Atmospheric \( \text{CO}_2 \) and \( \text{O}_3 \) alter competition for soil nitrogen in developing forests

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Abstract

Plant growth responses to rising atmospheric \( \text{CO}_2 \) and \( \text{O}_3 \) 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 \( \text{CO}_2 \) and \( \text{O}_3 \) should shift as juvenile individuals mature, thereby altering competitive hierarchies and forest composition. In a 12-year-long forest FACE experiment, we used tracer \( ^{15}\text{N} \) and whole-plant N content to assess belowground competitive interactions among five \( \text{Populus tremuloides} \) genotypes, between a single \( \text{P. tremuloides} \) genotype and \( \text{Betula papyrifera} \), as well as between the same single \( \text{P. tremuloides} \) genotype and \( \text{Acer saccharum} \). Under elevated \( \text{CO}_2 \), the amount of soil N and \( ^{15}\text{N} \) obtained by the \( \text{P. tremuloides} \) 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 \( ^{15}\text{N} \) under ambient and elevated \( \text{CO}_2 \); however, its acquisition of soil N under elevated \( \text{CO}_2 \) increased by a significant margin when grown in competition with \( \text{B. papyrifera} \) (+30%) and \( \text{A. saccharum} \) (+60%). In contrast, elevated \( \text{O}_3 \) had no effect on soil N and \( ^{15}\text{N} \) acquisition by the \( \text{P. tremuloides} \) genotype common in each community, regardless of competitive interactions. Under elevated \( \text{CO}_2 \), the rank order of N acquisition among \( \text{P. tremuloides} \) genotypes shifted over time, indicating that growth responses to \( \text{CO}_2 \) change during ontogeny; this was not the case under elevated \( \text{O}_3 \). In the aspen-birch community, the competitive advantage elevated \( \text{CO}_2 \) initially conveyed on birch diminished over time, whereas maple was a poor competitor for soil N in all regards. The extent to which elevated \( \text{CO}_2 \) and \( \text{O}_3 \) 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 \( \text{CO}_2 \), elevated \( \text{O}_3 \), interspecific competition, intraspecific competition, soil N

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Introduction

Although elevated atmospheric \( \text{CO}_2 \) and \( \text{O}_3 \) 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 \( \text{CO}_2 \) or \( \text{O}_3 \) 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 \( \text{CO}_2 \) and \( \text{O}_3 \) 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 \( \text{CO}_2 \) and \( \text{O}_3 \) 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 \( \text{CO}_2 \) and \( \text{O}_3 \) 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 \( \text{CO}_2 \) (\( \text{eCO}_2 \)) and elevated \( \text{O}_3 \) (\( \text{eO}_3 \)) has been derived from short-term chamber studies with

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juveniles (1- to 2-year-old seedlings), and there are reasons to suspect that the strength of competitive interactions, and thus growth responses to eCO$_2$ and eO$_3$ 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 eCO$_2$ and eO$_3$ 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 eCO$_2$ and eO$_3$. Furthermore, relatively few studies have assessed belowground competition by directly measuring resource acquisition from soil (e.g., uptake of $^{15}$NH$_4^+$ or $^{15}$NO$_3^-$; Berntson & Wayne, 2000; Bartelheimer et al., 2008).

Here, we report the results of a decade-long FACE experiment in which we used $^{15}$N and whole-plant N content to assess resource acquisition by five contrasting trembling aspen genotypes ($Populus tremuloides$ Michx.) exposed to future concentrations of atmospheric CO$_2$ and O$_3$. 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, eCO$_2$ modified the competitive hierarchy among aspen genotypes for soil N (i.e., relative to ambient CO$_2$) and the same was true for eO$_3$ (Zak et al., 2007). In addition, eCO$_2$ disproportionally increased the amount of tracer $^{15}$N paper birch obtained from soil, indicating that it became more competitive for soil N than aspen under eCO$_2$ (Zak et al., 2007). Because belowground 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 eCO$_2$ and eO$_3$. To test this idea, we assessed the amount of N and $^{15}$N obtained by aspen genotypes and by the individuals growing in the mixed species communities after 11 years of eCO$_2$ and eO$_3$ exposure. This enabled us to determine whether eCO$_2$, eO$_3$, or both, exerted interactive effects on soil N acquisition by the genotypes and species growing in our experiment. We then compared the rank order of N and $^{15}$N 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 eCO$_2$ and eO$_3$ 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 CO$_2$ and O$_3$ 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 CO$_2$ (ambient and 560 µmol mol$^{-1}$) and O$_3$ (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 CO$_2$ responsiveness and O$_3$ 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 CO$_2$ and O$_3$ 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 $^{15}$N 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 NH$_4^+$ 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 $^{15}$N 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 dispense (0.034 L m⁻²) a dilute solution of ¹⁵NH₄Cl (99.98% ¹⁵N) over the forest floor. The isotope was applied at the rate of 15 mg ¹⁵N 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 ¹⁵N into mineral soil. Here, we evaluate the uptake of ¹⁵N tracer into plants 6 years after its application.

Plant N and ¹⁵N content

We quantified the amount of N and ¹⁵N residing in above- and belowground plant components to determine whether CO₂ or O₃ 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 eCO₂ and eO₃ (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 ¹⁵N. 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 powder. Leaves and twigs were analyzed for N concentration and δ¹⁵N 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 δ¹⁵N. 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 ¹⁵N 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 mm) contain a small fraction of whole-plant N (<3%) and ¹⁵N (<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 halts by excavating 1-m-deep soil pits (2 m x 5 m). In the mixed community ring quarters, pits were 1-m deep and 2 m x 3 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 ¹⁵N 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 ¹⁵N contained in the biomass of each genotype and species, we first determined the % atom excess ¹⁵N using the initial and final amount of ¹⁵N contained in each plant component (Zak et al., 2007). We then estimated the recovery of tracer ¹⁵N (mg ¹⁵N m⁻²) as the summed product of the N content (g N m⁻²) and atom percent excess ¹⁵N of each plant component. We used the N and ¹⁵N 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 CO₂ and O₃. 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 ¹⁵N (mg ¹⁵N 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 eCO₂ or eO₃ 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 CO₂ and O₃ treatments. Our hypothesis would be supported by the occurrence of a significant interaction between species or genotype and the CO₂–O₃ treatment combinations. It also would be supported by a change in the rank order of N and ¹⁵N acquisition by aspen genotypes, as well as a change over time in the amounts of N and ¹⁵N 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 ¹⁵N) 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 α = 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 \(^{15}\text{N}\) \((P = 0.009)\) 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 \(^{15}\text{N}\) 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). 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, \(P = 0.263\); \(^{15}\text{N}\) 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 community 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 \(^{15}\text{N}\) 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 \(^{15}\text{N}\) 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, \(P = 0.503\); \(^{15}\text{N}\) 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 \(^{15}\text{N}\) 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 \(^{15}\text{N}\) 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, \(P = 0.513\); \(^{15}\text{N}\) 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 \(^{15}\text{N}\) content of aspen genotypes, as well as the species occurring in the mixed species

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**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 \(^{15}\text{N}\) 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 \(^{15}\text{N}\) displayed a much different response to elevated O₃ (panels c and d), relative to elevated CO₂.

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...

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).

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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 thalia, 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 216 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 15N) displayed in Fig. 1. The recent acquisition of soil N, assessed by the 15N 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 eCO2 and eO3; (ii) under eCO2, 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 eCO2 and have contributed equivalent NPP under aO3 and eO3. More importantly, these observations reveal that genetic diversity is a central component of ecosystem response to rising concentrations of CO2 and O3 in the Earth’s atmosphere, wherein genotypic variation in resource acquisition and growth sustained greater NPP under eCO2 and buffered NPP from the negative effect of eO3 on the growth of some genotypes.

Interspecific competition for soil N under eCO2 and eO3

The nature of competition (intra- vs. interspecific competition) modified the response of aspen to eCO2 and eO3, 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 15N under aCO2 and eCO2. In contrast, eCO2 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 interspecific competition is greater than interspecific competition, the acquisition of soil N under eCO2 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 eO3, wherein aspen genotype 216 was generally unresponsive to eO3 regardless of whether it was competing for soil N with aspen, birch, or maple. Interestingly, eO3 can alter competitive interactions in several directions. For example, eO3 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 (Pfleeger et al., 2010); however, we have no evidence that eO3 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 eCO2 disproportionately increased the amount of N and 15N birch acquired from soil, indicating that eCO2 had increased its competitive ability over that of aspen (Zak et al., 2007). In our earlier report, for example, eCO2 increased the acquisition of 15N 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 eCO2 (Fig. 2a and b). Although eCO2 had no significant influence on the N or 15N content of these co-occurring species (Fig. 2a and b), each species individually contained ~30% more N under eCO2. The consistently greater amounts of soil N obtained by aspen and birch appear to be a critical factor sustaining the eCO2-enhancement of NPP during the later stages of our experiment (Zak et al., 2011).

The initial negative effect of eO3 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, eO3 had no effect on the amount of N or 15N acquired by these co-occurring species. Furthermore, 1 year following 15N addition, equivalent amounts of the isotope were individually taken up by aspen and birch exposed to aO3 and eO3 suggesting recent N acquisition was equivalent, despite the fact that whole-plant N content of each species was significantly lower under eO3 (Zak et al., 2007). Taken together, these observations indicate that the negative effects of eO3 on plant growth and N acquisition were lessening during our experiment. The fact that the amount of N and 15N 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 eO3 at the end of our experiment (Zak et al., 2011), a response that appears to arise from compensatory growth and N uptake by eO3-tolerant birch genotypes and the eO3-tolerance of aspen genotype 216, further emphasizing the importance of diversity in moderating ecosystem response to rising O3 concentrations.

The competitiveness of aspen genotype 216 for soil N was substantially enhanced by eCO2 when it co-occurred with maple, evidenced by a significant interaction between species and CO2 (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 (Krug & 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.

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