

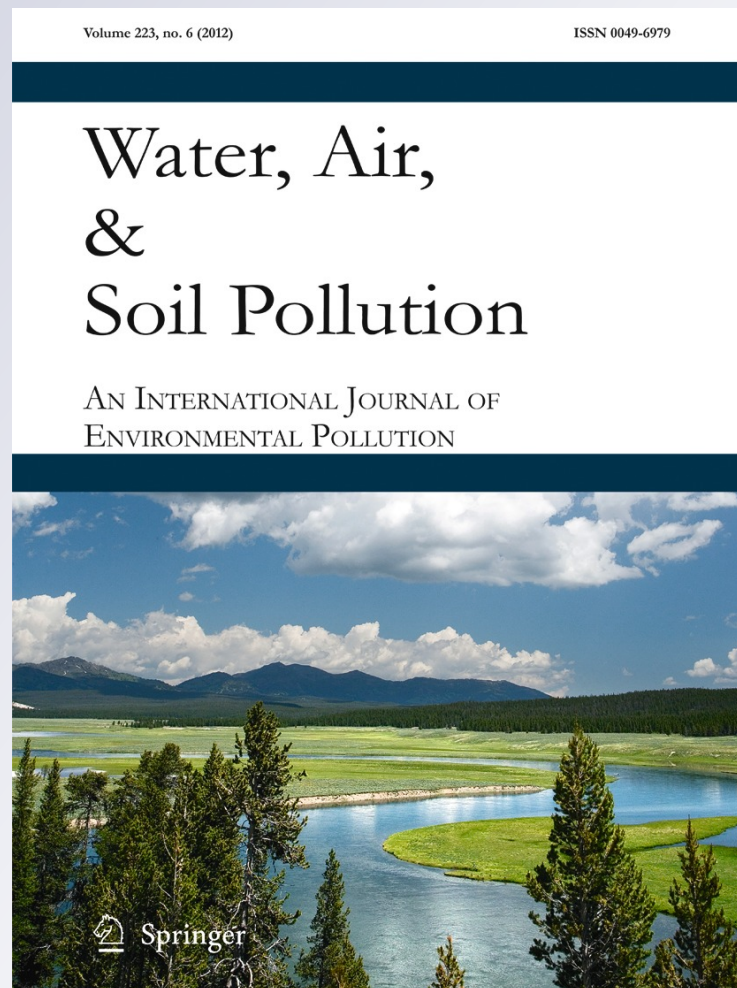
# *Potential for Phosphate Mitigation from Agricultural Runoff by Three Aquatic Macrophytes*

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# Potential for Phosphate Mitigation from Agricultural Runoff by Three Aquatic Macrophytes

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**Abstract** Phosphate from agricultural runoff is a major contributor to eutrophication in aquatic systems. Vegetated drainage ditches lining agricultural fields have been investigated for their potential to mitigate runoff, acting similarly to a wetland as they filter contaminants. It is hypothesized that some aquatic macrophytes will be more effective at removing phosphate than others. In a mesocosm study, three aquatic macrophyte species, cutgrass (*Leersia oryzoides*), cattail (*Typha latifolia*), and bur-reed (*Sparganium americanum*), were investigated for their ability to mitigate phosphate from water. Mesocosms were exposed to flowing phosphate-enriched water ( $10 \text{ mg L}^{-1}$ ) for 6 h, left stagnant for 42 h, and then flushed with non-nutrient enriched water for an additional 6 h to simulate flushing effects of a second storm event. Both *L. oryzoides* and *T. latifolia* decreased the load of dissolved phosphate (DP) in outflows by greater than 50 %, significantly more than *S. americanum*, which only decreased DP by  $15 \pm 6 \%$  ( $p \leq 0.002$ ). All treatments decreased concentrations inside mesocosms by 90 % or more after 1 week, though the decrease

occurred more rapidly in *T. latifolia* and *L. oryzoides* mesocosms. By discovering which species are better at mitigating phosphate in agricultural runoff, planning the community composition of vegetation in drainage ditches and constructed wetlands can be improved for optimal remediation results.

**Keywords** Phosphate · Phytoremediation · Mesocosms

## 1 Introduction

Eutrophication, caused by excessive concentrations of nitrogen (N) and phosphorus (P), is a major problem in aquatic ecosystems. The high nutrient concentrations can result in toxic algal blooms, low oxygen levels, fish kills, changes in macrophyte diversity, and a variety of other problems (Carpenter et al. 1998). Nonpoint source pollution is the major contributor to eutrophication in US surface waters, and of all nonpoint sources, runoff from agricultural lands is responsible for the largest input of P (Carpenter et al. 1998). Models indicate that more than 70 % of the P delivered to the Gulf of Mexico from the Mississippi River basin is from agricultural sources (Alexander et al. 2008).

Wetlands have been employed as a means to mitigate contaminated waters for several decades, and constructed wetlands have been reported to remove up to 99 % of P from effluent waters (Hammer 1992; Hey et al. 1994; Gu and Dreschel 2008). Unfortunately, the utilization of wetlands in mitigating nonpoint

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source pollution from agriculture is not always feasible, as their construction requires large areas of land to be taken out of production. An alternative to constructed wetlands are vegetated drainage ditches, which are an integral part of agricultural landscapes and a link between agricultural fields and aquatic receiving systems (Moore et al. 2001). Previous studies have demonstrated that agricultural drainage ditches have the potential to mitigate pesticides and nutrients from agricultural runoff (Moore et al. 2001, 2010; Cooper et al. 2004; Kröger et al. 2008b).

In the past, vegetation in drainage ditches was viewed as an obstruction (Ramser 1947). However, subsequent studies have demonstrated that removal of vegetation by dredging can adversely impact the ability of ditches to remove high concentrations of P from runoff (Shigaki et al. 2008). Both vegetated and unvegetated ditches are capable of reducing nutrient loads in runoff, though vegetated ditches have been shown to reduce P loads significantly more than unvegetated ones (Moore et al. 2010). It is hypothesized that plant species may differ in their ability to mitigate nutrients from agricultural runoff, either by direct uptake, adsorption to plant tissue, settlement of suspended particulates, or root zone effects that stimulate microbial communities in the sediment. Previous studies have been conducted to examine the potential of different plant species for nutrient uptake, biomass production, and/or load reductions (Reddy and De Busk 1985; Tanner 1996; Deaver et al. 2005). In the current study, three emergent aquatic macrophytes were evaluated for their ability to lower phosphate loads from a simulated storm runoff in a mesocosm experiment.

## 2 Materials and Methods

### 2.1 Experimental Setup

Mesocosms (1.25×0.6×0.8 m) were constructed outdoors in Rubbermaid tubs (1.25×0.6×0.8 m) at the US Department of Agriculture—Agricultural Research Service, National Sedimentation Laboratory in Oxford, Mississippi, 8 weeks prior to initiation of the experiment. The base of each tub was filled with 50 cm of sand, on top of which 20 cm of sediment was layered. Mesocosms were populated with one of three rooted, emergent, aquatic plant species: cutgrass

(*Leersia oryzoides*), cattails (*Typha latifolia*), or bur-reed (*Sparganium americanum*). Plant stocks and sediments were collected from the University of Mississippi Field Station (Abbeville, MS, USA). All plant species are common in agricultural drainage ditches in the Mississippi Delta and classified as obligate wetland plants for the southeast region (US Department of Agriculture 1991). Three replicate mesocosms per plant species and unvegetated sediment controls were arranged randomly.

### 2.2 Simulated Runoff

Mesocosms were dosed with orthophosphate ( $\text{PO}_4^{3-}$ ) enriched Oxford, Mississippi, municipal well water to simulate an agricultural runoff event in June of 2010. Prior to dosing, the water depth of each mesocosm was drawn down to 2/3 of the original volume in order to simulate the effect of drainage management systems commonly employed in agricultural lands in the Mississippi Delta (Kröger et al. 2008a). Phosphate-enriched water was prepared in mixing chambers using potassium phosphate dibasic to yield a  $\text{PO}_4^{3-}$  target concentration of 10 mg L<sup>-1</sup>. The target concentration was selected based on previous field observations and the need to distinguish decreases in dosed  $\text{PO}_4^{3-}$  from background concentrations in the water. Separate mixing chambers were set up for each mesocosm, and the  $\text{PO}_4^{3-}$  enriched water was pumped into individual mesocosms using Fluid Metering Inc. (FMI™) piston pumps, models QD-1 and QD-2. Water flowed through each mesocosm and exited at the surface through a discharge hose on the opposite end of the mesocosm. Pump flow rates were adjusted so that all mesocosms had a 6-h hydraulic retention time. Mesocosms were exposed to a constant flow of nutrient enriched water for 6 h, after which they were allowed to sit undisturbed for 42 h. Forty-eight hours after initiation of the  $\text{PO}_4^{3-}$  dose, mesocosms were flushed with clean water for an additional 6 h in order to simulate flushing effects of a second storm event.

### 2.3 Sample Collection and Analysis

Water samples were collected in 230 mL polyethylene cups before exposure and at 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 24, 48, 49, 51, 54, 72, and 168 h after initiation of  $\text{PO}_4^{3-}$  amendment. All water samples were analyzed to determine concentrations of dissolved

phosphate (DP), total orthophosphate (TOP), and particulate phosphate (PP). Dissolved phosphate and TOP were determined using the ascorbic acid and persulfate methods, respectively, according to standard methods (Murphy and Riley 1962; APHA 1998). All analyses were performed using a ThermoSpectronic Genesys 10 ultraviolet spectrophotometer. Particulate phosphate was calculated by subtracting DP from TOP. Water quality parameters (dissolved oxygen, temperature, pH, and conductivity) were measured in each mesocosm before the experiment and at 4, 9, 12, 24, 48, 72, and 168 h after initiation of the experiment using an Oakton pH meter and an YSI-85 multi-probe meter.

## 2.4 Statistical Analysis

Influent loads were calculated by multiplying the inflow concentration (milligrams per liter) by the volume of water passing through each mesocosm during the given time. Effluent loads were estimated by multiplying outflow concentrations by the amount of water exiting each system over associated periods of time. Percent decrease in P loads exiting mesocosms after the 6-h simulated runoff, percent of P load released from mesocosms during the clean water flush, and total percentage decrease in P loads exiting the mesocosms were calculated from the total influent loads and amount of each nutrient in the effluents over the given time frames. Percent decreases in concentration were calculated for periods when water was not flowing through the mesocosms (6 to 48 and 54 to 168 h after initiation of the experiment) when inflow and effluent loads could not be calculated. Significant differences between treatments were determined using one-way analysis of variance and Student's *t* test between individual treatments, with an alpha of 0.05.

## 3 Results

Phosphate concentrations in the mixing chambers were not significantly different across treatments, and concentrations in clean water used to flush the mesocosms were negligible in comparison to target dosage concentrations (Table S1). Thus, differences observed between mesocosms were not the result of variations in dosing or flushing. Background concentrations of DP within mesocosms prior to initiation of the experiment

were similar between all treatments ( $p=0.0731$ ), while PP and TOP were three or more times greater in *L. oryzoides* and *T. latifolia* mesocosms ( $p<0.003$ ) (Table 1). However, the amounts of PP and TOP present in mesocosms prior to initiation of the experiment were minor in comparison to the amounts loaded onto each mesocosm during dosing. Vegetated mesocosms had lower dissolved oxygen and pH values than unvegetated mesocosms, which could potentially influence phosphate removal from the water column (Table S2).

Distinct differences were observed in the macrophytes' ability to mitigate DP. During the 6-h  $\text{PO}_4^{3-}$  dosage, *L. oryzoides* and *T. latifolia* decreased DP load by greater than 50 %, significantly more than unvegetated and *S. americanum* mesocosms ( $p<0.029$ ) (Table 2). When flushed with clean water to simulate a second storm event, the amount of DP released from the mesocosms varied significantly between treatments ( $p=0.001$ ). While the DP released from *L. oryzoides* and *T. latifolia* mesocosms was  $\leq 5$  % of the total DP load, *S. americanum* and unvegetated mesocosms released  $27\pm 8$  and  $42\pm 4$  %, respectively (Table 2). By the end of the experiment, *L. oryzoides* and *T. latifolia* were the most efficient at retaining soluble P, reducing total DP loads  $51\pm 6$  and  $53\pm 6$  %, respectively (Table 2). In comparison, *S. americanum* reduced loads by  $15\pm 6$  % (Table 2). Unvegetated mesocosms were the least efficient at mitigating DP, increasing loads exiting mesocosms rather than decreasing them.

Reductions in TOP load were less pronounced than those observed in DP. The decrease in TOP loads during the 6-h  $\text{PO}_4^{3-}$  dosed runoff event ranged from  $33\pm 2$  to  $45\pm 1$  % (Table 3). Most of the TOP retained by mesocosms during the 6-h dose was released during the second simulated storm event with clean water. As a result, total reductions in TOP loads were lower than DP loads. For example, *S. americanum* mesocosms removed  $35\pm 3$  % of the TOP load during the 6-h dose but released  $32\pm 5$  % when flushed with clean water, resulting in a net decrease of only  $3\pm 5$  % of the total TOP load. The total percent decrease in TOP load at the end of the experiment was the greatest in *L. oryzoides* ( $16\pm 3$  %) and *T. latifolia* ( $18\pm 5$  %) mesocosms ( $p<0.032$ ) (Table 3). As was the case with DP, the amount of TOP exiting unvegetated mesocosms was higher than the amount in the total inflow, resulting in an increase of TOP load exiting the mesocosms. In contrast to DP and TOP, all mesocosms released

**Table 1** Background nutrient concentrations in mesocosms ( $\text{mg L}^{-1} \pm \text{SE}$ )

Nutrient	<i>S. americanum</i>	<i>L. oryzoides</i>	<i>T. latifolia</i>	Unvegetated
Dissolved phosphate	0.09±0.02	0.05±0.02	0.03±0.01	0.05±0.01
Total orthophosphate	0.88±0.05	2.93±0.74	3.70±0.33	1.02±0.17
Particulate phosphate	0.79±0.06	2.89±0.73	3.67±0.34	0.97±0.16

more PP than was delivered during the simulated runoff event, and differences between mesocosms were not significantly different ( $p > 0.232$ ) (Table 4).

*S. americanum* and unvegetated mesocosms tended to have the highest  $\text{PO}_4^{3-}$  concentration throughout the majority of the experiment, remaining high after termination of the 6-h dose and only dropping after 10–12 h (Fig. 1a, b). In contrast, concentrations in *L. oryzoides* and *T. latifolia* mesocosms tended to be lower and peak earlier, at around 6–8 h, corresponding to when dosing was completed (Fig. 1a, b).  $\text{PO}_4^{3-}$  concentrations also dropped off more rapidly in the *L. oryzoides* and *T. latifolia* mesocosms compared to *S. americanum* and unvegetated treatments. However, spikes in TOP and PP concentrations were observed in mesocosms by the 168-h time point (Fig. 1b, c). These spikes were not observed in DP concentrations, indicating they were the result of particulate resuspension during sample collection.

In order to evaluate the potential role of the different macrophyte species to mitigate phosphate from runoff during times of water stagnation (between and after storm events), percent decreases in nutrient concentrations after termination of  $\text{PO}_4^{3-}$  treatment were calculated for periods of stagnation from 6 to 48 and 51 to 168 h after initiation of the experiment. Differences in percent DP reduction between the treatments were more distinct after 48 h, with both *L. oryzoides* and *T. latifolia* significantly decreasing concentrations more than *S. americanum* and unvegetated treatments ( $p < 0.0016$ ) (Table 5). However, after 168 h, none of

the treatments differed significantly in regards to DP decrease ( $p = 0.3385$ ). Owing in part to the high variability in their values, there were no significant differences between treatments in TOP after 48 h. While decreases in TOP were different between treatments after 168 h ( $p = 0.0086$ ), unvegetated mesocosms actually had the highest percent decrease, which contrasts with DP results (Table 5). As stated above, the high variability in PP and TOP could be a result of particulates being resuspended due to agitation during sampling.

#### 4 Discussion

Agricultural drainage ditches play an important role in reducing nutrient loads and concentrations in runoff. Previous studies have demonstrated vegetation enhances rates of  $\text{PO}_4^{3-}$  removal (Silvan et al. 2004; Moore et al. 2010), and the current study confirms that sediment planted with vegetation can reduce phosphate load and concentration more than unvegetated sediment. Of the vegetated mesocosms, *L. oryzoides* and *T. latifolia* were the most efficient at lowering  $\text{PO}_4^{3-}$  loads from effluent waters. These results complement those from studies by Gu and Dreschel (2008) and Deaver et al. (2005), who found *L. oryzoides* and *T. latifolia*, respectively, performed better than other aquatic macrophytes at removing  $\text{PO}_4^{3-}$ .

In addition to enhancing the removal of nutrients from runoff during storm events, vegetation also

**Table 2** Loads (milligrams) and percent decrease of loads of dissolved phosphate entering and exiting mesocosms ( $\pm \text{SE}$ )

	<i>S. americanum</i>	<i>L. oryzoides</i>	<i>T. latifolia</i>	Unvegetated
Total inflow (mg)	1,202±36	963.1±81	798.9±170	1,017±198
0–6 h outflow (mg)	699.9±44	436.3±73	348.6±91	645.3±113
48–51 h flush outflow (mg)	320.7±95	38.09±8.5	41.41±19	426.8±104
Total outflow (mg)	1,021±58	474.4±81.51	390.0±109	1,072±210
% decrease after 6 h	42±2	55±5	57±4	36±2
% released after flush	27±8	4±0.7	5±2	42±4
Total % decrease	14±6	51±6	53±6	−6±4

**Table 3** Loads (milligrams) and percent decrease of loads of total orthophosphate entering and exiting mesocosms ( $\pm$ SE)

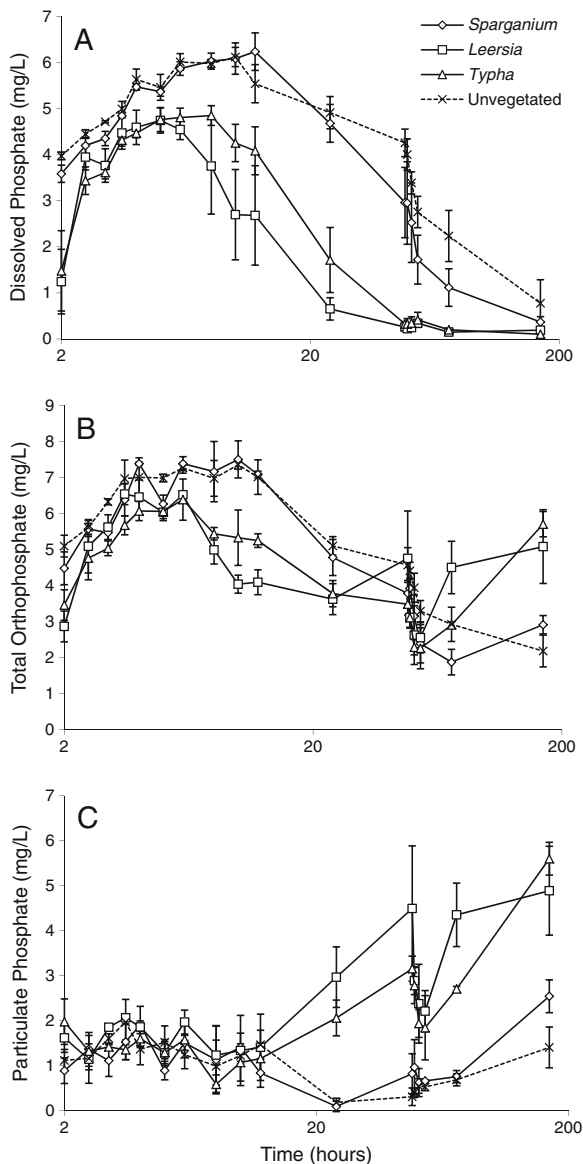
	<i>S. americanum</i>	<i>L. oryzoides</i>	<i>T. latifolia</i>	Unvegetated
Total inflow (mg)	1,346 $\pm$ 40	1,175 $\pm$ 54	926 $\pm$ 215	1,226 $\pm$ 224
0–6 h outflow (mg)	879.3 $\pm$ 57	647.8 $\pm$ 76	508.6 $\pm$ 122	816.5 $\pm$ 131
48–51 h flush outflow (mg)	429.2 $\pm$ 60	335.4 $\pm$ 42	227 $\pm$ 32	496.0 $\pm$ 118
Total outflow (mg)	1,308 $\pm$ 41	983.2 $\pm$ 60	735.6 $\pm$ 143	1,313 $\pm$ 242
% decrease after 6 h	35 $\pm$ 3	45 $\pm$ 4	45 $\pm$ 1	33 $\pm$ 2
% released after flush	32 $\pm$ 5	29 $\pm$ 5	27 $\pm$ 6	40 $\pm$ 3
Total % decrease	3 $\pm$ 5	16 $\pm$ 3	18 $\pm$ 5	-7 $\pm$ 1

influences nutrient mitigation during longer periods of low flow and stagnation. Rainfall following application of fertilizers can lead to increased concentrations of  $\text{PO}_4^{3-}$  in runoff that collects in agricultural drainage ditches. However, as the amount of time between storm events decreases, so does the concentration of DP in runoff (Sharpley 1980). It is not uncommon during the growing season in the Mississippi Delta for storm events to be separated by 2 days or less. While concentrations in runoff from a second event may be lower, any  $\text{PO}_4^{3-}$  still in the water column of drainage ditches can be flushed into downstream receiving systems by the excess runoff. In order to investigate the influence of multiple storm events on P mitigation, the current study flushed the mesocosms with water 48 h after initiation of the experiment to simulate a second storm event. *L. oryzoides* and *T. latifolia* acted more rapidly to reduce DP levels than *S. americanum* and unvegetated mesocosms. As a result, the percentage of the DP load released from *L. oryzoides* and *T. latifolia* during the second simulated storm event was much lower than the amount released by the other two treatments (Table 2), most likely due to the fact that, 48 h after initiation of the experiment, the DP concentrations in those mesocosms were still relatively high in relation to *L. oryzoides* and *T. latifolia* (Fig. 1a).

**Table 4** Loads (milligrams) and percent decrease of loads of particulate phosphate entering and exiting mesocosms ( $\pm$ SE)

	<i>S. americanum</i>	<i>L. oryzoides</i>	<i>T. latifolia</i>	Unvegetated
Total inflow (mg)	144.3 $\pm$ 29	212.2 $\pm$ 59	126.9 $\pm$ 52	209.2 $\pm$ 46
0–6 h outflow (mg)	179.4 $\pm$ 14	211.4 $\pm$ 24	160 $\pm$ 32	171 $\pm$ 18
48–51 h flush outflow (mg)	108.4 $\pm$ 37	297.3 $\pm$ 49	185.6 $\pm$ 35	69.24 $\pm$ 15
Total outflow (mg)	287.8 $\pm$ 48	508.8 $\pm$ 64	345.5 $\pm$ 55	240.5 $\pm$ 33
% decrease after 6 h	-40 $\pm$ 39	-13 $\pm$ 26	-80 $\pm$ 66	13 $\pm$ 14
% released after flush	79 $\pm$ 23	153 $\pm$ 26	267 $\pm$ 149	35 $\pm$ 8
Total % decrease	-119 $\pm$ 54	-166 $\pm$ 48	-347 $\pm$ 215	-21 $\pm$ 22

Mechanisms of P retention include physical chemical reactions such as sorption, precipitation, and sedimentation, as well as biological uptake by plants and microorganisms (Reddy et al. 1999). Dissolved  $\text{PO}_4^{3-}$  is more bioavailable for uptake by plants and microorganisms. In contrast, particulate forms of  $\text{PO}_4^{3-}$  are more likely to be removed by precipitation or sedimentation, which may be enhanced by decreased flow rates resulting from the presence of aquatic vegetation. All plant species included in the current study were rooted, emergent plants that uptake nutrients primarily from the sediments, though they still possess potential to remove nutrients from the water column. As demonstrated by Martin et al. (2003), transpiration by *T. latifolia* can increase the movement of water and dissolved nutrients into sediments, stimulating nutrient removal from the water column. *T. latifolia* has also been shown to outperform submerged aquatic macrophytes in removing dissolved  $\text{PO}_4^{3-}$  from the water column (Gu and Dreschel 2008). In addition to direct uptake, aquatic macrophytes may indirectly enhance P mitigation through the rhizosphere, the zone of soil surrounding plants roots where root exudates and oxygenation of sediments result in enhanced microbial numbers and activity (Brix 1997; Faulwetter et al. 2009). Several studies have demonstrated microbial communities



**Fig. 1** Phosphate concentrations in mesocosms. Concentrations of dissolved phosphate (a), total orthophosphate (b), and particulate phosphate (c) in mesocosms of *S. americanum*, *L. oryzoides*, *T. latifolia*, and unvegetated controls. Error bars represent standard errors

contribute to the total amount of P removed from water (McDowell and Sharpley 2003; Sharpley et al. 2007; Huang et al. 2011). Thus, microorganism in the plant rhizosphere may contribute to increased P uptake.

The data collected in the current study is a measure of the initial P mitigating potential of each plant species. Sorption and uptake by plant tissue do not remove P from the drainage system. As a result, this P can be released back into the water during plant senescence. Richardson and Marshall (1986) found that plant dieback resulted in a 5-fold increase in P flux in the water column. Thus, it is important to consider how well certain plant species can retain the nutrients they initially remove from storm runoff. Kao et al. (2003) found that while *S. americanum* accumulated the most P in its above ground biomass, most of that retained P was lost during decomposition, resulting in *S. americanum*'s net retention of P falling below that of other plant species examined. In another study, Kröger et al. (2007) found that while *L. oryzoides* plants exposed to P enriched water incorporated more P into their biomass, they also released significantly more P into the water column during senescence of above ground biomass compared to non-enriched plants. Thus, while initial removal rates are important for immediate mitigation of nutrients in runoff, plant species that retain P for longer time periods, even after decomposition, are more desirable in vegetated ditches. As the current study only looked at  $PO_4^{3-}$  concentrations in the water and not plant tissue or sediment, it is unclear which mechanisms contributed the most to P removal, or the potential for long-term P mitigation. Further studies are needed to determine how each plant species enhances P mitigation, by either direct uptake, enhancing microbial soil communities, altering the chemical conditions in soil for optimal sorption, or a combination of these mechanisms.

**Table 5** Percent decrease of DP, PP, and TOP concentrations in mesocosms after periods of stagnation ( $\pm$ SE)

	Percent decrease 48 h after initiation			Total percent decrease 168 h after initiation		
	DP	PP	TOP	DP	PP	TOP
<i>S. americanum</i>	63 $\pm$ 9	6.8 $\pm$ 46	58 $\pm$ 4	95 $\pm$ 1	-195 $\pm$ 74	67 $\pm$ 4
<i>L. oryzoides</i>	96 $\pm$ 1	-191 $\pm$ 71	49 $\pm$ 13	97 $\pm$ 0.6	-209 $\pm$ 32	45 $\pm$ 11
<i>T. latifolia</i>	96 $\pm$ 0.6	-257 $\pm$ 134	62 $\pm$ 4	99 $\pm$ 0.2	-526 $\pm$ 226	37 $\pm$ 5
Unvegetated	44 $\pm$ 4	84 $\pm$ 11	51 $\pm$ 5	90 $\pm$ 7	13 $\pm$ 20	77 $\pm$ 5

DP dissolved phosphate, PP particulate phosphate, TOP total orthophosphate



## 5 Conclusions

All aquatic macrophytes examined here were more efficient at reducing the amount of  $\text{PO}_4^{3-}$  exiting mesocosms than unvegetated controls. Of the three plant species examined, *L. oryzoides* and *T. latifolia* possess the greatest potential for mitigating  $\text{PO}_4^{3-}$  from runoff in drainage ditches, as they consistently outperformed *S. americanum*, reducing  $\text{PO}_4^{3-}$  loads to a greater extent and acting more rapidly to lower concentrations in the water column. However, a greater understanding of the role these species play in  $\text{PO}_4^{3-}$  mitigation, including which mechanisms of  $\text{PO}_4^{3-}$  removal are enhanced and the amount of  $\text{PO}_4^{3-}$  released when vegetation senesces, is needed in order to confidently incorporate them into drainage ditches for the control of  $\text{PO}_4^{3-}$  in runoff. Such information will aid efforts to develop management practices involving macrophyte communities in drainage ditches for optimal mitigation efficiency.

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