11-1-2010

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## Recommended Citation

Holdredge C, Bertness MD, Von Wettberg E, Silliman BR. Nutrient enrichment enhances hidden differences in phenotype to drive a cryptic plant invasion. Oikos. 2010 Nov;119(11):1776-84.

Research

**Oikos 119: 1776–1784, 2010**
doi: 10.1111/j.1600-0706.2010.18647.x

Subject Editor: Tiffany Knight. Accepted 9 March 2010

# Nutrient enrichment enhances hidden differences in phenotype to drive a cryptic plant invasion

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Many mechanisms of invasive species success have been elucidated, but those driving cryptic invasions of non-native genotypes remain least understood. In one of the most successful cryptic plant invasions in North America, we investigate the mechanisms underlying the displacement of native *Phragmites australis* by its Eurasian counterpart. Since invasive *Phragmites*' populations have been especially prolific along eutrophic shorelines, we conducted a two-year field experiment involving native and invasive genotypes that manipulated nutrient level and competitor identity (inter- and intra-genotypic competition) to assess their relative importance in driving the loss of native *Phragmites*. Inter-genotypic competition suppressed aboveground biomass of both native and invasive plants regardless of nutrient treatment (~ 27%), while nutrient addition disproportionately enhanced the aboveground biomass (by 67%) and lateral expansion (by $> 3 \times$ farther) of invasive *Phragmites*. Excavation of experimental plots indicated that nutrient addition generates these differences in aboveground growth by differentially affecting rhizome production in invasive vs native plants; invasive rhizome biomass and rhizome length increased by 595% and 32% with nutrient addition, respectively, while natives increased by only 278% and 15%. Regardless of nutrient level, native rhizomes produced twice as many roots compared to invasives, which field surveys revealed are heavily infected with mycorrhizal symbionts. These results suggest that native *Phragmites* competes well under nutrient-limited conditions because its rhizomes are laden with nutrient-harvesting roots and mycorrhizae. Invasive *Phragmites*' vigorous aboveground response to nutrients and scarcity of lateral roots, in contrast, may reflect its historic distribution in eutrophic Eurasian wetlands and correspond to its prevalence in New England marshes characterized by elevated nutrient availability and relaxed nutrient competition. These findings reveal that discrete differences in phenotype can interact with anthropogenic modification of environmental conditions to help explain the success of cryptic invaders.

Understanding the mechanisms driving biological invasions has become an important theoretical and pragmatic goal of ecology. Great strides have been made in elucidating the conditions that make environments susceptible to invasion and the traits of invading species that lead to their success. Human alteration of habitats commonly facilitates species invasions and successful invaders often have weedy, fast-growing life histories (Dukes and Mooney 1999, Byers 2002, Stachowicz et al. 2002). Few studies, however, have investigated mechanisms underlying cryptic invasions, where non-native invaders become established and proliferate without detection because they are phenotypically similar to native conspecifics (Geller 1999, Saltonstall 2002, Blakeslee et al. 2008, Mabuchi et al. 2008). The takeover of native genotypes in cryptic invasions poses the question: how can an invader dominate and displace a native counterpart when they are morphologically indistinguishable?

Shallow water marine and estuarine ecosystems have been particularly vulnerable to biological invasions due to their proximity to ports that act as transoceanic dispersal corridors and extensive human degradation (Carlton 1996, Grosholz 2002, Lotze et al. 2006). Since cryptic marine invasions are difficult to detect, their frequency and impact have been underestimated in these vulnerable ecosystems. In San Francisco Bay, California, for instance, cryptic species are thought to account for 40% of all invaders (Carlton 1996). Across North America, elusive invasions of cryptic and hybridized species have transformed plant communities in many salt, brackish and fresh water marsh landscapes. The Eurasian reed *Phragmites australis australis* (hereafter, invasive *Phragmites*), cattail hybrid *Typha* × *glauca* (Woo and Zedler 2002) and cordgrass hybrid *Spartina alterniflora* × *S. foliosa* (Tyler et al. 2007) are all marsh invaders that are phenotypically similar but competitively dominant to native conspecifics, and are notoriously linked with local losses in biodiversity (Zedler and Kercher 2004). Historically, native counterparts to these invaders were common, but rapid changes in species distributions within and across marshes justified suspicion that an introduction or hybridization had occurred.

Like many prolific plant invaders, *Phragmites, Typha* and *Spartina* are associated with eutrophic environments where nutrient enrichment has altered competitive dynamics (Silliman and Bertness 2004, Zedler and Kercher 2004, Tyler et al. 2007). In New England marshes where the cryptic *Phragmites*

invasion has occurred (Saltonstall 2002), the distribution of native grasses is mediated by competition for nutrients and species-specific tolerance of salinity and inundation stress which arrange species in zones of dominance parallel to shore (Levine et al. 1998). Species with superior nutrient-harvesting roots and rhizomes dominate the physically benign high marsh while inferior nutrient competitors are displaced to stressful, waterlogged low marsh habitats (Levine et al. 1998). In eutrophic systems, however, nutrient competition is relaxed and competition for light is intensified. This shift in resource limitation favors highly productive plants, like the low-marsh dominant, *Spartina alterniflora,* which expands into higher marsh zones, and invasive *Phragmites,* which shades out and overgrows the native plant community (Bertness et al. 2002).

What remains unclear is why native *Phragmites australis americanus* (hereafter native) is not also proliferating in eutrophic systems (but see Lynch and Saltonstall 2002). Native *Phragmites* is now rare in New England (Saltonstall 2002) despite its common distribution along the terrestrial border of fresh and oligohaline marshes for the past few thousand years (Niering et al. 1977, Orson et al. 1987). In contrast, invasive *Phragmites* is prevalent throughout New England with monospecific stands often covering entire marshes (Chambers et al. 1999, Farnsworth and Meyerson 2003). In this study, we use *Phragmites* as a model system to explore the mechanisms that underlie cryptic invasions and test the hypothesis that anthropogenic modification of environmental conditions interacts with discrete phenotypic differences between genotypes (i.e. in stem characteristics, root and rhizome architecture) to promote the displacement of native by invasive *Phragmites*.

Clonal plants, like *Phragmites*, can shift biomass allocation plastically between root, rhizome and stem structures in response to environmental cues (De Kroon and Hutchings 1995, Wolfer and Stralie 2004), including elevated nutrients, space or light. Furthermore, previous studies comparing the morphology of native and invasive *Phragmites* in adjacent populations (League et al. 2006) and seedlings (Saltonstall and Stevenson 2007) have documented variation in their shoot-to-rhizome ratios, biomass production and a number of other parameters (i.e. shoot height, shoot and rhizome internode length), although the traits used to evaluate the genetic identity of populations in the field have been limited to more subtle characteristics, such as ligule length (K. Saltonstall pers. comm.). Work to date has not examined the relative plasticity of these phenotypic traits across different nutrient regimes, however, or whether this variation gives rise to differences in competitive ability (i.e. growth rate, stand expansion) in natural field conditions. To evaluate differences in phenotypic traits and the functional response of *Phragmites* genotypes to eutrophic conditions, we transplanted native and invasive *Phragmites* in a fully factored genotype $\times$ competitor identity $\times$ nutrient level field experiment and examined the lateral expansion, biomass allocation, and root architecture of plants after two growing seasons.

Arbuscular mycorrhizae (hereafter AM) may also affect variation in belowground phenotypic traits and the success of cryptic plant invaders as AM has been shown to influence root architecture (Yano et al. 1996, Hart et al. 2003, Scheublin et al. 2007) and mediate marsh plant competition (Daleo et al. 2008). Under nutrient-limited conditions, AM typically enhance plant growth by providing the plant host with nutrients, primarily phosphorus, in exchange for carbon resources (Smith and Read 1997, Hart et al. 2003). Some studies indicate that under low soil fertility conditions AM can also induce branching of lateral roots which increases exploration of the soil and the surface area available for mycorrhizal infection (Hetrick et al. 1988, Yano et al. 1996). When phosphorus concentrations are elevated, AM may become parasitic to the host plant, which can reduce its association with AM by minimizing further fungal colonization and altering root architecture to reduce the surface area vulnerable to infection (Ratnayake et al. 1978). Commonly, aerenchymatous rhizomes thicken, lateral roots elongate and root hairs proliferate when mycorrhizal plants are no longer phosphorus limited (Hetrick et al. 1988). Both native and invasive *Phragmites* host AM (Cooke and Lefor 1998, Oliveira et al. 2001, Wirsel 2004), but it is not known whether they vary in their susceptibility to infection. Here we quantify natural AM colonization rates in sympatric populations across central and northern New England and relate these findings to the genotypic variation in root architecture and response to nutrient enrichment observed in our field experiment.

By exposing both the strength and nature of the response of native and invasive genotypes to nutrient enrichment, this study provides new insight into interaction between physiological traits and environmental conditions that may underlie the success of other cryptic plant invaders. Furthermore, the asymmetrical response of genotypes to nutrient enrichment in our natural field experiment provides convincing evidence that eutrophication is playing a central role in the aggressive expansion of invasive clonal plants, like *Phragmites,* and loss of native plant assemblages. Consequently, we promote the protection of pristine, low nutrient wetlands that can harbor native biodiversity and aggressive reduction of anthropogenic nutrient sources to these ecosystems.

## Material and methods

### Fully factored field experiment

In November 2005, we collected rhizomes from adjacent ($<$ 200 m apart) native and invasive *Phragmites* stands (each a single continuous stand, $>$ 1 ha in area) located in a tidal, brackish marsh at the Great Bay National Estuarine Research Reserve in Greenland, New Hampshire, USA. The native stand at this site is a large, vigorous clone relative to native stands identified in other Atlantic coastal marshes that exhibit lower stem densities and heights (unpubl.). Due to the protected status of native stands, however, we were unable to extract rhizomes from multiple native populations. Since the previously described differences between native and invasive *Phragmites* in relative biomass, stem density, rhizome internode length, etc. (Vasquez et al. 2005, League et al. 2006, Saltonstall and Stevenson 2007) were represented in these source stands, however, our results may be generalized cautiously across populations.

Within each stand, we extracted 40–25 × 25 × 20 cm (l × w × d, 7–10 remnant stems/plug) rhizome plugs from positions distributed across each stand (> 2 m apart to reduce impact on the stand and capture potential genetic variation within each stand). Rhizome plugs were then transplanted into trays with generic potting medium in the Brown Univ. glasshouse, watered daily and grown under the same conditions with supplemental light (14 h $day^{-1}$) for seven months. Rhizome plugs were used in the experiment rather than seeds or seedlings (as in Saltonstall and Stevenson 2007) because ecologically relevant encounters among native and invasive *Phragmites* (one genotype replacing the other on a landscape) likely occur on a clone-to-clone rather than seedling-to-seedling scale, making rhizome plugs a more appropriate proxy to make inferences about competitive interactions between genotypes.

We conducted our field experiment in a tidal, oligohaline (salinity ~ 0–12 ppt) marsh at the Adolf Rotundo Wildlife Refuge in Rehoboth, Massachusetts, USA. Currently, invasive *Phragmites* covers ~30% of the marsh area and we positioned the experiment in a 100 × 75 m high marsh area dominated by the rush, *Juncus gerardii,* that had not yet been overtaken by *Phragmites* and was protected from nutrient run-off by woody buffer on the marsh/terrestrial border (Bertness et al. 2002). In March 2006, we marked 30 1-$m^2$ plots, positioned to minimize variation in elevation and distance from creeks (> 15 m) and spaced > 3 m apart, at the site. We then used a line trimmer to clear vegetation and debris from plots and covered each with black landscaping cloth for two months to inhibit the reemergence of natural vegetation. Each plot was then randomly assigned to one of six treatments: 1) native-only, control, 2) native-only, fertilized, 3) invasive-only, control, 4) invasive-only, fertilized, 5) competition, control, and 6) competition, fertilized. In May 2006, we transplanted rhizome plugs (25 × 25 cm, l × w) into the center of cleared plots in a 2 × 2 checkerboard design. Native- and invasive-only, or 'intra-genotypic,' competition plots received two rhizome plugs in opposite quadrants and unvegetated, peat plugs in the remaining quadrants. In 'inter-genotypic' competition plots, two native and two invasive plugs were transplanted in alternate quadrants (two plugs per genotype × five replicates per treatment = 10 plugs per genotype per treatment). Plugs assigned to each treatment were selected at random from our original 40 transplant plugs. To standardize plug size, we trimmed plugs so that each contained 7–10 live tillers and rhizome material was within ± 10% (g plant material) across treatments. We assigned each plug a unique label and placed colored toothpicks adjacent to tillers to identify plugs over time. All new, emergent tillers were also labeled with toothpicks. In competition treatments, we identified the genotype of emergent tillers based on the length and density of ligule fibers, which are visibly longer and thicker on native tillers than invasive, and the tiller's source plug as the most proximate plug of the correct genotype.

After six weeks we initiated fertilization treatments. Biweekly from July to September 2006 and May to August 2007, we sprinkled 25 g of fertilizer (29:3:4 N: P: K) evenly over fertilized plots. This fertilization level has been used to release plants from nutrient limitation and mimic eutrophication (Bertness et al. 2007). We also installed 0.5 × 1 m (ht × diam.) cages of 7-mm wire mesh around all plots that excluded herbivorous mammals (Gedan et al. 2009) but did not influence the movement or distribution of arthropods (e.g. aphids, spiders, grasshoppers, beetles).

In May 2007, we counted the number of tillers within the initial plug boundaries (in-plot) and expansion tillers, operationally defined as tillers that emerged > 5 cm from the edge of the original 25 × 25 cm plug boundary. In our counts, we included all tillers found within a 1.5 m radius of each plot. To estimate average foraging distance of expansion tillers, we measured the distance between the center of each source plug and five expansion tillers per plug (if < five expansion tillers, we recorded the distance to each tiller). We also recorded the maximum foraging distance of expansion tillers for each plug.

In August 2007, we counted and harvested all the stems per plug. If we could not verify the origin of a stem, primarily those along plug borders, we excluded them from tiller counts and harvested samples. Unidentified stems were rare, < five per plot. We then oven-dried and weighed each plug to assess aboveground biomass. We also excavated plugs to quantify belowground biomass and investment in root and rhizome structures. Since harvesting expansion tiller rhizomes would have been highly destructive to the marsh, we only extracted rhizomes in the initial 2 × 2 checkerboard area, such that excavated plots were 50 × 50 × 40 cm (l × w × d), below which *Phragmites* roots and rhizomes were sparse. For each plug, we randomly selected eight rhizome sections and measured: lateral root length and density (no. roots / 10 cm of rhizome), root hair density (no. root hairs / 5 cm of lateral root), and rhizome internode length and diameter. The whole plug was then separated into roots and rhizomes, oven-dried and weighed. To estimate root and rhizome biomass in expansion regions, we performed the following calculations for each plot: expansion root biomass = [(root biomass in plot/ no. live tillers in plot) × no. expansion tillers] and expansion rhizome biomass = [(rhizome biomass in plot/ no. live tillers in plot) × no. expansion tillers]. Acknowledging that root: tiller and rhizome: tiller ratios in expansion regions likely differ from ratios within plots where tillers and belowground structures were more concentrated, we calculated expansion biomass values to provide relative, not absolute, differences in belowground biomass across treatments and should be interpreted accordingly. Total root and rhizome biomass values were then estimated as the sum of in-plot and expansion biomass values. Due to overgrowth along plug boundaries leading to interspersed roots, we excluded belowground data from competition plots and pooled plugs from native-only and invasive-only plots in statistical analyses.

## Mycorrhizae survey

To quantify AM colonization in natural populations, we collected root samples from five native and invasive *Phragmites*' stands paired within marshes (< 300 m apart). We selected sites and stands to minimize differences in salinity, tidal inundation and other abiotic conditions that influence AM colonization (Carvalho et al. 2001, McHugh and Dighton 2004). Native and invasive *Phragmites* rhizomes were collected from one marsh in New Hampshire, where our experimental plant material was collected, and four marshes

located throughout southern and central Maine (contact CH for site details). Within each stand, we extracted 5–7 × 10 cm (diam. × depth) root cores, rinsed them and stored live roots in 60% ethanol until staining with Chlorazol Black E (Brundrett et al. 1984). Roots were mounted with glycerin and AM colonization was quantified by a cross-hair intersect method at 400× magnification (McGonigle et al. 1990). Approximately 100 cross-sections were scanned for infection from each stand within each site. Due to the protected status of native stands, we could not collect samples large enough to assess root architecture, but differences between native and invasive plants were evident and consistent with experimental transplants (unpubl.). We did not assess AM colonization of roots in our field experiment because they were infected prior to the start of the experiment and, consequently, could not accurately gauge the response of AM to experimental treatments.

### Statistical analysis

In-plot tiller density, expansion tiller density, mean and maximum tiller foraging distance and aboveground biomass data were log-transformed as necessary to meet the assumptions of parametric statistics and analyzed using a fully crossed genotype × competitor identity × nutrient level analysis of variance (ANOVA) model (JMP ver. 7.0.2, SAS Inc. 2007). Belowground biomass (root, rhizome and total biomass) and root architecture (root hair density, lateral root density and length, rhizome internode length) data were analyzed using genotype × fertilization ANOVA, with inter-genotypic competition plots excluded from analysis. Plugs were evaluated as independent replicates since there was no indication that plant response differed across experimental plots. AM colonization rates were evaluated as the percent of root sections colonized (no. root sections colonized / no. total root sections viewed) for each stand at each site and averaged across sites to assess the relative susceptibility of native and invasive genotypes to AM infection.

## Results

### Field experiment

While native transplants produced slightly higher in-plot tiller densities across all treatment combinations (Fig. 1a), nutrient addition triggered explosive lateral growth of invasive plugs, which produced five times more expansion tillers than natives (genotype × fertilization, $p = 0.0007$). The mean foraging distance of invasive tillers was three times greater than that of native tillers in fertilized plots (genotype × fertilization, $p = 0.0132$, Fig. 1b) and the maximum foraging distance of fertilized invasive tillers also dwarfed maximum distances observed for natives (genotype × fertilization, $p = 0.0474$, Fig. 1c). Nutrient addition also enhanced the aboveground biomass of invasives significantly more than natives (genotype × fertilization, $p = 0.0005$, Fig. 2a), boosting invasive biomass by 67% more than the increase detected in native biomass. Aboveground differences between genotypes were less conspicuous in nutrient control plots, where the total density of tillers (in plot + expansion, 12.2 ± 1.0 and 14.4 ± 1.0 stems plug$^{-1}$) and aboveground biomass (Fig. 2a) of natives and invasives, respectively, were similar.

We also detected a main effect of competitor identity, such that plugs in inter-genotypic plots generated fewer in-plot and expansion tillers on average than plugs grown in intra-genotypic plots regardless of genotype or nutrient treatment (competitor identity, $p \leq 0.0358$, Fig 1a). Expansion tillers foraged shorter mean and maximum distances from inter-than intra-genotypic competition plots, although this effect was not statistically significant ($p \geq 0.0954$). Inter-genotype competition also reduced aboveground biomass of native and invasive plugs by 34% and 21%, respectively, relative to those grown in native- or invasive-only plots (Fig. 2a).

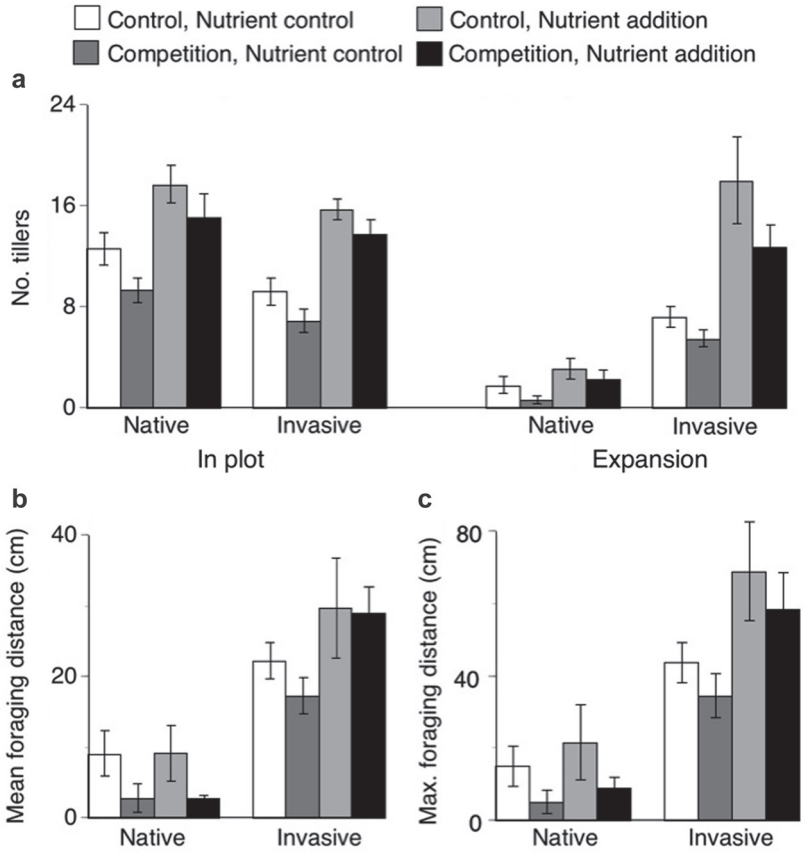
Examination of excavated plugs revealed natives produced twice as much in-plot root biomass compared to invasives regardless of fertilization treatment (Table 1). Inclusion of root biomass estimates in expansion regions where invasive plugs foraged much more extensively, however, suggests that the total root biomass (in-plot + expansion) was not significantly different for native and invasive plugs and increased similarly for each genotype in response to fertilization (Fig. 2b). In general, the proportion of biomass allocated to roots (root biomass/ total belowground biomass) was two times greater for native than invasive plugs and decreased in response to fertilization (Table 1).

In contrast, the proportion of belowground biomass allocated to rhizomes (rhizome biomass/total belowground biomass) was greater for invasive than native plugs (genotype, $F_{1,16} = 7.68$, $p < 0.0001$) and increased in response to fertilization (nutrient, $F_{1,16} = 3.25$, $p = 0.0053$, data not shown on Table 1). In plots, rhizome biomass was similar for native and invasive plants under low-nutrient conditions (Table 1), but different under high nutrient conditions where invasives produced nearly 45% more rhizome biomass than natives, although the interactive effect of genotype and nutrient treatments was not statistically significant ($p = 0.1245$). Accounting for rhizomes foraging in expansion regions, the total rhizome biomass of invasive plugs was 595% greater in fertilized than control plots, while native biomass only increased 278%, although, again, the interactive effect of genotype and nutrients was not statistically significant ($p = 0.3299$, Fig. 2c).

The phenotypic traits of belowground plant material also varied as a function of genotype and these differences in root architecture were evident in all experimental plots and surveyed sites. In general, natives produced two times more lateral roots per length of rhizome than invasives, while invasive transplants had double the density of fine root hairs and produced thicker rhizomes with longer internodes, a difference that was enhanced in fertilized plots (Table 1).

### Mycorrhizae survey

AM percent colonization of *Phragmites*' roots was high (> 32% root sections colonized) and similar for native (51.3 ± 3.7%, mean ± SE) and invasive (54.9 ± 7.7%) populations when averaged across all sites surveyed. Within sites, there also was not a consistent pattern of AM percent colonization of roots between genotypes (Fig. 3). However, the density of lateral roots and proportion of biomass allocated to lateral roots




| | In-plot tillers | | Expansion tillers | | Mean foraging distance (cm) | | Maximum foraging distance (cm) | |
|---|---|---|---|---|---|---|---|---|
| Effect | F | p | F | p | F | p | F | p |
| Genotype (G) | 2.59 | **0.0115** | 8.46 | **<0.0001** | 9.43 | **<0.0001** | 9.06 | **<0.0001** |
| Competitor (C) | 2.88 | **0.0053** | 2.14 | **0.0358** | 1.69 | 0.0954 | 1.58 | 0.1182 |
| Nutrient (N) | 6.83 | **<0.0001** | 4.99 | **<0.0001** | 2.57 | **0.0122** | 3.05 | **0.0032** |
| G × C | 0.48 | 0.6295 | 1.19 | 0.2386 | 1.18 | 0.2434 | 0.57 | 0.5716 |
| G × N | 0.71 | 0.4784 | 3.57 | **0.0007** | 2.54 | **0.0132** | 2.02 | **0.0474** |
| C × N | 0.31 | 0.7548 | 0.76 | 0.4494 | 0.44 | 0.6625 | 0.15 | 0.8815 |
| G × C × N | 0.09 | 0.9321 | 0.95 | 0.3449 | 0.54 | 0.5905 | 0.46 | 0.6475 |

Figure 1. The density of tillers in the initial plot and expansion region (a), and mean (b) and maximum (c) distances foraged by native and invasive *Phragmites* tillers in Spring 2007 after one full growing season. Data are shown as mean ± SE pooled across 10 replicate plugs. Effects table summarizing three-way genotype × competitor identity × nutrient level ANOVA tests are shown below.

(i.e. surface area of belowground structures able to be colonized by AM symbionts, Fig. 2b, Table 1) was much higher for native than invasive genotypes in both the large-scale AM survey and smaller-scale field experiment.

## Discussion

Our experimental and survey findings add further mechanistic evidence to an increasing body of literature demonstrating that eutrophication plays a domineering role in driving the cryptic displacement of native *Phragmites* by its Eurasian counterpart in New England salt marshes (Chambers et al. 1999, Bertness et al. 2002, Silliman and Bertness 2004). By reducing the benefits of nutrient acquisition structures and favoring plants that excel in aboveground and vegetative growth, nutrient enrichment disproportionately promotes the more productive and rapidly expanding invasive *Phragmites* and is fueling its invasion in freshwater and non-tidal marshes scattered throughout the interior of North America. Given these findings, conservation managers should have a rigorous understanding of the distinguishing phenotypic traits of cryptic species and how these features interact with environmental conditions to reduce future cryptic invader impacts.

### Eutrophication effects on dominance of *Phragmites*

Previous research has shown that eutrophication can trigger the proliferation of fast-growing primary producers at the expense of more diverse, but less productive, plants and seaweeds in wetland, estuarine and shallow marine habitats (Newman et al. 1996, Bertness et al. 2002, Childers et al. 2003, Zedler and Kercher 2004). In eutrophic salt and fresh water marshes, the most productive species are often invasive (Zedler and Kercher 2004). For example, tall, monotypic stands of invasive cattails, *Typha* × *glauca*, have displaced native plant communities throughout the heavily impacted Great Lakes region of the United States. In a 2002 study, Woo and Zedler found fertilization disproportionately enhanced

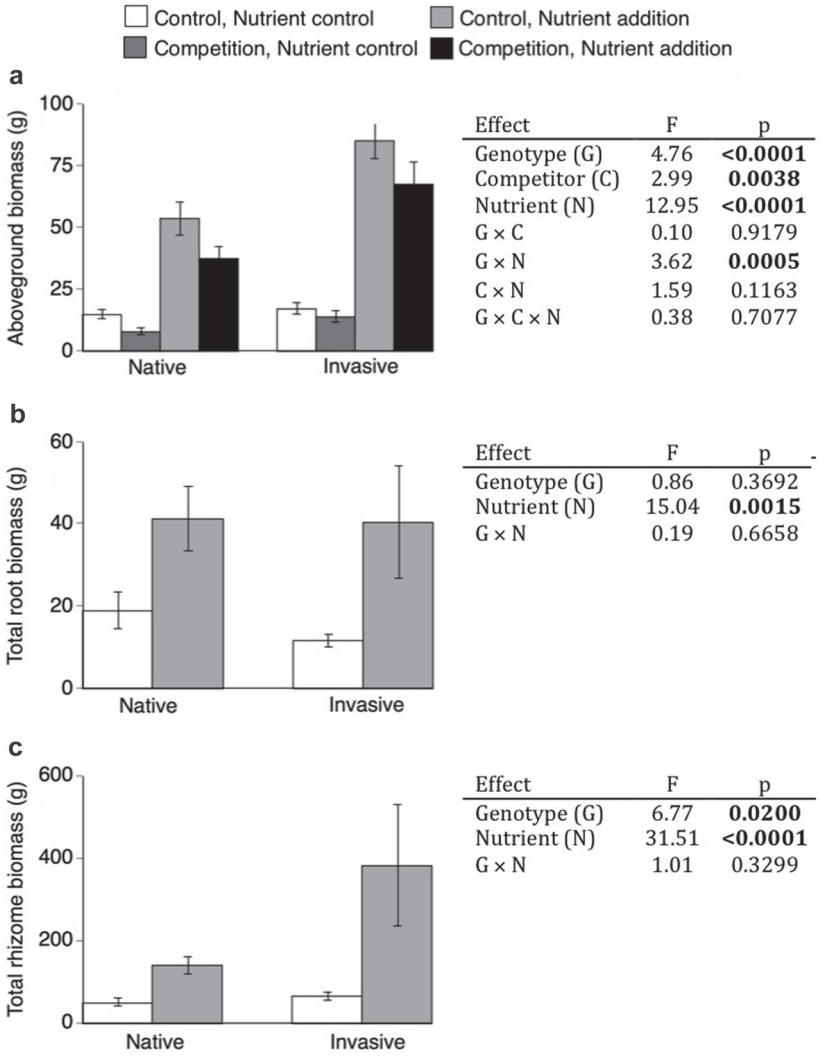



Figure 2. The aboveground biomass (a) and estimated total root biomass (b) and total rhizome biomass (c) of experimental native and invasive *Phragmites* plugs harvested after two full growing seasons. Data are shown as mean ± SE pooled across 10 replicate plugs. Effects table summarizing three- and two-way ANOVA tests are shown beside corresponding figures.

*T. glauca* production compared to native sedges and concluded that nutrients alone could shift competitive dominance from sedges to cattails in this system. In the northeastern United States, *Phragmites* is the tallest, most productive species in invaded marshes and is highly associated with eutrophic conditions (Chambers et al. 1999, Silliman and Bertness 2004). The aggressive vegetative response of invasive *Phragmites* to fertilization found in this study suggests that nutrient enrichment is a critical driver of its expansion and corresponding loss of the native plant community in New England marshes.

In our field experiment, under nutrient-limited (control) conditions, native plants matched invasives in total tiller density and above- and belowground biomass (Fig. 1, 2), suggesting that natives may keep pace and coexist with invasive *Phragmites* when competition for nutrients is strong. In fact, all five marshes where we were able to find sympatric stands of native and invasive *Phragmites* were in protected reserves where adjacent development was minimal and the potential for nutrient enrichment low. Due to the scarcity of native *Phragmites* and the difficulty in identifying genotypes in the field, the geographic distribution and long-term demography of native *Phragmites* are unknown. Our findings, however, suggest that native *Phragmites* can persist within pristine, nutrient-limited marshes even in the presence of invasive stands because it is a strong nutrient competitor.

When competition for nutrients was relaxed in fertilized plots, the growth rate of native and invasive *Phragmites* diverged dramatically. In only two growing seasons, fertilization led to the rapid expansion of invasive *Phragmites* but did not have a significant effect on the density or distance traveled by native tillers (Fig. 1). Invasive *Phragmites* produced nearly 70% more aboveground biomass in fertilized plots than natives (Fig. 2) and had already begun to surround native transplants in competition treatments (unpubl.). Although we did not see significant inter-genotypic suppression of native *Phragmites* by invasive *Phragmites* in our two-year experiment, our results suggest that invasive *Phragmites* displaces native *Phragmites* over time by producing more biomass and expanding at a faster rate. In the eutrophic marshes of Narragansett Bay, Rhode Island (Bertness et al. 2002, Silliman and

Table 1. Summary table of the phenotypic traits of and allocation of biomass to root and rhizome structures harvested in August 2007. Biomass measurements are presented as those collected from initial transplant plugs and estimates for total root and rhizome biomass (in plot and expansion) are presented in Fig. 2 (see Methods for details). Data are shown as mean ± SE. Effects table summarizing two-way genotype × nutrient level ANOVA tests are shown below corresponding response variables.

| Genotype, treatment | Lateral roots/ 10 cm rhizome | Root length (cm) | Internode length (cm) | Rhizome diameter (cm) | Root biomass, in plot (g) | Rhizome biomass, in plot (g) | Root/ total belowground biomass % | No. root hairs / 5 cm lateral root |
|---|---|---|---|---|---|---|---|---|
| Native, control | 12.5 ± 0.9 | 14.8 ± 0.6 | 5.8 ± 0.1 | 2.3 ± 0.2 | 15.3 ± 3.2 | 40.8 ± 7.9 | 27.2 ± 1.9 | 5.8 ± 1.9 |
| Native, fertilized | 14.6 ± 1.1 | 17.7 ± 1.6 | 6.7 ± 0.4 | 2.0 ± 0.3 | 35.4 ± 7.9 | 117.9 ± 19.1 | 22.5 ± 1.3 | 3.8 ± 1.1 |
| Invasive, control | 6.4 ± 1.0 | 11.9 ± 1.0 | 6.7 ± 0.2 | 4.5 ± 0.4 | 6.54 ± 1.0 | 36.5 ± 4.8 | 15.4 ± 1.4 | 10.3 ± 0.8 |
| Invasive fertilized | 6.5 ± 0.5 | 16.7 ± 1.3 | 8.8 ± 0.2 | 4.8 ± 0.4 | 18.8 ± 3.0 | 169.9 ± 25.6 | 10.0 ± 0.6 | 12.0 ± 0.7 |
| ANOVA | | | | | | | | |
| Genotype | *** | ns | *** | *** | * | ns | *** | ** |
| Nutrient | ns | ** | *** | ns | ** | *** | ** | ns |
| Genotype × Nutrient | ns | ns | * | ns | ns | ns | ns | ns |

Bertness 2004) where we have 25 years of field experience, small native stands of *Phragmites* which were common in the 1980s have almost all disappeared and in their place are expansive stands of invasive *Phragmites* (unpubl.).

## Root architecture, mycorrhizae and natural history of *Phragmites*

The different aboveground response of native and invasive *Phragmites* to fertilization corresponded to underlying phenotypic differences in root architecture between genotypes. Native *Phragmites* is likely a strong nutrient competitor because of its highly developed network of lateral roots that hang from thin, compact rhizomes (Table 1). This morphology is conducive to nutrient acquisition since the lateral roots provide ample surface area for mycorrhizal symbionts that are capable of harvesting nutrients from large volumes of soil (Hetrick et al. 1988). Invasive *Phragmites*, in contrast, has fewer lateral roots, but higher densities of root hairs, which can absorb nutrients directly from the soil, and thick, long rhizomes that foraged well beyond the boundaries of the plot. When nutrients were added to the system, both genotypes reduced the proportion of biomass allocated to lateral roots and increased biomass investment in rhizomes, but invasive plants, predisposed with thick rhizomes and few roots, did this to a much greater degree (Table 1, Fig. 2).

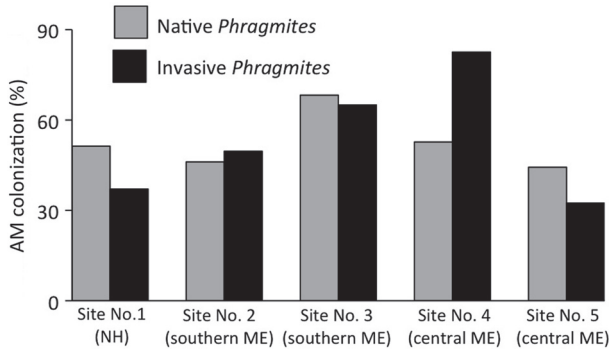



Figure 3. Results of a five-marsh survey of arbuscular mycorrhizae (AM) colonization of roots harvested from sympatric native and invasive *Phragmites* in New Hampshire and Maine, USA. AM colonization (%) represents the percent of root cross-sections viewed colonized by AM based on standard scoring methods.

There is the potential that these differences in root architecture among genotypes were induced by environmental cues, such that our transplant plugs exhibited disparities in structural traits because they were extracted from source populations positioned in environments that varied in important, but unaccounted for, ways. If environmentally-induced, we would expect that the observed differences in traits would fade if we were to propagate multiple generations of each *Phragmites* genotype by seed in a controlled environment (Salgado and Pennings 2004). It is more likely, however, that this phenotypic variation in root architecture is constitutive, or under genetic control, as it has been observed in other studies conducted in a range of salinities (Vasquez et al. 2005, League et al. 2006, Saltonstall and Stevenson 2007), and corresponds to the historic distribution of each genotype (Salgado and Pennings 2005). Invasive *Phragmites* was introduced from Eurasia where habitat degradation and eutrophication have impacted coastal ecosystems for centuries longer than in North America (Davy et al. 2009). Within its home range, invasive *Phragmites* is prolific in disturbed, eutrophic marshes (Ostendorp 1989, Romero et al. 1999) and is so successful in these environments that it is commonly used to process sewage effluent in constructed wetlands (Hardej and Ozimek 2002). Thus, it is not surprising that the phenotypic response of invasive *Phragmites* to high nutrients was not to produce nutrient-harvesting lateral roots but more, long, thick rhizomes that efficiently spread across marsh landscapes.

The phenotypic traits of native *Phragmites*, by comparison, correspond to its distribution in New England wetlands that have been historically nutrient-limited. In all five native stands surveyed, we observed a high density of elongate roots that were heavily colonized by mycorrhizae. The proportion of belowground structures suitable for AM colonization [root biomass/ total belowground biomass (%), as mycorrhizae tend to be sparse on thick rhizomes (Yano et al. 1996)] was also two-times greater for native than invasive plants and natives produced fewer fine root hairs relative to invasives (Table 1, Fig. 2). This pattern suggests that native *Phragmites* heavily utilizes AM symbionts, a strategy efficient in low-nutrient

environments, while invasive *Phragmites* may absorb more of its nutrients directly through root hairs, a strategy more efficient in eutrophic environments (Fitter 1985, Fitter and Strickland 1991). Although further studies are required to assess whether these phenotypic traits are environmentally-induced or constitutive and gauge the relative importance of *Phragmites*– mycorrhizae interactions across genotypes, our results indicate that genetic differences in root architecture and utilization of mycorrhizal symbionts likely contribute to the success of native and invasive populations in low nutrient and eutrophic marshes.

## Conclusions

As genetic techniques that distinguish native from non-native genotypes improve, the prevalence and impact of cryptic invaders are emerging (Saltonstall 2003). Our findings reveal that underlying phenotypic differences can determine how and where cryptic invaders are successful. Consequently, we propose that identifying these critical traits and understanding how they interact with human alteration of ecosystems will be essential to managing the spread of cryptic invaders. Our results also suggest that pervasive eutrophication may be reducing the role played by mycorrhizal fungi and the competitive advantage of species that utilize these symbionts in wetland ecosystems that historically have been nitrogen-limited.

*Acknowledgements* – We thank Great Bay NERR and Rachel Carson National Wildlife Refuge for providing rhizome samples, E. Hazelton for assistance, K. B. Gedan for comments on the manuscript and N. Sala, W. Goldenheim and A. Irving for help in the field. Funding was provided by an Andrew Mellon Foundation Young Investigator Grant awarded to BS, Brown Univ. UTRA program and Rhode Island Sea Grant.

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