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SPECIAL ISSUE: *Phragmites australis* in North America and Europe

Maximal stomatal conductance to water and plasticity in stomatal traits differ between native and invasive introduced lineages of *Phragmites australis* in North America

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Abstract. The fitness costs of reproduction by clonal growth can include a limited ability to adapt to environmental and temporal heterogeneity. Paradoxically, some facultatively clonal species are not only able to survive, but colonize, thrive and expand in heterogeneous environments. This is likely due to the capacity for acclimation (*sensu stricto*) that compensates for the fitness costs and complements the ecological advantages of clonality. Introduced *Phragmites australis* demonstrates great phenotypic plasticity in response to temperature, nutrient availability, geographic gradient, water depths, habitat fertility, atmospheric CO_2, interspecific competition and intraspecific competition for light. However, no *in situ* comparative subspecies studies have explored the difference in plasticity between the non-invasive native lineage and the highly invasive introduced lineage. Clonality of the native and introduced lineages makes it possible to control for genetic variation, making *P. australis* a unique system for the comparative study of plasticity. Using previously identified clonal genotypes, we investigated differences in their phenotypic plasticity through measurements of the lengths and densities of stomata on both the abaxial (lower) and adaxial (upper) surfaces of leaves, and synthesized these measurements to estimate impacts on maximum stomatal conductance to water (g_{wmax}). Results demonstrated that at three marsh sites, invasive lineages have consistently greater g_{wmax} than their native congeners, as a result of greater stomatal densities and smaller stomata. Our analysis also suggests that phenotypic plasticity, determined as within-genotype variation in g_{wmax}, of the invasive lineage is similar to, or exceeds, that shown by the native lineage.

Keywords: Clonal plant; invasive; *Phragmites*; plasticity; stomata.

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Introduction

The capacity for clonal growth is often given as an explanation for the invasive character of many introduced species (Thompson *et al.* 1995). Clonal growth affords species a capacity for reproduction despite small initial population sizes. It also offers competitive advantages such as the ability to nurse new ramets (sprouts), share resources between ramets and avoid the costly risks involved in sexual reproduction. However, the fitness costs of reproduction by clonal growth can include a limited ability to adapt to environmental and temporal heterogeneity (Alpert and Simms 2002). Recombination of genetic material and associated natural selection are not available for the rapid innovation and trial of new genotypes in clones, suggesting that the range of habitats invaded by clonal lineages should be more limited than that inhabited by competitors exhibiting more frequent sexual reproduction. Paradoxically, some facultatively clonal species are not only able to survive, but colonize, thrive and expand in heterogeneous environments. What factors underlie the success of particularly invasive clonal lineages? We hypothesize that these lineages are able to compete with, and ultimately outcompete, species with more diverse gene pools, greater rates of recombination or longer history of local adaptation, through the process of acclimation (*sensu stricto*) and a potentially greater range of phenotypic plasticity, which compensates for the fitness costs and complements the ecological advantages of clonality.

Phragmites australis is a large stature clonal grass that is found in a wide range of wetland and marsh-like ecosystems and occurs on every continent but Antarctica. In North America, several lineages have been recognized, while two are most prevalent: *P. australis* (Trin. × Steud.) is an invasive lineage (introduced), and *P. australis* subspecies *americanus* (Saltonstall, PM Peterson and Soreng) is a native lineage (native) (Saltonstall *et al.* 2004). Both the native and introduced lineages have the capacity for extensive clonal growth (Douhovnikoff and Hazelton 2014). However, the introduced lineage is expanding its range and outcompeting many native species across a broad range of local conditions and wetland types throughout North America.

Introduced *P. australis* demonstrates great phenotypic plasticity in response to temperature and nutrient availability (Eller and Brix 2012), geographic gradient (Bastlova *et al.* 2004), water depths (Vretare *et al.* 2001), habitat fertility (Clevering 1999), atmospheric CO_2 (Mozdzer and Megonigal 2012), interspecific competition (Bellavance and Brisson 2010) and intraspecific competition for light (Bellavance and Brisson 2010). However, the majority of prior work focussed on common garden studies with the European ancestral lineage, and not plants collected in North America. Further, no *in situ* comparative lineage studies have explored the difference in plasticity between the invasive introduced and non-invasive native lineages (reviewed in Mozdzer *et al.* 2013). Despite the obvious comparative potential, such closely related groups have rarely been examined with respect to the ecology of invasion. Among 93 comparative studies of plasticity in invasive plants identified by Palacio-López and Gianoli (2011), the closest shared taxon was at the genus level.

In addition to closely related (conspecific) lineages, the clonality of the native and introduced lineages make *P. australis* a unique system for the comparative study of phenotypic plasticity. Phenotype is a result of genetic (G) × environmental (E) interactions (Via and Lande 1985). Clonal plants are powerful model systems as they control for genetics (G). Assuming moderate mutation rates and developmental differences among compared groups, observed variation would largely be explained by plastic responses to environmental (E) conditions. Naturally occurring replicates (ramets) of a given genotype (genet) make it possible to measure and compare the reaction norms within and between genotypes permitting a better understanding of the role plasticity plays in plant ecology (Douhovnikoff and Dodd 2015) from the ramet to the lineage scale (Gianoli and Valladares 2012).

The size and spacing of stomata on leaves are simple measurements that provide a strong framework within which to explore phenotypic plasticity linked with physiological performance (Hetherington and Woodward 2003). Stomata permit and regulate gas exchange between the inner plant and the atmosphere, facilitating the exchange of gases necessary for photosynthesis and transpiration. In moving air, stomatal conductance is the principal control over leaf gas exchange with direct consequences for both leaf metabolism and energy balance (Schulze *et al.* 1994). Stomatal morphometrics provide an accurate representation of the capacity for leaf gas exchange through the calculation of maximal conductance (g_{max}, Dow *et al.* 2014), which incorporates the influences of stomatal pore area and pore depth (Brown and Escombe 1900). The multi-dimensional framework for the assessment of stomatal variation provided by g_{max} has been used to demonstrate both heritable variation and environmental plasticity (Franks *et al.* 2009; Fanourakis *et al.* 2015). Differences in stomatal morphometrics have previously been identified for *P. australis* lineages (Hansen *et al.* 2007; Saltonstall *et al.* 2007). Differences in plasticity of stomatal morphology could further permit a single genotype to acclimate to a range of conditions, making it a strong competitor in heterogeneous environments such as tidal wetlands.

Introduced *Phragmites* produces biomass more quickly, metabolizes carbon and nitrogen more quickly, and it is suspected that the introduced lineage has a photosynthetic advantage over its native conspecific (Mozdzer *et al.* 2013). Using previously identified clonal genotypes (Douhovnikoff and Hazelton 2014), we took advantage of the g_{max} framework to investigate variation in stomatal conductance and its dependence on stomatal morphometrics within and between *P. australis* lineages, stands and genets. We quantified maximum stomatal conductance to water, g_{wmax}, and its plasticity, through measurements of the lengths and densities of stomata on the abaxial (lower) and adaxial (upper) surfaces of leaves. We tested the hypotheses that (i) there are genetic effects on g_{wmax} differentiating native and introduced *P. australis* lineages and genotypes and (ii) variation in g_{wmax} in response to local site conditions is greater in clones of introduced *P. australis*, indicating greater physiological plasticity that may contribute to the invasive character of this lineage.

Methods

Site description

Three marshes in Southern Maine were systematically surveyed for stand scale *P. australis* clonal structure, which was mapped on a 5×5 m grid (Douhovnikoff and Hazelton 2014). Marsh sites were Libby (70.310W, 43.563N), Spurwink (70.250W, 43.589N) and the more distant Webhannet (70.585W, 43.286N). Maximum and minimum marsh-to-marsh distances were 43.2 and 5.6 km, respectively. The marshes are back barrier dune systems, and are well suited for comparisons of lineages among stands within the respective marshes; both native and introduced *P. australis* were present, in proximity to each other, at all sites. In the case of the Libby marsh, the introduced and native stands abut each other and overlap in some areas (E. L. G. Hazelton, pers. obs.). The most developed of these sites is the Webhannet marsh, the Spurwink marsh abuts agricultural land and the Libby marsh occupies a watershed with relatively little development or agriculture.

Sample collection and DNA extraction

Samples were collected in the summer of 2011. The most apical fully expanded leaves were collected from the nearest stem to each sample grid point. Earlier work had determined that the 5×5 m sampling grid was ideal for the efficient mapping of genotypic diversity at the sites (Douhovnikoff and Hazelton 2014). Lineages were differentiated by morphological characteristics (Swearingen and Saltonstall 2010), and microsatellite markers (Saltonstall 2003) were used to establish clonal identities (detailed methods in Douhovnikoff and Hazelton 2014).

Stomatal morphometrics and g_{wmax}

Leaf material was stored at -20 °C prior to analysis. Epidermal impressions were made using clear nail polish (ethyl acetate) applied directly to the leaf surface, and were mounted on slides. Preliminary measurements indicated that stomatal traits varied systematically along the length of leaves, so middle-adaxial and middle-abaxial leaf surfaces were sampled for consistency. Slides were viewed on Olympus BX-51 microscopes and stomatal morphometrics were determined from images captured at $\times 400$ total magnification using QCapture software (QImaging). ImageJ software (Abramoff *et al.* 2004) was used to count the total number of stomata and measure the lengths of five randomly chosen stomata within a standardized 200×200 μm area within each image.

Maximum stomatal conductance to water vapour (mol m^{-2} s^{-1}) was calculated using the formula of Brown and Escombe (1900, see also Weyers and Meidner 1990; Franks and Farquhar 2006) parameterized for grass stomata (Taylor *et al.* 2012). Briefly, g_{wmax} for each leaf is the sum of maximum conductance values for leaf surfaces ($g_{wmax,i}$, where i is abaxial or adaxial), calculated as:

$$g_{wmax,i} = \frac{d}{v} \times D \times \frac{a_{max}}{l + (\pi/2)\sqrt{a_{max}/\pi}}$$

The diffusivity of water in air (d, m^2 s^{-1}, at 25 °C), the molar volume of air (v, m^3 mol^{-1}, at 25 °C) and π are physical and geometric constants. Stomatal density (D, m^{-2}) and stomatal length (L, m) were determined from our measurements and used to derive (i) stomatal size (S, m^2), as $0.25L^2$ (stomatal width $= 0.25L$, Taylor *et al.* (2012)); (ii) depth of stomatal pores (l, m), as $0.125L$ (equal to guard cell width, Franks *et al.* 2009) and (iii) the maximum stomatal pore area (a_{max}, m^2), as $0.4S$ (an empirical relationship for grass stomata determined by Taylor *et al.* 2012). Calculations were made using R Language and Environment (version 3.1.3, R Development Core Team 2015).

Statistical analysis

We $\log_e$ transformed g_{wmax} prior to statistical analysis. We employed standard approaches for an unbalanced nested 2×2 analysis of variance, using the R Language and Environment (version 3.1.3, R Development Core Team 2015), as follows. We performed a Type III conditioning procedure (Fox 2008), initially testing for interactions between the two putative fixed effects, site and lineage, holding the clones as random effects. We detected no significant

interactions in the complete data set, though we did find weak but statistically significant interactions when several highly variable clones were excluded from the data. We inferred the significant effects using the complete data set, employing a Type II procedure to ensure full power to determine effects (Langsrud 2003): all factors (site, lineage and clone) exhibited effects with *P*-values $<10^{-16}$. We also employed a more advanced model selection machinery available to Bayesian approaches to calculate the Bayes factors across a wide variety of possible analytic frameworks (Rouder *et al.* 2012), garnering additional support for our choice of analysis. For clones with $N > 11$ ramets, robust estimates of within-clone spatial variation, mean and standard deviation (SD) in $\log_e(g_{wmax})$ were made using a permutation test that preserved the variation intrinsic to the data accounting for the variable number of ramets within each clone. This test proceeds by generating two distributions of statistics, a null distribution reflecting the correlation expected under no spatial effect but accounting for unevenness in the underlying spatial distribution of ramets and a corresponding distribution reflecting the correlation observed within the data. The first was generated by randomly permuting which g_{wmax} values associate with which (*x*,*y*) position pair for a given ramet, and repeating 10 000 times; for each permutation, a subset of size 10 was taken and a simple Spearman (rank order) correlation was calculated between the pairwise distance between ramets and the difference in their g_{wmax} values. The latter distribution was generated to represent the observed data by sampling 10 000 size 10 subsets and again calculating the Spearman correlation. A *P*-value was calculated by finding the fraction of replicates in the observed distribution that were more extreme than all values in the null distribution. While similar in concept to a Mantel test, this permutation approach is significantly more conservative in its *P*-value calculation while still sensitive to even mild (correlation values of 0.1) levels of spatial structure. To ensure that the results were independent of coordinate frame, the test was repeated having rotated the axes by 45°.

Results

Site, lineage and clone as factors influencing g_{wmax}

Our model of $\log_e(g_{wmax})$ identified significant additive effects of site, lineage and clone (clones having been identified as unique to each site, i.e. completely nested; *F* values 48.06, 495.70 and 4.50 with df = 2, 1 and 68, respectively, $P < 10^{-16}$ for all). At the three sites, *P. australis* showed greater mean $\log_e(g_{wmax})$ at Webhannet (2.28) and Libby (2.26) than at Spurwink (1.93). When grand means for the native and introduced lineages were compared, $\log_e(g_{wmax})$ of the introduced lineage was 21 % greater than the native lineage (Fig. 1A), equivalent to an increase of 54 % when back-transformed to the original scale (mean (2.5–97.5 % quantile): native, 7.5 (4.5–12.1) mol m^{-2} s^{-1}; introduced,

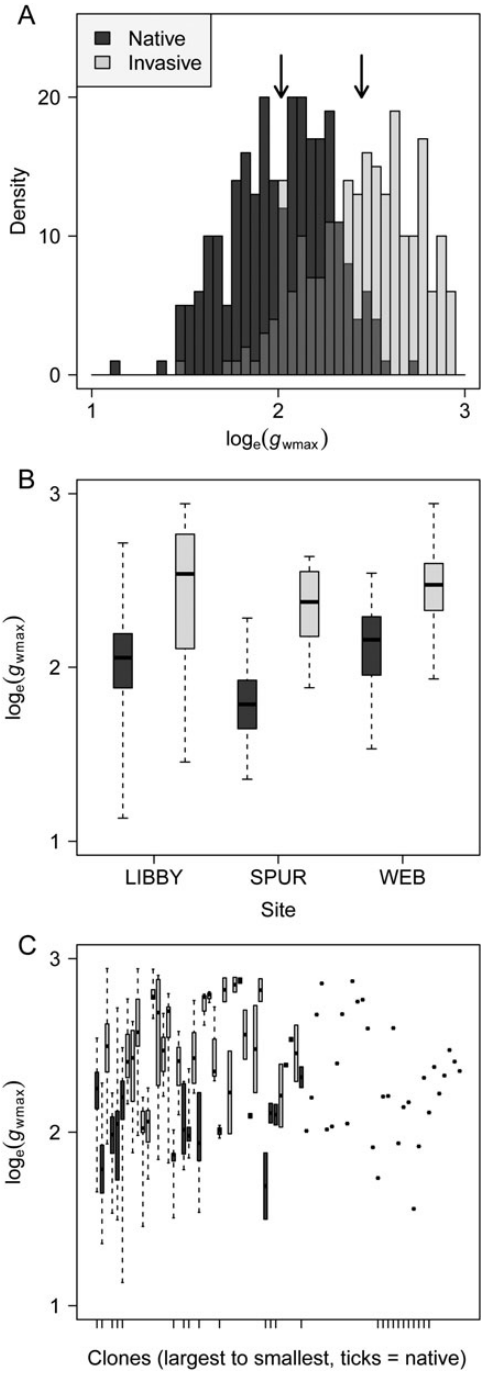

Figure 1. (A) Native and invasive lineages of *P. australis* show significantly different g_{wmax} determined on the basis of stomatal morphometrics. (B) Differences in g_{wmax} between native and invasive lineages of *P. australis* are consistent between marsh sites in Maine, and are substantially greater than differences in g_{wmax} between sites. (C) When comparing unique clones of *P. australis* across three marsh sites in Maine, g_{wmax} differentiates clones belonging to native and invasive lineages.

11.5 (6.7–18.1) mol m^{-2} s^{-1}). This substantial difference between the lineages was relatively consistent across the three sites (16–31 % increase on $\log_e$ scale depending on site; Fig. 1B). When clones were treated as independent of their classification by site and lineage, and when lineage was excluded from consideration, among-clone variation explained the majority of variance in $\log_e(g_{wmax})$ (55 %). Differences among clones were, however, strongly structured by contrasts between native and introduced lineages and sites (Fig. 1C).

Plasticity (within-clone variation) in g_{wmax}

Using our entire data set, plasticity in $\log_e(g_{wmax})$, determined as the SD of $\log_e(g_{wmax})$ conditioned for clone identity (Fig. 2), was greater within the introduced lineage at the Libby (SD: introduced, 0.36; native, 0.27) and Spurwink (SD: introduced, 0.22; native, 0.18) marshes. At the Webhannet marsh, the opposite was true (Fig. 2), but the lineages were also more similar (SD: introduced, 0.20; native, 0.22).

Our investigation of both spatial variation and phenotypic variation in $\log_e(g_{wmax})$ within the 10 clones having $N > 11$ ramets found no evidence for significant within-clone spatial structure (permutation test null distribution construction described in Methods with 9999 degrees of freedom, $P > 0.291$). The test used does not rule out spatial autocorrelation as a determinant of finer-scale patterns. Distributions of SDs for $\log_e(g_{wmax})$ within large clones at the Libby site, in particular, were multimodal (Fig. 3). The permutation distributions shown in Fig. 3 were realized for each clone by holding the number of ramets to 10 and resampling from the full collection of observed values with replacement: for each clone, 1000 resamplings were made, with the sample mean and sample SD calculated for each sample. This analysis indicates that within these large, genetically homogeneous clones, subsets of ramets showed uniquely identifiable levels of plasticity, perhaps linked by epi-genotype.

Lineage differences in stomatal morphometrics underpinning g_{wmax}

The consistently greater g_{wmax} of introduced lineages of *Phragmites* was a result of increases in both adaxial and abaxial g_{wmax} (Fig. 4A). Size (*S*)–density (*D*) plots indicated that differences in *S* and *D* between the lineages were broadly consistent with a size–density trade-off: the introduced lineage had relatively smaller and more abundant stomata than the native lineage (Fig. 4B and C). Shifts in *S* and *D* among native ramets resulted in conservation of g_{wmax} (data for native ramets fall along g_{wmax} isoclines in Fig. 4B and C). Among ramets of the introduced lineage, variation in g_{wmax} arose from variation in *D* that was not matched by shifts in *S* (Fig. 4B and C).

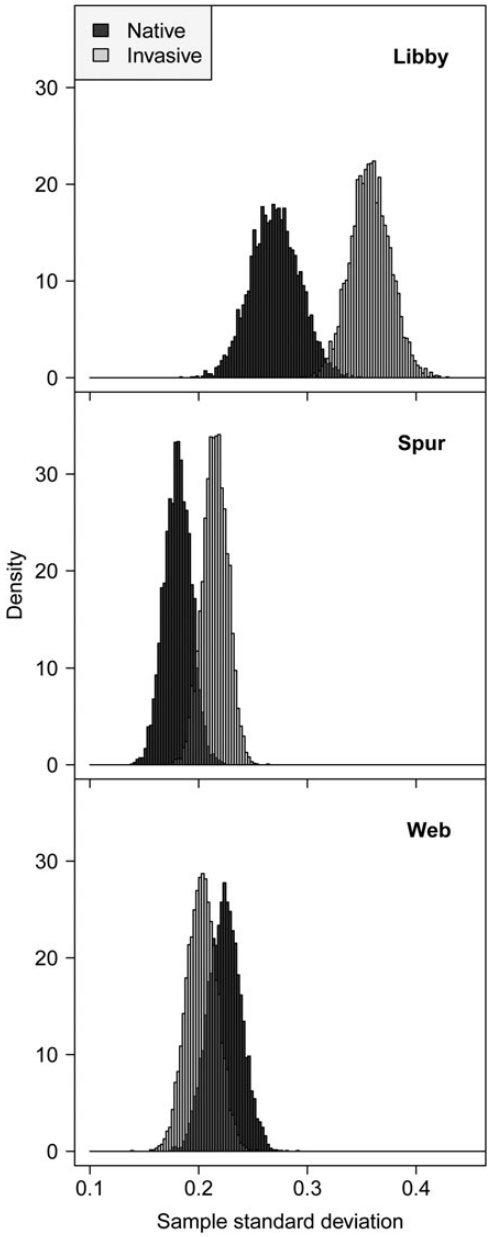

Figure 2. Plasticity (SD) in g_{wmax} within the invasive lineage of *P. australis* is similar to, or exceeds, plasticity measured within the native lineage at Libby, Spurwink and Webhannet marshes, in Maine.

Discussion

Previous demonstrations that g_{wmax} is reliably linked with gas exchange performance (Dow *et al.* 2014) and demonstrates both heritable variation and environmental plasticity (Franks *et al.* 2009; Fanourakis *et al.* 2015) suggested that simple measurements of the size and spacing of stomata on leaves would provide a strong framework within which to explore phenotypic plasticity in *P. australis*. Our results confirm this expectation; we were able to characterize plasticity in stomatal morphometrics that

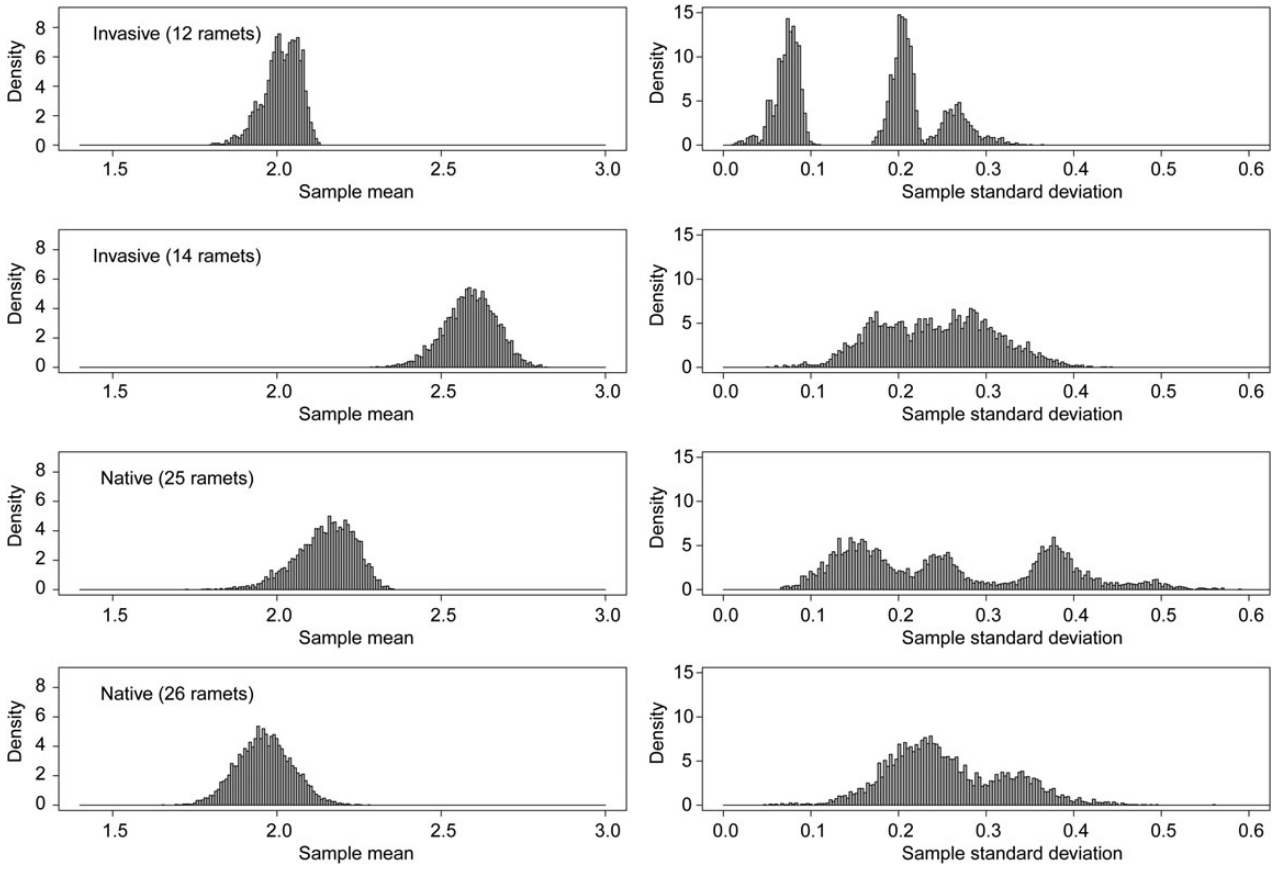

Figure 3. Permutation analysis demonstrates that unimodal distributions for means of g_{wmax} within clones of *P. australis* (left column) are linked with multimodal distributions for SDs (right column); clusters of ramets within each genet show unique levels of variability. Results shown are for invasive (BI24, BI51) and native (BN11, BN8) clones at the Libby marsh.

contributed to differences in g_{wmax} between native and invasive lineages. We found that at three marsh sites separated by as much as 43 km, introduced lineages have consistently greater g_{wmax} than their native congeners. Thus, g_{wmax} can be added to an already extensive list of functional traits that distinguish these genetic variants (stem densities, heights, above ground biomass, leaf area, leaf nitrogen and chlorophyll content, rates of photosynthesis, relative growth rates (RGR) and carbon fixation; reviewed in Mozdzer *et al.* 2013). Our analysis also indicates that plasticity of the introduced lineage, determined as within-genotype variation in g_{wmax}, is similar to or exceeds that shown by the native lineage. These results provide insights that scale up from stomatal morphometrics to community dynamics.

Phenotypic variation in stomatal morphometrics

We observed inverse relationships between stomatal size and density, as have been commonly reported in the literature for multiple taxa (Kawamitsu *et al.* 1996; Hetherington and Woodward 2003; Franks *et al.* 2009). The derivation of g_{wmax} based on the work of Brown and Escombe (1900) suggests that a trade-off between stomate size and density will be broadly linked with conservation of g_{wmax}; decreases in stomatal size without a compensatory increase in density should result in decreases in g_{wmax} (the relative effect of decreased stomatal size on g_{wmax} is smaller when stomata are large because while pore resistance is increased by declines in pore area, parallel decreases in pore depth act to decrease pore resistance; see discussion by Franks *et al.* 2009). We interpret our results as pointing to size–density trade-offs linked with conservation of g_{wmax} among leaves from native *P. australis*. Meanwhile, plasticity in g_{wmax} among ramets of introduced *P. australis* was linked with greater plasticity in densities of stomata and was sometimes greater than for native clones.

Smaller stomata, as observed for the introduced lineage of *P. australis*, may improve water use efficiency. They are expected to be capable of opening and closing more rapidly (Aasamaa *et al.* 2001; Drake *et al.* 2013); in combination with lower resistance offered by shorter diffusion paths through smaller pores, rapid adjustment should lead to tighter linkage between stomatal responses and the need to regulate transpiration (Knapp 1993). In the

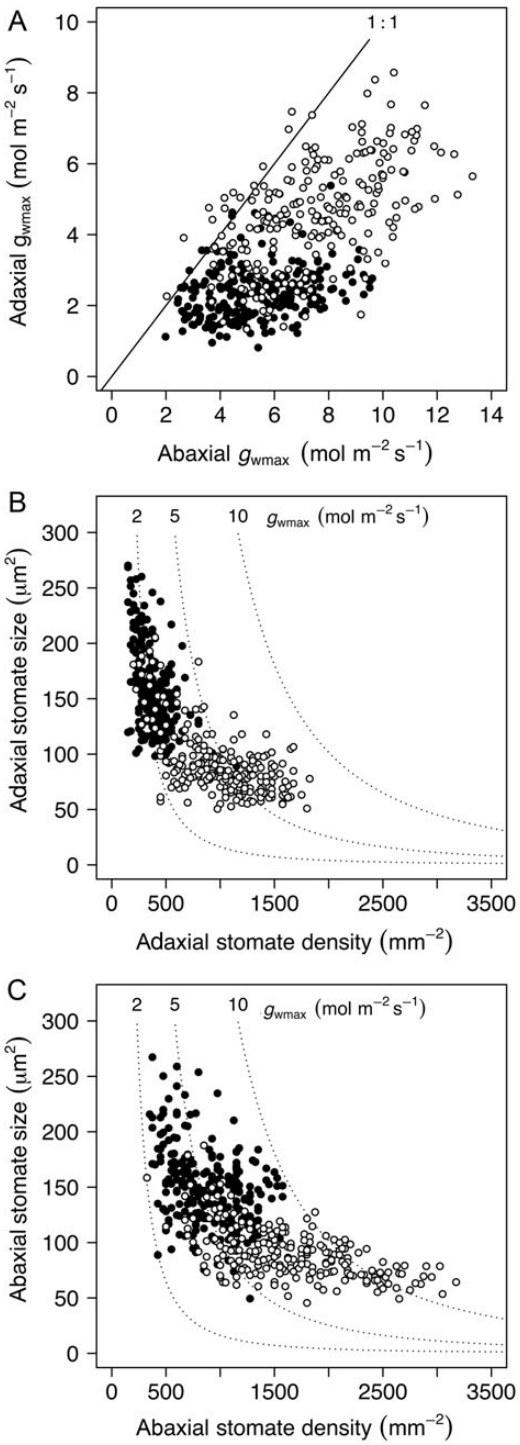

Figure 4. Components of g_{wmax} for lineages of *P. australis* native (filled symbols), and invasive (open symbols), to North America. (A) Leaf g_{wmax} is the sum of g_{wmax} for the adaxial and abaxial leaf surfaces; higher leaf g_{wmax} among invasive lineages is a result of increases in both adaxial and abaxial g_{wmax}. Stomate size shows a negative relationship with stomate density on both the adaxial (B) and abaxial (C) leaf surfaces: higher g_{wmax} on abaxial surfaces are linked with greater stomate densities, and the higher stomate densities among invasive *P. australis* are linked with reduced stomate size compared with the native lineage.

case of *P. australis*, improvements in stomatal feedback could allow introduced lineage access to more exposed ground with less reliable water supply, contributing to their observed capacity to reduce soil moisture levels (by accretion, Rooth *et al.* 2003; by transpiration, Windham 2001; by Venturi Effect ventilation, Armstrong and Armstrong 1991). Detailed physiological work assessing the components of leaf gas exchange and hydraulics will be necessary to fully resolve whether differences in water use efficiency are mechanistically linked with stomatal morphometrics in these *Phragmites* lineages.

The g_{wmax} values we determined for *P. australis* in Maine, particularly the introduced lineage, were very high (Table 1). They exceeded measurements made by one of the authors in a previous pot-based greenhouse study (Taylor *et al.* 2012). A broad survey of other grass species (Kawamitsu *et al.* 1996) indicates that stomatal morphometrics of cultivated rice (*Oryza sativa*) were most similar to *P. australis*, but g_{wmax} values for *P. australis* were higher. This is despite the expectation that a hydrophytic habit and selection for high productivity in rice would be expected to have maximized g_{wmax}. The g_{wmax} values we determined are underpinned by similar stomatal morphometrics to those demonstrated in a previous study that addressed the potential for ploidy level variation of stomatal traits in field collected samples across north-eastern North America (Saltonstall *et al.* 2007; Table 1). Indeed, the stomatal traits reported by Saltonstall *et al.* (2007) suggest even more extreme values for g_{wmax} than in our sample (Table 1). Although our study is limited to three marshes in Maine, our results parallel those from a broader set of populations support differences in mean g_{wmax} between native and introduced lineages as a general feature of *P. australis*, at least across its north-eastern North American range. Comparison of our measurements, those made by Saltonstall *et al.* (2007), and material of a European origin (Table 1, Taylor *et al.* 2012) also suggests a strong conservation of between-lineage differences in stomatal size while density is more variable (Table 1): plastic responses of g_{wmax} in *P. australis* may depend strongly on variation in density of stomata.

Phragmites australis is a water-loving species characteristic of marshes and wetlands. Reliable availability of water can relax selection against increases in transpiration (Dudley 1996), allowing for improved net carbon gain or nutrient acquisition (Donovan *et al.* 2007). In hot environments, increased transpiration can improve photosynthetic efficiency and leaf survival by helping to decrease leaf temperatures (Lu *et al.* 1998). In the cool climate of New England, it seems likely that the principal advantage of high stomatal conductances would be to decrease resistance to CO_2 diffusion into leaves and improve net carbon gain, consistent with observations

Table 1. *Phragmites australis* as a species show exceptionally high g_{wmax}. [1] Saltonstall *et al.* (2007), [2] Taylor *et al.* (2012) and [3] Kawamitsu *et al.* (1996). [1]Saltonstall *et al.* did not determine lengths of adaxial stomata for most populations, there being no significant difference between surfaces in a subset. [2]Most extreme among 41 cultivars.

Species/lineage	Mean length of stomata (μm)		Mean density of stomata (mm^{-2})		g_{wmax} ($mol\ m^{-2}\ s^{-1}$) predicted from mean values	Location, data source
	Adaxial	Abaxial	Adaxial	Abaxial		
P. australis native	25	24	394	924	7.94	Maine, this study
P. australis invasive	19	19	1002	1635	12.42	
P. australis native	25[1]	25	804	1147	12.09	Northeast USA and Canada [1]
P. australis invasive	19[1]	19	1726	2167	18.34	
P. australis	19	19	419	510	4.38	Glasshouse, UK [2]
Oryza sativa cv. Raikei[2]	18	18	646	844	6.65	Japan [3]

that the introduced lineage shows greater productivity, responsiveness to carbon enrichment (Mozdzer and Megonigal 2012) and higher RGR, the latter being a proposed factor driving invasion (Mozdzer *et al.* 2013). More broadly, high rates of productivity and the capacity for local habitat modification, e.g. by drying, are traits common to many invasive plants (Cuddington and Hastings 2004); our demonstration that g_{wmax} values for introduced *Phragmites* stands exceed those for native stands fits with reports of local drying effects linked the introduced lineage, mediated by both evapotranspiration and sediment accretion (Rooth *et al.* 2003). Summarizing, advantages under a variety of field conditions could arise from increases in transpiration linked with higher g_{wmax} that would provide for increased conductance to CO_2 and reduction in leaf temperature, or improved water use efficiency linked with decreases in stomatal size.

Community dynamics

High levels of plasticity in stomatal traits support the description of introduced *P. australis* as a 'Jack-and-master' of change (Mozdzer and Megonigal 2012; Mozdzer *et al.* 2013). Plasticity in stomatal morphology would be expected to permit a single genotype to acclimate to a range of conditions and make it a strong competitor in a heterogeneous environment. Marsh systems susceptible to Phragmites invasion are starkly heterogeneous in many factors, for example sharp gradients from waterline to bank in salinity, aeration, nutrient availability and water depth (reviewed in Engloner 2009). Comparing North American lineages, Holdredge *et al.* (2010) described a cline ranging from lower elevation associated with waterlogged soils up to higher elevation characterized by high levels of interspecific competition. A single clonal genotype of *P. australis* might span multiple microhabitat transitions in this setting. Genotypes with a plastic localized response at the scale of the ramet could minimize the risks, costs or genetic resources associated with adaptation through sexual reproduction while best optimizing potential opportunities for resource sharing and economies of scale inherent in integrated clonality.

Indeed, 'Theory predicts that plasticity in . . . morphologies of plants can transmit heterogeneity from the environment to the population or community' (Callaway *et al.* 2003). Thus, we can predict that significant variation should be identifiable from the among-lineage down to the among-ramet scales dependent upon local conditions. The lack of spatial structure to our data suggests that drivers of heterogeneity in stands of *P. australis* operate at a scale smaller than the 5 × 5 m scale measured here.

Plasticity is important for both lineages (Mozdzer and Megonigal 2012) and worth comparison against other non-clonal species. However, the lower levels of native plasticity suggest that there may be a cost involved. Net fitness, which synthesizes survival, growth and fecundity, does not necessarily benefit from plasticity (Palacio-López and Gianoli 2011; Pichancourt and Van Klinken 2012). In some circumstances, plasticity can be disadvantageous, for example, when there are costs of inappropriate specialized phenotypes, when environmental cues are unreliable, when the environment is not variable or when the plastic response lags too far behind environmental change (Vretare *et al.* 2001; Callaway *et al.* 2003). Thus, narrower plasticity in the native lineage may constrain optimal microhabitat range or reflect the more homogeneous sites it occupies.

A frequent assertion in invasive plant literature is that phenotypic plasticity is common in invasive species, making possible a broader ecological niche through the expression of site-specific advantageous phenotypes (Richards *et al.* 2006; Davidson *et al.* 2011). Previous work has shown that invading populations have the potential for rapid adaptive evolution (Dlugosch and Parker 2008),

which would select for a 'general purpose genotype' (Moroney *et al.* 2013). There is some evidence that introduced *P. australis* may be less plastic in its native range (Rolletschek *et al.* 1999) warranting further study of reaction norms in common gardens (e.g. Křiváčková-Suchá *et al.* 2007; Achenbach *et al.* 2012).

After within-genotype variation (plasticity), genetic variation (diversity) was the most important contributor to heterogeneity in phenotypes in this study, with relatively little variation being explained by among-site comparisons. Limited variation among sites may result from an emphasis on clonal reproduction, with limited sexual reproduction, natural selection and genetic drift. Initial models of *P. australis* establishment focussed on the transport of vegetative propagules and would lead to low genet richness at a given site (Bart *et al.* 2006); however, recent research indicates a greater role for sexual reproduction (McCormick *et al.* 2010) with clonal growth clearly important on a local scale (Kettenring and Mock 2012; Douhovnikoff and Hazelton 2014). Instead local genetic diversity can remain relatively high due to long lifespans and mechanisms such as remnant regional dynamics (Douhovnikoff and Hazelton 2014).

Conclusions

Plasticity in the introduced lineage of *P. australis* is similar to or exceeds that of native stands, both in our results and other reports (Mozdzer and Megonigal 2012; Mozdzer *et al.* 2013). This suggests that capacity for greater plasticity may be a major driver in the introduced lineage's invasiveness. Nonetheless, native *P. australis* does demonstrate considerable plasticity, which may underpin observations of long-term resistance to invasion, resilience and site consolidation. For example, the native lineage is well adapted to both low nutrient environments and exploitation of increasing nitrogen (sensu Hazelton *et al.* 2010). In contrast, the invader consistently outperforms the native in biomass production, nitrogen assimilation and various aspects of carbon metabolism (Mozdzer *et al.* 2013). These differences in physiological traits and trait plasticity may be indicators of different life-history strategies underpinning the ecological success and evolutionary maintenance of the two *P. australis* lineages in North America.

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Contributions by the Authors

V.D. was involved in all stages of research execution, data analysis and manuscript preparation. J.O.B. and S.H.T. were involved in data analysis and manuscript preparation. E.L.G.H. and C.S. were involved in research execution and data analysis.

Conflict of Interest Statement

None declared.

Literature Cited

Aasamaa K, Sõber A, Rahi M. 2001. Leaf anatomical characteristics associated with shoot hydraulic conductance, stomatal conductance and stomatal sensitivity to changes of leaf water status in temperate deciduous trees. *Australian Journal of Plant Physiology* **28**:765–774.

Abramoff MD, Magalhaes PJ, Ram SJ. 2004. Image processing with ImageJ. *Biophotonics International* **11**:36–42.

Achenbach L, Lambertini C, Brix H. 2012. Phenotypic traits of *Phragmites australis* clones are not related to ploidy level and distribution range. *AoB PLANTS* **2012**: pls017; doi:10.1093/aobpla/pls017.

Alpert P, Simms EL. 2002. The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? *Evolutionary Ecology* **16**:285–297.

Armstrong J, Armstrong W. 1991. A convective through-flow of gases in *Phragmites australis* (Cav.) Trin. ex Steud. *Aquatic Botany* **39**: 75–88.

Bastlova D, Čížková H, Bastl M, Kvet J. 2004. Growth of *Lythrum salicaria* and *Phragmites australis* plants originating from a wide geographical area: response to nutrient and water supply. *Global Ecology and Biogeography* **13**:259–271.

Bart D, Burdick D, Chambers R, Hartman JM. 2006. Human facilitation of *Phragmites australis* invasions in tidal marshes: A review and synthesis. *Wetlands Ecology and Management* **14**:53–65.

Bellavance M-E, Brisson J. 2010. Spatial dynamics and morphological plasticity of common reed (*Phragmites australis*) and cattails (*Typha* sp.) in freshwater marshes and roadside ditches. *Aquatic Botany* **93**:129–134.

Brown HT, Escombe F. 1900. Static diffusion of gases and liquids in relation to the assimilation of carbon and translocation in plants. *Philosophical Transactions of the Royal Society B: Biological Sciences* **193**:223–291.

Callaway RM, Pennings SC, Richards CL. 2003. Phenotypic plasticity and interactions among plants. *Ecology* **84**:1115–1128.

Clevering OA. 1999. The effects of litter on growth and plasticity of *Phragmites australis* clones originating from infertile, fertile or eutrophicated habitats. *Aquatic Botany* **64**:35–50.

Cuddington K, Hastings A. 2004. Invasive engineers. *Ecological Modelling* **178**:335–347.

Davidson AM, Jennions M, Nicotra AB. 2011. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecology Letters* **14**:419–431.

Dlugosch KM, Parker IM. 2008. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology* **17**:431–449.

Donovan LA, Dudley SA, Rosenthal DM, Ludwig F. 2007. Phenotypic selection on leaf water use efficiency and related ecophysiological traits for natural populations of desert sunflowers. *Oecologia* **152**:13–25.

Douhovnikoff V, Dodd RS. 2015. Epigenetics: a potential mechanism for clonal plant success. *Plant Ecology* **216**:227–233.

Douhovnikoff V, Hazelton ELG. 2014. Clonal growth: invasion or stability? A comparative study of clonal architecture and diversity in native and introduced lineages of *Phragmites australis* (Poaceae). *American Journal of Botany* **101**:1577–1584.

Dow GJ, Bergmann DC, Berry JA. 2014. An integrated model of stomatal development and leaf physiology. *New Phytologist* **201**: 1218–1226.

Drake PL, Froend RH, Franks PJ. 2013. Smaller, faster stomata: scaling of stomatal size, rate of response, and stomatal conductance. *Journal of Experimental Botany* **64**:495–505.

Dudley SA. 1996. Differing selection on plant physiological traits in response to environmental water availability: a test of adaptive hypotheses. *Evolution* **50**:92–102.

Eller F, Brix H. 2012. Different genotypes of *Phragmites australis* show distinct phenotypic plasticity in response to nutrient availability and temperature. *Aquatic Botany* **103**:89–97.

Engloner AI. 2009. Structure, growth dynamics and biomass of reed (*Phragmites australis*)—a review. *Flora - Morphology, Distribution, Functional Ecology of Plants* **204**:331–346.

Fanourakis D, Giday H, Milla R, Pieruschka R, Kjaer KH, Bolger M, Vasilevski A, Nunes-Nesi A, Fiorani F, Ottosen C-O. 2015. Pore size regulates operating stomatal conductance, while stomatal densities drive the partitioning of conductance between leaf sides. *Annals of Botany* **115**:555–565.

Fox J. 2008. *Applied regression analysis and generalized linear models*, 2nd edn. New York, NY: Sage.

Franks PJ, Farquhar GD. 2006. The mechanical diversity of stomata and its significance in gas-exchange control. *Plant Physiology* **143**:78–87.

Franks PJ, Drake PL, Beerling DJ. 2009. Plasticity in maximum stomatal conductance constrained by negative correlation between stomatal size and density: an analysis using *Eucalyptus globulus*. *Plant, Cell and Environment* **32**:1737–1748.

Gianoli E, Valladares F. 2012. Studying phenotypic plasticity: the advantages of a broad approach. *Biological Journal of the Linnean Society* **105**:1–7.

Hansen DL, Lambertini C, Jampeetong A, Brix H. 2007. Clone-specific differences in *Phragmites australis*: effects of ploidy level and geographic origin. *Aquatic Botany* **86**:269–279.

Hazelton ELG, Knight TJ, Theodose TA. 2010. Glutamine synthetase partitioning in native and introduced salt marsh grasses. *Marine Ecology Progress Series* **414**:57–64.

Hetherington AM, Woodward FI. 2003. The role of stomata in sensing and driving environmental change. *Nature* **424**:901–908.

Holdredge C, Bertness MD, Von Wettberg E, Silliman BR. 2010. Nutrient enrichment enhances hidden differences in phenotype to drive a cryptic plant invasion. *Oikos* **119**:1776–1784.

Kawamitsu Y, Agata W, Hiyane S, Murayama S, Nose A, Shinjyo C. 1996. Relation between leaf gas exchange rate and stomata I. Stomatal frequency and guard cell length in C3 and C4 grass species. *Japanese Journal of Crop Science* **65**:626–633.

Kettenring KM, Mock KE. 2012. Genetic diversity, reproductive mode, and dispersal differ between the cryptic invader, *Phragmites australis*, and its native conspecific. *Biological Invasions* **14**:2489–2504.

Knapp AK. 1993. Gas exchange dynamics in C_3 and C_4 grasses: consequence of differences in stomatal conductance. *Ecology* **74**: 113–123.

Křiváčková-Suchá O, Vávřová P, Čížková H, Čurn V, Kubátová B. 2007. Phenotypic and genotypic variation of *Phragmites australis*: a comparative study of clones originating from two populations of different age. *Aquatic Botany* **86**:361–368.

Langsrud O. 2003. ANOVA for unbalanced data: use Type II instead of Type III sums of squares. *Statistics and Computing* **13**: 163–167.

Lu Z, Percy RG, Qualset CO, Zeiger E. 1998. Stomatal conductance predicts yields in irrigated Pima cotton and bread wheat grown at high temperatures. *Journal of Experimental Botany* **49**: 453–460.

Mccormick MK, Kettenring KM, Baron HM, Whigham DF. 2010. Spread of invasive *Phragmites australis* in estuaries with differing degrees of development: genetic patterns, Allee effects and interpretation. *Journal of Ecology* **98**:1369–1378.

Moroney JR, Rundel PW, Sork VL. 2013. Phenotypic plasticity and differentiation in fitness-related traits in invasive populations of the Mediterranean forb *Centaurea melitensis* (Asteraceae). *American Journal of Botany* **100**:2040–2051.

Mozdzer TJ, Megonigal JP. 2012. Jack-and-master trait responses to elevated CO_2 and N: a comparison of native and introduced *Phragmites australis*. *PLoS ONE* **7**:e42794.

Mozdzer TJ, Brisson J, Hazelton EL. 2013. Physiological ecology and functional traits of North American native and Eurasian introduced *Phragmites australis* lineages. *AoB PLANTS* **5**: plt048; doi:10.1093/aobpla/plt048.

Palacio-López K, Gianoli E. 2011. Invasive plants do not display greater phenotypic plasticity than their native or non-invasive counterparts: a meta-analysis. *Oikos* **120**:1393–1401.

Pichancourt J-B, Van Klinken RD. 2012. Phenotypic plasticity influences the size, shape and dynamics of the geographic distribution of an invasive plant. *PLoS ONE* **7**:e32323.

R Development Core Team. 2015. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org (March 2015).

Richards CL, Bossdorf O, Muth NZ, Gurevitch J, Pigliucci M. 2006. Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecology Letters* **9**:981–993.

Rolletschek H, Rolletschek A, Kühl H, Kohl JG. 1999. Clone specific differences in a *Phragmites australis* stand: II. Seasonal development of morphological and physiological characteristics at the natural site and after transplantation. *Aquatic Botany* **64**:247–260.

Rooth JE, Stevenson JC, Cornwell JC. 2003. Increased sediment accretion rates following invasion by *Phragmites australis*: the role of litter. *Estuaries* **26**:475–483.

Rouder JN, Morey RD, Speckman PL, Province JM. 2012. Default Bayes factors for ANOVA designs. *Journal of Mathematical Psychology* **56**:356–374.

Saltonstall K. 2003. Microsatellite variation within and among North American lineages of Phragmites australis. *Molecular Ecology* **12**: 1689–1702.

Saltonstall K, Peterson PM, Soreng RJ. 2004. Recognition of *Phragmites australis* subsp. americanus (Poaceae: Arundinoideae) in North America: evidence from morphological and genetic analysis. *SIDA, Contributions to Botany* **21**:683–692.

Saltonstall K, Glennon K, Burnett A, Hunter RB, Hunter KL. 2007. Comparison of morphological variation indicative of ploidy level in *Phragmites Australis* (Poaceae) from Eastern North America. *Rhodora* **109**:415–429.

Schulze E, Kelliher FM, Körner C, Lloyd J, Leuning R. 1994. Relationships among maximum stomatal conductance, ecosystem surface conductance, carbon assimilation rate, and plant nitrogen nutrition: a global ecology scaling exercise. *Annual Review of Ecology and Systematics* **25**:629–662.

Swearingen J, Saltonstall K. 2010. Phragmites field guide: distinguishing native and exotic forms of common reed (*Phragmites australis*) in the United States. Plant Conservation Alliance, Weeds Gone Wild. http://www.nps.gov/plants/alien/fact/pdf/phau1-powerpoint.pdf.

Taylor SH, Franks PJ, Hulme SP, Spriggs E, Christin P-A, Edwards EJ, Woodward FI, Osborne CP. 2012. Photosynthetic pathway and ecological adaptation explain stomatal trait diversity amongst grasses. *New Phytologist* **193**:387–396.

Thompson K, Hodgson JG, Rich TC. 1995. Native and alien invasive plants: more of the same? *Ecography* **18**:390–402.

Via S, Lande R. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* **39**:505–522.

Vretare V, Weisner SEB, Strand JA, Granéli W. 2001. Phenotypic plasticity in *Phragmites australis* as a functional response to water depth. *Aquatic Botany* **69**:127–145.

Weyers JDB, Meidner H. 1990. *Methods in stomatal research*. Harlow: Longman Scientific and Technical.

Windham L. 2001. Comparison of biomass production and decomposition between *Phragmites australis* (common reed) and *Spartina patens* (salt hay grass) in brackish tidal marshes of New Jersey, USA. *Wetlands* **21**:179–188.

