

Direct and legacy effects of long-term elevated CO₂ on fine root growth and plant–insect interactions

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Summary

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Received: 29 January 2013

Accepted: 18 March 2013

New Phytologist (2013)

doi: 10.1111/nph.12295

Key words: fine root growth, Florida, herbivory, legacy effects, long-term effects of elevated CO₂, scrub oaks.

- Increasing atmospheric CO₂ concentrations alter leaf physiology, with effects that cascade to communities and ecosystems. Yet, responses over cycles of disturbance and recovery are not well known, because most experiments span limited ecological time. We examined the effects of CO₂ on root growth, herbivory and arthropod biodiversity in a woodland from 1996 to 2006, and the legacy of CO₂ enrichment on these processes during the year after the CO₂ treatment ceased.
- We used minirhizotrons to study root growth, leaf censuses to study herbivory and pitfall traps to determine the effects of elevated CO₂ on arthropod biodiversity.
- Elevated CO₂ increased fine root biomass, but decreased foliar nitrogen and herbivory on all plant species. Insect biodiversity was unchanged in elevated CO₂. Legacy effects of elevated CO₂ disappeared quickly as fine root growth, foliar nitrogen and herbivory levels recovered in the next growing season following the cessation of elevated CO₂.
- Although the effects of elevated CO₂ cascade through plants to herbivores, they do not reach other trophic levels, and biodiversity remains unchanged. The legacy of 10 yr of elevated CO₂ on plant–herbivore interactions in this system appear to be minimal, indicating that the effects of elevated CO₂ may not accumulate over cycles of disturbance and recovery.

Introduction

Global atmospheric carbon dioxide (CO₂) levels continue to increase rapidly, mainly because of the burning of fossil fuels. The atmospheric concentration of CO₂ has increased from a pre-industrial level (*c.* 1750) of *c.* 270 ppm to a current level of *c.* 394 ppm, an increase of 124 ppm, or 45%. Most studies indicate that CO₂ levels will at least double from preindustrial levels over the next five to ten decades. This increase represents one of the most large-scale and wide-reaching perturbations to the environment (IPCC, 2007).

Many studies have shown an increase in above- and below-ground plant growth in elevated relative to ambient CO₂ (Curtis & Wang, 1998; Norby *et al.*, 1999; Long *et al.*, 2004; Ainsworth & Long, 2005; Jackson *et al.*, 2009; Seiler *et al.*, 2009; Day *et al.*, 2013). However, elevated CO₂ inhibits the assimilation of nitrate into organic nitrogen compounds (Bloom *et al.*, 2010) and usually reduces plant nitrogen concentrations and increases secondary metabolites (Lincoln *et al.*, 1993; Poorter *et al.*, 1997; Curtis & Wang, 1998; Bidart-Bouzat & Imeh-Nathaniel, 2008; Zavala *et al.*, 2013). This decreased plant quality decreases herbivore performance and levels of herbivory in many communities (Stiling & Cornelissen, 2007; Lindroth, 2010; Robinson *et al.*,

2012). Theoretically, the effects of elevated CO₂ on insect herbivores may cascade up to impact species feeding at higher trophic levels, such as spiders and parasitic wasps. In addition, increased biomass, and therefore litter production, could provide increased resources for detritivores. These effects could lead to changes in arthropod biodiversity.

Most studies investigating the effects of elevated CO₂ on plants and plant–herbivore interactions have used short-term experiments comparing the digestion and performance of insect herbivores fed foliage grown in elevated or ambient CO₂ (Barbehenn *et al.*, 2004; Sudderth *et al.*, 2005; Agrell *et al.*, 2006). Fewer studies have used elevated CO₂ levels in the field and measured changes in plant growth, herbivory and biodiversity, despite the greater validity of this technique to natural conditions (Sanders *et al.*, 2004; Hillstrom & Lindroth, 2008; Stiling *et al.*, 2010). Finally, no studies have investigated the legacy effects of elevated CO₂ on plant growth and plant–insect interactions. What happens to plant growth, plant quality and herbivory when elevated CO₂ levels are terminated? Extrapolations from previous work suggest that legacy effects of elevated CO₂ could last for a considerable period of time. For example, a substantial below-ground carbon sink develops that can affect plant re-growth for many years (Canadell *et al.*, 1996; Lousteau *et al.*, 2001). In

addition, increased microbial turnover in elevated CO₂, together with nitrogen accumulation in above-ground plant biomass, can cause progressive nutrient limitation over long time periods, depressing plant growth (Gifford *et al.*, 1996; Cannell & Thornley, 1998; Hungate *et al.*, 2006). Thus, it is possible that long-term legacy effects of elevated CO₂ could affect plant growth and plant–insect interactions for many years after the cessation of elevated CO₂.

This article provides data on the effects of elevated CO₂ on plants and insect herbivory in a Florida scrub oak forest after many years of continuously elevated CO₂. In addition, we provide data on the richness of insect orders and of beetle families and functional groups to examine whether there are noticeable changes in biodiversity under elevated CO₂. Finally, we examine the legacy effects of elevated CO₂ on plant re-growth and insect herbivory by measuring fine root re-growth and herbivory levels after elevated CO₂ is discontinued.

Materials and Methods

This study was conducted at NASA's Kennedy Space Center, in a scrub-oak, palmetto ecosystem (Schmalzer & Hinkle, 1992). The study site was dominated by three species of oak: myrtle oak, *Quercus myrtifolia* Willd, sand-live oak, *Q. geminata* Small, and Chapman oak, *Q. chapmanii* Sargent, which together accounted for 85–90% of the plant biomass (Seiler *et al.*, 2009). Of the three species of oak, *Q. myrtifolia* was the most common, accounting for *c.* 77% of the oak biomass, whereas *Q. geminata* accounted for *c.* 17% and *Q. chapmanii* for 6% (Dijkstra *et al.*, 2002). These oaks rely on stored below-ground resources for many years during stand development (Langley *et al.*, 2002). Twenty-seven additional plant species were identified in the area with Elliott's milk pea, *Galactia elliotii*, Nuthall, a nitrogen-fixing legume, the most common. Scrub-oak is a fire-dependent system and, at Kennedy Space Center, the community exists on a fire return cycle of between 10 and 15 yr.

In January 1996, a 0.8-ha area of scrub-oak forest was burned. During the following spring, 16 open top chambers (OTCs), each 2.5 m in height with octagonal sides of 1.4 m in width, were erected in the study area. Each side consisted of a 10-cm PVC pipe frame covered with Mylar (Melinex 071; Courtaulds Performance Films, Martinsville, VA, USA). Panels were easily removable to facilitate entry into the chambers. Eight OTCs were maintained at elevated CO₂ (ambient + 350 ppm CO₂) and eight at ambient CO₂ (*c.* 350 ppm in 1996 to *c.* 380 ppm in 2007). Ambient or CO₂-enriched air was blown into each chamber via four 20.5-cm-diameter ducts at a rate of 24–30 m³ min⁻¹. The blower speed was reduced at night to one-third of the daytime values. These CO₂ concentrations were continuously maintained inside the chambers from mid-May 1996 through mid-June 2007 (except for small periods in 1999 and 2005 when the chambers were damaged by hurricanes). The effects of elevated CO₂ on above- and below-ground plant growth, and on nutrient cycling, are presented elsewhere (Seiler *et al.*, 2009; Day *et al.*, 2013). Here, we present previously unpublished data on the effects of elevated CO₂ on plant–herbivore interactions, insect

biodiversity and the legacy effects of elevated CO₂ on fine root growth and herbivory.

Herbivory

During August 2001 and 2002, we counted the numbers of leaf miners, leaf tiers, chewed leaves, eyespot galls, leaf galls and leaves with pathogens per 200 haphazardly selected leaves on each oak species in each chamber and on the legume *Galactia elliotii*. Among the most common leaf mining genera were *Cameraria*, *Stigmella*, *Stilbosis* and *Buccatrix*. Leaf damage was caused by various chewing lepidopteran and orthopteran species, leaf tying by various lepidopterans, eye spot galls by cecidomyiids and other leaf galls by cecidomyiids, including *Belonocnema quercusvirens*, *Neuroterus quercusverrucarum*, *Sphaeroteris carolina*, *S. melleum* and others. Leaf pathogens were not identified.

Statistical analyses of the effects of CO₂ treatment on damaged leaves for 2001 and 2002 were performed using split-plot ANOVAs on the total numbers of leaves damaged by each herbivore guild or pathogen, with CO₂ as the main factor, guild and plant species as the subplot factors and chamber as a random effect.

Biodiversity

Pitfall trap catches are considered to be good indicators of biodiversity in most terrestrial habitats (Duelli *et al.*, 1999; Hillstrom & Lindroth, 2008). We installed two 8.5-cm-diameter × 6-cm-deep pitfall traps per chamber. Traps were half filled with antifreeze to keep insects from crawling out and to minimize fluid loss through evaporation. Traps were installed at the end of 2002 and were replaced approximately bimonthly for 3 yr, until 2005. All arthropods were identified to order under a dissection scope. In addition, in 2004 samples, all beetles were identified to family. The biodiversity of trap catches was analyzed using repeated-measures ANOVA of bimonthly totals of arthropod orders or beetle families. In addition, in 2004, we scored beetles as herbivores, detritivores, insectivores and fungivores and analyzed treatment effects using Wilk's lambda MANOVA.

Legacy effects

The chambers were dismantled and all vegetation was harvested in July 2007 to determine species-specific and community biomass responses to 11 yr of elevated CO₂ (Seiler *et al.*, 2009). During the remainder of 2007, and in 2008, the vegetation began to re-grow from the remaining roots under ambient atmospheric CO₂ levels.

To estimate the legacy effects of elevated CO₂ on fine root growth, images from minirhizotrons installed in the former chamber plots were collected in August 2007 (*c.* 1 month after above-ground vegetation removal) and May 2008 (*c.* 10 months after removal) using the methods described in Day *et al.* (2013). Digital jpeg images were captured from the video recordings. Fine root biomass (g m⁻² to a depth of 1 m) was calculated from root length and width values for all roots < 2 mm in diameter, following the methods detailed by Day

et al. (2013). For statistical analyses, the data were log-transformed to meet the assumptions for ANOVA. Fine root biomass was tested with a four-factor repeated-measures ANOVA using SAS Proc GLM (SAS version 9.1; SAS Institute Inc., Cary, NC, USA), with plot as the random effect and CO₂ treatment, depth and date as fixed effects. A three-factor nested ANOVA was run on each individual date to test for CO₂ treatment effects; plot was the random effect and treatment and depth were fixed effects.

During September 2008, we counted the numbers of leaf mines and chewed leaves per 200 haphazardly selected leaves on *Q. myrtifolia*, *Q. chapmanii*, *Q. geminata* and *G. elliotii* in each ambient or elevated CO₂ legacy plot. In addition, leaves of each species were collected haphazardly throughout the plots and oven dried at 70°C, and then ground and analyzed for percentage nitrogen. Statistical analyses of the legacy effects of CO₂ treatment on the numbers of leaf mines per 200 leaves, number of chewed leaves per 200 leaves and percentage leaf nitrogen were performed using split-plot ANOVAs with CO₂ as the main plot factor and chamber as a random effect. Three chambers were omitted from the percentage leaf nitrogen analyses because no *Galactia* was collected.

Results

Herbivory

There was a significant effect of CO₂ on leaf damage: elevated CO₂ reduced the numbers of leaves damaged by leaf miners, leaf tiers, leaf chewers, eyespot galls and other leaf galls for all four plant species in both 2001 (Fig. 1, $P < 0.001$) and 2002 (Fig. 2, $P < 0.001$). There was also a significant effect of tree species in both years, as the amount of herbivore damage varied between host plant species ($P < 0.001$ for both years), but there was no interaction of CO₂ with plant species, meaning that elevated CO₂ depressed leaf damage on all plant species (2001, $P = 0.793$; 2002, $P = 0.808$). There was also a significant effect of guild on leaf damage, because damage by some guilds, such as leaf miners and leaf tiers, was more common than by others (Figs 1, 2, $P < 0.001$ for both years). There was an interaction of CO₂ with guild (2001, $P = 0.002$; 2002, $P = 0.030$), as pathogen-damaged leaves were not consistently depressed in elevated CO₂, but all other types of insect-damaged leaves were. Finally, there was a significant interaction of guild and tree species (2001 and 2002, both $P < 0.001$), as the abundance of leaves damaged by different guilds varied according to tree species, but there was no three-way interaction between CO₂ level, guild and tree species (2001, $P = 0.966$; 2002, $P = 0.924$).

Biodiversity

Arthropods from 25 orders were found in pitfall traps, but there was no significant effect of CO₂ treatment on arthropod order richness ($P = 1.000$), although richness varied through time (Fig. 3a, $P < 0.001$). Beetles from 39 families were found in pitfall traps. There was also no significant effect of elevated CO₂ on

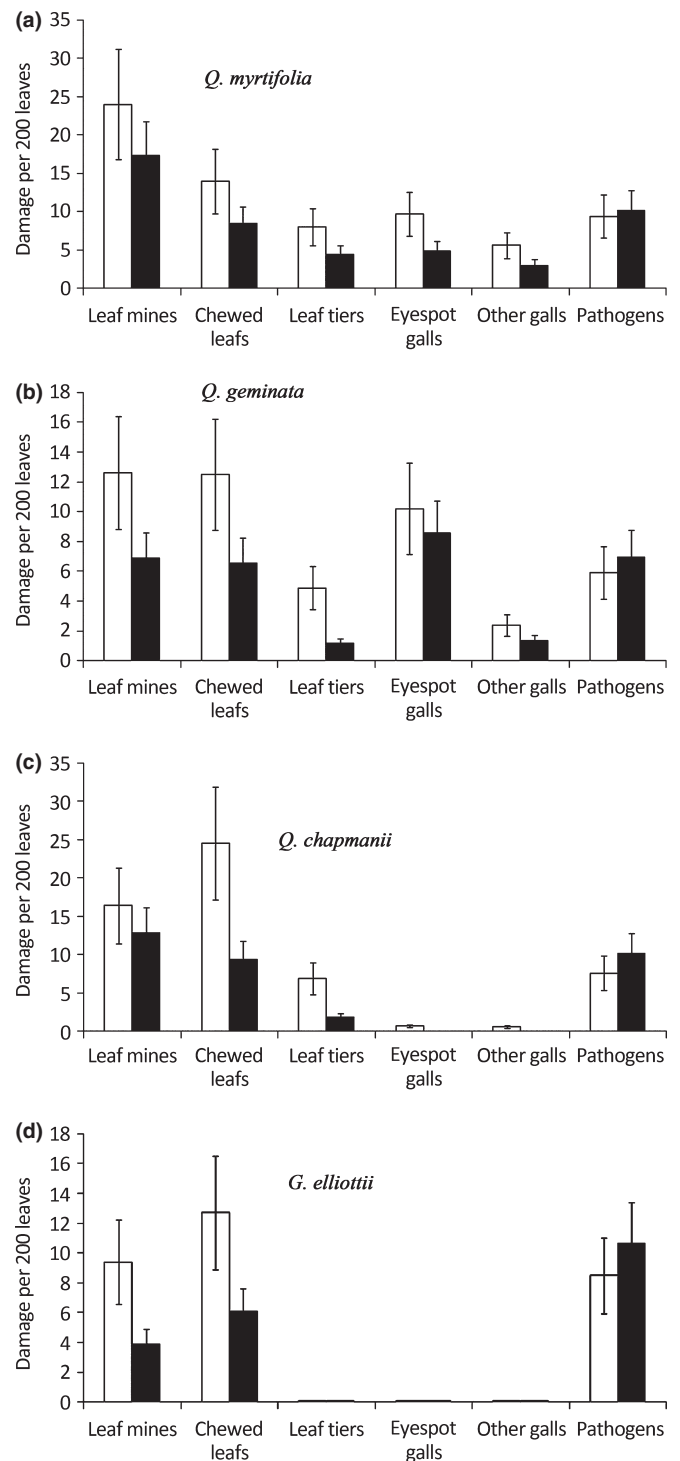


Fig. 1 Frequency of damage per 200 leaves, ± SEM, September 2001, in ambient (open bars) and elevated (closed bars) CO₂ at Kennedy Space Center, FL, USA. (a) *Quercus myrtifolia*; (b) *Q. geminata*; (c) *Q. chapmanii*; (d) *Galactia elliotii*.

beetle family richness in pitfall traps in 2004 ($P = 1.000$), although beetle richness also varied over time (Fig. 3b, $P < 0.001$). For beetles, there were no significant effects of elevated CO₂ on any guild or interaction of time with CO₂ (Fig. 4, $P > 0.05$ in all cases).

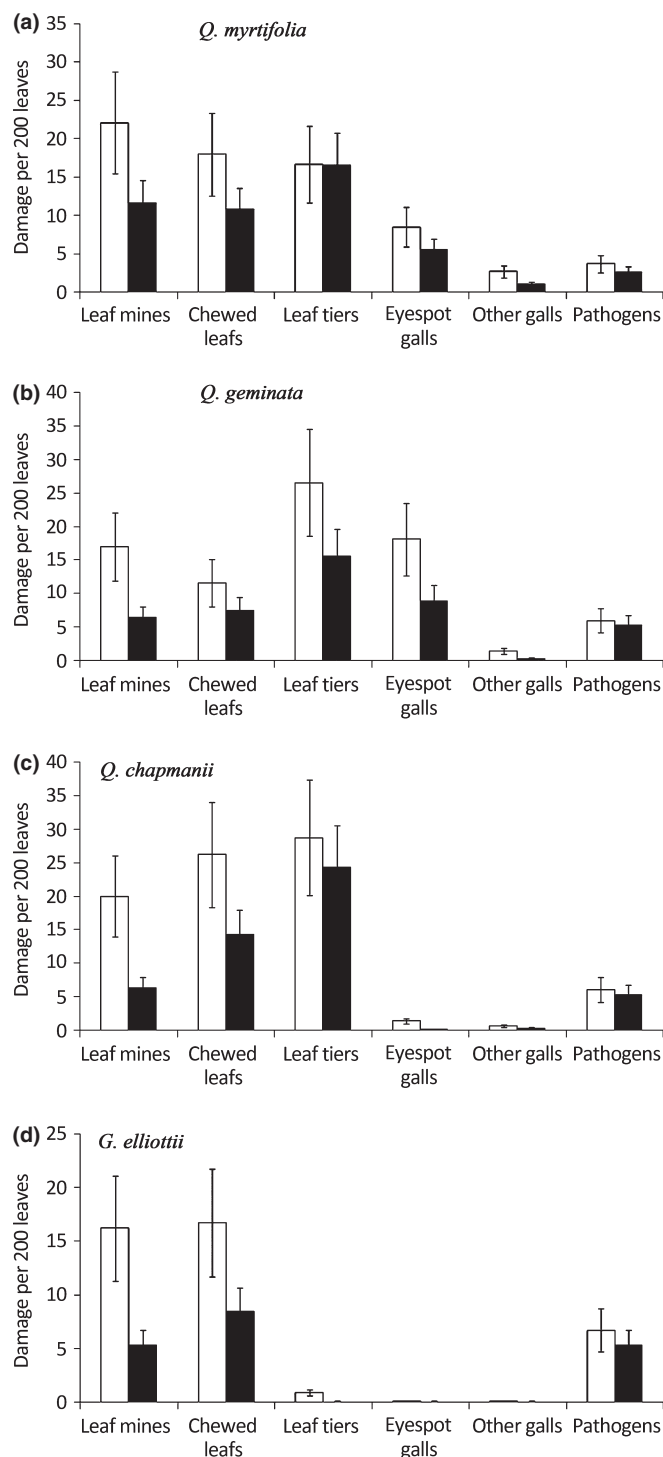


Fig. 2 Frequency of damage per 200 leaves, \pm SEM, September 2002, in ambient (open bars) and elevated (closed bars) CO₂ at Kennedy Space Center, FL, USA. (a) *Quercus myrtifolia*; (b) *Q. geminata*; (c) *Q. chapmanii*; (d) *Galactia elliotii*.

Legacy effects

Fine root biomass values in ambient CO₂ plots were 1644, 1620 and 1687 g m⁻² for March 2007, August 2007 and May 2008, respectively. In elevated CO₂ plots, fine root biomass values were 1942, 1852 and 2078 g m⁻² for the same time series (Fig. 5). No

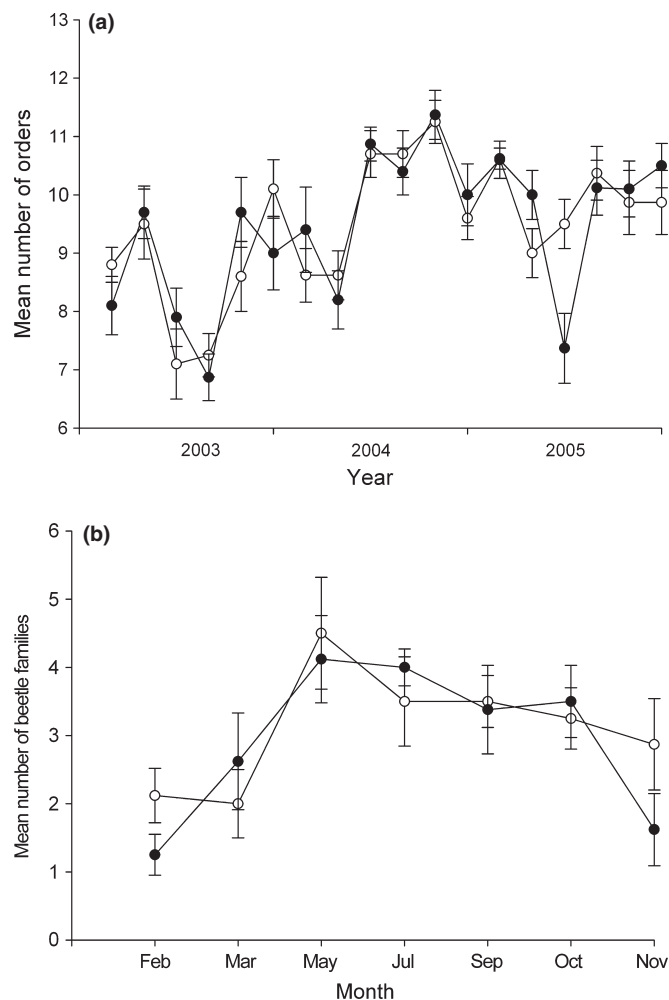


Fig. 3 Arthropod richness in pitfall traps, \pm SEM, under ambient (open circles) and elevated (closed circles) CO₂ at Kennedy Space Center, FL, USA for (a) arthropod orders and (b) beetle families.

statistically significant CO₂ treatment effect on fine root biomass was detected on any given date (March 2007, $P=0.31$; August 2007, $P=0.57$; May 2008, $P=0.39$), although fine root biomass was consistently higher in plots previously under elevated CO₂ for all three sample dates. However, there was a significant difference among the three sampling dates ($P<0.0001$) in the previously elevated CO₂ plots. There was minimal change in fine root biomass in the ambient plots over the three sample dates. Fine root biomass increased by only 4% in the ambient plots between August 2007 and May 2008, but increased by 12% in the formerly elevated CO₂ plots, indicating significant recovery of fine root biomass in the elevated plots, but not in the ambient plots.

Leaf nitrogen was unaffected by previous CO₂ treatment (Fig. 6, $P=0.760$) and, although there was an effect of plant species on foliar nitrogen ($P<0.001$), there was no interaction between treatment and plant species ($P=0.890$). Levels of damage by the two most common herbivore guilds, leaf-mining moths and leaf chewers, primarily larval lepidopterans and grasshoppers, were unaffected by previous CO₂ treatment, for all four plant species (Figs 7, 8, $P=0.975$ for leaf miners, $P=0.811$ for leaf chewers). Although the amount of leaf mining and leaf

Coleoptera guilds (2004)

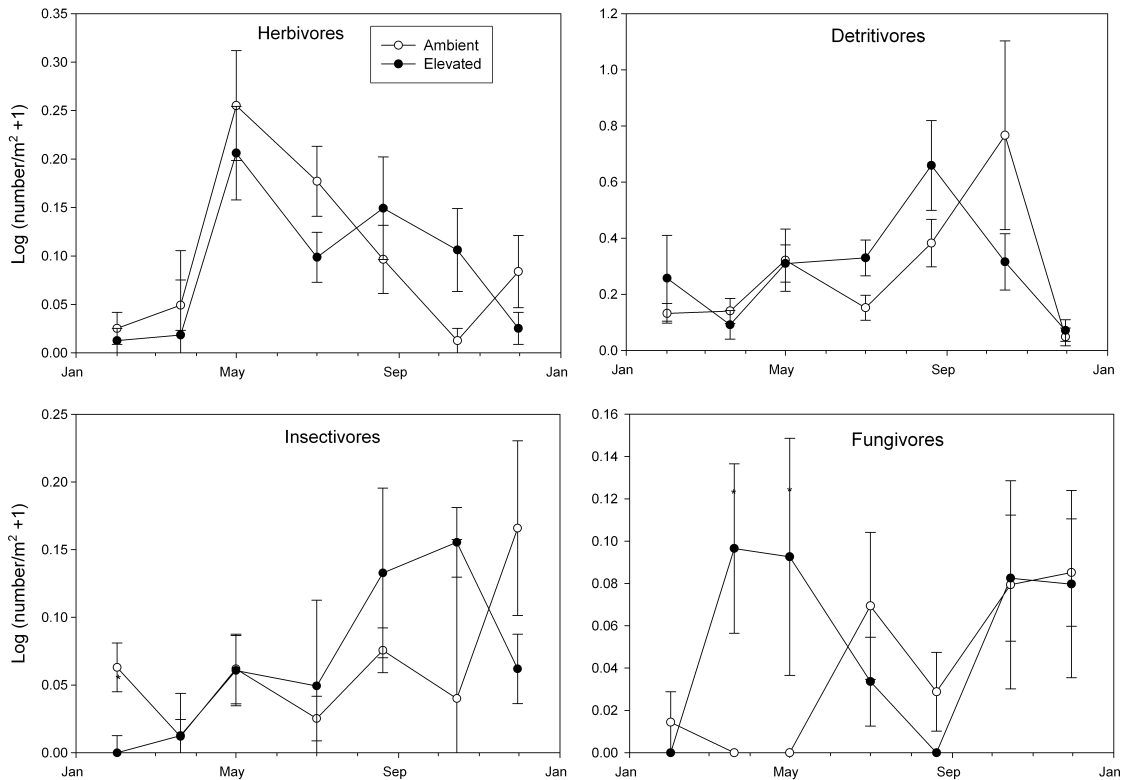


Fig. 4 Beetle densities per chamber, ± SEM, during 2004 for herbivores, detritivores, insectivores and fungivores. Open circles, ambient CO₂; closed circles, elevated CO₂.

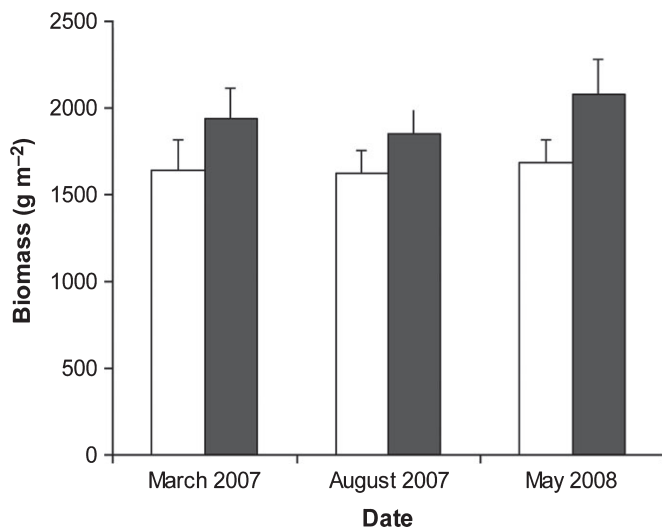


Fig. 5 Fine root biomass (g m⁻²) to 100-cm depth estimated using minirhizotrons for sampling dates before and after complete above-ground vegetation removal. Values are means + SE. Open bars, ambient CO₂; closed bars, elevated CO₂.

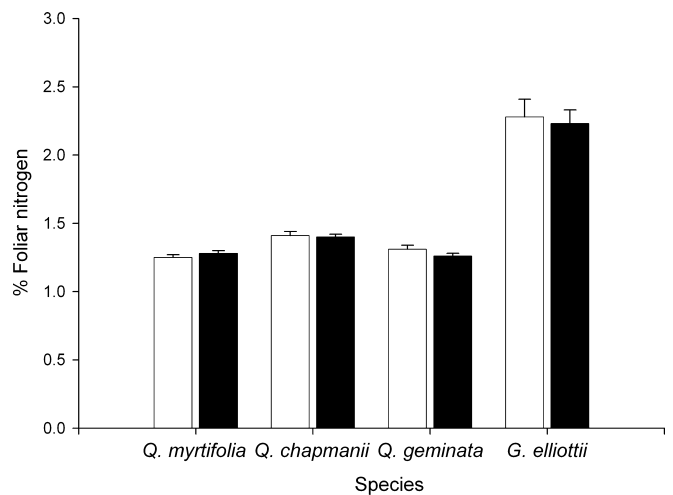


Fig. 6 Foliar nitrogen concentration, ± SEM, in elevated CO₂ and ambient CO₂ legacy plots at Kennedy Space Center, FL, USA. Open bars, ambient CO₂; closed bars, elevated CO₂.

chewing differed between plant species ($P < 0.001$ and $P = 0.001$, respectively), there was no interaction between previous CO₂ level and plant species, indicating that the response to previously elevated CO₂ was the same across all plant species ($P = 0.647$, leaf miners; $P = 0.944$, leaf chewers).

Discussion

Herbivore damage and biodiversity

Elevated CO₂ reduced the densities of all herbivore-damaged leaves, which included damage produced by leaf miners, leaf tiers, leaf chewers and leaf galls, on all host plant species, including the nitrogen-fixing legume, *Galactia*. Only pathogen damage was

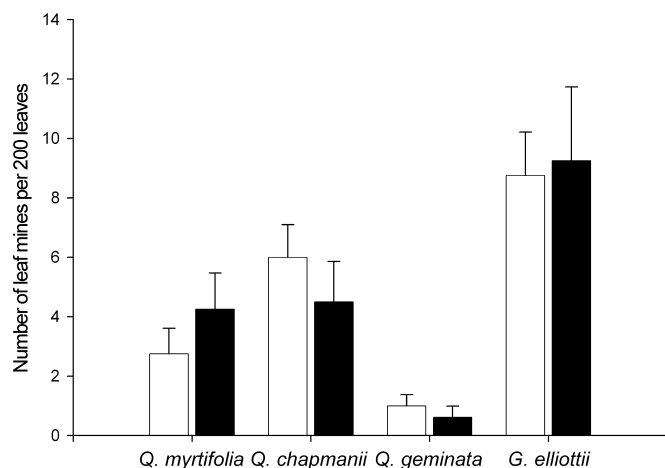


Fig. 7 Density of leaf mines per 200 leaves, \pm SEM, in elevated CO₂ (closed bars) and ambient CO₂ (open bars) legacy plots at Kennedy Space Center, FL, USA.

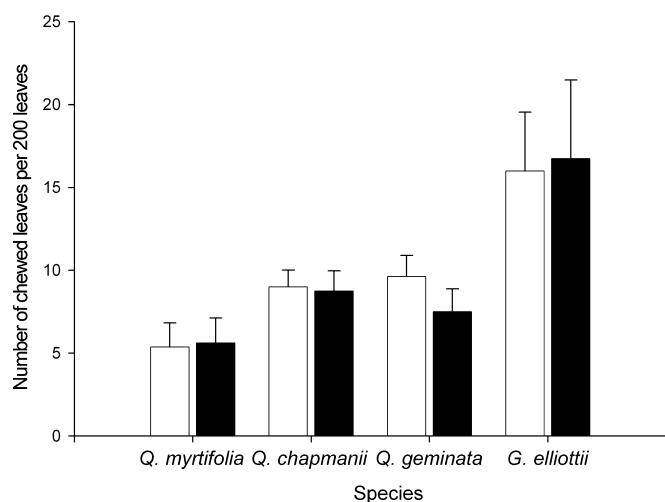


Fig. 8 Density of chewed leaves per 200 leaves, \pm SEM, in elevated CO₂ (closed bars) and ambient CO₂ (open bars) legacy plots at Kennedy Space Center, FL, USA.

not consistently depressed in elevated CO₂. Although longer term counts revealed that numbers of leaf miners and leaf tiers per 200 leaves in elevated CO₂ were decreased in nearly all years (Stiling *et al.*, 2009), this is the first time we have shown similar reductions for other herbivores, such as leaf galls and for herbivory by leaf chewers. Our results are similar to those of other studies, most of which have also found reductions in insect herbivory under elevated CO₂ (reviewed in Lincoln *et al.*, 1993; Watt *et al.*, 1995; Bezemer & Jones, 1998; Hunter, 2001; Whittaker, 2001; Stiling & Cornelissen, 2007; Lindroth, 2010; Robinson *et al.*, 2012). Several mechanisms are responsible for this decline. First, elevated CO₂ inhibits the assimilation of nitrate into organic nitrogen compounds (Bloom *et al.*, 2010). This tissue nitrogen reduction causes reduced insect herbivore survival and reproduction. Foliar nitrogen reductions in our oaks averaged between 7% and 10% across all years, and reductions in *Galactia* averaged 15% (Stiling *et al.*, 2009). Second, elevated

CO₂ can cause increases in allocations to carbon-based secondary metabolites, such as condensed and hydrolyzable tannins (Peñuelas & Estiarte, 1998). Earlier studies in our system showed a trend towards increased total phenolics, condensed and hydrolyzable tannins (Rossi *et al.*, 2004; Hall *et al.*, 2005). Third, reduced leaf quality often delays insect development (Stiling & Cornelissen, 2007; Robinson *et al.*, 2012) and, in our system, this exposes herbivores longer to natural enemies, increasing herbivore death rates (Stiling *et al.*, 1999), although such increases in mortality are not always evident (Lindroth, 2010).

Earlier results from our pitfall traps showed that, although there was a significant increase in herbivore catches in pitfall traps in elevated relative to ambient CO₂, these increases were not evident at other trophic levels, such as insectivores, parasitoids and predators, or decomposers (Stiling *et al.*, 2010). Because of the limited trophic cascade of CO₂ from plants to other trophic levels, it is not surprising that biodiversity was not affected at the level of either insect order or beetle family. Perhaps a more detailed examination would reveal finer scale changes, but this would involve the identification of insects to family or species, which would be logistically difficult. Other studies that have examined the influence of elevated CO₂ on insect biodiversity have also failed to find many significant effects (Sanders *et al.*, 2004; Hillstrom & Lindroth, 2008). This may be because such studies have focused on species-rich communities, where reductions in some species may be offset by increases in others. Only in communities dominated by a few species might biodiversity be affected by elevated CO₂ (Altermatt, 2003). However, it is possible that studies over much longer time frames would reveal changes in biodiversity. Previous studies in our system have shown increases in acorn production under elevated CO₂ for *Q. myrtifolia* and *Q. chapmanii*, but not for *Q. geminata* (Stiling *et al.*, 2004). Over long time periods, such effects would almost certainly affect plant diversity, and thus insect diversity, given that the different oak species support different herbivore species, albeit from the same or similar genera.

Legacy effects

There was evidence of legacy effects on fine root growth, because fine root growth in the previously elevated CO₂ plots was greater than that in the ambient CO₂ plots. However, levels of foliar nitrogen in previously elevated CO₂ and ambient CO₂ plots were statistically indistinguishable. As a result, there was no legacy of elevated CO₂ on herbivory in our scrub oak forests. Shortly after the CO₂ treatment ceased, herbivory increased to normal levels. The legacy of below-ground carbon accumulation and progressive nutrient limitation does not appear as important as the short-term effects of changes in foliar nitrogen. One clear implication of this is that our current generation of global change experiments may reasonably capture the dominant effects of elevated CO₂, even over longer time scales than those over which we are currently capable of running experiments.

An abrupt return of atmospheric CO₂ levels to 'normal' is no more unrealistic an analog for a future scenario than is the abrupt increase in CO₂ concentrations used in typical step-change

experiments. Yet, both simulations provide insight into the nature of ecosystem responses to this chronic global environmental change. Specifically, the examination of the legacy of CO₂ effects in ambient conditions gauges the inertia of the ecosystem to CO₂ enrichment, without the confounding influence of ongoing CO₂ treatment. Thus, responses after CO₂ enrichment ceases can be ascribed unequivocally to CO₂-induced changes in ecosystem structure and functioning that occurred earlier, and to responses that persist beyond the cessation of CO₂ exposure. The lack of substantial legacy effects provides a unique insight into critical and currently poorly understood mechanisms of ecosystem responses to elevated CO₂ over cycles of disturbance and recovery. In short, we offer this analysis, not as a direct analog for future CO₂ reduction scenarios, which will obviously occur on a different time scale, but rather to test hypotheses about the nature of ecosystem responses to elevated CO₂. Will there be a similar lack of CO₂ legacy effects in other systems? At present, we cannot be sure, because there have not been any similar studies. It is possible that legacy effects may be more likely in systems with better developed soils, higher nitrogen and a more pronounced carbon sink. However, the results of our study suggest that other fire-dependent systems dominated by perennial plants may show a similar dearth of legacy effects. How do the legacy effects of elevated CO₂ compare with those of other environmental perturbations, such as acid rain or deforestation? Dobson *et al.* (1997) suggested a linear relationship between spatial scale of disturbance and community recovery time. In this model, the recovery time for large-scale environmental perturbations, such as acid rain and groundwater exploitation, is much longer than that of small-scale perturbations, such as tree falls and lightning strikes. This scenario might not hold for the effects of elevated CO₂, where changes over large spatial scales could have few substantial legacy effects. We encourage scientists to tackle these and other questions we have raised here during the course of our studies.

Acknowledgements

This research was supported by the Office of Science (BER), US Department of Energy, through Southeast Regional Center of the National Institute for Global Environmental Change grants to P.S., by a National Science Foundation (NSF) grant to B.A.H. and by Department of Energy grants to B.D. Jamie Colson-Moon helped collect the pitfall traps. Thanks are due to Sylvia Lukasiewicz, Terri Albarricin, Kerry Bohl, Heather Jezorek, Kara Winston, Christina Harris, Georgina Johnson, Heather Faulkner, Toni Gordon, Arnaldo Villafranca, Ciro Vasquez, Samvid Owivedi, Shawn Simmons, Andy Paluch, Matt Dumouchel, Crystal Bernarducci, Shalane Ponsell, Pauline Thai, Jessica Allen, Amanda Ditson, Hamid Hoveida, Caitlyn Palmy, Carl Franconi, Craig Beatty and Dianne Harshberger for help in sorting pitfall samples and leaf litter. Ben Duval, Paul Dijkstra and Rick Doucett helped with the nitrogen analyses. We acknowledge the support and encouragement of the NASA Kennedy Space Center and Dynamac Corporation, especially Ross Hinkle. Dave Johnson, Hans Anderson, Tom Powell and Graham Hymus provided a happy working environment at the field site.

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