Title:

Elevated tropospheric CO₂ and O₃ may not alter initial wood decomposition rate or wood-decaying fungal community composition of northern hardwoods

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ABSTRACT

We examined the effects of elevated CO₂ and/or O₃ on the wood-decaying basidiomycete fungal community and wood decomposition rates at the Aspen Free-Air CO₂ and O₃ Enrichment (Aspen FACE) project. Mass loss rates were determined after one year of log decomposition on the soil surface, and wood-decaying basidiomycetes were isolated from decaying wood and identified via DNA sequencing. Aspen (*Populus tremuloides* Michx.) and birch (*Betula papyrifera* Marshall) wood differed significantly in wood-decaying basidiomycete fungal communities and decomposition rate. Twelve years of site exposure to elevated CO₂ and/or O₃ did not have significant effects on wood-decaying fungal communities. Growth under elevated CO₂ and/or O₃ did not produce wood that differed in decay rate from that grown under ambient atmospheric conditions. Similarly, wood decay rate was not altered significantly when decomposition occurred in elevated CO₂ and/or O₃ environments. Our results suggest that wood-decaying fungal community composition and decomposition rates of northern hardwoods may not be directly affected by elevated tropospheric CO₂ and O₃.

Keywords: Elevated atmospheric CO₂, Elevated atmospheric O₃, wood decomposition, fungal community, northern hardwoods

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1 Introduction

Atmospheric CO₂ now exceeds 401 ppm (NOAA 2016). Concurrently, the concentration of tropospheric O₃ has increased by 38% within the last century (IPCC 2007). Elevated CO₂ and/or O₃ can alter biochemistry of plant tissues (Kostiainen et al. 2004; Parsons et al. 2008), fungal community composition (Edwards and Zak 2011), and leaf litter decomposition rates (Parsons et al. 2008). However, specific effects of elevated CO₂ and/or O₃ on wood-decaying basidiomycete community composition remain uncertain. Furthermore, most litter decomposition studies involving materials produced under elevated CO₂ and/or O₃ were not performed in the environment in which the litter was produced (Norby et al. 2001), hence results may not apply to future situations in which both production and decomposition of plant material will occur under elevated CO₂ and O₃. We therefore investigated effects of elevated CO₂ and/or O₃ on wood decay at Aspen FACE, to provide an *in vivo* investigation. Our specific objectives were to: (1) determine if twelve years of elevated CO₂ and/or O₃ treatments altered the wood-decaying basidiomycete fungal communities; and (2) determine if growth or subsequent decomposition under elevated CO₂ and/or O₃ environments impacted decay rates of birch or aspen wood. The experiment also let us assess effects of wood type (*P. tremuloides* clones and *B. papyrifera*) on wood-decaying fungal community composition and decay rate.

2 Materials and Methods

The Aspen FACE experiment was a 2 x 2 factorial randomized complete block design with each treatment replicated three times. The treatments were ambient CO₂ and O₃ as the control and elevated CO₂ (ambient + 200 ppm) with and without elevated O₃ (1.5 × ambient). Treatments were applied to aspen, birch and maple trees, planted as seedlings at 1 m x 1 m spacing in 1997, in 30-m diameter rings. Birch and maple were from local seed sources. Aspen clones used in the FACE experiment were selected based on differences in responsiveness to O₃ and CO₂ observed for natural clones from northern Wisconsin and northern Michigan utilized in earlier open-top chamber experiments (Karnosky et al. 1992; Kubiske et al. 1998). Further details on the original sources of clones 42 and 271 as well as their propagation for use in Aspen FACE can be found in Dickson et al. (2000); Zak et al. (2011) and Kubiske et al. (2015). After 12 years of treatment all surviving trees were harvested during the winter of 2009 / 2010.

Six trees each of birch and aspen genotypes 42 and 271 were randomly sampled from each treatment for this study (72 trees total). Five discs (2.5 cm thick) and four billets (50 cm long stem sections) were cut alternately from the lower 2.1 m portion of each tree. Mean diameter of the five discs per tree was 8.9 cm (5.7 – 14.5 cm) for birch; 9.7 cm (6.9 – 13.8 cm) for aspen 42; and 9.4 cm (7.1 – 13.6 cm) for aspen 271. Two billets of birch and each aspen genotype from trees grown under each of the four treatments were placed on the soil surface of all 12 treatments rings in a full factorial design (288 billets total). The rings were fumigated with elevated CO₂ and/or O₃ during the entire growing season (May–October 2010). One half of the billets were removed in May 2011 and wood-decaying basidiomycetes fungi were isolated following standard isolation methods (Eaton and Hale 1993). DNA was extracted from fungal cultures following Lindner and Banik (2009), modified for use with 200 µL strip tubes (Lorch et al. (2013)). Amplification of the resulting DNA was accomplished using the fungal specific primer pair ITS1F/ITS4 (Lindner and Banik (2009)). Fungal identifications were based on the nearest BLAST match in GenBank, using similarities of ≥ 97% to denote species identification and 90 – 97% for genus. Similarities less than 90% were tentatively identified to higher level taxa that provided the best match (i.e., order or family).

Percent loss in wood density: $\left(\frac{\text{initial wood density} - \text{final wood density}}{\text{initial wood density}} \right) \times 100$, was used as a measure of decomposition rate (Rajala et al. 2010). Initial wood density was determined from the 360 discs cut from the 72 trees. Average density of the two discs located at the ends of each billet was used as that billet's initial density. Final wood density was determined from additional discs taken from each billet after removal from the field in May 2011. A water displacement method (Williamson and Wiemann 2010) was used for disc volume. Samples were then oven-dried at 65°C, and density was computed as oven dry mass divided by sample moist volume.

Permutational multivariate analysis of variance (PerMANOVA) was used to determine the effects of wood species and elevated CO₂ and / or O₃ (during decomposition) on wood-decaying basidiomycete fungal community composition. PerMANOVA is a multi-response permutation procedure (MRPP) and requires neither the normal distributions nor equal variances of the general ANOVA assumptions (Anderson 2001; McCune et al. 2002). However, decomposition data was analyzed using ANOVA. A five way ANOVA for species (spp); growth CO₂ environment (gCO₂); growth O₃ environment (gO₃); decomposition CO₂ environment (dCO₂); and decomposition O₃ environment (dO₃) showed only a significant ($P < 0.0001$) species effect and a significant ($P = 0.031$) four way interaction spp*gO₃*dCO₂*dO₃. As a result all interactions were dropped and ANOVA was run again for the main factors spp, gCO₂, gO₃, dCO₂ and dO₃. Percent density loss was arcsine square root transformed before ANOVA was performed.

3 Results and Discussions

We found 11 fungal species present in the wood of aspen and 13 in birch (Table 1), with fungal community composition differing significantly between tree species ($P = 0.0009$). This is attributed to substrate-specificity of the wood-decaying fungi (Eaton and Hale 1993). Within a tree species, treatments tended to reduce the number of fungal species (Table 1). Rare fungal species with frequency occurrence less than n (in this case 3 out of 12) were excluded before PerMANOVA analysis as recommended for multivariate analysis (McCune et al. 2002). As a result, despite a trend for fewer fungal species under elevated CO₂ and O₃, twelve years of these treatments at the study site did not significantly alter the wood-decaying fungal community colonizing either species ($P = 0.1029$ for aspen; $P = 0.2567$ for birch), consistent with the findings of Strnadová et al. (2004) for microfungi. To our knowledge, this is the first time effects of elevated CO₂ and/or O₃ on the wood decaying fungal community have been investigated *in vivo*.

Woody litter input to the experimental site was minimal during the twelve-year FACE study, with only small tree mortality and branch loss occurring. Furthermore, fungal community development occurs in successional stages (Frankland 1998; Boddy and Heilmann-Clausen 2008). This study was conducted after a relatively short period of decay, and as a result, most of the fungal species isolated, *Chondostereum purpureum*, *Trametes* spp, *Stereum* spp, *Peniophora* sp, *Cylindrobasidium* sp, *Bjerkandera adusta*, and *Schizophyllum commune*, are from the primary or secondary successional stages of wood decomposition (Boddy and Heilmann-Clausen 2008). It is not known how a tertiary suite of fungal species would respond to elevated CO₂ and/or O₃ fumigations in a longer-term study.

Decay rates of birch and aspen wood were not significantly affected by placement in elevated CO₂ and/or O₃ concentration environments, as indicated by a lack of treatment effects on percent density loss (Table 2). Growth environment of wood (Table 2) also did not significantly affect its subsequent decomposition. This suggests that biochemical and anatomical changes in the wood caused by growth under elevated CO₂ and/or O₃ were minor relative to those needed to alter decomposition or the agents of decomposition. Birch and the aspen genotypes differed significantly in wood decomposition rates (Table 2). Birch exhibited the highest percent density loss (12.8 ± 0.7), aspen clone 271 had the lowest (7.8 ± 0.8), and aspen clone 42 was intermediate (10.6 ± 0.7), presumably due to effects on wood decomposition of species specific variation in physical, chemical and anatomical characteristics (Panshin and Zeeuw 1980; Cornwell et al. 2009). At Aspen FACE, changes in forest community composition (proportions of birch, maple and aspen genotypes) occurred during the experiment due to differential growth and survival under elevated CO₂ and elevated O₃ (Kubiske et al. 2007). Hence it is possible that future high levels of elevated CO₂ and O₃ may affect wood decomposition at the ecosystem scale via changes in species or genotype composition of natural stands and the total amount of woody detritus produced, but not due to altered wood biochemistry within a species.

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Table 1 Fungal species present (1) or absent (0) with respect to elevated CO₂ and / or O₃ treatments (during decomposition) and species.

[illegible]

Table 2 Effects of growth environment and decomposition environment on percent wood density loss. Only the *P* – values for the main effects are presented here. Treatment codes are for ambient CO₂ (aCO₂); elevated CO₂ (eCO₂); ambient O₃ (aO₃); and elevated O₃ (eO₃). Highlighted P-value is significant (P-values < 0.05). Values are mean (±1 SE), n =3.

Main Effect	Species/Genotype	% density loss for treatment			
		aCO ₂	eCO ₂	aO ₃	eO ₃
Growth environment (source)	Birch	13.4 ± 1.2	12.2 ± 1.1	12.5 ±1.5	13.1 ± 0.8
	Aspen clone 42	11.3 ± 0.7	10.0 ± 2.5	11.0 ± 1.1	10.3 ±1.4
	Aspen clone 271	8.5 ±1.1	7.2 ± 0.5	7.6 ± 0.8	8.0 ± 0.4
	<i>P – values</i>	<0.0001	0.1087	0.9003	
Decomposition environment	Birch	12.8 ±1.6	12.8 ± 0.7	11.7 ±1.4	14.0 ± 1.0
	Aspen clone 42	11.0 ±1.8	10.5 ± 0.2	10.6 ±1.6	10.7 ± 0.4
	Aspen clone 271	9.0 ±1.0	6.7 ±1.1	7.4 ± 0.6	8.3 ± 0.5
	<i>P – values</i>	<0.0001	0.2413	0.2029	