Influence of Three Aquatic Macrophytes on Mitigation of Nitrogen Species from Agricultural Runoff

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Abstract Agricultural runoff containing nitrogen fertilizer is a major contributor to eutrophication in aquatic systems. One method of decreasing amounts of nitrogen entering rivers or lakes is the transport of runoff through vegetated drainage ditches. Vegetated drainage ditches can enhance the mitigation of nutrients from runoff; however, the efficiency of nitrogen removal can vary between plant species. The efficiency of three aquatic macrophytes, cutgrass (*Leersia oryzoides*), cattail (*Typha latifolia*), and burreed (*Sparganium americanum*), to mitigate dissolved and total nitrogen from water was investigated. Replicate mesocosms of each plant species were exposed to flowing water enriched with ammonium and nitrate for 6 h, allowed to remain stagnant for 42 h, and then flushed with non-enriched water for an additional 6 h to simulate a second storm event. After termination of the final simulated runoff, all vegetated treatments lowered total nitrogen loads exiting mesocosms by greater than 50%, significantly more than unvegetated controls, which only decreased concentrations by 26.9% ($p \leq 0.0023$). *L. oryzoides* and *T. latifolia* were more efficient at lowering dissolved nitrogen, decreasing ammonium by $42 \pm 9\%$ and $59 \pm 4\%$ and nitrate by $67 \pm 6\%$ and $64 \pm 7\%$, respectively. All treatments decreased ammonium and nitrate concentrations within mesocosms by more than 86% after 1 week. However, *T. latifolia* and *L. oryzoides* absorbed nitrogen more rapidly, lowering concentrations by greater than 98% within 48 h. By determining the nitrogen mitigation efficiency of different vegetative species, plant communities in agricultural drainage ditches can be managed to significantly increase their remediation potential.

Keywords Ammonium · Nitrate · Phytoremediation · Mesocosms

1 Introduction

Agriculture is one of the largest contributors of nonpoint source pollution to US surface waters (Carpenter et al. 1998). As the world population continues to grow, agriculture is becoming increasingly dependent on the use of nitrogen (N) fertilizers to sustain crops. As only a portion of applied N fertilizer is incorporated by crops, increased application has resulted in higher amounts of ammonium (NH_4) and nitrate (NO_3^-) leached from soils during storm events and carried away in agricultural runoff (Tilman 1999; Prakasa Rao and Puttanna 2000). These high N concentrations contribute to increased eutrophication in downstream aquatic ecosystems and hypoxic zones in the Gulf of Mexico (Carpenter et al. 1998). Thus, greater attention is being paid to

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developing best management practices to minimize the amount of nutrients leaving agricultural lands.

Constructed wetland treatment systems are known to efficiently decrease a variety of pollutants from contaminated waters (Hammer 1992). Numerous studies have demonstrated treatment wetlands were effective at lowering the amounts of N from wastewater (Gersberg et al. 1983, 1986; Hey et al. 1994; Braskerud 2002). However, constructed wetlands also require large areas of land that would remove land from potential crop production. In lieu of constructed wetlands, agricultural drainage ditches have been investigated for their potential to mitigate pollutants from agricultural runoff. Drainage ditches are already present in many agricultural settings, lining fields to channel runoff away from crops. Ditches are well placed to mitigate contaminants in a manner similar to a constructed wetland, filtering excess nutrients from runoff before exiting into downstream receiving systems. Previous studies have demonstrated that agricultural drainage ditches have the potential to mitigate pesticides from agricultural runoff (Moore et al. 2001, 2008, 2011 Cooper et al. 2004). More recently, studies have demonstrated a potential for ditches to decrease N loads exiting agricultural systems (Kröger et al. 2007; Moore et al. 2010).

Aquatic vegetation can improve the removal efficiency of N from water both directly and indirectly. In the former instance, vegetation lowers N loads by direct uptake and incorporation into plant biomass (Hoagland et al. 2001; Silvan et al. 2004). In the latter, aquatic vegetation can also provide ideal environments for microbial nitrification [conversion of ammonium (NH_4) to NO_3^-] and denitrification [conversion of NO_3^- to nitrogen gas (N_2)]. The presence of vegetation in the water column provides surfaces for bacterial attachment and biofilm formation, where nitrification and denitrification can occur (Eriksson and Weisner 1997, 1999; Bastviken et al. 2003). In water-saturated soil, plants also create an aerobic environment in the area around the roots, known as the rhizosphere, while the surrounding soil remains largely anaerobic. As nitrification is an aerobic process and denitrification is anaerobic, this combination of aerobic and anaerobic soils provides an ideal environment for the mitigation of nitrogenous compounds (Hammer 1992). Plants can also contribute organic carbon to soil, which is also important for denitrification (Burford and Bremner 1975; Craft et al. 1988).

Given the number of ways aquatic vegetation can enhance the removal of N from the water, it is likely that some plant species are more effective at mitigating NH_4 and NO_3^- than others. Therefore, when planning the use of vegetated ditches for nutrient mitigation, a greater knowledge of individual plant species' capabilities to process NH_4 and NO_3^- is needed in order to select and maintain macrophyte communities for optimal performance. In the current study, three emergent aquatic macrophytes were evaluated for their ability to lower NH_4 and NO_3^- loads from a simulated agricultural runoff in a mesocosm experiment.

## 2 Materials and Methods

2.1 Experimental Setup

Mesocosms were constructed outdoors in Rubbermaid™ tubs ($1.25 \times 0.6 \times 0.8$ m) at the US Department of Agriculture (USDA)–Agricultural Research Service (ARS), National Sedimentation Laboratory (NSL) in Oxford, MS, 8 weeks prior to initiation of the experiment, as depicted in Fig. 1. The base of each tub was filled with 22 cm of sand, on top of which, 16 cm of sediment (Lexington silt loam) was layered. Mesocosms were populated with one of three rooted, emergent, aquatic plant species: cutgrass (*Leersia oryzoides*), cattail (*Typha latifolia*), or bur-reed (*Sparganium americanum*). Plant stocks and sediments were collected from the University of Mississippi Field Station, Abbeville, MS. All three plant species are common in agricultural drainage ditches in the Mississippi River alluvial plain and are classified as obligate wetland plants for the southeast region (US Department of Agriculture). Three replicate mesocosms per plant species and three replicate unvegetated sediment controls were arranged randomly.

2.2 Simulated Runoff

Mesocosms were dosed with NH_4- and NO_3^--enriched Oxford, MS, well water in June 2010 to simulate an agricultural runoff event. The water depth in each mesocosm was reduced to two third of the original volume prior to dosing in order to simulate the effect of controlled drainage systems commonly used in the Mississippi Delta (Kröger et al. 2008). Nitrogen-

Fig. 1 Diagram of meso-cosm setup
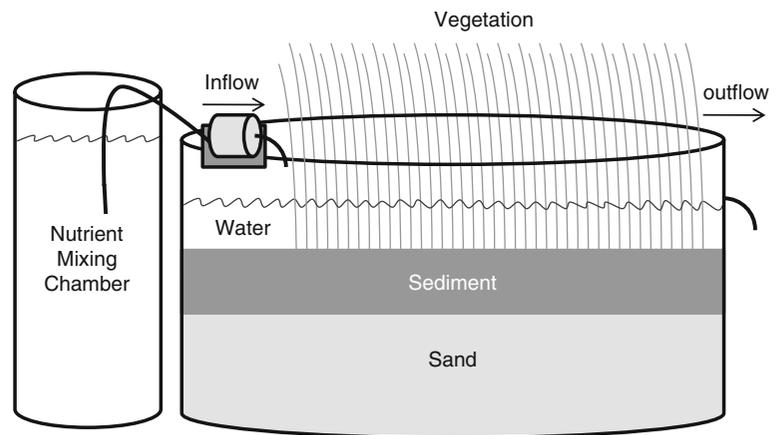
enriched water was prepared in 208-L drum mixing chambers with Oxford, MS, municipal well water using ammonium sulfate and sodium nitrate (Fisher Scientific) to yield target NH_4 and NO_3^- concentrations of 0.6 and 10 mg L^{-1}, respectively. Target concentrations were selected in order to distinguish decreases in NH_4 and NO_3^- from background concentrations found in municipal well water. N-enriched water was pumped into individual mesocosms using Fluid Metering Inc. (FMI[TM]) piston pumps, models QD-1 and QD-2 connected with 0.95 cm (o.d.)× 0.64 cm (i.d.) vinyl tubing. Water traveled through each mesocosm, exiting at the surface through a discharge hose (0.95×0.64 cm) at 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 flowing N-enriched water for 6 h, allowed to remain stagnant for 42 h, then exposed to flowing clean water for an additional 6 h to simulate flushing effects of a second storm event (Fig. 2).

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 N amendment from an outflow tube at the opposite

end from the inflow. During still phases when water was not being pumped, the samples were collected by dipping cups inside the tubs. Samples were also collected from each of the 12 mixing chambers immediately prior to initiation of the experiment to confirm target concentrations and that no differences were present in the amounts of NH_4 and NO_3^- delivered to each treatment. All water samples were analyzed in the Water Quality Laboratory of the USDA–ARS NSL to determine concentrations of NH_4, NO_3^-, total Kjeldahl nitrogen (TKN), total nitrogen (TN), and total organic carbon (TOC). The cadmium reduction method was used to analyze NO_3^-, whereas NH_4 was analyzed using the phenate method according to standard methods (APHA 1998). Nitrate and NH_4 analyses were performed using a ThermoSpectronic Genesys 10 ultraviolet spectrophotometer. TKN concentrations were determined as described by Moore et al. (2010) and TN was calculated as the sum of TKN and NO_3^- concentrations. TOC was determined by using standard methods (APHA 1998). Water quality parameters [dissolved oxygen (DO), 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 a YSI™-85 multi-probe meter. All water quality measurements were determined by 8:30 a.

Fig. 2 Timeline of meso-cosm exposures
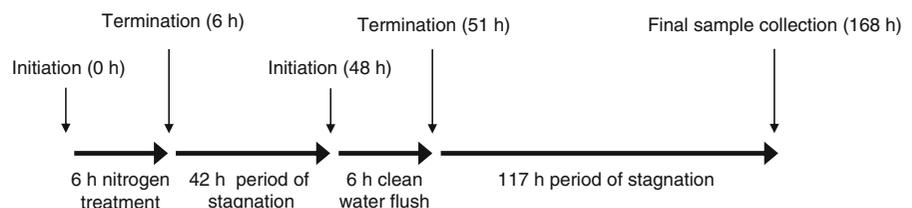
m., with the exception of the 4, 9, and 12-h samplings, which were measured at 12:00, 5:00, and 8:00 p.m.

Influent loads were calculated by multiplying the inflow concentration (in milligrams per liter) by the FMI pump rate for each mesocosm during the given time. Effluent loads were estimated by multiplying outflow concentrations by the amount of water exiting each tub over associated periods of time. Percent decrease in nutrient loads exiting mesocosms after the 6-h simulated runoff, percent of nutrient load released from mesocosms during the clean water flush, and total percentage decrease in N loads exiting the mesocosms were calculated from the total influent loads and amount of each N species in the effluent over the given time frames. In order to evaluate the potential of vegetation to mitigate N species from the water column during times of stagnation, percent decreases in concentration were calculated for periods when water was not flowing through the mesocosms (6–48 h and 54 to 168 h after initiation of the experiment) when inflow and effluent loads could not be calculated. Significant differences in effluent N loads between replicates of each treatment were determined using analysis of variance and Student's t test between individual treatments, with an alpha level of 0.05.

3 Results

Nitrogen concentrations in the mixing chambers used to dose the mesocosms confirmed that there were no significant differences in the amount of NH_4 or NO_3^- delivered to each mesocosm ($p \geq 0.9066$). Concentrations in the clean water used to flush the mesocosms from 48 to 51 h were low relative to target nutrient concentrations in amendments, with concentrations of 0.8 and 0.001 mg L^{-1} NO_3^- and NH_4, respectively. Background concentrations of NO_3^- and NH_4 in mesocosms were also negligible, less than 0.006 and 0.025 mg L^{-1}, respectively, and they did not differ significantly between treatments ($p \geq 0.1568$). However, there were notable variations in DO between mesocosms. DO levels in *L. oryzoides* and *T. latifolia* fell to ≤ 1.5 mg L^{-1} several times during the course of the experiment, at 0, 12, 24, 72, and 168 h, while levels in unvegetated mesocosms never dropped below 4.7 (Table 1).

By the end of the experiment, all vegetated mesocosms decreased TN loads by greater than 50%,

Table 1 pH and dissolved oxygen measurements in mesocosms during experiment (±SE)

Time (h)	Vegetation	pH	Dissolved oxygen (mg L^{-1})
0	*S. americanum*	5.7±0.1	2.51±0.22
	L. oryzoides	5.7±0.1	0.52±0.16
	T. latifolia	5.6±0.0	0.90±0.33
	Unvegetated	5.8±0.0	4.72±0.52
4	*S. americanum*	6.6±0.1	6.38±0.87
	L. oryzoides	6.3±0.1	3.36±0.51
	T. latifolia	6.4±0.2	8.58±0.81
	Unvegetated	7.6±0.0	9.97±0.03
9	*S. americanum*	6.6±0.1	5.95±0.81
	L. oryzoides	5.9±0.2	1.52±0.74
	T. latifolia	6.1±0.3	5.87±0.53
	Unvegetated	8.1±0.1	12.09±0.66
12	*S. americanum*	5.8±0.3	1.48±0.32
	L. oryzoides	5.8±0.4	0.26±0.13
	T. latifolia	5.8±0.1	1.43±0.18
	Unvegetated	6.5±0.2	6.95±0.88
24	*S. americanum*	5.8±0.2	1.39±0.18
	L. oryzoides	5.9±0.1	0.63±0.31
	T. latifolia	5.9±0.1	1.52±0.24
	Unvegetated	6.7±0.1	5.13±1.10
48	*S. americanum*	6.7±0.2	8.38±1.39
	L. oryzoides	6.2±0.2	3.14±0.94
	T. latifolia	6.8±0.3	10.79±0.36
	Unvegetated	8.7±0.2	12.77±1.01
72	*S. americanum*	6.0±0.2	1.31±0.06
	L. oryzoides	6.0±0.2	0.33±0.09
	T. latifolia	6.1±0.1	0.72±0.08
	Unvegetated	6.6±0.3	5.40±0.82
168	*S. americanum*	6.4±0.2	1.57±0.77
	L. oryzoides	6.3±0.1	0.07±0.01
	T. latifolia	6.4±0.0	1.37±0.45
	Unvegetated	6.5±0.0	5.21±0.78

significantly more than unvegetated mesocosms, which only decreased loads by 26.9% ($p \leq 0.0023$) (Table 2). Vegetated mesocosms also decreased NH_4 loads significantly more than unvegetated mesocosms ($p \leq 0.0022$), which actually increased the amount of NH_4 exiting mesocosms compared to inflow loads (Table 3). *T. latifolia* mesocosms removed the most NH_4, decreasing loads by 59±4%, significantly more than *S. americanum* mesocosms, which only decreased NH_4 by 33.7±4% ($p=0.0371$). In regard to

Table 2 Loads and percent decrease of loads of total nitrogen entering and exiting mesocosms (±SE)

	S. americanum	L. oryzoides	T. latifolia	Unvegetated
Total inflow (mg)	2,885.4±176.78	2,603.24±121.82	1,919.37±339.24	2,633.96±520.77
0–6 h outflow (mg)	1,078.34±123.31	821.21±107.74	612.22±147.55	1,118.58±163.43
48–51 h flush outflow (mg)	367.9±36.31	151.52±28.64	105.2±5.43	785.73±198.46
Total outflow (mg)	1,446.25±159.62	972.74±115.72	717.42±142.13	1,904.31±356.47
% decrease after 6 h	62.88±2.06	68.64±3.23	69.03±2.62	56.39±3.16
% released after flush	12.69±0.49	5.89±1.2	6.05±1.62	29.49±4.33
Total % decrease	50.19±2.55	62.75±3.73	62.97±1.03	26.91±5.9

NO_3^-, only *L. oryzoides* and *T. latifolia* differed significantly from unvegetated mesocosms ($p \leq 0.0096$), decreasing the total loads exiting the system by 67±6% and 64±7%, respectively, compared to decreases of 45±2% and 29±11% by *S. americanum* and unvegetated mesocosms, respectively (Table 4).

The amount of each N species released during the flush with clean water greatly influenced the mitigation potential of mesocosm treatments. All mesocosms decreased TN loads greater than 55% during the 6-h dosage (Table 2). However, vegetated and unvegetated treatments differed greatly in the amount of TN released during the 48- to 5-h flush ($p \leq 0.0049$). Unvegetated mesocosms released 29.5% of TN loads, while all vegetated mesocosms released less than 13% during this time frame (Table 2). As a result, unvegetated mesocosms only decreased total TN loads by 26.9%, significantly less than vegetated treatments ($p \leq 0.0023$), which all lowered TN loads by greater than 50% by the end of the simulated runoff events (Table 2). A similar trend was observed with NO_3^- and NH_4 loads. While all mesocosms reduced NO_3^- loads by 61% to 70% during the 6-h dosage, differences between treatments during the flush with freshwater resulted in greater variation in total NO_3^- load reductions between treatments by the

conclusion of the experiment. *L. oryzoides* and *T. latifolia* mesocosms only released 3±1% of their NO_3^- loads when flushed with freshwater, while *S. americanum* and unvegetated mesocosms released 18±0.2% and 33±8%, respectively (Table 4). As a result, only *L. oryzoides* and *T. latifolia* NO_3^- load reductions remained greater than 60% by the end of the experiment. When flushed with clean water, all vegetated mesocosms released less than 10% of the NH_4 load, significantly less than unvegetated mesocosms, which released 39% ($p < 0.0001$) (Table 3). Thus, all three species of vegetation are capable of retaining N species after initial exposure and minimizing the amounts released from sediments and biomass into overlying water during subsequent storm events.

In regard to concentrations in the water column, both NH_4 and NO_3^- decreased earliest and most rapidly in *L. oryzoides* and *T. latifolia* mesocosms (Fig. 3a, b). By 48 h, concentrations in *L. oryzoides* and *T. latifolia* mesocosms were more than 30-fold lower than unvegetated mesocosms. NH_4 and NO_3^- concentrations in *S. americanum* mesocosms were also more than 25-fold higher than *L. oryzoides* and *T. latifolia* mesocosms. However, after 1 week, neither NH_4 nor NO_3^- concentrations differed significantly

Table 3 Loads and percent decrease of loads of ammonium entering and exiting mesocosms (±SE)

	S. americanum	L. oryzoides	T. latifolia	Unvegetated
Total inflow (mg)	88.83±8.96	74.97±10.15	58.99±9.86	76.96±18.03
0–6 h outflow (mg)	51.99±7.19	41.6±5.13	24.72±5.95	53.36±8.94
48–51 h flush outflow (mg)	7.69±3.33	0.55±0.4	0.12±0.06	29.56±6.14
Total outflow (mg)	59.67±9.57	42.15±4.74	24.84±5.93	82.92±15.05
% decrease after 6 h	41.88±2.37	42.97±9.76	59.35±4.4	27.71±6.52
% released after flush	8.2±3.52	0.8±0.6	0.23±0.13	39.16±2.98
Total % decrease	33.68±4.46	42.17±9.46	59.12±4.28	−11.44±8.95

Table 4 Loads and percent decrease of loads of nitrate entering and exiting mesocosms (±SE)

	S. americanum	L. oryzoides	T. latifolia	Unvegetated
Total inflow (mg)	1,359.76±154.58	1,241.56±62	875.82±131.46	1,233.74±280.8
0–6 h outflow (mg)	491.35±57.98	373.14±51.57	301.53±87.35	441.38±41.01
48–51 h flush outflow (mg)	250.25±26.14	34.97±12.45	30.89±7.74	393.95±105.45
Total outflow (mg)	741.6±82.36	408.1±62.16	332.42±94.25	835.33±143.81
% decrease after 6 h	63.81±2.27	69.59±5.15	67.19±6.19	61.65±5.74
% released after flush	18.44±0.19	2.82±0.95	3.4±0.43	32.8±7.9
Total % decrease	45.37±2.4	66.77±5.87	63.79±6.51	28.84±11.45

between vegetated and unvegetated mesocosms ($p \geq$ 0.2606). While concentrations in all mesocosms were similar by the end of the experiment, the differences in concentration observed between treatments are still noteworthy, as any additional storm event could flush out N that has not yet been sorbed by plants and sediments or denitrified.

TN concentrations were more variable and less stable than NH_4 or NO_3^-, with several fluctuations occurring after nutrient amendment ended (Fig. 3c). One such fluctuation in unvegetated mesocosms at 10 h was the result of a single outlier with a concentration fivefold higher than found in the other replicates and likely the result of debris in the sample. TN concentrations in the overlying water of all the vegetated mesocosms had increased by the 168-h sampling event. The variability and spikes in TN concentration were likely due to the fact that TKN was measured from unfiltered samples that would have included particulate-bound N. As the water level in mesocosms was much lower by the end of the experiment due to evaporation, the likelihood of particulates being resuspended as a result of sampling was increased, accounting for the spike in TN at the 168-h time point.
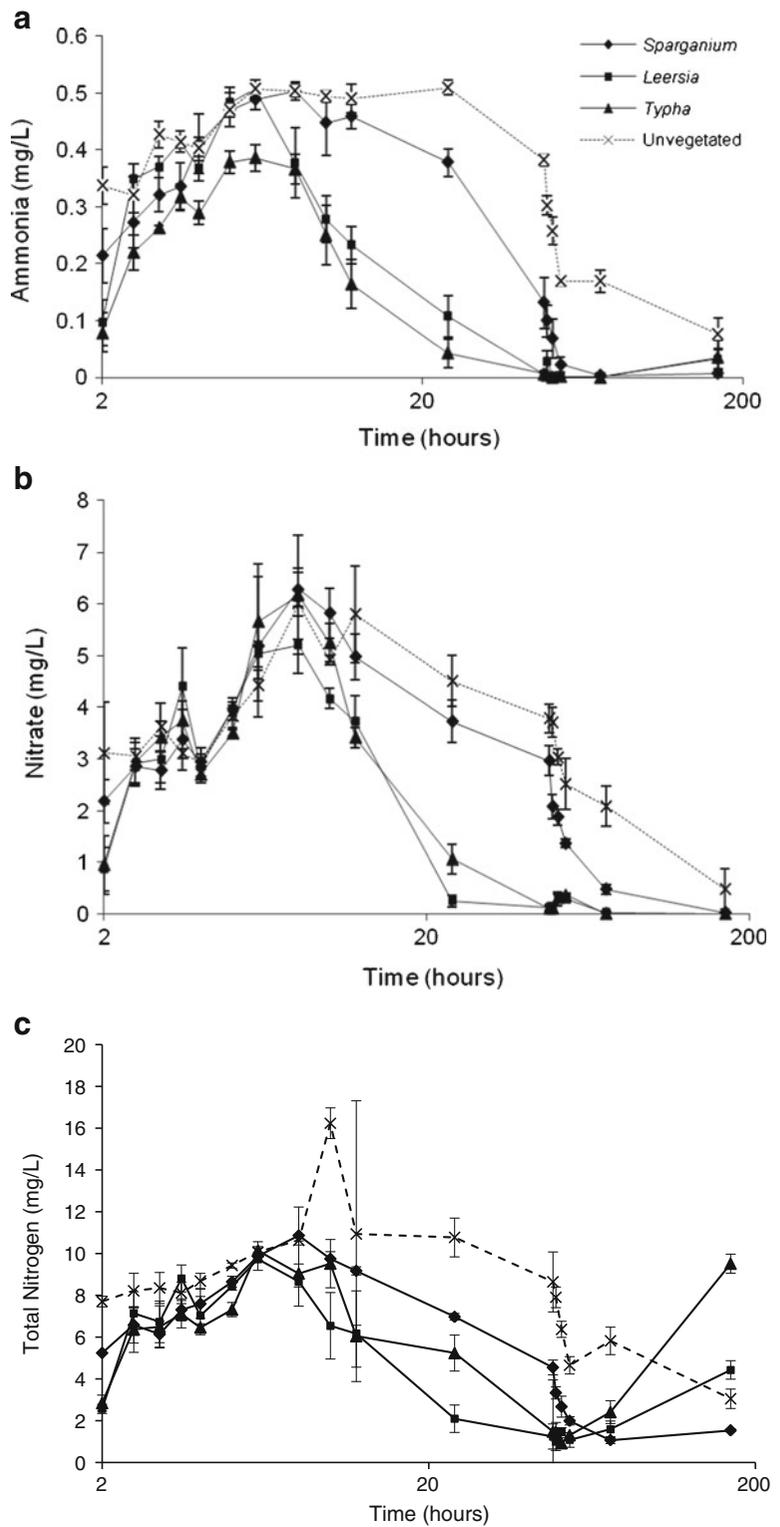
Percent decreases in both NH_4 and NO_3^- concentrations were greatest in *L. oryzoides* and *T. latifolia* mesocosms during the 42-h stagnant period between dosing and flushing with clean water ($p \leq 0.028$), while there were no significant differences in total percent decrease between treatments by the end of the experiment ($p \geq 0.3057$) (Table 5). This observation indicates that *L. oryzoides* and *T. latifolia* were more efficient at lowering NH_4 and NO_3^- from the water column than *S. americanum* or unvegetated systems. While all treatments eventually decreased NH_4 and NO_3^- concentrations to a similar extent, the more time it takes to do so provides a longer period when these

excess nutrients can be flushed out of the system by additional storm events, as demonstrated by the significantly lower NH_4 and NO_3^- loads released from *L. oryzoides* and *T. latifolia* mesocosms after flushing with clean water (Tables 2 and 3). In the case of TN, all three plant species lowered concentrations significantly more than unvegetated mesocosms after 48 h ($p <$ 0.0001). However, *L. oryzoides* and *T. latifolia* mesocosms decreased concentrations by more than 90%, significantly greater than *S. americanum* treatments, which only decreased concentrations by 76.3% after 48 h ($p \leq 0.0003$). In contrast to NH_4 and NO_3^-, decreases in TN at the end of experiment were not similar between treatments, likely due to particulates in the water column disturbed during sampling, as stated previously. Given the observations of N concentrations after 48 h, it is apparent that vegetated mesocosms are poised to react more rapidly to decrease N species than unvegetated controls.

4 Discussion

Passage through vegetated drainage ditches has the potential to decrease nutrient loads and concentrations in agricultural runoff. Several studies have observed higher rates of nutrient removal from vegetated systems compared to unvegetated ones (Gersberg et al. 1986; Rogers et al. 1991; Zhu and Sikora 1995; Tanner et al. 1999; Deaver et al. 2005). *L. oryzoides* and *T. latifolia* were the most efficient at removing NO_3^- and NH_4 loads from effluent waters, although *T. latifolia* performed slightly better at reducing NH_4. These results are in agreement with a previous study that found *T. latifolia* performed better than *Scirpus* spp. (bulrush) at removing NO_3^- (Bachand and Horne 2000). In another study, Pierce et al. (2009) observed decreases in NO_3^-

Fig. 3 Nitrogen concentrations in mesocosms. Concentrations of ammonium (**a**), nitrate (**b**), and total Kjeldahl nitrogen (**c**) in mesocosms of *S. americanum*, *L. oryzoides*, *T. latifolia*, and unvegetated controls over the time course. *Error bars* represent standard errors. X-axis displayed in log scale

concentration of greater than 80% after 48 h in stagnant *L. oryzoides* planted mesocosms, similar to the greater than 98% decreases in NO_3^- concentration observed in the current study.

Table 5 Percent decrease of ammonium (NH$_4$), nitrate (NO$_3^-$), and total nitrogen (TN) concentrations in mesocosms after periods of stagnation (±SE)

	% decrease 48 h after dosage			Total % decrease after 168 h		
	NH4	NO$_3^-$	TN	NH4	NO$_3^-$	TN
S. americanum	78.46±6.77	66.32±5.06	76.29±1.14	98.79±1.06	99.77±0.23	91.94±1.16
L. oryzoides	99.26±0.37	98.67±0.64	94.02±1.87	94.2±2.97	99.97±0.03	77.61±12.17
T. latifolia	99.1±0.9	98.87±0.82	92.38±2.44	93.39±6.16	100±0	51.57±3.47
Unvegetated	32.12±4.28	58.54±4.62	56.39±1.64	86.59±4.81	93.72±5.55	84.48±1.56

While vegetation can enhance removal of nutrients from agricultural runoff during storm events, it can also contribute to total nutrient load reductions during longer periods of low flow and stagnation. Thus, NH$_4$ and NO$_3^-$ concentrations within mesocosms were followed after termination of the simulated runoff events when the water was not flowing. Results indicate that while *S. americanum* and unvegetated mesocosms eventually lower nutrient concentrations similar to *L. oryzoides* and *T. latifolia*, they do so at a slower rate. This lag in nutrient reduction provides a longer window of time when these nutrients can be flushed out of drainage ditch systems by a subsequent storm event. In the current experiment, a second storm event was simulated by flushing mesocosms with water 48 h following the first event. Consistent with the observations of concentration data, the NH$_4$ and NO$_3^-$ loads released from *L. oryzoides* and *T. latifolia* during the flush with clean water were much lower than the amount released by the other two mesocosm treatments, most likely due to the fact that the concentrations in those mesocosm waters were still relatively high in relation to *L. oryzoides* and *T. latifolia* at the 48-h time point. These results highlight the potential importance of plant uptake and microbial activity on plant tissue and in the sediment during periods of low flow and stagnation. Therefore, knowing the rate at which different plant species lower nutrient concentrations after a storm event is pivotal when considering types of vegetation for drainage ditches. Any nutrients not sorbed and removed from the water column can be further washed out into downstream receiving systems by subsequent storm events.

There are various mechanisms by which N can be removed from the water column, including direct plant uptake or stimulation of nitrifying and denitrifying microorganisms. The extent to which each of these mechanisms contributes to N removal depends on the environmental conditions and plant species present in a given system. Pierce et al. (2009) found that both flooding and plant species could influence soil oxidation–reduction potential and N uptake in mesocosm systems. Silvan et al. (2004) attributed the high rate of NO$_3^-$ retention observed in a constructed wetland buffer to vigorous vegetation growth and colonization. Along those same lines, Hoagland et al. (2001) postulated that plant uptake only made a small contribution to N removal in another constructed wetland, due to a lack of synchronization between plant growth and tile drainage from an adjacent agricultural field.

One possible mechanism by which plants could stimulate NO$_3^-$ removal is transpiration. Martin et al. (2003) postulated that higher rates of transpiration of water from above ground plant tissues results in faster movement of water into the soil, thus increasing the transport of dissolved nutrients into soil zones where conditions are favorable for nitrification and denitrification by soil bacteria. In corroborating this idea, Martin et al. (2003) found decreased rates of *T. latifolia* transpiration led to concomitant decreases in NO$_3^-$ removal from the water column.

The mechanism of N removal utilized in a wetland can also fluctuate due to environmental and seasonal changes. Seasonal changes in nutrient mitigation can be influenced by the macrophyte community composition present in a wetland. For instance, Reddy and DeBusk (1985) found that water hyacinth had the highest rates of N removal in the summer, while pennywort had the highest rates during the winter. Therefore, maintaining a variety of different species with complementary nutrient mitigating abilities in drainage ditches may be necessary for sustaining efficiency of nutrient reductions year round.

Diversity of flora in aquatic systems can contribute greatly to nutrient mitigation. Community

composition of aquatic vegetation growing in drainage ditches can respond to changes in nutrient levels in the water column, shifting toward plant species that absorb nutrients directly from the water column as opposed to from sediments (Janse 1998). While these shifts could lead to populations in ditches capable of increased nutrient mitigation, such changes occur over the course of years. Thus, selectively planting ditches with macrophytes known to have potential to decrease nutrient concentrations from the water column could speed the process of optimizing ditches for maximum nutrient load reduction. All species included in the current study were emergent plants with root systems capable of uptaking nutrients primarily from the sediments, though they still possess potential to remove nutrients from the water column.

Even in cases where plant uptake does not play a major role in nutrient mitigation, the presence of vegetation can stimulate microbial processes. For instance, aeration of soil in the root zone enhances rates of NH_4 removal by nitrification (Reddy et al. 1990; Yang et al. 2001), which could alter rates of NO_3^- retention. Vegetation can also enhance rates of nitrification and denitrification by providing surfaces for bacterial attachment and biofilm formation (Eriksson and Weisner 1997, 1999; Bastviken et al. 2003). However, there is still little known about the role different plant species play in stimulating nitrifying and denitrifying bacterial communities. Bachand and Horne (2000) found that denitrification, rather than plant uptake, was the primary mechanism of NO_3^- removal, and that rates of denitrification varied greatly between plant treatments. However, they were unable to determine why these rates differed between plant species or the mechanisms at play. In the current study, DO levels were lowest in vegetated mesocosms, particularly *L. oryzoides* and *T. latifolia* mesocosms, which were also the most efficient at lowering NO_3^- levels. As denitrification is an anaerobic process requiring conditions of low or no oxygen (Seitzinger 1988; Korner and Zumft 1989), it is possible that these differences in DO levels between mesocosms influenced denitrification rates in vegetated treatments, contributing to their overall effectiveness at removing NO_3^- relative to unvegetated treatments. However, further experiments are needed to confirm the rates of denitrification in vegetated mesocosms with different DO levels in the soil and water column.

In conclusion, *L. oryzoides* and *T. latifolia* showed the greatest overall potential at lowering NH_4 and NO_3^- from runoff. However, further research is needed to better understand the roles these plants play in nutrient mitigation over the long term. The mechanism(s) responsible for the N mitigation by these species, the extent to which N is released when above ground vegetation senesces, and variations in their N mitigation capacity during different seasons are still unknown. With a greater understanding of the mechanism(s) and optimal conditions for mitigation by these specific aquatic macrophytes, farmers will be better prepared to manage macrophyte communities in drainage ditches for optimal mitigation efficiency year round.

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