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Contrasting Nutrient Mitigation and Denitrification Potential of Agricultural Drainage Environments with Different Emergent Aquatic Macrophytes

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Abstract

Remediation of excess nitrogen (N) in agricultural runoff can be enhanced by establishing wetland vegetation, but the role of denitrification in N removal is not well understood in drainage ditches. We quantified differences in N retention during experimental runoff events followed by stagnant periods in mesocosms planted in three different vegetation treatments: unvegetated, cutgrass [*Leersia oryzoides* (L.) Sw.], and common cattail (*Typha latifolia* L.). We also quantified denitrification rates using membrane inlet mass spectrometry from intact cores extracted from each mesocosm treatment. All treatments retained 60% or more of NO_3^-–N loads during the 6-h experimental runoff event, but mesocosms planted with cutgrass had significantly higher (68%) retention than the cattail (60%) or unvegetated (61%) treatments. After the runoff event, mesocosms planted in cattail reduced NO_3^-–N concentrations by >95% within 24 h and cutgrass achieved similar reductions within 48 h, whereas reductions in the unvegetated mesocosms were significantly less (65%). Cores from cutgrass mesocosms had significantly higher average denitrification rates (5.93 mg m^{-2} h^{-1}), accounting for as much as 56% of the immobilized NO_3^-–N within 48 h, whereas denitrification rates were minimal in cores from the unvegetated (–0.19 mg m^{-2} h^{-1}) and cattail (0.2 mg m^{-2} h^{-1}) mesocosms. Our findings have implications for mitigating excess NO_3^-–N in agricultural runoff. While vegetated treatments removed excess NO_3^-–N from the water column at similar and significantly higher rates than unvegetated treatments, the high denitrification rates observed for cutgrass highlight the potential for permanent removal of excess N from agricultural runoff in vegetated ditches and wetlands.

Significant advances in modern agriculture have increased food production capabilities to meet the growing global population demand. Intensification of fertilizer use associated with agricultural advances has also led to significant environmental impacts. In particular, losses of reactive N via leaching from soils into surface waters is an area of significant agronomic and environmental concern because inputs to freshwaters have increased and impacted water quality in downstream ecosystems, particularly coastal waters (Vitousek et al., 1997; Carpenter et al., 1998; Hoeft, 2003). For example, changes in land use and agricultural practices within the Mississippi River basin have increased N outputs during the last 200 yr and led to seasonal hypoxia in the Gulf of Mexico (Turner and Rabalais, 2003; Alexander et al., 2008). Dagg and Breed (2003) reported that 53% of the annual average dissolved N within the Mississippi River was NO_3^-. As the global population continues to increase, agriculture will become even more dependent on N fertilizers to sustain crop production. Widespread implementation of management practices designed to reduce N leaching and subsequent loading to surface waters is needed.

Within the Lower Mississippi River Valley, constructed drainage ditches provide access to productive alluvial soils and represent an integral component of the agricultural landscape (Moore et al., 2001). Agricultural ditches are designed to move surface water off agricultural lands quickly and can result in high NO_3^- loads exported to downstream ecosystems (Needelman et al., 2007; Stock et al., 2007). During runoff events, nutrient removal efficiencies, particularly for NO_3^-–N, in ditches are probably regulated by factors similar to those that inhibit efficient nutrient removal in channelized agricultural streams of the Upper Mississippi River basin, namely high nutrient inputs, low biotic interactions, and low residence times (Royer et al., 2004; Sheibley et al., 2014). However, unlike channelized agricultural streams, constructed agricultural ditches within the Lower Mississippi River Valley are very low gradient, do not maintain perennial flow, and possess many of the same key characteristics that define wetlands, including hydric soils and the ability to

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Abbreviations: DNRA, dissimilatory nitrate reduction to ammonium; DO, dissolved oxygen; MIMS, membrane inlet mass spectrometer; NSL, National Sedimentation Laboratory.

support aquatic emergent vegetation (Cooper and Moore, 2003). Wetlands provide longer residence times and more opportunities for biotic interactions in vegetated sediments. There is a large body of research on the use of constructed wetlands as a remediation tool for nutrients in wastewater and agricultural runoff, with relatively high NO_3^- removal efficiencies of 41 to 86% reported for several studies investigating horizontal-flow constructed wetlands (Fink and Mitsch, 2004, 2007; Vymazal, 2007; Kadlec, 2010). Wetland characteristics of ditches can be enhanced by increasing hydraulic residence times and holding water with flow control structures, including slotted pipes and low-head weirs, allowing greater nutrient removal (Stock et al., 2007; Kröger et al., 2011). Additionally, maintaining vegetation enhances biotic interactions to increase nutrient mitigation in agricultural ditches (Stock et al., 2007). For example, a 57% reduction in dissolved inorganic N has been reported from runoff flowing through northern Mississippi vegetated ditches during a 2-yr monitoring period (Kröger et al., 2007a). While there is growing evidence that vegetated ditches may develop a natural nutrient mitigation capacity that is potentially enhanced by flow control structures, more research on how vegetation influences different N cycling pathways within low-gradient agricultural ditches is needed (Stock et al., 2007).

Nitrogen cycling is complex, and many of the N transformations that contribute to cycling and removal of N in horizontal-flow constructed wetlands are probably at work in ditches that are managed to increase biotic interactions. Two major removal pathways for NO_3^-–N entering agricultural ditches include biological assimilation by plants, algae, and microbial biomass and microbial-mediated denitrification (Stock et al., 2007). Denitrification occurs under anaerobic conditions, where NO_3^-–N acts as the terminal electron acceptor for the oxidation of organic matter (Knowles, 1982). This process can convert significant amounts of NO_3^-–N to N_2 gas, with a variable but small fraction escaping as N_2O (Burgin and Hamilton, 2007). Dissimilatory nitrate reduction to ammonium (DNRA) may also account for significant portions of NO_3^-–N reduction in wetlands (Scott et al., 2008), but under aerobic conditions, NH_4^+–N may be rapidly nitrified back to NO_3^-–N (Burgin and Hamilton, 2007). However, anaerobic conditions can also stimulate mineralization of organic N as well as P release from sediments, potentially exacerbating nutrient impacts to downstream aquatic systems (Burgin and Hamilton, 2007; Sharpley et al., 2007).

Denitrification rates are controlled by the supply of NO_3^-–N, O_2, and organic C but are also regulated by the presence of denitrifying bacteria and environmental factors including pH and temperature (Seitzinger, 1988; Burgin and Hamilton, 2007; Vymazal, 2007). Aquatic emergent vegetation potentially provides a more stable C and N pool for maintaining denitrifying microbes at the sediment–water interface (Reddy et al., 1989; Weisner et al., 1994; Brix, 1997). Higher microbial activity associated with the breakdown of plant-derived organic matter can also maintain anoxic conditions conducive to denitrification of NO_3^-–N. Alternatively, aquatic emergent vegetation may limit sediment anoxia through adaptations for living in inundated and anaerobic sediments. Internal pressurization and convective gas transport mechanisms move O_2 from shoots to roots and increasing internal O_2 concentrations in rhizomes (Brix et al., 1992; Konnerup et al., 2011). Subsequent release of O_2 from roots into the rhizosphere may inhibit denitrification by increasing O_2 within the sediments, as it is the preferred electron acceptor for respiration of C (Knowles, 1982), or create adjacent anaerobic and aerobic sediments that enable coupled nitrification–denitrification of NH_4^+ (Reddy et al., 1989; Risgaard-Petersen and Jensen, 1997).

The primary objective of this study was to determine if denitrification varied between unvegetated sediments and those planted in one of two common emergent plant species, rice cutgrass and common cattail. While previous research has established that maintaining these two emergent species in agricultural ditches can significantly reduce nutrient export from agricultural systems, the relative role of denitrification has not been compared between these two common but functionally different species (Tyler et al., 2012). We hypothesized that nutrient retention and uptake during and after an experimental runoff event would be greater in vegetated vs. unvegetated mesocosms. Due to the potential for increased C availability and higher microbial activity that can create anoxic conditions in vegetated sediments, we hypothesized that vegetated sediments would convert more NO_3^-–N to N_2 gas through the microbial-mediated denitrification pathway than unvegetated sediments. We also hypothesized that cutgrass would have higher denitrification rates than cattail treatments because higher biomass turnover in cutgrass probably results in more stable C and N pools (Farnsworth and Meyerson, 2003). Additionally, published convective flow rates for *Typha* spp. are an order of magnitude higher than *Leersia* spp. (Brix et al., 1992; Bendix et al., 1994; Sorrell and Hawes, 2010; Konnerup et al., 2011), increasing the likelihood for reduced denitrification activity in cattail mesocosms due to oxygenated root zones.

Materials and Methods

Experimental Design

Our experimental design followed Tyler et al. (2012), where mesocosms were constructed outdoors in Rubbermaid tubs (1.25 by 0.6 by 0.8 m) at the USDA–ARS National Sedimentation Laboratory (NSL) in Oxford, MS. Mesocosms were established by filling each tub with 22 cm of sand overlaid with 16 cm of sediment (Lexington silt loam) and planted in one of three different vegetation treatments. Experimental treatments included mesocosms planted with rooted, emergent, aquatic plant species, either cutgrass or cattail, and unvegetated sediment controls (Fig. 1). Plant stocks and sediments were collected from the University of Mississippi Field Station (UMFS) located in Abbeville, MS. Representative plants and sediment were collected from Ponds 86 to 88, which are shallow, permanently inundated ponds. These ponds were chosen because UMFS records going back 25 yr indicated that no exposure to wastewater or agricultural effluents had occurred. Plants were hand collected in early spring, with careful attention paid to collecting intact root systems and minimizing stress during transplantation. Plants were evenly distributed randomly across three replicate mesocosms per treatment. Mesocosms were allowed to equilibrate for 12 mo to establish plant communities and presumably establish detrital and microbial resources within the benthos before initiating the experiment. Inundation of

Fig. 1. Experimental mesocosms under different vegetation treatments. From left to right: unvegetated control, cutgrass [*Leersia oryzoides* (L.) Sw.], and cattail (*Typha latifolia* L.). Each treatment had three total replicate mesocosms.

mesocosms was maintained at depths of approximately 15 cm by rain and supplemental watering with Oxford municipal well water throughout the equilibration period. Before starting the experiment, plant stem counts were done for the entire mesocosm and were 0, 622 ± 44, and 40 ± 3 m^{-2} for control, cutgrass, and cattail treatments, respectively.

Experimental Runoff Event

We simulated an agricultural runoff event by dosing mesocosms with NO_3^-–N and PO_4–P enriched Oxford municipal well water in June 2014. The average depth between the sediment layer and outflow was 17 ± 0.5, 13.3 ± 1.3, and 15.2 ± 0.5 cm for the control, cutgrass, and cattail treatments, respectively. To simulate the effect of controlled drainage systems commonly used in the Mississippi Delta (Kröger et al., 2008), the water depth of each mesocosm was drawn down to two-thirds of the original standing water volume before dosing. We enriched Oxford municipal well water with sodium nitrate and potassium phosphate monobasic (Fisher Scientific) to yield target NO_3^-–N and PO_4–P concentrations of 6 and 1 mg L^{-1}, respectively. Target concentrations were similar to the observed mean concentrations in small agricultural streams and ditches within the Mississippi Delta (Shields et al., 2009) and within the range observed in storm runoff sampling currently being conducted in delta agricultural ditches (USDA–ARS, unpublished data, 2008–2011). We pumped nutrient-enriched water into individual mesocosms using Fluid Metering Inc. (FMI) piston pumps, Models QD-1 and QD-2, connected with 0.95-cm (o.d.) by 0.64-cm (i.d.) vinyl tubing. Pump flow rates (mean ± SE) were adjusted so that hydraulic retention times of the inflow water in each mesocosm were within a time frame that minimizes the impact of reduced drainage on working farms (6 h) before exiting at the surface through a discharge hose (0.95 by 0.64 cm) at the opposite end of the mesocosm. Mesocosms were exposed to flowing, nutrient-enriched water for 6 h.

Sample Collection and Analysis

Water samples were collected in 230-mL polyethylene cups before initiation of the runoff event, at first outflow (99 ± 4 min), and at 2.5, 3, 4, 5, and 6 h after its initiation. Runoff samples were collected from the discharge hose. Pre-runoff sampling and continued sampling after the runoff event at 9, 12, 24, 48, 72, and 168 h occurred by dipping sample cups inside the tubs near the outflow. Evaporation was minimal during the sampling period, and water removal via sampling was negligible (1–1.6% of the total volume). Samples were also collected from each of the nine mixing chambers to confirm target concentrations and equivalent delivery of NO_3^-–N and PO_4–P to each treatment, as well as to calculate inflow loads of each nutrient. USDA–ARS NSL water quality laboratories were used to analyze all water samples for concentrations of dissolved nutrients. The NH_4^+–N, NO_3^-–N, and PO_4–P concentrations were determined after filtration (0.45 μm) using the phenate, Cd reduction, and molybdate methods, respectively (American Public Health Association, 1998). All aqueous nutrient samples were stored frozen until they could be run on a Lachat QuickChem 8599 autoanalyzer (Lachat Instruments). Water quality parameters including dissolved oxygen (DO), temperature, pH, and conductivity were measured in each mesocosm before the experiment and at multiple time intervals throughout the day after initiation of the experiment using an Oakton pH meter and a YSI 85 multiprobe meter. At the beginning and end of the experiment we collected surface sediment samples and plant tissue samples from each mesocosm. Aboveground plant tissues were collected and prepared for analysis by clipping stems at the sediment interface, drying to a constant weight at 50°C, and grinding the material using a Thomas Wiley Mini-Mill (Thomas Scientific). We measured the C and N content of the soil and plant materials using a Vario Max CNS elemental analyzer (Elementar).

Intact Core Denitrification Experiment

Intact sediment cores were collected from within the mesocosms after the 6-h experimental runoff event. Sediments including one cattail stem or, in the case of cutgrass, a mass of stems and the associated root–rhizome mat associated with the sediments. One core with approximately 10 cm of overlying water was collected in clear plastic tubes (surface area = 40.6 cm^2, height = 30 cm) from each mesocosm by manually pushing the cores into sediments that included vegetation, clipping rhizomes, and lightly tapping the core approximately 20 cm into the sediments. Cores open to the water column above were installed before the initiation of runoff and removed shortly after. During removal, vegetation extending beyond the top of the cores was clipped, and the cores were capped on both ends for transport to the adjacent laboratory. Mesocosm outflow water was collected from all treatments throughout the experimental runoff event, combined into one mixing chamber, and spiked with NO_3^- to a concentration of 3.17 ± 0.05 mg L^{-1} (PO_4–P = 0.35 ± 0.01 mg L^{-1}, NH_4^+–N = 0.02 ± 0.00 mg L^{-1}) to supply inflow water for continuous-flow core incubations.

Continuous-flow sediment core incubations were conducted to directly measure denitrification rates from intact sediment cores (Scott et al., 2008; Grantz et al., 2012). In the laboratory, upper core caps were removed, and the cores were sealed airtight with rubber stoppers. Rubber stoppers were outfitted with two pieces of Teflon tubing inserted through each stopper to provide inflow and outflow paths for incubation water. Inflow tubing extended to the overlying water just above the sediment–water interface, and outflow tubing was flush with the stopper on the interior of the core. Changes in inflow gas concentrations may prevent accurate N_2 and O_2 flux measurements in flow-through core incubations (Kana et al., 1994), so incubation water was constantly aerated to maintain saturated conditions and constant inflow N_2–N (13.47 ± 0.02 mg L^{-1}) and O_2 (7.86 ± 0.2 mg L^{-1}) gas concentrations. Incubation water was pumped into cores at a mean rate of 0.83 ± 0.001 mL min^{-1} using an ISMATEC MV peristaltic pump (Model 7332-00). Control chambers (10-cm cores without sediment) were also established to correct for any changes in dissolved gases and solutes that could be attributed to biogeochemical transformation in the overlying water column rather than within the sediment cores, as well as potential physical effects related to reaction with core chamber materials. Flow-through core incubations were conducted in complete darkness to prevent photosynthesis and the production of O_2 bubbles, which can confound dissolved N_2 gas measurements in closed-core systems (Kana et al., 1994; Gardner et al., 2006). All cores were incubated within a Powers Scientific diurnal growth chamber (Model DS33SD) set at average ambient temperature (23.7°C) for the study location in late May to early June, which was similar to the average in situ temperature observed within the mesocosms during the experiment (control = 25.5°C, cutgrass = 25.3°C, cattail = 25.5°C).

Cores were allowed to flow continuously for 12 to 18 h before sampling the effluent from each core chamber and influent from the incubation water reservoir. Samples were collected in 20-mL glass vials and immediately preserved by adding 0.26 mL of 50% (w/v) $ZnCl_2$ (Grantz et al., 2012). Vials were then capped with ground-glass stoppers and wrapped with Parafilm to prevent gas exchange with the outside atmosphere. We collected three samples for analysis from each core on three consecutive days. The sealed vials were wrapped in protective wrapping, packed in 1-L Nalgene bottles filled with deionized water to further prevent any gas exchange, and shipped on ice to the University of Arkansas in Fayetteville for dissolved gas analysis.

Dissolved gas samples were analyzed for their N_2/Ar and O_2/Ar gas ratios using a membrane inlet mass spectrometer (MIMS) equipped with a Pfeiffer Prisma mass spectrometer and a Bay Instruments DGA membrane inlet S-25-75. The full MIMS setup was described in detail by Kana et al. (1994). Potential instrument-specific O_2 interference in N_2/Ar determination was previously ruled out on the MIMS by comparing the N_2–N concentration of replicate (oxic) samples measured both with and without O_2 removal using a Cu reduction column heated to 600°C (Eyre et al., 2002). Sample temperatures were brought back to the in situ temperature, and the temperature of the MIMS standard solution was adjusted to match each sample before MIMS analysis. The MIMS method assumes 100% Ar saturation, which varies with temperature and salinity but not due to biological production or consumption. Thus, biological effects on the N_2 and O_2 pools of the samples can be separated from physical effects using the Ar signal. Sample N_2/Ar and O_2/Ar ratios for each sample were converted to N_2–N and O_2–O concentrations based on

$$[\mathrm{DG}]_{\mathrm{sample}} = \left(\mathrm{DG/Ar}_{\mathrm{sample}} \times [\mathrm{Ar}]_{\mathrm{exp}}\right)\left(\frac{[\mathrm{DG}]/[\mathrm{Ar}]_{\mathrm{exp}}}{\mathrm{DG/Ar}_{\mathrm{standard}}}\right) \quad [1]$$

where $\mathrm{DG/Ar}_{\mathrm{sample}}$ is the measured dissolved gas sample signal and $\mathrm{DG/Ar}_{\mathrm{standard}}$ is the measured dissolved gas signal for well-mixed, deionized water open to the atmosphere at the same temperature as the samples. The terms $[\mathrm{Ar}]_{\mathrm{exp}}$ and $[\mathrm{DG}]/[\mathrm{Ar}]_{\mathrm{exp}}$ are the theoretical saturated concentration and ratio, respectively, calculated for each in situ sample temperature using gas solubility tables (Weiss, 1970). Areal sediment denitrification (K_{dnf}, mg m^{-2} h^{-1}) for each core was calculated as

$$K_{\mathrm{dnf}} = \frac{\left([\mathrm{DG}]_{\mathrm{out}} - [\mathrm{DG}]_{\mathrm{in}}\right)Q_{\mathrm{core}}}{A} - \frac{\left([\mathrm{DG}]_{\mathrm{control}} - [\mathrm{DG}]_{\mathrm{in}}\right)Q_{\mathrm{control}}}{A} \quad [2]$$

where $[\mathrm{DG}]_{\mathrm{out}}$, $[\mathrm{DG}]_{\mathrm{in}}$, and $[\mathrm{DG}]_{\mathrm{control}}$ are the core chamber outflow and inflow and control chamber outflow dissolved gas concentrations (N_2, O_2) (in μmol L^{-1}), respectively; Q_{core} and Q_{control} are the measured flow rates through the core and control chambers (in L h^{-1}); and A is the core surface area (in m^2). The solution to this equation will yield an aerial denitrification estimate for each independent intact core.

Whole-System 48-Hour Nitrogen Budget

Results were summarized by estimating 48-h areal whole-system N budgets using measured changes in NO_3^-–N, denitrification rates, and mass balance equations for comparison among vegetation treatments. The NO_3^-–N inputs (mg) during the 6-h experimental runoff event were estimated as

$$\mathrm{NO_3^-N_{input}} = \mathrm{MT_{conc}} \times t \times Q \quad [3]$$

where MT_{conc} is the mixing tank concentrations, t is the number of hours, and Q is the flow rate (in L h^{-1}). The NO_3^-–N outputs (mg) during the experimental runoff event were calculated as

$$NO_3^-\text{–}N_{output} = Q\sum_{i=1}^{j}(Conc_i \times t_i) \quad [4]$$

where $Conc_i$ is the NO_3–N concentration corresponding to the ith time interval, t_i is the time (in h) corresponding to the ith time interval, and $j = 6$. Hydraulic NO_3^-–N retention during the runoff event was estimated as

$$NO_3^-\text{–}N_{ret} = \left[NO_3^-\text{–}N_{input} + \left(Conc_{bg} \times V\right)\right] - NO_3^-\text{–}N_{output} \quad [5]$$

where $Conc_{bg}$ is the background NO_3^-–N concentration (in mg L^{-1}) and V is the initial volume in the mesocosm (in L) before dosing. After the experimental runoff event, 48-h uptake was calculated as

$$NO_3^-\text{–}N_{upt48} = NO_3^-\text{–}N_{ret} - \left(Conc_{48} \times V_{48}\right) \quad [6]$$

where $Conc_{48}$ is the NO_3^-–N concentration at 48 h and V_{48} is the standing water volume at 48 h.

Uptake efficiency, which we are treating here as the percentage of NO_3^-–N retained that was removed from the water column during the first 48 h of the experiment, was estimated as

$$\text{Uptake efficiency} = \left(\frac{NO_3^-\text{–}N_{upt48}}{NO_3^-\text{–}N_{ret}}\right)100 \quad [7]$$

Denitrification rates from intact cores were assumed to represent the average K_{dnf} in mesocosms of the respective treatments during the experiment and were used to estimate the amount of N denitrified:

$$DNF_{48} = K_{dnf} \times t \times A \quad [8]$$

where K_{dnf} is based on Eq. [2], time $t = 48$ h, and A represents the surface area of sediment in mesocosms. Denitrification efficiency was estimated as

$$\text{DNF efficiency} = \left(\frac{DNF_{48}}{NO_3^-\text{–}N_{upt48}}\right)100 \quad [9]$$

and represents the percentage of immobilized N that was denitrified during the first 48 h of the experiment. All estimates in the budget were standardized to 1 m^2 to facilitate comparisons with other studies.

Statistical Analysis

We used a combination of generalized least squares (GLS) and linear mixed effects (LME) models (Zuur et al., 2009) to compute F statistics and test for differences in nutrient load reductions during the experimental runoff event, changes in nutrient concentrations with time during the stagnant period, changes in DO concentrations with time, and differences in denitrification and O_2 consumption rates between treatments. The GLS models were used to compare background nutrients, mixing tank nutrients, and nutrient reductions during the experimental runoff event between vegetation treatments. We used LME models that included random effects to account for repeated measures within individual mesocosms ($\sim$1|mesocosm) when comparing changes in nutrient or DO concentrations with time or nested samples ($\sim$1|mesocosm core) when combining multiple measurements from one core to analyze differences in denitrification rates between treatments. The restricted maximum likelihood criterion was used to fit all models. We assessed the assumptions of all models visually with normality plots (*qqnorm*) and standardized residual plots across treatments (Zuur et al., 2009). If error variances differed across levels of treatments, this heterogeneity was incorporated into our model by modeling variance separately among treatments with the *VarIdent* command (Zuur et al., 2009). We used Tukey's honestly significant difference (HSD) multiple comparison of means to test for significant differences among levels of fixed factors or multiple comparison of least-squares means (LSmean) to perform predetermined contrasts of factors within different time segments. The GLS and LME models were run in the *nlme* package (Pinheiro and Bates, 2000), Tukey HSD tests were run in the *multcomp* package (Bretz et al., 2010), and LSmean multiple comparisons were computed with the *lsmeans* package (Lenth, 2013) in R (Version 3.0.1; R Core Team, 2013).

Results

Background Nutrient Concentrations

There were no differences among treatments for any of the dissolved nutrient concentrations within the mixing chambers used to dose mesocosms during the experimental runoff event (Table 1). However, mesocosms planted with cattail had significantly lower background PO_4–P and NH_4^+–N concentrations than the other treatments (Table 1). Unvegetated mesocosms had higher initial NO_3^-–N concentrations than either vegetated treatment (Table 1).

Sediment and Plant Carbon/Nitrogen Ratios

Sediment C/N ratios in mesocosms varied with vegetation treatment (Table 2). Mesocosms planted with cutgrass had higher C/N ratios than unvegetated mesocosms but did not differ significantly from cattail (Table 3). Mesocosms planted with cattail had marginally significantly higher C/N values than unvegetated treatments (Table 3; Tukey's HSD $P = 0.07$). Sediment C/N ratios did not change between pre- and post-experiment periods (Table 2). Plant tissue C/N ratios increased with time but did not vary with treatment (Table 2). Despite a pattern of C/N ratios increasing more in cattail than cutgrass (Table 3), there were no significant interactions between vegetation type and time (Table 2).

Nutrient Load Retention during Runoff Event

Retention of NH_4^+–N loads in cattail treatments (31.3 $\pm$ 10.58%) trended higher than unvegetated (11.91 $\pm$ 27.71%) or cutgrass treatments (8.77 $\pm$ 10.81%) but were highly variable and not significantly different among treatments during the 6-h experimental runoff event (Supplemental Table S2). In contrast, all treatments retained 60% or more of NO_3^-–N loads during the 6-h experimental runoff event, but cutgrass mesocosms had

Table 1. Dissolved nutrient concentrations in mixing chambers and mesocosms before the runoff event, with *F* values and associated *p* values based on generalized least squares models.

Variable	Dissolved nutrient concentration			$F_{2,6}$	*p*
	Unvegetated	Cutgrass	Cattail		
	mg L^{-1}				
Mixing chamber					
NH_4^+–N	0.032 ± 0.007†	0.037 ± 0.005	0.034 ± 0.005	0.15	0.867
NO_3^-–N‡	5.956 ± 0.155	6.089 ± 0.035	6.006 ± 0.082	0.72	0.526
PO_4–P‡	1.110 ± 0.025	1.133 ± 0.007	1.123 ± 0.020	0.48	0.640
Background					
NH_4^+–N	0.069 ± 0.007 b§	0.069 ± 0.004 b	0.042 ± 0.005 a	8.27	0.018
NO_3^-–N	0.044 ± 0.005 a	0.026 ± 0.000 b	0.031 ± 0.001 b	10.94	0.010
PO_4–P‡	0.160 ± 0.073 b	0.025 ± 0.008 b	0.008 ± 0.003 a	6.60	0.031

† Mean ± 1 SE.

‡ Different variances for each vegetation treatment were incorporated into the model.

§ Different letters indicate significant differences between treatments based on Tukey's honestly significant difference at $p < 0.05$.

significantly higher (68%) retention compared with cattail (60%) and unvegetated (61%) treatments (Table 4; Fig. 2A). Measured NO_3^-–N load retention was similar to mass balance estimates for whole mesocosms (Table 5). Vegetated treatments retained >70% of PO_4–P loads during the 6-h treatment, significantly more than unvegetated treatments (54.5%) (Supplemental Table S2).

Nutrient Uptake after Runoff Event

After the experimental runoff event, differences in reduction of the NO_3^-–N concentration varied among treatments with time (Table 4; Fig. 2B). Significantly higher reductions in NO_3^-–N concentrations occurred in mesocosms planted with cattail than in those planted with cutgrass or left unvegetated at 12 and 24 h (Fig. 2B). Mesocosms planted in cattail reduced NO_3^-–N concentrations by >95% within 24 h. Within 48 h, NO_3^-–N concentrations were reduced by >90% in both cattail and cutgrass, which were not statistically different (Fig. 2B). Unvegetated mesocosms reduced NO_3^-–N concentrations by significantly less (65%) within 48 h. Mass balance estimates for NO_3^-–N uptake during 48 h agreed with measured changes in NO_3^-–N concentrations (Table 5). Within 72 h, unvegetated mesocosms had reduced NO_3^-–N by 85% but still significantly less than cattail or cutgrass (Fig. 2B). Observations 1 wk after dosing indicated that all treatments had reduced NO_3^-–N concentrations by >99% (Fig. 2B). Patterns in NH_4^+–N concentrations were variable and did not demonstrate a gradual decline in the mesocosms (Supplemental Tables S1 and S2). Reductions in PO_4–P concentrations followed a pattern similar to NO_3^-–N, with vegetated treatments demonstrating rapid uptake within the first 48 to 72 h and a significant lag in PO_4–P removal within unvegetated mesocosms (Supplemental Table S2).

Table 2. Effects of vegetation on sediment and plant molar C/N ratios before and after the experiment (time), with *F* values and associated *p* values based on linear mixed effects models that incorporated random effects to account for repeated measures within each mesocosm.

Source of variation	*F*	*p*
Sediment C/N ratios		
$Vegetation_{2,6}$	8.20	0.019
$Time_{1,6}$	0.01	0.935
Vegetation × $time_{2,6}$	1.16	0.375
Plant C/N ratios		
$Vegetation_{2,4}$	3.78	0.124
$Time_{1,4}$	8.82	0.041
Vegetation × $time_{2,4}$	4.49	0.101

Table 3. Sediment and plant tissue C/N ratios for each vegetation treatment before and after the experiment.

Treatment	Initial C/N ratio	Final C/N ratio
Sediment		
Unvegetated	11.60 ± 0.25 b†	12.21 ± 0.93 b
Cutgrass	16.372 ± 1.37 a	15.91 ± 0.72 a
Cattail	14.55 ± 0.77 ab	14.86 ± 0.68 ab
Plant tissues		
Cutgrass	57.06 ± 2.47	65.97 ± 1.85
Cattail	57.08 ± 7.53	110.48 ± 20.40

† Mean ±1 SE. Means followed by different letters indicate significant differences between treatments based on Tukey's honestly significant difference at $p < 0.05$.

Denitrification Core Experiment

Net denitrification was always observed in intact cores collected from mesocosms planted with cutgrass but was highly variable for cores collected from unvegetated and cattail mesocosms, resulting in no overall net denitrification (Fig. 3A). Cores from cutgrass mesocosms had significantly higher average denitrification rates (5.93 ± 0.62 mg m^{-2} h^{-1}) than cores from unvegetated mesocosms (−0.19 ± 0.49 mg m^{-2} h^{-1}), whereas

Table 4. Effects of vegetation on NO_3^-–N retention during the runoff event, NO_3^-–N uptake after runoff, and areal sediment denitrification rate (K_{dnf}) throughout the experiment, with *F* values and associated *p* values based on a generalized least squares model for NO_3^-–N retention. Linear mixed effects models that incorporated random effects to account for repeated measures within each mesocosm or nested measurements within each core were used to calculate *F* values and associated *p* values for NO_3^-–N uptake and denitrification rates (K_{dnf}), respectively.

Response	Source of variation	*F*	*p*
NO_3^-–N retention†	$Vegetation_{2,6}$	12.42	0.007
NO_3^-–N uptake (stagnant)‡	$Vegetation_{2,6}$	2.50	0.161
	$Time_{4,24}$	391.60	<0.001
	Vegetation × $time_{8,24}$	24.60	<0.001
K_{dnf}†	$Vegetation_{2,6}$	24.86	0.001

† Different variances for each vegetation treatment were incorporated into the model.

‡ Different variances for each time were incorporated into the model.

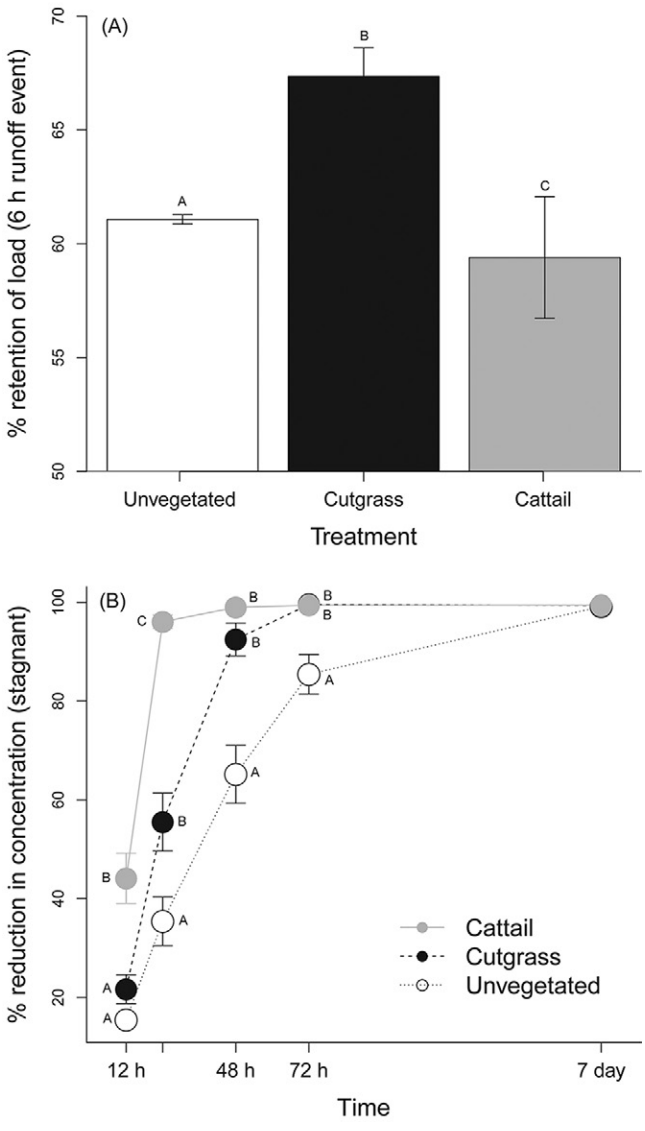

Fig. 2. Mean (±1 SE) reduction in (A) NO_3^-–N load during 6-h runoff event through different vegetation treatments, and (B) NO_3^-–N concentration within the different vegetation treatments during the 7-d stagnant period immediately after the runoff event. Letters represent significant differences between treatments based on Tukey's honestly significant difference post-hoc tests ($p < 0.05$) (A) or treatments within each time period based on LSmeans comparisons ($p < 0.05$) (B).

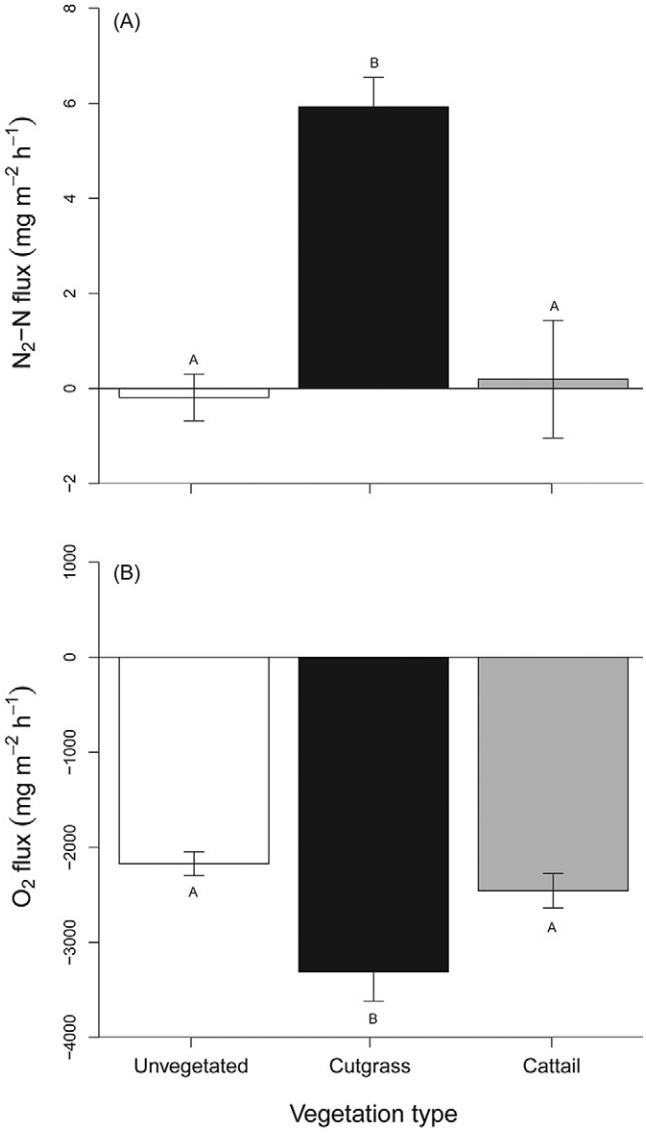

Fig. 3. Mean (± 1SE) (A) denitrification rates and (B) sediment O_2 demand from intact cores extracted from mesocosms representing different vegetation treatments. Letters represent significant differences between treatments based on Tukey's honestly significant difference post-hoc tests ($p < 0.05$).

Table 5. Forty-eight-hour NO_3^-–N budget based on mass balance estimates for mesocosms under different vegetation treatments.

Mesocosm fluxes	Unvegetated	Cutgrass	Cattail
	mg N m^{-2}		
		6-h runoff event budget	
Background†	5.20 ± 0.53‡	2.80 ± 0.29	3.82 ± 0.28
NO_3^-–N input (Eq. [3])	1056.39 ± 32.27	823.83 ± 78.52	991.35 ± 80.81
NO_3^-–N output (Eq. [4])	397.89 ± 18.23	266.40 ± 23.81	394.04 ± 47.94
NO_3^-–N retention (Eq. [5])	663.71 ± 19.93 (63%)	560.23 ± 57.07 (68%)	601.13 ± 43.39 (60%)
		48-h budget	
NO_3^-–N uptake (Eq. [6])	429.11 ± 54.47 (65%)	512.49 ± 43.72 (92%)	598.15 ± 40.82 (100%)
Denitrification (Eq. [8])	−8.96 ± 23.54 (−2%)	284.48 ± 29.69 (56%)	9.41 ± 59.35 (2%)
NO_3^-–N unaccounted for§	438.07 ± 65.33	228.01 ± 16.98	588.74 ± 76.03

† Background NO_3^-–N concentration × initial mesocosm standing water volume.

‡ Mean ± SE.

§ Estimated based on NO_3^-–N uptake minus denitrification.

denitrification rates for cores from cattail mesocosms (0.20 ± 1.24 mg m^{-2} h^{-1}) were not different from the unvegetated mesocosms (Table 4; Fig. 3A). Applying measured denitrification rates to mass balance estimates indicated that denitrification accounted for as much as 56% of the NO_3^-–N immobilized during 48 h in the cutgrass mesocosms (Table 5). In contrast, the mean observed denitrification rates accounted for very little, if any, of the observed uptake or removal of NO_3^-–N in the unvegetated or cattail mesocosms, although high standard errors around the means indicated that some mesocosms exhibited positive but comparatively low N_2 flux.

Dissolved Oxygen Dynamics

All treatments had high O_2 demand in core incubations, but cores from cutgrass mesocosms had significantly greater reductions in O_2 (−3311.65 ± 307.37 mg m^{-2} h^{-1}) than cores from unvegetated (−2172.68 ± 126.69 mg m^{-2} h^{-1}) or cattail mesocosms (−2458.18 ± 181.26 mg m^{-2} h^{-1}) (Table 6; Fig. 3B). Differences in overall mesocosm DO concentrations among vegetation treatments varied with time of day (Table 6). Mesocosms planted with cutgrass had significantly lower DO concentrations than unvegetated mesocosms throughout the day (Fig. 4). During early morning hours (0700 h), mesocosms planted with cattail also had DO concentrations that were statistically lower than unvegetated mesocosms (Fig. 4); however, throughout the rest of the day (0930–1830 h), DO concentrations in mesocosms planted with cattail were significantly higher than within cutgrass mesocosms and lower than within unvegetated mesocosms (Fig. 4). Early morning sampling on Days 2, 3, 4, and 7 indicated that differences in DO concentration observed on Day 1 for early morning were similar throughout the experiment; i.e., cutgrass and cattail were significantly less than unvegetated mesocosms but similar to each other (data not shown).

Table 6. Effects of vegetation on sediment O_2 demand and whole mesocosm dissolved O_2 concentrations, with *F* values and associated *p* values based on linear mixed effects models that incorporated individual cores as random effects to account for nested measurements (*n* = 3) within each core or repeated measurements with time within each mesocosm.

Response	Source of variation	*F*	*p*
Sediment O_2 demand†	$Vegetation_{2,6}$	7.74	0.022
	$Vegetation_{2,6}$	391.47	<0.001
Dissolved O_2‡	$Time_{9,54}$	586.22	<0.001
	Vegetation × $time_{18,54}$	28.21	<0.001

† Different variances for each vegetation treatment were incorporated into the model.

‡ Different variances for each time were incorporated into the model.

Discussion

The objective of our study was to assess how aquatic emergent vegetation influenced nutrient mitigation and denitrification in mesocosms set up to represent ditch environments managed to function more like wetlands. We hypothesized that: (i) nutrient retention and uptake during and after the experimental runoff event would be greater in vegetated mesocosms, and (ii) denitrification would contribute significantly more to NO_3^-–N removal in vegetated mesocosms, potentially more so in mesocosms planted with cutgrass. In support of our first hypothesis, emergent vegetation comprised of either cattail or cutgrass captured and removed significantly more NO_3^-–N from surface water runoff within the first 48 h than unvegetated mesocosms. However, unvegetated mesocosms assimilated all of the retained NO_3^-–N within 7 d, suggesting that the longer term mitigation potential is similar among treatments. Intact core incubations indicated that substantial amounts of retained NO_3^-–N were denitrified in mesocosms planted with cutgrass, whereas minimal denitrification occurred in mesocosms planted with cattail or left unvegetated, providing support for our second hypothesis. Collectively, our results provide evidence that emergent wetland vegetation can retain and remove significant amounts of nutrients from agricultural runoff, but the role of denitrification in NO_3^-–N mitigation varies with vegetation type.

Plant and associated microbial uptake can be a significant nutrient removal mechanism in vegetated aquatic systems (Vymazal, 2007). This may be especially true under low flow or standing water conditions created by vegetative barriers or low-grade weirs in ditches after storm flow events. In this study, >90% of the retained NO_3^-–N was removed from the water column within a 48-h period in vegetated mesocosms (Table 5), similar to previously observed removal rates during standing water periods in vegetated ditch mesocosms (Tyler et al., 2012). While our results were constrained by the small temporal and spatial scale of the experiment, previous long-term studies on whole wetlands have shown that increasing nutrient loads can enhance wetland plant biomass and nutrient content, providing evidence that

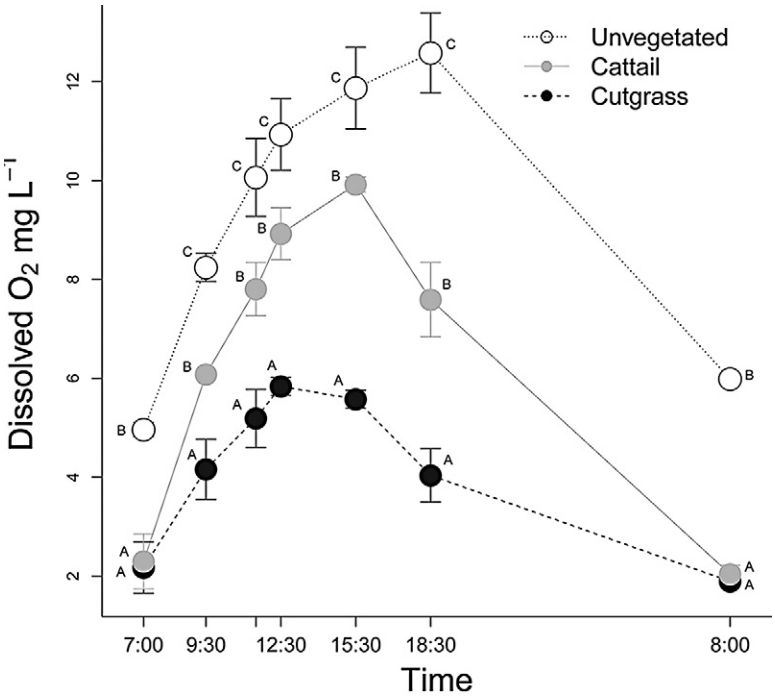

Fig. 4. Mean (±1 SE) dissolved O_2 (DO) concentrations within different vegetation treatments during the first 24 h of the experiment. Morning DO concentrations for each treatment on Days 3, 4, and 7 were similar to those observed on Days 1 and 2 and are not plotted. Letters represent significant differences between treatments within each time period based on post-hoc LSmeans comparisons ($p < 0.05$).

plant uptake and incorporation of excess nutrients occurs, with uptake accounting for up to 52% of N removal (Hoagland et al., 2001; Silvan et al., 2004; Wu et al., 2011). Movement of water into sediments due to macrophyte transpiration can stimulate NO_3^- removal in wetlands (Martin et al., 2003). Cutgrass exhibits more conservative water use than cattail (Farnsworth and Meyerson, 2003), and probable differences in transpiration rates between the two species may explain the higher initial NO_3^-–N uptake rates observed for cattail in this study.

Despite early differences in nutrient mitigation among treatments, NO_3^-–N and PO_4–P concentrations declined to background concentrations in unvegetated mesocosms within 7 d, evidence that significant, albeit slower nutrient mitigation occurred. Given that we observed minimal denitrification in sediment cores from unvegetated mesocosms, benthic and sestonic algae may explain NO_3^-–N removal in the unvegetated mesocosms. The time lags associated with N uptake observed during our experiment may be related to sestonic algae being flushed during the runoff event. Removal of nutrients from the water column over 7 d could be due to the reestablishment of algae. While these results suggest that significant N mitigation may also occur in unvegetated ditches, differences in timing and storage compartments among treatments probably influence the overall nutrient mitigation potential. In vegetated environments, N is rapidly removed from the water column into longer term storage compartments (plant tissue, sediments) or denitrified before it can be flushed downstream as organic or inorganic N in subsequent runoff events. However, follow-up tracer studies are needed to assess N storage in vegetated vs. unvegetated ditch environments to confirm this potential explanation of our data.

We observed dramatic differences in measured denitrification rates among our vegetation treatments (Fig. 3A), which, when applied to 48-h N budgets, suggest that denitrification may have been responsible for as much as 56% of the observed NO_3^-–N uptake within the first 48 h in mesocosms planted with cutgrass (Table 5). Similar losses of NO_3^-–N to denitrification in Danish agricultural ditches were reported by de Klein (2008). Previous studies have demonstrated differences in denitrification rates associated with macrophyte species (Bachand and Horne, 1999; Bastviken et al., 2005; Veraart et al., 2011). Variations in denitrification among the vegetation treatments may be attributed to differences in O_2 production or consumption associated with plants and microbes (Vymazal, 2007). Mesocosms planted with cutgrass had lower DO than cattail mesocosms throughout the day, a good indication that higher C mineralization was occurring in cutgrass beds or higher aeration of root zones occurred within cattail mesocosms.

Emergent vegetation can enhance denitrification by increasing C availability for microbes and creating anoxic conditions. Mesocosms planted with cutgrass in our experiment had higher sediment C/N ratios than unvegetated mesocosms, and both plant species had lower minimum DO concentrations. Additionally, sediment cores from cutgrass mesocosms had significantly higher sediment O_2 demand and net denitrification rates than the other two treatments, evidence that higher denitrification rates may be related to lower O_2 conditions in sediments of cutgrass beds. Thus, vegetation inputs of C may represent a significant controlling factor for denitrification within managed agricultural ditches. Increased dissolved organic C (DOC) availability fuels overall microbial activity and provides potentially limiting resources to the denitrification pathway. In particular, C and N released during senescence may fuel denitrification (McMillan et al., 2010) and maintain denitrifier microbial communities within plant beds. Farnsworth and Meyerson (2003) reported higher biomass turnover in cutgrass than cattail, an indication that more leaf input and breakdown probably occurs throughout the year in cutgrass stands. High biomass turnover and breakdown creates an ideal environment for denitrification by increasing the supply of potentially limiting organic C and NO_3^- to denitrifying bacteria (Reddy et al., 1989; Weisner et al., 1994; Brix, 1997).

Internal pressurization and convective gas flow in aerenchyma are an important aeration adaptation for many emergent and floating-leaved aquatic plants in waterlogged, anoxic sediments and can result in oxygenated zones around roots surrounded by anoxic zones, creating ideal conditions for coupled nitrification–denitrification of NH_4^+ (Reddy et al., 1989). In NO_3^-–N-limited systems, denitrification rates can be dependent on nitrification, but in systems where it is not limited, diel patterns in plant photosynthesis can inhibit denitrification by oxygenating the sediments (Christensen and Sørensen, 1986; Risgaard-Petersen and Jensen, 1997). Published convective flow rates for cattail and its congeners are much higher (3.4–8 mL min^{-1}) than those reported for *Leersia* spp. (0.15 ± 07 mL min^{-1}) (Brix et al., 1992; Bendix et al., 1994; Sorrell and Hawes, 2010; Konnerup et al., 2011), and one study has directly observed more reduced soils in cutgrass beds compared with another emergent plant [*Bacopa monnieri* (L.) Pennell] due to greater rhizosphere oxidation in the latter (Pierce et al., 2009).

While our results suggest that differences in denitrification between cutgrass, cattail, and unvegetated sediments were probably due to differences in C availability and anoxic conditions within the sediment–water interface, both factors can be influenced by diel patterns in light and photosynthesis (Christensen and Sørensen, 1986; Veraart et al., 2011). Light conditions may enhance denitrification by increasing the delivery of organic C to sediments and the associated microbial community via root exudates (Christensen and Sørensen, 1986). Zhai et al. (2013) observed that the average DOC release rate of three emergent wetland plants [*Phragmites australis* (Cav.) Trin. ex Steud., *Iris pseudacorus* L., and *Juncus effusus* L.) was two times higher under light conditions. However, diel light conditions and associated photosynthesis-driven flux of O_2 from shoots to root zones may also inhibit denitrification during the day, particularly for cattail, where known convective flow rates are much higher than in cutgrass (Brix et al., 1992; Bendix et al., 1994; Sorrell and Hawes, 2010; Konnerup et al., 2011). While this process can also stimulate nitrification, it is unlikely that diel light conditions would have enhanced our measured denitrification rates by providing oxygenated root zones for coupled nitrification–denitrification of NH_4^+ because the available NO_3^-–N exceeded concentrations at which this pathway contributes significantly to overall denitrification rates (Seitzinger et al., 2006). Our denitrification measurement method probably uncoupled the effects of photosynthesis on DOC and O_2 dynamics from denitrification rates, and the balance between these opposing effects is unknown; therefore, we do not know if the MIMS measurements are biased high or low relative to our 48-h budget.

An additional factor that may have influenced our denitrification rates was poor replication. One disadvantage of using flow-through core experiments to determine denitrification rates is that replication within treatments is limited due to the more elaborate setup and greater incubation times compared with the more commonly used denitrification enzyme activity assay (Groffman et al., 2006). While mean denitrification rates for both control and cattail treatments overlapped zero, in both cases this was driven by one out of three cores having a negative N_2 flux. Omitting negative values provides denitrification estimates of 0.30 and 1.43 mg m^{-2} h^{-1} for control and cattail, respectively, compared with 5.93 mg m^{-2} h^{-1} for cutgrass. It is possible that some denitrification occurred in all treatments but was still considerably less for the control (~20× lower) and cattail (~4× lower) compared with measured rates from cutgrass sediment cores.

Despite evidence for denitrification in cutgrass beds, anoxic conditions can also promote unfavorable transformations of N and P, and the benefits of denitrification need to be considered within the context of potential ecosystem disservices (Burgin et al., 2013). Some of the unexplained N loss may have been as N_2O, a potent greenhouse gas (Reay et al., 2003; Beaulieu et al., 2009). Nitrous oxide production in agricultural streams can be significant and related to NO_3^-–N availability (Beaulieu et al., 2009), but factors such as higher soil O_2 content may promote higher N_2O vs. N_2 as the end product of denitrification in wetland soils (Burgin and Groffman, 2012). This may be an important control on denitrification end products in drying and wetting cycles of natural ditches and needs further investigation. In the current study, NH_4^+–N concentrations varied with time, with a temporary increase in NH_4^+–N early in the morning after dosing cutgrass mesocosms, as well as lower background NH_4^+–N in cattail treatments (Supplemental Table S1, 24 h). Ammonium concentrations within our mesocosms may have been controlled by variable inhibition of nitrification related to differences in diel O_2 dynamics between treatments or by DNRA, another potentially important but poorly studied NO_3^- reduction pathway (Burgin and Hamilton, 2007). Scott et al. (2008) observed higher DNRA during summer when NO_3^-–N was low and sediment O_2 demand was high. Anoxia may promote more NH_4^+ production via DNRA in cutgrass beds but was unlikely to have been a major factor in our study due to the lack of NO_3^-–N limitation (Burgin and Hamilton, 2007; Scott et al., 2008). We observed net uptake of >90% of retained PO_4–P in our mesocosms, although unvegetated mesocosms captured significantly less P during runoff. Despite this, longer term mitigation of P in agricultural ditches is regulated by other factors like adsorption–desorption relationships between soils, DO, and P and the release of plant-bound nutrients during senescence (Kröger et al., 2007b, Sharpley et al., 2007).

Conclusions

Our study provides experimental evidence that establishing cattail and cutgrass in agricultural ditches can reduce nutrient impacts to receiving water bodies in agricultural landscapes, but the fate of excess NO_3^-–N in vegetated ditches probably varies with vegetation type. Denitrification was an important N sink in treatments planted with cutgrass capable of removing significant portions of retained N. Managing a combination of both vegetation species in ditches probably provides significant nutrient mitigation benefits through short-term NO_3^-–N and PO_4–P retention via plant uptake and immediate long-term N sinks through denitrification. These results have important implications for managing NO_3^-–N in agricultural landscapes, but more experimental studies designed to isolate alternative mechanisms involved in N processing in agricultural ditches, along with field studies conducted in ditches at larger spatial and temporal scales, are needed to fully understand the potential of managing agricultural ditches for enhanced denitrification efficiency and N mitigation.

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