the top of the core exposed to the bath. An OA solution of equal parts of lactic, oxalic, and citric acids at 0, 5, 50, 500, and $5000 \mu\text{mol L}^{-1}$ total OA concentrations and an HCl solution of $1500 \mu\text{mol L}^{-1}$ (all in Instant Ocean) were injected into triplicate cores in 3-mL aliquots. Each solution was removed after 60 min using the pore-water probe, which was not removed after the initial injection. Each replicate and solution for each incubation period was injected into its own core. The removed samples were analyzed for P_i and OA as described above.

Statistical analysis—The four replicate measurements for pore-water analysis from each depth, sediment type, and site were averaged prior to subsequent analysis. Analysis of variance (ANOVA) tests were used to examine differences between sites and sediment types. p -values of <0.05 were used for significant differences and Tukey–Kramer post-tests were used to further investigate interactions between sites, sediments types, and sediment types across sites with p -values <0.05.

Results

Site characterization—Characteristics of the vegetated sediment at each site, averaged across depths and seasons, are summarized in Table 1. There was a gradient in both the sediment and pore-water P_i content in vegetated sites across Florida Bay, with the highest concentrations in the southwest (Rabbit) and lowest in the northeast (Duck; Table 1). Ammonium pore-water concentrations in vegetated sediments had the opposite trend with concentrations lowest in the southwest. The organic content of the sediments across sites was significantly different, with the highest organic content in the southwest and the lowest in the northeast.

Seagrass shoot-specific (increase in growth in mg per short shoot per day) and areal productivity (increase in growth in $\text{g m}^{-2} \text{d}^{-1}$) also varied, with the most productive sites in the southwest and the least productive in the northeast. Root biomass was greatest at a depth of 10–20 cm in the sediment at all sites. There was a difference in root and rhizome biomass across sites with highest amounts in the southwest. The root-tips cm^{-2} (actual number of root tips per sediment surface area) was calculated from the average dry weight per root, average root length, and total root dry weight, which yielded 0.16 ± 0.03 , 0.27 ± 0.04 , and 0.64 ± 0.07 individual root tips cm^{-2} for Duck, Bob Allen, and Rabbit sites, respectively.

Pore-water characterization—The pH of pore water in the vegetated and nonvegetated sediments (averaged across depths and sites) was different, with the vegetated sediments consistently having a lower pH ($F_1 = 245.45$, $p < 0.0001$; Fig. 3). The pH also was correlated with productivity, with the highest productivity site of Rabbit having the lowest pH (Fig. 3). The pH also varied seasonally with a summer pH of 7.19 ± 0.02 and a winter pH of 7.47 ± 0.02 in bulk-vegetated sediments. The pH in the root zone was not determined due to insufficient sample volume.

Fluoride concentrations in the root zone were significantly higher than both the vegetated and nonvegetated sediments (Fig. 4), which were not different from each other. Fluoride concentrations in bulk-vegetated sediments were 22% lower in the winter than in the summer (30.7 ± 0.3 and $39.2 \pm 0.6 \mu\text{mol L}^{-1}$, respectively). Sulfide

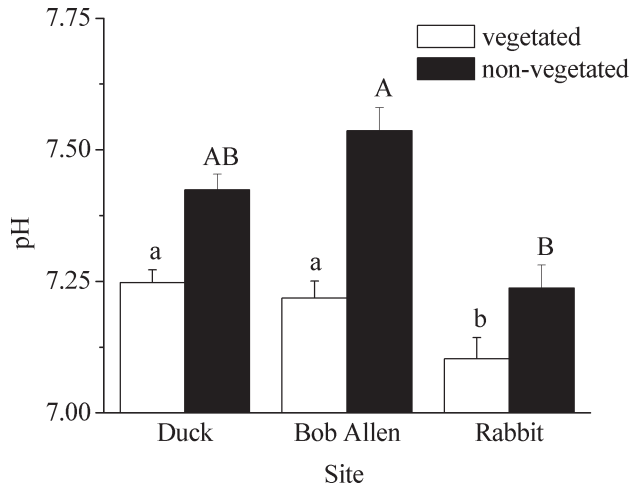


Fig. 3. The changes in pH across sites in vegetated and nonvegetated sediments. Error bars represent \pm standard errors. Letters above bars represent differences determined by ANOVA Tukey post-test with $p = 0.05$ where cases are different groups and different letters represent significant differences. (nonvegetated \times site ANOVA, $F_2 = 11.51$, $p < 0.0001$; vegetated \times site ANOVA, $F_2 = 4.62$, $p = 0.013$).

concentrations also were not significantly different between vegetated and nonvegetated sediments, but there was a large reduction in sulfides (66%) in the root zone (Fig. 5). Sulfide concentrations increased across the bay from northeast to southwest, and were consistent with increasing organic matter. Sulfide concentrations also varied seasonally with concentrations 34% lower in the winter than in the summer in vegetated sediments (347 ± 26 and $529.1 \pm 23 \mu\text{mol L}^{-1}$, respectively).

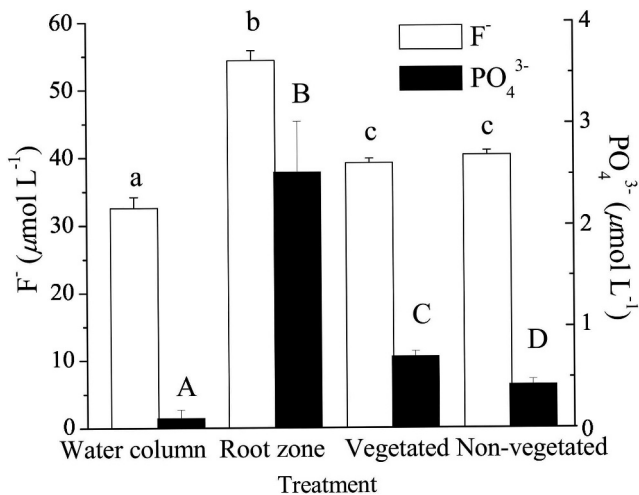


Fig. 4. The changes in F^- and PO_4^{3-} across treatments (averaged across sites). Error bars represent \pm standard errors. The correlation between these averaged F^- and PO_4^{3-} concentrations results in an R^2 of 0.97. Letters above bars represent differences determined by ANOVA Tukey post-test with $p = 0.05$ where cases are different groups and different letters represent significant differences. (F^- ANOVA, $F_3 = 21.33$, $p < 0.0001$; P_i ANOVA, $F_3 = 42.21$, $p < 0.0001$).

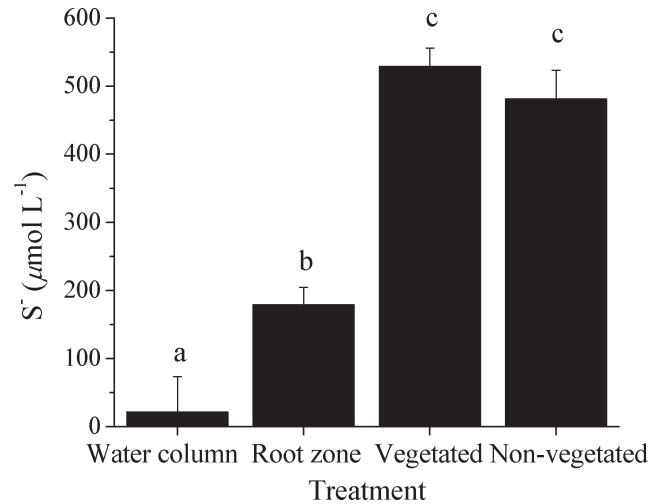


Fig. 5. The changes in S^- concentrations across treatments (averaged across sites). Error bars represent \pm standard errors. Letters above bars represent differences determined by ANOVA Tukey post-test with $p = 0.05$ where cases are different groups and different letters represent significant differences. (ANOVA, $F_3 = 12.65$, $p < 0.0001$).

Water-column P_i concentrations were consistently low across the bay with an average concentration of $0.1 \pm 0.08 \mu\text{mol L}^{-1}$. Pore-water P_i concentrations were significantly different between all sediments, with the root-zone concentrations being 4 \times and 7 \times higher than vegetated and nonvegetated bulk samplings, respectively (Fig. 4).

Organic acids—The seven-fold increase in P_i concentrations was accompanied by a seven-fold increase in

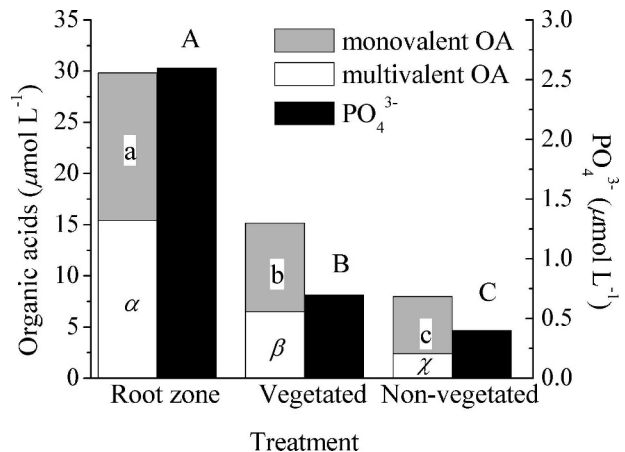


Fig. 6. The changes in multivalent and monovalent OA and PO_4^{3-} across treatments (averaged across sites). The correlation between these averaged monovalent and multivalent OA and PO_4^{3-} concentrations results in an R^2 of 0.95 and 0.96, respectively. Letters in or above bars represent differences determined by ANOVA Tukey post-test with $p = 0.05$ where cases are different groups and different letters represent significant differences. (multivalent OA \times sediment type ANOVA, $F_3 = 21.73$, $p < 0.0001$; monovalent OA \times sediment type ANOVA, $F_3 = 26.83$, $p < 0.0001$).

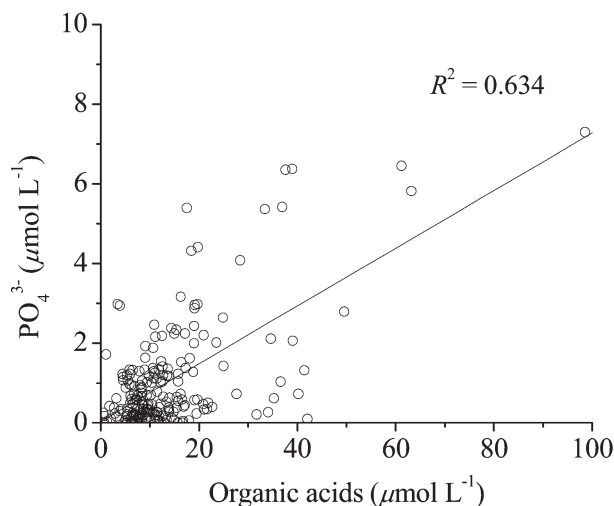


Fig. 7. Linear regression of OA concentrations vs. PO_4^{3-} concentrations for individual measurements in all treatments and sites ($n = 261$). $R^2 = 0.634$ represents a best-fit linear regression.

multivalent OA and a three-fold increase in monovalent OA (Fig. 6). A linear fit of individual samplings of P_i and total OA concentrations produced a correlation of $R^2 = 0.634$ (Fig. 7). Total OA concentrations showed a consistent trend across sites with the lowest concentrations in the bulk samples from the nonvegetated sites and the highest in the root-zone samples (Fig. 8). The average total OA concentrations in the root zone, vegetated-bulk sampling, and nonvegetated bulk sampling were 29.8 ± 1.8 , 15.5 ± 1.9 and $7.5 \pm 0.6 \mu\text{mol L}^{-1}$, respectively ($F_3 = 40.29$, $p < 0.0001$). The concentrations in the root zone were 23.9 ± 2.1 , 29.2 ± 3.1 , and $36.2 \pm 3.2 \mu\text{mol L}^{-1}$ for the sites of Duck, Bob Allen, and Rabbit, respectively, indicating a gradient from northeast to southwest (Table 2). There were significant differences in OA concentrations in the vegetated sediments across sites, with concentrations highest in the southwest, where production was also the highest. There were no differences in OA concentrations between sites for the nonvegetated sediments. The concentrations for each individual OA between the root-zone and vegetated sediments were all different ($p = 0.05$, Tukey post-test). In the vegetated and nonvegetated sediments the only OA that were different were citric and oxalic acid, which were also the multivalent OA that had the highest concentration in the root zone ($p = 0.05$, Tukey post-test). Organic acid concentrations also varied seasonally with bulk-vegetated winter concentrations 23% lower than summer concentrations (12 ± 0.7 and $15.5 \pm 1.9 \mu\text{mol L}^{-1}$, respectively) and root-zone concentrations 30% lower than summer concentrations (21 ± 3.0 and $29.8 \pm 1.8 \mu\text{mol L}^{-1}$, respectively).

Internal concentrations in seagrass tissue were higher for all OA relative to external concentrations found in the root zone (Fig. 9). On average, OA were 420× more abundant in the root material than in the root zone for all acids. Certain OA were highly conserved (meaning internal concentrations were much higher than pore-water concentrations), such as lactic, propionic, succinic, and malic acids, with internal vs. root-zone ratios of 1948, 1991, 1989, and 2003, while most multivalent acids such as maleic,

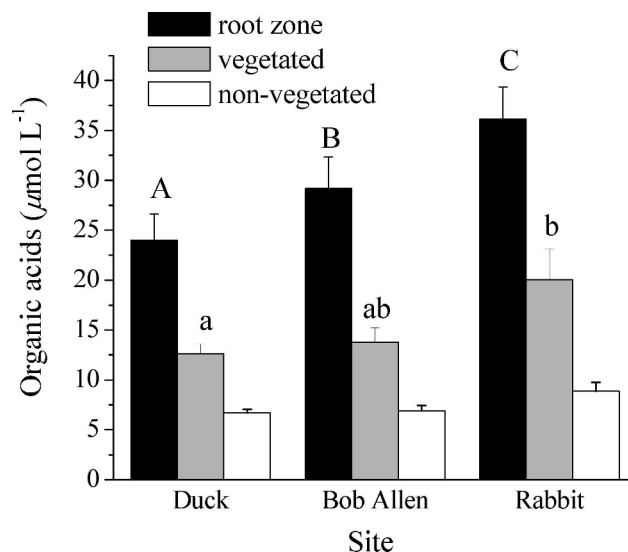


Fig. 8. The changes in total OA across sites in vegetated, nonvegetated, and root-zone sediments. Error bars represent \pm standard errors. Letters above bars represent differences determined by ANOVA Tukey post-test with $p = 0.05$ where cases are different groups and different letters represent significant differences, no letters represent no difference. (root zone \times site ANOVA, $F_2 = 9.43$, $p = 0.0006$; vegetated \times site ANOVA, $F_2 = 3.43$, $p = 0.037$; nonvegetated \times site ANOVA, $F_2 = 2.25$, $p = 0.136$).

fumaric, oxalic, and citric were less conserved with ratios of 20, 15, 17, and 10. Acetic and formic acids were also less conserved with internal vs. root-zone ratios of four and six, respectively. Monovalent OA were found in much greater concentrations than multivalent OA in plant biomass (9.2 ± 0.2 vs. $1.4 \pm 0.1 \text{ mmol L}^{-1}$, respectively). The similar concentrations of monovalent and multivalent acid concentrations in the root zone (14.4 ± 1.1 and $15.4 \pm 1.4 \mu\text{mol L}^{-1}$, respectively) indicated that monovalent OA were generally much more conserved than multivalent OA.

There were large differences in internal OA concentrations in different plant tissues, with the highest concentrations generally found in the apical meristem, followed by the root, rhizome, and leaf tissues. All individual OA, except propionic and acetic acids, were different among the different plant tissues (Fig. 10). All OA except formic (highest in leaf), fumaric (highest in root), and acetic (highest in root) were found to be greatest in the apical meristem. Monovalent OA were elevated in the leaf material due to high concentrations of lactic and formic acid (Fig. 10). Internal root concentrations of OA represented 1.55% of the total root dry weight.

Dissolution experiment—Dissolution experiments showed an increase in both P_i and F^- concentrations with increasing OA concentrations (Fig. 11). Significant increases in P_i concentrations were found at a $50 \mu\text{mol L}^{-1}$ acid concentration, which is a reasonable estimate of OA concentrations in the field. A significant increase in F^- was not seen until $500 \mu\text{mol L}^{-1}$, which is not a concentration that can be reproduced in situ. The thresholds at which significant increases in F^- and P_i were found are

Table 2. Summarization of site characteristics of root zone, vegetated, and nonvegetated samplings between all sites, sampled in June 2005 and January, June, and August 2006. Averages ($n = 3$) and standard errors (\pm values) are reported. F_2 - and p -values were determined from ANOVA tests across sites for each sampling type.

Treatment	Duck	Bob	Rabbit	Average	F_2 -value	p -value
Total organic acid ($\mu\text{mol L}^{-1}$)						
Root zone	23.9 \pm 2.1	29.2 \pm 3.1	36.2 \pm 3.2	29.8 \pm 1.8	9.43	0.0006
Vegetated	12.6 \pm 1.0	13.7 \pm 1.5	20.0 \pm 3.1	15.5 \pm 1.9	3.43	0.037
Nonvegetated	6.7 \pm 0.4	6.9 \pm 0.7	8.9 \pm 0.9	7.5 \pm 0.6	2.25	0.136
Multivalent organic acid ($\mu\text{mol L}^{-1}$)						
Root zone	11.9 \pm 2.7	14.0 \pm 1.9	20.8 \pm 2.7	15.4 \pm 2.5	3.16	0.058
Vegetated	4.3 \pm 0.7	6.3 \pm 1.3	10.8 \pm 2.2	7.1 \pm 1.7	2.47	0.090
Nonvegetated	1.8 \pm 0.3	1.8 \pm 0.4	3.0 \pm 0.6	2.2 \pm 0.4	2.07	0.134
Monovalent organic acid ($\mu\text{mol L}^{-1}$)						
Root zone	12.1 \pm 1.5	15.2 \pm 1.1	16.3 \pm 1.9	14.4 \pm 1.5	0.86	0.436
Vegetated	8.3 \pm 0.9	7.4 \pm 0.8	9.2 \pm 1.1	8.3 \pm 0.9	0.85	0.430
Nonvegetated	4.9 \pm 0.3	5.0 \pm 0.5	5.9 \pm 0.6	5.3 \pm 0.5	0.74	0.479
PO_4^{3-} ($\mu\text{mol L}^{-1}$)						
Root zone	1.4 \pm 0.65	3.3 \pm 0.39	3.0 \pm 0.56	2.6 \pm 0.5	2.83	0.086
Vegetated	0.5 \pm 0.03	0.7 \pm 0.03	0.9 \pm 0.11	0.7 \pm 0.1	3.35	0.040
Nonvegetated	0.3 \pm 0.06	0.3 \pm 0.05	0.67 \pm 0.11	0.4 \pm 0.1	5.03	0.010
F^- ($\mu\text{mol L}^{-1}$)						
Root zone	53.8 \pm 1.6	52.5 \pm 7.5	56.7 \pm 2.8	54.3 \pm 1.5	0.52	0.503
Vegetated	37.9 \pm 1.5	37.9 \pm 1.2	41.7 \pm 1.6	39.2 \pm 0.7	2.23	0.113
Nonvegetated	31.0 \pm 0.8	41.7 \pm 0.9	47.7 \pm 0.9	40.1 \pm 0.7	58.29	<0.0001
S^- ($\mu\text{mol L}^{-1}$)						
Root zone	128.0 \pm 44.3	122.5 \pm 10.2	286.6 \pm 62.8	179.0 \pm 39.1	2.8	0.045
Vegetated	418.6 \pm 50.0	445.1 \pm 128.8	723.5 \pm 126.5	529.1 \pm 27.1	11.18	<0.0001
Nonvegetated	169.8 \pm 24.0	229.3 \pm 21.7	1044.6 \pm 102.8	481.2 \pm 62.2	45.7	<0.0001
pH						
Vegetated	7.25 \pm 0.02	7.21 \pm 0.03	7.10 \pm 0.04	7.19 \pm 0.03	4.62	0.013
Nonvegetated	7.42 \pm 0.03	7.53 \pm 0.04	7.23 \pm 0.04	7.4 \pm 0.04	11.51	<0.0001

likely lower than the above values, due to the large increments between experimental OA concentrations. No significant increases in either P_i or F^- were found with the addition of HCl, in a conservative concentration compa-

table to the acidity contributed by a 500 $\mu\text{mol L}^{-1}$ trivalent OA solution.

Discussion

Sampling at plant root tips clearly indicates that seagrasses modify sediment processes through root exudation. The observed patterns of higher OA concentrations immediately adjacent to seagrass roots, compared to bulk measures in vegetated and nonvegetated sediments, and the high amounts of OA in plant below-ground biomass indicates that seagrass roots are a significant source of OA to the surrounding sediments. The increases in P_i and F^- concentrations and the reduced concentrations of sulfides in the root zone, compared to the bulk sediment also indicate that there is a unique microenvironment present around the root tips. The examination of O_2 dynamics in the root zone was originally part of our research design, but proved to be too extensive for the scope of this study. However, previous studies have found that significant amounts of O_2 are released from seagrass roots and quickly consumed in the root zone (Perderson et al. 1998; Frederiksen and Glud 2006). This root-zone microenvironment is likely mediated by the exudation of OA and O_2 from root tips and the geochemical and microbial processes that utilize them. This exudation, combined with the correlation between high OA and P_i and their correlation with high F^- concentrations, suggests

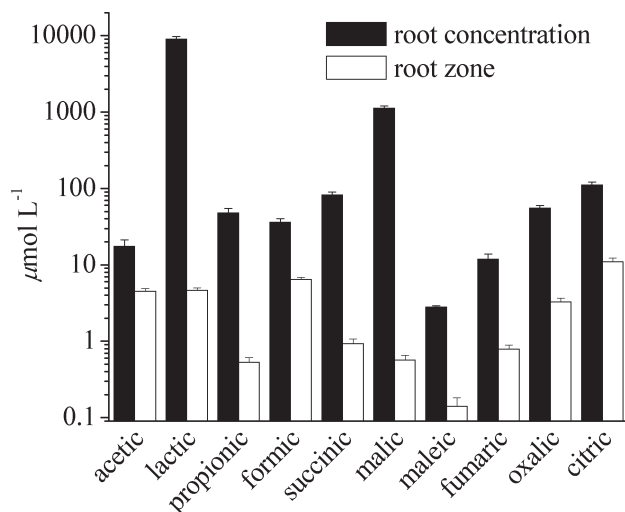


Fig. 9. The internal root concentrations vs. external root-zone concentrations of specific OA on a logarithmic scale. Internal root concentrations were determined by root extractions and external root concentrations were determined from root-zone samples. Error bars represent \pm standard errors.

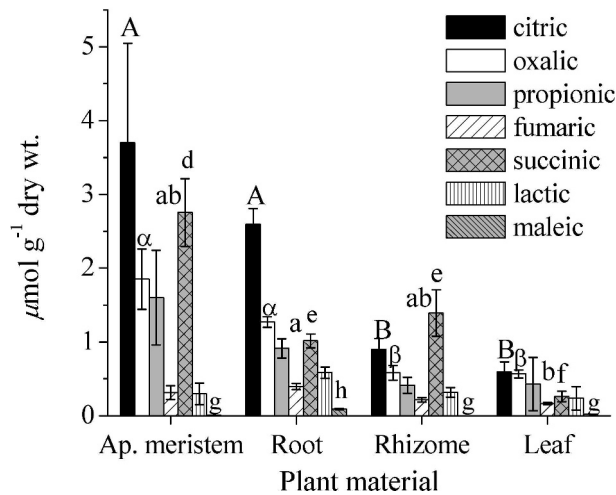
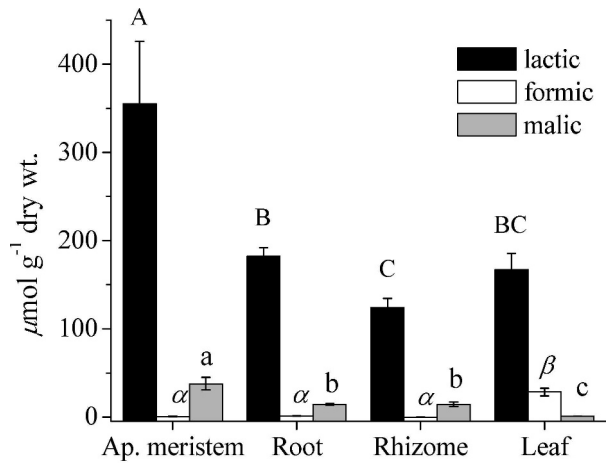


Fig. 10. Specific OA concentrations in different seagrass biomass, determined by root extractions. Notice high amounts of acetic and formic acids in the leaf materials compared to below-ground biomass. Also notice the difference in the y-axis of each panel. Error bars represent \pm standard errors. Letters above bars represent differences determined by ANOVA Tukey post-test with $p = 0.05$ where cases are different groups and different letters represent significant differences, no letters represent no difference. (lactic ANOVA, $F_3 = 13.34$, $p < 0.0001$; formic ANOVA, $F_3 = 62.32$, $p < 0.0001$; malic ANOVA, $F_3 = 27.25$, $p < 0.0001$; citric ANOVA, $F_3 = 16.39$, $p < 0.0001$; succinic ANOVA, $F_3 = 13.81$, $p < 0.0001$; oxalic ANOVA, $F_3 = 15.94$, $p < 0.0001$; propionic ANOVA, $F_3 = 2.71$, $p = 0.0508$; fumaric ANOVA, $F_3 = 4.08$, $p = 0.0095$; acetic ANOVA, $F_3 = 2.35$, $p = 0.0787$; maleic ANOVA, $F_3 = 9.75$, $p < 0.0001$).

that OA and O_2 exudation by seagrass roots is an effective mechanism for liberating P from carbonate sediments. Taken together, these results indicate that our view of carbonate sediments as a sink for reactive P must be modified to incorporate localized interactions on a millimeter scale around plant roots.

Evidence for organic acid exudation—In terrestrial plants, root OA exudation occurs at the root tip in response to

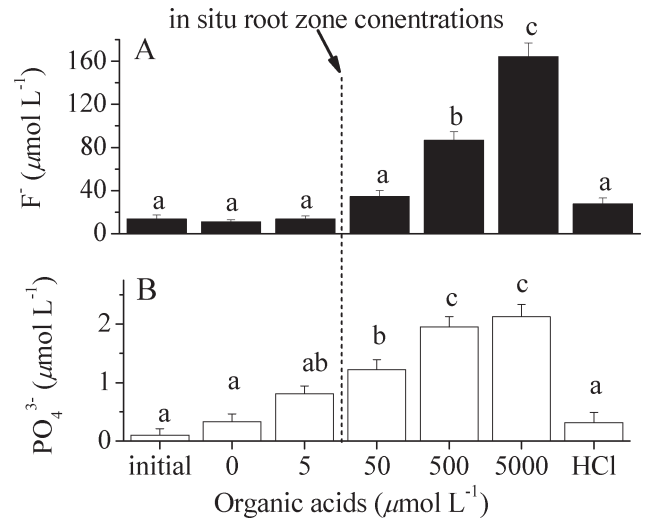


Fig. 11. (A) F^- liberated and (B) PO_4^{3-} released from sediments from increasing concentrations of OA after 60-min incubation. Initial measurements represent the pore water present in cores after incubation period. The HCl treatment is $1500 \mu\text{mol L}^{-1}$ and was used to represent the acidity contributed by a trivalent acid at $500 \mu\text{mol L}^{-1}$. The dashed line represents the actual root-zone concentrations, which were determined in situ in the root zone of *T. testudinum* in Florida Bay. Error bars represent \pm standard errors. Letters above bars represent differences determined by ANOVA Tukey post-test with $p = 0.05$ where cases are different groups and different letters represent significant differences. (F^- ANOVA $F_6 = 64.85$, $p < 0.0001$; PO_4^{3-} ANOVA $F_6 = 19.48$, $p < 0.0001$).

environmental stressors, such as P-limitation, as a mechanism for liberating P from carbonate soils (Strom et al. 2002; Jones et al. 2003). Our study provides two lines of evidence to suggest that this mechanism also exists for P-limited seagrasses growing in marine carbonate sediments. First, we found high internal OA concentrations in *T. testudinum*, especially in the apical meristem and roots where OA exudation has been shown to occur in terrestrial plants (Strom et al. 2002). Second, we found high concentrations immediately adjacent to the roots compared to the bulk samples from vegetated and nonvegetated sediments, suggesting that *T. testudinum* is actively releasing OA to the root zone. The average internal root OA concentration of 1.55% is consistent with terrestrial plants (1–4%; Jones et al. 2003) that are known to exude OA. The higher concentrations in the bulk samples from the vegetated sediments compared to the nonvegetated samples (Fig. 6) is likely due to the diffusion of OA from the root zone, and further indicates seagrasses as the main source of OA. Organic acids in the sediment typically have $t_{1/2}$ (half-life) of 1–12 h (van Hees et al. 2005), and the fact that we found them in significant concentrations in the root zone suggests that seagrass roots provide a constant supply of OA to the sediment. Our data also provide clear evidence of a link between OA exudation and seagrass metabolism. Since OA are products of photosynthesis, we would expect more productive seagrasses to support higher concentrations of OA. Indeed, we found significant increases in OA concentrations in both root-zone and bulk samples from

vegetated sediments as seagrass productivity increased across the gradient in Florida Bay, and significant seasonal variation with lower OA concentrations found in the winter when seagrasses are less productive.

Seagrasses were also expected to increase OA exudation in response to increased P-limitation. We found a significant response to P-limitation, which is evident in the ratio of OA concentrations to productivity: 36, 33, and 23 for Duck, Bob Allen, and Rabbit sites, respectively (based on root-zone OA concentrations and short-shoot-specific productivity). There were no similar increases in OA concentrations along either the P-limitation or seagrass productivity gradient in nonvegetated samples, further supporting that *T. testudinum* is the source of OA. This lack of variation in nonvegetated sediments with P or seagrass productivity gradients and the low concentrations of OA found there suggest that microbes and organic matter interactions are not significant sources of OA, but may only provide a low background concentration in the sediment pore water.

The higher concentrations of multivalent OA we observed in the root zone, compared to monovalent OA, are important for P liberation because multivalent OA are much more efficient at sediment dissolution and are excellent substrates for microbial processes (Bolan et al. 1994; Strom et al. 2002; Jones et al. 2003). The influence of multivalent OA is also seen in the correlation between increased concentrations of P_i and increased concentrations of multivalent OA. In the nonvegetated sediments, the lower concentrations of multivalent vs. monovalent OA suggests that microbial and organic matter interactions do not influence the multivalent OA pool as drastically as seagrasses do, and that microbes and organic matter mineralization are not significant sources of multivalent OA.

Internal Thalassia testudinum organic acid concentrations—Although we do not know the exact mechanisms linking OA efflux with internal OA concentrations, studies on P-limited terrestrial plants have shown a link between internal concentrations and the exudation of OA (Ryan et al. 2001). Organic acid extractions from *T. testudinum* plant material showed that high concentrations exist in root tips, compared to other plant tissues, suggesting that OA are concentrated in root structures for release. The high ratio of internal vs. pore-water OA concentrations (420) would favor their diffusion into the sediment and suggests that this may be an efficient and deliberate mechanism for the acquisition of P from the sediments. The apical meristem (root tip) tissue also has high growth rates and, thus, a high demand for P.

Monovalent acids were much more conserved than multivalent acids. This suggests that multivalent OA may be released preferentially over monovalent OA, which provides a positive feedback because multivalent OA are more effective at liberating P_i from sediments (Bolan et al. 1994; Strom et al. 2002; Jones et al. 2003). Multivalent OA concentrations have been shown to increase at the root apex (Jones 1998) and our data show that multivalent OA showed a much stronger gradient across plant materials,

Table 3. *T. testudinum* oxygen flux based on reduction of sulfides in the root zone. Delta S^- -values are differences between root-zone and bulk-vegetated sampling over the 14-d deployment and were determined over area by the number of individual root tips m^{-2} . Frederiksen and Glud (2006) values were determined from planar optodes and Burdige and Zimmerman (2002) values were calculated from bulk O_2 measures.

Site	delta S^- ($\mu\text{mol L}^{-1}$)	mmol O_2 $m^{-2} d^{-1}$
Duck	290.7	0.46
Bob Allen	322.6	0.68
Rabbit	436.9	2.00
Site average	350.0	0.96
Frederiksen and Glud (2006) on <i>Z. marina</i>		2.30
Burdige and Zimmerman (2002) on <i>T. testudinum</i> (calculated)		0.47

compared to monovalent acids, which suggests that multivalent OA are concentrated in the root for release. High concentrations of lactic acid were found throughout the plant, which can be attributed to fermentative metabolism. Why such high concentrations of lactic and other monovalent OA were not found in the root zone is unknown, but may be due to a rapid biodegradation by microorganisms (Jones 1998). The high concentrations of formic acid found in leaves may be due to the reduction of photosynthetically derived CO_2 , a mechanism described by Trezl et al. (2005), to explain high formic acid concentrations in terrestrial plant leaves.

Mechanisms of sediment dissolution—The dissolution of carbonate sediments can be mediated by a number of mechanisms including sulfur oxidation and sulfate reduction (Ku et al. 1999), organic matter mineralization (Burdige and Zimmerman 2002), and OA exudates. Although we cannot specifically quantify the magnitude of the different mechanisms by which dissolution occurs in the sediments, our data indicate that it occurs at least in part as a result of OA exudation from seagrass roots. Organic acids can function through two main mechanisms, through biotic interactions where OA stimulate the microbial processes of organic matter oxidation, fermentation, and sulfate reduction (the latter of which is tightly coupled to sulfide oxidation) resulting in the production of acidity and by reacting abiotically with carbonate minerals and organic matter to directly release P_i .

Organic matter (here OA) oxidation may represent a sink for OA due to the release of O_2 in the root zone. The oxidation of OA would produce CO_2 , and would therefore result in the acidification of the root zone and carbonate undersaturation (Burdige and Zimmerman 2002; Hu and Burdige 2007). However, our data suggests that the fate of a large fraction of O_2 in these highly sulfidic sediments is sulfide oxidation. We used the large decrease in sulfides found in the root zone to approximate the O_2 release from roots based on stoichiometric ratios, individual root tips m^{-2} and sulfide oxidation area $^{-1} d^{-1}$. This calculation yielded a daily flux of O_2 from the roots of 0.96 mmol

O_2 $\text{m}^{-2} \text{d}^{-1}$ in the vegetated sediments (Table 3), which is within the range of other studies by Frederiksen and Glud (2006) on *Zostera marina* and Burdige and Zimmerman (2002) on *T. testudinum*. Due to the diel changes in O_2 (and presumably OA release; Pedersen et al. 1998; Frederiksen and Glud 2006) and the tight coupling between sulfate reduction and sulfide oxidation, these processes would result in the net production of acidity (Burdige and Zimmerman 2002; Hu and Burdige 2007). The resulting acid produced by sulfide oxidation and sulfate reduction is likely influenced by the release of OA, because OA can provide the organic matter necessary for sulfate reduction. Fermentation may also consume OA, which would produce CO_2 , but the lack of high concentrations of the metabolic products of fermentation in our pore-water samples, such as acetic and lactic acids, suggests that this process may not be significant. However, due to diurnal fluctuations in O_2 release, these products may be quickly consumed by the sulfate reduction and organic matter oxidation. The consumption of OA by microbial processes likely occurs, and the result of this consumption is the acidification of the root zone. The magnitude of OA utilization by microbes is important to the abiotic reactions of OA with carbonate sediments (such as P_i binding-site competition and complexing Ca^{2+}), because it would reduce the amount of OA available for the abiotic mechanisms of OA sediment dissolution. The rate and amount of OA degradation by microbial processes, as well as how this varies spatially and temporally, is needed in future studies to evaluate the relative importance of the abiotic and biotic functions of OA surrounding the root tips of seagrasses.

Although OA are thought to directly increase sediment acidity by being exuded in their protonated form, the mechanisms of their release are largely unknown (Ryan et al. 2001; Jones et al. 2003). This makes the determination of their direct contribution to sediment pH difficult to verify and to separate from other acid-producing mechanisms. If OA are released in their anion form, or another anion is taken up to balance the internal root charge, their direct contribution to sediment pH may be negligible and would act mainly to fuel microbial processes, compete with P_i for binding sites, and precipitate out the end products of dissolution (Delgado and Torrent 2000; Ryan et al. 2001). If the OA is released as an acid or is exchanged across the root surface with cations, such as H^+ via H^+ -ATPases (Jones 1998), it would also act to decrease directly the pH at the point of exudation. Plants experiencing P-limitation may also increase acidity around roots because the P deficiency leads to a loss of integrity of the plant membranes. This may allow for the diffusion of OA and H^+ ions into the sediment in the fastest growing (and highest P demand) root tips (Dinkelaker et al. 1989; Strom et al. 2002). While pH was not determined in the root zone due to the low volume of individual samples, it is hypothesized that the pH would decrease immediately around the roots due to organic matter mineralization, sulfur reduction and oxidation, and the effects of OA release. Our data suggest that OA have the potential to influence local sediment pH in the root zone; however, further work is needed on the biochemical pathway of OA

release to determine accurately their direct contribution to sediment acidity.

The most important abiotic functions of OA may be to compete with P_i for binding sites on CaCO_3 minerals and organic matter and to complex dissolution end products, especially when these processes are examined on a finite scale (Ryan et al. 2001; Strom et al. 2002; Drouillon and Merckx 2003). Multivalent OA are much more efficient than monovalent OA at competing with P_i and complexing dissolution end products due to their increased number of ligand binding sites. The ability of multivalent OA to shift dissolution equilibria (through the complexing of end products) is very important in marine systems compared to terrestrial systems, as in terrestrial systems the leaching of soils results in the removal of dissolution end products such as Ca^{2+} (Kirk 1999; Delgado and Torrent 2000; Jones et al. 2003). In marine sediments where the main hydrodynamic is molecular diffusion, there is little exchange of pore water (compared to terrestrial leaching), and the complexing of dissolution end products by multivalent OA may have large implications in allowing for increased rates of dissolution through the removal of dissolution end products.

Pore-water pH and F^- changes are suggested as proxies for sediment (specifically carbonate fluoroapatite) dissolution and have been used in laboratory experiments and in the field to determine P release (Rude and Aller 1991; Jensen et al. 1998; Burdige and Zimmerman 2002). Our dissolution experiments showed that F^- was liberated from sediment cores at high OA concentrations, but that increased F^- concentrations were not present with equal H^+ additions in the form of HCl, suggesting that OA are more efficient at dissolving carbonate fluoroapatite minerals than simple changes in acidity (such as the acidity provided by OA-biotic mechanisms). Our laboratory experiments show that carbonate sediments rapidly buffer the acidity changes and that this is likely done by the most easily dissolved material such as CaCO_3 , and not the more recalcitrant F^- - and P_i -containing carbonate apatite minerals. We believe that F^- concentrations may represent the dissolution of apatite minerals, but additional P_i is also released from the dissolution of other carbonate minerals that have more loosely bound P_i and do not contain F^- . However, the elevated F^- concentrations we found in the root zone suggest that a significant amount of carbonate dissolution must be occurring in the root-tip microenvironment to dissolve the more recalcitrant F^- -containing species.

The importance of sampling on small spatial scales is critical to the study of plant-mediated sediment dissolution via OA and O_2 exudation, because bulk sediment measures do not capture the observed fluctuations caused by the rapid reaction and consumption of OA and O_2 in the sediments surrounding seagrass roots. Our data show that OA exudates from *T. testudinum* represent a newly described and significant mechanism that can increase sediment dissolution and available P_i concentrations, turning carbonate sediments into sources rather than sinks of P_i to P-limited seagrasses. The abiotic reactions of OA with carbonate minerals and the microbial mechanisms

that are fueled by OA have the potential to be very important for the acquisition of P by seagrasses inhabiting carbonate sediments. The question still remains, however, of why seagrasses are typically P-limited in carbonate sediments if they can mobilize sediment-bound P. We believe that this is due to either the limited availability of dissolved P_i at the plant surface, relative to plant demand, or to energetic constraints on the release of OA and O_2 from the plant roots. More investigation into the consumption of OA by microbes, the biochemical pathway of OA release and the energetic expense of exudation to *T. testudinum* is needed to elucidate the mechanisms that govern OA exudation, OA degradation, and P availability in marine carbonate sediments.

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