

Drought and increased CO₂ alter floral visual and olfactory traits with context-dependent effects on pollinator visitation

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Summary

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- Climate change can alter species interactions essential for maintaining biodiversity and ecosystem function, such as pollination. Understanding the interactive effects of multiple abiotic conditions on floral traits and pollinator visitation are important to anticipate the implications of climate change on pollinator services.
- Floral visual and olfactory traits were measured from individuals of four forb species subjected to drought or normal water availability, and elevated or ambient concentrations of CO₂ in a factorial design. Pollinator visitation rates and community composition were observed in single-species and multi-species forb assemblages.
- Drought decreased floral visual traits and pollinator visitation rates but increased volatile organic compound (VOC) emissions, whereas elevated CO₂ positively affected floral visual traits, VOC emissions and pollinator visitation rates. There was little evidence of interactive effects of drought and CO₂ on floral traits and pollinator visitation. Interestingly, the effects of climate treatments on pollinator visitation depended on whether plants were in single- or multi-species assemblages.
- Components of climate change altered floral traits and pollinator visitation, but effects were modulated by plant community context. Investigating the response of floral traits, including VOCs, and context-dependency of pollinator attraction provides additional insights and may aid in understanding the overall effects of climate change on plant–pollinator interactions.

Introduction

Mutualistic interactions between plants and pollinators provide critical services in agriculture, and are essential for the maintenance of biodiversity and functioning of natural ecosystems (Bascompte *et al.*, 2006). However, climate change can influence these important plant–pollinator interactions (Memmott *et al.*, 2007; Tylianakis *et al.*, 2008) by altering plant phenologies (Hegland *et al.*, 2009; Forrest *et al.*, 2010), pollinator physiology (Bartomeus *et al.*, 2011; Scaven & Rafferty, 2013), and the spatial distribution of plants and pollinators (Hegland *et al.*, 2009; Burkle *et al.*, 2013). Another fundamental route by which environmental change can alter plant–pollinator interactions is by influencing floral cues important for pollinator attraction (Schweiger *et al.*, 2010; Hoover *et al.*, 2012; Scaven & Rafferty, 2013; Burkle & Runyon, 2016). For example, water availability can strongly influence flower size, nectar volume, floral scent, pollinator visitation rates and pollinator community composition for several plant species (Carroll *et al.*, 2001; Burkle & Runyon, 2016; Gallagher & Campbell, 2017). Thus, examining how floral traits, pollinator visitation rates and pollinator community composition are affected by climate factors is key to

understanding and predicting how global change will impact pollination services.

Patterns of plant–pollinator interactions are influenced by floral traits that appeal to multiple pollinator sensory abilities. In addition to the roles of floral visual traits (i.e. morphology, pigmentation and floral display) and floral rewards (i.e. quality and quantity of nectar and pollen) in mediating interactions between plants and pollinators, the importance of floral olfactory traits, like volatile organic compounds (VOCs), are increasingly appreciated (Raguso, 2008; Junker & Parachnowitsch, 2015; Schiestl, 2015). Pollinator responses to floral VOCs can increase pollinator fidelity to flowers (Schiestl & Peakall, 2005; Dudareva *et al.*, 2006; Raguso, 2008; Xiao *et al.*, 2012), increase pollinator foraging efficiency (Howell & Alarcon, 2007), drive the diversification of floral traits (Gervasi & Schiestl, 2017) or serve as deterrents to potential nectar thieves (Junker & Bluthgen, 2008). Floral VOCs also can structure pollinator resource partitioning and flower specialization at a community level through both positive and negative responses of pollinators towards floral scents (Junker & Bluthgen, 2010; Junker *et al.*, 2010). Thus, floral visual traits and VOCs are important biological signals that influence patterns of plant–pollinator interactions and should be taken into consideration when evaluating the response of plant–pollinator community interactions to climate change (Burkle & Runyon, 2017).

See also the Editorial by Kessler, 220: 655–658.

Drought and increased concentrations of atmospheric carbon dioxide (hereafter 'CO₂') are two principal components of climate change that have the potential to alter floral visual and olfactory traits important for pollinator attraction through main and interactive effects. CO₂ fertilization of plants as a result of increases in atmospheric CO₂ concentration (Pachauri *et al.*, 2014) can increase the efficiency of plant resource use (Korner, 2006) and the production of flowers, fruits and seeds (Jablonski *et al.*, 2002). The documented effects of elevated CO₂ on VOC emissions from plant leaves (Loreto *et al.*, 2001; Penuelas & Staudt, 2010) suggests that elevated CO₂ has the potential to alter floral VOCs by changing total emission rates and VOC bouquet composition. In addition, regionally reduced precipitation will likely result in drought conditions for some plant species (Pederson *et al.*, 2010; Dai, 2013; Cook *et al.*, 2015), which can impact floral traits important for pollinator attraction (Carroll *et al.*, 2001). For instance, peak floral abundance, corolla width, nectar volume and seed-set were reduced in naturally occurring *Mertensia ciliata* plants growing in plots manipulated to receive less rainfall (Gallagher & Campbell, 2017). Additionally, vegetative VOC emissions can increase from drought-affected plants (Bertin & Staudt, 1996; Loreto & Schnitzler, 2010; Penuelas & Staudt, 2010) and rapidly decline after reaching extreme drought conditions (Ormeno *et al.*, 2007; Lavoie *et al.*, 2009). Consequently, the responses of plant metabolism and biosynthetic pathways to drought could lead to novel blends of VOCs with community-level implications for pollinator visitation (Junker *et al.*, 2010; Farre-Armengol *et al.*, 2013; Burkle & Runyon, 2016; Larue *et al.*, 2016). Climate change factors can also interact to affect floral traits and pollinator behavior (Hoover *et al.*, 2012). Therefore, studying the influence of abiotic conditions and their interactions on floral traits will help to better understand the mechanisms leading to shifts in plant–pollinator interactions that result from environmental change.

Climate change can modify species interactions by altering floral traits important to pollinator attraction, but it is unclear how altered floral traits will affect plant–pollinator interactions in multi-species assemblages because of the influence of co-occurring forbs on pollinator attraction (Gornish & Tylianakis, 2013). Co-occurring forbs with similar floral morphologies can be more pollen-limited than when the forbs occur separately, demonstrating that some forb species in communities compete with each other for pollinators (Caruso, 2000). Alternatively, pollination can be facilitated by co-occurring forbs with dissimilar (i.e. complementary) floral traits in a plant community (Moeller, 2004; Ghazoul, 2006). Therefore, examining pollinator attraction in single- and multi-species plant assemblages is needed to more realistically assess the effects of climate change on plant–pollinator interactions.

This work aimed to evaluate the main and interactive effects of drought and CO₂ fertilization on: floral visual and olfactory traits; and the community composition and rates of pollinators visiting plants in single-species and multi-species forb assemblages. We subjected individuals of four forb species to normal or decreased water availability and ambient or increased CO₂ concentrations in a fully-crossed, factorial experimental design.

Drought was expected to decrease floral visual traits (plant size, mean petal area, floral display) and alter floral olfactory traits by increasing total VOC emissions and changing the composition of the floral VOC bouquet. We expected CO₂ fertilization to increase floral visual traits and alter floral olfactory traits by increasing total VOC emissions and/or changing the composition of the VOC bouquet, given that CO₂ fertilization generally increases resource use efficiency, rates of photosynthesis and plant growth (Ainsworth & Rogers, 2007). In combination, we expected the effects of CO₂ fertilization to oppose those of drought, resulting in no change in floral visual and olfactory traits. We anticipated that the response of pollinator visitation rates to drought and CO₂ would reflect changes in floral traits, such that pollinator visitation rates would be reduced by drought, increased by CO₂ fertilization, and remain unchanged by the combination of drought and CO₂ fertilization.

Materials and Methods

Source and materials for plants

Twenty-four individuals of each of three native forb species (*Campanula rotundifolia* L., harebell; *Heterotheca villosa* (Pursh) Shinnars, hairy false goldenaster; and *Phacelia hastata* Douglas ex Lehm., silver leaf phacelia) and one invasive forb species (*Potentilla recta* L., sulphur cinquefoil) were grown from seeds collected locally from the Mt Ellis site (see 'Pollinator visitation' section below). These plant species were chosen because they co-occur in the Mt Ellis meadow and can be readily grown from seeds. *Potentilla recta* was of further interest because it is non-native and has increased in abundance at Mt Ellis in recent years (J. R. Runyon, pers. obs.). These plant species are widespread, common across a range of abiotic conditions and can occur in disturbed, rocky, dry soils, suggesting some degree of drought tolerance (Shetler, 1979; Nesom, 2006; McIver & Erickson, 2012; Bujak & Dougher, 2017). Insect pollination is known to increase the reproductive output for three of the forb species. *Heterotheca villosa* and *P. recta* are self-compatible, but produce fewer seeds in the absence of insect visitors (McIver & Erickson, 2012; Herron-Sweet *et al.*, 2016). *Campanula rotundifolia* is self-incompatible and relies on bees for pollination (Shetler, 1979). Less is known about the pollination ecology of *P. hastata*, but it is visited by a diversity of pollinator species (Burkle & Runyon, 2016; Cane & Love, 2016).

Seeds were planted in the fall and allowed to grow and establish for 5–6 wk in a glasshouse. Plants were then transplanted into cone-tainers (6.5 cm wide by 25 cm tall) with Sunshine Mix #1 soil (Sun Gro Horticulture, Agawam, MA, USA) with 1-teaspoon (5 ml) of Osmocote fertilizer per pot (The Scotts Company, Marysville, OH, USA). Transplanted plants were vernalized in a climate-controlled chamber at c. 4°C with a 12-h photoperiod for 100–130 d. Plants were then returned to the glasshouse with a day : night temperature regime of 26°C : 15°C and a 16-h photoperiod with metal halide lamps providing supplemental light. Plants began flowering within 4–6 wk of being transferred into the glasshouse.

Drought and CO₂ treatments

Once flowering, plants were randomly assigned one of four treatments ($n=5$ –6 individual plants of each forb species per treatment) and moved into growth chambers (Model: PGC-6L, Percival Scientific, Perry, IA, USA) on the same day. Treatment combinations were: (1) well-watered + ambient CO₂ (control); (2) drought + ambient CO₂; (3) well-watered + CO₂ fertilization; and (4) drought + CO₂ fertilization. CO₂ concentrations inside growth chambers were set to reflect current approximate atmospheric CO₂ concentrations (400 ppm) in the ambient CO₂ treatment, or the predicted future atmospheric CO₂ concentration for the year 2100 (800 ppm) in the CO₂ fertilization treatment (Pachauri *et al.*, 2014). In order to simulate drought, water was withheld from plants until the first signs of wilting were observed. After wilting, plants were watered until water drained freely through the holes in the bottom of the containers. Wilting cycles were repeated for 25 d before measuring floral traits and pollinator field observations (Huberty & Denno, 2004). Pulsed drought treatments are thought to mimic natural conditions better than continuous drought (Huberty & Denno, 2004), and plants subjected to continuous drought did not produce flowers (W. R. Glenny *et al.*, unpublished data). The time it took each species to wilt varied with species: *C. rotundifolia* and *H. villosa* wilted after *c.* 3 d, *P. hastata* wilted in *c.* 2 d, and *P. recta* wilted within 1 d after water was withheld.

Floral visual traits

After 25 d of climate treatments, floral visual traits important for pollinator attraction were measured, including plant size, mean petal area and the number of open flowers at the time of measurement (i.e. floral display) (Kearns & Inouye, 1993). Floral visual traits were measured immediately before field observations.

Plant size was measured to assess the degree of plant visibility by pollinators. Plant size was estimated for each individual of *C. rotundifolia*, *H. villosa* and *P. recta* as the distance from the surface of the soil to the tallest part of the plant (in cm). Plant size was estimated for each individual of *P. hastata* as the major axis (i.e. greatest plant width) $\times$ the minor axis (in cm²), to measure the area over which flowers were displayed. Area is a better measure of plant size for *P. hastata* due to its prostrate, sprawling growth form.

In order to estimate mean petal area, up to five flowers (range: 0–5 flowers) were randomly measured from each plant using digital calipers (Absolute Digimatic, Mitutoyo, Takatsu-ku, Kawasaki, Japan). Petal area was estimated for bell-shaped flowers (*C. rotundifolia* and *P. hastata*) by measuring the flower's width (and dividing by two to get the radius, r), and depth (h) (in mm), and calculating the surface area of a cone ($A = \pi r(r + \sqrt{h^2 + r^2})$). Petal area was estimated for flat flowers (*H. villosa* and *P. recta*) by measuring the width of the flower (d) (in mm) and calculating the area of a circle ($A = \pi(1/2)d^2$). For *H. villosa*, the petal area of flower heads (capitula) were measured rather than of individual flowers.

Floral display was determined for *C. rotundifolia* and *P. recta* by counting the number of open flowers on each plant, and for *H. villosa* by counting the number of flower heads. Floral display was estimated for *P. hastata* by multiplying the number of open flowers counted on one randomly selected branch by the number of branches on each plant.

Floral olfactory traits

After the 25 d of climate treatments, we also measured floral VOCs within 2–3 h after moving plants from growth chambers into the glasshouse. Floral volatiles were collected by enclosing female phase flowers in a 950-ml clear polyethylene cup with a clear dome lid (Dart Container Corp., Mason, MI, USA) and by pulling air for 1 h at 0.5 l min⁻¹ through a volatile trap containing 30 mg of the adsorbent HayeSep-Q (Restek, Bellefonte, PA, USA) using a portable volatile collection system (Volatile Assay Systems, Rensselaer, New York, USA) or AirLite air sampling pump (SKC Inc., Eighty Four, PA, USA). Volatiles were collected for 1 h once from each plant during peak pollinator activity (1000–1500 h) on one of the two days before field observations. Due to plant architecture, the numbers of flowers from which volatiles were sampled varied among forb species. Therefore, we standardized volatile emissions by the total mean petal area in the cup, and are reported as VOC emissions per petal area per hour (ng mm⁻² h⁻¹). Plants were returned to growth chambers following VOC collection.

Volatiles were eluted from traps with 150 μ l of dichloromethane, and 10 μ l of 50 ng μ l⁻¹ *n*-nonyl-acetate was added as an internal standard. Samples were analyzed using an Agilent 7890A gas chromatograph (GC) coupled with a 5975C mass spectrometer and separated on a HP-1 ms column (30 m $\times$ 0.25 mm inside diameter, 0.25 μ m film thickness); helium was used as the carrier gas. The GC oven was maintained at 35°C for 3 min and then increased by 5°C min⁻¹ to 125°C, then 25°C min⁻¹ to 250°C. Quantifications were made relative to the internal standard using ChemStation software (Agilent Technologies, Wilmington, DE, USA). Identifications of compounds were confirmed by comparing mass spectra and retention times with commercial standards, when available, using the NIST 08 Mass Spectral Search Program (National Institute of Standards and Technology, Gaithersburg, MD, USA). Compounds without commercial standards were named if the NIST 08 Mass Spectral Search Program estimated a 75% or greater match to a compound. The remaining unidentified compounds were labeled as unidentified monoterpenoids (MT1, MT2, etc.) sesquiterpenoids (ST1, ST2, etc.), nitrogen containing compounds (NCC), green leaf volatiles (GLVs), benzenoids (BZN), aliphatics (ALI), or irregular terpenes (IT) and are presented in Supporting Information Tables S1–S4.

Pollinator visitation

In order to investigate how climate change might affect the community composition and rates of pollinators visiting plants

through changes in floral visual and olfactory traits, treated plants were transported to a diverse meadow community five miles southeast of Bozeman, Montana, USA (45.627181°N, 110.960287°W) at the base of Mt Ellis. Typical to this region, plants begin blooming after snowmelt (April–May) and continue through August. The timing of field observations was near peak-bloom in the field system.

Six plants of the same species and treatment combination were grouped into a single-species assemblage (*c.* 1 m² area) (four treatment combinations of each of the four forb species, totaling 16 assemblages). Assemblages were separated by at least 8 m at random positions in a 32 m × 32 m grid at the field site. Thus, pollinators were presented with the choice of visiting plants of a focal forb species subjected to any of the four treatment combinations. Within each assemblage, pollinator visitation was recorded to individual plants by hand netting and counting all floral visitors contacting the reproductive parts of flowers for 1 h during peak pollinator activity on two days (4 June and 6 June 2016), and 0.5 h on a third day (7 June 2016) (2.5 total observation hours per plant, 235 total observation-hours for all plants) (Gibson *et al.*, 2011). Plants were returned to growth chambers at the end of each day of observations. Pollinator visitation rates are reported as the number of visitors per plant and the number of visitors per flower for each plant to account for the potential effects of floral display on pollinator visitation rates. Pollinators were identified to species or morphospecies for community analysis. Although the effective role of floral visitors for plant reproduction was not assessed in this study, we hereafter refer to all floral visitors as ‘pollinators’.

In order to determine the potential of climate change to affect patterns of pollinator visitation differently in single- vs multi-species plant assemblages, three plants of each forb species that received the same treatment combination were randomly selected and reassembled to create a multi-species assemblage of 12 individuals (*n* = 8 total assemblages, each *c.* 1 m² area). Using the observation methods described for the single-species assemblages, individual plants within each multi-species assemblage were observed for 1 h during peak pollinator activity on two days (4 June and 6 June 2016), and 0.5 h on a third day (7 June 2016) (2.5 total observation hours per assemblage, 235 total observation-hours for all plants).

Analytical methods

Separate two-way MANCOVA tests were performed for each forb species to test for the main and interactive effects of water and CO₂ treatments on multiple potentially correlated response variables (i.e. plant size, mean petal area, floral display, total VOC emissions per petal area per hour, visits per plant and visits per flower) measured from the same plant individuals within the single-species assemblages. Significant MANCOVAs ($\alpha < 0.05$) were followed by two-way ANOVA tests to test for the main and interactive effects of drought and CO₂ fertilization on each trait for each forb species. Significant ANOVAs ($\alpha < 0.05$) were followed by a Tukey’s honest significant difference (HSD) *post-hoc*

test to identify pairwise differences between climate treatments for each trait.

In order to account for repeated observations of the same plant individuals used in the single- and multi-species assemblages, a generalized linear mixed effects model (GLMM) with a Poisson family distribution and a random effect for individual plant was used to test for the main and interactive effects of drought, CO₂ and assemblage type on pollinator visitation rates. Given that noninteger response variables cannot be modeled using a Poisson family distribution, an offset term of floral display (log-transformed) was added to the GLMM to test for differences in pollinator visitation rate per flower (Zuur *et al.*, 2009).

A permutational ANOVA (PERMANOVA) was used for each forb species to test for the main and interactive effects of water and CO₂ treatments on volatile composition. Significant PERMANOVAs ($\alpha < 0.05$) were followed by a similarity-percentage test (SIMPER) to identify specific volatile compounds that contributed the most to differences in floral VOCs between treatments. For each forb species, we evaluated differences among treatments in floral VOC variability using homogeneity of dispersions tests (‘betadisper’), followed by ANOVAs to compare the mean distance-to-centroid of floral VOCs among treatments for each forb species.

We compared the magnitude and direction of the effects of climate treatments on floral traits to those on pollinator visitation rates among species by calculating mean effect sizes. Standardized mean effect sizes (Hedges *g* to account for small sample sizes) with 95% confidence intervals for each response variable were calculated by contrasting a trait’s mean value in each treatment group to the control groups (well-watered + ambient CO₂) and dividing by a pooled standard deviation using bias-corrected bootstrapping. Significant differences are indicated by 95% confidence intervals that do not overlap zero. For clarity, we note that there were occasional discrepancies in the significance of responses between the effect size analyses and the two-way ANOVAs (above), although the magnitude and direction of responses were similar. This is expected because the comparisons are distinct, and we interpret these results accordingly.

A PERMANOVA was used for each forb species to test whether the composition of pollinators visiting assemblages was influenced by drought and CO₂ treatments, assemblage type (i.e. single- or multi-species), or their interactions. Additionally, to evaluate differences in the variability of pollinator composition among treatments and forb species, we used separate homogeneity of dispersion tests and ANOVAs to compare the mean distance-to-centroid of pollinator communities among forb species. Significant ANOVAs ($\alpha < 0.05$) were followed by a Tukey’s HSD *post-hoc* test to identify pairwise differences in pollinator community dispersion between forb species.

Statistical software used

All analyses were performed in the statistical software, ‘R’ (v.3.1.2: Smooth Sidewalk) (R Development Core Team, 2014). Multi-variate analyses used the VEGAN package (Oksanen *et al.*, 2007), effect sizes were calculated using the BOOTES package (Kirby &

Gerlanc, 2013) and GLMMs were performed using the LME4 package (Bates *et al.*, 2014).

Results

Overall results of climate treatment effects on floral visual and olfactory traits and subsequent pollinator visitation are summarized in (Fig. 1).

Floral visual traits and total VOC emissions

Campanula rotundifolia Floral traits were affected by drought and CO₂ treatments (whole model MANCOVA, $F_{18,37.3} = 4.40$, $P = 0.001$). Specifically, drought-treated plants were 22% shorter (Table 1; Fig. 2a), produced flowers with 32% less petal area (Table 1; Fig. 2b), and emitted 98% more total VOC emissions per petal area per hour (Table 1; Fig. 2d) compared to plants in the control treatment. Additionally, there was an interactive effect of drought and CO₂ fertilization on total VOC emission rate (Table 1; Fig. 2d), but not floral visual traits (Table 1).

Heterotheca villosa Floral traits were affected by drought and CO₂ treatments (whole model MANCOVA, $F_{18,25.9} = 5.91$, $P = 0.009$). Specifically, drought-treated plants produced flowers with 18% less petal area compared to control plants (Table 1; Fig. 2h), whereas plants in the CO₂ fertilization treatment produced flowers with 10% greater petal area than plants in the control treatment (Table 1; Fig. 2h). We did not detect any interactive effects of drought and CO₂ on floral visual traits or total VOC emission rate (Table 1).

Phacelia hastata Floral traits were affected by drought and CO₂ treatments (whole model MANCOVA, $F_{18,42.9} = 5.06$, $P = 0.004$). Drought-treated plants produced flowers with 35% less petal area than plants in the control treatment (Table 1; Fig. 2n), whereas CO₂-fertilized plants grew 37% larger (Table 1; Fig. 2m) and produced 33% more flowers (Table 1; Fig. 2o) than plants in the control treatment. CO₂-fertilized plants emitted 42% more total VOCs per petal area per hour (Table 1; Fig. 2p) compared to plants in the control treatment. We did not detect any interactive effects of drought and CO₂ treatments on floral visual traits or total VOC emission rate (Table 1).

	Floral visual traits			Floral olfactory traits			Pollinator visitation rate	
	Plant size	Mean petal area	Floral display	Total VOC emission	VOC composition	VOC dispersion	Pollinator visits per plant	Pollinator visits per flower
 <i>Campanula rotundifolia</i>	↓	—	—	↑ Drought ↓ CO ₂	* Drought ↓ CO ₂	—	—	—
 <i>Heterotheca villosa</i>	—	↓ Drought ↑ CO ₂	—	—	—	—	—	—
 <i>Phacelia hastata</i>	↑	↓	↑	↑	* Drought ↓ CO ₂	—	—	—
 <i>Potentilla recta</i>	↑	↓	↓	↑	* Drought ↓ CO ₂	—	↓	—
 Change driven by drought Change driven by CO₂ enrichment ↑ Increased ↓ Decreased * Altered Composition — No effect								

Fig. 1 Summarized effects of drought and CO₂ fertilization treatments on floral traits and pollinator visitation rates in the four focal plant species. The effects of drought (brown) and CO₂ fertilization (green) on floral traits and pollinator visitation rates are indicated by the direction of arrows. Boxes with both brown and green indicate an interactive effect of drought and CO₂ fertilization. Asterisks designate changes in floral volatile organic compound (VOC) composition.

Table 1 Two-way ANOVA results testing for differences in mean floral visual traits (plant size, petal area, floral display) and total volatile organic compound (VOC) emissions in response to the main and interactive effects of drought and CO₂ fertilization treatments for each of the four forb species

Source	Plant size*			Mean petal area [†]			Floral display			Total VOC emissions [‡] (ng mm ⁻² h ⁻¹)		
	df	F	P	df	F	P	df	F	P	df	F	P
<i>Campanula rotundifolia</i>												
Whole model	3, 18	3.35	0.04	3, 18	2.79	0.07	3, 18	0.80	0.57	3, 18	5.04	< 0.01
Drought	1, 18	9.37	0.01	1, 18	7.87	0.01	1, 18	0.01	0.93	1, 18	6.56	< 0.01
CO ₂	1, 18	0.15	0.70	1, 18	0.49	0.49	1, 18	1.57	0.23	1, 18	1.19	0.29
Drought × CO ₂	1, 18	0.51	0.48	1, 18	0.01	0.91	1, 18	0.84	0.37	1, 18	7.35	< 0.01
<i>Heterotheca villosa</i>												
Whole model	3, 20	3.00	0.05	3, 16	7.32	< 0.01	3, 20	0.76	0.53	3, 17	1.36	0.29
Drought	1, 20	2.30	0.14	1, 16	5.34	< 0.01	1, 20	1.15	0.30	1, 17	2.72	0.11
CO ₂	1, 20	2.70	0.12	1, 16	13.97	< 0.01	1, 20	0.91	0.35	1, 17	0.58	0.46
Drought × CO ₂	1, 20	4.01	0.06	1, 16	2.69	0.12	1, 20	0.23	0.64	1, 17	0.80	0.38
<i>Phacelia hastata</i>												
Whole model	3, 20	3.51	0.03	3, 20	5.71	< 0.01	3, 20	2.91	0.06	3, 20	3.19	0.05
Drought	1, 20	0.68	0.42	1, 20	13.74	< 0.01	1, 20	1.28	0.27	1, 20	1.85	0.19
CO ₂	1, 20	7.79	0.01	1, 20	0.52	0.48	1, 20	7.11	< 0.01	1, 20	6.02	< 0.01
Drought × CO ₂	1, 20	2.04	0.17	1, 20	2.87	0.11	1, 20	0.34	0.57	1, 20	1.68	0.21
<i>Potentilla recta</i>												
Whole model	3, 20	1.97	0.15	3, 20	9.06	< 0.01	3, 20	9.43	< 0.01	3, 20	3.89	< 0.01
Drought	1, 20	0.49	0.49	1, 20	26.25	< 0.01	1, 20	26.85	< 0.01	1, 20	10.84	< 0.01
CO ₂	1, 20	5.40	0.03	1, 20	0.00	0.95	1, 20	0.03	0.96	1, 20	0.84	0.37
Drought × CO ₂	1, 20	0.02	0.89	1, 20	0.93	0.35	1, 20	1.43	0.25	1, 20	0.00	0.97

Bold text indicates significant differences in means following a significant whole model MANOVA ($\alpha < 0.05$).

*Plant size was measured as height for all forbs except *Phacelia hastata* for which area was measured.

[†]Mean petal area was estimated using the equation for area of a circle for *Heterotheca villosa* and *Potentilla recta*, and the area of a cone for *Campanula rotundifolia* and *Phacelia hastata*.

[‡]Standardized by mean petal area per hour.

Potentilla recta Floral traits were affected by drought and CO₂ treatments (whole model MANCOVA, $F_{18,42.9} = 2.98$, $P = 0.002$). CO₂-fertilized plants were 6% larger compared to plants grown in ambient CO₂ concentrations (Table 1; Fig. 2s). Drought-treated plants produced flowers with 18% less petal area (Table 1; Fig. 2t) and 47% fewer flowers (Table 1; Fig. 2u) compared to control plants. Drought-treated plants emitted 129% more total VOCs per petal area per hour than plants in the control treatment (Table 1; Fig. 2v). We did not detect any interactive effects of drought and CO₂ on floral visual traits or total VOC emission rate (Table 1).

Floral VOC composition

Campanula rotundifolia Drought-treated plants (Fig. 3a; PERMANOVA, $F_{1,21} = 3.37$, $P = 0.009$), CO₂-fertilized plants (Fig. 3b; PERMANOVA, $F_{1,21} = 3.43$, $P = 0.013$), and plants in the combination treatment (i.e. drought + CO₂ fertilization) (PERMANOVA, $F_{1,21} = 8.49$, $P = 0.001$) emitted different compositions of floral VOCs than control plants. Terpenoids and a benzenoid accounted for the most dissimilarity in floral VOC composition between treatments. For example, 6-methyl-5-hepten-2-one, β -pinene and benzaldehyde were emitted at 50%, 147% and 96% greater rates, respectively, from drought-treated plants compared to control plants. Additionally,

6-methyl-5-hepten-2-one, β -pinene and β -myrcene were emitted at 72%, 255% and 24% greater rates, respectively, from CO₂-fertilized plants compared to control plants (Table S1).

Heterotheca villosa We did not find evidence for main or interactive effects of drought and CO₂ treatments on floral VOC composition (Tables 1, S2).

Phacelia hastata CO₂-fertilized plants emitted different compositions of floral VOCs compared to plants in the control treatment (Fig. 3c; PERMANOVA, $F_{1,23} = 2.47$, $P = 0.030$). A combination of monoterpenes and green leaf volatiles (GLVs) primarily accounted for differences in floral VOC composition between CO₂ treatments. Specifically, linalool, limonene and (*Z*)-3-hexenyl-acetate contributed the most towards the dissimilarity in floral VOC composition and were emitted at 75%, 272% and 42% greater rates in the CO₂-fertilized plants compared to plants in the control treatment, respectively (Table S3). Neither drought (PERMANOVA, $F_{1,23} = 1.33$, $P = 0.210$), nor the combination of drought and CO₂ fertilization (PERMANOVA, $F_{1,23} = 0.90$, $P = 0.450$) affected the composition of floral VOCs.

Potentilla recta Drought-treated plants emitted a different composition of floral VOCs compared to well-watered control plants (Fig. 3d, PERMANOVA, $F_{1,23} = 6.45$, $P = 0.001$). Two GLVs and an irregular terpene contributed the most towards the

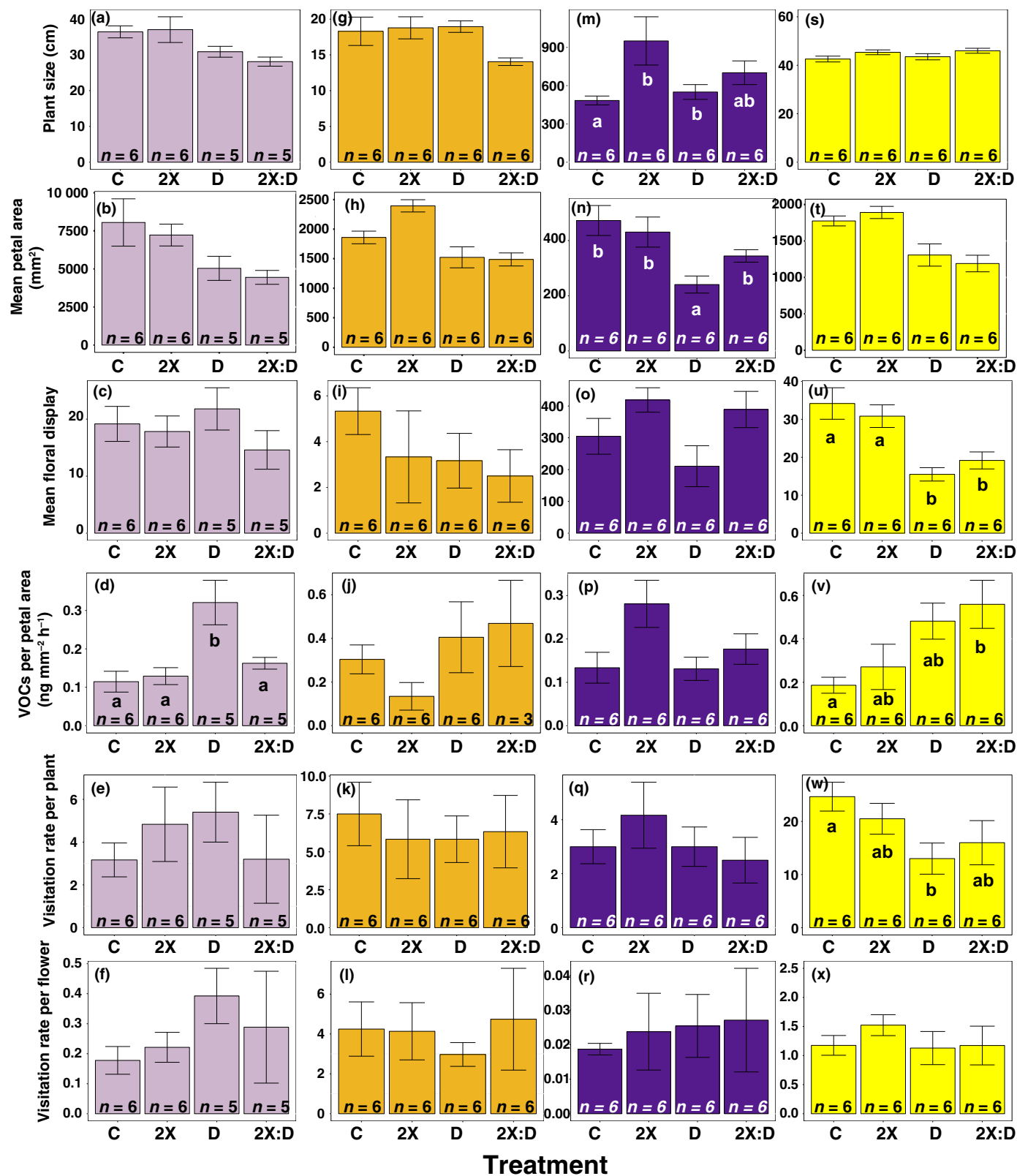


Fig. 2 Mean values of floral traits and pollinator visitation rates in each climate treatment (C, well-watered + ambient CO₂; 2X, well-watered + CO₂ fertilization; D, drought + ambient CO₂; 2X:D, drought + CO₂ fertilization). The bars represent the mean (± 1 SE) value of response variables for *Campanula rotundifolia* (light purple, a–f), *Heterotheca villosa* (orange, g–l), *Phacelia hastata* (dark purple, m–r), and *Potentilla recta* (yellow, s–x). Lowercase letters indicate significant differences in means between treatments. Sample sizes are reported on the bars.

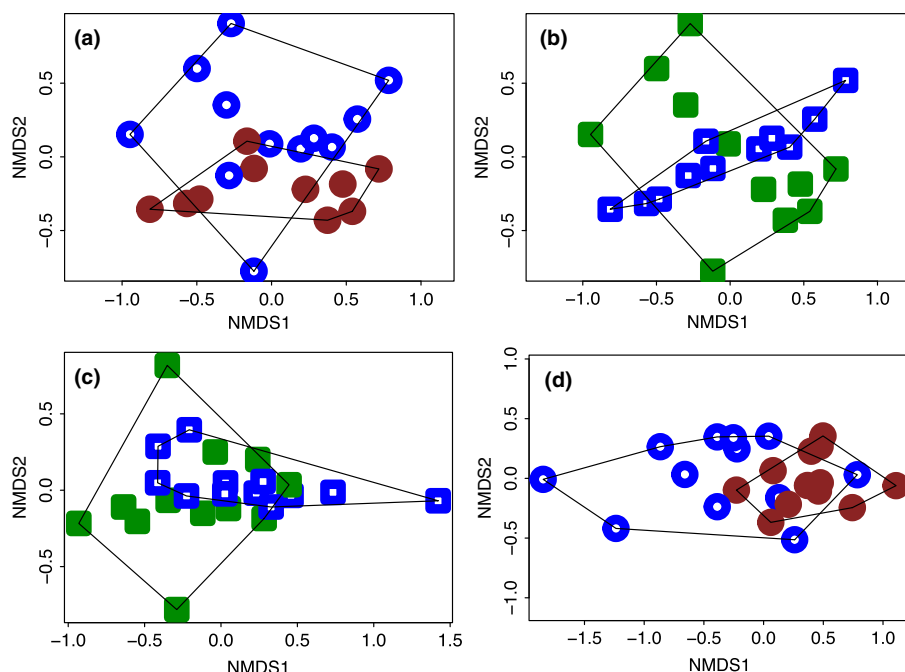


Fig. 3 Climate treatment effects on floral volatile composition. Nonmetric multidimensional scaling (NMDS) of the volatile organic compound (VOC) composition of (a) *Campanula rotundifolia*, (b) *Campanula rotundifolia*, (c) *Phacelia hastata* and (d) *Potentilla recta* plants, treated with drought (red circles) and CO₂ fertilization (green squares) compared to control plants (blue symbols). Polygons encircle the relative VOC composition of all of the plant individuals in the same treatment group.

dissimilarity of floral VOC composition between drought treatments. (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol, and 6-methyl-5-hepten-2-one were emitted at 106%, 61%, and 30% greater rates, respectively, in drought-treated plants compared to control plants. We did not find evidence for an interactive effect of drought and CO₂ on floral VOC composition (PERMANOVA, $F_{1,23} = 1.28$, $P = 0.25$) (Table S4).

Pollinator visitation

Campanula rotundifolia Pollinator visitation rates per plant and per flower were 89% greater to plants in the multi-species assemblage, compared to plants in the single-species assemblage (Table 2).

Heterotheca villosa Pollinator visitation rate per plant and per flower were not different between treatment groups or assemblage types (Table 2).

Phacelia hastata Pollinator visitation rate per plant and per flower were not different between treatment groups or assemblage types (Table 2).

Potentilla recta Pollinator visitation rate per plant was 52% lower to drought-treated plants compared to control plants (Table 2). Additionally, pollinator visitation rate per plant and per flower were 34% higher to plants in the multi-species assemblage, compared to plants in the single-species assemblage (Table 2).

Composition of pollinators visiting treated plants in single- and multi-species assemblages Within the single-species assemblages, 36 species of pollinators including 30 bee (Hymenoptera:

Apidae) and six fly (Diptera) species totaling 408 specimens (95% bees, 5% flies) were collected (Table S5). There were no effects of drought (PERMANOVA, $F_{1,15} = 0.31$, $P = 0.950$), CO₂ fertilization (PERMANOVA, $F_{1,15} = 0.42$, $P = 0.910$), or the combination of drought and CO₂ fertilization (PERMANOVA, $F_{1,15} = 1.12$, $P = 0.390$) on the composition of visiting pollinators for any forb species. However, the dispersion of visiting pollinator communities across treatments differed significantly depending on the forb species ('betadisper', ANOVA, $F_{3,12} = 5.70$, $P = 0.012$). Specifically, pollinator communities visiting *P. recta* treatments were less variable than pollinator communities visiting *P. hastata* treatments (Tukey's HSD, 95% CI = -0.22 to -0.39 , $P < 0.001$) (Fig. 4).

Within the multi-species assemblages, 35 species of pollinators (31 bees, four flies) were collected, totaling 418 specimens (97% bees, 3% flies) (Table S6). There were no effects of drought (PERMANOVA, $F_{1,15} = 0.46$, $P = 0.870$), CO₂ fertilization (PERMANOVA, $F_{1,15} = 0.45$, $P = 0.900$), or the combination of drought and CO₂ fertilization (PERMANOVA, $F_{1,15} = 0.67$, $P = 0.710$) on the composition of visiting pollinators. Additionally, there were no differences in the dispersion of pollinator communities visiting each treatment in the multi-species assemblages (ANOVA, $F_{3,12} = 0.061$, $P = 0.980$).

Climate treatment effect sizes

Campanula rotundifolia Pollinator visitation rate per plant and per flower were consistent across treatments despite VOC emission rates increasing and plant size and petal area decreasing in drought-treated plants compared to control plants (Fig. 5a).

Heterotheca villosa In the multi-species assemblages, petal area was reduced by a similar magnitude as pollinator visitation rate

Table 2 Back-transformed results of generalized linear mixed effects models (GLMMs) testing for main and interactive effects of drought, CO₂ fertilization and assemblage type on per-plant and per-flower pollinator visitation rates for each focal forb species

Source	Per-plant visitation rate					Per-flower visitation rate				
	df	Estimate	SE	z-value	P	df	Estimate	SE	z-value	P
<i>Campanula rotundifolia</i>										
Drought	123	1.44	1.43	1.03	0.30	123	1.15	1.39	0.44	0.66
CO ₂	123	1.29	1.42	0.73	0.46	123	1.15	1.27	0.44	0.66
Assemblage type	123	1.89	1.33	2.27	0.02	123	1.89	1.32	2.26	0.02
Drought × CO ₂	123	0.46	1.68	−1.49	0.14	123	0.79	1.63	−0.47	0.64
Drought × assemblage type	123	0.73	1.48	−0.79	0.43	123	0.73	1.48	−0.77	0.43
CO ₂ × assemblage type	123	0.63	1.48	−1.17	0.24	123	0.63	1.48	−1.17	0.24
Drought × CO ₂ × assemblage type	123	2.55	1.78	1.67	0.09	123	2.59	1.78	1.66	0.09
<i>Heterotheca villosa</i>										
Drought	135	0.74	1.39	−0.91	0.36	135	1.12	1.39	0.35	0.73
CO ₂	135	0.70	1.40	−1.07	0.29	135	0.95	1.40	−0.16	0.87
Assemblage type	135	1.05	1.25	0.23	0.82	135	1.05	1.25	0.23	0.82
Drought × CO ₂	135	1.69	1.61	1.11	0.27	135	1.44	1.60	0.77	0.44
Drought × assemblage type	135	0.71	1.44	−0.93	0.35	135	0.71	1.44	−0.93	0.35
CO ₂ × assemblage type	135	1.26	1.40	−0.70	0.48	135	1.27	1.40	0.70	0.48
Drought × CO ₂ × assemblage type	135	1.02	1.65	0.04	0.96	135	1.02	1.65	0.04	0.96
<i>Phacelia hastata</i>										
Drought	135	1.07	1.45	0.19	0.85	135	1.04	1.45	0.11	0.91
CO ₂	135	1.50	1.41	1.18	0.24	135	1.10	1.41	0.28	0.78
Assemblage type	135	1.50	4.41	1.18	0.24	135	1.50	1.41	1.18	0.24
Drought × CO ₂	135	0.49	1.69	−1.36	0.17	135	0.48	1.69	−1.41	0.16
Drought × assemblage type	135	1.29	1.59	0.54	0.59	135	1.29	1.60	0.54	0.59
CO ₂ × assemblage type	135	0.98	1.56	−0.04	0.97	135	0.98	1.57	−0.04	0.97
Drought × CO ₂ × assemblage type	135	1.29	1.92	0.39	0.69	135	1.29	1.92	0.39	0.70
<i>Potentilla recta</i>										
Drought	135	0.48	1.19	−4.13	0.01	135	0.81	1.18	−1.24	0.22
CO ₂	135	0.79	1.17	−1.50	0.13	135	0.95	1.16	−0.32	0.75
Assemblage type	135	1.34	1.12	2.58	0.01	135	1.34	1.12	2.58	0.01
Drought × CO ₂	135	1.54	1.29	1.73	0.08	135	1.22	1.27	0.84	0.40
Drought × assemblage type	135	1.20	1.21	0.96	0.34	135	1.20	1.21	0.96	0.34
CO ₂ × assemblage type	135	0.99	1.19	−0.01	0.99	135	0.99	1.19	−0.01	0.97
Drought × CO ₂ × assemblage type	135	0.99	1.31	−0.04	0.97	135	0.99	1.31	−0.04	0.97

Bold text indicates significant differences in mean pollinator visitation rates between treatment groups.

per plant in drought-treated plants (Fig. 5b). Drought reduced pollinator visitation rate per plant by 240% more in the multi-species assemblage, than in the single-species assemblage (Fig. 5b). The combination of drought and CO₂ reduced petal area and plant size by 126–129% more than pollinator visitation rate (per plant and per flower) in both the single- and multi-species assemblages (Fig. 5b).

Phacelia hastata CO₂ fertilization increased plant size, total VOC emission rate and pollinator visitation rate per plant by similar magnitudes in the multi-species assemblage only (Fig. 5c). Additionally, drought reduced petal area 62% more than drought increased pollinator visitation rate per plant in the multi-species assemblage (Fig. 5c).

Potentilla recta Drought reduced petal area, floral display, and pollinator visitation rates per plant 35–40% more than drought increased total VOC emission rate (Fig. 5d). Additionally, drought reduced pollinator visitation rate per plant by 18% more in the multi-species assemblage than in the single-species

assemblage (Fig. 5d). The combination of drought and CO₂ reduced pollinator visitation rate per plant by 17–22% less than the drought treatment alone in both the single-species and multi-species assemblages (Fig. 5d).

Discussion

Our findings provide evidence that drought has the potential to reduce floral visual traits and increase floral volatile organic compound (VOC) emissions, whereas CO₂ fertilization can increase floral visual traits and have mixed effects on floral VOCs. Despite frequent main effects of drought and CO₂ fertilization on floral visual and olfactory traits, an interaction between drought and CO₂ fertilization was observed rarely on VOCs and never on floral traits and pollinator visitation rates. Interestingly, the effects of drought and CO₂ on pollinator visitation were influenced by plant community context. Drought decreased pollinator visitation to two forb species and CO₂ fertilization increased pollinator visitation to one forb species more so in the multi-species assemblages than in the single-species assemblages, indicating that the

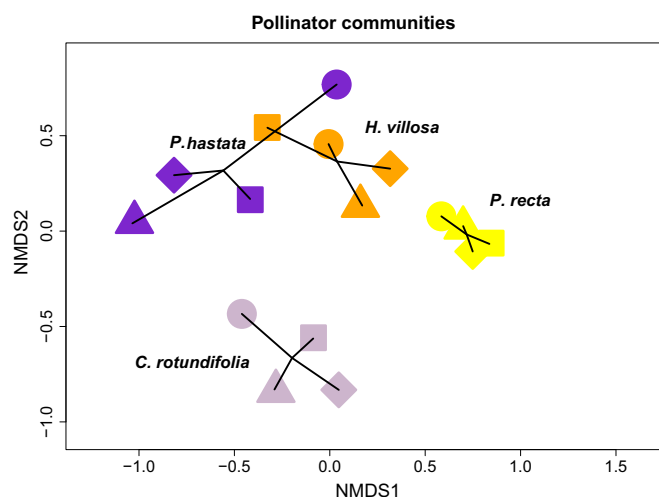


Fig. 4 Nonmetric multidimensional scaling (NMDS) of the composition of visiting pollinators to assemblages of focal plant species (light purple, *Campanula rotundifolia*; orange, *Heterotheca villosa*; dark purple, *Phacelia hastata*; yellow, *Potentilla recta*). Symbols represent the relative composition of visiting pollinators to treated assemblages of plant species (diamond, well-watered + ambient CO₂; circle, drought + ambient CO₂; triangle, well-watered + CO₂ fertilization; square, drought + CO₂ fertilization). Lines connect the relative composition of visiting pollinator communities to treated assemblages of the same focal species, to the species centroid.

effects of climate change are likely to influence the competitive or facilitative effects of neighboring plants on pollination of focal forb species.

For both *Potentilla recta* and *Campanula rotundifolia*, drought reduced floral visual trait values but increased floral VOC emissions. Such opposing effects of drought on floral visual traits and VOCs may help explain the observed patterns of pollinator visitation to these forb species. Drought reduced pollinator visitation rates to *P. recta* plants by a similar magnitude as floral visual traits and in spite of an increase in total VOC emissions. This is somewhat surprising because we predicted that pollinator visitation rates would increase following an increase in floral VOC emissions, assuming that these VOCs improve pollinator foraging efficiency and fidelity to flowers (Howell & Alarcon, 2007; Schiestl, 2015). However, it is possible that drought increased emissions of volatile compounds deterrent to pollinators, thereby negatively affecting pollinator visitation (Junker & Bluthgen, 2010). Nevertheless, pollinator attraction is more likely to be influenced by the composition of volatile compounds emitted by a flower (i.e. the floral 'bouquet'), not the total quantity of scent emitted by a flower (Raguso, 2008; Burkle & Runyon, 2017). In addition, drought reduced floral visual traits, which are strong determinants of pollinator attraction (Nilsson, 1988; Aspi *et al.*, 2003; Hegland & Totland, 2005; Hegland *et al.*, 2009). Consequently, the combination of a strong reduction of drought on floral visual traits, and a weak enhancement of drought on floral VOCs likely reduce pollinator visitation rates to *P. recta*. For *C. rotundifolia*, pollinator visitation rates were unaffected by drought treatments, potentially because the high floral VOC emission rate may have offset reductions in floral visual traits.

This result suggests that pollinators could increase their reliance on olfactory cues to locate flowers under drought conditions (*sensu* Kessler & Halitschke, 2009).

The composition of floral VOCs in response to climate change – as opposed to total floral VOC emissions – likely have stronger effects on pollinator attraction (Raguso, 2008; Burkle & Runyon, 2017). Pollinators might detect changes in the composition of VOCs to avoid drought-affected plants. For example, pollinator visitation rates were reduced to drought-treated *P. recta* plants, which also had VOC bouquets that were altered primarily as a result of increased GLV emissions. Although undamaged plants typically produce trace amounts of GLVs, larger amounts of GLVs are emitted in response to various biotic or abiotic stresses (Loreto & Schnitzler, 2010). Thus, pollinators might use the presence or abundance of GLVs in the VOC bouquet of drought-stressed plants as a cue for avoidance. Alternatively, drought-induced compositional shifts of floral VOCs could have a positive effect on plant–pollinator interactions for other forb species. This may have been the case in drought-treated *C. rotundifolia* plants where VOC compositional shifts were largely a result of increased monoterpene emissions, which are compounds sometimes associated with increased pollinator foraging ability and fidelity to flowers (Schiestl & Roubik, 2003; Theis, 2006; Byers *et al.*, 2014). Examining changes in floral VOC composition, in addition to total VOC emissions, helps provide a more comprehensive understanding of the effects of climate change on plant–pollinator interactions.

Elevated CO₂ generally increased floral visual trait values and had species-specific effects on floral VOC emission and composition, but had no measurable effect on pollinator visitation rates in the single-species forb assemblages. It is surprising that pollinator visitation rates did not increase to CO₂-fertilized *Phacelia hastata* plants despite increases in floral visual traits (plant size and floral display) and total floral VOCs emitted. However, VOC composition was affected, which could alter pollinator visitation patterns in ways not consistent with the effects of climate treatments on floral visual traits and VOC emission rate, as discussed in the preceding discussion paragraphs. Additionally, it is possible that elevated CO₂ affected other, unmeasured floral traits important for pollinator foraging decisions, such as the quantity and quality of pollen and nectar. For example, increased CO₂ concentration can increase or decrease the amount of nectar produced by flowers (reviewed in Jamieson *et al.*, 2017). Changes in the abundance of floral rewards could affect floral VOC composition because both nectar and pollen emit VOCs that pollinators can use to make foraging decisions (Dobson *et al.*, 1999; Raguso, 2008; Wright & Schiestl, 2009). Potentially of additional importance for pollinator foraging decisions are the effects of elevated CO₂ on the quality of floral rewards (i.e. nectar sugar, amino acids and secondary metabolite concentration) and, in turn, on VOC composition. A better understanding of the contribution of nectar and pollen to VOC blends will help clarify the mechanisms by which CO₂ fertilization can affect floral traits important for pollinator visitation.

The species-specific responses of forbs to climate treatments have the potential to alter visiting pollinator community

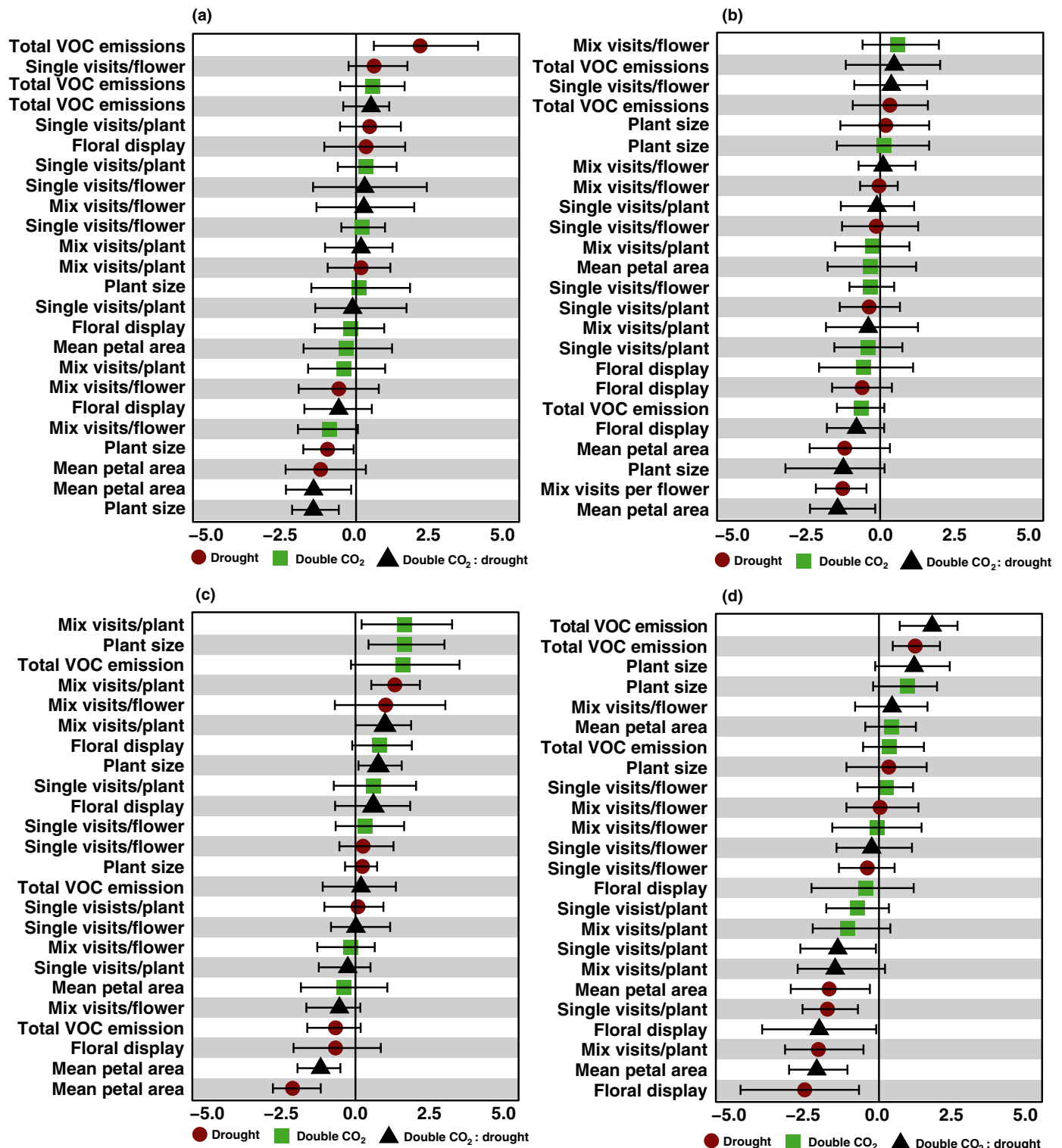


Fig. 5 Standardized effect sizes for each response variable. Mean effect sizes (Hedges g) of plant and pollinator responses in drought (red circle), CO_2 fertilization (green square), and drought + CO_2 fertilization (black triangle) treatments, relative to the control treatment (well-watered + ambient CO_2) for (a) *Campanula rotundifolia*, (b) *Heterotheca villosa*, (c) *Phacelia hastata* and (d) *Potentilla recta*. Plant size, petal area and floral display were measured as visual traits, whereas total volatile organic compound (VOC) emissions were measured as olfactory traits. Pollinator visitation rates were measured per flower and per plant in both single- and multi-species (mix) plant assemblages. Mean effect sizes are sorted by the most negative on bottom to the most positive on top, and are surrounded by bias-corrected 95% confidence intervals. Differences were significant when 95% confidence intervals did not overlap zero.

composition in a multi-species plant community context. Pollinator communities visiting *P. hastata* were more variable (i.e. greater dispersion) across climate treatments, compared to those visiting *P. recta*. These results indicate that pollinator communities visiting *P. hastata* were more responsive to the relatively weak effects of climate treatments on floral traits, whereas pollinator communities visiting *P. recta* were consistent despite relatively strong effects of climate treatments on floral traits. Lower turnover of pollinator visitors (i.e. intraspecific dispersion) between climate treatments for *P. recta* compared to *P. hastata* could indicate that the composition of pollinator communities will be more consistent for some forb species experiencing climate change than others. Climate-induced variation in floral VOCs of *P. hastata* individuals could reduce pollinator attraction or constancy if altered VOC signals are unrecognizable by pollinator species (Wright & Schiestl, 2009). However, the implications of pollinator community consistency for the delivery of pollination services and plant reproductive success are unclear. Different pollinator communities could be more or less capable of delivering sufficient pollination services to plants in the future depending on if taxonomically different pollinators deliver better, worse, or similar pollination services to those plants (Zamora, 2000). Importantly, we did not evaluate differences in reproduction of plants in different climate treatments that were visited by different pollinator communities within this experiment. Overall, pollinators respond to changes in floral traits accompanying drought and CO₂ fertilization, which have the potential to drive variability in the composition of pollinators visiting a forb species.

Despite frequently observing main effects of drought and CO₂ fertilization on floral visual and olfactory traits, interactive effects between drought and CO₂ fertilization were rare. Several possibilities may explain the lack of these interactive effects. First, as noted earlier, the forb species included in this study differ in drought tolerances, which can affect the potential for interactive effects with CO₂ fertilization (Beerling *et al.*, 1996). Second, plants may have been limited by resources other than water and CO₂ (i.e. rooting space, light, soil nutrients), which can influence resource allocation (Korner, 2006). Thus, CO₂ and drought may have had interactive effects on other plant structures that might not influence pollinator visitation, such as belowground biomass or leaf carbohydrates. Future experiments may consider measuring a more comprehensive suite of floral traits to increase our understanding of the interactive effects of drought and CO₂ fertilization on plant–pollinator interactions.

We found that drought and CO₂ have the potential to increase competition for pollinators or facilitate pollinator attraction to forbs depending on the community context in which the plant occurs (i.e. single- or multi-species forb assemblages). Pollinator visitation was reduced to drought-treated *P. recta* and *Heterotheca villosa* (which both display flat, yellow flowers that were reduced in size) in multi-species assemblages relative to single-species assemblages, suggesting that competition for pollinators could increase among plants with similar floral visual traits negatively affected by drought (Caruso, 2000; Ghazoul, 2006). By contrast, pollinator visitation increased to CO₂-treated *P. hastata* plants

(which increased in plant size and floral display) in the multi-species assemblages relative to single-species assemblages. This result suggests that CO₂ fertilization could enhance the facilitative effects of complementary floral visual traits on pollinator visitation to other forbs co-occurring within the multi-species assemblage (Moeller, 2004; Ghazoul, 2006). Taken together, these results suggest that the effects of environmental change on co-occurring floral visual traits influences pollinator visitation, and studying single forb species in isolation likely does not represent the full range of effects of climate change on plant–pollinator interactions (Gornish & Tylianakis, 2013).

Pollinators are essential for the maintenance of biodiversity and functioning of natural ecosystems (Bascompte *et al.*, 2006), but the global decline in pollinator populations threatens ecosystem services provided by pollinators upon which humans and wildlife depend (Potts *et al.*, 2010; Burkle *et al.*, 2013; Kerr *et al.*, 2015). Here we provided evidence from a field experiment demonstrating that climate change can affect plant–pollinator interactions via altered floral visual traits and floral VOCs, depending on the plant community context. Although floral morphological traits have dominated studies evaluating the structure of plant–pollinator interactions, plant–pollinator interactions can be better understood after incorporating floral VOC composition and total floral VOC emissions into traditional frameworks (Raguso, 2008; Junker & Parachnowitsch, 2015). Given the multitude of sensory information that flowers emit to inform pollinator foraging decisions, observing the effects of climate change on a holistic set of floral traits will contribute to a mechanistic understanding of how climate change will affect plant–pollinator interactions.

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Author contributions

W.R.G., J.B.R. and L.A.B. planned and designed the research, performed experiments, conducted fieldwork, analyzed data and wrote the manuscript. W.R.G., J.B.R. and L.A.B. have agreed to final approval of this manuscript and to be accountable for this work.

References

- Ainsworth EA, Rogers A. 2007. The response of photosynthesis and stomatal conductance to rising CO₂: mechanisms and environmental interactions. *Plant, Cell & Environment* 30: 258–270.
- Aspi J, Jakalanemi A, Tuomi J, Siikamäki P. 2003. Multilevel phenotypic selection on morphological characters in a metapopulation of *Silene tatarica*. *Evolution* 57: 509–517.

- Bartomeus I, Ascher JS, Wagner D, Danforth BN, Colla S, Kornbluth S, Winfree R. 2011. Climate-associated phenological advances in bee pollinators and bee-pollinated plants. *Proceedings of the National Academy of Sciences, USA* 108: 20645–20649.
- Bascompte J, Jordano P, Olesen JM. 2006. Asymmetric coevolutionary networks facilitate biodiversity maintenance. *Science* 312: 431–433.
- Bates D, Maechler M, Bolker B, Walker S. 2014. lme4: Linear mixed-effects models using Eigen and S4. *R Package v. 1.1-15* [WWW document] URL <https://cran.r-project.org/web/packages/lme4/index.html>. 1: 1–23.
- Beerling DJ, Heath J, Woodward FI, Mansfield TA. 1996. Drought–CO₂ interactions in trees: observations and mechanisms. *New Phytologist* 134: 235–242.
- Bertin N, Staudt M. 1996. Effect of water stress on monoterpene emissions from young potted holm oak (*Quercus ilex* L.) trees. *Oecologia* 107: 456–462.
- Bujak CM, Dougher TA. 2017. Improved germination of silverleaf phacelia (*Phacelia hastata* Douglas ex Lehm. var. *hastata*). *Native Plants Journal* 18: 42–49.
- Burkle LA, Marlin JC, Knight TM. 2013. Plant–pollinator interactions over 120 years: loss of species, co-occurrence, and function. *Science* 339: 1611–1615.
- Burkle LA, Runyon JB. 2016. Drought and leaf herbivory influence floral volatiles and pollinator attraction. *Global Change Biology* 22: 1644–1654.
- Burkle LA, Runyon JB. 2017. The smell of environmental change: using floral scent to explain shifts in pollinator attraction. *Applications in Plant Sciences* 5: 1600123.
- Byers KJRP, Bradshaw HD, Riffell JA. 2014. Three floral volatiles contribute to differential pollinator attraction in monkeyflowers (*Mimulus*). *Journal of Experimental Biology* 217: 614–623.
- Cane JH, Love B. 2016. Floral guilds of bees in sagebrush steppe: comparing bee usage of wildflowers available for postfire restoration. *Natural Areas Journal* 36: 377–391.
- Carroll AB, Pallardy SG, Galen C. 2001. Drought stress, plant water status, and floral trait expression in fireweed, *Epilobium angustifolium* (Onagraceae). *American Journal of Botany* 88: 438–446.
- Caruso CM. 2000. Competition for pollination influences selection on floral traits of *Ipomopsis aggregata*. *Evolution* 54: 1546–1557.
- Cook BI, Ault TR, Smerdon JE. 2015. Unprecedented 21st century drought risk in the American Southwest and Central Plains. *Science Advances* 1: e1400082.
- Dai AG. 2013. Increasing drought under global warming in observations and models. *Nature Climate Change* 3: 52–58.
- Dobson HE, Danielson EM, Wesep IDV. 1999. Pollen odor chemicals as modulators of bumble bee foraging on *Rosa rugosa* Thunb. (Rosaceae). *Plant Species Biology* 14: 153–166.
- Dudareva N, Negre F, Nagegowda DA, Orlova I. 2006. Plant volatiles: recent advances and future perspectives. *Critical Reviews in Plant Sciences* 25: 417–440.
- Farre-Armengol G, Filella I, Llusia J, Penuelas J. 2013. Floral volatile organic compounds: between attraction and deterrence of visitors under global change. *Perspectives in Plant Ecology Evolution and Systematics* 15: 56–67.
- Forrest J, Inouye DW, Thomson JD. 2010. Flowering phenology in subalpine meadows: does climate variation influence community co-flowering patterns? *Ecology* 91: 431–440.
- Gallagher MK, Campbell DR. 2017. Shifts in water availability mediate plant–pollinator interactions. *New Phytologist* 215: 792–802.
- Gervasi DDL, Schiestl FP. 2017. Real-time divergent evolution in plants driven by pollinators. *Nature Communications* 8: 14691.
- Ghazoul J. 2006. Floral diversity and the facilitation of pollination. *Journal of Ecology* 94: 295–304.
- Gibson RH, Knott B, Eberlein T, Memmott J. 2011. Sampling method influences the structure of plant–pollinator networks. *Oikos* 120: 822–831.
- Gornish ES, Tylianakis JM. 2013. Community shifts under climate change: mechanisms at multiple scales. *American Journal of Botany* 100: 1422–1434.
- Hegland SJ, Nielsen A, Lazaro A, Bjerknes AL, Totland O. 2009. How does climate warming affect plant–pollinator interactions? *Ecology Letters* 12: 184–195.
- Hegland SJ, Totland O. 2005. Relationships between species' floral traits and pollinator visitation in a temperate grassland. *Oecologia* 145: 586–594.
- Herron-Sweet CR, Lehnhoff EA, Burkle LA, Littlefield JL, Mangold JM. 2016. Temporal- and density-dependent impacts of an invasive plant on pollinators and pollination services to a native plant. *Ecosphere* 7: e01233.
- Hoover SER, Ladley JJ, Shchepetkina AA, Tisch M, Gieseg SP, Tylianakis JM. 2012. Warming, CO₂, and nitrogen deposition interactively affect a plant–pollinator mutualism. *Ecology Letters* 15: 227–234.
- Howell AD, Alarcon R. 2007. Osmia bees (Hymenoptera: Megachilidae) can detect nectar-rewarding flowers using olfactory cues. *Animal Behaviour* 74: 199–205.
- Huberty AF, Denno RF. 2004. Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecology* 85: 1383–1398.
- Jablonski LM, Wang XZ, Curtis PS. 2002. Plant reproduction under elevated CO₂ conditions: a meta-analysis of reports on 79 crop and wild species. *New Phytologist* 156: 9–26.
- Jamieson MA, Burkle LA, Manson JS, Runyon JB, Trowbridge AM, Zientek J. 2017. Global change effects on plant–insect interactions: the role of phytochemistry. *Current Opinion in Insect Science* 23: 70–80.
- Junker RR, Bluthgen N. 2008. Floral scents repel potentially nectar-thieving ants. *Evolutionary Ecology Research* 10: 295–308.
- Junker RR, Bluthgen N. 2010. Floral scents repel facultative flower visitors, but attract obligate ones. *Annals of Botany* 105: 777–782.
- Junker RR, Hocherl N, Bluthgen N. 2010. Responses to olfactory signals reflect network structure of flower–visitor interactions. *Journal of Animal Ecology* 79: 818–823.
- Junker RR, Parachnowitsch AL. 2015. Working towards a holistic view on flower traits – how floral scents mediate plant–animal interactions in concert with other floral characters. *Journal of the Indian Institute of Science* 95: 43–67.
- Kearns CA, Inouye DW. 1993. *Techniques for pollination biologists*. Louisville, CO, USA: University Press of Colorado.
- Kerr JT, Pindar A, Galpern P, Packer L, Potts SG, Roberts SM, Rasmont P, Schweiger O, Colla SR, Richardson LL et al. 2015. Climate change impacts on bumblebees converge across continents. *Science* 349: 177–180.
- Kessler A, Halitschke R. 2009. Testing the potential for conflicting selection on floral chemical traits by pollinators and herbivores: predictions and case study. *Functional Ecology* 23: 901–912.
- Kirby KN, Gerlanc D. 2013. BootES: an R package for bootstrap confidence intervals on effect sizes. *Behavior Research Methods* 45: 905–927.
- Korner C. 2006. Plant CO₂ responses: an issue of definition, time and resource supply. *New Phytologist* 172: 393–411.
- Larue AAC, Raguso RA, Junker RR. 2016. Experimental manipulation of floral scent bouquets restructures flower–visitor interactions in the field. *Journal of Animal Ecology* 85: 396–408.
- Lavory AV, Staudt M, Schnitzler JP, Landais D, Massol F, Rocheteau A, Rodriguez R, Zimmer I, Rambal S. 2009. Drought reduced monoterpene emissions from the evergreen Mediterranean oak *Quercus ilex*: results from a throughfall displacement experiment. *Biogeosciences* 6: 1167–1180.
- Loreto F, Fischbach RJ, Schnitzler JP, Ciccioli P, Brancaleoni E, Calfapietra C, Seufert G. 2001. Monoterpene emission and monoterpene synthase activities in the Mediterranean evergreen oak *Quercus ilex* L. grown at elevated CO₂ concentrations. *Global Change Biology* 7: 709–717.
- Loreto F, Schnitzler JP. 2010. Abiotic stresses and induced BVOCs. *Trends in Plant Science* 15: 154–166.
- McIver J, Erickson K. 2012. Pollination biology of *Potentilla recta* (sulfur cinquefoil) and its cooccurring native congener *Potentilla gracilis* in Northeastern Oregon. *Psyche: A Journal of Entomology* 2012: 281732.
- Memmott J, Craze PG, Waser NM, Price MV. 2007. Global warming and the disruption of plant–pollinator interactions. *Ecology Letters* 10: 710–717.
- Moeller DA. 2004. Facilitative interactions among plants via shared pollinators. *Ecology* 85: 3289–3301.
- Nesom GL. 2006. Taxonomic overview of the *Heterotheca villosa* complex (Asteraceae: Astereae). *SIDA, Contributions to Botany* 22: 367–380.
- Nilsson LA. 1988. The evolution of flowers with deep corolla tubes. *Nature* 334: 147–149.
- Oksanen J, Kindt R, Legendre P, O'Hara B, Stevens MHH, Oksanen MJ, Suggests M. 2007. The vegan package. *Community Ecology Package* 10: 631–637.

- Ormeno E, Mevy JP, Vila B, Bousquet-Melou A, Greff S, Bonin G, Fernandez C. 2007. Water deficit stress induces different monoterpene and sesquiterpene emission changes in Mediterranean species. Relationship between terpene emissions and plant water potential. *Chemosphere* 67: 276–284.
- Pachauri RK, Allen MR, Barros VR, Broome J, Cramer W, Christ R, Church JA, Clarke L, Dahe Q, Dasgupta P. 2014. *Climate change 2014: synthesis report. Contribution of Working Groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change*. Geneva, Switzerland: IPCC.
- Pederson GT, Graumlich LJ, Fagre DB, Kipfer T, Muhlfeld CC. 2010. A century of climate and ecosystem change in Western Montana: what do temperature trends portend? *Climatic Change* 98: 133–154.
- Penuelas J, Staudt M. 2010. Bvocs and global change. *Trends in Plant Science* 15: 133–144.
- Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE. 2010. Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution* 25: 345–353.
- R Development Core Team. 2014. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Raguso RA. 2008. Wake up and smell the roses: the ecology and evolution of floral scent. *Annual Review of Ecology Evolution and Systematics* 39: 549–569.
- Scaven VL, Rafferty NE. 2013. Physiological effects of climate warming on flowering plants and insect pollinators and potential consequences for their interactions. *Current Zoology* 59: 418–426.
- Schiestl FP. 2015. Ecology and evolution of floral volatile-mediated information transfer in plants. *New Phytologist* 206: 571–577.
- Schiestl FP, Peakall R. 2005. Two orchids attract different pollinators with the same floral odour compound: ecological and evolutionary implications. *Functional Ecology* 19: 674–680.
- Schiestl FP, Roubik DW. 2003. Odor compound detection in male euglossine bees. *Journal of Chemical Ecology* 29: 253–257.
- Schweiger O, Biesmeijer JC, Bommarco R, Hickler T, Hulme PE, Klotz S, Kühn I, Moora M, Nielsen A, Ohlemüller R. 2010. Multiple stressors on biotic interactions: how climate change and alien species interact to affect pollination. *Biological Reviews* 85: 777–795.
- Shetler SG. 1979. Pollen-collecting hairs of *Campanula* (Campanulaceae). I - Historical review. *Taxon* 28: 205–215.
- Theis N. 2006. Fragrance of canada thistle (*Cirsium arvense*) attracts both floral herbivores and pollinators. *Journal of Chemical Ecology* 32: 917–927.
- Tylianakis JM, Didham RK, Bascompte J, Wardle DA. 2008. Global change and species interactions in terrestrial ecosystems. *Ecology Letters* 11: 1351–1363.
- Wright GA, Schiestl FP. 2009. The evolution of floral scent: the influence of olfactory learning by insect pollinators on the honest signalling of floral rewards. *Functional Ecology* 23: 841–851.
- Xiao Y, Wang Q, Erb M, Turlings TCJ, Ge L, Hu L, Li J, Han X, Zhang T, Lu J *et al.* 2012. Specific herbivore-induced volatiles defend plants and determine insect community composition in the field. *Ecology Letters* 15: 1130–1139.
- Zamora R. 2000. Functional equivalence in plant–animal interactions: ecological and evolutionary consequences. *Oikos* 88: 442–447.
- Zuur A, Ieno E, Walker N, Saveliev A, Smith G. 2009. GLM and GAM for Count Data. In: Gail M, Krickeberg K, Samet JM, Tsiatis A, Wong W, eds. *Mixed effects models and extensions in ecology with R*. New York, NY, USA: Springer Science and Business Media, 209–239.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Table S1 Volatile compounds released by *Campanula rotundifolia* flowers subjected to the control, drought, CO₂ fertilization, and drought + CO₂ fertilization treatments

Table S2 Volatile compounds released by *Heterotheca villosa* flowers subjected to the control, drought, CO₂ fertilization, and drought + CO₂ fertilization treatments

Table S3 Volatile compounds released by *Phacelia hastata* flowers subjected to the control, drought, CO₂ fertilization, and drought + CO₂ fertilization treatments

Table S4 Volatile compounds released by *Potentilla recta* flowers subjected to the control, drought, CO₂ fertilization, and drought + CO₂ fertilization treatments

Table S5 Summary of pollinator species observed visiting each of the treatment plant species in the single species assemblage

Table S6 Summary of pollinator species observed visiting each of the treatment plant species in the mixed species assemblage

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