

Flowering phenology in a species-rich temperate grassland is sensitive to warming but not elevated CO₂

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Summary

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- Flowering is a critical stage in plant life cycles, and changes might alter processes at the species, community and ecosystem levels. Therefore, likely flowering-time responses to global change drivers are needed for predictions of global change impacts on natural and managed ecosystems.
- Here, the impact of elevated atmospheric CO₂ concentration ([CO₂]) (550 μmol mol⁻¹) and warming (+2°C) is reported on flowering times in a native, species-rich, temperate grassland in Tasmania, Australia in both 2004 and 2005.
- Elevated [CO₂] did not affect average time of first flowering in either year, only affecting three out of 23 species. Warming reduced time to first flowering by an average of 19.1 d in 2004, acting on most species, but did not significantly alter flowering time in 2005, which might be related to the timing of rainfall. Elevated [CO₂] and warming treatments did not interact on flowering time.
- These results show elevated [CO₂] did not alter average flowering time or duration in this grassland; neither did it alter the response to warming. Therefore, flowering phenology appears insensitive to increasing [CO₂] in this ecosystem, although the response to warming varies between years but can be strong.

Key words: climate change, elevated CO₂, flowering, free-air CO₂ enrichment (FACE), global warming, grassland, phenology.

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Introduction

There is now sufficient evidence to be certain that the timing of recurring, or phenological, biological events is shifting in response to global changes (Penuelas & Filella, 2001; Sparks & Menzel, 2002; Beaubien & Hall-Beyer, 2003; Menzel, 2003; Badeck *et al.*, 2004; Penuelas *et al.*, 2004; Primack *et al.*, 2004; Walther, 2004; Menzel *et al.*, 2006). This evidence comes from both experimental climate-manipulation studies (Price & Waser, 1998; Saavedra *et al.*, 2003; Cleland *et al.*, 2006; Sherry *et al.*, 2007) and long-term observations (Myneni *et al.*, 1997; Ahas *et al.*, 2002; Fitter & Fitter, 2002; Penuelas *et al.*, 2002; Walther *et al.*, 2002; Beaubien & Hall-Beyer, 2003; Scheifinger *et al.*, 2003; Stefanescu *et al.*, 2003; Badeck *et al.*, 2004; Primack *et al.*, 2004), as well as from sophisticated, combined observational-modeling studies

(Osborne *et al.*, 2000; Badeck *et al.*, 2004; Dose & Menzel, 2006; Estrella *et al.*, 2006; Schleip *et al.*, 2006). It is clear from the results of these studies that rising temperatures have already accelerated many spring phenomena including bud-break of deciduous trees (Badeck *et al.*, 2004), ecosystem primary productivity increase (Keeling *et al.*, 1996; Randerson *et al.*, 1999; Zhou *et al.*, 2001) and flowering of alpine and arctic plants (Price & Waser, 1998; Arft *et al.*, 1999; Suzuki & Kudo, 2000; De Valpine & Harte, 2001; Dunne *et al.*, 2003) and crops (Estrella *et al.*, 2007). However, current global changes involve more than just an increasing mean surface temperature. Especially important in this regard, particularly when considering plant phenological patterns, is the increasing atmospheric concentration of CO₂ ([CO₂]) which has the potential to alter the strength and timing of plant biomass allocation patterns, including flower production (Badeck

et al., 2004). Because temperature and $[\text{CO}_2]$ are linked in nature, it is difficult to disentangle their relative effects on plant phenology, especially as other factors such as rainfall can also affect phenology (Guak *et al.*, 1998; Norby *et al.*, 2003; Penuelas *et al.*, 2004). Therefore, experimental manipulations are particularly valuable in determining the causative factors of shifting phenological events (Badeck *et al.*, 2004; Cleland *et al.*, 2006). Despite the strength of a multifactor experimental approach, relatively few reports exist of phenological responses to more than one global change factor (Guak *et al.*, 1998; Norby *et al.*, 2003; Cleland *et al.*, 2006). There are also few reports, either observational or experimental, of phenological patterns from the southern hemisphere. This is of concern since temperate latitudes in the southern hemisphere are different from similar latitudes in the northern hemisphere, mostly as a result of the lack of large landmasses at temperate latitudes in the southern hemisphere. Thus, southern temperate ecosystems tend to have milder winters and, apart from coastal areas, are generally drier than similar latitudes in the northern hemisphere (Reid *et al.*, 1999).

Timing of flowering is a crucial aspect of a plant's life cycle. Pollination success, particularly in animal-pollinated species, depends upon the timing of flower opening. Changes in flowering commencement are also likely to lead to changes in fruit ripening and seed dispersal, with impacts not only on a plant and its competitors, but on many other trophic levels. Thus, changes in flowering times are likely to have major ramifications for ecosystem function (Arft *et al.*, 1999; Walther *et al.*, 2002; Badeck *et al.*, 2004; Cleland *et al.*, 2007).

Here, we investigate the effects of experimental warming and elevated CO_2 , both singly and interactively, on flowering phenology of a range of native and exotic species of various growth forms in an Australian temperate grassland over 2 yr.

Materials and Methods

Study site and experimental design

Flowering was monitored throughout the year from March (autumn) 2004 until March 2007 in the TasFACE climate change impacts experiment at Pontville in southeastern Tasmania, Australia ($42^\circ 42' \text{S}$ $147^\circ 16' \text{E}$, 40 m above sea-level (asl)). This experiment exposed a species-rich temperate grassland to both pure- CO_2 free-air CO_2 enrichment (FACE) and infrared warming. Treatment commenced in February 2002. Elevated CO_2 ($550 \mu\text{mol mol}^{-1}$) and warming ($+2^\circ\text{C}$) were applied in 1.5-m diameter circular plots with three replicate plots of each $\text{CO}_2 \times$ warming combination, giving 12 experimental plots in total. The $[\text{CO}_2]$ in the elevated CO_2 plots was controlled by pure- CO_2 fumigation free-air CO_2 enrichment (FACE, Miglietta *et al.*, 2001), operating from sunrise to sunset. The system uses proportional control of $[\text{CO}_2]$ in the centre of each plot by manipulating CO_2

supply with electropneumatic flow control valves (CKD USA, Rolling Meadows, IL, USA). Valves are controlled via a microprocessor-based control system running a proportional integration device algorithm (Hendrey *et al.*, 1993, 1999). The control of CO_2 is excellent, with the central $[\text{CO}_2]$ in all plots being within 10% of the set-point over 85% of the time. Full details of the experimental design and system performance were provided in Hovenden *et al.* (2006).

Warming of 2.0°C was achieved by using overhead ceramic infrared heat lamps (Salamanda ESE250 240 V 250 W Emerson Solid Ceramic Infrared Emitter; Delta-T, Melbourne, Australia) that operated continuously, supplying 140 W m^{-2} of infrared radiation. The lamps produced no visible radiation and on average during the growing season (Mar–Dec) elevated canopy temperature by $1.98 \pm 0.05^\circ\text{C}$ and soil temperature at 5 mm by $0.82 \pm 0.04^\circ\text{C}$. Night-time warming was more pronounced than daytime warming because of the influence of direct solar radiation. Thus, canopy temperature was elevated by an average of $1.54 \pm 0.20^\circ\text{C}$ during the day and $2.61 \pm 0.14^\circ\text{C}$ during the night. Similarly, soil warming at 5 mm depth was $1.79 \pm 0.10^\circ\text{C}$ during the night but only $0.14 \pm 0.013^\circ\text{C}$ during the day. Warming and elevated CO_2 treatments interacted to affect soil moisture content with the mean soil water potential for the period March 2004 until March 2007 being $-113 \pm 19 \text{ kPa}$ in unwarmed control plots, $-112 \pm 18 \text{ kPa}$ in warmed control plots, $-91 \pm 13 \text{ kPa}$ in unwarmed elevated CO_2 plots and $-126 \pm 12 \text{ kPa}$ in warmed elevated CO_2 plots. Differences in soil moisture were most pronounced during periods of intermediate water availability and disappeared under wet or dry conditions.

The vegetation at the site is dominated by the C_4 perennial grass *Themeda triandra*, which is the only C_4 species found at the site. The C_3 perennial grasses *Austrodanthonia* spp. and *Austrostipa* spp. are also abundant (nomenclature follows Buchanan, 1999). Exotic annual grasses include *Vulpia myuros*, *Briza minor* and *Aira praecox*. Abundant herbaceous dicots include the native species *Calocephalus citreus*, *Leptorhynchus squamatus* and *Solenogyne dominii* and the exotic species *Hypochaeris glabra*, *Hypochaeris radicata* and *Leontodon taraxacoides*. Nitrogen-fixing forbs, which include *Trifolium subterraneum* and *Trifolium striatum*, are rare and form an extremely small fraction of the biomass ($< 0.05\%$). The grassland community also contains the woody species *Hibbertia hirsuta* and *Bossiaea prostrata*, which is a nitrogen-fixing species but again forms a small proportion of the biomass ($< 1\%$). There is evidence that species dominance at the site is changing as a result of the experimental manipulations (Williams *et al.*, 2007). The soil at the site is a black vertisol formed of basaltic clay, pH *c.* 6.5, with low total nitrogen (N) (approx. 0.2%) and extractable phosphorus (P) (approx. 20 mg kg^{-1}). The site has a mean annual temperature of 11.6°C , a mean annual rainfall of 560 mm and experiences substantial summer drought between December and March/April.

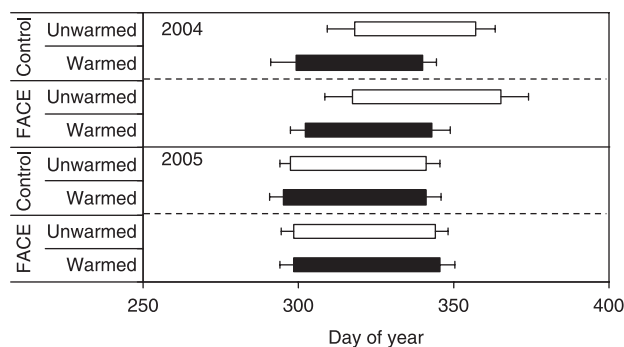


Fig. 1 Average flowering times in unwarmed (open bars) and warmed (closed bars) plots in 2004 and 2005 in the Tasmanian free-air CO₂ enrichment experiment (TasFACE) experiment exposed to both free-air CO₂ enrichment (FACE) and control atmospheric CO₂ concentration ([CO₂]). Bars indicate flowering duration from mean flowering commencement to mean flowering conclusion, error bars indicate \pm SE.

Table 1 Results of ANOVA showing effects of elevated CO₂, warming, year and all interactions on flowering commencement and conclusion times and flowering duration in the Tasmanian free-air CO₂ enrichment experiment (TasFACE) during 2004 and 2005

Effect	Variable		
	First flowering	Last flowering	Duration
CO ₂	0.42	0.80	0.66
Warming (W)	< 0.0001	< 0.0001	0.87
CO ₂ × W	0.40	0.19	0.63
Year (Y)	0.0002	0.03	< 0.0001
CO ₂ × Y	0.55	0.51	0.92
W × Y	< 0.0001	0.007	0.18
C × W × Y	0.78	0.87	0.94

Values shown are probabilities associated with the *F* ratio; significant effects ($P < 0.05$) are shown in bold type.

Climate measurements

All climate measurements were made using a Campbell Scientific automatic weather station with hourly measurements logged to a CRX-10 data logger (Campbell Scientific Australia, Townsville, Australia). Degree-days were calculated using hourly air temperature data and were summed from 01:00 h on 1 July in each year.

Flowering measurements

Flowering of all species in each plot was recorded from March 2004 until March 2006 and thus data cover two complete flowering seasons. During this period, the number of flowering individuals and the number of inflorescences produced was recorded each week during periods of low flower production and two to three times per week during peak flowering

periods (Sept–Dec). Flowering was defined as the presence of open flowers or exposed stamens and/or styles for grasses. From these observations, the time to first flowering and the time of last flowering were calculated separately for each species in each plot as the number of days after 1 January in each year. As flowering times of some species extend into the subsequent calendar year, the day for last flowering for these species was calculated as the number of days from 1 January of the previous year. The duration of flowering was calculated as the difference between last flowering and first flowering for each species in each plot.

Data analyses

Data were analysed by mixed model repeated measures analysis of variance (ANOVA) using general linear model procedures (PROC GLM) in the SAS statistical software package, version 9.1 (SAS Institute Inc., 2003). The model used had CO₂ and warming as fixed, orthogonal factors with repeated years. Warming and CO₂ effects were also analysed by repeated measures ANOVA separately for each species.

Results

Flowering commencement

Flowering commencement date averaged across all species was not affected by CO₂ concentration (Fig. 1; Table 1); neither was there any significant interaction between CO₂ and any of the other factors (Table 1). This lack of any CO₂ effect was consistent across years, as demonstrated by the nonsignificant CO₂ × year interaction (Table 1). In 2004, mean first flowering day averaged across all species was 308.9 ± 3.8 (mean \pm SE throughout) (Fig. 1). Flowering was slightly earlier in 2005 ($P < 0.0002$; Fig. 1), with first flowers appearing, on average, on day 297.5 ± 2.0 .

In close agreement with the average response, there were few significant effects at the species level, with first flowering date being significantly affected by elevated CO₂ in only two species in 2004 and three in 2005 (see the Supplementary Material, Table S1). Furthermore, the responses of these few species to elevated CO₂ were inconsistent across years (Fig. 2). Indeed, time to first flowering was significantly affected by elevated CO₂ in both years in both *L. squamatus* and *V. myuros*, but the direction of the effect differed between years. In *V. myuros*, elevated CO₂ accelerated flowering in 2004 ($P < 0.05$) but delayed flowering in 2005 ($P < 0.04$; Fig. 2), whereas the situation was reversed for *L. squamatus* ($P < 0.02$ both years; Fig. 2). Flowering of *H. birsuta* was accelerated in 2005 by 24.5 d ($P < 0.01$, Fig. 2), but was unaffected by CO₂ concentration in 2004 ($P < 0.21$; Fig. 2).

In agreement with many previous reports, the time to first flowering was substantially affected by both warming (Fig. 1; Table 1) and a warming × year interaction (Fig. 1; Table 1).

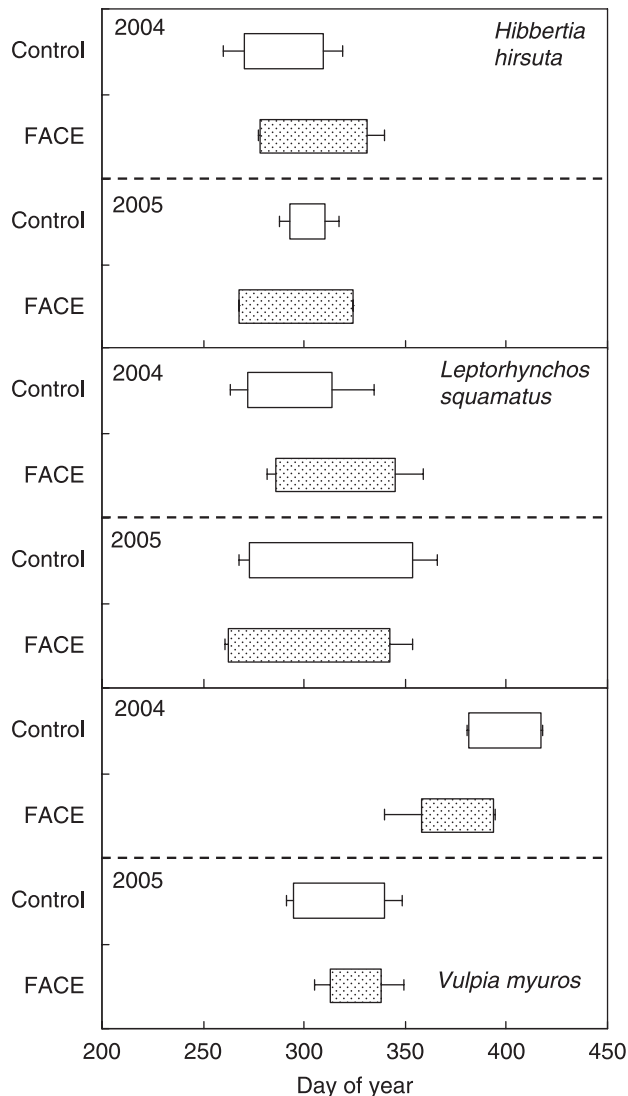


Fig. 2 Flowering times of *Hibbertia hirsuta*, *Leptorhynchus squamatus* and *Vulpia myuros* at control (open bars) and elevated CO₂ (free-air CO₂ enrichment (FACE); stippled bars) during 2004 and 2005 in the Tasmanian free-air CO₂ enrichment experiment (TasFACE). Bars indicate flowering duration from mean flowering commencement to mean flowering conclusion, error bars indicate \pm SE.

In 2004, mean day of first flowering was 316.0 ± 5.2 in unwarmed plots and 296.9 ± 3.7 in warmed plots, whereas in 2005, first flowering occurred on day 299.6 ± 1.8 in unwarmed plots and 296.6 ± 2.2 in warmed plots (Fig. 1). Thus, flowering was substantially earlier in warmed plots than in unwarmed plots in 2004 but not in 2005 (Fig. 1). Not surprisingly, the species-level results reflect the overall trends, with warming accelerating the commencement of flowering to a greater degree in 2004 than in 2005 (Table S1). Thus, warming significantly accelerated flowering in 11 out of 19 species in 2004 but only in one out of 21 species in 2005 (Table S1).

Flowering conclusion and flowering duration

Treatment impacts on flowering conclusion were very similar to those on flowering commencement, with a significant warming \times year interaction and no effect of elevated CO₂ (Fig. 1; Table 1). Thus, in 2004, flowering concluded on day 352.5 ± 5.9 in unwarmed plots, on day 327.8 ± 4.3 in warmed plots compared with the situation in 2005, in which flowering concluded on day 343.9 ± 2.4 in unwarmed plots and on day 338.8 ± 2.7 in warmed plots (Fig. 1). Given the similar effect of warming on flowering commencement and cessation, there was no overall effect of warming on flowering duration (Fig. 1; Table 1) or any warming \times year interaction (Fig. 1; Table 1). There was, however, a significant effect of year, with plants flowering for an average of 33.9 ± 2.7 d in 2004 and 44.2 ± 1.8 d in 2005 (Fig. 1; Table 1). As with flowering commencement, CO₂ concentration did not affect the overall timing of flowering completion or the overall flowering duration (Fig. 1; Table 1).

At the species level, elevated CO₂ did not significantly alter flowering conclusion date in any species in either year but it did significantly affect flowering duration of two species. In 2005, the only year in which the species flowered, elevated CO₂ significantly increased flowering duration of *E. scaber* from 8.6 ± 2.7 d to 36.8 ± 5.7 d ($P < 0.003$). Similarly, elevated CO₂ increased flowering duration of *H. hirsuta* in 2004 from 38.7 ± 10.2 d to 53.0 ± 9.0 d ($P < 0.17$; Fig. 2) and in 2005 from 17.5 ± 7.3 d to 56.0 ± 0.0 d ($P < 0.01$; Fig. 2).

Interannual variation in climate

There was significantly greater rainfall during winter in 2004 than there was in 2005 at the study site (Fig. 3). By contrast, 2005 was substantially warmer in winter than was 2004, particularly during July and August (Fig. 3). Since spring flowering date is often strongly correlated with a thermal sum, such as degree-days, we calculated the number of degree-days from 1 July until the average commencement of flowering in both warmed and unwarmed plots for 2004 and 2005. In 2004, average first flowering occurred at a thermal sum of 1143 degree-days in unwarmed plots compared with 1142 degree-days in warmed plots. By contrast, in 2005 first flowering occurred at a thermal sum of 1153 degree-days in unwarmed plots and at 1326 degree-days in warmed plots. Thus, the warming influence on flowering time in 2004 was very tightly linked with treatment-induced effects on the thermal sum, but this was not the case in 2005, in which flowering occurred at a substantially higher thermal sum in warmed than in unwarmed plots.

Discussion

In a recent review, Springer & Ward (2007) assessed the impacts of elevated CO₂ on flowering times of both wild and

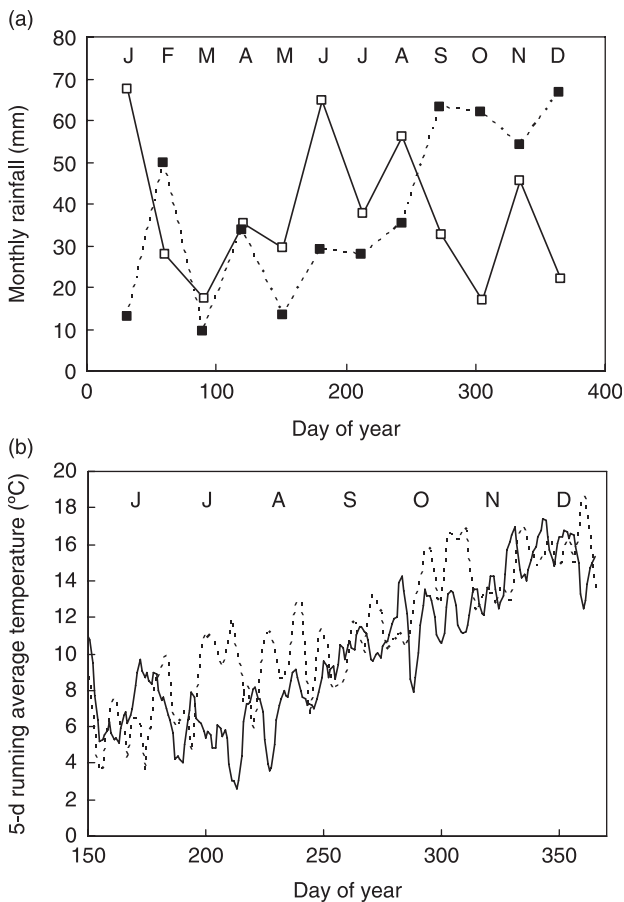


Fig. 3 Monthly rainfall (a) and 5-d running average temperature (b) at the Tasmanian free-air CO_2 enrichment experiment (TasFACE) site during 2004 (open squares, solid lines) and 2005 (closed squares, dashed lines). Letters at the top of each graph indicate month.

crop species, showing that while elevated CO_2 tended to accelerate flowering of crop species, the responses of wild species were remarkably evenly distributed, with similar proportions of species responding positively, negatively or not at all to elevated CO_2 . As well as this variation among species, substantial variation within species in the responsiveness of flowering time to elevated CO_2 has been demonstrated, both among and within individual studies (Springer & Ward, 2007). One problem with comparing responses of different species to experimental manipulation is variation among experiments in growth conditions such as nutrient and water availability, light levels, etc., thereby limiting the utility of meta-analyses and similar comparisons of multiple experiments (Körner, 2006). Therefore, experiments such as the TasFACE experiment and other similar experiments (Cleland *et al.*, 2006; Sherry *et al.*, 2007) are extremely useful in that a large number of species are exposed to simulated future conditions at the same time in the same place. Thus, experiments such as this allow valid analyses of the impacts of environmental manipulation on various species.

The results presented here show that while flowering time was very sensitive to experimental warming in at least 1 yr, it was remarkably unaffected by $[\text{CO}_2]$ in this southern temperate grassland. Importantly, we found no evidence for any interaction between warming and elevated CO_2 on flowering time. Since elevated CO_2 can cause substantial changes in plant productivity (Navas *et al.*, 1995), water use (Jackson *et al.*, 1994) and biomass allocation (Cipollini *et al.*, 1993; Dippert *et al.*, 1995; Cotrufo & Gorissen, 1997), it seems reasonable to expect some influence on reproductive output. Meta-analyses have indicated that elevated CO_2 tends to increase total flower and seed production, although the response is strongly dependent upon species (Jablonski *et al.*, 2002). It is possible, therefore, that stimulation of carbon assimilation by elevated CO_2 leads to an alteration of flowering time (Springer & Ward, 2007). However, a survey of published work indicates that there is no general or average response of flowering phenology to elevated CO_2 (Springer & Ward, 2007). As discussed by Springer & Ward (2007), the mechanisms whereby $[\text{CO}_2]$ affects flowering phenology are poorly understood and seem to vary substantially both among and within species. The lack of response of flowering time to elevated CO_2 seen here, and the lack of any $\text{CO}_2 \times$ warming interaction, indicates that elevated CO_2 is unlikely to substantially alter flowering times in southern temperate grasslands in the near future. The TasFACE site is nutrient-poor and flower and seed production of these species are largely unresponsive to $[\text{CO}_2]$ (Hovenden *et al.*, 2007). Therefore, it is possible that nutrient limitations restrict the species' ability to take advantage of the elevated CO_2 , thus preventing any elevated CO_2 -induced alterations of flowering phenology or reproductive output (Körner, 2003; Körner, 2006). The few species that were responsive to elevated CO_2 treatment did not show a consistent response across the 2 yr, indicating that either the responses were related to other factors or that the impact of elevated CO_2 on phenology in these species varies from year to year. It is interesting that the two species in which elevated CO_2 affected flowering phenology in both years, namely *L. squamatus* and *V. myuros*, are very early- and late-flowering species, respectively. Indeed, *L. squamatus* is the earliest flowering of all the forbs in the experiment. Whether flowering time has an influence on the sensitivity of flowering phenology to CO_2 concentration, as appears to be the case with warming in some communities (Sherry *et al.*, 2007), is currently unknown. As elevated CO_2 reduces stomatal conductance, FACE treatment can increase leaf temperature (Long *et al.*, 2004) and this might explain why *V. myuros* plants flowered earlier in elevated CO_2 plots in 2004, the wetter, cooler year, but this does not explain the retardation of flowering in the co-occurring *L. squamatus*; neither does it explain why warming did not alter the flowering time of either species in either year. Clearly, more work is needed to elucidate the mechanisms whereby $[\text{CO}_2]$ influences flowering time.

The large difference observed here in average flowering response to warming between years might have been due to differences in rainfall and hence water availability between the 2 yr (Fig. 3). In 2004, the mechanism responsible for the warming-induced acceleration of flowering was likely to be an increase in developmental rates that were limited by sub-optimal ambient temperatures. This is supported by the very close agreement of the thermal sum at mean flowering time in both warmed and unwarmed plots. In 2005, however, warming did not have a similar effect, such that mean flowering occurred at almost exactly the same time in warmed and unwarmed plots, despite differences in their thermal sum. Since there was substantially less winter rainfall in 2005 than in 2004, it would appear that accelerating reproductive developmental rates did not lead to earlier flowering since flowering was probably constrained by water availability, as has been demonstrated previously (Brando *et al.*, 2006; Brearley *et al.*, 2007). Close analysis of the mean flowering times over the 2 yr, however, suggests an alternative hypothesis. Mean first flowering in warmed plots occurred on precisely the same day in both years (day 296.9 ± 3.7 in 2004 and on day 296.6 ± 2.2 in 2005) but in unwarmed plots mean day of first flowering was substantially earlier in 2005 than it was in 2004 (day 316.0 ± 5.2 in 2004 and day 299.6 ± 1.8 in 2005). This might be related to the warmer ambient temperatures in 2005 (Fig. 3), as flowering of unwarmed plants occurred at a similar thermal sum in both years (1143 degree-days in 2004, 1153 degree-days in 2005). It is possible that beyond a certain point, additional warming cannot accelerate developmental rates, either because optimal developmental temperature has already been reached or because flower production is limited by other factors, such as day length (Rivera & Borchert, 2001; Keller & Körner, 2003; Crepinsek *et al.*, 2006). This indicates that warming will cause a finite acceleration in flowering time, to a point where flower induction is limited by factors other than temperature, particularly in areas prone to strongly seasonal rainfall. If this is true, then it is likely that the rate of change of phenological events will reduce as the globe warms.

Direct observations of trends in plant phenology have shown that, on average, 'spring' has been advancing by between 2.3 (Parmesan & Yohe, 2003) and 5.1 (Root *et al.*, 2003) days per decade during the twentieth century. Similarly, flowering of 385 British plants was 4.5 d earlier in the 1990s than in the previous four decades (Fitter & Fitter, 2002), representing a shift in spring flowering corresponding to 4.3–6.0 d advancement per degree of warming. Similar figures have been produced from various observational studies (Menzel, 2003; Menzel *et al.*, 2006; Estrella *et al.*, 2007). Most previous experimental work has shown an acceleration of flowering that is somewhat less than these direct observations. For example, experimental warming of 4.2°C in an American tallgrass prairie accelerated mean flowering by 7.6 d, corresponding to 1.8 d advancement per degree, which is similar to the 1.3–3.3 d per degree reported for a Californian annual

grassland (Cleland *et al.*, 2006). Chambered warming treatments in a range of arctic sites produced a mean acceleration of flowering of 1.7–5.0 d per degree, depending upon year (Arft *et al.*, 1999). Our results show a mean acceleration of flowering of 11.0 d per degree of warming (overall mean using all species in both years), which is a larger response than most previous reports. Unfortunately, there is very little published flowering phenology data available for Australian species and none from Australian grasslands. In a 23 yr study of four co-occurring *Eucalyptus* trees from southeastern Australia, it was found that flowering times had not changed measurably in two species, flowering of one species had advanced by 33.0 d per degree of warming and flowering of one species had been delayed by 21.1 d per degree of warming (Keatley *et al.*, 2002). It is unclear why the acceleration of flowering was greater in the TasFACE experiment than in similar warming experiments using infrared heaters, but it is possible that timing of productivity plays some role. The experimental site is prone to severe and prolonged summer drought and thus has highest productivity during the cool months when warming might be expected to play a greater role. The winter months are also reasonably mild such that plants may be metabolically active in all winter months, particularly when warmed. It is possible therefore that global warming will have a pronounced effect on spring flowering in this community.

Our results indicate that while mean date of first flowering is very sensitive to experimental warming in some years, the duration of flowering is not. We found no significant treatment effects on mean flowering duration indicating that warming merely shifted flowering in 2004, rather than extending it. This differs with results from subalpine meadows in which earlier flowering led to longer flowering duration (Dunne *et al.*, 2003). It is likely that flowering responses to warming will differ between different climatic regions. In the American tallgrass prairie, experimental warming significantly reduced flowering duration in some species but increased it in others (Sherry *et al.*, 2007), indicating that the response of flowering duration to warming is species-specific.

Since flowering is such an important event in the lifecycle of most flowering plant species, alterations of flowering time by global changes are likely to have important implications for ecological interactions such as pollination, competition for pollinators and seed dispersal times. Changes in these interactions are likely to produce strong selective pressures that act on both the plant species themselves and on other organisms that rely on these plants (Sherry *et al.*, 2007). Ultimately, such changes may affect ecosystem productivity and carbon sequestration ability (Springer & Ward, 2007). Springer & Ward (2007) stressed the need for work examining the developmental responses of plants to environmental manipulation in natural settings, particularly to multiple experimental factors. Here we have presented results from a comparatively large number of co-occurring species, all growing in a natural setting with natural climatic variation and limiting resources such as

nutrients. Our results indicate that in this ecosystem flowering time is sensitive to both warming and interannual variation in climate but is insensitive to elevated CO₂. Therefore, the lack of response to manipulation of [CO₂] does not arise from the flowering times of these species being unresponsive in general, rather being unresponsive to [CO₂] specifically. Thus, in this ecosystem, at least, the rising atmospheric concentration of CO₂ appears unlikely to either ameliorate or accentuate the impacts of global warming on flowering phenology. Just why elevated CO₂ fails to elicit any response in flowering time of the vast majority of species in this ecosystem is an area requiring further investigation.

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Supplementary Material

The following supplementary material is available for this article online:

Table S1 The impact of elevated CO₂ and warming as main effects on flowering commencement (days) of species in the TasFACE experiment during 2004 and 2005

This material is available as part of the online article from: <http://www.blackwell-synergy.com/doi/abs/10.1111/j.1469-8137.2008.02419.x>
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