Warming, CO$_2$, and nitrogen deposition interactively affect a plant-pollinator mutualism

Abstract
Environmental changes threaten plant-pollinator mutualisms and their critical ecosystem service. Drivers such as land use, invasions and climate change can affect pollinator diversity or species encounter rates. However, nitrogen deposition, climate warming and CO$_2$ enrichment could interact to disrupt this crucial mutualism by altering plant chemistry in ways that alter floral attractiveness or even nutritional rewards for pollinators. Using a pumpkin model system, we show that these drivers non-additively affect flower morphology, phenology, flower sex ratios and nectar chemistry (sugar and amino acids), thereby altering the attractiveness of nectar to bumble bee pollinators and reducing worker longevity. Alarmingly, bees were attracted to, and consumed more, nectar from a treatment that reduced their survival by 22%. Thus, three of the five major drivers of global environmental change have previously unknown interactive effects on plant-pollinator mutualisms that could not be predicted from studies of individual drivers in isolation.

Keywords
Bombus, bottom-up, carbon dioxide, Cucurbita, ecosystem service, global change, global warming, higher-order effects, pollination, species interactions, temperature.

INTRODUCTION
Ecosystems worldwide are undergoing unprecedented environmental change (MEA 2005) caused by habitat fragmentation, nitrogen (N) deposition, biotic invasions, increasing atmospheric carbon dioxide (CO$_2$) and climatic changes. Each of these drivers threatens biodiversity (Sala et al. 2000), but disruptions to crucial interactions between species may even precede biodiversity loss (Tylianakis et al. 2008), even though the arrangement of interactions within a whole community can confer system stability. Moreover, it is becoming increasingly apparent that important interaction effects cannot be predicted from the independent effect of each driver (Tylianakis et al. 2008; Schweiger et al. 2010). For example, N and temperature both affect plant physiological responses to elevated CO$_2$ (Reich et al. 2006; Zvereva & Kozlov 2006), with potential cascading effects on species that interact with plants (Tylianakis et al. 2008).

Species interactions underpin numerous ecosystem services on which human well-being depends. For example, animal pollination of plants is crucial for maintaining plant diversity (Ollerton et al. 2011), and provides an essential ecosystem service through pollination of three quarters of global food crops (Klein et al. 2007). However, studies have shown that changes such as habitat modification and pesticide use (Aguilar et al. 2006; Brittain et al. 2010), plant and pollinator invasions (Lopezaraiza-Mikel et al. 2007; Aizen et al. 2008), or phenological mismatches due to climate change (Memmott et al. 2007) can potentially threaten this mutualism, either by reducing pollinator diversity or by altering the ability of native pollinators to encounter and successfully pollinate plants. Consequently, the decline of pollinators may be paralleled by declines in the wild and agricultural plants that rely on them (Biesmeijer et al. 2006; Potts et al. 2010).

Despite widespread evidence of pollinator decline due to the above drivers, the results of previous studies are surprisingly variable (Tylianakis et al. 2008; Winfree et al. 2011). This may be due to the correlative, non-experimental approaches used (of around 670 studies recently reviewed by Winfree et al. (2011), only a handful were experimental), which cannot reveal mechanisms behind pollinator responses. Furthermore, studies testing effects of a single driver cannot detect interactions. Yet, these and other examples of the effects of single global change drivers on plant-pollinator mutualisms and pollinator fitness (Rusterholz & Erhardt 1998; Mevi-Schutz & Erhardt 2005; Schweiger et al. 2010) raise concerns that a suite of drivers acting simultaneously could further alter this relationship, and that altered attractiveness of flowers or nutritional rewards to pollinators could dramatically alter pollinator fitness and plant-pollinator mutualisms.

Herein we examine the interactive effects of three global change drivers (CO$_2$ enrichment, N deposition and increased temperature) on a pollinator-plant mutualism, using a series of experiments to test hypotheses specific to various mechanistic pathways (Fig. 1). Single-driver studies suggest that elevated CO$_2$ (and its fertilisation effects) could affect pollination interactions by altering plant morphology, phenology and nectar production (Rusterholz & Erhardt 1998), thereby causing a general increase in reproductive investment and floral abundance (Jablonski et al. 2002), although gene suppression at elevated CO$_2$ may delay flowering (Springer et al. 2008). In addition to higher carbohydrate levels from CO$_2$ enrichment, nectar volume and concentration may be affected by temperature (Pacini et al. 2003) through more rapid evaporation and changes to plant physiology. Furthermore, enhanced plant growth through N enrichment can increase flower abundance, duration and size (Burkle & Irwin 2009a), and N enrichment can affect the concentration and composition of amino acids in nectar (Gardener & Gillman 2001), potentially altering pollinator preferences (Inouye & Waller 1984; Gardener & Gillman 2002). We test for changes to flower morphology and phenology, but do not test the known effects of these variables on pollinator visitation (Fig. 1). Subsequently, we use controlled laboratory experiments to
Figure 1 Pathways through which global environmental change drivers (climate, nitrogen deposition, CO2 and their interactions) may affect pollination mutualisms. Dashed arrows represent pathways (4, 5) examined in other studies (see Fig. S3 for further details), solid arrows represent pathways examined in this study. (1) Multiple impacts of the drivers on flower size, abundance, nectar volume and sex ratios. (2) Flowering phenology was affected by all three drivers (3) The drivers interactively affected nectar chemistry (sugar and amino acid concentration and sugar composition). Bees tended to more frequently visit (6) and consume nectar (7) from the elevated N treatment, and this effect interacted with CO2 and temperature. (8) Nectar from the elevated N and elevated CO2 treatments significantly reduced worker bee longevity.

test whether or not interactive effects of the global change drivers alter nectar chemistry in ways that affect pollinator visitation, nectar consumption and longevity.

We use pumpkin as a model system because (1) it depends strongly on effective bee pollination for fruit production (Hoehn et al. 2008), (2) cucurbits are cultivated over a wide geographic range for their food and economic value, and (3) their large unisexual flowers produce enough nectar to allow detailed biochemical analysis. We show experimentally that the three drivers have main and interactive effects on various attributes of flower structure and nectar chemistry, attractiveness of the nectar to pollinators and worker longevity. We chose the bumble bee Bombus terrestris (L.) as a model pollinator because of its near global distribution, importance for the pollination of both wild and cultivated plants (Velthuis & van Doorn 2006) and the commercial availability of colonies to facilitate experimentation. Despite the importance of bumble bees as generalist pollinators, many wild populations are facing decline (Goulson et al. 2008). We show that N deposition, warming and elevated CO2 have previously unknown effects on a pollination mutualism, with interactions that could not have been predicted from studies of any of these drivers in isolation.

MATERIALS AND METHODS

Flower and nectar analyses

Pumpkin plants (Cucurbita maxima Var. ‘Little Cutie’) were grown in mineral soil (Supplementary Material S1) in pots within custom-built (60 × 60 × 60 cm) controlled-environment chambers under factorial combinations of three global change treatments, each with two levels (ambient vs. elevated according to estimates for the end of this century). The three drivers were: CO2 (360 vs. 700 ppm), N (0.19 vs. 0.57 g N added per pot in the form of ammonium nitrate, elevated level equivalent to 100 kg N ha⁻¹, or 2 years at typical global deposition levels; Vitousek et al. 1997) and temperature (19 °C or 23 °C, equivalent to the average current and predicted future summer temperature in the study region; Mullan et al. 2008). The 0.19 g N per pot added to the control treatment ensured that control plants were not too N limited to produce flowers (a preliminary trial in which we deprived plants of additional N produced no flowers). The factorial design produced eight treatment combinations, each with two replicate plants. The limit of two replicate plants was for logistical reasons, although the fully factorial design maximised power, as effects were tested over an error term with degrees of freedom arising from the total number of replicates across all treatments (15 total d.f.). Nevertheless, low power would, if anything, provide a more conservative test, reducing the likelihood of finding any significant effects. Plants were lightly watered daily to keep soil moist, and grown under ‘Grolux’ tubes with a 16:8 h light : dark regime until they ceased flowering. The number of days to onset of flowering, flower diameter and the total number and sex of flowers produced were measured. To avoid contaminating the nectar with pollen, stamens were removed from the flowers (by cutting the tip of the flower at an appropriate point along its length) on the afternoon prior to flower
opening. Cut flowers were not used for flower size analyses. Nectar was then extracted from open flowers using microcapillary tubes on the following morning, and the volume of nectar produced per flower was recorded (Supplementary Material S1). For nectar composition analyses, nectar from the first male flower from each plant was used. Sugar and amino acid composition and concentration were measured using High Pressure Liquid Chromatography. Amino acids were derivatised using the AccQtag protocol (see Supplementary Material S1) and sugars analysed according to AOAC International protocols (OMA, 18th Edition; method 977.20; analyses conducted by the Cawthron Institute, Nelson, New Zealand).

To test the response of nectar amino acid composition (presence and concentration of each amino acid) to the three global change drivers, we first used a principal component analysis to reduce the number of variables from concentrations of 21 amino acids to the four principal component axes that each explained over 5% of the variance in the data. These four orthogonal axes explained a cumulative 86.93% of the variance in amino acid composition (Table S2). We used the scores of these four axes as response variables in a multivariate analysis of variance (MANOVA), with N, CO₂ and temperature treatments (current vs. elevated) as predictors, and all interactions included. We also tested for changes to sugar composition using a MANOVA with the same predictor variables, and the concentrations of sucrose, fructose and glucose as the multivariate response. After testing the overall response of sugar composition to the global change treatments, we tested for changes in the ratios of glucose to fructose, and of sucrose to fructose and glucose combined, as these are reported to be important determinants of attractiveness to pollinators (Baker & Baker 1983). We tested for effects of the three global change treatments on these sugar ratios, as well as total sugar and amino acid concentrations and various aspects of flower morphology and phenology using generalised linear models. In the case of sugar ratios, and flower sex ratios, we used a binomial error distribution with a logit link function. For the number of flowers per plant we used a Poisson error and log link, and for the nectar volume, total sugars, total amino acids, time to flowering and flower diameter (average of all the uncut male flowers per plant) we used Gaussian (normal) errors. In all cases, initial models included all interaction terms, and we simplified models by removing all non-significant interactions and main effects, retaining only those main effects involved in higher order interactions to satisfy the principal of marginality. The best-fitting model was obtained by minimising the Akaike Information Criterion (AIC), and final models were checked for overdispersion. These analyses were conducted in the base and ade4 (Dray & Dufour 2007) packages of the R v.2.10.1 environment (R Development Core Team 2006).

**Bumble bee preferences**

To test whether or not the global change drivers altered the attractiveness of nectar to pollinators, and to isolate the pathway through which this occurs, we synthesised nectar solutions and offered these to bumble bees in a choice experiment. Nectar solutions were generated by adding sugar and amino acid standards to water, in concentrations that mimicked the average concentrations of amino acids and sugars produced by the plants grown under each of the eight global change treatment combinations (see Table S1). We used synthetic nectar to ensure that any effects on pollinators were caused by the changes in nectar components that we measured (sugars and amino acids), thereby excluding the possibility that any unknown compounds in nectar confounded these effects. We then tested the preference of bumble bees (12 colonies of *Bombus terrestris* Zonda Resources, New Zealand) for synthetic nectar based on each of the global change treatments. The experimental nectar solutions were presented to the bees in a flight cage (175 × 175 × 175 cm) comprising three white polyester/nylon mesh sides and roof, one clear vinyl observation panel and a black mesh floor panel (Bioquip Inc, Rancho Dominguez, CA, USA). Nectar solutions were offered in an array (50 × 50 cm) containing eight randomised ‘flower clusters’ (each cluster comprised four artificial ‘flowers’ representing one of the eight nectar treatment combinations), with 7.5 cm between each cluster and its nearest neighbour. Artificial flowers were constructed from 1.5 mL clear centrifuge tubes with lids removed, containing 10 µL of ‘nectar’ solution embedded in a polystyrene base covered with green paper. All treatments were presented to the bees concurrently, and the position of each treatment cluster was randomised for each colony. Bee colony boxes were attached by a mesh tunnel to a flight cage, and a gate in the tunnel allowed us to control access of the bees to the flight cages. Flight cages and bee colonies were kept in a glasshouse (night time minimum temperature 15 °C, daytime maximum 23 °C, good flight temperatures for bumble bees), both during and between experiments. All experiments were conducted between 10:00 h and 12:00 h on sunny days, with three colonies tested simultaneously (in separate cages) per day. All colony boxes had their internal sugar solution feeder removed 36 h prior to the experiment to encourage foraging, but still had access to internal ‘honey pots’ containing sugar solution and pollen they had stored.

Arrays were presented to a naive colony the afternoon prior to the experiment. The foragers were allowed access to the array for 2 h to learn to forage on the experimental flowers. Arrays presented during this learning period had the same arrangement of treatments as in the experimental period. To test nectar preferences, bees were allowed to forage on a newly filled array for 10 min after the first forager landed on a ‘flower’. The number of visits to each flower was recorded for the duration of the experimental period. After 10 min, the volume of nectar consumed from each flower was recorded. Each colony was tested once in this manner. In each experiment at least five bees visited at least one experimental flower, and visitation rates and nectar consumption were pooled for the four flowers per treatment cluster. On average, each nectar treatment received between 2.4 and 4.5 visits per replicate (i.e. per 10 min of colony exposure), making an average of 29 visits across all treatments within a replicate.

We used generalised linear mixed effects models (GLMs), conducted in the lme4 package (Bates & Maechler 2009) in R, to test how visitation frequency and nectar consumption responded to driver-induced changes to nectar. Response variables were either the proportion of available nectar consumed (using binomial errors) or the number of visits (using Poisson errors). These predictors were significantly correlated ($P = 0.002$), so elevated nectar consumption could represent a greater number of visits with a constant consumption rate per visit. However, this possibility could not be tested, because the amount of nectar consumed was not measured after each visit, and any averaged measure (total consumption divided by total number of visits) would be confounded by a lack of replenishment of nectar (i.e. later visitors would not be able to consume large quantities once nectar was already depleted), and by an elevated number of visits if bees ‘took turns’ at foraging. Day and colony identity were included as crossed random effects, to account for the possibility that multiple colonies tested on a single day may have responded to specific conditions (e.g. weather), and also to
incorporate non-independence of consumption of different nectar treatments by a given colony. We also included N, CO2 and temperature treatments (current vs. elevated) and their interactions as fixed effects, simplifying models using the procedure outlined above. We used several additional models (replacing the fixed effects of drivers with those of sugar ratios or total sugar and amino acid concentrations) to understand better which specific aspects of nectar had the greatest effect on attractiveness to pollinators.

**Bumble bee longevity**

The effects of changes to nectar on pollinator fitness were tested by measuring the longevity of caged *B. terrestris* workers, each fed on one of the eight nectar treatments. Newly-emerged bees were collected from a total of 16 colonies (Zonda Resources, New Zealand), marked with a unique identifier tag, and placed into (30 × 30 × 30 cm) mesh cages. Each cage contained bees from a minimum of five different source colonies, and the experiment consisted of four replicates of each of the eight synthetic nectar treatments (Table S1). One replicate was conducted with ten bees per cage, then due to limited supplies of colonies, five bees were used for the subsequent three replicates. Each cage had both water and nectar treatment feeders, and bees had access to both nectar and water *ad libitum*. The nectar in each cage was changed every 3 days to prevent fermentation. Cages were monitored daily for bee mortality. Since bees were unable to return to their source colony, the longevity we recorded may not represent their longevity under natural conditions when they have access to colony resources. However, the purpose of this experiment was to determine relative changes in longevity across treatments, so we were confident that keeping groups of bees isolated from other colony members would not bias any observed differences between treatments.

The effects of nectar treatments on bee longevity were tested using GLMMs, with survival days of each bee as the response, normal (Gaussian) errors and nectar treatments representing the eight combinations of global change drivers and their interactions as levels of a fixed factor. The number of bees per cage was included as a covariate to control for potential biases arising from one replicate of each treatment having 10 bees and the remaining replicates five bees. Cage number and the identity of the colony from which a bee was sourced were also included as crossed random effects, to account for the non-independence of bees within a cage, and for potential genetic (colony-specific) traits affecting longevity. This maximal model was simplified by removing non-significant fixed terms until no further reduction in AIC score could be obtained. Due to issues associated with calculating *P*-values from mixed effects models with a Gaussian error structure, we used Markov Chain Monte Carlo (MCMC) resampling to estimate *P*-values, though these gave qualitatively identical results to the less-conservative *P*-values obtained from the traditional *t*-test for each parameter estimate using the upper bounds of degrees of freedom. The MCMC procedure was carried out using the pvals.fnc function in the languageR package (Baayen 2010) for the R environment.

**RESULTS**

**Flower growth**

The experimental drivers significantly influenced plant growth, C : N ratio (Supplementary Material S2.1), and flowering attributes such as phenology (timing of first flowers) and morphology (Table S3a). The number of flowers produced by each plant increased with increasing N (*Z* = 6.55, *P* < 0.001) and temperature (*Z* = 3.01, *P* = 0.003), whereas elevated CO2 slightly weakened the temperature effect (*Z* = −1.79, *P* < 0.073) and drove production of smaller flowers, with less nectar (effect of CO2 on flower diameter *F* _1,11_ = 5.06, *P* = 0.046, nectar volume *F* _1,8_ = 11.95, *P* = 0.0086, number of flowers *Z* = 0.25, *P* = 0.803, Fig. 1). Elevated temperature (T) reduced the negative effect of CO2 on the number of flowers produced (*T* × CO2 interaction: *F* _1,10_ = 5.08, *P* = 0.048). Nitrogen addition increased flower diameter (*F* _1,11_ = 6.02, *P* = 0.032), whereas elevated temperature had a negative effect (*F* _1,10_ = 16.13, *P* = 0.002). Elevated temperature also caused a decrease in the ratio of female (fruit-producing) to male flowers (*F* _1,8_ = 11.78, *P* = 0.040). Furthermore, elevated N and temperature both accelerated the onset of flowering (by an average of 15.8 and 7.5 days respectively, *T*: *F* _1,8_ = 26.03, *P* = 0.009; N: *F* _1,8_ = 5.90, *P* = 0.041; whereas CO2 delayed the onset of flowering by an average of 10.8 days (*F* _1,8_ = 12.12, *P* = 0.008). Nectar volume was greater under elevated temperature (*F* _1,8_ = 10.62, *P* < 0.012), but lower under CO2 (*F* _1,8_ = 11.95, *P* = 0.0086), however, the positive effect of elevated temperature was greater at low N levels (negative N × T interaction: *F* _1,8_ = 5.78, *P* = 0.043; Fig. S2.2a).

**Nectar composition**

The drivers interactively affected various aspects of nectar chemistry (Table S3b). Total amino acid and sugar concentrations were not affected by the separate effects of temperature and N, but the effect of temperature became positive under elevated N (T × N interaction: total sugars *F* _1,9_ = 6.18, *P* = 0.035; total amino acids *F* _1,11_ = 4.78, *P* = 0.049; Figs S2.2b and S2.2c). Elevated CO2 also made the effect of temperature on sugar concentration become positive (T × CO2 interaction: *F* _1,9_ = 7.69, *P* = 0.022). Despite this change in total concentration, the relative composition of amino acids did not change significantly under any of the global change treatments (all predictors were removed during model simplification). There was a net effect of CO2 on nectar sugar composition, but when the concentrations of specific sugars were analysed individually, there was no effect of any drivers on sucrose (the largest sugar constituent in nectar). However, there was a significant positive effect of elevated CO2 on the concentrations of glucose and fructose (glucose *F* _1,9_ = 6.13, *P* = 0.035; fructose *F* _1,9_ = 5.16, *P* = 0.049) although this effect was reduced by elevated temperature (T × CO2 interaction: glucose *F* _1,9_ = 11.95, *P* = 0.007; fructose *F* _1,9_ = 11.85, *P* = 0.007). There was also a negative interaction between temperature and N (N × T interaction: glucose *F* _1,9_ = 11.62, *P* = 0.008; fructose *F* _1,9_ = 8.03 *P* = 0.02). There were significant negative effects of both N and temperature on the ratio of glucose to fructose (effect of N: *F* _1,8_ = 10.33, *P* = 0.0123, effect of T: *F* _1,8_ = 21.58, *P* = 0.0017, and a nearly significant positive effect of N on the ratio of sucrose to glucose + fructose (N: *F* _1,10_ = 4.79, *P* = 0.053). In contrast, CO2 had a negative effect on the ratio of sucrose to glucose + fructose, and there was a significant temperature × CO2 interaction (CO2: *F* _1,10_ = 7.885, *P* = 0.0123, effect of T: *F* _1,10_ = 23.0, *P* = 0.0017).

**Bumble bee preferences and longevity**

There was a non-significant (*P* < 0.07) tendency for bees to visit the artificial flowers containing the nectar recipe from the elevated N treatment more frequently, and they consumed significantly more nectar...
Elevated consumption by pollinators (Fig. 3). At ambient CO2 levels, elevated N effect on the volume of nectar was marginally non-significant (CO2 x temperature interaction: $t_{10} = 1.75$, $P_{MCMC} = 0.086$). Finally, total sugar concentration had a negative effect on bee longevity ($t_{10} = -2.03$, $P_{MCMC} = 0.032$).

**DISCUSSION**

Global environmental changes are predicted to continue and accelerate (MEA 2005). We have demonstrated that plant responses to these conditions can directly and indirectly affect the benefits associated with a pollination mutualism through various pathways (Fig. S3). The three global change drivers – climate warming, N deposition, and carbon dioxide enrichment – all had significant, non-additive impacts on both plants and their interactions with pollinators. Plant reproductive traits were affected, with resulting impacts on pollinator nectar preferences and consumption, and pollinator longevity (Supplemental Material S4). The high frequency of interaction effects we observed among different drivers may partly explain the variability of previously reported effects of single drivers on characters such as flowering times (Springer & Ward 2007) and nectar volume (Rusterholz & Erhardt 1998; Dag & Eisikowitch 2000).

The three drivers each affected flower timing, with CO2 enrichment delaying flowering (possibly through altered gene expression; Springer et al. 2008), whereas N enrichment and increased temperatures both accelerated the date of first flowering. Phenological mismatches between plants and pollinators are a commonly-predicted result of climate change (Memmott et al. 2007; Hegland et al. 2009), potentially driving pollen limitation in some early-flowering plants (Rafferty & Ives 2011). Our results suggest that other drivers of environmental change (namely CO2 enrichment and N deposition) may similarly disturb flowering phenology.

Floral attributes, such as the number of flowers and corolla size, influence pollinator attraction to plants (with bumble bees in particular preferring larger flowers; Goulson 2003), and these attributes were significantly affected by all three drivers. Furthermore, we found a

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**Figure 2** Impact of nitrogen enrichment on a plant-pollinator mutualism. Changes to pumpkin (Cucurbita maxima) nectar constituents alter pollinator (Bombus terrestris) visitation to artificial flowers, nectar consumption and longevity. The nectar from the elevated nitrogen treatment increased bumble bee visitation and nectar consumption; however, it decreased worker bee longevity.

![Nitrogen Levels](image)

**Figure 3** Interactive effects of drivers on nectar consumption. Interaction plots showing the impact of environmental change drivers (ambient vs. elevated levels of nitrogen, CO2 and temperature) on the amount of nectar consumed by bumble bees. (a) ambient CO2 levels (360 ppm) and (b) elevated CO2 levels (700 ppm). Mean values (±SEM) are calculated from parameter estimates (with an inverse link function applied) of a generalised linear mixed effects model, after controlling for random effects of bee colony and cage. Elevated nitrogen generally increased nectar consumption (significant main effect of N), however, this effect was modified by both temperature and CO2, as there was a significant three-way interaction among the drivers.
number of main and interactive effects of the drivers on the composition of nutrients in nectar (sugar and amino acid concentrations). The relative importance of flowering traits (e.g. size, phenology) vs. nectar composition in attracting pollinators is unclear. Therefore, even though previous studies (e.g. Inouye & Waller 1984) have also used synthetic nectar to test specific bee preferences, it is important to consider that they may be overwhelmed in nature by the well-known effects of changes to flower morphology or phenology. The subtle interactive effects of the drivers on nectar sugar concentration contrast with observations that single drivers tend to affect the total amount of sugars in nectar through changes to nectar volume, rather than sugar concentration (Rusterholz & Erhardt 1998; Dag & Eisikowitch 2000). However, total sugar and amino acid concentration may be less important for stimulating or repelling insect nectar feeding than are concentrations of particular amino acids (Petanidou et al. 2006). Alarmingly, the nectar that was most frequently visited by bumble bees, and consumed in the greatest quantity by the colony, was that from the elevated N treatments, which caused a significant reduction in bee longevity. The elevated N treatment did not have higher concentrations of phenylalanine (which stimulates feeding; Inouye & Waller 1984) or lower concentrations of amino acids such as asparagine, which behave as general repellents (Petanidou et al. 2006) (Table S1). However, it did have higher sucrose to hexose (fructose and glucose) ratios, which previous work (Petanidou 2005) and our results suggest can be more attractive to bees. Consumption of nectar is traditionally thought to be correlated with bee survival (Barker & Lehner 1974). Thus, altered preference for nectar under rapidly-changing environmental conditions may lead pollinators to select less-nutritious floral resources, potentially reducing their fitness.

Nectar sugar composition is known to be related to the type, number and diversity of pollinator species attracted to a particular plant (Petanidou et al. 2006) (i.e. pollination ‘syndrome’), and the duration of pollinator visits received by flowers (Baker & Baker 1983). For example, sucrose-dominated nectars, such as that of Cucurbita, are preferred by large bees (Baker & Baker 1983), potentially explaining the preference of B. terrestris for higher sucrose : hexose ratios. Although the composition of floral nectar amino acids is thought to be consistent within a species (Baker & Baker 1977), the total concentration is more variable (Gardener & Gillman 2001), and our results indicate that this concentration can be altered by interactive effects of global environmental changes. We found that the concentration of both glucose and fructose were increased by CO2 and N, thereby reducing the ratio of sucrose to hexoses and attractiveness to bumble bees. Changes in the attractiveness of nectar to different species carry strong implications for plant fitness, which depends on pollinator diversity and abundance (Hoehn et al. 2008). Previous work found that elevated N altered the relative visitation frequency of different plant species by pollinators (Burkle & Irwin 2009b), and suggested that the effects of multiple interacting drivers on plant attractiveness to pollinators required further attention. Although we only found a marginally non-significant effect of N on pollinator visitation, we found a large number of interactions among the drivers in their effect on nectar consumption (Table S3C).

We found an interactive effect of elevated temperature and N on the total amino acid concentration, and amino acids in nectar attract a variety of pollinators, such as butterflies, birds, bats and bees (Erhardt & Rusterholz 1998). The amino acid content of nectar is particularly important for strictly nectar-feeding pollinators that do not utilise pollen or insect prey as a source of protein. Thus, nectar amino acid content affects butterfly fecundity (Mevi-Schutz & Erhardt 2005), and is higher in flowers that do not offer additional extra-floral protein sources (Baker & Baker 1986). In contrast, bumble bees require ongoing access to proteinaceous food, making them dependent on pollen consumption for maximum survival (Smeets & Duchateau 2003). Despite this requirement, and the absence of pollen in the diet of caged bees in this study, we found no effect of amino acid concentration on bee longevity. This suggests that nectar amino acids may not play such a large role for pollen feeders as for strict nectar feeders, although global change drivers may also affect bumble bee longevity through changes to pollen amount or nutritious value, and this requires further investigation.

There are a number of caveats to the findings presented herein. First, practical restrictions meant that changes to nectar composition were based on few replicates of each treatment combination, so caution must be exercised when generalising these results. Second, we measured effects on worker bees, which may not necessarily translate to similar effects at the colony level. For example, colonies may adjust their foraging patterns and intensity to compensate for altered food quality (Molet et al. 2008), and interactions among colony members may affect visitation frequency of artificial flowers. Similarly, our experiments did not allow pollinators to compensate for altered nectar quality by foraging on pollen or different plant species, thus they may have magnified effects of nectar changes on worker longevity. Finally, if all plant species are affected in a similar way, pollinators would never have to choose between different nectar compositions. Thus, future studies should examine differences in the response of multiple plant and pollinator species.

The total economic value of crop pollination worldwide is estimated to be over €153 billion annually (Gallai et al. 2009), and 70% of agricultural crops depend on pollinators (Klein et al. 2007). There is growing concern about global decline in many pollinator species (Potts et al. 2010), and our results highlight novel mechanisms through which human changes to the environment may alter plant-pollinator mutualisms. The high frequency of interaction effects we found indicates that current environmental changes will have manifold effects on pollination services. These interactions among drivers may be as important as the main effects, and studies of multiple drivers will continue to reveal complex and unanticipated outcomes.

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AUTHORSHIP

S.H. and J.M.T. designed the experiments, conducted analyses and wrote the manuscript. S.H. and J.L. conducted the nectar experiments with some help from J.M.T. S.H. conducted the bee experiments. S.G.

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and A.S. adapted the method for amino acid detection, and wrote methods for this analysis. A.S. and M.T. conducted the amino acid analyses. J.M.T. obtained research funding, S.H. obtained fellowship support. All authors commented on the manuscript.

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