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# Elevated atmospheric carbon dioxide and ozone alter forest insect abundance and community composition

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**Abstract.** 1. Human-induced climate changes threaten the health of forest ecosystems. In particular, carbon dioxide ($CO_2$) and tropospheric ozone ($O_3$) will likely have significant but opposing impacts on forests and their associated insect communities. Compared with other animal groups, insect communities are expected to be especially sensitive to changes in global climate.

2. This study examined the effects of elevated $CO_2$ and $O_3$ ($eCO_2$ and $eO_2$) individually and in combination on the abundance, diversity and composition of forest insect communities. Insects were sampled using yellow pan traps in an aggrading aspen-birch forest at the Aspen Free Air $CO_2$ Enrichment (FACE) site in northern Wisconsin, USA. We trapped for 24 h every 10–15 days throughout the summers (June to September) of 2000–2003.

3. We examined 47 415 insects from 4 orders and 83 families. Elevated $CO_2$ reduced abundance of phloem-feeding herbivores and increased abundance of chewing herbivores, although results were not statistically significant. Enriched $CO_2$ increased numbers of some parasitoids. The effects of $eO_3$ on insect abundance were generally opposite those of $eCO_2$. No significant differences in arthropod family richness were found among treatments. However, $eCO_2$, $eO_3$, or both significantly affected insect community composition in all years.

4. Carbon dioxide and tropospheric ozone have the potential to alter significantly forest insect communities. Feeding guild may strongly influence insect response to environmental change and may provide the best opportunity to generalise for conservation efforts. Because insect communities influence forest health and ecosystem services, continued research on their response to global change is critically important to forest management and conservation.

**Key words.** Biodiversity, carbon dioxide, climate change, FACE, forest, insect community, NMDS, pan trap, ozone.

## Introduction

As the evidence for human-induced climate change mounts, conservation of natural communities has become a scientific, social and political priority. Ecosystems face not only increases in carbon dioxide levels, temperature, and altered precipitation, but also increased habitat fragmentation and loss, making adaptation increasingly difficult (Lovejoy & Hannah, 2005). Responses of organisms to global change will be species-specific and occur at different rates, potentially disaggregating communities and causing alteration or loss of ecosystem services critical to human existence (Lovejoy & Hannah, 2005).

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Biodiversity will play a key role in how ecosystems respond to global change (Hansen & Dale, 2001) as loss of species can lead to reduced ecosystem function (Cardinale *et al*., 2006). Much of the planet's biodiversity resides in two groups of organisms: plants and insects. Forests are of particular interest as they cover one third of Earth's land mass (Ozanne *et al*., 2003), host millions of species and are expected to be especially sensitive to changes in the global environment (Fowler *et al*., 1999). Forest insects play key ecological roles, influencing forest productivity, species composition, energy flow, and nutrient cycling (Mattson & Addy, 1975; Schowalter *et al*., 1986; Veblen *et al*., 1991). Insects are also important herbivores, pollinators, predators and parasitoids in forests (Altermatt, 2003). Thus, changes in insect abundance, diversity or community composition have the potential to alter forest ecosystems and the services they provide.

Two gases in particular, carbon dioxide ($CO_2$) and tropospheric ozone ($O_3$), will likely have significant but opposing impacts on forest ecosystems (Dickson *et al*., 1998; Saxe *et al*., 1998). Since the beginning of the industrial revolution, $CO_2$ levels have increased from 280 to 380 ppm (IPCC, 2007). Over this same time period, ambient tropospheric $O_3$ levels have risen to over 40 ppb (from ~10 ppb in 1850) in many regions of the world and are projected to reach 70 ppb (mean monthly 24-h ozone concentrations) during the summer in the USA, Europe and East Asia by 2100 (Sitch *et al*., 2007). Elevated $CO_2$ and $eO_3$ have been shown to alter tree growth and chemical characteristics (reviewed for Aspen Free Air $CO_2$ Enrichment (FACE) in Lindroth *et al*., 2001; Karnosky *et al*., 2003). Numerous studies have demonstrated changes in insect performance at $eCO_2$ and $eO_3$ (Bezemer & Jones, 1998; Coviella & Trumble, 1999), but more recent work suggests that population and community responses may differ from individual responses (Percy *et al*., 2002; Awmack *et al*., 2004). Likewise, the artificial conditions of many of the early studies confound population and community level predictions because natural communities experience many indirect biotic and abiotic interactions and feedbacks that cannot be accounted for in studies with simplified environments (Bailey & Whitham, 2002). Recent work at FACE facilities has provided some evidence for the effects of $CO_2$ and $O_3$ on natural insect communities, but no study to date has examined the effects of $CO_2$ and $O_3$ simultaneously on above-ground forest insect communities. Multifactor, ecologically realistic studies of environmental change are necessary for conservation strategies to be developed.

The effects of $eCO_2$ or $eO_3$ on insect communities are likely to be indirect. For example, effects on herbivorous insect populations will depend on the magnitude of change in host plant quality, natural enemy impact and changes in the physical environment (tree architecture, etc.). Elevated $CO_2$ and $eO_3$ may change natural enemy populations via shifts in the diversity, abundance and quality of prey or changes in host-finding mechanisms. Natural enemies may be particularly sensitive to $eCO_2$ or $eO_3$ through changes in searching behaviour (Gate *et al*., 1995; Vuorinen *et al*., 2004; Pinto *et al*., 2007) or the behaviour of their prey (Mondor *et al*., 2004).

This study was designed to examine the effects of $eCO_2$ and $eO_3$, individually and in combination, on the abundance, biodiversity and composition of forest insect communities. Insects were sampled using yellow pan traps in planted aspen-birch stands at the Aspen FACE site in northern Wisconsin. Yellow pan traps are known to preferentially attract predators and parasitoids (Duelli *et al*., 1999), two of the most under-represented groups in climate change research. We recognise that pan traps sample only a subset of the insect community but chose them because they allow for easy collection of large quantities of insects across a broad range of families.

## Methods

### *Aspen FACE*

The Aspen FACE site is a 32-ha experimental station located near Rhinelander, Wisconsin. The site consists of 12 rings each 30 m in diameter planted with trees spaced 1 m apart. Sampling occurred in the interplanted trembling aspen (*Populus tremuloides* Michx.) and paper birch (*Betula papyrifera* Marsh.) section of each ring. The aspen-birch community is one of the most widespread communities in northern forests and contains the primary pulpwood species of the Great Lakes region (Piva, 2006). Trees were planted as seedlings in 1997 and fumigation began in 1998 (Dickson *et al*., 2000; Karnosky *et al*., 2003).

Aspen FACE is set up as a randomised complete block design with three blocked sets of four treatment rings: control (ambient $CO_2$, ambient $O_3$), elevated $CO_2$ ($+CO_2$; 560 $\mu M\ M^{-1}$ $CO_2$), elevated $O_3$ ($+O_3$; 1.5 × ambient) and elevated $CO_2$ and $O_3$ ($+CO_2+O_3$). Elevated $CO_2$ and $O_3$ levels represent concentrations predicted for 2060. Ambient $O_3$ concentration is monitored daily and the elevated rings are fumigated based on a diurnal concentration profile approximately 1.5 times that of ambient levels (Dickson *et al*., 2000).

### *Insect sampling*

The intent of this study was to sample a large number of insects across a broad range of families. We choose pan traps to accomplish this task although we recognise the limitations of this methodology. Pan traps do not attract all families of insects equally but are particularly good at attracting predators and parasitoids (Duelli *et al*., 1999; Triplehorn & Johnson, 2005), the intended focus of this study. Pan traps are also passive traps so location of the trap will affect the insects captured. To account for this sampling effect, we always placed the traps in the same location in each ring and in similar surrounding vegetation. It is also possible that some percentage of the insects collected were only flying through the rings and were unaffected by the treatment. We accounted for this by analyzing only the 15 most abundant families (each accounting for greater than one percent of total abundance) in both the community and individual analyses. Each of the 15 families had over 500 individuals collected over the 4 years so they are unlikely to be merely passing through. Although pan traps have limitations, we accounted for these concerns in both sampling technique and data analysis.

We placed one yellow pan trap (22 cm in diameter) on the ground in the aspen-birch section of each FACE ring for 24-h collections during the summers of 2000–2003. Sampling occurred approximately every 10 days from June to September. Traps contained 0.5% scentless detergent solution 1 cm deep and were placed at fixed locations each time to preclude spatial differences in catches among sample dates. Insects were removed from the traps by filtering the detergent solution through a fine strainer. Samples were stored in 70% ethyl alcohol. Insects were identified under a dissecting microscope to order and family. Identifications and feeding guild assignment were made using Triplehorn & Johnson (2005).

### *Insect community analysis*

Arthropod abundance was analysed two ways. First, total abundance of individuals across all insect families collected

 

**Table 1.** Average (±1 SE) total number of insects collected per pan trap per year, by treatment, for the 15 most abundant families of arthropods. Statistics represent the results of two-way crossed repeated measures ANOVA tests (df 1,8). Significant *P*-values (< 0.05) are indicated in bold.

| | | | | | $CO_2$ | | $O_3$ | | $CO_2 \times O_3$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| Feeding guild/family | Control | $+CO_2$ | $+O_3$ | $+CO_2+O_3$ | *F* | *P* | *F* | *P* | *F* | *P* |
| Herbivores – phloem feeders | | | | | | | | | | |
| Aphididae | 51 ± 15 | 58 ± 15 | 83 ± 15 | 56 ± 15 | 1.1 | 0.334 | 0.6 | 0.449 | 6.6 | **0.033** |
| Cicadellidae | 57 ± 10 | 32 ± 10 | 78 ± 10 | 56 ± 10 | 3.8 | 0.087 | 3.8 | 0.087 | 0.6 | 0.463 |
| Miridae | 10 ± 3 | 9 ± 3 | 24 ± 3 | 13 ± 3 | 4.1 | 0.077 | 7.8 | **0.024** | 2.1 | 0.185 |
| Herbivores – chewers | | | | | | | | | | |
| Curculionidae | 14 ± 2 | 16 ± 2 | 9 ± 2 | 13 ± 2 | 3.8 | 0.087 | 4.7 | 0.062 | 0.4 | 0.553 |
| Anthomyiidae | 101 ± 23 | 139 ± 23 | 76 ± 23 | 141 ± 23 | 5.1 | 0.054 | 0.3 | 0.612 | 0.3 | 0.576 |
| Parasitoids | | | | | | | | | | |
| Braconidae | 51 ± 5 | 49 ± 5 | 32 ± 5 | 35 ± 5 | 0.2 | 0.649 | 10.6 | **0.011** | 0 | 0.961 |
| Chalcidoidea | 97 ± 6 | 93 ± 6 | 76 ± 6 | 65 ± 6 | 1.6 | 0.248 | 15 | **0.005** | 0.1 | 0.765 |
| Figitidae | 23 ± 6 | 52 ± 6 | 12 ± 6 | 19 ± 6 | 9.3 | **0.016** | 13.5 | **0.006** | 3.4 | 0.102 |
| Ichneumonidae | 99 ± 15 | 122 ± 15 | 55 ± 15 | 75 ± 15 | 6 | **0.040** | 14 | **0.006** | 0 | 0.921 |
| Predators | | | | | | | | | | |
| Dolicopodidae | 41 ± 5 | 33 ± 5 | 35 ± 5 | 28 ± 5 | 2 | 0.191 | 1.1 | 0.320 | 0 | 0.945 |
| Saprophages | | | | | | | | | | |
| Drosophilidae | 16 ± 4 | 20 ± 4 | 9 ± 4 | 12 ± 4 | 0.9 | 0.376 | 4 | 0.079 | 0 | 0.874 |
| Phoridae | 191 ± 27 | 143 ± 27 | 90 ± 27 | 141 ± 27 | 0 | 0.955 | 3.6 | 0.100 | 3.3 | 0.108 |
| Sciaridae | 160 ± 30 | 162 ± 30 | 141 ± 30 | 94 ± 30 | 0.6 | 0.455 | 2.4 | 0.159 | 1.1 | 0.332 |
| Herbivores/Predators | | | | | | | | | | |
| Formicidae | 31 ± 6 | 12 ± 6 | 30 ± 6 | 21 ± 6 | 7.3 | **0.027** | 1.3 | 0.292 | 0.9 | 0.362 |
| Predators/Saprophages | | | | | | | | | | |
| Sarcophagidae | 43 ± 8 | 42 ± 8 | 48 ± 8 | 30 ± 8 | 5.1 | 0.054 | 0.2 | 0.708 | 2.1 | 0.183 |
| **Total** | **1094 ± 90** | **1069 ± 90** | **903 ± 90** | **887 ± 90** | 0.1 | 0.807 | 5.3 | **0.050** | 0 | 0.956 |

(= total arthropod abundance) was evaluated. Second, the 15 most abundant families were examined individually in more detail (Table 1). All data were pooled across an equal number of sample dates for each of the 4 years of this study. The data were normalised using a square root or log transformation until the Shapiro–Wilks W test statistic exceeded 0.85. Treatment effects were analysed using two-way crossed repeated measures ANOVA with block, $CO_2$ (= $eCO_2$) and $O_3$ (= $eO_3$) as the main effects, year as the repeated measures term and year by main effect interactions as the subplot terms. Year is considered a repeated measure because traps were located in the same spot for each of the 4 years. We also included the interaction of the main effects ($CO_2 \times O_3$). $CO_2$ main effects ($eCO_2$) represent differences between plots with enriched $CO_2$ ($+CO_2$ and $+CO_2+O_3$) and plots with ambient $CO_2$ (control and $+O_3$). Similarly, $O_3$ main effects ($eO_3$) represent differences between plots with enriched $O_3$ ($+O_3$ and $+CO_2+O_3$) and plots with ambient $O_3$ (control and $+CO_2$). *P*-values are considered significant at $\alpha = 0.05$ for individual family tests. We did not employ Bonferroni corrections because these individual family tests are almost certainly not independent of one another due to direct and indirect biotic interactions among the insects and host plants. If the tests are not independent, then Bonferroni correction is excessively conservative leading to high Type II error rates (Gotelli & Ellison, 2004; Moran, 2003). Nevertheless, we interpret the results with caution.

We analysed arthropod family richness using sample-based rarefaction curves produced with the program ESTIMATES 7.5.0 (Colwell, 2006). Rarefaction curves account for differential sampling effort whereas other commonly used alpha diversity measures (e.g. Shannon–Wiener index, Simpson's index) do not, producing potentially misleading results (Colwell *et al*., 2004; Buddle *et al*., 2005). Curves were re-scaled to individuals to account for differing densities of individuals in samples (Colwell *et al*., 2004). For each year, samples were pooled across all sample dates and Mao Tau species richness and 95% confidence intervals were calculated for each treatment as suggested in Colwell *et al*. (2004). Treatment differences were tested using a two-way crossed ANOVA for each year. Treatments were compared at an abundance shared by all treatments on the asymptotic part of the rarefaction curves, where species richness had stabilised. All data met normality and equal variance assumptions.

We assessed arthropod community composition (which accounts for abundance, richness and family identity) using non-metric multidimensional scaling (NMDS) (Shepard, 1962; Kruskal, 1964). NMDS is commonly used in arthropod community analysis (e.g. Wimp *et al*., 2005) for several reasons: (i) it makes few assumptions about the nature of the data (e.g. it does not require a Gaussian distribution), (ii) it relies on only the rank order of similarities rather then their actual values (results are easy to understand), and (iii) it provides robust results (Clark & Warwick, 2001; McCune & Grace, 2002). We pooled data across sample dates within each year and analysed years separately using PRIMER 6. Pooled raw abundance data for each year were square root transformed and all families that did not comprise at least 1% of total abundance were excluded (Clark & Warwick, 2001). We then produced a dissimilarity matrix using the Bray–Curtis

 

dissimilarity coefficient (Faith *et al*., 1987). NMDS was run and stress levels were evaluated to select the best representation of the data by assessing how well the distances between samples were maintained from the dissimilarity matrix to the ordination. A two-dimensional solution was selected because it maintained a consistently low stress (< 0.2) across multiple runs. We tested for differences in community composition using a two-way crossed ANOVA on the NMDS scores. NMDS scores represent the relative similarity of each of the 12 FACE rings to each other (points in ordination plots that are near one another are more similar than those that are farther apart). Because NMDS plots are based only on the relative similarities of the specific data points, axes have no intrinsic meaning.

We used indicator analysis (Dufrene & Legendre, 1997) in PC-ORD5 to examine whether particular families were significant indicators of control, $+CO_2$, $+O_3$, or $+CO_2+O_3$ treatments. This analysis is specific to treatments because we could not test both $CO_2$ and $O_3$ main effects simultaneously as we did for our abundance and community composition data. Indicator analysis accounts for each family's fidelity to a treatment as well as its abundance within that treatment. Values range from zero (no indication) to 100 (perfect indication) (McCune & Grace, 2002). Perfect indication for this data set would mean a family was found in only one treatment. Results for data pooled across all 4 years are presented.

## Results

In total, we examined 47 415 insects from 4 orders and 83 families. Over half of the specimens collected were Diptera (54.8%), followed by Hymenoptera (28.4%), Hemiptera (13.9%) and Coleoptera (2.9%). The 15 most abundant families consisted of six Diptera, five Hymenoptera, three Hemiptera and one Coleoptera.

### *Arthropod abundance*

Changes in total arthropod abundance were determined by changes in individual family abundance. Elevated $CO_2$ had no effect on total abundance. Elevated $O_3$, alternatively, reduced total abundance by 17% compared with ambient $O_3$ conditions, largely as a result of the negative effects on parasitoids (Table 1). Total abundance also varied by collection year, with the highest abundances in 2000 and 2003 and much lower abundance in 2001 [raw abundance (no. of individuals) 2000: 15 442; 2001: 6923; 2002: 9793; 2003: 15 257] ($F_{3,24} = 37.33$, $P < 0.001$).

Analysis of individual family abundance reveals that $eCO_2$ and $eO_3$ had significant effects on numbers of some insect families (Table 1). Elevated $CO_2$ substantially decreased the abundance of Formicidae (46%) compared with ambient $CO_2$. In contrast, $eCO_2$ increased numbers of Figitidae (103%) and Ichneumonidae (28%) parasitoids. Trends for decreased numbers of Cicadellidae (35%), Miridae (35%) and Sarcophagidae (21%) and increased numbers of Curculionidae (26%) and Anthomyiidae (58%) were apparent but these results were not statistically significant. Overall, the trend under $eCO_2$ was for the abundance of sucking herbivores and families using resources similarly to sucking herbivores (e.g. honeydew and nectaries) to decrease whereas parasitoids tended to increase. Relative changes in abundance due to $eCO_2$ were generally consistent across years.

**Table 2.** Mean (±1 SE) number of insect families collected per pan trap per year.

| Year | Control | $+CO_2$ | $+O_3$ | $+CO_2+O_3$ |
|---|---|---|---|---|
| 2000 | 38 ± 2 | 34 ± 1 | 35 ± 1 | 37 ± 1 |
| 2001 | 35 ± 2 | 34 ± 3 | 35 ± 1 | 34 ± 2 |
| 2002 | 34 ± 1 | 35 ± 4 | 39 ± 2 | 34 ± 1 |
| 2003 | 40 ± 1 | 37 ± 4 | 41 ± 2 | 38 ± 2 |

The effect of $eO_3$ on insect abundance was generally opposite that of $eCO_2$. Elevated $O_3$ had a strong negative effect on parasitoid abundances, reducing Braconidae by 33%, Chalcidoidea by 26%, Figitidae by 59% and Ichneumonidae by 41% (Table 1). A pronounced $O_3 \times$ year interaction occurred, however, for Figitidae ($F_{3,24} = 4.23$, $P = 0.034$). From 2000–2002, Figitidae abundance was decreased 22–59% but in 2003, numbers increased 66%. Elevated $O_3$ also decreased Curculionidae (27%) and Drosophilidae (42%) but these changes were not statistically significant. In contrast to the largely negative effects of $eO_3$ on other families, Miridae abundance increased 95%. Cicadellidae abundance increased 51% but again the change was not significant. Generally, $eO_3$ had the strongest impact on parasitoids (negative) but also influenced some sucking herbivores (positive). Families other than Figitidae showed more consistent relative changes in abundance due to $eO_3$ across years.

The effects of $CO_2$ and $O_3$ on insect abundance were independent of one another. The single exception was for the family Aphididae, for which $eCO_2$ increased abundance under $aO_3$, but decreased abundance under $eO_3$ (Table 1).

### *Arthropod family richness*

We found no significant differences in arthropod family richness among fumigation treatments for any year (Table 2). FACE rings contained 34–41 families across $CO_2$ and $O_3$ treatments and years.

### *Arthropod community composition*

Arthropod community composition shifted in response to $CO_2$ and $O_3$ treatments in all 4 years of this study. Elevated $CO_2$ affected composition in 2001, 2002 and 2003, whereas $eO_3$ affected composition in 2000 and 2003 (Fig. 1). Overall model $P$-values were significant in 2001 ($F_{3,8} = 4.00$, $P = 0.050$) and 2003 ($F_{3,8} = 12.30$, $P = 0.002$) but not significant in 2000 ($F_{3,8} = 3.30$, $P = 0.080$) and 2002 ($F_{3,8} = 2.70$, $P = 0.119$). $CO_2$ and $O_3$ affected the insect community independently (no significant $CO_2 \times O_3$ interactions). Thus, although family richness was not altered by $eCO_2$ or $eO_3$, shifts in insect abundance due the treatments led to changes in community composition that varied among years.

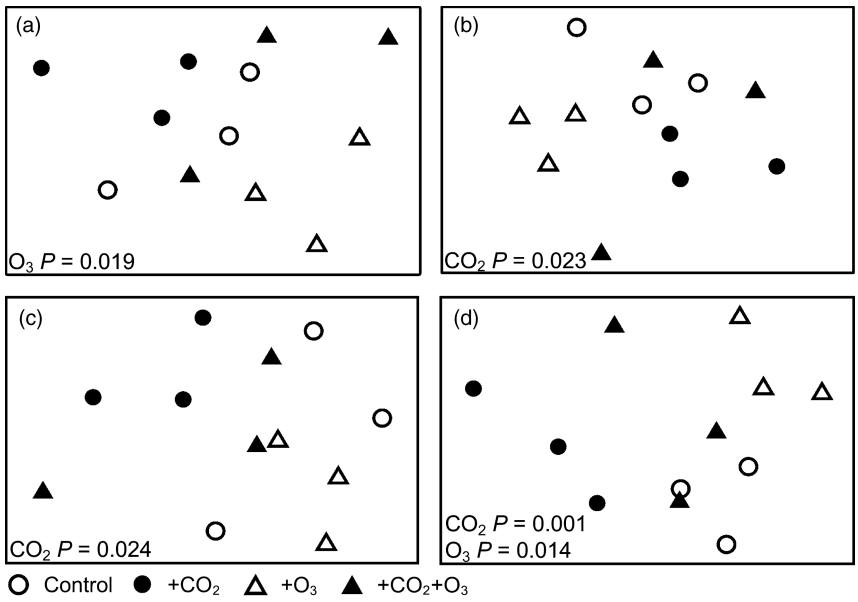


**Fig. 1.** 2000–2003 non-metric multidimensional scaling (NMDS) ordinations of community composition. Differences among treatments were tested using two-way crossed ANOVA on NMDS axis 1. A $CO_2$ main effect tests filled versus unfilled symbols, whereas an $O_3$ main effect tests circles versus triangles.
(a) 2000
(b) 2001
(c) 2002
(d) 2003

**Table 3.** Indicator analysis (Dufrene & Legendre, 1997) across 2000–2003 for the 15 most abundant families. Indicator values can range from 0–100, with 100 signifying complete fidelity to the indicated treatment.

| Feeding guild/family | Indicator value | Indicated treatment | *P* |
|---|---|---|---|
| Herbivores – phloem feeders | | | |
| Aphididae | 26.5 | +$O_3$ | 0.082 |
| Cicadellidae | 28.7 | +$O_3$ | **0.025** |
| Miridae | 23.3 | +$O_3$ | **0.001** |
| Herbivores – chewers | | | |
| Curculionidae | 15 | +$CO_2$ | 0.447 |
| Anthomyiidae | 29.7 | +$CO_2$+$O_3$ | **0.025** |
| Parasitoids | | | |
| Braconidae | 25 | Control | 0.1 |
| Chalcidoidea | 27.6 | Control | 0.085 |
| Figitidae | 30.3 | +$CO_2$ | **0.001** |
| Ichneumonidae | 33.2 | +$CO_2$ | **0.001** |
| Predators | | | |
| Dolicopodidae | 20.7 | Control | 0.315 |
| Saprophages | | | |
| Drosophilidae | 14 | +$CO_2$ | 0.163 |
| Phoridae | 32.2 | Control | **0.002** |
| Sciaridae | 28 | +$CO_2$ | 0.122 |
| Herbivores/Predators | | | |
| Formicidae | 25.2 | +$O_3$ | **0.014** |
| Predators/Saprophages | | | |
| Sarcophagidae | 18.1 | +$O_3$ | 0.344 |

*Community composition indicator analysis*

Indicator analysis designated 7 of the 15 most abundant families as significant indicators of particular treatments (Table 3). The +$CO_2$ treatment was indicated by two families, Figitidae and Ichneumonidae, in agreement with their increased abundance at e$CO_2$. Increased abundance at e$O_3$, similarly, led to three families that indicated +$O_3$ treatments: Cicadellidae, Miridae and Formicidae. Aphididae also tended to increase when exposed to e$O_3$ but the trend was not significant. Current atmospheric concentrations of $CO_2$ and $O_3$ (control) allowed for the greatest abundances of Phoridae. The combined elevated treatment (+$CO_2$+$O_3$), in contrast, was indicated by Anthomyiidae. Anthomyiidae may have indicated +$CO_2$+$O_3$ due to e$CO_2$ significantly increasing Anthomyiidae abundance, but doing so slightly more in the +$CO_2$+$O_3$ treatment than in the +$CO_2$ treatment. In general, indicator analysis results reflect the changes in family abundances.

## Discussion

Insect communities in the second half of the 21st century will likely be significantly different from present forest insect communities due to elevated concentrations of $CO_2$ and $O_3$. Our results suggest that e$CO_2$ may favour some parasitoid families but limit populations of sucking insects. In contrast, forests exposed to e$O_3$ may have significantly fewer insects, especially parasitoids, although phloem-feeding insects may benefit. $CO_2$ and $O_3$ effects may be expected to influence families/feeding guilds differently, leading to altered community composition. Insects exposed to both e$CO_2$ and e$O_3$ appear to respond to each gas independently. Therefore, offsetting effects of e$CO_2$ and e$O_3$ may occur for insect families affected positively by one gas and negatively by the other. The lack of interactions suggests that, at least for some global change factors, accurate predictions may be drawn from studies manipulating single variables.

*Abundance*

Total insect abundance did not change under e$CO_2$ but was reduced under e$O_3$. Similarly, total insect abundance was unaffected

by $eCO_2$ at the Oak Ridge FACE site (Sanders *et al*., 2004) and at SoyFACE (minus a large effect on Japanese beetles for one sample date; Hamilton *et al*., 2005). Total insect abundance at SoyFACE was also unaffected by $eO_3$ (Hamilton *et al*., 2005). Our $eO_3$ results likely differ from those of Hamilton *et al*., (2005) because they sampled only herbivores and our major reduction in abundance was due to the strongly reduced abundance of parasitoids. Changes in total insect abundance appear to depend on the families or feeding guilds sampled.

Abundances of four of the five herbivore families we examined exhibited strong mean responses to $eCO_2$, $eO_3$ or both, but low replication precluded statistical significance. Changes in abundance due to $eCO_2$ and $eO_3$ were dependent on herbivore feeding guild (chewing vs. phloem feeding) as suggested in reviews by Bezemer & Jones (1998) and Koricheva *et al*. (1998). We found that chewing herbivores increased in abundance under $eCO_2$ and decreased in abundance under $eO_3$, while phloem-feeders showed the converse. Bezemer & Jones (1998) and Stiling & Cornelissen (2007) suggested the opposite pattern for $eCO_2$. Our results may differ from their predictions because we sampled families with feeding habits different from those of previous work, as well as families that were not previously examined. For example, species of the two chewing herbivore families we trapped, Curculionidae and Anthomyiidae, feed on roots below ground as larvae. Indeed, our results match those of Altermatt (2003) for weevil abundance. We also recognise that pan traps are not an effective means of capturing many families of herbivorous insects, which limits our conclusions. Broader sampling of many herbivore families will be necessary to determine if our trends hold across multiple feeding guilds.

Global change is predicted to have larger impacts at higher trophic levels (Voight *et al*., 2003) and our data support that perspective. Parasitoid abundance increased under $eCO_2$ for two of the four families we examined and decreased under $eO_3$ for all four families. Previous work suggested that $eCO_2$ and $eO_3$ can increase or decrease parasitoid abundance, respectively, for short periods, but have no effect on parasitoid abundance over an entire season (Percy *et al*., 2002; Awmack *et al*., 2004). Furthermore, predation and parasitism rates under $eCO_2$ have been found to increase (Stiling *et al*., 1999, 2003), decrease (Gate *et al*., 1995) or not change (Stacy & Fellowes, 2002). While increased predation at $eCO_2$ could benefit pest control, the particularly strong negative impacts of $O_3$ on parasitoids could have major negative implications for pest control. Reduced ability to find prey, or the host plants of prey, is one possible explanation for the substantial reduction of parasitoids at $eO_3$. A 1995 study by Gate *et al.* found that $eO_3$ interferes with wasp searching behaviour. Furthermore, recent work suggests that $eCO_2$ and $eO_3$ inhibit the ability of wasps to detect damaged plants (Vuorinen *et al*., 2004; Pinto *et al*., 2007). Parasitoids could also be influenced by changes in herbivore behaviour at $eCO_2$ and $eO_3$ (Mondor *et al*., 2004).

Saprophagous insects showed the fewest significant responses to the treatments, despite previous work at Aspen FACE suggesting strong influences of $eCO_2$ and $eO_3$ on litter microarthropods (Loranger *et al*., 2004). Our results likely differ because Loranger *et al*. (2004) focused on Collembola which feed on bacteria whereas we examined saprophagous species feeding on decaying organic material or fungi. In agreement with our results, Sanders *et al*. (2004) found no effect of $CO_2$ on detritivore abundance.

### *Insect species richness*

Neither the $CO_2$ nor $O_3$ treatments affected species richness. Similarly, insect species richness was unaffected by both $CO_2$ in a sweetgum understorey at the Oak Ridge National Laboratory FACE site (Sanders *et al*., 2004) and a natural $O_3$ pollution gradient in California forests (Jones & Paine, 2006). In contrast, $eCO_2$ did decrease insect species richness on beech and oak trees, although sample sizes were small (Altermatt, 2003). Together, these studies suggest that $eCO_2$ and $eO_3$ will have negligible impacts on species richness via direct or plant-mediated effects, at least over the short term. The effects of $CO_2$ and $O_3$ may, however, magnify over longer timescales. The strong negative effect of $O_3$ on parasitoids could increase extinction risk and reduce diversity over time. $CO_2$ and $O_3$ may also alter diversity by changing insect species dominance (e.g. making the most abundant species more abundant) in the community (Jones & Paine, 2006). Finally, the effects of $CO_2$ and $O_3$ may be mediated more strongly via their function as greenhouse gases. Decreases in insect diversity over the past three decades appear to be linked to elevated temperatures (Lovejoy & Hannah, 2005).

We acknowledge that the taxonomic level of family may be too coarse to evaluate fully the richness for this system. However, species level analyses have also revealed no change in richness in response to $eCO_2$ and $eO_3$ (Sanders *et al*., 2004; Jones & Paine, 2006).

### *Arthropod community composition*

Few studies have examined the effects of $eCO_2$ or $eO_3$ on insect community composition. We found that $eCO_2$ altered forest insect communities in 3 of 4 years, while $eO_3$ modified communities in 2 of 4 years. Previous work at another forest FACE site showed that $eCO_2$ had no effect on understorey arthropod community composition (Sanders *et al*., 2004). Elevated $CO_2$ has, however, been shown to cause the most abundant herbivores to become more abundant on forest trees, (Altermatt, 2003) an effect that could alter community composition. We found increased abundance of the most abundant insects in some years but not in others. Insect communities could also be modified by changes in the ratio of chewing to sucking herbivores. Jones & Paine (2006) found that insect communities exposed to elevated $O_3$ concentrations may shift from phloem-feeding to chewing-dominated herbivore communities. Our results suggest the opposite effect, with phloem-feeders benefiting from $eO_3$ environments.

### *Mechanisms and implications*

Changes in insect abundance and community composition at $eCO_2$ and $eO_3$ are likely due to a combination of bottom-up, top-down and abiotic effects. Meta-analysis has clearly shown

that both plant quality and herbivore performance are altered by $CO_2$ and $O_3$ (Zvereva & Kozlov, 2006; Valkama *et al*., 2007). Altered herbivore quality may in turn influence predator fitness (Ode, 2006), altering top-down effects on the herbivore community. Insect communities also likely differ due to $eCO_2$- and $eO_3$-mediated alterations in forest environment. For example, forest stand development in $eCO_2$ rings reached canopy closure over the 4 years of this study, which may be responsible for the strengthening $eCO_2$ effect. The forest environment along with insect populations and plant quality are also affected directly and indirectly by major weather patterns (e.g. temperature, precipitation) each year.

Predicting abiotic conditions of the future is critical to conservation efforts. New regional climates, in combination with individualistic species responses, may mean disassembly of current communities and establishment of 'no-analogue' communities (Williams & Jackson, 2007). Under environmental conditions of the future, many plants and insects will likely be forced to adapt, move to new areas, or go extinct. Plants will respond directly and indirectly to changes in the abiotic environment, whereas insects will respond to both changes in plant community composition and climate (Schaffers *et al*., 2008). Additionally, insects may respond to new conditions with altered feeding preferences (Goverde & Erhardt, 2003; Agrell *et al*., 2006) and food consumption (Hamilton *et al*., 2004; Hamilton *et al*., 2005; Knepp *et al*., 2005). The consequences of potential 'no-analogue' communities for ecosystem function remains to be determined.

### *Conclusions*

Elevated $CO_2$ and $eO_3$ altered insect abundance and community composition but not family level richness. Feeding guild may strongly influence insect response to environmental change and may provide the best opportunity to generalise for conservation efforts. Because insects play key roles in forest ecosystems, changes in insect abundance, diversity or community composition have the potential to alter forest ecosystems and the services they provide. Continued multifactor research and increased public awareness will be essential to forest management and conservation efforts.

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