

Atmospheric CO₂ and O₃ alter competition for soil nitrogen in developing forests

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Abstract

Plant growth responses to rising atmospheric CO₂ and O₃ vary among genotypes and between species, which could plausibly influence the strength of competitive interactions for soil N. Ascribable to the size-symmetric nature of belowground competition, we reasoned that differential growth responses to CO₂ and O₃ should shift as juvenile individuals mature, thereby altering competitive hierarchies and forest composition. In a 12-year-long forest FACE experiment, we used tracer ¹⁵N and whole-plant N content to assess belowground competitive interactions among five *Populus tremuloides* genotypes, between a single *P. tremuloides* genotype and *Betula papyrifera*, as well as between the same single *P. tremuloides* genotype and *Acer saccharum*. Under elevated CO₂, the amount of soil N and ¹⁵N obtained by the *P. tremuloides* genotype common to each community was contingent on the nature of belowground competition. When this genotype competed with its congeners, it obtained equivalent amounts of soil N and tracer ¹⁵N under ambient and elevated CO₂; however, its acquisition of soil N under elevated CO₂ increased by a significant margin when grown in competition with *B. papyrifera* (+30%) and *A. saccharum* (+60%). In contrast, elevated O₃ had no effect on soil N and ¹⁵N acquisition by the *P. tremuloides* genotype common in each community, regardless of competitive interactions. Under elevated CO₂, the rank order of N acquisition among *P. tremuloides* genotypes shifted over time, indicating that growth responses to CO₂ change during ontogeny; this was not the case under elevated O₃. In the aspen-birch community, the competitive advantage elevated CO₂ initially conveyed on birch diminished over time, whereas maple was a poor competitor for soil N in all regards. The extent to which elevated CO₂ and O₃ will shape the genetic structure and composition of future forests is, in part, contingent on the time-dependent effects of belowground competition on plant growth response.

Keywords: belowground competition, elevated CO₂, elevated O₃, interspecific competition, intraspecific competition, soil N

Received 20 September 2011 and accepted 19 October 2011

Introduction

Although elevated atmospheric CO₂ and O₃ influence photosynthesis and plant growth to varying degrees (Ainsworth & Long, 2005; Karnosky *et al.*, 2005), it remains uncertain how this variation will influence intra- and interspecific competition among temperate forest trees (Brooker, 2006), especially for growth-limiting soil N. Moreover, accumulating evidence indicates that the extent to which atmospheric CO₂ or O₃ modify plant growth and the acquisition of soil N is, in turn, contingent on the strength of competitive interactions among individuals (intra- vs. interspecific competition; Liu *et al.*, 2004; Kozovits *et al.*, 2005). Thus, in the absence of competitive interactions, the manner in which individual trees respond to atmospheric CO₂

and O₃ may not be predictive of their performance in mixed-species communities where competition for soil N is keen. As a limited number of studies have focused on the interactive effects of atmospheric CO₂ and O₃ on competition among and within tree species (e.g., Kozovits *et al.*, 2005; Kubiske *et al.*, 2007; Vapaavuori *et al.*, 2009), this remains an important gap in our knowledge of forest response to anthropogenic environmental change. Moreover, projecting how the accumulation of CO₂ and O₃ in the Earth's lower atmosphere will shape the composition of future forests is contingent on understanding the extent to which intra- and interspecific competition constrains, enhances, or has no effect on species-specific growth responses (*sensu* Poorter & Navas, 2003; Bradley & Pregitzer, 2007).

Our present knowledge of how competitive interactions influence the growth response of northern tree species to elevated CO₂ (eCO₂) and elevated O₃ (eO₃) has been derived from short-term chamber studies with

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juvenile individuals (1- to 2-year-old seedlings), and there are reasons to suspect that the strength of competitive interactions, and thus growth responses to eCO₂ and eO₃, will vary during ontogeny. For example, as development progresses from seedling through sapling stages, competitive hierarchies for light change among northern temperate tree species due to interactions among shade tolerance, seed size, and relative growth rate (Sack & Grubb, 2001; Kneeshaw *et al.*, 2006; Niinemets, 2006). These observations present the possibility that similar responses occur belowground for growth-limiting soil resources, and accumulating evidence indicates that soil resource acquisition is proportional to plant size (i.e., size-symmetric competition; Casper & Jackson, 1997; Weiner *et al.*, 1997; Cahill & Casper, 2000). Because plant growth responses to both eCO₂ and eO₃ vary among genotypes and between species, it is plausible this variability is translated into the strength of belowground competitive interactions due to the size-symmetric nature of belowground competition. However, whether the belowground competitive ability changes during ontogeny remains an open question, especially under eCO₂ and eO₃. Furthermore, relatively few studies have assessed belowground competition by directly measuring resource acquisition from soil (e.g., uptake of ¹⁵NH₄⁺ or ¹⁵NO₃⁻; Berntson & Wayne, 2000; Bartelheimer *et al.*, 2008).

Here, we report the results of a decade-long FACE experiment in which we used ¹⁵N and whole-plant N content to assess resource acquisition by five contrasting trembling aspen genotypes (*Populus tremuloides* Michx.) exposed to future concentrations of atmospheric CO₂ and O₃. We also assessed interspecific competition for soil N in mixed stands of paper birch (*Betula papyrifera* Marsh.) and a single genotype of aspen, as well as in mixed stands of sugar maple (*Acer saccharum* Marsh.) and the same single aspen genotype. Previously, we have reported that, after 7 years of growth, eCO₂ modified the competitive hierarchy among aspen genotypes for soil N (i.e., relative to ambient CO₂) and the same was true for eO₃ (Zak *et al.*, 2007). In addition, eCO₂ disproportionally increased the amount of tracer ¹⁵N paper birch obtained from soil, indicating that it became more competitive for soil N than aspen under eCO₂ (Zak *et al.*, 2007). Because aboveground competitive hierarchies can change during ontogeny, we hypothesized that, over time (7 vs. 11 years), belowground competitive hierarchies for soil N also would shift under both eCO₂ and eO₃. To test this idea, we assessed the amount of N and ¹⁵N obtained by aspen genotypes and by the individuals growing in the mixed species communities after 11 years of eCO₂ and eO₃ exposure. This enabled us to determine whether eCO₂, eO₃, or both, exerted interac-

tive effects on soil N acquisition by the genotypes and species growing in our experiment. We then compared the rank order of N and ¹⁵N acquisition by aspen genotypes to assess whether the strength of intraspecific belowground competition has changed over the duration of our experiment (i.e., 7 vs. 11 years). We further assessed whether the strength of interspecific competition between aspen and birch for soil N also has changed, and, report for the first time, how a decade of exposure to eCO₂ and eO₃ has influenced belowground competition in mixed stands of maple and aspen. To our knowledge, no other experiment has evaluated the influences of genetic diversity and species composition on competitive dynamics in developing forests exposed to future concentrations of CO₂ and O₃ in the Earth's lower atmosphere, information necessary to assess how these trace gases will shape future forests.

Methods

Experimental design

The Rhinelander FACE experiment (49°40.5' N, 89°37.5' E; 490 m elevation) was composed of factorial CO₂ (ambient and 560 μmol mol⁻¹) and O₃ (ambient and 50–60 nmol mol⁻¹) treatments arranged in a split-plot, randomized complete block (*n* = 3) design. The treatment combinations were delivered in twelve 30-m FACE rings, each of which was divided into three plant communities (split plot). Our experiment was established on bare ground into which we planted seedlings or ramets that were <1 year old. In 1997, one half of each FACE ring was planted with trembling aspen genotypes (8, 42, 216, 259, and 271) of differing CO₂ responsiveness and O₃ sensitivity (Dickson *et al.*, 2000; Isebrands *et al.*, 2001); one quarter of each ring was planted with a single aspen genotype (216; moderate sensitivity to CO₂ and O₃) and paper birch; the remaining ring quarter was planted with the same aspen genotype (216) and sugar maple. All plant communities were established at a density of one stem m⁻² (Dickson *et al.*, 2000; Karnosky *et al.*, 2005). They contained an equal number of aspen genotypes in the mixed aspen community. Aspen and birch, as well as aspen and maple, also occurred in equal numbers in the mixed species communities; genotypes and species were planted in an alternating manner (see http://aspenface.mtu.edu/ring_maps.htm). Stem density in our experiment (1 stems m⁻²) was similar to that in some naturally occurring 9-year-old aspen stands (Fraser *et al.*, 2006), although variability can be high (Mulak *et al.*, 2006).

In June 2003, 7 years after establishment of our experiment, each 30 m diameter FACE ring was labeled with tracer quantities of ¹⁵N to follow the flow of N in the plant–soil system (Zak *et al.*, 2007). Our purpose was to trace the movement into plants of NH₄⁺ released during the microbial mineralization of organic matter as well as into the microbial community and soil organic matter. Prior to isotope addition, we quantified the natural abundance of ¹⁵N in leaves, branches, stems, and coarse roots for each genotype in the aspen community and

each species growing in the mixed communities (Zak *et al.*, 2007); this analysis was conducted in each FACE ring. Backpack sprayers were then used to evenly disperse (0.034 L m^{-2}) a dilute solution of $^{15}\text{NH}_4\text{Cl}$ (99.98% ^{15}N) over the forest floor. The isotope was applied at the rate of $15 \text{ mg } ^{15}\text{N m}^{-2}$, which represented 3% of the inorganic N pool in mineral soil (0–10 cm depth) at the time of application. Immediately following isotope addition to the forest floor, 1.6 L m^{-2} of water was applied to move ^{15}N into mineral soil. Here, we evaluate the uptake of ^{15}N tracer into plants 6 years after its application.

Plant N and ^{15}N content

We quantified the amount of N and ^{15}N residing in above- and belowground plant components to determine whether CO_2 or O_3 differentially altered the acquisition of soil N by the genotypes and species, using the exact methods of Zak *et al.* (2007). After 11 years of exposure to eCO_2 and eO_3 (i.e., August 2008), we collected leaf, branch, stem, and coarse root samples from two individuals of each aspen genotype growing in the aspen community, and from two individuals of each species growing in the aspen-birch and aspen-maple communities. From each randomly selected individual tree, we collected new leaves, the first four to six mature leaves, and appending fine twigs from four canopy levels: 75% to maximum canopy height, 50–75%, 25–50%, and below 25%. These samples were collected from a scaffold extending into the canopy of each FACE ring; a height pole was used to measure canopy depth. Leaf samples collected from individuals in each plant community were frozen on site until they could be analyzed for N and ^{15}N . In the laboratory, leaves were removed from each appending twig, and each plant component was oven dried at 70°C and then ground to a fine power. Leaves and twigs were analyzed for N concentration and $\delta^{15}\text{N}$ using a Delta Plus isotope ratio mass spectrometer (Thermo-Finnigan, San Jose, CA, USA) interfaced to a NC2500 elemental analyzer (CE Elantech, Lakewood, NJ, USA).

From each randomly selected tree used for leaf and twig collection, we collected a stem sample to quantify its N concentration and $\delta^{15}\text{N}$. Stem samples consisted of a 2.5 cm diameter core collected at 3.8 m above the ground surface; cores extended into the center of each stem. Following collection of the stem sample, two coarse roots were excavated from the base of each tree. The coarse roots were severed and a 2.5 cm section of each was removed. Stem and coarse root samples were taken to the laboratory and prepared for N and ^{15}N analysis as described above. Although fine roots were collected for other purposes, fine root data were not included in the analyses reported here for two reasons: we could not assign fine roots to an individual tree, and fine roots ($< 2 \text{ mm}$) contain a small fraction of whole-plant N ($< 3\%$) and ^{15}N ($< 1\%$) in our experiment (Zak *et al.*, 2007).

Individual tree biomass

In October 2008, the diameter of each tree in each FACE ring was measured. Leaf, branch, and stem biomass were

estimated using allometric equations developed from a complete destructive harvest of the experiment in 2009. We developed separate equations for each genotype and species in our experiment (M. E. Kubiske, unpublished data). During the 2009 destructive harvest, coarse roots were also recovered from aspen ring halves by excavating 1-m-deep soil pits ($2 \text{ m} \times 5 \text{ m}$). In the mixed community ring quarters, pits were 1-m deep and $2 \text{ m} \times 3 \text{ m}$ in size; coarse roots were recovered from each pit using a mechanical sieve. Coarse root biomass in 2008 was estimated as the product of the 2009 coarse-root-to-aboveground-biomass ratio and 2008 aboveground biomass (leaves, branches, and stem).

Plant N and ^{15}N content

We calculated the amount of N contained (g N m^{-2}) in each genotype and species as the summed product of the N concentration (mg N kg^{-1}) and biomass (kg m^{-2}) of each plant component. To calculate the amount of ^{15}N contained in the biomass of each genotype and species, we first determined the % atom excess ^{15}N using the initial and final amount of ^{15}N contained in each plant component (Zak *et al.*, 2007). We then estimated the recovery of tracer ^{15}N ($\text{mg } ^{15}\text{N m}^{-2}$) as the summed product of the N content (g N m^{-2}) and atom percent excess ^{15}N of each plant component. We used the N and ^{15}N content of the genotypes and species in our experiment as a measure of their competitive ability for soil N under ambient and elevated levels of atmospheric CO_2 and O_3 . We used whole-plant N content (g N m^{-2}) as a measure of cumulative N acquisition over the duration of our experiment, and we used recovery of tracer ^{15}N ($\text{mg } ^{15}\text{N m}^{-2}$) as a measure of the N plants acquired following isotope addition (i.e., recent N acquisition).

Statistical analyses

For each plant community, we used an ANOVA for a randomized complete block design to determine whether eCO_2 , eO_3 or both altered the acquisition of soil N by the genotypes and species in our experiment. In these analyses, genotype or species were fixed effects, as were CO_2 and O_3 treatments. Our hypothesis would be supported by the occurrence of a significant interaction between species or genotype and the CO_2 – O_3 treatment combinations. It also would be supported by a change in the rank order of N and ^{15}N acquisition by aspen genotypes, as well as by a change over time in the amounts of N and ^{15}N obtained by aspen and birch when grown together. Because we previously did not analyze soil N acquisition in the mixed aspen-maple stands, we are unable to draw inference regarding whether the strength of competitive interactions had changed over time. Using the same ANOVA model, we analyzed tissue N concentrations to discern whether the amount of N (and ^{15}N) contained in plants resulted from a change in concentration, a change in biomass, or both. Treatment means were compared using a protected Fisher's least significant difference *post hoc* test, and significance was accepted at $\alpha = 0.05$.

Results

Intraspecific competition for soil N under eCO₂ and eO₃

In support of our hypothesis, we observed a significant two-way interaction between aspen genotype and both atmospheric CO₂ and O₃ in the mixed-aspen community, and this was true for both the N ($P = 0.050$) and ¹⁵N ($P = 0.009$) content of the individual aspen genotypes (Fig. 1). For example, eCO₂ significantly increased the N content of genotype 42 and 271, whereas the N content of genotype 8, 216, and 259 was not influenced by this trace gas (Fig. 1a). The same relationship also occurred for the amount of tracer ¹⁵N recovered 5 years following isotope application (i.e., 2008) in the aspen genotypes (Fig. 1b). Elevated O₃ elicited a much different response, wherein genotype 8 became more competitive for soil N (i.e., greater N content), genotype 271 became less competitive for soil N, and genotypes 42, 216, and 259 showed no response (Fig. 1c). The amount of ¹⁵N recovered in these genotypes also displayed the same pattern (Fig. 1d). We observed no three-way interaction among genotype, CO₂, and O₃; therefore, genotypes responded similarly to CO₂ regardless of O₃ concentration, and the reverse is also true (N content, $P = 0.263$; ¹⁵N content, $P = 0.932$).

Interspecific competition for soil N under eCO₂ and eO₃

Although eCO₂ increased the amount of N contained in aspen (genotype 216) and birch growing in the commu-

nity composed by these species, this increase was not statistically significant ($P = 0.589$; Fig. 2a); eCO₂ also had no significant effect on the amount of ¹⁵N residing in the biomass of either species in the aspen-birch community (Fig. 2b). Similarly, species and atmospheric O₃ did not interact to influence the N content of individual species composing the aspen-birch community (Fig. 2c; $P = 0.814$), nor did species and O₃ interact to influence the amount of ¹⁵N recovered in them (Fig. 2d; $P = 0.664$). Consequently, neither atmospheric CO₂ nor O₃ significantly altered belowground competitive interactions between aspen (genotype 216) and birch when these species co-occurred. We also found no three-way interaction among species, CO₂ and O₃ in the aspen-birch community (N content, $P = 0.503$; ¹⁵N content, $P = 0.488$).

In the aspen-maple community, eCO₂ significantly increased the N content of aspen (genotype 216), at the expense of sugar maple (Fig. 3a), and the same was true for the amount of ¹⁵N individually acquired by these co-occurring species (Fig. 3b). Although eO₃ had no influence on the N content of either aspen or maple growing in this mixed community (Fig. 3c), it did significantly decrease the amount of ¹⁵N recovered in aspen; maple showed no response (Fig. 3d). We observed no three-way interaction among species, CO₂ and O₃ in the aspen-maple community (N content, $P = 0.513$; ¹⁵N content, $P = 0.517$), indicating that response to CO₂ is not contingent on O₃, and *vice versa*.

The manner in which atmospheric CO₂ or O₃ influenced the N and ¹⁵N content of aspen genotypes, as well as the species occurring in the mixed species

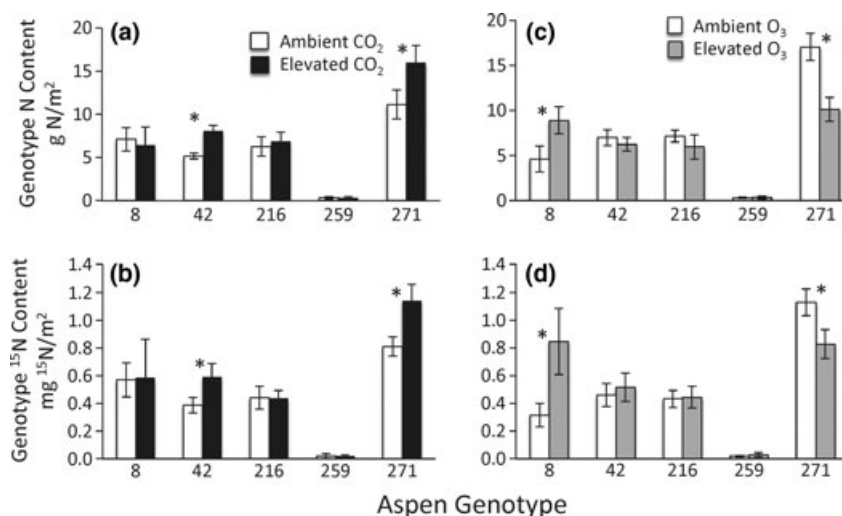


Fig. 1 The interactive response of aspen genotypes to atmospheric CO₂ and O₃ assessed after 11 years of growth. Elevated CO₂ differentially modified the amount of N and ¹⁵N acquired by aspen genotypes over the course of our experiment (panels a and b). Aspen genotypes also differentially responded to elevated O₃, but the acquisition of N and ¹⁵N displayed a much different response to elevated O₃ (panels c and d), relative to elevated CO₂.

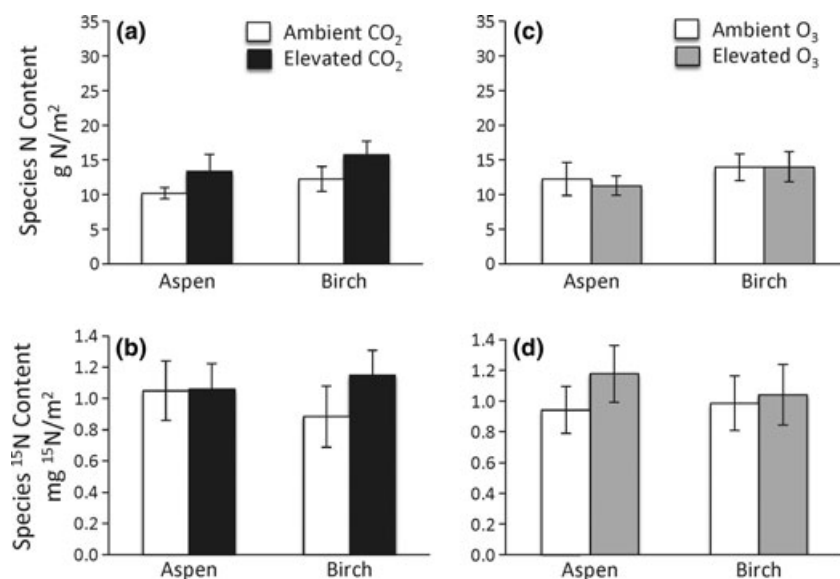


Fig. 2 The N content of aspen genotype 216 and birch growing in the mixed aspen-birch community exposed to CO₂ (panel a) and O₃ (panel c) treatments; neither trace gas interacted with species to influence the amount of N contained in either aspen or birch. In addition, neither did these trace gases interact with species to influence the ¹⁵N content of aspen or birch when grown together (panels b and d).

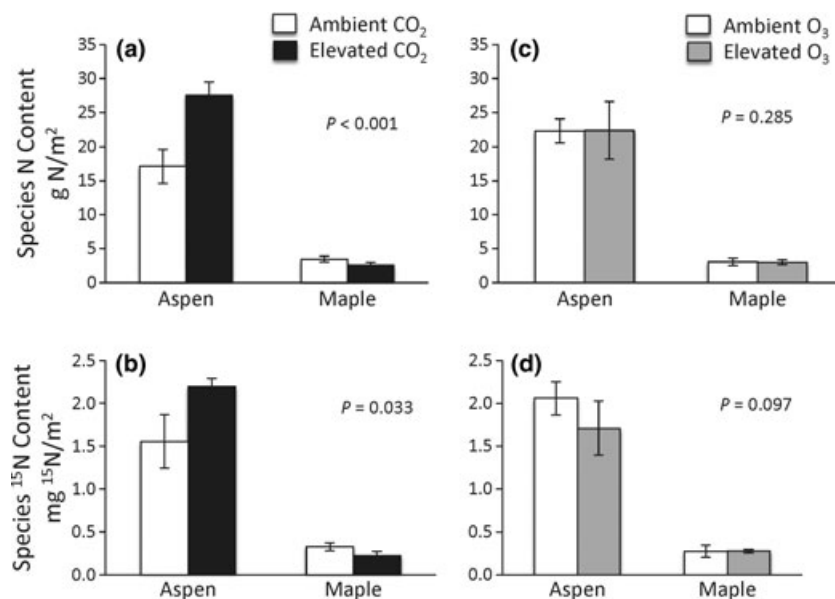


Fig. 3 In the aspen-maple community, there was a significant interaction between species and CO₂, wherein elevated CO₂ differentially increase N acquisition by aspen genotype 216 (panel a). There was no significant interaction between species and O₃ in the aspen-maple community on the N content of either species (panel c). Both CO₂ and O₃ interacted with species in the aspen-maple community, wherein elevated CO₂ increased the ¹⁵N content of aspen; maple showed no response (panel c). Elevated O₃ decreased the ¹⁵N content of aspen growing in the aspen-maple community, but this response was not statistically significant ($P = 0.097$); maple did not respond to elevated O₃ (panel b).

communities, did not arise from large changes in tissue N concentration. To calculate whole-plant N and ¹⁵N content, we determined the N concentration

(mg N g⁻¹) of each plant tissue (i.e., leaves, branches, stems, and coarse roots), and when we analyzed these data, we found very little evidence that either trace gas

had altered tissue N concentration. Although some statistically significant changes did occur, leaf N concentrations, for example, exhibited a <1% relative difference between ambient and elevated levels of CO₂ and O₃. Therefore, the response of whole-plant N and ¹⁵N content to eCO₂ or eO₃ occurred due to changes in biomass (e.g., change in growth, but no change in N concentration), and not from large changes in tissue N (or ¹⁵N) concentration.

Discussion

We hypothesized that eCO₂ and eO₃ would differentially influence the acquisition of growth-limiting soil N among aspen genotypes and between species in our experiment and that competitive abilities for soil resources would change during ontogeny. Previously, we reported that eCO₂ and eO₃ had modified belowground competition among aspen genotypes as well as between aspen and birch (Zak *et al.*, 2007). Here, our evidence indicates that this response was sustained among some aspen genotypes, but not others, and that competitive differences between aspen and paper birch had diminished over time. Although we did not initially analyze competitive interactions between aspen and sugar maple (Zak *et al.*, 2007), it is evident that eCO₂ increased the competitive strength of aspen for soil N when it co-occurred with sugar maple; in contrast, eO₃ had no effect on N acquisition by these co-occurring species. If developing forests respond in a similar manner under field conditions, then eCO₂ and eO₃ have the potential to change the genetic structure of tree populations, the abundance of ecologically important species, as well as the composition of future forests. Most importantly, our results indicate that aspects of biodiversity, like genetic variation and species composition, are integral components of forest response to anthropogenic environmental change.

Intraspecific competition for soil N under eCO₂ and eO₃

After 11 years of exposure, eCO₂ differentially influenced intraspecific competition for growth-limiting soil N among aspen genotypes, but this response shifted over time (Zak *et al.*, 2007). For example, after 7 years of growth in 2004, eCO₂ significantly increased the N content of genotypes 42, 216, and 271 (Zak *et al.*, 2007). However, after 11 years of growth in 2008, the N content of genotype 216 was equivalent under aCO₂ and eCO₂, whereas the N content of genotypes 42 and 271 was still significantly greater under eCO₂; these responses changed the rank order of N content among genotypes over the duration of our experiment. For example, in 2004, genotype 271 contained the greatest

amount of N under eCO₂, followed by genotypes 216, 8, 42, and 259. In 2008, genotype 271 still had the greatest N content under eCO₂, but it was now followed by genotypes 8, 42, 216, and 259 (Fig. 1a). Consequently, the competitive advantage that eCO₂ conveyed on genotype 216 for soil N had diminished over the course of our experiment. Interestingly, under aCO₂, the rank order of aspen genotypes did not change from 2004 to 2008 (highest to lowest N content = 271, 8, 216, 42, 259; Zak *et al.*, 2007; Fig. 1a). In combination, these observations indicate that eCO₂ had a variable influence on the belowground competitive abilities of aspen genotypes, and for those genotypes that initially respond positively, such a response may not be sustained as intraspecific competition strengthens over time. Our results contrast with those of Lau *et al.* (2010) wherein eCO₂ lessened the strength of intraspecific competition for *Arabidopsis thaliana*, *Bromus inermis*, and *Andropogon gerardii*, suggesting that plant life-history traits (herb vs. grass vs. tree) may be an important component of how competition modifies plant growth responses to eCO₂.

The influence of eO₃ on N content changed over time among aspen genotypes, further indicating that the strength of belowground competitive interactions varies during development. Previously, we reported that eO₃ significantly reduced N and ¹⁵N acquisition by genotypes 216 and 271, whereas eO₃ significantly increased them in genotype 8 (Zak *et al.*, 2007). Here, we found that the negative effect of eO₃ on genotype 216 had diminished, whereas the opposing responses by genotypes 8 and 271 have been sustained (Fig. 1c and d). Unlike the response of aspen genotypes to eCO₂, the rank order of N and ¹⁵N content did not change over time (highest to lowest = 271, 8, 42, 216, 259) under eO₃, even though the negative effect of eO₃ on genotype 216 lessened over time.

In the aspen community as a whole, the genotypic-specific responses we described above have sustained the enhancement of net primary productivity (NPP) under eCO₂ as well as resulted in equivalent rates of NPP under eO₃ at the end of our experiment (Zak *et al.*, 2011). For example, aspen community NPP under eCO₂ was 24–35% (2006–2008) greater than rates under aCO₂ (Zak *et al.*, 2011), and this response appears to result from increased N acquisition and growth by genotypes 271 and 42. However, because soil N availability increased under eCO₂, it remains unclear which of these factors underlies greater growth by these genotypes (Zak *et al.*, 2011). Despite initial declines under eO₃ (King *et al.*, 2005), NPP in the aspen community was equivalent under aO₃ and eO₃ in the final years of our experiment (Zak *et al.*, 2011). It appears that compensatory growth by eO₃-tolerant genotypes had offset growth declines in those sensitive to eO₃, and this

response was evident in the growth and acquisition of soil N (and ^{15}N) displayed in Fig. 1. The recent acquisition of soil N, assessed by the ^{15}N content of aspen genotypes (Zak *et al.*, 2007), revealed that increased N acquisition by genotype 8 nearly compensated for declines in N acquisition by genotype 271 (Fig. 1d), a response that directly paralleled that of growth (M. E. Kubiske, unpublished data). Taken together, the aforementioned observations demonstrate that: (i) the strength of intraspecific competition for soil N displayed substantial genotypic variation, which was further modified by eCO_2 and eO_3 , (ii) under eCO_2 , the rank order of N acquisition changed over time, implying that competitive strength for belowground resources also changed during the course of our experiment, and (iii) that compensatory N acquisition and growth by aspen genotypes that were superior competitors have sustained greater NPP under eCO_2 and have contributed equivalent NPP under aO_3 and eO_3 . More importantly, these observations reveal that genetic diversity is a central component of ecosystem response to rising concentrations of CO_2 and O_3 in the Earth's atmosphere, wherein genotypic variation in resource acquisition and growth sustained greater NPP under eCO_2 and buffered NPP from the negative effect of eO_3 on the growth of some genotypes.

Interspecific competition for soil N under eCO_2 and eO_3

The nature of competition (intra- vs. interspecific competition) modified the response of aspen to eCO_2 and eO_3 , as illustrated by genotype 216 which co-occurred with aspen as well as with birch and maple. For example, when genotype 216 occurred with congeners, it contained equivalent amounts of N and ^{15}N under aCO_2 and eCO_2 . In contrast, eCO_2 increased the N content of genotype 216 by 30% when it occurred with paper birch and by 60% when it occurred with sugar maple (Figs 1a, 2a and 3a). Although the strength of intraspecific competition is greater than interspecific competition, the acquisition of soil N under eCO_2 by genotype 216 was clearly contingent on the nature of belowground competitive interactions, especially by the identity of co-occurring species. However, this was not the case for eO_3 , wherein aspen genotype 216 was generally unresponsive to eO_3 regardless of whether it was competing for soil N with aspen, birch, or maple. Interestingly, eO_3 can alter competitive interactions in several directions. For example, eO_3 can lessen competition between seedlings of *Fagus sylvatica* and *Viburnum lantana* (Novak *et al.*, 2008), increase the competitiveness of *Cyperus esculentus* when it co-occurred with *Lycopersicon esculentum* in agriculture fields (Shrestha & Grantz, 2005), as well as modify

competitive interactions and community competition in old-fields (Pfleger *et al.*, 2010); however, we have no evidence that eO_3 exerted a similar effect in the developing forest stands in our experiment.

Competitive differences for soil N between aspen (genotype 216) and paper birch appear to have diminished over time, supporting the idea that the strength of competitive interactions can change during ontogeny. Previously, we reported that eCO_2 disproportionately increased the amount of N and ^{15}N birch acquired from soil, indicating that eCO_2 had increased its competitive ability over that of aspen (Zak *et al.*, 2007). In our earlier report, for example, eCO_2 increased the acquisition of ^{15}N by 68% in birch and by 19% in aspen, a measure of recent N acquisition (Zak *et al.*, 2007). However, at the end of our experiment, the disproportionate increase by birch was no longer apparent under eCO_2 (Fig. 2a and b). Although eCO_2 had no significant influence on the N or ^{15}N content of these co-occurring species (Fig. 2a and b), each species individually contained ~30% more N under eCO_2 . The consistently greater amounts of soil N obtained by aspen and birch appear to be a critical factor sustaining the eCO_2 -enhancement of NPP during the later stages of our experiment (Zak *et al.*, 2011).

The initial negative effect of eO_3 on the amount of N acquired by co-occurring aspen and birch also dissipated over the course of our experiment. In the final year of our experiment, eO_3 had no effect on the amount of N or ^{15}N acquired by these co-occurring species. Furthermore, 1 year following ^{15}N addition, equivalent amounts of the isotope were individually taken up by aspen and birch exposed to aO_3 and eO_3 suggesting recent N acquisition was equivalent, despite the fact that whole-plant N content of each species was significantly lower under eO_3 (Zak *et al.*, 2007). Taken together, these observations indicate that the negative effects of eO_3 on plant growth and N acquisition were lessening during our experiment. The fact that the amount of N and ^{15}N acquired by these species at the end of our experiment was comparable further supports this idea. Moreover, NPP of the aspen-birch community was also equivalent under eO_3 at the end of our experiment (Zak *et al.*, 2011), a response that appears to arise from compensatory growth and N uptake by eO_3 -tolerant birch genotypes and the eO_3 -tolerance of aspen genotype 216, further emphasizing the importance of diversity in moderating ecosystem response to rising O_3 concentrations.

The competitiveness of aspen genotype 216 for soil N was substantially enhanced by eCO_2 when it co-occurred with maple, evidenced by a significant interaction between species and CO_2 (Fig. 3a and b). Unlike its response when competing with congeners, genotype

216 increased its acquisition of soil N (61%) and ¹⁵N (42%) by a substantial margin in the aspen-maple community; it did not respond to eO₃, which is consistent with its response in the other communities. Moreover, maple was generally unresponsive to either trace gas, evidenced by the equivalent amounts of N and ¹⁵N maple contained under ambient and elevated levels of CO₂ or O₃. In addition, maple obtained the smallest quantities of soil N over the duration of our experiment, relative to the other genotypes and species. These observations suggest that, in these developing stands, maple was a relatively poor competitor for soil resources, most likely due to its slow relative growth rate (Kruger & Reich, 1997; Kubiske *et al.*, 2007). Nevertheless, the compensatory growth of aspen genotype 216 was sufficient to sustain the CO₂ enhancement of NPP in the aspen-maple community during the later phase of our experiment as well as maintain NPP under eO₃ at rates comparable to that under aO₃ (Zak *et al.*, 2011).

Conclusions

Understanding how the accumulation of anthropogenic CO₂ and O₃ in the Earth's lower atmosphere will shape the composition of future forests is contingent on understanding how competitive interactions will modify growth responses to these trace gases. A growing body of evidence indicates that the response of tree species to eCO₂ and eO₃, when grown in the absence of intra- and interspecific competition, can provide misleading insights into how they will respond in competition with individuals of the same or different species (Liu *et al.*, 2004; Kozovits *et al.*, 2005; Novak *et al.*, 2008). If we are to accurately predict how eCO₂ and eO₃ will shape the genetic structure of individual tree population as well as their relative abundance in mixed forests, then models of future forest composition should include the time-dependent, interactive effects of competition as a component of growth responses to eCO₂ and eO₃. More importantly, aspects of biodiversity, like genetic diversity and species composition, are important components of ecosystem response to rising atmospheric CO₂ and O₃.

Acknowledgments

This work was supported by grants from the U.S. Department of Energy, Division of Environmental Biology and by the U.S.D.A. Forest Service, North Central Experiment Station. Dave Karnosky played an integral role in the design, initiation, and implementation of this experiment; we miss him as a colleague, collaborator, and friend. Jud Isebrands, George Hendry, Richard Dickson, John Nagy, and Keith Lewin all have provided invaluable and integral contributions over the past decade of this

experiment; we sincerely thank them. Courtney Campamy, Sarah Eisenlord, Pat Micks, Sierra Patterson, Angela Pickett, Alaina Ritter, Alan Talhelm, Rima Upchurch, Jennifer Eikenberry, Wendy Loya and Scott Jacobsen provided assistance in the field and laboratory. Ines Ibanez and Lauren Cline provided insightful comments on an earlier draft of this article.

References

- Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. *New Phytologist*, **165**, 351–372.
- Bartelheimer M, Steinlein T, Beyschlag W (2008) ¹⁵N-nitrate-labeling demonstrates a size symmetric competitive effect on belowground resource uptake. *Plant Ecology*, **199**, 243–253.
- Berntson GM, Wayne PM (2000) Characterizing the size dependence of resource acquisition within crowded plant populations. *Ecology*, **81**, 1072–1085.
- Bradley KL, Pregitzer KS (2007) Ecosystem assembly and terrestrial carbon balance under elevated CO₂. *Trends in Ecology and Evolution*, **22**, 538–547.
- Brooker RW (2006) Plant-plant interactions and environmental change. *New Phytologist*, **171**, 271–284.
- Cahill JF, Casper BB (2000) Investigating the relationship between neighbor root biomass and belowground competition: field evidence for symmetric competition belowground. *Oikos*, **90**, 311–320.
- Casper BB, Jackson RB (1997) Plant competition belowground. *Annual Review of Ecology and Systematics*, **28**, 545–570.
- Dickson RE, Lewin KF, Isebrands JG *et al.* (2000) *Forest Atmosphere Carbon Transfer Storage-II (FACTS II) - The Aspen Free-Air CO₂ and O₃ Enrichment (FACE) Project: An Overview*. U.S. Department of Agriculture Forest Service North Central Experiment Station, General Technical Report NC-214, St. Paul, MN.
- Fraser EC, Landhäusser J, Leiffers VJ (2006) Does mechanical site preparation affect trembling aspen density and growth 9–12 years after treatment? *New Forests*, **32**, 299–306.
- Isebrands JG, McDonald EP, Kruger E *et al.* (2001) Growth of *Populus tremuloides* clones to interacting elevated carbon dioxide and tropospheric ozone. *Environmental Pollution*, **115**, 359–371.
- Karnosky DF, Pregitzer KS, Zak DR *et al.* (2005) Scaling ozone responses of forest trees to the ecosystem level in a changing climate. *Plant Cell Environment*, **28**, 965–981.
- King JS, Kubiske ME, Pregitzer KS *et al.* (2005) Tropospheric O₃ compromises net primary production in young stands of trembling aspen, paper birch and sugar maple in response to elevated atmospheric CO₂. *New Phytologist*, **168**, 623–635.
- Kneeshaw DD, Kobe RK, Coates D, Messier C (2006) Sapling size influences shade tolerance rankings among southern boreal tree species. *Journal of Ecology*, **94**, 471–480.
- Kozovits AR, Matyssek R, Blaschke H, Göttslein A, Grams TEE (2005) Competition increasingly dominates the responsiveness of juvenile beech and spruce to elevated CO₂ and/or O₃ concentrations throughout two subsequent growing seasons. *Global Change Biology*, **11**, 1387–1401.
- Kruger EL, Reich PB (1997) Response of hardwood regeneration to fire in mesic forest openings. III. Whole-plant growth, biomass distribution, nitrogen and carbohydrate relations. *Canadian Journal of Forest Research*, **27**, 1841–1850.
- Kubiske ME, Quinn VS, Heilman W E, Marquardt PE, Karnosky DE (2007) Effects of elevated atmospheric CO₂ and/or O₃ on intra- and interspecific competition. *Plant Biology*, **9**, 342–355.
- Lau JA, Shaw RG, Reich PB, Tiffin P (2010) Species interactions in a changing environment: elevated CO₂ alters the ecological and potential evolutionary consequences of competition. *Evolutionary Ecology Research*, **12**, 435–455.
- Liu X, Kozovits AR, Grams TEE *et al.* (2004) Competition modifies effects of enhanced ozone/carbon dioxide concentrations on carbohydrate and biomass accumulation in juvenile Norway spruce and European beech. *Tree Physiology*, **24**, 1045–1055.
- Mulak T, Landhäusser SM, Leiffers VT (2006) Effects of timing of cleaning and residual density on regeneration of juvenile aspen stands. *Forest Ecology & Management*, **232**, 198–204.
- Niinemetts U (2006) The controversy over traits conferring shade-tolerance in trees: ontogenetic changes revisited. *Journal of Ecology*, **94**, 464–470.
- Novak K, Schaub M, Fuhrer J *et al.* (2008) Ozone effects on visible foliar injury and growth of *Fagus sylvatica* and *Virburnum lantana* seedlings grown in monoculture or in mixture. *Environmental and Experimental Botany*, **62**, 212–220.

- Pfleeger TG, Plocher M, Bichel P (2010) Response of pioneer plant communities to elevated ozone exposure. *Agriculture Ecosystems & Environment*, **138**, 116–126.
- Poorter H, Navas M-L (2003) Plant growth and competition at elevated CO₂: on winners, losers and functional groups. *New Phytologist*, **157**, 175–198.
- Sack L, Grubb PJ (2001) Why do species of wood seedlings change rank in relative growth rate between low and high irradiance. *Functional Ecology*, **15**, 145–154.
- Shrestha A, Grantz DA (2005) Ozone impacts on competition between tomato and yellow nutsedge: above- and below-ground effects. *Crop Science*, **45**, 1587–1595.
- Vapaavuori E, Holopainen JK, Holopainen T *et al.* (2009) Rising atmospheric CO₂ concentration partially masks the negative effects of elevated O₃ on silver birch. *Ambio*, **38**, 418–424.
- Weiner J, Wright DB, Castro S (1997) Symmetry of belowground competition between *Kochia scoparia* individuals. *Oikos*, **79**, 85–91.
- Zak DR, Holmes WE, Pregitzer KS *et al.* (2007) Belowground competition and the response of developing forest communities to atmospheric CO₂ and O₃. *Global Change Biology*, **13**, 2230–2238.
- Zak DR, Kubiske ME, Pregitzer KS, Burton AJ (2011) Forest productivity under elevated CO₂ and O₃: positive feedbacks to soil N cycling sustain decade-long NPP enhancement by CO₂. *Ecology Letters*, doi: 10.1111/j.1461-0248.2011.01692.x.