New Phytologist

Review

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A meta-analytical review of the effects of elevated CO_2 on plant–arthropod interactions highlights the importance of interacting environmental and biological variables

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Received: *5 December 2011*
Accepted: *16 January 2012*

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New Phytologist (2012) **194**: 321–336
doi: 10.1111/j.1469-8137.2012.04074.x

Key words: carbon dioxide (CO_2), climate change, global warming, insect–plant interactions, meta-analysis, plant defences, plant–animal interactions, review.

Summary

We conducted the most extensive meta-analysis of plant and animal responses to elevated CO_2 to date. We analysed > 5000 data points extracted from 270 papers published between 1979 and 2009. We examined the changes in 19 animal response variables to the main effect of elevated CO_2. We found strong evidence for significant variation among arthropod orders and feeding guilds, including interactions in the direction of response. We also examined the main effects of elevated CO_2 on: six plant growth and allocation responses, seven primary metabolite responses, eight secondary metabolite responses, and four physical defence responses. We examined these response variable changes under two-way and three-way interactions between CO_2 and: soil nitrogen, ambient temperature, drought, light availability, photosynthetic pathway, reproductive system, plant growth rate, plant growth form, tissue type, and nitrogen fixation. In general we found smaller effect sizes for many response variables than have been previously reported. We also found that many of the oft-reported main effects of CO_2 obscure the presence of significant two- and three-way interactions, which may help better explain the relationships between the response variables and elevated CO_2.

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I. Introduction

In the most recent meta-analytical attempt to synthesize our knowledge of plant–animal interactions under elevated CO_2, Stiling & Cornelissen (2007) examined 59 studies of plant responses and 75 studies of herbivore responses conducted up to 2003. They concluded that: '… elevated CO_2 significantly decreased herbivore abundance (−21.6%), increased relative consumption rates (+16.5%), development time (+3.87%) and total consumption (+9.2%), and significantly decreased relative growth rate (−8.3%), conversion efficiency (−19.9%) and pupal weight (−5.03%). No significant differences were observed among herbivore guilds. Host plants growing under enriched CO_2 environments exhibited significantly larger biomass (+38.4%), increased C : N ratio (+26.57%), and decreased nitrogen concentration (−16.4%), as well as increased concentrations of tannins (+29.9%) and other phenolics.'

While there is support for these conclusions, *on average*, there are nevertheless many individual experiments in which there is either no effect of CO_2, or the results are in the opposite direction. As the experimental increases in CO_2 are unlikely to directly affect arthropods, any changes, positive or negative, are likely to be caused indirectly via changes in the quality of the host plants as a food source. Changes in nutritional quality and/or plant defences that result from the alteration of the carbon (C) and nitrogen (N) economy within the plant will translate into benefits or detriments for their arthropod herbivores. We think that more progress will be made in understanding, and so predicting, arthropod responses to elevated CO_2 if we more closely consider plant quality responses to elevated CO_2.

1. Changes in host plant quality

Plant biochemistry under ambient and elevated CO_2 has been studied extensively. Some general responses are frequently observed. For example, it is very common for the C : N ratio in the leaf tissue to greatly increase. As N is thought to be the limiting nutrient for arthropods (Mattson, 1980), it is generally thought that this will result in increased *per capita* herbivore consumption and/or decreased herbivore fitness (Coviella & Trumble, 1999). And there is generally support for this view from the Stiling & Cornelissen (2007) study, as discussed above. Beyond these very general nutritional responses, 'plant quality' is something that is relative to the needs and susceptibilities of the herbivore in question, but generally depends on the plant's size, its nutritional status, and its chemical and physical defences (Awmack & Leather, 2002).

The Carbon–Nutrient Balance Hypothesis (CNBH; Bryant *et al.*, 1983) has been the primary working hypothesis in this field, and predicts that carbon-based defence compounds such as phenolics and terpenoids will increase as a result of the 'excess' C under elevated CO_2, and that N-based defence compounds such as alkaloids, cyanogenic glycosides and glucosinolates will decrease as a result of the scarce N. In a recent semi-quantitative (vote counting) review of this hypothesis, Ryan *et al.* (2010) analysed 608 data points from plant secondary metabolites under elevated CO_2 with measurements taken from 102 species. They found that, all things being equal, with the possible exception of phenolics, these predictions were not supported. Under elevated CO_2, N-based compounds increased (18% of cases) about as often as they decreased (16% of cases). For the C-based terpenoids, concentrations increased in 11% of cases and decreased in 27%. The same was true for the C-based volatile class, with increases in 17% of cases and decreases in 23% of cases. In the phenolic class, however, allelochemicals increased in 50% of cases with decreases in only 7% of cases.

The CNBH is, of course, context-dependent, and Ryan *et al.* considered the N context to be most relevant. They examined 378 cases of C-based allelochemical changes under elevated CO_2, for which N concentrations were measured simultaneously. In only 32% of the 261 cases where the N concentration decreased under elevated CO_2 did C-based allelochemicals also increase. When only tannins were considered, 52 out of 106 cases reported simultaneous decreases in N and increases in tannins. Thus, even when N concentrations are considered, the results of empirical studies of allelochemical allocation under elevated CO_2 are only weakly predicted by existing frameworks such as the CNBH (Ryan *et al.*, 2010).

In addition to chemical defences, plants may also have physical characteristics that contribute to host plant quality for arthropod herbivores. Surface waxes, trichomes, secretory canals and general plant toughness (increased indigestible polymers such as cellulose and lignin) can produce physical barriers to herbivore feeding (Walters, 2011). The challenge posed by these physical barriers to arthropod herbivore feeding can be dependent upon the feeding guild in question. For example, increased plant pubescence (trichome density) may be particularly effective against small, sap-feeding insects but may be less effective against folivores. Leaf toughness can be dependent on plant functional group where certain morphologies may deter herbivore feeding. Grasses are generally tougher than herbaceous plants as a result of the deposition of silica (constitutes 2–5% of dry leaf mass; Massey *et al.*, 2006; Walters, 2011), thus increasing abrasiveness and reducing digestibility. As a physical defence, silica deposition may be more effective against folivores than it is against phloem feeders (Massey *et al.*, 2006). C_4 plants tend to be tougher than C_3 plants as a consequence of Kranz anatomy (starch-rich bundle sheath cells which surround plant vascular bundles).

Plant physical defences have received far less attention than plant chemistry in studies of insect nutritional ecology and elevated CO_2. Leaf toughness can be measured by punch strength (units of force) or fracture toughness, which is highly correlated with the index of sclerophylly (ratio of fibre to protein) and thus is a useful food quality determinant (Choong *et al.*, 1992). Only a handful of studies have measured leaf toughness (see Supporting Information Notes S2) under elevated CO_2 and this parameter has generally been shown to increase, thus decreasing the food quality of herbivores. However, in the absence of toughness measurements, specific leaf weight (or its inverse, specific leaf area) and leaf thickness may serve as a reasonable approximation of this parameter (Lincoln *et al.*, 1993). In a review of plant

structure under elevated CO_2, Pritchard *et al.* (1999) reported that specific leaf area (m^2 leaf g^{-1} DM) decreased for trees, wild non-trees, and crop species (−14%, −20%, and −6%, respectively) under elevated CO_2. Again, this suggests an increase in plant tissue toughness and subsequent decrease in host plant quality under elevated CO_2; however, the effects of this on herbivores may be dependent on feeding guild. Studies of trichome density are fewer still, although two studies conducted to date have reported decreases in trichome density under elevated CO_2 (Masle, 2000; Bidart-Bouzat *et al.*, 2005).

2. The importance of interactions

There are good biological reasons to expect that the magnitude, and/or direction, of the effects of elevated CO_2 concentrations on plant growth and quality might depend upon any or all of these environmental conditions: soil nutrient availability, the temperature at which the comparisons are made, and the availability of either water or light. We expect that many of the primary and secondary metabolite responses, involving nitrogen, to elevated CO_2 (as discussed in section I.1) might be reduced by the addition of soil N. As the rate of photosynthesis depends both on the concentration of atmospheric CO_2 and on the ambient temperature, there is every reason to suppose that this proximate mechanism will also influence the plant's primary and secondary metabolism. Photosynthesis is also limited by water availability, although it is unclear whether this limitation is mainly attributable to stomatal regulation or to metabolic changes in ATP synthesis. In any case, like temperature, there is every reason to believe that the plant's photosynthetic response, and as a result its primary and secondary metabolism, will be dependent upon the interaction between elevated CO_2 and drought status (for a review see e.g. Newman *et al.*, 2011).

Despite these expectations, experiments that combine manipulation of CO_2 concentrations and these other environmental variables are relatively uncommon, probably as a consequence of the technical challenges inherent in conducting such research. Nevertheless, the presence of interactions makes it difficult to interpret the impacts of elevated CO_2 *per se*, in experiments that do not manipulate all, or at least some, of these environmental conditions. In this review, we show that the available research demonstrates that the responses of plants to elevated CO_2 regularly depend upon such interactions, and thus we suggest that more experiments that manipulate *only* CO_2, will add little to what we already know.

In this review we used meta-analysis to examine the responses of arthropods and host plants to elevated CO_2 and the interactions between elevated CO_2 and temperature, soil N, water availability and light intensities. We purposefully ignored CO_2 by O_3 interactions, which have been well studied, and recently reviewed elsewhere (Valkama *et al.*, 2007; Bidart-Bouzat & Imeh-Nathaniel, 2008; Lindroth, 2010). For the animal and plant responses, we examined the hypotheses shown in Table 1. For about half of these hypotheses we had some prior expectation based on experimental work from the literature.

II. Methods

1. Herbivore responses database

The implications of elevated CO_2 for herbivore performance were investigated by performing the following search for papers published from 1998 to 2009 in Web of Science: '(elevated, increased) + (CO_2, carbon dioxide) + (insect, herbivor*, parasit*, predator*)'. In order to expand the search for non-insect arthropod herbivores and arthropod decomposers of foliage, the following searches were later performed: '(elevated, increased) + (CO_2, carbon dioxide) + (herbivor*) + (snail, gastropod*, mite, Acarina, spider, Araneae)' and '(elevated, increased) + (CO_2, carbon dioxide) + decomposer'. Papers published before 1998 and cited in reviews by Coviella & Trumble (1999) and Stiling & Cornelissen (2007) were also included in our database. We included only those studies that reported means, variances (standard deviation or standard error), and sample sizes at both ambient and elevated CO_2 concentrations. When a study had herbivore response data for more than one elevated concentration of CO_2, we recorded the difference between the ambient concentration and the highest concentration of CO_2. The lowest sample size within a range was used, along with the mean and variances from the last time-point in a time series (see section II.2 on plant responses for reasoning). Where subjects were divided into groups (e.g. by gender or growth form) for a given response, effect sizes were calculated separately for each group. In the case where data were grouped by gender, with results for males and females being recorded separately but with only a total sample size being given, a sex ratio of 1 : 1 was assumed to determine the male and female sample sizes.

Although many of these data are not strictly independent (e.g. gender, generation and species data within a single study) we included this information rather than sacrifice valuable data and possibly bias our results by excluding them, as has been suggested by Koricheva *et al.* (1998) and Stiling & Cornelissen (2007). For studies that manipulated factors other than CO_2 (e.g. temperature, fertilization, light, etc.), values from the treatment level that most closely represented subjective 'ambient conditions' were used in the analysis. In total, 122 studies met our criteria (a list of definitions and variables extracted from each study is given in Supporting Information Notes S3, and complete references are listed in Notes S4), providing data on 19 herbivore response variables.

2. Plant responses database

To first consider the phytochemical and growth responses of plants to an increase in atmospheric CO_2 concentration in combination with an increase in temperature, an increase in N availability, drought, or shade, we used the papers that were included in a recent review (Ryan *et al.*, 2010) and then performed a literature search in Google Scholar to expand on this database using the search terms '(plant) + (nitrogen, temperature, light, drought, irrigation) + (elevated, increase, enrichment) + (CO_2,

Table 1 (a) *A priori* hypotheses regarding arthropod responses to elevated CO_2 and interactions among different groups of arthropods; (b) *a priori* hypotheses regarding plant responses to elevated CO_2 and interactions involving other environmental variables and different groups (see footnotes)

(a)

Insect response	CO_2	CO_2 × order	CO_2 × guild	CO_2 × specialization
Relative growth rate	Expect ⇓ at high CO_2	No hypothesis	Chewers ⇓ , others do not	No hypothesis
References	Roth & Lindroth (1994), Lawler *et al.* (1996), Brooks & Whittaker (1998)		Stiling & Cornelissen (2007)	
We found	Confirmed ***	Lepidoptera ⇓, others do not	Confirmed ***	Not evaluated
Relative consumption rate	Expect ⇑ at high CO_2	No hypothesis	No hypothesis	No hypothesis
References	Fajer (1989), Marks & Lincoln (1996)			
We found	Confirmed ***	Coleoptera and Lepidoptera ⇑ , others do not	Folivores ⇑ , decomposers no change	Not evaluated
Total consumption	Expect ⇑ at high CO_2	No hypothesis	No hypothesis	No hypothesis
References	Fajer (1989), Marks & Lincoln (1996)			
We found	Confirmed ***	Lepidoptera ⇑ , others do not	No evidence of an interaction	Not evaluated
Conversion efficiency	Expect ⇓ at high CO_2	No hypothesis	No hypothesis	No hypothesis
References	Roth & Lindroth (1994), Lawler *et al.* (1996), Brooks & Whittaker (1998)			
We found	Confirmed ***	Lepidoptera ⇓ , others do not	Insufficient data	Not evaluated
Development time	Expect ⇑ at high CO_2	No hypothesis	Chewers ⇑ , others do not	No hypothesis
References	Roth & Lindroth (1994), Lawler *et al.* (1996), Brooks & Whittaker (1998)		Stiling & Cornelissen (2007)	
We found	Confirmed ***	Homoptera ⇓ , Lepidoptera ⇑ , others no change	Phloem feeders ⇓ , Folivores ⇑ , others no change	Not evaluated
Life span	Expect ⇓ at high CO_2 (1)	No hypothesis	No hypothesis	No hypothesis
References	Fajer (1989), Stiling *et al.* (2003)			
We found	Not supported	Homoptera ⇓ , Lepidoptera no change	Insufficient data	Not evaluated
Fecundity	Expect ⇓ at high CO_2	No hypothesis	No hypothesis	No hypothesis
References	Wu *et al.* (2006)			
We found	Not supported	Homoptera ⇑ , Lepidoptera, Orthoptera, and Coleoptera ⇓	Phloem feeders ⇑ , Folivores ⇓ , others no change	Not evaluated
Abundance	Expect ⇓ at high CO_2	Aphids may ⇑ while others ⇓	No hypothesis	Specialists ⇓ , others do not change
References	Stiling *et al.* (1999), Kopper & Lindroth (2003b)	Bezemer & Jones (1998)		Stiling & Cornelissen (2007)
We found	Not supported	Acari and Hompotera ⇑ , Lepidoptera ⇓, others no change	Phloem feeders and scrapper ⇑ , leafminers ⇓	Not evaluated

Table 1 (*Continued*)

(b) Plant response	CO_2	$CO_2 \times N$	$CO_2 \times H_2O$	$CO_2 \times T$	$CO_2 \times$ light
[C]	Expect ⇓ at high CO_2 (2)	No hypothesis	No hypothesis	No hypothesis	No hypothesis
References	Bazzaz (1990)				
We found	Not supported	No evidence of interaction	No evidence of interaction	No evidence of interaction	Insufficient data
[N]	Expect ⇓ at high CO_2 (3)	>⇓ at low N	No hypothesis	No hypothesis	No hypothesis
References	Bazzaz (1990), Bowes (1993), Cotrufo *et al.* (1998), Stitt & Krapp (1999)	Stitt & Krapp (1999)			
We found	Confirmed***	Confirmed*	No evidence of interaction	No evidence of interaction	No evidence of interaction
[Starch]	Expect ⇑ at high CO_2 (4)	>⇑ at low N	No hypothesis	<⇑ at high *T*	No hypothesis
References	Bazzaz (1990), Bowes (1993)	Stitt & Krapp (1999)		Farrar & Williams (1991), Zvereva & Kozlov (2006)	
We found	Confirmed***	No evidence of interaction	No evidence of interaction	No evidence of interaction	Insufficient data
[Soluble CHO]	Expect ⇑ at high CO_2 (4)	No hypothesis	No hypothesis	>⇑ at high *T*	No hypothesis
References	Bazzaz (1990), Stitt & Krapp (1999)			Farrar & Williams (1991)	
We found	Confirmed**	No evidence of interaction	No evidence of interaction	No evidence of interaction	No evidence of interaction
[TNC]	Expect ⇑ at high CO_2 (4)	>⇑ at low N	No hypothesis	<⇑ at high *T*	No hypothesis
References	Stitt & Krapp (1999)	Stitt & Krapp (1999)		Zvereva & Kozlov (2006)	
We found	Confirmed***	No evidence of interaction	Insufficient data	Insufficient data	Insufficient data
[Amino acids]	Expect ⇓ at high CO_2	>⇓ at low N	No hypothesis	No hypothesis	No hypothesis
References	Stitt & Krapp (1999), Ziska & Bunce (2006)	Stitt & Krapp (1999)			
We found	Confirmed***	No evidence of interaction	Insufficient data	No evidence of interaction	Insufficient data
[Protein]	Expect ⇓ at high CO_2	>⇓ at low N	No hypothesis	No hypothesis	No hypothesis
References	Stitt & Krapp (1999), Ziska & Bunce (2006)	Stitt & Krapp (1999)			
We found	Confirmed***	No evidence of interaction	No evidence of interaction	No evidence of interaction	Insufficient data
Leaf toughness	Expect ⇑ at high CO_2	No hypothesis	No hypothesis	No hypothesis	No hypothesis
References	Bazzaz (1990), Stitt & Krapp (1999)				
We found	Confirmed***	No evidence of interaction	Insufficient data	No evidence of interaction	Insufficient data
Biomass	Expect ⇑ at high CO_2 (5–8)	>⇑ at high N	<⇑ at low H_2O	>⇑ at high *T*	>⇑ at high light
References	Bazzaz (1990), Bowes (1993), Stitt & Krapp (1999), Ziska & Bunce (2006)	Bazzaz (1990), Stitt & Krapp (1999)	Asseng (2004)	Bazzaz (1990), Bowes (1993), Poorter & Navas (2003)	Bazzaz (1990) Or no difference Bowes (1993)
We found	Confirmed***	Confirmed***	No evidence of interaction	No evidence of interaction	No evidence of interaction
C : N	Expect ⇑ at high CO_2	>⇑ at low N	No hypothesis	No hypothesis	No hypothesis
References	Bowes (1993), Stitt & Krapp (1999)	Stitt & Krapp (1999)			
We found	Confirmed***	No evidence of interaction	Insufficient data	No evidence of interaction	No evidence of interaction
Water	Expect WUE ⇑ at high CO_2	No hypothesis	WUE <⇑ at low H_2O	No hypothesis	No hypothesis
References	Bazzaz (1990)		Asseng (2004)		
We found	Water content declines; WUE not evaluated	No evidence of interaction	No evidence of interaction	No evidence of interaction	No evidence of interaction

Table 1 (*Continued*)

(b) Plant response	CO_2	$CO_2 \times N$	$CO_2 \times H_2O$	$CO_2 \times T$	$CO_2 \times$ light
Root:shoot	Expect ⇑ at high CO_2	>⇑ at low N	>⇑ at low H_2O	No hypothesis	No hypothesis
References	Bazzaz (1990), Stitt & Krapp (1999)	Bazzaz (1990), Stitt & Krapp (1999)	Bazzaz (1990)		
We found	Not supported	No evidence of interaction	No evidence of interaction	Insufficient data	Insufficient data
N-based 2° metabolites	Expect ⇓ at high CO_2	>⇓ at low N	No hypothesis	No hypothesis	No hypothesis
References	Ryan *et al.* (2010)	Stitt & Krapp (1999)			
We found	Confirmed***	No evidence of interaction	No evidence of interaction	Total glycosides ⇑ under elevated temperature	Insufficient data
C-based 2° metabolites	Expect ⇑ at high CO_2 (4)	No hypothesis	No hypothesis	<⇑ at high *T*	No hypothesis
References	Bidart-Bouzat & Imeh-Nathaniel (2008), Ryan *et al.* (2010)			Zvereva & Kozlov (2006)	
We found	Phenolics ⇑ , terpenes ⇓	No evidence of interaction for total phenolics or terpenes, but under elevated CO_2 terpene emission rates ⇑ under high N and ⇓ under low N	No evidence of interaction for total phenolics or terpenes, but under elevated CO_2 terpene emission rates ⇓ more under high water	No evidence of interaction	Insufficient data

Blue text denotes where the evidence apparently confirms the hypothesis (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$); red text denotes where the evidence apparently contradicts the hypothesis; and green text denotes new hypotheses arising from this analysis.
(1) Perhaps because of increased mortality imposed by natural enemies on larval and nymphal stages.
(2) Increased [C] greater in C_3 plants than C_4 plants (Bazzaz, 1990; Bowes, 1993; Poorter & Navas, 2003; Ziska & Bunce, 2006). We found: confirmed* for total carbohydrates and starch, but no evidence of an interaction for total non-structural carbohydrates (TNC) or soluble CHO.
(3) Decreased [N] greater in C_3 plants than C_4 plants (Cotrufo *et al.*, 1998). We found: confirmed*.
(4) Starch, soluble CHO and TNC increases greater for slow-growing plants than fast-growing plants (Pritchard *et al.*, 1999). We found: no evidence for an interaction for soluble CHO, total CHO or TNC. We found an interaction for starch but it was in the opposite direction (i.e. increase in [starch] was greater for fast-growing plants than for slow- or moderate-growing plants).
(5) Increase in biomass greater for fast-growing plants than slow-growing plants (Poorter, 1993; Pritchard *et al.*, 1999). We found: no evidence of an interaction.
(6) Increase in biomass greater for nitrogen fixers (Poorter, 1993; Poorter & Navas, 2003). We found: no evidence of an interaction.
(7) Increase in biomass greater for herbaceous plants than for woody plants (Poorter & Navas, 2003). We found: significant interaction, but in the opposite direction (i.e. trees had a larger positive cumulative effect size than herbaceous plants).
(8) Increase in biomass greater for angiosperms than gymnosperms (Poorter & Navas, 2003). We found: significant interaction, but in the opposite direction (i.e. gymnosperms had a larger positive cumulative effect size than angiosperm plants).
T, temperature; WUE, water use efficiency; TNC, total non-structural carbohydrates.

climate change)'. Studies that gave means, variances (standard deviation or standard error) and sample sizes for full factorial CO_2 × temperature, N, drought/irrigation, or light/shade experimental manipulations were included in the database. In total, 170 studies met our criteria for inclusion in the meta-analysis and numerous phytochemical and growth parameters were extracted from these studies. A detailed list of the variables that were extracted from these studies can be found in Notes S1; however, because of small sample sizes, many of these variables had to be pooled for analysis. Some of these studies looked at the interaction of CO_2 with more than one of the other factors but, when considered separately, 40 looked at the interaction of CO_2 with temperature, 98 with N, 45 with drought, and six with shade (a list of variables extracted from each study is given in Notes S2, and complete references are given in Notes S4). For studies that reported plant response to more than one elevated level of a factor (CO_2, temperature, N, water, or light), we used the difference between the control and the highest level of the factor to calculate effect sizes. When a range of sample sizes was given in a study, the lowest value from the range was used in the meta-analysis. We believed this to be the most conservative use of these data, as individual effect sizes are weighted based on sample sizes. For studies that reported plant response over time, values from the last time-point in the series were included as they would more closely represent the longer term effects of elevated CO_2. Where data were presented graphically, we measured the number of pixels in Adobe Photoshop Elements 8.0 and converted these values to the correct units.

3. Statistical analysis

For our meta-analyses of plant and herbivore responses to elevated CO_2 and, in the case of plants, to other changes in environmental conditions, we used the program METAWIN 2.1 (Rosenberg *et al.*, 2000). The natural log of the response ratio was used as a measure of effect size ($\log_e R = \log_e$ (elevated/ambient)) as it can be easily interpreted. A negative proportion indicates a decrease in a variable under elevated CO_2 concentrations compared with ambient and a positive proportion represents an increase (Rosenberg *et al.*, 2000). For each plant and herbivore response variable, we first performed a meta-analysis using a random effects model to look at the main effects of elevated CO_2. This model assumes that there is one true effect size but that, in addition to sampling error, there is also random variation in effect sizes between studies (Rosenberg *et al.*, 2000).

Many meta-analyses consider cumulative effect sizes to be significant if, and only if, their confidence intervals do not overlap zero. That is a simplistic but conservative approach, as significant results are possible even when confidence intervals do overlap zero. Instead, we therefore performed *Z*-tests (Bland & Peacock, 2002) for each response variable for the main effects of CO_2, to determine more rigorously their significance. The *Z*-values were calculated by dividing the cumulative effect size by the standard error of that effect size (Bland & Peacock, 2002).

As only published studies could be included in our meta-analyses, we performed a fail-safe analysis for each response variable using Rosenthal's method in order to determine how many missed or unpublished studies with nonsignificant results would need to be added to our analysis in order to make significant cumulative effect sizes nonsignificant. If the fail-safe number was large relative to the sample size ($\geq 5x + 10$, where x is the sample size) then we assumed that the results of the meta-analysis reliably estimated the true effect (Rosenthal, 1979; Rosenberg *et al.*, 2000). Throughout, we express this as the ratio of our calculated fail-safe value to Rosenthal's critical value. Thus, ratios larger than 1 exceed Rosenthal's criterion.

Categorical variables We used a random model with categorical structure for each response variable (also referred to as a mixed model) to investigate interactions between CO_2 and various biological and environmental categorizations. For these categorical models, the heterogeneity between groups (Q_B) and the heterogeneity within groups (Q_W) were tested against a χ^2 distribution, with a significant Q_B indicating differences in effect sizes between groups and a significant Q_W indicating remaining heterogeneity among effect sizes that had not been explained by the model (Rosenberg *et al.*, 2000).

For plant responses, we investigated interactions between CO_2 and: temperature (ambient or elevated), drought (well watered or drought), N concentration (low N or high N), and light intensity (light or shade). We divided the plant responses by growth form (grass, herb/forb, sedge, shrub or tree), growth rate (fast, moderate or slow), reproductive system (angiosperm or gymnosperm), whether or not it was a leguminous plant (N-fixer or non-N-fixer), and photosynthetic mechanism (C_3 or C_4). With the exception of photosynthetic mechanism, these classifications were obtained from the United States Department of Agriculture (USDA) Natural Resources Conservation Service online 'Plants Database' (http://plants.usda.gov/java/factSheet). We categorized the herbivores by feeding guild (foliage feeders, leaf-miners, leaf-tiers, phloem feeders, decomposers, cell-feeders, and scrapers) and by order, or subclass for mites (Acari, Coleoptera, Diptera, Gastropoda, Homoptera, Hymenoptera, Isopoda, Lepidoptera, and Orthoptera).

Three-way interactions For plant response variables only, we investigated whether the biological categorizations interacted with the environmental variables, to determine the effect of elevated CO_2. To do this, for each level of the biological category, we performed two meta-analyses, one for each level of the environmental variable, using a random effects model. In one we estimated the mean effect of elevated CO_2 at the *ambient* level of the environmental variable (temperature, N, water, or light) and, in the other, we estimated the mean effect at the *elevated* level of the environmental variable. For each level of the biological category, we determined the statistical significance of the environmental interaction using a technique borrowed from biomedical research (Altman & Bland, 2003). We first found the difference (d) between the cumulative effect size at the ambient level of the environmental variable and the cumulative effect size at the elevated level of that variable. We calculated the standard error of the difference, SE(d), using the width of the confidence intervals

for these two cumulative effect sizes, and we then performed a Z-test where $Z = d/\mathrm{SE}(d)$ (Altman & Bland, 2003). If only some of the levels within a plant biological category showed significant environmental interactions then we considered this a three-way interaction.

By way of illustration of this method, consider the interaction between N fertilization (high or low), N fixation (N-fixers or non-N-fixers) and CO_2 (ambient or elevated) on the concentration of N in plant leaves. For each level of N fixation we performed two meta-analyses, one for each level of N fertilization, and calculated the difference between the effect sizes and the standard error of these differences. For the four combinations of N fertilization and N fixation we found (mean effect size ± standard error of the mean): [low N, non-N-fixer] = −0.1766 ± 0.0103; [high N, non-N-fixer] = −0.1308 ± 0.0133; [low N, N-fixer] = −0.1116 ± 0.0310; [high N, N-fixer] = −0.0879 ± 0.0415. The standard error for the differences is the square root of the sum of the individual squared standard errors. So for non-N-fixers, the difference in effect sizes of elevated CO_2 at low and high N is −0.0458 ± 0.0168, which yields a Z-test of $Z = -2.73$ and $P = 0.007$. For N-fixers this difference is −0.0237 ± 0.0518, which yields $Z = -0.46$ and $P = 0.65$. These results suggest that the difference in the effect of elevated CO_2 between levels of N fertilization depends upon whether or not the plant is an N-fixer, and hence indicate the presence of a three-way interaction.

Caution should be taken when interpreting these three-way interactions because of differences in sample sizes between biological groups. A CO_2 by environmental factor interaction may be significant in one group, but not in another, simply as a result of the discrepancy in sample sizes, with the group with a small sample size showing no significant interaction (i.e. a possible Type II error). For example, in the illustration given above, the sample size for the non-N-fixing plants was 157, while for the N-fixers it was just 18. One way to reduce the possibility of the Type II error is to use less stringent α levels (e.g. $\alpha = 0.1$) for the differences based on smaller sample sizes. For a Z-test this is equivalent to using a one-tailed rather than two-tailed hypothesis test. In the case of the example described above, such an adjustment does not change our interpretation. Throughout the results section we utilize the standard two-tailed hypothesis test (i.e. we assume $\alpha = 0.05$), but in the Supporting Information (Tables S1–S3) we report probabilities based on both one-tailed and two-tailed tests to allow readers to decide for themselves if such an adjustment is appropriate. While the adjustment does make a difference in some cases, many of the results reported below are robust to this choice.

III. Herbivore responses to elevated CO_2

When the data from all of the insect orders and feeding guilds were combined, there was a significant decrease in relative growth rate (−4.5%) and a significant increase in relative consumption rate (+14%) under elevated CO_2 (Fig. 1). Conversion efficiencies for both ingested and digested food also decreased under elevated CO_2 (−17% and −12%, respectively). While there was no change in larval/nymphal weight, there were decreases in both pupal weight and adult weight (−5.5%). However, the fail-safe number for adult weight was less than Rosenthal's critical value

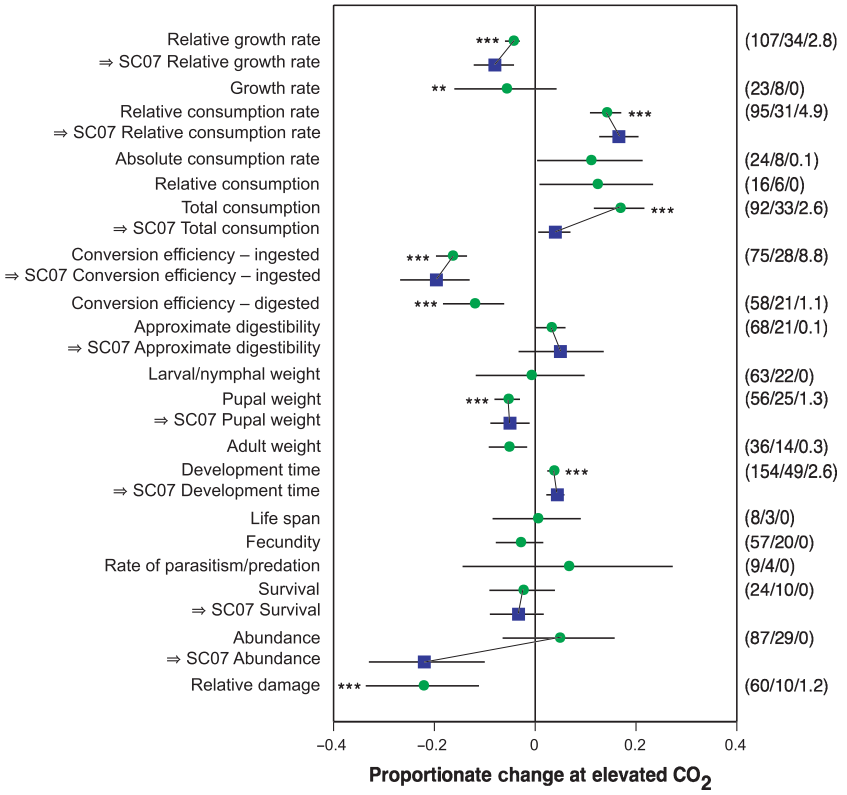

Fig. 1 Herbivore responses to elevated CO_2. '⇒ SC07' and corresponding blue symbols denote the comparable results from Stiling & Cornelissen (2007). The SC07 results are joined, by a thin line, to our results to denote the appropriate comparison. Numbers to the right denote (sample size/number of studies/fail-safe ratio). The fail-safe ratio is the fail-safe number divided by Rosenthal's critical value ($\geq 5x + 10$, where x is the sample size). Fail-safe ratios > 1 pass Rosenthal's test. Significance levels were determined by Z-tests and are denoted by: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Complete details for all statistical tests are contained in Supporting Information Table S1(a). Variable definitions and literature sources are given in Notes S3.

(Rosenthal, 1979). Herbivores exposed to elevated concentrations of CO_2 had longer development times (+3.5%). Even though there was no effect of CO_2 on survival or abundance, relative damage to plants was greater (+22%) under elevated CO_2. Some of the responses reported in Fig. 1 are based on too few studies to enable meaningful statistical inferences to be made, most notably life span and rate of parasitism/predation. See Table S1(a) for complete results.

Dividing the data based on insect order revealed significant responses to elevated CO_2 for variables that were obscured when the data were combined. While fecundity decreased under elevated CO_2 for Coleoptera, Lepidoptera, and Orthoptera (−13%, −13%, and −34%, respectively), it increased significantly in Homoptera (+8.5%; see Fig. 2b). Both survival (Table S1b) and abundance (Fig. 2a) significantly increased in Homoptera under elevated CO_2 (+16% and +22%, respectively), while these two variables decreased in Lepidoptera (−7% and −65%, respectively). The abundance of mites (Acari) also increased under elevated CO_2 (+59%), but there was no significant effect on Coleoptera or Thysanoptera (Fig. 2a). The results for all other variables divided based on orders can be found in Table S1(b).

Fig. 2 also shows the most important interactions between feeding guild and CO_2 for arthropod performance. The remainder of the effects are given in Table S1(c). Significant heterogeneity between feeding guilds was found for development time (Table S1c), with development time increasing significantly in foliage feeders (+5%) but decreasing in phloem feeders (−3%). For fecundity (Fig. 2b) we see decreases in foliage feeders (−14%), increases in phloem feeders (+8%), and no significant effect on scrapers or decomposers. Finally, for abundance (Fig. 2a), we see increases for phloem feeders (+22%) and scrapers (+59%), decreases for leaf-miners (−70%), and no significant differences for folivores (see Table S1c for details).

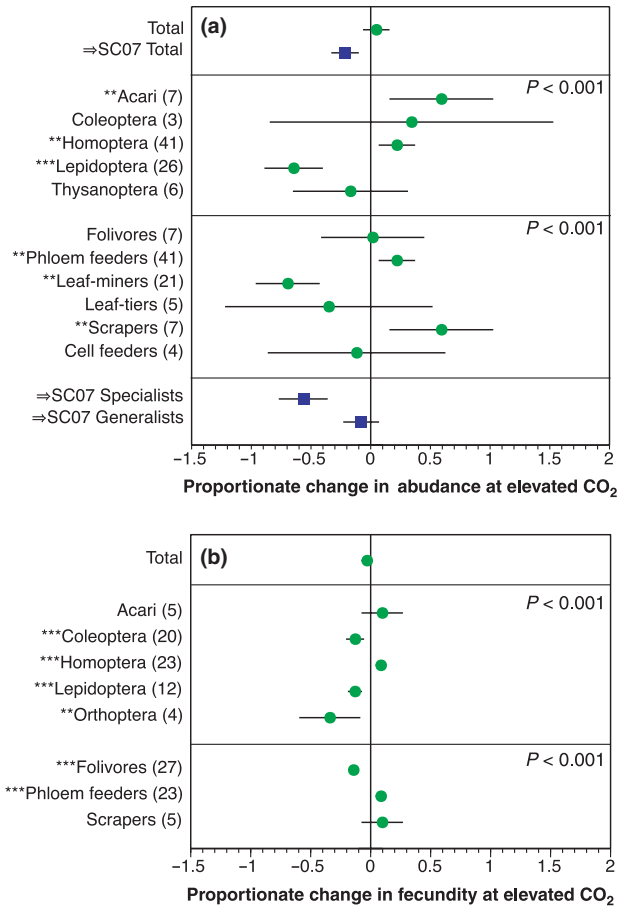

Fig. 2 Differences in herbivore order and feeding guild responses to elevated CO_2 for (a) abundance and (b) fecundity. '⇒ SC07' and corresponding blue symbols denote the comparable results from Stiling & Cornelissen (2007). Bracketed numbers to the right of the group name denote sample size. Fecundity data for Diptera are not presented because of wide confidence intervals that interfered with an examination of the effect sizes for other orders. The significance level for each order was determined by a *Z*-test and is denoted by: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. The significance level for the overall interaction was determined by χ^2 tests for the Q_B values, and is denoted by the *P*-values in the upper right corners. Complete details of all statistical tests are contained in Supporting Information Table S1(b and c). Variable definitions and literature sources are given in Notes S3.

IV. Plant responses

1. Plant responses: main effects

Fig. 3 shows the main effects of elevated CO_2 on all plant response variables measured. For comparison, the results from Stiling & Cornelissen (2007) and Zvereva & Kozlov (2006) are also shown. In general, the growth and allocation response followed common predictions of plant response to elevated CO_2. Both plant biomass and the C : N ratio increased under elevated CO_2 (+25% and +19%, respectively), while N concentration decreased (−15%). Protein (−10%) and amino acids decreased, but the fail-safe number for amino acids was below Rosenthal's critical value, probably as a result in part of the small sample sizes. Total carbohydrates, starch, soluble sugars and total nonstructural carbohydrates increased under elevated CO_2 (+23%, +50%, +8% and +39%, respectively), but structural carbohydrates decreased significantly (−13%). Under elevated CO_2, N-based secondary metabolites decreased (−16%), total phenolics, condensed tannins and flavonoids increased (+19%, +22%, +27%, respectively), and plant terpenoid concentrations decreased (−13%). Several plant physical characteristics commonly used in estimates of 'toughness' showed consistent responses under elevated CO_2. Increases in leaf toughness and specific leaf weight (+11% and +18%, respectively) and a nonsignificant decrease in the specific leaf area all suggest an increase in general 'toughness' under elevated CO_2. Further main effects and test details are shown in Table S2(a–e).

2. Plant responses: interactions

Although the plant responses reported in the main effects subsection appear clear enough, a more thorough analysis reveals that many of these responses actually depend upon interactions between CO_2 and other conditions. In this section we explore some of these two-way and three-way interactions. Effect sizes,

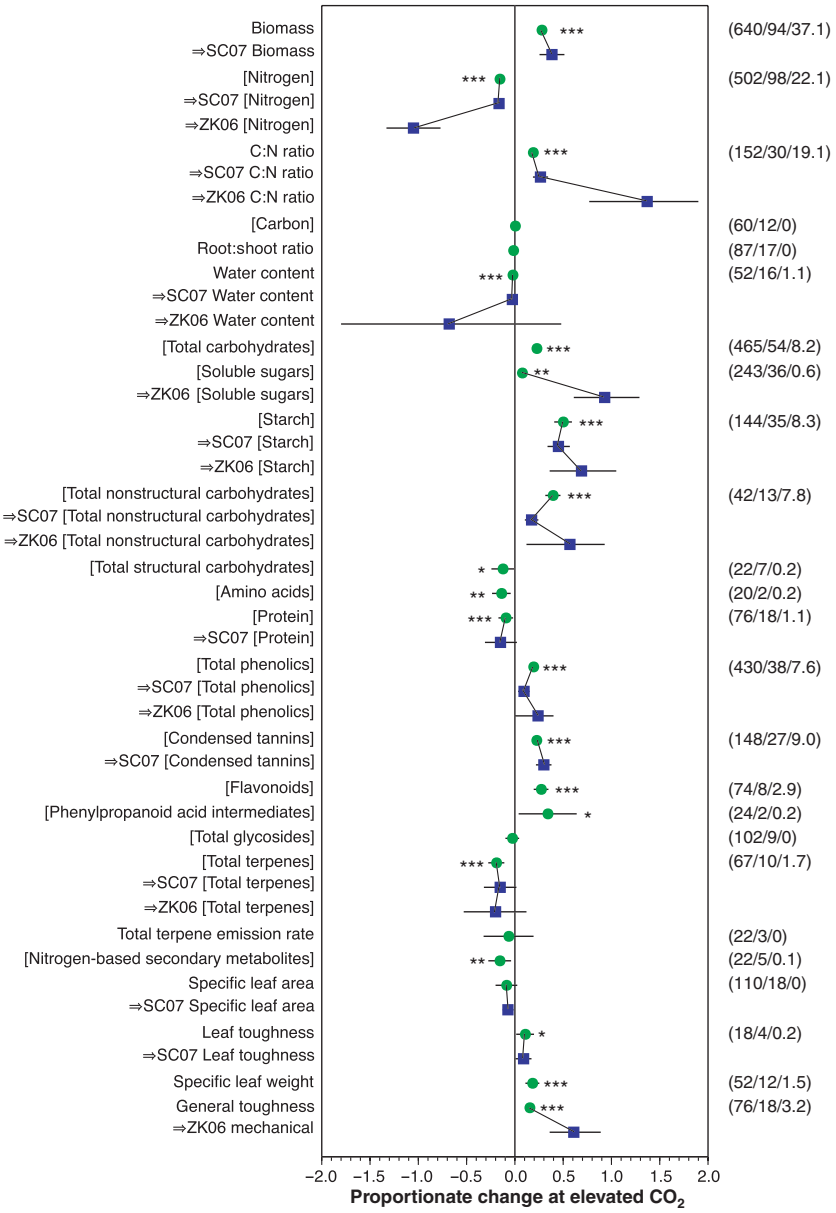

Fig. 3 Plant responses to elevated CO_2. '⇒ SC07' and corresponding blue symbols denote the comparable results from Stiling & Cornelissen (2007). '⇒ ZK06' and corresponding blue symbols denote the comparable results from Zvereva & Kozlov (2006). The SC07 and ZK06 results are joined, by a thin line, to our results to denote the appropriate comparisons. Numbers to the right denote (sample size/number of studies/fail-safe ratio). The fail-safe ratio is the fail-safe number divided by Rosenthal's critical value (≥5*x* + 10, where *x* is the sample size). Fail-safe ratios > 1 pass Rosenthal's test. Significance levels were determined by *Z*-tests and are denoted by: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Complete details of all statistical tests are contained in Supporting Information Table S2(a–e). Variable definitions are given in Notes S1 and literature sources of the extracted results are given in Notes S2. Note that ZK06's 'mechanical' response variable included leaf toughness, specific leaf weight or mass, specific leaf area, and leaf thickness, and is comparable to our 'general toughness' response variable.

sample sizes, and significance tests for all interactions between CO_2 and biotic and environmental variables can be found in Tables S2 and S3.

Biological variable interactions When main effects of CO_2 on plant response variables were divided into various categorical groups based on biological variables, we tended to find that the general result held for some, but not all of those groups. For example, although there was a strong and significant decrease in N concentrations under elevated CO_2 (−16%; see Fig. 3), that effect was only true for C_3 plants. C_4 plants, on average, showed no change in N concentrations (see Table S2a). In other cases, although all the categories changed in the same *direction*, the *magnitude* of change was dramatically different. For example, the overall decrease in N concentration just considered is about twice as strong for aboveground tissue as it is for belowground tissue (−17% vs −7%, respectively). In this section we highlight some of these more striking interactions. The complete results of this analysis are presented in the Supporting Information Tables S2 and S3.

Many of the main effects of elevated CO_2 hide differing responses by grasses, shrubs, herbs/forbs and trees. This was particularly true for the secondary metabolites (see Table S2d for the complete analysis). Fig. 4 shows that herbs/forbs were more responsive than the other groups for the total phenolics, total glycosides, phenylpropanoid acid intermediates, and total flavonoids. The overall significant decrease in total terpenes (Fig. 3) actually comprises a significant positive response by shrubs and a negative response by trees (Fig. 4). The overall nonsignificant response of the total glycosides (Fig. 3) hides a significant positive response by the trees and a significant negative response by the herbs/forbs (Fig. 4). There were also large differences among these groups in their responses to biomass, root : shoot

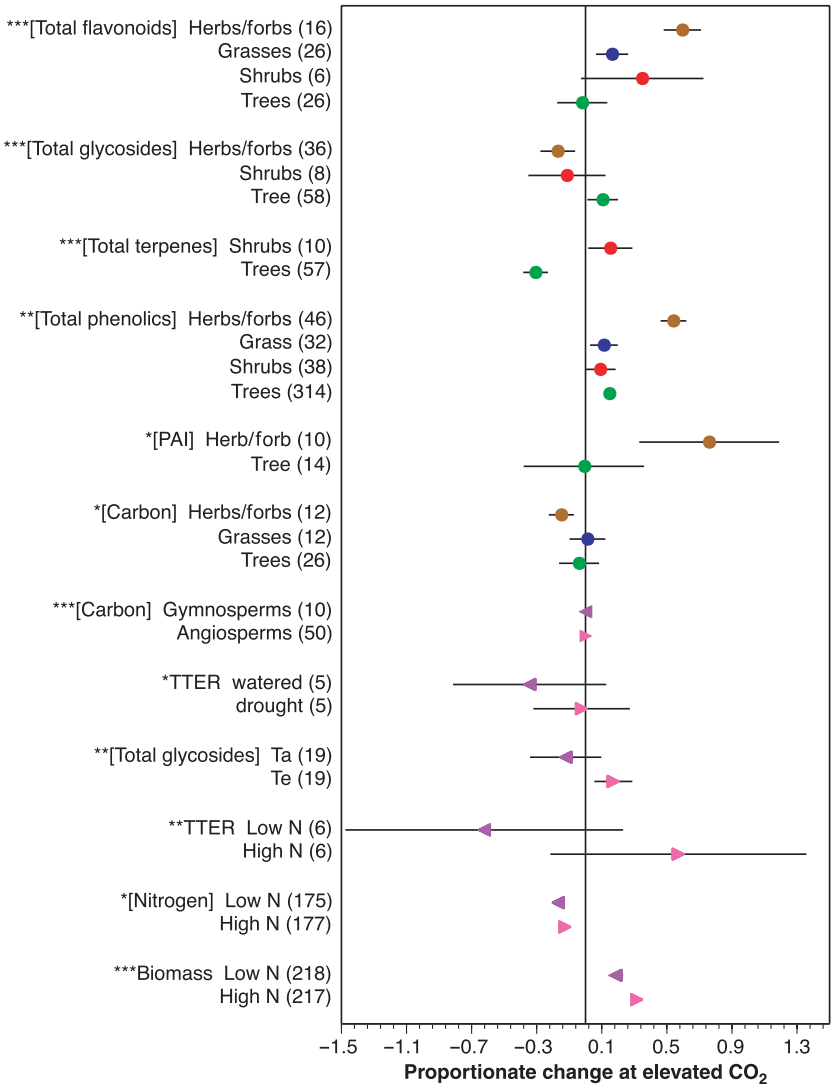

Fig. 4 Plant responses to elevated CO_2 at two levels of the interacting environmental factor or differences in the direction of responses between plant functional groups. Ta, ambient temperature; Te, elevated temperature; PAI, phenylpropanoid acid intermediates; TTER, total terpene emission rate. Bracketed numbers to the right of the group name denote sample size. Significance levels were determined by χ^2 tests for the Q_B values, and are denoted by the asterisks preceding the response variable name. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Complete details of all statistical tests are contained in Supporting Information Tables S2(d) and S3(a,c,g). Variable definitions are given in Notes S1 and literature sources of the extracted results are given in Notes S2.

ratio, protein, C : N ratio, total carbohydrates, starch, total non-structural carbohydrates, and total structural carbohydrates (Table S2).

Comparing N-fixing plants to non-N-fixing plants, there were several large differences. Plant N concentration declined more under elevated CO_2 for the non-N-fixing plants (−17%) than for the N-fixers (−10%). The increase in total carbohydrates under elevated CO_2 was greater for N-fixers (+30%) than for non-N-fixers (+20%). Surprisingly, protein concentrations declined twice as much for N-fixers as for non-N-fixers (−16% vs −8%, respectively). Total phenolics increased twice as much under elevated CO_2 for the N-fixers as for non-N-fixers (+35% vs +16%, respectively). Total flavonoids increased more than four times as much under elevated CO_2 for the N-fixers than for non-N-fixers (+60% vs +13%, respectively). The complete results of this analysis are presented in Table S2.

Gymnosperms were more responsive than angiosperms for biomass (+40% vs +27%, respectively) and soluble sugars (+43% vs +5%, respectively). Angiosperms were more responsive than gymnosperms for starch (+58% vs +10%, respectively), total phenolics (+23% vs +10%, respectively) and condensed tannins (+27% vs +13%, respectively). Gymnosperms and angiosperms responded in opposite directions for total terpenes (−30% vs +13%, respectively). The complete results of this analysis are presented in Table S2.

Some large differences also emerged when comparing 'fast', 'moderate', and 'slow' growth forms. C : N ratios increased about twice as much under elevated CO_2 for moderate growth forms as for fast or slow growth forms (+28%, +18% and +13%, respectively). However, starch was more responsive in fast growth forms than in slow or moderate forms (+60%, +35% and +28%, respectively). Total terpenes were significantly depressed under elevated CO_2 in both the slow growth forms (−17%) and the fast growth forms (−50%), while the moderate growth forms showed a small increase (+9%). The complete results of this analysis are presented in Table S2.

Environmental variable interactions Not surprisingly, soil N and CO_2 interacted in their effects on several plant variables (Fig. 4). Total plant biomass responses to elevated CO_2 were

stronger under high N compared with low N treatments (+32% vs +19%, respectively). Plant N concentrations declined under elevated CO_2, but the effect was more pronounced in low N than in high N (−17% vs −13%, respectively), suggesting that fertilization may buffer decreases in plant N. Total terpene emission rates did not significantly differ under the main effect of elevated CO_2. However, there was a significant interaction with soil N such that these rates increased under elevated CO_2 and high soil N, but decreased under elevated CO_2 and low soil N.

A significant CO_2 by temperature interaction was observed for total glycosides (Fig. 4). Although there was no significant main effect, an interaction with temperature shows that there was a significant increase when both CO_2 and temperature were elevated (Te = +17% vs Ta = −12%).

Finally, there was only one response variable that depended on the interaction between CO_2 and drought: total terpene emission rates. As mentioned above, these emission rates did not depend on the main effect of elevated CO_2, but we saw a decline in these rates under well-watered conditions but not under drought conditions (−34% vs −2%, respectively).

On the one hand, the relative paucity of two-way interactions between CO_2 and these seemingly important environmental variables is encouraging, as it suggests that for many variables just studying the main effects will be sufficient. On the other hand, this encouraging interpretation loses some clarity when we consider three-way interactions later in this review.

Environmental by biological by CO_2 interactions There were many three-way interactions between the effects of CO_2, temperature and one or more biological variables (Supporting Information Table S3) on plant N concentrations (Fig. 5a), plant biomass (Fig. 5b), and phenolic concentrations (Fig. 5c,d). The particular biological groupings involved in the interactions varied by response variable and did not seem to generalize. Surprisingly there was little evidence of CO_2 × drought × biological variable interactions. We found significant evidence of a three-way interaction only for soluble carbohydrates (angiosperms: well watered = +2%, drought = +10%; gymnosperms: well watered = +49%, drought = +150%). Finally, and perhaps not surprisingly, the richest source of three-way interactions was those involving CO_2 × soil N. Many, but not all of these three-way interactions are depicted in Fig. 6. Again, the particular biological groupings involved varied by response variable. See Table S3 for the complete results.

V. Searching for general responses to elevated CO_2

Of the 122 studies we examined that documented herbivore responses to elevated CO_2, 98 (80%) of these also measured some plant parameters, most often biomass, leaf N concentration, or C : N ratio. Only 26 (21%) of studies examined interactions with abiotic parameters (temperature, light, water and N fertilization). Because of the limited number of studies of plant

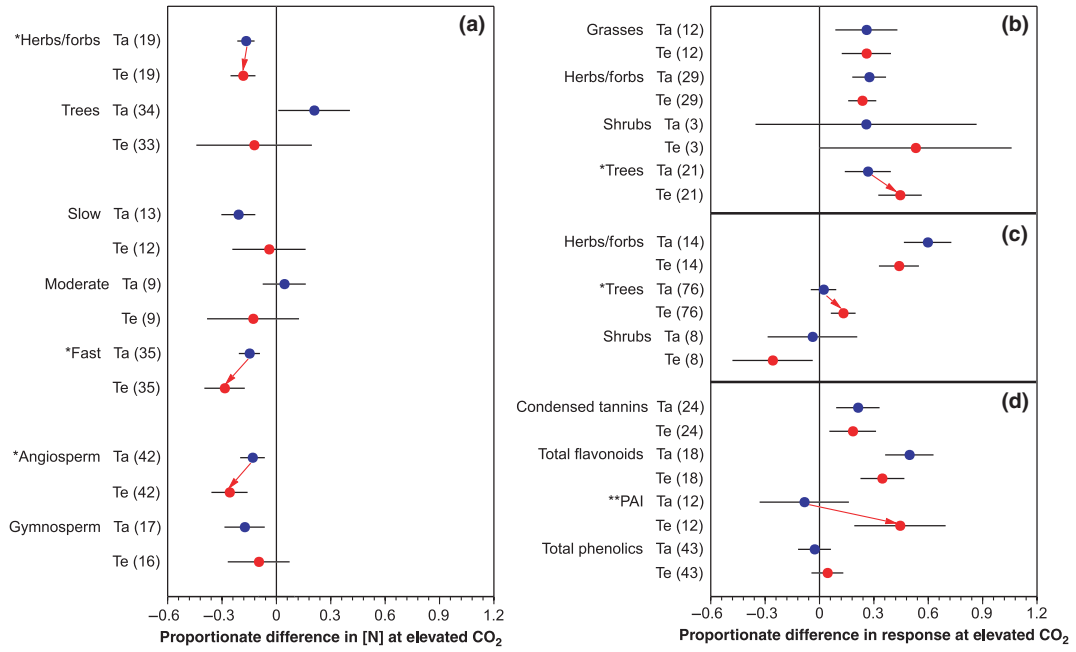

Fig. 5 Effects of elevated CO_2 on (a) plant nitrogen concentration, (b) biomass, (c) [total phenolics] by plant growth form, and (d) [total phenolics] by class of phenolic compound, under two levels of temperature (Ta, ambient temperature, blue symbols; Te, elevated temperature, red symbols), showing differences in the presence of two-way interactions between plant functional groups. Numbers to the right of the group name denote the sample sizes at each level of temperature for each plant functional group. Significance levels for the difference in the effect of elevated CO_2 between the two temperature conditions were determined by *Z*-tests for each plant functional group and are denoted by: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Where significant differences exist, the two levels of temperature are also connected by a red arrow for fast visual reference. PAI, phenylpropanoid acid intermediates. Complete details of all statistical tests are given in Supporting Information Table S3(h). Variable definitions are given in Notes S1 and literature sources of the extracted results are given in Notes S2.

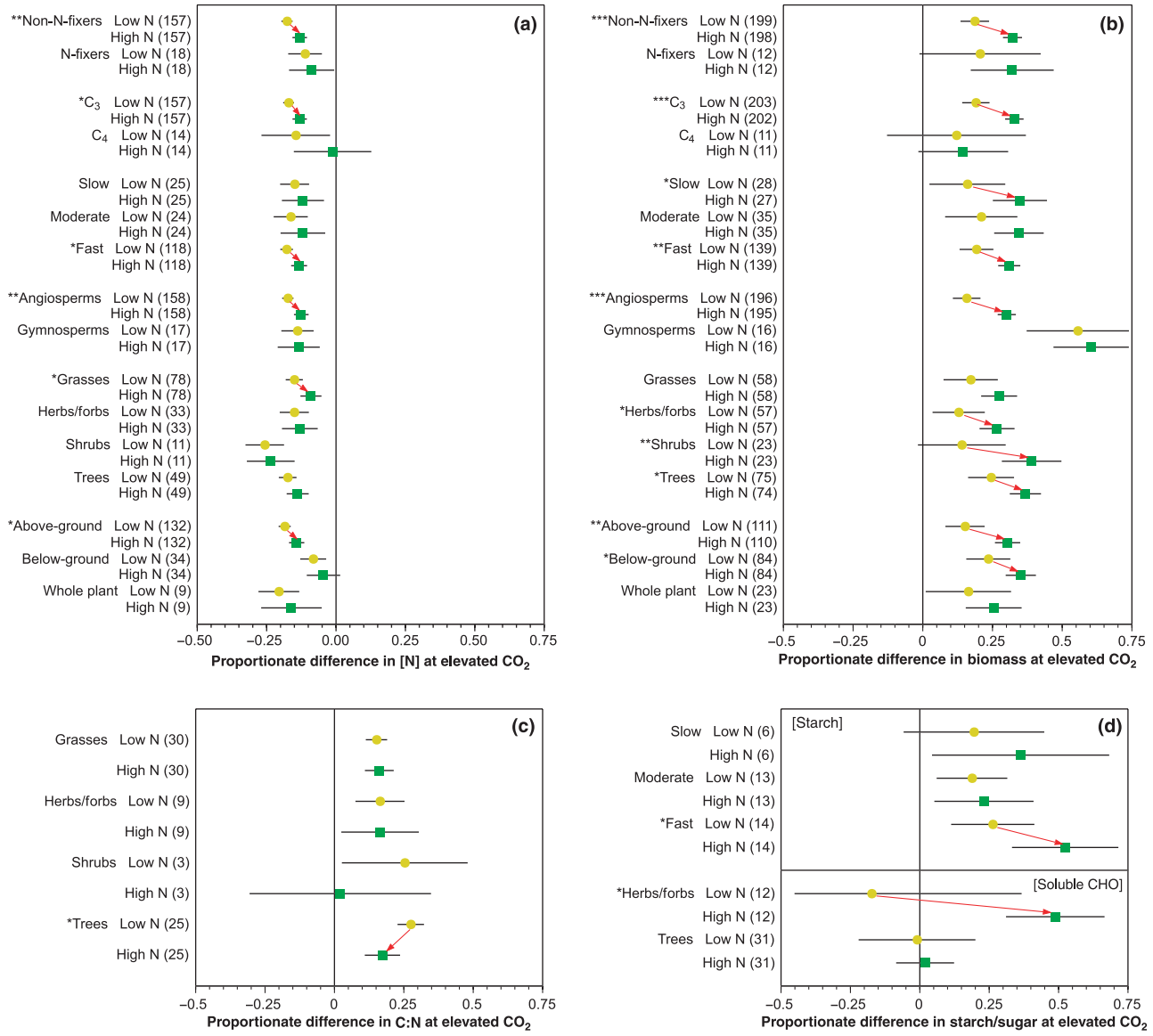

Fig. 6 Effects of elevated CO_2 on (a) plant nitrogen concentration, (b) biomass, (c) carbon:nitrogen ratio, (d) [starch] and [soluble sugars] under two levels of nitrogen availability (low N, brown symbols; high N, green symbols), showing differences in the presence of two-way interactions between plant functional groups. Numbers to the right of the group name denote the sample sizes at each level of nitrogen availability for each plant functional group. Significance levels for the difference in the effect of elevated CO_2 between the two levels of nitrogen availability were determined by *Z*-tests for each plant functional group and are denoted by: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Where significant differences exist, the two levels of nitrogen are also connected by a red arrow for fast visual reference. Complete details of all statistical tests are given in Supporting Information Table S3(b). Variable definitions are given in Notes S1 and literature sources of the extracted results are given in Notes S2.

chemistry in the herbivore literature, the limited number of plant parameters measured, and the fact that interactions between abiotic factors and CO_2 have been rarely studied in the context of herbivory, we expanded our search to include studies of elevated CO_2 and plant chemistry, resulting in an additional 148 studies beyond the herbivore data set. Plant parameters known to have an effect on herbivore success were extracted in the hope that an examination of these parameters may help to provide a mechanistic framework for herbivore responses to elevated CO_2.

The data presented in this meta-analysis suggest that elevated CO_2 will induce changes in plant chemistry, physiology and morphology that are likely to impact the nutritional quality of host plants for insect herbivores. We observed a general increase in total carbohydrates (+23%) under elevated CO_2; we saw increases in starch (+50%), total nonstructural carbohydrates (+39%) and soluble sugars (+8%). The only carbohydrate group that decreased under elevated CO_2 was the structural carbohydrates (−13%), although fewer studies (22 data points from seven independent studies) measured structural carbohydrates compared with other forms. While increased carbohydrates may act as an additional energy resource or phagostimulant for insect herbivores (Bernays & Chapman, 1994), the increased concentrations of carbohydrates observed under elevated CO_2 can also dilute more limiting nutrients such as N-based metabolites like

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soluble protein and free amino acids. This dilution effect is evidenced by the highly significant increase in the C : N ratio (+19%) observed here. Total plant N (−16%), amino acids (−14%) and soluble protein (−10%) were also reduced by elevated CO_2. The efficiency with which a herbivore can convert ingested plant tissue into its own biomass is positively correlated with plant N content (Mattson, 1980), suggesting that, in general, elevated CO_2 may change plant nutrient dynamics in a way that will negatively impact insect herbivores. This is consistent with the herbivore results presented here where the efficiency of conversion of both ingested and digested food decreased with elevated CO_2 (−17% and −12%, respectively). However, it is worth noting that all of the studies in this meta-analysis that examined efficiency of conversion did so only for foliage feeders; information about these parameters for other feeding guilds is absent from this literature. This highlights the need for caution in making generalized interpretations of such data – where different performance parameters are more easily or commonly applied to different feeding guilds.

One of the difficulties in relating general plant chemistry responses to insect herbivory is the fact that different plant tissues may have differential responses to elevated CO_2 and the subsequent effects on herbivores will depend on the feeding guild involved. Indeed, here we observe notable differences in herbivore responses when divided into feeding guild or arthropod order. On average it appears that phloem feeders, such as Homoptera, respond positively to elevated CO_2 while foliage feeders/Lepidoptera, on average, respond negatively. However, in this data set, of the 15 papers that related phloem feeder performance to plant chemistry, only a single paper actually measured CO_2-induced changes in phloem composition, while the rest related performance to whole-tissue chemistry. This is probably a reflection of the difficulty of extracting pure phloem from plant tissues. As it is not clear to what extent whole-plant changes are related to changes in individual tissues, examining whole-tissue chemistry in the context of phloem feeders may be of limited value. This disparity in the literature suggests that we know much less about the plant mechanisms that drive phloem feeder/Homoptera responses to elevated CO_2 than we do for other feeding guilds.

The main effects of CO_2 on herbivores (see Fig. 1) may be dependent on interacting abiotic factors, though this has not been widely studied. For example, fertilization can bring about changes in N quality and quantity (see e.g. Newman *et al.*, 2003) and has the greatest effect on soluble nitrogenous compounds (amino acids, soluble proteins, and amides) that are likely to be crucial limiting nutrients for herbivores (Mattson, 1980). This may have important implications for how herbivores respond to elevated CO_2 in natural (N-limited) vs agricultural (N-rich) ecosystems. In this meta-analysis we observed that N fertilization resulted in a smaller CO_2-induced reduction in total N (–12.6% vs –16.9%, respectively), soluble protein (−11% vs −19%, respectively) and amino acids (−9.7% vs −25.1%, respectively) and a smaller increase in the C : N ratio (+16% vs +20%, respectively). While these changes suggest that fertilization may lessen the negative impacts of CO_2 on herbivore performance, N fertilization here was also shown to lessen the CO_2-induced reduction in N-based secondary metabolites (−7.9% vs −27.0%, respectively).

Interactions between CO_2 and temperature, light and drought, and their effects on plant chemistry and insect herbivores have been much less well studied. These variables can significantly alter plant responses to CO_2, although our results are only really convincing for the effects of temperature. Indeed, the general lack of interactions involving drought or light was rather surprising. Even the often-discussed interaction between the effects of elevated CO_2 and drought on biomass was not significant in this meta-analysis. The effect of elevated CO_2 was nearly identical averaged over the 85 results comparing well-watered plants and those experiencing drought conditions (+29% vs +31%, respectively). It is not clear what we should make of this result. There are good theoretical reasons to expect this interaction, based on basic principles of plant physiology, and yet the data do not support this expectation.

VI. Limitations and future studies

One limitation of this review is the same as was encountered by Coviella & Trumble (1999) in one of the first ever attempts to review plant–insect interactions under elevated CO_2: the insects studied are heavily dominated by a few groups. Herbivore responses are far better studied for Lepidoptera than for any other order and so our view of arthropod responses is strongly biased by this one order.

We have suggested that perhaps more progress can be made in understanding, and so predicting, herbivore responses to elevated CO_2 if we incorporate more information about changes in plant nutritional quality and defences. We showed that many potential indicators of quality and defence depend upon interactions between CO_2 and other environmental variables such as temperature and nutrient status. The obvious next step would be to link these to changes to herbivore performance, but this is problematic for at least three reasons. First, there is a reasonably large sample size for herbivore responses, which we might hope to relate to changes in indicators of plant quality. However, we have shown that these herbivore responses depend upon herbivore guild (Fig. 2), and for many variables, each guild is represented by relatively few studies. It will therefore be difficult to make a quantitative connection between herbivore response and plant quality indicator. A second, related problem is that often the plant quality indicator is measured in a tissue that is not relevant to all herbivore guilds. For example, while Fig. 2 shows sample sizes for phloem feeders that range from 23 to 41, there was only a single study that estimated changes in plant phloem quality (Sun *et al.*, 2009b). And finally, attempting to relate *average* responses in plant quality to *average* herbivore responses is probably an inappropriate use of the results of meta-analyses. 'Plant quality' is not an absolute measure, but one that is *relative* to the particular herbivore in question, its nutritional needs, its sensitivity to various metabolites, and so on (Awmack & Leather, 2002). So while our results show that plant responses that will be important for at least some herbivores depend upon interactions between CO_2 and other variables, environmental and biological, we are not yet able to complete the linkage of interactions affecting plant

quality and plant quality affecting arthropod responses. Nevertheless, our analysis does suggest particular hypotheses that might be fruitful to pursue in the future.

Finally, two related criticisms of meta-analysis deserve some consideration here: the 'apples and oranges' criticism, and the 'Flat Earth Society' criticism (Glass, 1999). Neither criticism negates the value of conducting meta-analyses, but they are important reminders to us to not over-interpret the results of such analyses. We consciously ignored one other potentially important criticism, in the context of meta-analyses of elevated CO_2 research, the 'quality' of the research (long-term vs short-term experiments, controlled chambers vs free-air CO_2 enrichment (FACE) systems, etc.). However, we note that Stiling & Cornelissen (2007) did conduct such an analysis, but did not find large differences, except perhaps for arthropod abundance between closed chambers and FACE systems.

Graphs such as Fig. 1, in seeking to draw general conclusions about arthropod responses, will necessarily be averaging over the responses of many different species. Just as we would pause to think about the rationale for comparing apples and oranges, we ought to pause and think about comparing aphids and butterflies. Comparing apples and oranges makes sense when we are seeking to draw general conclusions about 'fruit', and so comparing aphids and butterflies makes sense when we are seeking to draw conclusions about arthropods. However, we must ask ourselves how meaningful such conclusions are biologically. Of course mechanically we can calculate such average effect sizes and their confidence intervals, but they only really make sense if we expect such disparate taxonomic groups to react similarly to elevated CO_2. Perhaps we shouldn't have such an expectation, and Fig. 2 suggests, *a posteriori*, why. Also, imagine that we are indeed trying to draw conclusions about fruit, but of all of our samples, 75% were from apples, 20% from oranges, and the remaining 5% from a smattering of other fruits. Would the average effect size really represent 'fruit'? This is the problem we face in trying to characterize arthropod responses. Notice too that, although Fig. 2 seemingly addresses the apples and oranges criticism, it only lessens that criticism, it does not dispense with it, as within these orders and guilds we will again be averaging over many species of arthropods, many of which are disproportionately represented in the data.

The 'Flat Earth Society' is a criticism, attributed to Lee Cronbach, that says meta-analysis seeks to bury complex hypotheses with an empirical bulldozer (Glass, 1999). Figs 4–6 show more complex hypotheses that are buried in analyses like that in Fig. 3. Meta-analysis encourages us to ignore results that are clearly statistically significant, but in the opposite direction of the average effect size (e.g. Newman *et al.*, 1999). Unless we believe that these less common responses are all Type I errors of statistical inference, we risk ignoring some interesting biology if we don't follow meta-analyses with attempts at explaining such contradictory results.

Acknowledgements

We thank the Canadian Natural Sciences and Engineering Research Council (NSERC) and the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) for funding. We also thank three anonymous referees and the Editor for helpful comments on an earlier version of this manuscript.

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Supporting Information

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

Notes S1 Definitions of abbreviations used in Notes S2.

Notes S2 List of sources and variables extracted for plant responses.

Notes S3 Definitions of abbreviations, and list of sources and variables extracted for arthropod responses.

Notes S4 References for sources of data extracted in Notes S2 and S3.

Table S1 Herbivore meta-analyses

Table S2 Plant main effects and biological variable interaction meta-analyses

Table S3 Plant three-way interactions meta-analyses

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