Final Technical Report

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Distribution limitation notices

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Executive summary

Rising concentrations of atmospheric CO₂ and O₃ are altering the structure and function of forest ecosystems. Herbivorous insects are the major consumers in temperate deciduous forests, with the capacity to dramatically alter tree growth (via outbreaks), forest community composition and ecosystem dynamics (e.g., nutrient cycling). Until recently, however, experimental quantification of the impacts of CO₂ and O₃ on canopy herbivore communities and rates of defoliation and nutrient flux has not been addressed. This research, conducted at the Aspen FACE (Free Air CO₂ Enrichment) facility in northern Wisconsin, U.S.A., evaluated the independent and interactive effects of CO₂ and O₃ on 1) the abundance and diversity of forest canopy insect communities, and 2) rates of insect herbivory and transfer of material (leaf greenfall and insect frass) from the canopy to the forest floor.

Results of studies of individual insects revealed that elevated CO₂ and O₃ influence the performance of individual species of damaging insect pests, but the magnitude of impact is influenced by both insect species and their host tree species. Censuses of canopy insects showed that some species were positively affected, some negatively affected, and some not affected by elevated CO₂ and O₃. Moreover, overall species diversity was generally not strongly affected by CO₂ and O₃. In summary, the effects of CO₂ and O₃ on forest insects is highly variable among species and over time, and thus difficult to generalize across broad taxonomic groups.

Estimates of foliar damage revealed that CO₂ and O₃ have pronounced effects on canopy damage by insect herbivores. Averaged over three years, foliar biomass lost to insect feeding increased 86% in high CO₂ environments and decreased 12% in high O₃ environments. The increases/decreases were greater for aspen than for birch, indicating that the selective pressure of insects will shift across tree species in forests of the future.

Herbivore-mediated material (green leaf tissue, insect frass) transfer from the canopy to the forest floor increased 37% in elevated CO₂ and decreased 21% in elevated O₃. Nitrogen transfers paralleled those results: 39% increase in elevated CO₂ and 19% decrease in elevated O₃.

In summary, this body of work indicates that in future environments with elevated CO₂ and O₃, the performance of individual species of tree-feeding insects will change, but that effects will be highly variable among insect and tree species. Overall insect diversity and abundance may not be strongly affected. In contrast, the foliar damage caused by tree-feeding insects will likely increase significantly under elevated CO₂ conditions, but decrease under elevated O₃ conditions.

Comparison of accomplishments with goals and objectives

The objectives of this research were to evaluate the independent and interactive effects of CO₂ and O₃ on 1) the abundance and diversity of forest canopy insect communities, and 2) rates of insect herbivory and transfer of material (leaf greenfall and insect frass) from the canopy to the forest floor. The original goal was to conduct this work in three forest community types: aspen-birch, aspen-maple, and mixed aspen genotype. Due to the magnitude of work involved, and small stature of maple trees at the site, we excluded the aspen-maple community type from our research activities. In addition to the work originally proposed, we conducted targeted performance studies with several species of tree-feeding insects, and also evaluated the effects of CO₂- and O₃-mediated changes in leaf litter chemistry on performance of litter-feeding invertebrates.

Summary of project activities and results

The overall objective of this research program was to assess the independent and interactive effects of CO₂ and O₃ on herbivorous insect communities (abundance/ biodiversity), and feedback effects of insects on rates of herbivory and conversion of primary production to organic substrates, in forest canopies at the Aspen FACE (Free Air CO₂ Enrichment) site in northern Wisconsin, U.S.A. Activities and results are summarized below, organized by original hypothesis.

Hypothesis 1. Elevated CO₂ and O₃ will alter the abundance and biodiversity (richness and relative composition) of herbivorous canopy insects at Aspen FACE.

Insect abundance, diversity and community composition were monitored via routine trapping and visual censuses in two forest types (aspen-birch and mixed aspen genotype) at the Aspen FACE facility during the summers of 2005-07. The number of insects in the visual censuses alone numbered over 36,000. Data were analyzed via standard statistical time-series analyses (abundance data) and state-of-the-art community analyses (e.g., nonmetric multidimensional scaling [NMDS] and analysis of similarity [ANOSIM]).

Controlled bioassays with individual insects revealed that elevated CO₂ and O₃ influence the performance of individual species of damaging insect pests, but the magnitude of impact is influenced by both insect species and their host tree species. Field censuses of insect abundance revealed that elevated CO₂ tended to increase the abundance of phloem-feeding insects and decrease the abundance of the chewing and galling insects. Insect abundance under elevated O₃ typically had the opposite response of insect abundance under elevated CO₂. However, despite some general patterns, insect responses to elevated CO₂ and O₃ varied considerably depending on the insect species and sample year. Analyses for idiosyncratic effects revealed multiple strong

two- and three-way interactions between CO₂, O₃, arthropod species, and year indicating that the effects of eCO₂ and eO₃ on arthropod abundance are species-specific and temporally variable. The total abundance of insects surveyed was rarely affected by elevated CO₂ and O₃ because family/species responses were offsetting and idiosyncratic. Similarly, elevated CO₂ and O₃ rarely altered the species composition of forest canopy insect communities. A consistent finding in this research is that the effects of elevated CO₂ and O₃ vary among tree species, insect species, and over time, such that population- and community-level responses appear to be idiosyncratic and species-specific.

Complementary bioassays with detritus-feeding invertebrates (earthworms and springtails) showed that elevated carbon dioxide reduced nitrogen and increased condensed tannin concentrations in leaf litter. These changes were associated with decreases in earthworm individual growth, earthworm growth efficiency, and springtail population growth. Elevated ozone increased fiber and lignin concentrations of leaf litter. These changes did not influence earthworm consumption or growth, but accelerated springtail population growth. These results suggest that changes in litter chemistry caused by increased carbon dioxide concentrations will have negative impacts on the productivity of diverse detritivore taxa, whereas those caused by increased ozone concentrations will have variable, species-specific effects.

Hypothesis 2. Elevated CO₂ and O₃ will alter herbivory rates and reapportion foliar herbivory among tree species and genotypes at Aspen FACE.

To evaluate the effects of CO₂ and O₃ on rates of canopy herbivory, we measured foliar damage in two forest types (aspen-birch and mixed aspen genotype) at Aspen FACE during the summers of 2006-08. A sample of 10-12 randomly selected leaves was collected from each of two canopy levels, for each of four trees, for each of four genotypes/species (3 aspen genotypes & birch) for each FACE ring, three times during the growing season (June, July, August), for a total of ~14,000 leaves/year. All leaves were digitally scanned and leaf damage estimates were assessed via image analysis (WinFolia software). The sets of 10-12 leaves were then processed for subsequent chemical analysis, including carbon, nitrogen, simple sugars, starch, fiber, lignin, tannins and phenolic glycosides.

Estimates of foliar damage revealed that CO₂ and O₃ have pronounced effects on canopy damage by insect herbivores. Averaged over three years, foliar biomass lost to insect feeding increased 86% in high CO₂ environments and decreased 12% in high O₃ environments. The increases/decreases were greater for aspen than for birch, indicating that the selective pressure of insects will shift across tree species in forests of the future.

Changes in phytochemistry under elevated CO₂ and O₃ explained variation in gypsy moth and forest tent caterpillar performance in insect bioassays. Variation in phytochemistry also explained overall canopy damage patterns in aspen, but not birch. This finding suggests that combinations of factors (i.e., biotic *and* abiotic) will drive patterns of canopy damage in northern temperate forests in the future, and will vary among tree species.

Hypotheses 3. Elevated CO₂ and O₃ will alter the quantity and quality of organic substrates (frass and green leaf litter) produced by insects and transferred from the canopy to the forest floor.

To determine the effects of CO₂ and O₃ on the rates of insect-mediated transfer of organic substrates (frass and greenfall, i.e., “herbivore inputs”) from the canopy to the forest floor, we measured frass and greenfall deposition during the summers of 2006-08. Inputs were collected using laundry baskets covered with muslin cloth that captured inputs and allowed drainage of precipitation. In each FACE ring, four baskets were placed on the forest floor in both aspen-birch and mixed aspen stands for five, 10-day periods during the growing season. Herbivore inputs collected from the muslin filters were air-dried, sorted, freeze-dried, and weighed. Frass nitrogen concentration was measured with an elemental analyzer and multiplied by mass of collected frass to estimate insect-mediated nitrogen deposition via frassfall. Nitrogen concentrations of greenfall were taken from data on foliar chemistry (described above) and multiplied by the mass of collected greenfall to estimate insect-mediated nitrogen deposition via greenfall. Total herbivore input and nitrogen deposition were estimated for the growing season by integrating an input per time curve for each basket, where the curve was anchored at 0 g m⁻¹ d⁻¹ on 15 May and 15 September.

Herbivore-mediated organic substrate (frass, greenfall) transfer from the canopy to the forest floor increased 37% in elevated CO₂ and decreased 21% in elevated O₃. Nitrogen transfers paralleled those results: 39% increase in elevated CO₂ (0.098 to 0.136 g/m²/yr) and 19% decrease in elevated O₃ (0.129 to 0.105 g/m²/yr). These results reveal that in future, elevated CO₂ and O₃ environments, herbivorous insects will shift the timing and magnitude of nutrient fluxes from the forest canopy to the forest floor.

Products developed

a. Publications

- Meehan, T.D. and R.L. Lindroth. 2007. Modeling nitrogen flux by larval insect herbivores from a temperate hardwood forest. *Oecologia* 153:833-843.
- Habeck, C.W. and T.D. Meehan. 2008. Mass invariance of population nitrogen flux by terrestrial mammalian herbivores: an extension of the energetic equivalence rule. *Ecology Letters* 11:898-903.
- Hillstrom, M.L. and R.L. Lindroth. 2008. Elevated atmospheric carbon dioxide and ozone alter forest insect abundance and community composition. *Insect Conservation and Diversity* 1:233-241.
- Meehan, T.D., and R.L. Lindroth. 2009. Scaling of individual phosphorus flux by caterpillars of the whitemarked tussock moth, *Orygia leucostigma*. *Journal of Insect Science* 9:42 (8 pp.).
- Pelini, S.L., K.M. Prior, D.J. Parker, J.D.K. Dzurisin, R.L. Lindroth, and J.J. Hellmann. 2009. Climate change and temporal and spatial mismatches in insect communities. Pp. 215-231,

in *Climate Change: Observed Impacts on Planet Earth*. (T. Letcher, ed.) Elsevier, Inc. Amsterdam.

Lindroth, R.L. 2010. Impacts of elevated atmospheric CO₂ and O₃ on forests: phytochemistry, trophic interactions, and ecosystem dynamics. *Journal of Chemical Ecology* 36:2-21.

Vigue, L.M., and R.L. Lindroth. 2010. Effects of genotype, elevated CO₂, and elevated O₃ on aspen phytochemistry and aspen leaf beetle *Chrysomela crotchii* performance. *Agricultural and Forest Entomology* 12:267-276.

Hillstrom, M.L., L.M. Vigue, D.R. Coyle, K.F. Raffa and R.L. Lindroth. 2010. Performance of the invasive weevil *Polydrusus sericeus* is influenced by atmospheric CO₂ and host species. *Agricultural and Forest Entomology* 12:285-292.

Meehan, T.D., M.S. Crossley, and R.L. Lindroth. 2010. Impacts of elevated CO₂ and O₃ on aspen leaf litter chemistry and earthworm and springtail productivity. *Soil Biology and Biochemistry* 42:1132-1137.

Hillstrom, M.L., T.D. Meehan, K. Kelly, and R.L. Lindroth. 2010. Soil carbon and nitrogen mineralization following deposition of insect frass and greenfall from forests under elevated CO₂ and O₃. *Plant and Soil* 336:75-85.

Couture, J.J., T.D. Meehan, and R.L. Lindroth. 2011. Atmospheric change alters foliar quality of host trees and performance of two outbreak insect species. *Oecologia* (in press).

Lindroth, R.L. 2012. Atmospheric change, plant secondary metabolites, and ecological interactions. Pp. XX-XX, in *The Ecology of Plant Secondary Metabolites: Genes to Global Processes*. Ecological Reviews (G.R. Iason, M. Dicke and S.E. Hartley, eds.). Cambridge University Press, Cambridge.

b. Collaborations fostered

This research facilitated ongoing collaborations among Aspen FACE scientists from multiple universities and federal research laboratories. It also helped to initiate a developing collaboration between the Lindroth research group and forest geneticists and ecologists at the Umea Plant Sciences Research Centre in Umea, Sweden. That collaboration will, in part, address the impacts of environmental change on *Populus* trees in northern temperate forests.

c. Technologies

This research contributed to development of NIRS (near infrared reflectance spectroscopy) for characterization of the effects of CO₂ and O₃ on the chemical composition of tree foliage. NIRS affords rapid and precise determination of foliar constituents such as nitrogen and tannins. This work is being written up for publication.

Modeling nitrogen flux by larval insect herbivores from a temperate hardwood forest

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Abstract Herbivorous insects flux considerable amounts of nitrogen from the forest canopy to the soil in the form of frass. The amount of nitrogen fluxed varies depending on the characteristics of the herbivores, their food resources, and their physical environment. We used concepts from metabolic ecology and ecological stoichiometry to develop a general model of individual nitrogen flux via frass fall for moth and sawfly larvae from a temperate hardwood forest in northern Wisconsin, USA. We found that individual nitrogen flux (Q_N , mg N/day) was related to larval body mass (M_B , mg dry), short-term variation in environmental temperature (T , K), and larval nitrogen concentration (N_B , proportion dry mass) as $Q_N = e^{25.75} M_B^{0.77} e^{-0.83/kT} N_B^{-1.56}$, where k is Boltzmann's constant (8.62×10^{-5} eV/K). We also found that larval nitrogen flux did not vary with the nitrogen concentration of food, and suggest that this was due to compensatory feeding by larvae living on low-quality leaves. With further work, models of individual N flux could be used to scale individual fluxes to population and community levels, and thus link the characteristics of insect herbivore communities with the flow of nitrogen through forested ecosystems.

Keywords Body mass · Consumer-driven nutrient cycling · Ecological stoichiometry · Environmental temperature · Forest insects · Hymenoptera · Lepidoptera · Metabolic theory of ecology

Introduction

Herbivores play important roles in nutrient cycling in both aquatic and terrestrial ecosystems (Mattson and Addy 1975; Carpenter and Kitchell 1988; McNaughton et al. 1988; Belovsky and Slade 2000; Vanni 2002). In forested biomes, in particular, larval insect herbivores are responsible for transforming and translocating (sensu Vanni 2002) considerable amounts of nitrogen (N) from the canopy to the soil in the form of frass (Fogal and Slansky 1985; Hollinger 1986; Reynolds and Hunter 2001; Lovett et al. 2002; Hunter et al. 2003). Frass-derived N typically falls at the peak of the growing season and is in a highly labile form (Lovett and Ruesink 1995). As a result, it is rapidly transformed by microbes, absorbed by plants, or flushed from the local system during precipitation events (Swank et al. 1981; Webb et al. 1995; Eshleman et al. 1998; Frost and Hunter 2007).

Nitrogen inputs from insect frass are typically quantified by collecting frass in trays placed on the forest floor, weighing and analyzing the N concentration of the frass, and calculating the amount of N deposited per unit area and time (Fogal and Slansky 1985; Hunter et al. 2003). A complementary approach to estimating frass N inputs is to scale individual N flux to the community level using general models of individual flux and information about community structure. This strategy has been used to study N flux by a variety of aquatic (Peters and Rigler 1973; Ejsmont-Karabin 1984; Grimm 1988; Wen and Peters 1994; Vanni et al. 2002) and mammalian herbivores (Clark et al. 2005), but this approach has not been used with herbivorous insects.

Our primary objective in the present study was to develop a general model of individual N flux via frass production for larval insect herbivores using concepts from

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ecological energetics and metabolic ecology (Grodzinski et al. 1975; Peters 1983; Gillooly et al. 2001, 2005; Enquist et al. 2003; Brown et al. 2004; Allen et al. 2005) and nutritional ecology and ecological stoichiometry (Stern et al. 1992; Elser et al. 1996; Elser and Urabe 1999; Stern et al. 2002). The scaling of individual fluxes to the community level will be addressed elsewhere.

Model

The quantity of N fluxed via frassfall by an individual insect herbivore per unit time (Q_N , mg N/day) should be related to the individual frass production rate (M_F , mg dry frass/day) and the N concentration of frass (N_F , proportion of frass dry mass) as:

$$Q_N = M_F N_F \quad (1)$$

From metabolic ecology, we expect individual frass production to be proportional to the rate of ingestion, which, in turn, should be proportional to whole organism metabolic rate when animals are not using internal stores of energy (Lavigne 1982; Peters 1983; Peters et al. 1996; Brown et al. 2004). If frass production and metabolic rate are approximately proportional to one another, then frass production should be related to larval body mass (M_B , mg dry) as a power function (Kleiber 1932; Hemmingsen 1960; Peters 1983; Gillooly et al. 2001) and to short-term variation in environmental temperature (from hours to days, where minimal temperature acclimation occurs; T , K) as an exponential function (Crozier 1924; Robinson et al. 1983; Clarke and Johnston 1999; Gillooly et al. 2001). The combined effects of body mass and temperature on frass production can thus be modeled using an equation similar to the metabolic rate equation of Gillooly et al. (2001):

$$M_F \propto M_B^b e^{-c/kT} \quad (2)$$

Here, b is a mass-scaling exponent, c is a coefficient describing the temperature dependence of frass production, sometimes called the “critical thermal increment” or “apparent activation energy” for a complex biological process (Withers 1992), and k is Boltzmann’s constant (8.62×10^{-5} eV/K) (Gillooly et al. 2001). Given the proportionality of metabolic rate and frass production, values for b and c should fall within ranges generally observed for metabolic rate, and thus $0.65 < b < 0.85$ (Peters 1983; Glazier 2005) and $0.25 < c < 0.80$ eV (Vasseur and McCann 2005; Meehan 2006). Metabolic scaling theory predicts that values for b and c should be 0.75 and 0.65 eV, specifically (West et al. 1997; Gillooly et al. 2001; Banavar et al. 2002; Gillooly et al. 2006).

The N concentration of frass is influenced by a complex suite of physiological factors (Nation 2002). It can be approximated, however, using abstractions provided by ecological stoichiometry (Stern et al. 1992; Elser and Urabe 1999; Stern et al. 2002). First, the N concentration of frass is expected to be a positive function of the N concentration of food (N_L , proportion of leaf dry mass), simply because a large fraction of N in food is not absorbed by larvae. Second, ecological stoichiometry predicts that consumers showing elemental homeostasis should excrete absorbed N above their physiological demands. Most insect herbivores studied have demonstrated partial to complete N homeostasis (Fox and Macauley 1977; Slansky and Feeny 1977; Raubenheimer and Simpson 2004; Kagata and Ohgushi 2006). Thus, N excretion should reinforce the positive relationship between frass and leaf N concentration that is due to lack of absorption. Third, according to ecological stoichiometry, the physiological demands for N are proportional to the N concentration of an organism’s body (N_B , proportion of body dry mass). Thus, frass N concentration should be inversely related to body N concentration. These relationships can be formalized simply as:

$$N_F \propto N_B^{-d} N_L^g \quad (3)$$

We approximate the relationships between the N concentration of frass, food, and larvae using power functions because they are simple and flexible mathematical forms, i.e., depending on the values of d and g , the functions can fit relationships that are positive or negative, accelerating or decelerating.

Substituting Eqs. 2, 3 into Eq. 1 gives:

$$Q_N = a M_B^b e^{-c/kT} N_B^{-d} N_L^g \quad (4)$$

Here, a is a normalization constant with units of mg N $\times$ day $^{-1} \times$ mg dry body mass $^{-b}$. Equation 4 is a general model for individual N flux via frass fall by a larval insect herbivore. The model is an intentional simplification of many complex physiological processes that vary across age, sex, and taxonomic groups. This simplification, admittedly, reduces the precision of the model when predicting N flux for any particular group. However, by creating a general model, our intention is to trade precision for applicability, and to provide a means to approximate frass-derived N inputs across a diverse community of larval insect herbivores. Equation 4 is based on principles from metabolic ecology and ecological stoichiometry. In the process of testing this model, we hoped to simultaneously: (1) evaluate a new tool for studying insect-derived N inputs, and (2) assess the generality of several expectations from ecological theory.

Materials and methods

To evaluate the individual N flux model described above, we assembled a dataset that included information on frass production, frass N concentration, larval body mass, environmental temperature, larval N concentration, and leaf N concentration for a variety of larval insect herbivores common to the hardwood forests of the Great Lakes region. The dataset was compiled from two different studies of individual N flux. One study was of moth and sawfly larvae collected from the field (hereafter, “field animals”) and a second study was of moth larvae that were reared in the laboratory (hereafter, “lab animals”).

Field animals

Field animals included 87 larvae, from 22 species of moths, butterflies, and sawflies (Table 1). Larvae were collected opportunistically from the lower branches of eight dominant tree species (Table 1) as they were encountered during walks through forest stands in Onieda County, Wisconsin, USA. We collected larvae, along with corresponding host plant foliage, placed them into plastic bags, and stored them in a cooler for 1–3 h until they were transported to the laboratory.

At the lab, larvae were transferred, along with their food and a moist piece of tissue paper, into 20-ml polyethylene vials for 24-h feeding trials. In all, 60 feeding trials were conducted using the 87 field animals. The number of animals was greater than the number of trials because, on ten occasions, 2–7 similarly sized animals were placed into one vial to increase the quantity of frass produced during the trial. Before each trial, we weighed larvae and divided the total mass by the number of larvae to estimate the average pre-trial wet mass. During trials, animals were kept at room temperature, 22 °C, under a natural light cycle of 15:9 light:dark hours. After the feeding trial, we collected frass from the bottom of the vial for frass production and N concentration estimates, reweighed larvae, calculated a post-trial wet mass, calculated a midpoint wet mass as the average of pre- and post-trial wet masses, and transferred larvae and leaves to a new vial for an additional 24-h period so that additional frass could be collected for chemical analysis. Afterwards, we reweighed larvae and promptly placed frass, larvae, and foliage into a freezer.

We dried frass, larvae, and foliage in an oven (55 °C) and weighed frass and larvae. We converted midpoint wet mass for each trial to midpoint dry mass using the following conversion equations: lepidopteran dry mass = $0.15 \times \text{wet mass}^{1.05}$ ($R^2 = 0.99$); hymenopteran dry mass = $0.16 \times \text{wet mass}^{1.05}$ ($R^2 = 0.98$). Midpoint dry mass was then converted to a final larval mass per trial by multiplying midpoint dry mass by a factor of 0.90 to account for the mass of

gut contents (Bowers et al. 1991). Per capita frass production rate was calculated as the dry mass of frass produced over the one-day trial divided by the number of animals in the trial.

Frass, foliage, and larvae were then homogenized using a mortar and pestle and 2–10 mg samples were packed into tin capsules for N analysis on either a Carlo Erba (Milan, Italy) NV 2100 or a Thermo Finnigan (San Jose, CA, USA) Flash 1112 elemental analyzer. Larval N concentration was corrected for that of gut contents using the equation: larval concentration = (measured concentration – $(0.10 \times \text{average concentration of leaf and frass})$)/0.90, after Fagan et al. (2002). The coefficient, 0.10, represented the approximate fraction of larval dry mass that is gut contents (Bowers et al. 1991), and was multiplied by the average concentration of the leaf and frass because that was our best estimate of the N concentration of the total gut contents. Larval N flux per feeding trial was calculated as the per capita frass production rate multiplied by frass N concentration.

On six occasions, larvae, frass, or leaf samples from a given feeding trial were too small for N analysis. As a result, materials from 2–3 feeding trials were pooled before N analysis and the same larval, frass, and leaf N concentration was used to represent the two or three trials included in that pool (Table 1). This caused a minor lack of independence in our N concentration data. The N flux data from pooled trials were not entirely correlated, however, because N flux was the product of both N concentration and frass production, and all trials produced independent frass production measurements. Our decision to use a common set of N concentrations across 2–3 trials on six occasions had no effect on the conclusions of this study.

Lab animals

Lab animals used in this study were whitemarked tussock moth (*Orygia leucostigma*) larvae. Larvae were raised from eggs purchased from the Canadian Forest Service (Sault Ste. Marie, ON, Canada). Egg masses were divided into two groups and put into two rearing dishes in a growth chamber set to 22 °C and a 14:10 light:dark hour cycle. Eggs hatched after two weeks of incubation and half of the larvae were fed aspen leaves with a high nitrogen concentration, while half were fed aspen leaves with a low nitrogen concentration. Leaves came from ten potted trees propagated from a single aspen clone; five of the ten trees were given 4.5 g/L soil of slow release fertilizer (18:6:12, N:P:K without micronutrients) in May 2004 and 2006 to increase nitrogen content of foliage. At the time of this study, the trees were in their fourth growing season.

As above, N flux was measured during 24-h feeding trials in 20-ml vials. Trials were conducted at various

Table 1 Data used in this analysis

Larva				Leaf		Frass		Flux
Species	<i>n</i>	Mean body mass (mg)	N	Species	N	Per capita frass production (mg/day)	N	Per capita N flux (mg/day)
Field animals								
Hymenoptera								
<i>Arge pectoralis</i>	7	0.18	0.106	<i>Corylus cornuta</i>	0.023	0.91	0.019	0.02
<i>Arge pectoralis</i>	3	1.33	0.100	<i>Corylus cornuta</i>	0.025	4.13	0.019	0.08
<i>Arge pectoralis</i>	3	1.65	0.105	<i>Corylus cornuta</i>	0.027	4.44	0.021	0.09
<i>Arge pectoralis</i>	3	1.78	0.093	<i>Corylus cornuta</i>	0.022	4.72	0.021	0.10
<i>Cimbex americana</i>	1	204.49	0.077	<i>Betula papyrifera</i>	0.020	219.39	0.017	3.79
<i>Cimbex americana</i>	1	268.52	0.075	<i>Betula papyrifera</i>	0.025	322.61	0.016	5.23
<i>Cimbex americana</i>	1	75.06	0.082	<i>Tilia americana</i>	0.026	43.30	0.020	0.86
Lepidoptera								
<i>Achatia distincta</i>	1	53.84	0.089	<i>Populus tremuloides</i>	0.034	30.94	0.025	0.78
<i>Acronicta leporine</i>	1	76.77	0.082	<i>Populus tremuloides</i>	0.022	96.12	0.024	2.33
<i>Archips cerasivorana</i>	1	6.63	0.081	<i>Quercus rubra</i>	0.024	7.09	0.023	0.16
<i>Bucculatrix ainselella</i>	3	0.65	0.096	<i>Quercus rubra</i>	0.029	1.10	0.028	0.03
<i>Bucculatrix ainselella</i>	4	0.66	0.096	<i>Quercus rubra</i>	0.029	1.33	0.028	0.04
<i>Bucculatrix ainselella</i>	1	1.17	0.096	<i>Quercus rubra</i>	0.029	4.80	0.028	0.13
<i>Bucculatrix ainselella</i>	1	1.60	0.100	<i>Quercus rubra</i>	0.025	3.85	0.020	0.08
<i>Bucculatrix ainselella</i>	2	1.09	0.102	<i>Quercus rubra</i>	0.027	2.70	0.024	0.06
<i>Bucculatrix ainselella</i>	1	1.36	0.102	<i>Quercus rubra</i>	0.027	3.39	0.024	0.08
<i>Bucculatrix ainselella</i>	3	1.43	0.102	<i>Quercus rubra</i>	0.027	3.67	0.024	0.09
<i>Ellida caniplaga</i>	1	14.39	0.106	<i>Tilia americana</i>	0.024	27.00	0.021	0.57
<i>Erranis tiliaria</i>	1	37.22	0.081	<i>Acer rubrum</i>	0.016	28.62	0.020	0.57
<i>Erranis tiliaria</i>	1	31.76	0.086	<i>Betula papyrifera</i>	0.024	25.98	0.020	0.52
<i>Erranis tiliaria</i>	1	20.90	0.088	<i>Betula papyrifera</i>	0.022	25.67	0.020	0.51
<i>Erranis tiliaria</i>	1	46.44	0.101	<i>Quercus rubra</i>	0.021	23.83	0.025	0.60
<i>Erranis tiliaria</i>	1	42.40	0.101	<i>Populus tremuloides</i>	0.024	34.32	0.020	0.70
<i>Erranis tiliaria</i>	1	43.52	0.103	<i>Betula papyrifera</i>	0.029	10.70	0.038	0.41
<i>Erranis tiliaria</i>	1	43.06	0.107	<i>Betula papyrifera</i>	0.026	22.49	0.025	0.55
<i>Erranis tiliaria</i>	1	53.26	0.107	<i>Betula papyrifera</i>	0.027	22.46	0.024	0.54
<i>Erranis tiliaria</i>	1	45.75	0.108	<i>Betula papyrifera</i>	0.028	27.35	0.019	0.51
<i>Erranis tiliaria</i>	1	12.33	0.112	<i>Betula papyrifera</i>	0.023	7.38	0.021	0.15
<i>Erranis tiliaria</i>	1	53.23	0.117	<i>Betula papyrifera</i>	0.028	28.31	0.028	0.80
<i>Erranis tiliaria</i>	1	42.44	0.130	<i>Acer rubrum</i>	0.020	12.72	0.029	0.36
<i>Heterocampa guttivitta</i>	1	113.16	0.091	<i>Betula papyrifera</i>	0.018	228.91	0.023	5.34
<i>Hyphantria cunea</i>	1	2.26	0.117	<i>Prunus virginiana</i>	0.025	2.96	0.013	0.04
<i>Hyphantria cunea</i>	1	1.81	0.128	<i>Prunus virginiana</i>	0.026	1.28	0.015	0.02
<i>Hyphantria cunea</i>	2	2.39	0.128	<i>Prunus virginiana</i>	0.026	1.40	0.015	0.02
<i>Lambdina fiscellaria</i>	1	4.22	0.095	<i>Betula papyrifera</i>	0.024	3.14	0.016	0.05
<i>Nadata gibbosa</i>	1	105.73	0.091	<i>Betula papyrifera</i>	0.024	130.93	0.017	2.25
<i>Orgyia leucostigma</i>	1	18.43	0.090	<i>Corylus cornuta</i>	0.024	33.40	0.018	0.59
<i>Orgyia leucostigma</i>	1	45.60	0.093	<i>Betula papyrifera</i>	0.019	65.34	0.020	1.30
<i>Orgyia leucostigma</i>	1	1.52	0.107	<i>Acer rubrum</i>	0.012	2.79	0.013	0.04
<i>Orgyia leucostigma</i>	1	1.18	0.111	<i>Alnus incana</i>	0.019	2.85	0.012	0.03
<i>Orgyia leucostigma</i>	2	0.76	0.116	<i>Alnus incana</i>	0.022	2.06	0.015	0.03
<i>Orgyia leucostigma</i>	1	0.96	0.121	<i>Acer rubrum</i>	0.016	1.15	0.016	0.02
<i>Polyphemus sp.</i>	1	23.11	0.103	<i>Quercus rubra</i>	0.025	15.04	0.020	0.30

Table 1 continued

Larva				Leaf		Frass		Flux
Species	<i>n</i>	Mean body mass (mg)	N	Species	N	Per capita frass production (mg/day)	N	Per capita N flux (mg/day)
Unknown Lepidoptera A	1	2.55	0.089	<i>Tilia americana</i>	0.027	6.40	0.019	0.12
Unknown Lepidoptera B	1	1.04	0.105	<i>Tilia americana</i>	0.030	2.70	0.018	0.05
Unknown Lepidoptera C	1	0.50	0.105	<i>Tilia americana</i>	0.030	0.76	0.018	0.01
Unknown Lepidoptera D	1	9.84	0.105	<i>Populus tremuloides</i>	0.028	9.80	0.012	0.11
Unknown Lepidoptera D	1	1.93	0.097	<i>Populus tremuloides</i>	0.026	2.09	0.010	0.02
Unknown Lepidoptera E	1	19.32	0.130	<i>Betula papyrifera</i>	0.028	9.06	0.018	0.16
Unknown Lepidoptera E	1	19.79	0.084	<i>Betula papyrifera</i>	0.023	8.70	0.030	0.26
Unknown Lepidoptera E	1	20.33	0.111	<i>Betula papyrifera</i>	0.027	8.41	0.030	0.25
Unknown Lepidoptera E	1	65.44	0.078	<i>Betula papyrifera</i>	0.019	52.70	0.021	1.08
Unknown Lepidoptera F	1	7.40	0.076	<i>Acer rubrum</i>	0.019	6.63	0.017	0.12
Unknown Lepidoptera F	1	8.94	0.078	<i>Acer rubrum</i>	0.020	9.00	0.015	0.13
Unknown Lepidoptera G	1	2.36	0.085	<i>Betula papyrifera</i>	0.034	2.58	0.031	0.08
Unknown Lepidoptera G	1	4.94	0.085	<i>Betula papyrifera</i>	0.034	6.78	0.031	0.21
Unknown Lepidoptera G	1	7.01	0.116	<i>Betula papyrifera</i>	0.026	6.30	0.014	0.09
Unknown Lepidoptera H	1	3.02	0.080	<i>Quercus rubra</i>	0.024	5.43	0.015	0.08
Unknown Lepidoptera H	1	3.52	0.100	<i>Quercus rubra</i>	0.025	5.22	0.020	0.10
Unknown Lepidoptera H	1	5.48	0.093	<i>Quercus rubra</i>	0.030	4.47	0.024	0.10
Lab animals								
Lepidoptera								
<i>Orgyia leucostigma</i> ^a	11	0.47	0.116	<i>Populus tremuloides</i>	0.014	2.58	0.007	0.02
<i>Orgyia leucostigma</i> ^b	10	0.57	0.111	<i>Populus tremuloides</i>	0.011	1.37	0.006	0.01
<i>Orgyia leucostigma</i> ^a	9	0.89	0.132	<i>Populus tremuloides</i>	0.027	4.10	0.021	0.08
<i>Orgyia leucostigma</i> ^a	2	7.03	0.099	<i>Populus tremuloides</i>	0.014	29.79	0.006	0.18
<i>Orgyia leucostigma</i> ^a	2	7.52	0.102	<i>Populus tremuloides</i>	0.013	33.39	0.007	0.24
<i>Orgyia leucostigma</i> ^b	1	7.58	0.097	<i>Populus tremuloides</i>	0.015	11.98	0.007	0.08
<i>Orgyia leucostigma</i> ^b	1	15.38	0.094	<i>Populus tremuloides</i>	0.013	21.61	0.009	0.19
<i>Orgyia leucostigma</i> ^a	1	23.89	0.114	<i>Populus tremuloides</i>	0.028	59.84	0.025	1.50
<i>Orgyia leucostigma</i> ^a	1	25.28	0.112	<i>Populus tremuloides</i>	0.025	74.45	0.021	1.57
<i>Orgyia leucostigma</i> ^a	1	28.11	0.104	<i>Populus tremuloides</i>	0.029	71.23	0.025	1.79
<i>Orgyia leucostigma</i> ^b	1	41.48	0.094	<i>Populus tremuloides</i>	0.013	43.55	0.009	0.39

Each row is a replicate feeding trial. Masses are dry mass and N concentrations are proportion dry mass

Ambient temperature for trials was 22 °C except for those denoted by ^a(30 °C) and ^b(15 °C)

Bolded N concentrations indicate pooled samples

masses over the course of larval development. In total, 11 trials were conducted using 39 larvae. The number of animals was greater than the number of trials because, in five cases, 2–11 similarly sized animals were placed into one vial for a feeding trial. For each trial, tussock moth larvae were weighed, an average pre-trial wet mass was calculated as described above, and larvae, fresh aspen foliage, and a moist piece of tissue paper were placed into a vial. During feeding trials, vials were placed into environmental chambers at 15 or 30 °C. After 24 h, the vials were removed from chambers and placed directly into a freezer.

Samples were then freeze-dried and larvae and frass were weighed. Post-trial dry mass per larva was calculated for each trial as the total dry mass of all larvae divided by the number of larvae in vial. A pre-trial dry mass was estimated for each trial using pre-trial wet mass and the function: larval dry mass = $0.17 \times \text{wet mass}^{0.98}$ ($R^2 = 0.99$). A midpoint dry mass was then calculated as the average of pre- and post-trial dry masses. A final larval mass for each trial was calculated by correcting midpoint average dry mass for gut contents using the equation: gut-content-free dry mass = $0.78 \times \text{midpoint dry mass}^{1.01}$ ($R^2 > 0.99$). The gut content correction function was pro-

duced for the tussock moths in our study following the fasting method of Bowers et al. (1991). Per capita frass production rate for each of these trials was calculated as described previously for field animals. The N concentrations of frass, foliage, and larvae were also quantified as described previously. Larval N concentration was corrected for gut contents using the equation given for field animals. Here, however, we used a gut content proportion of 0.22, which was calculated from our fasting tussock moth larvae. Finally, larval N flux per feeding trial was calculated as described previously.

Data analysis

We linearized the N flux model by taking the natural logarithm of both sides of Eq. 4 and fitted it to natural-log-transformed data using multiple regression. The full linear model used in our analysis was $\ln(Q_N) = b_0 + b_1 \ln(M_B) + b_2 1/kT + b_3 \ln(N_B) + b_4 \ln(N_L)$, where the regression coefficients b_0 , b_1 , b_2 , b_3 , and b_4 corresponded with $\ln(a)$, b , c , d , and g , respectively, in Eq. 4. We used ANOVA to evaluate the contribution of each term, and calculated 95% confidence intervals to assess the uncertainty around estimated model coefficients.

Results

Larval body masses of field animals ranged from 0.18 to 268.52 and averaged 28.16 mg dry, while larval N concentration ranged from 7.5 to 13 and averaged 9.9%, leaf N concentration ranged from 1.2 to 3.4 and averaged 2.5%, and N fluxes ranged from 0.01 to 5.34 and averaged 0.56 mg N/day (Table 1). Larval body mass of lab animals ranged from 0.47 to 41.48 and averaged 14.38 mg dry, while larval N concentration ranged from 9.4 to 13.2 and averaged 10.7%, leaf N concentration ranged from 1.1 to 2.9 and averaged 1.8%, and N fluxes ranged from 0.008 to 1.79 and averaged 0.55 mg N/day (Table 1).

When we combined information from studies of field and lab animals, we had data for 71 feeding trials. When we fit these data to the linear version of Eq. 4, we found that body mass, temperature, and body N concentration terms were related to N flux as:

$$\ln(Q_N) = 25.75 + 0.77 \ln(M_B) - \frac{0.83}{kT} - 1.56 \ln(N_B) \quad (5)$$

The model fit the N flux data well, with a whole model R^2 of 0.89. The coefficients for the intercept, body mass ($F_{(1,67)} = 424.23$; $P < 0.001$), temperature ($F_{(1,67)} = 28.00$;

$P < 0.001$), and body N concentration ($F_{(1,67)} = 10.83$; $P = 0.002$) terms had 95% confidence intervals of 13.74–37.77, 0.69–0.84, –1.14 to –0.52, and –2.50 to –0.61, respectively. Contrary to our expectations, ANOVA indicated that the leaf N term ($F_{(1,66)} = 1.85$; $P = 0.18$) was not a significant predictor of N flux.

Figure 1 depicts the relationships described in Eq. 5. For each panel in Fig. 1, we standardized N flux rates for two of the independent variables in order to demonstrate the partial relationship between N flux and the third independent variable. In panel a, N flux was standardized for a temperature of 293 K (20 °C) and a larval N concentration of 0.10 using the equation: standardized $Q_N = Q_N (e^{0.83/kT} N_B^{1.56}) (e^{-0.83/k293} 0.10^{-1.56})$. In panel b, N flux was standardized for a larval mass of 20 mg and an N concentration of 0.10 using the equation: standardized $Q_N = Q_N (M_B^{0.77} N_B^{1.56}) (20^{0.77} 0.10^{-1.56})$. In panel c, N flux was standardized for a larval mass of 20 mg and a temperature of 293 K using the equation: standardized $Q_N = Q_N (M_B^{0.77} e^{0.83/kT}) (20^{0.77} e^{-0.83/k293})$. Several patterns are evident in Fig. 1. First, body mass accounted for more variation (partial $R^2 = 0.86$) in individual N flux than did environmental temperature (partial $R^2 = 0.29$) or body N concentration (partial $R^2 = 0.14$). Second, the relationships between N flux and body mass and N flux and body N concentration were reasonably represented by power functions, i.e., straight lines could be fitted to the data on log–log axes. Third, the relationship between N flux and temperature was reasonably represented by an exponential function, i.e., a straight line could be fit to the data on log–linear axes.

Discussion

Our primary objective in this study was to construct a general model of individual N flux for a novel group of herbivores using concepts from metabolic ecology and ecological stoichiometry. We found that a model that included larval mass, environmental temperature, and larval N concentration explained nearly 90% of the variation in the flux of egested and excreted N. We evaluated the model using data from a wide variety of insect (22) and tree (8) species, and over a broad range in body mass (0.18–268.52 mg dry), temperature (15–30 °C), larval N concentration (7.5–13.2%), and leaf N concentration (1.1–3.4%). The model should yield reasonable N flux predictions for moth and sawfly larvae from hardwood forests of the upper Great Lakes region. However, the model should be considered a quantitative hypothesis to be tested before it is used in systems where species composition, body mass range, temperature, or leaf N concentration differs markedly.

Larval body mass

Our analysis showed that the relationship between larval N flux and larval mass was well fit by a power function with a mass-scaling exponent of 0.77 (Fig. 1a). Thus, for a given environmental temperature and larval N concentration, a 100-fold increase in body mass corresponded with a 35-fold increase in N flux. Figure 1a illustrates how this pattern appeared to hold across multiple field-collected species and within lab-reared whitemarked tussock moths. We are aware of only one previous report on the mass dependence of total N flux across terrestrial animals of varying species and sizes. Brody (1945) showed that the flux of excreted and egested N by domesticated mammals and birds scaled with body mass raised to the 0.74 power. Studies conducted on aquatic organisms have shown that N excretion, on its own, scales with body mass raised to the powers of 0.85 (Brett and Groves 1979), 0.78 (Schaus et al. 1997), and approximately 0.79 (from Fig. 2 in Vanni et al. 2002) for fish, and 0.79 for zooplankton (Wen and Peters 1994).

In developing the individual N flux model, we reasoned that N flux would be proportional to egestion rate, which, in turn, would be proportional to metabolic rate. Accordingly, the mass-scaling exponent of 0.77 was similar to scaling exponents for egestion rates from other animal studies, which have ranged from 0.59 for crabs (Cammen et al. 1980), to 0.63 for mammals (Blueweiss et al. 1978), 0.68 for insects (Peters et al. 1996), 0.79 for birds and mammals (Peters et al. 1996), 0.91 and 0.81 for benthic invertebrates (McDiffett 1970; Hargrave 1972), 0.93 for lepidopteran larvae (Smith 1972), and 1.18 for reptiles and amphibians (Peters et al. 1996). The mass-scaling exponent of 0.77 was also centered within the range of 0.65–0.85 generally observed for metabolic allometries (Peters 1983; Glazier 2005), was identical to the mass-scaling exponent of 0.77 observed for larval lepidopteran metabolic rate (Smith 1972), and was very close to and not significantly different from the value of 0.75 predicted by metabolic scaling theory (West et al. 1997; Banavar et al. 2002; West and Brown 2005).

Environmental temperature

We found that short-term variation in environmental temperature was significantly related to variation in individual N flux. The relationship between temperature and N flux was reasonably represented by the Boltzmann–Arrhenius equation with a critical thermal increment of 0.83 eV (Fig. 1b). Given this functional form and temperature coefficient, an increase in environmental temperature from 20 to 25 °C would result in a 75% increase in individual N flux. Relatively little has been published on the effects of

Fig. 1a–c Relationship between standardized larval nitrogen flux (excreted and egested nitrogen, mg N day⁻¹) and **a** larval body mass (mg dry), **b** environmental temperature ($1/kT$, where temperature, T , is in K and k is 8.62×10^{-5} eV/K), and **c** larval N concentration (proportion of dry mass) for field-collected (open triangles) and lab-reared (filled circles) larvae. All axes are on a logarithmic scale except for temperature. Standard larval masses, temperatures, and larval N concentrations are given per panel. See text for further details

temperature on herbivore nutrient fluxes, and all of the information available relates to the temperature dependence of N excretion by aquatic organisms. Wen and Peters (1994) assessed N excretion by zooplankton using data compiled from the literature. They found that Q_{10} values (the factorial increase in a rate with a temperature increase of 10 °C) for N excretion averaged 2.0, and reported that

other reviews (Ejsmont-Karabin 1984) have given Q_{10} values as high as 2.8. The critical thermal increment from our study can be converted to a Q_{10} value using the equation $Q_{10} = e^{c/0.1kT_0^2}$, where T_0 is the median of the range over which Q_{10} was measured (Gillooly et al. 2001; Vasseur and McCann 2005). Using this equation, the observed thermal increment of 0.83 gives a Q_{10} of 3.07, which is slightly higher than the value observed in other studies. However, given that the 95% confidence interval for the thermal increment extended to 0.52 ($Q_{10} = 2.02$), the temperature dependence observed here was not significantly different from that seen in studies of other organisms.

Regarding the proportionality of N flux, egestion rate, and metabolic rate, the temperature sensitivity observed here was similar to that noted for sawfly frass production, where Q_{10} values range from 2.43 to 2.93 and average 2.64 (Green and deFreitas 1955; Simandl 1993). The temperature sensitivity of N flux was also comparable to that of metabolic rate, where empirical Q_{10} values typically range from 2 to 3 (Withers 1992; Hill et al. 2004) and critical thermal increments typically range from 0.25 to 0.80 eV (Vasseur and McCann 2005; Meehan 2006). Concerning ecological theory, the critical thermal increment of 0.83 was not significantly different from the value of 0.65 predicted by metabolic scaling theory (Gillooly et al. 2001, 2006).

There are two additional aspects of the temperature component of this study that are worth noting. First, the temperature coefficient in Eq. 5 was estimated mainly from the data on whitemarked tussock moth larvae. We recognize the shortcomings of this approach, and are conducting additional studies on other species to assess the generality of the temperature effect. Second, larvae were not acclimated to experimental temperatures before onset of the feeding trials. This method was consistent with our intention to assess larval responses to temperature changes that occur at diurnal time scales. Temperature variations over larger time scales (e.g., months to years) could lead to temperature acclimation that might alter the apparent relationship between temperature and N flux.

Larval N concentration

We found that larval N flux scaled with larval N concentration as $N_B^{-1.56}$ (Fig. 1c). In quantitative terms, this indicated that a larva that was 8% N fluxed N at twice the rate of a larva that was 12% N. The dependence of nutrient release on body composition was expected from ecological stoichiometry, and has been previously observed for aquatic herbivores (Elser and Urabe 1999; Vanni et al. 2002; Evans-White and Lamberti 2006). To our knowledge, however, it has not been documented for terrestrial herbivores.

Fig. 2a–b **a** Distribution of values for the normalization constant, a , from Eq. 6. The mean of this distribution was $e^{26.16}$. **b** Relationship between observed larval nitrogen flux (mg N day^{-1}) and that predicted by Eq. 6 with $a = e^{26.16}$ for field-collected (open triangles) and lab-reared (filled circles) larvae. The dashed line is the line of equality and axes are on a logarithmic scale

Leaf N and compensatory feeding

Ecological stoichiometry predicted a positive relationship between larval N flux and leaf N concentration (Sterner et al. 1992; Elser and Urabe 1999). A similar pattern has been found for N excretion by aquatic herbivores (Sterner and Elser 2002) and for total N flux by locusts (Raubenheimer and Simpson 2004). Given the theoretical prediction and previous findings, we were surprised that leaf N was not included in the final model of individual N flux. This result was likely due to a combination of factors related to the relationships between leaf N concentration, frass N concentration, and frass production rate.

For example, when we looked at the relationship between leaf N and frass N concentration, we found that, as expected, the two were positively related ($N_F \propto N_L^{1.10}$). However, increases in leaf N concentration were also accompanied by decreases in frass production ($M_F \propto M_B^{0.76} e^{-0.69/kT} N_L^{-0.79}$). The inverse relationship between leaf N concentration and frass production was (1) observed

across multiple field-collected species and within laboratory-reared whitemarked tussock moths, and (2) indirect evidence for compensatory feeding, which has been demonstrated for terrestrial (Slansky and Feeny 1977; Raubenheimer 1992; Kingsolver and Woods 1998; Lavoie and Oberhauser 2004) and aquatic (Cruz-Rivera and Hay 2000; Fink and Von Elert 2006) herbivores. Given these relationships, an increase in leaf N concentration from 1.3 to 2.5% resulted in an approximate doubling of frass N concentration and an approximate halving of frass production. This finding suggests that compensatory feeding should have a role in the development of future stoichiometric theory.

Other theoretical considerations

In developing the N flux model, we borrowed functional forms from metabolic ecology for the mass and temperature terms and used flexible power function forms to represent qualitative relationships suggested by ecological stoichiometry. The coefficients associated with the functional forms were left as free parameters that were estimated using standard regression techniques. As noted previously, we found that estimated coefficients for the mass, temperature, and stoichiometric terms were similar to those quantitatively or qualitatively predicted by theory. A different way to assess the value of these theories would be to test the explanatory power of a model where coefficients were forced to hold theoretical values. We attempted this using the model:

$$Q_N = a M_B^{3/4} e^{-0.65/kT} N_B^{-1} N_L. \quad (6)$$

Here, the mass-scaling exponent was fixed at a theoretical value of 0.75 (West et al. 1997; Gillooly et al. 2001; West and Brown 2005), the temperature coefficient was fixed at 0.65 eV (Gillooly et al. 2001, 2006), and stoichiometric terms were linear functions, which might be expected for organisms that are not nutrient-stressed, have constant assimilation efficiencies, and are strictly homeostatic. The only free parameter in this model was the normalization constant, a . We calculated values for a using our data by rearranging the equation such that $a = Q_N / (M_B^{3/4} e^{-0.65/kT} N_B^{-1} N_L)$. When this was done, we obtained the distribution shown in Fig. 2a. The mean of this distribution was $e^{26.16}$; the exponent, 26.16, was close to and not significantly different from the value of 25.75 from Eq. 5. When $e^{26.16}$ was placed into Eq. 6 and N flux was predicted for the larvae in our study, we found that the regression of observed $\ln(Q_N)$ against predicted $\ln(Q_N)$ had a slope not significantly different from 1, an intercept not different from 0, and an R^2 of 0.87 (Fig. 2b). Thus, with the addition of an empirical normalization constant,

a theoretical model predicted larval N flux nearly as well as our empirical model. Future work could involve use of the framework proposed by Gillooly et al. (2005) to derive normalization constants for N flux models from first principles.

Future work

The next step in this research will be to explore the scaling of larval N flux models from the individual level to population and community levels. This exercise will have its own set of challenges, such as developing valid, spatially and temporally-integrated estimates of herbivore body mass, temperature, stoichiometry, and abundance. The reward for this effort will be a new set of tools for exploring the contribution of insect larvae to N cycling in forested ecosystems. These tools may also be useful for forecasting the role of insects in N cycling under different scenarios of environmental change. For example, human introduction of invasive species (Lovett et al. 2006) and alteration of forest structure (Cunningham and Murray 2007) can affect the abundances, body mass distributions, and elemental profiles of canopy herbivore communities. Additionally, increases in atmospheric carbon dioxide concentrations are expected to alter environmental temperatures (IPCC 2001) and the elemental ratios of foliage (Throop and Lerdau 2004). Nutrient flux models that incorporate these key variables may provide a means to predict the impact of anthropogenic changes on the role of herbivorous insects in future ecosystem function.

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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 (CO₂) and tropospheric ozone (O₃) 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 CO₂ and O₃ (eCO₂ and eO₃) 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 CO₂ 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 CO₂ reduced abundance of phloem-feeding herbivores and increased abundance of chewing herbivores, although results were not statistically significant. Enriched CO₂ increased numbers of some parasitoids. The effects of eO₃ on insect abundance were generally opposite those of eCO₂. No significant differences in arthropod family richness were found among treatments. However, eCO₂, eO₃, 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).

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 (Alternatt, 2003). Thus, changes in insect abundance, diversity or community composition have the potential to alter forest ecosystems and the services they provide.

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Two gases in particular, carbon dioxide (CO₂) and tropospheric ozone (O₃), 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, CO₂ levels have increased from 280 to 380 ppm (IPCC, 2007). Over this same time period, ambient tropospheric O₃ 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 CO₂ and eO₃ have been shown to alter tree growth and chemical characteristics (reviewed for Aspen Free Air CO₂ Enrichment (FACE) in Lindroth *et al.*, 2001; Karnosky *et al.*, 2003). Numerous studies have demonstrated changes in insect performance at eCO₂ and eO₃ (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 CO₂ and O₃ on natural insect communities, but no study to date has examined the effects of CO₂ and O₃ 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 eCO₂ or eO₃ 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 CO₂ and eO₃ 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 eCO₂ or eO₃ 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 eCO₂ and eO₃, 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 CO₂, ambient O₃), elevated CO₂ (+CO₂; 560 µM M⁻¹ CO₂), elevated O₃ (+O₃; 1.5 × ambient) and elevated CO₂ and O₃ (+CO₂+O₃). Elevated CO₂ and O₃ levels represent concentrations predicted for 2060. Ambient O₃ 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.

Feeding guild/family	Control	+CO ₂	+O ₃	+CO ₂ +O ₃	CO ₂		O ₃		CO ₂ ×O ₃	
					<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Herbivores – phloem feeders										
Aphididae	51 $\pm$ 15	58 $\pm$ 15	83 $\pm$ 15	56 $\pm$ 15	1.1	0.334	0.6	0.449	6.6	0.033
Cicadellidae	57 $\pm$ 10	32 $\pm$ 10	78 $\pm$ 10	56 $\pm$ 10	3.8	0.087	3.8	0.087	0.6	0.463
Miridae	10 $\pm$ 3	9 $\pm$ 3	24 $\pm$ 3	13 $\pm$ 3	4.1	0.077	7.8	0.024	2.1	0.185
Herbivores – chewers										
Curculionidae	14 $\pm$ 2	16 $\pm$ 2	9 $\pm$ 2	13 $\pm$ 2	3.8	0.087	4.7	0.062	0.4	0.553
Anthomyiidae	101 $\pm$ 23	139 $\pm$ 23	76 $\pm$ 23	141 $\pm$ 23	5.1	0.054	0.3	0.612	0.3	0.576
Parasitoids										
Braconidae	51 $\pm$ 5	49 $\pm$ 5	32 $\pm$ 5	35 $\pm$ 5	0.2	0.649	10.6	0.011	0	0.961
Chalcidoidea	97 $\pm$ 6	93 $\pm$ 6	76 $\pm$ 6	65 $\pm$ 6	1.6	0.248	15	0.005	0.1	0.765
Figitidae	23 $\pm$ 6	52 $\pm$ 6	12 $\pm$ 6	19 $\pm$ 6	9.3	0.016	13.5	0.006	3.4	0.102
Ichneumonidae	99 $\pm$ 15	122 $\pm$ 15	55 $\pm$ 15	75 $\pm$ 15	6	0.040	14	0.006	0	0.921
Predators										
Dolichopodidae	41 $\pm$ 5	33 $\pm$ 5	35 $\pm$ 5	28 $\pm$ 5	2	0.191	1.1	0.320	0	0.945
Saprophages										
Drosophilidae	16 $\pm$ 4	20 $\pm$ 4	9 $\pm$ 4	12 $\pm$ 4	0.9	0.376	4	0.079	0	0.874
Phoridae	191 $\pm$ 27	143 $\pm$ 27	90 $\pm$ 27	141 $\pm$ 27	0	0.955	3.6	0.100	3.3	0.108
Sciaridae	160 $\pm$ 30	162 $\pm$ 30	141 $\pm$ 30	94 $\pm$ 30	0.6	0.455	2.4	0.159	1.1	0.332
Herbivores/Predators										
Formicidae	31 $\pm$ 6	12 $\pm$ 6	30 $\pm$ 6	21 $\pm$ 6	7.3	0.027	1.3	0.292	0.9	0.362
Predators/Saprophages										
Sarcophagidae	43 $\pm$ 8	42 $\pm$ 8	48 $\pm$ 8	30 $\pm$ 8	5.1	0.054	0.2	0.708	2.1	0.183
Total	1094 $\pm$ 90	1069 $\pm$ 90	903 $\pm$ 90	887 $\pm$ 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, CO₂ (= eCO₂) and O₃ (= eO₃) 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 (CO₂ × O₃). CO₂ main effects (eCO₂) represent differences between plots with enriched CO₂ (+CO₂ and +CO₂+O₃) and plots with ambient CO₂ (control and +O₃). Similarly, O₃ main effects (eO₃) represent differences between plots with enriched O₃ (+O₃ and +CO₂+O₃) and plots with ambient O₃ (control and +CO₂). *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 than 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 (Dufréne & Legendre, 1997) in PC-ORD5 to examine whether particular families were significant indicators of control, +CO₂, +O₃, or +CO₂+O₃ treatments. This analysis is specific to treatments because we could not test both CO₂ and O₃ 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 CO₂ had no effect on total abundance. Elevated O₃, alternatively, reduced total abundance by 17% compared with ambient O₃ 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 eCO₂ and eO₃ had significant effects on numbers of some insect families (Table 1). Elevated CO₂ substantially decreased the abundance of Formicidae (46%) compared with ambient CO₂. In contrast, eCO₂ 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 eCO₂ was for the abundance of sucking

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 $\pm$ 2	34 $\pm$ 1	35 $\pm$ 1	37 $\pm$ 1
2001	35 $\pm$ 2	34 $\pm$ 3	35 $\pm$ 1	34 $\pm$ 2
2002	34 $\pm$ 1	35 $\pm$ 4	39 $\pm$ 2	34 $\pm$ 1
2003	40 $\pm$ 1	37 $\pm$ 4	41 $\pm$ 2	38 $\pm$ 2

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 eCO₂ were generally consistent across years.

The effect of eO₃ on insect abundance was generally opposite that of eCO₂. Elevated O₃ 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 O₃ $\times$ 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 O₃ also decreased Curculionidae (27%) and Drosophilidae (42%) but these changes were not statistically significant. In contrast to the largely negative effects of eO₃ on other families, Miridae abundance increased 95%. Cicadellidae abundance increased 51% but again the change was not significant. Generally, eO₃ 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 eO₃ across years.

The effects of CO₂ and O₃ on insect abundance were independent of one another. The single exception was for the family Aphididae, for which eCO₂ increased abundance under aO₃, but decreased abundance under eO₃ (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 CO₂ and O₃ treatments and years.

Arthropod community composition

Arthropod community composition shifted in response to CO₂ and O₃ treatments in all 4 years of this study. Elevated CO₂ affected composition in 2001, 2002 and 2003, whereas eO₃ 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 ($F_{3,8} = 2.70$, $P = 0.119$). CO₂ and O₃ affected the insect community independently (no significant CO₂ $\times$ O₃ interactions). Thus, although family richness was not altered by eCO₂ or eO₃, 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			
Dolichopodidae	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 eCO_2 . Increased abundance at eO_3 , similarly, led to three

families that indicated + O_3 treatments: Cicadellidae, Miridae and Formicidae. Aphididae also tended to increase when exposed to eO_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 eCO_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 eCO_2 may favour some parasitoid families but limit populations of sucking insects. In contrast, forests exposed to eO_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 eCO_2 and eO_3 appear to respond to each gas independently. Therefore, offsetting effects of eCO_2 and eO_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 eCO_2 but was reduced under eO_3 . Similarly, total insect abundance was unaffected

by eCO₂ 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 eO₃ (Hamilton *et al.*, 2005). Our eO₃ 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 eCO₂, eO₃ or both, but low replication precluded statistical significance. Changes in abundance due to eCO₂ and eO₃ 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 eCO₂ and decreased in abundance under eO₃, while phloem-feeders showed the converse. Bezemer & Jones (1998) and Stiling & Cornelissen (2007) suggested the opposite pattern for eCO₂. 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 eCO₂ for two of the four families we examined and decreased under eO₃ for all four families. Previous work suggested that eCO₂ and eO₃ 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 eCO₂ 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 eCO₂ could benefit pest control, the particularly strong negative impacts of O₃ 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 eO₃. A 1995 study by Gate *et al.* found that eO₃ interferes with wasp searching behaviour. Furthermore, recent work suggests that eCO₂ and eO₃ 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 eCO₂ and eO₃ (Mondor *et al.*, 2004).

Saprophagous insects showed the fewest significant responses to the treatments, despite previous work at Aspen FACE suggesting strong influences of eCO₂ and eO₃ 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 CO₂ on detritivore abundance.

Insect species richness

Neither the CO₂ nor O₃ treatments affected species richness. Similarly, insect species richness was unaffected by both CO₂ in a sweetgum understorey at the Oak Ridge National Laboratory FACE site (Sanders *et al.*, 2004) and a natural O₃ pollution gradient in California forests (Jones & Paine, 2006). In contrast, eCO₂ did decrease insect species richness on beech and oak trees, although sample sizes were small (Altermatt, 2003). Together, these studies suggest that eCO₂ and eO₃ will have negligible impacts on species richness via direct or plant-mediated effects, at least over the short term. The effects of CO₂ and O₃ may, however, magnify over longer timescales. The strong negative effect of O₃ on parasitoids could increase extinction risk and reduce diversity over time. CO₂ and O₃ 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 CO₂ and O₃ 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 eCO₂ and eO₃ (Sanders *et al.*, 2004; Jones & Paine, 2006).

Arthropod community composition

Few studies have examined the effects of eCO₂ or eO₃ on insect community composition. We found that eCO₂ altered forest insect communities in 3 of 4 years, while eO₃ modified communities in 2 of 4 years. Previous work at another forest FACE site showed that eCO₂ had no effect on understorey arthropod community composition (Sanders *et al.*, 2004). Elevated CO₂ 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 O₃ concentrations may shift from phloem-feeding to chewing-dominated herbivore communities. Our results suggest the opposite effect, with phloem-feeders benefiting from eO₃ environments.

Mechanisms and implications

Changes in insect abundance and community composition at eCO₂ and eO₃ 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 CO₂ and O₃ (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 eCO₂- and eO₃-mediated alterations in forest environment. For example, forest stand development in eCO₂ rings reached canopy closure over the 4 years of this study, which may be responsible for the strengthening eCO₂ 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 CO₂ and eO₃ 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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Scaling of individual phosphorus flux by caterpillars of the whitemarked tussock moth, *Orygia leucostigma*

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Abstract

We conducted a laboratory study to evaluate the effects of body mass, environmental temperature, and food quality on phosphorus (P) efflux by caterpillars of the whitemarked tussock moth, *Orygia leucostigma*, J. E. Smith (Lepidoptera: Lymantriidae). We found that individual phosphorus efflux rate (Q , the rate at which excreted and unassimilated P was egested in frass, mg P/day) was related to larval mass (M , mg dry) and environmental temperature (T , K) as $Q = e^{14.69} M^{1.00} e^{-0.54/kT}$, where k is Boltzmann's constant (8.62×10^{-5} eV/K, $1 \text{ eV} = 1.60 \times 10^{-19}$ J). We also found that P efflux was not related to food phosphorous concentration, and suggest that this result was due to compensatory feeding by larvae eating low quality leaves. The P efflux model resulting from this analysis was simple and powerful. Thus, it appears that this type of model can be used to scale P flux from individual larvae to the population level and link species of insect herbivores to ecosystem processes.

Keywords: allometry, body mass, compensatory feeding, consumer-driven nutrient recycling, ecological stoichiometry, environmental temperature, excretion, Lepidoptera, phosphorus, tussock moth

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Introduction

Herbivorous insects play important roles in nutrient cycling in forest ecosystems. They consume canopy foliage, transform materials through digestive processes, and translocate portions of the canopy to the soil as frass (Mattson and Addy 1975; Hunter 2001; Schowalter 2006). The compounds in herbivore frass are then taken up by plants or microbes, stimulating microbial activity in the soil and affecting microbe-mediated ecosystem processes such as carbon and nutrient mineralization (Lovett and Ruesink 1995; Reynolds and Hunter 2001; Christenson et al. 2002; Frost and Hunter 2004).

Previous work on herbivore-mediated nutrient fluxes from the canopy to the soil has focused mainly on the movement of nitrogen (Swank et al. 1981; Lovett et al. 2002; Townsend et al. 2004). Relatively few studies have considered phosphorus flux by insect herbivores (Fogal and Slansky 1985; Hollinger 1986), despite the fact that phosphorus has been shown to limit primary production in both aquatic and terrestrial ecosystems (Elser et al. 2000; Elser et al. 2007).

A common approach to studying elemental fluxes via herbivore frass is to collect frass in baskets placed on the forest floor, weigh it, determine its elemental concentration, and calculate the total flux from the canopy to the soil (e.g., Hunter et al. 2003). This approach provides a flux estimate for the community as a whole, but is not useful for estimating the contribution of particular species. Alternatively, it may be possible to compute species-level estimates of elemental flux by combining predictions of individual fluxes with information on the characteristics of populations. This approach would facilitate linking the activities of a particular species to fluxes of elements in forested ecosystems.

In this study we use principles from animal physiology to develop a simple model of phosphorus efflux via frass that can be used to predict deposition by individuals over a range of body sizes, body temperatures, and food qualities. The model is evaluated using data on phosphorus efflux by caterpillars of the whitemarked tussock moth, *Orygia leucostigma*, J. E. Smith (Lepidoptera: Lymantriidae).

Materials and Methods

Model

Individual phosphorus efflux rate (Q , mg P/day in frass) was modeled as a function of larval body mass (M , mg dry mass), environmental temperature (T , K), and leaf phosphorus concentration (P_l , % dry mass). The rates of several physiological processes, including elemental fluxes, frequently are related to body mass as a power function with a scaling exponent, b , between 0.65 and

0.85 (Peters 1983; Calder 1984; Schmidt-Nielsen 1984; Brown 1995; Brown et al. 2004). Thus, phosphorus efflux was modeled as $Q \propto M^b$ and b was expected to fall near 0.75.

Physiological rates also are known to increase exponentially with body temperature (Crozier 1924; Cossins and Bowler 1987; Gillooly et al. 2001; Hochachka and Somero 2002). Therefore, an exponential temperature term was added to the phosphorus efflux model so that $Q \propto M^b e^{-E/kT}$ (Gillooly et al. 2001). This temperature term is referred to as the Arrhenius or Boltzmann factor (Arrhenius 1915; Gillooly et al. 2001). Although its roots are in physical chemistry (for describing the temperature dependence of simple chemical reactions), it has also been shown to be useful for describing the temperature dependence of many whole-organism physiological rates (Crozier 1924; Gillooly et al. 2001; Gillooly et al. 2002; Gillooly et al. 2005b). Here, k represents Boltzmann's constant (8.62×10^{-5} eV/K, $1 \text{ eV} = 1.60 \times 10^{-19}$ J) and E reflects the average activation energy of the biochemical reactions involved in aerobic respiration (approximately 0.6 to 0.7 eV) (Gillooly et al. 2006).

Individual phosphorus efflux was expected to be a function of the phosphorus concentration of leaves for two reasons. First, this relationship would be expected if a fraction of the ingested phosphorus was never absorbed as food passed through the gut. Second, a surplus of phosphorus in food should lead to a decrease in phosphorus absorption or an increase in phosphorus excretion (Sternner et al. 1992; Elser and Urabe 1999; Sternner and Elser 2002; Woods et al. 2002). Together, these factors should result in a positive relationship between individual phosphorus flux and leaf phosphorus concentration that can be represented as $Q \propto P_l^c$. A power function relationship with scaling exponent, c , is used because it is a simple and flexible function that can accommodate the potentially nonlinear relationship between individual flux and leaf phosphorus. Combining this relationship with those above gives a simple equation for predicting phosphorus efflux by an individual insect herbivore, $Q = a M^b e^{-E/kT} P_l^c$, where a is a normalization constant with units $\text{mg P day}^{-1} \text{ mg larva}^{-b}$.

Evaluation

To evaluate the individual phosphorus efflux model, a dataset was assembled that included information on larval mass, leaf phosphorus concentration, frass phosphorus concentration (P_f , % dry mass), dry-matter egestion rate (E , mg dry/day), and individual phosphorus efflux rate (the product of frass phosphorus concentration and egestion rate) for larval *O. leucostigma*. *Orygia leucostigma* was used in this study because it is a widely distributed native herbivore, and because it has similar feeding behavior, gut chemistry, and frass chemistry (Frost and

Hunter 2007) as other economically important species such as the gypsy moth, *Lymantria dispar*.

Orygia leucostigma larvae were raised from eggs purchased from the Canadian Forest Service (Sault St. Marie, Ontario, Canada). Egg masses were divided into two groups and put into two rearing dishes in a growth chamber set to 22° C and a 14:10 hr L:D cycle. Eggs hatched after two weeks of incubation, whereupon half of the larvae were fed high-nutrient leaves, while the other half were fed low-nutrient leaves. Leaves came from 10 potted aspen trees, *Populus tremuloides* Michx. (Malpighiales: Salicaceae), propagated from a single aspen clone (Waushara County, Wisconsin, USA). Trees were grown in 40 L pots, in a soil medium consisting of 70% sand and 30% silt-loam soil. Five of the 10 trees (high-nutrient trees) were given 4.5 g/L soil of slow-release fertilizer (18:6:12, N:P:K, without micronutrients) in May 2004 and 2007. Low nutrient trees were not given fertilizer. Trees were in their fourth growing season during summer 2006, when this study was conducted.

Phosphorus efflux data were collected during 24-hr feeding trials, which were conducted at various body masses over the course of larval development. In total, 25 trials were conducted using 129 larvae. The number of animals was greater than the number of trials because, in 15 cases, 2 to 11 similarly-sized individuals were placed into one vial for a feeding trial in order to collect enough frass for subsequent chemical analyses. For each trial, *O. leucostigma* larvae were weighed and a pre-trial wet mass was calculated as the total mass of larvae divided by the number of larvae. Then larvae were placed into a 20-ml polyethylene vial with a moist piece of tissue paper and 200 to 300 mg of high- or low-nutrient aspen leaves. Vials were then placed into environmental chambers set at 15 or 30° C and a 14:10 hr L:D cycle. After 24 hours, the vials were removed from chambers and placed directly into a freezer.

Samples were then freeze-dried and larvae and frass were weighed with a microbalance. Post-trial dry mass was calculated for each trial as the total dry mass of all larvae divided by the number of larvae in the vial. A pre-trial dry mass was estimated using pre-trial wet mass and the function: larval dry mass = $0.17 \times \text{wet mass}^{0.98}$ ($R^2 = 0.99$, $n = 25$). A midpoint dry mass was then calculated as the average of pre- and post-trial dry masses. A final larval mass for each trial was calculated by correcting midpoint average dry mass for gut contents using the equation: gut-content-free dry mass = $0.78 \times \text{midpoint dry mass}^{1.01}$ ($R^2 > 0.99$, $n = 18$). The gut content correction function was produced for the larvae in this study following the fasting method of Bowers et al. (1991). Dry-matter egestion rate for each trial was calculated as the total dry mass of frass divided by the number of animals in a vial.

Dried leaf and frass samples were homogenized using a mortar and pestle, and phosphorus concentrations were quantified using the following modified version of the Murphy-Riley method (Murphy and Riley 1962; Olsen and Sommers 1982; Avila-Segura et al. 2004). First, samples of 3 mg of leaves, or 4 mg of frass, were combusted in 100 mm × 13 mm diameter glass culturing vials in a muffle furnace for 3 hours at 500° C. After cooling, 4 ml (for leaves) or 2 ml (for frass) of 1 M H₂SO₄ were added to ashes in vials to dissolve phosphorus in samples. After approximately 3 hr at room temperature, vials were centrifuged at 3500 rpm for 8 min. Next, a 100-μl sample of supernatant was combined with 100 μl of 2 M NaOH and 50 μl of freshly made Murphy-Riley reagent (Reagent 2, Olsen and Sommers, 1982) in the well of a 96-well microplate. On each plate, we included 2 replicates from each sample vial and 2 replicates from each of 5 levels of P standards (0, 0.5, 1, 2.5, 5 mg P/L, made from K₂PO₄ dissolved in 1 M H₂SO₄). Plates were then allowed to develop for 30 min at room temperature and were read at 850 nm using a 96-well-plate-compatible spectrophotometer. Repeated analysis of an internal leaf standard yielded an analytical coefficient of variation of 2.08 %.

As noted above, individual phosphorus efflux was expected to relate to larval mass, temperature, and leaf P concentration as $Q = a M^b e^{-E/kT} P_i^c$, where b was expected to be near 0.75, E was expected to fall near 0.65 eV, and c was expected to be a positive number. We linearized this equation by taking natural logarithms of both sides and evaluated expectations by fitting the equation to natural log-transformed data using multiple linear regression. We used partial F-tests to evaluate the contribution of each term to the model, and calculated 95% confidence intervals to assess the uncertainty around estimated model coefficients.

Results and Discussion

The phosphorus concentrations of the aspen leaves included in this study ranged from 0.11 to 0.20%, and averaged 0.15%. Phosphorus concentrations of tree foliage generally fall between 0.05 and 0.25% (Reich and Oleksyn 2004; Kerkhoff et al. 2005), so the leaves included in this study had phosphorus concentrations that were representative of the natural range of variation. The phosphorus concentrations of larval frass included in this study ranged from 0.03 to 0.13 % and averaged 0.07%. Rates of individual phosphorus efflux observed during the study ranged from 0.00032 to 0.076 mg P/day, while body masses and environmental temperatures ranged from 0.34–41.48 mg dry and 15–30° C, respectively.

Individual phosphorus efflux rate was found to be related to larval mass and environmental temperature as $Q = e^{14.69} M^{1.00} e^{-0.54/kT}$ ($R^2 = 0.94$, Figure 1). The 95%

confidence intervals for the mass ($F_{1,22} = 324.40$, $P < 0.001$) and temperature ($F_{1,22} = 34.87$, $P < 0.001$) coefficients were 0.88 to 1.11 and -0.73 to -0.35 , respectively. Phosphorus flux was related to leaf phosphorus concentration as $Q \propto P_l^{-0.17}$, but the 95% confidence interval for the scaling exponent (-1.30 to 0.95) and partial F-test ($F_{1,21} = 0.75$, $P = 0.75$) indicated that leaf phosphorus was not a significant predictor of larval phosphorus flux (Figure 1).

The temperature coefficient of 0.54 eV for individual phosphorus efflux was not significantly different from the range of 0.6 to 0.7 eV that is expected for metabolic rate (Gillooly et al. 2001; Gillooly et al. 2006). This coefficient can be translated into a Q_{10} value (factorial rate increase with a 10°C increase in temperature) using the equation $Q_{10} = e^{E/0.1(kT_o^2)}$, where T_o is the median of the temperature range (K) over which temperature dependence was measured (Gillooly et al. 2001; Vasseur and McCann 2005). When this was done, a Q_{10} value of 2.05 was obtained for individual phosphorus flux, which falls within the range of 2–3 that is typically reported for metabolic rate (Withers 1992; Hill et al. 2004).

Relatively little has been published on the temperature dependence of individual elemental flux rates, *per se*, and most of the available information is on the temperature dependence of nutrient excretion by aquatic organisms. Wen and Peters (1994) conducted an analysis of the determinants of phosphorus excretion by zooplankton using data compiled from the literature. They found an average Q_{10} value of 1.6 for phosphorus excretion rates, and reported that other reviews (Ejlsmont-Karabin 1984) have given Q_{10} values as high as 2.6. Whether these results are comparable to ours is not clear, however, because studies of aquatic herbivores generally have not quantified phosphorus fluxed via feces.

The mass-scaling exponent of 1.00 for individual phosphorus efflux rate was steeper than the range of 0.65 to 0.85 that is commonly reported for the metabolic rate of animals, in general (Hemmingsen 1960; Peters 1983; Gillooly et al. 2001; Savage et al. 2004; Glazier 2005; White et al. 2007), and for larval lepidopterans, in particular (Smith 1972). When possible causes for the steep mass-scaling coefficient were explored, it was found that the phosphorus concentration of frass was related to body mass as $P_f \propto M^{0.13}$, i.e., frass phosphorus concentration increased with larval mass. In addition, total body phosphorus concentration of larvae, P_b , was related to body mass as $P_b \propto M^{-0.04}$, i.e., large larvae had relatively low body phosphorus concentrations (Woods et al. 2004; Bertram et al. 2008). Together, these results lead us to hypothesize that the mass scaling of phosphorus flux was steeper than expected because (1) larger larvae have lower concentrations of, and requirements for, phosphorus (Sturner and Elser 2002), (2) lower phosphorus

requirements allow for lower phosphorus absorption or higher excretion, leading to (3) relatively high phosphorus concentrations of frass and overall phosphorus flux rates for relatively large larvae (Gillooly et al. 2005a). Further studies are necessary to evaluate this possibility.

We are not aware of previous studies on the mass-dependence of phosphorus flux by terrestrial herbivores, although several studies have addressed the allometry of phosphorus excretion by aquatic herbivores. Those studies have reported mass-scaling exponents between 0.62 and 0.69 for zooplankton (Johannes 1964; Peters and Rigler 1973; Wen and Peters 1994) and between 0.78 and 0.89 for fish (Schaus et al. 1997; Vanni et al. 2002). Again, the degree to which these results are comparable to ours is not known because studies of aquatic herbivores generally have not quantified phosphorus fluxed via feces.

We were somewhat surprised to see that individual phosphorus efflux rate was unrelated to leaf phosphorus concentration. This likely occurred because a decrease in leaf phosphorus was accompanied by both a decrease in frass phosphorus concentration ($P_f \propto P_l^{0.74}$) and an increase in larval ingestion and egestion rates ($E \propto P_l^{-0.95}$), with the net result being an approximate independence of individual phosphorus flux and leaf phosphorus concentration ($Q \propto E P_f \propto P_l^{-0.95} P_l^{0.74} \approx P_l^0$).

The increase in ingestion and egestion rates by larvae given low-nutrient food is an example of compensatory feeding (Figure 2). Compensatory feeding in response to low food nitrogen concentrations has been observed in both terrestrial (Slansky and Feeny 1977; Raubenheimer 1992; Lindroth et al. 1997; Kingsolver and Woods 1998; Lavoie and Oberhauser 2004) and aquatic (Cruz-Rivera and Hay 2000; Fink and Von Elert 2006) organisms. Compensatory feeding in response to low food phosphorus concentrations has been explored for a variety of organisms, including lepidopterans (Woods et al. 2002; Perkins et al. 2004), though it has been clearly demonstrated only for snails (Fink and Von Elert 2006). In our study, the phosphorus and nitrogen concentrations of leaves were strongly correlated ($r = 0.98$), because high leaf phosphorus concentrations were achieved by adding a combined N, P, and K fertilizer to potted aspen trees. Consequently, it is quite possible that the compensatory feeding that was observed was a behavioral response to shortages in leaf nitrogen. Nonetheless, nitrogen and phosphorus concentrations of foliage tend to be correlated in the wild ($r = 0.84$, Garten 1976). Thus, our results may reflect the relationship between food quality and phosphorus flux in natural settings, and suggest that compensatory feeding should be considered in more detail in future theory on consumer-driven nutrient recycling (Elser and Urabe 1999; Sturner and Elser 2002).

Figure 1. (A) Relationship between the natural log of larval body mass (mg dry) and the natural log of larval phosphorus flux (mg P/day) after accounting for the effect of environmental temperature (T , in K; k is Boltzmann's constant, 8.62×10^{-5} eV/K, $1 \text{ eV} = 1.60 \times 10^{-19}$ J). (B) Relationship between the inverse of temperature multiplied by Boltzmann's constant and the natural log of larval phosphorus flux after accounting for the effect of larval body mass. (C) Relationship between the natural log of leaf phosphorus concentration (% dry mass) and the natural log of larval phosphorus flux after accounting for the effects of larval body mass and temperature.

Figure 2. Relationship between the natural log of leaf phosphorus concentration (% dry mass) and the natural log of leaf consumption (C, mg dry/day) after accounting for the effects of larval body mass (mg dry) and environmental temperature (T, in K; k is Boltzmann's constant, 8.62×10^{-5} eV/K, $1 \text{ eV} = 1.60 \times 10^{-19}$ J). Consumption was calculated as dry mass of leaves before trial minus dry mass after trial divided by the number of individuals in trial. The negative relationship ($F_{1,21} = 19.21$, $P < 0.001$) suggests compensatory feeding by larvae in response to nutrient content of leaves.

The model for individual phosphorus efflux derived and evaluated here was relatively simple and powerful, in that it incorporated two easily measured predictor variables and explained 94% of the variation in individual phosphorus efflux rates. Thus, simple individual flux models such as these may provide a practical means for scaling nutrient deposition rates from individual larvae to population levels. With further work, it may be possible to (1) generalize the model for other species, (2) scale from individual elemental flux rates to whole communities of lepidopteran larvae, and (3) use these estimates to better understand the role of these organisms in the functioning of forest ecosystems.

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Chapter 11

Climate Change and Temporal and Spatial Mismatches in Insect Communities

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1. INTRODUCTION

As ectotherms, insect performance is heavily dependent on climate. Warming from climate change will alter insect development time, voltinism, foraging behaviour, emergence time and survivorship [1]. These changes, which alter population size and distribution, will affect the temporal and spatial dynamics of insect communities. Many insects provide important ecosystem services

(e.g. pollination, decomposition, etc.) or affect human activities (e.g. via pest activity) and the effects of climate change could alter these services or exacerbate these effects. To date, geographic range shifts and early emergence are the best documented responses of insects to climate change, but some species have experienced population increases, changes in cyclical population dynamics and local extinctions or genetic and phenotypic changes that may be examples of rapid evolution. Further, more research is needed to understand how species-level changes affect interacting species. Overall, the sensitivity of insects to climatic factors makes them ideal for tracking and understanding the effects of climate change on biodiversity.

This chapter provides an overview of the effects of climate change on insects and their interactions with host plants and predators. We provide examples from the literature using herbivorous insects and their host plants and predators because herbivores are a large, well-studied group of insects that affect human activities and provide important ecosystem services. While the effects of increasing carbon dioxide levels are also important, particularly the resulting changes in host plant chemistry [2,3], the focus of this review is on the effects of warming and increased variability in precipitation associated with climate change. We also offer a novel analysis of northern range limit comparisons between North American butterflies and their host plant species to delineate the potential for spatial disassociations (i.e. differential range shifts between herbivorous insects and their host plants under climate change). Finally, we discuss potential impacts on future communities of insects and altered community effects on ecosystem services.

Life history traits such as resource specificity, geographic location, trophic level and dispersal ability are potentially good predictors of the magnitude and direction of the response of insect species to climate change. For example, habitat and/or resource specificity may limit the tolerance of specialists to changing conditions, potentially leaving habitats dominated by generalists. Warren et al. [4] found that butterflies with strong habitat specificity and limited mobility have reduced distributions and have fared worse under changing climatic conditions than generalists that share the same geographic range. Another study on beetles identified climate change response groups based on host plant specificity and distribution size, and the authors project that cosmopolitan species may be the most resilient to climate change while specialists will be faced with extinction if they do not move with their host plants [5]. Furthermore, Deutsch et al. [6] concluded that tropical species that have narrow thermal limits are more likely to be negatively affected by climate change. Yet Bale et al. [1] also highlight other vulnerable groups: insects that are cold-adapted, restricted to montane areas and also those in polar areas, where proportionally larger temperature increases are expected. In addition, Voigt et al. [7] report that species in higher trophic levels are sensitive to climatic change due to the combined indirect effects of climate change on lower trophic groups. When possible, we relate our article back to life history traits

to allow for generalisations because insects as a taxonomic group are too diverse to be studied exhaustively. Our only hope for understanding insect responses to climate change is through generalisations from well-studied species to others.

Climate change can decouple interactions between insects and their resources and/or predators. Variable responses of these interacting species to climate change can lead to differential changes in the geographic ranges of species (herein 'spatial mismatches') as well as differential changes in the phenology, or timing, of species ('temporal mismatches'). These mismatches could lead to places where and times when insect populations could greatly decline towards local extinction. In this review, we identify studies that demonstrate such mismatches, focusing, where possible, on those that have yielded negative effects on ecosystem services provided by insects.

2. DIRECT EFFECTS OF CLIMATE CHANGE ON INSECTS

The impact of climate change on insects is multifaceted. We begin by reviewing the direct effects of climate change on the temporal and spatial dynamics of insect populations. Thus far, enhanced individual and population growth in warmer temperatures have resulted in early emergence and changes in the location, through geographic range shifts, of insect species. A few studies also have linked climate change to genetic and phenotypic changes in insect populations, which we discuss here, but this response is not anticipated due to a number of constraints in evolution under human-caused, rapid climate change.

2.1. Temporal Changes

Insect life cycles depend on climatic variables such as degree days, minimum winter temperatures, average maximum summer temperatures, total precipitation and aridity. Generally, higher temperatures result in faster development, increased number of generations and increased overwinter survivorship [1]. As a result of enhanced and accelerated growth, climate change has been linked to phenological advances in many insects [8,9]. Gordo and Sanz [10] found earlier spring emergence in honey bee *Apis mellifera* (L.) and small white *Pieris rapae* (L.) populations in the Iberian Peninsula over the past 50 a. Several other studies of butterflies have documented early emergence [11–14], and it also has been observed in aphids [15,16] and in members of Heteroptera [17]. Decreased generation time due to warming has been observed in the mountain pine beetle (*Dendroctonus ponderosae*), leading to increases in the abundance of this pest species [18]. In some cases, the direct effect of increased temperature on insect development leads to simple population increases (e.g., *D. ponderosae*). In other cases, however, it can change synchrony of herbivores, host plants and predators, producing more complex community effects.

2.2. Spatial Changes

Increased overwinter survivorship, growth rates and generations have led to range shifts in many insect species. Many insects, including some pest species (e.g. the pine processionary moth (*Thaumetopoea pityocampa*)), have shifted their distributions poleward and to higher elevations to track recent climatic changes [19–27]. Still others have been unable to track the changing climate, and some have experienced range contractions or local extinctions [18,28–30]. These results and others have helped to establish a simple paradigm for the responses of species to climate change: populations will contract and go extinct in the equatorial portion of the range and poleward populations will expand and colonise new locations as the climate warms.

The ability of a species to shift its distribution is determined largely by its ability to disperse into newly suitable areas and the availability of suitable resources, that is breeding habitat and host plants, in those sites [4,31]. Thomas et al. [22] examined four insect species that recently expanded their ranges northward and found that two butterfly species increased the range of habitat types that they historically used while newly established populations of two bush cricket species had more longer-winged individuals than their source populations, suggesting that only the best dispersers were able to reach new habitat. Similarly, Warren et al. [4] have argued that climate change will leave habitats dominated by mobile generalists. Later, we discuss how limitations in resource use may lead to spatial mismatches between herbivorous insects and their host plants.

2.3. Genetic and Phenotypic Changes

Climate change has altered the selection pressures on insect populations, and some insects have responded via genetic and phenotypic change. Several groups have found that *Drosophila* populations are tracking climate change through genetic changes, for example, with genotypes characteristic of equatorial latitudes increasing in frequency with warming over the past few decades [32–34]. Rodríguez-Trelles and Rodríguez [35] found a decrease in diversity of a chromosome polymorphism in *Drosophila*, and they argue that this alteration correlates with climatic change. Rank and Dahlhoff [36] found allele frequency shifts in an enzyme related to heat stress in the leaf beetle (*Chrysomela aeneicollis*) that they linked to climatic changes occurring in the Sierra Nevada during the 1990s.

Phenotypic changes in insects also have been linked to recent climate change. For example, Bradshaw and Holzapfel [37] found that northern populations of the pitcher plant mosquito (*Wyeomyia smithii*) have shifted their critical diapause photoperiod towards that of their southern counterparts over the past 24 a. de Jong and Brakefield [38] found changes in melanism clines in the two-spot ladybird (*Adalia bipunctata*) that they also linked to recent

climatic change. Such rapid evolutionary response under climate change, however, may be rare since high genetic diversity is required for adaptation to occur and co-variation among traits can slow the process [39,40]. Gienapp et al. [41] also caution that other studies claiming evidence of microevolution under climate change fail to separate genetic change from phenotypic plasticity.

Now that we have examined the direct effects of climate change on insect populations, we will explore the indirect effects of climate change. These indirect mechanisms of change can lead to spatial and temporal mismatches between insects and their food or predators. These mismatches ultimately affect the ecosystem services that we gain or lose due to insects.

3. HOST PLANT-MEDIATED EFFECTS ON INSECTS

The persistence of herbivorous insects depends highly on interactions with plants. Host plants impact herbivore populations directly through phenological and nutritional conditions [42–46]. For example, Nzekwu and Akingbohungre [47] showed that utilisation of different types of host plants has significant effects on insect development. Previous studies on butterflies also have shown that larvae need long-lasting host plants with the proper phenology to be able to withstand environmental changes [48]. Any variation in resources may influence the dynamics and abundances of herbivores [49]. Mismatches between herbivorous insects and their food plants are caused by differential responses in the two groups; for example, earlier emergence and range shifts in insects can occur at a faster rate than those of plants. These mismatches can lead to declines in insect populations. For those species that affect processes such as pollination, this could lead to a reduction in ecosystem services.

3.1. Temporal Mismatches Between Insects and Their Host Plants

The indirect effects of climate change on insects are largely occurring because of altered host plant phenologies and quality [50–53]. Reduced host plant quality results in increased mortality because insect larvae compensate for the decrease in nutritional value by increasing consumption and development time, which in turn increases their exposure to predators and other environmental stresses [2,54]. In addition, species that feed on ephemeral resources are more likely to be sensitive (e.g. increased population variability) to asynchronies with host plants [55]. For example, insects whose eggs hatch before bud burst in their host plants will likely starve while those that require young foliage that hatch late will be forced to eat leaves that are more heavily defended [56].

Experiments have shown that temperature increases are altering insect development proportionately more than that of their host plants [57,58]. In fact, asynchrony has already occurred between the winter moth (*Operophtera brumata*)

and its host plant, pedunculate oak (*Quercus robur*) because *O. brumata* egg hatch has advanced more so than bud burst in *Q. robur* [59]. In this particular case, however, the authors speculate that high levels of genetic variation in *O. brumata* may allow rapid adjustment to phenology of *Q. robur* over time. Such information about the plasticity of species is lacking for most taxa; therefore, it is difficult to predict if this will be a general response (see Visser [60] for a discussion of adaptation to climate change).

Insects that provide important pollination services, for example, butterflies, are particularly vulnerable to temporal mismatches with their food resources. On average, butterfly species are advancing faster than herbs [61]. For example, in Britain, the migratory red admiral butterfly (*Vanessa atalanta*) has advanced its return flight over the past couple of decades, but its host plant, stinging nettle (*Urtica dioica*), has not advanced its flowering time, creating a mismatch in phenology [62]. In another study, McLaughlin et al. [28] linked increased variability in precipitation associated with climate change to extinctions in two populations of the Bay checkerspot butterfly (*Euphydryas editha bayensis*). This variability caused extinctions by accelerating plant senescence relative to larval development [28,48]. Simulations by Memmott et al. [63] concluded that 17–50% of pollinators, including insects, will suffer a disassociation with their food, and, as with other cases, small diet breadth was a greater risk factor for asynchrony with food. Later, we will discuss how other species that negatively affect human activities are benefiting from temporal mismatches with predators.

3.2. Spatial Mismatches Between Insects and Their Host Plants

In many insect systems, the dispersal ability of the insect is greater than its host plant. Such differential dispersal capacity of specialist herbivores, if they are to remain host plant-limited, could cause range contractions. Many insects have the ability to move long distances. For example, some butterflies in Europe have shifted their distributions northward 240 km in the past 30 a [19]. Recent shifts are not as well documented for plants, but post-glacial range expansions with past climate change were up to 100 km over 100 a with a median of only 20–40 km per century [64]. This is not surprising since plants, especially large trees, typically have long generation times and lower recruitment than insects [65]. These differences between plants and insects are further complicated by habitat fragmentation, which may be especially restrictive for plant migrations because of their limited dispersal ability [66–68].

To illustrate the potential impact of spatial mismatches under climate change, we analysed the relationship of specialist butterflies reaching a northern geographic limit in the United States with that of their primary host plants. A total of 74 butterfly species from 15 subfamilies have both a northern range boundary within the United States and utilise a single host species. Of these, 59 species have county-level distribution records for both butterfly and host plant.

FIGURE 1 Categorisation of spatial distances between the northern-most geographic distributions of host-specific butterflies and their larval host plant in North America. Distances based on county-level centroid locations of observational butterfly and vegetation records (ET SpatialTechniques, ArcGIS, Version 9.0) [71]. White bars are totals within each category and black bars represent the additive totals across categories.

Geographic centroids were calculated (ET SpatialTechniques, ArcGIS, Version 9.0, ESRI) for the northern-most county within each species' range [69] and that of its host plant [70]. Spatial discontinuity distances were calculated by subtracting the centroid latitude of the host plant from that of the butterfly species (Fig. 1).

Of the 59 specialist species assessed, 46% show a northern-most geographic range distribution within 100 km, 76% within 500 km, 93% within 1000 km and 7% extend more than 1000 km beyond the range edge of their host (Fig. 1). Of these, ten species (17%) reach their current range limit in the same location as their host. Furthermore, nine species (15%) show distributions extending beyond their current food plant range margin (negative values in Fig. 1). This is likely a result of sampling error arising from the scale of the observational data used, broad longitudinal distributions leading to multiple northern range fronts, inaccurate assessments of range boundaries due to occasional migrants into sink habitats or undocumented range extensions of species.

Spatial differences between butterfly and host range margins will determine how far butterfly ranges can expand under climate change, assuming relatively static host plant margins and consistent host preference for specialist herbivores. Where the geographic distance between the boundary of the butterfly and its host is small, there may be a small distance available for butterfly range expansion. Given the extent of range change seen in European butterflies (upwards of 240 km over 30 a) [19], we would estimate that up

to 59% of specialist butterflies in North America will reach the range limit of their host by 2050, assuming no change in host plant distribution and unlimited dispersal. For example, the Florida Leafwing, *Anaea floridalis*, which occurs less than 100 km from the edge of its host distribution, will approach host boundaries in just over a decade, as will other insect species. Up to 4% of the total butterfly fauna of North America could experience contracting geographic ranges where limiting food resource distributions interact with future climate warming to prevent adequate climate tracking. Simple risk assessments such as this procedure of comparing insect versus host plant poleward distribution boundaries can help identify species of greatest risk under climate change.

Now that we have explored how climate change can cause temporal and spatial mismatches between herbivorous insects and their food, we turn to examine how climate change can decouple interactions between insects and their predators, potentially leading to increases in and movement of species that diminish habitats, that is via outbreaks.

4. PREDATOR-MEDIATED EFFECTS ON INSECT POPULATIONS

Few studies have considered the influence of climate change on higher trophic levels (i.e. predators, parasitoids and pathogens) and its subsequent effects on insect herbivore populations. Climate change is expected to affect the relationship between predators and their prey by independently altering the dynamics of each trophic group [71]. In addition, higher trophic levels have been found to be more sensitive to climate change, likely due to the combined effects of climate change on lower trophic levels [7] and greater sensitivity to abiotic stress among higher trophic levels [72–74]. Changes in top trophic levels can cause trophic cascades. The loss of predators, therefore, can disrupt interactions between lower trophic levels including herbivores and primary producers [75,76].

Predators, particularly parasitoids, play an important role in controlling insect populations [77–82]. In some cases, they have been found to cause cyclical dynamics of herbivorous insects ([82,83]; but see Refs. [80,84] for exceptions). Recent studies of forest insects have linked population outbreaks to climate change as a result of phenological changes in insect life histories [85–87]. The role of higher trophic levels in causing insect outbreaks under climate change, however, has only started to be addressed [88]. We review the current observations and evidence to date of climate change causing temporal and spatial mismatches in predator–prey relationships and its subsequent effects on herbivore outbreaks.

4.1. Temporal Mismatches Between Insects and Their Predators

Herbivore development often changes rapidly and dramatically in response to changing climatic conditions, and this leads to large temporal variation in their populations. Parasitoids also may be affected by both climate variation

and its effect on host dynamics. For example, a study by Stireman et al. [88] used data from multi-year inventories of caterpillars reared from natural ecosystems to relate parasitism frequency to interannual climate variability. They found that parasitism frequency was negatively related to variability in precipitation. This suggests that there will be increased outbreaks of herbivores occurring under climate change as a result of increased climate variability causing mismatches in parasitoid–herbivore interactions.

Temporal mismatches could occur if predators and their insect herbivores respond differentially to changes in climate. Hosts and parasitoids may have different thermal preferences or different capacities to survive extreme temperatures and differential responses to climate could disrupt synchronisation [89]. For example, there is evidence that parasitoids often have lower temperature tolerances than their hosts [90, and references therein]. Models predict that increasing phenological asynchrony between predators and prey due to differential responses to climate can cause destabilisation of their population dynamics and extinction of the system [91,92].

Few studies, however, have empirically shown differential responses of predators and prey to changes in climate. Van Nouhuys and Lei [93] found that the light-coloured parasitoid *Cotesia melitaearum*, which is restricted to shaded habitats, develops slowly, while the dark-coloured larvae of its butterfly host, *Melitaea cinxia*, seeks out warm microhabitats where the sun increases body temperature, allowing individuals to develop faster. In cool but sunny springs, therefore, parasitoids emerged after most of their larval hosts had already pupated. In warmer springs, the synchrony between host and parasitoid was maintained and parasitism rate was high. In this study, the authors detected no effect of synchrony on local host population size, but disruption of synchrony is likely important for overall host metapopulation dynamics.

There is evidence of increasing sensitivity to climatic variation and other forms of abiotic stress in higher trophic levels [7,73,74,94]. This could be due to combined effects of climate variation on lower trophic levels [7]. Menge and Sutherland's [72] theory of community regulation predicts that higher trophic levels are more sensitive to abiotic stress as organisms in high trophic levels are likely to be larger and more mobile, enabling them to disperse from suboptimal areas. Species in higher trophic levels have been found to have slower recovery rates after catastrophic events [95,96], for example. In addition, Spiller and Schoener [94] found reductions in predators after two hurricanes in 1999 and 2001 on ten small Caribbean islands that likely explained observed increases in herbivory. Preisser and Strong [74] also observed mass mortality of lupine, *Lupinus arboreus*, off of the coast of California after 2 years of below average precipitation that was associated with El Niño events. Lupine die-offs were due to an outbreak of a root herbivore, *Hepialus californicus*. *H. californicus* populations are regulated by the nematode *Heterorhabditis marelatus* [97], which is dependent on soil moisture for movement. In their study, Preisser and Strong experimentally increased soil

moisture content to levels of wet El Niño years (above average precipitation levels) and found that increases in moisture levels directly affected the predatory nematode but not lupine or the ghost moth. Increases in the predator suppressed *H. californicus*, indirectly protecting lupines. Climate change is expected to increase such extreme events [98].

4.2. Spatial Mismatches Between Insects and Their Predators

Species are shifting their ranges in response to climate change, but as discussed above for the movement of herbivores and their plants, insect herbivores and their predators can shift at different rates. Predators that rely on close associations with their hosts such as parasites and parasitoids are expected to be especially affected by changes in the spatial distribution of their hosts. Colonising hosts could lack parasites or parasitoids and/or hosts that are infected could be less fit and therefore less successful in establishing in new locales [99]. In addition, native predators in newly colonised areas could switch from native hosts; however, some native predators may need to evolve phenological, behavioural or ecological specialisations before they can affect the colonising species. Predator species complexes, therefore, may be less rich with lower attack rates than those in the native range [100–102]. Reductions in parasitoids or other predators at the edges of expanding ranges of herbivorous insects could give expanding species an advantage (e.g. increases in population size) through release from predator control.

Only one study to date has investigated differential rates of spread of insect herbivores and their parasitoids under climate change. Menéndez et al. [103] found that the Brown Argus butterfly (*Aricia agestis*), a species that has shifted its range northward due to climate warming, experienced lower mortality from parasitoids in newly colonised areas. Other studies of invasive insects that have been introduced into new areas or of insects that have expanded their ranges as a result of their host plants being introduced also have found lower parasitoid rates in newly colonised areas [100,104]. Reductions in enemies of invasive species in their invaded ranges have been documented for a number of other plant and animal species as well (e.g. [105,106]).

Menéndez et al. [103] found no difference in parasitoid richness in the expanded and native ranges of *A. agestis*; they did, however, find a difference in parasitoid species composition. The majority of species attacking *A. agestis* in its newly colonised range were generalists that were already present in that area attacking *Polyommatus icarus*, a native butterfly. Other studies have found that specialist parasitoids were lost when insect hosts expanded their ranges and that the majority of species attacking hosts in their invaded range were generalists that switched from alternative native hosts [100,104,107].

Specialist parasitoids that follow hosts are likely to be more efficient predators than generalist parasitoids that have switched from native hosts. Although more generalist parasitoids attacked *A. agestis* in its expanded

range, the most abundant parasitoid was one that is believed not to have been present historically and therefore is likely a specialist that expanded its range with *A. agestis* [103]. Other studies of invading hosts have found similar results—that the most abundant predator in the invaded range are specialist predators that followed from the native range [106].

Generalist species in the expanded range could be less effective predators on new hosts that have undergone a range expansion. For example, while parasitoid species richness was similar in the native and expanded range of *A. agestis*, attack rates were lower for *A. agestis* compared to the native host *P. icarus* [103]. Similar results were found for a recent invasion of the variegated leafhopper (*Erythroneura variabilis*) into California's San Joaquin Valley. *E. variabilis* experienced lower attack rates from a shared parasitoid than the native grape leafhopper, *E. elegantula* [108]. Therefore, there is evidence that native parasitoids are slow to shift to new hosts because they are locally adapted to native hosts.

Over time, assemblages of predators and hosts in the invaded range would be expected to become similar to assemblages in the native range as enemies catch up to expanded host distributions. Most studies have found an increase in predator richness over time in the hosts' invaded or expanded range, however, resulting in different species composition than in the hosts' native range. Schonrogge et al. [104] found that for several cynipid wasps that were expanding their ranges, parasitoid assemblages in the introduced range were determined more by the parasitoid assemblages attacking existing cynipid species than by the parasitoids in their previous ranges.

No study to date has linked reduced predation after a host range shift to increases in populations of species that have undergone an expansion. Experimental manipulation of predators of species undergoing range expansions in different portions of their ranges is needed to link reductions in parasitoids in expanding ranges to changes in population dynamics. The invasion biology literature provides frameworks to test a reduction in enemies to host release in expanded ranges [99,109,110]. However, no tests to date have been conducted for insects expanding their range in response to climate change. Since many insects are important pests, we now discuss how climate change could favour native and non-native pests that impact important human activities and ecosystem services, including loss of forests and reduction in carbon sequestration.

5. CLIMATE CHANGE AND INSECT PESTS

Traits that enable a species to respond favourably to climate change are those very traits that pest species (native and non-native) often possess. For example, pests often have wide diet breadth, are multi-voltine, have rapid growth rates, are highly mobile and/or are phenotypically plastic [111–113]. Since many pest species have these traits, they are likely to respond favourably to climate change, perhaps more so than those that are specialised and/or rare.

Chown et al. [114], for example, found that invasive springtails were more phenotypically plastic in their ability to cope with desiccation under warming conditions compared to indigenous springtails.

Insect pests affect many human activities and ecosystem services. Many insect species are already damaging, and some may become more so under climate change. Insect pests are the most important agents of disturbance in North American forests, affecting areas almost 50 times larger than fire [18,115,116] with an estimated average annual economic cost of 2.1 billion dollars in the United States [117]. Many insect pests are non-native; up to 40% of major insect pest species in North America are invasive [118] with 62% of 29 major insect forestry pests being non-native [117]. Insect pests, both native and non-native, not only have economic impacts, but also have significant ecological impacts. For example, the invasive balsam woolly adelgid (*Adelges piceae*) has caused extensive death in relict stands of Fraser fir (*Abies fraseri*) and threatens other native species that depend on *A. fraseri* [119].

Many pest species have expanded their ranges as a result of climate change. The pine processionary moth (*T. pityocampa*), a pest of *Pinus* spp. and other conifers in southern Europe, has shifted 87 km north and to higher altitudes as a result of increased winter survival due to increased winter temperatures in the region over the last 30 a [26]. The mountain pine beetle (*D. ponderosae*) has also moved northward and to higher elevations in western Canada due to increased summer and winter temperatures and reduced precipitation [120]. In addition to expanding their ranges, these and other pests also are outbreaks more frequently and for longer durations [85–87]. Although causes of insect outbreaks are complex, observed increases in outbreak severity have been linked to both the direct effect of climate change on insect physiology and indirect effects through changes in their host plants (e.g., if their host plants are more drought stressed) and predators (e.g. [85–88,121–123]).

Finally, the effect of climate change on forest insect pests can affect the current balance of forest budgets. Widespread tree mortality due to *D. ponderosae* causes forests to have low primary productivity and increased carbon emissions as a result of tree decay. Over the next 20 a, it is predicted that pine forests in British Columbia, Canada will become net carbon sources rather than net carbon sinks mediated by increases in the severity of *D. ponderosae* outbreaks from recent climate change [86,87,124].

6. CONCLUSION

In this review, we demonstrate that the direction and magnitude of the effects of climate change on insect species are multifaceted. Changes are occurring both spatially and temporally, and these changes result directly from changing climate and indirectly through interactions with species in lower (i.e. plants) and upper (i.e. predators) trophic levels. In addition, trophic cascades can

occur such that changes in interactions between herbivores and their host plants affect higher trophic levels, and changes between predators and herbivorous prey can affect primary producers.

Further, species traits such as dispersal ability, trophic level and degree of specialisation are potentially good predictors of the effects of climate change. Our prediction is that future communities will be dominated by mobile generalists, species with fast generation times, those that have high dispersal capabilities or those that have been dispersed around the globe by humans. Many of these species are already pests, and new species could become pests on new host plants or in new locations under climate change. These opportunists may affect important human activities, such as forestry and agriculture, with extensions into the role of forests as carbon sinks. Insects that provide important ecosystem services such as pollination or biological control could not fare as well.

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Performance of the invasive weevil *Polydrusus sericeus* is influenced by atmospheric CO₂ and host species

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- Abstract**
- 1 Natural forest systems constitute a major portion of the world's land area, and are subject to the potentially negative effects of both global climate change and invasion by exotic insects. A suite of invasive weevils has become established in the northern hardwood forests of North America. How these insects will respond to increasing CO₂ or O₃ is unknown.
 - 2 The present study examined the effects of elevated atmospheric CO₂ and O₃ on the invasive weevil *Polydrusus sericeus* Schaller at the Aspen Free Air CO₂ Enrichment (FACE) site near Rhinelander, Wisconsin. A performance assay was conducted in the laboratory during the summer of 2007 using mated pairs of *P. sericeus* fed a combination of aspen, birch and maple foliage. We recorded leaf area consumption, oviposition and adult longevity. We also conducted visual abundance surveys in the field from 2004 to 2007 on aspen and birch at Aspen FACE.
 - 3 Elevated CO₂, but not O₃, significantly affected *P. sericeus* performance. Female, but not male, longevity was reduced under elevated CO₂. *Polydrusus sericeus* also produced fewer eggs under elevated CO₂ conditions compared with ambient conditions. Adult *P. sericeus* strongly preferred birch over both aspen and maple, regardless of fumigation treatment.
 - 4 The effects of elevated CO₂ on *P. sericeus* populations at Aspen FACE were minimal, and varied among years and host tree species. *Polydrusus sericeus* abundance was significantly greater on birch than aspen. Over the long term, elevated CO₂ may reduce adult female longevity and fecundity of *P. sericeus*. Further studies are needed to evaluate how this information may scale to ecosystem impacts.

Keywords *Acer saccharum*, *Betula papyrifera*, carbon dioxide, coleoptera, curculionidae, feeding trials, global change, invasive species, ozone, *Polydrusus sericeus*, *Populus tremuloides*.

Introduction

Invasive insects and increasing concentrations of tropospheric CO₂ and O₃ are joint threats to forest ecosystems. Elevated CO₂ tends to increase photosynthesis and overall plant biomass (Will & Ceulemans, 1997; Isebrands *et al.*, 2001; King *et al.*, 2001), at the same time as reducing host plant quality for herbivores as a result of an increase in total foliar carbon, including some carbon-based secondary compounds (Zvereva & Kozlov, 2006; Stiling & Cornelissen, 2007), and a decrease in nitrogen (Lawler *et al.*, 1997; Norby *et al.*, 1999; Kopper & Lindroth, 2003a, b). Altered phytochemistry typically results in increased

plant consumption by herbivores to compensate for lower food quality (Lindroth & Dearing, 2005; Stiling & Cornelissen, 2007). Tropospheric O₃ generally decreases plant health by reducing photosynthesis and biomass production (Heath, 1994; Coleman *et al.*, 1995) and tends to increase the production of carbon-based secondary compounds (Koricheva *et al.*, 1998). As a result of the stimulation of carbon-based secondary compounds, elevated O₃ may decrease host plant palatability to insect herbivores. Nonetheless, a range of responses has been observed, depending on the particular insect, host species and chemical group (Valkama *et al.*, 2007).

As globalization increases, so does the risk of new invasive species becoming established (Jenkins, 1996). Biological invasions are a major threat to the biodiversity of native flora

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and fauna (Gurevitch & Padilla, 2004; Hendrix *et al.*, 2006) and cost the U.S.A. billions of dollars annually (Pimentel *et al.*, 2005). Invasive species may persist for years before their discovery, especially in forest systems (Liebhold *et al.*, 1995; Mattson *et al.*, 2007). Over 400 documented invasive forest insect species exist in North America (Mattson *et al.*, 1994), and their invasions can alter natural trophic interactions or lead to changes in tree species composition, forest structure and ecosystem processes (Orwig & Foster, 1998; Hoebeke *et al.*, 2005).

Understanding how elevated CO₂ and O₃ will influence herbivore performance and abundance is critical to predicting the future state of forest ecosystems. Most research on the effects of elevated CO₂ and/or O₃ on insect herbivores has focused on Lepidoptera. Few studies have monitored coleopteran performance under elevated CO₂ and O₃ conditions (Coviella & Trumble, 1999; Valkama *et al.*, 2007; Vigue & Lindroth, 2010), even though almost 30% of invasive species are beetles (Mattson *et al.*, 1994). A complex of invasive weevils (Coleoptera: Curculionidae) of European origin has established in northern hardwood forest systems of North America (Coyle *et al.*, 2008). This complex is the most common above- and below-ground arthropod mesofauna in this ecosystem (Pinski *et al.*, 2005a). *Polydrusus sericeus* Schaller is the second most abundant invasive weevil species in northern hardwood forests and its distribution range spans much of eastern North America (Coyle *et al.*, 2008). Adults are polyphagous folivores, emerging from the soil in May. Mating occurs after an obligatory feeding period, and eggs are deposited in or near the soil. Larvae feed on fine roots of various woody plants from July to April, at which time pupation occurs. All of the invasive weevils, including *P. sericeus*, are univoltine. Currently, little is known regarding the effect of *P. sericeus* on northern forest ecosystems, although their impacts are suspected to be substantial (Coyle *et al.*, 2008).

Given the potentially large impact of invasive species, it is important to understand how global change may influence their ecology. The first objective of the present study was to assess *P. sericeus* performance on paper birch (*Betula papyrifera* Marsh.), trembling aspen (*Populus tremuloides* Michx.) and sugar maple (*Acer saccharum* Marsh.) foliage (comprising three ecologically and economically important tree species in northern hardwood forests) produced under elevated CO₂ and O₃ conditions. Because *P. sericeus* is a generalist feeder and foliar quality typically declines slightly under both elevated CO₂ and O₃ concentrations (e.g. reduced nitrogen, increased phenolics), we hypothesized that individual weevil performance would decrease in both treatments. We also expected weevils to perform better on birch than aspen or maple, based on prior studies (Pinski *et al.*, 2005a, b). We also examined the abundance of weevils at the Aspen Free Air CO₂ Enrichment (FACE) site to determine whether the effects of elevated CO₂ and O₃ on weevil consumption and performance in the laboratory would match population responses to elevated CO₂ and O₃. We hypothesized that reduced *P. sericeus* performance on trees receiving elevated CO₂ and O₃ concentrations would decrease weevil abundance compared with trees receiving ambient CO₂ and O₃ concentrations. We hypothesized that

P. sericeus would be more abundant on birch compared with aspen or maple.

Materials and methods

Study location and design

The study was conducted at the Aspen FACE experimental site (89.5°W, 45.7°N) located in Oneida Co., Wisconsin. Soils are Pandus sandy loam with occasional clay layers approximately 30 cm deep (Dickson *et al.*, 2000). Twelve treatment rings (diameter 30 m), each separated by a minimum of 100 m, were arranged in a split-plot randomized complete block design. Rings were divided into three blocks from north to south, and received one of four treatments ($n = 3$ per treatment): control, +CO₂ (target concentration = 560 p.p.m.); +O₃ (target concentration = 1.5 × ambient); and +CO₂+O₃ (measured CO₂ and O₃ levels are shown in Table 1) (<http://aspenface.mtu.edu/results.htm>). Gases were dispensed between 07.00 h and 19.00 h from budbreak to budset (average growing season length from 2004 to 2007 was 139 days). Unfortunately, the ozone generator malfunctioned for approximately 2 weeks at the beginning of the study, although the residual effects of elevated O₃ on tree chemistry persisted through this period (J. Couture and R. L. Lindroth, unpublished data). One half of each ring was planted with a combination of five aspen genotypes (clones 8 L, 42E, 216, 259, 271), one fourth of each ring was planted with a mixture of birch and aspen, and the remaining fourth with a mixture of maple and aspen. Additional details about the design and fumigation processes of the Aspen FACE are provided in Dickson *et al.* (2000).

Laboratory assay

We monitored weevil emergence visually, and began collecting weevils in the field as emergence began. We hand collected 200 mating pairs of *P. sericeus* from birch trees growing on the perimeter of the FACE site on 21 June 2007. Each weevil pair was placed into a separate vial and starved for 24 h prior to the experiment.

The next day, three trees each of birch, aspen (clone 216), and maple were marked in each FACE ring for foliage collection (three sets of three trees per ring). We collected several sun-exposed leaves from each tree, placed them into a zip lock bag, and transported them to the laboratory in a cooler. Sun leaves were chosen because visual surveys revealed that weevils were most commonly found on the present year's growth exposed to high sunlight (Pinski *et al.*, 2005a; M. L. Hillstrom, personal observation). One or two leaves of each species were grouped together, with petioles wrapped in wet cotton, and remoistened every 3–4 days to prevent desiccation. Leaves were placed into a single Petri dish (15 × 1.5 cm) lined with Whatman #1 filter paper (Whatman, U.K.). Leaves were replaced every 7 days to maintain a consistent leaf appearance (green) and quality (moisture). One pair of mating *P. sericeus* was added to each dish along with one half of a Kimwipe tissue (Kimberly-Clark, Roswell, Georgia), which served as an

Table 1 May, June and July mean air temperatures, precipitation, and CO₂ and O₃ levels (daytime p.p.m.) when *Polydrusus sericeus* adults were present from 2004 to 2007 at the Aspen Free Air Carbon dioxide Enrichment site near Rhinelander, Wisconsin

Year	Month	Average maximum temperature (°C)	Average minimum temperature (°C)	Total precipitation (cm)	Ambient CO ₂ (p.p.m.)	Elevated CO ₂ (p.p.m.)	Ambient O ₃ (p.p.b.)	Elevated O ₃ (p.p.b.)
2004	May	16	3	3.2	378	516	36	38
	June	21	8	2.7	376	518	34	47
	July	24	11	3.6	368	533	35	43
2005	May	16	4	1.1	383	531	34	45
	June	26	13	1.0	374	524	40	53
	July	27	13	1.8	365	528	37	53
2006	May	18	6	4.5	385	500	45	52
	June	24	9	0.6	377	540	42	51
	July	29	13	4.4	379	545	40	49
2007	May	20	6	2.0	393	520	48	57
	June	25	10	3.6	402	520	45	50
	July	26	13	4.2	390	493	34	39

oviposition site (Pinski *et al.*, 2005b). Kimwipes tissues with egg masses were kept moist and replaced every 3–4 days, and stored in separate Petri dishes until eggs could be counted. All Petri dishes were kept on a two-tiered plant rack in front of an open sliding door. Weevils were exposed to outdoor day/night conditions (Table 1) for the duration of the experiment and were rotated daily to prevent differences in sunlight exposure. In total, we used five Petri dishes for each of three sets of three trees (birch, aspen, maple) per treatment ring, for a total of 45 experimental units per control, +CO₂, +O₃, and +CO₂+O₃ treatment.

Leaf area consumed for each dish was divided by the number of weevil days for that dish. One weevil day was equivalent to each 24-h period that an individual weevil survived. To calculate leaf area consumed, leaves were scanned (Winfolia, Regent Instruments, Inc., Canada) after removal from Petri dishes to determine leaf area remaining after beetle feeding. We then digitally filled in the missing leaf portions and calculated the original leaf area (Hamilton *et al.*, 2005).

Weevil mortality was recorded every 3–4 days. In every pairing, the female was larger than the male, allowing when the male and female had died to be determined. Eggs were counted approximately twice weekly. Eggs collected on 7, 10 and 14 July were kept at approximately 20°C, sprayed with water every 3–4 days to keep them moist, and allowed to hatch. We recorded the proportion of eggs that hatched to determine differences in hatching rate among treatments. We did not measure initial female size, which may or may not influence adult fecundity (Leather, 1988), but suggest that female size may not have a strong effect on the number of eggs produced by leaf feeding beetles that feed extensively as adults and oviposit over a long period (Coyle *et al.*, 1999).

Field visual surveys

Aspen and birch trees were visually inspected for *P. sericeus* in mid-July from 2004 to 2007. Forty-eight trees per species were sampled each year. In 2004 and 2005, birch and aspen (genotype 216) were sampled in the inter-planted aspen-birch section of each ring. In 2006 and 2007, we sampled the same

birch trees but aspen (genotype 216) trees were sampled in the mixed aspen genotype section. Each tree was visually inspected for 10 min and the number of weevils recorded. To account for differences in tree size (number and size of branches), we surveyed random branches for approximately 3.3 min at the bottom, middle and top of each tree without inspecting any branch or leaf more than once. Surveying visually also allowed us to account for any differences in leaf area as a result of species or treatments because inspection takes the same amount of time per unit leaf area (i.e. trees with more leaves per branch take longer to inspect, so fewer total branches, but the same total leaf area per tree, is inspected).

Statistical analysis

Leaf area consumed per Petri dish was summed for the duration of the experiment. Mean leaf area consumed across dishes and trees was calculated for each tree species in each ring (unit of replication), log transformed to satisfy assumptions of normality and equal variance, and analysed as a two-way crossed split plot analysis of variance (ANOVA). The main effects were block, CO₂ and O₃, with a CO₂ × O₃ interaction term. Subplot effects were tree species and tree species by main effects. We recognize that measuring the amount of leaf area consumed does not account for potential differences in leaf mass per area as a result of elevated CO₂ or O₃. We examined data from neighbouring aspen and birch trees from July 2007 and found that elevated CO₂ did not affect leaf mass per area. Elevated O₃ increased mass per area by 10%, although this effect was not significant (J. Couture and R. L. Lindroth, unpublished data). Accounting for the increased mass per area resulted in no significant change to our estimates of leaf consumption under elevated O₃. Thus, we will refer to leaf area consumed, rather than mass consumed.

Longevity data met normality and equal variance assumptions, and were analysed as a two-way crossed split plot ANOVA with block, CO₂ and O₃ main effects and the CO₂ × O₃ interaction term. Subplot effects included sex and sex by main effects.

Table 2 Effects of ambient and elevated CO₂ and O₃ on leaf consumption by *P. sericeus*

Tree species	Treatment				Species means
	Control	+CO ₂	+O ₃	+CO ₂ +O ₃	
Birch	0.472 ± 0.044	0.450 ± 0.040	0.495 ± 0.144	0.450 ± 0.062	0.467 ± 0.003a
Aspen	0.038 ± 0.006	0.056 ± 0.008	0.041 ± 0.006	0.038 ± 0.004	0.043 ± 0.003b
Maple	0.019 ± 0.004	0.023 ± 0.004	0.020 ± 0.004	0.020 ± 0.006	0.020 ± 0.002c

Data represent the mean ± SE leaf area consumed (cm²) per weevil per day, for $n = 3$ FACE rings per fumigation treatment. Tree species means sharing a letter are not significantly different at $\alpha = 0.05$ (Tukey's honestly significant difference).

Egg production rate (i.e. the number of eggs produced per female per day) and total egg production (i.e. the number of eggs produced by a mating pair of weevils throughout the duration of the experiment) were log transformed to meet assumptions of normality and equal variance. Both eggs per day and total number of eggs produced were analysed using a two-way crossed ANOVA with block, CO₂, O₃ and CO₂ × O₃. Egg hatch rate was analysed using the same model as egg production.

Weevil abundance data were log transformed and analysed as a two-way, crossed, split-split plot ANOVA with block, CO₂ and O₃ as the main effects and an interaction term (CO₂ × O₃). Tree species and tree species crossed with each main effect were the first subplot factors, and the second subplot factors were year and year crossed with each main effect and first subplot effects. Significant terms were examined in more detail using Tukey's honestly significant difference.

Results

Leaf area consumption

Elevated CO₂ and O₃ did not significantly affect the leaf area consumed by *P. sericeus* (all $P > 0.431$). Weevils strongly preferred birch foliage regardless of fumigation treatment, and also preferred aspen over maple ($F_{2,16} = 385.39$, $P < 0.001$) (Table 2). Across fumigation treatments, the average leaf area consumption per dish was 90% birch, 7% aspen and 3% maple.

Longevity

Feeding on enriched CO₂ foliage reduced *P. sericeus* female longevity by 19% but male longevity was unaffected (sex × CO₂: $F_{1,8} = 7.94$, $P = 0.023$; Fig. 1). Elevated O₃ and the CO₂ × O₃ interaction did not significantly affect *P. sericeus* longevity (all $P > 0.535$).

Fecundity

Polydrusus sericeus egg production rate declined by 23% ($F_1 = 7.46$, $P = 0.034$) and total egg production declined by 29% ($F_1 = 6.08$, $P = 0.049$) for females fed foliage produced under elevated CO₂ compared with ambient CO₂ (Fig. 2). We observed no significant O₃ or CO₂ × O₃ interaction for either fecundity parameter (all $P > 0.788$). Eight female *P. sericeus* laid more than 1000 eggs over their lifetime, with a maximum

Figure 1 Effects of ambient and elevated CO₂ on female and male *Polydrusus sericeus* longevity. Means ± SE sharing the same letter are not significantly different at $\alpha = 0.05$ (Tukey's honestly significant difference).

production of 1342 eggs by a single female. Females continued to oviposit after males had died. Egg hatch rate was consistent among treatments (model: $P = 0.671$, CO₂: $P = 0.611$, O₃: $P = 0.445$), in the range 93–96%.

Abundance

Although the main effects of elevated CO₂ and O₃ on *P. sericeus* abundance were not significant (all $P > 0.897$), we did observe a significant CO₂ × tree species × year interaction ($F_{3,280} = 3.28$, $P = 0.022$). In summary, CO₂ had little effect on weevil abundance in 2004 and 2006 but increased the abundance of weevils on aspen and decreased the abundance of weevils on birch in 2005. The pattern for 2005 was reversed for 2007. We found no significant CO₂ × O₃ interaction ($P = 0.574$). *Polydrusus sericeus* was 3.9-fold more abundant on birch than on aspen across the 4 years of this study ($F_{1,280} = 265.28$, $P < 0.001$; Fig. 3). Weevil abundance varied 2.1-fold among years ($F_{3,280} = 16.06$, $P < 0.001$; Fig. 3).

Discussion

Fumigation effects

Performance. Elevated CO₂ did not alter foliar consumption by *P. sericeus* but did reduce female longevity and oviposition rate. Birch leaf chemistry data from a concurrent study indicate that elevated CO₂ had minimal effects on nitrogen and condensed tannin concentrations (relative increases of only

Figure 2 Effects of CO₂-treated foliage on (A) egg production rate and (B) total eggs produced. Means $\pm$ SE within a figure sharing the same letter are not significantly different at $\alpha = 0.05$ (Tukey's honestly significant difference).

Figure 3 Mean $\pm$ SE abundance of *Polydrusus sericeus* (A) for birch and aspen, pooled across fumigation treatments and years, and (B) for each year of the study, pooled across fumigation treatments and tree species. Means sharing the same letter are not significantly different at $\alpha = 0.05$ (Tukey's honestly significant difference).

1% and 6%, respectively; J. Couture and R. L. Lindroth, unpublished data), which are comparable with previous changes in birch chemistry at Aspen FACE (Agrell *et al.*, 2005). Such small increases appear unlikely to alter feeding and

performance. Likewise, elevated CO₂ does not increase the toughness of birch leaves at Aspen FACE (Oksanen *et al.*, 2001). Thus, the cause of reduced female longevity and fecundity remains unknown. Elevated CO₂ reduced oviposition of *Phyllonorycter tremuloidiella* Braun on aspen trees at Aspen FACE (Kopper & Lindroth, 2003b) but *Popillia japonica* Newman at SoyFACE lived 8–25% longer and laid approximately two-fold more eggs on soybean plants exposed to elevated CO₂ compared with ambient conditions (O'Neill *et al.*, 2008).

No significant elevated O₃ effects were evident on *P. sericeus* performance. Elevated O₃ had minimal effects on nitrogen and condensed tannin concentrations (relative reduction of 10% and 22%, respectively) in birch at Aspen FACE in July 2007 (J. Couture and R. L. Lindroth, unpublished data). Friewald *et al.* (2008) found that *Phyllobius pyri* L. weevils feeding on hybrid aspen preferred younger (later budburst), less thick elevated O₃ leaves. Trees at Aspen FACE experience later budburst when exposed to elevated O₃ (Karnosky *et al.*, 2003). Therefore, the increased quality associated with younger leaves may offset or surmount the reduced quality as a result of decreased nitrogen and increased condensed tannins. Changes in foliar chemistry under elevated O₃ in the present study agree with previous results at Aspen FACE (Kopper *et al.*, 2001; Agrell *et al.*, 2005). Other studies have found no change in insect performance on elevated O₃ foliage (Trumble *et al.*, 1987; Whittaker *et al.*, 1989; Costa *et al.*, 2001; Kopper & Lindroth, 2003b; Awmack *et al.*, 2004; O'Neill *et al.*, 2008). Additional studies, however, show that elevated O₃ may increase insect preference (Jeffords & Endress, 1984; Jones & Coleman, 1988; Endress *et al.*, 1991; Fortin *et al.*, 1997; Kopper & Lindroth, 2003a; Agrell *et al.*, 2005; Friewald *et al.*, 2008) and performance (Coleman & Jones, 1988; Whittaker *et al.*, 1989; Holton *et al.*, 2003). Differences in fumigation setup (FACE, open top chamber, etc.), insect and plant species, plant age and O₃ concentration probably contribute to this diversity of responses.

Abundance. Elevated CO₂ increased the abundance of weevils on aspen and decreased the abundance of weevils on birch in 2005, decreased the abundance of weevils on aspen and increased the abundance of weevils on birch in 2007, and had little effect on weevil abundance in 2004 and 2006. Yearly variability (1997–2003) in tree growth responses to elevated CO₂ at Aspen FACE has been linked to the amount of photosynthetically active radiation in July and temperature in October (Kubiske *et al.*, 2006). Similarly, variability in abiotic conditions probably affected weevil abundance. Previous work suggests that elevated CO₂ may increase weevil abundance (Altermatt, 2003; Hillstrom & Lindroth, 2008) but decrease general folivore abundance (Bezemer & Jones, 1998; Stiling & Cornelissen, 2007). Differences in the taxonomy or feeding guild of the insect, experimental design, fumigation concentrations, or plant species and plant age may explain the contrasting abundance responses of chewing herbivores to elevated CO₂ across studies.

Elevated O₃ had no effect on *P. sericeus* abundance, in agreement with the lack of change in foliar consumption or

insect performance. Over the last decade, elevated O₃ has reduced the abundance of most insect species examined at Aspen FACE (Loranger *et al.*, 2004; Hillstrom & Lindroth, 2008). Elevated O₃ may, however, increase the abundance of species, such as bark beetles, that preferentially attack O₃-stressed host plants (Cobb *et al.*, 1968; Grodzki *et al.*, 2004). More studies are necessary to determine whether responses differ among insect orders or feeding guilds.

Host species

Polydrusus sericeus were substantially more abundant on birch than aspen, probably because birch is their preferred food (Pinski *et al.*, 2005b). Previous research at Aspen FACE has shown that control birch and aspen leaves have almost equal nitrogen content but birch foliage has approximately half the concentration of condensed tannins relative to aspen (Agrell *et al.*, 2005). Fewer structural compounds (e.g. 25% less fibre in birch leaves compared with aspen leaves in July 2006) (J. Couture and R. L. Lindroth, unpublished data) could also make birch more palatable than aspen or maple.

Conclusions

As documented for other herbivores (Hillstrom & Lindroth, 2008), CO₂ and O₃ acted independently on performance and abundance of *P. sericeus*. Concentrations of elevated CO₂ above 500 p.p.m. have the potential to decrease *P. sericeus* populations by reducing female longevity and fecundity. By contrast, elevated O₃ alone or in combination with elevated CO₂ did not alter *P. sericeus* performance or abundance. Atmospheric CO₂ concentrations interact with weather factors to produce variable plant responses among years; it remains unknown whether such effects may also influence abundance and performance of herbivorous insects. Finally, the results obtained in the present study also support the findings of Pinski *et al.* (2005b) indicating that *P. sericeus* prefer birch foliage to aspen or maple foliage.

Damage to trees by adult *P. sericeus* may not change with the concentrations of CO₂ and O₃ predicted for 2060. If concentrations continue to rise to those predicted for 2100, however, the changes we found in longevity and fecundity at elevated CO₂ could be exacerbated, leading to significant impacts on non-indigenous weevil populations. Reductions in *P. sericeus* abundance could also change competitive interactions, leading to increased populations of other weevils in the complex or further invasion of northern hardwood forests by other insects. Future studies should consider the effects of atmospheric change on other invasive weevil species, both individually and as a guild, in northern hardwood forests.

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Soil carbon and nitrogen mineralization following deposition of insect frass and greenfall from forests under elevated CO₂ and O₃

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Abstract Elevated CO₂ and O₃ alter tree quality and the quality of herbivore inputs, such as frass, to forest soil. Altered quality or quantity of herbivore inputs to the forest floor can have large impacts on below-ground processes. We collected green leaves and frass from whitemarked tussock moth caterpillars from aspen-birch stands at the Aspen Free Air CO₂ Enrichment (FACE) site near Rhinelander, WI, USA. Small or large quantities of frass, greenfall, or a 1:1 ratio of frass and greenfall were added to microcosms for each FACE treatment (control, +CO₂, +O₃, +CO₂+O₃). We measured initial frass and greenfall quality, and recorded microbial respiration, and nitrate leaching over 40 days. Elevated carbon dioxide (eCO₂) and tropospheric ozone (eO₃) significantly altered the carbon, nitrogen, and condensed tannin content of insect frass and green leaves. Although FACE treatments affected input quality, they had minimal effect on microbial respiration and no effect on nitrogen leaching. In contrast, input quantity substantially influenced microbial respiration and nitrate leaching. Respiratory carbon loss and nitrate immobilization were nearly double in microcosms

receiving large amounts of herbivore inputs than those receiving no herbivore inputs. Small amounts of herbivore inputs, however, did not significantly alter microbial respiration or immobilization, suggesting that effects of herbivore inputs on soil processes will be detected only at moderate to high herbivory/input levels. These results suggest that subtle changes in frass and greenfall quality may not affect soil nutrient cycling. In contrast, environmental change induced increases in insect population size or frass and greenfall inputs to the soil may substantially impact nutrient cycling.

Keywords Carbon and nitrogen mineralization · Aspen FACE · *Orgyia leucostigma* · Canopy herbivore inputs · Soil respiration · Carbon dioxide · Ozone

Introduction

Forest ecosystems of the 21st century face a multitude of global change factors that will alter their structure and function, as well as the services they provide. Fragmentation, invasion of alien species and changes in the atmosphere will alter forests directly through effects on plants and indirectly through impacts on higher trophic levels (e.g., herbivores). Changes in atmospheric concentrations of two gases, carbon dioxide and tropospheric ozone, are expected to have significant impacts on forest ecosystems (Karnosky et al. 2003). Annual human-caused CO₂ emissions have increased more

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than 80% since 1970 (IPCC 2007) and tropospheric ozone concentrations are expected to increase from an average ambient background concentration of 40 ppb today to 68 ppb by 2050 and 85 ppb by 2100 (Akimoto 2003; Wittig et al. 2009). Numerous studies have evaluated the effects of both elevated CO₂ (eCO₂) and elevated O₃ (eO₃) on tree health, nutrient status and defense characteristics. Generally, forest trees respond to eCO₂ with increased photosynthesis and growth above- and below-ground (Saxe et al. 1998; Karnosky et al. 2003), decreased foliar nitrogen and increased foliar carbohydrates and phenolics, resulting in increased carbon to nitrogen ratios (Zvereva and Kozlov 2006; Stiling and Cornelissen 2007; Lindroth 2010). Unlike the fertilizing effects of CO₂, tropospheric O₃ causes significant damage to plants, inducing foliar injury (Karnosky 1976; Karnosky et al. 2003) and decreasing photosynthesis and growth (Karnosky et al. 2003). Changes in phytochemistry of trees exposed to eO₃ can shift in magnitude or direction depending on the timing of the sampling (season), and the species and genotypes (O₃ sensitive vs. O₃ resistant) involved (Lindroth et al. 2001; Holton et al. 2003; Kopper and Lindroth 2003; Lindroth 2010). Although the effects of eCO₂ and eO₃ on plant quality vary, the implications are clear: eCO₂ and eO₃ alter the quality of forest trees for herbivores.

Changes in tree quality due to eCO₂ and eO₃ have been shown to affect herbivore growth, survivorship and abundance (Bezemer and Jones 1998; Zvereva and Kozlov 2006; Valkama et al. 2007; Lindroth 2010), but the implications for herbivore-mediated ecosystem processes have received little attention. Canopy herbivory can strongly affect nutrient cycling (Belovsky and Slade 2000; Schowalter 2000; Hunter 2001). Canopy insects may influence soil nutrient dynamics through four types of soil surface inputs: frass deposition, turnover of insect cadavers, changes in throughfall chemistry, or changes in quality or quantity of leaf litter that falls to the forest floor (e.g., greenfall deposition) (Hunter 2001). Frass and greenfall inputs, in particular, are important because they occur during the growing season and frass inputs have been shown to influence tree nutrient levels (Christenson et al. 2002; Frost and Hunter 2007) and soil carbon and nitrogen cycling within the same growing season (Frost and Hunter 2004, 2007). This fast processing of organic matter is possible because, unlike senesced leaves that have much of their

nutrient content resorbed, frass and greenfall inputs are “higher quality” inputs that may contain 74% more available nitrogen than senescent leaves (Risely and Crossley 1993; Fonte and Schowalter 2004; Madritch et al. 2007a). Further, soil nitrogen inputs from frass can exceed those from leaf litter during severe outbreaks (Fogal and Slansky 1984; Grace 1986; Hollinger 1986). Frass and greenfall inputs to the soil may result in increased nitrogen mineralization (Constantinides and Fownes 1994; Lightfoot and Whitford 1990; Reynolds et al. 2000) leading to enhanced microbial immobilization (Lovett and Ruesink 1995; Christenson et al. 2002; Lovett et al. 2002) or export from the system (Swank and Crossley 1988; Eshleman et al. 1998). Nitrogen export may occur rapidly (e.g., within 1 week) if timed with a rainfall event, but in most situations the nitrogen is immobilized by microbes or fungi near the soil surface (top 0–30 cm) and is not available for plant uptake (Christenson et al. 2002; Lovett et al. 2002; Frost and Hunter 2007).

The effects of herbivore inputs on soil carbon and nitrogen dynamics depend on both input quality and quantity. Input quality may change due to the direct effects of atmospheric chemistry on foliar chemistry, which, in turn, affects the chemistry of both frass and greenfall. Knepp et al. (2007) demonstrated that under eCO₂ *Polyphemus* caterpillars have altered carbon and nitrogen concentrations and caterpillar frass has altered elemental composition, phenolic content and carbon to nitrogen ratios. Input quantity may change due to the effects of foliar chemistry on the ingestion and egestion rates of individual herbivores (Lindroth 1996), as well as on herbivore population density (Liebhold and Elkinton 1988). Of these, changes in population densities are likely particularly important, as research has documented increased effects of herbivore inputs at higher population densities (Hunter et al. 2003).

Given that atmospheres of the future are likely to alter the quality and quantity of frass and greenfall inputs, we investigated how eCO₂ and eO₃ would affect carbon respiration of soil microorganisms and soil nitrate leaching. We employed an experimental microcosm approach, using different quantities of whitemarked tussock moth (*Orgyia leucostigma* Fitch) frass and greenfall, both of which varied in quality due to atmospheric chemistry treatments administered at the Aspen Free Air CO₂ Enrichment (FACE) facility near Rhinelander, WI, USA.

Methods

Aspen FACE facility

The Aspen FACE site consists of 12 circular plots (rings), each 30 m in diameter. During the growing season, each ring receives one of two levels of CO₂ (ambient ~350 ppm, aCO₂; elevated ~550 ppm) and O₃ (ambient ~35 ppb, aO₃; elevated ~50 ppb). Thus, at the level of atmospheric gas treatment, this is a two-way factorial experiment with three replicates of each of the four treatment combinations. Fumigation of trees began in 1998 with eCO₂ and eO₃ dispensed at concentrations predicted for 2060. Elevated O₃ is fumigated at approximately 1.5 times that of monitored, daily, ambient levels (Dickson et al. 2000).

Frass and greenfall

In August 2006, we reared whitemarked tussock moth larvae from eggs obtained from the Canadian Forest Service (Sault St. Marie, ON, Canada), at 22°C in a Percival® growth chamber. The whitemarked tussock moth was chosen because it is a common generalist, native herbivore in this system. This tussock moth is not considered an outbreak species, but has similar feeding behavior and gut and frass chemistry to the gypsy moth (*Lymantria dispar* L.) (Kopper et al. 2002; Lovett et al. 1998; Frost and Hunter 2007). Larvae were fed paper birch foliage from trees at Aspen FACE that were not exposed to the fumigation treatments. Once larvae had reached the third instar, 10 individuals were put into a mesh enclosure on each of four aspen (*Populus tremuloides* Michx., clone 216) and four birch (*Betula papyrifera* Marsh.) trees in each of the 12 rings. Larvae were then allowed to feed until pupation. Frass was collected from fifth-instar caterpillars, air-dried, and weighed. Equal amounts (1:1 mix by dry mass) of frass from aspen- and birch-fed caterpillars were then combined into a single aggregate pool for each FACE ring (three replicate FACE rings per fumigation treatment). “Greenfall” was artificially generated for this study using green leaves collected from each FACE ring in August 2006. To preserve chemical characteristics, field collected leaves were stored on crushed ice while in the field, flash frozen in liquid nitrogen, and freeze-dried. Freeze-dried leaves easily crumbled into small pieces. We selected pieces roughly 0.1 to

0.5 cm² for this experiment. Equal amounts of aspen and birch foliage were weighed and pooled (1:1 mix by dry mass) for each FACE ring.

Subsamples of frass and composite greenfall were measured for carbon and nitrogen concentration (% dry mass) with a Thermo Finnigan (San Jose, CA, USA) Flash 1112 elemental analyzer. Condensed tannins of frass and greenfall were extracted with 70% acetone at 4°C and quantified by the acid butanol method of Porter et al. (1986), with a purified 1:1 mix of aspen and birch tannin standards. We do not report lignin concentrations in this study because the fine particulate nature of frass caused too much loss of material during the standard lignin digestion procedure.

Microcosms

We set up seven microcosms for each of the 12 FACE rings. Microcosms consisted of pieces of clear acrylic plastic (18 cm in height and 6 cm in diameter) enclosed at the bottom by 1 mm nylon mesh and a clear plastic funnel. Each microcosm was filled to 10 cm depth with 150 g (dry mass) of field moist soil mix. This mix contained 100 g of topsoil from a non-fumigated portion of the Aspen FACE site and 50 g of sand, added to improve drainage. On top of the soil, we placed 1.5 g of chopped, air-dried leaf litter to simulate natural soil conditions at Aspen FACE. This litter was also collected from a non-fumigated portion of the FACE facility during August 2006, and was a mixture of partially decomposed aspen and birch leaves from previous growing seasons. Soil and old leaf litter were defaunated by repeated (three times) freezing and thawing. Sand was sterilized using an autoclave.

After adding soil and old leaf litter, each microcosm was flushed with 30 ml of double distilled water to rehydrate the soil and litter. After four days, either a low or high quantity of frass, greenfall, or a mix of frass and greenfall (1:1 mix by dry mass) was added to each microcosm (Table 1). Input quantities were based on estimates of gypsy moth herbivory in mature aspen stands calculated from aspen specific leaf area, leaf area index, litterfall and gypsy moth digestion efficiencies (Raich and Nadelhoffer 1989; Lindroth and Hwang 1996; Steele et al. 1997; Roth et al. 1998; Burrows et al. 2002; Davidson et al. 2002; Madritch et al. 2007a). The 100 mg treatments represent frass and greenfall inputs from background canopy herbivory of less than 20% leaf area consumed while the

Table 1 Frass and greenfall treatments applied to the soil microcosms. Each of the seven treatments was applied to one microcosm for each of the 12 FACE rings for a total of 84 microcosms

	Treatment	Abbreviation	Quantity (mg)
1	Control	C	0
2	Frass (low)	FL	100
3	Greenfall (low)	GL	100
4	Frass + Greenfall (low)	FGL	50+50
5	Frass (high)	FH	700
6	Greenfall (high)	GH	700
7	Frass + Greenfall (high)	FGH	350+350

700 mg treatments represent frass and greenfall inputs from outbreak herbivory levels of greater than 70% leaf area consumed. After substrates were added to the microcosms, we added 20 ml of distilled water to rehydrate the inputs. Microcosms were incubated at room temperature (19°C) for 40 days.

Soil carbon and nitrogen mineralization

On days 1–5, 7, 9, 11, 16, 18, 21, 24, 30, and 37 we measured CO₂ efflux from each of the 84 microcosms (12 rings × 7 microcosms) for two minutes using a PP Systems (Amesbury, MA) infrared gas analyzer. Five milliliters of water were added to each microcosm approximately 24 h before each CO₂ measurement and as necessary between measurements to maintain soil moisture levels. The amount of water added to each microcosm over the entirety of the study approximated the average summer monthly rainfall per 37 days in northern Wisconsin. We report CO₂ respired in conventional units of g/m²/hr.

After the 40 day incubation period, microcosms were flushed with 50 ml distilled water. Leachate was collected by drawing a vacuum through each microcosm and was analyzed for combined NO₂ and NO₃ concentration using a modified version of the vanadium chloride method (Miranda et al. 2001; Doane and Horwath 2003; Allison et al. 2008). Leachate was analyzed for NH₄ concentration using a modified version of the Berthelot-salicylate method (Weatherburn 1967; Madritch et al. 2007a; Allison et al. 2008). Ammonium concentration is typically much lower than nitrate concentration in leachate due to lower mobility. Nitrogen concentra-

tion of leachate is reported as NO₃-N and NH₄-N loss (mg/L) per liter of leachate.

Statistical analysis

Average carbon, nitrogen, and condensed tannins in frass and greenfall pools from the 12 FACE rings were analyzed with a blocked, two-way ANOVA, where effects were block, CO₂, O₃, and CO₂ × O₃ interaction. CO₂ respiration and nitrogen leaching results were analyzed using a blocked, split-plot ANOVA. Whole plot effects were related to input quality and included experimental block, CO₂, O₃, and CO₂ × O₃ interaction. At the sub-plot level, effects were microcosm treatment (Table 1), CO₂ × microcosm treatment, O₃ × microcosm treatment, and CO₂ × O₃ × microcosm treatment. Correlation analysis was used to reveal the pattern of relationship between nitrate leaching and soil respiration. Analyses were conducted using JMP® 8.0.1 software (SAS Institute, Inc, Cary, NC, USA). P-values are considered statistically significant at $\alpha \leq 0.05$ and marginally significant for $0.05 < \alpha < 0.1$ (Filion et al. 2000).

Results

Input quality

FACE treatments significantly affected frass quality (Table 2). Frass carbon concentration was 1% lower, nitrogen concentration was unaltered, and condensed tannin concentration was 41% higher in eCO₂ plots compared with aCO₂ plots. Frass carbon concentration was not affected by eO₃, but nitrogen concentration was reduced 12% and condensed tannin concentration was increased 30% at eO₃ compared with aO₃. However, the 30% increase in tannins at eO₃ was not statistically significant.

Elevated CO₂ and O₃ also affected green leaf quality (Table 2). Greenfall carbon concentration was 1% lower in eCO₂ plots compared with aCO₂ plots. Greenfall nitrogen and condensed tannin concentrations were not altered by eCO₂. Elevated O₃ increased greenfall carbon concentration 1%. Greenfall nitrogen concentration decreased 9% and condensed tannin concentration increased 13% compared with aO₃ but the decrease in tannins was not significant. For comparison, greenfall, frass, and leaf litter collected

Table 2 Mean (± 1 SE) percent carbon, nitrogen, and condensed tannins of frass and greenfall added to microcosms. Statistics represent the results of two-way crossed ANOVA tests (df 1,6). Significant P-values (<0.10) are indicated in bold

	Control	+CO ₂	+O ₃	+CO ₂ +O ₃	CO ₂		O ₃		CO ₂ $\times$ O ₃	
					F	P	F	P	F	P
Frass carbon	50.21 $\pm$ 0.25	49.61 $\pm$ 0.16	50.24 $\pm$ 0.09	49.61 $\pm$ 0.21	10.52	0.02	0.01	0.94	0.01	0.95
Frass nitrogen	1.18 $\pm$ 0.07	1.12 $\pm$ 0.06	0.99 $\pm$ 0.04	1.05 $\pm$ 0.05	0.00	0.99	5.34	0.06	1.12	0.33
Frass tannins	6.83 $\pm$ 0.62	11.32 $\pm$ 1.53	10.49 $\pm$ 2.20	12.93 $\pm$ 1.96	4.00	0.09	2.33	0.17	0.38	0.56
Greenfall carbon	49.95 $\pm$ 0.11	49.5 $\pm$ 0.11	50.36 $\pm$ 0.07	50.1 $\pm$ 0.23	4.91	0.07	10.23	0.02	0.39	0.56
Greenfall nitrogen	1.87 $\pm$ 0.02	1.88 $\pm$ 0.04	1.59 $\pm$ 0.04	1.83 $\pm$ 0.13	2.77	0.15	5.51	0.06	2.48	0.17
Greenfall tannins	17.32 $\pm$ 2.37	20.01 $\pm$ 3.34	23.44 $\pm$ 4.43	18.82 $\pm$ 2.53	0.08	0.79	0.5	0.51	1.09	0.34

from the FACE rings had carbon concentrations of 50%, 50%, and 49%, respectively, and nitrogen concentrations of 1.8%, 1.0%, and 0.9%, respectively.

Soil processes

Consistent with the minimal effects of FACE CO₂ and O₃ treatments on input quality, we found only minor effects on microbial respiration (Table 3; Fig. 1a, c). We found no CO₂ or O₃ main effects on microbial respiration averaged across microcosm treatments.

Table 3 Two-way crossed split-plot ANOVA of microbial respiration and nitrate leaching from microcosms. P-values in bold are significant at $\alpha \leq 0.05$. Microcosm trt. (= treatment) includes the seven microcosm treatments from Table 1

Source	F	df	P
Microbial respiration			
CO ₂	1.92	1,6	0.21
O ₃	1.04	1,6	0.35
CO ₂ $\times$ O ₃	0.99	1,6	0.36
Microcosm trt.	21.57	6,48	<0.01
CO ₂ $\times$ microcosm trt.	2.05	6,48	0.08
O ₃ $\times$ microcosm trt.	0.30	6,48	0.93
CO ₂ $\times$ O ₃ $\times$ microcosm trt.	1.91	6,48	0.10
Nitrate-N leaching			
CO ₂	0.53	1,6	0.50
O ₃	0.14	1,6	0.72
CO ₂ $\times$ O ₃	0.10	1,6	0.77
Microcosm trt.	6.40	6,48	<0.01
CO ₂ $\times$ microcosm trt.	0.92	6,48	0.49
O ₃ $\times$ microcosm trt.	1.60	6,48	0.17
CO ₂ $\times$ O ₃ $\times$ microcosm trt.	0.90	6,48	0.50

Microbial respiration was marginally affected by the interaction of CO₂ and microcosm treatment ($F_{6,48} = 2.05$, $P=0.08$). We found a trend for larger differences in CO₂ respiration between low and high inputs at eCO₂ compared with aCO₂. Microcosms with the FGL treatment had greater carbon respiration than control microcosms at aCO₂ but similar carbon respiration to FGL microcosms at eCO₂. Microbial respiration was not significantly different between greenfall, frass, and combinations of the two at the same quantity of input (Fig. 1b, c) even though the substrates differed slightly in terms of carbon and nitrogen concentrations.

In contrast to input quality, input quantity had a substantial positive effect on microbial respiration (Table 3, Fig. 1b, c). Losses from microcosms receiving small amounts of herbivore inputs were larger (0.079 ± 0.006 g CO₂/m²/hr) but not significantly different from those receiving no herbivore inputs (0.066 ± 0.004 g CO₂/m²/hr; Fig. 1b). However, respiratory carbon losses from microcosms receiving large amounts of herbivore inputs were nearly double (0.126 ± 0.007 g CO₂/m²/hr) those receiving no herbivore inputs (Table 3, Fig. 1b).

Nitrate loss from microcosms was unaffected by the CO₂ or O₃ treatments (Table 3, Fig. 2b, c) but was negatively related to input quantity. Nitrate concentrations of leachate from microcosms receiving small amounts of herbivore inputs was lower, but not significantly different from those receiving no herbivore inputs (72.5 ± 8.9 vs. 80.3 ± 6.9 mg/L; Fig. 2b). Nitrate concentrations of leachate from microcosms receiving large amounts of herbivore inputs were approximately half (38.5 ± 8.0 mg/L) those receiving low herbivore inputs. However, only GH and FGH

Fig. 1 Effects of FACE fumigation treatments (CO₂, O₃) and organic inputs (frass, greenfall) on soil respiration (± 1 SE). ANOVA results are presented in Table 3. **a** effects of fumigation treatments, pooled across microcosm treatments. **b** effects of microcosm treatment, pooled across fumigation treatments. **c** effects of individual fumigation and microcosm treatments. Abbreviations for microcosm treatments are provided in Table 1

inputs (not FH) were significantly different from no input (Table 3, Fig. 2b). We found a trend for a greater reduction in nitrate leaching in microcosms with greenfall compared with frass organic inputs across fumigation treatments (Fig. 2b, c). We also found a clear negative correlation between respiratory carbon loss and nitrate leaching across microcosm treatments (Fig. 3).

Relatively little ammonium nitrogen leached from the soil microcosms. The average concentration of NH₄-N in leachate across all microcosms was 0.058 ± 0.003 mg/L, only 1% of the concentration of nitrate. Neither input quality nor quantity influenced rates of ammonium leaching.

Fig. 2 Effects of FACE fumigation treatments (CO₂, O₃) and organic inputs (frass, greenfall) on nitrate concentration of leachate (± 1 SE). ANOVA results are presented in Table 3. **a** effects of fumigation treatments, pooled across microcosm treatments. **b** effects of microcosm treatment, pooled across fumigation treatments. **c** effects of individual fumigation and microcosm treatments. Abbreviations for microcosm treatments are provided in Table 1

Discussion

Effects of input quality and quantity on soil processes

Changes in frass and greenfall quality due to changes in atmospheric chemistry were sufficiently subtle that they did not significantly affect carbon and nitrogen mineralization. Differences in input quality, in terms of whether it was frass or greenfall, also had minor effects on soil carbon and nitrogen mineralization. In contrast, variation in the quantity of herbivore inputs strongly influenced carbon and nitrogen mineralization rates.

Fig. 3 Correlation of microbial respiration and nitrate leached for the 84 soil microcosms. Each point represents one microcosm

Elevated CO_2 and O_3 had relatively minor effects on frass and greenfall carbon and nitrogen concentrations. Condensed tannin levels increased in leaves exposed to eCO_2 and eO_3 . Caterpillar frass also had increased amounts of tannins at eCO_2 and eO_3 . Previous work with gypsy moth and forest tent caterpillars found that frass carbon to nitrogen ratios and condensed tannin levels reflected the carbon to nitrogen ratio and condensed tannin levels of the aspen foliage on which they fed (Madritch et al. 2007a). Frass of whitemarked tussock moth caterpillars fed birch condensed tannins also reflected the concentration of tannins in their food source (Kopper et al. 2002). These results suggest that if eCO_2 or eO_3 alter host plant chemical composition, frass chemical composition will be similarly altered. Altered chemical composition of frass, especially altered condensed tannin levels, could significantly alter soil processes because condensed tannins have been shown to reduce nutrient availability and slow leaf litter decomposition (Schweitzer et al. 2008) and alter soil respiration (Madritch et al. 2007b). Elevated CO_2 has been shown to reduce leaf litter quality (e.g., increased tannin concentrations and carbon to nitrogen ratios) at Aspen FACE, resulting in slower decomposition (Parsons et al. 2004, 2008). Similarly, lower frass or greenfall quality could impact soil processes, although the mostly weak effects of eCO_2 and eO_3 on input quality in this study were insufficient to do so.

Our results show that low levels of herbivore inputs (inputs from <20% leaf area removed) had a minor effect on soil microbial respiration, but high levels of herbivore inputs (inputs from >70% leaf area removed) nearly doubled microbial respiration. Pre-

vious studies suggest that small input levels can significantly alter soil processes, but do so less often than when inputs are large (e.g., during outbreaks) (Hunter et al. 2003). Only moderate or high input levels may provide enough labile carbon to allow detection of increased soil respiration. Both our results and those of Lovett and Ruesink (1995) support the concept that soil microbes do not respond unless frass inputs reach some carbon threshold. However, the response at even high input levels is likely to be short-lived (Frost and Hunter 2004). Soil temperature and precipitation events likely set the stage for the strength of the effect of herbivore inputs on soil microbe respiration rates. For example, high humidity or precipitation leaches frass carbon and nitrogen into the soil, while high temperature will increase microbial activity once inputs become available (Reynolds and Hunter 2001). Although we focused on microbial respiration, we recognize that part of the respiration we measured could be due to the frass microbial community and not the soil microbial community. We also recognize that the inclusion of leaf litter in our microcosms may have resulted in an early flush of CO_2 that masked smaller fumigation treatment effects on respiration.

Variation in input quantity also strongly influenced nitrate leaching rates. Nitrate leaching was relatively high at low-input levels, while microbial immobilization occurred at high-input levels. Frost and Hunter (2007) suggest that significant amounts of frass nitrogen may be leached if frass inputs are timed with precipitation, but otherwise the nitrogen will be incorporated into soil organic matter. Indeed, frass nitrogen may rapidly move into nonexchangeable soil pools near the soil surface (top 30 cm) (Christenson et al. 2002). It appears, therefore, that leaching losses likely occur shortly after deposition but most frass nitrogen is rapidly immobilized (Frost and Hunter 2007). Our results add that the quantity of organic input may alter whether nitrate is leached from, or immobilized in, the soil. The clear negative correlation we found between respiratory carbon loss and nitrate leaching across microcosm treatments strongly supports the concept that only moderate to high carbon inputs supply enough labile carbon for soil microbial populations to grow and increase nitrate immobilization (Fig. 3). Regardless, frass nitrogen inputs change the timing of nitrogen input to the forest floor and can alter the ultimate distribution of

nitrogen, even within the season of defoliation (Frost and Hunter 2004). For example, Frost and Hunter (2007) found that nitrogen from frass deposited early in the summer was taken up by plants within the same growing season. Approximately 1.2% of the frass nitrogen ended up in senescent leaves of the same trees at the end of the growing season, linking the fast (frass) and slow (senescent leaves) nutrient cycles. However, plants may acquire senesced leaf nitrogen more easily than frass nitrogen (Christenson et al. 2002).

Greenfall, respiration, and nitrate leaching

Our results suggest that, similar to large frass inputs, microbial respiration is enhanced by large inputs of greenfall. In another study, Reynolds and Hunter (2001) found reduced microbial respiration in forest soils where greenfall was excluded. While soil respiration was similar after frass and greenfall inputs, nitrate immobilization appeared to be slightly, although not significantly greater, for greenfall than for frass. The 65% higher nitrogen concentration of greenfall compared with frass in our study may have afforded greater microbial population growth and, thereby, increased immobilization. Reynolds and Hunter (2001) found no leaching of nitrate in their greenfall exclusion plots and suggested this may have been due to immobilization by roots or microbes. ^{15}N isotope studies have been useful for tracking frass N cycling and should be performed for greenfall given its high resource quality for microbes. However, as for frass, greenfall effects in the soil may be short-lived and dependent on temperature and precipitation.

Limitations

Microcosm studies are useful because they reduce environmental complexity and facilitate interpretation of results, but they are also less ecologically realistic (Carpenter 1996; Verhoef 1996). For example, our microcosms did not include detritivores or plant roots, and both will have important effects on soil respiration and nitrogen dynamics. Also, we did not consider other herbivore inputs, such as throughfall, which can influence soil processes (Stadler et al. 2001, 2006). We also recognize that some nutrients may have leached from our frass in the rain storm that occurred the day we collected frass. However, no other rain

events occurred during caterpillar rearing so very little leaching should have occurred. The nitrogen concentration of frass used in our study was approximately 1.0%, which is in line with values reported by Frost and Hunter (2007), but lower than the 2.4% documented by Lovett and Ruesink (1995) and the 1.8% reported by Madritch et al. (2007a). Our numbers may differ because results vary depending on the herbivore species used (Madritch et al. 2007a) and the time of sampling within a year. Insects feeding on early season foliage with greater concentrations of nitrogen and water and lower concentrations of defensive compounds (e.g., expanding leaves) could produce different results.

Implications of changes in herbivore input quantity

We found that soil processes were much more sensitive to the quantity of herbivore inputs than to the qualitative changes due to altered atmospheric chemistry. Liu et al. (2005) found that eCO_2 increased leaf litter inputs to the forest floor, and more recently that the increased inputs were more important to soil carbon and nitrogen cycles than were changes in litter chemistry due to eCO_2 (Liu et al. 2009). Larger inputs from the canopy also resulted in greater microbial respiration (King et al. 2004) and soil nitrogen concentration (Liu et al. 2009), in parallel with our large frass and greenfall inputs. These results suggest that altered atmospheric chemistry may have indirect effects on soil processes via changes in input quantity. Canopy damage rates at Aspen FACE have increased over 300% under eCO_2 , resulting in a more than 40% increase in frass and greenfall (Couture, Meehan, and Lindroth unpublished data). Thus, continued increases in eCO_2 in the future may increase the quantity of herbivore inputs, which may substantially impact nutrient cycling in forest soils.

Summary

Elevated CO_2 and eO_3 affected frass and greenfall quality, but the changes were minimal, producing little effect on short-term microbial respiration and no effect on nitrogen leaching. In contrast, large quantities of herbivore inputs nearly doubled microbial respiration and nitrogen immobilization compared with no inputs. Although microbial response to herbivore inputs may be relatively short-lived, herbi-

vore inputs are very important for carbon and nitrogen cycling, at least within the season of deposition. Changes in the abundance or feeding habits of canopy herbivores due to environmental change could lead to changes in soil carbon sequestration and nitrogen balance in forests of the future.

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Impacts of Elevated Atmospheric CO₂ and O₃ on Forests: Phytochemistry, Trophic Interactions, and Ecosystem Dynamics

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Abstract Prominent among the many factors now affecting the sustainability of forest ecosystems are anthropogenically-generated carbon dioxide (CO₂) and ozone (O₃). CO₂ is the substrate for photosynthesis and thus can accelerate tree growth, whereas O₃ is a highly reactive oxygen species and interferes with basic physiological functions. This review summarizes the impacts of CO₂ and O₃ on tree chemical composition and highlights the consequences thereof for trophic interactions and ecosystem dynamics. CO₂ and O₃ influence phytochemical composition by altering substrate availability and biochemical/physiological processes such as photosynthesis and defense signaling pathways. Growth of trees under enriched CO₂ generally leads to an increase in the C/N ratio, due to a decline in foliar nitrogen and concomitant increases in carbohydrates and phenolics. Terpenoid levels generally are not affected by atmospheric CO₂ concentration. O₃ triggers up-regulation of antioxidant defense pathways, leading to the production of simple phenolics and flavonoids (more so in angiosperms than gymnosperms). Tannins levels generally are unaffected, while terpenoids exhibit variable responses. In combination, CO₂ and O₃ exert both additive and interactive effects on tree chemical composition. CO₂- and O₃-mediated changes in plant chemistry influence host selection, individual performance (development, growth, reproduction), and population densities of herbivores (primarily phytophagous insects) and soil invertebrates. These changes can effect shifts in the amount and temporal pattern of forest canopy damage and organic substrate deposition. Decomposition rates of leaf

litter produced under elevated CO₂ and O₃ may or may not be altered, and can respond to both the independent and interactive effects of the pollutants. Overall, however, CO₂ and O₃ effects on decomposition will be influenced more by their impacts on the quantity, rather than quality, of litter produced. A prominent theme to emerge from this and related reviews is that the effects of elevated CO₂ and O₃ on plant chemistry and ecological interactions are highly context- and species-specific, thus frustrating attempts to identify general, global patterns. Many of the interactions that govern above- and below-ground community and ecosystem processes are chemically mediated, ultimately influencing terrestrial carbon sequestration and feeding back to influence atmospheric composition. Thus, the discipline of chemical ecology is fundamentally important for elucidating the impacts of humans on the health and sustainability of forest ecosystems. Future research should seek to increase the diversity of natural products, species, and biomes studied; incorporate long-term, multi-factor experiments; and employ a comprehensive “genes to ecosystems” perspective that couples genetic/genomic tools with the approaches of evolutionary and ecosystem ecology.

Keywords CO₂ · Decomposition · Ecosystem processes · Forest · Global change · Herbivory · Human impacts · Nutrient cycling · Ozone · Phenolics · Phytochemistry · Plant-insect interactions · Tannins · Terpenoids

Introduction

Forest ecosystems have played roles of unparalleled importance in the evolution and ecology of life on Earth. Currently, forests cover ~30% of the earth's land surface, store ~45% of terrestrial carbon, and contribute ~50% of

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terrestrial net primary production (Bonan 2008). They provide vital ecological, economic and social services, including regulation of biogeochemical cycles, production of food and fiber, preservation of biodiversity, governing of climate systems, and buffering against natural and anthropogenic disasters (Hassan et al. 2005; Millenium Ecosystem Assessment 2005).

Earth's forests are now subject to a veritable Pandora's Box of environmental evils, the magnitude and diversity of which have never occurred previously in human history. Worldwide, climate and land use changes, invasive species, pest outbreaks, and pollution are exacting an increasing toll on the health, biological diversity, and sustainability of forests (Laurence and Peres 2006; Hari and Kulmala 2008; Kurz et al. 2008; Raffa et al. 2008; van Mantgem et al. 2009). Against this backdrop of massive and seemingly intractably complex socio-environmental ills, the question arises: "of what relevance is chemical ecology?"

In a word, plenty. Naturally-produced chemicals, particularly phytochemicals, govern many, if not most, of the interactions that characterize community and ecosystem function. They *respond* to global environmental change; they *perpetuate*, via interaction networks, the consequences of global change; and they *feed back* to influence future global change. Consider, for example, the carbon cycle (Fig. 1). Enriched atmospheric CO₂ influences plant physiology, with consequences for plant production and chemical composition. Plant chemical composition in turn influences trophic interactions and decomposition, which ultimately feed back to affect atmospheric CO₂ concentrations (Peñuelas and Estiarte 1998; Beedlow et al. 2004; Lindroth and Dearing 2005). Or, consider the isoprenoids and related biogenic volatile organic carbons (BVOCs) released in large quantities by some species of trees (e.g., eucalypts, poplars, and conifers). These compounds are precursors for the formation of tropospheric ozone (O₃), which in turn negatively impacts plant production (Fig. 1) and human health (Fowler et al. 1999; Lerdau and Gray 2003; Monson 2003; Laothawornkitkul et al. 2009). An understanding of the roles of natural products as responders to, and effectors of, global change will improve our ability to predict ecosystem responses to future change, and inform management decisions with respect to mitigation and adaptation strategies.

Analogous in some respects to intestinal villi, forest trees provide structurally expansive and heterogeneous surfaces through which the exchange of chemicals between the atmosphere and biosphere is effected. The same properties enhance exposure of forest trees to gaseous pollutants. Two such gases, CO₂ and O₃, are considered the most important and ubiquitous, affecting forests worldwide (Saxe et al. 1998; Fowler et al. 1999; Felzer et al. 2004; Karnosky et al. 2005, 2007). Independent of their function as greenhouse

Fig. 1 Carbon cycling and storage in forest ecosystems. Solid boxes represent major pools; dashed boxes represent major plant physiological processes (adapted from Lindroth and Dearing 2005)

gases, CO₂ and O₃ are directly and indirectly affecting forest ecosystems now, with increasingly pronounced impacts predicted for the future (Fig. 2). (Note that although both CO₂ and O₃ occur naturally, they are appropriately considered "pollutants" because of their anthropogenic production and effects on living systems.)

The purpose of this review is to assess the impacts of enriched atmospheric CO₂ and O₃, independently and interactively, on the chemical ecology of forest ecosystems. Several recent reviews provide an assessment of the effects of CO₂ and O₃ on plant chemistry; that information will be summarized and updated here. I will then explore the consequences of CO₂- and O₃-mediated changes in tree chemistry for trophic interactions (plant—animal—natural enemy) and ecosystem dynamics (rates of plant damage, organic substrate deposition and decomposition) (Fig. 1). I will not detail the links between ozone and plant volatiles, as those have been covered in several very recent reviews, including elsewhere in this issue (Laothawornkitkul et al. 2009; Pinto et al. 2010; Yuan et al. 2009). Finally, although CO₂ and O₃ are both greenhouse gases, the effects of climate change per se on ecological interactions (e.g., Bale et al. 2002; Parmesan 2006; Berggren et al. 2009; Dukes et al. 2009; Pelini et al. 2009) are beyond the scope of this review.

Temporal Trends in Concentrations of CO₂ and O₃

Concentrations of atmospheric CO₂ now approach 386 ppm, higher than at any time in the last 26 million years (Pearson and Palmer 2000) and 40% higher than at the dawn of the industrial revolution. Emissions scenarios predict a range of substantially elevated concentrations by the end of this century, from a low of 550 ppm to a high of 900 ppm (Karl et al. 2009). Over the last several decades, combustion of fossil fuels, coupled with a smaller contribution from manufacture of cement, have accounted for

Fig. 2 Complex of interacting factors that feed forward and feed back to influence food webs, community and ecosystem structure and function, and atmospheric composition

approximately 80% of human-caused emissions. The remaining 20% has resulted from changes in land use, primarily deforestation (Denman et al. 2007; Karl et al. 2009).

Since the evolution and spread of large, vascular plants, about 380 MYA, forests have played prominent, regulating roles with respect to atmospheric CO₂ concentrations. They were largely responsible for the massive drawdown of atmospheric CO₂ (peaking at upwards of 5,000 ppm) in the late Devonian and Carboniferous Periods. Forests exerted two major effects on the global carbon cycle, both of which reduced atmospheric CO₂ concentrations (Berner 2005). First, they produced massive amounts of lignified tissue that was refractory to decomposition. This organic material, upon sedimentary burial, led to the formation of enormous coal deposits. Second, large and deep root systems greatly accelerated nutrient uptake from rock substrates, enhancing the weathering of Ca-Mg silicate minerals. Ca and Mg cations, in the presence of carbonic acid (derived from atmospheric CO₂ and water), formed Ca-Mg carbonates, which precipitated in ocean sediments to form limestone and dolomite.

Tropospheric ozone is recognized as the most damaging and widespread pollutant affecting agricultural and forest ecosystems in North America and Europe (Chameides et al. 1994; Ollinger et al. 1997; Skärby et al. 1998; Fuhrer and Booker 2003; Ashmore 2005; Karnosky et al. 2007; Wittig

et al. 2009). Concentrations of O₃ have increased an average of 38% (range of 20–50%) since the pre-industrial age (Denman et al. 2007). Although O₃ is a regional pollutant, large areas of the earth already are impacted, background levels are steadily increasing, and 60% of the world's forests are expected to be affected detrimentally by the end of this century (Fowler et al. 1999; Vingarzan 2004; Denman et al. 2007).

O₃ is a secondary pollutant, produced via the catalytic oxidation of hydrocarbons (e.g., VOCs) by OH radicals, in the presence of nitrogen oxides and sunlight (Wennberg and Dabdub 2008). Because O₃ is highly reactive, it persists for short periods (hours to several weeks) in the atmosphere. Rapid degradation, coupled with substantial spatial and temporal variation in production, contribute to highly variable distribution of this pollutant. Regional VOC and NO_x emission control policies are expected to reduce peak O₃ levels in North American and Europe, whereas in Asia and other regions, emissions of these precursors are increasing (Ashmore 2005). Moreover, background levels of O₃ (not attributable to local origin) are increasing steadily worldwide (Vingarzan 2004).

Plant-derived, biogenic VOCs can be important contributors to the formation of O₃ (Sharkey and Lerdau 1999; Lerdau and Gray 2003; Laothawornkitkul et al. 2009; Yuan et al. 2009). When BVOCs such as isoprene, monoterpenes or methylbutenol are oxidized in the presence of NO_x,

substantial amounts of O_3 can be produced. Indeed, large inputs of BVOCs may handicap efforts to reduce ozone pollution through controlling emissions of anthropogenic VOCs. Moreover, ecological interactions may amplify ozone production. A modeling study by Litvak et al. (1999) indicated that even modest (10%) damage by defoliating insects in coniferous forests is sufficient to increase local concentrations of O_3 .

In contrast, in the presence of low NO_x concentrations, BVOCs are oxidized by O_3 , leading to a decrease in atmospheric O_3 and a concomitant increase in secondary organic aerosols (Laothawornkitkul et al. 2009). Thus, forest ecosystems can serve as effective quenchers of anthropogenic O_3 , but in the process contribute to atmospheric aerosol loading that influences climate. Kurpius and Goldstein (2003) documented that for a *Pinus ponderosa* forest during summer, 51% of atmospheric O_3 is broken down via gas phase chemistry (reactions with BVOCs), 30% is lost to stomatal uptake, and 19% is lost to surface deposition.

Effects of CO_2 and O_3 on Plant Physiology and Growth

Carbon Dioxide

As the substrate for photosynthesis, and ultimate source of all carbon fixed via primary production, atmospheric CO_2 strongly influences plant physiology and growth. Photosynthesis is catalyzed by Rubisco (ribulose-1,5-bisphosphate carboxylase oxygenase), which fixes carbon to form carbohydrates. In C_3 plants, including most forest tree species, enriched CO_2 atmospheres increase the intracellular $CO_2:O_2$ ratio, thus reducing photorespiration (oxygenation) and increasing photosynthesis (carboxylation). Carbohydrates then are transported throughout the plant and subsequently converted to various carbon-containing compounds required for plant metabolism, structure, storage and defense (Fig. 3).

The effects of elevated atmospheric CO_2 on tree physiology and growth have been covered in numerous reviews (e.g., McGuire et al. 1995; Saxe et al. 1998; Long et al. 2004; Ainsworth and Long 2005; Norby et al. 2005; Körner 2006; Leakey et al. 2009). In brief, N use efficiency improves as the N allocated to Rubisco (the largest single pool of N in leaves) can be re-allocated to other metabolic processes. Stomatal conductance generally declines, decreasing transpiration and improving water use efficiency. Enhanced photosynthesis, coupled with improved nitrogen and water use efficiencies, often accelerates plant growth, i.e., the “fertilizer effect” of enriched CO_2 (Beedlow et al. 2004). The growth stimulation afforded by enriched CO_2 is especially strong in young forest stands, characterized by abundant soil resources and open canopies. In contrast,

studies in mature stands, where tree growth is tightly coupled to soil nutrients and canopies are closed, indicate smaller to zero growth enhancement (Asshoff et al. 2006; Körner 2006).

A key, unresolved issue in global change biology is to what extent improved tree growth can be sustained into the future, affording greater carbon sequestration and thereby mitigating climate change. To date, open-air FACE studies generally indicate that accelerated rates of photosynthesis and growth are sustained over the short to medium term and across a broad range of productivity (Long et al. 2004; Norby et al. 2005; Huang et al. 2007; but see Körner et al. 2005 for a contrary example with mature trees). Still, the duration of these studies is short compared with the lifespan of trees. The Progressive Nitrogen Limitation (PNL) hypothesis (Luo et al. 2004) posits that CO_2 -enhanced growth and carbon sequestration will diminish over time: as storage of N in plant biomass and soil organic matter increases, available soil N becomes limiting to growth. If not nitrogen, then a host of other factors (climate change, fire, insects, ozone, etc.) is predicted to constrain the fertilization effect of atmospheric CO_2 enrichment (Beedlow et al. 2004; Huang et al. 2007). For example, Cole et al. (2009) reported that atmospheric CO_2 enrichment over the last 50 years has increased growth rates of natural stands of *Populus tremuloides* by an astonishing 53%, but enhancement is diminished in periods of low precipitation. Finally, elevated atmospheric CO_2 may, via climate change impacts, ultimately lead to reduced carbon sequestration at the landscape level. In Canada, for example, extensive areas of forest recently have transitioned from CO_2 sinks to CO_2 sources, due to insect outbreaks and fires (Kurz et al. 2008).

Ozone

Reactions of tropospheric O_3 with plant chemistry can occur in gaseous, solid, or liquid phases (Fuhrer and Booker 2003). Gaseous reactions with plant VOCs are described by Pinto et al. (2010). Solid phase reactions may occur, for example, with plant cuticular components. The most important reactions for plants, however, occur in the liquid phase, after ozone has entered through the stomata and diffused into the aqueous media of intercellular spaces (Fuhrer and Booker 2003).

O_3 elicits a cascade of damaging physiological consequences in plants, described in detail in numerous recent reviews (e.g., Andersen 2003; Fuhrer and Booker 2003; Ashmore 2005; Kangasjärvi et al. 2005; Wittig et al. 2007, 2009). Because it is so highly reactive, O_3 is minimally distributed following dissolution into intercellular fluids. It reacts with lipid and protein components of cell walls and plasma membranes, leading to the formation of aldehydes, peroxides and assorted reactive oxygen species (ROS). These products

Fig. 3 Biosynthetic pathways for the production of the major classes of plant secondary compounds (adapted from Taiz and Zeiger 2002). Dashed lines indicate a control (signal transduction) pathway. PAR = photosynthetically active radiation

can then cause cell death, or activate various transduction pathways for defense responses, such as stomatal closure, production of anti-oxidants such as ascorbate and phenolics, and programmed cell death (a component of the hypersensitive response) (Fuhrer and Booker 2003; Valkama et al. 2007; Fig. 3). Reduced stomatal conductance, compromised photosynthesis, consumption of carbohydrates in localized tissue repair, and inhibition of phloem transport at the leaf level combine to decrease the supply of carbohydrates throughout the plant (Andersen 2003; Wittig et al. 2007, 2009). As a consequence, senescence is accelerated and whole-plant growth is inhibited.

A recent, comprehensive meta-analysis by Wittig et al. (2009) evaluated the effects of O_3 on the growth of trees representative of northern temperate and boreal forests. They found that, relative to preindustrial levels, current levels of tropospheric O_3 account for an average 7% reduction in tree biomass growth. Moreover, as O_3 levels continue to rise, further decreases of 11% and 17% are predicted for 2050 and 2100, respectively.

Understanding of the interactive effects of CO_2 and O_3 on tree growth is critically important to our ability to predict the efficacy of forests to serve as global carbon sinks in the future (Beedlow et al. 2004). A modeling study by Sitch et al. (2007) indicated that tropospheric O_3 may reduce terrestrial carbon sinks, and thereby effect indirect radiative forcing of climate change. This conclusion was subsequently corroborated by the work of Wittig

et al. (2009), which indicated increasingly pronounced reductions in tree growth under elevated concentrations of O_3 in the future. To date, only one experiment has addressed the long-term (10+ years) effects of independent and combined CO_2 and O_3 treatments on forest trees. Karnosky et al. (2005) reported that at Aspen FACE (Wisconsin, U.S.A.), O_3 reduced the growth-enhancement effect of CO_2 , although responses differed among tree species.

Effects of CO_2 and O_3 on Phytochemistry

CO_2 and O_3 directly and indirectly influence carbon assimilation and nutrient (e.g., N) acquisition, and thereby the pools and fluxes of key precursors for the production of secondary metabolites (Fig. 3). They also may influence the signal transduction pathways that govern gene expression and subsequent synthesis of secondary compounds. Not surprisingly then, growth under CO_2 - or O_3 -enriched atmospheres typically alters the chemical composition of forest trees.

Numerous reviews have addressed the effects of CO_2 and O_3 on plant chemistry (Kangasjärvi et al. 1994; Peñuelas et al. 1997; Bezemer and Jones 1998; Cotrufo et al. 1998a; Koricheva et al. 1998a; Peñuelas and Estiarte 1998; Norby et al. 2001; Zvereva and Kozlov 2006; Stiling and Cornelissen 2007; Valkama et al. 2007; Bidart-Bouzat and Imeh-

Nathaniel 2008; Wittig et al. 2009). Unless noted otherwise, the general trends for forest trees, summarized below, are drawn from that comprehensive set of reviews. Specific studies will be mentioned to illustrate particular patterns or highlight recent findings.

To date, research on the effects of CO₂ and O₃ on forest trees has been restricted to a small set of primarily temperate species. Most commonly studied are species of *Acer*, *Betula*, *Fagus*, *Pinus*, *Populus*, and *Quercus*. Although these are prominent, if not “foundation,” genera, a major challenge to the global change research community is to broaden the taxonomic and geographic representation of trees, especially tropical species.

Carbon Dioxide

Enriched atmospheric CO₂ produces generally consistent effects on concentrations of primary metabolites in trees (Cotrufo et al. 1998a; Koricheva et al. 1998a; Saxe et al. 1998; Stiling and Cornelissen 2007). Foliar concentrations of nitrogen typically decline (on average, 14–17% relative to ambient CO₂), due to reductions in Rubisco concentrations and dilution by accumulating carbohydrates. Levels of simple sugars may or may not increase, whereas levels of starch generally increase substantially.

Studies of the effects of CO₂ on secondary metabolites in trees have been restricted to “carbon-based” compounds, particularly phenolics and terpenoids. Consequently, this research often has been framed in the context of source-sink balance hypotheses [e.g., carbon-nutrient balance hypothesis (Bryant et al. 1983), growth-differentiation balance hypothesis (Herms and Mattson 1992)]. These hypotheses predict that elevated CO₂ will increase plant concentrations of carbon-based secondary compounds, especially if soil nutrient availability is limiting to growth (Peñuelas and Estiarte 1998).

In general, results have not been as readily predictable as the hypotheses would suggest (reviewed in Koricheva et al. 1998a; Peñuelas and Estiarte 1998; Zvereva and Kozlov 2006; Stiling and Cornelissen 2007; Bidart-Bouzat and Imeh-Nathaniel 2008). Moreover, the shikimic acid pathway, leading to production of phenolics, is more strongly influenced by CO₂ levels than are the mevalonic acid and methylerythritol phosphate pathways, leading to production of terpenoids (Fig. 3). Enriched atmospheric CO₂ typically, but not invariably, increases tannin concentrations. Common, but less consistent, are increases in simple phenolics, including phenolic acids and glycosides (e.g., salicylates). Growth under elevated CO₂ also tends to increase lignin concentrations in tree leaves (Coûteaux et al. 1999; Norby et al. 2001), although more recent results, from long-term FACE studies, have shown no such effects (Finzi et al. 2001; Parsons et al. 2008; Liu et al. 2009). Overall, effects

of CO₂ on terpenoid concentrations are minimal; although a few studies have revealed increases, most have found no change or decreases in concentrations.

The C/N ratio is a widely recognized, general index of overall tissue quality, relevant to both herbivory and decomposition. Enriched CO₂ atmospheres almost invariably increase C/N ratios in tree foliage (Coûteaux et al. 1999), due to a combination of decreased N concentrations and increased carbohydrate and phenolic concentrations.

Aside from such general indices, however, empirical studies have frustrated attempts to develop general, predictive models of the impacts of CO₂ on plant secondary chemistry. In short, a host of interacting factors have, over evolutionary time, combined to determine the chemical profiles of trees, and the effects of any one environmental factor can be masked by the direct or interactive effects of others.

One of the major confounding factors is taxon-specificity. Phytochemical responses of trees to CO₂ enrichment differ at the level of class (e.g., gymnosperm vs. angiosperm), species, and genotype (Julkunen-Tiitto et al. 1993; Lindroth et al. 2001, 2002; Zvereva and Kozlov 2006; Stiling and Cornelissen 2007; Bidart-Bouzat and Imeh-Nathaniel 2008). These differences represent taxonomic variation in evolutionary strategies that optimize solutions for the conflicting demands of carbon allocation toward growth, reproduction, and defense. For example, Cseke et al. (2009) recently compared leaf transcription profiles, physiology, and biochemistry between CO₂-responsive and unresponsive clones of *Populus tremuloides* grown at Aspen FACE. They found that the CO₂-responsive clone partitions carbon into pathways associated with “active” defense and stress responses, carbohydrate synthesis, and subsequent growth, whereas the unresponsive clone partitions carbon into pathways associated with cell wall compounds such as lignin, derived from the shikimic acid pathway.

Interactions with abiotic environmental factors also influence chemical responses of trees to CO₂. The availability of key resources (e.g., nutrients, water, light) can strongly shape plant chemical responses to CO₂, but, again, the magnitude and even direction of responses differ among chemical constituents and tree species (e.g., Lavola and Julkunen-Tiitto 1994; Kinney et al. 1997; McDonald et al. 1999; Booker and Maier 2001; Coley et al. 2002; Koike et al. 2006). Of particular importance in the context of global environmental change are potential interactions between atmospheric CO₂ and temperature. To that end, Zvereva and Kozlov (2006) conducted a meta-analysis of studies that assessed the simultaneous elevation of CO₂ and temperature on plant quality. Again, results were compound- and taxon-specific. Thus, responses to enriched CO₂ may be: 1) independent of temperature (e.g., foliar nitrogen and phenolics in Angiosperms); 2) offset by elevated temperature

(e.g., carbohydrates; foliar terpenes in gymnosperms); or 3) apparent only with elevated temperature (e.g., foliar nitrogen in gymnosperms, phenolics, and terpenoids in woody tissues). More recently, Veteli et al. (2007) reported for three tree species (*Betula* and *Salix*) that carbon allocation to phenolics was increased under elevated CO₂, decreased under elevated temperature, and unchanged under a combination of elevated CO₂ and temperature.

Interactions with biotic agents also may influence chemical responses of trees to CO₂, although much less research has been conducted with biotic than with abiotic factors. Herbivory frequently elicits induced chemical changes in trees, particularly in levels of phenolics (Nykänen and Koricheva 2004). Thus, enriched CO₂ atmospheres have been predicted to enhance induced chemical responses. Evidence to date, however, suggests that CO₂ does not modify induced chemical responses to defoliation in trees, although only a few studies have been conducted (Lindroth and Kinney 1998; Roth et al. 1998; Agrell et al. 1999; Rossi et al. 2004; Hall et al. 2005; Huttunen et al. 2008).

A final factor complicating attempts to draw general conclusions about the effects of CO₂ on tree chemistry is ontogeny (genetically-determined developmental changes). Many species of trees exhibit strong ontogenetic shifts in chemical composition (e.g., Bryant and Julkunen-Tiitto 1995; Donaldson et al. 2006), which reflect shifting physiological demands and defense strategies as trees mature (Boege and Marquis 2005). How ontogeny may interact with CO₂ to affect chemical profiles in trees is unknown. Such interactions may be one reason, however, for why chemical responses of plants in long-term FACE studies appear to be less pronounced than in short-term pot studies (Ainsworth and Long 2005; Lindroth, unpublished data).

Ozone

Elevated levels of tropospheric O₃ can modify plant chemical composition via several mechanisms. By disrupting photosynthesis, O₃ inhibits production of carbohydrates and the flow of precursors into pathways of primary and secondary metabolism (Fig. 3). Ozone also functions as a general abiotic elicitor of defense signaling pathways, particularly of phenolic compounds (Kangasjärvi et al. 1994; Heath 2008; Betz et al. 2009a). The two mechanisms may, of course, interact, as reductions in carbohydrate precursors for the shikimic acid pathway may constrain induced defensive responses. Moreover, these mechanisms may play out differently over different time scales, resulting in varying responses to acute versus chronic ozone stress.

The effects of O₃ on levels of primary metabolites vary among compounds and tree species. Levels of nutrients

(e.g., N, P, K, Ca) may increase, decrease, or not change in response to O₃ fumigation. Levels of simple sugars generally are unaffected, whereas levels of starch typically decline in angiosperms, but not gymnosperms.

As is true for CO₂, studies of the effects of O₃ on secondary compounds in trees have been restricted almost entirely to phenolics and terpenoids. In general, elevated O₃ leads to increases in concentrations of phenolic acids and flavonoids in angiosperms but not in gymnosperms, and no overall change in concentrations of tannins. Foliar lignin concentrations also are generally unresponsive to O₃ fumigation (Boerner and Rebbeck 1995; Booker et al. 1996; Liu et al. 2005; Oksanen et al. 2005). Effects of O₃ on terpenoid levels have been studied in only a few tree species, and results are species-specific: increases in *Pinus sylvestris* and *Populus* species, and no change in *Picea abies* (Valkama et al. 2007; Blande et al. 2007). Moreover, the magnitude of response varies among types of terpenes, with diterpenes responding more strongly to O₃ than mono- or sesquiterpenes.

That O₃ elicits increases in the levels of some carbon-based secondary metabolites without corresponding increases in carbohydrates runs counter to the predictions of source-sink balance hypotheses (e.g., Bryant et al. 1983), and may be explained by the fact that ozone triggers up-regulation of antioxidant defense systems linked to the shikimic acid pathway (Valkama et al. 2007). Ozone causes oxidative damage, coupled to the proliferation of oxygen radicals. Gene transcription and activity of numerous enzymes in the shikimic acid pathway are elevated in response to O₃ exposure, leading to the production of antioxidants such as flavonoids and other simple phenolics, and shifts in the monomeric composition of lignin (Kangasjärvi et al. 1994; Heath 2008; Betz et al. 2009a, b). Indeed, ozone-induced phenolics have been considered potential bioindicators of O₃ stress in plants under natural conditions (Sager et al. 2005; Bidart-Bouzat and Imeh-Nathaniel 2008).

Ozone exposure also influences the amount and chemical composition (e.g., alkyl esters and fatty acids vs. alkanes and alkanols) of cuticular waxes in tree leaves (Karnosky et al. 2002; Kontunen-Soppela et al. 2007; Percy et al. 2009). These changes in turn determine the physical structure of the cuticular surface, with repercussions for leaf surface properties such as wettability.

In contrast to a large body of research with CO₂, little work has addressed how O₃ pollution interacts with other environmental factors to shape plant chemical profiles. Lindroth et al. (1993a) evaluated the effects of elevated O₃ and light intensity on several foliar constituents of hybrid poplar and sugar maple (*Acer saccharum*), and found that O₃ and light interacted to affect only concentrations of N in maple. Given that forest ecosystems are exposed to

multiple, simultaneous stress factors (e.g., climate change, insect outbreaks) in addition to increasing O₃ damage, and that some of those stressors induce the same chemical defense pathway as does O₃, such research is sorely needed.

Interactions Between CO₂ and O₃

The environmental factor that has been investigated most thoroughly in combination with O₃ pollution is CO₂. Enriched CO₂ may reduce ozone damage to trees both by decreasing stomatal conductance (and consequent oxidative stress) and by increasing carbohydrate pools for the synthesis of antioxidant compounds. The recent meta-analysis by Valkama et al. (2007) evaluated the effects of O₃, and O₃ combined with CO₂, on foliar chemistry of 22 species of trees. They found that enriched CO₂ can offset O₃-induced chemical changes, but their analysis did not differentiate between simple additive, versus interactive, effects of CO₂ and O₃.

Indeed, CO₂ and O₃ exert both independent (additive) and interactive effects on tree chemical profiles. Elevated O₃ concentrations exacerbated the CO₂-mediated reductions in N concentrations in *Betula papyrifera* (Kopper et al. 2001) and *Populus tremuloides* (Holton et al. 2003), but offset reductions in N in *Betula pendula* (significant CO₂ × O₃ interactions). Peltonen et al. (2005) assessed the effects of CO₂ and O₃ on over thirty simple phenolics in *B. pendula*. They identified interactive effects in approximately three-quarters, and independent effects in one-quarter, of the cases. The interactive effects revealed that O₃-mediated induction of phenolics disappeared under elevated CO₂. Tannin levels also are influenced by both additive and interactive effects of CO₂ and O₃. Condensed tannin concentrations responded to the additive, independent effects of CO₂ and O₃ in *Fagus crenata* and various species of *Betula* (Kopper et al. 2001; Peltonen et al. 2005; Karonen et al. 2006), but to the interactive effects of CO₂ and O₃ in *Populus tremuloides* (Holton et al. 2003). Similarly, the effects of CO₂ and O₃ on monoterpenes and sesquiterpenes in *Pinus sylvestris* sometimes were additive and sometimes interactive (Sallas et al. 2001). In short, whether CO₂ and O₃ function in an additive or interactive manner depends on the particular chemical constituent and tree species studied.

Effects of CO₂ and O₃ on Trophic Interactions

Plant chemical composition is a strong ecological and evolutionary driver of trophic interactions, particularly between plants and herbivores. Given that green plants and phytophagous insects comprise nearly half of Earth's

recognized species (Strong et al. 1984), it is not surprising that these taxa occupy the vast majority of all studies on plant-herbivore interactions. Several recent reviews have addressed the effects of CO₂ and O₃ on plant-insect interactions (Zvereva and Kozlov 2006; Stiling and Cornelissen 2007; Valkama et al. 2007; Bidart-Bouzat and Imeh-Nathaniel 2008). Composite results will be summarized below and supplemented with additional, relevant studies of tree-feeding herbivores.

Carbon Dioxide

Little evidence exists to suggest that elevated CO₂, upwards of 1,000 ppm predicted for this century, will directly affect insects (Coviella and Trumble 1999). Work by Stange (1997) showed that atmospheric CO₂ concentrations influence host location by *Cactoblastis cactorum*, but no similar work has been done with forest insects. Rather, the major effect of enriched CO₂ on herbivores will be indirect, mediated via changes in plant quality (Fig. 2). Overall, elevated CO₂ reduces the quality of tree foliage as food, due to decreases in nitrogen (an index of protein) and minerals, and increases in carbohydrates and phenolics. These changes typically, but not always, alter the preference and reduce the performance of herbivores.

CO₂ concentration influences selection of plants by insects and mammals. Elevated CO₂ reduced leafminer oviposition on *Populus tremuloides* (Kopper and Lindroth 2003a), but increased birch aphid oviposition on one of two clones of *B. pendula* (Peltonen et al. 2006). The few feeding studies conducted to date suggest that preferences of insects for ambient versus elevated CO₂ foliage are not easily predicted, as shifts in both directions, and no change, have been reported (Traw et al. 1996; Kuokkanen et al. 2003; Agrell et al. 2005; Knepp et al. 2007). Elevated CO₂ decreased the palatability of winter-dormant *Betula* species to hares and rabbits (Mattson et al. 2004), but not to voles (Kuokkanen et al. 2004). Of greater relevance in the universally CO₂-enriched world of the future, however, is whether enrichment alters relative preferences of herbivores for various host species. Minimal evidence to date suggests that such is the case. For example, Agrell et al. (2005) demonstrated that forest tent caterpillars switch relative preferences between *Populus tremuloides* and *Betula papyrifera*, as well as between *P. tremuloides* genotypes, grown in high CO₂ environments.

Elevated CO₂ also influences the individual performance of herbivores, although the effects are highly species-specific for both trees and insects. Among the more uniform (though still variable) of responses is food consumption. In general, insects increase both consumption rates, and total consumption, on CO₂-enriched foliage, ostensibly as a means to compensate for reduced N concentrations. Compensatory

feeding responses may be constrained, however, by the simultaneous consumption of higher concentrations of secondary metabolites such as phenolics. Approximate digestibility of food typically is not significantly affected, but conversion efficiencies of digested food into biomass generally decline. Little is known about how CO₂ environment affects insect detoxication capacities; a study with gypsy moths revealed enhanced oxidase, reductase, and esterase activities in larvae fed CO₂-enriched *P. tremuloides*, but not *Acer saccharum* (Lindroth et al. 1993b). Development times usually are prolonged, while growth rates and final pupal and adult weights decline or are unaffected, but rarely improve. Effects on reproduction have rarely been investigated. Lindroth et al. (1997) found no effect of CO₂ on the number and mass of eggs produced by gypsy moths, whereas Awmack et al. (2004) reported decreased fecundity in aphids on *B. papyrifera* under elevated CO₂.

Species-specificity in terms of insect herbivore responses to enriched CO₂ may be linked to differences among feeding guilds (e.g., chewers, miners, sap-feeders, seed-feeders). Bezemer and Jones (1998) identified such guild effects for plants and phytophagous insects in general. Too few studies have been conducted with tree-feeding insects to evaluate differences among guilds. However, the minimal, if not positive effects of CO₂ on tree-feeding aphids (Docherty et al. 1997; Awmack et al. 2004), in contrast to effects on chewing insects, suggest that guild-specific responses to CO₂ may exist for forest insects.

Just as interactions with various environmental factors influence the effects of CO₂ on plant chemistry, so may they modulate effects on herbivores. For example, the effects of elevated CO₂ on herbivore performance can be ameliorated or exacerbated by the availability of light, water, and soil nutrients (e.g., Kinney et al. 1997; Lawler et al. 1997; Roth et al. 1997; Hättenschwiler and Schafellner 1999; Agrell et al. 2000). Potential interactions with climate warming are of particular interest, as warm temperatures generally affect insects in a manner opposite that of CO₂, i.e., accelerated development and enhanced growth and reproductive performance (Bale et al. 2002). Indeed, Zvereva and Kozlov (2006) conclude from their recent meta-analysis that temperature increases are likely to mitigate predicted negative effects of enriched CO₂ on insects, and emphasize the need for future research to address the two factors in parallel.

In addition to the bottom-up effects of altered plant chemistry, elevated CO₂ may influence the top-down effects of natural enemies (predators, parasitoids, and pathogens) on insect herbivores (Fig. 2). Enriched CO₂ could affect natural enemies by altering the behavioral or physiological defenses of prey, modifying the quality of prey (e.g., insect size, concentrations of plant-derived toxins), or by altering the efficacy of host location and disease transmission. Few studies have addressed these

possibilities, however, especially with forest systems. Elevated atmospheric CO₂ dramatically diminished the pheromone-mediated, predator escape behavior of aphids on *Populus tremuloides* (Mondor et al. 2004). In contrast, two studies have found the bottom-up effects of CO₂ on parasitoid performance (survivorship, development, and growth) to be minimal to nil (Roth and Lindroth 1995; Holton et al. 2003). The virulence of pathogens such as nucleopolyhedrosis virus (NPV) is linked to host plant chemistry [e.g., tannins (Foster et al. 1992)], so CO₂-enriched atmospheres may influence the dynamics of disease outbreaks. CO₂-induced increases in condensed tannins in *P. tremuloides* did not, however, alter the susceptibility of gypsy moths to NPV (Lindroth et al. 1997). At this time insufficient information is available to predict whether the bottom-up effects of CO₂ on natural enemies will in general be buffered, amplified, or, more likely, context- and species-specific.

The impacts of elevated CO₂ concentrations on forest insects at the levels of populations and communities will be determined by a combination of bottom-up, top-down, and abiotic factors (Fig. 2). Historically, inferences about population-level responses have been drawn from studies of individual insects, but such conclusions can be misleading (Awmack et al. 2004). To date, however, few studies have addressed the impacts of elevated CO₂ at higher levels of organization. Several studies with tree-feeding aphids have shown no population-level effects (Docherty et al. 1997; Awmack et al. 2004), while another revealed positive effects of enriched CO₂ on aphid population size (Percy et al. 2002). In contrast, Stiling et al. (1999, 2002, 2003, 2009) found reduced densities (number per leaf) of herbivores, especially leafminers and leaf-tiers, in several woody species of a scrub-oak community exposed to elevated CO₂. Reduced herbivore densities were attributed to higher rates of both plant- and parasitoid-induced mortality, and were offset in several *Quercus* species by increased foliar production under high CO₂ (Stiling et al. 2009). Monitoring of aspen blotch leafminers (*Phyllonorycter tremuloidiella*) at Aspen-FACE indicated that elevated CO₂ roughly doubled plant-mediated mortality, substantially reduced predator-mediated mortality, and minimally influenced parasitoid-mediated mortality (Fig. 4).

Even fewer studies have addressed the effects of enriched CO₂ on speciose insect communities. Following 4 years of pan-trapping at Aspen FACE, Hillstrom and Lindroth (2008) reported that elevated CO₂ reduced abundance of phloem-feeding herbivores and increased abundance of chewing herbivores, although results were only marginally significant. Enriched CO₂ increased numbers of figitid and ichneumonid parasitoids, but not of braconid or chalcid parasitoids. Insect community composition differed between ambient and elevated CO₂ plots in 3 out of 4 years. In the most comprehensive study to

Fig. 4 Effects of CO₂ and O₃, in isolation and combination, on plant-, predator-, and parasitoid-induced mortality of aspen blotch leafminers (*Phyllonorycter tremuloidiella*). Data reveal major sources of mortality, prior to adult emergence, for miners on *Populus tremuloides*. Numerals below bars indicate aspen genotypes. Observations recorded in July 2001 at the Aspen FACE research site (Awmack and Lindroth, unpublished data)

date for forest canopy insects, Hillstrom and Lindroth (unpublished data) visually monitored insects on *Populus tremuloides* and *Betula papyrifera* over 3 years at Aspen FACE, cataloging over 36,000 insects from nearly 300 species. Overall, enriched CO₂ tended to increase the abundance of phloem-feeders and decrease the abundance of chewers and gallers on *P. tremuloides*, although effects were variable among species and over time. Few consistent effects on abundance were found, however, for insects on *B. papyrifera*. Elevated CO₂ did not affect species diversity of canopy insects, except for insects on *P. tremuloides* in 1 year. Elevated CO₂ also did not influence insect community composition, except for insects on *B. papyrifera* in 1 year.

Ozone

Increasing evidence suggests that tropospheric ozone pollution may affect directly insects. Gate et al. (1995) found that under elevated O₃, searching efficiency of a hymenopteran parasitoid, as well as the proportion of hosts parasitized, declined. O₃ likely interacts with the complex of BVOCs that function as “infochemicals” to govern key interactions between insects and both their hosts (plant or insect) and natural enemies (predators and parasitoids) (Laotawornkitkul et al. 2009; Yuan et al. 2009; Pinto et al. 2010, this volume). Chemical degradation or transformation of BVOCs by O₃ is likely to increasingly disrupt olfactory cues in atmospheres of the future.

Selection of plants as oviposition or food hosts is influenced by O₃ concentrations. O₃-treated foliage reduced

leaf beetle oviposition onto *Populus deltoides* (Jones and Coleman 1988) and leafminer oviposition onto *Populus tremuloides* (Kopper and Lindroth 2003a). Few feeding studies have compared the preferences of insects for fumigated vs. nonfumigated foliage, and those indicate a range of responses. Leaf beetles preferred O₃-treated *P. deltoides* (Jones and Coleman 1988) and common leaf weevils preferred O₃-treated hybrid aspen (Freiwald et al. 2008), whereas forest tent caterpillars preferred untreated *P. tremuloides* (Agrell et al. 2005). Forest tent caterpillars greatly increased their preference for *Betula papyrifera*, relative to *P. tremuloides*, under elevated O₃, and also shifted their preferences among *P. tremuloides* genotypes (Agrell et al. 2005).

O₃ fumigation changes the individual performance of phytophagous insects, although, as for CO₂, effects are highly species-specific. The meta-analysis by Valkama et al. (2007) summarized results for 22 species of trees and ten species of insects. Food consumption rates typically are unaffected, as are survival and reproduction rates. Development times usually are reduced, and pupal masses increased, under elevated O₃. For example, Kopper and Lindroth (2003b) reported that for forest tent caterpillars reared on high-O₃ *Populus tremuloides*, development times decreased an average of 14% and pupal weights increased an average of 31% compared with insects on control trees. Relative growth rates of chewing, but not sucking, insects generally increase under elevated O₃. On average, insect fecundity is not affected by O₃, although Awmack et al. (2004) reported decreased fecundity for aphids on *Betula papyrifera*.

Under natural conditions, interactions between trees and herbivores likely are influenced by multiple, interacting stressors, including O₃ (Koricheva et al. 1998b). With the exception of interactions with CO₂ (addressed below), however, exceedingly little is known about how O₃ may interact with other environmental factors to influence trophic interactions. Lindroth et al. (1993a) reported that gypsy moth larval performance was influenced by the independent, but not interactive, effects of O₃ and light intensity. With the likelihood of increased pest damage to forests under future scenarios of global environmental change, assessments of the combined effects of ozone and other stressors should be a high priority.

A growing body of literature reveals that O₃ pollution likely will affect interactions between tree-feeding insects and their natural enemies. As indicated above and described elsewhere in detail (Yuan et al. 2009; Pinto et al. 2010), O₃ may directly affect the behavior and fitness of predators and parasitoids. Alternatively, elevated O₃ may alter the behavioral or physiological defenses of prey, or their quality as food. For example, Mondor et al. (2004) reported that at Aspen FACE, high O₃ environments markedly

enhanced predator escape behaviors of aphids on *Populus tremuloides*. Also at Aspen FACE, O₃ fumigation reduced survivorship of the parasitoid *Compsilura concinnata* (Holton et al. 2003). Decreased fitness was consistent with improved performance of its forest tent caterpillar host, which in turn was linked to decreased levels of phenolic glycosides in *P. tremuloides*.

Despite accumulating evidence for impacts of O₃ at the level of individual insect performance, little work has addressed impacts at the level of populations. Awmack et al. (2004) found that O₃ fumigation at Aspen FACE increased population densities of aphids on *Betula papyrifera* when protected from natural enemies, but not when unprotected. Also at Aspen FACE, elevated O₃ levels tended to increase plant-mediated mortality, reduce predator-mediated mortality, and increase parasitoid-mediated mortality of aspen blotch leafminers (*Phyllonorycter tremuloidiella*), although magnitudes of effects varied among insects on different aspen genotypes (Fig. 4).

At the level of insect communities, pan-trapping at Aspen FACE revealed positive effects of elevated O₃ on abundance of some phloem-feeding insects, and strong suppressive effects on a diversity of parasitoids (Hillstrom and Lindroth 2008). O₃ influenced the community composition of insects captured by traps in 2 out of 4 years. In our more recent visual censuses of canopy insects at Aspen FACE, we found that O₃ fumigation increased abundances of several species of chewing insects, but decreased numbers of several phloem-feeders, on *Populus tremuloides* (Hillstrom and Lindroth, unpublished data). O₃ did not, however, consistently affect abundances of insects on *Betula papyrifera*. O₃ also had generally little impact on the species diversity and community composition of canopy insects on several tree species, and across multiple years, at Aspen FACE.

Interactions Between CO₂ and O₃

Valkama et al. (2007) sought to differentiate the effects of O₃ alone, versus O₃ in combination with CO₂, on the performance of tree-feeding insects. Their meta-analysis revealed that many of the positive effects of O₃ on insect performance, especially for leaf chewers, are negated with simultaneous exposure to enriched CO₂. Their review did not, however, differentiate between simple additive, versus truly interactive, effects of CO₂ and O₃ on insects.

Indeed, relatively few studies have been designed to detect potential interactive effects of CO₂ and O₃ on forest insect performance, and those have shown mixed results. Several studies (Awmack et al. 2004; Peltonen et al. 2006) have assessed the independent and combined effects of CO₂ and O₃ on feeding, growth, and reproductive performance of aphids, and revealed no significant interactions. Similar-

ly, no significant interactions were observed with respect to CO₂ and O₃ effects on leafminers on *Populus tremuloides* (Kopper and Lindroth 2003a), whitemarked tussock moths feeding on *Betula papyrifera* (Kopper et al. 2001), or forest tent caterpillar feeding preferences for *P. tremuloides* and *B. papyrifera* (Agrell et al. 2005). In contrast, CO₂ and O₃ interacted to influence development and growth of forest tent caterpillars on *P. tremuloides*: O₃-mediated enhancements in performance were greater at ambient than elevated CO₂. In short, the limited evidence to date suggests that the combined effects of CO₂ and O₃ on insect herbivores will be primarily additive, although the standard caveat of context- and species-specificity applies.

Effects of CO₂ and O₃ on Ecosystem Dynamics

At the level of forest ecosystems, plant chemistry plays a host of important roles, the most prominent of which arguably is regulation of the transfer of fixed carbon to herbivores and decomposers—the dominant pathways of material flow (Fig. 1). Accumulating evidence suggests that elevated concentrations of CO₂ and O₃ will modify the complex of physiological and ecological interactions that determine rates of plant damage, organic substrate deposition, and nutrient cycling. In short, the effects of CO₂ and O₃ on plant chemistry will in turn influence the sequestration and cycling of carbon and nutrients in forest ecosystems.

Forest Canopy Damage

Phytophagous insects generally remove 2–15% of primary production in temperate deciduous forests (Cebrian 1999; Cyr and Pace 1993; Cebrian and Lartigue 2004), although during outbreaks, nearly 100% can be consumed (e.g., Donaldson and Lindroth 2008). Canopy damage rates are a function of the host preferences, individual consumption rates, population densities, and community composition of herbivorous insects, all of which can be influenced by levels of atmospheric CO₂ and O₃.

The effects of CO₂ on loss of primary production to herbivores have been explored in only a few forest systems. In a Florida scrub oak community, enriched CO₂ decreased the frequency of damage to *Quercus myrtifolia* (Stiling et al. 2002). Later work in the same system documented a decline in the frequency of damage by 4 of 6 insect feeding groups, across three *Quercus* species, under elevated CO₂ (Hall et al. 2005). Working with four understory tree species at the FACTS-1 FACE site (loblolly pine—hardwood forest), Hamilton et al. (2004) reported declines of 10–46% in foliar damage rates in high CO₂ plots. These declines were driven largely, however, by the

response of one species (*Ulmus alata*); damage rates in other species were not statistically significant from those of control trees. Knepp et al. (2005) followed up that work by censusing foliar damage rates in twelve species of potted saplings at FACTS-1 FACE. Enriched CO₂ reduced damage rates across all species in 2001, but not in 2002–2003. In the most comprehensive study to date, Couture, Meehan, and Lindroth (unpublished data) evaluated damage rates on leaves collected from *Populus tremuloides* and *Betula papyrifera* over 3 years at Aspen FACE. Elevated CO₂ concentrations did not markedly affect herbivory rates in 1 year, but increased damage rates by 2–3-fold in 2 years of the study. Overall, these studies indicate that canopy damage rates are likely to change under CO₂ concentrations of the future. But, consistent with effects on individual insects, the magnitude and direction of change will be both species-specific and temporally variable.

To date, no published study has evaluated the effects of elevated tropospheric O₃ on community-wide, canopy damage in forest ecosystems. Our work at Aspen FACE (Couture, Meehan and Lindroth, unpublished data) revealed that high levels of O₃ reduced damage to both *Populus tremuloides* and *Betula papyrifera* in 2 of 3 years.

Calculations of losses to primary production based on leaf areas damaged by herbivores may significantly underestimate true reductions in production. Aldea et al. (2006) showed that in a variety of deciduous tree species, chewing damage caused modest and localized suppression of photosynthetic efficiency in adjacent, undamaged leaf tissue, whereas galling damage caused large and extensive suppression. The authors suggest that the spatial propagation of reduced photosynthesis may be influenced by enriched CO₂, but did not observe such effects in their study. More recently, however, P. Nabity and M. Hillstrom (pers. comm.) found that elevated CO₂ decreased the propagation of reduced photosynthesis caused by insects feeding on *Populus tremuloides* and *Betula papyrifera* at Aspen FACE.

Organic Substrate Deposition

Herbivorous insects mediate the transfer of organic materials from the forest canopy to the forest floor by depositing frass, greenfall, and insect biomass, and altering the timing and amounts of leachate throughfall and leaf litterfall. These processes have received little attention in the context of global change research.

We recently evaluated the effects of fumigation treatments at Aspen FACE on the quality of frass produced by whitemarked tussock moth larvae feeding on *Populus tremuloides*, and the effects of both frass and greenfall on soil microbial respiration and nitrogen leaching (Hillstrom, Meehan, Kelly and Lindroth, unpublished data). Insect frass

had slightly decreased N levels in high O₃ environments, and increased tannin levels in high CO₂ environments. However, neither frass nor greenfall quality affected microbial respiration (CO₂ efflux) or nitrogen (NO₃[−]) leaching in soil microcosms. In contrast, the quantity of frass and greenfall added to microcosms markedly affected both respiration and N leaching, under all atmospheric conditions. Thus, insects may affect carbon and nitrogen mineralization in forests of the future more through their effects on the quantity (and timing), than quality, of substrate deposition.

Indeed, atmospheric CO₂ and O₃ concentrations appear to influence rates of insect-mediated organic substrate deposition in forests. At Aspen FACE, rates of combined frass and greenfall deposition were 35% higher in elevated vs. ambient CO₂ stands, and 13% lower in elevated vs. ambient O₃ stands, over three field seasons (Meehan, Couture, Bennett and Lindroth, unpublished data).

Finally, insect feeding and atmospheric composition both may alter the timing of litterfall in forest ecosystems. In general, feeding damage accelerates leaf abscission, whereas enriched CO₂ may either accelerate (Stiling et al. 2002) or decelerate (Karnosky et al. 2003) abscission rates. How herbivory and atmospheric chemistry may interact to influence the magnitude and timing of leaf abscission is unknown.

Soil Invertebrates

Soil invertebrates play important roles in the nutrient cycling dynamics of forest ecosystems, from the comminution and microbial inoculation of plant litter to the vertical transport of organic matter through soil horizons. These processes are governed both by the quality and quantity of litterfall, which in turn are influenced by atmospheric composition (Fig. 1).

Several studies have evaluated the effects of leaf litter from elevated CO₂ plots on feeding preferences of isopods. Using short-term (3 day) feeding choice studies, Cotrufo et al. (1998b) found that isopods (*Oniscus asellus*) consumed 16% less high-CO₂ litter than ambient-CO₂ litter from *Fraxinus excelsior*. Subsequently, Cotrufo et al. (2005a) fed isopods (*Porcellio* sp.) for 2 weeks on litter from six tree species grown under ambient and elevated CO₂, and found no difference in palatability for five species, and increased preference for high-CO₂ litter for one species. Using no-choice feeding studies and two species of isopods, Hättenschwiler et al. (1999) found increased consumption of high-CO₂ beech litter, but no effect of CO₂ on consumption of spruce litter. Hättenschwiler and Bretscher (2001) reported that growth of trees under elevated CO₂ altered the relative preference of *O. asellus* for different litter species—increasing preference for one, decreasing

preference for another, and not affecting yet another. Thus, as is the case for insects feeding on green leaves, the consequences of CO₂ for detritus-feeding invertebrates are species-specific.

Additional studies have assessed the effects of leaf litter from elevated CO₂ or O₃ environments on fitness indices and population growth of soil invertebrates. Individual growth of juvenile earthworms (*Lumbricus terrestris*) was reduced when fed high-CO₂ or high-O₃ birch (*Betula pendula*) litter (Kasurinen et al. 2007), and high-CO₂ (but not high-O₃) aspen (*P. tremuloides*) litter (Meehan, Crossley and Lindroth, unpublished data). Growth and mortality rates of isopods, however, generally have been shown not to be affected by CO₂ or O₃ treatments (Hättenschwiler and Bretscher 2001; Kasurinen et al. 2007). Population growth of collembola (*Sinella curviseta*) decreased when reared on high-CO₂ aspen litter, but increased when reared on high-O₃ aspen litter (Meehan, Crossley and Lindroth, unpublished data).

Several studies have evaluated the effects of CO₂ and O₃ on soil invertebrate communities under field conditions. Hansen et al. (2001) investigated the effects of elevated CO₂ concentrations on soil microarthropods at the FACTS-1 FACE site. Over the first 19 months of CO₂ treatment, total microarthropod abundance declined by 34% in high-CO₂ plots, relative to ambient plots. The decline was driven by changes in abundance of oribatid mites, and was attributed to factors other than changes in litter quality. Haimi et al. (2005) censused populations of enchytraeids, mites and collembola in soil samples surrounding chambered *Pinus sylvestris* trees. Elevated CO₂ concentrations contributed to low numbers of acaridid mites, but did not significantly affect other soil fauna. Loranger et al. (2004) assessed responses of soil invertebrates to CO₂ and O₃ enrichment at Aspen FACE. They found that numbers of collembola and Oribatid mites, but not various other soil animals, declined in elevated CO₂ plots. Mites, but not other fauna, also decreased in high O₃ plots.

In summary, CO₂- and O₃-mediated changes in litter quality are likely to influence the performance of soil invertebrates of forests in the future. Some evidence exists to suggest that reduced litter quality will negatively affect the fitness of soil animals. In turn, their roles in nutrient cycling and soil carbon sequestration are likely to be altered. Overall, however, as noted by Couteaux and Bolger (2000), no general patterns of response have yet emerged, likely due to the species- and context-specific nature of these interactions.

Litter Decomposition

Rates of litter decomposition and attendant nutrient mineralization are governed primarily by climate, litter chemistry,

and soil organisms. Climate (especially temperature and moisture) is the most important factor on a global scale, whereas chemical composition is most influential within a geographic region (Aerts 1997). All of these regulating factors are influenced directly, or indirectly, by CO₂ and O₃ (Fig. 1).

Litter quality is determined by carbon availability (which ranges from labile sugars to recalcitrant lignin), mineral content, and chemical modifiers such as tannins (Swift et al. 1979; Anderson 1991). Tannins and related phenolic constituents alter decomposition by complexing with other litter components (e.g., amino acids) and/or by inhibiting the degradative activity of enzymes and soil fauna (Horner et al. 1988; Anderson 1991; Couteaux et al. 1995). For similar litter types in common environments, nitrogen, lignin, and tannin concentrations appear to act as “a hierarchical series of controls” (Anderson 1991). In high quality, readily decomposable litters, tannins may be rate retardants, whereas in low quality, slowly decomposable litters, lignin concentration, or lignin: N ratio typically determine decomposition rates (Anderson 1991). Any factor that markedly affects the nitrogen or C-based secondary metabolite concentration of litter also will affect decomposition, and consequently, nutrient availability (Chapin 1991).

The “litter quality” hypothesis, introduced by Strain and Bazzaz (1983), proposed that CO₂ enrichment will decrease plant nitrogen (relative to carbon) concentrations, and thereby reduce litter decomposition and soil N availability. Progressive soil nitrogen limitation is anticipated to eventually feed back to reduce plant production (Luo et al. 2004; Johnson 2006). Thus, CO₂-mediated changes in the chemical quality of litter have been of key interest with respect to understanding the cycling and storage of carbon in forest ecosystems. Indeed, litter from trees grown under elevated CO₂ characteristically exhibits reduced N concentrations and increased lignin concentrations and C:N ratios (Ceulemans et al. 1999; Norby et al. 2001).

To date, however, studies of the effects of elevated CO₂ on litter decomposition (measured as mass loss or microbial respiration) have not demonstrated a consistent direction of response. A comprehensive meta-analysis by Norby et al. (2001), including data for 33 woody species, showed that data do not support the “litter quality” hypothesis of reduced decomposition rates for litter produced under elevated CO₂. Decomposition studies published since that review have been increasingly realistic, employing more long-term and field-based approaches with open-top chambers or FACE sites. Still, a consistent pattern has yet to emerge. Several studies have documented reduced decomposition of leaf litter produced under elevated CO₂. For example, litter from three *Populus* species grown under elevated CO₂ at POPFACE exhibited reduced decomposi-

tion rates (Cotrufo et al. 2005b), as did litter from *Betula papyrifera* and *Populus tremuloides* from young (sapling) forest stands grown under elevated CO₂ at Aspen FACE (Parsons et al. 2004, 2008). In contrast, CO₂ growth environment did not alter subsequent litter decomposition rates for *Quercus myrtifolia* in a scrub oak community (Hall et al. 2006), for five tree species at the FACTS-1 FACE site (Finzi et al. 2001), or for *B. papyrifera* and *P. tremuloides* in older, closed-canopy stands at Aspen FACE (Liu et al. 2009). Diverse responses among experiments have been attributed to a number of factors, including soil fertility, decomposition environment, CO₂ exposure mechanism, study duration, stand development, and, genotype- and species-specific effects (Norby et al. 2001; Kasurinen et al. 2006; Hall et al. 2006; Liu et al. 2009).

Relatively little research has investigated the effects of atmospheric O₃ on litter quality and decomposition. Growth of three species of deciduous tree seedlings (Boerner and Rebbeck 1995; Scherzer et al. 1998) and two species of *Pinus* seedlings and saplings (Scherzer et al. 1998; Kainulainen et al. 2003) under elevated O₃ did not influence subsequent decomposition rates of leaf litter. Findlay et al. (1996), however, found that cottonwood leaves produced under high O₃ levels exhibited reduced decomposition rates (in aquatic microcosms), which were linked to increased phenolic concentrations. Similarly, Kasurinen et al. (2006) observed reduced decomposition in *Betula pendula* leaves produced under high O₃ concentrations. Parsons et al. (2008) found that early in stand development at Aspen FACE, growth under elevated O₃ reduced 2-year decomposition rates of *Populus tremuloides* litter, independent of CO₂ treatment. In contrast, elevated O₃ accelerated decomposition of *Betula papyrifera* litter from ambient CO₂ treatments, but decreased decomposition of litter from enriched CO₂ treatments (a significant O₃ × CO₂ interaction). Later work at Aspen FACE, however, showed that elevated O₃ decreased the decomposition of *P. tremuloides* and *B. papyrifera* litter from closed-canopy stands during only the first year of a 2.6-year study (Liu et al. 2009). As is the case for atmospheric CO₂, the effects of elevated O₃ on litter decomposition appear to be species-, age-, and context-specific.

Although too early for definitive conclusions, the developing scientific consensus is that atmospheric CO₂ and O₃ will affect litter decomposition and subsequent nutrient cycling more through their effects on the quantity, than quality, of foliage produced, and by shifting the species composition of forests (Finzi and Schlesinger 2002; Cotrufo et al. 2005b; Liu et al. 2007, 2009; Hillstrom, Meehan, Kelly and Lindroth, unpublished data). For example, soil carbon sequestration at Aspen FACE appears to be more closely related to CO₂- and O₃-mediated changes in input quantity and species composition than to

qualitative changes in litter chemical composition (Loya et al. 2003; Liu et al. 2007, 2009).

Conclusions and Recommendations for Future Research

Human-induced changes in the levels of atmospheric CO₂ and O₃ clearly influence the quantity and quality of primary production. In turn, changes in plant chemical composition cascade through above- and below-ground ecological interactions (e.g., herbivory, decomposition) to alter ecosystem structure and function, ultimately feeding back to affect terrestrial carbon sequestration and atmospheric composition. Many of these interactions are governed by processes that are fundamentally chemical in nature (double entendre intended). Thus, chemical ecologists have much to offer in terms of elucidating the mechanisms that integrate biological organization—from genomes to ecosystems—in a globally changing world.

An admittedly frustrating theme to emerge from this and other recent reviews (e.g., Stiling and Cornelissen 2007; Bidart-Bouzat and Imeh-Nathaniel 2008; Tylianakis et al. 2008) of global environmental change is that the effects of any single change factor on any particular ecological interaction are highly variable. Responses tend to be species- and context-specific, and thus appear idiosyncratic. Global change science must advance considerably further before predictions about impacts on natural systems can be made with accuracy and precision. The work of chemical ecologists should be central to such efforts: it will provide mechanistic insight into ecosystem function and provide data critical to the accurate parameterization of global change and risk assessment models.

Below I provide a non-exhaustive list of some of the more pressing needs for the contributions of chemical ecologists toward furthering our understanding of the effects of CO₂ and O₃ on forest ecosystems. Funding of large, long-term, multi-investigator projects is essential to the success of these efforts.

1. Increase the diversity of natural products studied. Our understanding of the effects of global change on the phytochemistry of forest ecosystems is based on a few classes of chemical compounds, primarily phenolics and terpenoids. Almost nothing is known about effects on other classes of secondary metabolites important to at least some tree species, e.g., alkaloids, cyanogenic glycosides, nonprotein amino acids, coumarins, and iridoid glycosides.
2. Broaden the representation of species and biomes investigated. To date, research has focused on a narrow range of deciduous and coniferous tree species and biomes, and mostly lepidopteran herbivores. Both

tropical and boreal forests are critically important to the global carbon cycle, and poorly represented in global change research. Future studies should emphasize foundation tree species (Ellison et al. 2005) and functional groups (e.g., insect borers, miners) and taxa (e.g., mammals) known to be important to the health and sustainability of forest ecosystems. The coniferous forests and irruptive bark beetles of western North America are a prime example (Raffa et al. 2008). In a similar vein, future research should address non-exploitative ecological interactions, such as mutualistic associations of plants with pollinators and mycorrhizae.

3. Emphasize simultaneous investigation of multiple global change drivers. As documented in this review and others (e.g., Tylianakis et al. 2008), the effects of any particular global change factor on ecological interactions are context-dependent. Multiple drivers should be investigated simultaneously to more realistically approximate future environments and to provide a diversity of conditions under which to test the effects of individual drivers (Tylianakis et al. 2008). Altered temperature and precipitation are arguably the most important additional factors to evaluate in the context of CO₂ and O₃ effects on forests.
4. Conduct long-term experiments. The majority of studies published to date have been executed over short time frames (up to several years), with only a few approaching 10 years—considerably less than the lifespan of forest trees. Some ecosystem processes (e.g., soil nutrient depletion and carbon storage) require assessment over the course of a decade or longer, logistical and funding constraints notwithstanding. Long-term experiments also could allow for studies of evolutionary responses of insects to CO₂- and O₃-mediated changes in tree chemistry, which are conspicuously absent in the published literature and important to understanding the effects of global change on forest ecosystems.
5. Employ “genes to ecosystems” research perspectives. “Genes to ecosystems” research has emerged as a new disciplinary frontier in genetics and ecology (Whitham et al. 2006). It combines genetic/genomic tools with the approaches of evolutionary ecology to understand the functional significance of genes in ecological context and the dynamics of ecosystems in evolutionary context. The discipline of chemical ecology can play a key role in elucidating the “metabolomes” that link gene expression to ecosystem processes in the context of global environmental change.

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Impacts of elevated CO₂ and O₃ on aspen leaf litter chemistry and earthworm and springtail productivity

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ABSTRACT

Human alteration of atmospheric composition affects foliar chemistry and has possible implications for the structure and functioning of detrital communities. In this study, we explored the impacts of elevated carbon dioxide and ozone on aspen (*Populus tremuloides*) leaf litter chemistry, earthworm (*Lumbricus terrestris*) individual consumption and growth, and springtail (*Sinella curviseta*) population growth. We found that elevated carbon dioxide reduced nitrogen and increased condensed-tannin concentrations in leaf litter. These changes were associated with decreases in earthworm individual growth, earthworm growth efficiency, and springtail population growth. Elevated ozone increased fiber and lignin concentrations of leaf litter. These changes were not associated with earthworm consumption or growth, but were associated with increased springtail population growth. Our results suggest that changes in litter chemistry caused by increased carbon dioxide concentrations will have negative impacts on the productivity of diverse detritivore taxa, whereas those caused by increased ozone concentrations will have variable, taxon-specific effects.

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1. Introduction

Tropospheric concentrations of carbon dioxide and ozone are increasing at rates unparalleled in human history (Marenco et al., 1994; Vingarzan, 2004; IPCC, 2007). These increases are expected to alter the chemical characteristics of tree foliage and leaf litter (Norby et al., 2001; Valkama et al., 2007; Bidart-Bouzat and Imeh-Nathaniel, 2008; Lindroth, 2010). Changes in leaf litter chemistry will likely lead to changes in soil carbon and nutrient dynamics, as litter chemistry reflects resource quality for organisms that are responsible for decomposition (Cotrufo et al., 1995; Hättenschwiler et al., 1999; Parsons et al., 2004, 2008; Lindroth, 2010).

Soil invertebrates play important roles in litter decomposition. Although the fraction of litter carbon respired by soil invertebrates is fairly small, invertebrate exclusion studies have shown that litter processing by soil animals causes disproportionately large increases in decomposition rates, especially in temperate and tropical regions (Seastedt, 1984; Wall et al., 2008; Powers et al., 2009). Earthworms and springtails are major components of the detritivore

community in many ecosystems. In temperate hardwood forests, earthworm biomass can reach 1 Mg ha⁻¹, and springtails often reach densities of 100,000 individuals m⁻² (Coleman et al., 2004). Furthermore, both taxa are known to have important impacts on soil processes (Lavelle et al., 1998; Filser, 2002). These impacts are clearly demonstrated in forests of the Great Lakes region, where introduction of earthworms, in particular, has resulted in the complete elimination of the litter layer, development of topsoil, and marked changes in soil carbon, nitrogen, and phosphorus dynamics (Alban and Berry, 1994; Bohlen et al., 2004; Hale et al., 2005; Madritch and Lindroth, 2009). Invertebrate-mediated changes in soil processes, in turn, have had cascading effects on plant establishment and community structure (Hale et al., 2006, 2008).

Despite the importance of soil invertebrates for decomposition and nutrient cycling, relatively few studies have considered how CO₂- and O₃-induced changes in litter quality will affect their activities. In short-term feeding trials, Cotrufo et al. (1998) found that isopods (*Oniscus asellus*) avoided consuming litter from trees grown under high CO₂. They suggested that this result was due to a decrease in nitrogen and an increase in lignin concentrations in high-CO₂ leaves. In long-term feeding trials, Hättenschwiler et al. (1999) found that isopods (*O. asellus* and *Porcellio scaber*) increased their consumption rates of litter from trees grown under high CO₂. These authors suggested that consumption was increased

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over the long-term to compensate for low nitrogen concentrations in high-CO₂ leaves. Kasurinen et al. (2007) reported a limited and variable influence of atmospheric chemistry and leaf litter quality on isopod (*P. scaber*) consumption and growth. However, these authors also found that earthworm (*Lumbricus terrestris*) growth rates were reduced when worms were fed leaves from trees grown under increased CO₂ or O₃. Reduced growth rates were attributed to the relatively high concentrations of condensed tannins and lignin, and low concentrations of nitrogen, in leaf litter. Altogether, previous studies suggest that increases in atmospheric CO₂ and O₃ reduce food quality for soil invertebrates by increasing concentrations of structural-carbon and phenolic compounds and decreasing concentrations of nitrogen. Reductions in resource quality presumably cause a decrease in individual growth, with possible implications for detritivore community structure and function.

In this study, we collected leaf litter from the Aspen Free Air CO₂ Enrichment (Aspen FACE) facility and conducted a laboratory microcosm experiment to determine how atmosphere-induced changes in litter quality affect the individual growth of earthworms (*L. terrestris*) and the population growth of springtails (*Sinella curviseta*). We predicted, based on previous studies using litter from greenhouses (Cotrufo et al., 1998) and open-top chambers (Hättenschwiler et al., 1999; Kasurinen et al., 2007), that 10 years of exposure to increased CO₂ and O₃ at the Aspen FACE facility would lead to leaf litter of lower quality, and that reduced leaf litter quality would have direct (via leaf quality) and indirect (via litter microbes) negative effects on earthworm growth and springtail populations.

2. Methods

2.1. Leaf litter collection

Our study was conducted using leaf litter from the Aspen FACE facility, which is located near Rhinelander, WI, USA (N 45.678, W –89.628). The facility is comprised of 12, 30-m diameter, open-air, circular plots (rings). In these rings, two factors, CO₂ concentration and O₃ concentration, are maintained at one of two levels: current ambient concentrations and elevated concentrations forecasted for the middle of this century. The 12 rings are divided into 3 rings per 4 treatment combinations: aCO₂/aO₃ rings have ambient concentrations of CO₂ and O₃ (monthly averages for daytime concentrations ranged from 365 to 406 ppm for CO₂ and 32–48 ppb for O₃ during the 2007 growing season); eCO₂/aO₃ rings have elevated CO₂ (454–556 ppm) and ambient O₃ concentrations; aCO₂/eO₃ rings have ambient CO₂ and elevated O₃ concentrations (37–57 ppb); and eCO₂/eO₃ rings have elevated concentrations of both CO₂ and O₃.

Each ring is partitioned into three community types, including interplanted aspen genotypes (*Populus tremuloides*), aspen and birch (*Betula papyrifera*), and aspen and maple (*Acer saccharum*). Trees were planted at the site in 1997; atmospheric treatments were begun in 1998 and have continued to present. For our study, four litter samples were collected during the last two weeks of September 2007 from the mixed aspen genotype portion of each ring, using laundry baskets placed on the forest floor. Baskets had an opening of 57 × 41 cm and had holes drilled into the bottom to provide drainage of precipitation. Collected leaf litter, representing a mix of five genotypes, was air-dried in the lab and stored for approximately 1 month under ambient conditions until chemical analyses and microcosm studies were begun.

2.2. Litter chemical analyses

A portion of the leaf litter from each basket was freeze-dried, ground, and assayed for total carbon, total nitrogen, condensed tannin, acid-detergent fiber, and lignin concentration (% dry mass).

Carbon and nitrogen analysis was performed with a Thermo Finnigan Flash 1112 elemental analyzer (Thermo Finnigan, San Jose, CA, USA). Condensed tannins were extracted with 70% acetone and assayed by the acid-butanol method of Porter et al. (1986) using purified aspen standards. Acid-detergent fiber and lignin were estimated gravimetrically using an Ankom 2000 fiber analyzer (Ankom Technology, Macedon, NY, USA).

2.3. Earthworm consumption and growth

Young earthworms were obtained from a fishing supply company (Knutson's Recreational Sales, Brooklyn, MI, USA). Upon arrival to our laboratory, individuals were placed in petri dishes with moist paper towels and fasted for 24 h to eliminate residual digestive material before determining initial live masses.

Forty-eight earthworm microcosms (corresponding with 12 FACE rings × 4 litter basket subsamples) were prepared by placing 800 mL of silt loam topsoil in 1 L plastic containers. Soil was collected from the Eagle Heights Community Garden in Madison, WI, USA during November 2007, passed through a 2 mm sieve, and then defaunated by three rounds of rapid freezing and thawing.

Each of the microcosms received 2 g of leaf litter, cut into 1-cm² pieces, from a single litter-collection basket. We then added one fasted and weighed earthworm to each microcosm, moistened litter with a spray of distilled water, covered microcosms with a perforated plastic lid, and placed them into a growth chamber (Percival Scientific, Perry, IA, USA) set to a 12:12 h day (20 °C) to night (15 °C) cycle. Volumetric water content of microcosm soil was monitored with a soil moisture meter (HydroSense Soil Water Measurement System, Campbell Scientific, Logan, UT, USA) and kept at 20% throughout the duration of the experiment.

After six weeks in the growth chamber, earthworms and unconsumed leaf litter fragments were extracted from microcosms using soil sieves. Worms were again fasted for 24 h and reweighed to attain a final live mass. Litter fragments were air-dried and weighed to give a rough estimate of the amount of litter consumed during the 6-week period.

2.4. Springtail population growth

A population of *S. curviseta* was obtained from a laboratory culture established by D. A. Crossley, Jr., University of Georgia. Springtails were propagated in 1 L plastic containers on a moist 2:1 plaster/charcoal substrate and fed baker's yeast for one month until the beginning of the experiment.

Forty-eight springtail microcosms were prepared as described above for earthworms. Each of these microcosms received 800 mL of silt loam topsoil and 2 g of leaf litter, cut into 1-cm² pieces, from a single litter-collection basket. We then transferred 10 adult springtails to each microcosm and moistened the litter with a spray of distilled water. Microcosms were then covered with a perforated plastic lid and placed into a growth chamber set to the same light and temperature parameters as described above. Volumetric water content of soil was kept at 20% throughout the duration of the experiment.

After 10 weeks in the growth chamber, springtails were extracted from microcosms using a modified salt-floatation method (Edwards and Fletcher, 1971). First, microcosms were placed in a freezer at –20 °C for 1 h to immobilize springtails. Then the top 6 cm of litter and soil from each microcosm was transferred to another 1 L container and soaked in 700 mL of saturated NaCl solution to separate springtails and litter from soil. The salt solution, along with floating material, was decanted through stacked 1500 μm and 75 μm sieves. The 1500 μm sieve was rinsed with tap water to dislodge any remaining springtails from organic material.

Springtails that accumulated in the 75 μm sieve were rinsed and stored in ethanol, and then counted with a dissecting microscope to determine population growth over the 10-week period.

2.5. Statistical analyses

We used two-factor ANOVAs to determine the effects of CO_2 , O_3 , and their interaction on leaf litter chemistry and invertebrate consumption and production. Dependent variables included in the analyses were total carbon, total nitrogen, condensed tannin, acid-detergent fiber, lignin, carbon-to-nitrogen (C:N) ratio, lignin-to-nitrogen (L:N) ratio, individual earthworm consumption (g dry mass/6 wk), individual earthworm growth (Δ g dry mass/6 wk), earthworm growth efficiency (g biomass/g litter consumed), and springtail population growth (new individuals accrued/10 wk). We did not standardize earthworm consumption and growth rates for initial worm size because average initial masses did not differ significantly across treatments. Air-dried litter mass was multiplied by a conversion factor of 0.89 (derived by oven-drying a set of air-dried litter samples) to attain litter dry mass. Worm live mass was multiplied by 0.18 to attain worm dry mass (Satchell, 1971).

We used general linear modeling to determine how litter-chemistry variables were related to detritivore production. This process involved regressing all possible combinations of litter quality variables against earthworm consumption, earthworm individual growth, earthworm growth efficiency, and springtail population growth, individually (127 combinations per dependent variable). For each dependent variable, best models were determined as those with $\Delta\text{AIC}_c \leq 2$ (Burnham and Anderson, 1998). Results from these extensive analyses indicated that detritivore production was best explained by univariate litter-chemistry models. Thus, for simplicity, we illustrate relationships between litter chemistry and detritivore production variables in a correlation matrix.

For all analyses, the results of multiple subsamples per FACE ring were averaged prior to analysis, and ring was the unit of replication ($N = 12$ rings, 3 per CO_2 by O_3 treatment combination). Ring was used as the unit of replication in our analyses because it is the unit upon which the atmospheric treatments were administered, and to use subsample or experimental animal as the replicate for analyses would constitute pseudoreplication with respect to atmospheric treatments. Given the low replication at the Aspen FACE experiment, we follow the suggestion by Filion et al. (2000), that P -values should be considered significant at $P < 0.10$. All analyses were conducted using JMP, Version 7 software (SAS Institute Inc., Cary, NC, USA).

3. Results

Altered CO_2 and O_3 concentrations had a variety of effects on aspen leaf litter chemistry. The main effect of elevated CO_2 (averaged across O_3 treatments) was a minor reduction in total carbon concentrations, a 10% reduction in total nitrogen concentrations, a doubling of condensed-tannin concentrations, and a 10% increase in C:N ratios (Table 1). The main effect of elevated O_3 (averaged across CO_2 treatments) was a negligible reduction in total carbon concentrations, a 7% increase in fiber concentrations, a 15% increase in lignin concentrations, and a 17% increase in L:N ratios (Table 1). Interactions between CO_2 and O_3 were not common or pronounced, but were detectable for litter nitrogen concentrations and C:N ratios. In both cases, the effects of elevated CO_2 (i.e., reducing N concentration and increasing C:N ratio) were marginally stronger under ambient O_3 than elevated O_3 (Table 1).

Earthworm consumption was not influenced by CO_2 or O_3 treatments at the Aspen FACE site (Fig. 1a). Earthworm growth rates

decreased by 38% when animals were fed litter from CO_2 -enriched rings (Fig. 1b), but were unaffected by O_3 concentration and the interaction between CO_2 and O_3 (Fig. 1b). Because growth was related to CO_2 concentrations while consumption was not, earthworm growth efficiency decreased by 61% when worms were fed litter from rings with elevated CO_2 (Fig. 1c). As with growth, earthworm growth efficiency was not related to O_3 concentration or the interaction between CO_2 and O_3 (Fig. 1c).

Elevated CO_2 and O_3 concentrations had opposite effects on springtail population growth. Population growth rate decreased by 31% when springtails were provided with litter from rings with elevated CO_2 (Fig. 2). In contrast, population growth increased by 56% when springtails were fed litter from elevated O_3 plots (Fig. 2). The opposing effects of elevated CO_2 and O_3 on springtail population growth roughly offset one another, and population growth was similar in the aCO_2/aO_3 and eCO_2/eO_3 treatments (Fig. 2).

There were few statistically significant relationships between aspen leaf litter chemistry and detritivore production variables (Table 2). However, a few general patterns in the data warrant mention. Earthworm growth, earthworm growth efficiency, and springtail population growth tended to be positively related to nitrogen concentrations and negatively related to condensed-tannin concentrations and C:N ratios of leaf litter. Earthworm growth and growth efficiency were also positively related with litter carbon concentrations, whereas springtail population growth was positively related to litter fiber and lignin concentrations.

4. Discussion

4.1. Leaf litter chemistry

Multiple chemical characteristics of aspen leaf litter were altered via changes in atmospheric composition at the Aspen FACE site (Table 1). First, litter nitrogen concentrations were considerably lower in rings with elevated CO_2 , which agrees with results from several previous studies (Hättenschwiler et al., 1999; Norby et al., 2001; Parsons et al., 2004, 2008; Liu et al., 2005; Kasurinen et al., 2006; Liu et al., 2007). Second, litter condensed-tannin concentrations and C:N ratios were considerably higher in rings with elevated CO_2 , which is also consistent with previous studies (Norby et al., 1986; Parsons et al., 2004, 2008; Kasurinen et al., 2007). Third, structural-carbon compounds, such as fiber and lignin, were notably higher in litter from rings with elevated O_3 . This result agrees with those from other research sites (Norby et al., 2001), although it has not been observed previously at the Aspen FACE site (Parsons et al., 2004, 2008). The changes that we observed in litter chemistry were largely as expected, and we predicted that these changes would have implications for detritivore consumption and production.

4.2. Earthworm consumption and growth

Elevated CO_2 and O_3 concentrations did not influence litter consumption by earthworms, which consumed an average of 64% of the food provided (Fig. 1a). The fact that consumption rates did not vary across treatments indicates that the earthworms did not employ compensatory feeding to cope with food of low nutritional quality. We recognize that our measurements of consumption were only approximate, and are probably overestimates; some leaf litter fragments may have been small enough to pass through our sieves, and some of the litter mass loss was likely due to microbial respiration. Nonetheless, earthworm growth efficiencies calculated in this study were comparable to those of previous studies (e.g., Whalen and Parmelee, 2000).

Table 1

Summary statistics (mean $\pm$ 1 SD, $N = 3$) and ANOVA P -values for litter chemistry responses to Aspen FACE treatments. Superscripts denote statistically different means within a column where there is a significant interaction term.

	Carbon (C) (% dry mass)	Nitrogen (N) (% dry mass)	Condensed tannin (% dry mass)	Fiber (% dry mass)	Lignin (L) (% dry mass)	C:N	L:N
FACE treatment combination							
Ambient CO ₂ /ambient O ₃	49.3 $\pm$ 0.5	1.24 $\pm$ 0.02 ^a	0.6 $\pm$ 0.2	52.1 $\pm$ 2.5	29.4 $\pm$ 2.2	39.8 $\pm$ 0.4 ^a	23.7 $\pm$ 2.0
Elevated CO ₂ /ambient O ₃	48.7 $\pm$ 0.7	1.06 $\pm$ 0.09 ^b	1.7 $\pm$ 0.6	49.6 $\pm$ 3.5	28.0 $\pm$ 4.0	46.2 $\pm$ 3.3 ^b	26.4 $\pm$ 4.3
Ambient CO ₂ /elevated O ₃	48.9 $\pm$ 0.3	1.15 $\pm$ 0.02 ^{ab}	0.7 $\pm$ 0.3	54.2 $\pm$ 2.2	32.7 $\pm$ 1.5	42.6 $\pm$ 0.4 ^{ab}	28.3 $\pm$ 0.8
Elevated CO ₂ /elevated O ₃	48.0 $\pm$ 0.2	1.09 $\pm$ 0.05 ^b	0.9 $\pm$ 0.5	54.6 $\pm$ 1.3	33.1 $\pm$ 1.3	44.3 $\pm$ 2.0 ^b	30.5 $\pm$ 2.3
Two-way ANOVA source							
CO ₂	0.028	0.004	0.040	0.470	0.740	0.007	0.153
O ₃	0.089	0.322	0.226	0.039	0.019	0.696	0.023
CO ₂ $\times$ O ₃	0.695	0.097	0.140	0.353	0.554	0.064	0.843

Fig. 1. Effects of elevated CO₂ and O₃ on (a) individual earthworm consumption (g dry mass/6 wk), (b) individual earthworm growth (g dry mass/6 wk), and (c) earthworm growth efficiency (g growth/g consumed). Error bars represent $\pm$ 1 SE.

Although consumption did not vary across atmospheric treatments, earthworms fed high-CO₂ litter exhibited markedly reduced growth (Fig. 1b). This growth reduction corresponded with low nitrogen concentrations in high-CO₂ litter (Table 1). When we related earthworm growth directly to litter nitrogen, we found a reasonably strong positive correlation ($r = 0.41$, Table 2). The relationship was not statistically significant; however, a power analysis revealed that a significant correlation would have been detected if our total sample size had been 18 rather than 12 replicates. Regardless, the overall patterns in our data are consistent with the suggestion by Kasurinen et al. (2007), that low mass gain by earthworms fed high-CO₂ litter is partly due to decreased litter nitrogen content. The patterns also agree with those from other studies that show positive correlations between the nitrogen content of food and earthworm mass gain (e.g., Shipitalo et al., 1988).

The reduced earthworm growth rates observed in this study also corresponded with high condensed-tannin concentrations in high-CO₂ litter (Table 1). When we related earthworm growth directly to litter condensed tannins, we found a reasonably strong negative correlation ($r = -0.44$, Table 2). Again, the relationship was not statistically significant, although power analysis indicated that a significant relationship would have been detected if our samples size had been 15 rather than 12 replicates. Mechanisms by which dietary tannins can reduce growth include feeding deterrence (not observed in this study), chemical binding of dietary proteins or digestive enzymes, increases in costly excretory processes, formation of gut lesions, and inhibition of gut microbial activity (discussed in Simpson and Raubenheimer, 2001). A full exploration of

Fig. 2. Effects of elevated CO₂ and O₃ on the growth of springtail populations (Δ abundance/10 wk). Error bars represent $\pm$ 1 SE.

Table 2Correlations between litter chemistry and invertebrate consumption and production variables. * $P < 0.10$, ** $P < 0.05$, *** $P < 0.01$.

	Carbon	Nitrogen	Condensed tannin	Fiber	Lignin	C:N	L:N
Nitrogen	0.74***						
Condensed tannin	−0.05	−0.61**					
Fiber	0.02	0.13	−0.17				
Lignin	−0.07	−0.02	−0.04	0.96***			
C:N	−0.62**	−0.98***	0.68**	−0.19	−0.03		
L:N	−0.46	−0.59**	0.35	0.70**	0.82***	0.54*	
Earthworm consumption	0.18	0.15	−0.10	−0.39	−0.38	−0.11	−0.43
Earthworm growth	0.42	0.41	−0.44	0.16	0.14	−0.37	−0.16
Earthworm growth efficiency	0.54*	0.45	−0.36	−0.01	−0.08	−0.40	−0.35
Springtail population growth	0.00	0.22	−0.54*	0.38	0.40	−0.26	0.18

these mechanisms was beyond the scope of this study, and could be the topic of future research.

4.3. Springtail population growth

As with earthworm individual growth, springtail population growth was considerably lower when populations were given litter from rings with elevated CO_2 (Fig. 2). Springtails, in general, are flexible consumers that can ingest leaf litter and the microbes that live on or within leaf litter (Chen et al., 1996; Ponge, 2000). The feeding habits of *S. curviseta*, in particular, are not well documented for wild populations, although the population used in this study had subsisted on fungi (baker's yeast) for approximately 50 years. Given uncertainty about the feeding habits of the springtails in our experiment, we do not know exactly how altered atmospheric chemistry affected springtail population growth; this will be a subject of further study. Reduced population growth might have followed the causal chain described above for earthworms, i.e., elevated CO_2 led to litter or litter-associated microbes with low nitrogen and high condensed-tannin concentrations, which led to slow springtail growth, low fecundity, or high mortality (e.g., Booth and Anderson, 1979). It is also possible that litter with low nitrogen and high condensed-tannin concentrations yielded low microbial biomass (Harrison, 1971; Swift et al., 1979), which, in turn, caused a shortage of resources for springtail population growth. A third potential explanation is that litter chemistry may have elicited a shift in microbial community composition (e.g., Chung et al., 2006), such that microbes dominating high- CO_2 litter were not consumed by springtails. We did not monitor microbial biomass or community structure, so we cannot evaluate these mechanisms.

We did not expect to see increased springtail population growth in microcosms receiving litter from rings with elevated O_3 , especially because litter from these rings had relatively high concentrations of structural carbohydrates such as fiber and lignin (Table 1). Increased population growth rates associated with elevated O_3 may have resulted from chemical changes in leaf litter that were not measured. Growth rates of leaf-chewing herbivores show a similar unexplained increase under elevated ozone (Valkama et al., 2007). A second possible explanation for increased population growth is that the O_3 -induced changes in litter chemistry led to a change in microbial community composition (e.g., Chung et al., 2006) that was favorable for springtail production.

4.4. Scaling up

Our study showed that earthworm individual growth and springtail population growth are negatively affected when animals are fed aspen leaf litter grown under elevated CO_2 . If all other factors were equal, this result would imply that productivity of these organisms will be reduced under predicted increases in atmospheric CO_2 in aspen-dominated forests of the Great Lakes

region. We also found that increases in O_3 have a stimulating effect on springtail population growth. Thus, if atmospheric concentrations of both CO_2 and O_3 increase at rates similar to those employed in this study, we would expect a net-negative effect on earthworm productivity, but a net-neutral effect on springtail productivity.

To better understand the implications of our results, it is necessary to consider environmental temperature, an additional environmental factor that will change with increased CO_2 and affect invertebrate productivity (David and Gillon, 2009). Climate models predict that, under likely emission scenarios, elevated CO_2 will cause a 2 °C increase in average annual temperature in the Great Lakes region by 2050 (IPCC, 2007). Assuming minor temperature acclimation and a Q_{10} temperature coefficient between 1.5 and 3 for production rates (Savage et al., 2004; Frazier et al., 2006), a temperature increase of 2 °C would increase the production rates of litter invertebrates by approximately 10–25%. In the present study, we found that CO_2 -induced changes in litter quality decreased invertebrate productivity by approximately 35%. If we further assume that the effects of temperature and litter quality are additive (e.g., David and Gillon, 2009), we can hypothesize that projected increases in CO_2 alone will have an overall negative effect on invertebrate productivity (+10 to +25% temperature effect, combined with a −35% litter quality effect, yields a −10 to −25% overall effect). Using the same logic, we hypothesize that projected increases in both CO_2 and O_3 will have a net-negative effect on earthworms (as above), but a net positive effect on springtails (+10 to +25% temperature effect, combined with no litter quality effect, yields a +10 to +25% overall effect). Thus, taxon-specific effects of elevated CO_2 and O_3 could lead to shifts in detritivore community structure. Changes in detritivore community structure could lead to changes in ecological patterns and processes in aspen-dominated forests of the Great Lakes region. A fuller understanding of the effects of elevated CO_2 and O_3 on detritivore community structure and function will be attained when assumption-laden projections, like those above, can be replaced with empirical results from experiments that simultaneously manipulate resource quality and environmental temperature (e.g., David and Gillon, 2009) for a wide variety of functionally important taxa.

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Effects of genotype, elevated CO₂ and elevated O₃ on aspen phytochemistry and aspen leaf beetle *Chrysomela crotchii* performance

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- Abstract**
- 1 Trembling aspen *Populus tremuloides* Michaux is an important forest species in the Great Lakes region and displays tremendous genetic variation in foliar chemistry. Elevated carbon dioxide (CO₂) and ozone (O₃) may also influence phytochemistry and thereby alter the performance of insect herbivores such as the aspen leaf beetle *Chrysomela crotchii* Brown.
 - 2 The present study aimed to relate genetic- and atmospheric-based variation in aspen phytochemistry to *C. crotchii* performance (larval development time, adult mass, survivorship). The experiment was conducted at the Aspen Free-Air CO₂ Enrichment (FACE) site in northern Wisconsin. Beetles were reared on three aspen genotypes under elevated CO₂ and/or O₃. Leaves were collected to determine chemical characteristics.
 - 3 The foliage exhibited significant variation in nitrogen, condensed tannins and phenolic glycosides among genotypes. CO₂ and O₃, however, had little effect on phytochemistry. Nonetheless, elevated CO₂ decreased beetle performance on one aspen genotype and had inconsistent effects on beetles reared on two other genotypes. Elevated O₃ decreased beetle performance, especially for beetles reared on an O₃-sensitive genotype. Regression analyses indicated that phenolic glycosides and nitrogen explain a substantial amount (27–45%) of the variation in herbivore performance.
 - 4 By contrast to the negative effects that are typically observed with generalist herbivores, aspen leaf beetles appear to benefit from phenolic glycosides, chemical components that are largely genetically-determined in aspen. The results obtained in the present study indicate that host genetic variation and atmospheric concentrations of greenhouse gases will be important factors in the performance of specialist herbivores, such as *C. crotchii*, in future climates.

Keywords Carbon dioxide, FACE, herbivore performance, ozone, ozone-tolerance, phenolic glycosides, plant–insect interactions, *Populus tremuloides*, specialist, trembling aspen.

Introduction

Trembling aspen *Populus tremuloides* Michaux is the most widely distributed tree in North America and an important woody crop in the Great Lakes region (Piva, 1996; Coyle

et al., 2001; Isebrands *et al.*, 2001). Aspen is also one of the most genetically variable tree species in North America (Perala, 1990). Substantial clonal variation is present in foliar chemical attributes such as condensed tannin and phenolic glycoside concentrations (Hwang & Lindroth, 1997; Osier & Lindroth, 2001, 2004; Donaldson & Lindroth, 2007). Some aspen secondary compounds, such as phenolic glycosides, can negatively alter insect herbivore performance (Hemming & Lindroth, 1995; Hwang & Lindroth, 1998; Donaldson & Lindroth, 2007).

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Intraspecific genetic variation in the concentrations of these constituents contributes to differential performance of insect herbivores on various aspen genotypes (Hwang & Lindroth, 1997; Osier *et al.*, 2000; Osier & Lindroth, 2006; Donaldson & Lindroth, 2007). Such changes in herbivore performance are likely to influence the success of specific aspen genotypes during herbivore outbreaks (Gruppe *et al.*, 1999; Donaldson & Lindroth, 2007).

Herbivore performance may also be influenced by atmosphere-induced alterations in plant phytochemistry. Concentrations of atmospheric CO₂ and O₃ have been increasing subsequent to the industrial revolution (Solomon *et al.*, 2007) and are expected to continue rising in the future (Fowler *et al.*, 1999; Sitch *et al.*, 2007). Previous research has shown that elevated concentrations of CO₂ and O₃ may significantly affect plant physiology and foliar chemistry, thereby affecting plant–herbivore interactions. For example, foliar concentrations of nitrogen are typically reduced under elevated CO₂ (Lawler *et al.*, 1997; Roth *et al.*, 1997; Norby *et al.*, 1999; Kopper & Lindroth, 2003a). Elevated CO₂ also generally causes an increase in plant carbon uptake, thereby increasing carbon-based secondary compounds such as condensed tannins and phenolic glycosides (Roth *et al.*, 1997; Saxe *et al.*, 1998; Lindroth *et al.*, 2002). Leaf-chewing insects reared on plants exposed to elevated CO₂ typically exhibit increased consumption rates, longer development times and reduced pupal masses (Lindroth & Dearing, 2005). The effects of elevated O₃ on foliar nitrogen are somewhat inconsistent in the literature (Koricheva *et al.*, 1998; Holton *et al.*, 2003; Kopper & Lindroth, 2003a, b; Agrell *et al.*, 2005). However, research indicates that O₃ often increases carbon-based secondary compounds such as phenolics (Runeckles & Krupa, 1994; Koricheva *et al.*, 1998). Herbivorous insects reared on plants grown under elevated O₃ show variable responses in performance, depending on insect species and host species or genotype (Agrell *et al.*, 2000; Holton *et al.*, 2003; Kopper & Lindroth, 2003a, b; Agrell *et al.*, 2005). Variation in plant chemistry and herbivore responses as a result of a changing climate may have considerable impacts on future aspen populations.

Most previous research on the effects of elevated CO₂ and O₃ on aspen phytochemistry and chewing insect performance has focused on Lepidoptera herbivores. Surprisingly, few studies have addressed the effects of elevated CO₂ (Coviella & Trumble, 1999; Johns *et al.*, 2003) or elevated O₃ (Valkama *et al.*, 2007; Freiwald *et al.*, 2008) on Coleoptera herbivores. Several Coleoptera species are well-adapted to host plants, such as aspen, that contain high concentrations of salicylates (phenolic glycosides). The larvae of these specialists typically employ plant-derived salicylaldehyde in their own defence secretions (Rowell-Rahier & Pasteels, 1982; Pasteels *et al.*, 1983). Indeed, many studies have addressed the effects of poplar phytochemistry on the feeding preferences of chrysomelids and have found that phenolic glycoside concentrations influence host specificity (Tahvanainen *et al.*, 1985; Matsuki & MacLean, 1994; Orians *et al.*, 1997; Ikonen *et al.*, 2001). Although phenolic glycosides may negatively affect the performance of generalist chrysomelids, they generally have little to no negative impact, and may improve the performance

of specialist chrysomelids (Matsuki & MacLean, 1994; Orians *et al.*, 1997; Rank *et al.*, 1998). Condensed tannins, however, negatively affect the performance (e.g. growth) of chrysomelids (Donaldson & Lindroth, 2004).

The aspen leaf beetle *Chrysomela crotchii* Brown is a common chrysomelid beetle that specializes on poplars. Females lay eggs during June and July and the larvae can persist from July to September. One generation is produced per year (Smereka, 1965). Larvae feed on the lower surface of the leaf, skeletonizing the structure and leaving only the veins behind. Adults also feed on foliage, leaving only major veins uneaten. In outbreak years, these beetles can defoliate aspen trees in localized areas (U.S. Department of Agriculture, 2000).

The present study aimed to relate *C. crotchii* performance to genetic-, CO₂- and O₃-based variation in *Populus tremuloides* phytochemistry *in situ*. Specifically, we examined the relationships of beetle larval development time, adult mass and survivorship to foliar nitrogen, condensed tannin and phenolic glycoside concentrations. We predicted that aspen phytochemistry and beetle performance would vary among aspen genotypes. In particular, we expected that beetle performance would improve as foliar nitrogen and phenolic glycoside concentrations increased and tannin concentrations decreased. We expected CO₂ and O₃ to decrease foliar quality (e.g. decrease nitrogen, increase tannins) and subsequently decrease beetle performance as well.

Materials and methods

This research was conducted during the 2003 growing season at the Aspen Free Air CO₂ Enrichment (FACE) site (89.5°W, 45.7°N) near Rhinelander, Wisconsin. Aspen FACE is a 32-ha site consisting of twelve 30-m diameter rings. The overall experimental design at Aspen FACE is a split-plot randomized complete block. The Aspen FACE site is divided into three blocks, north to south, and each block contains four FACE rings, one each of four experimental treatments: ambient CO₂ and O₃ (control); elevated CO₂, ambient O₃ (+CO₂); ambient CO₂, elevated O₃ (+O₃); and elevated CO₂ and O₃ (+CO₂+O₃). The whole-plot treatments include the CO₂ and O₃ (2 × 2) factors; the subplot factor is aspen genotype (see below). Ambient levels of CO₂ averaged 384 µL/L and 32 nL/L for O₃. Elevated target levels for CO₂ were 560 µL/L and 1.5 × ambient for O₃. These levels were chosen based on predictions of CO₂ and O₃ concentrations in the northern Great Lakes Region for the year 2060 (Dickson *et al.*, 2000). Actual levels of elevated CO₂ averaged 537 ± 77 µL/L, and 51 ± 22 nL/L for elevated O₃, in 2003 (J. Sober, personal communication). Fumigants were applied during daylight hours of the growing season (21 May to 12 October). As a result of the photochemical properties of O₃ production and to mimic actual O₃ fluctuations, target concentrations of O₃ were adjusted daily according to current weather conditions. No O₃ treatment was administered on cool (<15°C) days or when leaf surfaces were wet due to precipitation or condensation. Additional details about the design and operation of the Aspen FACE are provided in Dickson *et al.* (2000).

One section of each Aspen FACE ring contains a mix of five trembling aspen genotypes. At the time of this experiment, the

trees were 7 years old and in their sixth year of fumigation. For the present study, we chose to work with aspen genotypes 216, 271 and 42E because of the range of O₃ sensitivity demonstrated by these trees. Genotypes 216 and 271 are relatively O₃-tolerant, whereas 42E is O₃-sensitive (Karnosky *et al.*, 1996). Three aspen trees per genotype per ring were used for this experiment (108 total trees).

Approximately 40 female beetles were collected from the Aspen FACE site (outside of FACE rings) and allowed to mate with a similar number of males in cages containing fresh aspen shoots in water picks. Each female produces multiple clusters of eggs, with the number of eggs per cluster in the range 1–150. Egg clusters were removed daily and stored in a refrigerator (4°C) until enough eggs had been collected to proceed with the experiment. A typical egg cluster contained approximately 20–30 eggs. We created additional clusters of 20–30 eggs by grouping small clusters together or dividing large clusters, until we had a total of 108 clusters (one for each experimental tree). The eggs were transferred to a 25°C environmental growth chamber (Percival Scientific Inc., Boone, Iowa) until eclosion. Upon eclosion, egg clusters were randomly assigned across genotypes and fumigation treatments and were placed on fresh, detached Aspen FACE leaves. Once larvae had crawled onto the leaves, the leaves were paper-clipped onto the FACE trees from which they had derived. The branch including the larvae was enclosed in a fine, double mesh sleeve (No-See-Um insect netting, approximately 100 holes/cm²; Quest Outfitters, Sarasota, Florida) to exclude predators and parasitoids. We placed larvae onto each of three trees per aspen genotype, in each of three FACE rings per CO₂ and O₃ treatment, resulting in 180–270 insects used per genotype × fumigation treatment combination.

The beetle larvae were monitored daily and moved to new branches (on the same tree) as the food source was depleted. Upon pupation, larval development time and beetle survivorship were recorded. Pupae were removed from the tree and placed in a Percival environmental chamber set at 25/21°C (day/night) under an LD 15 : 9 h photoperiod to monitor development from pupae to adults. Sex and adult weight were determined within 24 h after adults emerged.

Because Aspen FACE is an open-air facility, trees are exposed to endemic levels of insect feeding. Although induction of aspen defensive chemistry, especially tannins (Osier & Lindroth, 2001), could have been possible, it was unlikely given the minimal damage (well below 10% at the time of this study), and so was not addressed in the present study.

Approximately 15 leaves (not fed on by aspen leaf beetles) were collected from each experimental tree midway through the larval development period. Leaves were collected from multiple branches and canopy heights to ensure an accurate representation of leaf chemistry for each tree crown. Leaves were clipped at the petiole and placed on ice for transport to the laboratory. In the laboratory, leaves were flash frozen in liquid nitrogen, freeze-dried (at –20°C), ground through a no. 40 mesh in a Wiley Mill (Arthur H. Thomas Co., Scientific Apparatus, Philadelphia, Pennsylvania), and stored at –20°C until analysed. Leaves were analysed for nitrogen, condensed tannins and phenolic glycosides. Nitrogen concentration was determined with a LECO nitrogen analyser (LECO Corporation, St. Joseph,

Michigan), using glycine *p*-toluenesulfonate (Hach Company, Loveland, Colorado) as the reference standard. Condensed tannins were extracted and quantified with the butanol-HCl method (Porter *et al.*, 1986), using purified aspen condensed tannins as the reference standard. Salicortin and tremulacin concentrations were determined via high-performance thin layer chromatography, using purified aspen salicortin and tremulacin reference standards (Lindroth *et al.*, 1993).

Analysis of variance (PROC Mixed; SAS, version 8.0; SAS Institute, Cary, North Carolina) was used for statistical analysis of treatment effects on phytochemistry and beetle performance. The statistical model employed was:

$$Y_{ijkl} = \mu + B_i + C_j + O_k + CO_{jk} + e_{ijk} + G_l + CG_{jl} + OG_{jl} + COG_{jkl} + \varepsilon_{ijkl}$$

where Y_{ijkl} is the average response of block i , CO₂ level j , O₃ level k and genotype l . Fixed effects were CO₂ level (C_j), O₃ level (O_k), genotype (G_l) and their interaction terms [(CO_{jk}) , (CG_{jl}) , (OG_{jl}) , (COG_{jkl})]. Random effects were block (B_i), whole plot error (e_{ijk}) and subplot error (ε_{ijkl}). Simple correlation and regression analysis were used to relate beetle performance to quantitative phytochemistry variables (R: A Language and Environment for Statistical Computing, version 2.4.1; R Foundation for Statistical Computing, Austria). Concentrations of salicortin and tremulacin were summed and presented in the regression analysis as 'total phenolic glycosides' as a result of their chemical similarities and parallel results when analysed separately. Partial correlation coefficients were calculated using the equation:

$r_{Y.X/T}^2 = R_{Y.XT}^2 - r_{Y.T}^2$ where r is the coefficient of correlation, Y is the dependent (beetle performance) variable, X and T are independent (phytochemistry) variables, and R^2 is the multiple coefficient of correlation (Abdi, 2007). The notation ' $Y.X$ ' signifies the correlation coefficient between variables Y and X ; the notation ' $/T$ ' signifies that variable T is included in the regression model. The term $R_{Y.XT}^2$ was determined with regression analysis, and $r_{Y.T}^2$ was determined with simple correlation. Outliers identified using Bonferroni corrected p -values were removed from the analyses. Also, beetles that did not survive to pupation were excluded from the analyses of development time and adult mass.

Low statistical power is inherent to FACE experimental designs. Thus, when a conventional significance level of 0.05 is employed, the probability of type II errors increases, even when potentially important treatment effects occur (Filion *et al.*, 2000). We therefore report significant values in the range $0.05 < P < 0.10$ as 'marginally significant.'

Results

Foliar chemistry

CO₂ and O₃ had little to no effect on aspen chemistry (Fig. 1 and Table 1). Nitrogen concentrations varied among genotypes, but the magnitude of variation was very small (1.7–1.8% dry mass, averaged across fumigation treatments). No significant CO₂ or O₃ effect on nitrogen was detected.

Figure 1 Effects of CO₂ and O₃ on foliar chemistry in aspen genotypes 216, 271 and 42E. Bars and vertical lines indicate the mean $\pm$ SE.

Table 1 Summary of *F* values, degrees of freedom and *P* values for the effects of genotype, CO₂ and O₃ on foliar chemistry of aspen leaves

Main effects and interactions	Nitrogen		Condensed tannins		Salicortin		Tremulacin	
	<i>F</i> (d.f.)	<i>P</i>	<i>F</i> (d.f.)	<i>P</i>	<i>F</i> (d.f.)	<i>P</i>	<i>F</i> (d.f.)	<i>P</i>
Genotype	4.51 (2.88)	0.014	52.82 (2.88)	<0.001	127.83 (2.88)	<0.001	146.26 (2.87)	<0.001
CO ₂	1.23 (1.6)	0.310	4.73 (1.6)	0.073	3.21 (1.6)	0.123	0.26 (1.6)	0.631
O ₃	2.54 (1.6)	0.162	2.46 (1.6)	0.168	2.53 (1.6)	0.163	2.50 (1.6)	0.166
Genotype $\times$ CO ₂	0.67 (2.88)	0.517	0.11 (2.88)	0.896	2.28 (2.88)	0.108	1.13 (2.87)	0.329
Genotype $\times$ O ₃	1.77 (2.88)	0.176	0.78 (2.88)	0.462	0.29 (2.88)	0.750	0.02 (2.87)	0.976
CO ₂ $\times$ O ₃	0.09 (1.6)	0.770	0.79 (1.6)	0.409	0.23 (1.6)	0.648	0.11 (1.6)	0.750
Genotype $\times$ CO ₂ $\times$ O ₃	1.32 (2.88)	0.273	1.48 (2.88)	0.234	0.36 (2.88)	0.696	0.20 (2.87)	0.817

Condensed tannin concentrations ranged from 11% to 19% among genotypes. Genotypes 216 and 42E had similar condensed tannin concentrations, whereas levels in genotype 271 were 38% lower. Elevated CO₂ increased condensed tannin concentrations by 17% (marginally significant) across all genotypes.

Aspen genotypes exhibited striking variation in concentrations of phenolic glycosides, with concentrations highest in genotype 271 and lowest in 42E (Fig. 1). Elevated CO₂ and O₃ had no significant independent or interactive effects on salicortin and tremulacin concentrations.

Beetle performance

Aspen genotype, CO₂ and O₃ influenced the performance of aspen leaf beetles (Fig. 2 and Table 2). Female larval development time averaged 31, 30 and 36 days when reared on genotypes 216, 271 and 42E, respectively (pooled across fumigation treatments). Similarly, male larval development time

averaged 31, 29 and 35 days when reared on genotypes 216, 271 and 42E, respectively. Female larvae reared on genotype 271 experienced a slight (8%) increase in development time under elevated CO₂ conditions, whereas females on genotypes 216 and 42E experienced no change or minor decreases in development times under elevated CO₂ conditions (significant genotype $\times$ CO₂ interaction). No significant change in development time was observed for male larvae under elevated CO₂ conditions. Both female and male larvae experienced increases in development time across genotypes under elevated O₃, although more so for females reared on 42E (23%) than those on 216 (7%) or 271 (6%) (significant genotype $\times$ O₃ interaction). No significant CO₂ $\times$ O₃ interaction was detected for larval development time. We observed a 15% and 10% increase in development time for female and male larvae, respectively (pooled across genotypes), under the combined elevated CO₂ and elevated O₃ plots compared with ambient treatments.

Genotype, CO₂ and O₃ independently or interactively influenced adult beetle mass. Females reared on genotypes 216,

Figure 2 Effects of CO₂ and O₃ on performance of beetles reared on aspen genotypes 216, 271 and 42E. Bars and vertical lines indicate the mean $\pm$ SE. Dev. time, development time.

Table 2 Summary of *F* values, degrees of freedom and *P* values for the effects of genotype, CO₂ and O₃ on aspen leaf beetle performance

Main effects and interactions	Development time				Adult mass				Survivorship	
	Female		Male		Female		Male			
	<i>F</i> (d.f.)	<i>P</i>	<i>F</i> (d.f.)	<i>P</i>	<i>F</i> (d.f.)	<i>P</i>	<i>F</i> (d.f.)	<i>P</i>	<i>F</i> (d.f.)	<i>P</i>
Genotype	67.01 (2.70)	<0.001	52.66 (2.70)	<0.001	33.28 (2.73)	<0.001	28.42 (2.72)	<0.001	8.47 (2.87)	<0.001
CO ₂	1.06 (1.6)	0.344	0.21 (1.6)	0.663	0.00 (1.6)	0.989	3.58 (1.6)	0.106	0.25 (1.6)	0.632
O ₃	15.20 (1.6)	0.008	21.55 (1.6)	0.003	23.30 (1.6)	0.003	16.06 (1.6)	0.007	15.03 (1.6)	0.008
Genotype × CO ₂	5.21 (2.70)	0.008	1.67 (2.70)	0.195	3.52 (2.73)	0.035	4.62 (2.72)	0.013	2.73 (2.87)	0.071
Genotype × O ₃	10.82 (2.70)	<0.001	4.79 (2.70)	0.011	3.96 (2.73)	0.023	1.41 (2.72)	0.252	0.18 (2.87)	0.837
CO ₂ × O ₃	0.17 (1.6)	0.698	0.27 (1.6)	0.624	0.00 (1.6)	0.955	0.08 (1.6)	0.786	1.45 (1.6)	0.275
Genotype × CO ₂ × O ₃	1.11 (2.70)	0.335	0.81 (2.70)	0.451	2.15 (2.73)	0.123	2.16 (2.72)	0.123	0.82 (2.87)	0.443

Table 3 Phytochemicals accounting for variation in beetle performance (multiple regressions using backward elimination; the selection criterion for remaining in the model was $\alpha = 0.10$)

Parameter	Regression model				Partial coefficients			
	Equation	Adjusted R^2	F (d.f.)	P	Variable	r^2	t -value	$P(t)$
Development time								
Females	$Y = 45.09 - 0.56 (\text{PG}) - 5.35 (\text{N})$	0.445	35.88 (2.85)	<0.001	PG	0.321	7.149	<0.001
					N	0.118	4.230	<0.001
Males	$Y = 39.98 - 0.50 (\text{PG}) - 3.13 (\text{N})$	0.416	31.23 (2.83)	<0.001	PG	0.349	-7.121	<0.001
					N	0.057	-2.887	0.005
Adult mass								
Females	$Y = 10.64 + 0.76 (\text{PG}) + 8.36 (\text{N})$	0.367	26.82 (2.87)	<0.001	PG	0.253	5.904	<0.001
					N	0.114	4.007	<0.001
Males	$Y = 15.95 + 0.52 (\text{PG}) + 2.95 (\text{N})$	0.271	17.14 (2.85)	<0.001	PG	0.241	5.314	<0.001
					N	0.032	1.960	0.053
Survivorship	$Y = 27.67 + 2.21 (\text{PG})$	0.088	11.02 (1.103)	0.001	PG	0.097	3.320	0.001

PG, total phenolic glycoside; N, nitrogen

271 and 42E averaged 31, 33 and 25 mg, respectively (pooled across fumigation treatments). Males reared on genotypes 216, 271 and 42E averaged 25, 26 and 21 mg, respectively. Elevated CO_2 increased beetle mass for females on genotype 216 (10%), but decreased mass for females on genotypes 271 (6%) and 42E (5%) (significant genotype $\times$ CO_2 interaction). Elevated CO_2 increased beetle mass for males on genotypes 216 (12%) and 42E (11%) but decreased mass for those on genotype 271 (4%) (significant genotype $\times$ CO_2 interaction). Elevated O_3 decreased beetle mass across all aspen genotypes (16% and 12% decreases for females and males, respectively, pooled across genotypes). Elevated O_3 decreased mass by 14%, 8% and 29% for female beetles reared on genotypes 216, 271 and 42E, respectively (significant genotype $\times$ O_3 interaction). By contrast, the effect of elevated O_3 on male mass was consistent across genotypes. No significant $\text{CO}_2 \times \text{O}_3$ interaction was detected for beetle mass. We observed a 17% decrease in female mass and 7% decrease in male mass under the combined fumigation treatment compared with ambient (pooled across genotypes).

Aspen genotype strongly affected beetle survivorship. Averaged across all treatments, 48%, 51% and 28% of beetles survived on genotypes 216, 271 and 42E, respectively. Elevated CO_2 did not affect the survivorship of beetles reared on genotype 216, decreased survivorship on genotype 271 (21%) and increased survivorship on 42E (87%) (marginally significant genotype $\times$ CO_2 interaction). By contrast, elevated O_3 decreased survivorship consistently (27%) across all genotypes. No significant $\text{CO}_2 \times \text{O}_3$ interaction was found. The combined fumigation treatment decreased survivorship by 19% and 46% for beetles reared on genotypes 216 and 271, respectively, and increased survivorship by 23% for beetles on genotype 42E compared with ambient conditions.

In summary, all measures of beetle performance (larval development time, adult mass and survivorship) were best on aspen genotype 271 and worst on genotype 42E. Elevated CO_2 negatively affected beetles reared on 271 but had inconsistent effects on beetles reared on 216 or 42E. Overall, elevated O_3 decreased beetle performance across genotypes; however, elevated O_3 had the most negative impact on beetles reared on 42E.

Beetle performance in relation to foliar chemistry

Aspen phytochemistry explained a substantial amount (9–45%) of the variation in leaf beetle performance (Table 3). Total foliar phenolic glycosides and nitrogen concentration were inversely related to larval development time for both sexes, indicating that higher concentrations of foliar phenolic glycosides and nitrogen are correlated with shorter development times. Furthermore, total phenolic glycosides and nitrogen concentration positively affected adult mass of both males and females (Table 3). Total phenolic glycoside concentrations explained a minimal amount (9%) of the variation in beetle survivorship, having a positive influence on survivorship.

Discussion

As predicted, aspen genotype had a significant impact on foliar chemistry and beetle performance. Nitrogen and phenolic glycosides, in particular, were the most influential with respect to beetle performance, with both having a positive influence on the specialist herbivore. Aspen leaf beetles performed best on aspen genotype 271 and worst on genotype 42E. Although elevated CO_2 and O_3 had minimal effects on the phytochemical constituents analysed in the present study, the gases influenced beetle performance nonetheless. Elevated CO_2 negatively affected the performance of beetles on genotype 271 but had inconsistent effects across performance variables for beetles reared on genotypes 216 and 42E. Elevated O_3 negatively affected beetle performance across all genotypes, and most strongly for beetles reared on aspen genotype 42E, a relatively O_3 -sensitive genotype.

Foliar chemistry

As predicted, phytochemistry varied significantly among aspen genotypes. Similar to the results of several previous studies (Hemming & Lindroth, 1995; Lindroth *et al.*, 2002; Osier & Lindroth, 2004), aspen genotype had a significant effect on foliar nitrogen concentration. A large genetic effect was observed for condensed tannin concentrations, consistent with previous research (Hwang & Lindroth, 1998; Kopper &

Lindroth, 2003b; Donaldson & Lindroth, 2004). We also observed notable variation in salicortin and tremulacin concentrations among aspen genotypes, consistent with the findings reported by Hwang and Lindroth (1997) and Osier and Lindroth (2004).

Elevated CO₂ and O₃ had minimal effects on aspen phytochemistry, in contrast to our prediction and the results obtained in several previous Aspen FACE studies (Agrell *et al.*, 2000; Holton *et al.*, 2003; Kopper & Lindroth, 2003b). Nonetheless, elevated CO₂ increased condensed tannins, consistent with the findings of Peltonen *et al.* (2005) for birch foliage under elevated CO₂ conditions. Although previous studies have reported an increase in phenolic concentrations under elevated O₃ (Yamaji *et al.*, 2003; Peltonen *et al.*, 2005; Valkama *et al.*, 2007), these effects are not typically observed when O₃ levels are 1.5 × ambient or lower (Valkama *et al.*, 2007). Because Aspen FACE ozone levels are 1.5 × ambient, the absence of significant O₃ effects on phenolic concentrations is not surprising. Furthermore, the meta-analysis of Valkama *et al.* (2007) revealed no significant effects of elevated O₃ on tannins or combined elevated CO₂ and O₃ on overall phenolic concentrations.

Several factors may help explain the minimal effects of CO₂ and O₃ observed in the present study, relative to earlier work at Aspen FACE. First, the effects of CO₂ and O₃ on foliar chemistry can shift temporally through a season (Gifford *et al.*, 2000; Kopper & Lindroth, 2003b; Marinari *et al.*, 2007). Kopper and Lindroth (2003b) found that the major impact of fumigation treatment on nitrogen concentrations, for example, occurred earlier in the growing season compared with when the present study was conducted. Second, the diminution of fumigant impact in the present study could possibly reflect ontogenetic variation in tree responsiveness to CO₂ and O₃: younger, rapidly-growing trees in open stands may be more responsive than older trees in closed-canopy stands.

Beetle performance

Effects of genetic variation in aspen. As predicted, genetic variation in aspen significantly influenced *C. crotchii* performance in the present study. *Chrysomela crotchii* larvae developed fastest on the genotype (271) with the lowest concentrations of condensed tannins and the highest concentrations of phenolic glycosides, as predicted, which supports the notion that specialist herbivores on *Populus* avoid condensed tannins and prefer high concentrations of phenolic glycosides. Phenolic glycosides are probably a valuable resource for specialist leaf beetles such as *C. crotchii*, perhaps serving as a precursor for a defence chemical, such as salicylaldehyde (Rowell-Rahier & Pasteels, 1982), or providing glucose for energy metabolism (Rowell-Rahier & Pasteels, 1986).

Adult beetle mass was also influenced by aspen genotype in our study. The results obtained in the present study are in contrast to several studies (Orians *et al.*, 1997; Coyle *et al.*, 2001; Donaldson & Lindroth, 2004) that report no significant differences in leaf beetle mass among multiple host genotypes. However, Augustin *et al.* (1997) and Glynn *et al.* (2004) did find genetic effects on *Chrysomela scripta* and *Phratora vulgatissima* pupal mass, respectively.

Finally, aspen genotype also significantly influenced *C. crotchii* survivorship. In a study by Coyle *et al.* (2001) that examined the effects of eight *Populus* clones on *C. scripta* performance, larval survivorship was differentially influenced by *Populus* genotype when assayed over multiple beetle generations. Similarly, Augustin *et al.* (1997) reported that *C. scripta* survival varied among five *Populus* clones. The results of the present study suggest that *C. crotchii* survivorship increases with increasing phenolic glycoside concentrations, which are predominantly genetically-determined.

Aspen leaf beetles may use salicylates to synthesize their own defence secretions to combat predation, an adaptation that is common among salicylate-feeding chrysomelids (Rowell-Rahier & Pasteels, 1982; Pasteels *et al.*, 1983). The results obtained in the present study are consistent with this hypothesis because beetle survivorship increased with increasing phenolic glycoside concentrations. Although we had intended to prevent predation with mesh enclosures, we occasionally found that stink bugs had successfully pierced through the mesh enclosures and killed beetle larvae. Less frequently, we observed that cryptic syrphid fly larvae had been accidentally enclosed with the larvae, resulting in additional beetle predation. Predation by stink bugs and syrphid fly larvae across genotypes and fumigation treatments accounted for 0–33% of the total beetle mortality (data not shown). Beetles feeding on genotype 42E grown in control and +O₃ FACE rings experienced the highest predation (33% of mortality was a result of predation). These patterns of mortality suggest that leaf beetles feeding on hosts with higher phenolic glycoside concentrations (i.e. genotypes 216 and 271) may have experienced reduced predation because more salicylates were available for defence.

Host plant quality explains many of the observed differences in beetle performance among aspen genotypes in the present study. In particular, *C. crotchii* appear to perform better on aspen genotypes with higher nitrogen and phenolic glycoside concentrations, as we predicted. These results are similar to those of previous studies on *Populus* and *Salix* leaf beetle specialists. Rowell-Rahier (1984) found that willow containing phenolic glycosides were often fed upon by specialists and avoided by generalist feeders. Rank (1992) found that larvae and adult *Chrysomela aeneicollis* preferred feeding on salicylate-rich willows rather than salicylate-poor willows. Rank *et al.* (1998) showed that larvae of the leaf beetle *Phratora vitellinae* developed faster on salicylate-rich willows than salicylate-poor willows. Moreover, previous studies have shown that salicylates may stimulate feeding by leaf beetles (Rank, 1992; Orians *et al.*, 1997), which may explain the shortened development time and increased beetle mass observed for those larvae reared on aspen genotypes with higher phenolic glycoside concentrations.

Effects of elevated CO₂. We expected to observe a decrease in plant quality and, subsequently, beetle performance under elevated CO₂; however, we observed no significant change in plant quality. We did observe a change in beetle performance, and these changes differed among aspen genotypes. In particular, we observed a decrease in beetle performance on genotype 271 under elevated CO₂. In a study on beetle larvae (*Phratora vitellinae*) reared on willow, Veteli *et al.* (2002) found that elevated

CO₂ reduced relative growth rates, probably as a result of a decrease in food quality (nitrogen content). Furthermore, Johns and Hughes (2002) observed that elevated CO₂ and temperature decreased survivorship of leaf miners. However, O'Neill *et al.* (2008) observed increased longevity and fecundity of Japanese beetles fed soybeans grown under elevated CO₂. In the present study, beetles performed best on genotype 271 under ambient CO₂, causing this genotype to be more susceptible to aspen leaf beetle damage than the other genotypes tested. However, this genotype may be less susceptible to leaf beetle damage in future high-CO₂ environments (as indicated by decreased beetle performance), and thus better able to compete with other genotypes. Because elevated CO₂ had little effect on aspen phytochemistry in the present study, the factors contributing to changes in beetle performance remain unresolved. Nonetheless, the trends in our data indicate that elevated CO₂ may lower the concentration of salicortin in some genotypes (e.g. genotype 271), and this effect may negatively influence the performance of specialist leaf beetles.

Effects of elevated O₃. As we predicted, elevated O₃ generally decreased beetle performance. Previous research (Kopper & Lindroth, 2003a) documented an increase in development time of male blotch leafminers under elevated O₃. By contrast, the effects of elevated O₃ on the forest tent caterpillar have been somewhat inconsistent, causing development time to decrease (Kopper & Lindroth 2003b), increase or remain the same (Fortin *et al.*, 1997). However, the aspen blotch leafminer and *C. crotchii* are specialist herbivores that are likely adapted to species (such as aspen) rich in phenolic glycosides, whereas the forest tent caterpillar is a generalist herbivore that is typically deterred by high concentrations of phenolic glycosides (Lindroth & Bloomer, 1991; Hemming & Lindroth, 1995; Hwang & Lindroth, 1997). Thus, the influence of atmospheric pollutants on herbivore performance may be dependent on the herbivore's feeding behaviour (generalist or specialist) and the specific chemical fingerprint of each host genotype.

Elevated O₃ typically decreased adult mass, and the magnitude of change was genotype-specific in females. Lyytikäinen *et al.* (1996) and Fortin *et al.* (1997) observed a decrease in larval mass of sawflies and male pupal mass of forest tent caterpillars, respectively, when reared on hosts produced under elevated O₃. However, Kopper and Lindroth (2003b) reported an O₃-induced increase in pupal mass of forest tent caterpillars, probably as a result of decreased foliar concentrations of phenolic glycosides and slightly increased concentrations of nitrogen. By contrast, elevated O₃ did not affect pupal mass of aspen blotch leafminers (Kopper & Lindroth, 2003a). In the present study, a combination of factors, including a decrease in nitrogen and a decrease in phenolic glycoside concentrations (although not significant), may have contributed to the decline in aspen leaf beetle mass under elevated O₃. Freiwald *et al.* (2008) documented the importance of phenolic glycosides to leaf beetles that feed on salicaceous species such as aspen. These authors suggest that *Phyllobius pyri* preferred feeding on O₃-exposed leaves because these leaves were physiologically younger (O₃ delays bud burst), and therefore had lower leaf mass per area and higher concentrations of phenolic glycosides than control leaves. Thus, fumigation treatments such as

O₃ can influence leaf structure as well as composition, which may have contributed to the differences in beetle performance observed in the present study.

Elevated O₃ reduced survivorship across all genotypes. Few studies have researched the effects of elevated O₃ on herbivore survivorship on perennial plants. No significant O₃ effects were observed by Lyytikäinen *et al.* (1996), Fortin *et al.* (1997), or Kopper and Lindroth (2003a).

Aspen genotypes 216 and 271 are relatively O₃-tolerant, whereas genotype 42E is relatively O₃-sensitive (Karnosky *et al.*, 2003). Under elevated O₃, beetles performed better on less sensitive genotypes (216 and 271) than on a more sensitive genotype (42E). In high O₃ environments, O₃-tolerant aspen may be more susceptible to outbreaks by specialist herbivores and thus be less successful than predicted based on physiological responses to O₃ alone. Conversely, when herbivore interactions are considered, O₃-sensitive aspen genotypes may be less compromised under future atmospheric conditions than previously predicted.

Conclusions

Substantial genetic variation in trembling aspen may differentially influence the performance of specialist herbivores, such as the aspen leaf beetle, under both ambient and altered atmospheric conditions. Indeed, aspen genotype affected all aspen phytochemistry and leaf beetle performance parameters measured in the present study. Not surprisingly, the most important chemical factors that influenced beetle performance (i.e. phenolic glycosides and nitrogen) are largely genetically-determined.

Many previous studies have shown that phenolic glycosides negatively impact the performance of insect herbivores. The present study, however, shows that phenolic glycoside concentrations positively influence the performance of aspen leaf beetles. This result is congruent with the specialized adaptation of *C. crotchii* to feeding on salicylate-rich species, such as aspen, although the adaptive advantage of this specialization (defence or improved nutrition) remains unexplored.

Despite the minimal atmospheric effects on foliar chemistry, elevated CO₂ and O₃ did alter the performance of aspen leaf beetles. Elevated CO₂ influenced development time (females only), mass and survivorship differently depending on genotype. In general, elevated O₃ negatively influenced beetle performance. However, beetles performed better on O₃-tolerant genotypes than on the O₃-sensitive genotype, which has important implications for the future genetic structure of aspen populations. Indeed, aspen are likely to show differential susceptibility to leaf beetle specialists (e.g. *C. crotchii*) both among and within genotypes under future atmospheric conditions. Coupled with changes in clonal feeding preference that may occur under elevated CO₂ and/or O₃ (Agrell *et al.*, 2005), aspen populations will experience changing impacts by insect herbivores under future atmospheric conditions.

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