



Does wet prairie vegetation retain more nitrogen with or without *Phalaris arundinacea* invasion?

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Received 1 September 2004. Accepted in revised form 8 November 2004

Key words: invasive, mesocosm, nitrogen retention, reed canary grass, wetland

Abstract

Elevated nitrogen (N) levels accelerate expansion of *Phalaris arundinacea* L. (reed canary grass), a highly aggressive invader that displaces native vegetation and forms monotypes. Hence, *Phalaris* is commonly presumed to have high nutrient uptake that contributes to higher N retention in a wetland. We compared the capability of wet prairie vegetation with and without invading *Phalaris* under low-N and high-N treatments to (1) accumulate N in plant tissues, (2) retain N in soil and (3) remove N from water flowing through mesocosms. With high-N treatment, above-ground biomass increased by >90% ($P < 0.0004$; yrs. 1–2) and percent total N in above-ground tissues increased by >46% ($P = 0.0005$; yrs. 1–2). Consequently, there was ~3 times as much total N accumulation in above-ground tissue (calculated from biomass and percent total N in tissues) with high-N treatment vs. low-N treatment ($P < 0.0001$; yrs. 1–2). Without invading *Phalaris*, wet prairie vegetation produced over 49% more above-ground biomass ($P \leq 0.022$; yrs. 1–2) and accumulated over 38% more N in its above-ground tissues ($P = 0.009$; yrs. 1–2), compared to invaded mesocosms. The high-N treatment increased concentrations of soil ammonium (NH₄-N) up to 157% ($P = 0.0001$) and soil nitrate (NO₃-N) up to 549% ($P < 0.001$). After N treatments began, we found no differences in total N or NO₃-N in soils ($P > 0.05$) or in concentrations of NH₄-N or NO₃-N released in the discharged water ($P > 0.1$) from wet prairie mesocosms with and without invading *Phalaris*. Soil NH₄-N did not differ between the wet prairie mesocosms with and without *Phalaris* invasions on five dates ($P > 0.05$); the one exception was in August 2004 (27% greater with invasion; $P = 0.02$). Our results from wet prairie mesocosms do not support the presumption that *Phalaris* retains more N than native plant assemblages.

Abbreviations: NH₄-N – ammonium–nitrogen; NO₃-N – nitrate–nitrogen; N – nitrogen

Introduction

The conversion of native wetland vegetation to an invasive species following eutrophication (Green and Galatowitsch, 2002; Kadlec and Bevis, 1990; Kercher and Zedler, 2004; Maurer

and Zedler, 2002; Miao and Sklar, 1998; Windham and Ehrenfeld, 2003) suggests that the invader can take up more nutrients than the displaced resident species. Although aggressive invaders are recommended for use in “treatment” wetlands designed to reduce nutrients from secondary wastewater (Cooper and Findlater, 1990; Hammer, 1989; Kadlec and Knight, 1996), little is known of the comparative ability of native

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assemblages vs. invasive-dominated vegetation to remove nutrients from through-flowing water. We compared N-retention capabilities of wet prairie vegetation as it converted to dominance by a well-known aggressive plant in experimental systems of known nutrient status.

Elevated nutrient levels accelerate the expansion of *Phalaris arundinacea* L. (reed canary grass), a highly aggressive invader of temperate North American wetlands (Galatowitsch et al., 1999), in experimental settings (Kercher and Zedler, 2004; Maurer and Zedler, 2002). Increased N alone facilitates its suppression of native wetland vegetation (Green and Galatowitsch, 2002). Thus, *Phalaris* is presumed to have high N uptake and to increase retention of N within a wetland. Conchou and Fustec (1988) describe *Phalaris* as a “nutrient pump” that is useful in reducing nutrients released to downstream systems if its aerial biomass is harvested before senescence.

Phalaris has been recommended for use in treatment wetlands, because harvesting its above-ground biomass could remove a high proportion of the N entering a wetland (Dubois, 1994). Hurry and Bellinger (1990) found that harvesting *Phalaris* shoots every 3 months from a treatment wetland receiving continual flow of sewage effluent removed nearly $50 \text{ g N m}^{-2} \text{ year}^{-1}$, which approximated 11% of the site’s annual N loading. Bernard and Lauve (1995) report that *Phalaris* accumulated more biomass and N in its tissues in a constructed wetland receiving nutrient-rich landfill leachate than in an unpolluted control site. N uptake approximated 35 g m^{-2} or about 10% of the annual N input to the polluted site. However, evidence that it is capable of greater accumulation of N in its biomass than other wetland plants is lacking (Kao et al., 2003; McJannet et al., 1995). In experimental wastewater treatment wetlands in Sweden, the amount of N in harvested biomass of *Phalaris* did not differ from other grasses (Geber, 2000).

If *Phalaris* accumulates more N in its biomass than other wetland vegetation, it could affect N accumulation in soils and N leakage in discharged waters. Geber (2000) reported that soil and porewater N associated with *Phalaris* in treatment wetlands were low throughout the growing season and speculated that the risk of N leakage in discharged waters was low. No comparisons were made of soil and porewater N

associated with other species in the wetland (ibid.). Invasive species can alter soil N, but patterns and degrees of change vary among species, for the same species at different sites, and among ecological processes (Ehrenfeld, 2003; Meyerson et al., 2000; Otto et al., 1999; Templer et al., 1998; Windham 2001). Few studies examine changes in the leaching of N from wetlands dominated by invasive species (Ehrenfeld, 2003). Yet, if wetlands are to be restored to reduce N loading locally and downstream (e.g., from the US Midwest to the Gulf of Mexico; Hey, 2002; Mitsch et al., 2001), it will be prudent to encourage growth of species that can remove large amounts of N. Managers will need to know if native species or invasive macrophytes remove more N.

Phalaris commonly invades wetlands in disturbed landscapes impacted by urban development and changes in hydraulic conditions (Galatowitsch et al., 2000; Kercher et al., 2004; Werner and Zedler, 2002). Although this clonal C3 grass is considered a native species to North America, cultivars were extensively introduced from northern Europe for streambank erosion control and pasture forage (Galatowitsch et al., 1999; Merigliano and Lesica, 1998). In the 1950s, Curtis (1959) documented *Phalaris* in wet prairies and sedge meadows in southern Wisconsin. Currently, it dominates (>50% cover) over 40,000 ha of southern Wisconsin wetlands (Bernthal and Willis, 2004). Understanding if there is a tradeoff of higher N retention when *Phalaris* displaces native species becomes critical when deciding to control its invasions or justify its use in treatment wetlands.

We used experimental mesocosms to test the assumption that wet prairie vegetation retains more N after invasion by *Phalaris*. Mesocosms have been used to demonstrate how plant species richness affects phosphorus leakage in water (Engelhardt and Ritchie, 2001). They are a useful tool for comparing N retention, because N inputs and hydrologic loading and retention times can be regulated without the extensive structures that would be required in the field. Controlling hydrologic conditions is important because frequency and duration of flooding affects N removal in wetland systems (Busnardo et al., 1992). We tested the assumption with experimental systems that had well-established wet prairie vegetation and followed responses in the 2nd and 3rd years

after the invader was introduced. We hypothesized that experimental systems with wet prairie vegetation would show:

- (1) Greater accumulation of N in biomass with *Phalaris* invasion.
- (2) Greater total N and inorganic N in soil with *Phalaris* invasion.
- (3) Less release of inorganic N to water flowing through the mesocosms with *Phalaris* invasion.

Materials and methods

Experimental design

A two-factor experiment that varied N addition and vegetation type used 20 mesocosms arranged in a randomized complete block design. The mesocosms were imbedded in a large outdoor experiment that tested the relationship and interactions of disturbances on *Phalaris* invasions (Kercher and Zedler, 2004). In the larger experiment, five blocks, double rows of 16 mesocosms running north to south, differed in shade cast by trees along the eastern edge

of the University of Wisconsin Arboretum facility. We compared N retention capabilities of species-rich wet prairie vegetation with no *Phalaris* (resident treatment) and *Phalaris*-dominated (invasion treatment) vegetation under high- and low-N additions. Each treatment was replicated once per block and was randomly assigned within each block of the larger experiment.

On May 19, 2000, black plastic oval-shaped stock tanks measuring 1.25 m × 0.92 m × 0.65 m deep with approximately 1.1 m² in surface area (Freeland Industries, Portage, Wisconsin, USA) were filled to a depth of 15 cm with locally quarried, screened sandstone (St. Peter's sandstone) and topped with 30 cm of screened, pulverized loamy topsoil from a supplier in Verona, Wisconsin, USA. Twenty-five species were selected to represent a wet prairie assemblage from southern Wisconsin (Curtis, 1959). On July 4, 2000, after 2 months of cold-moist stratification, seeds were sown at a rate of 650 seeds m⁻² in each mesocosm. Four grasses comprised 50% of the total number of seeds and 21 forbs and sedges comprised the remainder. Mesocosms were watered daily and weeded four times in 2000 and

Table 1. Mean percent of intercepts ± 1 S.E. for each species in the 20 mesocosms from the line intercept sampling in June 2001 and June 2002 prior to treatment initiation

Species planted	Percent of intercepts	
	June 2001	June 2002
Graminoids		
<i>Agrostis gigantea</i> Roth ^a	28.5 ± 0.9	27.5 ± 0.8
<i>Andropogon gerardii</i> Vitman	17.2 ± 1.2	17.4 ± 1.3
<i>Glyceria striata</i> (Lam.) A.S. Hitchc.	7.8 ± 1.1	4.3 ± 0.8
<i>Spartina pectinata</i> Bosc ex Link	2.6 ± 0.5	4.2 ± 0.9
<i>Phalaris arundinacea</i> L.	0.0	3.4 ± 0.8
<i>Carex vulpinoidea</i> Michx.	1.5 ± 0.4	2.5 ± 0.4
Forbs		
<i>Desmodium canadense</i> (L.) DC.	10.7 ± 1.8	10.3 ± 1.4
<i>Verbena hastata</i> L.	14.1 ± 1.2	6.9 ± 0.8
<i>Silphium perfoliatum</i> L.	6.5 ± 0.9	6.0 ± 0.9
<i>Symphyotrichum novae-angliae</i> (L.) Nesom	3.0 ± 0.5	4.5 ± 0.8
<i>Helenium autumnale</i> L.	3.2 ± 0.5	4.4 ± 0.6
<i>Pycnanthemum virginianum</i> (L.) T. Dur. & B.D. Jackson ex B.L. Robins & Fern.	1.0 ± 0.5	1.7 ± 0.4
<i>Asclepias incarnata</i> L.	2.6 ± 0.5	1.6 ± 0.3

Species were seeded into the mesocosms in July 2000, except for *Phalaris* which was introduced as small plants and seeds in May and June of 2002. Species with a mean percent of intercepts < 1.0 for both sampling episodes are excluded from this table. Nomenclature follows the USDA, NRCS (2002).

^aThe wet prairie seed mix sown in Summer 2000 was intended to have *Calamagrostis canadensis*, but when the plants flowered in 2001, the grass provided by the seed company was identified as *Agrostis gigantea*, a naturalized species native to Eurasia (Fassett, 1951).

2001. By June 2002, the mesocosms contained a 2-year-old diverse wet prairie assemblage composed of native and one naturalized species (Table 1).

Treatments

Phalaris arundinacea L. seeds (Olds Seed Solutions, Madison, Wisconsin, USA) were sown in flats on March 7, 2002, transplanted to plug trays 4 weeks later and grown in a greenhouse. On May 21, 2002, we transplanted four 10-week-old *Phalaris* plants into each of the 10 mesocosms randomly selected for invasions, placing them 20 cm from the adjacent “corner” walls at a depth of 10 cm, covering the roots. On June 5, 2002, *Phalaris* was seeded in the same mesocosms at a rate of 11 g m^{-2} (twice the supplier’s recommended rate) and the wet prairie species were clipped at the soil level to facilitate *Phalaris* germination (Lindig-Cisneros and Zedler, 2001, 2002) and expansion of the young transplants (Maurer and Zedler, 2002; Morrison and Molofsky, 1998). The remaining 10 mesocosms, which retained the resident vegetation, received equalized soil disturbance but no *Phalaris* introductions. Clipping the resident treatment would have risked loss of native species, thereby jeopardizing the experimental comparison of species-rich vs. *Phalaris*-dominated vegetation.

We applied Forever Green Lawn Builder Turf Food (Eau Claire Crop Oil Company, Eau Claire, Wisconsin, USA), containing 27.0% N (1.2% $\text{NH}_4\text{-N}$; 25.8% urea-N), 3% P_2O_5 , 4% K_2O , 1% S, and 1% Fe, to 10 of the mesocosms, of which 5 were invasion and 5 were resident treatments. The nutrient treatment matched the recommended rate of the fertilizer manufacturer and totaled 14.2 g N , 1.6 g P , and 2.1 g K m^{-2} in 2002. The remaining 10 mesocosms received no nutrient additions. Between June 17 and September 23, 2002 mesocosms were flooded on an intermittent cycle with 2 days flooding followed by 12 days of draw-down during which mesocosms were watered as needed. The flooding regime was based on hydrograph data showing flashy hydroperiods in stormwater basins in southern Wisconsin and northern Illinois (Miller, 2001; Veltman, 2002). The intermittent flooding did not reduce species richness of the wet prairie vegetation (Kercher and Zedler, 2004). *Phalaris* expanded in the invasion treatments during the 2002 growing season.

Because conversion to a *Phalaris* monotype was incomplete in 2002, additional protocols were applied in 2003 to facilitate its dominance in the invasion treatment. Standing dead material was removed from all 20 mesocosms in February 2003 to equalize growing conditions. On April 23, 2003, the invasion treatments were sown with *Phalaris* seeds at a rate of 11 g m^{-2} . On May 28–29, 2003, we clipped the wet prairie species to facilitate the spread of *Phalaris* clones, as in 2002, and we added 8-week-old *Phalaris* transplants that were grown in the greenhouse. Nine *Phalaris* transplants were evenly spaced along three transects within each mesocosm. Clipped vegetation was cut into small pieces and distributed along the outer edge of each mesocosm to retain each system’s N. The resident treatment received equalized soil disturbance but no *Phalaris* introductions. Vegetation in the resident treatment was unclipped in May 2003 to retain native species. Standing dead material was removed from all 20 mesocosms in February 2004 to equalize growing conditions, but no protocols were applied to induce *Phalaris* dominance in 2004.

The fertilizer used in 2002 was replaced with a granular ammonium–nitrate fertilizer (Royster-Clark Inc., Norfolk, Virginia, USA) applied at high ($48 \text{ g N m}^{-2} \text{ year}^{-1}$) and low ($12 \text{ g N m}^{-2} \text{ year}^{-1}$) treatment levels in 2003 and 2004. The high rate induces *Phalaris* dominance and is representative of the nitrate levels common in agricultural landscapes (Green and Galatowitsch, 2002). The N treatment was divided into five applications per year and added on June 2, June 16, June 30, July 14 and July 28, 2003 and on May 31, June 14, June 28, July 12 and July 26, 2004. The high-N treatment was applied to the mesocosms that received nutrients in 2002; the low-N treatment was applied to mesocosms that had no nutrient addition in 2002. During each N application, mesocosms were flooded for 2 days (75 L of water), followed by 12 days during which the mesocosms were drained but watered as needed.

Data collection

Species composition. We used a line intercept method to sample the vegetation within each mesocosm and calculate similarity among treatments. On June 4–7, 2001, June 3–4, 2002, September 18–24, 2002, August 5–7, 2003, and July 29–30, 2004,

two 1-m long dowels marked at 10-cm intervals were placed parallel to the long axis of the mesocosm 30 cm from the sides (32 cm apart) and equidistant from the ends. We recorded the total number of intervals intercepted by each species along each line and calculated the proportion of intervals intercepted by each species within a mesocosm. Similarity was calculated as

$$S = \sum \min(p_{1i}, p_{2i})$$

where p_{1i} is the average proportion of intervals intercepted by species i in treatment 1 and p_{2i} is the average proportion of intervals intercepted by species i in treatment 2 (Whittaker, 1975).

Soil. We collected four soil cores (2.54 cm diameter \times 10 cm deep) from each mesocosm on May 29, August 13 and October 29, 2003 and on June 3, July 29, August 17, and September 30, 2004. Homogenized composite soil samples for each mesocosm were analyzed by the University of Wisconsin Soil and Plant Analysis Lab for concentrations of extractable $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ in 2003 and 2004 and total N in 2004. Dried and ground samples from 2003 were analyzed for total N using a LECO CNS 2000 Elemental Combustion Analyzer (St. Joseph, Michigan, USA). Soil samples were analyzed for pH using a Barnant 20 Digital pH/mV/ORD meter (Barrington, IL, USA) and soil moisture by weight loss after oven drying for 48 h at 110 °C.

We measured soil redox potential on May 30, 2003, prior to treatment initiation and on August 12 and October 28, 2003 and on June 1, July 27, August 16, and September 29, 2004, when the mesocosms were flooded prior to water sampling. Redox was measured in five blocks, except October 28, 2003 and July 27, 2004 when measurements were limited to two and three blocks, respectively. Redox was measured using four platinum electrodes, a calomel reference electrode and a Barnant 20 Digital pH/mV/ORD meter (Barrington, IL, USA). In each mesocosm, platinum electrodes were placed \sim 30 cm from opposite sides along the two central axes and the calomel reference electrode was placed in the center. Electrodes were inserted into the soil to a depth of 10 cm and allowed to stabilize before a reading was taken. We adjusted the readings by adding the potential of the calomel reference electrode (244 mV).

Water. We collected water samples to test for N leakage on August 12 and 15, 2003 when plants were at peak growth and on October 28, 2003 when plants were senescing. In the second year, we collected water samples on June 1, 2004 during early season growth, on July 27 and August 16, 2004 when plants were at peak growth, and on September 29, 2004 when plants were senescing. Mesocosms were flooded with 75 L of water 24 h prior to sampling. On August 14, 2003, ammonium–nitrate fertilizer was added at the same rates used for the treatment applications (31.0 and 7.75 g of fertilizer in the high and low treatments, respectively). On May 31 and July 26, 2004, ammonium–nitrate fertilizer was added during treatment applications. No fertilizer was applied during flooding for August 12 and October 28, 2003 and August 16 and September 29, 2004 samples. Water samples were collected from outflow valves at the base of each mesocosm and kept on ice until submitted to the UW Soil and Plant Analysis Lab for analysis of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations. Water samples were analyzed for pH using a Barnant 20 Digital pH/mV/ORD meter (Barrington, IL, USA).

Plant tissues. On August 20–21, 2003 and on August 18–19, 2004, above-ground biomass was collected from a random quadrant (0.27 m²) in each mesocosm. Biomass was separated into *Phalaris*, other graminoids, and forbs and chopped into 20-cm segments starting at the base. Canopy segments were homogenized and random samples were separated for N analysis. Biomass was oven dried at 60 °C for 48 h, weighed, and the remainder not used for N analysis was returned to the mesocosms 2 days after clipping to retain N within each system and to reduce the impact of removing vegetation on the soil.

On August 22, 2003 and August 19, 2004, below-ground biomass samples were collected using a 10.2-cm-diameter soil core to a depth of 10 cm. Soil cores were taken from the center of the quadrant used to sample above-ground biomass in 12 of the 20 mesocosms (blocks 1, 3 and 5) in order to limit soil disturbance and compare N levels from disturbed and undisturbed mesocosms in late season. Cores were washed over a 2-mm mesh to separate soil particles and stones from below-ground biomass. Biomass was oven dried at 60 °C for 48 h and weighed. Separate above-ground biomass

samples of *Phalaris*, other graminoids, and forbs and composite below-ground biomass samples were ground and analyzed by the UW Soil and Plant Analysis lab for tissue N concentration.

Data analysis

Two-way ANOVAs with N and vegetation treatments as independent variables were run using R (version 1.7.1; R Development Core Team, 2003). Our response variables were $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ and total N in soil samples, $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ in water samples, biomass, and average N concentrations and total N accumulation in plant tissues. Net changes in N concentrations in water samples between August 12 and October 28, 2003 and between June 1 and July 27, 2004 and in soil samples between August 13 and October 29, 2003 and between June 3 and July 29, 2004 were tested for treatment effects and interactions.

Boxcox analyses indicated that log transformations were appropriate to correct for non-normality. We tested the robustness of using one-half the lower detection limit for water samples that had $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations below the detection limit. ANOVAs were run with and without extreme data points for $\text{NO}_3\text{-N}$ in water samples from August 2003 and June and July 2004, $\text{NH}_4\text{-N}$ in water samples from July 2004, and $\text{NO}_3\text{-N}$ in soil samples from August and October 2003. Extreme points were up to $25 \times$ greater than the next highest data point from the same sampling date. In water samples from July 2004, two outliers, one from the high-N resident treatment and one from the low-N invasion treatment, had $\text{NO}_3\text{-N}$ concentrations $> 19 \times$ greater than the average of the other mesocosms from the same treatment. We used Tukey's Honest Significant Difference to separate treatment means if an ANOVA was significant. Differences in tissue N concentrations of the forbs, *Phalaris*, and other graminoids were tested using paired *t*-tests and a Bonferonni correction for multiple tests.

Results

Because species composition is the primary treatment factor in the experiment, the vegetation is described first. We expected the vegetation in the

resident and invasion treatments to diverge as *Phalaris* invaded. The similarity index provides an indication of how similar the vegetation was among the four treatments and whether *Phalaris* was dominant in the invasion treatments. Physical conditions are described second, followed by N concentrations in the soil, in the water discharged from the mesocosms, and in plant tissues. Data mentioned in text are means \pm S.E. Significant differences in N are reported as "X% more" N in soil, water or plant tissue [e.g., if treatment A has 100% more soil N than treatment B, then soil N in treatment A = soil N in treatment B + (100% \times soil N in treatment B)].

Species composition

In June 2002, 2 weeks prior to nutrient and flooding treatments and two weeks after adding the first *Phalaris* transplants, the mesocosms averaged 12.7 ± 0.5 species, with 5.6 ± 0.2 graminoids and 7.1 ± 0.3 forbs. Graminoids averaged $61.7 \pm 0.8\%$ of the line intercepts, with *Agrostis gigantea* having the highest percent in all treatments (Table 1). *Phalaris* comprised $6.7 \pm 0.5\%$ of the intercepts in the invasion treatments. As desired, mesocosms were initially similar for the resident and invasion treatments and the high-N and low-N treatments (Table 2).

In September 2002, overall similarity between the resident and invasion treatments was lower, but *Phalaris* was not yet dominant in the invasion treatment. The resident treatment was no more than 0.66 similar to the high-N invasion treatment (Table 2). The percent of *Phalaris* intercepts was $1.5 \times$ greater with high-N treatment ($18.2 \pm 3.1\%$) than with low-N treatment ($11.9 \pm 0.2\%$). At the end of the first-year treatments, mesocosms were similar for the high-N and low-N treatments and between the resident treatment and the low-N invasion treatment, warranting additional protocols in the second year to induce *Phalaris* dominance in the invasion treatment.

In August 2003, *Phalaris* was dominant in the invasion treatment and the overall similarity between the resident and invasion treatments had dropped (Table 2). Graminoids were less common in the resident treatment ($51.4 \pm 2.2\%$ of the line intercepts) but more common in the invasion treatment ($88.1 \pm 2.5\%$ of the line intercepts). *Phalaris* comprised $25.6 \pm 1.0\%$ of

Table 2. Similarity between treatments in June and September 2002, August 2003, and July 2004

Treatment comparisons	June 2002	Sept. 2002	Aug. 2003	July 2004
Invasion, high N vs. resident, high N	0.85	0.66	0.42	0.26
Invasion, high N vs. resident, low N	0.86	0.65	0.46	0.38
Invasion, low N vs. resident, high N	0.86	0.80	0.63	0.43
Invasion, low N vs. resident, low N	0.84	0.82	0.67	0.51
Invasion, high N vs. invasion, low N	0.90	0.80	0.75	0.71
Resident, high N vs. resident, low N	0.88	0.88	0.83	0.75
Invasion vs. resident	0.88	0.75	0.55	0.40
High N vs. low N	0.92	0.86	0.83	0.78

Similarity was calculated using the minimum mean proportion of intercepts by species within a treatment. *Phalaris* was added in the invasion treatments as transplants in May 2002 and May 2003 and as seeds in June 2002 and April 2003.

the intercepts in the low-N and $46.9 \pm 5.6\%$ in the high-N invasion treatments, intercepting 100% of the intervals in each mesocosm.

In July 2004, *Phalaris* remained dominant in the invasion treatments, comprising $28.9 \pm 1.0\%$ of the intercepts in the low-N and $43.6 \pm 4.9\%$ in the high-N invasion treatments and intercepting $>90\%$ of the intervals in each mesocosm. The resident treatment was less similar to the invasion treatment, being no more than 0.38 similar to the high-N invasion treatment and 0.51 similar to the low-N invasion treatment (Table 2). The percent of graminoid intercepts continued to decrease in the resident treatment ($29.8 \pm 2.3\%$), while remaining high in the invasion treatment ($83.5 \pm 2.0\%$).

Physical parameters

Two-way ANOVAs showed occasional treatment effects in the physical parameters of the mesocosms. The pH of discharged water was higher from the high-N than low-N treatment in October 2003 (7.59 ± 0.01 vs. 7.54 ± 0.01 , respectively; $P = 0.003$; Table 3) and from the resident than invasion treatment in July 2004 (7.13 ± 0.03 vs. 7.00 ± 0.03 ; $P = 0.017$). We found vegetation and N-treatment effects on soil pH in May 2003 ($P = 0.0035$ and 0.039 , respectively) and in June 2004 ($P = 0.0003$ and 0.009 , respectively). Soil pH in the invasion treatment was higher than the resident treatment in May 2003 (7.91 ± 0.03 vs. 7.80 ± 0.04 , respectively) but lower than the resident treatment in June 2004 (7.36 ± 0.02 vs. 7.51 ± 0.03 , respectively). The high-N treatment had lower soil pH than

the low-N treatment in May 2003 and June 2004. Soil moisture was 6% greater in the invasion vs. resident treatment in May 2003 ($18.9 \pm 0.2\%$ vs. $17.8 \pm 0.4\%$; $P = 0.017$) and 5% greater in the invasion vs. resident treatment in August 2004 ($20.2 \pm 0.2\%$ vs. $19.2 \pm 0.3\%$; $P = 0.011$), when more soil was visible in the resident treatment. No other treatment effects were detected in the physical parameters. We do not report sporadic block effects for some physical parameters, for total soil N, or for $\text{NH}_4\text{-N}$ concentrations in discharged water, as none were strong or interpretable.

Soil nitrogen

Baseline soil $\text{NH}_4\text{-N}$ concentrations in May 2003, 1-week before N treatments, showed significant treatment effects ($P < 0.05$). The resident treatment had 32% more soil $\text{NH}_4\text{-N}$ than the invasion treatment (12.33 ± 0.71 and 9.35 ± 0.44 mg $\text{NH}_4\text{-N kg}^{-1}$ soil, respectively; Figure 1a). No further differences in soil $\text{NH}_4\text{-N}$ ($P > 0.2$) were detected in the resident and invasion treatments after N treatments began except in August 2004. The invasion treatment had 27% more soil $\text{NH}_4\text{-N}$ than the resident treatment (3.68 ± 0.24 vs. 2.90 ± 0.31 mg $\text{NH}_4\text{-N kg}^{-1}$ soil, respectively; $P = 0.017$) in August 2004, three weeks after the final N application when the vegetation was near peak biomass, but no differences in soil $\text{NH}_4\text{-N}$ were detected in September 2004. The high-N treatment had more soil $\text{NH}_4\text{-N}$ in May, August and October 2003 and June and August 2004 ($P = 0.042$, 0.003 ,

Table 3. Environmental conditions of the four treatments on water and soil sampling dates

	High-N Resident	High-N Invasion	Low-N Resident	Low-N Invasion
Water pH				
Aug. 12, 2003	7.65 ± 0.09	7.57 ± 0.10	7.66 ± 0.10	7.62 ± 0.16
Aug. 15, 2003	7.07 ± 0.03	7.14 ± 0.08	7.04 ± 0.01	7.08 ± 0.04
Oct. 28, 2003	7.60 ± 0.01 ^a	7.59 ± 0.01 ^{a,b}	7.55 ± 0.01 ^{a,b}	7.53 ± 0.02 ^b
June 1, 2004	7.16 ± 0.02	7.18 ± 0.02	7.11 ± 0.03	7.16 ± 0.03
July 27, 2004	7.16 ± 0.05	6.97 ± 0.04	7.11 ± 0.05	7.03 ± 0.05
Aug. 16, 2004	7.17 ± 0.02	7.15 ± 0.02	7.18 ± 0.02	7.09 ± 0.04
Sept. 29, 2004	7.38 ± 0.02	7.31 ± 0.03	7.30 ± 0.03	7.35 ± 0.04
Soil pH				
May 2003	7.79 ± 0.05 ^a	7.85 ± 0.05 ^{a,b}	7.82 ± 0.06 ^a	7.96 ± 0.03 ^b
Aug. 2003	7.78 ± 0.02	7.87 ± 0.08	7.83 ± 0.02	7.80 ± 0.03
Oct. 2003	8.46 ± 0.03	8.41 ± 0.02	8.40 ± 0.01	8.41 ± 0.04
June 2004	7.46 ± 0.04 ^{c,d}	7.32 ± 0.02 ^c	7.56 ± 0.02 ^c	7.40 ± 0.02 ^{d,e}
July 2004	7.37 ± 0.02	7.41 ± 0.03	7.32 ± 0.03	7.36 ± 0.01
Aug. 2004	7.61 ± 0.05	7.65 ± 0.05	7.69 ± 0.06	7.67 ± 0.07
Sept. 2004	7.86 ± 0.04	7.82 ± 0.02	7.79 ± 0.04	7.82 ± 0.05
Soil moisture (%)				
May 2003	17.8 ± 0.5	19.0 ± 0.4	17.8 ± 0.6	18.7 ± 0.2
Aug. 2003	18.9 ± 0.4	18.9 ± 0.3	19.1 ± 0.2	19.5 ± 0.5
Oct. 2003	18.2 ± 0.5	18.3 ± 0.3	18.1 ± 0.4	18.6 ± 0.4
June 2004	19.4 ± 0.2	19.1 ± 0.2	19.7 ± 0.7	19.5 ± 0.4
July 2004	20.0 ± 0.2	19.7 ± 0.3	19.4 ± 0.2	19.6 ± 0.3
Aug. 2004	19.4 ± 0.5	20.0 ± 0.4	19.0 ± 0.3	20.0 ± 0.3
Sept. 2004	19.8 ± 0.2	20.1 ± 0.2	19.7 ± 0.2	20.0 ± 0.3
Soil redox (mV)				
May 2003	529.7 ± 9.8	513.1 ± 8.4	525.5 ± 6.3	499.3 ± 9.5
Aug. 2003	280.8 ± 8.6	288.7 ± 8.7	275.0 ± 7.2	287.6 ± 5.1
Oct. 2003	487.3 ± 5.7	492.8 ± 9.2	501.0 ± 11.3	511.6 ± 11.5
June 2004	319.0 ± 9.3	321.4 ± 7.3	311.5 ± 15.6	305.2 ± 9.3
July 2004	303.9 ± 3.8	311.6 ± 23.7	296.3 ± 8.6	308.3 ± 10.2
Aug. 2004	291.3 ± 12.1	302.6 ± 14.6	299.8 ± 12.3	307.3 ± 19.1
Sept. 2004	350.2 ± 6.0	353.8 ± 6.3	346.0 ± 3.5	347.2 ± 8.4

Data are mean ± 1 S.E. Within dates, treatment means that differed significantly following Tukey's Honest Significant Difference ($P < 0.05$) are indicated by different letters. Water samples were collected 24 h after flooding but no N pulse on August 12 and October 28, 2003 and August 16 and September 29, 2004, and 24 h after flooding with a N pulse on the remaining dates. Mean soil redox was based on 8 mesocosms in 2 blocks on October 2003 and 12 mesocosms in 3 blocks on July 2004.

0.037, 0.0001, and 0.008, respectively). The high-N treatment had 18% more soil $\text{NH}_4\text{-N}$ than low-N treatment (11.74 ± 0.89 vs. 9.93 ± 0.46 mg $\text{NH}_4\text{-N}$ kg^{-1} soil, respectively) in May 2003, increasing to 157% more soil $\text{NH}_4\text{-N}$ (17.28 ± 2.13 vs. 6.73 ± 0.39 mg $\text{NH}_4\text{-N}$ kg^{-1} soil, respectively) in June 2004, and declining to no significant difference in July and September 2004.

No differences in soil $\text{NO}_3\text{-N}$ concentrations ($P > 0.3$; Figure 1b) were detected between the resident and invasion treatments on any of the dates sampled in 2003 and 2004. The high-N

treatment had more soil $\text{NO}_3\text{-N}$ on all dates sampled ($P \leq 0.001$). Baseline $\text{NO}_3\text{-N}$ in May 2003 was 165% greater in the high-N (1.09 ± 0.12 mg $\text{NO}_3\text{-N}$ kg^{-1} soil) than low-N treatment (0.41 ± 0.03 mg $\text{NO}_3\text{-N}$ kg^{-1} soil). In August 2004, after the final N application, $\text{NO}_3\text{-N}$ was 549% greater in the high-N (2.79 ± 0.69 mg $\text{NO}_3\text{-N}$ kg^{-1} soil) than low-N treatment (0.43 ± 0.04 mg $\text{NO}_3\text{-N}$ kg^{-1} soil). A two-way ANOVA showed a N-treatment effect on the net change in soil $\text{NO}_3\text{-N}$ from August to October, 2003 ($P = 0.002$) with soil $\text{NO}_3\text{-N}$ increasing 58%

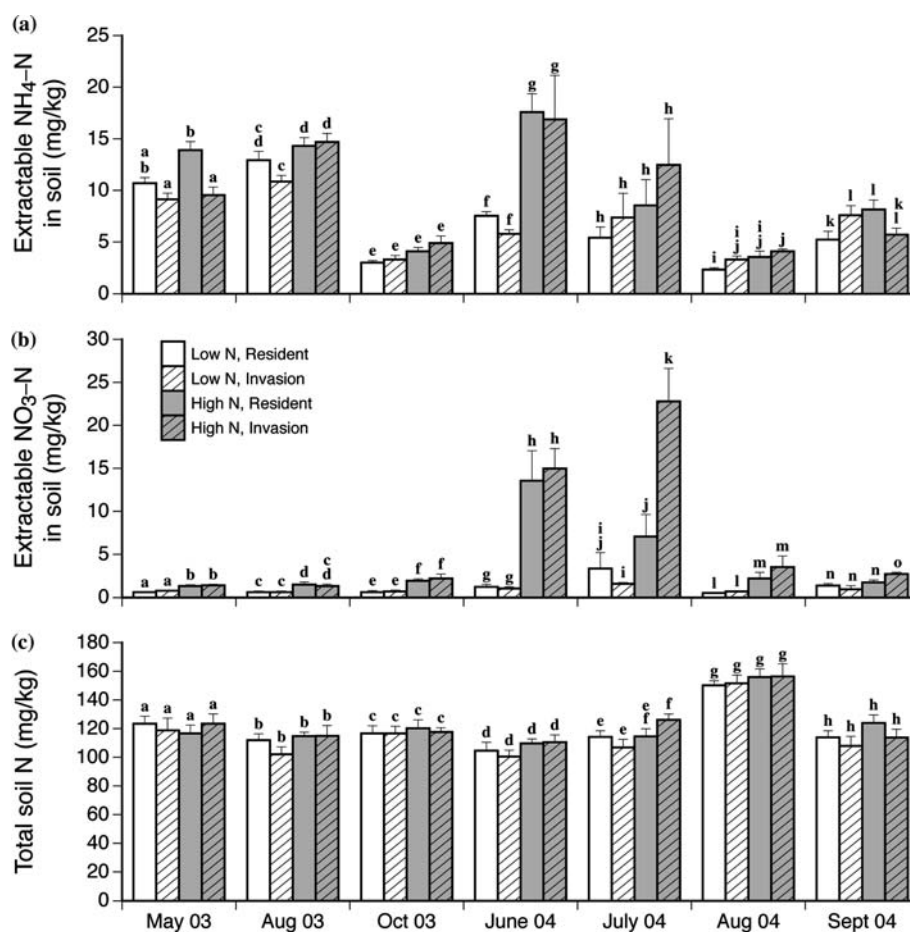


Figure 1. Concentrations of extractable soil NH₄-N (a), extractable soil NO₃-N (b), and total soil N (c) from the four treatments sampled on May 29, August 13 and October 29, 2003 and June 3, July 29, and August 17, and September 30, 2004. Within dates, treatment means that differed significantly following Tukey's Honest Significant Difference ($P < 0.05$) do not share a common letter. Data are mean + 1 S.E. Treatment means and S.E. exclude extreme points that were removed from the two-way ANOVAs.

in the high-N mesocosms and decreasing 12% in the low-N mesocosms.

Two-way ANOVAs of total soil N concentrations showed no treatment differences in 2003 and no differences between the resident and invasion treatments in 2004 ($P > 0.05$; Figure 1c). The high-N treatment had 7% more total soil N than the low-N treatment (110 ± 3 vs. 102 ± 3 mg N kg⁻¹ soil, respectively; $P = 0.003$) in June 2004 and 10% more total soil N (120 ± 3 vs. 109 ± 3 mg N kg⁻¹ soil, respectively; $P = 0.027$) in July 2004, coinciding with differences in soil NO₃-N.

Nitrogen in discharged water

No differences were detected in NH₄-N concentrations in discharged water from the resident and

invasion treatments in 2003 or 2004 ($P > 0.1$; Figure 2a). There were occasional differences in NH₄-N in discharged water from the high-N and low-N treatments. The discharged water from the high-N treatment had 100% more NH₄-N than the low-N treatment (0.18 ± 0.06 vs. 0.09 ± 0.02 mg NH₄-N L⁻¹, respectively; $P = 0.017$) on August 12, 2003, 24 h after flooding without N application, and 628% more NH₄-N (1.82 ± 0.73 vs. 0.25 ± 0.23 mg NH₄-N L⁻¹, respectively; $P = 0.011$) on July 27, 2004, 24 h after flooding and the final N application. NH₄-N concentrations in the discharged water from the high-N and low-N treatments did not differ on the other sampling dates ($P > 0.1$).

No differences were detected in NO₃-N concentrations in water discharged from the resident and

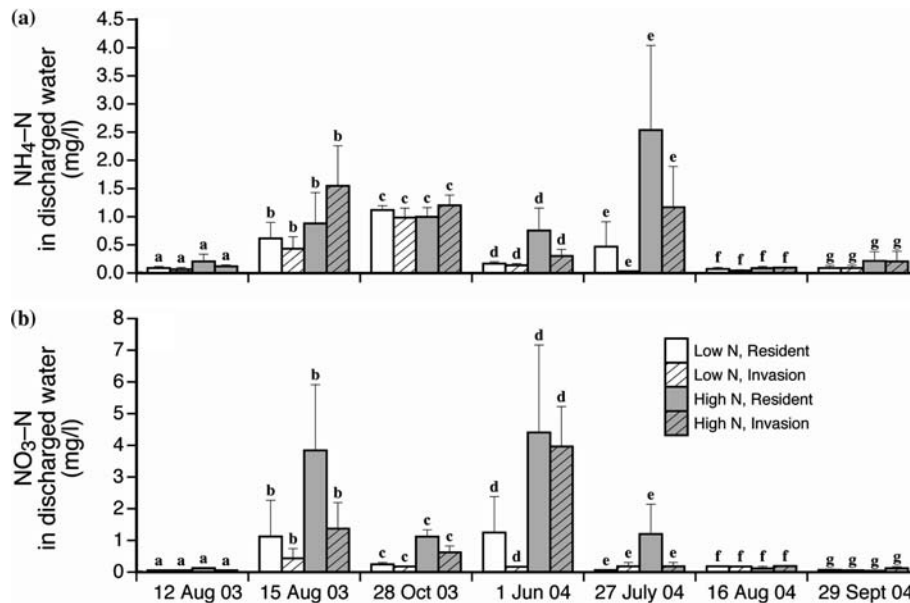


Figure 2. Concentrations of $\text{NH}_4\text{-N}$ (a) and $\text{NO}_3\text{-N}$ (b) in the discharged waters from the four treatments sampled 24 h after flooding with no N addition on August 12 and October 28, 2003 and on August 16 and September 29, 2004 and 24 h after flooding with a N addition on August 15, 2003 and on June 1 and July 27, 2004. Within dates, treatment means that differed significantly following Tukey's Honest Significant Difference ($P < 0.05$) do not share a common letter. Data are mean + 1 S.E. We used half the lower detection limit for water samples with concentrations below the detection limit of the analysis. Treatment means and S.E. exclude extreme points that were removed from the two-way ANOVAs.

invasion treatments in 2003 or 2004 ($P > 0.1$; Figure 2b). Differences in $\text{NO}_3\text{-N}$ in discharged water from the high-N and low-N treatments were not consistent. Discharged water from the high-N treatment had over 100% more $\text{NO}_3\text{-N}$ than the low-N treatment 24 h after flooding without N application on August 12 and October 28, 2003 ($P = 0.022$ and 0.0002 , respectively) and 480% more $\text{NO}_3\text{-N}$ 24 h after flooding and a N application on June 1, 2004 ($P = 0.013$). No differences in $\text{NO}_3\text{-N}$ in discharged water from the high-N and low-N treatments were detected on the other sampling dates ($P > 0.05$). Outliers in $\text{NO}_3\text{-N}$ concentrations did not always come from the same mesocosm. A two-way ANOVA showed a N-treatment effect on the net change in $\text{NO}_3\text{-N}$ concentrations from August 12 and October 28, 2003 ($P = 0.0005$), with $\text{NO}_3\text{-N}$ increasing 1000% in the water from the high-N treatments and 425% in the water from the low-N treatments.

Plant tissue nitrogen

Total above-ground biomass was significantly affected by N treatment in August 2003 and August

2004 ($P < 0.0001$ and $P < 0.0007$, respectively; Figure 3a) and by vegetation treatments in August 2003 and August 2004 ($P = 0.0004$ and 0.022 , respectively). We report above-ground biomass by plot (0.27 m^2), collected from a random quadrant in a mesocosm. The high-N treatment had over 91% more above-ground biomass than the low-N treatment (458.3 ± 35.2 vs. $236.1 \pm 31.9 \text{ g plot}^{-1}$, respectively in August 2003 and 732.5 ± 101.1 vs. $382.5 \pm 40.9 \text{ g plot}^{-1}$, respectively in August 2004). Above-ground biomass was 52% greater in the resident vs. invasion treatment (418.5 ± 43.8 vs. $275.9 \pm 44.1 \text{ g plot}^{-1}$, respectively) in August 2003 and 49.7% greater in the resident vs. invasion treatments (668.4 ± 112.6 vs. $446.5 \pm 57.2 \text{ g plot}^{-1}$, respectively) in August 2004.

The high-N resident treatment had at least 120% more forb biomass than the low-N resident treatment in August 2003 (297.0 ± 68.3 vs. $134.2 \pm 35.9 \text{ g plot}^{-1}$, respectively) and August 2004 (641.5 ± 138.6 vs. $233.9 \pm 39.6 \text{ g plot}^{-1}$, respectively). In contrast, the high-N resident treatment had 27% more graminoid biomass than low-N resident treatment in 2003 (227.0 ± 34.4 vs. $178.7 \pm 41.6 \text{ g plot}^{-1}$, respectively) and

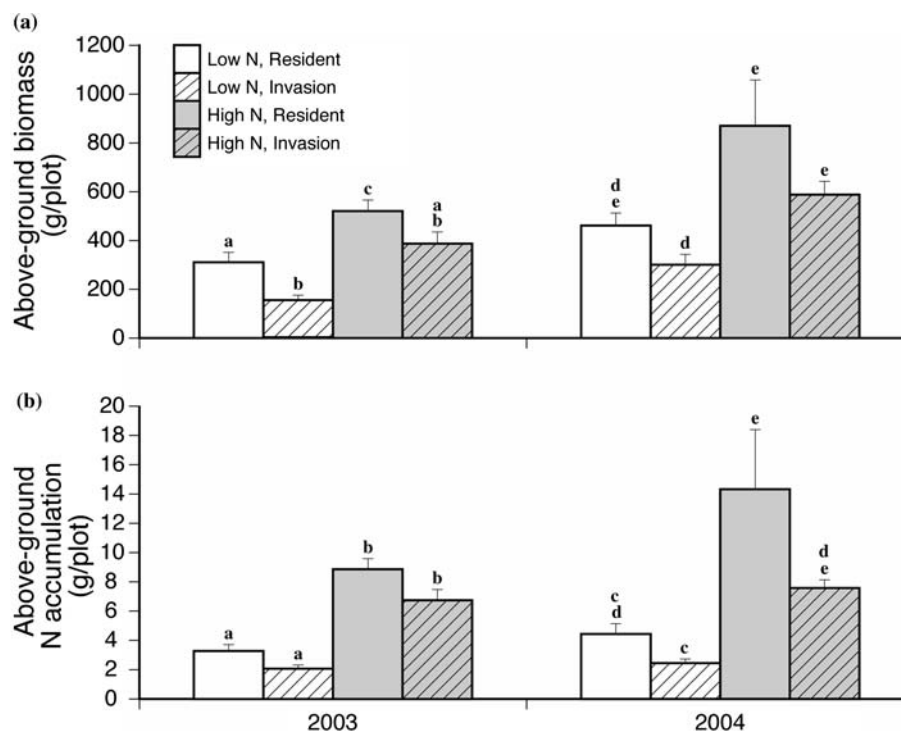


Figure 3. Above-ground biomass (a) and N accumulation in above-ground tissues (b) from the four treatments sampled in August 2003 and August 2004. Within dates, treatment means that differed significantly following Tukey's Honest Significant Difference ($P < 0.05$) do not share a common letter. Data are mean + 1 S.E.

only 2% more in 2004 (233.7 ± 76.7 vs. 227.7 ± 41.5 g plot⁻¹, respectively). Between 2003 and 2004, forbs increased from 55% to 75% of the above-ground biomass in the high-N resident treatment and from 43% to 51% of the above-ground biomass in the low-N resident treatment.

In the invasion treatment, *Phalaris* above-ground biomass was more than 400% greater with the high-N treatment than the low-N treatment in both 2003 and 2004. From August 2003 and August 2004, *Phalaris* biomass increased 56% in the high-N treatment (from 247.2 ± 29.6 to 385.6 ± 49.3 g plot⁻¹) and 58% in the low-N treatment (from 48.4 ± 6.5 to 76.8 ± 21.6 g plot⁻¹). In both years, *Phalaris* comprised 66% of the total above-ground biomass in the high-N treatment. In the low-N treatment, *Phalaris* comprised 30% of the biomass in August 2003 and 25% in August 2004. From August 2003 and August 2004, the biomass of other graminoids increased more in the low-N invasion treatment (81%) than in the high-N invasion treatment (18%). Consequently, the biomass of other graminoids, which was 36% greater in the high-N than low-N

invasion treatment in 2003, was 13% greater in the low-N than high-N invasion treatment in 2004. Forbs produced <5% of the above-ground biomass in the invasion treatment in 2003 and <6% of the above-ground biomass in the high-N and <13% in the low-N invasion treatments in 2004.

Mean above-ground tissue N concentration was 46% greater ($P = 0.0005$) in the high-N ($1.76 \pm 0.12\%$ N) than in the low-N treatment ($1.20 \pm 0.08\%$ N), but did not differ between resident and invasion treatments in August 2003 ($P > 0.1$). Consequently, there was 196% more total N accumulation in above-ground tissues (calculated from biomass and concentration of total N in tissues), with high-N vs. low-N treatments (7.7 ± 0.6 vs. 2.6 ± 0.3 g N plot⁻¹, respectively; $P < 0.0001$; Figure 3b) and 39% more with the resident vs. invasion treatments (6.0 ± 1.0 vs. 4.3 ± 0.9 g N plot⁻¹, respectively; $P = 0.009$) in August 2003. In August 2004, mean above-ground tissue N concentration was 63% greater ($P < 0.0001$) in the high-N ($1.41 \pm 0.09\%$ N) than in the low-N treatment ($0.86 \pm 0.04\%$ N)

and 20% greater ($P = 0.03$) in the resident ($1.24 \pm 0.13\%$ N) than in the invasion treatment ($1.03 \pm 0.08\%$ N). Paired t -tests showed greater tissue N concentration in forbs ($1.46 \pm 0.09\%$ N) than *Phalaris* ($1.07 \pm 0.07\%$ N; $P = 0.002$) or other graminoids ($0.84 \pm 0.04\%$ N; $P < 0.0001$). Accumulation of N in above-ground tissues was 218% greater in the high-N vs. low-N treatment (10.8 ± 2.2 vs. 3.4 ± 0.5 g N plot⁻¹, respectively; $P < 0.0001$; Figure 3b) and 90% greater in the resident vs. invasion treatments (9.3 ± 2.5 vs. 4.9 ± 0.9 g N plot⁻¹, respectively; $P = 0.009$) in August 2004.

Because the data were highly variable, no treatment effects were found for below-ground biomass in August 2003 or August 2004 ($P > 0.3$). The high-N treatment had over 90% greater N concentration in below-ground tissues than low-N treatment ($1.51 \pm 0.11\%$ vs. $0.79 \pm 0.08\%$ N, respectively; $P = 0.004$) in August 2003 and ($1.38 \pm 0.13\%$ vs. $0.70 \pm 0.04\%$ N, respectively; $P = 0.0015$) in August 2004 and 100% more N accumulation in below-ground tissues than the low-N treatment (0.2 ± 0 vs. 0.1 ± 0 g N core⁻¹, respectively; $P = 0.025$) in August 2004. No differences were detected between the resident and invasion treatments.

Discussion

Because elevated N levels accelerate the expansion of *Phalaris*, this aggressive invader is presumed to have high N uptake and to increase retention of N within a wetland. Wetland managers (e.g., the Wetland Team of Wisconsin Department of Natural Resources) often ask if this species retains more N than the native wetland vegetation it displaces when deciding to control its invasions or, alternatively, to allow its use in treatment wetlands. Our comparison of wet prairie vegetation in mesocosms showed that *Phalaris* invasion did not (1) increase N accumulation in wet prairie vegetation, (2) increase N retention in soil of wet prairie vegetation, or (3) decrease N leakage in water discharged from wet prairie vegetation.

Invasion of Phalaris did not increase N accumulation in plant tissues

As expected, above-ground biomass and N accumulation in plant tissues increased with high-N

treatment in both the resident and invasion treatments (Bernard and Lauve, 1995; Figiel et al., 1995; Green and Galatowitsch, 2002; Kline and Boersma, 1983; Wetzel and van der Valk, 1998). We also expected *Phalaris*' suppression of wet prairie vegetation to increase with the high-N treatment, because Green and Galatowitsch (2002) found that the above-ground biomass of *Phalaris* was more than doubled, while the native wetland vegetation was reduced by nearly 50%, as NO₃ additions increased from 12 to 48 g N m⁻² yr⁻¹. In response to our 4-fold increase in N additions, *Phalaris* biomass increased >400%, more than doubling the proportion of *Phalaris* to total biomass. In contrast, the biomass of the other graminoids in the invasion treatment was only 36% greater with high-N treatment in 2003 and dropped to 13% greater with low-N treatment in 2004. In wetland communities with and without *Phalaris* invasions, Green and Galatowitsch (2002) found that native graminoid biomass declined while native forb biomass increased as NO₃ additions increased over 2 years. While we did not find a decrease in graminoid biomass between 2003 and 2004 in any of the treatments, graminoid biomass increased more with low-N than the high-N treatment in the mesocosms with and without *Phalaris* invasions.

In contrast to *Hypothesis 1*, wet prairie vegetation accumulated more N in its plant tissues without *Phalaris* invasions. Because tissue N concentration did not differ between the resident and invasion treatments in 2003, the accumulation of N in plant tissues was greater in the vegetation with no invading *Phalaris* as a result of more above-ground biomass. We expected tissue N concentration to be similar between the resident and invasion treatments, because we have no evidence that *Phalaris* concentrates more N its tissues than other wetland species. In an outdoor experiment using small pots, McJannet et al. (1995) found that the tissue N concentration of *Phalaris* (~0.7% to 1.2% N) was near the middle of the range for 41 wetland species. Further support for the suggestion that *Phalaris* lacks unusually high N uptake ability comes from Kao et al. (2003), who found no differences in tissue N concentration or accumulation of N in biomass between *Phalaris* (~1.5% N and 11.8 g N m⁻²) and the four native species they tested in the field. Our tissue N concentration averaged 1.0%

in the low-N and 1.5% in the high-N treatment over 2 years.

The invasion process continued in 2004, but none of the changes supported *Hypothesis 1*. There was no evidence that our 2002–2003 clipping of natives in the invasion treatments reduced biomass. On the contrary, another experiment (Kercher and Zedler, 2004) showed that reducing the canopy of native competitors released *Phalaris* and increased overall biomass, with a strong positive correlation between light penetration through the canopy and *Phalaris* biomass. The above-ground biomass increased in both the resident and invasion treatments between 2003 and 2004, and the percent difference remained similar (~50% more above-ground biomass in the resident treatment in both years despite clipping in 2003 and no clipping in 2004).

In August 2004, more N accumulated in the above-ground plant tissues in the resident treatment where the vegetation had ~50% more above-ground biomass and 20% greater mean tissue N concentration. The resident treatment had a greater abundance of native forbs, which had considerably more N in their tissues than the graminoid species. If we extrapolate to a wastewater wetland situation, one could harvest species-rich vegetation and remove substantial amounts of N.

Invasion of Phalaris had little effect on soil N

Phalaris invasions had much less effect on soil N concentrations than the N treatments, which increased inorganic soil N on all of the sampling dates. The addition of 14 g N m⁻² in the high-N treatment in 2002 was sufficient to increase soil NH₄-N and NO₃-N in May 2003 prior to N treatments. We expected the high-N treatment to have more soil NH₄-N, especially on the sampling dates following a N application, because NH₄⁺ are readily immobilized through ion exchange onto negatively charged soil particles (Mitsch and Gosselink, 1993) and because, for 2 days preceding soil sampling, the mesocosms were flooded. Anaerobic conditions reduce nitrification (Mitsch and Gosselink, 1993). The high-N treatment had more soil NH₄-N for nearly 2 years; we detected a drop during the 2 days after the last N application in July 2004. Soil

NH₄-N was dynamic in time; however, and in August 2004 it was greater in the high-N treatment but in September 2004 it was not different between the high-N and low-N treatments. Between August and October 2003 during which time the mesocosms were not flooded, soil NH₄-N decreased and soil NO₃-N increased. These changes may have occurred from increases in nitrification and decreases in denitrification during aerobic conditions, and less N uptake by plants late in the growing season (Mitsch and Gosselink, 1993; Richardson and Vepraskas, 2001). Total soil N, which was composed of >80% organic N in 2003, did not differ among the treatments in part because differences in inorganic N were low. The high-N treatment had more total soil N only when soil samples were collected 2 days after a N application.

In contrast to *Hypothesis 2*, *Phalaris* did not increase soil total N or NO₃-N and had little effect on soil NH₄-N during 2003 and 2004. Although the resident treatment had more soil NH₄-N prior to the N treatments in 2003, this difference did not persist after N treatments were applied and *Phalaris* became more dominant in the invasion treatment. Soil NH₄-N was greater in the invasion treatment only on 1 of the 6 sampling dates when *Phalaris* was dominant. Soil NH₄-N is dynamic in these systems. The difference between the invasion and resident treatments in August 2004 was small (<1 mg NH₄-N kg⁻¹ soil) and did not persist in September 2004. Plant uptake may account for lower soil NH₄-N in the resident treatment, which had ~50% more above-ground biomass. Greater accumulation of N in plant tissues does not necessarily coincide with lower soil NH₄-N, however. For example, Otto et al. (1999) found more accumulation of N in the above-ground tissues of invasive *Phragmites australis* than in the native *Typha latifolia* but no differences in soil NH₄-N between the two species.

Plant species can develop positive feedbacks to nutrient cycles within wetlands. For example, fast-growing species in N-rich systems tend to have faster tissue turnover rates, lower nutrient resorption efficiency, and shorter residence times of nutrients (Aerts, 1999; Hobbie, 1992; van der Krift and Berendse, 2001, 2002; Vazquez de Aldana and Berendse, 1997), which can result in increased releases of N into a wetland

and increased nutrient losses from wetland ecosystems (Shaver and Mellilo, 1984). Longer-term N accumulation in plant tissues may be reduced by shorter residence time of N in the plants and faster biomass turnover (Ryser, 1996). Consequently, higher soil N concentrations have been associated with fast-growing, N-demanding species (Tilman and Wedin 1991). Although we harvested vegetation to calculate biomass and N accumulation within the mesocosms, most of the biomass was returned to the mesocosms to retain N within each system. The remaining vegetation was left intact to measure N releases in discharged waters when the plants were senescing. While we found that higher N additions accelerate the expansion of *Phalaris*, we found no consistent evidence that invasions of *Phalaris* increase soil N concentrations within these experimental systems.

Invasion of Phalaris did not decrease N in discharged water

Several results lead us to reject *Hypothesis 3*, that invaded wet prairie vegetation would release less inorganic N in through-flowing water. First, $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations in the water discharged from the mesocosms were similar between the resident and invasion treatments on all sampling dates, usually coinciding with similar soil inorganic N concentrations. Secondly, more soil $\text{NH}_4\text{-N}$ with invading *Phalaris* in August 2004 did not result in more $\text{NH}_4\text{-N}$ released to water discharged from the mesocosms. Thirdly, the noise in the data was stronger than the treatment effects; i.e., high variability and outliers did not always come from the same mesocosm. While the high-N treatment generally increased soil $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$, the release of inorganic N to the water discharged from the high-N mesocosms was not consistently greater. $\text{NO}_3\text{-N}$ concentrations in the soil and in the discharged water were higher in October 2003, when the plants were senescing, than in August 2003, when plants were at peak biomass. NO_3^- is mobile in soil and can be quickly depleted through assimilation by plants or microbes, denitrification or leaching (Mitsch and Gosselink, 1993).

General points and suggestions

Although the resident treatment had more above-ground biomass and tissue N accumulation, it did not exceed the *Phalaris* invasion treatment in total soil N, extractable inorganic soil N, or inorganic N released in the discharged water. Furthermore, differences in soil $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ did not consistently coincide with differences in inorganic N leakage in the water. These findings demonstrate the need to test multiple parameters to determine whether patterns in N retention affect N leakage to downstream systems. High variability, especially in the leakage of N to the discharged water, warrants additional investigation of N retention patterns of native vegetation with and without *Phalaris* invasions at larger spatial scales, where sampling can be more extensive.

Our study tested one wetland type (wet prairie) under selected environmental conditions (low hydrologic disturbance, namely, intermittent flooding), so we do not generalize to other situations. With other disturbance regimes, *Phalaris* may alter N retention. For example, Kercher and Zedler (2004) found that *Phalaris* invades most strongly with increasing disturbance (e.g., prolonged flooding and nutrient additions). Longer flooding episodes, however, resulted in loss of wet prairie species (ibid.) and would not allow comparison of invasions with species-rich wet prairies. Elevated nutrient levels lead to shifts in plant community composition, decreases in plant species diversity and losses of rare or uncommon species in wetlands of western Europe and North America (Bedford et al., 1999). Further investigations are needed to compare the effects of *Phalaris* invasions on N retention in wetlands subjected to alternative disturbance regimes, including harvest cycles typical of treatment wetlands, and to identify native vegetation tolerant of such disturbances.

Despite the limitations of our study, the findings establish doubt that this aggressive invader retains more N than the native species it displaces. Overall there was no evidence that vegetation invaded by *Phalaris* had greater N-removal capacity than the wet prairie it displaced. In the absence of evidence that native species cannot remove sufficient N, we suggest that treatment wetlands employ aggressive native species instead of *Phalaris*. If managers of such wetlands can

indeed sustain native species, their efforts would serve biodiversity conservation without impairing water treatment. At the least, the concept deserves field testing.

Acknowledgements

This research was supported by USGS Eastern Region's FY 2002 State Partnership Program (Joy B. Zedler and Eileen Kirsch, principal investigators). Funding from the UW Aldo Leopold Endowment helped set up and maintain the mesocosm facility. Additional funding was received from the Ruth Dickie-Sigma Delta Epsilon Grants-in-Aid Award and the Anna Grant Birge Memorial Award. We thank the staffs of the UW Walnut Street Greenhouse, Biotron, and Arboretum for providing facilities. We thank Bret Larget for statistical advice, Kandis Elliot for preparing the figures, Suzanne Kercher, Aaron Boers, Christin Frieswyk, Michael Healy, Roberto Lindig-Cisneros, Debbie Maurer, Becky Miller, Hem Nalini Morzaria-Luna, Kara Jorstad, Laura Ladwig, Sara Green, Brenda Castillo-Navarro, Charles Campbell, Kevin McCartney, Jennifer Kao, Ryo Fujinuma, and Kristy Goodman for assistance in maintaining the mesocosms and collecting data, Jessica Mentzer for analyzing total soil N in 2003, and three anonymous reviewers whose comments improved our manuscript.

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