

Research Article

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

Belowground advantages in construction cost facilitate a cryptic plant invasion

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Abstract. The energetic cost of plant organ construction is a functional trait that is useful for understanding carbon investment during growth (e.g. the resource acquisition vs. tissue longevity tradeoff), as well as in response to global change factors like elevated CO₂ and N. Despite the enormous importance of roots and rhizomes in acquiring soil resources and responding to global change, construction costs have been studied almost exclusively in leaves. We sought to determine how construction costs of aboveground and belowground organs differed between native and introduced lineages of a geographically widely dispersed wetland plant species (*Phragmites australis*) under varying levels of CO₂ and N. We grew plants under ambient and elevated atmospheric CO₂, as well as under two levels of soil nitrogen. We determined construction costs for leaves, stems, rhizomes and roots, as well as for whole plants. Across all treatment conditions, the introduced lineage of *Phragmites* had a 4.3 % lower mean rhizome construction cost than the native. Whole-plant construction costs were also smaller for the introduced lineage, with the largest difference in sample means (3.3 %) occurring under ambient conditions. In having lower rhizome and plant-scale construction costs, the introduced lineage can recoup its investment in tissue construction more quickly, enabling it to generate additional biomass with the same energetic investment. Our results suggest that introduced *Phragmites* has had an advantageous tissue investment strategy under historic CO₂ and N levels, which has facilitated key rhizome processes, such as clonal spread. We recommend that construction costs for multiple organ types be included in future studies of plant carbon economy, especially those investigating global change.

Keywords: Carbon dioxide; common reed; construction cost; eutrophication; intraspecific; invasion ecology; *Phragmites*; plant functional traits; rhizomes; wetlands.

Introduction

The energetic requirement of plant tissue biosynthesis, or construction cost (CC), has proven to be a valuable functional trait in investigations of the carbon economy of plants. Research on leaf CC and associated traits has

yielded insights into the strategies used by plants for carbon acquisition (investment in leaf longevity, payback time for the investment, light harvesting area, etc.) and has thereby helped to explain patterns in growth at the

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individual and population levels (Wright et al. 2004; Poorter and Bongers 2006). For instance, a number of studies on invasive species have found lower leaf CCs, higher specific leaf areas (SLAs) and more rapid growth rates relative to co-occurring non-invasive species across life forms (Baruch and Goldstein 1999; Nagel and Griffin 2001; Deng et al. 2004; Feng et al. 2008; Osunkoya et al. 2010; Shen et al. 2011). Research on leaf CC has also identified ways in which plants will adjust leaf structure and function as changes in global climate intensify. In prior studies, most species decreased leaf CCs in response to elevated CO₂ (Poorter et al. 1997; Lei et al. 2012), while leaf CC rose in response to higher nitrogen availability (Griffin et al. 1993).

Although functional trait studies that have included CC have almost exclusively used it to gain insight into the carbon economy of leaves, CC is not a trait specific to leaves. The few studies that have addressed CCs of roots, rhizomes or other organs have shown that high investment in one organ does not necessarily correspond to high investment in another (Wullschlegel et al. 1997; Nagel et al. 2005; Osunkoya et al. 2008). Given that changes in biomass allocation and tissue composition have been observed in many species following CO₂ and nitrogen manipulation (Poorter et al. 1997, 2012; Curtis and Wang 1998; Booker et al. 2000; Booker and Maier 2001; Kraus et al. 2004), changes in the CCs of organs other than leaves are probably common as well. Research explicitly investigating the CC of belowground organs in response to additions of CO₂ and inorganic nitrogen would be especially useful in understanding how global change will affect the trajectory of plant populations as resource regimes shift.

Phragmites australis, or common reed (hereafter *Phragmites*), is well suited for an investigation of how plants may adjust tissue construction in response to global change. *Phragmites* has a cosmopolitan distribution, with dozens of genetic lineages in the species (Saltonstall 2002; Lambertini et al. 2012). It is therefore possible to tightly constrain phylogeny while comparing CCs between lineages that co-occur in natural ecosystems. The most well-studied case is that of two lineages that occur in tidal wetlands along the Atlantic coast of North America. One lineage was introduced from Eurasia to North America in the mid-1800s (haplotype M; *P. australis* subsp. *australis*; hereafter ‘introduced *Phragmites*’) (Saltonstall 2002). It has invaded wetlands across the Atlantic coast of North America, dramatically changing both ecosystem structure and function (Marks et al. 1994; Chambers et al. 1999; Kettenring et al. 2012). The other lineage present is a haplotype native to the region (haplotype F; *P. australis* subsp. *americanus*; hereafter ‘native *Phragmites*’) (Saltonstall 2002).

Strong differences in physiology, growth (aboveground and belowground) and abundance have been observed between native and introduced *Phragmites* (Saltonstall 2007; Saltonstall and Stevenson 2007; Park and Blossey 2008; Mozdzer and Zieman 2010; Mozdzer et al. 2010, 2013). Further, differences in growth rate between the lineages are known to become exacerbated in response to eutrophication and elevated atmospheric CO₂ (Saltonstall and Stevenson 2007; Holdredge et al. 2010; Mozdzer and Magonigal 2012; Tulbure et al. 2012; Mozdzer et al. 2013). Eutrophication is probably one of the primary drivers of the introduced lineage spreading rapidly in many wetland ecosystems. For instance, its abundance is correlated with shoreline development (King et al. 2007), a process that combines elevated nutrient availability, habitat modification and diminished salinity (Silliman and Bertness 2004). Introduced *Phragmites* is able to achieve particularly high rates of seedling establishment and growth in such environments, and also experiences higher rates of outcrossing (rather than self-pollination; McCormick et al. 2010). Because outcrossing is associated with greater seedling production, the availability of eutrophied environments is hypothesized to accelerate invasion dramatically (McCormick et al. 2010; Hazelton et al. 2014). In the context of rising atmospheric CO₂ and intensifying anthropogenic disturbance in wetland systems, information on how introduced *Phragmites* invests in tissue construction, and how it adjusts this investment in response to the environment, could be highly relevant in understanding the ecological processes driving the invasion, as well as in formulating strategies to manage it.

We sought to determine how CCs of plant organs in introduced and native *Phragmites* lineages would vary in response to alterations to CO₂, nitrogen (N) and the combination of these factors. We measured leaf, stem, rhizome and root CCs in greenhouse-grown plants, and compared organ-specific and whole-plant CCs with other functional traits related to growth and morphology. In keeping with prior observations of leaves in invasive species, we hypothesized that CCs of all organ types, as well as whole plants, would be lower for introduced vs. native *Phragmites*. Further, we hypothesized that the difference in CCs between lineages would increase when plants grew under levels of CO₂ or inorganic N expected in the coming century (Hopkinson and Giblin 2008; Meinshausen et al. 2011), with the greatest difference being in plants that experienced higher CO₂ and N simultaneously.

Methods

Phragmites australis plant material was originally collected from marshes on the Delmarva Peninsula, USA (38.5°N, 75.5°W); populations of native and introduced

Phragmites were sampled from stands that were located within 50 km of one another. Samples were genetically confirmed to belong to haplotypes F and M, which correspond to North American Atlantic coast native and Eurasian introduced lineages, respectively. Clones from this material were subsequently grown in a common garden at the University of Rhode Island, where they experienced identical abiotic conditions for 3 years (2006–09). We therefore attribute any differences in functional trait expressions between lineages from this experiment strictly to the genetic source. Plants for the experiment described herein were propagated from rhizome fragments at the Smithsonian Environmental Research Center in Edgewater, MD, USA in 2009, where the experiment also took place. Rhizome fragments contained 3–5 intact internodes, which was equivalent to 1.29 ± 0.07 and 1.10 ± 0.70 g (mean \pm SE) dry mass for native and introduced lineages, respectively. Rhizomes were planted individually in plastic pots (15 L; 24 \times 24 \times 33 cm) that contained reed-sedge peat (Baccto, Houston, TX, USA) on 11–12 June 2009.

The experiment had a three-way factorial design, which included two levels of atmospheric CO₂, two levels of soil N and the two *Phragmites* lineages. Plants from each lineage were randomly distributed among six transparent chambers, in which CO₂ was either not added or elevated to ~ 330 ppm above ambient air (Mozdzer and Megonigal 2012). This is a conservative estimate of rise in global mean CO₂ concentration by the latter part of the 21st century (Meinshausen et al. 2011). Plants were placed in chambers when new growth became visible at the soil surface; the first plant emerged on 19 June 2009. Within each chamber, half of the plants from each lineage received supplemental N at a rate equivalent to 25 g m⁻² year⁻¹, while the remaining half were unfertilized. The higher N level is typical of those seen in eutrophied tidal marsh ecosystems (Hopkinson and Giblin 2008). Nitrogen was delivered bi-weekly via a solution of NH₄Cl. A sufficient quantity of tapwater to maintain at least 3 cm of standing water was added to each pot daily. To allow for water movement through the potting medium, four macropores were inserted vertically using PVC tubing (1.25 cm i.d.).

Plants were destructively harvested after ~ 2 months of exposure to treatment conditions (20–27 August 2009). Material from each individual ($N = 52$) was carefully separated into leaf, stem (culm plus leaf sheath), rhizome and root categories. All plant material was oven dried at 60 °C to constant mass, weighed and finely ground. Samples of ground tissue were analysed at the University of Virginia for elemental carbon and nitrogen content (Carlo Erba Instruments, NA2500, Milan, Italy). Tissue mineral content was determined via loss-on-ignition using a separate set

of samples; ~ 0.5 g of each sample was ashed in a muffle furnace for 6 h at 550 °C.

Organ-specific CCs were determined using a method based on the production value of dry matter. Construction cost is defined specifically as the mass of glucose required to synthesize a given mass of plant tissue, but can be determined from the carbon (C_{dm}) and ash (Ash_{dm}) content of dried organic material as follows (Vertregt and Penning de Vries 1987):

$$CC = \frac{5.39C_{dm} + 0.80Ash_{dm} - 1191}{1000}$$

While estimates of CC are more complicated when the N source available to plants includes NO₃ (Vertregt and Penning de Vries 1987; Poorter et al. 1997), NH₄ was the sole N source in this experiment. Further, very little of the NH₄ could have oxidized given that soils were constantly inundated; measurements of redox potential confirmed that soils were predominantly anaerobic (Mozdzer and Megonigal 2013). After calculating CCs for each organ type (CC_{org}, where org is alternately leaf, stem, rhizome or root), we determined the contribution of organ-specific CCs (Contrib_{org}) to plant-scale CCs (CC_{plant}) by weighting CC_{org} by the corresponding mass fraction (MF_{org}; organ mass per plant mass) and summing the contributions:

$$\begin{aligned} \text{Contrib}_{org} &= CC_{org} \times MF_{org}, \\ CC_{plant} &= \text{Contrib}_{leaf} + \text{Contrib}_{stem} + \text{Contrib}_{root} \\ &\quad + \text{Contrib}_{rhizome}. \end{aligned}$$

Additional functional traits were measured for each plant. Relative growth rate (RGR) was based on the accumulation of dry biomass between planting (M_p) and harvest (M_h):

$$RGR = \frac{(\ln(M_h) - \ln(M_p))}{t},$$

where t is the number of days between emergence and harvest (mean \pm SE: 58 ± 1 days). Masses at the time of planting (M_p) were determined from the fresh masses of rhizome fragments used to propagate plants; the water content of rhizome fragments that were not used in the study was used to estimate dry masses. Dry masses at harvest (M_h) were sums of leaf, stem, rhizome and root masses. Stem heights and diameters were calculated as the mean of all stems in individual pots, with diameters measured at the soil surface. Stem density was a count of the number of stems per pot. Specific leaf area was calculated as the ratio of the area of the leaf blade to the dry mass of the third-most apical, fully developed leaf. Leaf blade areas were measured with an LI-3000 leaf scanner (LI-COR Biosciences, Lincoln, NE, USA). Additional procedural details are provided elsewhere (Mozdzer and Megonigal 2012, 2013).

Differences among experimental factors (CO₂, N and lineage) with respect to CCs (plant scale and organ specific) were evaluated with ANOVA-type linear models in R version 3.0.2. Transformations to response variables (square root or natural log) were made if residuals were not normal and homoscedastic. Models initially contained terms for all main effects and interactions; when *F* statistics for individual terms (especially interactions) or the model itself were non-significant (using $\alpha = 0.05$), simpler models were sought by sequentially removing non-significant terms. If an interaction term was significant, all lower-order terms were retained regardless of significance. Tukey's honestly significant difference (HSD) tests were used to evaluate pairwise differences among means based on terms in the final models. We assessed the correlation (Pearson coefficient, ρ) between CCs and other functional traits using mean values for each lineage within each

combination of treatments. Variables for which $\rho > 0.7$ were considered strongly correlated, as this level of correlation corresponds to ~50 % of the variation in CCs being explained by the functional traits in question (Sokal and Rohlf 1995).

Results

The influence of lineage and environmental manipulations on CCs was strongly organ specific. Aboveground, leaf CCs were influenced by both N and CO₂ treatment, but the magnitude of these effects depended on lineage (Fig. 1A, Table 1). Specifically, N fertilization induced an increase in leaf CC for native *Phragmites*, but this effect was independent of the CO₂ level. In contrast, introduced *Phragmites* only increased its leaf CC with fertilization if CO₂ was elevated as well. When averaging across environmental treatments, leaf CCs were similar for

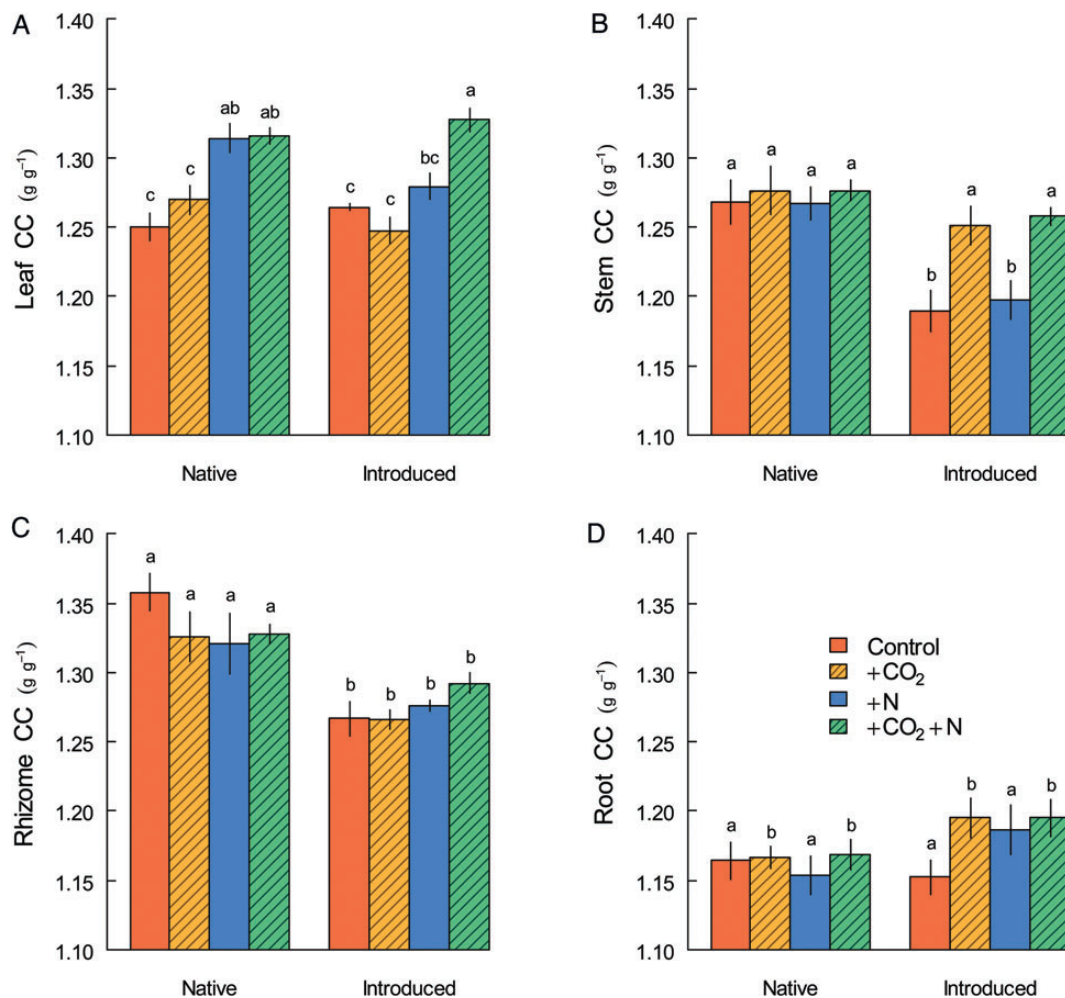


Figure 1. Organ-specific construction costs (CCs) for *Phragmites* lineages native to the North American North Atlantic coast ('Native') and introduced from Eurasia ('Introduced'). Bar heights represent mean (\pm SE) CC for all plants grown in a combination of CO₂ and N fertilization treatments. Within each panel, lowercase letters above bars differ when Tukey's HSD tests for the best-fitting model identified statistically significant differences in means. Units are grams of glucose required per gram of biomass produced.

Table 1. Statistical results for the linear models best describing CCs as a function of *Phragmites* lineage and environmental treatments. Values in the top row for each model correspond to *F*-tests of each model as a whole, while the remaining values correspond to *F*-tests of individual terms.

Model	d.f.	F	P
Plant CC	4	16.07	<0.001
Lineage	1	31.99	<0.001
CO ₂	1	13.05	<0.001
N	1	14.30	<0.001
Lineage × CO ₂	1	4.96	0.031
Residuals	46		
Leaf CC	7	13.85	<0.001
Lineage	1	1.68	0.20
CO ₂	1	6.98	0.011
N	1	74.43	<0.001
Lineage × CO ₂	1	0.012	0.91
Lineage × N	1	0.059	0.81
CO ₂ × N	1	2.67	0.11
Lineage × CO ₂ × N	1	11.11	0.002
Residuals	43		
Stem CC	3	16.88	<0.001
Lineage	1	27.04	<0.001
CO ₂	1	14.65	<0.001
Lineage × CO ₂	1	8.95	0.004
Residuals	47		
Rhizome CC	1	36.35	<0.001
Lineage	1	36.35	<0.001
Residuals	49		
Root CC	2	3.91	0.027
Lineage	1	3.57	0.065
CO ₂	1	4.24	0.045
Residuals	48		

the two lineages. Unlike leaves, the CC of stems was unaffected by N fertilization, but did differ by lineage and CO₂ status (Fig. 1B, Table 1). Specifically, under ambient CO₂ conditions, introduced *Phragmites* generated stems that had 5.8 % lower CCs than did native *Phragmites*.

The largest difference in CC between lineages was seen belowground, specifically in rhizomes. Rhizome CCs were 4.3 % lower for introduced *Phragmites* than for the native, and this difference was not significantly influenced by environmental treatments (Fig. 1C, Table 1). Root CCs were notably lower than they were for any other organ (Fig. 1D). Elevated CO₂ induced slight increases in root

CC for both lineages, while N fertilization had no measurable effect (Table 1). Although there was a trend towards higher root CC for introduced *Phragmites* compared with the native, this effect was not significant.

At the level of the whole plant, CCs differed by lineage and by environmental conditions. Introduced *Phragmites* had a lower mean CC than did the native (Table 1); the magnitude of this effect ranged from 0.6 to 3.3 % depending on the CO₂ and N treatment levels, and was 2.3 % for all treatments pooled (Fig. 2A). Native and introduced *Phragmites* also differed markedly in the size of contribution that each type of organ made to whole-plant CCs. Under all environmental conditions, introduced *Phragmites* had smaller rhizome and root contributions, but larger stem and leaf contributions compared with the native. Relative to unfertilized conditions, elevated nitrogen raised the contribution of belowground organs to whole-plant CCs in both lineages. These differences

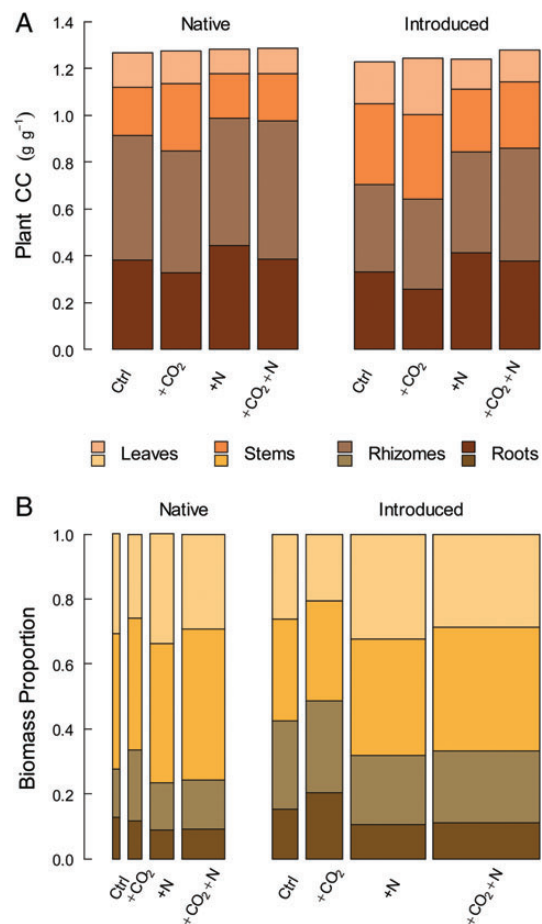


Figure 2. Partitioning of (A) plant-level CCs and (B) plant biomass by organ type for native and introduced *Phragmites* under each of the environmental treatment combinations evaluated in this experiment. Mean values for the replicate individuals within each treatment are shown. The widths of bars in (B) are scaled to the total biomass produced across treatments.

in aboveground vs. belowground contributions were driven by CCs and not biomass distributions, as organ mass fractions were higher belowground for the introduced lineage and under fertilized conditions (Fig. 2B). The addition of CO₂ also raised the contribution of aboveground organs to plant CCs over ambient conditions.

Across environmental treatments, higher leaf, rhizome and whole-plant CCs corresponded to introduced *Phragmites* plants growing faster, more densely and larger (taller, more massive and having wider stems; Table 2, Fig. S1 [see Supporting Information]). Correlation coefficients were consistently strongest for rhizome CCs in all of

Table 2. Correlation coefficients between whole-plant or organ-specific CCs and other functional traits. The underlying data were the means for each combination of environmental treatments (Control, +CO₂, +N and +CO₂+N) within each lineage. Pearson coefficients >0.7 are shown; all coefficients and plots of the underlying data appear in Fig. S1 [see Supporting Information]. RGR, aboveground relative growth rate; Height, mean height of stems; Stem diam., mean basal diameter of stems; Biomass, total plant biomass; Density, number of stems per pot; SLA, specific leaf area; N:C, nitrogen to carbon ratio in tissue. All traits were measured at harvest except RGR.

	Plant CC	Leaf CC	Stem CC	Rhizome CC	Root CC
<i>Introduced Phragmites</i>					
RGR	0.86	0.87	–	0.94	–
Height	0.93	0.92	–	0.98	–
Stem diam.	0.88	0.97	–	1.00	–
Biomass	0.86	0.91	–	0.97	–
Density	0.79	0.84	–	0.91	–
SLA	–0.87	–	–0.98	–	–
N : C _{lf}	–	–	–	–	–
N : C _{stem}	–	–	–	–	–
N : C _{rhiz}	–	0.89	–	0.91	–
N : C _{root}	–	0.78	–	0.79	–
<i>Native Phragmites</i>					
RGR	0.99	0.96	–	–0.75	–
Height	0.73	–	0.88	–0.73	–
Stem diam.	0.92	0.80	–	–0.72	–
Biomass	0.94	0.89	–	–	–
Density	0.93	0.94	–	–	–
SLA	0.90	0.86	–	–0.98	–
N : C _{lf}	0.76	0.88	–	–	–
N : C _{stem}	0.87	0.93	–	–	–
N : C _{rhiz}	0.88	0.93	–	–	–
N : C _{root}	0.83	0.94	–	–	–

these relationships. Native *Phragmites* likewise grew more rapidly, more densely and larger as plant and leaf CCs increased. In contrast to the introduced lineage, rhizome CC in the native was oppositely, and generally more weakly, correlated to these and other traits than were leaf and whole-plant costs (Table 2). While SLA was negatively correlated with plant and stem CCs for the introduced lineage, it was positively correlated with plant and leaf CCs in the native (Table 2). Finally, for introduced *Phragmites*, leaf and rhizome CCs were strongly and positively associated with the N:C ratio of belowground organs, but only weakly associated with the N:C ratio of aboveground organs ($\rho \approx 0.6$). The native lineage had strong positive associations between leaf and plant CCs and the N:C ratio of all organ types (Table 2).

Discussion

Rhizome construction costs

This study demonstrates that CCs for organs not typically measured (rhizomes, roots and stems) can reveal patterns of plant adaptation well beyond those that can be gleaned from leaf CC alone. Most strikingly, our results identified key advantages in rhizome investment for introduced *Phragmites* over the native lineage that likely contribute to its invasion as a perennial, clonal grass. By maintaining lower CCs under all combinations of CO₂ and nitrogen, introduced *Phragmites* can recoup its investment in tissue construction more quickly (Poorter et al. 2006), enabling it to generate additional rhizome biomass and potentially other organs as well. Prior research supports this explanation; under multiple CO₂ and N conditions and in multiple studies, introduced *Phragmites* had greater absolute rhizome mass, higher rhizome mass fractions, higher ramet densities and greater leaf areas compared with the native (League et al. 2006; Holdredge et al. 2010; Mozdzer and Magonigal 2012).

We suggest that the lower rhizome CC of introduced *Phragmites* ultimately contributes to advantages in belowground dynamics that are known to facilitate its invasion in North American tidal marshes. More specifically, we suggest that lower rhizome CC and shorter payback times allow introduced *Phragmites* to build more extensive rhizome systems (e.g. greater biomass, as seen in this study, as well as greater total length) than it would if CCs were higher. Low rhizome CC may also yield thicker rhizomes (i.e. higher masses per unit length, which could come from greater diameters, as seen by Holdredge et al. (2010), and/or from more dense rhizome tissue). Given that clonal expansion occurs by stems emerging from laterally extending rhizomes (Amsberry et al. 2000), the favourable carbon economics of rhizome generation may

facilitate higher rates of ramet production, spatially and/or temporally, as reported for introduced vs. native lineages previously (Vasquez et al. 2005; League et al. 2006; Holdredge et al. 2010; Mozdzer and Magonigal 2012). By building and maintaining a network of stems that are connected by rhizomes, *Phragmites* clones can draw oxygen into belowground organs, aiding respiration and nutrient uptake (Brix et al. 1992; Vretare and Weisner 2000; Tulbure et al. 2012). Introduced *Phragmites* is able to induce 4 × the rate of airflow per unit of pressure differential and stand area than native *Phragmites* (Tulbure et al. 2012). This efficiency is due, in part, to the higher stem densities of its clones (Rolletschek et al. 1999; Tulbure et al. 2012). In addition, the ability of introduced *Phragmites* to tolerate substantially higher salinity than native *Phragmites* contributes to its ability to invade habitats that the native lineage is unable to colonize (Vasquez et al. 2005). Tolerance to relatively high salinity (≤ 0.40 M NaCl) has been attributed to larger rhizome sizes, greater rhizosphere oxygenation and more rapid clonal growth by the introduced lineage (Vretare and Weisner 2000; Bart and Hartman 2003; Vasquez et al. 2005).

The advantages that introduced *Phragmites* exhibits in connection with low rhizome CC and short payback times, compared with native *Phragmites* and likely other species, are magnified by its rapid photosynthetic rates. The photosynthetic capacity (A_{\max}) of the introduced lineage has been measured as being 12–80 % higher than that of the native (Hansen et al. 2007; Mozdzer and Zieman 2010; Mozdzer et al. 2013). This translated into the introduced lineage producing more than twice as much rhizome biomass as native *Phragmites* in both field and greenhouse settings (Holdredge et al. 2010; Mozdzer and Zieman 2010; Mozdzer and Magonigal 2012). As past studies of photosynthetic traits did not manipulate CO₂ or N, ecophysiological data collected under predicted future conditions would be extremely valuable in assessing the carbon economy of *Phragmites* as global change intensifies.

Our results also suggest that introduced *Phragmites* may avoid a tradeoff in photosynthate allocation between rhizomes and leaves. This is supported by the fact that rhizome CCs were positively correlated with leaf CCs and metrics of plant size in the introduced lineage, but negatively correlated in the native lineage. An ability to make a large investment in rhizomes may lead to greater root production and nutrient acquisition rates for introduced *Phragmites* (Holdredge et al. 2010), positively feeding back to growth and tissue quality both aboveground and below. We suspect that the native lineage is not sufficiently productive to support the initial investment in rhizome biomass needed to make such feedback possible.

There are several possible changes in rhizome tissue composition that could contribute to the observed differences in CCs between lineages. One possibility is that introduced *Phragmites* invests in a lower proportion of energetically expensive compounds like lignins, proteins and phenolics in rhizomes. Because they have larger diameters (Holdredge et al. 2010), rhizomes of introduced *Phragmites* may require less structural support via lignification. As described above, synthesis of fewer expensive compounds in rhizome tissue would lead to lower longevity, but a faster payback time, and a more rapid growth rate (Poorter et al. 2006). It is also possible that introduced *Phragmites* incorporates a greater proportion of inexpensive compounds than the native, such as non-structural carbohydrates or organic acids (Poorter et al. 2006). For instance, it may synthesize a larger surplus of starch via photosynthesis, much of which it may allocate belowground for immediate growth or storage (Granéli et al. 1992). Through this mechanism as well, introduced *Phragmites* would be able to achieve a rapid return on the energetic investment in rhizome tissue, facilitating its further growth.

Response to global change factors

In direct contrast to prior studies (Poorter et al. 1997; Wullschlegel et al. 1997; Nagel et al. 2004, 2005), all statistically separable comparisons of mean CCs for ambient vs. elevated CO₂, as well as most of the non-significant comparisons, involved increases in CCs. However, the vast majority of past studies focused specifically on CCs of leaves. As seen in other studies that manipulated CO₂ (Poorter et al. 2012; Langley et al. 2013; Madhu and Hatfield 2013), belowground production increased under elevated CO₂ for both *Phragmites* lineages. The concomitant rise in root CCs may have been due to shifts in root morphology or architecture, such as larger diameters, higher tissue density or more frequent branching (Madhu and Hatfield 2013). Such shifts allow for increased nutrient uptake, soil penetration ability and resistance to pathogens and herbivores, but require increased synthesis of energetically expensive compounds like lignin and suberin (Vance et al. 1980; Soukup et al. 2002; Baxter et al. 2009). Consistent with this possibility, prior studies have found higher lignin concentrations in fine roots under elevated CO₂ (Booker et al. 2000; George et al. 2003). The strong correlation of stem CCs with plant height in native *Phragmites* raises the possibility that stems were also more lignified under elevated CO₂. Introduced *Phragmites* likewise exhibited a positive correlation between these factors, though it was only moderate in strength ($\rho = 0.58$).

Despite the literature's enormous emphasis on leaf CCs, we found no differences in leaf CC between lineages.

While both lineages adjusted leaf CC in response to nitrogen addition, the magnitude of response was similar. Higher N availability probably corresponded to a greater investment in rubisco and other compounds associated with photosynthetic capacity (Griffin et al. 1993; Poorter and Bongers 2006). Other studies have also found negative correlations between leaf CC and SLA (e.g. Feng et al. 2008), whereas we found a positive correlation. We attribute this discrepancy to the fact that most other studies describe variation among species grown under similar environmental conditions, while our analysis portrays phenotypic plasticity in leaf construction to strongly varying environmental conditions. If we had only investigated leaf CCs for these *Phragmites* lineages, we would have overlooked key differences belowground, and determined little about the carbon economy or differences in invasiveness between lineages.

Our findings on whole-plant CCs suggest that an ability to generate biomass with a relatively short return time on the energetic investment has facilitated introduced *Phragmites* colonizing wetlands in North America over the past century (Saltonstall 2002). Modest differences in CCs, like the 3.3 % difference seen in this study, have previously been linked with large differences in abundance (Nagel and Griffin 2001). In combination with its relatively high photosynthetic rates (Mozdzer and Zieman 2010; Mozdzer et al. 2013) and plastic nutrient use efficiency (Mozdzer and Megonigal 2012), introduced *Phragmites* has had an energetic advantage from its establishment to the present day that could have contributed to its invasiveness.

In contrast to our expectations, and unlike most performance metrics measured in introduced *Phragmites* under global change conditions (Holdredge et al. 2010; Mozdzer and Megonigal 2012; Eller et al. 2014), our plant-scale data suggest that advantages due to CC will diminish with rising atmospheric CO₂ and nutrient proliferation. If efficient tissue construction and short payback time are particularly strong components of introduced *Phragmites* invasiveness, as global change intensifies, the competitive dynamics of these lineages may shift such that introduced *Phragmites* is less able to dominate ecosystems. However, other factors may allow for a continued competitive advantage by introduced *Phragmites*, especially if the increased investment in tissues improves their performance. Such factors include photosynthetic capacity (Mozdzer and Zieman 2010), salinity tolerance (Vasquez et al. 2005), production of litter that suppresses competing plants (Holdredge and Bertness 2011) and a propensity to outcross and generate greater numbers of seedlings at eutrophied sites (McCormick et al. 2010). In addition, like the processes that are selecting for genotypes well adapted to eutrophied conditions (McCormick

et al. 2010), shifts in CO₂ and N may similarly select for more efficient tissue construction in populations of introduced *Phragmites*.

Conclusions

Leaf CCs alone do not provide an adequate representation of the energy required to produce biomass for *Phragmites*. Accounting for all major plant organs enabled us to identify key patterns in CCs, particularly belowground, that are likely associated with the invasive ability of the introduced lineage. In future studies attempting to address questions of plant carbon economy using CCs, we recommend that organs other than leaves be investigated, especially those belowground. In addition to gaining insight into invasion dynamics associated with rhizome and whole-plant CC patterns, these traits allowed us to identify responses to global change that are not well described in the literature. For instance, we observed greater root and stem CCs under elevated CO₂ and greater leaf CC under high N. Given the critical nature of understanding plant responses to global change, scientists should use the full array of tools available.

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Contributions by the Authors

T.J.M. designed the experiment, T.J.M. and C.N.W. collected data, and J.S.C. analysed the data. All authors contributed to writing, led by J.S.C.

Conflicts of Interest Statement

None declared.

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Supporting Information

The following Supporting Information is available in the online version of this article –

Figure S1. Construction costs vs. other functional traits by treatment, for native and introduced *Phragmites australis* (mean \pm SE). See [Table 2](#) for descriptions of traits.

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