Oecologia (2012) 168:863–876
DOI 10.1007/s00442-011-2139-1

GLOBAL CHANGE ECOLOGY - ORIGINAL PAPER

# Atmospheric change alters foliar quality of host trees and performance of two outbreak insect species

**John J. Couture · Timothy D. Meehan ·**
**Richard L. Lindroth**

**Abstract** This study examined the independent and interactive effects of elevated carbon dioxide ($CO_2$) and ozone ($O_3$) on the foliar quality of two deciduous trees species and the performance of two outbreak herbivore species. Trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*) were grown at the Aspen FACE research site in northern Wisconsin, USA, under four combinations of ambient and elevated $CO_2$ and $O_3$. We measured the effects of elevated $CO_2$ and $O_3$ on aspen and birch phytochemistry and on gypsy moth (*Lymantria dispar*) and forest tent caterpillar (*Malacosoma disstria*) performance. Elevated $CO_2$ nominally affected foliar quality for both tree species. Elevated $O_3$ negatively affected aspen foliar quality, but only marginally influenced birch foliar quality. Elevated $CO_2$ slightly improved herbivore performance, while elevated $O_3$ decreased herbivore performance, and both responses were stronger on aspen than birch. Interestingly, elevated $CO_2$ largely offset decreased herbivore performance under elevated $O_3$. Nitrogen, lignin, and C:N were identified as having strong influences on herbivore performance when larvae were fed aspen, but no significant relationships were observed for insects fed birch. Our results support the notion that herbivore performance can be affected by atmospheric change through altered foliar quality, but how herbivores will respond will depend on interactions among $CO_2$, $O_3$, and tree species. An emergent finding from this study is that tree age and longevity of exposure to pollutants may influence the effects of elevated $CO_2$ and $O_3$ on plant–herbivore interactions, highlighting the need to continue long-term atmospheric change research.

**Keywords** Aspen FACE · Atmospheric change · Phytochemistry · Plant–insect interactions

## Introduction

Anthropogenic inputs have substantially increased levels of atmospheric carbon dioxide ($CO_2$) and tropospheric ozone ($O_3$) (IPCC 2007). Concentrations of carbon dioxide are currently ~37% higher (386 ppm) than pre-industrial levels (280 ppm) and are predicted to increase to 550 ppm by 2050 (IPCC 2007). Concurrently, background levels of tropospheric ozone have also increased by 40% (IPCC 2007). Nearly 25% of global forests were exposed to $O_3$ concentrations exceeding 60 ppb in 1990, and 50% of global forests are expected to be exposed to these damaging levels by 2100 (Fowler et al. 1999). How atmospheric change influences forest ecosystems is critically important considering that forests currently cover ~30% of the terrestrial area, contribute ~50% of terrestrial net primary productivity, and store ~45% of terrestrial carbon (Bonan 2008).

Increases in atmospheric $CO_2$ and tropospheric $O_3$ can alter multiple components of forest ecosystem processes, including tree growth, physiology, and phytochemistry, which in turn can influence plant–herbivore interactions (Lincoln et al. 1993; Lindroth 1996a, b, 2010; Bezemer and Jones 1998). Elevated $CO_2$ generally increases plant

---

Communicated by Roland Brandl.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00442-011-2139-1) contains supplementary material, which is available to authorized users.

J. J. Couture (✉) · T. D. Meehan · R. L. Lindroth
Department of Entomology, University of Wisconsin,
Russell Laboratories, 1630 Linden Dr., Madison,
WI 53706, USA
e-mail: jjcouture@wisc.edu

growth (Ainsworth and Long 2005), although this response depends on forest stand age and nutrient availability (Körner 2006). Elevated $CO_2$ also generally decreases nitrogen concentrations and increases phenolic and carbohydrate concentrations and C:N ratios (Lindroth 2010). Conversely, elevated $O_3$ is potentially the most damaging air pollutant to plants, generally reducing plant growth (Karnosky et al. 2007; Wittig et al. 2009). The effects of $O_3$ on foliar nutrient concentrations are variable and depend on the tree species studied (Valkama et al. 2007), longevity of exposure (Oksanen 2003), and tree sensitivity (Karnosky et al. 1999). Prolonged exposure can compound the negative effects of $O_3$, leading to reduced foliar nutrient concentrations (Oksanen 2003). Elevated $O_3$ also generally increases the production of phenolic compounds, potentially as an antioxidant response (Valkama et al. 2007).

Insect herbivores are the primary consumers in forest ecosystems, and are important regulators of nutrient cycling, biomass production, succession patterns, and energy flow (Mattson and Addy 1975; Fajvan and Wood 1996; Lindroth 1996a, b, 2010; Hunter 2001; Lovett et al. 2006). Elevated $CO_2$ generally decreases herbivore growth (Zvereva and Kozlov 2006). However, compensatory mechanisms, such as increased consumption or prolonged development, can ameliorate reduced growth under elevated $CO_2$. Elevated $O_3$ generally has beneficial effects on herbivore growth, but these benefits are reduced when elevated $O_3$ co-occurs with elevated $CO_2$ (Valkama et al. 2007).

Outbreak herbivores can increase population sizes exponentially, and have the potential to cause widespread damage to forest ecosystems. Two outbreak herbivores that have historically caused widespread defoliation in the United States are the gypsy moth, *Lymantria dispar*, and the forest tent caterpillar, *Malacosoma disstria* (Mattson et al. 1991). Combined damage across 2007–2008 by gypsy moths in the eastern United States was over 1.2 million hectares of forest land (United States Department of Agriculture, Forest Service 2009). Forest tent caterpillars erupt to outbreak levels every 7–12 years (Fitzgerald 1995), and in 2001 defoliated over 6 million hectares in the Great Lakes region alone (United States Department of Agriculture, Forest Service 2003).

Trembling aspen, *Populus tremuloides*, and paper birch, *Betula papyrifera*, are preferred hosts for gypsy moths and forest tent caterpillars, and are major components of forest ecosystems in the Great Lakes region, representing approximately 16% of commercial forest lands in this area (Dickson et al. 2000). Consequently, aspen–birch stands represent a significant carbon pool in the Great Lakes region. How gypsy moths and forest tent caterpillars respond to future atmospheric conditions is important, considering that climate change is predicted to increase the frequency and intensity of insect outbreaks (Stireman et al. 2005; Jepsen et al. 2008).

The goal of this study was to determine whether levels of $CO_2$ and $O_3$ predicted for the year 2050 alter the foliar quality of aspen and birch trees, and whether changes in foliar quality influence the performance of two outbreak insect herbivores. Specifically, we predicted that: (1) elevated $CO_2$ will decrease foliar quality by increasing C:N ratios through reductions in nitrogen concentrations and increases in structural and nonstructural carbohydrate and phenolic concentrations, (2) elevated $O_3$ will also reduce foliar quality via the same mechanisms as proposed for $CO_2$, (3) differential sensitivities of tree species to elevated $CO_2$ and $O_3$ will result in foliar quality responses that differ between species, (4) reductions in foliar quality will decrease herbivore performance unless compensatory mechanisms (e.g., increased feeding or digestion efficiency) offset reductions in insect performance, and (5) herbivore performance will differ between both herbivore species and tree species.

## Materials and methods

### Experimental design

This experiment was conducted in northern Wisconsin, USA (W89.5°, N45.7°) at the Aspen Free Air $CO_2$ and $O_3$ Enrichment (FACE) research facility. The Aspen FACE site is a 32 ha research facility located near Rhinelander, WI. The site contained twelve experimental rings, 30 m in diameter, with three blocks of four treatments. The full-factorial design allowed for all possible treatment combinations of ambient and elevated (560 ppm) $CO_2$ and ambient and elevated (1.5× ambient) $O_3$ levels. Detailed information about the experimental design, set-up, and operation of the Aspen FACE research facility can be found in Dickson et al. (2000).

One half of each experimental ring contained multiple genotypes of aspen, one quarter of each ring contained a mixture of aspen and paper birch, and one quarter of each ring contained a mixture of aspen and sugar maple, *Acer saccharum*. One-year old seedlings were planted in the rings in 1997, and fumigation treatments began in 1998. We restricted our experiment to the aspen and aspen–birch sections. Within each of the 12 rings, we randomly selected three trees each of aspen and birch for foliar collections and herbivore bioassays. We used aspen genotype 42E because it had emerged as the dominant genotype within the aspen community under elevated $CO_2$, ambient $O_3$ environments (Kubiske et al. 2007), and is relatively $O_3$ sensitive (McGrath et al. 2010). At the time of the experiment (2007), the pole-stage trees were 11 years old and the canopy had closed.

## Foliar quality analysis

Leaves were collected for phytochemical analyses at the midpoint of the insect bioassays from similar canopy positions (lower and middle interior canopy) as the leaves collected for the bioassays. A parallel study revealed minimal phytochemical variation (<5% relative difference) across multiple canopy levels of aspen and birch at this research site (Couture 2011). We used pole pruners to clip short shoots from trees, excised leaves from petioles, and stored them on ice while in the field. Leaves were subsequently flash frozen in liquid nitrogen, freeze-dried, ground, and stored at −20°C until chemical analysis. Aspen and birch leaves were assayed for carbon, nitrogen, simple sugars (i.e., hexoses and sucrose), starch, fiber, lignin, and condensed tannins. In addition, aspen leaves were analyzed for the phenolic glycoside tremulacin. Carbon and nitrogen were determined using a Thermo Finnigan (San Jose, CA, USA) Flash 1112 elemental analyzer. Simple sugars and starch were determined spectrophotometrically using a modified dinitrosalicyclic acid assay (Lindroth et al. 2002). Fiber (acid detergent fiber, determined as cellulose + lignin) and lignin were determined gravimetrically using sequential extraction in a hot acid-detergent solution in an Ankom 200 Fiber Analyzer and incubation in 72% $H_2SO_4$. Condensed tannins were determined spectrophotometrically using a butanol–HCl method (Porter et al. 1986), with condensed tannins purified from aspen and birch leaves as standards. Tremulacin was determined by high-performance thin layer chromatography (HPTLC) using methods described by Lindroth et al. (1993), with tremulacin purified from aspen leaves as a standard. While other phenolic glycosides (e.g., salicin, tremuloidin, and salicortin) exist in aspen leaves, tremulacin and salicortin are the most biologically active (Lindroth et al. 1988) and represent the majority concentration (generally >90%) of these compounds in foliar tissue of *P. tremuloides* (Lindroth and Hwang 1996). We report concentrations of only tremulacin, because salicortin occurred in very low levels and interfering compounds made quantification problematic. However, concentrations of salicortin and tremulacin are highly (positively) correlated (Lindroth and Hwang 1996), so measurements of tremulacin provide a suitable index of the total phenolic glycoside pool in aspen foliage.

## Insect bioassays

Bioassays were conducted with fourth stadium gypsy moths and forest tent caterpillars to determine the effects of $CO_2$ and $O_3$ on multiple insect performance variables, including growth, consumption, and food processing efficiencies. Low populations of gypsy moth and forest tent caterpillars and logistical difficulties in collecting egg masses from the area around the research site precluded us from using insects collected from native populations near Aspen FACE in this study. Gypsy moth egg masses were obtained from USDA–APHIS (Otis Air National Guard Base, MA), and forest tent caterpillar egg bands were collected from the field in central New York State, USA. After hatching, gypsy moths and forest tent caterpillars were reared in a Percival® growth chamber under a 24:18°C and 15:9 h light:dark cycle in 2.5 × 15 cm plastic rearing dishes. We reared larvae on the same host plant foliage that they would receive during the bioassay. By rearing larvae on foliage from the same host plant, we were able to examine how elevated $CO_2$ and $O_3$ influenced foliar quality, and subsequent herbivore performance, while avoiding any effects of host-plant switching.

Five recently molted (≤18 h, with access to nonexperimental foliage), fourth instar larvae were randomly selected, weighed, placed together into a 4 × 15 cm rearing dish, and fed aspen or birch foliage from an Aspen FACE treatment (i.e., ambient $CO_2$, ambient $O_3$; elevated $CO_2$, ambient $O_3$; ambient $CO_2$, elevated $O_3$; elevated $CO_2$, elevated $O_3$). As the larvae had access to nonexperimental foliage prior to beginning the bioassay, the individuals did not have an empty gut upon initiation of the study. However, the insects used were seen feeding at the beginning and the end of the bioassay (J. Couture, personal observation), indicating that the results we report are more likely to be dependent on the larval responses to variation in foliar quality under the different fumigation treatments and less likely to be influenced by larval gut contents at the onset of the study. We treated each rearing dish as an experimental unit and used a total of 144 rearing dishes (2 levels of $CO_2$ × 2 levels of $O_3$ × 3 blocks × 2 tree species × 3 replicate trees/tree spp. × 2 insect species = 144 rearing dishes) and 720 larvae (5/dish). Larvae were maintained in a Percival® growth chamber under a 24:18°C and 15:9 h light:dark cycle at the Aspen FACE site. We used detached leaves, as opposed to in situ feeding on trees, to focus on how $CO_2$- and $O_3$-mediated effects on foliar quality influence herbivore performance independent of their effects on environmental conditions (e.g., temperature, humidity).

Experimental foliage was collected between 1400 and 1800 h in the same manner as foliage for chemical analysis and fed to the larvae on the same day as collected. Foliage was kept hydrated by inserting the petioles into 6 ml florist water piks filled with water. All rearing dishes were examined daily to ensure adequate foliage was present; additional leaves were provided if more than 50% of the existing foliage was consumed. Foliage was replaced every second day regardless of consumption to ensure that foliage quality resembled foliage collected for chemical analysis (Hemming and Lindroth 1999). Uneaten leaves and frass from each rearing dish were collected every 2–3 days, air dried, and stored in a freezer until further processing. Bioassay trials were run for 7 days, whereupon dishes containing leaves, frass, and larvae were frozen. The duration of the bioassay resulted in a small percentage (8%) of gypsy moth larvae molting into their fifth instar; no forest tent caterpillars molted. However, the number of gypsy moths molting into their fifth instar was not influenced by the fumigation treatments for either aspen ($P = 1.00$) or birch ($P = 0.493$). Thus, it is unlikely that our results were skewed by differences in herbivore performance due to altered molting patterns.

After freezing, the uneaten leaves, frass, and larvae of each rearing dish were air dried, transferred into scintillation vials, and lyophilized. Larval growth was determined gravimetrically, as the difference between final and initial dry masses. Initial dry masses were calculated based on a wet:dry mass ratio determined from a set of ten similar-sized larvae of each insect species from each host plant species. Consumption was also determined gravimetrically, as the difference between the dry masses of leaf material provided and material remaining. We used a subset of leaves from each collection to calculate a wet:dry mass ratio, and used that ratio to estimate the initial dry masses of leaves. Frass produced was measured gravimetrically. Individual herbivore performance responses were calculated as the total response within a dish divided by the number of larvae in the dish.

## Statistical analysis

We analyzed tree foliar quality by analysis of variance with a split-plot design, using the model $Y_{ijkl} = b_i + C_j + O_k + CO_{jk} + e_{ijk} + S_l + CS_{jl} + OS_{kl} + COS_{jkl} + \varepsilon_{ijkl}$. In this model, $b$ represents block $i$, $C$ represents $CO_2$ level $j$, $O$ represents $O_3$ level $k$, $e_{ijk}$ represents the whole-plot error, $S$ represents tree species $l$, and $\varepsilon_{ijkl}$ represents the subplot error. $Y_{ijkl}$ represents the average response of block $i$, $CO_2$ level $j$, $O_3$ level $k$, and tree species $l$. $F$ tests were conducted with degrees of freedom assigned using the Satterthwaite approximation. Means and pooled standard errors are reported for all combinations of $CO_2$, $O_3$, and tree species.

Insect performance variables were analyzed separately for each insect species using a similar analysis of variance to that described above, with the exception that covariates were included in the model as recommended by Raubenheimer and Simpson (1992). Specific covariates included in the model varied according to the response variable measured. For larval growth and consumption, initial larval dry mass was included as a covariate. As a measure of approximate digestibility (AD), we used frass production with consumption as a covariate (Barbehenn et al. 2009). Efficiency of conversion of digested food (ECD) was measured as growth, with "use" (use = total mass consumed − total frass produced) as a covariate, whereas efficiency of conversion of ingested food (ECI) was measured as growth, with consumption included as a covariate (Knepp et al. 2007). $F$ tests were conducted with degrees of freedom assigned using the Satterthwaite approximation. Means and standard errors are reported for all combinations of $CO_2$, $O_3$, tree species, and insect species.

To interpret the influence of phytochemical variables on growth, total consumption, and frass produced, we used partial least squares regression (PLSR) analysis. In cases where predictor variables are highly correlated with each other (e.g., many variables of foliar quality), the use of multiple linear regression produces unreliable coefficients, because collinear predictor variables contribute similar information to the response variable, ultimately affecting the ability of the model to produce reliable regression coefficients. PLSR is particularly useful because it can reduce a large number of collinear variables into relatively few, uncorrelated latent variables. PLSR identifies a select number of latent variables from an independent data matrix able to generate score values that capture predictor variation and are highly correlated with the response variable. An important part of PLSR is deciding the number of latent variables to use. It is possible to use as many latent variables as predictor variables, although doing so may lead to overfitting, so initially a smaller number of variables is typically used. We determined the number of latent variables extracted iteratively by cross-validation through reduction of the predictive residual sum of squares (PRESS). The latent variables were added into the model until the currently added latent variable did not improve the PRESS score, whereupon the preceding number of latent variables was selected (Wold et al. 1984). Additionally, we reduced the number of predictor variables in the model using variable importance for the projection (VIP) selection (Wold et al. 1984), with 1.0 used as a cutoff for variable selection. This procedure selects predictor variables (e.g., foliar quality) that explain the greatest variation in both response and predictor matrices (Wold et al. 2001), and thus retains predictor variables that demonstrate the greatest influence on the response variables and explain variation in the predictor matrix structure (i.e., have strong treatment responses). The final set of extracted factors was then transformed into a linear regression model for the response variable. Larger regression coefficients indicate greater contributions by predictor variables to the response, and the sign indicates the direction of the influence of the foliar quality variable on the larval performance variable. We examined residuals to determine distributions of response and predictor variables. Aspen foliar nitrogen, starch, and tremulacin concentrations, birch starch and lignin concentrations and C:N ratios, and gypsy moth frass when insects were fed aspen were all log transformed, after which all variables met assumptions of normality. All predictor and response variables were centered (i.e., the mean was subtracted from each variable), to ensure that criteria for selecting successive factors was based on the amount of variation they explain, and scaled (i.e., variables were divided by the standard deviation), to weight the variation in predictors and response variables relative to variation in the data, thus producing centered and scaled coefficients. We validated our models by examining the relationship between the observed and predicted responses ($n = 36$). Because of the small sample size for each model validation, we used a more stringent cutoff ($P < 0.01$) to exclude models from our inferences relating insect performance to foliar quality.

Statistical analyses were performed with JMP v. 8.0 statistical software (SAS Institute Inc., 2008). The low replication of the Aspen FACE design increases the potential for type II errors. To balance between potential type I and II errors, we report $P$ values $0.05 < 0.10$ as being "marginally significant" and $P$ values $<0.05$ as "significant" (Filion et al. 2000); exact $P$ values provided by the statistical analyses are shown in Tables 1 and 3.

## Results

### Foliar quality

Aspen and birch foliar quality was influenced by elevated $CO_2$, $O_3$, tree species, and their interactions (Tables 1, 2). The effects of elevated $CO_2$ on nutrient quality were minimal, and the response was apparent only in aspen (significant $CO_2$ × tree spp. interaction, Table 1). Aspen nitrogen concentrations decreased by 6% and C:N ratios and sugar concentrations increased by 11 and 9%, respectively, under elevated $CO_2$. Elevated $O_3$ had a negative effect on aspen and birch nutrient quality, and this response was greater for aspen than birch (Tables 1, 2). Elevated $O_3$ decreased nitrogen concentrations by 17 and 10% and increased foliar C:N ratios by 21 and 12% in aspen and birch foliage, respectively. Elevated $O_3$ increased sugar concentrations in aspen and birch foliage, but the response was greater in aspen (24%) compared with birch (10%; $O_3$ × tree spp. interaction, Table 1).

Elevated $CO_2$, $O_3$, tree species, and their interactions also influenced the concentrations of phenolic and structural compounds (Tables 1, 2). Elevated $CO_2$ increased condensed tannin concentrations by 18 and 21% in aspen and birch, respectively. It did not, however, affect concentrations of phenolic glycosides in aspen, or fiber and lignin concentrations in either species (Tables 1, 2).

**Table 1** Summary of *F* and *P* values for the effects of $CO_2$, $O_3$, tree species, and their interactions on aspen and birch foliar quality

| Treatments and interactions | | N | | C:N | | Sugar | | Starch | | Condensed tannins | | Fiber | | Lignin | | Tremulacin | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *df* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *df* | *F* | *P* |
| $CO_2$ | 1,6 | 0.22 | 0.65 | 0.64 | 0.45 | 0.64 | 0.45 | 0.74 | 0.42 | 39.64 | **<0.001** | 0.14 | 0.71 | 1.41 | 0.27 | 1,5.4 | 2.25 | 0.18 |
| $O_3$ | 1,6 | 13.63 | **0.010** | 12.96 | **0.011** | 27.02 | **0.002** | 3.74 | 0.10 | 3.19 | 0.12 | 0.07 | 0.79 | 6.73 | **0.041** | 1,5.4 | 0.01 | 0.92 |
| $CO_2$ × $O_3$ | 1,6 | 1.31 | 0.29 | 1.15 | 0.32 | 0.83 | 0.39 | 0.96 | 0.36 | 4.76 | *0.072* | 0.45 | 0.52 | 0.27 | 0.62 | 1,5.4 | 0.01 | 0.95 |
| Tree spp. | 1,56 | 3.27 | *0.076* | 11.45 | **<0.001** | 54.69 | **<0.001** | 19.36 | **<0.001** | 0.48 | 0.49 | 94.41 | **<0.001** | 46.27 | **<0.001** | na | na | na |
| $CO_2$ × tree spp. | 1,56 | 3.73 | *0.059* | 3.97 | *0.051* | 4.16 | **0.046** | 2.25 | 0.12 | 0.12 | 0.73 | 0.31 | 0.58 | 0.07 | 0.79 | na | na | na |
| $O_3$ × tree spp. | 1,56 | 1.78 | 0.18 | 3.38 | *0.071* | 4.30 | **0.048** | 0.13 | 0.71 | 8.26 | **0.005** | 0.11 | 0.74 | 9.83 | **0.002** | na | na | na |
| $CO_2$ × $O_3$ × tree spp. | 1,56 | 0.08 | 0.77 | 0.10 | 0.75 | 1.58 | 0.21 | 0.43 | 0.51 | 4.03 | **0.049** | 0.82 | 0.36 | 1.13 | 0.29 | na | na | na |

Numerator and denominator degrees of freedom (*df* numerator, denominator) were calculated using the Satterthwaite approximation

*P* values <0.05 are bolded and *P* values $0.05 < P < 0.10$ are italicized

*N* nitrogen, *C:N* ratio of carbon to nitrogen, *na* not applicable

**Table 2** Effects of $CO_2$, $O_3$, and their interaction on aspen and birch foliar quality

| Tree species and treatments | N | C:N | Sugar | Starch | Condensed tannins | Fiber | Lignin | Tremulacin |
|---|---|---|---|---|---|---|---|---|
| Aspen | | | | | | | | |
| Control | 2.6 ± 0.1 | 17.8 ± 0.9 | 19.1 ± 1.3 | 2.2 ± 0.5 | 12.8 ± 1.3 | 27.5 ± 0.9 | 10.1 ± 0.9 | 0.5 ± 0.1 |
| $CO_2$ | 2.3 ± 0.1 | 20.3 ± 0.9 | 22.1 ± 1.3 | 2.9 ± 0.5 | 17.9 ± 1.3 | 26.5 ± 0.9 | 9.1 ± 0.9 | 0.4 ± 0.1 |
| $O_3$ | 2.0 ± 0.1 | 22.2 ± 0.9 | 25.8 ± 1.3 | 2.7 ± 0.5 | 17.7 ± 1.3 | 26.8 ± 0.9 | 13.8 ± 0.9 | 0.5 ± 0.1 |
| $CO_2 + O_3$ | 2.0 ± 0.1 | 23.5 ± 0.9 | 26.4 ± 1.3 | 3.8 ± 0.5 | 18.2 ± 1.3 | 26.1 ± 0.9 | 12.6 ± 0.9 | 0.4 ± 0.1 |
| Birch | | | | | | | | |
| Control | 2.5 ± 0.1 | 17.9 ± 1.1 | 17.4 ± 1.1 | 4.4 ± 0.6 | 16.1 ± 1.0 | 20.1 ± 0.9 | 8.7 ± 1.1 | na |
| $CO_2$ | 2.5 ± 0.1 | 18.6 ± 1.1 | 15.7 ± 1.1 | 3.6 ± 0.6 | 19.0 ± 1.0 | 18.6 ± 0.9 | 6.9 ± 1.1 | na |
| $O_3$ | 2.2 ± 0.1 | 21.1 ± 1.1 | 18.5 ± 1.1 | 4.4 ± 0.6 | 14.9 ± 1.0 | 18.5 ± 0.9 | 8.5 ± 1.1 | na |
| $CO_2 + O_3$ | 2.4 ± 0.1 | 19.7 ± 1.1 | 17.8 ± 1.1 | 5.3 ± 0.6 | 18.3 ± 1.0 | 20.0 ± 0.9 | 8.5 ± 1.1 | na |

Values are mean (% dry mass) ± 1SE

*N* nitrogen, *C:N* ratio of carbon to nitrogen, *na* not applicable

Elevated $O_3$ increased condensed tannin concentrations in aspen by 16%, but decreased concentrations in birch by 5% ($O_3$ × tree spp. interaction, Table 1). Elevated $O_3$ increased lignin concentrations in aspen and birch foliage, but the response was greater in aspen (39%) compared with birch (7%; $O_3$ × tree spp. interaction, Table 1). It did not, however, affect concentrations of phenolic glycosides in aspen, or fiber concentrations in either species (Tables 1, 2).

## Insect performance

Elevated $CO_2$, $O_3$, tree species, and their interactions influenced gypsy moth and forest tent caterpillar performance. Gypsy moth growth increased 11 and 5% when fed aspen and birch foliage, respectively, from elevated $CO_2$, but the changes were not statistically significant (Fig. 1, Table 3). Forest tent caterpillar growth increased when fed aspen and birch foliage from elevated $CO_2$, and the response was greater on aspen (22%) than on birch (11%; $CO_2$ × tree spp. interaction, Table 3). Gypsy moth and forest tent caterpillar growth decreased when fed foliage from elevated $O_3$, relative to foliage from ambient $O_3$, but the response depended on tree species and $CO_2$ level (Fig. 1, Table 3). Gypsy moth growth decreased when larvae were fed aspen and birch foliage from elevated $O_3$, and the response was greater on aspen (21%) than on birch (10%; $O_3$ × tree spp. interaction, Table 3). Additionally, elevated $CO_2$ ameliorated the reduction in growth of gypsy moth larvae reared on foliage from elevated $O_3$ ($CO_2$ × $O_3$ interaction, Table 3). Forest tent caterpillar growth also decreased when larvae were fed aspen and birch foliage from elevated $O_3$ (Fig. 1). Again, the response was greater on aspen (32%) than on birch (7%; $O_3$ × tree spp. interaction, Table 3). Interestingly, elevated $CO_2$ did not offset reductions in forest tent caterpillar growth from elevated $O_3$ (no significant $CO_2$ × $O_3$ interaction, Table 3).

Gypsy moths and forest tent caterpillars altered consumption differently when fed foliage from elevated $CO_2$ (Fig. 1, Table 3). Gypsy moths consumed less foliage, but forest tent caterpillar consumption was unaffected when fed foliage from elevated $CO_2$ (Fig. 1, Table 3). Gypsy moths increased the consumption of foliage from elevated $O_3$ by 10% (Fig. 1, Table 3), and the response was consistent across both tree species. Elevated $CO_2$, however, offset the increased consumption by gypsy moths when they were fed foliage from elevated $O_3$ ($CO_2$ × $O_3$ interaction, Table 3). Forest tent caterpillars increased the consumption of foliage from elevated $O_3$, but the response was greater on aspen (37%) than on birch (15%; $O_3$ × tree spp. interaction, Table 3). Again, elevated $CO_2$ offset increased consumption by forest tent caterpillars when fed foliage from elevated $O_3$ ($CO_2$ × $O_3$ interaction, Table 3).

Gypsy moths produced 8% less frass when fed foliage from elevated $CO_2$, and the response was consistent across both tree species (Fig. 1, Table 3). Forest tent caterpillars also produced less frass when fed foliage from elevated $CO_2$, but the response was greater for insects on aspen (11%) compared with birch (4%; $CO_2$ × tree spp. interaction; Table 3). Gypsy moths produced more frass when fed foliage from elevated $O_3$, but the response was greater on aspen (11%) than on birch (3%; $O_3$ × tree spp. interaction, Table 3). Additionally, elevated $CO_2$ offset increased gypsy moth frass production under elevated $O_3$ ($CO_2$ × $O_3$ interaction; Table 3). Elevated $O_3$ altered forest tent caterpillar frass production, but this response depended on tree species and $CO_2$ level. When fed aspen foliage from the elevated $O_3$ only treatment, compared with

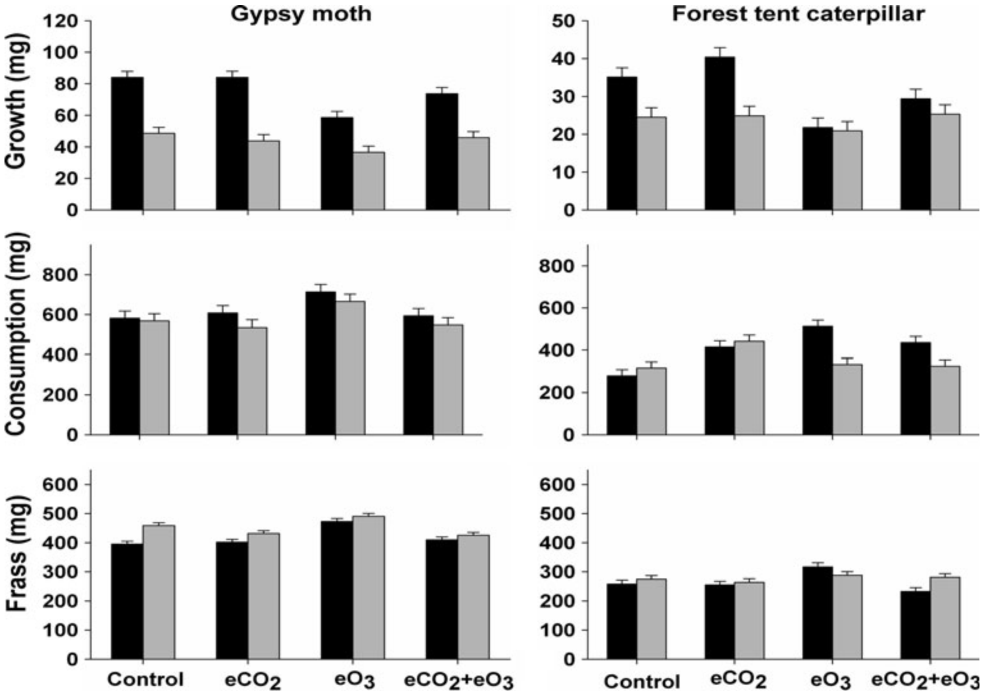

**Fig. 1** *Left panel*: gypsy moth growth, consumption, and frass production on aspen (*dark bars*) and birch (*light bars*); *Right panel*: forest tent caterpillar growth, consumption, and frass production on aspen (*dark bars*) and birch (*light bars*). *Error bars* are +1SE

control foliage, forest tent caterpillars increased frass production by 23%, but this response was offset by elevated $CO_2$ ($CO_2 \times O_3 \times$ tree spp. interaction; Table 3).

Gypsy moths slightly increased the efficiency of digested foliage when fed aspen, but not birch, from elevated $CO_2$ ($CO_2 \times$ tree spp. interaction; Table 3). Additionally, conversion of ingested foliage tended to increase on foliage from elevated $CO_2$, and more so on aspen compared with birch, but the response was not statistically significant (Table 3). Forest tent caterpillars marginally increased both efficiency of digested and ingested foliage from elevated $CO_2$, and the response was greater on aspen than birch ($CO_2 \times$ tree spp. interactions; Table 3). Gypsy moth conversion of digested foliage into biomass decreased when larvae were fed foliage from elevated $O_3$, and the response was greater on aspen (20%) than birch (8%; $O_3 \times$ tree spp. interactions, Tables 3, 4). Gypsy moth conversion of ingested foliage into biomass also decreased when larvae were fed foliage from elevated $O_3$, and again the response was greater on aspen (22%) than on birch (9%; $O_3 \times$ tree spp. interactions, Tables 3, 4). Similarly, forest tent caterpillar conversion of digested foliage into biomass decreased when larvae were fed foliage from elevated $O_3$, and the response was greater on aspen (31%) than on birch (8%; $O_3 \times$ tree spp. interaction, Tables 3, 4). Forest tent caterpillar conversion of ingested foliage into biomass also decreased when larvae were fed foliage from elevated $O_3$, and again the response was greater on aspen (31%) than on birch (7%; $O_3 \times$ tree spp. interactions, Tables 3, 4).

## Identification of relationships between foliar quality and insect performance via PLSR

PLSR can be a useful tool for relating single or multiple response variables to a large number of correlated predictor variables (e.g., foliar quality factors). Models relating insect performance and aspen foliar quality all produced significant relationships between observed and predicted responses (Electronic supplementary material 1). Gypsy moth and forest tent caterpillar growth was most positively and negatively related to aspen nitrogen and lignin concentrations, respectively (Table 5). Gypsy moth and forest tent caterpillar consumption and frass production were most negatively related to aspen nitrogen concentrations (Table 5). Gypsy moth consumption and frass production were most positively related to aspen lignin concentrations, and forest tent caterpillar consumption and frass production were most positively related to aspen C:N ratios (Table 5). PLSR was unable to generate significant models for the actual versus predicted data on relationships between herbivore performance and birch foliar quality, given the more stringent cutoff ($P < 0.01$) for significance we used (Electronic supplementary material 2). However, weak relationships were detected between foliar quality and growth for both insect species, and between foliar quality and frass production for gypsy moths. The inability of PLSR to produce significant models for the relationships between insect performance and birch foliar quality was likely a result of the nominal response of birch foliar quality to the fumigation treatments, and the subsequently

**Table 3** Summary of *F* and *P* values for the analysis of covariance of the effects of $CO_2$, $O_3$, tree species, and their interactions on growth, consumption, and food processing efficiencies of gypsy moths and forest tent caterpillars

| Insect species and treatments | Growth | | | Consumption | | | Frass produced | | | ECD | | | ECI | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *df* | *F* | *P* | *df* | *F* | *P* | *df* | *F* | *P* | *df* | *F* | *P* | *df* | *F* | *P* |
| Gypsy moth | | | | | | | | | | | | | | | |
| $CO_2$ | 1,5.9 | 2.20 | 0.18 | 1,6.1 | 4.95 | *0.067* | 1,6.8 | 22.36 | **0.002** | 1,6.2 | 1.79 | 0.22 | 1,6.4 | 3.63 | 0.10 |
| $O_3$ | 1,6.1 | 11.78 | **0.014** | 1,6.3 | 4.23 | *0.083* | 1,6.7 | 12.78 | **0.001** | 1,5.9 | 12.43 | **0.013** | 1,6.3 | 15.81 | **0.007** |
| $CO_2 \times O_3$ | 1,5.9 | 4.94 | *0.068* | 1,6.1 | 4.38 | *0.081* | 1,6.7 | 11.57 | **0.012** | 1,5.9 | 6.57 | **0.044** | 1,6.3 | 8.94 | **0.023** |
| Covariate | 1,58.4 | 4.29 | **0.043** | 1,60.7 | 0.13 | 0.72 | 1,61.0 | 395.02 | **<0.001** | 1,59.9 | 1.39 | 0.24 | 1,60.3 | 2.19 | 0.14 |
| Tree spp. | 1,56.0 | 205.74 | **<0.001** | 1,57.1 | 2.74 | 0.10 | 1,55.6 | 25.13 | **<0.001** | 1,56.6 | 199.25 | **<0.001** | 1,55.3 | 278.30 | **<0.001** |
| $CO_2$ × tree spp. | 1,55.1 | 2.00 | 0.16 | 1,55.4 | 0.39 | 0.53 | 1,55.2 | 2.08 | 0.15 | 1,54.9 | 3.35 | *0.072* | 1,55.0 | 2.70 | 0.10 |
| $O_3$ × tree spp. | 1,55.1 | 11.69 | **<0.001** | 1,55.3 | 0.01 | 0.94 | 1,55.2 | 5.86 | **0.019** | 1,55.1 | 11.30 | **<0.001** | 1,54.9 | 13.75 | **<0.001** |
| $CO_2 \times O_3$ × tree spp. | 1,55.2 | 0.02 | 0.89 | 1,55.5 | 0.42 | 0.51 | 1,55.2 | 1.64 | 0.20 | 1,54.9 | 0.16 | 0.69 | 1,55.0 | 0.05 | 0.82 |
| Forest tent caterpillar | | | | | | | | | | | | | | | |
| $CO_2$ | 1,6.1 | 4.09 | *0.089* | 1,6.1 | 2.83 | 0.14 | 1,6.5 | 5.68 | *0.051* | 1,6.2 | 4.82 | *0.062* | 1,7.5 | 4.25 | *0.083* |
| $O_3$ | 1,6.0 | 9.90 | **0.019** | 1,6.0 | 2.22 | 0.18 | 1,6.3 | 2.39 | 0.17 | 1,6.1 | 10.44 | **0.018** | 1,6.0 | 9.57 | **0.021** |
| $CO_2 \times O_3$ | 1,6.1 | 0.51 | 0.50 | 1,6.1 | 11.11 | **0.015** | 1,8.0 | 2.71 | 0.13 | 1,7.6 | 0.48 | 0.51 | 1,6.0 | 0.31 | 0.59 |
| Covariate | 1,57.0 | 0.14 | 0.71 | 1,57.3 | 5.14 | **0.027** | 1,61.0 | 187.75 | **<0.001** | 1,60.9 | 0.75 | 0.39 | 1,61.0 | 0.13 | 0.71 |
| Tree spp. | 1,55.1 | 46.73 | **<0.001** | 1,55.1 | 15.09 | **<0.001** | 1,57.2 | 3.02 | *0.087* | 1,56.9 | 42.08 | **<0.001** | 1,57.5 | 38.19 | **<0.001** |
| $CO_2$ × tree spp. | 1,55.0 | 3.27 | *0.076* | 1,55.0 | 1.11 | 0.29 | 1,55.2 | 9.07 | **0.004** | 1,55.0 | 3.82 | *0.056* | 1,55.8 | 3.00 | *0.089* |
| $O_3$ × tree spp. | 1,55.1 | 22.58 | **<0.001** | 1,55.1 | 38.66 | **<0.001** | 1,58.4 | 0.03 | 0.85 | 1,58.1 | 15.92 | **<0.001** | 1,57.1 | 12.09 | **<0.001** |
| $CO_2 \times O_3$ × tree spp. | 1,55.0 | 0.14 | 0.70 | 1,55.0 | 1.94 | 0.16 | 1,55.4 | 13.53 | **<0.001** | 1,55.2 | 0.01 | 0.90 | 1,55.9 | 0.18 | 0.67 |

Numerator and denominator degrees of freedom (*df* numerator, denominator) were calculated using the Satterthwaite approximation

*P* values <0.05 are bolded and *P* values $0.05 < P < 0.10$ are italicized

Growth (mass gained with initial insect mass as covariate); consumption (total consumption with initial insect mass as covariate); frass produced (with total consumption as covariate); ECD (efficiency of conversion of digested food; growth with "use" as covariate); ECI (efficiency of conversion of ingested food; growth with total consumption as covariate)

**Table 4** Effects of $CO_2$, $O_3$, and their interaction on the efficiency of conversion of digested food (ECD; growth with "use" as covariate) and the efficiency of conversion of ingested food (ECI; growth with total consumption as covariate) of gypsy moths and forest tent caterpillars feeding on aspen or birch

| Insect and tree species | ECD | | | | ECI | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | $CO_2$ | $O_3$ | $CO_2 + O_3$ | Control | $CO_2$ | $O_3$ | $CO_2 + O_3$ |
| Gypsy moth | | | | | | | | |
| Aspen | 83.7 ± 3.6 | 84.5 ± 3.6 | 60.1 ± 3.5 | 74.2 ± 3.5 | 85.1 ± 3.4 | 85.6 ± 3.4 | 58.1 ± 3.6 | 75.1 ± 3.4 |
| Birch | 48.9 ± 3.6 | 41.1 ± 3.5 | 37.1 ± 3.6 | 45.7 ± 3.5 | 48.4 ± 3.4 | 41.6 ± 3.5 | 34.9 ± 3.5 | 46.4 ± 3.5 |
| Forest tent caterpillar | | | | | | | | |
| Aspen | 34.8 ± 2.5 | 40.7 ± 2.5 | 21.6 ± 2.4 | 30.3 ± 2.7 | 34.8 ± 2.6 | 40.4 ± 2.5 | 22.2 ± 2.8 | 29.5 ± 2.5 |
| Birch | 24.2 ± 2.5 | 25.4 ± 2.5 | 20.4 ± 2.5 | 24.9 ± 2.5 | 24.3 ± 2.5 | 25.2 ± 2.5 | 20.7 ± 2.5 | 25.2 ± 2.5 |

Values are means ±1SE

**Table 5** Standardized coefficients calculated using partial least squares regression relating aspen foliar quality to growth, consumption, and frass produced

| Foliar quality variable | Gypsy moth | | | Forest tent caterpillar | | |
|---|---|---|---|---|---|---|
| | Growth | Consumption | Frass produced | Growth | Consumption | Frass produced |
| Nitrogen | **0.031** | **−0.119** | **−0.123** | **0.120** | **−0.252** | **−0.206** |
| C:N | −0.114 | 0.119 | 0.131 | −0.087 | **0.283** | **0.227** |
| Condensed tannins | ns | ns | 0.094 | ns | ns | ns |
| Sugar | ns | 0.089 | ns | ns | 0.248 | 0.207 |
| Starch | ns | ns | ns | ns | ns | ns |
| Fiber | ns | ns | ns | ns | ns | ns |
| Lignin | **−0.629** | **0.142** | **0.235** | **−0.510** | ns | ns |
| Tremulacin | ns | ns | ns | ns | ns | ns |

Predictor variables included in the final model were selected using variable importance for the projection criteria from Wold et al. (1984)

Negative or positive weighted coefficients indicate negative or positive contributions, respectively, of foliar quality on herbivore performance. The largest positive and negative weighted coefficients are bolded

*C:N* ratio of carbon to nitrogen; *ns* not selected for final model

limited influence that birch foliar quality had on herbivore performance.

## Discussion

At the Aspen FACE research site, elevated $CO_2$ and $O_3$ altered the foliar quality of both aspen and birch. Overall, the effects of elevated $CO_2$ were much smaller than those of elevated $O_3$, and aspen was more responsive than birch. Insect performance was influenced by changes in foliar quality, and the responses were strongly dependent upon species-specific responses of trees to elevated $CO_2$ and $O_3$. PLSR identified multiple foliar quality variables that influenced insect performance when larvae were fed aspen, and these foliar quality variables were altered by FACE treatments. Birch foliar quality was only nominally affected by the treatments at Aspen FACE, and thus PLSR was unable to generate significant relationships relating insect performance and foliar quality. These findings demonstrate that herbivore performance can be indirectly affected by atmospheric change, through altered foliar quality, but how herbivores will respond will depend on levels of $CO_2$ and $O_3$, and interactions among $CO_2$, $O_3$, and tree species.

### Foliar quality

Contrary to our first hypothesis, elevated $CO_2$ had relatively little effect on foliar quality. Elevated $CO_2$ marginally decreased nitrogen concentrations and increased C:N ratios and sugar concentrations in aspen. That elevated $CO_2$ did not have a more pronounced effect on nutrient composition is surprising, but not without precedent in this study system (Agrell et al. 2005; Vigue and Lindroth 2010). Generally, a consistent trend of decreased foliar nitrogen, coupled with increasing C:N ratios, has been reported for plants grown under elevated $CO_2$ (Zvereva and Kozlov 2006; Stiling and Cornelissen 2007). Elevated $CO_2$ increased concentrations of condensed tannins in aspen and birch, but did not influence concentrations of tremulacin in aspen or concentrations of fiber and lignin in either tree species.

Our observation of the nominal effect of elevated $CO_2$ on foliar quality is likely a product of the potentially numerous ways elevated $CO_2$ influences forest ecosystems. Elevated $CO_2$ increases the ability of plants to forage for and uptake nitrogen (Finzi et al. 2007), and if nitrogen uptake increases proportionally with growth, foliar nutrient quality may not decrease. Resource availability can also influence the responses of foliar quality to elevated $CO_2$ (Körner 2006). Additionally, forest developmental stage can influence foliar quality. For example, condensed tannins and phenolic glycosides exhibit apparent ontogenetic shifts in aspen, with relatively low levels of condensed tannins and high levels of phenolic glycosides in juvenile trees and the opposite relationship in mature trees (Donaldson et al. 2006). While little is known about how ontogeny interacts with atmospheric change to influence foliar quality, a decade of research examining aspen secondary chemistry at Aspen FACE suggests that phytochemical responses to atmospheric change are likely greatest in juvenile trees at this site, and that phytochemical responses to atmospheric change potentially increase parallel to investment by trees into specific compounds. Finally, the aspen genotype used in this study is emerging as the dominant genotype relative to all other aspen clones in elevated $CO_2$ environments at Aspen FACE, with reduced mortality and increased growth (Kubiske et al. 2007). Additional rooting space, due to competitor mortality or enhanced competitive ability, may increase localized resource availability and potentially influence foliar quality.

Consistent with our second and third hypotheses, elevated $O_3$ reduced foliar quality, and the magnitude of response differed among tree species. Elevated $O_3$ decreased nitrogen concentrations and increased C:N ratios and sugar concentrations in both aspen and birch, but the responses were much greater in aspen than birch. While our findings contrast with a recent meta-analysis showing that elevated $O_3$ generally does not influence foliar nutrient composition (Valkama et al. 2007), our results agree with numerous reports of reductions in nitrogen concentrations under elevated $O_3$ at this study site (Kopper et al. 2001; Kopper and Lindroth 2003a, b; Holton et al. 2003). Elevated $O_3$ can affect the synthesis of Rubisco (Bortier et al. 2000), potentially reducing nitrogen concentrations and altering C:N ratios. Increases in soluble sugars under elevated $O_3$ may be a result of the inhibition of photosynthate conversion to storage carbohydrates or a product of the hydrolysis of starch to sugars to aid in the repair of $O_3$-induced damage (Lavola et al. 1994).

Also consistent with our second and third hypotheses, elevated $O_3$ altered concentrations of secondary metabolites, and differently so among tree species. Elevated $O_3$ increased levels of condensed tannins and lignin in aspen but did not affect levels in birch. Previous studies have reported increases in condensed tannin concentrations in aspen under elevated $O_3$ (Holton et al. 2003; Agrell et al. 2005). Elevated $O_3$ can alter the enzymatic activity of the shikimic acid pathway, enhancing the production of a number of phenolic compounds involved in stress responses (Close and McArthur 2002; Cabané et al. 2004). High molecular mass polyphenolics, such as condensed tannins, have been reported to be effective antioxidants (Hagerman et al. 1997), and increases in phenolic compounds under elevated $O_3$ may be a stress-defensive antioxidant response (Heath 2008; Betz et al. 2009). Also, increased lignin accumulation in poplar under elevated $O_3$ is suggested to provide a structural barrier and/or antioxidant activity to reactive oxygen species, thereby increasing tolerance to $O_3$ (Cabané et al. 2004).

## Herbivore performance

Gypsy moths and forest tent caterpillars increased growth when fed foliage from elevated $CO_2$. This finding is in contrast with recent meta-analyses showing that herbivore growth is often reduced when insects are fed foliage from elevated $CO_2$ (Zvereva and Kozlov 2006; Stiling and Cornelissen 2007). However, insect performance under elevated $CO_2$ has been shown to vary depending on both the tree and insect species studied, and findings of unchanged growth or even increased growth of herbivores feeding on foliage from elevated $CO_2$ environments have been reported multiple times (Lindroth et al. 1993; Roth and Lindroth 1995; Kinney et al. 1997; Kopper et al. 2001; Holton et al. 2003; Williams et al. 2003; Kopper and Lindroth 2003a; Hättenschwiler and Schafellner 2004; Knepp et al. 2007; Peltonen et al. 2010; Vigue and Lindroth 2010). Our findings of increased growth by gypsy moths and forest tent caterpillars fed aspen foliage from elevated $CO_2$ also contrast with previous studies of aspen–herbivore interactions (Lindroth et al. 1993; Roth and Lindroth 1995). The reason for differences between past studies and the current study is potentially a function of the stronger phytochemical response exhibited by aspen in the earlier studies, due to the use of much younger trees and higher levels of $CO_2$. Additionally, genotypic variation in phenolic glycoside production can reduce the ability of nonadapted herbivores to utilize aspen as a resource (Osier et al. 2000). The genotype we used in this study has relatively low levels of phenolic glycosides compared with other aspen genotypes at Aspen FACE, and in contrast to previous studies, levels were not influenced by elevated $CO_2$.

Consistent with our fourth hypothesis, reductions in foliar quality decreased gypsy moth and forest tent caterpillar performance. Gypsy moths and forest tent caterpillars grew less, consumed more, and had lower digestibility and conversion efficiencies on foliage from elevated $O_3$. Compensatory feeding is a common response to reductions in nitrogen concentrations and increases in C:N ratios (Mattson 1980; Scriber and Slansky 1981). Neither herbivore species was able to fully compensate for reduced foliar quality, but, consistent with our fifth hypothesis, gypsy moths were able to more fully compensate than forest tent caterpillars. Frass production increased and efficiency of conversion of ingested foliage into biomass decreased for both herbivores when fed foliage from elevated $O_3$. However, efficiency of conversion of digested foliage into biomass was reduced more for forest tent caterpillars than gypsy moths, potentially explaining the more pronounced effect of elevated $O_3$ on forest tent caterpillars, compared with gypsy moths. Our results agree with a recent study finding that elevated $O_3$ reduced the performance of multiple early season folivores feeding on silver birch (Peltonen et al. 2010). Additionally, our performance results are consistent with findings that forest tent caterpillars fed ozonated foliage preferred birch to aspen, and that aspen foliage from elevated $O_3$ increases consumption (Agrell et al. 2005). However, our findings contrast with previous studies from Aspen FACE demonstrating that elevated $O_3$ either does not affect or it increases herbivore performance (Kopper et al. 2001; Kopper and Lindroth 2003a; Holton et al. 2003). This disparity is likely a result of the use of juvenile trees in the previous studies and the longevity of exposure by trees to $O_3$ in the current study. The negative effects of $O_3$ on plants are cumulative and longer exposure times increase $O_3$ sensitivity and the magnitude of negative responses (Oksanen 2003). Preliminary examination of long-term responses of foliar quality to elevated $O_3$ suggests that chronic $O_3$ exposure for almost a decade at Aspen FACE can produce trees with lower quality foliage (i.e., lower nitrogen and higher phenolic glycoside concentrations), compared with foliar quality responses to elevated $O_3$ from earlier experiments (Couture and Lindroth, unpublished data).

Elevated $CO_2$ ameliorated most of the reductions in performance for gypsy moths and forest tent caterpillars fed foliage from elevated $O_3$. This finding contrasts with the results of Valkama et al. (2007), who found that elevated $CO_2$ offset improved chewing herbivore performance under elevated $O_3$, and has significant implications for how outbreak herbivores will respond to atmospheric change in forest ecosystems in the future. The consequences of interactions between elevated $CO_2$ and $O_3$ for herbivore performance are important considering that increases in $CO_2$ are occurring globally, while increases in tropospheric $O_3$ are temporally and spatially variable (Wittig et al. 2009).

We used PLSR analysis to relate foliar quality to insect performance. The value of PLSR is its ability to reveal the influence of multiple, highly correlated predictor variables on response variables. Nitrogen and lignin concentrations and C:N ratios were identified as the variables explaining the greatest amount of variation in gypsy moth and forest tent caterpillar performance when fed aspen. Nitrogen consistently produced the most positive relationship with growth and the most negative relationship with consumption and frass produced for both herbivores. These results are similar to those of Peltonen et al. (2010) who, using PLSR, found that nitrogen had the highest positive and negative relationship with growth and consumption, respectively, for the autumnal moth, *Epirrita autumnata*. Lignin had the strongest negative relationship with growth for both herbivores. Lignin also had the most positive relationship with consumption and frass production for gypsy moths, while C:N ratios had the strongest positive relationship with consumption and frass production for forest tent caterpillars. Gypsy moths and forest tent caterpillars experienced reduced growth and conversion efficiencies on aspen from elevated $O_3$, compared with ambient $O_3$, and nitrogen and lignin concentrations decreased and increased, respectively, in aspen leaves from elevated $O_3$, relative to ambient $O_3$. Additionally, we identified nitrogen and lignin concentrations as having strong influences on frass production, and by association digestibility and conversion efficiencies. This relationship between foliar quality and herbivore performance suggests that $O_3$-induced changes in foliar quality reduced conversion efficiencies, and ultimately the growth, of both gypsy moths and forest tent caterpillars. The existence of weak or non-significant relationships between herbivore performance and birch foliar quality indicates that, in this study, elevated $CO_2$ and $O_3$ did not alter birch phytochemistry sufficiently to influence insect behavior.

Although extrapolating population responses from individual performance measurements is risky (Awmack and Leather 2002), a positive relationship generally exists between insect mass and fecundity (Honěk 1993). While multiple factors (e.g., natural enemies, stochastic events) influence population growth, marginal increases in growth under elevated $CO_2$ and decreases in growth under elevated $O_3$ for insects suggest that atmospheric change may potentially contribute to altered populations of these herbivores in areas where aspen and birch commonly occur (e.g., the Great Lakes region). Aspen phytochemistry was more responsive to the fumigation treatments than birch, and herbivore growth was affected more by aspen than birch under elevated $CO_2$ and $O_3$. This finding is in line with Agrell et al. (2005), who found a preference shift by forest tent caterpillars from birch to aspen under elevated

$CO_2$ and the opposite preference shift under elevated $O_3$. These results also suggest that populations of folivores may not be as adversely affected in mixed aspen–birch, compared with only aspen, forested areas under elevated $O_3$, because of potential host shifting from aspen to birch. Additionally, greater background population levels of outbreak herbivores under elevated levels of $CO_2$ may contribute to a predicted increase in the frequency and duration of insect outbreaks (Stireman et al. 2005; Jepsen et al. 2008)

In summary, two outbreak herbivores performed marginally better on foliage exposed to elevated $CO_2$, compared with ambient $CO_2$, and worse on foliage from elevated $O_3$, compared with ambient $O_3$. Additionally, elevated $CO_2$ largely offset reduced performance by both herbivores under elevated $O_3$, but more so for gypsy moths than forest tent caterpillars. Although both herbivores increased consumption when fed aspen from elevated $O_3$, neither herbivore was able to fully compensate for reductions in foliar quality. These results suggest that gypsy moths and forest tent caterpillars may have greater impacts on aspen than birch under future predicted levels of $CO_2$. Our finding that elevated $O_3$ negatively affected herbivore performance contrasts with most earlier findings from Aspen FACE (but see Agrell et al. 2005), suggesting that tree developmental stage and longevity of exposure to pollutants may influence foliar quality and plant–herbivore interactions in future atmospheres. Also, this contrast highlights the need to continue long-term global change research in ecologically relevant settings. Herbivore populations encounter not only multiple tree species but temporal variation in forest stand structure and age that can influence foliar quality (Körner 2006; Donaldson et al. 2006), and ultimately herbivore performance. Long-term studies using experimental manipulation in ecologically relevant settings provide insight into not only the specificity of herbivore–tree species interactions (Bezemer and Jones 1997; Lindroth 2010), but how temporal variation influences those interactions.

**Acknowledgments** We are grateful to T.D. Fitzgerald for generously providing forest tent caterpillar egg masses. We thank K.F. Rubert-Nason for assistance with the chemical analysis of phenolic glycosides, and M. Bushell for laboratory assistance. We also thank P.A. Townsend, P.T. Wolter, and S.P. Serbin for assistance with PLSR analysis. One reviewer provided particularly constructive comments on the manuscript. Aspen FACE was principally supported by the Office of Science (BER), U.S. Department of Energy, grant no. DE-FG02-95ER62125 to Michigan Technological University, and contract no. DE-AC02-98CH10886 to Brookhaven National Laboratory, the US Forest Service Northern Global Change Program and North Central Research Station, Michigan Technological University, and Natural Resources Canada—Canadian Forest Service. This work was supported by the U.S. Department of Energy (Office of Science, BER), grant DE-FG02-06ER64232, to RL.

## References

Agrell J, Kopper BJ, McDonald EP, Lindroth RL (2005) $CO_2$ and $O_3$ effects on host plant preferences of the forest tent caterpillar (*Malacosoma disstria*). Glob Change Biol 11:588–599

Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air $CO_2$ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising $CO_2$. New Phytol 165:351–372

Awmack CS, Leather SR (2002) Host plant quality and fecundity in herbivorous insects. Annu Rev Entomol 47:817–844

Barbehenn RV, Jaros A, Lee G, Mozola C, Weihr Q, Salminen J-P (2009) Tree resistance to *Lymantria dispar* caterpillars: importance and limitations of foliar tannin composition. Oecologia 159:777–788

Betz GA, Gerstner E, Stich S, Winkler B, Welzi G, Kremmer E, Langebartles C, Heller W, Sandermann H, Ernst D (2009) Ozone affects shikimate pathway genes and secondary metabolites in saplings of European birch (*Fagus sylvatica* L.) grown under greenhouse conditions. Trees Struct Funct 23:539–553

Bezemer TM, Jones TH (1998) Plant–insect herbivore interactions in elevated $CO_2$: quantitative analysis and guild effects. Oikos 82:212–222

Bonan GB (2008) Forests and climate change: Forcings, feedbacks, and the climate benefits of forests. Science 320:1444–1449

Bortier K, Ceulemans R, de Temmerman L (2000) Effects of tropospheric ozone on woody plants. In: Agrawal SB, Agrawal M (eds) Environmental pollution and plant responses. CRC Press, Boca Raton, pp 153–182

Cabané M, Pireaux J-C, Léger E, Weber E, Dizengremel P, Pollet B, Lapierre C (2004) Condensed lignins are synthesized in poplar leaves exposed to ozone. Plant Physiol 134:586–594

Close DC, McArthur C (2002) Rethinking the role of many plant phenolics—protection from photodamage not herbivores. Oikos 99:166–172

Couture JJ (2011) Impact of elevated $CO_2$ and $O_3$ on community herbivory in a northern temperate forest (Ph.D. dissertation). University of Wisconsin, Madison

Dickson RE, Lewin KF, Isebrands JG, Coleman MD, Heilman WE, Riemenschneider DE, Sober J, Host GE, Zak DR, Pregitzer KS, Karnosky DF (2000) Forest atmosphere carbon transfer storage II (FACTS II)—the Aspen Free-air $CO_2$ and $O_3$ Enrichment (FACE) Project: an overview (Gen Tech Rep NC-214). USDA Forest Service, North Central Research Station, Rhinelander

Donaldson JR, Stevens MT, Barnhill HR, Lindroth RL (2006) Age-related shifts in leaf chemistry of clonal aspen. J Chem Ecol 32:1415–1429

Fajvan MA, Wood JM (1996) Stand structure and development after gypsy moth defoliation in the Appalachian Plateau. Forest Ecol Manag 89:79–88

Filion M, Dutilleul P, Potvin C (2000) Optimum experimental design for Free-Air Carbon dioxide Enrichment (FACE) studies. Glob Change Biol 6:843–854

Finzi A, Norby R, Carlo C, Gallet-Budynek A, Gielen B, Holmes WE, Hoosbeek MR, Iversen CM, Jackson RB, Kubiske ME, Ledford J, Liberloo M, Oren R, Polle A, Pritchard S, Zak DR, Schlesinger WH, Ceulemans R (2007) Increases in nitrogen uptake rather than nitrogen-use efficiency support higher rates of temperate forest productivity under elevated $CO_2$. Proc Natl Acad Sci USA 104:14014–14019

Fitzgerald TD (1995) The tent caterpillars. Cornell University Press, New York

Fowler D, Cape JN, Coyle M, Flechard C, Kuylenstierna J, Hicks K, Derwent D, Johnson C, Stevenson D (1999) The global exposure of forests to air pollutants. Water Air Soil Poll 116:5–32

Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, Riechel L (1997) High molecular weight polyphenolics (tannins) as antioxidants. J Agr Food Chem 46:1887–1892

Hättenschwiler S, Schafellner C (2004) Gypsy moth feeding in the canopy of a $CO_2$-enriched mature forest. Glob Change Biol 10:1899–1908

Heath RL (2008) Modification of the biochemical pathways of plants induced by ozone: What are the varied routes to change? Enviro Pollut 155:453–463

Hemming JDC, Lindroth RL (1999) Effects of light and nutrient availability on aspen: growth, phytochemistry, and insect performance. J Chem Ecol 25:1687–1714

Holton MK, Lindroth RL, Nordheim EV (2003) Foliar quality influences tree-herbivore–parasitoid interactions: effects of elevated $CO_2$, $O_3$, and plant genotype. Oecologia 137:233–244

Honěk A (1993) Intraspecific variation in body size and fecundity in insects: a general relationship. Oikos 66:483–492

Hunter MD (2001) Effects of elevated atmospheric carbon dioxide on insect–plant interactions. Agric Forest Entomol 3:153–159

Intergovernmental Panel on Climate Change (IPCC) (2007) In: Solomon S et al. (eds) Climate Change 2007: the scientific basis (Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change). Cambridge University Press, New York

Jepsen JU, Hagen SB, Ims RA, Yoccoz NG (2008) Climate change and outbreaks of the geometrids *Operophtera brumata* and *Epirrita autumnata* in subarctic birch forest: evidence of a recent outbreak range expansion. J Anim Ecol 77:257–264

Karnosky DF, Mankovska B, Percy K, Dickson RE, Podila GK, Sober J, Noormets A, Hendrey G, Coleman MD, Kubiske M, Pregitzer KS, Isebrands JG (1999) Effects of tropospheric $O_3$ on trembling aspen and interaction with $CO_2$: results from an $O_3$-gradient and a FACE experiment. Water Air Soil Poll 116:311–332

Karnosky DF, Werner H, Holopainen T, Percy K, Oksanen T, Oksanen E, Heerdt C, Fabian P, Nagy J, Heilman W, Cox R, Nelson N, Matyssek R (2007) Free-air exposure systems to scale up ozone research to mature trees. Plant Biol 9:181–190

Kinney KK, Lindroth RL, Jung SM, Nordheim EV (1997) Effects of $CO_2$ and $NO_3$ availability on deciduous trees: phytochemistry and insect performance. Ecology 78:215–230

Knepp RG, Hamilton JG, Zangerl AR, Berenbaum MR, DeLucia EH (2007) Foliage of oaks grown under elevated $CO_2$ reduces performance of *Antheraea polyphemus* (Lepidoptera: Saturniidae). Environ Entomol 36:609–617

Kopper BJ, Lindroth RL (2003a) Effects of elevated carbon dioxide and ozone on the phytochemistry of aspen and performance of an herbivore. Oecologia 134:95–103

Kopper BJ, Lindroth RL (2003b) Response of trembling aspen (*Populus tremuloides*) phytochemistry and aspen blotch leafminer (*Phyllonorycter tremuloidiella*) performance to elevated levels of atmospheric $CO_2$ and $O_3$. Agric Forest Entomol 5:17–26

Kopper BJ, Lindroth RL, Nordheim EV (2001) $CO_2$ and $O_3$ effects on paper birch (Betulaceae: *Betula papyrifera*) phytochemistry and whitemarked tussock moth (Lymantriidae: *Orgyia leucostigma*) performance. Environ Entomol 30:1119–1126

Körner C (2006) Plant $CO_2$ responses: an issue of definition, time, and resources supply. New Phytol 172:393–411

Kubiske ME, Quinn VS, Marquardt PE, Karnosky DF (2007) Effects of elevated atmospheric $CO_2$ and/or $O_3$ on intra- and interspecific competitive ability of aspen. Plant Biol 9:342–355

Lavola A, Julkunen-Tiitto R, Paakkonen E (1994) Does ozone stress change the primary or secondary metabolites of birch (*Betula pendula* Roth)? New Phytol 126:637–642

Lincoln DE, Fajer ED, Johnson RH (1993) Plant–insect herbivore interactions in elevated $CO_2$ environments. Trends Ecol Evol 8:64–68

Lindroth RL (1996a) $CO_2$-mediated changes in tree chemistry and tree–Lepidoptera interactions. In: Koch GW, Mooney HA (eds) Carbon dioxide and terrestrial ecosystems. Academic, San Diego, pp 105–120

Lindroth RL (1996b) Consequences of elevated atmospheric $CO_2$ for forest insects. In: Körner C, Bazzaz FA (eds) Carbon dioxide, populations, and communities. Academic, San Diego, pp 347–361

Lindroth RL (2010) Impacts of elevated atmospheric $CO_2$ and $O_3$ on forests: phytochemistry, trophic interactions, and ecosystem dynamics. J Chem Ecol 36:2–21

Lindroth RL, Hwang S-Y (1996) Diversity, redundancy, and multiplicity in chemical defense systems of aspen. In: Romero JT, Saunders JA, Barbosa P (eds) Recent advances in phytochemistry: phytochemical diversity and redundancy in ecological interactions. Plenum, New York, pp 26–51

Lindroth RL, Scriber JM, Hsia MTS (1988) Chemical ecology of the tiger swallowtail: mediation of host use by phenolic glycosides. Ecology 69:814–822

Lindroth RL, Kinney KK, Platz CP (1993) Responses of deciduous trees to elevated atmospheric $CO_2$: productivity, phytochemistry, and insect performance. Ecology 74:763–777

Lindroth RL, Wood SA, Kopper BJ (2002) Responses of quaking aspen genotypes to enriched $CO_2$: foliar chemistry and tussock moth performance. Agric Forest Entomol 4:315–323

Lovett GM, Canham CD, Arthur MA, Weathers KC, Fitzhugh RD (2006) Forest ecosystem response to exotic pests and pathogens in eastern North America. Bioscience 56:395–405

Mattson WJ (1980) Herbivory in relation to plant nitrogen content. Annu Rev Ecol Syst 11:119–161

Mattson WJ, Addy ND (1975) Phytophagous insects as regulators of forest primary production. Science 190:515–522

Mattson WJ, Herms DA, Witter JA, Allen DC (1991) Woody plant grazing systems: North American outbreak folivores and their host plants. In: Baranchikov YN, Mattson WJ, Hain FP, Payne TL (eds) Forest insect guilds: patterns of interactions with host trees (Gen Tech Rep NE-153). USDA Forest Service, North Central Research Station, Rhinelander

McGrath GM, Karnosky DF, Ainsworth EA (2010) Spring leaf flush in aspen (*Populus tremuloides*) clones is altered by long-term growth at elevated carbon dioxide and elevated ozone concentration. Environ Pollut 105:1023–1028

Oksanen EA (2003) Physiological responses of birch to ozone a comparison between open-soil-grown trees exposed for six growing seasons and potted seedlings exposed for one season. Tree Physiol 23:603–614

Osier TL, Hwang T-Y, Lindroth RL (2000) Effects of phytochemical variation in quaking aspen *Populus tremuloides* clones on gypsy moth *Lymantria dispar* performance in the field and laboratory. Ecol Entomol 25:197–207

Peltonen PA, Vapaavuori E, Heionen J, Julkunen-Tiitto R, Holopainen JK (2010) Do elevated atmospheric $CO_2$ and $O_3$ affect food quality and performance of folivorous insects on silver birch? Glob Change Biol 16:918–935

Porter LJ, Hrstich LN, Chan BG (1986) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry 25:223–230

Raubenheimer D, Simpson SJ (1992) Analysis of covariance: an alternative to nutritional indices. Entomol Exp Appl 62:221–231

Roth SK, Lindroth RL (1995) Elevated atmospheric $CO_2$: effects on phytochemistry, insect performance and insect–parasitoid interactions. Glob Change Biol 1:173–182

Scriber JM, Slansky F (1981) The nutritional ecology of immature insects. Annu Rev Entomol 26:183–211

Stiling P, Cornelissen T (2007) How does elevated carbon dioxide ($CO_2$) affect plant–herbivore interactions? A field experiment and meta-analysis of $CO_2$-mediated changes on plant chemistry and herbivore performance. Glob Change Biol 13:1–20

Stireman JO III, Dyer LA, Janzen DH, Singer MS, Lill JT, Marquis RJ, Rickelfs RE, Gentry GL, Hallwachs W, Coley PD, Barone JA, Greeney HF, Connahas H, Barbosa P, Morais HC, Diniz IR (2005) Climatic unpredictability and caterpillar parasitism: implications of global warming. Proc Natl Acad Sci USA 102:17384–17387

United States Department of Agriculture, Forest Service (2003) Forest insect and disease conditions in the United States 2001. http://www.fs.fed.us/foresthealth/publications/ConditionsReport_01_final.pdf

United States Department of Agriculture, Forest Service (2009) Forest insect and disease conditions in the United States 2008. http://www.fs.fed.us/foresthealth/publications/ConditionsReport_08_final.pdf

Valkama E, Koricheva J, Oksanen E (2007) Effects of elevated $O_3$, alone and in combination with elevated $CO_2$, on tree leaf chemistry and insect herbivore performance: a meta-analysis. Glob Change Biol 13:184–201

Vigue LM, Lindroth RL (2010) Effects of genotype, elevated $CO_2$ and elevated $O_3$ on aspen phytochemistry and leaf beetle *Chrysomela crotchi* performance. Agric Forest Entomol 12:267–276

Williams RS, Lincoln DE, Norby RJ (2003) Development of gypsy moth larvae feeding on red maple saplings at elevated $CO_2$ and temperature. Oecologia 137:114–122

Wittig VE, Ainsworth EA, Naidu SL, Karnosky DF, Long SP (2009) Quantifying the impact of current and future tropospheric ozone on tree biomass, growth, physiology and biochemistry: a quantitative meta-analysis. Glob Change Biol 15:396–424

Wold S, Ruhe A, Wold H, Dunn WJ (1984) The collinearity problem in linear regression. The partial least squares (PLS) approach to generalized inverses. SIAM J Sci Stat Comp 5:735–743

Wold S, Sjöström M, Eriksson L (2001) PLS-regression: a basic tool of chemometrics. Chemometr Intell Lab 58:109–130

Zvereva EL, Kozlov MV (2006) Consequences of simultaneous elevation of carbon dioxide and temperature for plant–herbivore interactions: a metaanalysis. Glob Change Biol 12:27–41