



# Strong effects of hydrologic environment and weak effects of elevated CO<sub>2</sub> on the invasive weed *Alternanthera philoxeroides* and the biocontrol beetle *Agasicles hygrophila*

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Received: 5 July 2017 / Accepted: 24 May 2018 / Published online: 11 June 2018  
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## Abstract

Global change, such as elevated CO<sub>2</sub>, may alter interactions between invasive plants and biocontrol agents, impacting biocontrol efficacy. Here, we conducted four experiments in Texas, USA to test how elevated CO<sub>2</sub> influences an invasive plant (*Alternanthera philoxeroides*) and its interactions with an introduced biocontrol beetle (*Agasicles hygrophila*) in terrestrial (well-watered) and flooded environments. We grew plants for 9 months in ambient or elevated CO<sub>2</sub> (800 ppm) chambers in continuously flooded or well-watered conditions. In no-choice trials, flooding increased leaf toughness and decreased beetle consumption but beetles only oviposited on ambient CO<sub>2</sub> leaves. In choice trials, beetles preferred to feed and oviposit on terrestrial plants but were also less likely to damage elevated CO<sub>2</sub> leaves. Caged beetle populations were larger in terrestrial conditions than aquatic conditions for a second set of plants grown in the chambers. With a third set of plants grown in the ambient or elevated CO<sub>2</sub> chambers, damage for plants placed in the field (aquatic setting) was higher for plants grown in terrestrial conditions vs. flooded conditions at ambient CO<sub>2</sub>. Our results suggest that elevated CO<sub>2</sub> will have minor effects on the efficacy of this biocontrol agent by decreasing oviposition and number of leaves damaged, and hydrologic environment may affect invasive plant performance by altering herbivore oviposition and feeding preferences. A broader understanding of the effects of global change on biocontrol will help prevent and manage future spread of invasive plants.

**Keywords** Alligator weed · Alligator weed flea beetle · Biocontrol · Carbon dioxide · Climate change

## Introduction

Increased global trade and travel have brought a wide variety of exotic plants into new environments, and a small proportion of them become invasive and compete with native species (Hulme 2015). Invasive plants disrupt both community

composition and nutrient cycling in a wide variety of different ecosystems across the globe (Vila et al. 2011). Part of the effort to combat invasive plants has been the introduction of biocontrol agents (Carson et al. 2008). These are organisms with host-specific herbivory in the target plant's native range (Clewley et al. 2012). Though there have been many successes, use of biocontrol could be improved by a better understanding of the ecological interactions between control agents, target plants, and non-target plants, and how these interactions respond to environmental change (Thomas and Reid 2007; Aguilar-Fenollosa and Jacas 2014). Perhaps, even more worrying is the possibility of climate change altering interactions in unpredicted ways (Facey et al. 2014; Lu et al. 2015).

While research on the effects of anthropogenic climate change often focuses on temperature change, there are also potential direct effects of increased CO<sub>2</sub> concentrations, particularly pertaining to plant growth. Plants respond differently to increased CO<sub>2</sub> concentrations depending on how they fix carbon. C<sub>3</sub> plants typically show strongly enhanced

Handling Editor: Livy Williams.

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growth under CO<sub>2</sub>-enriched conditions, while C<sub>4</sub> plants are typically less responsive to changes in CO<sub>2</sub> concentration (DeLucia et al. 2012; Zavala et al. 2013). The increased growth of plants exposed to elevated CO<sub>2</sub> can vary with soil nitrogen availability with plant growth responses to CO<sub>2</sub> often limited when soil resources are low (de Graaff et al. 2006). Increased CO<sub>2</sub> also affects plant reproduction by increasing the number of flowers, fruits, and seeds (Jablonski et al. 2002; DeLucia et al. 2012; Zavala et al. 2013). Plants exposed to elevated CO<sub>2</sub> generally show an increase in leaf toughness, which may affect how palatable the leaves are to herbivores (DeLucia et al. 2012).

Effects of increased CO<sub>2</sub> concentrations on insects are probably largely indirect as the changes in atmospheric CO<sub>2</sub> concentrations brought on by anthropogenic climate change are typically too small to have any significant direct effect on insects (Facey et al. 2014) with only minor effects if any being reported (Xie et al. 2015; Liu et al. 2017). Herbivorous insects are, however, indirectly affected by changes to plants due to increased CO<sub>2</sub> concentrations such as reductions in leaf palatability or nutritional quality (DeLucia et al. 2012; Zavala et al. 2013; Dader et al. 2016). This can lead to decreased larval survival rates, decreased pupal mass, and altered oviposition preferences through decreases in foliar nitrogen and/or increases in toughness (Agrell et al. 2000; DeLucia et al. 2012; Zavala et al. 2013). Increased CO<sub>2</sub> may also alter plant's chemical defenses (Landosky and Karowe 2014; Sharma et al. 2016; Jamieson et al. 2017), likely through changes in plant hormones (Zavala et al. 2017), and indirectly affect associated herbivores (DeLucia et al. 2012). However, field evidence is still limited for the effects of elevated CO<sub>2</sub> on plant insect interactions (Robinson et al. 2012; Gherlenda et al. 2016), especially for biocontrol agents. One field study with the biocontrol weevil *Larinus minutus* on *Centaurea diffusa* found that the benefits of CO<sub>2</sub> to plants were reduced by increased feeding on seeds (Reeves et al. 2015). The effects of elevated CO<sub>2</sub> on plant-herbivore interactions may also be amplified in coastal areas, where increased sea levels will change topography and increase salinity, an important consideration for controlling invasive aquatic plants in coastal riparian areas (Caplan et al. 2015).

*Alternanthera philoxeroides* (Alligator weed—Amaranthaceae) is an invasive C<sub>3</sub> weed (Sage et al. 2007) native to South America that has been introduced to many parts of the world (Spencer and Coulson 1976). In its native range, *A. philoxeroides* reproduces mostly vegetatively, but it only reproduces vegetatively in its introduced ranges (Julien et al. 1995). In parts of its introduced ranges, it forms dense mats that clog waterways, displacing native plants and preventing recreation and economic activity (Fleming and Dibble 2015). In Australia, New Zealand, and the USA, *A. philoxeroides* is mainly found in an aquatic form, which forms dense, floating mats anchored to banks (Julien et al. 1995).

In introduced populations in China, terrestrial and aquatic forms are both common (Lu et al. 2015; Wu et al. 2017).

*Agasicles hygrophila* (alligator weed flea beetle—Coleoptera: Chrysomelidae) is an herbivorous beetle that feeds on the leaves and stems of *A. philoxeroides* in its native range (Spencer and Coulson 1976). The beetles lay eggs on the surface (usually on the underside) of *A. philoxeroides* leaves and pupate within its hollow stems. It has been deliberately introduced across the United States in 1964 (Spencer and Coulson 1976), and later to China, New Zealand, and Australia (Julien et al. 1995). Previous work has shown that warming leads to increased population size for the beetle and causes a geographic shift in ranges of the beetle and the invasive plant in China (Lu et al. 2015). Here, we examined the impacts of increased atmospheric CO<sub>2</sub> concentrations on the invasive plant *A. philoxeroides*, biocontrol beetle *A. hygrophila*, and their interactions in terrestrial and flooded conditions.

We conducted four experiments with *A. philoxeroides* and *A. hygrophila* to investigate the following questions: (1) What are the direct effects of elevated CO<sub>2</sub> on *A. philoxeroides*? (2) What are the effects of elevated CO<sub>2</sub> on *A. hygrophila* at individual (e.g., host preference) and population (e.g., population dynamics) levels? (3) How do these effects vary between terrestrial and flooded conditions?

## Materials and methods

### No-choice experiment

We randomly assigned 48 chambers (60 × 60 × 90 cm<sup>3</sup> tall) in the Rice University greenhouse to ambient (ca. 400 ppm, 24 chambers) or elevated CO<sub>2</sub> (800 ppm, 24 chambers). Each chamber was a nylon mesh cage (“jumbo cage”, Live-Monarch.com, Live Monarch Foundation, Blairsville, GA, USA) set inside a clear plastic bag with the top open to allow air to readily exit the chamber and each received air from an 8 mm inner diameter plastic tube through the bottom of the chamber. We placed a pump (Gast 1423-103Q-G626X, Benton Harbor, MI, USA) with the air intake outside the greenhouse to draw in air unaffected by the experiment. The pump output was split into two sets of tubes. One fed air into a system of pipes and tubes that supplied the 24 ambient chambers and a 25th chamber without plants. The other fed an identical set of pipes, tubes, and 25 chambers (24 experimental plus a plant free one). In the elevated CO<sub>2</sub> plant-free chamber, we placed a CO<sub>2</sub> monitor/controller (Atlas 3, Titan Controls, Vancouver, WA USA) that regulated a valve that injected CO<sub>2</sub> from a compressed gas cylinder such that the air supply was kept at 800 ppm CO<sub>2</sub>. We monitored CO<sub>2</sub> in the plant-free chambers with data loggers (SD800, Extech, Nashua NH, USA). Chambers had approximately one air

change per hour. In fall 2014, we field collected *A. philoxeroides* (Pasadena, TX) and planted them into a pot in each chamber that was filled with hydric clay soils collected from a riverbank (La Marque, TX) where *A. philoxeroides* was not present.

We assigned each pot to a flooding treatment creating a factorial design. Flooded pots had approximately 10 cm of standing water kept above the soil and non-flooded pots were kept well watered by adding water until it began to run out of the holes in the bottom of the pot. The pot for one ambient CO<sub>2</sub> pot initially assigned to a flooded treatment would not hold water and we switched it to be a non-flooded treatment. We grew plants at 20–30 °C for approximately 8 months before the feeding trials.

In March 2015, we collected *A. hygrophila* larvae from a natural field population (Pasadena, TX) and reared them on *A. philoxeroides*. We used adults for the feeding trials. We excised the largest leaf from each plant, immediately brought them to the lab, measured its area and mass and calculated leaf area ratio [LAR], and placed it in a petri dish (15 cm diameter) on moist filter paper with one adult *A. hygrophila*. We allowed the beetles to consume their leaf for 24 h and replaced leaves that were mostly consumed with a new leaf from the same plant. At 48 h, we replaced the leaf of each beetle with one from the same plant. After 120 h, we removed the leaf. We scanned leaves (2 or 3 per beetle) and measured leaf area remaining and leaf area consumed (using Image J, NIH USA) summed across leaves, and counted the number of egg clutches laid on the dish or the leaves.

We tested leaf toughness from additional leaves on each plant, using seven leaves for each plant. Using a metal C-clamp, we secured leaves between two boards (15 × 8.9 × 1.9 cm) with a hole (4 mm diameter) drilled between them which the leaf overlapped (avoiding the midrib for leaves that were large enough). We added sand to a cup attached to a bolt (no. 8) placed in the hole in the top board until it punctured the leaf. We used the weight of the bolt, washer, cup, and sand to estimate the tissue strength of each leaf.

We analyzed the dependence of LAR, leaf toughness, leaf area consumed, leaf mass consumed, and number of egg clutches laid (on leaf and total) on CO<sub>2</sub>, flooding, and their interactions in ANOVAs (Proc Mixed). For response variables with significant interactive effects, we used adjusted means partial difference tests to distinguish among treatment means. We performed all analyses for this and the other experiments with SAS 9.4 (SAS 2012).

### Choice experiment

We used the plants from the no-choice experiment to conduct a choice experiment between pairs of plants that were grown for more than 9 months in different CO<sub>2</sub> and flooding

conditions. In May, we placed pairs of pots into ambient CO<sub>2</sub> chambers and maintained their previous flooding treatments. Pairs of plants in four sub-experiments tested (1) the effect of CO<sub>2</sub> in terrestrial conditions (five pairs of terrestrial and ambient CO<sub>2</sub> with terrestrial and elevated CO<sub>2</sub>), (2) the effect of CO<sub>2</sub> in flooded conditions (five pairs of flooded and ambient CO<sub>2</sub> with flooded and elevated CO<sub>2</sub>), (3) the effect of flooding in ambient CO<sub>2</sub> (six pairs of terrestrial and ambient CO<sub>2</sub> with flooded and ambient CO<sub>2</sub>), and (4) the effect of flooding in elevated CO<sub>2</sub> (five pairs of terrestrial and elevated CO<sub>2</sub> with flooded and elevated CO<sub>2</sub>). We excluded several plants due to aphid infestations that began after the no-choice experiment was completed resulting in the unbalanced design.

We estimated plant size (leaf area, stem width, height, and leaf count) and assigned plants of similar sizes to pairs. One mating pair of recently emerged *A. hygrophila* adults was added on 13 May and again on 14 May. These adults were offspring from a greenhouse colony collected for the no-choice experiment. On 18 May, we counted eggs laid on each of the plants. We collected damaged leaves, scanned and analyzed them for percent of damage (using Image J), then dried and weighed them. We clipped plants at the soil surface and dried and weighed undamaged leaves and stems separately.

We analyzed the dependence of plant aboveground mass and plant leaf area on CO<sub>2</sub>, flooding, and CO<sub>2</sub> × flooding. We analyzed the dependence of leaf mass eaten, leaf area eaten, and number of eggs on plants on flooding, CO<sub>2</sub>, and sub-experiment nested in (CO<sub>2</sub> × flooding) as fixed effects and chamber as a random effect. This tested for effects of CO<sub>2</sub> and flooding treatments across the experiment as well as for non-additive effects of pairing in sub-experiments while controlling for the non-independence of plants being in pairs. We used adjusted means partial difference tests for means within a sub-experiment to test for differences in preference when plants were in the same chamber. We performed an additional analysis to examine the dependence of the odds of a leaf being damaged vs. undamaged using a binomial model (Proc Glimmix).

### Population dynamics experiment

After the choice experiment, we thoroughly cleaned chambers. Then we planted beetle-free, field-collected *A. philoxeroides* (Pasadena, TX) in 48 pots filled with commercial potting soil. We assigned each pot to a flooding and CO<sub>2</sub> treatment in a factorial design. After plants had grown for 2 months, we collected egg masses of *A. hygrophila* from Pasadena, TX on 5 June 2015. We removed leaves with the attached unhatched eggs and brought them to the greenhouse. We clipped one leaf to the single plant in each chamber using plastic-coated office paperclips. If no larvae were

observed after 72 h, we added an additional leaf with eggs to the chamber. If no larvae were observed after an additional 72 h, we added another leaf. At this point, all chambers had larvae present. We estimated plant size when beetles were added: leaf area, stem width, stem height, and leaf count. The experiment ran from June to 12 August at Rice University with at least two generations in each chamber.

We counted adults, larvae, and eggs and estimated damage to plants weekly until 12 August. Then, we counted leaves, measured stem diameter at ground level, harvested aboveground biomass, washed roots from soil, dried plant shoots and roots, and weighed them.

We analyzed the dependence of plant attributes and the abundance of beetles (each life stage plus total) on CO<sub>2</sub>, flooding, and their interactions in ANOVAs.

### Field experiment

After the population dynamics experiment, we again thoroughly cleaned chambers. Then on 24 March 2017, we planted beetle-free, field-collected *A. philoxeroides* (Pasadena, TX) in 100 pots filled with commercial potting soil (3 terrestrial and 2 flooded in each of 20 chambers). We assigned each pot to a flooding and CO<sub>2</sub> treatment in a factorial design. After plants had grown for 6 weeks (4 May), we selected 8 plants from each treatment and transported them to the field (Armand Bayou, Pasadena, TX). We anchored them with stake flags in the edge of a patch of *A. philoxeroides* with moderate damage from *A. hygrophila* (<20% of leaf area removed). We placed plants between 0.5 and 1 m apart. The water level in this section of Armand Bayou varies with Galveston Bay tides and prevailing wind direction (Wundrow et al. 2012). At the time we placed plants in the field, water levels were low so plants were set on exposed mud. When we retrieved those 4 days later, the water level was above the pot rims. We selected eight plants from each treatment on 9 May, transported them to the field, and anchored them with stake flags in the edge of a patch of *A. philoxeroides* with high levels of damage from *A. hygrophila* (>50% of leaf area removed). We retrieved plants 7 days later. We anchored plants with the pot rims slightly above the water level and water levels varied 10 cm higher and lower than this while plants were in the field based on a nearby river gauge (<https://www.harriscountyfws.org/GageDetail/Index/210>).

When we retrieved plants, we scanned leaves that had beetle damage and estimated the proportion of area remaining using Image-J. We dried and weighed damaged and undamaged leaves separately. We estimated the mass of leaves lost to herbivory as (no. missing leaves × average mass of undamaged leaves + [(1/proportion remaining)−1] × mass of damaged leaves). We dried and weighed stems and roots. We analyzed the dependence of number

of leaves, estimated leaf mass lost, stem mass, and root mass on CO<sub>2</sub> (with chamber nested in CO<sub>2</sub> as a random variable), flooding, field trial, and their interactions in an ANOVA.

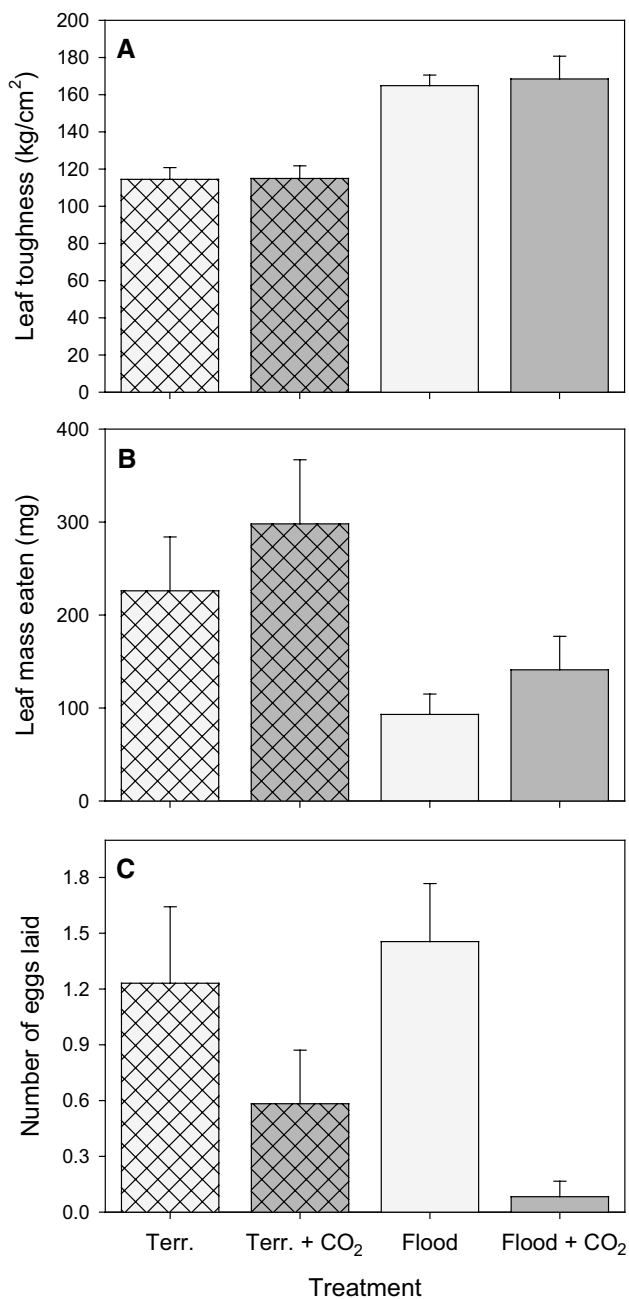
## Results

### No-choice experiment

Leaves from flooded plants were tougher (higher penetration resistance) than those from terrestrial plants (Fig. 1a), but toughness was independent of CO<sub>2</sub> treatment and the interaction of flooding and CO<sub>2</sub> (Table 1). Beetles consumed more area (terrestrial:  $7.50 \pm 0.68 \text{ cm}^2$ ; flooded:  $4.07 \pm 0.43 \text{ cm}^2$ ) and mass (Fig. 1b) of leaves from terrestrial plants than flooded plants, but CO<sub>2</sub> treatment did not affect beetle consumption (Table 1). Beetles laid more eggs in dishes containing ambient CO<sub>2</sub> leaves than in those containing elevated CO<sub>2</sub> leaves (Table 1; Fig. 1c). Beetles that laid eggs in dishes with leaves grown in elevated CO<sub>2</sub> laid all of their eggs on the coffee filter or dish itself (Fig. 1c); none laid eggs directly on the leaf. The number of eggs laid was independent of flooding treatment and the interaction of flooding and CO<sub>2</sub> (Table 1).

### Choice experiment

Flooded plants were smaller ( $4.80 \pm 0.66 \text{ g}$  mass;  $6.94 \pm 1.31 \text{ cm}^2$  area) than terrestrial plants ( $10.70 \pm 1.31 \text{ g}$ ;  $22.77 \pm 2.51 \text{ cm}^2$ ), but CO<sub>2</sub> treatment did not affect plant growth (Table 2). The pairing of plants into sub-experiments had no non-additive effects on insects (i.e., effects of CO<sub>2</sub> and flooding were additive; Table 2). Beetles consumed more leaf mass (Fig. 2a) and area (Fig. 2b) of terrestrial plants, laid more eggs on terrestrial plants (Fig. 2c), and more likely to damage leaves of terrestrial plants (Fig. 2d) than flooded plants (Table 2). When beetles were offered a flooded plant and a terrestrial plant in the same chamber, their preferences for terrestrial plants were significant when both plants were grown in ambient CO<sub>2</sub> (Fig. 2). Only leaf mass consumed was significantly higher for terrestrial vs. flooded plants when plants were grown in elevated CO<sub>2</sub> (Fig. 2). The mass and area of leaves eaten and egg clutches laid did not vary between ambient and elevated CO<sub>2</sub> plants, even when they were in the same chamber (Table 2; Fig. 2). However, the likelihood of a leaf being damaged was higher for plants grown in ambient CO<sub>2</sub> than for plants grown in elevated CO<sub>2</sub> (Table 2) but when plants were offered in the same chamber, this result was only significant when both were grown in flooded soils (Fig. 2d).



**Fig. 1** The dependence of **a** leaf toughness, **b** leaf mass eaten, and **c** number of eggs laid on CO<sub>2</sub> and flooding treatment in the no-choice experiment. Means + 1 SE. Light grey indicates ambient CO<sub>2</sub>, dark grey indicates elevated CO<sub>2</sub>, cross hatching indicates terrestrial, and open shading indicates flooded

### Population dynamics experiment

Of the plant parameters measured, only root mass and root-to-shoot ratio varied significantly between terrestrial and aquatic plants (Table 3). Terrestrial plants had more root mass and higher root-to-shoot ratio (Fig. 3a). Although the abundance of individual life stages did not vary with

treatments, there were significantly more beetles in total across all observed life stages present on terrestrial plants than on flooded plants (Table 3; Fig. 3b).

### Field experiment

Estimated leaf mass lost was higher in the second trial ( $0.213 \pm 0.021$  g) than the first trial ( $0.032 \pm 0.028$  g;  $F_{1,35} = 29.42$ ,  $P < 0.0001$ ) and it depended on CO<sub>2</sub> × flooded ( $F_{1,35} = 5.18$ ,  $P = 0.0291$ ) with terrestrial plants grown in ambient CO<sub>2</sub> losing more leaf mass than flooded plants grown in ambient CO<sub>2</sub> (Fig. 4). It was independent of other factors (CO<sub>2</sub>  $F_{1,18} = 0.07$ ,  $P = 0.7917$ ; flooded  $F_{1,35} = 2.20$ ,  $P = 0.1472$ ; field × CO<sub>2</sub>  $F_{1,35} = 2.82$ ,  $P = 0.1022$ ; field × flooded  $F_{1,35} = 0.01$ ,  $P = 0.9433$ ; field × CO<sub>2</sub> × flooded  $F_{1,35} = 0.07$ ,  $P = 0.7926$ ). Plants grown in terrestrial conditions (leaf no.:  $78.63 \pm 3.83$ ; stem mass:  $5.58 \pm 0.34$  g; root mass:  $1.91 \pm 0.14$  g) were larger than those grown in flooded conditions (leaf no.:  $78.63 \pm 3.83$ ,  $P = 0.0121$ ; stem mass:  $5.58 \pm 0.34$  g,  $P < 0.0001$ ; root mass:  $1.91 \pm 0.14$  g,  $P = 0.0399$ ) but plant size did not vary with CO<sub>2</sub> ( $P = 0.7100$ ,  $0.6963$ ,  $0.5962$ ) or the interaction of CO<sub>2</sub> and flooding treatment ( $P = 0.7132$ ,  $0.7387$ ,  $0.5975$ ).

### Discussion

Elevated CO<sub>2</sub> has the potential to change plant traits and the interactions of invasive plants and biocontrol agents (Reeves et al. 2015), but here we only observed relatively minor effects of CO<sub>2</sub> concentration on *A. philoxeroides* and its interaction with *A. hygrophila*. We did, however, observe dramatic differences between terrestrial and flooded plants and their interactions with the biocontrol beetle *A. hygrophila*. Specifically, we found that leaves of plants grown in the flooding treatment were physically tougher than leaves from terrestrially grown plants and that beetles ate more leaf mass from terrestrial plants.

We did not observe any differences in the growth of plants exposed to elevated CO<sub>2</sub>. This suggests that there were other limiting factors that prevented CO<sub>2</sub> treated plants from experiencing greater growth. For instance, nitrogen availability can impact the magnitude of elevated CO<sub>2</sub> effects on growth with little to no response when soil resources are limited (de Graaff et al. 2006; Terrer et al. 2018). However, the population dynamics and field experiments were conducted using commercial soil mix, so strong limitation by nitrogen or other soil nutrients seems unlikely. In the first experiment (plants for the no-choice and choice experiments), plant growth was slow in all conditions, perhaps due to the field soil used. Water was certainly not a limiting factor, since the terrestrial plants were regularly watered and the flooded plants were kept partially submerged. Nutrients can

**Table 1** The dependence of leaf attributes, beetle consumption, and beetle reproduction in the no-choice experiment on CO<sub>2</sub>, flooding, and their interaction in ANOVAs

Response variable	CO <sub>2</sub>		Flooded		CO <sub>2</sub> × flooded	
	F <sub>1,44</sub>	P	F <sub>1,44</sub>	P	F <sub>1,44</sub>	P
Leaf attributes						
LAR	0.19	0.6691	2.12	0.1529	0.16	0.6925
Toughness	0.06	0.8035	<b>41.92</b>	<b>&lt; 0.0001</b>	0.04	0.8490
Beetle consumption						
Area consumed	0.05	0.8251	<b>17.26</b>	<b>&lt; 0.0001</b>	1.48	0.2308
Mass consumed	0.23	0.6337	<b>15.41</b>	<b>0.0003</b>	0.54	0.4682
Beetle reproduction						
Total eggs	<b>10.97</b>	<b>0.0019</b>	0.21	0.6526	1.41	0.2414
Eggs on leaf	<b>9.57</b>	<b>0.0034</b>	0.63	0.4307	0.63	0.4307

Significant results shown in bold

**Table 2** The dependence of plant mass, beetle consumption, and beetle reproduction in the choice experiment on CO<sub>2</sub>, flooding, and chamber sub-experiment in ANOVAs

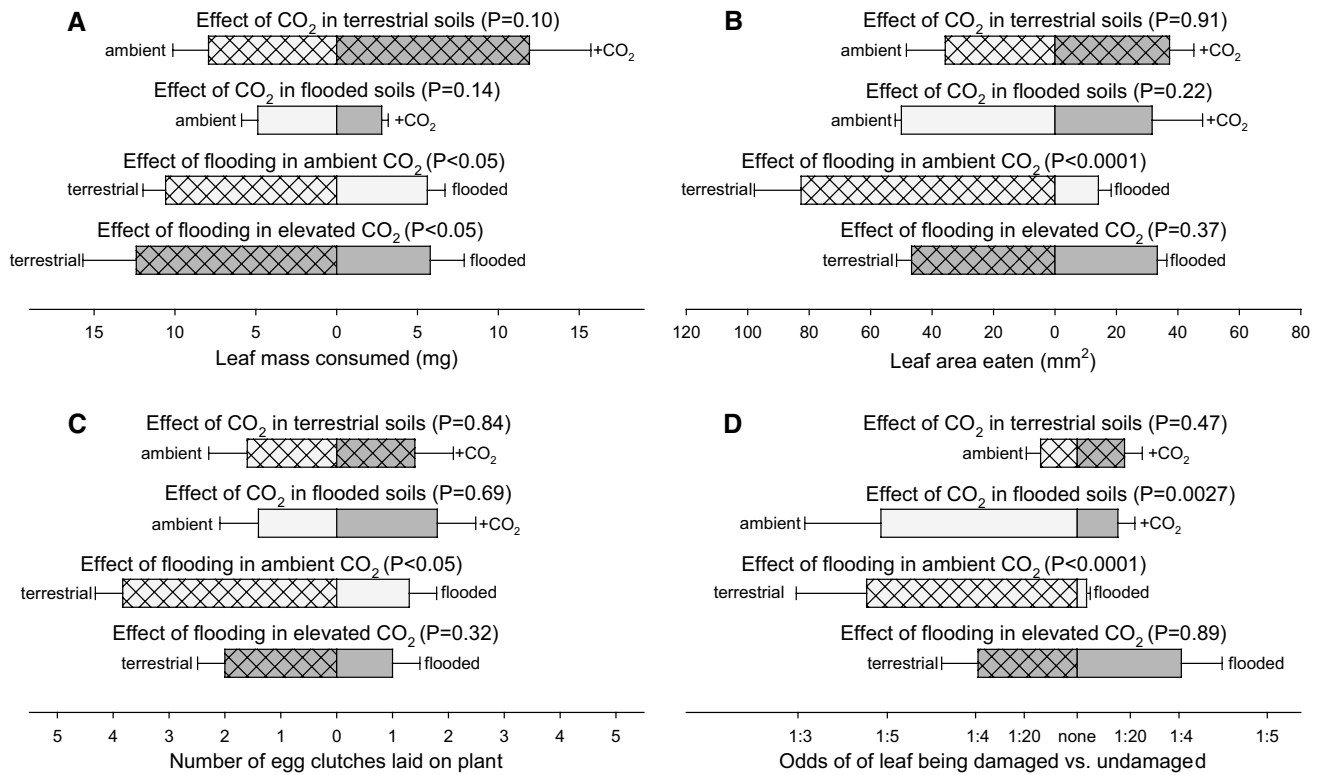
Plant variables	CO <sub>2</sub>		Flooded		CO <sub>2</sub> × flooded	
	F <sub>1,38</sub>	P	F <sub>1,38</sub>	P	F <sub>1,38</sub>	P
Leaf mass	0.37	0.5443	<b>16.56</b>	<b>0.0002</b>	1.64	0.2079
Leaf area	0.34	0.5644	<b>31.66</b>	<b>&lt; 0.0001</b>	0.40	0.5321
Beetle variables	CO <sub>2</sub>		Flooded		Sub-experiment	
	F <sub>2,17</sub>	P	F <sub>2,17</sub>	P	F <sub>3,17</sub>	P
Leaf mass eaten (g)	0.72	0.5537	<b>15.86</b>	<b>&lt; 0.0001</b>	0.72	0.5537
Leaf area eaten (cm <sup>2</sup> )	0.80	0.4657	<b>13.67</b>	<b>0.0003</b>	0.53	0.6676
Eggs laid on plants (no.)	0.10	0.9027	<b>4.38</b>	<b>0.0293</b>	1.31	0.3048
Damaged vs. total leaves	<b>6.41</b>	<b>0.0084</b>	<b>35.35</b>	<b>&lt; 0.0001</b>	1.09	0.3800

Significant results shown in bold

be more limiting in anaerobic soils but this would likely lead to a flooding × CO<sub>2</sub> effect on plant growth, which we did not observe. A previous study with *A. philoxeroides* cuttings grown in clear bags with sand, water, and nutrients did find a growth response to CO<sub>2</sub> but with higher CO<sub>2</sub> concentrations (1000–3000 ppm) and extreme nutrient loads (Xu et al. 2009). Light limitation is another possibility, as the light energy amounts plants received here were two-thirds those in Xu et al. (2009), even though the plants here were grown in clear plastic-topped, nylon mesh cages and received approximately 35% of sunlight. While our results showing no effect of CO<sub>2</sub> for this C<sub>3</sub> plant were not what we had predicted, other studies have found elevated CO<sub>2</sub> did not increase growth of C<sub>3</sub> plants (Zavala et al. 2013).

Beetles in the no-choice experiment laid more eggs when they were with a leaf from plants grown at ambient CO<sub>2</sub> levels and they laid eggs only on the petri dish, but not on the leaf, when they were with a leaf from an elevated CO<sub>2</sub> plant. It is unlikely that there was a strong role for direct chemical defenses in the leaves because beetle leaf consumption and beetle survival did not depend on plant CO<sub>2</sub> treatment.

In addition, leaves of plants grown in flooded ambient CO<sub>2</sub> conditions had higher odds of being damaged in the choice experiment than those of plants grown in flooded elevated CO<sub>2</sub> conditions. Although we did not measure volatile organic compounds (VOCs), they could be contributing to oviposition and feeding choices, especially since they appear to be important for *A. hygrophila* host identification (Li et al. 2017). Specifically, they found that higher ratios of (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) to (Z)-3-hexenol encouraged oviposition and low ratios discouraged oviposition on *A. philoxeroides*. More generally, volatile emissions can inhibit host choice as well as encourage it (Hammack 1996; Khelfane-Goucem et al. 2014), and these effects can be influenced by environmental conditions (Boullis et al. 2015; Block et al. 2017). However, effects of elevated CO<sub>2</sub> on VOC emissions are poorly understood and have not been widely studied (DeLucia et al. 2012; Facey et al. 2014). Emissions of different terpene compounds appear to react differently to increased CO<sub>2</sub> (Yuan et al. 2009). For example, the monoterpenes pinene and limonene were inhibited and enhanced in evergreen oaks (*Quercus ilex*), respectively



**Fig. 2** The dependence of **a** leaf mass consumed, **b** percent of leaves eaten, **c** egg clutches laid on plant, and **d** odds of a leaf being damaged in the choice experiment. Means+SE. Each horizontal bar indicates the results within a sub-

experiment in which plants were paired in chambers. *P* values indicate differences on plants within a chamber. Light grey indicates ambient CO<sub>2</sub>, dark grey indicates elevated CO<sub>2</sub>, cross hatching indicates terrestrial, and open shading indicates flooded

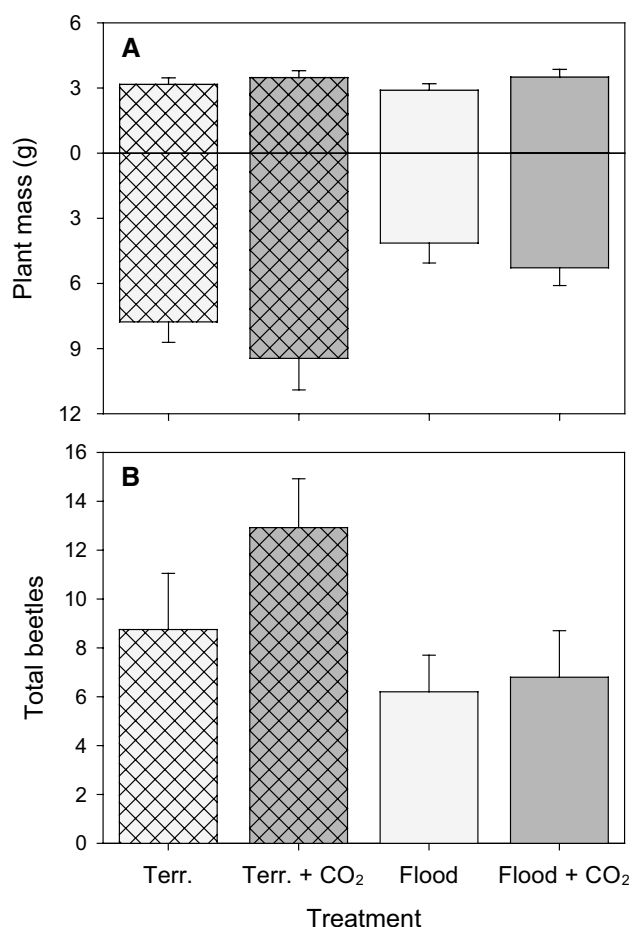
**Table 3** The dependence of plant attributes and beetle population parameters in the population dynamics experiment on CO<sub>2</sub>, flooding, and their interaction in ANOVAs

Response variable	CO <sub>2</sub>		Flooded		CO <sub>2</sub> ×flooded	
	<i>F</i> <sub>1,44</sub>	<i>P</i>	<i>F</i> <sub>1,44</sub>	<i>P</i>	<i>F</i> <sub>1,44</sub>	<i>P</i>
<b>Plant attributes</b>						
Stem diameter	0.81	0.3729	0.10	0.7530	0.05	0.8162
Shoot mass	2.06	0.1578	0.07	0.7920	0.22	0.6389
Root mass	1.78	0.1891	<b>13.53</b>	<b>0.0006</b>	0.07	0.7997
Mass	2.22	0.1435	2.08	0.1563	0.06	0.8009
Root to shoot	0.81	0.3731	<b>24.96</b>	<b>&lt;0.0001</b>	0.30	0.5848
<b>Beetle populations</b>						
Eggs	0.16	0.6867	1.91	0.1745	0.03	0.8717
Larvae	1.44	0.2371	2.97	0.0919	0.20	0.6553
Adults	0.54	0.4680	1.49	0.2290	1.74	0.1945
Total	1.45	0.2351	<b>4.92</b>	<b>0.0318</b>	0.82	0.3687

Significant results shown in bold

(Yuan et al. 2009). Terpene profiles play an important role in host identification by other chrysomelids (Fernandez et al. 2007; Wolf et al. 2012). Other studies have found dramatic (2- and 38-fold) increases in production of certain VOCs, such as terpenoids and ketones, by various plant species in response to elevated CO<sub>2</sub> (Jasoni et al. 2004; Himanen et al.

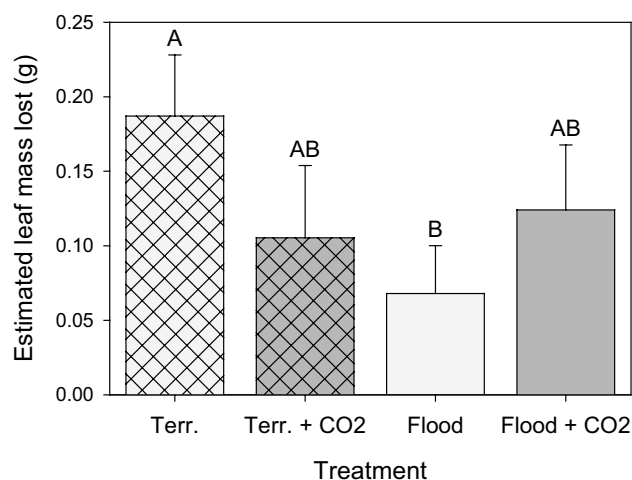
2009), while another study found reduced herbivore induced volatiles for *Zea mays* with elevated CO<sub>2</sub> (Block et al. 2017). Terpenoids, which saw a twofold increase in *Brassica napus* grown in elevated CO<sub>2</sub>, are known to occur in the Amaranthaceae (Himanen et al. 2009; Mroczek 2015). Such changes in VOCs with elevated CO<sub>2</sub> affect host finding by insects and



**Fig. 3** The dependence of **a** plant mass (above and belowground mass) and **b** total beetles in a chamber (summed across life stages) on CO<sub>2</sub> and flooding treatment in the population dynamics experiment. Means + 1 SE. Light grey indicates ambient CO<sub>2</sub>, dark grey indicates elevated CO<sub>2</sub>, cross hatching indicates terrestrial, and open shading indicates flooded

CO<sub>2</sub> levels may also affect insect responses to plant signals (Yuan et al. 2009) but beetles were never exposed to elevated CO<sub>2</sub> here. Underlying reasons for oviposition and feeding choices of the beetle still need to be clarified.

The strong effects of plant growth environment (terrestrial or flooded) on beetle feeding and reproduction together with *A. philoxeroides* occurring commonly only in flooded habitats in North America imply there could be differences in efficacy of biocontrol among environments across the weed's introduced ranges (Lu et al. 2015). A field experiment in China showed that *A. hygrophila* had high populations and reduced *A. philoxeroides* mass in well-watered compared to flooded conditions but *A. philoxeroides* performance was still higher in well-watered conditions with beetles than in flooded conditions without beetles (Wei et al. 2015) but no similar field experiment has been carried out in other introduced ranges. However, the introduction to China



**Fig. 4** The dependence of estimated plant leaf mass lost on CO<sub>2</sub> and flooding treatment in the field experiment. Means + 1 SE. Light grey indicates ambient CO<sub>2</sub>, dark grey indicates elevated CO<sub>2</sub>, cross hatching indicates terrestrial, and open shading indicates flooded

was deliberate (for livestock forage) versus accidental in the USA (ballast dumping) (Pan et al. 2006) and there is lower diversity of both *A. philoxeroides* (Wang et al. 2005) and *A. hygrophila* (Ma et al. 2013) in China compared to North America. So, plant habitat distributions and biocontrol efficacy may vary among introduced ranges in response to phenotypic variation of *A. philoxeroides* as well as differences in the genetic diversity of both the weed and the beetle.

There are limits to the application of our results to interactions between invasive weeds and biocontrol insects in general. For instance, C<sub>4</sub> plants are less sensitive to effects of elevated CO<sub>2</sub> (Zavala et al. 2013) which may limit effects on oviposition in such systems. Our study was largely conducted in a controlled environment with chambers in a greenhouse where population dynamics are limited. In natural field settings, *A. philoxeroides* is subject to competition from other plants, and *A. hygrophila* must contend with predators but our field trials were short to limit acclimation of greenhouse grown plants. In addition, all of our specimens were taken from a single source population in Texas, so genetic differences in *A. philoxeroides* and *A. hygrophila* across ranges may affect the extent to which our results can be generalized.

There were numerous strong effects of flooding on plants and beetles consistent with biocontrol herbivores facing challenges to nutrition, reproduction, pupation, or overwintering based on the environment in which the target weed grows. Although effects of elevated CO<sub>2</sub> were comparatively weaker compared to the strong effects of hydrologic conditions, the effects of increasing ambient CO<sub>2</sub> levels on arthropod-plant interactions should be taken into account when assessing the efficacy of weed biocontrol (Reeves



et al. 2015). This adds to other effects of global change on plant-arthropod interactions, such as changes in range due to warming (Lu et al. 2013), shifting the phenologies of plants and insects (Facey et al. 2014), and disruption of plant signaling (Yuan et al. 2009). As atmospheric CO<sub>2</sub> concentrations rise and climate changes, these and other changes affecting the biosphere will impact invasive species management programs and determine how biocontrol efficacy responds to global change.

**Acknowledgements** We would like to thank Stephen Truch, Kate Snyder, and Hong Wang for their help in field and lab work and financial support from NSF-China (NSF-C 31370547 & 31570540) and a Spurlino Fellowship.

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