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Invasive forb benefits from water savings by native plants and carbon fertilization under elevated CO_2 and warming

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Summary

- As global changes reorganize plant communities, invasive plants may benefit. We hypothesized that elevated CO_2 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 CO_2 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 C_3 grass *Pascopyrum smithii*.
- CO_2 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 CO_2 decreased stomatal conductance in *P. smithii*, contributing to higher soil water, but not in *L. dalmatica*. Elevated CO_2 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 CO_2. Water-limited ecosystems may therefore be particularly vulnerable to invasion as CO_2 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 noninvasive plants to changes that increase resource availability, such as elevated atmospheric CO_2, 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 CO_2 concentration and climate warming are the most global and predictable (Solomon *et al.*, 2007). Although both elevated CO_2 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 CO_2 commonly increases photosynthesis and growth of invasive plants (Ziska, 2003; Song *et al.*, 2009; Manea & Leishman, 2011). Whether or not elevated CO_2 favors invasive species over native species is less clear. Meta-analyses have found either similar CO_2 responses in invasive and noninvasive species (Dukes, 2000) or trends toward stronger CO_2 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; Hättenschwiler & Körner, 2003; Dukes *et al.*, 2011), but not all (Williams *et al.*, 2007), have found elevated CO_2 to favor invasive over native species. For example, in the Mojave Desert, elevated CO_2 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 CO_2. 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 CO_2 (Nagel *et al.*, 2004; Song *et al.*, 2009, 2010; Körner, 2011; Manea & Leishman, 2011). Fast-growing species tend to respond strongly to elevated CO_2, but this advantage is less apparent in competitive environments (Hunt *et al.*, 1993; Poorter & Navas, 2003; Manea & Leishman, 2011).

Warming is also expected to influence plant invasion. Both direct observation and niche modeling suggest that invasive species, like native species, will grow at higher latitudes and elevations than they did in the past (Richardson *et al.*, 2000; Walther *et al.*, 2002, 2009; Bradley *et al.*, 2010). Little is known, however, about how warming will influence the relative success of native and invasive species within plant communities. Particularly at higher latitudes and elevations, warming may favor invasive species from warmer regions (Verlinden & Nijs, 2010; Sandel & Dangremond, 2012), and invasive species that are able to rapidly adjust their phenology (Willis *et al.*, 2010; Hulme, 2011). Warming may also favor C_4 invaders (Dukes & Mooney, 1999). However, warming has been associated with both increased and decreased success of invasive C_3 forbs in C_4-dominated grasslands (Alward *et al.*, 1999; Williams *et al.*, 2007).

Only two studies have tested how the combination of warming and CO_2 influences invasive species, with opposing results. Warming strongly reduced the success of two invasive perennial forbs in a temperate grassland in New Zealand, as a result of reduced seed production and seedling recruitment, while elevated CO_2 had only minor stimulatory effects (Williams *et al.*, 2007). By contrast, warming had little effect on the annual forb *Centaurea solstitialis* in California grassland, while elevated CO_2 increased its growth six-fold (Dukes *et al.*, 2011).

In semi-arid ecosystems, warming and elevated CO_2 are likely to influence invasive species not only directly but also indirectly, through their combined effects on water (Dukes & Mooney, 1999). Elevated CO_2 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 CO_2 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 CO_2 responses for invasive species success in semi-arid ecosystems, however, have not been tested. We hypothesize that elevated CO_2 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 CO_2 enrichment and infrared warming experiment to address the following questions: How do elevated CO_2 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 CO_2 and warming effects on *L. dalmatica* explained by different physiological responses of *L. dalmatica* and the dominant, native C_3 grass *Pascopyrum smithii* (western wheatgrass) to direct or water-mediated, indirect effects of CO_2 and warming?

Materials and Methods

The study was conducted within the Prairie Heating and CO_2 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 C_3 grasses (particularly *P. smithii* (Rydb.) A. Love), which comprised 61.5% of above-ground plant biomass, and also included native C_4 grasses, forbs and subshrubs. *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 C_3 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 CO_2 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) CO_2 enrichment plus heating (CT). The 20 plots were randomized within two soil-type blocks: Ascalon Variant Loam (fine-loamy, mixed-mesic), and Altvan Loam (fine-loamy over sandy, mixed-mesic). Each circular 7-m^2 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 $CO_2 \pm 50.4$ (SD; monitored at 1-min intervals over a 40-d period) and $+1.6 \pm 0.3$°C (SD) during the day and $+3.0 \pm 0.3$°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-m^2 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 CO_2-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-m^2 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 responses 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 CO_2 concentrations (C_i) were measured at saturating light (1500 $\mu mol\ m^{-2}\ s^{-1}$), constant temperature (25°C) and the lowest vapor pressure deficit achievable in the field (1–3 kPa). Step changes in CO_2 were implemented following Long & Bernacchi (2003). Reported photosynthesis and stomatal conductance (g) are the initial point in A/C_i curves, always measured at growth CO_2. The maximum rate of carboxylation by Rubisco (V_{cmax}) was calculated following Sharkey *et al.* (2007). WUE was obtained from both leaf gas exchange measurements (instantaneous A/g) and leaf ^{13}C isotope discrimination ($\Delta^{13}C$), 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 $\Delta^{13}C$ 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 CO_2, temperature, and $CO_2 \times$ 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 $CO_2 \times$ 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 $CO_2 \times$ temperature, $CO_2 \times$ 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 CO_2 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 CO_2 and warming affect A or g. These models included CO_2, temperature, $CO_2 \times$ temperature, and water, with block as a random effect, and omitted nonsignificant interactions between water and CO_2 or temperature.

Results

Elevated CO_2 increased per-plot *L. dalmatica* biomass ($F_{1,15} = 22.2$; $P = 0.0003$), seed pod production ($F_{1,15} = 34.4$; $P < 0.0001$) and new shoot production ($F_{1,15} = 19.5$; $P = 0.0005$; Fig. 1, Table S1). By contrast, warming did not influence *L. dalmatica* biomass or reproduction, either alone or in combination with elevated CO_2. 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 CO_2 significantly increased above-ground biomass ($F_{1,14} = 7.7$; $P = 0.01$) and seed pod production ($F_{1,13} = 17.7$; $P = 0.001$) and had a marginally significant effect on new shoot production ($F_{1,14} = 4.5$; $P = 0.05$). Warming neither influenced *L. dalmatica* nor interacted with elevated CO_2. This indicates that treatment effects after the plants became established were similar to overall treatment effects, and the

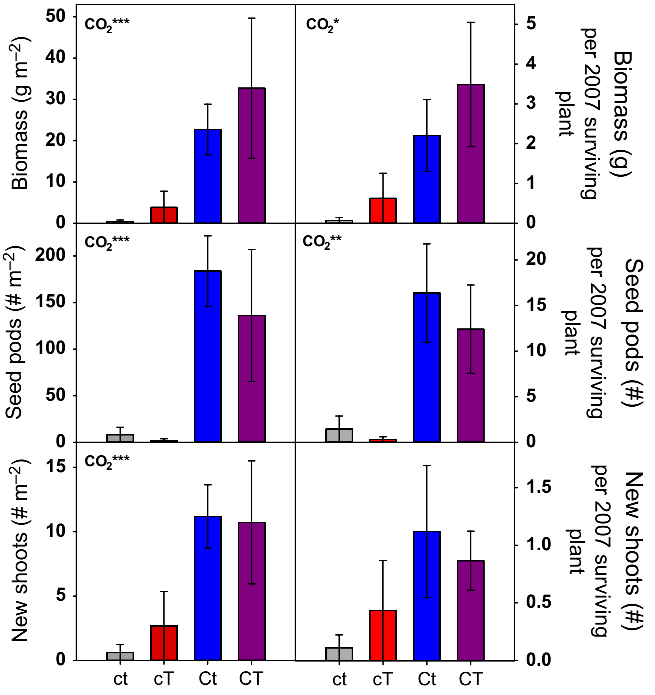

Fig. 1 Cumulative effects of elevated CO_2 (c, ambient; C, 600 ppmv) and warming (t, ambient; T, 1.5 : 3°C day : night warming) on *Linaria dalmatica* growth and reproduction per square meter (left panels), and per surviving plant in the second growing season, to exclude effects of first-year mortality (right panels). Biomass and new shoot production were measured at the end of the experiment (July 2010). Seed pod production, which continues into the autumn, was measured the previous year. Significant treatment effects are listed for each panel: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Error bars are ± 1 SE.

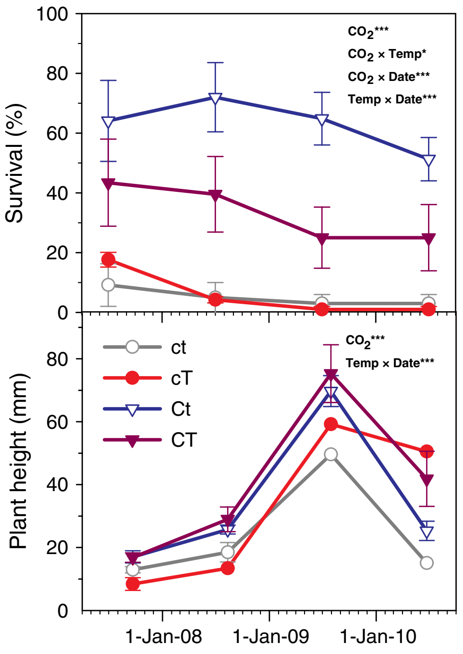

Fig. 2 Influence of CO_2 and warming on *Linaria dalmatica* survival and per-plant height (treatments: t, ambient temperature; T, warming; c, ambient CO_2; C, elevated CO_2). The exact location of each planted individual was permanently marked, allowing accurate determination of survival, despite the appearance of new stems. For each year, data (± 1 SE) are presented for the date with maximum total survival or height, respectively. The temporary increase in survival in Ct plots is a result of random variation associated with the decrease in plot size at the end of the 2007 growing season. Points without error bars represent treatment combinations with one replication containing surviving plants (note that surviving plants are necessary for measuring per-plant height but not survival), and should be interpreted cautiously. Significant treatment effects are listed for each panel: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

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 CO_2 (Fig. 2, Table S2). Elevated CO_2 consistently increased *L. dalmatica* percentage survival ($F_{1,15} = 36.9$; $P < 0.0001$; CO_2 × date: $F_{3,48} = 47.5$; $P < 0.0001$; post hoc CO_2 (date): $P < 0.001$ at all dates), and these differences accounted for much of the CO_2 effect on biomass and reproduction at the end of the experiment. Elevated CO_2 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 CO_2 (CO_2 × temperature: $F_{1,14} = 5.4$; $P = 0.04$; post hoc temperature within elevated CO_2: $F_{1,7} = 8.1$; $P = 0.02$), 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 CO_2 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 CO_2 within elevated temperature: $F_{1,12} = 22.9$; $P = 0.0004$) than in unwarmed plots (post hoc CO_2 within ambient temperature: $F_{1,15} = 9.4$; $P = 0.008$) for *L. dalmatica* (CO_2 × temperature: $F_{1,21} = 8.7$; $P = 0.008$). Elevated CO_2 decreased stomatal conductance only in *P. smithii* ($F_{1,19} = 7.4$; $P = 0.01$). Nevertheless, elevated CO_2 increased instantaneous WUE (*A/g*) for *L. dalmatica* (as a result of large increases in *A*), as well as *P. smithii* (*L. dalmatica*: CO_2 × date: $F_{5,34.7} = 2.7$; $P = 0.03$; post hoc CO_2: 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_{cmax}) decreased with elevated CO_2 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 CO_2 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 CO_2 (CO_2 × temperature: $F_{1,12} = 8.7$; $P = 0.01$; post hoc temperature within ambient CO_2: $F_{1,10} = 14.57$; $P = 0.004$), but had no effect on stomatal conductance with elevated CO_2. 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 Δ^{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$_2$ × 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$_2$ ($F_{1,56} = 28.3$; $P < 0.0001$).

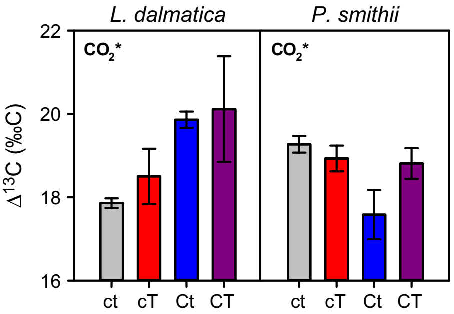

Fig. 4 Influence of elevated CO$_2$ and warming on leaf ^{13}C isotope discrimination (Δ^{13}C) of *Linaria dalmatica* and *Pascopyrum smithii* (± 1 SE) (treatments: t, ambient temperature; T, warming; c, ambient CO$_2$; C, elevated CO$_2$). Higher numbers indicate more discrimination against ^{13}C and lower integrated water use efficiency (*A/g*). Significant treatment effects are listed for each panel: *, $P < 0.05$.

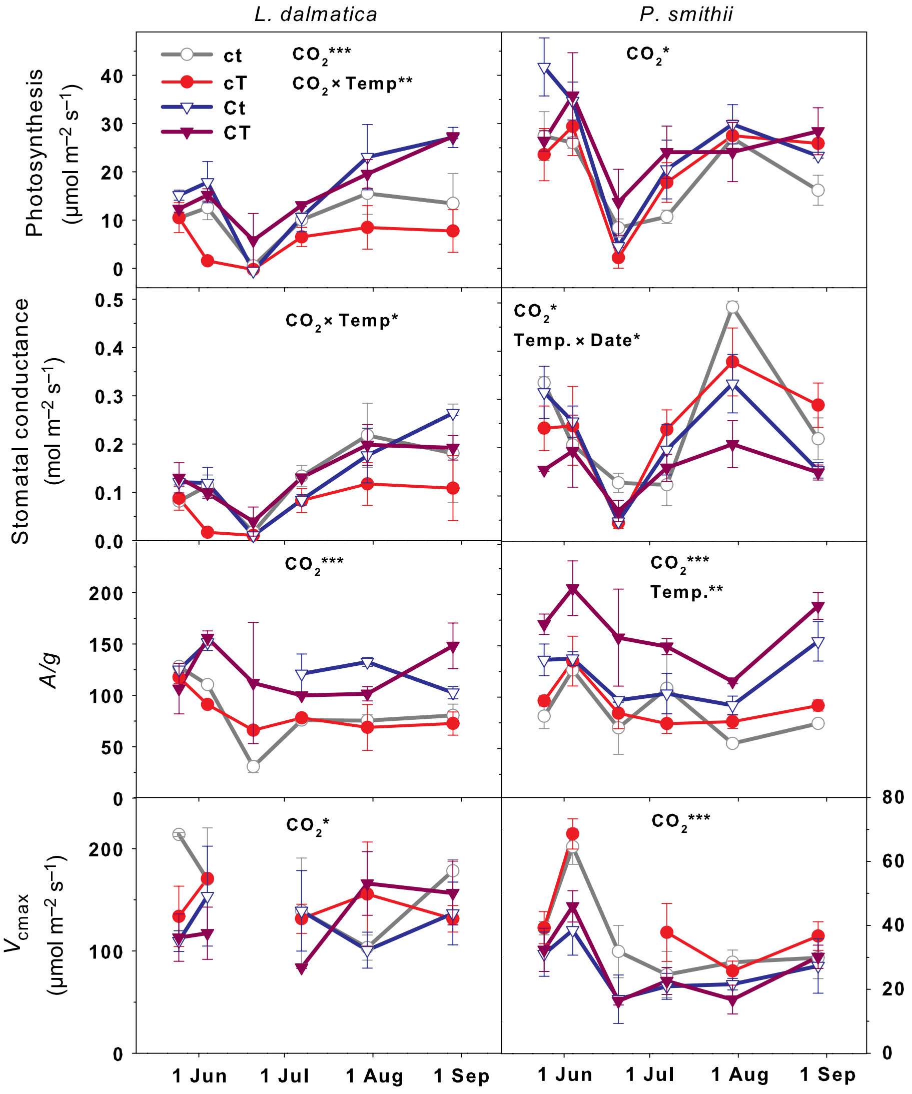

Fig. 3 Effects of elevated CO$_2$ and warming on photosynthesis (*A*), stomatal conductance (*g*), instantaneous water use efficiency (*A/g*) and photosynthetic capacity (V_{cmax}) of *Linaria dalmatica* and *Pascopyrum smithii* in 2007 (treatments: t, ambient temperature; T, warming; c, ambient CO$_2$; C, elevated CO$_2$). *A*, *g*, and *A/g* were measured at growth CO$_2$ concentrations. Water stress led to missing *A/g* (negative values omitted) and V_{cmax} data for some species–treatment combinations on 20 June. Significant treatment effects are listed for each panel: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Error bars are ± 1 SE.

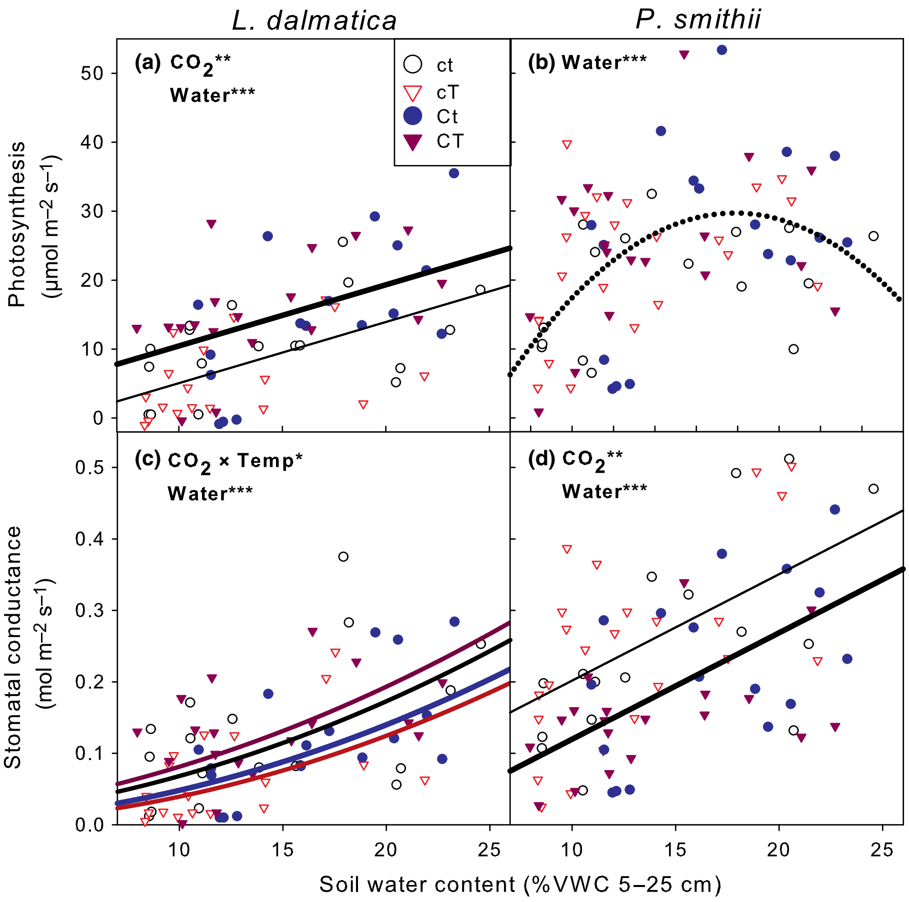

Fig. 5 Effects of elevated CO_2, warming, and soil volumetric water content (VWC) on photosynthesis and stomatal conductance (measured at growth CO_2 concentrations; treatments: t, ambient temperature; T, warming; c, ambient CO_2; C, elevated CO_2). Multiple lines with different intercepts depict significant CO_2, warming or CO_2 × warming effects. Because slopes did not vary by treatment (treatment × VWC interactions were not significant), regression equations were estimated with variable intercepts (for treatments that differed significantly) and fixed slopes. Line type denotes treatment: (a, d) thick, elevated CO_2; thin, ambient CO_2, across warming treatments; (b) dotted, all treatments; (c) black, control; red, warming; blue, elevated CO_2; purple, warming and elevated CO_2. *Linaria dalmatica* stomatal conductance lines were back-transformed from linear models of square-root-transformed data. Second-degree effects of water were included when they resulted in lower Akaike information criterion (AIC) values (*Pascopyrum smithii* photosynthesis). Significant treatment effects are listed for each panel: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

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 CO_2 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 CO_2 than without ($F_{1,23} = 11.1$; $P = 0.003$).

Discussion

We hypothesized that elevated CO_2 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 CO_2 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 CO_2 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 CO_2 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 CO_2), 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 CO_2 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 CO_2 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 CO_2 in this study? In accord with our hypothesis, *L. dalmatica*'s strong responses to elevated CO_2 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 CO_2. Increases in survival, in particular, accounted for much of the observed CO_2 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 CO_2, 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 CO_2. It is important to note that transplanting may have increased water stress, and therefore accentuated elevated CO_2 effects on growth and survival in the first year. Effects of elevated CO_2 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* benefitted from a combination of indirect CO_2 effects, mediated by plant and soil water relations, and direct CO_2 effects on photosynthesis and growth. In response to CO_2 enrichment, the dominant native C_3 grass, *P. smithii*, experienced a reduction in stomatal conductance (*g*), a moderate (23%) increase in photosynthesis (*A*), and an increase in instantaneous WUE (*A*/*g*), 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 $\Delta^{13}C$ with elevated CO_2 reflects an increase in *A*/*g* integrated over the growing season. Presumably as a result of decreased water use by *P. smithii* and other native species, elevated CO_2 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 CO_2 (rather, CO_2 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 CO_2 led to higher instantaneous *A*/*g*, as in *P. smithii*, despite the fact that stomatal conductance did not decrease. However, elevated CO_2 decreased *L. dalmatica* integrated *A*/*g* (leaf $\Delta^{13}C$ increased), opposite to its effect on *P. smithii*. Differences between instantaneous and integrated *A*/*g* in *L. dalmatica* may reflect temporal asynchrony in the measurements: $\Delta^{13}C$ 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*/*g* primarily later in the season. Alternatively, in some cases the expected correlation between $\Delta^{13}C$ and *A*/*g* may be compromised by variation in mesophyll conductance and photorespiration, each of which can influence $\Delta^{13}C$ independent of changes in C_i/C_a and *A*/*g* (Seibt *et al.*, 2008). In accord with our integrated *A*/*g* results, measurements of leaf $\Delta^{13}C$ and $\Delta^{18}O$ near a natural CO_2 vent suggested that stomatal conductance in *L. dalmatica* can increase and *A*/*g* can decrease with elevated CO_2 (Sharma & Williams, 2009). Thus, *L. dalmatica* appears to respond to elevated CO_2 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 A*/*g* and soil water with elevated CO_2, 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 CO_2 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 CO_2 responses of individual species (Poorter & Navas, 2003), here CO_2 responses of native species appear to have alleviated competition for water and facilitated *L. dalmatica* invasion.

The increase in *L. dalmatica* photosynthesis with elevated CO_2 was not solely attributable to increases in soil water, however. At a given soil water content, *L. dalmatica* photosynthesis was higher with elevated CO_2 than without, suggesting that elevated CO_2 also increased *L. dalmatica* photosynthesis directly. By contrast, reduced stomatal conductance but not increased photosynthesis with elevated CO_2 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 CO_2 through reductions in the maximum velocity of Rubisco carboxylation (V_{cmax}; Ellsworth *et al.*, 2004). A less consistent reduction in photosynthetic capacity with elevated CO_2 in *L. dalmatica* may have contributed to its stronger photosynthetic response.

Evidence for both direct and indirect, water-mediated CO_2 responses suggests that elevated CO_2 benefitted *L. dalmatica* so greatly because it increased two limiting resources, C and water. In turn, this suggests that CO_2 may have particularly strong effects on invasion under semi-arid conditions, where the water-saving effect of CO_2 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 CO_2 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 CO_2 often facilitates invasion. Differences in leaf gas exchange between native and invasive species have also been observed in desert shrubland, where elevated CO_2 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 CO_2

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 CO_2 on invasion. Direct effects of CO_2 may also be related to growth rates, as fast-growing species can have strong C sinks, relatively plastic construction costs, and strong responses to CO_2 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, CO_2 can strongly facilitate invasive species, with and without warming, by increasing availability of both C and water. As atmospheric CO_2 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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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 CO_2 and temperature on *L. dalmatica* biomass and reproduction

Table S2 ANOVA results for effects of elevated CO_2 and temperature on *L. dalmatica* survival and growth

Table S3 ANOVA results for effects of elevated CO_2 and temperature on photosynthesis (A), stomatal conductance (g), instantaneous water use efficiency (A/g) and the maximum rate of carboxylation (V_{cmax}) in *L. dalmatica* and *P. smithii*

Table S4 ANOVA results for effects of elevated CO_2 and temperature on $\Delta^{13}C$ for *L. dalmatica* and *P. smithii*

Table S5 ANCOVA results and regression coefficients for effects of elevated CO_2, temperature, and soil water content on photosynthesis (A) and stomatal conductance (g), in *L. dalmatica* and *P. smithii*

Notes S1 CO_2 and warming effects on soil volumetric water content.

Notes S2 Evaluation of how transplanting may have influenced effects of elevated CO_2 and warming on *L. dalmatica*.