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Original Article

Long-term exposure to elevated CO_2 and O_3 alters aspen foliar chemistry across developmental stages

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ABSTRACT

Anthropogenic activities are altering levels of greenhouse gases to the extent that multiple and diverse ecosystem processes are being affected. Two gases that substantially influence forest health are atmospheric carbon dioxide (CO_2) and tropospheric ozone (O_3). Plant chemistry will play an important role in regulating ecosystem processes in future environments, but little information exists about the longitudinal effects of elevated CO_2 and O_3 on phytochemistry, especially for long-lived species such as trees. To address this need, we analysed foliar chemical data from two genotypes of trembling aspen, *Populus tremuloides*, collected over 10 years of exposure to levels of CO_2 and O_3 predicted for the year 2050. Elevated CO_2 and O_3 altered both primary and secondary chemistry, and the magnitude and direction of the responses varied across developmental stages and between aspen genotypes. Our findings suggest that the effects of CO_2 and O_3 on phytochemical traits that influence forest processes will vary over tree developmental stages, highlighting the need to continue long-term, experimental atmospheric change research.

Key-words: Aspen FACE; CO_2; ontogeny; phytochemistry; secondary metabolism; tropospheric ozone.

INTRODUCTION

Atmospheric change is one of the most serious environmental issues confronting natural ecosystems. Anthropogenic activities, such as the combustion of fossil fuels and deforestation, are increasing levels of greenhouse gases (IPCC 2007) to the extent that multiple ecosystem processes are being altered (Norby *et al.* 2005; Holmes *et al.* 2006; Taneva *et al.* 2006; Zak *et al.* 2011, 2012; Talhelm, Pregitzer & Giardina 2012). Forest ecosystems, in particular, are being impacted by elevated concentrations of the greenhouse gases carbon dioxide (CO_2) and tropospheric ozone (O_3).

Elevated concentrations of CO_2 generally increase plant growth and alter the production and concentration of primary and secondary metabolites (Long *et al.* 2004; Ainsworth & Long 2005; Lindroth 2010; Robinson, Ryan & Newman 2012). In contrast, tropospheric O_3 is a highly reactive oxygen species considered to be one of the most damaging environmental pollutants affecting forest health (Karnosky *et al.* 2007; Wittig *et al.* 2009; Ainsworth *et al.* 2012). Ozone decreases plant growth by reducing photosynthesis and also alters production of secondary metabolites, potentially as a response to oxidative stress (Valkama, Koricheva & Oksanen 2007; Heath 2008; Betz *et al.* 2009a, 2009b; Lindroth 2010; Ainsworth *et al.* 2012). Increased duration of ozone exposure can exacerbate its negative effects (Oksanen 2003a, 2003b). Understanding how atmospheric change will affect foliar chemistry is critical to predict the influence of phytochemistry in forest ecosystem functioning in future environments (Lindroth 2010, 2011).

A wealth of information exists on the influence of CO_2 and O_3 on plant metabolism, chemistry and growth (Karnosky *et al.* 2003; Ainsworth & Long 2005; Kontunen-Sopela *et al.* 2007; Bidart-Bouzat & Imeh-Nathaniel 2008; Leakey *et al.* 2009; Lindroth 2010, 2011; Ainsworth *et al.* 2012). Most of this information, however, is based on data collected over relatively short exposure lengths or during a single plant developmental time period. In contrast, the longitudinal effects of elevated CO_2 and O_3 on phytochemical responses during stand development, especially in long-lived tree species, are poorly understood (Körner 2006). In addition to responses to environmental change, developmentally based ('ontogenetic') mechanisms alter phytochemical levels as trees age (Kearsley & Whitham 1989; Bryant & Julkunen-Tiitto 1995; Donaldson *et al.* 2006; Rehill *et al.* 2006; Holeski, Kearsley & Whitham 2009; Holeski *et al.* 2012). For example, leaves of young trembling aspen (*Populus tremuloides*) trees generally have considerably higher levels of phenolic glycosides and lower levels of condensed tannins than leaves of older trees (Donaldson *et al.* 2006; Smith *et al.* 2011). The interaction of environmental variables, such as atmospheric change, with these ontogenetic changes in phytochemistry is largely unknown (Kolb & Matyssek 2001; Karnosky *et al.* 2007; Lindroth 2010).

Taking advantage of multiple years of work by our research group at the Aspen Free-Air Carbon dioxide and ozone Enrichment (FACE) site, we analysed data collected from two genotypes of trembling aspen (*P. tremuloides*) grown for over a decade under levels of CO_2 and O_3 predicted for the year 2050. Our primary goal was to determine the consistency of aspen foliar chemical responses across multiple developmental stages to long-term exposure to

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Table 1. Average effect of CO_2, O_3, and their combination on aspen foliar nitrogen, phenolic glycoside and condensed tannin concentrations over three developmental stages (2–5, 6–10, 11–12 years)

Aspen genotype and treatment combinations	Chemical constituents								
	Nitrogen			Phenolic glycosides			Condensed tannins		
216	2–5	6–10	11–12	2–5	6–10	11–12	2–5	6–10	11–12
Control	2.7 ± 0.1	2.0 ± 0.1	2.4 ± 0.1	9.1 ± 0.4	6.3 ± 0.4	5.7 ± 0.4	17.1 ± 1.3	18.7 ± 1.3	14.6 ± 1.3
CO_2	2.6 ± 0.1	1.9 ± 0.1	2.3 ± 0.1	10.0 ± 0.4	6.2 ± 0.4	5.4 ± 0.4	14.1 ± 1.3	19.8 ± 1.3	16.7 ± 1.3
O_3	2.7 ± 0.1	1.7 ± 0.1	2.2 ± 0.1	7.2 ± 0.4	6.0 ± 0.4	7.7 ± 0.4	18.4 ± 1.3	21.9 ± 1.3	15.5 ± 1.3
$CO_2 + O_3$	2.2 ± 0.1	1.8 ± 0.1	2.3 ± 0.1	8.0 ± 0.4	5.3 ± 0.4	6.3 ± 0.4	20.5 ± 1.3	22.3 ± 1.3	16.2 ± 1.3
271									
Control	3.2 ± 0.1	2.1 ± 0.1	2.5 ± 0.1	9.7 ± 0.8	10.4 ± 0.6	8.1 ± 0.6	6.5 ± 1.5	11.8 ± 0.9	9.9 ± 0.9
CO_2	2.9 ± 0.1	2.0 ± 0.1	2.3 ± 0.1	10.4 ± 0.8	9.7 ± 0.6	7.9 ± 0.6	7.6 ± 1.5	14.4 ± 0.9	12.8 ± 0.9
O_3	3.5 ± 0.1	1.9 ± 0.1	2.3 ± 0.1	9.0 ± 0.8	10.3 ± 0.6	8.4 ± 0.6	7.9 ± 1.5	13.7 ± 0.9	12.1 ± 0.9
$CO_2 + O_3$	2.9 ± 0.1	1.8 ± 0.1	2.3 ± 0.1	9.9 ± 0.8	9.1 ± 0.6	8.4 ± 0.6	9.7 ± 1.5	15.9 ± 0.9	13.7 ± 0.9

Values are mean % dry mass ± 1 standard error.

enhanced levels of CO_2 and O_3. A secondary goal was to evaluate the influence of elevated levels of CO_2 and O_3 on the ontogeny of phytochemistry in two aspen genotypes.

METHODS

Data used in this analysis were collected at the Aspen FACE research facility in northern Wisconsin, USA (W 89.5°, N 45.7°). The 32 ha site contained 12, 30 m diameter experimental rings, with three blocks of four treatments. A full-factorial design allowed for all possible treatment combinations of ambient and elevated (~560 ppm) CO_2 and ambient and elevated (~1.5 × ambient) O_3 levels. A mixed-genotype aspen stand was planted with 1-year-old aspen seedlings in the east half of each ring in 1997 and fumigation treatments began in 1998. Detailed information about the experimental design, setup and operation of the Aspen-FACE research facility can be found in Dickson *et al*. (2000).

We analysed phytochemical data collected from four studies spanning 8 years at Aspen FACE for concentrations of primary (nitrogen) and secondary (phenolic glycosides and condensed tannins) chemical compounds in leaves of two aspen genotypes (216 and 271, both relatively O_3-tolerant genotypes; Karnosky *et al*. 1999). These chemical constituents mediate species interactions and govern ecosystem processes in aspen forests. Nitrogen is likely a growth-limiting factor in forests exposed to elevated CO_2 (Reich *et al*. 2006). Nitrogen (as an index of protein) is also a major limiting factor for insect growth and development (Mattson 1980) and plays an influential role in litter decomposition and nutrient cycling in forests (Melillo, Aber & Muratore 1982). Phenolic glycosides and condensed tannins are the major secondary compounds in aspen. Phenolic glycosides (located in cell vacuoles) defend aspen against insect and mammalian herbivores; condensed tannins (located in vacuoles and attached to cell walls) defend against some herbivores and pathogens, and influence nutrient cycling (Lindroth & Hwang 1996; Constabel & Lindroth 2010; Lindroth & St. Clair 2013).

While chemical data for other aspen genotypes had also been collected, only these two genotypes had phytochemical information collected for all time periods used in this analysis. All studies were conducted by our research group. We excluded three studies (Lindroth *et al*. 2001; Kopper & Lindroth 2003; Agrell *et al*. 2005) examining the phytochemical responses of genotype 216 during the first developmental stage. The information contained in these studies contributed similar chemical information as those used in the current analysis and inclusion of data from the three omitted studies did not alter the results.

Timing and techniques for foliar collections were similar for all studies. Briefly, collected leaves were removed from the petiole and stored on ice in the field. Leaves were then flash frozen in liquid nitrogen, lyophilized, ground and stored in a −20 °C freezer until chemical analysis. Methods employed for chemical quantification of each constituent can be found in the individual studies used in this analysis, whereas information regarding the timing of collections, types of leaves and canopy positions from which leaves were collected, can be found in the supporting information (Supporting Information Appendix 1). We categorized the phytochemical data into three distinct developmental stages (2–5, 6–10 and 11–12 years; Table 1), over which aspen is known to exhibit ontogenetic shifts in concentrations of condensed tannins and phenolic glycosides (Donaldson *et al*. 2006; Smith *et al*. 2011).

We analysed phytochemical responses separately for each genotype using an analysis of variance with a split-plot design, following the model $Y_{ijkl} = b_i + C_j + O_k + CO_{jk} + e_{ijk} + D_l + CD_{jl} + OD_{kl} + COD_{jkl} + \varepsilon_{ijkl}$. In this model, b represents block i, C represents CO_2 level j, O represents O_3 level k, e_{ijk} represents the whole-plot error, D represents developmental stage l, and ε_{ijkl} represents the sub-plot error. Y_{ijkl} represents the average response of block i, CO_2 level j, O_3 level k and developmental stage l. F tests were conducted with degrees of freedom assigned using the Satterthwaite approximation. Statistical analyses were performed with JMP v. 9.0 statistical software (SAS Institute Inc., Cary, NC, USA).

Effect sizes were used to illustrate the influence of elevated CO_2 and O_3 on aspen phytochemistry and were calculated separately for each genotype at each of the three developmental stages using Hedges d. This test statistic uses the

Table 2. Summary of *F* and *P* values for the effects of CO_2, O_3, developmental stage, and their interactions on aspen foliar nitrogen, phenolic glycoside and condensed tannin concentrations for genotypes 216 and 271

	Nitrogen			Phenolic glycosides			Condensed tannins		
Aspen genotype and treatments	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*
216									
CO_2	1.6	14.9	**<0.001**	1.6	0.4	0.548	1.6	0.7	0.409
O_3	1.6	62.5	**<0.001**	1.6	5.7	*0.053*	1.6	12.6	**0.012**
$CO_2 \times O_3$	1.6	0.0	0.950	1.6	3.0	0.129	1.6	0.6	0.467
Developmental stage	1.52	64.8	**<0.001**	1.52	40.4	**<0.001**	1.52	13.3	**<0.001**
$CO_2 \times$ developmental stage	1.52	4.1	**0.022**	1.52	3.7	**0.029**	1.52	0.4	0.612
$O_3 \times$ developmental stage	1.52	0.2	0.816	1.52	14.6	**<0.001**	1.52	1.9	0.146
$CO_2 \times O_3 \times$ developmental stage	1.52	3.0	*0.057*	1.52	0.3	0.752	1.52	1.7	0.186
271									
CO_2	1.9	26.8	**<0.001**	1.6	0.1	0.828	1.14	26.0	**<0.001**
O_3	1.9	0.8	0.372	1.6	0.2	0.694	1.14	17.4	**<0.001**
$CO_2 \times O_3$	1.9	0.5	0.463	1.6	0.0	0.932	1.14	0.1	0.701
Developmental stage	1.40	137.6	**<0.001**	1.40	11.7	**<0.001**	1.40	18.9	**<0.001**
$CO_2 \times$ developmental stage	1.40	4.1	**0.022**	1.40	1.8	0.172	1.40	0.1	0.898
$O_3 \times$ developmental stage	1.40	2.9	*0.061*	1.40	0.7	0.488	1.40	0.0	0.993
$CO_2 \times O_3 \times$ developmental stage	1.40	1.4	0.236	1.40	0.2	0.845	1.40	0.1	0.859

P values <0.05 are bolded and *P* values $0.05 < P < 0.10$ are italicized. Numerator and denominator degrees of freedom (d.f.: numerator, denominator) were calculated using the Satterthwaite approximation. d.f., degrees of freedom.

mean, standard deviation and sample size from each study to calculate the standardized mean difference between the control (i.e. ambient CO_2 or O_3) and the treatment (i.e. elevated CO_2 or O_3) response values (Gurevitch & Hedges 2001). Positive or negative Hedges *d* values reflect higher or lower levels, respectively, of a particular plant chemical constituent in the treatment group relative to the control group. We calculated bootstrapped 95% confidence intervals for the effect sizes using non-parametric variance and 999 resampling iterations. Hedges *d* and confidence intervals were calculated using MetaWin 2.1 (Rosenberg, Adams & Gurevitch 2002).

RESULTS

Aspen phytochemistry was influenced by both elevated CO_2 and O_3. Responses varied considerably, however, between genotypes and among tree developmental stages (Tables 1 & 2, Fig. 1).

Elevated CO_2

Overall, elevated CO_2 had little effect on nitrogen concentrations for genotype 261, but reduced nitrogen levels for genotype 271 (Table 2, Fig. 1). The effects of enriched CO_2, however, varied among tree developmental stages for both genotypes. Elevated CO_2 reduced nitrogen concentrations of leaves from younger, but not older, trees for both genotypes (significant $CO_2 \times$ developmental stage interactions, Table 2), although the diminished effects of CO_2 on leaf nitrogen concentrations in older trees occurred at different rates between genotypes (Fig. 1).

Enriched CO_2 increased phenolic glycoside concentrations in leaves from genotype 216 in younger trees, but decreased levels in leaves from older trees (significant $CO_2 \times$ developmental stage interaction, Table 2, Fig. 1). Elevated CO_2 had little effect on phenolic glycoside levels in trees of genotype 271 (Table 2, Fig. 1). Overall, enriched CO_2 had no effect on condensed tannin levels in genotype 216, but consistently increased condensed tannin concentrations in leaves from genotype 271 (Table 2, Fig. 1).

Elevated O_3

Elevated O_3 reduced nitrogen concentrations in leaves of genotype 216, and this response was consistent among tree developmental stages (Table 2, Fig. 1). In contrast, the effect of O_3 on foliar nitrogen for genotype 271 varied among tree developmental stages: nitrogen concentrations were higher in foliage of younger trees exposed to elevated O_3 and lower in foliage of older trees exposed to enriched O_3 (significant $O_3 \times$ developmental stage interaction, Table 2, Fig. 1).

Enriched O_3 decreased foliar phenolic glycoside levels in younger trees of genotype 216, but increased levels in foliage of older trees (significant $O_3 \times$ developmental stage interaction, Table 2, Fig. 1). In contrast, elevated O_3 had little influence on phenolic glycoside concentrations in genotype 271 (Table 2, Fig. 1). On average, enhanced O_3 increased levels of condensed tannins in genotype 216, with the response magnitude greatest in foliage from younger, compared with older, trees (Table 2, Fig. 1). Elevated O_3 consistently increased levels of condensed tannins in genotype 271 across developmental stages (Table 2, Fig. 1).

DISCUSSION

To our knowledge, this is the first study to evaluate the decadal effects of elevated levels of CO_2 and O_3 on

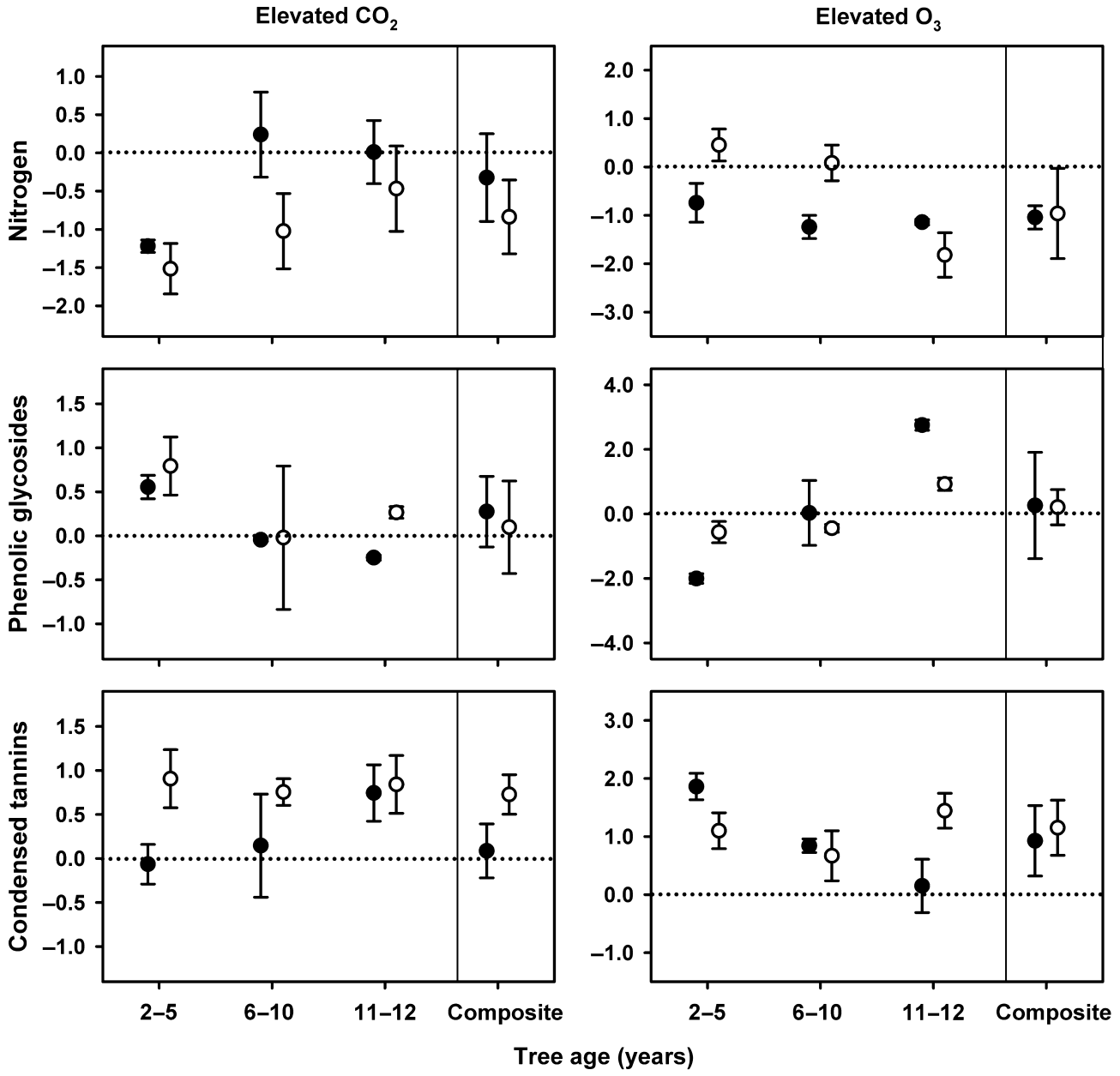

Figure 1. Effects of elevated CO_2 (left panel) and O_3 (right panel) on foliar nitrogen, phenolic glycoside and condensed tannin levels in aspen genotype 216 (closed circles) and 271 (open circles) at three separate tree developmental stages. Points are mean effect sizes calculated from Hedges *d* (except for time 2–5 for aspen genotype 271 whose point is the response ratio calculated from a single study) with bootstrapped 95% confidence intervals calculated from non-parametric variance. For within developmental stage responses $n = 2$ (except for time 2–5 for aspen genotype 271 where $n = 1$). For composite response for aspen genotype 216, $n = 6$, and for genotype 271, $n = 5$.

phytochemical constituents known to play key roles in forest community and ecosystem dynamics. In agreement with short-term studies, we found that levels of CO_2 and O_3 predicted for the year 2050 altered aspen phytochemistry, with substantial variation between genotypes. A novel result of our study is the demonstration that both the direction and magnitude of responses varied among tree developmental stages. Thus, the influence of phytochemistry on forest processes under future atmospheric conditions will depend greatly on forest stand age and composition, and therefore may not be readily extrapolated from the results of short-term studies.

Elevated CO_2 reduced foliar nitrogen concentrations in leaves of young trees of both aspen genotypes, but this response was muted in older trees. Reductions in nitrogen levels are commonly reported in foliage grown in elevated CO_2 (Zvereva & Kozlov 2006; Lindroth 2010; Robinson *et al.* 2012). Such reductions are effected via a number of mechanisms, including accumulation of carbohydrates, altered carbohydrate source–sink relationships and/or a down-regulation of

Rubisco (Long *et al*. 2004; Ainsworth & Long 2005). These findings, however, have been drawn predominantly from studies examining responses to enriched CO_2 levels over relatively short durations of exposure, from studies using young plants, or a combination of the two. Thus, while our report of reduced foliar nitrogen levels in young trees exposed to elevated CO_2 is largely in agreement with previous studies, our results also suggest that tree developmental stage will interact with environmental change to influence tree foliar quality, possibly to a greater extent than environmental influences alone.

Several explanations may underlie the influence of tree ontogeny on foliar nitrogen responses to enriched CO_2 environments. Enriched CO_2 alters competitive interactions for nutrients and other growth-limiting factors in forests (McDonald *et al*. 2002; Zak *et al*. 2012) and these interactions change throughout tree developmental stages (Körner 2006). Elevated CO_2 also increases the allocation of carbon to root production, allowing for greater exploration of soil for nutrients (King *et al*. 2005; Finzi *et al*. 2007; Zak *et al*. 2011). The flux of carbon to belowground microbial processes also increases under elevated CO_2, stimulating microbial activity, altering soil organic matter dynamics and rates of carbon and nutrient cycling, and ultimately facilitating greater rates of nutrient uptake by trees (Finzi *et al*. 2007; Drake *et al*. 2011; Phillips, Finzi & Bernhardt 2011; Zak *et al*. 2011). Additionally, enhanced CO_2 increases litter inputs, elevating the nutrient pool available to trees (Zak *et al*. 2011; Talhelm *et al*. 2012). The increased flux of carbon to belowground processes is suggested to play an important role in sustaining enhanced growth responses under enriched levels of CO_2. If nutrient uptake is able to increase proportionally with growth, than foliar nutrient concentrations are less likely to be affected under elevated CO_2. Plant competitive interactions, enhanced nutrient uptake, and increased nutrient turnover via greater litter inputs are suggested to ameliorate nutrient limitations that may ultimately constrain tree growth responses to elevated CO_2 (Finzi *et al*. 2007; Drake *et al*. 2011; Phillips *et al*. 2012; Zak *et al*. 2011, 2012). Our research suggests that the composite effects of these processes may in turn alter phytochemical concentrations and will do so differently across tree developmental stages.

Elevated O_3 affected nitrogen levels of the two aspen genotypes differently. Enriched O_3 levels consistently reduced nitrogen levels across developmental stages in genotype 216, but nitrogen levels increased in foliage of young trees and decreased in foliage of older trees in genotype 271 exposed to elevated O_3. Our findings contrast with the overall conclusions from a meta-analysis of relatively short-term studies (<3 years), reporting that elevated O_3 generally does not influence foliar nutrient composition (Valkama *et al*. 2007). However, our findings agree with the same report that substantial variation in nutrient responses to enriched O_3 exists among tree genotypes and/or species. In addition, responses to elevated O_3 are dependent upon longevity of exposure, such that prolonged exposure increases O_3 sensitivity and may compound the negative effects of enhanced levels of O_3 (Oksanen 2003a, 2003b). The effects of O_3 are also influenced by numerous environmental factors; drought, light availability, atmospheric CO_2 levels, tree competitive interactions, and nutrient availability can exacerbate or ameliorate tree responses to enriched O_3 levels (Matyssek *et al*. 2005; Kitao *et al*. 2007; Ainsworth *et al*. 2012; Zak *et al*. 2012). Phytochemical changes to enhanced O_3 are likely caused by a combination of factors affecting the molecular, biochemical, and physiological responses to damaging levels of O_3. The combination of these responses alters the balance of growth relative to chemical synthesis, ultimately affecting foliar chemical concentrations. Here we report the phytochemical responses by a tree species grown under the longest duration of exposure to date of experimentally enhanced O_3 levels. Our results agree with the notion that studies examining short- and medium-length exposures to elevated O_3 do not fully capture phytochemical responses that influence ecosystem processes in long-lived tree species (Matyssek *et al*. 2007, 2010).

Elevated CO_2 and O_3 also altered levels of secondary compounds, with variable responses between aspen genotypes and among tree developmental stages. Overall, one genotype (216) was more plastic than the other (271) in its phytochemical response to elevated CO_2 and O_3, with differences among developmental stages in the direction and extent of response.

Elevated CO_2 increased levels of phenolic glycosides in foliage of young trees, while leaves of older trees were less responsive. Elevated CO_2 affected tannin levels in genotype 216, predominantly in foliage from older trees, but consistently increased concentrations across developmental stages in genotype 271. Ontogenetic regulation of secondary metabolites occurs in leaves of aspen trees. For example, juvenile aspen foliage generally contains relatively low levels of condensed tannins and high levels of phenolic glycosides, whereas the opposite relationship exists in mature trees (Donaldson *et al*. 2006; Smith *et al*. 2011). Based upon the response of secondary phytochemistry in genotype 216 to elevated CO_2, it is possible that allocation of additional carbon parallels genetically controlled (ontogenetic) constitutive investment by trees into specific compounds. Additional work is needed to disentangle relationships between ontogeny and environmental change, but our results suggest that these two factors may interact to shape forest processes regulated by foliar chemistry.

Elevated O_3 had variable effects on secondary metabolites between and within genotypes. Elevated O_3 decreased phenolic glycoside levels in foliage from younger trees, but increased levels in foliage from older trees. Elevated O_3 also increased condensed tannin levels in both genotypes: the response by genotype 216 was greater in foliage from younger trees, while the response was generally consistent among developmental stages for genotype 271. Phenolic glycoside and condensed tannin production in aspen leaves is strongly influenced by genetic factors (Lindroth & Hwang 1996). Although plasticity in response to environmental variation does occur for both chemical constituents, phenolic glycosides are less environmentally responsive than condensed tannins (Lindroth & Hwang 1996; Osier & Lindroth 2006; Donaldson & Lindroth 2007). Our observed increases

in phenolic compounds in response to elevated O_3 are consistent with known effects of O_3 on the biosynthetic pathway for phenolics. Elevated O_3 increases activity of the shikimic acid pathway resulting in enhanced production of multiple compounds with antioxidant capabilities (Kangasjärvi *et al*. 1994; Gupta *et al*. 2005; Valkama *et al*. 2007; Heath 2008; Betz *et al*. 2009a, 2009b). Our findings support the notion that elevated O_3 alters aspen secondary chemistry and demonstrate that responses vary both among genotypes and in relation to duration of exposure. Additionally, the overall difference in levels of phenolic compounds measured, especially condensed tannins, between the two genotypes agrees with the idea that investment into specific phenolic compounds as antioxidant defences to O_3 varies among tree genotypes, even those of similar sensitivity to O_3 (Yamaji *et al*. 2003; Häikiö *et al*. 2009).

Secondary metabolites in aspen are known to influence multiple ecosystem processes. As foliar phenolic glycoside levels increase, generalist insect preference for and performance on these leaves declines and canopy damage is reduced (Lindroth & Hwang 1996; Donaldson & Lindroth 2007). Our findings demonstrate that phenolic glycosides may increase or decrease under elevated CO_2 and O_3, and that their response is influenced by tree genotype and developmental stage. We are unsure if the increase in phenolic glycoside concentrations in younger trees under elevated CO_2 was a genetically mediated response to enhanced resource availability or if the increase in phenolic glycosides in more mature trees exposed to enriched O_3 levels was a response to oxidative stress. Regardless of the mechanism, elevated phenolic glycoside levels under elevated CO_2 or O_3 could enhance protection to aspen against unadapted herbivores (Lindroth & Hwang 1996). Levels of foliar condensed tannins can influence nutrient cycling by altering litter decomposition and microbial respiration rates (Hättenschwiler & Vitousek 2000; Kraus, Dahlgren & Zasoski 2003; Madritch, Donaldson & Lindroth 2006; Madritch, Jordan & Lindroth 2007; Schweitzer *et al*. 2008). CO_2- and O_3-mediated changes in litter tannin levels are thus likely to influence these processes (Parsons, Bockheim & Lindroth 2008).

In conclusion, over a decade of exposure to experimentally elevated levels of CO_2 and O_3 influenced the phytochemistry of several aspen genotypes, with variation between genotypes and across tree developmental stages in the both magnitude and direction of response. Our findings suggest that CO_2 and O_3 effects on aspen phytochemistry known to influence forest ecosystem processes are temporally dynamic over decadal time periods.

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Supporting Information Appendix 1. References and foliar collection information for studies included in the analysis.