

Nutrient Loss Following *Phragmites australis* Removal in Controlled Soil Mesocosms

Kyle S. Herrman · Durelle T. Scott ·
John D. Lenters · Erkan Istanbuluoglu

Received: 11 April 2011 / Accepted: 14 February 2012 / Published online: 1 March 2012
© Springer Science+Business Media B.V. 2012

Abstract Mechanisms to remove Common reed (*Phragmites australis*) typically include a combination of herbicide applications and mechanical cutting or plowing of the soil. The objective of this study was to remove *P. australis* by various mechanisms and measure the subsequent short-term release of nutrients via simulated rain events. Three rain events of similar duration and intensity were conducted on a control subset and three treatments (above and belowground biomass removal, herbicide application, and basal cut) of soil mesocosms ($n=6$) that were designed to export excess water as either surface runoff or leachate through the soil profile. The dominant pathway for soluble reactive phosphorus ($p<0.001$) and ammonium

($p<0.001$) export were surface runoff while nitrate ($p<0.001$) was leached through the soil profile. More nitrate was exported in the vegetation removal treatments (i.e., biomass removal, herbicide, and basal cut) than the control ($p<0.001$) while more soluble reactive phosphorus was exported in the herbicide and basal cut treatment compared to the control ($p=0.010$). In regards to ammonium, a higher export was observed in the herbicide treatment compared to the control, biomass removal, and basal cut treatments ($p<0.001$). We attribute the higher amount of ammonium export in the herbicide treatment to the fact that the glyphosate herbicide used was in an isopropylamine salt form. After examining pre- and postmanipulation soil cores, there was a larger decrease in extractable ammonium in the control and all treatments compared to soil extractable nitrate, which displayed a smaller decrease and in some treatments actually increased during the course of the experiment. Ultimately, in this study, we observed a strong potential for nitrogen biogeochemistry to occur and the removal of vegetation-enhanced nutrient export.

K. S. Herrman (✉)
College of Natural Resources,
University of Wisconsin-Stevens Point,
Stevens Point, WI 54481, USA
e-mail: Kyle.Herrman@uwsp.edu

D. T. Scott
Biological Systems Engineering Department,
Virginia Polytechnic Institute and State University,
Blacksburg, VA 24061, USA

J. D. Lenters
School of Natural Resources, University of Nebraska,
Lincoln, NE 68583-0987, USA

E. Istanbuluoglu
Department of Civil and Environmental Engineering,
University of Washington,
Seattle, WA 98195-2700, USA

Keywords Nutrient export · *Phragmites australis* ·
Simulated rain event · Glyphosate · Isopropylamine

1 Introduction

Common reed (*Phragmites australis*) is a prolific invasive species in riparian areas and wetlands throughout the USA. *P. australis* has been present on the east

coast of the USA for >400 years; but in the last century, this species has begun invading aquatic ecosystems (Orson 1999). This sudden change in growth pattern has been attributed to the disturbance of wetland ecosystems and the introduction of specific genotypes of *P. australis* that outcompete native plant species (Chambers et al. 1999). *P. australis* forms monocultures as a result of vegetative reproduction through its dense rhizomes and its ability to quickly recover after damage to aboveground biomass (Meyerson et al. 1999). Typically, this species will more than double the aboveground biomass of the native species it outcompetes (Findlay et al. 2003) and can reach heights up to 5 m.

The impacts of *P. australis* invasion on aquatic ecosystems are variable and most likely depend on the sensitivity of the specific ecosystem being invaded. Invasion typically results in dense monocultures of *P. australis* and directly impacts wetlands by displacing native species (Chambers et al. 1999; Meyerson et al. 1999). In marine, tidal wetlands, where plant diversity is inherently low, invasion by *P. australis* slightly reduces biodiversity but does not alter wetland structure due to the invasive species having similar plant morphologies as the displaced, native species (Chambers et al. 1999). On the other hand, invasion of *P. australis* into freshwater wetlands, where biodiversity is high and plants display varied morphologies, results in reduced biodiversity and significantly altered ecosystem structure (Chambers et al. 1999). The indirect effects of *P. australis* invasion on wetland ecosystems are less clear. Porewater nutrient concentrations are affected by the presence of this invasive species (Chambers 1997; Templer et al. 1998) and studies suggest that *P. australis* removes higher amounts of heavy metals from surface water than native plant species (Bragato et al. 2001; Southichak et al. 2006). Carbon balance studies have determined that wetland areas dominated by *P. australis* can sequester more carbon than areas dominated by native species (Walters et al., in review), and as invaded wetlands mature, they can shift from a source of greenhouse gasses to a sink (Brix et al. 2001). Invasion reduces overall species richness of birds, mammals, and terrestrial insects (Hellings and Gallagher 1992; Benoit and Askins 1999; Chambers et al. 1999). *P. australis* invasion also lowers the abundance of some fish (Able and Hagan 2003) and decreases macroinvertebrate density and richness (Angradi et al. 2001). Conversely, other

research has indicated no measurable impact between *P. australis* and native vegetation for both fish and macroinvertebrate communities (Fell et al. 1998; Able and Hagan 2003; Kulesza et al. 2008).

Based on the aggressive nature of *P. australis* to quickly dominate ecosystems, many managers attempt to control the spread of this plant or completely eradicate it from the system. Dense rhizomes and a strong capacity to translocate nutrients make removal mechanisms such as burning and cutting ineffective. The most effective method in controlling *P. australis* is a combination of herbicide application followed by mechanical removal via mowing or burning (Turner and Warren 2003). Glyphosate, common names Roundup™ and Rodeo™, and imazapyr, common names Habitat™ and Arsenal™, are nonselective herbicides that are most effective against *P. australis*. Both of these herbicides, when used in aquatic ecosystems, are commonly used in an isopropylamine salt form, which increases absorption capacity. Death of the plant takes approximately 1–2 weeks, but the shoots may remain standing for over a year (Kulesza and Holomuzki 2006). Major *P. australis* removal projects have been active along the east coast of the USA (Findlay et al. 2003) and coastal areas near Lake Erie (Kulesza et al. 2008). In Nebraska, state agencies are attempting to eradicate *P. australis* and other invasive species along the riparian corridors of the Republican and Platte Rivers. Invasive species removal in the Republican River basin is intended to increase peak flows in the river, while along the Platte River, the primary goal of invasive species (mainly *P. australis*) is to restore stopover habitat necessary for migratory birds. However, these intense management projects are being completed with limited consideration of the potential water quality or ecological impacts.

The objective of this study is to measure short-term nutrient loss following *P. australis* removal using simulated rain events. Plants were grown in controlled soil mesocosms that forced excess water to either runoff the soil surface or drain through the soil profile. We hypothesized that the most aggressive removal technique (above and belowground biomass removal) would result in the highest amount of nutrient export. Also, we hypothesized that the majority of ammonium (NH_4^+) and soluble reactive phosphorus (SRP) would be exported via surface runoff while the majority of nitrate (NO_3^-) would be exported via leachate. We expect this study to provide a framework for discussion of the pathways of nutrient export, as well as the

most effective means of minimizing nutrient loss when removing invasive species such as *P. australis*.

2 Methods

2.1 Plant Mesocosms

Mature *P. australis* plants were extricated on 12 September 2007 from the littoral zone of Holmes Lake in southwest Lincoln, NE, USA. Thirty containers (approximately 30 cm tall and had a diameter of 30 cm) were filled with a silt loam top soil to a depth of 15 cm. Plants were established in each container (one plant per container) and these containers were then placed in a greenhouse on the University of Nebraska campus. Day and night temperatures in the greenhouse were set to $25\pm 2^\circ\text{C}$ and $20\pm 2^\circ\text{C}$, respectively, and relative humidity was maintained at $45\pm 5\%$ during the study. Containers were lined with plastic and had nylon barbs installed at the soil surface and at the bottom of the soil profile. The barbs were plugged, and the plants received approximately 500 mL of water every 3–5 days as they acclimated over an 8-week period in the greenhouse. This watering regime in combination with the plastic liner and plugged barbs resulted in fully saturated soils conditions and were done in part to mimic riparian conditions. Plants continued to receive this watering routine until the beginning of the experiment (i.e., the first simulated rain event). Growth on all the plant mesocosms was apparent, as new green material and shoots were observed during the 8-week period.

After 7 weeks, the 30 mesocosms were randomly divided into the following five subsets with six replicate mesocosms in each subset: (1) initial soil conditions, (2) control, (3) above and belowground biomass removal treatment (hereafter referred to as biomass removal), (4) herbicide application treatment (hereafter referred to as herbicide), and (5) basal cut treatment. Eight days before the first simulated rain event, the herbicide treatment was sprayed with Roundup™ Ready-to-Use herbicide (2% glyphosate, isopropylamine salt). The aboveground biomass of the plants was saturated with the herbicide according to directions and the herbicide was given 1 week to effectively kill the plants. One day before the simulated rain event, the biomass removal treatment had all plant material removed from the soil mesocosms, and the

basal cut treatment had all the plants cut approximately 5 cm above the soil surface. Immediately prior to the first simulated rain event, the initial soil conditions subset was cored instead of receiving a simulated rain event to determine the extractable nutrients present in the soil prior to any simulated rain events taking place. Finally, the control received no plant manipulations prior to the first simulated rain event and vegetation was left intact.

2.2 Simulated Rain Event

Simulated rain events took place inside the greenhouse and followed the design developed by Humphry et al. (2002). As originally intended, the rain simulator was designed to be a portable field unit; but for this study, we adapted the design to accommodate a more permanent greenhouse setting following the specifications set forth by Humphry et al. (2002). A standard garden hose was used, and water was first passed through inline redundant carbon filters to remove large particles (Fig. 1a), and then through a low-pressure regulator set to 4.1 psi (Fig. 1b). A Spraying Systems Fulljet HH50WSQ nozzle was attached at a height of 3 m (Fig. 1c). This specific nozzle under a constant pressure of 4.1 psi produces a rain event of 70 mm h^{-1}

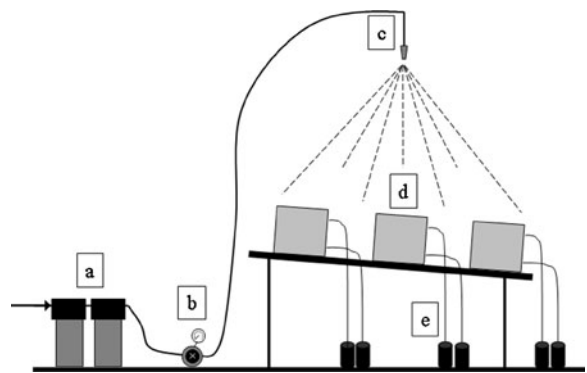


Fig. 1 Diagram showing the design of the simulated rain events that was adapted from Humphry et al. (2002). Using a standard garden hose, the flowpath of water was as follows: *a* initial treatment with redundant carbon filters to remove large particles, *b* a low pressure regulator set to 4.1 psi, *c* a Spraying Systems Fulljet HH50WSQ nozzle at a height of 3 m to create a 70 mm h^{-1} rain event, *d* soil mesocosms with barbs attached at the soil surface to export runoff and barbs at the bottom of the soil profile to export leachate, *e* tubing to deliver runoff and leachate to 1 L high-density polyethylene graduated bottles covered with parafilm

over a 1.5×2.0 m area (Humphry et al. 2002). This intensity was chosen because it produced runoff over a relatively short time period (~5 min) in all soil mesocosms and has a recurrence interval of less than 1 year in Lincoln, NE, USA. The soil mesocosms were placed ($n=6$), one treatment at a time, within a 1.5×2.0 m area beneath the nozzle (Fig. 1d). Each treatment received a 10-min duration rain event, after which the nozzle was moved to a different treatment. A total of three simulated rain events were conducted, each separated by 72 h (i.e., simulated rain events took place on days 1, 4, and 7). During the 72-h period, no water was administered to any treatment. Tubing was run from the barbs attached to each soil mesocosm down to 1-L high-density polyethylene graduated bottles covered with parafilm (Fig. 1e). One bottle captured runoff water, while the other bottle captured leachate water from each mesocosm. Therefore, the control and each treatment had six runoff samples collected and six leachate samples collected during each simulated rain event. Thirty minutes after the completion of each event, the total volume of water in each bottle (runoff and leachate) was recorded, and the water was filtered through a 0.45 μm membrane filter. Samples were stored at 4°C until water chemistry analyses could be performed (total hold time for water samples was less than 14 days).

2.3 Water and Soil Analysis

Water samples were analyzed for NH_4^+ , NO_3^- , and SRP using a Lachat QuikChem 8500. From each mesocosm, a mass export was determined by multiplying the nutrient concentration by the corresponding volume of runoff or leachate.

The extractable nutrients present in the soil prior to any simulated rain events occurring was determined by sacrificing one subset of soil mesocosms. The soil sampling procedure was conducted using the following protocol. In each mesocosm, three cores (1.75 cm in diameter) were randomly taken from the top 10 cm (after the 8-week incubation period the approximate level of soil in all mesocosms was 10 cm) and homogenized. Homogenized soil from each mesocosm was then sampled and used for extractable nutrient analyses. Mean values from this subset of mesocosms ($n=6$) were used to determine the soil conditions prior to any treatment effects or simulated rain events. Soil extractions were conducted to estimate plant available NH_4^+ , NO_3^- ,

and P following the methods of Craft and Chiang (2002). To determine plant available NH_4^+ and NO_3^- , 3 g of soil were extracted with 30 mL of 2 M potassium chloride. To determine plant available P, 3 g of soil were extracted with 30 mL of 0.5 M sodium bicarbonate. Soils and extract were shaken for 2 h, and the supernatant was filtered with 0.45 μm membrane filters and analyzed using a Lachat QuikChem 8500. Immediately after the final simulated rain event, the soil from each mesocosm was sampled using the same protocol as above. Post-experimental soil conditions for the control and treatment subsets were then based on mean values from all six replicate mesocosms. Extractable nutrient concentrations were multiplied by total soil weight from each mesocosm to determine the mass of extractable nutrients present in each mesocosm.

Soil cores were also taken from the initial soil conditions subset to determine physical and chemical soil characteristics. Bulk density ($1.16\pm 0.04 \text{ g cm}^{-3}$) was measured from 10 cm cores (1.75 cm in diameter), soil pH (6.7 ± 0.1) was measured on a slurry of 3 g of soil and 30 mL of deionized water, and organic matter content ($2.31\pm 0.06\%$) was measured using the loss on ignition method following Schulte and Hopkins (1996) from each mesocosm in the initial soil conditions subset ($n=6$).

2.4 Statistics

Individual datasets passed tests for normality and heterogeneity of variances. All values reported in this study represent the mean ± 1 SD. Analysis of variance (ANOVA) was run to determine differences in water volume export pathway, nutrient export pathway, total nutrient export, and changes in extractable nutrients among the control and the various treatments, and pairwise comparisons were made using the Holms–Sidak test and SigmaStat 3.1 ($\alpha=0.05$). To detect significant differences in daily nutrient export, repeated measures ANOVA was used, and pairwise comparisons were made using the Holms–Sidak test and SigmaStat 3.1 ($\alpha=0.05$).

3 Results

3.1 Water Export

In terms of water volume, the simulated rain events showed no significant differences among the control

and the various treatments, both for surface runoff and leachate. However, over twice as much water was exported via runoff ($2,270 \pm 660$ mL) compared to leachate (980 ± 590 mL; $F_{1,47} = 52.772$; $p < 0.001$). Pairwise comparisons also revealed that each of the treatments showed significantly more export of water via runoff as compared to leachate ($\alpha = 0.05$, Table 1).

3.2 Daily Nutrient Export

Comparison of treatments during the three simulated rain events showed significant differences in the export of NH_4^+ ($F_{2,71} = 20.187$; $p < 0.001$) and NO_3^- ($F_{2,71} = 3.380$; $p = 0.041$), but not in the export of SRP (Fig. 2). Specifically, NH_4^+ export was highest during the first rain event (day 1) compared to the last (day 7) for all the vegetation removal treatments (i.e., biomass removal, herbicide, and basal cut; Fig. 2). This general trend of NH_4^+ export, however, was not observed in the control treatment (Fig. 2). For NO_3^- export, significant differences were only observed in the basal cut treatment. Within that treatment, NO_3^- export was lowest during the first rain event (4.5 ± 2.2 mg N) compared to the second event (16.2 ± 9.8 mg N) and third event (11.8 ± 6.9 mg N; Fig. 2).

3.3 Total Nutrient Export

In order to examine cumulative treatment effects on nutrient export throughout the entire experiment, daily export was summed for both export pathways (i.e., leachate and runoff) and all three rain events to determine a total nutrient export value. Statistics were then run on total nutrient export values (Fig. 3). Significant differences in total nutrient export were observed in many treatments for NH_4^+ ($F_{3,23} = 25.555$; $p < 0.001$), NO_3^- ($F_{3,23} = 5.158$; $p < 0.001$), and SRP export

Table 1 Average volume of water (milliliter) collected for the three simulated rain events ($n = 18$) from each export pathway (i.e., runoff or leachate)

	Control (mL)	Biomass removal (mL)	Herbicide (mL)	Basal cut (mL)
Runoff	620±173	842±289	784±191	776±206
Leachate	230±77	454±237	362±255	266±114

In all treatments, significantly higher amounts of water were exported as runoff compared to leachate ($\alpha = 0.05$)

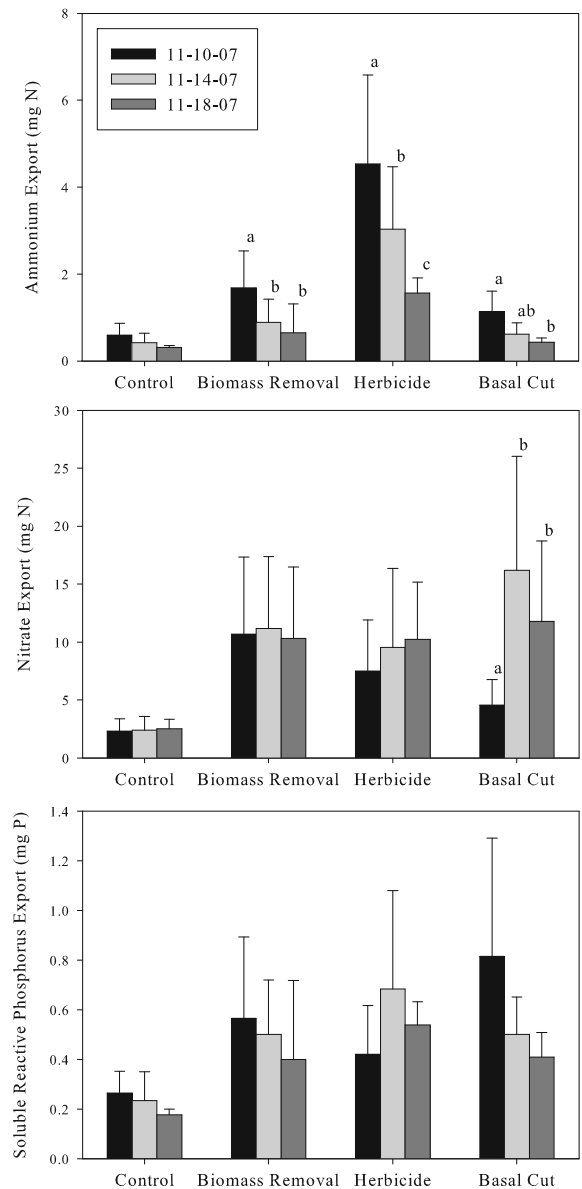


Fig. 2 Total export (i.e., runoff + leachate) of ammonium, nitrate, and soluble reactive phosphorus for each treatment ($n = 6$) during the three simulated rain events. Statistics were run on data within each treatment to determine if different levels of nutrient export occurred during each of the different rain events. Data represent mean ± 1 SD and bars with different letters are significantly different ($\alpha = 0.05$)

($F_{3,23} = 4.939$; $p = 0.010$; Fig. 3). In regards to NH_4^+ , the control (1.3 ± 0.5 mg N), basal cut (2.2 ± 0.8 mg N), and biomass removal treatments (3.2 ± 1.2 mg N) exported similar magnitudes while the herbicide treatment (9.1 ± 3.1) exported almost three times as much NH_4^+ as the next closest treatment (i.e., biomass

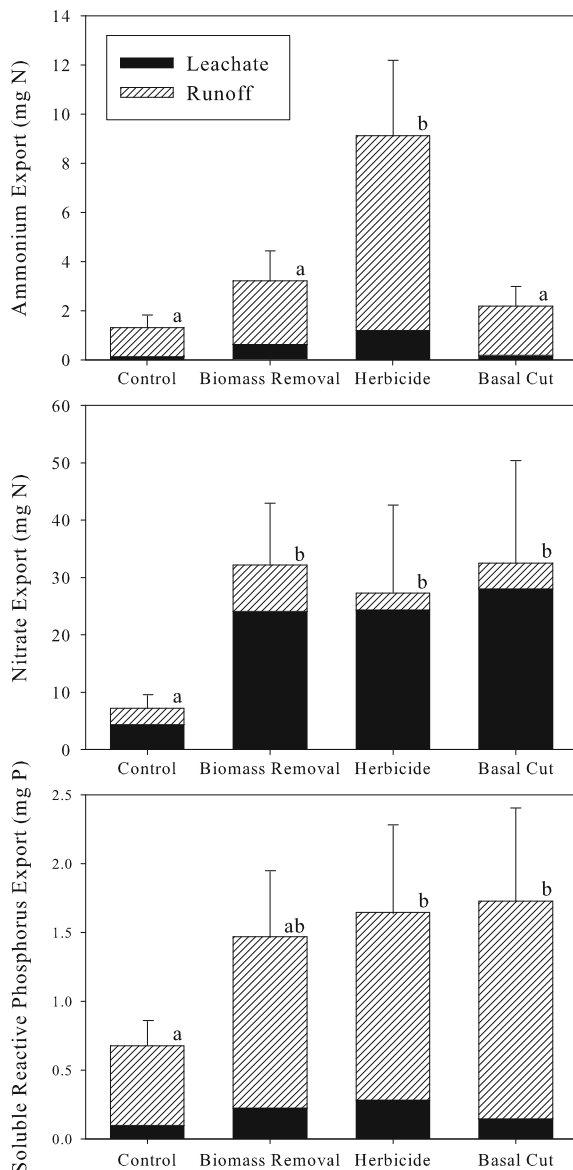


Fig. 3 Export of ammonium, nitrate, and soluble reactive phosphorus for each treatment ($n=6$) summed for all three simulated rain events. The striped portion of the bar represents runoff and the solid portion of the bar represents leachate. Statistics were run on total export data (i.e., runoff + leachate) to determine if different levels of nutrient export occurred among the treatments over the course of the entire experiment. Data represent mean \pm 1 SD and bars with different letters are significantly different ($\alpha=0.05$)

removal; Fig. 3). Nitrate export was lowest in the control treatment (7.2 ± 2.1 mg N) compared to the biomass removal, herbicide, and basal cut treatments (32.2 ± 10.8 , 27.3 ± 15.2 , and 32.5 ± 17.8 mg N, respectively; Fig. 3). Comparing the mean export of NO_3^-

(24.8 ± 16.0 mg N) to NH_4^+ (4.0 ± 3.5 mg N) across the control and all three treatments revealed that export of NO_3^- was over six times higher than NH_4^+ . Export of SRP was lowest in the control (0.7 ± 0.2 mg P) and biomass removal treatment (1.5 ± 0.5 mg P) compared to the herbicide (1.6 ± 0.6 mg P) and basal cut treatments (1.7 ± 0.7 mg P; Fig. 3).

3.4 Nutrient Export Pathway

To examine differences in export pathways (i.e., runoff vs. leachate), data were summed for all three rain events, and statistics were run to determine if nutrient export was different (within each treatment) between runoff and leachate (Table 2). Significant differences were observed in the export pathway of NH_4^+ ($F_{1,47}=61.656$; $p<0.001$), NO_3^- ($F_{1,47}=33.431$; $p<0.001$), and SRP ($F_{1,47}=110.027$; $p<0.001$). Specifically, across the control and all treatments surface runoff of NH_4^+ (3.4 ± 2.9) and SRP (1.2 ± 0.6 mg P) was higher than the leachate of NH_4^+ (0.5 ± 1.0) and SRP (0.2 ± 0.2 mg P; Table 2). The opposite was true for NO_3^- , however, as the mean of all the treatments except the control (which showed no statistical difference) revealed that leachate (25.5 ± 14.8 mg N) was the dominant pathway compared to runoff (5.2 ± 3.5 mg N; Table 2).

3.5 Extractable Nutrients

Analyses revealed that prior to treatments and simulated rain events, the concentration of soil extractable NH_4^+ , NO_3^- , and P were 25.8 ± 11.9 $\mu\text{g N g}^{-1}$, 18.0 ± 8.9 $\mu\text{g N g}^{-1}$, and 2.5 ± 0.7 $\mu\text{g P g}^{-1}$, respectively. Upon completion of the simulated rain events, soil cores were taken from the control and each of the treatments and concentrations were compared against the initial soil conditions. After converting concentration to a mass of extractable nutrients by multiplying by the total mass of soil in each mesocosm, statistics were run to determine if any significant differences were observed. Overall, the data revealed that soil extractable NH_4^+ decreased by 191.9 ± 12.0 , 182.4 ± 12.7 , 185.0 ± 16.3 , and 184.7 ± 15.4 mg N in the control, biomass removal, herbicide, and basal cut treatments, respectively, during the course of the experiment (an 85% reduction from initial conditions), but no significant differences were observed among the control and any of the treatments (Fig. 4). Significant differences were observed in the change in soil extractable NO_3^-

Table 2 Nutrient export data by pathway (i.e., runoff and leachate) summed over all three rain simulations ($n=6$)

Treatment	Ammonium (mg N)		Nitrate (mg N)		Soluble reactive phosphorus (mg P)	
	Runoff	Leachate	Runoff	Leachate	Runoff	Leachate
Control	1.19±0.46 a	0.13±0.08 b	2.83±0.93 a	4.37±1.81 a	0.58±0.16 a	0.10±0.04 b
Biomass removal	2.58±1.13 a	0.64±1.08 b	8.03±4.80 a	24.14±13.07 b	1.24±0.57 a	0.22±0.21 b
Herbicide	7.93±2.04 a	1.19±1.61 b	2.96±0.92 a	24.33±16.04 b	1.36±0.56 a	0.28±0.36 b
Basal cut	2.00±0.75 a	0.19±0.18 b	4.50±1.00 a	28.02±17.42 b	1.58±0.74 a	0.14±0.11 b

Data represent mean±1 SD, and statistics only analyze the difference between runoff and leachate within each treatment. Data with different letters are significantly different ($\alpha=0.05$)

($F_{3,23}=8.653$; $p<0.001$). In particular, soil in the control (51.5±53.4 mg N) and biomass removal treatment (52.2±15.8 mg N) experienced decreases, the basal cut treatment showed no change (0.2±40.6 mg N), and soil extractable NO_3^- actually *increased* in the herbicide treatment (36.9±21.3 mg N) over the course of the experiment (Fig. 4). Across the control and all treatments, the decrease in extractable NH_4^+ ranged from 155.5 to 204.4 mg N while extractable NO_3^- ranged from a decrease of 111.0 mg N to an increase of 58.3 mg N. A decrease in soil extractable P was observed in all treatments over the course of the experiment (Fig. 4). Although no statistical significance was detected at the 5% level, test statistics ($F_{3,23}=2.479$; $p=0.091$) suggest that the biomass removal, herbicide, and basal cut treatments most likely had a larger decrease in soil extractable P (5.4±0.7, 4.8±1.9, and 4.2±2.5 mg P, respectively) than the control (2.7±1.4 mg P; Fig. 4).

3.6 Inorganic Nitrogen

If nutrient export was controlled by physical processes and no additional input of nutrients occurred in any of the removal treatments, then the magnitude of the decrease in extractable nutrients should have been similar to the magnitude of nutrient export observed in leachate and runoff. A comparison of the decrease in soil extractable inorganic nitrogen (i.e., extractable NH_4^+ +extractable NO_3^-) with the amount of inorganic nitrogen exported via leachate and runoff (i.e., NH_4^+ export in leachate and runoff + NO_3^- export in leachate and runoff) revealed that significant amounts of inorganic nitrogen were unaccounted for in all treatments (Table 3). Specifically, the control (234.9±50.6 mg N) and the biomass removal treatment (199.1±14.3 mg N) had the highest amount of

unaccounted inorganic nitrogen, while the herbicide treatment (111.6±40.4 mg N) had the least amount of unaccounted inorganic nitrogen (Table 3; $F_{3,23}=9.378$; $p<0.001$). The basal cut treatment (149.9±55.9 mg N) had a similar amount of unaccounted for inorganic nitrogen as the herbicide and the biomass removal treatment but a lower amount than the control (Table 3).

4 Discussion

4.1 Inorganic Nitrogen

If physical action was the only mechanism that could remove nutrients from the soil mesocosms, then the decrease in extractable inorganic nitrogen observed from the soil should be equal to the amount of inorganic nitrogen exported in the leachate and surface runoff following the simulated rain events. Our mass balance revealed that there was a large difference between the decrease in extractable inorganic nitrogen and inorganic nitrogen exported via leachate and runoff. We do recognize that changes in soil extractable inorganic nitrogen were calculated using 2 M KCl as an extractant while export values were based on essentially water extractions. Thus, it is unrealistic to expect perfect closure in our mass balance, but by comparing the control and the vegetation removal treatments, we can make conjectures about the mechanisms that were occurring in the soil mesocosms during the experiment. Possible biological mechanisms that could have caused higher unexplained inorganic nitrogen in our mass balance (i.e., processes that would remove inorganic nitrogen from the soil of the mesocosms causing larger values of unaccounted

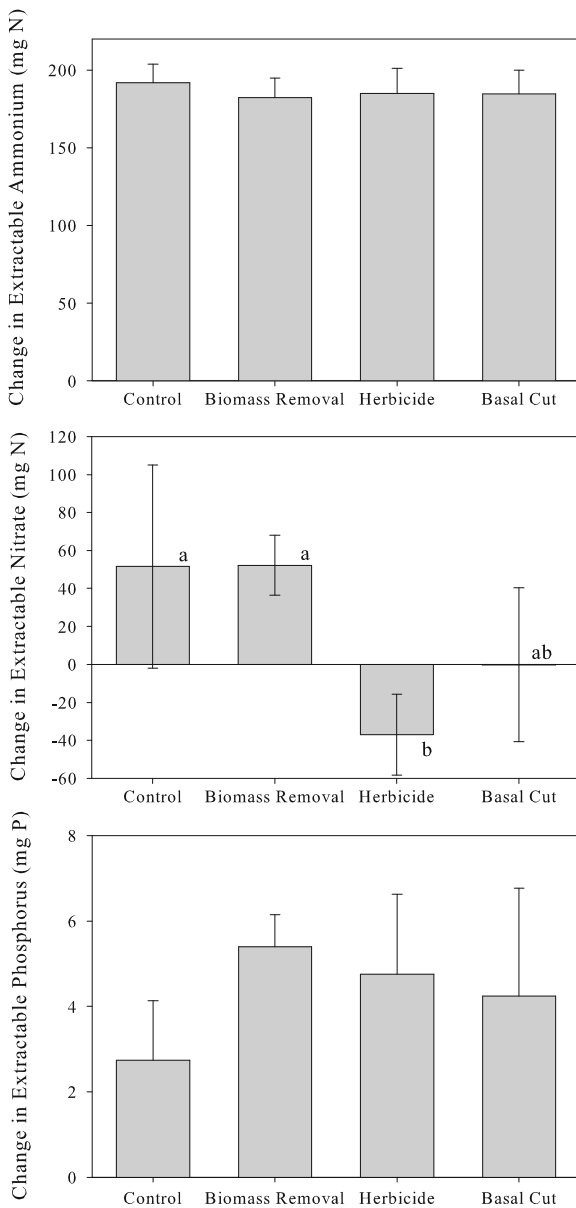


Fig. 4 Change in soil extractable ammonium, nitrate, and phosphorus. Changes in extractable nutrients were determined by taking the difference between the amount of soil extractable nutrients in the initial soil conditions subset (i.e., subset of mesocosms sacrificed prior to treatments and simulated rain events) and the amount of extractable nutrients remaining in the control and each treatment after all three simulated rain events had occurred ($n=6$). Positive values represent a decrease, while negative values represent an increase in extractable nutrients over the duration of the experiment. Data represent mean \pm 1 SD and bars with different letters are significantly different ($\alpha=0.05$)

for inorganic nitrogen in our mass balances) could be microbial immobilization, plant assimilation, and

denitrification. Site characteristics such as vegetation type, elevation, topography, and soil conditions such as organic carbon content, temperature, and moisture can all affect biological nitrogen transformations (Garten and Van Miegroet 1994; Garten et al. 1994). Thus, riparian soils where *P. australis* is typically found commonly have adequate organic carbon and undergo frequent periods of inundation and have a strong potential for biological nitrogen transformations. On the other hand, processes that could have been creating lower unexplained inorganic nitrogen in our mass balances (i.e., processes adding inorganic nitrogen to the soil of the mesocosms causing smaller values of unaccounted for inorganic nitrogen in our mass balance) could be mineralization, nutrient leakage from plants, and artificial nitrogen additions.

In our study, the majority of the decrease in extractable inorganic nitrogen was in the form of NH_4^+ compared to NO_3^- , yet more NO_3^- compared to NH_4^+ was exported following the simulated rain events. This pattern would suggest that a strong nitrification potential existed, and that NH_4^+ was being converted to NO_3^- . Further, our mass balance revealed that the magnitude of inorganic nitrogen exported was not high enough to account for the decrease in the extractable inorganic nitrogen from the soil. This would suggest that microbial immobilization, plant assimilation or denitrification was removing inorganic nitrogen or plant leakage or some other mechanism was adding inorganic nitrogen to the system. Closer examination revealed that the control treatment (with vegetation intact) and the biomass removal treatment (no vegetation remaining) had similar amounts of unaccounted for inorganic nitrogen. Thus, plant assimilation was most likely not the mechanism removing inorganic nitrogen from the soil mesocosms during our experiment. We also observed a significant treatment effect in our mass balance suggesting that the vegetation removal mechanism affected inorganic nitrogen cycling in the soil mesocosms. Specifically, we observed lower unaccounted for inorganic nitrogen in the herbicide and basal cut treatment compared to the biomass removal treatment. Either immobilization or denitrification was enhanced in the biomass removal treatment, which seems unlikely, or the more plausible explanation was that some other processes were adding inorganic nitrogen in the herbicide and basal cut treatments. Two possible processes that would add inorganic nitrogen would be plant leakage (which

Table 3 Mass balance of inorganic nitrogen ($\text{NH}_4^+ + \text{NO}_3^-$) for both soil extractable inorganic nitrogen and export from both leachate and runoff

	Control (mg N)	Biomass removal (mg N)	Herbicide (mg N)	Basal cut (mg N)
Change in extractable NH_4^+	191.9±12.0	182.4±12.7	185.0±16.3	184.7±15.4
Change in extractable NO_3^-	51.5±53.4	52.2±15.8	-36.9±21.3	-0.2±40.6
Change in extractable inorganic nitrogen	243.4±51.6	234.5±23.6	148.1±30.9	184.6±54.8
NH_4^+ export	1.3±0.5	3.2±1.2	9.1±3.1	2.2±0.8
NO_3^- export	7.2±2.1	32.2±10.8	27.3±15.2	32.5±17.8
Total inorganic nitrogen export	8.5±2.4	35.4±10.3	36.4±17.1	34.7±17.1
Difference between extractable inorganic nitrogen and inorganic nitrogen export	234.9±50.6 ^a	199.1±14.3 ^{a,b}	111.6±40.4 ^c	149.9±55.9 ^{b,c}

Soil extractable values were determined by taking the difference between extractable NH_4^+ and NO_3^- observed in the initial soil conditions and after simulated rain event conditions ($n=6$). Positive values represent a decrease while negative values represent an increase during the course of the experiment. Export values were summed for all three rain events and from both the leachate and runoff pathways ($n=6$). All data represent the mean±1 SD. Statistics were run only on the differences between the two loss mechanisms, and data with different letters are significantly different ($\alpha=0.05$)

could have occurred in both the herbicide and basal cut treatment) or the direct addition of inorganic nitrogen due to the herbicide mixture containing nitrogen (which would have only occurred in the herbicide treatment). The herbicide used in this study contained an isopropylamine (IPA) salt and in the herbicide treatment we observed higher NH_4^+ export compared to other treatments following the simulated rain events. We also suspect that some of this additional NH_4^+ added in the herbicide treatment was nitrified resulting in the increased extractable NO_3^- that we observed (Fig. 4). Root leakage may have also been occurring in the herbicide and basal cut treatments but we did not observe increased NH_4^+ export in the basal cut treatment. If root leakage was a substantial source of additional inorganic nitrogen, then we would have expected to observe increased inorganic nitrogen export in both the herbicide and basal cut treatments. Ultimately, similar to the work of Berger et al. (2009), we found that nitrogen cycling in our soil mesocosms was not simply driven by physical forces, but was a complex interaction between physical and biological mechanisms.

4.2 Herbicide Application

The use of herbicides in late spring has been shown to be the most effective removal mechanism for *P. australis* (Mozdzer et al. 2008). Caution must be taken, however,

to ensure that nontarget organisms are not impacted during a post-emergent treatment (Mozdzer et al. 2008). Both imazapyr and glyphosate have been cleared by the United States Environmental Protection Agency for use in aquatic ecosystems. However, herbicides are typically applied as mixtures in aquatic situations. These mixtures have surfactant additives such as polyethoxylated tallow-amine (POEA) to allow the herbicide to penetrate through the cuticles of plant leaves. Recent research has found that surfactants such as POEA are significantly more toxic than the active ingredient and can be toxic to aquatic organisms at environmentally relevant concentrations (Relyea 2005; Bringolf et al. 2007).

In addition to surfactants, herbicide mixtures will typically contain IPA salt, which allows the active ingredient to remain in an ionic form. One study concluded that IPA alone was toxic to freshwater mussels (Bringolf et al. 2007). The authors further concluded that the explanation for this toxicity was most likely the release of NH_4^+ from the IPA (Bringolf et al. 2007). In agreement with this previous study, we have found that the use of the IPA salt form of glyphosate (Roundup®) resulted in the export of significantly higher amounts of NH_4^+ compared to a control and various vegetation removal mechanisms. To our knowledge, the only other study that has examined nutrient dynamics following an herbicide treatment (Findlay et al. 2003), found significantly higher porewater NH_4^+ concentrations following

the treatment of *P. australis* with Rodeo® (an IPA salt form of glyphosate) in a tidal freshwater wetland. The authors concluded that the absence of the plant or the subsequent decay of plant material may have accounted for the accumulation of NH_4^+ to occur (Findlay et al. 2003). We propose that in addition to plant mineralization, the increase in porewater NH_4^+ observed by Findlay et al. (2003) was also due to the application of the IPA salt form of the herbicide. The lack of discussion regarding herbicides additions and surfactants in the literature may be due, in part, to the frequent changes in inactive ingredients used by pesticide manufacturers. Another possible explanation is based on the fact that pesticides manufacturers are only required to document on the label the active ingredients (i.e., the pesticide) being used in chemical mixtures.

4.3 Implications

Contrary to our hypothesis, the biomass removal treatment did not result in the highest amount of nutrient export following simulated rain events. Regardless of the removal mechanism, a higher amount of NO_3^- and SRP export was observed when vegetation was removed compared to the control treatment (except for SRP export in the biomass removal treatment). In terms of NH_4^+ , however, the herbicide application resulted in the highest amount of export, most likely due to the herbicide mixture containing an IPA salt. Treatment of *P. australis* in aquatic ecosystems such as riparian corridors, lakes, and wetlands of the USA typically consists of herbicide mixtures (either imazapyr or glyphosate) in an IPA salt form. Since this invasive plant is commonly found in standing water, spraying of herbicide mixtures directly into the water (i.e., overspray) is inevitable. In such cases, unionized ammonia (NH_3) could reach toxic concentrations, as has been suggested by Bringolf et al. (2007). Generally, NH_4^+ is nontoxic to aquatic organisms, while NH_3 is toxic to fish (Russo 1985; Constable et al. 2003), macroinvertebrates (Ankley et al. 1995; Schubaur-Berigan et al. 1995; Wang et al. 2008), freshwater mussels (Augspurger et al. 2003; Wang et al. 2008), and bacteria (Russo 1985). The relationship between NH_4^+ and NH_3 is controlled by pH and temperature, and at high pH values ($\text{pK}_a=8.3$) NH_3 becomes the dominant species. If *P. australis* is to be treated with herbicides in an IPA salt form and there is a high likelihood of overspray, then we suggest that the pH of the receiving water must be

analyzed. If pH is high, then there is a strong possibility that NH_3 could reach toxic concentrations and herbicide application should be discouraged.

Our second hypothesis was verified, in that SRP and NH_4^+ was exported via runoff while NO_3^- was exported by leaching through the soil profile. Research on biofilters have documented similar findings, namely that filter systems remove significant amounts of total phosphorus and NH_4^+ but remove little NO_3^- due to a strong capacity for NO_3^- to leach (Davis et al. 2001). Vegetated riparian zones have been shown to reduce the export of nutrients to adjacent aquatic ecosystems (Jordan et al. 1993; Lee et al. 2003; Mankin et al. 2007), yet little research is available on the impacts of riparian vegetation removal (Dosskey et al. 2010). The research that has been conducted documents that riparian vegetation removal results in increased sedimentation which negatively impacts fish communities (Jones et al. 1999), increased solutes in nearby streams (Novdin et al. 1988), and increased groundwater concentrations of NO_3^- (Yeakley et al. 2003). A recent review of riparian vegetation concludes that the overall impact of vegetation removal will vary on large timescales (years to decades) and will also vary depending on site characteristics and the specific nutrients being analyzed (Dosskey et al. 2010). We contend that the removal mechanism also impacts the amount of nutrient flux following riparian vegetation removal. Specifically, we observed that the use of glyphosate in an IPA salt form resulted in an unexpectedly high amount of NH_4^+ export. There is a large potential for the release of nutrients to nearby aquatic ecosystems when riparian vegetation is removed and in particular a large release of NH_4^+ when herbicides in an IPA salt form are used. As discussed previously, this additional nutrient input could cause toxicity issues, or it could also lead to the eutrophication of receiving waters. Inorganic nutrients can trigger enhanced primary production and respiration, which can stress aquatic organisms via low dissolved oxygen (Mallin et al. 2006).

Conclusions from this study are based on simulated rain events conducted in a greenhouse on soil mesocosms. Further research is needed to quantify the effects of herbicide treatments in the field and to examine how in situ treatments may impact aquatic ecosystems. Ultimately, our data analysis suggests that there may be enhanced NH_4^+ export following herbicide treatments when the herbicide is in an IPA salt

form, but further studies are needed to confirm these findings in the field. The original objective of this study was to compare nutrient flux from various removal mechanisms but was not intended to examine specific mechanisms responsible for the flux of nutrients. We stress that future studies quantify the addition of inorganic nitrogen from herbicide treatments and the addition of inorganic nitrogen from root exudates following the death of vegetation.

Acknowledgments Funding for this study was provided by the Nebraska Environmental Trust (NET), the University of Nebraska Water Resources Advisory Panel (WRAP), and the University of Nebraska Rural Initiative. We would also like to thank Stacy Adams in the Department of Agronomy and Horticulture at the University of Nebraska for access to greenhouse space to complete this research.

References

- Able, K. W., & Hagan, S. M. (2003). Impact of common reed, *Phragmites australis*, on essential fish habitat: Influence on reproduction, embryological development, and larval abundance of mummichog (*Fundulus heteroclitus*). *Estuaries and Coasts*, 26(1), 1559–2723.
- Angradi, T. R., Hagan, S. M., & Able, K. W. (2001). Vegetation type and the intertidal macroinvertebrate fauna of a brackish marsh: *Phragmites* vs. *Spartina*. *Wetlands*, 21(1), 75–92.
- Ankley, G. T., Schubauer-Berigan, M. K., & Monson, P. D. (1995). Influence of pH and hardness on toxicity of ammonia to the amphipod *Hyalella azteca*. *Canadian Journal of Fisheries and Aquatic Sciences*, 52(10), 2078–2083.
- Augsburger, T., Keller, A. E., Black, M. C., Cope, G., & Dwyer, F. J. (2003). Derivation of water quality guidance for protection of freshwater mussels (Unionidae) from ammonia exposure. *Environmental Toxicology and Chemistry*, 22(11), 2569–2575.
- Benoit, L. K., & Askins, R. A. (1999). Impact of the spread of *Phragmites* on the distribution of birds in Connecticut tidal marshes. *Wetlands*, 19(1), 194–208.
- Berger, T. W., Inselsbacher, E., Mutsch, F., & Pfeffer, M. (2009). Nutrient cycling and soil leaching in eighteen pure and mixed stands of beech (*Fagus sylvatica*) and spruce (*Picea abies*). *Forest Ecology and Management*, 258(11), 2578–2592.
- Bragato, C., Brix, H., & Malagoli, M. (2001). Accumulation of nutrients and heavy metals in *Phragmites australis* (Cav.) Trin. ex Steudel and *Bolboschoenus maritimus* (L.) Palla in a constructed wetland of the Venice lagoon watershed. *Environmental Pollution*, 144(3), 967–975.
- Bringolf, R. B., Cope, W. G., Mosher, S., Barnhart, M. C., & Shea, D. (2007). Acute and chronic toxicity of glyphosate compounds to glochidia and juveniles of *Lampsilis siliquoidea* (unionidae). *Environmental Toxicology and Chemistry*, 26(10), 2094–2100.
- Brix, H., Sorrell, B. K., & Lorenzen, B. (2001). Are *Phragmites*-dominated wetlands a net source or net sink of greenhouse gases? *Aquatic Botany*, 69(2–4), 313–324.
- Chambers, R. M. (1997). Porewater chemistry associated with *Phragmites* and *Spartina* in a Connecticut tidal marsh. *Wetlands*, 17(3), 360–367.
- Chambers, R. M., Meyerson, L. A., & Saltonstall, K. (1999). Expansion of *Phragmites australis* into tidal wetlands of North America. *Aquatic Botany*, 64(3–4), 261–273.
- Constable, M., Charlton, M., Jensen, F., McDonald, K., Craig, G., & Taylor, K. W. (2003). An ecological risk assessment of ammonia in the aquatic environment. *Human and Ecological Risk Assessment*, 9(2), 527–548.
- Craft, C. B., & Chiang, C. (2002). Forms and amounts of soil nitrogen and phosphorus across a longleaf pine-depressional wetland landscape. *Soil Science Society of America Journal*, 66(5), 1713–1721.
- Davis, A. P., Shokouhian, M., Sharma, H., & Minami, C. (2001). Laboratory study of biological retention (bioretention) for urban storm water management. *Water Environment Research*, 73(1), 5–14.
- Dosskey, M. G., Vidon, P., Gurwick, N. P., Allan, C. J., Duval, T. P., & Lowrance, R. (2010). The role of riparian vegetation in protecting and improving chemical water quality in streams. *Journal of the American Water Resources Association*, 46(2), 261–277.
- Fell, P. E., Weissbach, S. P., Jones, D. A., Fallon, M. A., Zeppieri, J. A., Faison, E. K., et al. (1998). Does invasion of oligohaline tidal marshes by reed grass, *Phragmites australis* (Cav.) Trin. ex Steud., affect the availability of prey resources for the mummichog, *Fundulus heteroclitus* L.? *Journal of Experimental Marine Biology and Ecology*, 222(1–2), 59–77.
- Findlay, S., Groffman, P., & Dye, S. (2003). Effects of *Phragmites australis* removal on marsh nutrient cycling. *Wetlands Ecology and Management*, 11(3), 157–165.
- Garten, C. T., & Van Miegroet, H. (1994). Relationships between soil nitrogen dynamics and natural ¹⁵N abundance in plant foliage from Great Smoky Mountains National Park. *Canadian Journal of Forest Research*, 24(8), 1636–1645.
- Garten, C. T., Huston, M. A., & Thoms, C. A. (1994). Topographic variation of soil nitrogen dynamics at Walker Branch Watershed, Tennessee. *Forest Science*, 40(3), 497–512.
- Hellings, S. E., & Gallagher, J. L. (1992). The effects of salinity and flooding on *Phragmites australis*. *Journal of Applied Ecology*, 29(1), 41–49.
- Humphry, J. B., Daniel, T. C., Edwards, D. R., & Sharpley, A. N. (2002). A portable rainfall simulator for plot-scale runoff studies. *Applied Engineering in Agriculture*, 18(2), 199–204.
- Jones, E. B. D., III, Helfman, G. S., Harper, J. O., & Bolstad, P. V. (1999). Effects of riparian forest removal on fish assemblages in Southern Appalachian streams. *Conservation Biology*, 13(6), 1454–1465.
- Jordan, T. E., Correll, D. L., & Weller, D. E. (1993). Nutrient interception by a riparian forest receiving inputs from adjacent cropland. *Journal of Environmental Quality*, 22(3), 467–473.
- Kulesza, A. E., & Holomuzki, J. R. (2006). Amphipod performance responses to decaying leaf litter of *Phragmites*

- australis* and *Typha angustifolia* from a Lake Erie coastal marsh. *Wetlands*, 26(4), 1079–1088.
- Kulesza, A. E., Holomuzki, J. R., & Klarer, D. M. (2008). Benthic community structure in stands of *Typha angustifolia* and herbicide-treated and untreated *Phragmites australis*. *Wetlands*, 28(1), 40–56.
- Lee, K. H., Isenhardt, T. M., & Schultz, R. C. (2003). Sediment and nutrient removal in an established multi-species riparian buffer. *Journal of Soil and Water Conservancy*, 58(1), 1–8.
- Mallin, M. A., Johnson, V. L., Ensign, S. H., & MacPherson, T. A. (2006). Factors contributing to hypoxia in rivers, lakes, and streams. *Limnology and Oceanography*, 51(1), 690–701.
- Mankin, K. R., Ngandu, D. M., Barden, C. J., Hutchinson, S. L., & Geyer, W. A. (2007). Grass-shrub riparian buffer removal of sediment, phosphorus, and nitrogen from simulated runoff. *Journal of the American Water Resources Association*, 43(5), 1108–1116.
- Meyerson, L. A., Chambers, R. M., & Vogt, K. A. (1999). The effects of *Phragmites* removal on nutrient pools in a freshwater tidal marsh ecosystem. *Biological Invasions*, 1(2–3), 129–136.
- Mozdzer, T. J., Hutto, C. J., Clarke, P. A., & Field, D. P. (2008). Efficacy of imazapyr and glyphosate in the control of non-native *Phragmites australis*. *Restoration Ecology*, 16(2), 221–224.
- Novdin, S. C., Driscoll, C. T., & Likens, G. E. (1988). Soil processes and sulfate loss at the Hubbard Brook Experimental Forest. *Biogeochemistry*, 5(2), 185–199.
- Orson, R. A. (1999). A Paleocological assessment of *Phragmites australis* in New England tidal marshes: changes in plant community structure during the last few millennia. *Biological Invasions*, 1(2–3), 149–158.
- Relyea, R. A. (2005). The lethal impacts of roundup and predator stress on six species of North American tadpoles. *Archives of Environmental Contamination and Toxicology*, 48(3), 351–357.
- Russo, R. C. (1985). Ammonia, nitrite and nitrate. In G. M. Rand & S. R. Petrocelli (Eds.), *Fundamentals of aquatic toxicology* (pp. 455–471). Washington DC: Hemisphere.
- Schubaur-Berigan, M. K., Monson, P. D., West, C. W., & Ankley, G. T. (1995). Influence of pH on the toxicity of ammonia to *Chironomus tentans* and *Lumbriculus variegates*. *Environmental Toxicology and Chemistry*, 14(4), 713–717.
- Schulte, E. E., & Hopkins, B. G. (1996). Estimation of organic matter by weight loss-on-ignition. In F. R. Magdoff et al. (Eds.), *Soil organic matter: Analysis and interpretation* (pp. 21–31). Madison: Soil Science Society of America.
- Southichak, B., Nakano, K., Nomura, M., Chiba, N., & Nishimura, O. (2006). *Phragmites australis*: a novel biosorbent for the removal of heavy metals from aqueous solution. *Water Research*, 40, 2295–2302.
- Templer, P., Findlay, S., & Wigand, C. (1998). Sediment chemistry associated with native and non-native emergent macrophytes of a Hudson River marsh ecosystem. *Wetlands*, 18(1), 70–78.
- Turner, R. E., & Warren, R. S. (2003). Valuation of continuous and intermittent *Phragmites* control. *Estuaries*, 26(2), 618–623.
- Wang, N., Erickson, R. J., Ingersoll, C. G., Ivey, C. D., Brunson, E. L., Augspurger, T., et al. (2008). Influence of pH on the acute toxicity of ammonia to juvenile freshwater mussels (fatmucket, *Lampsilis siliquoidea*). *Environmental Toxicology and Chemistry*, 27(5), 1141–1146.
- Yeakley, J. A., Coleman, D. C., Haines, B. L., Kloeppe, B. D., Meyer, J. L., Swank, W. T., et al. (2003). Hillslope nutrient dynamics following upland riparian vegetation disturbance. *Ecosystems*, 6(2), 154–167.