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LETTER

Productivity and community structure of ectomycorrhizal fungal sporocarps under increased atmospheric CO_2 and O_3

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Abstract

Sporocarp production is essential for ectomycorrhizal fungal recombination and dispersal, which influences fungal community dynamics. Increasing atmospheric carbon dioxide (CO_2) and ozone (O_3) affect host plant carbon gain and allocation, which may in turn influence ectomycorrhizal sporocarp production if the carbon available to the ectomycorrhizal fungus is dependant upon the quantity of carbon assimilated by the host. We measured sporocarp production of ectomycorrhizal fungi over 4 years at the Aspen FACE (free air CO_2 enrichment) site, which corresponded to stand ages seven to 10 years. Total mean sporocarp biomass was greatest under elevated CO_2, regardless of O_3 concentration, while it was generally lowest under elevated O_3 with ambient CO_2. Community composition differed significantly among the treatments, with less difference in the final year of the study. Whether this convergence was due to succession or environmental factors is uncertain. CO_2 and O_3 affect ectomycorrhizal sporocarp productivity and community composition, with likely effects on dispersal, colonization and sporocarp-dependent food webs.

Keywords

Aspen free air CO_2 enrichment, *Betula papyrifera,* carbon dioxide, ectomycorrhizal fungi, northern deciduous forests, ozone, *Populus tremuloides*, productivity, sporocarps.

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INTRODUCTION

Ectomycorrhizal fungi (EMF) may be particularly sensitive to the effects of changing atmospheric carbon dioxide (CO_2) and ozone (O_3) concentrations because they rely on their hosts to supply carbohydrates; therefore, any changes in host condition can affect them in turn. Sporocarps (the reproductive structures of fungi) can be significant carbon sinks for ectomycorrhizal fungi (Last *et al.* 1979; Högberg *et al.* 1999, 2001) and are last in the carbon flux pathway from the source of assimilation. Hence, they are likely to be especially sensitive to changes in the availability of fixed carbon. It is critical to understand how ectomycorrhizal fungal sporocarps are affected by elevated CO_2 and O_3 because sporocarps facilitate genetic recombination, permit long-distance dispersal and contribute to food webs (e.g. Maser *et al.* 1978; Fox 1983).

Tree production and growth typically respond positively to elevated CO_2 (Ainsworth & Long 2005), negatively to elevated O_3 (Andersen 2003), and elevated CO_2 typically mitigates the negative effects of elevated O_3 (Volin *et al.* 1998). As atmospheric CO_2 and O_3 levels continue to increase (Houghton *et al.* 2001; Mickley *et al.* 2001), carbon flux to ectomycorrhizal fungi may parallel the carbon assimilation or belowground carbon allocation of their host trees, especially when nutrients are not limiting (O'Neill 1994; Cairney & Meharg 1999; Andersen 2003).

There are two broad hypotheses for how CO_2 and O_3 may affect EMF community dynamics: first, ectomycorrhizal fungal community structure may be affected by elevated CO_2 or O_3 through effects on stand ontogeny (Rey & Jarvis 1997; Staddon & Fitter 1998; Gorissen & Kuyper 2000). Under this scenario, fungal communities would be similar once the hosts have attained an equivalent growth stage, although the time needed to reach this developmental stage may vary among treatments. Second, community dynamics may be driven more strongly by non-ontogenetic factors altered by elevated CO_2 or O_3, such as abiotic factors (N, P,

pH, etc.), belowground carbon allocation or root turnover rates (Andersen 2003). These two mechanisms are not mutually exclusive, with the first affecting the rate of fungal succession and the second affecting the trajectory of succession.

In order to understand how ectomycorrhizal fungi respond to elevated CO_2 and O_3, we sampled aboveground sporocarps for four years at the Aspen FACE (free air CO_2 enrichment) site near Rhinelander, WI, USA. Among the FACE experiments, this site provides the unique opportunity to examine the effects of both elevated CO_2 and O_3 on a forested ecosystem.

We proposed four testable hypotheses. First, ectomycorrhizal fungal sporocarp production would increase under elevated CO_2 and decrease under elevated O_3 (Hypothesis 1). Second, elevated CO_2 would ameliorate the effects of elevated O_3 on sporocarp production (Hypothesis 2). Third, communities would change as a result of divergent taxon-specific responses to elevated CO_2 and/or O_3 (Hypothesis 3). Fourth, if sporocarp community dynamics are driven by the effects of CO_2 and O_3 on stand ontogeny, this will result in a successional convergence of communities (Hypothesis 4a). Alternatively, if non-ontogenetic factors affected by CO_2 and O_3 drive sporocarp community dynamics more strongly, communities will not converge over time (Hypothesis 4b).

MATERIAL AND METHODS

Study area

The Aspen FACE site is located on the Harshaw Experimental Farm of the USDA Forest Service, near Cassian Township, Oneida County, WI, USA (T37N, R7E, Section 21). The soil is a level to gently rolling sandy loam. Dickson *et al.* (2000) provide a complete description of the Aspen FACE site and its soil characteristics. The land was a potato farm for half a century, followed by a forestry research site for short-rotation hybrid poplar and larch clones in 1972. The study trees were planted in early 1997 and the treatments were implemented in 1998. The study was designed as a randomized complete block design with two factors, CO_2 and O_3, at two levels replicated three times. Carbon dioxide levels were fixed at ambient (*c.* 360 ppm) and elevated (200 ppm + ambient; *c.* 560 ppm). Ozone levels were fixed at ambient (*c.* 33–67 ppb) and elevated (1.5x ambient; *c.* 50–100 ppb). Treatment occurred throughout the growing season, from approximately mid-May to mid-October. Canopy closure began in 2002.

Each treatment replicate contains a stand of 700 square metres in a 30 metre diameter ring. Sampling is confined to a core area of 531 square metres divided into three sections: the eastern half of the area contains four aspen clones (*Populus tremuloides* Michx.), the northwest quarter contains a mixture of one-half aspen and one-half maple (*Acer saccharum* Marshall var. saccharum), and the southwest quarter contains a mixture of one-half aspen and one-half birch (*Betula papyrifera* Marshall). The trees are spaced at one metre distances in a grid pattern.

Field methods

During the growing seasons of 2003–2006 (corresponding to stand age 7–10 years), sporocarps were collected biweekly within the aspen and aspen-birch sections. The aspen-maple sections were not surveyed due to the arbuscular mycorrhizal status of sugar maple trees. Collections occurred throughout the growing seasons, which were determined by tree leaf-out and senescence. Sporocarp collections were performed in belt transects, incorporating the entire core sampling area excluding boardwalks.

The number of sporocarps within each plot was recorded for all taxa. The sporocarps were placed in coolers with ice packs and taken back to the lab for identification. Taxa were defined as ectomycorrhizal through literature searches. After identification, the sporocarps were dried under forced air, cleaned of adhering leaves and soil, and weighed.

Data analyses

Statistical procedures were implemented in SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) and R version 2.3.0 (Venables & Ripley 2002; R Development Core Team 2006). If the data were not normally distributed or the variance was not homogeneous, the data were transformed to 1/sqrt(x+1). Sporocarp production (biomass, relative biomass, density, relative density) was analysed with repeated measures two-way ANOVA in SPSS. The factors CO_2, O_3, block and section (aspen or aspen-birch) were considered fixed. All interactions were modelled except the block interactions. If the section variable was significant, the data were analysed separately for each section.

In order to maintain the factorial design of the Aspen FACE site, we tested for shifts in sporocarp community composition with the programme PERMANOVA (Permutational multivariate analysis of variance; McArdle & Anderson 2001; Anderson 2001, 2005). This programme is a variant of multi-response permutation procedures (MRPP), nonparametric procedures that do not require the assumptions of normality or homogeneous variance. MRPP and its variants can statistically test for differences in the community compositions of groups (Anderson 2001; McCune & Grace 2002).

Analysis with relative and absolute biomass values, absolute and relative density values and the exclusion of rare species did not appreciably affect the results (Table 1).

Table 1 ANOVA table *P*-values of community analysis using PERMANOVA

	Biomass				Density			
	Aspen section		Aspen-birch section		Aspen section		Aspen-birch section	
	Relative biomass	Biomass ($g\ m^{-2}$)	Relative biomass	Biomass ($g\ m^{-2}$)	Relative density	Density (sporocarps m^{-2})	Relative density	Density (sporocarps m^{-2})
CO_2	0.0002**	0.0001**	0.0058**	0.0012**	0.0524*	0.0632*	0.0176**	0.0477**
O_3	0.0439**	0.1056	0.7232	0.4961	0.0054**	0.0573*	0.4044	0.3170
Year	0.0011**	0.0001**	0.0005**	0.0001**	0.0001**	0.0001**	0.0007**	0.0001**
$CO_2 \times O_3$	0.0140**	0.0449**	0.1496	0.0962*	0.1867	0.2484	0.0450**	0.0488**
$CO_2 \times$ year	0.0691*	0.0207**	0.4457	0.2649	0.6023	0.0720*	0.9469	0.8137
$O_3 \times$ year	0.4214	0.7866	0.9230	0.6299	0.1106	0.8055	0.2349	0.4903
$CO_2 \times O_3 \times$ year	0.6970	0.7312	0.7081	0.5689	0.9987	0.9995	0.7396	0.3510

*$P < 0.10$; **$P < 0.05$.

PERMANOVA, permutational multivariate analysis of variance; McArdle & Anderson 2001; Anderson 2001, 2005.
Absolute and relative biomass and density statistics for aspen and aspen-birch communities.
Analysis with rare species taken out gave equivalent results.

Here we present community response using relative biomass (biomass taxon A/ total biomass all taxa), and density (number of sporocarps per unit area) measures only when they provide additional information. Standardizing the data using relative biomass reduces heterogeneity in species abundance between stands while maintaining their rank in comparison to the other species present.

We used unrestricted permutation of the raw data with 9999 permutations and the Bray-Curtis coefficient index. The factors CO_2 and O_3 were analysed, as well as their interaction. Since there is no repeated measures module in PERMANOVA we accounted for repeated measures by including year as an additional fixed factor. Section (aspen or aspen-birch) was always significant ($P \leq 0.05$); the data were analysed separately to understand how the sections differed in response. Non-metric multidimensional scaling biplots of the distance matrices were produced in R vs. 2.3.0.

Species were generalized into categories of 'earlier successional' or 'later successional' (as defined by Fox 1983; Gibson & Deacon 1988; Cripps & Miller 1993; Hutchison & Piche 1995; Cripps 2001; Eaton & Ayers 2002; Nara 2006, 2009; Table 2). While these terms have their limitations (see Visser 1995; Taylor & Bruns 1999; Kranabetter *et al.* 2005; Twieg *et al.* 2007), they nonetheless provide a temporal scale to relate stand age to sporocarpic species presence. We use this term with the assumption that this binomial classification does not fully capture the continuous nature of species-specific community change over time. Earlier successional species tend to begin fruiting earlier on during stand ontogeny while later successional species tend to begin fruiting later on during stand ontogeny.

RESULTS

Total ectomycorrhizal sporocarp biomass

Mean ectomycorrhizal sporocarp biomass was consistently higher under elevated CO_2 ($P < 0.001$) despite differences in production between sections ($P = 0.021$) (Fig. 1). There was a non-significant trend towards decreased production with elevated O_3 ($P = 0.217$) during the first 2 years of sampling. The marginal interaction of CO_2 and O_3 ($P = 0.086$) caused production to be as high, or higher, under elevated CO_2 with or without elevated O_3, while elevated O_3 under ambient CO_2 caused a decrease in production. Production was also variable among sampling years ($P < 0.001$), with lower sporocarp production in 2003 and 2005 and the greatest amount of production in 2006. The interaction of year, CO_2 and O_3 ($P = 0.003$) appears to be driven by lower production in 2003 and 2006 under elevated CO_2 than under elevated CO_2 plus elevated O_3 while the inverse occurred in 2005 (Fig. 1). Total biomass for both sections, averaged across the years, was respectively 184%, 64%, and 183% of the ambient treatment biomass under elevated CO_2, elevated O_3, and elevated CO_2 and O_3.

The two sections exhibited similar results when they were analysed separately. In both the aspen and aspen-birch sections, sporocarp biomass increased under elevated CO_2 ($P = 0.015$ for aspen; $P = 0.039$ for aspen-birch), regardless of O_3 level (Fig. 1). Elevated O_3 resulted in decreased biomass production, although the effect of O_3 was not statistically significant for either section, nor was the interaction of CO_2 and O_3. Production was affected by the sampling year ($P < 0.001$ for aspen and $P = 0.002$ for aspen-birch). Sporocarp production increased under elevated CO_2 by, on average, 1.66 $g\ m^{-2}$ in the aspen sections

Table 2 Average relative biomass (%) of ectomycorrhizal sporocarps for all treatments pooled across 4 years at the Aspen FACE site, Rhinelander, WI, USA

	Generalized successional stage	Aspen sections				Aspen-birch sections			
		Ambient	CO_2	O_3	CO_2 & O_3	Ambient	CO_2	O_3	CO_2 & O_3
Amanita muscaria (L.) Lam.	Later	13.97 ± 13.73				9.96 ± 9.96	2.29 ± 2.29		
Cortinarius acutus (Pers.) Fr. group	Later		0.03 ± 0.02		< 0.01 ± < 0.01	0.05 ± 0.04	0.01 ± 0.01	0.35 ± 0.020	0.75 ± 0.75
Cortinarius bulbosus (Sowerby) Fr. group	Later	2.45 ± 1.81	19.15 ± 17.79	2.15 ± 2.15	0.66 ± 0.40	0.14 ± 0.01	1.92 ± 1.58	4.42 ± 4.42	0.30 ± 0.23
Cortinarius obtusus (Fr.) Fr. group	Later	0.02 ± 0.01	0.06 ± 0.05	0.13 ± 0.07	0.28 ± 0.13	< 0.01 ± 0.01	0.20 ± 0.16	0.04 ± 0.02	0.28 ± 0.24
Hebeloma crustuliniforme (Bull.) Quél. group	Earlier	1.27 ± 1.09	0.14 ± 0.05	6.19 ± 2.24	1.75 ± 1.50	2.67 ± 1.23	0.27 ± 0.15	4.64 ± 2.21	0.86 ± 0.75
Hebeloma mesophaeum (Pers.) Quél. group	Earlier	0.29 ± 0.24	0.50 ± 0.35	1.11 ± 0.37	1.58 ± 1.57	0.62 ± 0.41	0.49 ± 0.13	2.66 ± 0.92	0.52 ± 0.13
Inocybe flocculosa (Berk.) Sacc.	Earlier		0.05 ± 0.05	0.11 ± 0.11	0.77 ± 0.77		0.10 ± 0.10	0.84 ± 0.56	
Inocybe lacera (Fr.) P. Kumm.	Earlier	4.78 ± 1.28	01.31 ± 0.39	3.49 ± 1.84	1.17 ± 0.31	15.03 ± 4.14	2.19 ± 1.12	5.25 ± 2.28	6.01 ± 1.95
Inocybe sororia Kauffman	Earlier	0.01 ± 0.01	< 0.01 ± < 0.01	1.29 ± 1.02	0.26 ± 0.26			1.59 ± 0.83	0.04 ± 0.04
Inocybe sp. 4	Earlier	0.03 ± 0.03		< 0.01 ± < 0.01					
Laccaria laccata (Scop.) Cooke	Earlier	0.07 ± 0.06	0.21 ± 0.20	0.33 ± 0.33		2.72 ± 2.65	0.41 ± 0.27	0.49 ± 0.28	1.22 ± 1.01
Laccaria sp. 'C'	Earlier		< 0.01 ± < 0.01						
Laccaria tortilis (Bolton) Cooke	Earlier	0.03 ± 0.02	0.05 ± 0.03	0.17 ± 0.09	< 0.01 ± < 0.01	0.12 ± 0.12	0.08 ± 0.05	1.06 ± 0.53	0.20 ± 0.20
Lactarius pubescens (Fr.) Fr.	Later	0.02 ± 0.02	0.01 ± 0.01		0.02 ± 0.02		15.21 ± 11.13	5.64 ± 5.64	6.50 ± 3.80
Lactarius sp. 'B'	Later		0.18 ± 0.11	0.01 ± 0.01	0.03 ± 0.03			0.07 ± 0.07	0.06 ± 0.06
Leccinum c.f. *holopus* (Rostk.) Watling	Later		0.98 ± 0.76		1.08 ± 0.42		5.84 ± 3.52	1.17 ± 1.17	7.65 ± 3.88
Leccinum insigne A.H. Sm., Thiers & Watling	Later	69.82 ± 12.01	75.77 ± 18.11	63.24 ± 11.13	91.17 ± 3.48	65.93 ± 8.51	60.70 ± 7.75	58.36 ± 13.03	71.95 ± 8.53
Paxillus involutus (Batsch) Fr.	Earlier	6.95 ± 5.76	1.48 ± 0.79	21.77 ± 12.46	1.21 ± 0.51	2.72 ± 2.72	0.06 ± 0.06	10.11 ± 10.11	0.06 ± 0.06
Peziza c.f. *badia* Pers.	Earlier	0.06 ± 0.06	0.04 ± 0.04		< 0.01 ± < 0.01	0.04 ± 0.04	0.43 ± 0.40	3.08 ± 3.08	0.04 ± 0.01
Peziza c.f. *sylvestris* (Boud.) Sacc. & Traverso	Earlier		0.02 ± 0.02		< 0.01 ± < 0.01		0.09 ± 0.09	0.14 ± 0.14	
Russula sp.	Later		0.01 ± 0.01	0.01 ± 0.01			9.61 ± 4.82	0.09 ± 0.09	3.56 ± 2.84
Thelephora terrestris Ehrh.	Earlier	0.21 ± 0.21	< 0.01 ± 0.01				0.09 ± 0.08		0.01 ± 0.01
Pooled earlier-stage fungal species	Earlier	13.71 ± 5.71	3.80 ± 1.19	34.45 ± 12.43	6.75 ± 3.29	23.92 ± 1.77	4.21 ± 1.46	29.87 ± 9.71	8.96 ± 0.62
Pooled later-stage fungal species	Later	86.29 ± 5.71	96.20 ± 1.19	65.55 ± 12.43	93.25 ± 3.29	76.08 ± 1.77	95.79 ± 1.46	70.13 ± 9.71	91.04 ± 0.62

FACE, free air CO_2 enrichment.
Standard error (±) given.
Blanks indicate lack of taxa presence for that treatment.
The two sections, aspen and aspen-birch, are shown separately.
The generalized successional stage of the species is included as well (see text for references).
The average pooled per cent contribution by earlier- and later-stage groups is also provided.

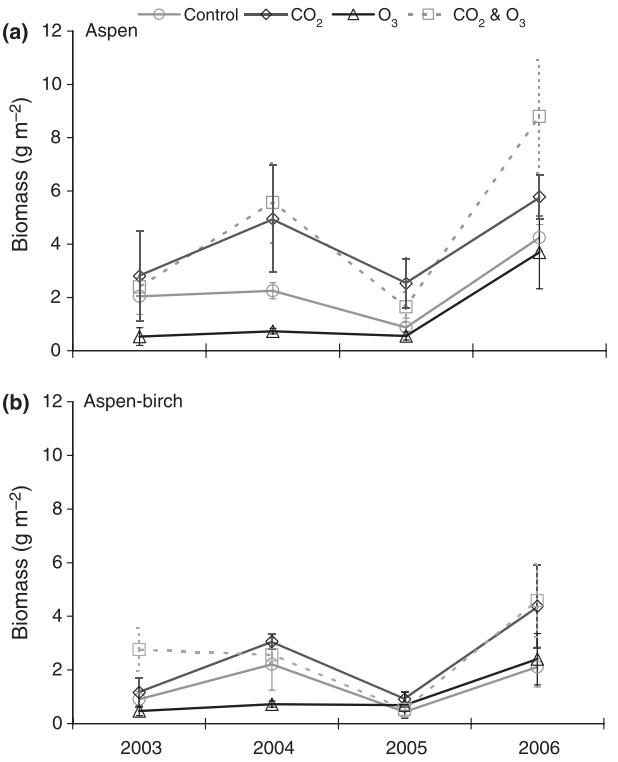

Figure 1 Mean biomass of ectomycorrhizal sporocarps for 4 years within (a) aspen sections and (b) aspen-birch sections at the Aspen FACE (free air CO_2 enrichment) site, Rhinelander, WI, USA. Each section was analysed separately, and standard error bars (±) are shown. Control is ambient CO_2+ambient O_3, CO_2 is elevated CO_2+ambient O_3, O_3 is ambient CO_2+elevated O_3, CO_2 & O_3 is elevated CO_2+elevated O_3.

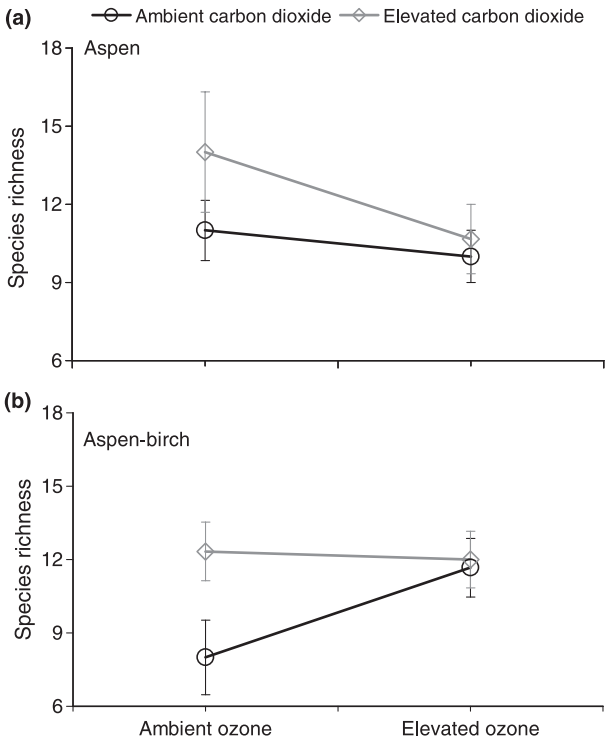

Figure 2 Average species richness of sporocarp producing ectomycorrhizal fungi within the (a) aspen sections and (b) aspen-birch sections at the Aspen FACE (free air CO_2 enrichment) site. Richness is calculated from the total number of species groups present over 4 years within each replicate. Standard error bars (±) are shown.

and 0.96 g m^{-2} in the aspen-birch sections, which was 196% and 170%, respectively, of the ambient treatment biomass. The average decrease in biomass under elevated O_3 was 0.97 g m^{-2} in the aspen sections and 0.35 g m^{-2} in the aspen-birch sections, which was 52% and 88%, respectively, of the ambient treatment biomass. The elevated CO_2 and O_3 treatment biomass increased by 2.25 g m^{-2} in the aspen sections and 1.19 g m^{-2} in the aspen-birch sections, which was 190% and 188%, respectively, of the ambient treatment biomass.

Species richness

Twenty-two species produced sporocarps in the aspen sections across the 4 years. Two of these species did not fruit in aspen-birch sections, leading to a total of 20 species in that section (Table 2). Species richness was marginally higher under elevated CO_2 within both sections ($P = 0.061$, Fig. 2). Richness was not significantly different between the two sections. The interaction of O_3 and section ($P = 0.082$) is likely due to dissimilar effects of elevated O_3 within the two sections. Richness decreased in the aspen sections and increased, or was equivalent, in the aspen-birch sections under elevated O_3. Within the aspen sections, mean total species richness was lower under elevated O_3 regardless of CO_2 level. In contrast, within the aspen-birch sections, richness was higher under the three non-ambient conditions and highest in the elevated CO_2 treatments. These trends were not statistically significant ($P > 0.100$ for all factors) when the two sections were analysed separately.

Community response

When analysed using relative biomass, CO_2 concentration affected the community composition of ectomycorrhizal sporocarps within both the aspen ($P < 0.001$) and aspen-birch ($P = 0.006$) sections (Fig. 3). There was an effect of year on composition in both sections as well ($P \leq 0.001$). Additionally, in the aspen section O_3 affected community composition ($P < 0.004$) and the effect of O_3 depended on the CO_2 concentration ($CO_2 \times O_3$ interaction, $P = 0.001$). Both sections exhibited a convergence over time, mainly due

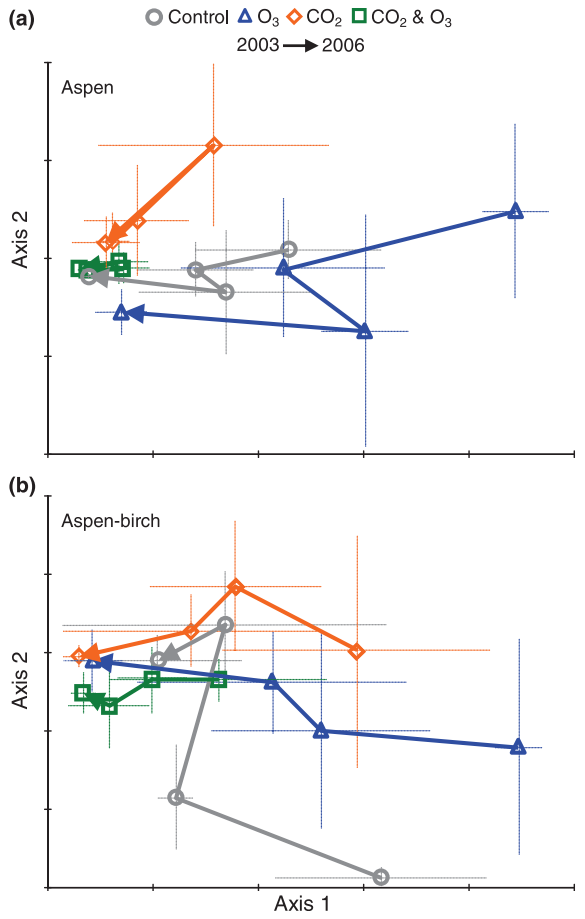

Figure 3 Trends in community similarity of ectomycorrhizal sporocarps at the Aspen FACE (free air CO_2 enrichment) site, Rhinelander, WI, USA within (a) aspen and (b) aspen-birch sections. The non-metric multidimensional scaling (NMDS) biplots display the first two axes of community responses to elevated and ambient CO_2 and O_3. Communities are more similar when they are proximal to one another. The arrows track communities across time. Sampling started in 2003 (beginning of lines) and ended in 2006 (tip of arrow). Grey circles are ambient, orange diamonds are elevated CO_2, blue triangles are elevated O_3 and green squares are elevated CO_2 with elevated O_3. Within-community variance is shown by the standard error bars (±). Axis orientation is arbitrary.

to greater similarity of the ambient and elevated CO_2 treatment communities in the last 2 years (Fig. 3).

Analysis with absolute biomass as the dependent variable yielded similar results in the aspen sections except that O_3 was only marginally significant (Table 1). In the aspen-birch sections, the interaction of CO_2 and O_3 was marginally significant. Analysis with density measures was also similar except that CO_2 was not as highly significant (Table 1).

The summed absolute biomass of earlier successional fungi remained unaffected by CO_2 and O_3 levels. The summed absolute biomass of later successional fungi mirrored the total ectomycorrhizal biomass response (Table 2). Later successional species were more dominant under elevated CO_2 (CO_2: $P = 0.018$ for aspen; $P = 0.009$ for aspen-birch), although there was variability between the years ($P < 0.001$ for aspen; $P = 0.011$ for aspen-birch). See Table 2 for the proportion each species contributed to relative biomass. Later successional *Leccinum* and *Cortinarius* species showed a positive response to CO_2. *Leccinum* c.f. *insigne* A.H. Sm., Thiers & Watling sporocarp productivity showed the strongest response to elevated CO_2 and O_3. This dominant species had greater absolute and relative biomass of sporocarps under elevated CO_2, regardless of O_3 concentration, in both aspen and aspen-birch sections. The average per cent of ectomycorrhizal sporocarp biomass attributable to *L.* c.f. *insigne*, pooled across the 4 years, ranged from 58 to 91 per cent of the average total biomass of all taxa. Likewise, the density of *Leccinum c.f. insigne* sporocarps was greater under elevated CO_2, regardless of O_3 concentration ($P = 0.028$ aspen; $P = 0.005$ aspen-birch). *Leccinum* c.f. *holopus* (Rostk.) Watling biomass was significantly higher under elevated CO_2 ($P = 0.004$ aspen; $P = 0.080$ aspen-birch) when pooled across years. The genus *Cortinarius* had higher relative biomass under ambient CO_2, regardless of O_3 level, within the aspen-birch section ($P = 0.003$). In contrast, earlier successional species in the genus *Hebeloma* exhibited higher relative biomass under ambient CO_2 than under elevated CO_2 in both sections ($P = 0.011$ aspen; $P = 0.096$ aspen-birch). While other taxa responded to elevated CO_2 and O_3 (see Appendices S1, S2, S3), most results were not statistically significant at the species or genus level.

DISCUSSION

Total ectomycorrhizal sporocarp biomass response to treatments

Hypothesis 1 regarding treatment effects on sporocarp biomass was generally supported by our analysis. As expected, sporocarp production increased under elevated CO_2 and decreased under elevated O_3. Hypothesis 2, which predicted that elevated CO_2 would ameliorate the effects of elevated O_3 on sporocarp production, was not entirely supported. In fact, the effects of CO_2 and O_3 often appeared to interact, resulting in a complete elimination of O_3 effects on sporocarp production when elevated CO_2 was added. The $CO_2 \times O_3$ interaction in sporocarp productivity contrasts with their documented additive effects on above-ground tree net primary production (ANPP; King *et al.* 2005), but is parallel to their interactive effects on soil respiration (Pregitzer *et al.* 2006). Perhaps sporocarp production is more strongly related to belowground carbon allocation than to ANPP. Pregitzer *et al.* (2006) noted that

respiration rates were highest in the combined elevated CO_2 and O_3 treatments. The authors postulated that roots may be turning over more rapidly under elevated CO_2 and O_3, which would increase respiration rates and belowground carbon allocation.

Under elevated O_3, the intraspecific variability in ozone tolerance of the aspen trees clones may affect carbon allocation, which could in turn affect ectomycorrhizal sporocarp production. At the Aspen FACE site, Kubiske *et al.* (2007) observed positive relative growth responses by ozone-tolerant aspen genotypes and negative relative growth responses by ozone-intolerant aspen genotypes to elevated O_3. Under the combination of elevated CO_2 and O_3, slower growth by the ozone-intolerant genotypes may be partially compensated for by increased growth by the ozone-tolerant genotypes (Pregitzer *et al.* 2006). In this case, ectomycorrhizal fungi may acquire more carbon from the ozone-tolerant clones when exposed to elevated CO_2 and O_3. This may partially explain why we observed such high sporocarp production under the combination of elevated CO_2 and O_3.

We expect that elevated O_3 affects ectomycorrhizal fungi indirectly via the host or the soils, but direct effects on the fungi are also possible, especially on sporocarps which are directly exposed to atmospheric O_3 concentrations. Although O_3 concentrations are typically lower near the soil surface (e.g. Skelly *et al.* 1996), the lack of strong barriers to ozone uptake by the sporocarps could lead to direct ozone effects on sporocarps. However, we have not examined direct ozone effects on sporocarps. The fact that an individual sporocarp size for a given species was not affected by O_3 level (C. Andrew & E. A. Lilleskov, Unpublished data) suggests that exposure does not lead to detectable reductions in individual sporocarp biomass accumulation. This can not exclude the possibility of higher C demand by individual sporocarps as a result of ozone damage and repair. However, there was no detectable increase in sporocarp respiration rates under elevated ozone (C. Andrew & E. A. Lilleskov, Unpublished data).

Finzi *et al.* (2007) have suggested that, since nitrogen uptake of trees at three FACE sites (including the Aspen FACE site) has increased without a change in nitrogen use efficiency, this may indicate that trees are allocating more carbon to ectomycorrhizal fungi under elevated CO_2. Consistent with this, some species of ectomycorrhizal fungi produced more sporocarps under elevated CO_2.

We can propose two hypotheses for why production was greater under elevated CO_2: (a) host trees, indeed, allocate more carbon to ectomycorrhizal fungi under elevated CO_2. In this case, not all fungal species invest this additional carbon into sporocarp production, but some species do (e.g. *Leccinum* c.f. *insigne*) and this results in our ability to see an aboveground response by ectomycorrhizal fungi to elevated CO_2. Alternatively, (b) ectomycorrhizal fungi may increase proportional allocation to sporocarps independent of the amount of carbon that is allocated to them through host trees. In this case, other factors that can be affected by elevated CO_2 (such as N, P, pH or litterfall) may more directly affect ectomycorrhizal fungi than the specific amount of carbon they acquire. These hypotheses are not mutually exclusive and could both be responsible for the effects we have documented, to greater or lesser extents depending upon the species. We cannot address these hypotheses with the data we present here, but future work that includes multiple biotic and abiotic factors and the belowground response of ectomycorrhizal fungi might shed light on this.

Community response to treatments

Hypothesis 3 regarding compositional distinction between the communities under elevated CO_2 and O_3 was supported during the 4 years of this study. Support for alternate hypotheses 4a and 4b, regarding community trends being attributable to stand ontogeny vs. other variable(s), was arguably equivocal. Although later successional species were more abundant under elevated CO_2 and communities converged during the study, differences did not seem to be solely related to ontogeny.

Sporocarp production by later successional species increased under elevated CO_2, regardless of O_3 level. The productivity trends by individual taxa to the treatments support this finding: the biomass of the later successional species *Leccinum* c.f. *insigne* (Gibson & Deacon 1988; Cripps 2001) was both dominant and consistently higher under elevated CO_2, although less so in the final sampling year. Likewise, within the aspen-birch sections, the presence of the later successional *Russula* sp. (*R. versicolor* Jul. Schäff. */R. odorata* Romagn. group) (Hutchison & Piche 1995; Nara 2009) was almost completely restricted to treatments with elevated CO_2 and the relative biomass of the later successional *Lactarius pubescens* (Fr.) Fr. and the *Cortinarius bulbosus* (Sowerby) Fr. group (Hutchison & Piche 1995; Nara 2009) was higher under elevated CO_2 without elevated O_3. Furthermore, the relative biomass of *Paxillus involutus* (Batsch) Fr. and *Inocybe lacera* (Fr.) Kummer, considered earlier successional taxa (Fox 1983; Hutchison & Piche 1995; Nara 2009), typically decreased under elevated CO_2 and contributed more under elevated O_3 with ambient CO_2 (see Appendix S1). This finding is partially in accord with Shaw *et al.* (1992), who noted that earlier successional ectomycorrhizal sporocarps were present for longer under slightly elevated O_3 and elevated sulphur dioxide (SO_2) than under ambient conditions, although this was stronger with elevated SO_2 than elevated O_3.

The decline in compositional differences in the last year (Fig. 3) could be an indication of community convergence

as the stands become more mature, suggesting that treatments affected the rate rather than the trajectory of succession, as Rey & Jarvis (1997), Staddon & Fitter (1998) and Gorissen & Kuyper (2000) have all hypothesized. Canopy closure had occurred within the CO_2 plots prior to this study (Hillstrom & Lindroth 2008), and canopy closure was achieved in the ambient and elevated O_3 plots during the first years of this study. Canopy closure can be a dynamic stage for ectomycorrhizal fungal species diversity and composition (Visser 1995; Twieg *et al.* 2007), and could partially explain the marginally greater species richness under elevated CO_2 (Fig. 2).

We would expect that if stand ontogeny alone were driving community dynamics then communities would be most similar in stands of similar ontogenetic stage. The stands treated with elevated CO_2 and O_3 were most similar to the ambient treatment in productivity (King *et al.* 2005) and time of canopy closure; we might expect EMF communities in these two treatments to be the most similar. We instead found that communities from the combined elevated CO_2 and O_3 treatment were the most divergent from other treatment communities through most of the course of the experiment. The elevated CO_2 and O_3 communities were most similar to the elevated CO_2 with ambient O_3 communities, and were quite divergent from those under ambient conditions (Fig. 3, Appendix S1). The discrepancy between communities in relation to stand ontogeny suggests that there is something additional which drives the process of sporocarp community dynamics.

Feedbacks, global change interactions and belowground responses

Some positive feedbacks that occur under global change scenarios may not be evident at the spatial scale of our treatments. Our results on sporocarp production indicate the potential for total ectomycorrhizal spore production to change, as well as the relative contributions by different taxa. Similarly, Klironomos *et al.* (1997) noted both an increase in fungal spore production and a compositional shift of those spores under elevated CO_2. Spores give rise to new genets and are therefore critical for fungal population maintenance and dispersal, especially into early successional areas (Nara 2009). Our results indicate that future regional to global changes in sporocarp production will likely affect spore production and dispersal, with potential feedbacks to new community assembly. The scale of the treatments in the present study likely minimized these feedbacks because spore transport from intact communities outside the treatments homogenized the treatments' spore rain. We would thus expect that the responses seen in the present study were conservative compared to those expected under regional to global changes in CO_2 and O_3.

Other global change factors besides elevated CO_2 and O_3 affect ectomycorrhizal sporocarp production. The responses we document here could become exacerbated or weakened when combined with other factors such as anthropogenic warming and nitrogen deposition via either additive or interactive effects. It is, however, difficult to predict how multiple global change factors will interact with each other to affect ectomycorrhizal fungal production without experimental testing.

It is important to keep in mind that although the reproductive responses by ectomycorrhizal fungal communities indicate their sensitivity to elevated CO_2 and O_3, these responses are unlikely to directly mirror those occurring belowground. Root colonization and extramatrical mycelial abundance of specific ectomycorrhizal taxa can be affected differently by elevated CO_2 (Fransson *et al.* 2005; Parrent *et al.* 2006; Parrent & Vilgalys 2007). At the FACE site, root tip and hyphal community compositions may not be affected as strongly by elevated CO_2 and O_3 as the sporocarp communities are (C. Andrew & E. A. Lilleskov, Unpublished data). Similarly, nitrogen deposition appears to drive ectomycorrhizal fungal community sporocarp responses more rapidly than root tip responses, but longer term (> 10 year) community responses to N inputs can be quite substantial (Lilleskov 2005 and references therein). Whether the same type of cumulative belowground changes occur in response to mid- to long-term CO_2 or O_3 exposure remains to be determined.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 Average relative biomass (proportion) of ectomycorrhizal sporocarps for all treatments by year.

Table S2 Average biomass (g m^{-2}) of ectomycorrhizal sporocarps for all treatments by year.

Table S3 Average density (sporocarps m^{-2}) of ectomycorrhizal sporocarps for all treatments by year.

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