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Short communication

Elevated tropospheric CO_2 and O_3 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 CO_2 and/or O_3 on the wood-decaying basidiomycete fungal community and wood decomposition rates at the Aspen Free-Air CO_2 and O_3 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 CO_2 and/or O_3 did not have significant effects on wood-decaying fungal communities. Growth under elevated CO_2 and/or O_3 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 CO_2 and/or O_3 environments. Our results suggest that wood-decaying fungal community composition and decomposition rates of northern hardwoods may not be directly affected by elevated tropospheric CO_2 and O_3.

1. Introduction

Atmospheric CO_2 now exceeds 401 ppm (NOAA, 2016). Concurrently, the concentration of tropospheric O_3 has increased by 38% within the last century (IPCC, 2007). Elevated CO_2 and/or O_3 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 CO_2 and/or O_3 on wood-decaying basidiomycete community composition remain uncertain. Furthermore, most litter decomposition studies involving materials produced under elevated CO_2 and/or O_3 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 CO_2 and O_3. We therefore investigated effects of elevated CO_2 and/or O_3 on wood decay at Aspen FACE, to provide an *in vivo* investigation. Our specific objectives were to: (1) determine if twelve years of elevated CO_2 and/or O_3 treatments altered the wood-decaying basidiomycete fungal communities; and (2) determine if growth or subsequent decomposition under elevated CO_2 and/or O_3 environments impacted decay rates of birch or aspen wood. The experiment also let us assess effects of wood type (*Populus tremuloides* clones and *Betula papyrifera*) on wood-decaying fungal community composition and decay rate.

2. Materials and methods

The Aspen FACE experiment was a 2 × 2 factorial randomized complete block design with each treatment replicated three times. The treatments were ambient CO_2 and O_3 as the control and elevated CO_2 (ambient + 200 ppm) with and without elevated O_3

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(1.5 × ambient). Treatments were applied to aspen, birch and maple trees, planted as seedlings at 1 m × 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 O_3 and CO_2 observed for natural clones from northern Wisconsin and norther 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 CO_2 and/or O_3 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: (*initial wood density* − *final wood density*/*initial wood density*) × 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 CO_2 and/or O_3 (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 CO_2 environment (gCO_2); growth O_3 environment (gO_3); decomposition CO_2 environment (dCO_2); and decomposition O_3 environment (dO_3) showed only a significant ($P < 0.0001$) species effect and a significant ($P = 0.031$) four way interaction spp*gO_3*dCO_2*dO_3. As a result all interactions were dropped and ANOVA was run again for the main factors spp, gCO_2, gO_3, dCO_2 and dO_3. 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 CO_2 and O_3, 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 CO_2 and/or O_3 on the wood-decaying fungal community have been investigated *in vivo*. (see Table 2)

Table 1
Fungal species present (1) or absent (0) with respect to elevated CO_2 and/or O_3 treatments (during decomposition) and species.

	Aspen				Birch			
	Treatments				Treatments			
Fungal species	Ambient	CO_2	O_3	$CO_2 + O_3$	Ambient	CO_2	O_3	$CO_2 + O_3$
Bjerkandera adusta (Willd.) P. Karst.	1	1	1	1	1	1	0	0
Cerrena unicolor (Bull.) Murrill	0	0	0	0	1	1	1	0
Chondrostereum purpureum (Pers.) Pouzar	1	1	1	1	1	0	0	0
Cylindrobasidium laeve (Pers.) Chamuris	1	1	1	1	1	1	1	1
Irpex lacteus (Fr.) Fr.	1	1	0	1	1	0	0	1
Peniophora aurantiaca (Bers.) Hohn. & Litsch.	1	1	1	0	1	1	1	1
Peniophora sp Cooke	1	0	1	0	1	0	1	1
Schizophyllum commune Fr.	1	0	0	0	0	0	0	0
Sistotrema brinkmannii (Bres.) J. Erikss.	0	0	0	0	1	0	0	0
Stereum rugosum Pers.	1	1	1	1	1	0	0	0
Stereum sp. Hill ex Pers.	1	1	1	1	0	0	0	1
Trametes gibbosa (Pers.) Fr.	1	1	0	1	1	1	1	0
Trametes versicolor (L.) Lloyd	1	1	1	1	1	0	1	0
Unidentified sp	0	0	0	0	1	0	0	1
Fungal species per treatment	11	9	8	8	12	5	6	6
Fungal species per community	Aspen = 11				Birch = 13			
Total number of isolated fungal species from birch and aspen = 14								

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 CO_2 (aCO_2); elevated CO_2 (eCO_2); ambient O_3 (aO_3); and elevated O_3 (eO_3). 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
P − values	***<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
P − values	***<0.0001***	*0.2413*		*0.2029*	

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 CO_2 and/or O_3 fumigations in a longer-term study.

Decay rates of birch and aspen wood were not significantly affected by placement in elevated CO_2 and/or O_3 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 CO_2 and/or O_3 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 CO_2 and elevated O_3 (Kubiske et al., 2007). Hence it is possible that future high levels of elevated CO_2 and O_3 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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