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Effects of Elevated Temperature and Carbon Dioxide Concentrations on the Response of Two Common Reed (*Phragmites australis*) Haplotypes to Glyphosate

Research Article

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Abstract

Common reed [*Phragmites australis* (Cav.) Trin. ex Steud.], an aggressive invader in North American wetlands, is likely to undergo a range expansion as the climate changes. Increased atmospheric [CO_2] and temperature have been shown to cause morphological and physiological changes in many species, sometimes altering the way they respond to herbicides. To understand how climate-related environmental parameters may impact *P. australis* management, we grew two *P. australis* haplotypes (the Gulf Coast type and the Eurasian type) under ambient (400 ppm CO_2, 32/21 C) or elevated (650 ppm CO_2, 35/24 C) climate conditions. After 6 wk, the Gulf Coast type had reduced leaf area, increased stomatal conductance, and increased transpiration under the elevated conditions. The Eurasian type had lower V_{cmax} (the maximum carboxylation rate of Rubisco) and lower J_{max} (the maximum electron transport rate of RuBP regeneration) under elevated climate conditions. Results likely reflected a greater impact of higher temperatures rather than increased [CO_2]. After the 6-wk period, plants were either treated with glyphosate (0.57 kg ae ha^{-1}) or remained an untreated control. Data were collected 30 d after treatment (DAT) and 60 DAT to evaluate herbicide efficacy. Overall, the Gulf Coast type was less responsive to glyphosate applications under the elevated climate conditions than under current climate conditions. The lower leaf area of the Gulf Coast type in these climate conditions may have resulted in less herbicide interception and uptake. Glyphosate efficacy was less impacted by climate treatment for the Eurasian type than for the Gulf Coast type.

Introduction

Common reed [*Phragmites australis* (Cav.) Trin. ex Steud.] is an aggressive invader of wetlands and anthropogenically disturbed areas in the United States (Amsberry et al. 2000; Brisson et al. 2010; Saltonstall 2002). *Phragmites australis* is a perennial rhizomatous grass that forms dense monocultures, allowing it to displace native plant communities and have ecosystem engineering effects on carbon storage, nutrient cycling, and hydrology (Peter and Burdick 2010; Rooth et al. 2003; Windham and Lathrop 1999; Windham and Meyerson 2003). This species is present on every continent except Antarctica and is separated into distinct phylogenetic lineages, which are further divided into haplotypes based on sequences of chloroplast DNA (Kettenring et al. 2012; Saltonstall 2002, 2003). Exotic haplotypes have been problematic throughout much of the United States during the past 150 yr but have only recently presented management concerns in the state of Florida.

A cryptogenic Gulf Coast lineage with haplotype I (referred to here as the Gulf Coast type) is often considered to be native to the region; however, genetic testing suggests that this type is instead a hybrid between *P. australis* and the South American *Phragmites mauritianus* Kunth (Lambertini et al. 2012). Regardless of origin, this haplotype has undergone a range expansion in recent years, possibly due to anthropogenic disturbance (Meyerson et al. 2010). There is also an introduced lineage comprising four haplotypes, the most common of which is haplotype M (referred to here as the Eurasian type) (Kettenring et al. 2012; Saltonstall 2002, 2003). This type, introduced to New England during the mid-1800s, was identified in Florida for the first time in 2013 (Overholt et al. 2014). Although this population has been eradicated, proximal populations of the Eurasian type in neighboring states will likely continue range expansion into Florida over the coming years, potentially co-mingling with the Gulf Coast type (Williams et al. 2012).

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Management Implications

As the climate changes, land managers will need to consider the effects of warming and increased [CO_2] on plant invasions. Certain species, such as *Phragmites australis*, are likely to see increased growth and range expansions under these conditions. In addition, changes in growth and physiology that can occur under increased [CO_2] and temperature may render current management strategies, most importantly herbicide use, less effective. In this study, both the Gulf Coast and Eurasian haplotypes of *P. australis* showed increased growth under our elevated climate treatment (based on moderate projections for the year 2100), supporting previous findings that *P. australis* invasions are likely to become more problematic by the end of the century. For managers, it is critical to prioritize prevention of new *P. australis* invasions and eradication of those that already exist before these effects of climate change occur.

Chemical control using glyphosate was not as effective for the Gulf Coast type under our elevated climate treatment, and increased belowground biomass under these conditions indicates superior ability to regenerate following herbicide application. Effects of our climate treatments on management of the Eurasian type were negligible. The Gulf Coast type is often considered to be native but can become aggressive in disturbed habitats; managers should closely monitor this haplotype and take action as needed to prevent it from displacing desirable species. In the future, managers will need to be aware of possible changes in herbicide efficacy for *P. australis* and possibly other species as climatic conditions continue to change.

Climate change is projected to have major impacts on invasive species (Hellmann et al. 2008). Increased hydrologic fluctuations are predicted to occur in rivers and freshwater wetlands, exposing large areas of land for *P. australis* seed germination (Tougas-Tellier et al. 2015). The direct effects of increased [CO_2] and temperature may also expand *P. australis* invasions; Eller et al. (2014) found that two exotic haplotypes in the Mississippi Delta region demonstrate increased salinity tolerance when grown under conditions of elevated [CO_2] and temperature, which would allow the species to advance further into coastal salt marshes.

Phragmites australis haplotypes have demonstrated a high phenotypic plasticity in response to altered environmental conditions, indicating an ability to rapidly adapt to changes in climate (Eller and Brix 2012; Mozdzer and Megonigal 2012). Increases in atmospheric [CO_2] can have a significant impact on the growth and physiological processes of plants through a "fertilization effect," particularly for C_3 species such as *P. australis* that photosynthesize at suboptimal CO_2 concentrations under current climate conditions (Eller et al. 2014; Ziska and McConnell 2016; Ziska and Teasdale 2000). Plant response to elevated atmospheric [CO_2] can include increases in biomass production, water-use efficiency, photosynthesis, and altered leaf traits such as specific leaf area and stomatal density (Erickson et al. 2007; Manea et al. 2011; Ziska and Teasdale 2000). Moreover, levels of plasticity vary between haplotypes, suggesting a differential response to climate change.

A growing body of research suggests that the physiological changes brought about by altered climate conditions may lessen the efficacy of herbicides on invasive plant species (Manea et al. 2011; Ziska 2010; Ziska and Teasdale 2000; Ziska et al. 2004). This may happen through a number of mechanisms, such as altered leaf characteristics reducing herbicide uptake (decreased stomatal number, altered leaf thickness, etc.) or increased belowground biomass allowing for quicker regeneration from rhizomes following herbicide applications (Ziska and George 2004; Ziska et al. 2004). However, there have been instances in which climate change impacts on plant growth have not yielded differences in response to herbicide (Marble et al. 2015). This makes it necessary to evaluate these effects on an individual species basis.

Over the next century, it seems likely that *P. australis* will undergo a range expansion in Florida and the Gulf Coast region that may be exacerbated by changes in atmospheric [CO_2] and temperature. Given that herbicides are often the most effective and common method of controlling *P. australis* (Derr 2008; Martin and Blossey 2013), it is important to study how climate change might impact chemical control efforts. Here, we had two main objectives: (1) to evaluate the growth response of two *P. australis* haplotypes (I and M) to simulated climate change conditions and (2) to evaluate the response of these haplotypes to a commonly used herbicide, glyphosate, under current and projected climate conditions. We hypothesized that the two haplotypes would show differential response to climate treatments and that plants grown under projected conditions would be less affected by the herbicide application.

Materials and Methods

Plant Material and Growth Conditions

Rhizome segments of the Gulf Coast type were collected from Lake Jesup, FL, USA. Leaf tissue samples from this population were assayed using the PCR-RFLP described by Saltonstall (2003) for haplotype confirmation. For the Eurasian type, rhizome segments were obtained from the population in Lake Seminole, FL, USA, that was identified and sequenced by Overholt et al. (2014). All plants were maintained in a common garden environment at a greenhouse in Gainesville, FL, for more than a year before initiation of this experiment.

Two- to three-node rhizome segments were planted in commercial potting soil (Professional Growing Mix, Sun Gro Horticulture Canada, Agawam, MA, USA) with slow-release fertilizer (Osmocote® Plus 15-9-12, Scotts Miracle-Gro, Marysville, OH, USA) and grown in climate-controlled greenhouse chambers set to one of two treatments: (1) an ambient climate treatment of 400 ppm atmospheric [CO_2] and temperature of 32/21 C (actual values of 457.0 ± 1.2 ppm [CO_2], $33.1 \pm 0.2/22.3 \pm 0.2$ C as recorded during the experiment); or (2) an elevated climate treatment of 650 ppm atmospheric [CO_2] and temperature of 35/24 C (actual values of 651.1 ± 0.15 ppm [CO_2], $35.6 \pm 0.1/24.1 \pm 0.04$ C as recorded during the experiment). All chambers were maintained under a 14-h photoperiod. The elevated climate treatment was chosen to reflect projected springtime temperatures in the southeastern United States during the year 2100, based on representative concentration pathway 4.5 from the latest Intergovernmental Panel on Climate Change report (IPCC 2013). As there can be an interactive effect of CO_2 and temperature on plants (as demonstrated by Eller et al. 2013), and these two factors are projected to increase simultaneously, we focused on their combined effects rather than individual effects. There were two

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greenhouse chambers per climate treatment, each with 14 pots per haplotype.

Initial Measurements

After 6 wk of growth, initial measurements were made on morphological and physiological traits. For each plant, height (cm) and stem number were recorded. Photosynthetic measurements were made on the second-highest fully extended leaf of the tallest culm, using an LI-6400 XT infrared gas analyzer (IRGA; Li-Cor Biosciences, Lincoln, NE, USA). Measurements were made on 4 plants per haplotype, per greenhouse chamber. Photosynthesis (A) versus leaf intercellular [CO_2] (C_i) curves were plotted for each plant, with a light level of 1,800 µmol m^{-1} s^{-1}, relative humidity of 50 ± 10%, and a flow rate of 500 µmol s^{-1}. Block temperature was adjusted to the temperature of the room (32 C for the ambient treatment, 35 C for the elevated treatment). Plots were used to solve for V_{cmax} (the maximum carboxylation rate of Rubisco) and J_{max} (the maximum electron transport rate of RuBP regeneration) following the methods of Farquhar et al. (1980), with measurements corrected to 25 C (per Bernacchi et al. [2001, 2003] and Long and Bernacchi [2003]). Stomatal conductance (g_s) and leaf transpiration (E) were autologged by the LI-6400 XT as it recorded the A–C_i curves; values recorded at 400 ppm CO_2 were used for data analysis. The second-tallest fully extended leaf was then harvested from all plants, and leaf area was measured using an LI-3100C Area Meter (Li-Cor Biosciences). Leaves were oven-dried at 60 C for 72 h and weighed. Leaf area and leaf weight were then used to calculate specific leaf area (SLA).

Herbicide Application and Final Measurements

Following initial measurements, herbicide applications were made on 7 plants per haplotype, per climate chamber (14 total per climate treatment). Glyphosate (560.4 g ae ha^{-1}) and a nonionic surfactant (0.25% v/v) were applied using a backpack sprayer at a rate of 187.1 L ha^{-1}. This sublethal rate of glyphosate was chosen to allow detection of subtle differences in haplotype response to treatment. Height (cm), stem and lateral branch number, and visual injury (%) were recorded 30 d after treatment (DAT). Aboveground biomass was then harvested, oven-dried at 60 C for 72 h, and weighed. Plants were allowed to regrow for an additional 30-d period, after which height, stem and lateral branch number, and above- and belowground dried biomass were measured.

Table 1. Initial differences between plants of the Gulf Coast type of *Phragmites australis* grown under ambient (400 ppm CO_2, 32/21 C) and elevated (650 ppm CO_2, 35/24 C) climate treatments.[a]

Trait	Ambient	Elevated
Height (cm)	110.95 ± 5.78	110.27 ± 5.01
Stem number	1.71 ± 0.15	1.86 ± 0.17
Leaf area (cm^2)	**44.35 ± 1.80***	**38.38 ± 2.12***
SLA (cm^2 g^{-1})	172.1 ± 5.03	159.99 ± 4.10
V_{cmax} (µmol m^{-2} s^{-1})	69.71 ± 3.79	74.66 ± 5.86
J_{max} (µmol m^{-2} s^{-1})	196.91 ± 15.90	175.01 ± 12.91
g_s (mmol H_2O m^{-2} s^{-1})	**127.40 ± 33.86***	**196.26 ± 79.20***
E (mmol H_2O m^{-2} s^{-1})	**3.65 ± 1.28***	**5.96 ± 2.04***

[a] Means with SEs are displayed for height, stem number, leaf area, specific leaf area (SLA), the maximum carboxylation rate of Rubisco (V_{cmax}), the maximum electron transport rate of RuBP regeneration (J_{max}), stomatal conductance (g_s) at 400 ppm CO_2, and leaf transpiration (E) at 400 ppm CO_2. Statistically significant values in bold: *$P < 0.05$.

Table 2. Initial differences between plants of the Eurasian type of *Phragmites australis* grown under ambient (400 ppm CO_2, 32/21 C) and elevated (650 ppm CO_2, 35/24 C) climate treatments.[a]

Trait	Ambient	Elevated
Height (cm)	67.26 ± 1.94	71.03 ± 2.29
Stem number	6.18 ± 0.37	5.71 ± 0.33
Leaf area (cm^2)	18.1 ± 1.01	18.49 ± 0.83
SLA (cm^2 g^{-1})	211.86 ± 6.20	199.61 ± 6.55
V_{cmax} (µmol m^{-2} s^{-1})	**87.43 ± 5.11***	**71.64 ± 4.92***
J_{max} (µmol m^{-2} s^{-1})	**191.76 ± 16.38***	**147.78 ± 8.99***
g_s (mmol H_2O m^{-2} s^{-1})	253.19 ± 56.52	218.46 ± 89.85
E (mmol H_2O m^{-2} s^{-1})	5.82 ± 1.12	6.18 ± 2.31

[a] Means with SEs are displayed for height, stem number, leaf area, specific leaf area (SLA), the maximum carboxylation rate of Rubisco (V_{cmax}), the maximum electron transport rate of RuBP regeneration (J_{max}), stomatal conductance (g_s) at 400 ppm CO_2, and leaf transpiration (E) at 400 ppm CO_2. Statistically significant values in bold: *$P < 0.05$.

Data Analysis

Data from each haplotype were analyzed separately using RStudio v. 1.0.136 (RStudio, Boston, MA, USA). An ANOVA was used to determine the effect of climate treatment on initial growth and photosynthetic characteristics, with mean

Table 3. *F*-ratios resulting from a two-way ANOVA of plant traits at 30 DAT, with glyphosate treatment (0.57 kg ae ha^{-1}) and climate treatment (ambient: 400 ppm CO_2, 32/21 C; or elevated: 650 ppm CO_2, 35/24 C) as factors.[a]

Haplotype	Trait	Climate	Glyphosate	Climate × glyphosate
Gulf Coast				
	Injury	1.27	**145.69*** **	1.26
	Height	1.90	**39.01*** **	0.54
	Stem number	2.52	**15.77*** **	0.07
	Lateral branch number	**6.22***	**127.79*** **	**6.22***
	Aboveground biomass	2.76	**45.25*** **	0.43
Eurasian				
	Injury	0.33	**15.38*** **	0.33
	Height	**9.40** **	**7.59** **	1.53
	Stem number	0.11	**12.97*** **	0.73
	Lateral branch number	**5.53***	**33.20*** **	**6.10***
	Aboveground biomass	1.37	0.40	0.55

[a] Results are shown for both the Gulf Coast and Eurasian haplotypes of *Phragmites australis*. Statistically significant values in bold: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

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separation at $P < 0.05$. A two-way ANOVA was used to determine the effect of herbicide application and climate treatment on measured characteristics at 30 DAT and 60 DAT. Residuals were tested for all model assumptions, and data were subjected to logarithmic transformation when necessary. Each pair of climate chambers was considered an experimental run. There was no significant run effect ($P \geq 0.05$), so data were pooled between experimental runs.

Results and Discussion

Initial Growth Response

For the Gulf Coast type (Table 1), there was no effect of climate treatment on height, stem number, SLA, or on the photosynthetic traits V_{cmax} and J_{max}. Although there was no significant difference in specific leaf area between treatments, there was an effect of climate on leaf area, with plants having smaller leaves when grown under elevated conditions (38.38 ± 2.12 cm^2 compared with 44.35 ± 1.8 cm^2 for the elevated and ambient climate treatments, respectively). Plants had higher g_s under elevated climate conditions (127.4 ± 33.86 and 196.26 ± 79.2 mmol H_2O m^{-2} s^{-1} for ambient and elevated climate treatments, respectively), as well as higher E (3.65 ± 1.28 and 5.96 ± 2.04 mmol H_2O m^{-2} s^{-1} for ambient and elevated climate treatments, respectively). This response is unusual in elevated [CO_2] conditions; typically, leaf area (as well as overall biomass production) for C_3 species is higher when atmospheric [CO_2] is increased, while g_s and E decrease (Ainsworth and Rogers 2007; Morison and Gifford 1984). However, these results are

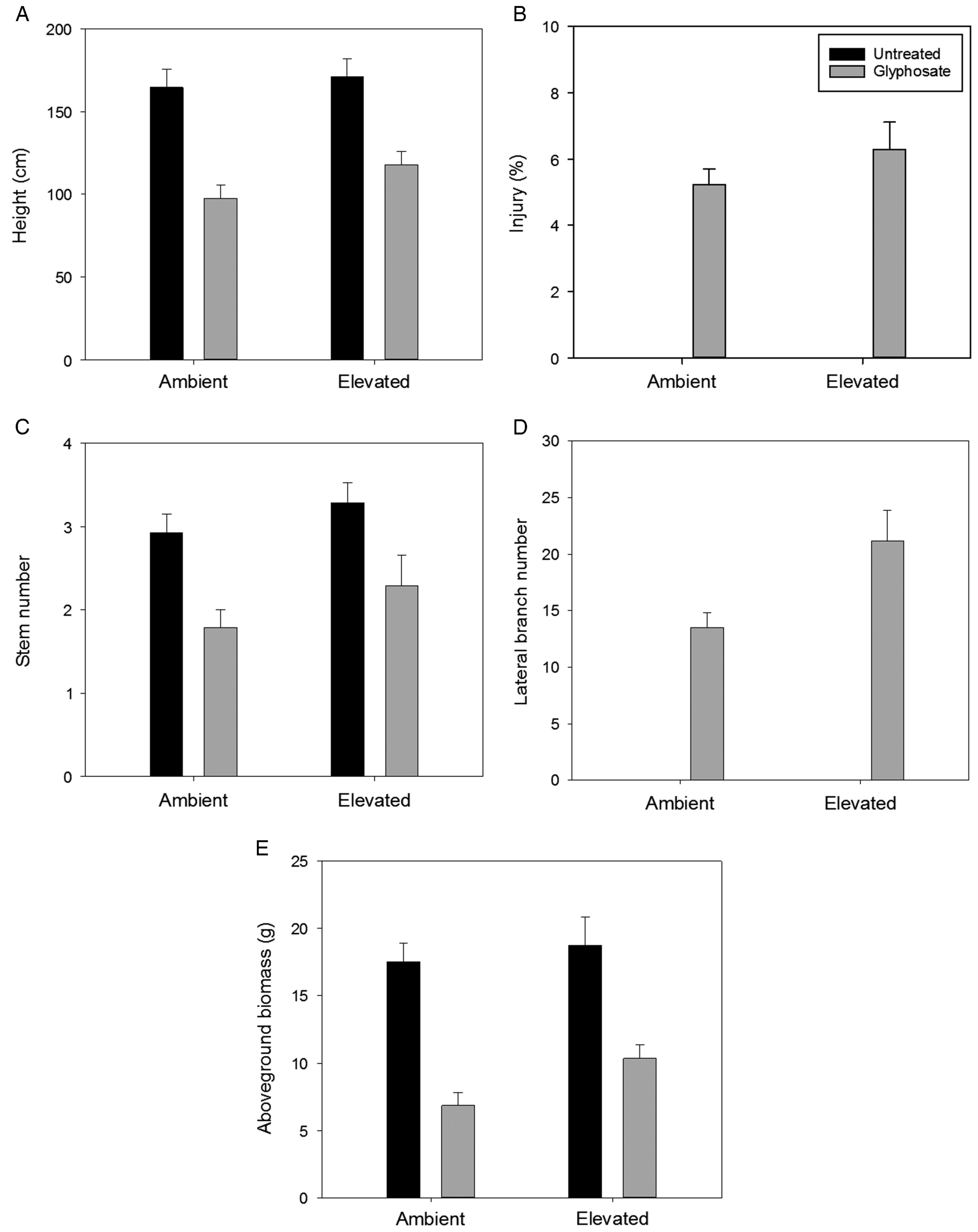

Figure 1. The response of the Gulf Coast type of *Phragmites australis* to glyphosate application at 30 d after treatment under ambient (400 ppm CO_2, 32/21 C) and elevated (650 ppm CO_2, 35/24 C) climate treatments. Displayed values represent the mean and SE of (A) height, (B) visual injury, (C) stem number, (D) lateral branch number, and (E) aboveground biomass.

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consistent with the effects of increased temperature. Plants under heat stress have been shown to produce smaller leaves, and in well-watered conditions often increase g_s and E to regulate leaf temperature through evaporative cooling (Crawford et al. 2012; Murata and Mori 2014). These responses may have implications for herbicide efficacy; for example, a lower leaf area can limit the amount of foliar-applied herbicide that is taken up by plants.

For the Eurasian type (Table 2), V_{cmax} was significantly higher under ambient climate conditions (87.43 ± 5.11 compared with 71.64 ± 4.92 μmol m^{-2} s^{-1} for ambient and elevated climate treatments, respectively). The same relationship was found for J_{max} (191.76 ± 16.68 compared with 147.78 ± 8.99 μmol m^{-2} s^{-1} for ambient and elevated climate treatments, respectively). There was no effect of climate on any other measured physical or physiological traits for this haplotype. Again, this is an unusual response for a C_3 species under increased atmospheric [CO_2]; typically, these values both increase under elevated climate conditions, and a previous study has demonstrated this with the Eurasian haplotype of *P. australis* (Eller et al. 2014). Increased atmospheric [CO_2] can exacerbate nitrogen-deficiency symptoms and lower V_{cmax} and J_{max}, and it is possible that this occurred in our study (Miglietta et al. 1996). However, lowered photosynthetic capacity can also result from exposure to high temperatures. Increased atmospheric [CO_2] can sometimes mitigate the effects of heat stress, although for some species this is not the case (Wang et al. 2016; Yu et al. 2012).

Previous research on *P. australis* response to climate change has found significant differences in growth and photosynthesis under altered climate regimes (Caplan et al. 2014; Eller et al. 2014;

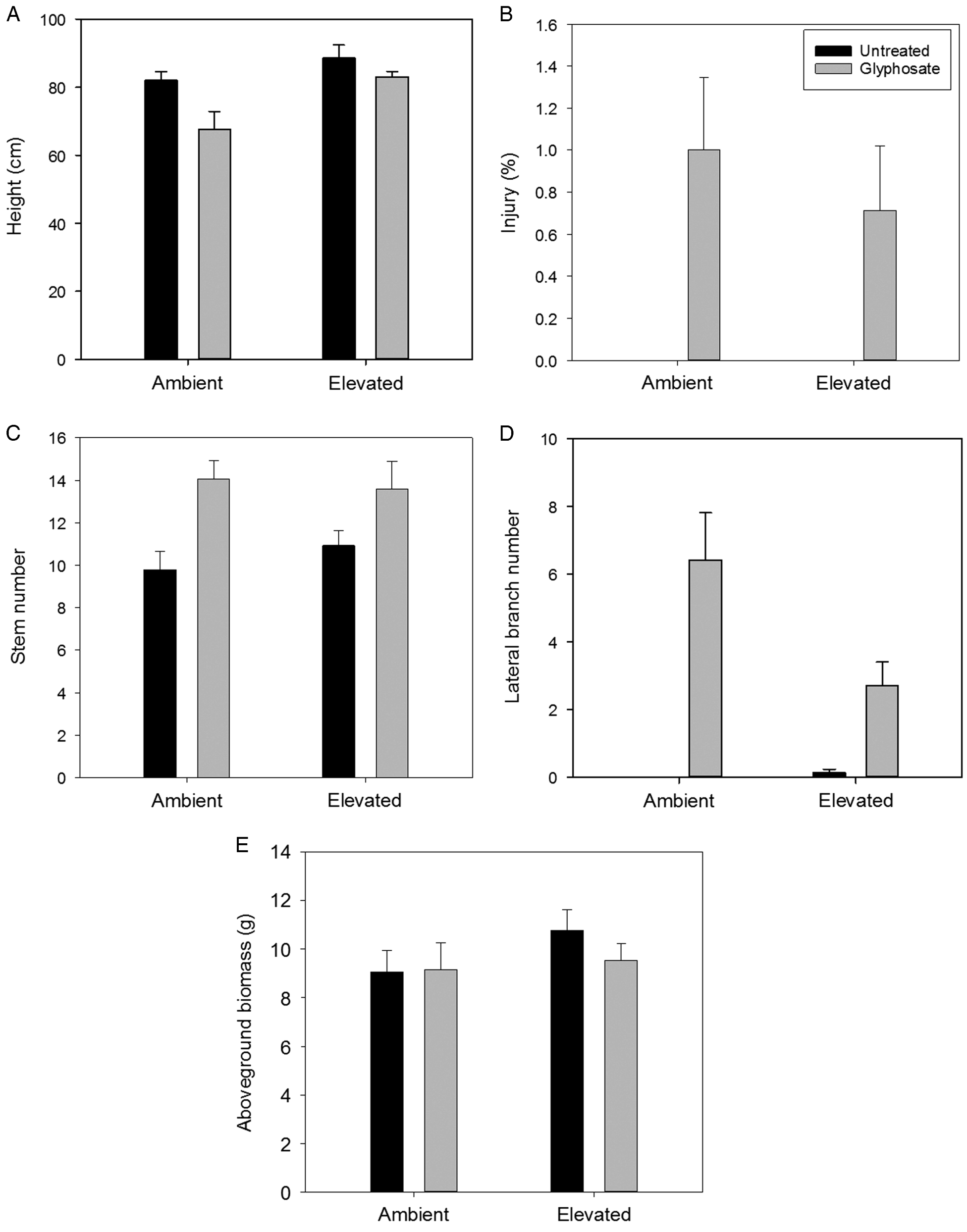

Figure 2. The response of the Eurasian type of *Phragmites australis* to glyphosate application at 30 d after treatment under ambient (400 ppm CO_2, 32/21 C) and elevated (650 ppm CO_2, 35/24 C) climate treatments. Displayed values represent the mean and SE of (A) height, (B) visual injury, (C) stem number, (D) lateral branch number, and (E) aboveground biomass.

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Mozdzer and Megonigal 2012). Mozdzer and Megonigal (2012) found that both the Eurasian type and a native North American haplotype exhibited increased productivity due to elevated atmospheric [CO_2] and simulated nitrogen pollution (although in this study there was not a temperature treatment), with the Eurasian type showing a greater overall response than the native. In addition, they showed the Eurasian type to have reduced specific leaf area and increased nitrogen productivity under elevated [CO_2] conditions. In this study, we found few initial differences between plants grown in ambient (400 ppm CO_2, 32/21 C) or elevated (650 ppm CO_2, 35/24 C) climate conditions for either haplotype.

It is possible that our [CO_2] treatment was not high enough to overcome the effects of temperature. Eller et al. (2014) also included increased temperature in their elevated climate treatment, but the magnitude of change between climate treatments was different than in ours (+310 ppm [CO_2] and + 5 C in their study compared with +250 ppm [CO_2] and + 3 C in ours). We chose a moderate climate projection for the year 2100; it is possible that had our climate treatment been more severe, we would have seen more differences related to the effects of increased [CO_2]. However, these results suggest that *P. australis* response to moderate changes in climate may be largely determined by temperature rather than [CO_2].

Initial Herbicide Response

Overall, initial response to glyphosate was limited for both haplotypes at 30 DAT. Injury rates were low, which is indicative of the low rate of glyphosate used in this study. Despite the low injury, there was still a significant effect of glyphosate application on most measured traits for both haplotypes (Table 3). For the Gulf Coast type, plants treated with glyphosate were significantly shorter, had fewer stems, lower aboveground biomass, and higher injury ratings than untreated plants regardless of climate treatment (Figure 1). For the Eurasian type, plants treated with glyphosate had higher injury ratings and lower height than untreated plants; however, there was no effect of glyphosate on aboveground biomass, and stem number was significantly increased by herbicide application at 30 DAT (Figure 2). These data suggest that the Gulf Coast type was more susceptible to glyphosate than the Eurasian type at 30 DAT. This is in contrast to a previous study by Cheshier et al. (2012), which found no significant differences in response to herbicide treatment between these two haplotypes; this may be due to differences in application rates between the two studies. Low rates of glyphosate have been shown to stimulate growth in certain plants, and the low rate used in this study may have had this effect on the Eurasian type (Velini et al. 2010).

For the Gulf Coast type, there was no effect of climate on most measured plant traits (Table 3). There was a significant effect of climate on lateral branch number posttreatment, as well as a significant interaction between climate and herbicide application (Table 3). Only plants treated with glyphosate produced lateral branches, and those grown under elevated climate conditions produced significantly more (21.1 ± 2.8) than those under ambient conditions (13.5 ± 1.3) (Figure 1D). Similarly, there was a significant effect of both climate and glyphosate application (as well as their interaction) on lateral branch production for the Eurasian type (Table 3). Unlike the Gulf Coast type, however, Eurasian type plants treated with glyphosate produced more lateral branches when grown under the ambient climate treatment (6.4 ± 1.4 compared with 2.7 ± 0.7 for ambient and elevated climate conditions, respectively) (Figure 2D). There was a significant effect of climate and glyphosate application on plant height as well, although the interaction was not significant; plants grown under the elevated climate treatment were taller than those under the ambient treatment, and plants treated with glyphosate were shorter than untreated plants (Figure 2A).

Lateral branching is a common symptom of sublethal glyphosate applications, indicating that for the Gulf Coast type, plants under the elevated climate conditions were showing a greater initial response than those in the ambient conditions. This could have one of two causes. First, glyphosate is translocated to areas of active growth; plants grown under the elevated climate conditions tended to produce more stems and had greater aboveground biomass than those in the ambient conditions (although this was not significant at 30 DAT), and it is possible that increased growth stimulated movement of the herbicide through the plant. Alternatively, this response could have resulted from heat stress; increased temperature has been correlated with increased herbicide efficacy in certain species (Hammerton 1967; Johnson and Young 2002). If the primary effect of the elevated climate treatment was due to increased temperature rather than [CO_2], it could have also resulted in increased herbicide symptoms.

For the Eurasian type, lateral branching was also increased by glyphosate, but to a lesser extent than for the Gulf Coast type. Additionally, lateral branching for this type was greater under ambient conditions. This differential response could be linked to photosynthesis. Photosynthesis affects movement of systemic herbicides such as glyphosate, and we observed lowered

Table 4. *F*-ratios resulting from a two-way ANOVA of plant traits at 60 DAT, with glyphosate treatment (0.57 kg ae ha^{-1}) and climate treatment (ambient: 400 ppm CO_2, 32/21 C; or elevated: 650 ppm CO_2, 35/24 C) as factors.[a]

Haplotype	Trait[a]	Climate	Glyphosate	Climate × glyphosate
Gulf Coast				
	Height	**7.59****	**32.07*****	**5.47***
	Stem number	**13.05***	2.64	0.29
	Lateral branch number	**5.51***	**23.55*****	**7.08***
	Aboveground biomass	**13.58*****	**15.29*****	0.22
	Belowground biomass	2.95	**13.40*****	0.24
Eurasian				
	Height	0.13	0.98	0.001
	Stem number	**17.59*****	**5.43***	0.04
	Lateral branch number	2.30	**6.12***	0.16
	Aboveground biomass	**11.46***	0.00	0.01
	Belowground biomass	**7.02***	0.28	0.27

[a] Results are shown for both the Gulf Coast and Eurasian haplotypes of *Phragmites australis*. Statistically significant values in bold: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

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photosynthetic rates in the Eurasian type under elevated climate conditions; this may result in slower or more limited herbicide efficacy under these conditions (Varanasi et al. 2016; Waltz et al. 2004). It may also be that for *P. australis*, the Eurasian type is less sensitive to increased temperatures compared with the Gulf Coast type. Genetic variability has been shown to play a role in plant response to climate change conditions; a recent study has found that rice (*Oryza sativa* L.) accessions respond differently to elevated atmospheric [CO_2] and temperature, with some accessions being more impacted by increases in temperature than others (Wang et al. 2016). Morphological and ecophysiological differences are known to exist between *P. australis* haplotypes, and it is not unlikely for these two haplotypes to respond differently to environmental stress (Eller and Brix 2012; League et al. 2006; Mozdzer and Zieman 2010; White et al. 2004).

Effects on Regrowth

For the Gulf Coast type, there was an effect on regrowth height by both climate treatment and glyphosate application, as well as an interactive effect of the two at 60 DAT (Table 4). Plants treated with glyphosate had less regrowth than untreated plants; however,

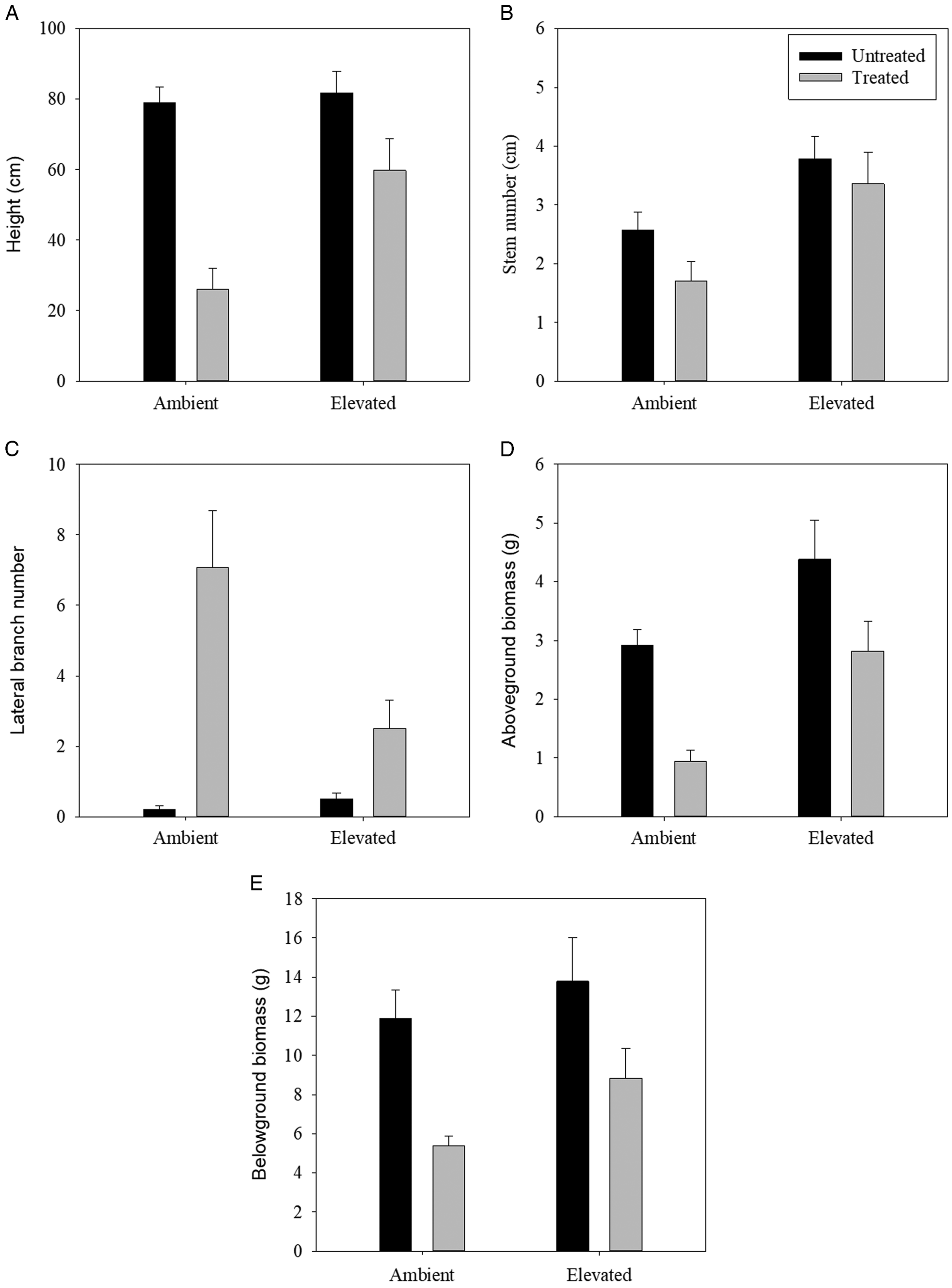

Figure 3. The response of the Gulf Coast type of *Phragmites australis* to glyphosate application at 60 d after treatment under ambient (400 ppm CO_2, 32/21 C) and elevated (650 ppm CO_2, 35/24 C) climate treatments. Displayed values represent the mean and SE of (A) height, (B) stem number, (C) lateral branch number, (D) aboveground biomass, and (E) belowground biomass.

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under the elevated climate treatment, this difference (81.8 ± 6.2 cm for untreated plants, 59.8 ± 9.1 cm for treated plants) was not significant. Plant height was more affected by herbicide application under the ambient climate treatment (79.1 ± 4.4 cm for untreated plants, 26.0 ± 6.0 cm for treated plants) (Figure 3A). This indicates that plants that had been treated with glyphosate were exhibiting a greater stress response under the ambient climate conditions. Stem number was affected by climate treatment, with plants in elevated climate conditions having more stems than those in the ambient conditions, although there was no effect of herbicide application (Table 4).

There was again an effect of both herbicide application and climate treatment, and an interactive effect on lateral branch production for the Gulf Coast type (Table 4). Glyphosate-treated plants grown in elevated climate conditions produced fewer branches (2.5 ± 0.8) than did those in ambient conditions (7.1 ± 1.6) (Figure 3C). Aboveground biomass was significantly affected by both climate and herbicide application as well (Table 4). Plants produced more aboveground biomass under elevated climate conditions, and less when treated with glyphosate (Figure 3D). For belowground biomass there was no effect of climate, only of herbicide application (with treated plants showing lower biomass than those that were untreated) (Figure 3E).

For the Eurasian type, there was no effect of climate or glyphosate application on plant height (Table 4). Stem number was significantly higher under elevated conditions, and plants produced more stems when treated with glyphosate (Figure 4B). There was no effect of climate on lateral branch production, although plants treated with glyphosate produced more branches

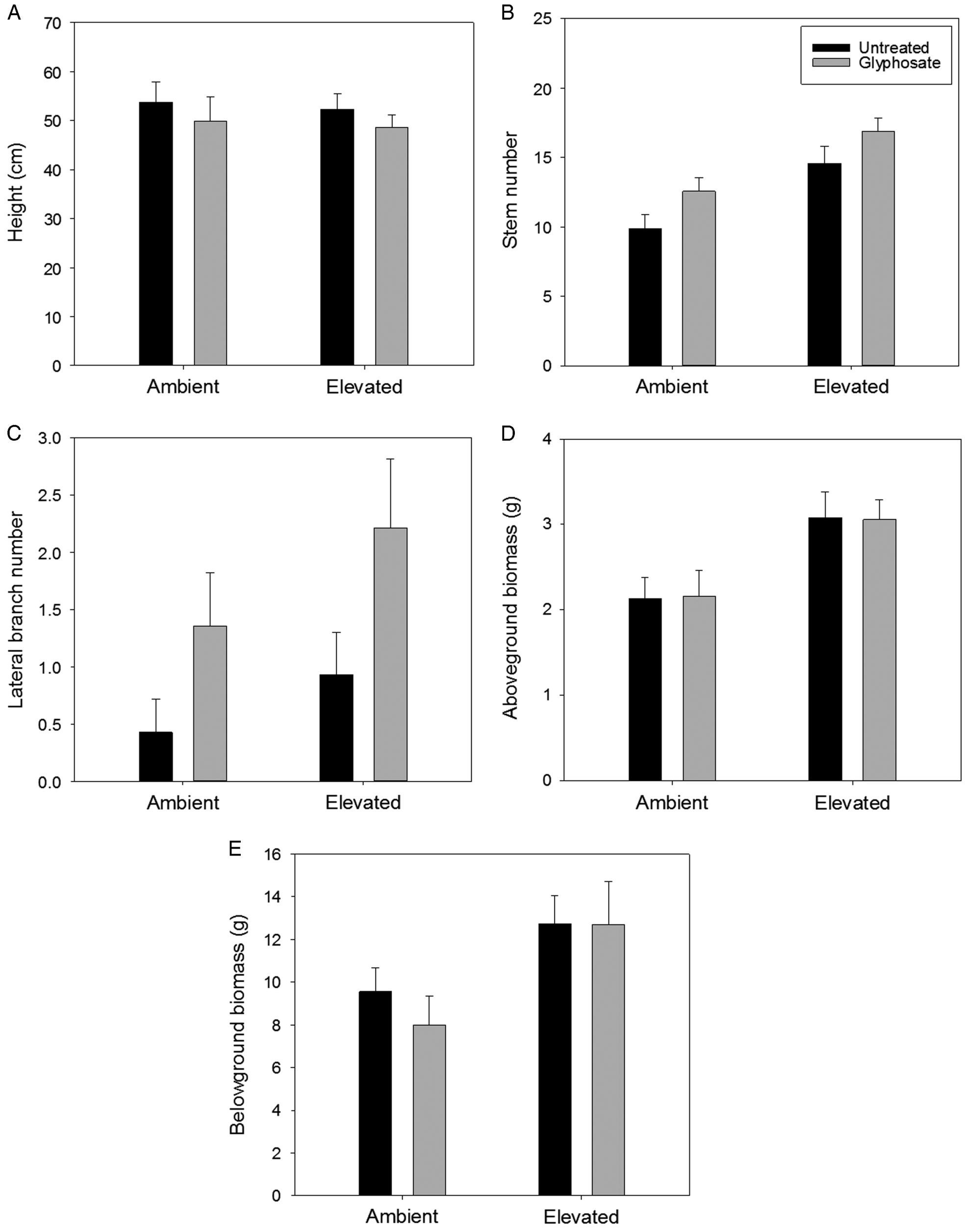

Figure 4. The response of the Eurasian type of *Phragmites australis* to glyphosate application at 60 d after treatment under ambient (400 ppm CO_2, 32/21 C) and elevated (650 ppm CO_2, 35/24 C) climate treatments. Displayed values represent the mean and SE of (A) height, (B) stem number, (C) lateral branch number, (D) aboveground biomass, and (E) belowground biomass.

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than the untreated controls (Figure 4C). Plants produced significantly lower aboveground and belowground biomass under ambient climate conditions, but there was no effect of glyphosate application for either trait (Table 4).

Plant regrowth at 60 DAT showed more effects of climate treatment than in the initial measurements. For the Gulf Coast type, stem number was higher under elevated climate conditions. Additionally, the elevated climate conditions increased belowground biomass production for both haplotypes, an effect of increased [CO_2] that has been observed for *P. australis* in other studies (Caplan et al. 2014; Mozdzer and Megonigal 2012). High belowground biomass facilitates shoot regeneration in rhizomatous species like *P. australis*, allowing for greater recovery potential following herbicide application. This resulted in plants growing in the elevated climate conditions having greater aboveground biomass than those in the ambient conditions for the Gulf Coast type.

Our data suggest that overall, increased temperature and atmospheric [CO_2] had a greater long-term effect on the Gulf Coast type's response to glyphosate, while there was only a limited initial impact on the Eurasian type. At the time of herbicide application, photosynthesis was lower for the Eurasian type under elevated climate conditions; although this may have slowed down glyphosate translocation, it was not enough to limit long-term efficacy. For the Gulf Coast type, differences in leaf area likely contributed to the differential effect of glyphosate under our two climate treatments; altered leaf traits are thought to be a primary mechanism by which climate change affects plant response to herbicides (Ziska 2008). Here, plants of the Gulf Coast type had a lower leaf area under elevated climate conditions, reducing the amount of herbicide taken up by the plants. There were no significant differences in leaf area between climate treatments for the Eurasian type.

The Gulf Coast type has been expanding its range in certain parts of the Gulf Coast region, including anthropogenically disturbed wetlands in Florida, where it frequently requires management. Glyphosate is currently one of the most effective tools for managing *P. australis* invasions; if climate change reduces its efficacy, this haplotype may become increasingly problematic in the coming years. Here, we conducted an initial study to determine whether elevated carbon dioxide concentrations and temperature have an effect on glyphosate efficacy for *P. australis*, and we used a sublethal rate to detect subtle differences. Further research is needed to evaluate alternative climate scenarios, lethal rates of glyphosate, and alternative herbicides and management strategies.

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