Invasive forb benefits from water savings by native plants and carbon fertilization under elevated CO2 and warming

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Summary

• As global changes reorganize plant communities, invasive plants may benefit. We hypothesized that elevated CO2 and warming would strongly influence invasive species success in a semi-arid grassland, as a result of both direct and water-mediated indirect effects.

• To test this hypothesis, we transplanted the invasive forb Linaria dalmatica into mixed-grass prairie treated with free-air CO2 enrichment and infrared warming, and followed survival, growth, and reproduction over 4 yr. We also measured leaf gas exchange and carbon isotopic composition in L. dalmatica and the dominant native C3 grass Pascopyrum smithii.

• CO2 enrichment increased L. dalmatica biomass 13-fold, seed production 32-fold, and clonal expansion seven-fold, while warming had little effect on L. dalmatica biomass or reproduction. Elevated CO2 decreased stomatal conductance in P. smithii, contributing to higher soil water, but not in L. dalmatica. Elevated CO2 also strongly increased L. dalmatica photosynthesis (87% versus 23% in P. smithii), as a result of both enhanced carbon supply and increased soil water.

• More broadly, rapid growth and less conservative water use may allow invasive species to take advantage of both carbon fertilization and water savings under elevated CO2. Water-limited ecosystems may therefore be particularly vulnerable to invasion as CO2 increases.

Introduction

Global change and biological invasion, which individually threaten biodiversity and economic productivity, are also likely to interact (Dukes & Mooney, 1999; Richardson et al., 2000; Vila et al., 2007). While these interactions will vary widely with the type of global change, invasive species, and ecosystem, global change may more often promote than inhibit plant invasion. Invasive plants have successfully colonized novel environments, and may therefore have traits that confer success in novel environments created by global change (Dukes & Mooney, 1999). Invasive plants also tend to respond more positively than non-invasive plants to changes that increase resource availability, such as elevated atmospheric CO2, increased nitrogen (N) deposition, and disturbances associated with land-use change (Davis et al., 2000; Daehler, 2003; Blumenthal et al., 2009; Bradley et al., 2010; Gonzalez et al., 2010; van Kleunen et al., 2010).

Among global changes, increases in atmospheric CO2 concentration and climate warming are the most global and predictable (Solomon et al., 2007). Although both elevated CO2 and warming can influence plant invasion, it remains difficult to predict the direction and magnitude of their effects, particularly their combined effects (Walther et al., 2009; Bradley et al., 2010; Sorte et al., 2013). Elevated CO2 commonly increases photosynthesis and growth of invasive plants (Ziska, 2003; Song et al., 2009; Manea & Leishman, 2011). Whether or not elevated CO2 favors invasive species over native species is less clear. Meta-analyses have found either similar CO2 responses in invasive and noninvasive species (Dukes, 2000) or trends toward stronger CO2 responses in invasive than co-occurring native species (Sorte et al., 2013). Of the few field studies conducted under competitive conditions, several (Smith et al., 2000; Härtenschwiler & Körner, 2003; Dukes et al., 2011), but not all (Williams et al., 2007), have found elevated CO2 to favor invasive over native species. For example, in the Mojave Desert, elevated CO2 more than doubled above-ground biomass and tripled seed production of the invasive grass Bromus madritensis, but had only minor effects on native annuals (Smith et al., 2000). It is not clear why some invasive species are so responsive to elevated CO2. Several potential mechanisms involve intrinsically high growth rates, which may allow invasive species to maintain strong carbon (C) sinks, high photosynthetic rates, and/or low construction costs under elevated CO2 (Nagel et al., 2004; Song et al., 2009, 2010; Körner, 2011; Manea & Leishman, 2011). Fast-growing species tend to respond strongly to elevated CO2, but this advantage is less apparent in
By contrast, warming had little effect on the annual forb mixed-grass prairie? Are elevated CO2 and warming effects on ssp. dry conditions (Williams et al., 1999). Elevated CO2 allows plants to decrease stomatal conductance while maintaining photosynthetic rates, thereby increasing water use efficiency (WUE) and conserving soil water (Volk et al., 2000; Niklaus & Körner, 2004; Ainsworth & Long, 2005; Morgan et al., 2011). Furthermore, these CO2 responses have been observed to differ between native and invasive species, with invasive species having relatively small decreases in stomatal conductance, and large increases in photosynthesis (Huxman & Smith, 2001; Song et al., 2009). The consequences of such distinct CO2 responses for invasive species success in semi-arid ecosystems, however, have not been tested. We hypothesize that elevated CO2 will strongly promote invasive species when water limits plant growth, by increasing the availability of both C and water. By contrast, warming reduces water availability by increasing evapotranspiration, and may inhibit invasive species under dry conditions (Williams et al., 2007; Bradley et al., 2010). This hypothesis also remains to be tested.

We used a free-air CO2 enrichment and infrared warming experiment to address the following questions: How do elevated CO2 and warming, alone and in combination, influence survival, growth and reproduction of the invasive perennial forb Linaria dalmatica ssp. dalmatica (Dalmatian toadflax) in semi-arid mixed-grass prairie? Are elevated CO2 and warming effects on L. dalmatica explained by different physiological responses of L. dalmatica and the dominant, native C3 grass Paspopyrum smithii (western wheatgrass) to direct or water-mediated, indirect effects of CO2 and warming?

Materials and Methods

The study was conducted within the Prairie Heating and CO2 Enrichment (PHACE) experiment, located in SE Wyoming (latitude 41°11'N, longitude 104°54'W) on undisturbed, native mixed-grass prairie (Morgan et al., 2011). Mean annual rainfall was 384 mm (Supporting Information Fig. S1). Mean July and January temperatures were 17.5 and −2.5°C, respectively. Plant growth and biomass production at this site are strongly limited by water (Derner & Hart, 2007; Chimner et al., 2010). The prairie was dominated by C3 grasses (particularly P. smithii (Ryd.) A. Love), which comprised 61.5% of above-ground plant biomass, and also included native C4 grasses, forbs and shrubs. Linaria dalmatica (L.) Mill. is an invasive forb, native to Eurasia, that is problematic throughout much of western North America, and is the most common invasive species in this part of the mixed-grass prairie (Vujnovic & Wein, 1997; Blumenthal et al., 2012). It is a C3 perennial that reproduces with both seeds and underground roots. It has high rates of both photosynthesis and growth relative to co-occurring native species (James & Drenovsky, 2007). As a seedling, L. dalmatica is a poor competitor, and experiences high mortality (70–100%) in competition with established perennials in general and mixed-grass prairie in particular (Robocker, 1970; Blumenthal et al., 2008).

The experiment contained five replications of four treatments: (1) control (ct); (2) free-air CO2 enrichment (FACE) to 600 ppmv (Ct); (3) infrared heating to increase canopy temperature by 1.5°C during the day and by 3°C at night (cT); and (4) CO2 enrichment plus heating (CT). The 20 plots were randomized within two soil-type blocks: Ascalon Variant Loam (fine-loamy, mixed-mesic), and Alvenan Loam (fine-loamy over sandy, mixed-mesic). Each circular 7-m² plot was hydrologically isolated from surrounding prairie by a 60-cm-deep plastic barrier. Dummy FACE rings and heaters around untreated plots controlled for potential infrastructure effects. Achieved treatment levels were 600.5 ppmv CO2 ± 50.4 (SD; monitored at 1-min intervals over a 40-d period) and +1.6 ± 0.3°C (SD) during the day and +3.0 ± 0.3°C (SD) at night (monitored at 1-h intervals over a 6-month period). Detailed information regarding the experimental site and performance of the treatments is provided in Morgan et al. (2011).

To create identical initial populations of L. dalmatica in each experimental ring, we transplanted 59 L. dalmatica seedlings into a 2.9-m² semicircular subplot within each plot (separated from the rest of the plot by a 30-cm-deep, below-ground metal flange). Although L. dalmatica regularly establishes from seed at this site, variability associated with seedling germination and survival makes these processes difficult to study in a CO2-by-warming experiment with limited replication. The use of transplants enabled us to control initial population size and therefore examine survival, growth, and reproduction with greater precision, but also limited inferences to post-recruitment phases of the L. dalmatica life history.

To minimize experimental artifacts associated with transplanting, we grew seedlings in Cone-tainers with removable inserts (164-ml; Stuewe & Sons, Tangent, OR, USA) filled with topsoil...
from the PHACE site, inserted root systems intact into precisely shaped holes, and removed Cone-tainer inserts after seedlings were in the ground. Seedlings were grown in the glasshouse for 3.5 months, until roots reached the bottom of the Cone-tainers, and hardened outside for 3 wk before transplanting. They were then planted in a 20-cm grid with permanently marked planting locations on 29–30 May 2006. To facilitate transplant survival during the dry summer following transplanting, whole rings were irrigated with 20 mm of water on eight dates: 31 May, 8 June, 13 June, 20 June, 27 June, 14 July, 20 July, and 4 August. Irrigation events occurred over 1–3 d to prevent runoff. After two growing seasons, in October 2007, two-thirds of each plot was removed to make way for other experiments; the remaining 0.97-m² plots were followed through July 2010.

We measured survival and height of each *L. dalmatica* plant approximately monthly during the growing season (May through October) from May 2007 to July 2010. Leaf gas exchange rates of *L. dalmatica* and *P. smithii* were measured on six dates in 2007, when surviving *L. dalmatica* plants were available in multiple replications for all treatments. *Pascopyrum smithii* measurements were conducted in the main plots, and reflect its presence in the absence of *L. dalmatica*. Measurements were made with a LI-6400 photosynthesis system (Li-Cor, Lincoln, NE, USA). Responses of photosynthesis (A) to increasing intercellular CO₂ concentrations (Ci) were measured at saturating light (1500 µmol m⁻² s⁻¹), constant temperature (25°C) and the lowest vapor pressure deficit achievable in the field (1–3 kPa). Step changes in CO₂ were implemented following Long & Bernacchi (2003). Reported photosynthesis and stomatal conductance (g) are the initial point in A/Ci curves, always measured at growth CO₂. The maximum rate of carboxylation by Rubisco (Vcmax) was calculated following Sharkey et al. (2007). WUE was obtained from both leaf gas exchange measurements (instantaneous A/g) and leaf ¹³C isotope discrimination (Δ¹³C), which integrates water use efficiency over the life span of the sampled leaves (integrated A/g). Leaves measured for gas exchange in July 2007 were collected and analyzed for bulk tissue Δ¹³C in a Finnigan Delta + XP continuous flow inlet isotope ratio mass spectrometer (Thermo Finnigan, Bremen, Germany) at the University of Wyoming Stable Isotope Facility. The precision was <0.1‰, and data were corrected to the Vienna Pee Dee Belemnite standard. Seed pods were collected July–November 2009, and all stems were counted, harvested, dried and weighed on 19–20 July 2010.

Soil water content was measured continuously (at 60-min intervals) with Sentek Envirosmart frequency domain reflectometry sensors (Sentek Sensor Technologies, Stepney, SA, Australia) placed at 10- and 20-cm depths, yielding 5–25-cm-deep soil volumetric water content. Precipitation was measured daily with an S-RGA-M002 rain gauge (Onset Corp., Pocasset, MA, USA). Measurements were taken adjacent to *L. dalmatica* subplots, within undisturbed mixed-grass prairie, and therefore reflect treatment responses of native species and direct effects of treatments in the absence of *L. dalmatica*.

Data were analyzed with two-way ANOVAs, including block as a random effect and CO₂, temperature, and CO₂ × temperature as fixed effects, in JMP (version 7.0.1; SAS Institute Inc., Cary, NC, USA). Repeated measures analyses for survival, height, and leaf gas exchange measurements also included date and date × treatment interactions within CO₂ × temperature, and were conducted in SAS (version 9.2) Proc Mixed or Proc Glimmix (for survival analyses, using a binomial distribution) (SAS Institute Inc.). Post hoc ANOVAs were used to further investigate significant CO₂ × temperature, CO₂ × date, or temperature × date interactions. Data were log- or square-root-transformed when necessary to meet assumptions of homoscedasticity and normality. Water stress led to some negative values of A on 20 June. These simply reflect measurement error when A was close to zero, and were included in analyses of A. The combination of these values with very low g in a ratio, however, magnified the measurement error, leading to unrealistically large negative A/g values. These points were therefore treated as missing data for analyses of A/g. To test how water availability influenced photosynthesis, stomatal conductance, and the treatment responses of these processes, we used mixed models (SAS 9.2, Proc Mixed) with volumetric soil water content as a continuous variable and repeated measures to account for multiple measurements (across the 2007 growing season) per plot. In these models, which control for effects of soil water, significant main effects would indicate influences of elevated CO₂ and warming on A or g beyond those mediated by soil water; significant interactions between soil water and treatments would indicate that water influences the degree to which elevated CO₂ and warming affect A or g. These models included CO₂, temperature, CO₂ × temperature, and water, with block as a random effect, and omitted nonsignificant interactions between water and CO₂ or temperature.

**Results**

Elevated CO₂ increased per-plot *L. dalmatica* biomass (F1,15 = 22.2; P = 0.0003), seed pod production (F1,15 = 34.4; P < 0.0001) and new shoot production (F1,15 = 19.5; P = 0.0005; Fig. 1, Table S1). By contrast, warming did not influence *L. dalmatica* biomass or reproduction, either alone or in combination with elevated CO₂. To test the influence of post-transplant mortality on *L. dalmatica* growth and reproduction treatment responses, we repeated analyses depicted in Fig. 1 but removed effects of first-year mortality by dividing 2009 reproduction and 2010 biomass values by the number of plants that survived into the second year of the experiment (2007). The right panels of Fig. 1 therefore depict the cumulative amounts of biomass, seed pods and new shoots produced by each plant present at the beginning of the second growing season. Treatment effects reflect differences in both survival (from spring 2007 onwards) and growth (from the start of the experiment). The results were very similar: elevated CO₂ significantly increased above-ground biomass (F1,14 = 7.7; P = 0.01) and seed pod production (F1,13 = 17.7; P = 0.001) and had a marginally significant effect on new shoot production (F1,14 = 4.5; P = 0.05). Warming neither influenced *L. dalmatica* nor interacted with elevated CO₂. This indicates that treatment effects after the plants became established were similar to overall treatment effects, and the
observed patterns were not caused by first-year mortality associated with transplant shock.

Increases in both survival and growth contributed to the success of *L. dalmatica* with elevated CO2 (Fig. 2, Table S2). Elevated CO2 consistently increased *L. dalmatica* percentage survival ($F_{1,15} = 36.9; P < 0.0001; CO2 × date: $F_{3,48} = 47.5; P < 0.0001$; post hoc CO2 (date): $P < 0.001$ at all dates), and these differences accounted for much of the CO2 effect on biomass and reproduction at the end of the experiment. Elevated CO2 also consistently increased per-plant height ($F_{1,14} = 5.4; P = 0.04$). Height, the mean length of the main stem, was closely correlated with biomass ($R^2 = 0.97$; data not shown). Warming reduced survival in plots treated with elevated CO2 (CO2 × temperature: $F_{1,14} = 5.4; P = 0.04$; post hoc temperature within elevated CO2: $F_{1,7} = 8.1; P = 0.02$), but increased height in 2010, following 2 yr with wet springs (temperature × date: $F_{3,22} = 5.8; P = 0.004$; post hoc temperature in 2010: $F_{1,6} = 15.6; P = 0.008$).

Leaf gas exchange responses, measured six times in 2007, revealed both similarities and differences between *L. dalmatica* and *P. smithii* (Fig. 3, Table S3). Elevated CO2 increased photosynthesis in both species (*L. dalmatica*: $F_{1,21} = 27.2; P < 0.0001; P. smithii$: $F_{1,16} = 4.5; P = 0.05$), and this effect was stronger in warmed (post hoc CO2 within elevated temperature: $F_{1,12} = 22.9; P = 0.0004$) than in unwarmed plots (post hoc CO2 within ambient temperature: $F_{1,15} = 9.4; P = 0.008$) for *L. dalmatica* (CO2 × temperature: $F_{1,21} = 8.7; P = 0.008$). Elevated CO2 decreased stomatal conductance only in *P. smithii* ($F_{1,19} = 7.4; P = 0.01$). Nevertheless, elevated CO2 increased instantaneous WUE (A/Δg) for *L. dalmatica* (as a result of large increases in A), as well as *P. smithii* (L. dalmatica: CO2 × date: $F_{3,34.7} = 2.7; P = 0.03$; post hoc CO2: 4 June: $F_{1,8} = 123.8; P < 0.0001$; 7 July: $F_{1,8} = 15.1; P = 0.005$; 30 July: $F_{1,8.4} = 1.03; P = 0.01$; 29 August: $F_{1,5,1} = 13.6; P = 0.01$; *P. smithii*: $F_{1,28} = 41.2; P < 0.0001$). Photosynthetic capacity ($V_{max}$) decreased with elevated CO2 in both species (*L. dalmatica*: $F_{1,28} = 4.4; P = 0.04$; *P. smithii*: $F_{1,21} = 21.2; P = 0.0002$). In *P. smithii*, warming further reduced stomatal conductance over CO2 alone on one date (temperature × date: $F_{5,40} = 2.7; P = 0.04$; post hoc temperature on 25 May: $F_{1,7} = 10.0; P = 0.02$) and further increased instantaneous WUE ($F_{1,28} = 9.8; P = 0.004$). In *L. dalmatica*, warming reduced stomatal conductance in the absence of elevated CO2 (CO2 × temperature: $F_{1,12} = 8.7; P = 0.01$; post hoc temperature within ambient CO2: $F_{1,10} = 14.57; P = 0.004$), but had no effect on stomatal conductance with elevated CO2. Although transplanting could have influenced physiological responses of *L. dalmatica*, by altering water stress or N availability, this seems unlikely given that water
limitation of photosynthesis and stomatal conductance was similar in the two species, and soil N availability was not positively related to *L. dalmatica* photosynthesis (Notes S2, Fig. S2).

Unlike instantaneous $A/g$, integrated $A/g$ differed between the two species (Fig. 4, Table S4). Elevated CO$_2$ led to more discrimination against $\Delta^{13}C$ (lower integrated $A/g$ in *L. dalmatica* ($F_{1,6} = 9.1; P = 0.02$), but less discrimination (higher integrated $A/g$) in *P. smithii* ($F_{1,15} = 5.4; P = 0.03$). Warming did not significantly influence integrated $A/g$ in either species.

Over the course of the experiment, volumetric soil water content consistently increased with elevated CO$_2$ and decreased with warming (Notes S1, Fig. S1; Dijkstra *et al.*, 2010; Morgan *et al.*, 2011). Including soil water content in analyses of leaf gas exchange to separate direct from water-mediated treatment effects accentuated differences between *L. dalmatica* and *P. smithii* (Fig. 5, Table S5). In *L. dalmatica*, photosynthesis increased with soil water ($F_{1,61} = 22.5; P < 0.0001$), and was greater at a given soil water content with elevated CO$_2$ than without ($F_{1,25} = 11.7; P = 0.002$). The effect of elevated CO$_2$ on *L. dalmatica* stomatal conductance varied with temperature ($CO_2 \times$ temperature: $F_{1,24} = 8.0; P = 0.01$), but post hoc tests of CO$_2$ effects within warmed and nonwarmed plots were not significant, despite a significant main effect of elevated CO$_2$ ($F_{1,56} = 28.3; P < 0.0001$).
In *P. smithii*, photosynthesis increased with soil water, reaching its maximum at intermediate soil water content ($F_{1,54.8} = 11.36; P = 0.001$), but did not increase with elevated CO2 at a given soil water content. *Pascopyrum smithii* stomatal conductance increased with soil water ($F_{1,56.4} = 32.4; P < 0.0001$), and was lower at a given soil water content with elevated CO2 than without ($F_{1,23} = 11.1; P = 0.003$).

**Discussion**

We hypothesized that elevated CO2 would strongly promote and warming would inhibit *L. dalmatica* in this semi-arid grassland, as a result of water-mediated as well as direct effects of these treatments. Over 4 yr, elevated CO2 dramatically increased *L. dalmatica* success, with and without warming, leading to 13-fold higher above-ground biomass, 32-fold higher seed production, and seven-fold higher vegetative reproduction. These CO2 effects occurred within mixed-grass prairie that was undisturbed apart from *L. dalmatica* transplanting, and were much larger than the increase in biomass observed for native species (Morgan et al., 2011). They are also the strongest CO2 effects that have been observed for an invasive species in the field (Smith et al., 2000; Hättenschwiler & Körner, 2003; Belote et al., 2004; Dukes et al., 2011).

In contrast to our hypothesis, warming had no net effect on final *L. dalmatica* biomass or reproduction. Although warming reduced *L. dalmatica* survival (in plots with elevated CO2), this was counterbalanced by the fact that it also increased the growth of surviving plants during wetter periods. Similarly, among previous experimental studies, effects of warming on invasion range from positive (Verlinden & Nijs, 2010), to neutral (Dukes et al., 2011), to negative (Williams et al., 2007) with declining water availability. Warming effects on invasion may therefore vary with precipitation, being more negative in periods and regions where warming-induced desiccation is more important (Bradley et al., 2010; Dukes et al., 2011).

The strong effects of CO2 relative to warming in this study are consistent with results from a California annual grassland (Dukes et al., 2011), but contrast with results from a New Zealand perennial grassland, in which warming strongly inhibited recruitment by invasive species (Williams et al., 2007). In our study, the combination of elevated CO2 and warming strongly increased *L. dalmatica* survival, growth, and reproduction. Together with previous studies showing that N deposition and altered precipitation can also increase invasion in mixed-grass prairie, these results suggest that this ecosystem may become more susceptible to invasion in the future, with concomitant losses of biological diversity and economic productivity (Brown, 2005; Maron & Marler, 2007; Blumenthal et al., 2008).

Why did *L. dalmatica* respond so strongly to CO2 in this study? In accord with our hypothesis, *L. dalmatica*’s strong responses to elevated CO2 appear to be attributable in part to less...
conservative physiological responses, which may have allowed it to take advantage of both C fertilization and water saved by native species (see below). These physiological responses probably contributed to consistent increases in both survival and growth of *L. dalmatica* with elevated CO₂. Increases in survival, in particular, accounted for much of the observed CO₂ effects on biomass and reproduction. Under ambient conditions, few toadflax plants survived to the end of the experiment. Such high seedling mortality is typical for *L. dalmatica* at this site, despite the fact that it is the most abundant invasive species (Blumenthal *et al.*, 2008). With elevated CO₂, however, survival increased to 51% without warming and 25% with warming. Increased survival, in turn, is probably attributable in part to increases in growth: across the 4 yr of the experiment, per-plant height increased by 44% with elevated CO₂. It is important to note that transplanting may have increased water stress, and therefore accentuated elevated CO₂ effects on growth and survival in the first year. Effects of elevated CO₂ on growth, survival, biomass and reproduction were similar when excluding effects of first-year mortality, however, suggesting that experimental artifacts associated with transplanting did not strongly influence the results.

Several lines of evidence suggest that *L. dalmatica* benefited from a combination of indirect CO₂ effects, mediated by plant and soil water relations, and direct CO₂ effects on photosynthesis and growth. In response to CO₂ enrichment, the dominant native C₃ grass, *P. smithii*, experienced a reduction in stomatal conductance (gₛ), a moderate (23%) increase in photosynthesis (A), and an increase in instantaneous WUE (A/gₛ), responses similar to those observed in a previous open-top-chamber study in shortgrass steppe (Lecain *et al.*, 2003). Furthermore, the decrease in *P. smithii* leaf Δ¹³C with elevated CO₂ reflects an increase in A/gₛ integrated over the growing season. Presumably as a result of decreased water use by *P. smithii* and other native species, elevated CO₂ also consistently increased soil water content (Fig. S1; Dijkstra *et al.*, 2010; Morgan *et al.*, 2011).

In contrast to *P. smithii*, *L. dalmatica* did not experience a reduction in stomatal conductance with elevated CO₂ (rather, CO₂ eliminated negative effects of warming on stomatal conductance), but did experience a larger increase (87%) in photosynthesis. The large increase in *L. dalmatica* photosynthesis with elevated CO₂ led to higher instantaneous A/gₛ as in *P. smithii*, despite the fact that stomatal conductance did not decrease. However, elevated CO₂ decreased *L. dalmatica* integrated A/gₛ (leaf Δ¹³C increased), opposite to its effect on *P. smithii*. Differences between instantaneous and integrated A/gₛ in *L. dalmatica* may reflect temporal asynchrony in the measurements: Δ¹³C of leaf bulk tissue is strongly influenced by responses early in the season, during leaf formation, while gas exchange measurements started after full leaf development, and showed decreases in instantaneous A/gₛ primarily later in the season. Alternatively, in some cases the expected correlation between Δ¹³C and A/gₛ may be compromised by variation in mesophyll conductance and photosynthesis, each of which can influence Δ¹³C independent of changes in Cᵢ/Cₒ and A/gₛ (Seibt *et al.*, 2008). In accord with our integrated A/gₛ results, measurements of leaf Δ¹³C and Δ¹⁸O near a natural CO₂ vent suggested that stomatal conductance in *L. dalmatica* can increase and Alg can decrease with elevated CO₂ (Sharma & Williams, 2009). Thus, *L. dalmatica* appears to respond to elevated CO₂ less conservatively, in terms of water use and growth, than *P. smithii* does.

Increases in stomatal conductance and photosynthesis with soil water availability in both species suggest that water strongly limited these processes. Together, the increases in both *P. smithii* Alg and soil water with elevated CO₂, and the increase in *L. dalmatica* photosynthesis with water, suggest that water saved by *P. smithii* and perhaps other native species (Lecain *et al.*, 2003) contributed to increased photosynthesis in *L. dalmatica*. Although high intrinsic plant WUE has long been considered adaptive in dry environments (Fischer & Turner, 1978), DeLucia & Schlesinger (1991) speculated that, in competitive environments, elevated CO₂ may allow plant species with less conservative water use to out-compete higher WUE species. This study supports that hypothesis. In contrast to situations where competition for nutrients limits CO₂ responses of individual species (Poorter & Navas, 2003), here CO₂ responses of native species appear to have alleviated competition for water and facilitated *L. dalmatica* invasion.

The increase in *L. dalmatica* photosynthesis with elevated CO₂ was not solely attributable to increases in soil water, however. At a given soil water content, *L. dalmatica* photosynthesis was higher with elevated CO₂ than without, suggesting that elevated CO₂ also increased *L. dalmatica* photosynthesis directly. By contrast, reduced stomatal conductance but not increased photosynthesis with elevated CO₂ at a given soil water content suggested that *P. smithii*’s photosynthetic response was driven largely by improved water status. Photosynthetic biochemistry in many species acclimates to growth at high CO₂ through reductions in the maximum velocity of Rubisco carboxylation (Vₐₚₙₙ; Ellsworth *et al.*, 2004). A less consistent reduction in photosynthetic capacity with elevated CO₂ in *L. dalmatica* may have contributed to its stronger photosynthetic response.

Evidence for both direct and indirect, water-mediated CO₂ responses suggests that elevated CO₂ benefitted *L. dalmatica* so greatly because it increased two limiting resources, C and water. In turn, this suggests that CO₂ may have particularly strong effects on invasion under semi-arid conditions, where the water-saving effect of CO₂ is most pronounced (Volk *et al.*, 2000; Morgan *et al.*, 2011). For deep-rooted species such as *L. dalmatica*, the effects of such water savings may also be magnified by the fact that elevated CO₂ can increase soil water at deep as well as shallow soil layers (Vujnovic & Wein, 1997; Nelson *et al.*, 2004; Morgan *et al.*, 2007).

More generally, and speculatively, less conservative stomatal regulation and photosynthesis may help explain why elevated CO₂ often facilitates invasion. Differences in leaf gas exchange between native and invasive species have also been observed in desert shrubland, where elevated CO₂ led to reduced stomatal conductance in the native forb *Eriogonum inflatum* and increased photosynthesis in the exotic grass *Bromus madritensis* (Huxman & Smith, 2001), and among vines of southern China, where several invasive species displayed smaller decreases in stomatal conductance and larger increases in photosynthesis with CO₂.
than did their native congeners (Song et al., 2009). Such differences may stem from high growth rates, stomatal conductance and water use in invasive relative to native species (Leishman et al., 2007; Cavaleri & Sack, 2010; van Kleunen et al., 2010; Penuelas et al., 2010; Manea & Leishman, 2011), and may lead to strong indirect water-mediated effects of CO₂ on invasion. Direct effects of CO₂ may also be related to growth rates, as fast-growing species can have strong C sinks, relatively plastic construction costs, and strong responses to CO₂ under favorable growing conditions (Hunt et al., 1993; Poorter & Navas, 2003; Nagel et al., 2004; Song et al., 2010; Körner, 2011). In sum, when and where water is limiting, CO₂ can strongly facilitate invasive species, with and without warming, by increasing availability of both C and water. As atmospheric CO₂ increases, water-limited ecosystems may be particularly vulnerable to colonization by fast-growing species with less conservative stomatal regulation, including many invasive species.

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References


**Supporting Information**

Additional supporting information may be found in the online version of this article.

**Fig. S1** Volumetric soil water content (VWC) at a depth of 5–25 cm averaged across the 4 months of maximum plant productivity (May–August), and monthly total precipitation.

**Fig. S2** Inorganic soil N in subplots with *L. dalmatica* and without.

**Table S1** ANOVA results for effects of elevated CO2 and temperature on *L. dalmatica* biomass and reproduction

**Table S2** ANOVA results for effects of elevated CO2 and temperature on *L. dalmatica* survival and growth

**Table S3** ANOVA results for effects of elevated CO2 and temperature on photosynthesis (*A*), stomatal conductance (*g*), instantaneous water use efficiency (*A/g*), and the maximum rate of carboxylation (*V_{max}*), in *L. dalmatica* and *P. smithii*.

**Table S4** ANOVA results for effects of elevated CO2 and temperature on Δ¹³C for *L. dalmatica* and *P. smithii*.
Table S5 ANCOVA results and regression coefficients for effects of elevated CO\textsubscript{2}, temperature, and soil water content on photosynthesis (A) and stomatal conductance (g\textsubscript{s}), in *L. dalmatica* and *P. smithii*.

Notes S1 CO\textsubscript{2} and warming effects on soil volumetric water content.

Notes S2 Evaluation of how transplanting may have influenced effects of elevated CO\textsubscript{2} and warming on *L. dalmatica*.

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