Global Change Biology (2007) **13,** 2230–2238, doi: 10.1111/j.1365-2486.2007.01436.x

Belowground competition and the response of developing forest communities to atmospheric CO_2 and O_3

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Abstract

As human activity continues to increase CO_2 and O_3, broad expanses of north temperate forests will be simultaneously exposed to elevated concentrations of these trace gases. Although both CO_2 and O_3 are potent modifiers of plant growth, we do not understand the extent to which they alter competition for limiting soil nutrients, like nitrogen (N). We quantified the acquisition of soil N in two 8-year-old communities composed of trembling aspen genotypes ($n = 5$) and trembling aspen–paper birch which were exposed to factorial combinations of CO_2 (ambient and 560 μL L^{-1}) and O_3 (ambient = 30–40 vs. 50–60 nL L^{-1}). Tracer amount of $^{15}NH_4^+$ were applied to soil to determine how these trace gases altered the competitive ability of genotypes and species to acquire soil N. One year after isotope addition, we assessed N acquisition by measuring the amount of ^{15}N tracer contained in the plant canopy (i.e. recent N acquisition), as well as the total amount of canopy N (i.e. cumulative N acquisition). Exposure to elevated CO_2 differentially altered recent and cumulative N acquisition among aspen genotypes, changing the rank order in which they obtained soil N. Elevated O_3 also altered the rank order in which aspen genotypes obtained soil N by eliciting increases, decreases and no response among genotypes. If aspen genotypes respond similarly under field conditions, then rising concentrations of CO_2 and O_3 could alter the structure of aspen populations. In the aspen–birch community, elevated CO_2 increased recent N (i.e. ^{15}N) acquisition in birch (68%) to a greater extent than aspen (19%), suggesting that, over the course of this experiment, birch had gained a competitive advantage over aspen. The response of genotypes and species to rising CO_2 and O_3 concentrations, and how these responses are modified by competitive interactions, has the potential to change the future composition and productivity of northern temperate forests.

Keywords: Betula papyrifera, carbon dioxide, competition, FACE, forest, nitrogen, nutrient acquisition, ozone, *Populus tremuloides*, species composition

Received 4 October 2006; revised version received 14 February 2007 and accepted 26 April 2007

Introduction

Currently, it is difficult to predict the manner in which elevated CO_2 and O_3 might alter the future composition of temperate forests, because we have a limited understanding of how these trace gases will modify competition for light, water, and nutrients among dominant tree species, as well as among genotypes of a particular species. For example, considerable variation exists among temperate tree taxa in the degree to which elevated CO_2 stimulates growth (Curtis & Wang, 1998; Wang *et al*., 2000; Ainsworth & Long, 2005), as well as

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the extent that phytotoxic levels of tropospheric O_3 reduce growth via foliar damage (Dickson *et al*., 1998; Karnosky *et al*., 1999, 2005). These differential responses could alter competitive interactions among individuals, leading to shifts in forest composition as CO_2 and O_3 increase in the Earth's lower atmosphere. However, the strength of competitive interactions can, in turn, modify the manner in which temperate trees respond to elevated CO_2 and O_3 (Liu *et al*., 2004). How these two atmospheric pollutants shape forest composition appears to depend on the response of individuals as well as how those individual responses are modified by competitive interactions (Kozovits *et al*., 2005).

Competition for soil nitrogen (N) is especially important because it is the nutrient most often constraining the growth of temperate forests (Vitousek & Howarth, 1991). Moreover, elevated CO_2 can differentially stimulate belowground growth and fine root turnover in some temperate trees (Matamala & Schlesinger, 2000; Norby *et al*., 2004; King *et al*., 2005), and it also can have species-specific effects on NH_4^+ and NO_3^- uptake rates (Zerihun & Bassirirad, 2001). In combination, these observations imply that future increases in atmospheric CO_2 could alter the acquisition of soil N to a different extent among temperate trees, presenting the possibility that it also could alter belowground competition for this limiting resource (Poorter & Navas, 2003). Although several studies have investigated the influence of elevated O_3 on intraspecific competition within intact forest stands (Dickson *et al*., 1998; Karnosky *et al*., 1999; Isebrands *et al*., 2001; McDonald *et al*., 2002), few have investigated the effect of CO_2, O_3 or both on nutrient acquisition in this context (Kytöviita *et al*., 2001).

In the Rhinelander free air CO_2–O_3 enrichment (FACE) experiment, communities composed of aspen genotypes (*Populus tremuloides* Michx.) and of aspen–paper birch (*Betula papyrifera* Marsh.) sustain higher rates of productivity under elevated CO_2 by more thoroughly exploiting soil for N (Zak *et al*., 2007). This response occurred despite no increase in either plant N-use efficiency or soil N availability (Holmes *et al*., 2006; Zak *et al*., 2007), and it appears to arise from larger root systems (King *et al*., 2005) which acquired greater amounts of soil N. In contrast, elevated O_3 exerted an opposite effect, wherein growth declined and plants acquired smaller amounts of soil N. Here, we explore the extent these community-level responses arise from the belowground competitive interactions among aspen genotypes and between aspen and paper birch. In the absence of competition, the genotypes and species in our experiment vary in their growth response to CO_2 and O_3 (Dickson *et al*., 2000; Wang *et al*., 2000; Isebrands *et al*., 2001), and we hypothesized these responses would differentially effect their belowground competitiveness in a developing forest community. Although the genotypes or species with the greatest growth under elevated CO_2 should also obtain the greatest amounts of soil N, it is uncertain how sensitivity to O_3 damage, as well as resource competition might alter this expectation (Liu *et al*., 2004; Kozovits *et al*., 2005). These interactions could differentially influence plant growth and dominance in developing forest communities, hence modifying species composition, as well as the genetic structure of tree populations. We analyzed the acquisition soil N among individual aspen genotypes and between aspen and birch by labelling soil with $^{15}NH_4^+$ and quantifying the amount of isotope plants obtained 1 year following application.

Methods

Study site

The Rhinelander FACE facility is located in northeastern Wisconsin, USA (49°40.5′N, 89°37.5′E, 490 m elevation); the site has level topography and a sandy loam soil (Dickson *et al*., 2000). The experiment was established in 1997 and consists of 12 30 m-diameter FACE rings assigned to factorial CO_2 and O_3 treatments in a randomized complete block ($n = 3$) design. The FACE control system maintains target concentrations of CO_2 and O_3 during the daylight hours of the growing season (May–October). The target concentration for elevated CO_2 was 560 μL L^{-1} (200 μL L^{-1} above ambient) and elevated O_3 was maintained at an average 50–60 nL L^{-1} (approximately 1.5 times ambient 30–40 nL L^{-1}; Karnosky *et al*., 2005).

Individual plants of similar size (ca. 10–15 cm in height) were planted at a 1 m × 1 m spacing in 1997 in each 30-m diameter FACE ring (i.e. 10 000 stems ha^{-1}). Aspen genotypes were propagated from root cuttings, whereas other species were propagated from seed. Root cuttings and seeds were collected from local populations in Wisconsin and Michigan, USA. In each FACE ring, one half of the ring was planted with five trembling aspen genotypes (8, 42, 216, 259, and 271) differing in O_3 sensitivity and CO_2 responsiveness (Dickson *et al*., 2000; Isebrands *et al*., 2001). One quarter of each ring was planted with paper birch and one trembling aspen genotype (216) of moderate responsiveness to CO_2 and O_3. The remaining ring quarter was planted with trembling aspen and sugar maple (*Acer saccharum* Marsh.), and it was not used in the present study. Individuals of each species were planted in an alternating manner within their respective ring sections; two to four individuals of each genotype were planted in an alternating pattern in the aspen ring section. For each species and

genotype, equal numbers of individuals occur in their respective ring section (see ring planting map at http://aspenface.mtu.edu/, Dickson *et al*., 2000). All trees were exposed to the experimental treatments during each growing season starting in 1997.

Stem density in our experiment (10 000 stems ha^{-1}) is 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). Following planting, tree biomass averaged 22 g m^{-2} in the mixed aspen community and 15 g m^{-2} in aspen–birch community, and by the end of 2003, tree biomass averaged 3421 g m^{-2} in mixed aspen and 3157 g m^{-2} in aspen–birch stands (King *et al*., 2005); canopy heights ranged from 5.2 m (O_3 treatment) to 6.7 m (CO_2 treatment) at that time. In 2003, canopy closure had occurred in mixed aspen and aspen–birch communities, with the exception of mixed-aspen that were exposed to elevated O_3; there was virtually no mortality in either community. In this phase of community development, we initiated an ^{15}N tracer study to determine whether CO_2, O_3 or the combination of both trace gases altered the competitive ability of genotypes and species to acquire soil N. One year after isotope addition, we assessed N acquisition by measuring the amount of ^{15}N tracer contained in the plant canopy (i.e. recent N acquisition), as well as the total amount of canopy N (i.e. cumulative N acquisition over 8 years of growth).

Isotope tracer experiment

In June 2003, each 30-m diameter FACE ring was labelled with tracer quantities of ^{15}N. Backpack sprayers were used to evenly dispense (0.034 L m^{-2}) a dilute solution of $^{15}NH_4Cl$ (99.98% ^{15}N) over the forest floor. We applied $^{15}NH_4^+$ to follow the movement of NH_4^+ released during microbial mineralization into plants, the soil microbial community, and soil organic matter (Zak *et al*., 2007). The isotope was applied at the rate of 15 mg ^{15}N m^{-2}, which represents approximately 3% of the inorganic N pool in mineral soil (0–10 cm). Immediately following application to the forest floor, 1.6 L m^{-2} of water was applied to rinse the ^{15}N into mineral soil. Soil and plant biomass were collected before, and 1 year following, ^{15}N application; this enabled us to determine the amount of N and ^{15}N in plant and soil pools before, and 1 year following, isotope addition.

In the Rhinelander FACE experiment, the amount of N contained in canopy leaves is 73 ± 16.8% (mean ± SD) of total overstory N content (Zak *et al*., 2007). Because neither CO_2 nor O_3 have altered biomass allocation in our experiment (King *et al*., 2005), we used canopy leaf N to estimate the amounts of soil N and ^{15}N acquired by mixed aspen and aspen–birch communities. We did not include roots because they compose a relatively small proportion of total plant N (ca. 3%); moreover, roots collected from soil cannot be attributed to individual trees growing in our experiment. Although the destructive harvest of individual trees would provide the most accurate estimate of plant N content and acquisition, doing so would have compromised the long-term nature of our experiment. Given these constraints and relationships, we used N in canopy leaves to estimate the cumulative amount of N plants acquired over the 8 year course of treatment exposure. Similarly, we used the amount of canopy ^{15}N 1 year following isotope application to estimate recent N acquisition from soil.

To estimate canopy N and ^{15}N, we collected shoots composed of 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%. We stratified sampling within the canopy to account for vertical variation in N content. Access to the canopy was gained with a scaffold system (15–17 m in height), which extended into aspen and aspen–birch communities. A height pole marked at 0.5 m intervals was then used to gauge canopy levels in each community. At each canopy level, we collected two to three shoots from one individual of each aspen genotype in the mixed aspen community, and two to three shoots from two individuals of aspen (genotype 216) and two individuals of birch in the aspen–birch community. The individuals of each genotype and species were randomly selected along the canopy access scaffold extending into each community. Following the collection of these samples, the vertical distribution of leaf area in aspen and aspen–birch communities was determined using a laser range finder (D.S. Ellsworth, unpublished data); leaf area profiles were measured in all FACE rings.

Shoot samples were frozen and processed at a later date. In the laboratory, shoot samples were thawed and 10 leaf disks (7 mm diameter) were collected from each leaf on each shoot; they were dried, and weighed used to determine leaf mass per area (LMA). Leaves and twigs were then separated, dried at 60 °C, weighed, and ground to a fine powder. These tissues were analyzed for N concentration and $\delta^{15}N$ using a Finnigan Delta Plus isotope ratio mass spectrometer connected to a CE Elantech NC2500 elemental analyzer with a Conflo II interface (Thermo Electron, San Jose, CA, USA).

Using the diameter of each tree in aspen and aspen–birch communities, leaf biomass was estimated using species-specific allometric equations derived from individuals in our experiment (King *et al*., 2005). We then used the vertical distribution of leaf area and LMA determined for each canopy level to partition total leaf biomass, generated via allomatric equations, among the

four canopy levels. In each canopy level, leaf N content ($g\,N\,m^{-2}$) was calculated as the product of tissue N concentration ($mg\,N\,g^{-1}$) and biomass ($g\,m^{-2}$). Additionally, we estimated ^{15}N in leaves in each canopy level as the product of atom% excess ^{15}N, tissue N concentration, and biomass ($g\,m^{-2}$). For each tissue, atom% excess ^{15}N was calculated as the difference between the atom % ^{15}N 1 year after isotope addition and the atom% ^{15}N before isotope addition. For each species and genotype, values were summed across canopy height levels to generate total canopy N (i.e. cumulative N acquisition) and ^{15}N content (recent N acquisition). We report cumulative N acquisition over the 8-year duration of exposure as the amount of canopy N in each genotype and species ($g\,N\,m^{-2}$). Similarly, we report recent N acquisition as the recovery (%) of ^{15}N in canopy leaves, relative to the amount we applied to soil ($15\,mg\,^{15}N\,m^{-2}$).

Statistical analyses

Leaf biomass, leaf N concentration, cumulative N acquisition, and recent N acquisition were analyzed with a general linear model ANOVA for a split-plot randomized complete block design ($n = 3$) with two factorial treatments (CO_2, $n = 2$; O_3, $n = 2$). Separate ANOVAs were used to test differences in treatment effects among genotypes ($n = 5$) in the mixed-aspen community, between species ($n = 2$) in the aspen–birch community, and between the community types ($n = 2$). We analyzed leaf biomass and N concentration to determine which of these parameters drive observed CO_2 or O_3 effects on N acquisition for each genotype and species. In order to assess how competitive interactions affect overall community-level responses, we summed N acquisition across genotypes and species in the mixed aspen and aspen–birch communities, respectively. *Post-hoc* Tukey HSD tests were performed for significant main and interaction effects ($P < 0.05$).

Results

Canopy biomass and N concentration

We observed no significant interaction between CO_2 and O_3 on leaf biomass or N concentration in any genotype or species. On average, elevated CO_2 increased leaf biomass by 50% in birch ($P = 0.009$) and aspen genotypes ($P = 0.001$–0.034), with the exception of genotype 8 which did not respond to CO_2. In the mixed-aspen community, elevated O_3 significantly decreased leaf biomass by 45% in aspen genotypes 216 and 271 ($P = 0.010$–0.001), increased leaf biomass by 42% in clone 8 (although effect was not significant, $P = 0.091$), and had no effect on leaf biomass in other aspen genotypes (42 and 259). In the aspen–birch community, O_3 had no effect on birch canopy leaf biomass, and decreased (−45%) canopy leaf biomass in co-occurring aspen clone 216.

Elevated CO_2 did not alter leaf N concentration in any aspen genotype or in birch ($P = 0.262$–0.899; Table 1). In the mixed-aspen community, elevated O_3 significantly decreased leaf N concentration in genotypes 8, 42, and 259 ($P = 0.005$–0.023) and had no significant effect on leaf N concentration in 216 and 271 (Table 1). In aspen–birch stands, elevated O_3 had no effect on aspen or birch leaf N concentration (Table 1). However, CO_2 and O_3 interacted ($P = 0.018$) to influence birch leaf N concentration, wherein leaf N concentration was lower under elevated O_3 ($17.8\,\mu g\,N\,mg^{-1}$) compared with the control treatment ($21.7\,\mu g\,N\,mg^{-1}$); there was no difference in N concentration between the $CO_2 + O_3$ ($21.6\,\mu g\,N\,mg^{-1}$) and control treatments ($21.7\,\mu g\,N\,mg^{-1}$). Because leaf N concentrations responded very little to our experimental treatments, differences in canopy N and ^{15}N among genotypes and species largely result from their growth response to CO_2 and O_3. That is, genotypes and species with the largest canopies also contained the greatest amounts of N.

Recent and cumulative N acquisition

In our analyses of recent and cumulative N acquisition, there was no significant interactions between CO_2 and O_3 ($P = 0.43$–0.98), indicating the effect of each gas was the same whether trees were exposed to one or both gases. Thus, we report main effect (CO_2, O_3) means by genotype for mixed-aspen stands and by species for aspen–birch stands (Figs 1 and 2).

Within mixed-aspen stands, there was a significant $CO_2 \times$ genotype interaction for recent N acquisition ($P = 0.036$, Fig. 1a) and cumulative N acquisition ($P < 0.006$, Fig. 1b), indicating that elevated CO_2 differentially altered the belowground competitive ability of aspen genotypes. For example, elevated CO_2 significantly increased recently acquired N in genotype 271 and decreased recently acquired N in genotype 8 (Fig. 1a). Elevated CO_2 significantly increased cumulative N acquisition in genotypes 42, 216, and 271 (Fig. 1b); 8 and 259 did not respond to elevated CO_2. These responses altered the rank order in which genotypes 8, 42, and 216 cumulatively obtained soil N, as well as the order in which they obtained soil N over the previous year (i.e. ^{15}N). For aspen genotypes, the $O_3 \times$ genotype interaction also was significant for recent N acquisition ($P < 0.001$, Fig. 1c) and cumulative N acquisition ($P < 0.001$, Fig. 1d). In response to elevated O_3, recent and cumulative N acquisition decreased significantly in

Table 1 In the mixed aspen community, elevated CO_2 had no main effect on the leaf N concentration of any aspen genotype; it also had no main effect on aspen or birch growing in the aspen–birch community

Community type	Ambient CO_2 ($\mu g\,N\,mg^{-1}$)	Elevated CO_2 ($\mu g\,N\,mg^{-1}$)	Ambient O_3 ($\mu g\,N\,mg^{-1}$)	Elevated O_3 ($\mu g\,N\,mg^{-1}$)
Mixed aspen				
Genotytpe 8	23.7a	22.9a	24.7a	22.0b
	(0.91)	(0.83)	(0.53)	(0.73)
Genotype 42	22.4a	22.6a	24.1a	20.9b
	(1.13)	(1.08)	(0.87)	(0.82)
Genotype 216	21.5a	23.0a	22.9a	21.6a
	(0.69)	(0.60)	(0.55)	(0.76)
Genotype 259	22.6a	23.0a	24.8a	19.8b
	(1.49)	(1.55)	(1.15)	(0.75)
Genotype 271	20.5a	20.7a	21.7a	19.4a
	(1.08)	(0.71)	(0.61)	(0.86)
Aspen–birch				
Aspen	22.6a	22.2a	22.8a	22.0a
	(0.31)	(0.58)	(0.48)	(0.39)
Birch	19.7a	21.3a	21.3a	19.7a
	(1.17)	(0.65)	(0.58)	(1.20)

Elevated O_3 (main effect) decrease leaf N concentration in several aspen genotypes in the mixed aspen community, but had no effect on aspen or birch growing in the aspen–birch community. Main effect means (i.e. ambient vs. elevated CO_2) with the same letter are not significantly different.
N, nitrogen.

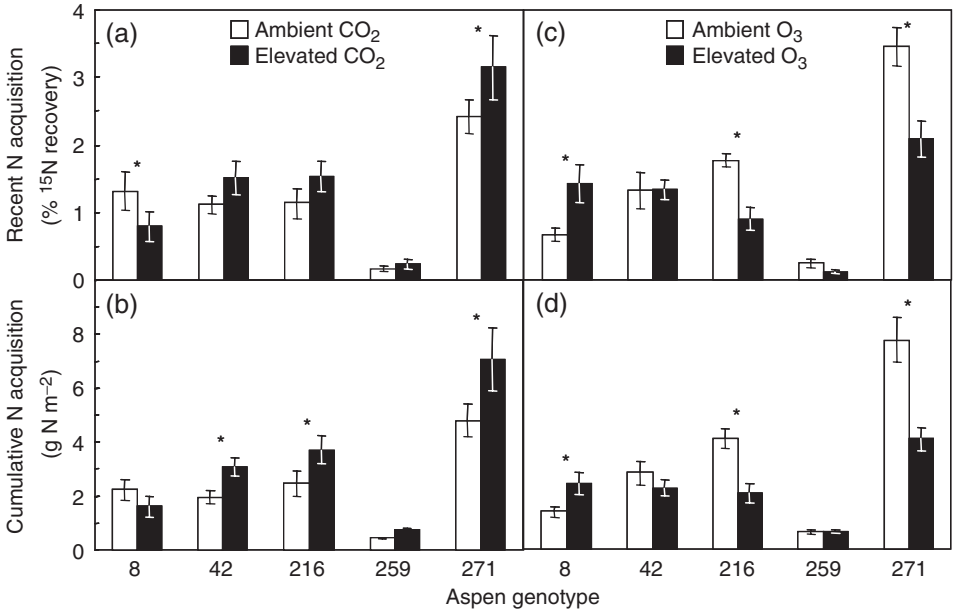

Fig. 1 Main effect of elevated CO_2 and O_3 and on recently acquired N (a, c) and cumulative N acquisition (b, d) in five trembling aspen clones within mixed-genotype aspen stands 1 year following ^{15}N tracer addition. Values are main effect means ± 1 SEM.

genotypes 216 and 271 and increased significantly in 8 (Fig. 1c and d).

Within aspen–birch stands, elevated CO_2 increased recent N acquisition ($P = 0.048$, Fig. 2a) and cumulative N acquisition ($P = 0.008$, Fig. 2b) in both aspen and birch; however, birch responded to a greater extent. For example, elevated CO_2 increased recent N acquisition in birch by 68%, but it increased by a much smaller margin in aspen (19%, Fig. 2a); this difference was significant (one tailed *t*-test; $n = 3$, $P < 0.001$). Elevated O_3 had no

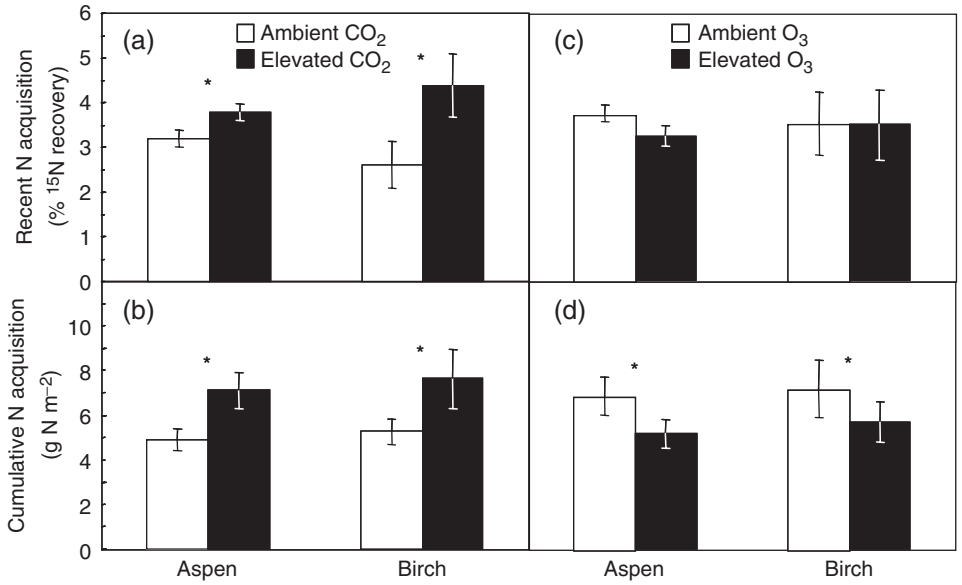

Fig. 2 Main effect of CO_2 and O_3 (right panels) on recently acquired N (a, c) and cumulative N acquisition (b, d) in aspen (clone 216) and paper birch canopies within aspen–birch stands. Values are main effect means ± 1 SEM.

Table 2 Community-level effects of CO_2 and O_3 on recently acquired N and cumulative N acquisition in mixed aspen stands and in aspen–birch communities

	Community type	Ambient CO_2	Elevated CO_2	*P*	Ambient O_3	Elevated O_3	*P*
Recent N acquisition (% ^{15}N recovery)	Mixed aspen	6.0	7.2	0.123	7.3	5.9	0.077
		(0.44)	(0.89)		(0.59)	(0.69)	
	Aspen–birch	5.8	8.2	0.057	7.3	6.7	0.618
		(0.81)	(0.81)		(1.05)	(1.14)	
Cumulative N acquisition ($g\,N\,m^{-2}$)	Mixed aspen	11.3	16.3	0.004	16.7	10.9	0.002
		(1.67)	(2.39)		(2.12)	(1.51)	
	Aspen–birch	10.1	14.7	0.008	14.0	10.8	0.033
		(1.17)	(1.56)		(1.79)	(1.65)	

Values are main effect means (standard error) within mixed aspen and aspen–birch communities. $CO_2 \times O_3$ interaction terms were not significant.
N, nitrogen.

effect on recent N acquisition by aspen or birch (Fig. 2c), and elevated O_3 decreased cumulative N acquisition to the same extent in both species ($P = 0.033$, Fig. 2d).

Using the allometric estimate of biomass for each individual tree within each forest community, we summed canopy N across tree species or genotypes to determine stand-level N acquisition responses to CO_2 and O_3 (Table 2). In both community types, CO_2 and O_3 did not interact to influence recent or cumulative N acquisition. The main effects of CO_2 and O_3 also had no effect on recent N acquisition in either community type However, cumulative N acquisition in the mixed aspen community significantly increased under elevated CO_2 (44%) and decreased significantly (−35%) under elevated O_3. In aspen–birch stands, elevated CO_2 also increased cumulative N acquisition (45%), whereas elevated O_3 elicited a 23% decline in cumulative N acquisition, relative to their respective ambient concentrations.

Discussion

Over the next century, human activity will continue to increase the concentration of CO_2 and O_3 in the Earth's lower atmosphere, both of which have the potential to influence plant growth in ways that can alter the outcome of competition in forest communities (McDonald *et al*., 2002; Liu *et al*., 2004; Kozovits *et al*., 2005). In our experiment, exposure to elevated CO_2 and O_3 differentially altered the ability of aspen genotypes to obtain soil N, wherein both trace gases changed the rank order

of recent and cumulative N acquisition among genotypes. Although aspen and birch responded similarly to CO_2 and O_3, N acquisition increased under elevated CO_2 to a much greater extent in birch, suggesting that this trace gas has increased the competitive ability of this species relative to aspen. These responses occurred in young, closed-canopy communities, in which stem densities and biomass are equivalent to those in naturally occurring stands (King *et al*., 2005; Fraser *et al*., 2006). Our results imply that singly, and in combination, greater concentrations of CO_2 and O_3 could alter the genetic structure of tree populations, as well as the dominance of particular species in north temperate forests.

Evidence supporting our conclusion is the significant interactions we observed between aspen genotypes and both CO_2 and O_3. Within mixed-aspen stands, competitive interactions among aspen genotypes resulted in the differential growth of genotypes under elevated CO_2 and O_3, resulting in differences in N acquisition among genotypes. For example, under ambient CO_2, cumulative and recent N acquisition was greatest in genotype 271, indicating it was a superior competitor for soil N under ambient conditions; genotype 8, 42, and 216 obtained intermediate amounts of soil N (Fig. 1a and b). The remaining genotype (259), which accounted for a small fraction of total canopy N acquisition, was a poor competitor for soil N from the initiation of the experiment (W. E. Holmes, personal observation) and responded little to CO_2 or O_3. Most importantly, elevated CO_2 altered the rank order of recent, as well as cumulative N acquisition among genotypes, wherein genotypes 42 and 216 became more competitive for soil N than 8. These observations suggest that genotypes 8 and 259 will decrease in abundance over the course of our experiment. If aspen growing under field conditions respond in a similar manner, then such a response could presage a change in the genetic structure of aspen populations as CO_2 increases in the atmosphere.

Exposure to elevated O_3 also altered the rank order of aspen genotypes to obtain soil N, resulting in both increases and decreases in N acquisition. For example, elevated O_3 decreased cumulative N acquisition ca. 50% in genotypes 216 and 271, whereas cumulative N acquisition increased by 75% in 8 and changed little in 42. These responses switched the rank order of genotypes 8 and 216 in acquiring soil N. Moreover, two genotypes (216 and 271) which were strong competitors for soil N under elevated CO_2 were those most negatively affected by elevated O_3 (Fig. 1b and d). Aspen genotype 8, which was a poor competitor in elevated CO_2, was able to take advantage of competitive release due to reduced growth of O_3-sensitive clones and actually increased N acquisition in response to O_3. The similarity between recent and cumulative N acquisition responses to CO_2 (Fig. 1a and b) and O_3 (Fig. 1c and d) further indicates that effects of these trace gases on plant N acquisition, and thus, belowground competition, have been sustained over the duration of this experiment. The differential responses we observed among aspen genotypes suggests that rising atmospheric CO_2 and O_3 will likely alter the competitive ability of aspen genotypes to acquire soil N, and hence, also altering their growth and dominance.

Within aspen–birch stands, elevated CO_2 increased cumulative N acquisition by aspen and birch to a similar degree (45%, Fig. 2b). However, the effect of CO_2 on recent N acquisition was notably greater in birch (68%) than aspen (19%, Fig. 2a), suggesting that, over the course of this experiment, birch has been gaining a competitive advantage over aspen in acquiring N. Under elevated O_3, cumulative N acquisition by aspen and birch declined 21–25% (Fig. 2d), but O_3 had no effect on recent N acquisition in either tree species (Fig. 2c). Although elevated O_3 was detrimental to plant N acquisition over an 8-year period of exposure, the negative effect of O_3 appears to be lessening over time. This agrees with observed decreases in O_3 impacts on NPP in these aspen–birch stands (King *et al*., 2005), which resulted from growth decline of O_3-sensitive trees and compensatory growth by those tolerant of O_3. The diminishing effect of O_3 on N acquisition by aspen trees within the aspen–birch community is surprising because this genotype, clone 216, was negatively affected by O_3 when growing in competition with other aspen genotypes in the mixed aspen community (Figs 1c and 2c). The difference in O_3 response of this genotype when grown with aspen or birch suggests that the strength of competitive interactions is as important as O_3 sensitivity in determining the response of the component species and genotypes to elevated O_3.

Several pieces of evidence indicate that increased N acquisition by CO_2-responsive genotypes and species exposed to elevated CO_2 resulted from greater root production. First, plants with the largest canopies also obtained the greatest amount of soil N. Because neither CO_2 nor O_3 have altered biomass allocation in aspen and birch, plants with the largest canopies also had the largest root systems. Moreover, a close relationship exists between root biomass (King *et al*., 2005) and canopy N in control and elevated CO_2 treatments ($R^2 = 0.94$, $P < 0.001$, data not shown). Theoretically, greater N acquisition under elevated CO_2 could occur through a larger absorptive area of roots and mycorrhizal hyphae, greater specific N uptake capacity of roots, or both. However, previous findings indicate that greater N acquisition by trembling aspen exposed to elevated CO_2 did not result from changes in specific N

uptake capacity of fine roots (Rothstein *et al*., 2000); rather, a larger root system under elevated CO_2 enabled plants to more effectively exploit soil for N. These results indicate that greater N acquisition under elevated CO_2 likely resulted from larger root system and a more thorough exploitation of soil (sensu Zak *et al*., 2007). It also is plausible that elevated CO_2 differentially altered the resorption of N from senescing leaves, which could further contribute to differences in N acquisition among the genotypes and species in our experiment. Although we are unable to determine the extent to which N resportion has influenced N acquisition, it is clear that a larger root system under elevated CO_2 is an important mechanism by which plants obtained greater amounts of soil N.

Although few studies have investigated the interactive effects of CO_2, O_3, and competition on resource acquisition, the responses we observed are surprisingly similar to those of competing *Fagus sylvatica* and *Picea abies* seedling exposed to these trace gases. For example, the growth response of *P. abies* to elevated CO_2 and its ability to acquire soil N was contingent on whether individuals were competing with con-specifics or with *F. sylvatica* (Kozovits *et al*., 2005). In this phytotron experiment, the presence of *P. abies* significantly lowered the N content of *F. sylvatica* when both seedlings were simultaneously exposed to CO_2 and O_3, relative to when monocultures of *F. sylvatica* were exposed to both trace gases. As such, intraspecific competition dramatically altered the manner in which each of these species responded to elevated CO_2 and O_3, further suggesting that responsiveness to these trace gases can be modified by competitive interactions. In the absence of competitive interactions, the manner in which individual trees respond to atmospheric CO_2 and O_3 may not be predictive of their performance in mixed-species communities growing under field conditions.

Community-level responses to elevated CO_2 and O_3 appear to depend on species and genotypic composition of forests, as well as outcomes of competitive interactions among individual trees. For example, cumulative N acquisition in mixed-aspen and aspen–birch communities increased 44–45% with elevated CO_2 and decreased 23–35% with elevated O_3 (Table 2). While cumulative effects were consistent among communities, the effects of CO_2 and O_3 on recent N acquisition differed between mixed-aspen and aspen–birch stands. Elevated CO_2 increased recent N acquisition by 41% in aspen–birch stands and had no effect in mixed-aspen stands (Table 2). In aspen–birch stands, both tree species are competitive for N and responsive to CO_2, resulting in consistently greater N acquisition through time under elevated CO_2. In mixed-aspen stands, competitive interactions under elevated CO_2 resulted in a decline in CO_2-enhancement of N acquisition through time, because greater N acquisition by competitive clones was offset by lower N acquisition by a less competitive clone (Fig. 1a). Elevated O_3 decreased recent N acquisition by 19% in mixed-aspen stands and had no effect in aspen–birch stands (Table 2). These observations indicate that elevated O_3 continued to have negative effects on O_3-sensitive genotypes in mixed-aspen stands, whereas effects of O_3 have diminished over time in aspen–birch stands. Therefore, the integrated effects of elevated CO_2 and O_3 on competition among species and genotypes were reflected in community-level responses, and they appear to have changed over the duration of the experiment.

Elevated tropospheric O_3 could limit forest NPP response to elevated CO_2. We found that moderate levels of O_3 exposure ($\sim$60 pbb), comparable with levels commonly occurring mid-summer in midwestern United States, were enough to substantially reduce N acquisition by some aspen genotypes. Our results suggest that shifts in forest composition resulting from intra- or interspecific competition could reduce deleterious effects of elevated O_3 at the stand level through decline of O_3-sensitive genotypes and compensatory growth of O_3-tolerant genotypes and species. Such changes in forest composition are important because aspen and birch are co-occurring species widely distributed throughout the Northern Hemisphere, which will likely be exposed to episodic high concentrations of O_3 in the future (Burns & Honkala, 1990; Fowler *et al*., 1999).

In summary, the accumulation of CO_2 and O_3 in the lower atmosphere has clear potential to alter the strength of competitive interactions within tree populations (e.g. aspen) as well as between tree species (e.g. aspen and birch). Although our experiment was of short duration relative to forest development, exposure to elevated CO_2 and O_3 differentially altered N acquisition among aspen genotypes, increasing it in some and decreasing it in others. In the aspen–birch community, elevated CO_2 enhanced the ability of birch to forage for N in soil to a greater extent than aspen, suggesting that birch had become more competitive for soil resources. In the design of our experiment, one aspen genotype (216) was grown in competition with con-specifics in the mixed aspen community and it also was grown in competition with birch in the aspen–birch community. The fact that this genotype became less responsive to the negative effects of elevated O_3 when competing with birch, suggests that the type and strength of competitive interaction can modify plant response to this trace gas. Inasmuch, plant response to elevated CO_2 and O_3 in the absence of competition may not provide an accurate prediction of performance in situations

where competition for soil resources is keen (i.e. in the field). Furthermore, if CO_2-responsive and O_3-tolerant species and genotypes attain greater dominance over time, then such a response could potentially sustain the CO_2 enhancement of forest NPP. The extent to which genotypes and species respond to rising CO_2 and O_3, as well as how competitive interactions alter these responses, have the potential to shape the future composition and productivity of forests, at least those dominated by organisms in our experiment.

Acknowledgements

This research was supported by the Office of Science (BER), US Department of Energy Grants DE-FG02-93ER61666 and DE-FG02-95ER62125, and by Contract DE-AC02-98CH10886 to Brookhaven National Laboratory, the US Forest Service Northern Global Change Program and North Central Research Station, Michigan Technological University, and Natural Resources Canada–Canadian Forest Service. G. Hendry, D. Karnosky, J. Isebrands, and J. Sober were instrumental in establishing and maintaining this experiment.

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