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Effects of nitrogen addition on the invasive grass *Phragmites australis* and a native competitor *Spartina pectinata*

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Summary

1. *Phragmites australis* is an invasive grass that has increased dramatically in distribution and abundance within the USA in the last 100 years. This study determined the effect of nitrogen addition on the growth of this invasive species compared with an indigenous competitor species, *Spartina pectinata*.

2. Twenty plants from each of three Illinois (USA) populations were collected and planted in the same garden in April 2001 and grown until August 2002. Following a year of growth in the garden, high-nitrogen (45 g N m^{-2}) and low-nitrogen (5 g N m^{-2}) treatments were applied to plants grown from paired rhizome cuttings from each plant. A single *S. pectinata* plant was grown with each *P. australis*. In August 2002, plants were harvested and above- and below-ground biomasses were determined for both species.

3. Mean (± SE) *P. australis* above- and below-ground biomasses were significantly higher in the high-nitrogen treatment (68·4 ± 2·6 g and 39·0 ± 4·5 g, above- and below-ground, respectively) than the low-nitrogen treatment (37·3 ± 2·0 g and 25·5 ± 4·5 g). There were no differences in *S. pectinata* above- and below-ground biomasses between high- (46·8 ± 3·2 g and 71·4 ± 9·6 g) and low- (45·4 ± 3·5 g and 50·3 ± 6·5 g) nitrogen treatments. The ratio of *P. australis* to *S. pectinata* biomass was used to compare the relative response of each species between nitrogen treatments; the mean ratio of *P. australis* to *S. pectinata* for the high-nitrogen treatment (2·72 ± 0·499) was significantly higher than the low-nitrogen treatment (1·83 ± 0·42).

4. *Synthesis and applications*. This study supports the hypothesis that *P. australis* benefits from increased nitrogen, and may be more likely to displace *S. pectinata* in nitrogen-rich environments. Our study also confirms the importance of nitrogen in affecting the interactions between invasive and native plants. Control of *P. australis* may be aided by nutrient management.

Key-words: common reed, invasive species, nutrient enrichment, wetlands

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Introduction

Biological invasions are a significant threat to biodiversity (Enserink 1999; Mack *et al*. 2000). Invasive species alter native ecosystems and disrupt commercial industries such as logging and agriculture (Vitousek *et al*. 1996). Invasive species can reduce biodiversity in native ecosystems by causing the local extinction of native plant and animal species (Savidge 1987). They also can alter community and ecosystem functioning (Vitousek *et al*. 1987; Vitousek 1990; Mack *et al*. 2000).

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Mack *et al*. (2000) define invasive species as exotic, aggressive species that have negative effects on their environment. Aggressive species that cause negative environmental effects are not limited to alien or exotic species, as some are indigenous to the area in which they are harmful. Such species are considered to be invasive in this paper because both exotic and indigenous invasive species have similar effects (Davis & Thompson 2000).

The environmental constraints hypothesis has been advanced to explain invasiveness in plants (Galatowitsch, Anderson & Ascher 1999) and proposes that limiting resources in the past prevented the invasion of some species. Removal of these constraints has allowed plants to invade habitats from which they were previously

excluded. Nitrogen is one such environmental constraint in many ecosystems (Fenn *et al*. 1998). Tilman (1990) found that nitrogen availability was the most significant environmental constraint on plant growth in sand prairies. In some ecosystems, however, this constraint has been removed because of anthropogenic nitrogen in agricultural run-off and acidic deposition (Wedin & Tilman 1996; Fenn *et al*. 1998; Reich *et al*. 2001).

When nitrogen is no longer a limiting resource, species previously limited may increase in abundance. For example, Brooks (2003) found that added nitrogen increased the growth of invasive plants in the Mojave Desert. Other species in the community that tolerate lower levels of resources or successfully compete for limiting resources may be unable to compete successfully against the newly released species. Tomassen *et al*. (2004) found that the invasive grass *Molinia caerulea* was stimulated by added nitrogen levels similar to levels of atmospheric nitrogen deposition. Release from constraints may cause local dominance of a native species or may allow an invasive species to become dominant.

Human activities, such as industrial fertilizer input into agricultural and urban areas, increased nitrogen fixation from growing legumes and fossil fuel burning, have altered the global nitrogen cycle (Jordan & Weller 1996; Vitousek *et al*. 1997). Of these nitrogen sources, agriculture is the most important for mid-western ecosystems in the USA (Hey 2002). Increased nitrogen in ecosystems can cause changes in the plant community (Wedin & Tilman 1996; Boyer & Zedler 1999; Morghan & Seastedt 1999; Green & Galatowitsch 2002), increase soil acidification, cause eutrophication of waterways and reduce drinking water quality (Fenn *et al*. 1998).

Under higher nitrogen levels, a poor competitor for nitrogen may be able to out-compete a better nitrogen competitor. The poor nitrogen competitor may be a superior competitor for another resource or may be more able to tolerate stress. Previous studies support this theory (Levine, Brewer & Bertness 1998; Emery, Ewanchuk & Bertness 2001); for example, plant species previously excluded from low marsh elevations due to stress colonized them under high nitrogen conditions.

The present study species *Phragmites australis* (Cav.) Trin. ex Steudel (Poaceae; common reed) is an invasive grass that has dramatically increased in distribution and abundance within the USA in the last 100 years (Marks, Lapin & Randall 1994; Rice, Rooth & Stevenson 2000). *Phragmites australis* is present in North America in several forms, a recently arrived non-indigenous haplotype M, from Europe and Asia, and haplotypes E and S, which are indigenous to North America (Saltonstall 2002). Currently, most *P. australis* on the east coast is haplotype M, which is indigenous in Europe and Asia. In North America, haplotype M has replaced native populations, suggesting that haplotype M is invasive (Saltonstall 2002).

Phragmites australis forms monospecific stands that can eradicate native wetland species such as *Typha* spp. (Ailstock, Norman & Bushman 2001) and *Spartina* spp. (Benoit & Askins 1999). *Phragmites australis* occurs in undisturbed and disturbed wetlands, along roadsides and in drainage ditches. Because *P. australis* is able to spread and forms monospecific stands, species diversity and richness decrease. Land managers are therefore attempting to control it using herbicides, cutting, burning and covering with plastic. All these options are either expensive, time consuming or need to be continued for many years, even indefinitely (Marks, Lapin & Randall 1994). Knowledge of the ecological factors that cause *P. australis* to expand aggressively could make its control easier (Ailstock, Norman & Bushman 2001).

Observational studies by Haslam (1965, 1972) found that *P. australis* spreads to deeper water depths under eutrophic conditions than under oligotrophic conditions. Eutrophication, however, has been implicated in the decline of *P. australis* in Europe (van der Putten 1997), where *P. australis* is a dominant plant in wet habitats. As haplotype M is the most common type in Europe today (Saltonstall 2002), this haplotype was probably involved. As well as *P. australis*, the growth of another invasive wetland grass, *Phalaris arundinaceae*, is enhanced by nitrogen (Green & Galatowitsch 2001; Green & Galatowitsch 2002; Maurer & Zedler 2002).

We aimed to determine the effect of nitrogen addition on the growth of haplotype M *P. australis* compared with an indigenous competitor species, *Spartina pectinata* Link. (Poaceae; prairie cordgrass). *Spartina pectinata* was chosen as a competitor because *P. australis* reduces the abundance of or eliminates *Spartina* spp. in wetlands (Benoit & Askins 1999). We hypothesized that haplotype M *P. australis* would respond positively to increased nitrogen availability while the native species, *Spartina pectinata*, would not. The ratio of the relative biomass of *P. australis* to *S. pectinata* would thus be greater under higher nitrogen conditions. To test our hypothesis, we determined whether addition of nitrogen increased the growth of plants from three Illinois (USA) *P. australis* populations relative to the competitor species *S. pectinata* in the same garden.

Methods

STUDY POPULATIONS

Three Illinois populations of *P. australis* were from Banner Marsh, Lincoln and Herrin. Analysis from non-coding chloroplast DNA for these populations indicated that all were the European/Asian haplotype M (Saltonstall 2002) and that individuals within each population were highly related (K. Saltonstall, personal communication).

The Banner Marsh population was located in Peoria County, Illinois (Fig. 1). Parts of this site has been disturbed by past strip mining or farming, but the site is currently owned the Department of Natural Resources of Illinois and is managed as a recreation area. This *P. australis* population appeared to be the most stable of

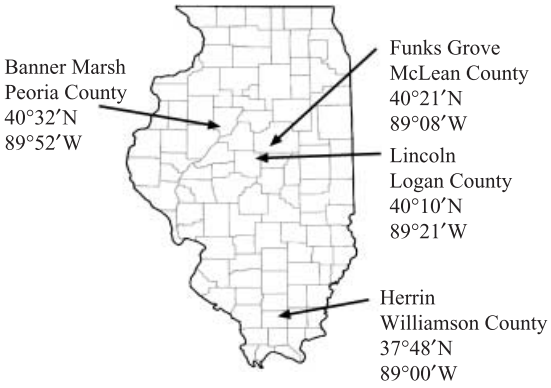

Fig. 1. Location in Illinois, USA, of research populations Banner Marsh, Lincoln and Herrin, and location of the research site for the garden experiment, Funks Grove. County name and latitude and longitude are given for each population and the research site.

the three because it occupied a relatively small area (approximately 32 × 23 m) and native wetland species (*Eupatorium perfoliatum*, *Leersia virginica*, *Typha latifolia*, *Impatiens capensis* and others) grow in association. The other sites had nearly complete dominance by *P. australis*, with other species excluded from most of the area occupied.

The Lincoln population occupied a drainage ditch in Logan County, Illinois, on interstate highway 55 northbound. The population extended for about 1 km in the ditch adjacent to the interstate highway. An agricultural field bordered the south edge of the population and *P. australis* plants were invading the field.

The Herrin population occupied a former strip mine site in southern Illinois near Herrin, Williamson County. *Phragmites australis* was growing in the drainage ditch adjacent to the road and on the drier soil over the entire strip mine. The strip mine was a monoculture of *P. australis*.

SOIL ANALYSIS

To determine nitrogen levels and other soil parameters on each site, six rhizosphere soil samples were collected randomly across each of the three study sites while collecting plants for the garden experiment. Rhizosphere soil was removed from the randomly selected plants and stored frozen in plastic bags at Illinois State University (ISU, Normal, IL). The frozen soil samples were packed in dry ice and sent to the Soil and Plant Analysis Laboratory (University of Wisconsin-Madison Extension). Soils were analysed for extractable organic matter, phosphorus, potassium and total nitrogen, nitrate-nitrogen, calcium, magnesium and pH.

THE GARDEN EXPERIMENT

Twenty rhizomes from each site were collected randomly by excavating below an area where a growing stem was visible. Rhizomes were collected during 2001 from Herrin on 8 April and 15 April, Lincoln on 11 April, and Banner Marsh on 15 April. Rhizomes with their attached root and rhizosphere soil were placed in plastic bags. The plants were transported to a walk-in cold room at ISU maintained at 8 °C.

The *P. australis* samples were planted at Funks Grove, McLean County, Illinois (Fig. 1) in 32-L (7-gallon) pots, 1·2 m apart from each other in a randomly assigned location, on 21 April 2001. Plants were under similar conditions in the same garden at Funks Grove for 1 year to help eliminate residual environmental effects and stored rhizome starch. The experimental plot at Funks Grove has tama silt loam (Windhorn 1998). The rhizomes and associated roots of each plant were planted in a potting mixture containing 1/4 Metro Mix 700, 1/4 Perlite and 1/2 soil from the Funks Grove plot. Pots were placed in a hole in the ground, exposing about 4–5 cm of the top of the pot above the ground. Plants were watered as needed during the summer, approximately once a week. Pots were weeded to remove plants other than *P. australis*. Six *P. australis* plants died within the first 2 months of the experiment. New samples from the respective sites were collected and planted to replace lost plants.

The propagules for the nitrogen experiment were pieces of the *P. australis* rhizomes grown in the garden during 2001. On 12 and 13 April 2002, rhizomes of each plant were dug, washed to remove all soil, and the roots removed. Each rhizome was divided into two pieces and each piece was used to make a 15–25-g ramet that had at least two rhizome buds. However, occasionally there was not enough viable rhizome for both pieces to weigh 15 g, and in these cases all material available was planted to make the ramet's biomass as close to 15 g as possible. Each ramet of the pair was randomly assigned to a high- or low-nitrogen treatment. Ramets were planted in 32-L pots filled with 3/4 Metro Mix 700 and 1/4 Perlite. One ramet was placed back into the original randomly assigned location, while the other was placed into a new randomly assigned location. The pot was placed inside a plastic bag to help retain moisture before it was placed in a hole.

Spartina pectinata seedlings were purchased from a nursery in Wisconsin (USA) that specializes in native plants; one *S. pectinata* seedling was then planted in each pot as a competitor species. The *S. pectinata* seedlings had been growing in Metro Mix, but any excess soil was washed off before planting. Seedlings ranged from 13 to 50 cm in height and were approximately 3 months old.

To achieve the high- and low-nitrogen treatments, nitrogen was added to the pots in the form of urea pellets by shaking the weighed urea equally over the pot and mixing the upper layer of the soil. In the high-nitrogen treatment, nitrogen was applied three times over the summer of 2002 at 15 g N m^{-2} per application, for a total of 45 g N m^{-2}. This amount is comparable to other nitrogen addition studies (Boyer & Zedler 1999; Green & Galatowitsch 2002). During July, the *P. australis* leaves in the low-nitrogen treatment began to yellow

and die-back, showing signs of nitrogen deficiency, so on 25 July 5 g N m^{-2} was added to all plants in the low-nitrogen treatment.

To measure soil nitrogen levels, soil samples were taken from pots in both treatments after each nitrogen application using a soil core to a depth of approximately 7 cm. Nitrogen was then extracted using the potassium chloride extractable nitrogen method (Maynard & Kalra 1993). Ammonia was determined using an Orion Research Expandable Ion Analyser EA 940 (Beverly, MA, USA). Nitrite was determined using the hydrazine method (American Public Health Association 1995).

To measure above-ground biomass, *P. australis* and *S. pectinata* stems were cut at soil level during 27 August–1 September 2002. Above-ground biomass was separated by species, and each species was separated into stems and inflorescences. Stems and inflorescences were dried at 70 °C for 48 h and weighed. Below-ground biomass was harvested for a subset of the replicates on 30 August and 1–2 September Five replicates were selected from each combined nitrogen and population treatment for a total of 30 replicates. Roots were separated by species, washed to remove soil particles, dried at 70 °C for 48 h and weighed.

STATISTICAL ANALYSIS

SAS® for Windows V.8 was used for all analyses (SAS Institute 2001). All data were tested to determine if they met the normality assumption of ANOVA with a Shapiro–Wilk test, and tested for the assumption of heterogeneity of variances with a plot of the residuals vs. the predicted values. When an ANCOVA was used, the homogeneity of slopes assumption was tested by looking at the interaction between the covariate and the main effects. An alpha value of 0·05 was used except when a Bonferroni correction was needed.

Results

SOIL ANALYSIS: FIELD SITES

Soil parameters were tested to determine if they were correlated; five pairs of soil parameters were significantly correlated while three were not. Despite the lack of complete correlation, MANOVA was used to analyse the soil parameters because they were determined from the same soil samples and therefore could not be considered independent. Potassium, total nitrogen, organic matter, nitrate-nitrogen and magnesium were transformed to meet the assumption of multivariate normality. The MANOVA showed significant differences among sites (Wilks' lambda, $\lambda = 0{\cdot}022$, $P < 0{\cdot}0006$, d.f. = 16,16). Univariate protected ANOVAs (Goldberg & Scheiner 1993) were used to determine which response variables contributed to the significance of the model. Potassium ($F = 18{\cdot}6$, $P < 0{\cdot}001$, d.f. = 2,15), phosphorus ($F = 5{\cdot}7$, $P < 0{\cdot}015$, d.f. = 2,15), total nitrogen ($F = 6{\cdot}6$, $P < 0{\cdot}009$, d.f. = 2,15), organic matter ($F = 3{\cdot}8$, $P < 0{\cdot}046$, 2,15), magnesium ($F = 7{\cdot}14$, $P < 0{\cdot}006$, d.f. = 2,15) and nitrate-nitrogen ($F = 4{\cdot}59$, $P < 0{\cdot}028$, d.f. = 2,15) were significantly different among sites. A Ryan–Einot–Gabriel–Welsch (REGWQ) (SAS Institute 1997) multiple range test was used to determine which sites accounted for the differences within soil variables. Banner Marsh had lower total nitrogen, phosphorus and potassium than Lincoln and Herrin (Table 1). Magnesium levels were significantly lower for Banner Marsh and Herrin than they were for Lincoln.

SOIL ANALYSIS: GARDEN

One-way ANOVAs indicated significant differences ($F = 102{\cdot}9$, $P < 0{\cdot}0001$, d.f. = 1,71) between nitrogen treatments in ammonia levels (high-nitrogen treatment = $50{\cdot}6 \pm 6{\cdot}3$ μg g^{-1} NH_3; low-nitrogen treatment = $3{\cdot}8 \pm 1{\cdot}5$ μg g^{-1} NH_3) and nitrate levels ($F = 10{\cdot}98$, $P < 0{\cdot}0022$, d.f. = 1,33) (high-nitrogen treatment = $1{\cdot}79 \pm 0{\cdot}20$ μg g^{-1} NO_3N; low-nitrogen treatment = $0{\cdot}77 \pm 0{\cdot}2$ μg g^{-1} NO_3N).

ABOVE-GROUND AND BELOW-GROUND BIOMASS

To compare the relative response of *P. australis* to *S. pectinata* under high- and low-nitrogen conditions, a ratio of *P. australis* above-ground biomass (stem plus flower biomass) to *S. pectinata* biomass was made. A two-way ANOVA was used to compare the ratio under the different nitrogen treatments and populations. The

Table 1. Mean (± 1 SE) soil parameters for the three study sites. Within a row means with the same letters are not significantly different

	Site		
Soil parameter	Herrin	Lincoln	Banner
Organic matter (%)	4·1 ± 1·1a	3·8 ± 0·6a	2·1 ± 0·1a
Total nitrogen (%)	0·2 ± 0·06a	0·2 ± 0·04a	0·08 ± 0·01b
Nitrate-nitrogen (p.p.m.)	3·7 ± 2·9a	3·2 ± 1·4a	0·2 ± 0·02a
Phosphorus (p.p.m.)	38·3 ± 12·5a	55·5 ± 12·6a	7·3 ± 0·7b
Magnesium (p.p.m.)	267·5 ± 73·0a	534·2 ± 41·8	321·7 ± 14·1a
Potassium (p.p.m.)	134·2 ± 15·9a	169·1 ± 25·5a	67·8 ± 1·9b
Calcium (p.p.m.)	3170·0 ± 360·8a	2975·0 ± 188·4a	3517·5 ± 26·1a
pH	7·7 ± 0·04a	7·5 ± 0·06a	7·8 ± 0·09a

data were log transformed, although normality could not be achieved ($W = 0{\cdot}97$, $P < 0{\cdot}012$). The ANOVA model was significant ($F = 4{\cdot}60$, $P < 0{\cdot}0007$, d.f. = 5,113) and the sources of nitrogen treatment ($F = 11{\cdot}02$, $P < 0{\cdot}0012$, d.f. = 1,113) and population ($F = 5{\cdot}24$, $P < 0{\cdot}006$, d.f. = 2,113) were significant, but the interaction was non-significant ($F = 0{\cdot}75$, $P < 0{\cdot}47$, d.f. = 2,113). The mean ratio of *P. australis* to *S. pectinata* above-ground biomass was $2{\cdot}72 \pm 0{\cdot}499$ for the high-nitrogen treatment and $1{\cdot}83 \pm 0{\cdot}42$ for the low-nitrogen treatment. The two-way ANOVA model used to compare the below-ground biomass ratio of *P. australis* to *S. pectinata* between nitrogen treatments and populations was non-significant ($F = 1{\cdot}94$, $P < 0{\cdot}12$, d.f. = 5,25).

Two MANCOVAs were used to determine if there were differences in *P. australis* and *S. pectinata* stem and inflorescence biomasses between nitrogen treatments (Scheiner 1993). Stem and inflorescence biomasses were correlated for *P. australis* (Pearson correlation, $r = 0{\cdot}21$, $P < 0{\cdot}019$) and *S. pectinata* (Pearson correlation, $r = 0{\cdot}52$, $P < 0{\cdot}0001$). *Phragmites australis* stem biomass and *S. pectinata* stem biomass were correlated (Pearson correlation, $r = -0{\cdot}25$, $P < 0{\cdot}0055$); however, *P. australis* inflorescence biomass and *S. pectinata* inflorescence biomass were not correlated. Due to the incomplete correlation between *P. australis* and *S. pectinata* response variables, they were analysed with separate MANCOVAs. The *P. australis* MANCOVA used *P. australis* stem and flower biomasses as main response variables and *P. australis* initial rhizome biomass as a covariate. The MANCOVA for *S. pectinata* used *S. pectinata* stem and inflorescence biomasses as response variables and initial height of longest leaf as a covariate. Nitrogen treatment and population were used as fixed-effect independent variables for both MANCOVAs. Although the *S. pectinata* plants were purchased from a nursery rather than being collected from different populations, population was included as a factor in the analysis because *S. pectinata* was grown with *P. australis* from different populations. The homogeneity of slopes assumption of ANCOVA was met for both analyses. A Bonferroni correction was used to compensate for the multiple (two) MANCOVAs; the accepted alpha level was 0·025 (Sokal & Rohlf 1995). Protected univariate ANCOVAs were used as follow-up tests for significant MANCOVAs and a REGWQ test was used to test for differences in means for the significant univariate ANCOVAs.

The overall model MANCOVA on *P. australis* stem and flower biomasses was significant for population (Wilks' lambda, $\lambda = 0{\cdot}840$, $P < 0{\cdot}0005$, d.f. = 4,228), and nitrogen treatment (Wilks' lambda, $\lambda = 0{\cdot}541$, $P < 0{\cdot}0001$, d.f. = 2,114), and the interaction between population and nitrogen was non-significant (Wilks' lambda, $\lambda = 0{\cdot}936$, $P < 0{\cdot}1109$, d.f. = 2,228); therefore univariate ANCOVAs were run. The overall univariate model for the *P. australis* stem biomass ANCOVA was significant ($F = 21{\cdot}9_{(\alpha=0{\cdot}025)}$, $P < 0{\cdot}0001$, d.f. = 6,115). There were significant differences in *P. australis* stem biomass with respect to population and nitrogen treatment, but there was no interaction between site and nitrogen treatment (Table 2). The REGWQ test revealed that mean stem biomass for *P. australis* from the Herrin site ($60{\cdot}7 \pm 3{\cdot}8$ g) was significantly higher than *P. australis* from the Lincoln ($50{\cdot}4 \pm 3{\cdot}9$ g) and Banner Marsh ($47{\cdot}4 \pm 3{\cdot}2$ g) sites. Lincoln and Banner Marsh were not significantly different from each other (Fig. 2). The REGWQ test also revealed significant differences between nitrogen treatments: *P. australis* stem biomass was significantly heavier in the high-nitrogen treatment ($68{\cdot}4 \pm 2{\cdot}6$ g) than the low-nitrogen treatment ($37{\cdot}3 \pm 2{\cdot}0$ g) (Fig. 2). The overall model for the univariate ANCOVA showed that there were marginal differences in *P. australis* inflorescence biomass ($F = 2{\cdot}43_{(\alpha=0{\cdot}025)}$, $P < 0{\cdot}03$, d.f. = 6,115). The mean inflorescence biomasses for the high- and low-nitrogen treatments were $2{\cdot}7 \pm 0{\cdot}43$ g and $2{\cdot}0 \pm 0{\cdot}33$ g, respectively. The inflorescence biomasses for Lincoln ($1{\cdot}4 \pm 0{\cdot}30$ g), Banner ($2{\cdot}9 \pm 0{\cdot}54$ g) and Herrin ($2{\cdot}8 \pm 0{\cdot}53$ g) were not significantly different. The Banner and Lincoln populations had greater biomasses in the high-nitrogen treatments than low-nitrogen treatments (Fig. 3), although the interaction between nitrogen treatment and population was non-significant ($F = 2{\cdot}96_{(\alpha=0{\cdot}025)}$, $P < 0{\cdot}055$, d.f. = 2,115).

Table 2. ANCOVA table for *P. australis* stem biomass, with initial rhizome weight as the covariate and population and nitrogen treatment as the main effects. A Bonferroni-corrected alpha value of 0·025 was used because two MANCOVAs were used to analyse *P. australis* and *S. pectinata* stem and flower biomasses

Source	d.f.	Mean square	*F*-ratio	*P*-value
Model	5	6 189	21·60	< 0·001
Population	2	2 086	6·2	0·003
Nitrogen	1	27 510	93·2	< 0·001
Population × nitrogen	2	140	0·5	0·601
Initial rhizome biomass	1	3 585	12·7	< 0·001
Error	116	282		

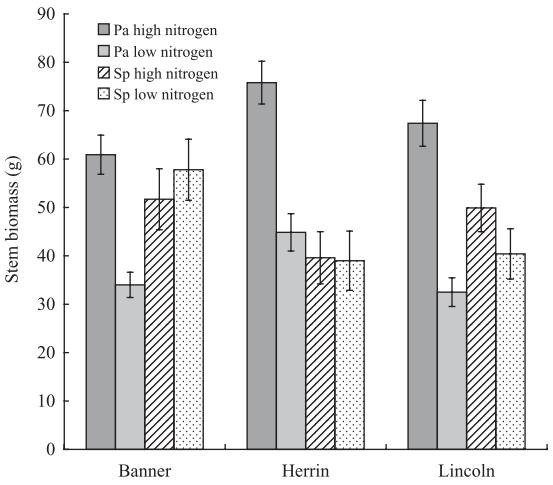

Fig. 2. Mean stem biomass ± 1 SE for *Phragmites australis* (Pa) and *Spartina pectinata* (Sp).

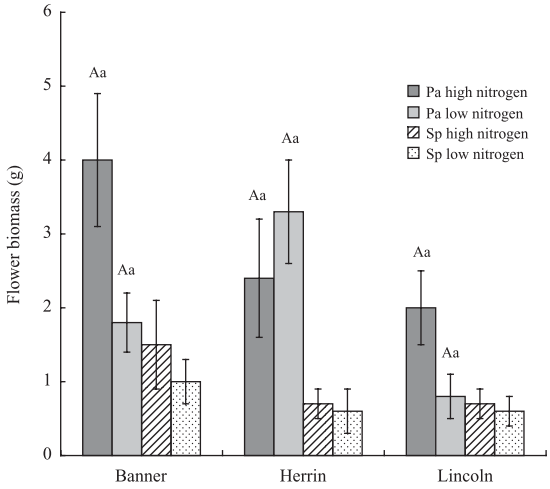

Fig. 3. Mean inflorescence biomass ± 1 SE for *Phragmites australis* (Pa) and *Spartina pectinata* (Sp). Capital letters represent significant differences among nitrogen treatments within species. Lower case letters represent significant differences among populations within species. *Spartina pectinata* means are not significantly different.

The MANCOVA on *S. pectinata* stem (Fig. 2) and flower biomass (Fig. 3) showed no significant differences in the overall models for site (Wilks' lambda, $\lambda = 0{\cdot}94$, $P < 0{\cdot}113$, d.f. = 4,228), nitrogen treatment (Wilks' lambda, $\lambda = 0{\cdot}98$, $P < 0{\cdot}310$, d.f. = 2,114) and the interaction term (Wilks' lambda, $\lambda = 0{\cdot}94$, $P < 0{\cdot}166$, d.f. = 4,228). *Spartina pectinata* stems and flowers were not heavier with respect to nitrogen treatment and population.

To determine if there were differences in *P. australis* and *S. pectinata* below-ground biomass among nitrogen treatments, two ANCOVAS were run. *P. australis* root biomass and *S. pectinata* biomass were not correlated (Pearson correlation, $r = -0{\cdot}32$, $P < 0{\cdot}08$), and were therefore analysed with separate ANCOVAS. However, because these measurements came from plants grown in the same pot, a Bonferroni correction was made and the accepted alpha level was 0·025.

The overall ANCOVA model for *P. australis* below-ground biomass was marginally different ($F = 2{\cdot}81_{(\alpha=0{\cdot}025)}$, $P < 0{\cdot}032$, d.f. = 6,24). Nitrogen was significant but population, the interaction between population, nitrogen and the initial rhizome weight were not significant (Table 3). REGWQ revealed that below-ground biomass was significantly higher in the high-nitrogen treatment (39·0 ± 4·5 g) than in the low-nitrogen treatment (25·5 ± 4·5 g) (Fig. 4).

The overall ANCOVA model for *S. pectinata* below-ground biomass was non-significant ($F = 1{\cdot}48_{(0{\cdot}025)}$, $P < 0{\cdot}49$, d.f. = 6,24). Nitrogen was the closest source to significance in the ANCOVA model ($F = 3{\cdot}31$, $P < 0{\cdot}08$, d.f. = 1,24). Although not significantly different, *S. pectinata* plants grown together with Banner Marsh *P. australis* had the greatest biomass (Fig. 4). *Spartina pectinata* plants grown under the high-nitrogen treatment had greater biomass than those grown in the low-nitrogen treatment, although not significantly higher.

Table 3. ANCOVA table for *P. australis* below-ground biomass, with initial rhizome weight as the covariate and population and nitrogen treatment as the main effects. A Bonferroni-corrected alpha value of 0·025 was used because two ANCOVAS were used to analyse *P. australis* and *S. pectinata* below-ground biomasses

Source	d.f.	Mean square	*F*-ratio	*P*-value
Model	6	0·28	2·8	0·032
Population	2	0·34	3·3	0·052
Nitrogen	1	0·8	7·9	0·0094
Population × nitrogen	2	0·06	0·06	0·55
Initial rhizome biomass	1	0·059	0·59	0·44
Error	24	0·1		

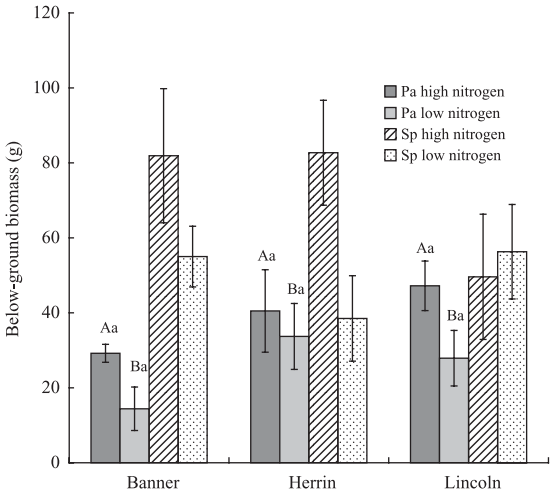

Fig. 4. Mean below-ground biomass ± 1 SE for *Phragmites australis* (Pa) and *Spartina pectinata* (Sp). Capital letters represent significant differences among nitrogen treatments within species. Lower case letters represent significant differences among populations within species. *Spartina pectinata* means are not significantly different.

Discussion

In this study, the relative response of *P. australis* to *S. pectinata* above-ground biomass was greater under high than low nitrogen conditions. *Phragmites australis* responded positively to nitrogen, whereas the native grass *S. pectinata* had no significant increase or decrease in the higher nitrogen treatment. This provides evidence that the growth of haplotype M *P. australis* increases with higher nitrogen levels, at least at nitrogen levels at or below 45 g N m^{-2}.

The response of *P. australis* inflorescence biomass was not as strong as the response of the stem biomass; *P. australis* may not invest as heavily in sexual reproduction because of its rhizome growth. When conditions are favourable, such as under high nitrogen levels, spreading by rhizomes may be more advantageous than by seed. Bart & Hartman (2002) found that, once

established, *P. australis* can spread to areas where it is unable to emerge. This suggests that if low nitrogen constrains *P. australis* establishment, it may be able to spread through rhizomes to less favourable habitats with lower nitrogen conditions (Bart & Hartman 2002).

The increase in *P. australis* below-ground biomass with higher nitrogen levels was not as great as that of the above-ground biomass. There was also no significant difference in the relative responses of below-ground *P. australis* and *S. pectinata* in the nitrogen treatments. The lack of significant response of the below-ground biomass is surprising in a species that spreads by rhizomes (Haslam 1965). Under high nitrogen levels *P. australis* can allocate more resources to above-ground growth than to rhizome growth (Minchinton & Bertness 2003). Rhizomes may be less important than above-ground growth under high nitrogen levels because there is little or no competition for nitrogen (Minchinton & Bertness 2003).

Nitrogen input has dramatically increased in mid-western USA ecosystems since the 1950s (Hey 2002). The abundance of *P. australis* has also increased dramatically in this time frame (Marks, Lapin & Randall 1994; Rice, Rooth & Stevenson 2000). Observational studies have shown that increased nutrient loads increase *P. australis* growth (Haslam 1965; Marks, Lapin & Randall 1994). The experimental work of Bertness, Ewanchuk & Silliman (2002) links increased nutrient loads to *P. australis* growth; this study found a significant relationship between nitrogen availability and the percentage of marsh border dominated by *P. australis*. Minchinton & Bertness (2003) also found that nitrogen enrichment and removal of surrounding vegetation enhance the spread of *P. australis*. The studies of Bertness, Ewanchuk & Silliman (2002) and Minchinton & Bertness (2003) were done in tidal marshes where nitrogen is not the only factor influencing *P. australis* distribution; sulphide concentration also limits *P. australis* distribution (Chambers, Mozdzer & Ambrose 1998). It is not clear what factors limit *P. australis* in the mid-west; the nitrogen levels found in the agricultural landscape of the mid-west are not likely to be limiting. In areas where nitrogen could be limiting establishment, *P. australis* may be able to establish in a more favourable area and spread to the low nitrogen area (Bart & Hartman 2002).

It is not known whether studies implicating nitrogen in the spread of *P. australis* (Haslam 1965; Bertness, Ewanchuk & Silliman 2002; Minchinton & Bertness 2003) were done on the alien haplotype M or one of the native haplotypes. It is also not known how other *P. australis* haplotypes respond to nitrogen; therefore we cannot rule out the hypothesis that the spread of *P. australis* is because of the existence of the new haplotype regardless of nitrogen levels. or because of an interaction between the new haplotype and nitrogen.

We found that providing additional nitrogen to an invasive species enhanced its growth and apparently released it from an environmentally limiting factor, supporting the environmental constraints hypothesis. Increased growth of *P. australis* under higher nitrogen conditions, however, did not suppress the growth of the native species. Resource limitations (time and labour) prevented growing *S. pectinata* without *P. australis* and vice versa; therefore it is not known how *S. pectinata* would respond without *P. australis*. Nitrogen addition could release an invasive species from a resource constraint and increase its growth, but this increased growth might not suppress a native species.

Our work supports the conclusions of Green & Galatowitsch (2001, 2002), who found that nitrogen addition increased the growth of the invasive grass *Phalaris arundinaceae. Phalaris arundinaceae* was able to suppress the growth of native species under all nitrogen levels (Green & Galatowitsch 2002), whereas *S. pectinata* growth was not suppressed by *P. australis* in our study. Green & Galatowitsch (2002) found that nitrogen suppressed the growth of native graminoid species while increasing the growth of native forb species. Differences between nitrogen treatments in *P. australis* were greater in the above-ground biomass than the below-ground biomass. *Spartina pectinata* below-ground biomass was greater in the high-nitrogen treatment, although this difference was not significant. From this observation, we conclude that nitrogen may increase the growth of the native species *S. pectinata* but not as much as it increases the growth of the invasive haplotype M *P. australis*.

In the mid-west USA, *P. australis* is often located on highways near agricultural fields, which leach nitrogen into the surrounding areas, including drainage ditches (Jordan & Weller 1996; Hey 2002). Consequently, *P. australis* is growing in nitrogen-rich areas and its success in these types of sites may be the result of a positive response to increased availability of nitrogen. Reducing nitrogen input into roadsides and natural areas may aid the control of *P. australis*.

Annually, nitrogen inputs from wet deposition are high in Illinois compared to other states in the USA, > 7·0 kg N ha^{-1} (0·7 g m^{-2}) (National Atmospheric Deposition Program (NRSP-3)/National Trends Network 2002). Total input of nitrogen in the upper Mississippi region is estimated to be 34 kg N ha^{-1} $year^{-1}$ (3·4 g N m^{-2} $year^{-1}$) (Jordan & Weller 1996). Therefore, the amount of nitrogen applied in this experiment is higher than the estimated total input of nitrogen in mid-western ecosystems. On a local scale, however, nitrogen amounts may be higher. In Illinois, maximum crop yields result from fertilizer application rates of 224 kg ha^{-1} (22·4 g N m^{-2}) (Hey 2002). In highly polluted areas of California, soil nitrogen concentrations can be as high as 90 µg N g^{-1} (Padgett & Allen 1999), higher than the average of 50·6 ± 6·3 µg NH_3 g^{-1} found in this experiment.

Morghan & Seastedt (1999) attempted to control two invasive species by reducing soil nitrogen levels by carbon amendment. Invasive species biomass declined

in reduced nitrogen plots; however, the authors were unable to conclude if reduced nitrogen increased the biomass of native species. Morghan & Seastedt (1999) did not recommend carbon amendments alone as a restoration technique because it must be repeated at regular intervals to keep up with nitrogen input. Reducing nitrogen input would be more effective than attempting to reduce nitrogen levels once they have already reached high levels.

Managers attempting to control *P. australis* should consider nitrogen input; however, this is difficult at the scale of individual sites. Nitrogen run-off from agricultural fields and acid deposition cannot be managed by one site or agency. Managing nitrogen input may be possible in a small watershed; however, managing a large watershed such as the Mississippi River would require large-scale government and citizen co-operation. A co-operative or innovative method, such as proposed by Hey (2002), will be needed to reduce nitrogen loads on roadsides and in natural areas. Hey (2002) recommended using wetlands to convert nitrate-nitrogen back into atmospheric nitrogen. With large inputs of nitrogen, however, these wetlands may become filled with invasive species such as *P. australis* and *Phalaris arundinaceae*. Therefore, attempts must be made to reduce the amount of nitrogen input by reducing fertilizer use and fossil fuel burning. Legislative approaches such as the Clean Air Act are already somewhat effective in reducing acidic deposition (Lynch, Bowersox & Grimm 2000). The UK Department for Environment, Food and Rural Affairs (Defra) is studying the effects of nitrogen enrichment and attempting to mitigate them through pollution swapping and sustainable agriculture (Dalton & Brand-Hardy 2003); such an approach would be beneficial in the USA.

This study was limited to the invasive type M *P. australis*. It is not known how native types E or S *P. australis* respond to nitrogen. Further studies comparing the nitrogen levels of habitats of all three North American haplotypes and their responses to nitrogen enrichment with and without competitor species should be conducted.

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