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Effects of elevated atmospheric CO₂ and O₃ on wood density, anatomical properties and decomposition of Northern Hardwoods

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EFFECTS OF ELEVATED ATMOSPHERIC CO₂ AND O₃ ON WOOD DENSITY, ANATOMICAL
PROPERTIES AND DECOMPOSITION OF NORTHERN HARDWOODS

By

Emmanuel Ebanyenle

A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

In Forest Science

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This dissertation has been approved in partial fulfillment of the requirements for the Degree of
DOCTOR OF PHILOSOPHY in Forest Science

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In loving memory

of

Professor David F. Karnosky

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PREFACE

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DISSERTATION ABSTRACT

Anthropogenic activities continue to drive atmospheric CO₂ and O₃ concentrations to levels higher than during the pre-industrial era. Accumulating evidence indicates that both elevated CO₂ and elevated O₃ could modify the quantity and biochemistry of woody plant biomass. Anatomical properties of woody plants are largely influenced by the activity of the cambium and the growth characteristics of wood cells, which are in turn influenced by a range of environmental factors. Hence, alterations in the concentrations of atmospheric CO₂ and / or O₃ could also impact wood anatomical properties. Many fungi derive their metabolic resources for growth from plant litter, including woody tissue, and therefore modifications in the quantity, biochemistry and anatomical properties of woody plants in response to elevated CO₂ and / or O₃ could impact the community of wood-decaying fungi and rates of wood decomposition. Consequently carbon and nutrient cycling and productivity of terrestrial ecosystem could also be impacted. Alterations in wood structure and biochemistry of woody plants could also impact wood density and subsequently impact wood quality. This dissertation examined the long term effects of elevated CO₂ and / or O₃ on wood anatomical properties, wood density, wood-decaying fungi and wood decomposition of northern hardwood tree species at the Aspen Free-Air CO₂ and O₃ Enrichment (Aspen FACE) project, near Rhinelander, WI, USA. Anatomical properties of wood varied significantly with species and aspen genotypes and radial position within the stem. Elevated CO₂ did not have significant effects on wood anatomical properties in trembling aspen, paper birch or sugar maple, except for marginally increasing ($P < 0.1$) the number of vessels per square millimeter. Elevated O₃ marginally or significantly altered vessel lumen diameter, cell wall area and vessel lumen area proportions depending on species and radial position. In line with the modifications in the anatomical properties, elevated CO₂ and O₃, alone, significantly modified wood density but effects were species and / or genotype specific. However, the effects of elevated CO₂ and O₃, alone, on wood anatomical properties and density were ameliorated when in combination. Wood species had a much greater impact on the wood-decaying fungal community and initial wood decomposition rate than did growth or decomposition of wood in elevated CO₂ and / or O₃.

Polyporales, Agaricales, and Russulales were the dominant orders of fungi isolated. Based on the current results, future higher levels of CO₂ and O₃ may have moderate effects on wood quality of northern hardwoods, but for utilization purposes these may not be considered significant. However, wood-decaying fungal community composition and decomposition of northern hardwoods may be altered via shifts in species and / or genotype composition under future higher levels of CO₂ and O₃.

CHAPTER 1: Introduction

The atmospheric concentration of CO₂ has increased to 394 ppm (NOAA, July 2012), which is about the highest in the last 25 million years (Pearson and Palmer 2000). At the same time, the concentration of tropospheric O₃ has increased by 38% within the last century (IPCC 2007).

Both greenhouse gases are predicted to rise further due to anthropogenic activities such as fossil fuel combustion and changing land use systems (IPCC 2007).

Both CO₂ and O₃ have been observed to modify growth rates and the biochemical composition of northern hardwood tree species (Karnosky et al. 2003; Kaakinen et al. 2004; Parsons et al. 2004; Liu et al. 2005; Karnosky et al. 2007; Kubiske et al. 2007; Liu et al. 2007; Kostianen et al. 2008; Parsons et al. 2008; Liu et al. 2009; Zak et al. 2011). Wood density largely depends on wood anatomical properties, which in turn are influenced by growth and biochemical characteristics of woody plants (Panshin and Zeeuw 1980; Barnett and Jeronimidis 2003; Grabner et al. 2005).

Hence the rising concentrations of atmospheric CO₂ and O₃ could impact wood density, which is a measure of wood quality for a variety of wood product uses. Additionally, the quantity, anatomical properties and chemical constituents of woody litter can influence the growth of wood-decaying basidiomycete fungi (Rayner and Boddy 1988; Sinsabaugh et al. 1993; Hattenschwiler et al. 2005; Cornwell et al. 2008; Cornwell et al. 2009; Weedon et al. 2009; Freschet et al. 2012; Talbot et al. 2012). Therefore, alterations in the production, chemical constituents and anatomical properties of woody plant biomass growth in elevated CO₂ and / or O₃ could cause changes in the wood-decaying basidiomycete fungal community and decomposition rates of woody litter. As a result, nutrient and carbon cycling and productivity in terrestrial ecosystems could also be impacted.

Studies on the effects of elevated CO₂ and / or O₃ on fungal community composition and function (Larson et al. 2002; Chung et al. 2006; Edwards and Zak 2011), and wood properties including density and anatomy have been accumulating (Telewski et al. 1999; Beismann et al. 2002; Kaakinen et al. 2004; Kostianen et al. 2008; Kostianen et al. 2009). Although wood density and

anatomical properties vary with position along the stem (Panshin and Zeeuw 1980; Zobel and Buijtenen 1989; Dickson 2000; Barnett and Jeronimidis 2003), the majority of studies focused on the lower portions (breast height, 1.37 m, or lower). Additionally, many of the studies were either performed on seedlings and saplings in greenhouses or in growth chambers for short periods (Rogers et al. 1983; Conroy et al. 1990; Hattenschwiler et al. 1996; Maherali and DeLucia 2000; Beismann et al. 2002; Ceulemans et al. 2002; Atwell et al. 2003; Kilpelainen et al. 2005; Qiao et al. 2008; Kostianinen et al. 2009). Results of these experiments have provided useful information, but they may not be applicable to naturally grown trees, since evidence from FACE studies has shown that some physiological processes of trees change during their ontogeny (Leakey et al. 2009; Norby and Zak 2011; Zak et al. 2012).

Furthermore, effects of elevated CO₂ and / or O₃ on wood-decaying fungal community composition and wood decomposition have received little or no attention. To our knowledge, no studies have investigated the specific impacts of elevated CO₂ and / or O₃ on wood-decaying basidiomycete community composition, despite their key role in nutrient and carbon cycling. Instead, decomposition studies have focused on leaf litter rather than wood (Norby et al. 2001a), even though the tissues differ in structure and composition. In addition, most decomposition studies were not performed in the environment in which the litter was produced (i.e. under elevated CO₂ and / or O₃) (Norby et al. 2001a), therefore, application of results to potential future field situations may not be appropriate.

The Aspen FACE project provided a more realistic field approach and an exceptional opportunity for investigating the long term effects of elevated CO₂ and /or O₃ on wood properties, wood decaying basidiomycete fungal community composition, and decomposition rates of wood from common northern hardwood tree species. Aspen FACE was located in Harshaw, near Rhinelander, WI. It was a unique, long term experiment evaluating the impact of elevated CO₂ and O₃, alone and in combination, on northern forest trees on a very large scale (Dickson et al. 2000). At Aspen FACE, an increase and a decrease in forest tree growth have been observed under elevated CO₂ and elevated O₃, respectively (Karnosky et al. 1996; Isebrands et al. 2001;

Karnosky et al. 2003; King et al. 2005; Kubiske et al. 2007). Additionally, alterations in wood anatomical properties and biochemistry of wood and leaf litter of aspen and birch under elevated CO₂ and O₃ have been observed (Kostiainen et al. 2004; Parsons et al. 2004; Liu et al. 2007; Kostiainen et al. 2008; Parsons et al. 2008). Alterations in soil and forest floor microbial community composition under elevated CO₂ and O₃ at the Aspen FACE site also have been documented (Chung et al. 2006; Andrew and Lilleskov 2009; Edwards and Zak 2011). Evidence of increased *N*-acetylglucosaminidase and cellobiohydrolase activity under elevated CO₂ and reduced cellobiohydrolase activity in soil under elevated O₃ has been reported (Larson et al. 2002; Chung et al. 2006; Edwards and Zak 2011).

The general goal of this dissertation was to evaluate the impact of elevated CO₂ and / or O₃ on the wood properties of common northern hardwood tree species (four *Populus tremuloides* Michx. clones, including three relatively O₃ tolerant (8, 216, 271) and one relatively O₃ sensitive (42) genotypes; *Acer saccharum* Marshall var. *saccharum*; and *Betula papyrifera* Marshall) after exposure for 12 growing seasons at the Aspen FACE experimental site to elevated CO₂ (ambient + 200 ppm), elevated O₃ (1.5 × ambient), and elevated CO₂ with elevated O₃. Additionally, the effects of twelve years of forest and soil development under elevated CO₂ and /or O₃ on the composition of the wood-decaying fungal community as well as initial rates of wood decomposition of aspen and birch were investigated under FACE conditions.

In Chapter 2, the long term effects of growth under elevated CO₂ and / or O₃ on wood density of three genotypes of trembling aspen and paper birch were examined. To capture the entire variation in wood density of the trees species, wood density was determined at five different positions along the longitudinal axis of the main stem. To our knowledge, this is the first time the combined long term effects of growth under elevated CO₂ and O₃ on wood density are being reported for these species.

In Chapter 3, the effects of 12 years of forest and soil development under elevated CO₂ and / or O₃ on the composition of the wood-decaying fungal community as well as initial rates of wood

decomposition of trembling aspen and paper birch were investigated. In line with the accruing evidence from Aspen FACE, we hypothesized that: (1) wood species effects would be observed in the wood-decaying basidiomycete fungal community composition and decomposition rates; (2) modification of soil and forest floor microbial communities resulting from alterations in the quantity and biochemistry of aspen and birch trees grown under elevated CO₂ and / or O₃ for 12 years would cause significant alterations in the wood-decaying basidiomycete fungal community; (3) elevated CO₂ and / or O₃ fumigation environment would have no direct impact on wood decomposition rates; and (4) rates of decomposition of aspen and birch wood produced under elevated CO₂ and O₃ would decrease significantly.

Chapter 4 examined the long term effects of growth under elevated CO₂ and /or O₃ on the wood anatomical properties of paper birch, sugar maple, and four clones of trembling aspen. Based on evidence from Aspen FACE, the following hypotheses were examined: (1) wood species /clonal effects would be observed in the wood anatomical properties of birch, sugar maple, and aspen (2) wood anatomical properties of birch, sugar maple and aspen were altered during growth under elevated CO₂ and O₃; and (3) the combined treatment (elevated CO₂ + elevated O₃) would have no effects on anatomical properties of birch, sugar maple and aspen.

Finally, a comprehensive synthesis of the dissertation findings, conclusions, implications, and limitations of the study and recommendations for future investigations are presented in Chapter 5.

The three distinct but strongly related investigations of this dissertation will contribute to an ability to predict wood quality and understand the dynamics and patterns of carbon storage and nutrient immobilization and mobilization in the woody detritus of forest ecosystems in the face of ever rising atmospheric CO₂ and O₃ concentrations.

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CHAPTER 2: Effects of elevated atmospheric CO₂ and / or O₃ on wood density of paper birch and trembling aspen¹

ABSTRACT

Current background concentrations of CO₂ and O₃ are about 40% higher than during the pre-industrial era. Mounting experimental evidence indicates that these greenhouse gases have opposing effects on the growth, biochemistry and wood structure of northern hardwood tree species. Wood density depends on the cellular structure of wood, but the effects of elevated CO₂ and / or O₃ on wood density of northern hardwoods are not well understood. We evaluated the effects of elevated CO₂ and O₃ on the wood density of birch (*Betula papyrifera* Marshall) and three aspen (*Populus tremuloides* Michx.) clones in 12 year old trees grown at the Aspen Free Air CO₂ and O₃ Enrichment (Aspen FACE) project near Rhinelander, WI, USA. Elevated CO₂ significantly decreased wood density of aspen clone 271, compared with trees grown under ambient conditions. In contrast, elevated O₃ increased wood density of aspen clone 42 and birch compared to the ambient. The combined effects of elevated CO₂ and elevated O₃ did not have any statistically significant impact on wood density across all species and clones investigated. However, the wood density of aspen clone 42 and birch tended to increase in either ambient or elevated O₃ in the presence of elevated CO₂. Our results were largely consistent with the differential growth patterns, biochemistry and structural changes which have been reported during the 12-year long Aspen FACE experiment. Based on our results, we hypothesize that wood density response of northern hardwood tree species to future higher levels of atmospheric CO₂ and /or O₃ will be species and / or genotype dependent.

INTRODUCTION

Atmospheric CO₂ concentration is currently higher than in any period in the last 25 million years (Pearson and Palmer 2000) and about 40% higher than during the pre-industrial era. It is

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predicted to increase further at the rate of 14-19 ppm every 10 years (IPCC 2007). The continuous increase in the background concentration of CO₂ is attributed to anthropogenic activities such as fossil fuel combustion (IPCC 2007). Atmospheric CO₂ is an important raw material for photosynthesis in woody plants, and therefore affects their growth and physiology. Photosynthesis is catalyzed by rubisco (ribulose-1-5-bisphosphate carboxylase oxygenase). In the presence of optimal supply of other environmental resources, elevated CO₂ increases photosynthesis in C3 plants by enhancing carboxylation by rubisco and reducing photorespiration. Generally, elevated CO₂ improves photosynthetic nitrogen use efficiency, enhances carbon uptake, increases water use efficiency and stimulates plant growth in young forests with adequate soil resources (Karnosky et al. 2003; Norby et al. 2005; Leakey et al. 2009; Norby and Zak 2011). However, the concomitant rising of tropospheric O₃ concentration may offset the stimulating growth effects of CO₂ (Karnosky et al. 2003) and reduce tree growth and carbon sinks in the future (Sitch et al. 2007; Wittig et al. 2009).

Volatile organic compounds and nitrogen oxides (NO_x) from fossil fuel combustion undergo photochemical reactions with oxygen to form O₃. This process is the major driving force for tropospheric O₃ increase (Fowler et al. 1999). The concentration of tropospheric O₃ has increased by 38% within the last century (IPCC 2007), and it is predicted that about a half of the Earth's forests will experience O₃ concentrations higher than 60 nL L⁻¹ by 2100 (Fowler et al. 1999; IPCC 2007). Unlike CO₂, ozone is injurious to woody plants (Karnosky et al. 1996; Karnosky et al. 2003; Karnosky et al. 2007). Ozone first enters the stomata and forms cytotoxic compounds such as aldehydes, peroxides and assorted radicals, which disrupt important physiological processes (Fuhrer and Booker 2003; Wittig et al. 2007; Wittig et al. 2009; Lindroth 2010; Street et al. 2011). This can reduce stomatal conductance and photosynthesis, induce leaf senescence (Karnosky et al. 1996) and lead to an overall reduction in plant growth and productivity (Karnosky et al. 2007; Wittig et al. 2009). Current concentrations of O₃ are causing reductions in biomass production of northern temperate and boreal forests by an estimated 7%,

and this reduction is expected to increase further to 11% and 17% by 2050 and 2100, respectively (Wittig et al. 2009).

The growth patterns of woody plants have long been recognized to influence wood quality (Zobel 1985; Zobel and Buijtenen 1989). Evidence from Aspen FACE has shown that 12 years of exposure of northern hardwood species to elevated O₃ and CO₂, alone, significantly affects growth (Karnosky et al. 2003; King et al. 2005; Kubiske et al. 2007; Norby and Zak 2011; Zak et al. 2011; Zak et al. 2012). The concurrent rising concentrations of atmospheric CO₂ and O₃ could impact wood quality via their effects on growth patterns. Consequently, evidence of the effects of rising concentrations of CO₂ and / or O₃ on wood quality are accumulating (Conroy et al. 1990; Telewski et al. 1999; Beismann et al. 2002; Kaakinen et al. 2004; Kostianen et al. 2004; Kostianen et al. 2006; Kostianen et al. 2008; Kostianen et al. 2009).

An easy-to-measure and reliable quality indicator of wood quality in the timber and pulp and paper industries is density (Barnett and Jeronimidis 2003). Wood density also has ecological importance (e.g. for use in biomass estimation). A growing number of studies have examined the effects of rising concentrations of greenhouse gases on wood density of conifers and hardwoods (Telewski et al. 1999; Beismann et al. 2002; Kostianen et al. 2009). However, conifers appear to be more studied than hardwoods. Additionally, a majority of the studies were either carried out on seedlings and saplings in greenhouses or in growth chambers for short periods (Rogers et al. 1983; Conroy et al. 1990; Hattenschwiler et al. 1996; Maherali and DeLucia 2000; Beismann et al. 2002; Ceulemans et al. 2002; Atwell et al. 2003; Kilpelainen et al. 2005; Qiao et al. 2008; Kostianen et al. 2009). Results of these experiments have provided useful information, but they may not be applicable to naturally grown trees, since evidence from FACE studies has shown that physiological processes of trees change during their ontogeny (Leakey et al. 2009; Norby and Zak 2011; Zak et al. 2012). Perhaps such ontogenic changes are the major underlying cause for the accumulating contradictory results both within and among different species. For example, elevated CO₂ had no significant effects on wood density of *Pinus taeda* after exposure for one year (Rogers et al. 1983) as well as four years (Telewski et al. 1999). Likewise, the wood

density of *Pinus sylvestris* did not change significantly after three (Ceulemans et al. 2002) and six (Kilpelainen et al. 2005) years of elevated CO₂ exposure in growth chambers. Beismann et al. (2002) and Kostianen et al. (2009) reported no effects of elevated CO₂ on the wood density of *Picea abies* in four-year long open top chamber and three-year long whole tree chamber experiments, respectively. Similarly, the wood density of *Pinus ponderosa* seedlings were not affected after being exposed to elevated CO₂ for a 24 month period (Maherali and DeLucia 2000). However, wood density was significantly enhanced in juvenile stems of *Pinus radiata* (Conroy et al. 1990; Atwell et al. 2003) and *Picea abies* (Hattenschwiler et al. 1996) grown under elevated CO₂. In contrast, *Abies faxoniana* seedlings exposed to elevated CO₂ in closed top chambers exhibited significantly lower wood density when compared to ambient conditions (Qiao et al. 2008). In a FACE experiment, elevated CO₂ reduced wood density of *Picea abies* when nutrient levels were improved via fertilization (Oren et al. 2001).

In contrast to conifers, there are very few experimental reports on the effects of either elevated CO₂ or O₃ on hardwoods. Elevated CO₂ significantly increased wood density of *Liquidambar styraciflua* in an open top chamber experiment with seedlings (Rogers et al. 1983), but no effects were observed when same species was tested under FACE conditions (Norby et al. 2001b). Similarly, elevated CO₂ had no observable effects on wood density of three species of *Populus* grown under FACE conditions (Calfapietra et al. 2003), seedlings of *Quercus ilex* grown in the greenhouse (Gartner et al. 2003) and saplings of *Fagus sylvatica* (Beismann et al. 2002) grown using open top chambers.

Although O₃ and CO₂ co-occur naturally, there are virtually no studies on the effects of elevated O₃, alone, or in combination with CO₂, on wood density for either hardwoods or softwoods. Moreover, wood anatomical and chemical properties which are known to influence wood density greatly (Panshin and Zeeuw 1980; Zobel 1981; Zobel and Buijtenen 1989; Dickison 2000; Barnett and Jeronimidis 2003), are reported to be significantly influenced by elevated O₃ and CO₂ alone or in combination (Kaakinen et al. 2004; Kostianen et al. 2004; Kostianen et al. 2006; Kostianen et al. 2008; Kostianen et al. 2009; Street et al. 2011).

The objective of this study was to determine the effects of elevated CO₂ and / or O₃ on wood density of paper birch and three clones of aspen (42, 216, and 271) after 12 years growth at Aspen FACE, near Rhinelander, WI, USA. To our knowledge, this reports the longest duration effects of elevated O₃, alone and in combination with elevated CO₂, on wood density of common northern hardwood tree species.

MATERIALS AND METHODS

Site description

Materials for this study were sampled from the Aspen Free-Air CO₂ and O₃ Enrichment (Aspen FACE) project (for detailed description of the site and experimental design for Aspen FACE, please see Dickson et al. (2000)). Aspen FACE research was conducted on a 32 ha USDA Forest Service Experimental Farm at Harshaw, near Rhinelander, in Wisconsin, USA (longitude 45.6° N, latitude 89.5° W). Potatoes and small grains were cultivated on the site for more than 50 years before the Forest Service acquired the land in 1972 to serve as a forest research station. Prior to the onset of the Aspen FACE research in 1997, the site was planted with poplar clones and larch. However, all the poplar clones and larch were cleared and stumps removed in 1996. The study site is nearly flat and the soil type is sandy loam. A thorough soil analyses was done for all treatment plots in 1997 and no significant differences were observed except for mean percent carbon and nitrogen, which were significantly greater in CO₂ rings than CO₂ + O₃ rings (Dickson et al. 2000).

The Aspen FACE experiment was a 2² factorial, randomized complete block design with each treatment level replicated three times, once each in the northern, central and southern blocks of the site. The main and crossed treatment factors were CO₂, O₃ and CO₂ + O₃, respectively. The four treatments were ambient CO₂ and O₃ as the control, elevated CO₂ (ambient + 200 ppm), elevated O₃ (1.5 × ambient), and elevated CO₂ with elevated O₃. The treatments were applied to twelve 30-m diameter rings, located at least 100 m apart (Appendix figure 2-1). Each ring was partitioned into east and west sections. The eastern portion was planted with five *Populus*

tremuloides Michx. (aspen) clones (8L, 42E, 216, 259 and 271) in random order with a planting spacing of 1 m × 1 m. The western portion was further divided into north and south subplots. The northwest and southwest subplots were mixed plantations of aspen clone 216 and *Acer saccharum* Marshall var. *saccharum* (sugar maple) and *Betula papyrifera* Marshall (paper birch), respectively. All planting was completed in 1997, and exposure of treatment rings to elevated CO₂ and O₃ was done during the growing seasons of 1998 to 2009 between 0700 hrs and 1700 hrs each day, unless foliage was wet.

Sampling and laboratory analysis

All trees in all treatment rings were harvested during the winter of 2009 / 2010. Six trees each of birch and aspen clones 271, 216 and 42E from each of the 12 rings were randomly selected from the harvested trees. From each sample tree, five 25-mm thick discs were removed at intervals of 0.5 m from the base of the tree. To prevent the wood discs from drying, they were placed in plastic bags and frozen at Michigan Technological University (MTU) until laboratory analysis was performed.

Wood density was determined from the discs using a water displacement method (Williamson and Wiemann 2010). The wood discs were suspended, completely immersed, in a water bath placed on an electronic balance, and the displacement of water, as indicated by the increase in mass measured by the balance, was taken as volume of the wood. Then the samples were oven dried at 105°C to a constant mass and the density computed as oven dry mass divided by the volume of sample (Williamson and Wiemann 2010).

Analysis of data

The experiment was considered a complete randomized design. Data analysis was carried out using the GLM procedure of SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA). Descriptive statistics (means and standard errors) were computed for wood density. In addition, data were examined for the normality and homogeneity of variance assumptions of analysis of variance (ANOVA), before repeated measures two-way ANOVA was employed to determine the

effects of the main factors (CO₂, O₃, and CO₂ + O₃) on the wood density of birch and the three aspen clones (42, 216 and 271). Position of wood along the tree stems and their interactions with either main factor and or clone were considered as the within-subject factors. When significant interactions were detected between species / clones and any of the treatment main factors, a separate analysis was done for each species / clone to identify the species / clone which exhibited statistically significant main treatment factor effects (Appendix table 2-1 to 2-3). Treatment effects were considered significant when the *P*-value of ANOVA *F*-test was less than 0.05.

RESULTS

Effects of species / clones and tree stem position on wood density

Independent of treatment and position along tree stems, wood densities of birch and aspen clones were significantly different (*P* = 0.0001). Birch and aspen clone 42 had the highest and lowest overall mean densities of 515.8 ± 4.3 and 360.2 ± 2.3 kg / m³, respectively, while overall mean wood density for aspen clones 216 and 271 were 409.2 ± 3.5 and 398.4 ± 3.3 kg / m³, respectively. Wood density varied considerably along the stem positions, being significantly higher and lower (*P* = 0.0001) at the lower and upper stem positions, respectively, in all species. With regards to position, overall mean wood density ranged from 509.5 ± 4.9 to 523 ± 4.9 kg / m³ in birch; 398.3 ± 3.9 to 427.2 ± 6.2 kg / m³ in clone 216; 384.3 ± 5.7 to 421.1 ± 4.4 kg / m³ in clone 271 and 350.9 ± 3.9 to 381.9 ± 4.7 kg / m³ in clone 42, independent of treatments.

Effects of elevated CO₂ and / or O₃ on wood density

Compared to the control, elevated CO₂ with ambient O₃ significantly reduced overall mean wood density in aspen clone 271 (*P* = 0.0041) but had no significant effects in birch and aspen clones 216 and 42 (Fig. 2-1). In contrast, elevated O₃ (with ambient CO₂) significantly increased wood density in birch (*p* = 0.0208) and aspen clone 42 (*P* = 0.0012) but had no significant effects in aspen clones 216 and 271, when compared to the control (Fig. 2-2). The combined treatment of elevated CO₂ and elevated O₃ did not have statistically detectable effects on overall wood density

across all species / clones, compared to the control. However, overall wood density of birch and aspen clone 42 tended to increase compared to the control (Fig. 2-3).

Elevated CO₂ with ambient O₃ treatment tended to increase wood density along the stems of birch and aspen clone 42 but the converse was true for aspen clones 216 and 271, compared to control treatment (Figs. 2-4 to 2-7). There was a marginal significant interactions effect between elevated CO₂ treatment and position ($P = 0.0606$) for all species. This was as a result of the marginal interactions ($P = 0.0780$) between position and elevated CO₂ treatment in aspen clone 42 (Appendix table 2-1), resulting in a marginal increase and decrease in wood density at the upper and lower positions, respectively in aspen 42 (Fig. 2-7). Elevated O₃ tended to increase wood density along tree stem positions in all species / clones without any significant interactions between position and treatment (Fig. 2-4 to 2-7; Appendix table 2-2). There was no significant interaction between position and the combined elevated CO₂ and O₃ treatments (Figs. 2-4 to 2-7).

DISCUSSION

This study demonstrates that elevated CO₂ and / or O₃, which have been known to influence growth and biochemistry of xylem (wood) cells, also impact wood density. Density is regulated by wood structure. Wood structure is determined by the activities of cambium and the developmental characteristics of wood cells. Available evidence indicates that the growth of wood cells can be affected by changing abiotic factors such as CO₂ and O₃ that in turn may affect wood density.

Effects of species / clones and tree stem position on wood density

Wood density is strongly under genetic control (Zobel and Buijtenen 1989; Zobel and Jett 1995; Barnett and Jeronimidis 2003). This may explain the statistically significant differences in the overall mean wood density of birch and aspen clones 42, 216 and 271, independent of treatment and tree stem positions. In agreement with our observation, Calfapietra et al. (2003), also reported significant differences among three *Populus* species grown under elevated and ambient

CO₂, with overall mean density ranging between 348 and 409 kg / m³. These values are comparable to the wood densities of the current study's aspen clones.

Tree stem position significantly influenced wood density in all species and clones. Typically, wood density varies considerable along the radial and longitudinal axis of the tree stems due to variations in age and physical and biochemical properties of individual cambial cells which control wood formation (Dickison 2000; Barnett and Jeronimidis 2003). In line with the wide inherent variability within woody plants, it is important that as much of this variation as possible is captured during sampling of woody plants for density studies. Hence the decision to sample five different positions along each tree stem of all species / clones for determination of the overall effects of elevated CO₂ and / or O₃ on wood density in this study.

Effects of elevated CO₂ and / or O₃ on wood density

The wood density of birch, aspen 42 and aspen 216, under elevated CO₂ was not different from the control. Calfapietra et al. (2003) also reported no effects of elevated CO₂ on wood density for three *Populus* species. Several studies also have shown no significant effects of elevated CO₂ for many woody plants species. For example elevated CO₂ had no influence on wood density of seedlings of *Quercus ilex* grown in the greenhouse (Gartner et al. 2003), saplings of *Fagus sylvatica* (Beismann et al. 2002) grown in open top chambers and *Liquidambar styraciflua* tested under FACE conditions (Norby et al. 2001b). However, the wood density of aspen 271 was significantly reduced under elevated CO₂ compared to the control in this study. Accruing evidence from the Aspen FACE experiment could help to explain the reduction in the wood density of aspen 271 under elevated CO₂. A persistent 26 % increase in ecosystem net primary productivity (NPP) occurred under elevated CO₂ fumigation during the 12 year long experiment. The sustained increase in NPP was partly attributed to enhanced microbial metabolism rates mediating rapid cycling of growth limiting nitrogen (Zak et al. 2011). Additionally, Zak et al. (2011) reported that elevated CO₂ increased NPP of the aspen clones community by 24-35% during the 10th-12th years of fumigation. The enhanced ecosystem productivity was attributed to the belowground competitive advantage of aspen clones 271 and 42 over the other congeners for the

growth limiting nutrient nitrogen (Zak et al. 2007a; Zak et al. 2012). This observation parallels evidence from Aspen FACE study, that growth of the aspen clones (8, 42, 216, 259 and 271) responded differentially to elevated CO₂ (Karnosky et al. 1996; Isebrands et al. 2001; Karnosky et al. 2003; King et al. 2005; Kubiske et al. 2007; Norby and Zak 2011; Zak et al. 2011; Zak et al. 2012), that aspen clones 271 and 42 grew faster than their congeners under elevated CO₂ (Isebrands et al. 2001; Kubiske et al. 2007), and that aspen 271 had the largest cumulative biomass production (Kubiske et al. 2007). Growth rates of woody plants influence wood quality including wood density (Zobel 1985; Zobel and Buijtenen 1989; Zobel and Jett 1995; Barnett and Jeronimidis 2003). The reduction in wood density of aspen 271 under elevated CO₂ therefore confirms the general notion that faster growth of woody plants could result in significant changes in anatomical characteristics and corresponding reductions in wood density and mechanical strength properties of wood. Aspen clone 271 was reported to have the largest fiber lumen diameter amongst all the clones under investigation (Kaakinen et al. 2004). Data pooled for all the clones of aspen showed that fiber lumen tended to increase under elevated CO₂ after 3 (Kaakinen et al. 2004) and 5 years (Kostiainen et al. 2008) of exposure at Aspen FACE. These observations suggest that the increase in growth of aspen clone 271 under elevated CO₂ (Isebrands et al. 2001; Kubiske et al. 2007; Zak et al. 2011) did not result from corresponding increases in cell wall materials but from an increase in the frequency of the vessel and fiber tissues (Kaakinen et al. 2004; Kostiainen et al. 2008) via enhancement of cambial activity (Yazaki et al. 2005). Luo et al. (2005) reported that elevated CO₂ alone either increased vessel diameters or reduced cell wall thickness in three *Populus* species, but nitrogen fertilization alone and in combination with elevated CO₂ significantly reduced cell wall area in all three *Populus* species. Yazaki et al. (2001) also observed increasing trends in cell lumen diameter of *Larix sibirica* grown under elevated CO₂ and enhanced nutrient availability. It is therefore likely that elevated CO₂ 'fertilization effects' coupled with high acquisition of growth limiting nitrogen stimulated longer duration of rapid cell division and expansion rather than cell wall deposition by aspen 271, thereby resulting in increased void space and subsequent reduction in wood density.

Another mechanism which might have contributed to reductions in wood density of aspen 271 under elevated CO₂ could be changes in its wood chemistry (Kaakinen et al. 2004; Kostianen et al. 2008). Under elevated CO₂ there was a significant increase in labile sugars and a reduction in α-cellulose concentrations in all aspen clones after 3 years of exposure at the Aspen FACE (Kaakinen et al. 2004). Additionally, after 5 years of exposure to elevated CO₂ aspen 271 was reported have reduced uronic acid (a constituent of hemicellulose) and significant increases in starch content (Kostianen et al. 2008). The reduction in cellulose and increase non-structural carbohydrates suggests that the products of photosynthesis were being used for storage and growth rather than cell wall development.

Wood density response to 12 years of elevated O₃ fumigation at the Aspen FACE experiment was genotype and species specific. Wood density of aspen clones 216 and 271 was unaffected by elevated O₃ treatment but significantly increased in birch and aspen clone 42 under elevated O₃ compared to the control. The increase in wood density of birch and aspen clone 42 is in agreement with previous studies at the Aspen FACE. Elevated O₃ significantly reduced diameter growth of all the aspen clones at the end of 3rd and 7th years of fumigation, but birch was not different from the control (Isebrands et al. 2001; Kubiske et al. 2007). Likewise, Kaakinen et al. (2004) and Kostianen et al. (2008) also observed reduction in radial growth and growth rings of aspen clones at the end of the 3rd and 5th year of elevated O₃ fumigation. They also reported a decrease and increase in the cell lumen and cell wall areas, respectively in aspen clones (Kaakinen et al. 2004; Kostianen et al. 2008). Implying that elevated O₃ may have stimulated cell wall thickening and dampened cell division and expansion during xylem cell development, resulting in reduction in radial growth (Isebrands et al. 2001; Karnosky et al. 2003; Kaakinen et al. 2004; Kubiske et al. 2007; Kostianen et al. 2008) and significant increase in wood density of aspen 42. Vessel lumen diameter of birch grown under elevated O₃ from Aspen FACE decreased marginally compared to the control (Chapter 4, this dissertation). Therefore changes in cellular structure in birch in response to elevated O₃ (Street et al. 2011) might have contributed to the observed increase in birch wood density. Kostianen et al. (2006) observed a decrease in

vessel percentage and an increase in percentage of cell wall area in silver birch clone 80 but not in birch clone 4 in response to elevated O₃ compared to the control. Typically, wood density increases with an increase in cell wall area and reduction in void space. The increase in total lignin, extractives and starch, as observed in birch under elevated O₃ at the same experimental site (Kaakinen et al. 2004; Kostianen et al. 2008), may have also contributed to the increase in birch wood density. Lignin, extractives, and accumulation of starch in the ray parenchyma positively influence wood density (Grabner et al. 2005).

Previous investigations at the Aspen FACE site on the combined effects of elevated O₃ and elevated CO₂ on growth parameters (Isebrands et al. 2001; Kubiske et al. 2007; Zak et al. 2011; Zak et al. 2012) and anatomical structure (Kaakinen et al. 2004; Kostianen et al. 2008) on birch and aspen clones 42, 216 and 271 observed no effects. In line with this study, there was no statistically significant effect of elevated CO₂ in combination with elevated O₃ on wood density compared to the control for birch and the three aspen clones under investigation. Implying that, the effects of either elevated CO₂ or elevated O₃ alone on wood density of birch and aspen clones are counteractive when in combination. Nonetheless, wood density of birch and aspen clone 42 tended to increase marginally in ambient O₃ and was largely enhanced in the elevated O₃ in combination with elevated CO₂ with a more pronounced impact in aspen 42 than birch. This observed trend suggest that lower concentrations (ambient) of O₃ have the tendency to influence cellular structure and / or biochemistry of birch and aspen clone 42, thereby causing slight increases in their density. Earlier investigations by Kaakinen et al. (2004) from same Aspen FACE site observed interaction effects of elevated CO₂ and elevated O₃ on biochemistry of the wood of aspen and birch. They reported that the combined effects of elevated CO₂ and elevated O₃ significantly increased aspen wood nitrogen by 15%, but in elevated CO₂ alone had no effects on aspen wood nitrogen. Likewise, Kostianen et al. (2006) indicated that elevated O₃ alone reduced vessel proportion, but in combination with elevated CO₂ led to significant increases of vessel percentage in the wood of silver birch clone 80. To our knowledge, only these observations (Kaakinen et al. 2004; Kostianen et al. 2006) have shown significant effects of a

combination of elevated O_3 and elevated CO_2 on wood chemistry and structure. In conjunction, with the tendency for ambient or elevated O_3 combined with elevated CO_2 to increase wood density of birch and aspen 42, is it possible for the concomitant rising of greenhouse gases to influence wood properties?. Perhaps more data and longer duration studies are needed to confirm or reject this reasoning.

Conclusions

Wood density is a very important wood quality parameter in the pulp and paper and timber industries and can aid in the wise use of wood resources. However wood density is largely influenced by xylem structure and cell growth. Secondary xylem cell development is in turn influenced by both biotic and abiotic environmental factors. Accumulating evidence indicates that wood structure could be affected by the concomitantly rising concentrations of CO_2 and O_3 . Hence knowledge in the effects of these gases on wood density could aid in the planning processes of future wood industries.

In agreement with mounting evidence, this study has demonstrated that mean wood density response to elevated CO_2 and / or O_3 was species and genotype specific after the 12-year-long fumigation at Aspen FACE. The wood density of aspen clone 216 was relatively stable across all treatments. Under the elevated CO_2 treatment, wood density of aspen clone 271, which showed the fastest growth during the 12 years long Aspen FACE experiment, decreased significantly, compared to the control. In contrast, under elevated O_3 , mean wood density of birch and aspen clone 42 increased significantly compared to the control. No statistically significant alterations were observed in the mean wood densities of all aspen clones and birch grown under elevated CO_2 in combination with elevated O_3 compared to the control. This suggests that the effects of elevated O_3 and elevated CO_2 are nullified when in combination. Interesting is the tendency for the wood of aspen clone 42 and birch to increase independent of the level of O_3 concentrations (ambient or elevated).

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Figures

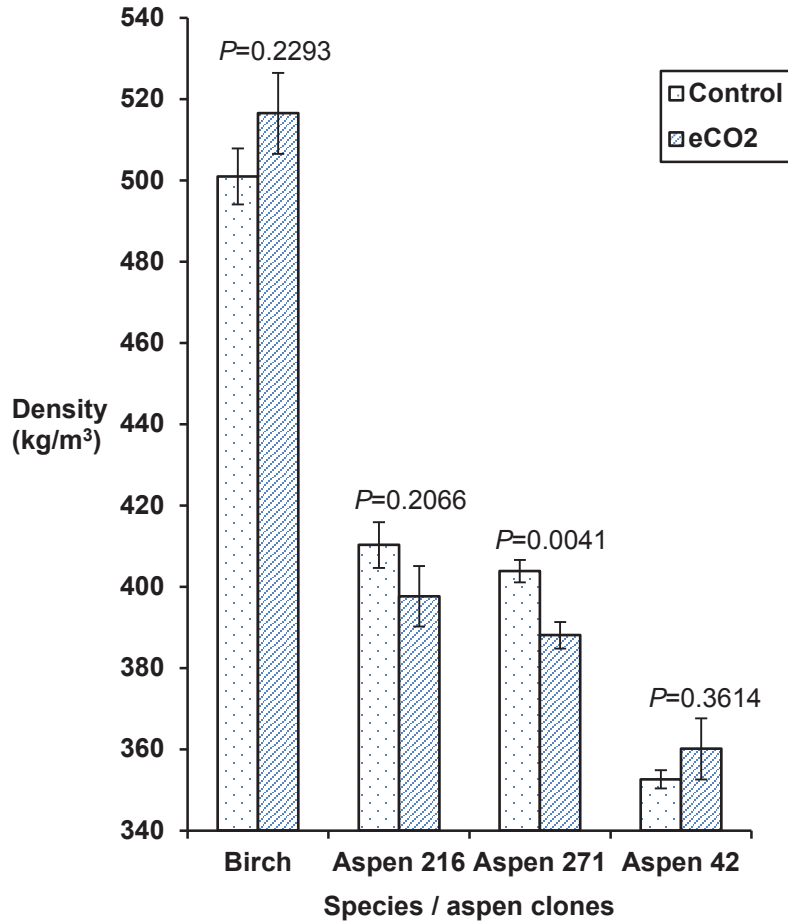


Figure 2-1 Wood density of *B. papyrifera* (birch) and *P. tremuloides* clones (aspen 216, 271 and 42) grown under either ambient CO₂ (control) or elevated CO₂ (eCO₂) for 12 years. Values shown are means ± SE, (n=6).

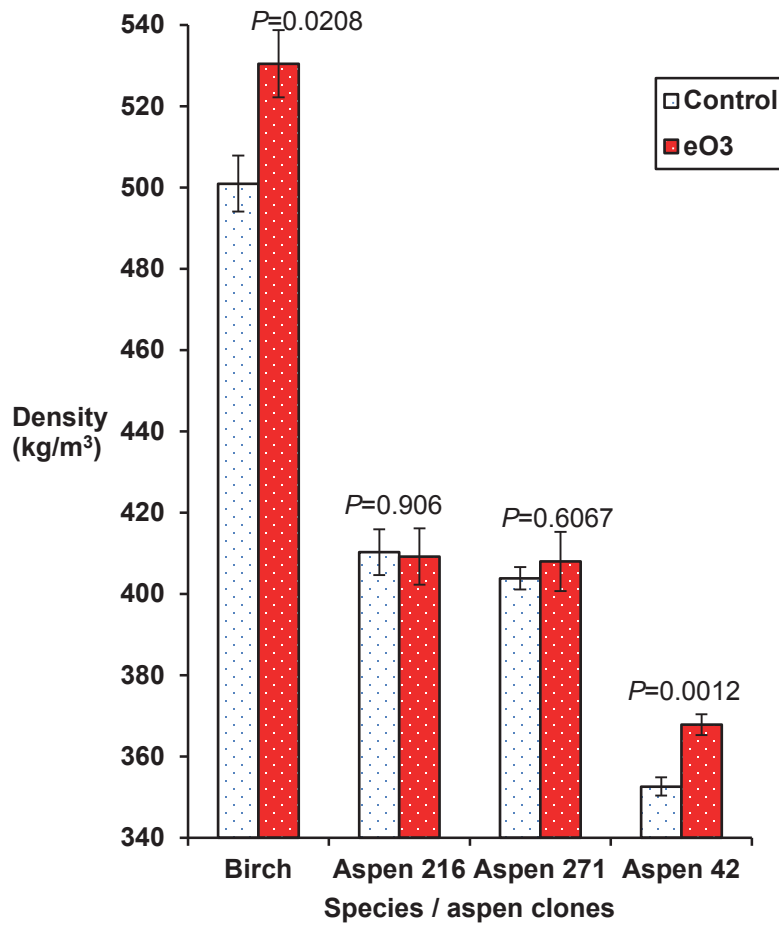


Figure 2-2 Wood density of *B. papyrifera* (birch) and *P. tremuloides* clones (aspen 216, 271 and 42) grown under either ambient O₃ (control) or elevated O₃ (eO3) for 12 years. Values shown are means ± SE, (n=6).

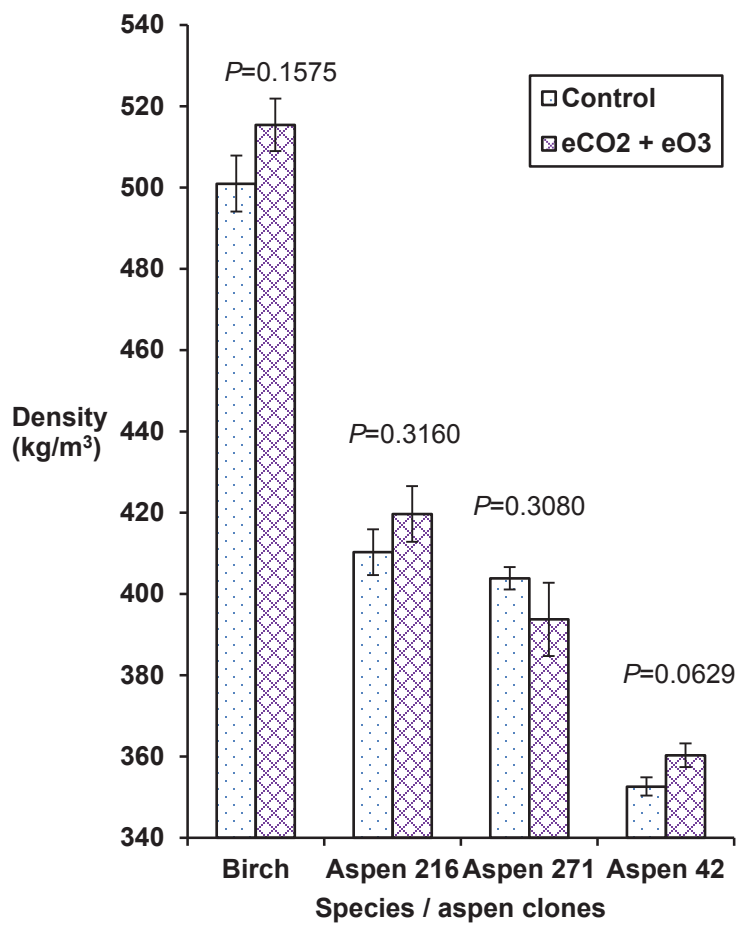


Figure 2-3 Wood density of *B. papyrifera* (birch) and *P. tremuloides* clones (aspen 216, 271 and 42) grown under either ambient CO₂ + O₃ (control) or elevated CO₂ + O₃ (eCO₂ + eO₃) for 12 years. Values shown are means ± SE, (n=6).

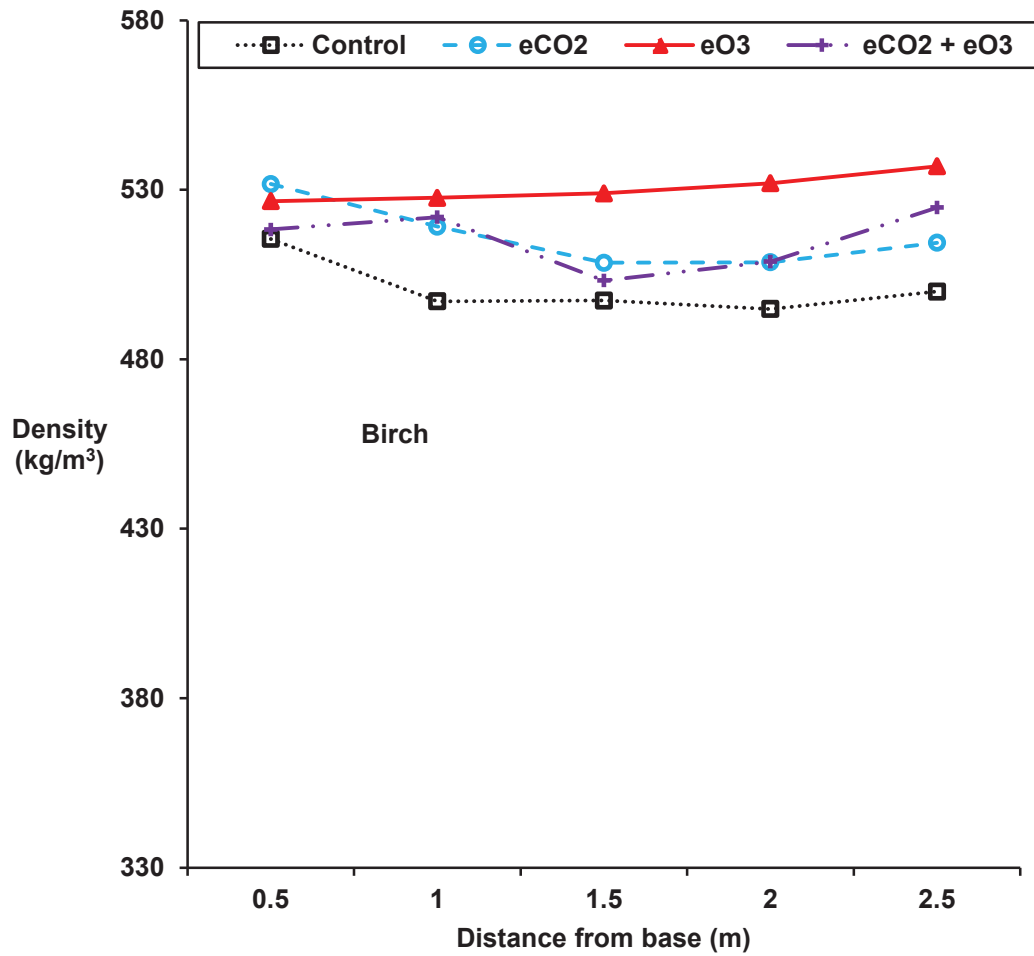


Figure 2-4 Wood density of *B. papyrifera* (birch) with respect to position along the stem, grown under either ambient CO₂ + O₃ (control) or elevated CO₂ (eCO₂), elevated O₃ (eO₃) and elevated CO₂ + O₃ (eCO₂ + eO₃) for 12 years. Values shown are means (n=6).

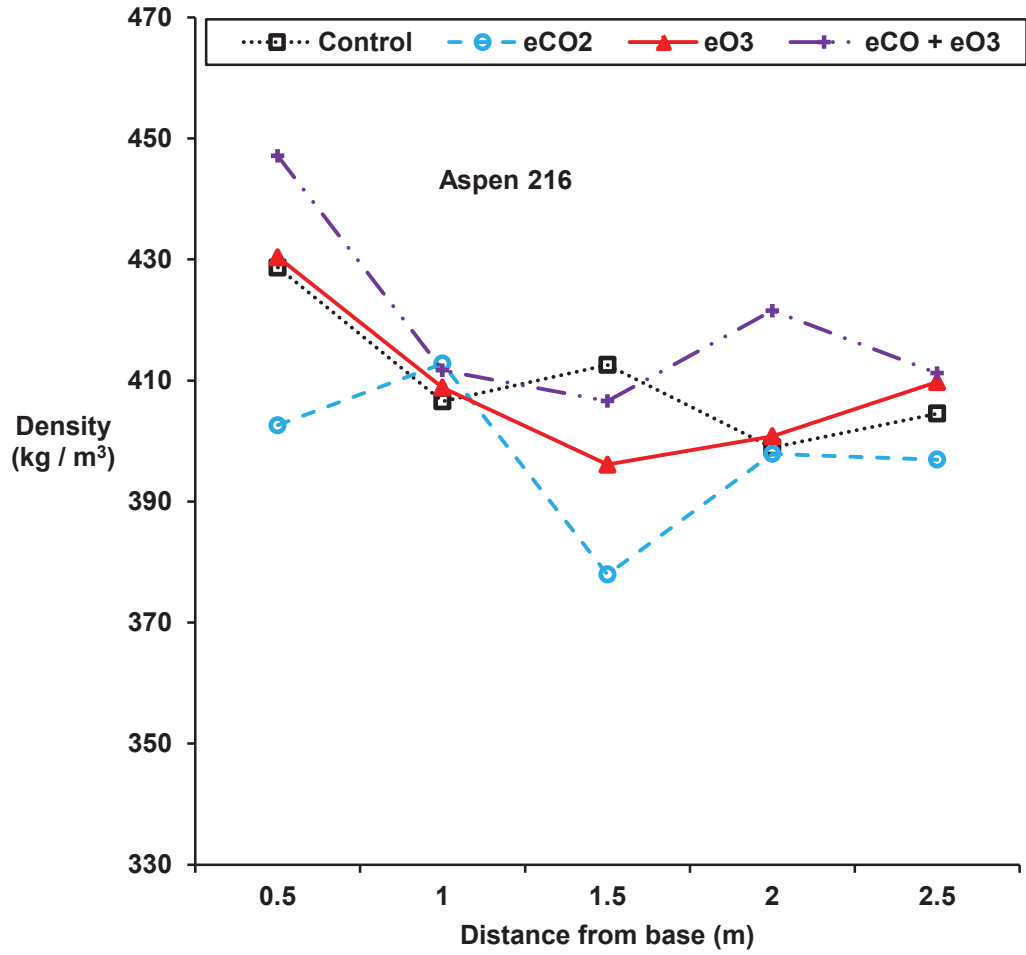


Figure 2-5 Wood density of *P. tremuloides* clone (aspen 216) with respect to position along the stem, grown under either ambient CO₂ + O₃ (control) or elevated CO₂ (eCO₂), elevated O₃ (eO₃) and elevated CO₂ + O₃ (eCO₂ + eO₃) for 12 years. Values shown are means (n=6).

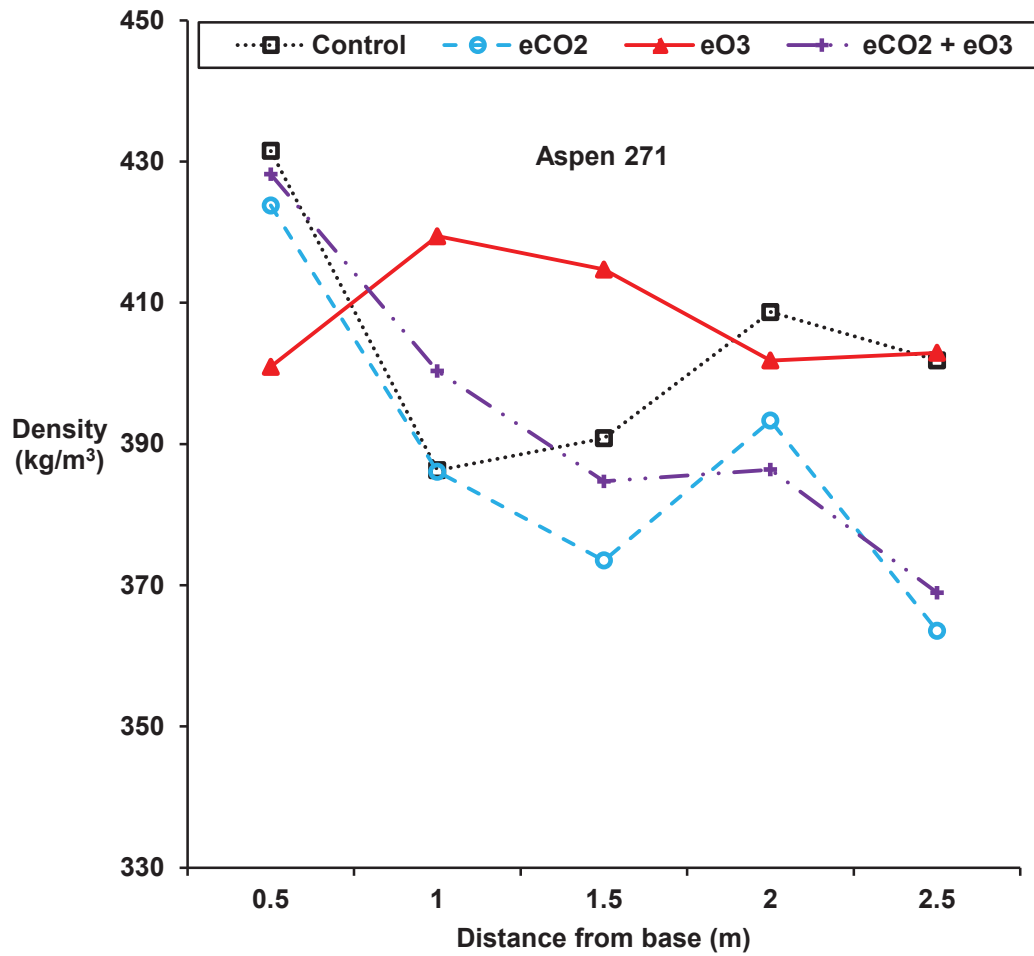


Figure 2-6 Wood density of *P. tremuloides* clone (aspen 271) with respect to position along the stem, grown under either ambient CO₂ + O₃ (control) or elevated CO₂ (eCO2), elevated O₃ (eO3) and elevated CO₂ + O₃ (eCO2 + eO3) for 12 years. Values shown are means (n=6).

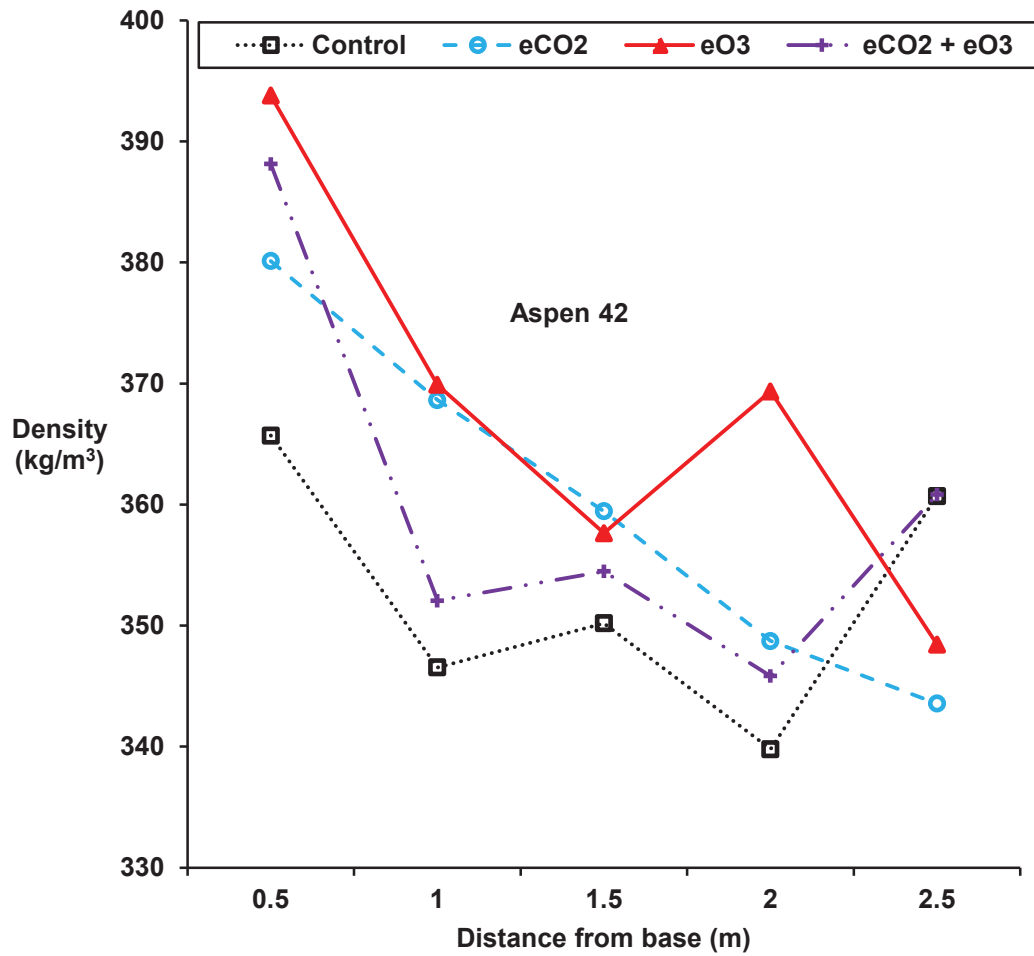


Figure 2-7 Wood density of *P. tremuloides* clone (aspen 42) with respect to position along the stem, grown under either ambient CO₂ + O₃ (control), or elevated CO₂ (eCO₂), elevated O₃ (eO₃) and elevated CO₂ + O₃ (eCO₂ + eO₃) for 12 years. Values shown are means (n=6).

CHAPTER 3: Effects of elevated CO₂ and O₃ on wood decomposition and wood-decaying fungal community composition²

ABSTRACT

Anthropogenic activities continue to drive atmospheric CO₂ and O₃ concentrations to levels higher than in the pre-industrial era. Accumulating evidence indicates that both elevated CO₂ and elevated O₃ could modify the productivity and biochemistry of terrestrial woody plants. Many fungi derive their metabolic resources for growth from plant litter, including woody tissue. Thus modifications in the production and biochemistry of woody plants in response to elevated CO₂ and / or O₃ could impact the community of wood-decaying fungi and rates of wood decomposition. Consequently carbon and nutrient cycling and productivity of terrestrial ecosystem could also be impacted. Although effects of elevated CO₂ and / or O₃ on soil microbial and forest floor fungal communities and functions have been studied, their effects on wood-decaying fungi and wood decomposition remain uncertain. We therefore examined the effects of elevated CO₂ and / or O₃ on the wood-decaying basidiomycete fungal community and initial rates of wood decomposition at the Aspen Free-Air CO₂ and O₃ Enrichment (Aspen FACE) project near Rhinelander, WI, USA. Stem sections from two genotypes of *Populus tremuloides* Michx. (aspen) and *Betula papyrifera* Marshall (paper birch), produced under elevated CO₂ and / or O₃ for 12 years, were reciprocally transplanted and fumigated with elevated CO₂ and / or O₃ at Aspen FACE. At the end of one growing season, initial wood decomposition rates were determined relative to initial wood density and wood-decaying basidiomycetes were isolated from the stem sections and identified via DNA sequencing. Polyporales, Agaricales, and Russulales were the dominant orders of fungi isolated. The wood-decaying basidiomycete fungal communities in aspen and birch wood were significantly different. Although, elevated CO₂ and / or O₃ fumigation tended to reduce the number of fungal species, the fungal communities under elevated CO₂ and / or O₃ were not statistically different from the fungal communities under the ambient conditions.

² Manuscript, in progress

Independent of origin of wood production and elevated CO₂ and / or O₃ fumigation, birch showed higher initial decomposition rate than the aspen clones. However, elevated CO₂ and / or O₃ fumigation environment and origin of wood production did not have significant impacts on wood decomposition. Our results suggest that wood species has a much greater impact on wood-decaying fungal community composition and initial wood decomposition rate than do either growth or decomposition of wood in elevated CO₂ and / or O₃.

INTRODUCTION

Atmospheric concentration of CO₂ has increased to 394 ppm (NOAA, July 2012), about the highest it has been in the last 25 million years (Pearson and Palmer 2000). At the same time, the concentration of tropospheric O₃ has increased by 38% within the last century (IPCC 2007). Both greenhouse gases are predicted to rise further due to anthropogenic activities including fossil fuel combustion and changing land use systems (IPCC 2007). The general body of evidence indicates that elevated CO₂ has stimulating effects on photosynthesis leading to increased biomass production (Ainsworth and Long 2005; Norby et al. 2005; Leakey et al. 2009; Dawes et al. 2011; Norby and Zak 2011). Unlike elevated CO₂, elevated O₃ disrupts important physiological processes (Fuhrer and Booker 2003; Wittig et al. 2007; Wittig et al. 2009; Lindroth 2010; Street et al. 2011), injures woody plants (Karnosky et al. 1996; Karnosky et al. 2003; Karnosky et al. 2007) and induces an overall reduction in plant growth and productivity (Karnosky et al. 2007; Wittig et al. 2009).

Furthermore, elevated CO₂ and / or O₃ are known to cause significant alterations in the chemical composition of leaf tissues (Parsons et al. 2004; Liu et al. 2005; 2007; Parsons et al. 2008; Liu et al. 2009) and woody tissues (Kaakinen et al. 2004; Kostianen et al. 2004; Kostianen et al. 2006; Kostianen et al. 2008). For example, Liu et al. (2005) observed a significant increase in the C:N ratio and tannin concentration under elevated CO₂ and O₃, respectively, in the leaf tissues of northern hardwoods (aspen and paper birch). Lignin concentration of the wood of aspen and

birch increased after 3 years (Kaakinen et al. 2004), but decreased in birch after 5 years of exposure to elevated O₃ (Kostiainen et al. 2008).

Wood litter constitutes a substantial amount of the total detrital inputs in many terrestrial ecosystems (Vogt et al. 1986). Compared to leaf litter, the annual nutrient release to the terrestrial forest ecosystem from decaying wood litter can be very small, but it is essential in the long term storage of carbon and nutrients (Rayner and Boddy 1988). The carbon and nutrients are made available to the ecosystem largely via wood-decaying basidiomycete fungi (Rayner and Boddy 1988; Boddy and Watkinson 1995). Therefore, wood-decaying basidiomycete fungi play an essential role in the retention and mobilization of carbon and growth limiting nutrients in forest ecosystems. The quantity and chemical constituents of wood-litter influence the growth of wood-decaying basidiomycete fungi (Rayner and Boddy 1988; Sinsabaugh et al. 1993; Hattenschwiler et al. 2005). Alterations in the production and chemical composition of woody plants due to the effects of elevated CO₂ and / or O₃ could cause changes in wood-decaying basidiomycete fungal community and decomposition rates of wood litter. As a result, nutrient and carbon cycling and productivity in terrestrial ecosystems could be impacted.

Evidence on the potential effects of global change, including the ever rising CO₂ and / or O₃ concentrations, on soil microbial communities and physiological activities is accumulating (Kilronomos et al. 1997; Klamer et al. 2002; Larson et al. 2002; Strnadova et al. 2004; Chung et al. 2006; Finzi et al. 2006; Parrent et al. 2006; Lesaulnier et al. 2008; Andrew and Lilleskov 2009; Edwards and Zak 2011; Gange et al. 2011; Norby and Zak 2011; Zak et al. 2011). Kilronomos et al. (1997) observed increased and decreased sporulation of depending on fungal species under elevated CO₂. Sporocarp biomass of ectomycorrhizal fungi increased under elevated CO₂ and decreased under elevated O₃ compared to the control (Andrew and Lilleskov 2009).

Ectomycorrhizal fungal community composition was significantly altered under elevated CO₂ and elevated O₃ but the effects diminished with time (Andrew and Lilleskov 2009).

Strnadova et al. (2004) observed no effect of elevated CO₂ on the saprotrophic fungal community. Likewise, Chung et al. (2006) did not detect significant effects of elevated CO₂ on fungal community composition, but found it was significantly modified under elevated O₃ at the Aspen FACE experiment. They also found no effects of either elevated CO₂ and / or eO₃ on the relative abundance of soil fungi. More recent results from Aspen FACE by Edwards and Zak (2011) indicated that plant communities and soil horizons appear to have greater impacts on fungal community composition and function than elevated CO₂ and elevated O₃. The metabolism of plant and fungal cell walls was augmented significantly under elevated CO₂ (Larson et al. 2002; Phillips et al. 2002; Chung et al. 2006) in the early years of Aspen FACE. Later in the experiment, however, plant cell wall metabolism was not affected by elevated CO₂ (Edwards and Zak 2011) but was significantly reduced by elevated O₃ (Larson et al. 2002; Phillips et al. 2002; Chung et al. 2006; Edwards and Zak 2011), and fungal cell wall metabolism was not affected by either elevated CO₂ or O₃ (Edwards and Zak 2011).

The outcomes of investigations aimed at evaluating the effects of the rising CO₂ and / or O₃ on litter decomposition have also had divergent results (Norby et al. 2001a; Lindroth 2010). For example, Strain and Bazzaz (1983) suggested that elevated CO₂ will result in production of poor quality litter and reduced decomposition rates. Accordingly, there was reduction in decomposition rates of leaf litter of *Betula papyrifera* (Parsons et al. 2004; Parsons et al. 2008) and *Populus* species (Cotrufo et al. 2005; Parsons et al. 2008) grown under elevated CO₂. Contrarily, elevated CO₂ had no effect on decomposition rates of leaf litter of similar species (Liu et al. 2009), twig and branch litter of *Fagus sylvatica* (Cotrufo and Ineson 2000) and leaf litter of some northern species (Finzi et al. 2001; Hall et al. 2006). Additionally, a meta-analysis of 33 species grown under elevated CO₂ showed no significant effects on litter decomposition (Norby et al. 2001a).

The effects of elevated ozone on decomposition also show contrasting results. Elevated O₃ increased decomposition rates in litter of *Betula papyrifera* (Parsons et al. 2008). However, elevated O₃ significantly reduced decomposition rates in aspen and birch (Kasurinen et al. 2006;

Parsons et al. 2008; Liu et al. 2009). No changes in decomposition rates were observed in needle litter of *Pinus* seedlings and saplings and leaves of *Liriodendron tulipifera* seedlings grown under elevated O₃ (Scherzer et al. 1998; Kainulainen et al. 2003).

The most important group of organisms that influences wood decomposition in the terrestrial environment are fungi (Rayner and Boddy 1988; Boddy and Watkinson 1995). Three classes of wood-decaying fungi are recognized based on the nature of their impact on wood: soft, brown and white rot fungi (Rayner and Boddy 1988; Boddy and Watkinson 1995; Worrall et al. 1997; Schmidt and Czeschlik 2006). White rot fungi decompose all components of wood, including lignin, but brown rot and soft rot fungi attack simple carbon-containing compounds and holocellulose, with minimal effects on lignin. Consequently, decomposition patterns vary with wood-decaying fungal species (Worrall et al. 1997) and richness (Chi et al. 2007; Rajala et al. 2010). Species richness may have retarding or stimulating effects on wood decomposition (Boddy 2000; Fukami et al. 2010; Rajala et al. 2010).

Until now, specific effects of elevated CO₂ and / O₃ on wood-decaying white-rot basidiomycete community composition remain uncertain, in spite of their key role in nutrient and carbon cycling. Meanwhile, Edwards and Zak (2011) showed that the effects of elevated CO₂ and O₃ could have different impacts on different groups within fungal communities and that the effects could fluctuate with time. Furthermore, most decomposition studies were not done in the same environment in which the litter was produced (Norby et al. 2001a), rendering applicability of results to field situations uncertain. The Aspen FACE project provided an *in vivo* field approach for such investigations. Aspen FACE was located in Harshaw, near Rhinelander, WI. It was a unique, long term experiment (about 13 years) evaluating the impact of elevated CO₂ and O₃ and their interaction on northern forest trees on very large scale. At Aspen FACE, a persistent 26% increase in net primary productivity was observed under elevated CO₂ for 12 years (Isebrands et al. 2001; Karnosky et al. 2003; King et al. 2005; Kubiske et al. 2007; Zak et al. 2011), and a reduction in productivity under elevated O₃ was also observed (Isebrands et al. 2001; Karnosky et al. 2003; King et al. 2005; Kubiske et al. 2007). Additionally, alterations in the biochemistry of

wood and leaf litter of aspen and birch produced under elevated CO₂ and elevated O₃ have been observed at Aspen FACE. Elevated CO₂ increased levels of condensed tannins, C:N, and lignin:N ratios in aspen and birch leaves (Parsons et al. 2004; Liu et al. 2005; 2007; Parsons et al. 2008; Liu et al. 2009). Elevated O₃ increased levels of lignin in aspen leaves, decreased C:N and lignin:N in birch leaves (Parsons et al. 2004; Parsons et al. 2008) and increased levels of soluble phenolics and condensed tannins in aspen and birch leaves (Liu et al. 2005; 2007; Liu et al. 2009). No statistically significant effect of elevated CO₂ and / or O₃ on the C:N and lignin:N levels were reported in birch and aspen wood (Kostiainen et al. 2008). However, concentrations of extractives in aspen wood increased and decreased under elevated CO₂ and O₃, respectively, and increased in birch wood under either elevated CO₂ or O₃ (Kostiainen et al. 2004; Kostiainen et al. 2008). Concomitant with the alterations in the production and biochemistry of litter, microbial community composition modifications have also been observed under elevated CO₂ and O₃ at the Aspen FACE site (Chung et al. 2006; Andrew and Lilleskov 2009; Edwards and Zak 2011). Evidence of increased *N*-acetylglucosaminidase and cellobiohydrolase activity under elevated CO₂ and reduced cellobiohydrolase activity in soil under elevated O₃ has been reported (Larson et al. 2002; Chung et al. 2006; Edwards and Zak 2011). Elevated CO₂ and / or O₃ fumigation environment had no direct impact on aspen and birch leaf litter decomposition rates. However, decomposition rates of leaf litter of aspen and birch produced under elevated CO₂ decreased significantly, while that of birch and aspen leaf produced under elevated O₃ increased and decreased respectively, after up to 23 months of field incubation (Parsons et al. 2004; Parsons et al. 2008). A longer duration study (up to 735 days) by Liu et al. (2007) suggested that the effects of elevated CO₂ and / or O₃ on decomposition rates via changes in biochemistry of leaf litter of aspen and birch could be transient. In line with the aforementioned evidence from Aspen FACE, we hypothesized that: (1) wood species effects would be observed in the wood-decaying basidiomycete fungal community composition and decomposition rates; (2) modification in soil and forest floor microbial communities resulting from alterations in the quantity and biochemistry of aspen and birch litter produced under elevated CO₂ and / or O₃ for 12 years would cause

significant alterations in the wood-decaying basidiomycete fungal communities; (3) elevated CO₂ and / or O₃ fumigation environment would have no statistically significant direct impact on wood decomposition rates; and (4) rates of decomposition of aspen and birch wood produced under either elevated CO₂ or O₃ would be significantly different than those for wood produced under ambient control conditions.

MATERIALS AND METHODS

Site description

Stem segments used in the study were obtained from the Aspen Free-Air CO₂ and O₃ Enrichment (Aspen FACE) project (for a detailed description of the site and experimental design of Aspen FACE see Dickson et al. (2000)). Aspen FACE research was conducted on a 32 ha USDA Forest Service Experimental Farm at Harshaw, near Rhinelander, in Wisconsin, USA (longitude 45.6° N, latitude 89.5° W). Potatoes and small grains were cultivated on the site for more than 50 years before the Forest Service acquired the land in 1972 to serve as a forest research station. Prior to the onset of the Aspen FACE research in 1997, the site was planted with poplar clones and larch. However, all the poplar clones and larch were cleared and stumps removed in 1996. The study site is nearly flat and the soil type is sandy loam. An initial soil analyses conducted for all treatment plots in 1997 showed no significant differences except for mean percent carbon and nitrogen, which were significantly greater in CO₂ rings than CO₂ + O₃ rings (Dickson et al. 2000).

The Aspen FACE experiment was a 2² factorial randomized complete block design with each treatment level replicated three times, once each in the northern, central and southern portions of the experimental site. The main and crossed factors were CO₂, O₃ and CO₂ + O₃, respectively. The treatment levels were ambient CO₂ and O₃ as the control and elevated CO₂ (ambient + 200 ppm) and elevated ozone (1.5 × ambient), respectively. The treatments were applied to twelve 30-m diameter rings, located at least 100 m apart (Appendix fig. 2-1). Each ring was partitioned into east and west regions. The eastern region was planted with five *Populus tremuloides* Michx. (aspen) clones (8L, 42E, 216, 259 and 271) in random order, with a 1 m × 1 m spacing. The

western region was further divided into north and south subplots. The northwest and southwest subplots were mixed plantations of aspen clone 216 and *Acer saccharum* Marshall var. *saccharum* (sugar maple) and *Betula papyrifera* Marshall (paper birch), respectively. All planting was completed in 1997, and exposure of treatment rings to elevated CO₂ and O₃, was done during the growing seasons of 1998 to 2009 between 0700 hrs and 1700 hrs each day, when foliage was not wet. In 2010, after the original experimental plantings had been harvested, CO₂ and O₃ treatments were continued for one growing season for the regenerating forest composed of aspen root suckers and maple and birch stump sprouts.

Sampling and laboratory analysis

All trees in all treatment rings were harvested during the winter of 2009/2010 (Fig. 3-1). Six trees of birch and aspen clones 271 and 42E from each treatment level were randomly sampled. From the lower stem of each tree, five 25-mm wide disc subsamples were cut at intervals of 0.5 m from the base to the top of the stem section. The four 0.5 m-log sections in between the discs together with the five 25-mm discs from all sampled trees were transported to Michigan Technological University for refrigeration and laboratory analysis.

The discs were used to determine initial wood density using a water displacement method (Williamson and Wiemann 2010). The wood discs were completely suspended under water in a beaker placed on an electronic balance, and the mass of displaced water was used to estimate volume of the wood. The samples were then oven dried at 105°C to a constant mass, and density was computed as oven dry mass divided by the moist volume of sample.

In early May 2010, all the 0.5 m log segments of birch and aspen clones 271 and 42 were redeployed onto the soil surfaces of the rings in a reciprocal transplanting manner and allowed to begin decaying (Table 3-1). Logs grown under each treatment were placed on the soil surface of every treatment in a full factorial design, with two logs per species / clone from each of the four treatments placed in each ring (Table 3-1). The rings were then fumigated with elevated CO₂, O₃ and CO₂ + O₃ at concentrations similar to the description above during the entire growing season

(May-October 2010). One log per species / clone and treatment from each ring (144 log samples in total) were removed from the rings in early May 2011 and frozen at MTU pending laboratory analysis.

To determine the effects of the treatments on fungal communities and rate of wood decomposition, one end of each 0.5 m-long log was first cleaned by cutting off a 6-mm disc. Then a 25-mm thick disc was sampled from all 144 samples from the cleaned edge. The 25-mm disc samples were then divided into two half discs. One half disc was used to estimate final wood density and the other for fungal isolation investigations. A water displacement method was used to measure final wood density as described above. The percent loss in

density: $\left(\frac{\text{initial density} - \text{final density}}{\text{initial density}} \right) \times 100$ was used as a measure of initial decomposition rate

(Fukasawa et al. 2009b; 2009a; Rajala et al. 2010).

The sample for fungal isolation studies was further subdivided into two sub-samples. From each of the fresh surfaces three wood chips of approximately 2 mm³ were aseptically removed and placed into three separate agar plates of two different media. One plate contained malt extract agar of 2% malt and 1.5% agar. The other two plates had malt extract agar containing benomyl (2 mg L⁻¹) and streptomycin (40 mg L⁻¹). The benomyl and streptomycin were added to suppress the growth of micro-fungi and bacteria, respectively (Eaton and Hale 1993). The plates were then incubated in the dark at room temperature and monitored for fungal growth. Pure culture isolates were transferred to 2% malt agar for long term storage and characterization. Characterizations were based on morphological (growth rate, nature of mat) and anatomical (propagative structures, nature of hyphae, presence or absence of clamps) features (Stalpers 1978; Rayner and Boddy 1988). Pure cultures were transferred to 2% malt agar slants and transported to the mycology lab of the USDA Forest Service at Madison, USA, for DNA sequencing and identification. A fungal species was recorded as present or absent in a 0.5 m log segment.

Analysis of data

Fungal community composition data analysis was done using R, version 2.15.0 (R Development Core Team 2012). Fungal species with frequency occurrence of less than 3 were excluded from analysis as recommended by McCune and Grace (2002) for multivariate analysis. Non-metric multidimensional scaling (NMDS) graphs of fungal community composition for the four treatments (control, elevated CO₂, elevated O₃ and elevated CO₂ + elevated O₃) and between birch and aspen were created using Bray Curtis distance measures. The NMDS graphs were done separately for aspen clones and the birch species independent of treatments. The minimum number of axes for the NMDS plots, were determined from scree plots, with the lowest amount of stress. Due to the multiple factors under investigation, permutational multivariate analysis of variance (PerMANOVA) was used to determine statistically significant effects of species and elevated CO₂ and / or O₃ on 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 and Grace 2002).

Split-split-plot ANOVA (Montgomery 2009) was used to analyze the data on initial wood decomposition rates using the GLM procedure of SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA). Each of the fixed main treatment factors of CO₂ (ambient vs. elevated CO₂); O₃ (ambient and elevated O₃), and CO₂ + O₃ (ambient vs. elevated CO₂ + elevated O₃) were considered as the whole plot factor, species (birch, aspen clones 42 and 271) as the sub-plot factor, and source of log (i.e. logs produced under ambient conditions, elevated CO₂ and / or O₃) as the sub-sub-plot factor. There were three replicate rings / blocks along the north, central and southern regions of the experimental site. The analyses were carried out separately for each main treatment factor on all species / clones and log sources. Error terms for testing block and main treatment effects were block × treatment with 2 degrees of freedom, where treatment was elevated CO₂ and or / O₃. The error term for testing for species, and species × treatment effects was block × species-nested-within-treatment, with 8 degrees of freedom. The error term for testing the effects of log source and its interaction with species and /or treatment was the total

error term of the split-split-plot ANOVA model, with 36 degrees of freedom (Appendix tables 3-4 to 3-6). Descriptive statistics (means and standard errors) were computed for each parameter. In addition, data were examined for the normality and homogeneity of variance assumptions of analysis of variance before analysis was performed. Data in percentages were arcsine transformed before the split-split-plot analysis was implemented.

RESULTS

Fungal community

A total of 123 out of the 144 log samples (85%) produced wood-decaying basidiomycete isolates. The remaining 21 log samples (15%) were either sterile or contaminated with bacteria and / or micro-fungi. A total of 14 wood-decaying basidiomycete were isolated and 13 were identified either to species or genus levels, with one isolate unidentified (Table 3-2). All isolated fungi were from the phylum Basidiomycota and class Agaricomycetes consisting of the orders Agaricales, Polyporales, Russulales and Cantharellales (Kirk et al. 2011). Five species were from two families of Polyporales (Meruliaceae, Polyporaceae); four species from two families of Russulales (Peniophoraceae, Stereaceae); three species from three families of Agaricales (Cyphellaceae, Physalacriaceae, Schizophyllaceae) and one species from the family Hydnaceae of the order Cantharellales (Table 3-2). Independent of treatment type, 11 and 13 fungal species were present in the aspen clones and birch logs, respectively (Table 3-3). The NMDS biplots separated the fungal community composition between the aspen clones and birch species along axis one (Fig. 3-2). A PerMANOVA further confirmed that the difference in fungal composition of aspen and birch logs was statistically significant ($P = 0.0009$, Table 3-4). An indicator species analysis revealed that *Bjerkandera adusta*, *Stereum rugosum* and *Trametes versicolor* were significant species indicators of the aspen logs ($P = 0.002$; $P = 0.010$; $P = 0.048$, respectively).

All 14 fungal species were present in the ambient rings with 10 each recorded under elevated CO₂ and elevated O₃, and 12 species present in their combination, independent of species of log (Table 3-3). The aspen logs under ambient conditions, elevated CO₂, elevated O₃ and elevated

CO₂ + O₃ had 11, 9, 8 and 8 fungal species, respectively. In addition the birch logs under ambient conditions, elevated CO₂, elevated O₃ and elevated CO₂ + elevated O₃ had 13, 5, 6 and 6 fungal species, respectively (Table 3-3). NMDS biplots did not separate the fungal community composition under the ambient and elevated CO₂ and / or O₃ for the aspen or birch logs (Figs. 3-3 and 3-4). The trends revealed by the NMDS biplots were confirmed by PerMANOVA (Table 3-4). There was no significant difference between fungal community compositions under ambient and either elevated CO₂ and / or O₃ for aspen ($P = 0.1279$) or birch logs ($P = 0.2438$).

Wood decomposition

Birch and aspen clone 271 exhibited the highest and lowest percent density loss, respectively, at the end of the study. Mean wood density across all treatments decreased from 518.1 ± 1.7 to 449.9 ± 4.7 kg / m³ with percent loss of 13.2 ± 1.1 in birch; (Table 3-5); from 357.4 ± 2.2 to 317.8 ± 4.9 Kg / m³ with percent loss of 11.1 ± 0.8 in aspen 42 (Table 3-6) and from 398.2 ± 8.2 to 363.8 ± 7.9 Kg / m³ with percent loss of 8.6 ± 0.1 in aspen 271 (Table 3-7). The observed differences in the percentage loss of wood density in birch and aspen clones 42 and 271 were statistically significant, independent of elevated CO₂ and / or O₃ fumigation and growth source of log (Table 3-8, Appendix tables 3-4 to 3-6). Independent of wood source, elevated CO₂ and / or O₃ fumigation (microenvironments) did not have any statistically significant impact on percent wood density loss in birch or aspen logs (Tables 3-8; Appendix tables 3-4 to 3-6) compared to the control. Independent of the differential microenvironments created by elevated CO₂ and / or O₃ fumigations, birch or aspen logs originally produced under either ambient conditions or elevated CO₂ and / or O₃ (source of wood) also did not show any statistically detectable effects on percent wood density loss compared to the control (Tables 3-5 to 3-8). There was a nearly significant 3-way-interraction effect among species, source of wood and elevated CO₂ treatment ($P = 0.1001$). This was the result of percent density loss in birch and aspen 271 wood produced under elevated CO₂ tending to decrease and that of aspen 42 wood produced under elevated CO₂ tending to increase elevated CO₂ treatment compared to the control.

DISCUSSION

We hypothesized that: (1) wood species effects would be observed in the wood-decaying basidiomycete fungal community composition and decomposition rates; (2) modification in soil and forest floor microbial communities resulting from alterations in the quantity and biochemistry of aspen and birch detritus produced under elevated CO₂ and / or O₃ for 12 years would cause significant alterations in wood-decaying basidiomycete fungal community (3) elevated CO₂ and / or O₃ fumigation environment would have no statistically significant direct impact on wood decomposition rates; and (4) rates of decomposition of aspen and birch wood produced under elevated CO₂ and O₃ alone would be significantly different than those for wood produced under ambient control conditions . Our results supported hypotheses 1 and 3 but not 2 and 4.

Fungal community

Three major orders, Polyporales, Agaricales and Russulales, dominated the isolated wood-decaying basidiomycetes fungi. Additionally one species of Cantharellales (*Sistotrema brinkmannii*) was isolated, but occurred only once. All isolated species are known to cause white rot except *Sistotrema brinkmannii*, which is known for causing brown rot on northern hardwoods in America (Lindsey and Gilbertson 1978; Gilbertson and Ryvarden 1986). The NMDS biplots separated the fungal community composition associated with birch and aspen clones logs along axis one, regardless of FACE treatment. A PerMANOVA analysis further indicated that fungal composition of aspen clones and birch logs were significantly different from each other. The difference was attributed to the greater relative abundance of *Bjerkandera adusta*, *Trametes versicolor* and *Stereum rugosum* in the aspen clones logs across all treatments. Additionally, *Chondostereum purpureum* and *Stereum* sp were very rare in the birch logs and one unknown species (unidentified sp) also tended to be very rare in the aspen logs. An earlier study at the same research site also identified plant community to be the major cause for differences in fungal community composition (Edwards and Zak 2011). A higher number of ascomycetes and basidiomycetes genotypes were reported for the aspen clones community than for the aspen-

birch mixed community (Edwards and Zak 2011). The importance of log species type on fungal community composition may be attributed to substrate-specificity effects of the different wood-decaying fungi occurring (Lodge and Cantrell 1995; Lindahl et al. 2007).

Elevated CO₂ and / or O₃ tended to reduce the number of wood-decaying fungal genotypes in both the aspen clones and aspen-birch communities. However, separate NMDS biplots for the two plant communities showed no clustering of wood-decaying fungal composition of the various treatments (Fig. 3-3 and 3-4). A PerMANOVA analysis also confirmed that elevated CO₂ and / or O₃ did not show statistically detectable effects on wood-decaying fungal communities in the aspen and the birch logs compared the control. Strnadova et al. (2004) also observed no significant effects of elevated CO₂ on saprotrophic micro-fungal community composition at the Swiss FACE experiment.

At Aspen FACE a number of studies on the effects of elevated CO₂ and /or O₃ on forest floor and soil microbial communities and function have been carried out. In one assessment, elevated CO₂ had no effect on soil fungal communities, but elevated O₃ did (Chung et al. 2006). Earlier in the Aspen FACE experiment the overall soil microbial community under elevated CO₂ was found to be different from ambient, but elevated O₃ diminished the effect (Phillips et al. 2002). Recent investigations indicated that different functional groups of fungi inhabiting the forest floor or soil responded differently to elevated CO₂ and / or eO₃ treatments (Edwards and Zak 2011). For example, elevated CO₂ and O₃ significantly altered ectomycorrhizal and Agaricomycetes fungal communities in the soil, but the Pezizomycotina fungal community in the forest floor and soil were not altered. Additionally elevated CO₂ altered the Agaricomycetes fungal community in the forest floor compared to ambient CO₂. Different fungal functional groups occupy different ecological niches (Lodge and Cantrell 1995; Lindahl et al. 2007; Edwards and Zak 2011) and respond differently to elevated CO₂ and / or O₃ (Edwards and Zak 2011).

Ectomycorrhizal fungi occupying the deeper depth of the soil are more efficient in harvesting nitrogen than carbon, but the Agaricomycetes occupying forest floor and litter are efficient in

depolymerizing carbon (Lindahl et al. 2007). Saprobic fungi, including wood-decaying basidiomycetes, utilize dead biological materials via the production of extracellular enzymes (Rayner and Boddy 1988; Baldrian 2008). Cellobiohydrolase and *N*-acetylglucosaminidase (NAG) are two major extracellular enzymes that saprobic fungi use in the degradation of plant cell wall (lignin) and fungal cell wall (chitin) materials, respectively. The activities of these two enzymes in soil were followed for ten years at Aspen FACE (Larson et al. 2002; Chung et al. 2006; Edwards and Zak 2011). Larson et al. (2002), reported an increased in cellobiohydrolase and *N*-acetylglucosaminidase activities under elevated CO₂, with no effects under elevated O₃ after three years of fumigation. At the end of five years of fumigation, elevated CO₂ was observed still to increase cellobiohydrolase and *N*-acetylglucosaminidase activities, while elevated O₃ reduced cellobiohydrolase and had no effect on *N*-acetylglucosaminidase activities (Chung et al. 2006). After ten years of fumigation, the effects of elevated CO₂ and O₃ on cellobiohydrolase and *N*-acetylglucosaminidase activities appeared to have dampened and were no longer statistically significant compared to the control (Edwards and Zak 2011).

Cellobiohydrolase and *N*-acetylglucosaminidase activities may reflect the presence of certain fungi (Baldrian 2008). For example, Chung et al. (2006), reported reduction of cellobiohydrolase activities with simultaneous alterations in fungal community composition under elevated O₃. It is therefore reasoned that the diminishing effect of elevated CO₂ and O₃ on cellobiohydrolase and *N*-acetylglucosaminidase activities after ten years of fumigation (Edwards and Zak 2011) is in agreement with the current studies observation which was conducted after the 12th year of Aspen FACE. To our knowledge, this is the first time effects of elevated CO₂ and / or O₃ on the wood decaying fungal community has been investigated. Woody litter input to the experimental site was virtually absent during the study, except for small tree mortality and branch loss during the experiment. Additionally, this study was performed after all trees were harvested and little canopy existed. As a result there were greater chances that fungal spores may have been blown or circulated in the experimental area and contributed to our current observation of no treatment effects on the wood decaying fungal community. Furthermore, fungal community development in

temperate decaying hardwood occurs in successional stages (Frankland 1998; Boddy and Heilmann-Clausen 2008), with the suite of fungal species changing over time (Eaton and Hale 1993; Boddy and Heilmann-Clausen 2008). This study was conducted after a relatively short period of decay (May 2010 – May 2011). As a result, most of the fungal species isolated, *Chondostereum purpureum*, *Trametes spp*, *Stereum spp*, *Peniophora sp*, *Cylindrobassidium sp*, *Bjerkandera adusta*, and *Schizophyllum commune*, are from the primary or secondary successional stages of wood decomposition (Boddy and Heilmann-Clausen 2008). It is therefore suggested that our observations should be interpreted with caution since we are not certain how a longer term study and a tertiary suite of fungal species would respond to elevated CO₂ and / or O₃ fumigations.

Wood decomposition

Wood species had a detectable impact on wood decomposition rates after one year at the Aspen FACE site, with birch decomposing faster than aspen. This is consistent with previous reports for a variety of tissues from the same species. At the Aspen FACE experiment, leaf litter of birch was found to decay faster than that of aspen (Liu et al. 2007; Parsons et al. 2008). Similarly, a lab decomposition assay found higher wood decay rates in birch than aspen wood from Aspen FACE (Richter, unpublished data). Species differences have long been recognized to have strong impact on wood decomposition (Rayner and Boddy 1988; Boddy and Watkinson 1995; Hattenschwiler et al. 2005; Freschet et al. 2012). Wood decomposition is influenced by species due to species specific variation in wood physical, chemical and anatomical characteristics (Panshin and Zeeuw 1980; Boddy and Watkinson 1995; Cornwell et al. 2008; Cornwell et al. 2009; Weedon et al. 2009; Freschet et al. 2012). For example, the relatively higher percentage of lignin in aspen (Kaakinen et al. 2004) could cause it to decay at a slower rate than birch. Lignin is a recalcitrant chemical compound and known to decompose at a slower rate (Eaton and Hale 1993; Talbot et al. 2012).

Independent of source of aspen or birch wood, elevated CO₂ and / or O₃ fumigation did not have any significant impact on percent density loss. This implies that elevated CO₂ and / or O₃

fumigation environment did not have direct effects on initial decomposition rates of birch and aspen wood. Elevated CO₂ and / or O₃ fumigation environment also did not alter rates of decomposition of aspen and birch leaf litter after 12 and 23 months of exposure at Aspen FACE (Parsons et al. 2004; Parsons et al. 2008).

Generally, elevated CO₂ and / or O₃ are expected to impact decomposition rates via changes in the biochemistry / quality of plant litter (Strain and Bazzaz 1983). Typically, poor quality litter (low N, P; high phenolics and extractives content) decomposes slowly and high quality litter (high N, P, low phenolics and extractives) decomposes at faster rates. Low quality litter induces agents of decomposition to divert metabolic resources into synthesizing enzymes for acquisition of growth limiting macronutrients such as N and P from exogenous sources and constrains the production of lignin and cellulose degrading enzymes, thereby dampening decomposition rates in poor quality substrates (Sinsabaugh et al. 1991; Sinsabaugh et al. 1992; 1993; Sinsabaugh and Linkins 1993). Plant litter, mainly leaf tissues produced under elevated CO₂, tended to have high C:N, lignin:N and condensed tannins (poor quality) and significant reductions in decomposition rates relative to litter generated under ambient conditions. Plant litter generated under elevated O₃ tended to have low C:N (high quality), but tannin or lignin concentrations also tended to increase, thereby reducing decomposition rates (Parsons et al. 2004; Liu et al. 2005; Parsons et al. 2008). Note however that Liu et al. (2007) indicated that the effects on decomposition rates could be transient. In contrast, beech wood (*Fagus sylvatica* L) generated under elevated CO₂ resulted in high C:N and lignin:N but did not result in reduction of decay rates (Cotrufo and Ineson 2000). Likewise, meta-analysis of 33 species grown under elevated CO₂ showed no significant effects on plant litter decomposition (Norby et al. 2001a).

We also did not find any statistically significant effects of the growth environment of wood on rates of decomposition in this study. The birch and aspen wood used in this study were grown under Aspen FACE conditions for 12 years, implying that biochemical changes in the wood of aspen and birch generated under elevated CO₂ and / or O₃ were minor with regard to those needed to alter decomposition. Percent density loss in aspen 271 produced under elevated CO₂

tended to decrease when placed in the elevated CO₂ treatment compared to the control. Surprisingly, percent density loss of aspen 271 produced under ambient or elevated CO₂ tended to increase under ambient, suggesting that the observed reduction in percent density loss in aspen 271 produced under elevated CO₂ and placed in elevated CO₂ treatment may not be directly explained by either source of wood (substrate quality) or elevated CO₂ fumigation environment.

Wood decomposition is influenced by the complex interactions of environment and decomposer fungal species, which may vary temporally and spatially (Rayner and Boddy 1988; Boddy and Watkinson 1995; Hattenschwiler et al. 2005). Additionally the anatomical, physical and chemical characteristics of the plant litter could also impact decomposition rates (Cornwell et al. 2008; Cornwell et al. 2009; Weedon et al. 2009). Leaf litter of birch and aspen clone 216 under native placement (fumigation environment same as during growth) at same experimental site (Aspen FACE, Rhinelander) exhibited significant trends in decomposition rates (Parsons et al. 2004; Parsons et al. 2008), which were comparable to the trends in percent density loss in aspen 271 and birch wood observed in this study. Under native placement, rates of decomposition of aspen leaf litter decreased significantly in all treatments, but those of birch decreased significantly under elevated CO₂ and increased under elevated O₃ and CO₂+O₃ (Parsons et al. 2008). They attributed the differential decomposition rates to differences in the biochemistry of the birch and aspen leaf litter. Although, data on the biochemistry of aspen and birch wood were not determined in this study, earlier reports from Aspen FACE showed that C:N tended to increase under elevated CO₂ and lignin and extractives increased and decreased, respectively, under elevated O₃ in aspen. In birch, wood extractives increased under elevated CO₂ and elevated O₃ and C:N tended to decrease in all treatments (Kaakinen et al. 2004; Kostianen et al. 2008). The relatively high C:N and lignin may have contributed to the decreasing trends in percent wood density loss in aspen 271 under elevated CO₂ native placement in this study. The low C:N in birch may explain high percent wood density loss under elevated O₃ and elevated CO₂ + elevated O₃

and high extractives may have contributed to the lower percent wood density loss under elevated CO₂.

Compared to leaf tissue, wood is inherently poor in quality and typically decomposes much more slowly. Additionally, mean moisture content at the end of the experiment for aspen 271, 42 and birch were 72, 68 and 61% respectively (data not shown). These levels were still high enough to potentially discriminate against the primary agents of wood decay (wood-decaying fungi). Wood-decaying basidiomycetes fungi are typically intolerant to high moisture content, but micro-fungi (Ascomycetes) are tolerant to high levels of moisture (Eaton and Hale 1993). Ascomycetes and early basidiomycetes do not forage on cellulose but on labile sugars and therefore do not cause appreciable wood mass loss.

Although, elevated CO₂ and / or O₃ did not have direct significant impacts on wood decomposition, species / aspen clone exhibited significant effects on wood decomposition rates. At Aspen FACE, changes in forest community composition (relative proportions of birch, maple and aspen genotypes) have occurred due to differential growth response to elevated CO₂ and elevated O₃ (Kubiske et al. 2007). Hence because wood decomposition varies with species or genotype, it is possible that future high levels of elevated CO₂ or elevated O₃ will affect decomposition via changes in species or genotype composition of natural stands and changes in the total amount of woody detritus produced due to effects on stand productivity.

Conclusions

Fungal community composition of aspen and birch logs were significantly different. Independent of treatments, birch logs had a significantly higher rate of initial decomposition than aspen logs. However, growth or decomposition in elevated CO₂ and / or O₃ had no significant effects on fungal community composition or decomposition rates of aspen and birch logs, compared to the control. Nevertheless, because the growth of northern hardwood species responds to elevated CO₂ and / or O₃ differentially, it is reasoned that, future higher levels of atmospheric CO₂ and O₃ will impact fungal community and decomposition via shifts in species and / or genotype

composition of forests. The experiment was done in an open canopy increasing the chance that fungal spores may have been blown or circulated in the experimental area and contributed to our observations. Furthermore, fungal community development in temperate dead hardwoods occurs in successional stages (Frankland 1998; Boddy and Heilmann-Clausen 2008), with a variable suite of fungal species shifting over time (Eaton and Hale 1993; Boddy and Heilmann-Clausen 2008). The duration for this study was relatively short (one year), and most of the fungal species isolated were primary or secondary successional stage members (Boddy and Heilmann-Clausen 2008). It is therefore suggested that our observations should be interpreted with caution since we are not certain how a longer term and tertiary suite of fungal species would respond to elevated CO₂ and / O₃ fumigations.

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Figures



Figure 3-1 A fully harvested treatment ring (Left) and stump in the ring (Right)

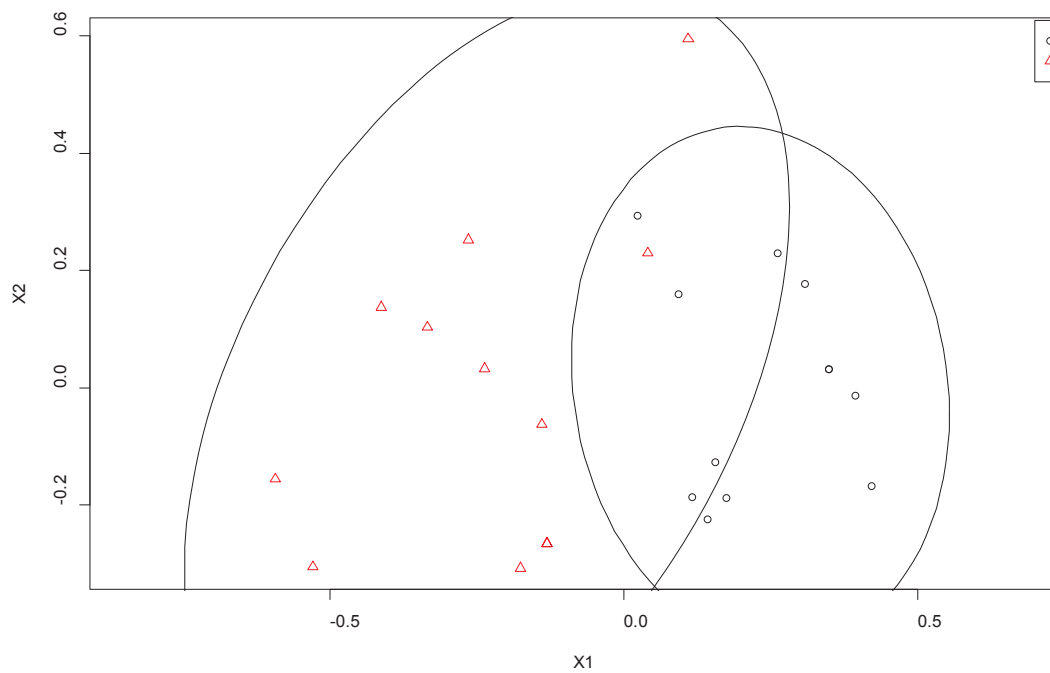


Figure 3-2 NMDS plot of wood-decaying basidiomycete fungal community composition, at Aspen FACE. Communities are displayed with respect to aspen logs and birch logs across all treatment rings and blocks. An ellipse is a 95% confidence level for each fungal community. The open circles and the red triangle represent aspen logs and birch logs respectively.

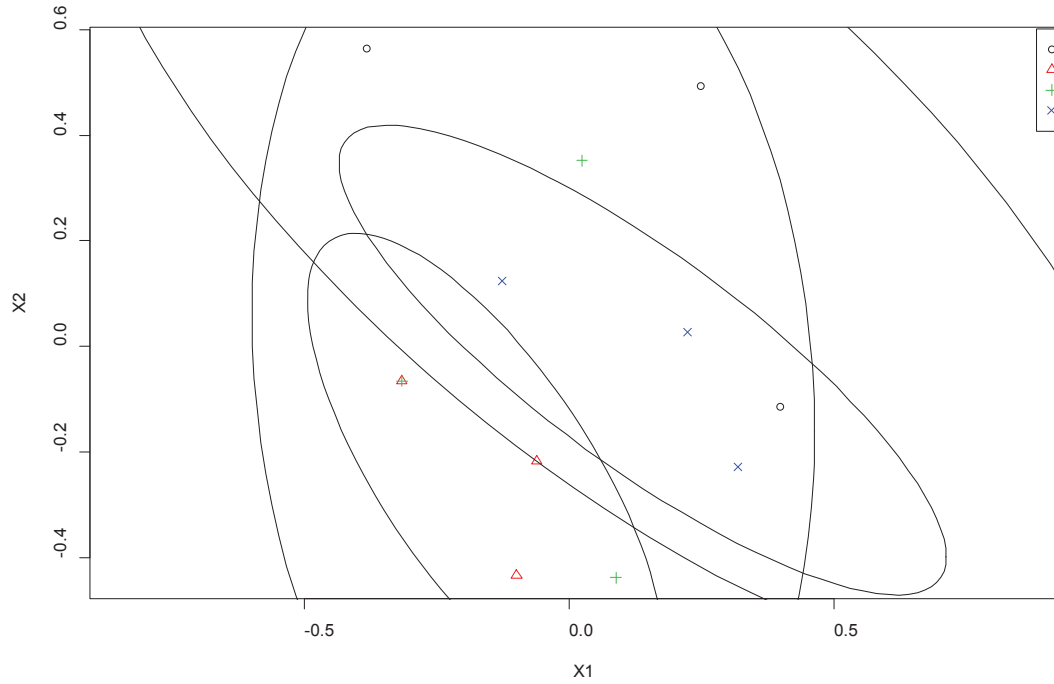


Figure 3-3 NMDS plot of wood-decaying basidiomycete fungal community composition for birch logs, at Aspen FACE. Communities are displayed with respect to ambient (open circle), elevated CO₂ (red triangle), elevated O₃ (green +) and elevated CO₂ + elevated O₃ (blue ×) across all treatment rings and blocks. An ellipse is a 95% confidence level for each fungal community.

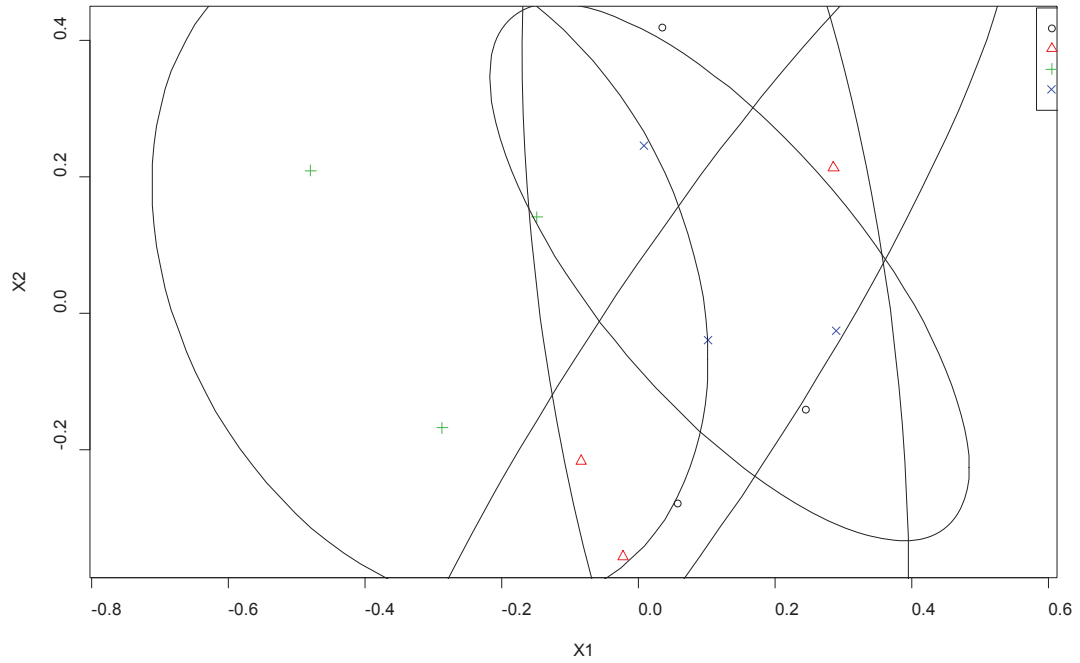


Figure 3-4 NMDS plot of wood-decaying basidiomycete fungal community composition for aspen logs, at Aspen FACE. Communities are displayed with respect to ambient (open circle), elevated CO₂ (red triangle), elevated O₃ (green +) and elevated CO₂ + elevated O₃ (blue ×) across all treatment rings and blocks. An ellipse is a 95% confidence level for each fungal community.

Tables

Table 3-1 Experimental design of the reciprocal transplanting of the 0.5 m log segments of aspen clones 42, 271 and birch produced or decomposing under elevated CO₂ and /or O₃. A total of 288 logs were deployed on the soil surface of the treatment rings. There were three replicates for each treatment factor. The plus symbol (+) indicates presence of two logs each for aspen 42, 271 and birch logs, one of which was removed for this study.

| Log Growth Source | Treatment Assigned for Log Decomposition | | | |
|---|--|--------------------------|-------------------------|---|
| | Ambient | Elevated CO ₂ | Elevated O ₃ | Elevated CO ₂ + O ₃ |
| Ambient | + | + | + | + |
| Elevated CO ₂ | + | + | + | + |
| Elevated O ₃ | + | + | + | + |
| Elevated CO ₂ + O ₃ | + | + | + | + |

Table 3-2 Taxonomic description of the isolated wood-decaying basidiomycete fungi. All isolated fungi were of the phylum Basidiomycota and class Agaricomycetes.

| <i>Fungus Species</i> | Genus | Family | Order |
|---------------------------------|------------------|------------------|----------------|
| <i>Bjerkandera adusta</i> | Bjerkandera | Meruliaceae | Polyporales |
| <i>Cerrena unicolor</i> | Cerrena | Polyporaceae | Polyporales |
| <i>Chondrostereum purpureum</i> | Chondrostereum | Cyphellaceae | Agaricales |
| <i>Cylindrobasidium laeve</i> | Cylindrobasidium | Physalacriaceae | Agaricales |
| <i>Irpex lacteus</i> | Irpex | Meruliaceae | Polyporales |
| <i>Peniophora aurantiaca</i> | Peniophora | Peniophoraceae | Russulales |
| <i>Peniophora sp</i> | Peniophora | Peniophoraceae | Russulales |
| <i>Schizophyllum commune</i> | Schizophyllum | Schizophyllaceae | Agaricales |
| <i>Sistotrema brinkmannii</i> | Sistotrema | Hydnaceae | Cantharellales |
| <i>Stereum rugosum</i> | Stereum | Stereaceae | Russulales |
| <i>Stereum sp.</i> | Stereum | Stereaceae | Russulales |
| <i>Trametes gibbosa</i> | Trametes | Polyporaceae | Polyporales |
| <i>Trametes versicolor</i> | Trametes | Polyporaceae | Polyporales |
| <i>Unidentified sp</i> | unknown | unknown | unknown |
| Total | 14 | 8 | 3 |

Table 3-3 Fungal species present (1) or absent (0) with respect to elevated CO₂ and / or O₃ treatments and log species.

| Fungal species | Aspen logs | | | | Birch logs | | | |
|---|-----------------|-----------------|----------------|-----------------|-----------------|-----------------|----------------|-----------------|
| | Treatments | | | | Treatments | | | |
| | Ambient | CO ₂ | O ₃ | CO ₂ | Ambient | CO ₂ | O ₃ | CO ₂ |
| | | | | + | | | | + |
| | | | | O ₃ | | | | O ₃ |
| <i>Bjerkandera adusta</i> | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| <i>Cerrena unicolor</i> | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| <i>Chondrostereum purpureum</i> | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| <i>Cylindrobasidium laeve</i> | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| <i>Irpex lacteus</i> | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 |
| <i>Peniophora aurantiaca</i> | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| <i>Peniophora sp</i> | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 |
| <i>Schizophyllum commune</i> | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Sistotrema brinkmannii</i> | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Stereum rugosum</i> | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| <i>Stereum sp.</i> | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| <i>Trametes gibbosa</i> | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 |
| <i>Trametes versicolor</i> | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 |
| <i>Unidentified sp</i> | 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 logs = 11 | | | | Birch logs = 13 | | | |
| Total number of isolated fungal species independent of treatments and wood species = 14 | | | | | | | | |

Table 3-4 PerMANOVA *P*-values for effects of wood species and elevated CO₂ and / or O₃ on wood-decaying basidiomycete fungal community composition. Highlighted *P*-value is significant (*P*-values ≤ 0.05).

| Parameter | Fungal community |
|--|------------------|
| Wood species | 0.0009 |
| <i>Aspen logs:-</i> | |
| Elevated CO ₂ and / or O ₃ | 0.1029 |
| <i>Birch logs:-</i> | |
| Elevated CO ₂ and / or O ₃ | 0.2567 |

Table 3-5 Mean density and percent density loss for birch in relation to log growth source and treatment environment assigned for log decomposition. Elevated CO₂ (= eCO₂); elevated O₃ (=eO₃). Values are mean (±1SE), n=3.

| Species | Log Source | Treatment | Density (Kg / m ³) | | | % Loss |
|---------|------------------------------------|------------------------------------|--------------------------------|--------------|-------------|------------|
| | | | Initial | Final | Loss | |
| Birch | Ambient | Ambient | 496.8 ± 11.5 | 430.9 ± 10.3 | 65.8 ± 1.2 | 13.3 ± 0.1 |
| | eCO ₂ | Ambient | 534.9 ± 7.4 | 480.2 ± 24 | 54.7 ± 31.4 | 10.1 ± 5.7 |
| | eO ₃ | Ambient | 534.4 ± 9.5 | 456.1 ± 20.8 | 78.3 ± 12.4 | 14.7 ± 2.6 |
| | eCO ₂ + eO ₃ | Ambient | 513.6 ± 16.3 | 432.7 ± 13.6 | 80.9 ± 12.4 | 15.7 ± 2.2 |
| | Ambient | eCO ₂ | 489.3 ± 10.3 | 425.7 ± 16 | 63.6 ± 5.7 | 13.1 ± 1.5 |
| | eCO ₂ | eCO ₂ | 528.9 ± 6.8 | 470 ± 1.5 | 58.9 ± 6.7 | 11.1 ± 1.1 |
| | eO ₃ | eCO ₂ | 524.6 ± 12.5 | 471.6 ± 17.6 | 53 ± 7.8 | 10.2 ± 1.7 |
| | eCO ₂ + eO ₃ | eCO ₂ | 511.9 ± 4.5 | 464.6 ± 6.3 | 47.3 ± 8.3 | 9.2 ± 1.6 |
| | Ambient | eO ₃ | 514.4 ± 5.4 | 442.2 ± 3.5 | 72.2 ± 7.4 | 14 ± 1.3 |
| | eCO ₂ | eO ₃ | 524.6 ± 12.3 | 465.7 ± 23.6 | 58.9 ± 11.7 | 11.3 ± 2.5 |
| | eO ₃ | eO ₃ | 526 ± 16.9 | 451.6 ± 10.8 | 74.5 ± 17.7 | 14 ± 3.1 |
| | eCO ₂ + eO ₃ | eO ₃ | 517.6 ± 3.1 | 449.4 ± 7.8 | 68.3 ± 7.7 | 13.2 ± 1.5 |
| | Ambient | eCO ₂ + eO ₃ | 501.2 ± 4.9 | 418.1 ± 26.7 | 83.1 ± 23.3 | 16.6 ± 4.8 |
| | eCO ₂ | eCO ₂ + eO ₃ | 507.8 ± 11.8 | 433.6 ± 22.8 | 74.1 ± 14.6 | 14.7 ± 3.1 |
| | eO ₃ | eCO ₂ + eO ₃ | 540.8 ± 15.5 | 469.4 ± 15.7 | 71.4 ± 1 | 13.2 ± 0.4 |
| | eCO ₂ + eO ₃ | eCO ₂ + eO ₃ | 523.3 ± 13.8 | 437.3 ± 17.7 | 86 ± 9.9 | 16.5 ± 2 |
| | Across Treatments Means | | | 518.1 ± 1.7 | 449.9 ± 4.7 | 68.2 ± 5.7 |

Table 3-6 Mean density and percent density loss for aspen 42 in relation to log growth source and treatment environment assigned for log decomposition. Elevated CO₂ (= eCO₂); elevated O₃ (=eO₃). Values are mean (±1SE), n=3.

| Species | Source | Treatment | Density (Kg / m ³) | | | |
|------------------------|-----------------------------------|-----------------------------------|--------------------------------|--------------|-------------|------------|
| | | | Initial | Final | Loss | % Loss |
| Aspen 42 | Ambient | Ambient | 345.5 ± 1.9 | 312.1 ± 11.1 | 33.4 ± 9.6 | 9.7 ± 2.8 |
| | eCO ₂ | Ambient | 379.4 ± 14 | 333.9 ± 36.1 | 45.5 ± 22.1 | 12.5 ± 6.1 |
| | eO ₃ | Ambient | 356.8 ± 10.3 | 318.9 ± 7.4 | 38 ± 2.9 | 10.6 ± 0.5 |
| | CO ₂ + eO ₃ | Ambient | 359.4 ± 12.1 | 315.4 ± 23.8 | 44 ± 11.8 | 12.5 ± 3.6 |
| | Ambient | eCO ₂ | 358.5 ± 8.7 | 307.5 ± 6.5 | 51 ± 14.3 | 14.1 ± 3.6 |
| | eCO ₂ | eCO ₂ | 354.7 ± 4.5 | 319.7 ± 9.5 | 35 ± 10.8 | 9.8 ± 3 |
| | eO ₃ | eCO ₂ | 366.3 ± 3.1 | 322.3 ± 1.9 | 44 ± 1.3 | 12 ± 0.3 |
| | CO ₂ + eO ₃ | eCO ₂ | 353.9 ± 4.8 | 322.6 ± 13.6 | 31.2 ± 8.7 | 8.9 ± 2.6 |
| | Ambient | eO ₃ | 349 ± 4.2 | 299 ± 6 | 50 ± 9.5 | 14.3 ± 2.5 |
| | eCO ₂ | eO ₃ | 345.3 ± 8.9 | 299.8 ± 8.6 | 45.5 ± 1.6 | 13.2 ± 0.5 |
| | eO ₃ | eO ₃ | 359.9 ± 8.5 | 322.2 ± 7.4 | 37.7 ± 8.6 | 10.4 ± 2.2 |
| | CO ₂ + eO ₃ | eO ₃ | 351.5 ± 2.5 | 322.3 ± 8.9 | 29.2 ± 8.7 | 8.3 ± 2.5 |
| | Ambient | CO ₂ + eO ₃ | 346.2 ± 3.8 | 314.2 ± 10.2 | 32 ± 8.5 | 9.3 ± 2.5 |
| | eCO ₂ | CO ₂ + eO ₃ | 359.3 ± 7.9 | 321.8 ± 3.4 | 37.5 ± 4.6 | 10.4 ± 1.1 |
| | eO ₃ | CO ₂ + eO ₃ | 374.2 ± 15.7 | 328 ± 15.6 | 46.2 ± 7.5 | 12.3 ± 1.9 |
| | CO ₂ + eO ₃ | CO ₂ + eO ₃ | 357.9 ± 11.9 | 325.1 ± 14.7 | 32.8 ± 6 | 9.2 ± 1.7 |
| Across Treatments Mean | | | 357.4 ± 2.2 | 317.8 ± 4.9 | 39.6 ± 2.8 | 11.1 ± 0.8 |

Table 3-7 Mean density and percent density loss for aspen 271 in relation to log growth source and treatment environment assigned for log decomposition. Elevated CO₂ (= eCO₂); elevated O₃ (=eO₃). Values are mean (±1SE), n=3.

| SPP | Source | Treatment | Density (Kg / m3) | | | |
|-------------------------|------------------------------------|------------------------------------|-------------------|--------------|-------------|------------|
| | | | Initial | Final | Loss | % Loss |
| Aspen | | | | | | |
| 271 | Ambient | Ambient | 413.2 ± 30.5 | 371.1 ± 28.8 | 42 ± 6.1 | 10.2 ± 1.5 |
| | eCO ₂ | Ambient | 400 ± 13.7 | 347.9 ± 12 | 52 ± 14 | 12.9 ± 3.3 |
| | eO ₃ | Ambient | 399.6 ± 3.9 | 374.4 ± 16.2 | 25.2 ± 12.3 | 6.4 ± 3.1 |
| | eCO ₂ + eO ₃ | Ambient | 391.8 ± 33.4 | 359.6 ± 22 | 32.3 ± 15 | 7.8 ± 3.1 |
| | Ambient | eCO ₂ | 412.4 ± 11.1 | 365.9 ± 28.4 | 46.5 ± 21.5 | 11.4 ± 5.2 |
| | eCO ₂ | eCO ₂ | 390.1 ± 11.2 | 386.4 ± 10.9 | 3.7 ± 1.9 | 0.9 ± 0.5 |
| | eO ₃ | eCO ₂ | 396.4 ± 3.5 | 359 ± 13 | 37.4 ± 9.8 | 9.5 ± 2.5 |
| | eCO ₂ + eO ₃ | eCO ₂ | 384.4 ± 19.1 | 351.3 ± 10.5 | 33.2 ± 10.3 | 8.4 ± 2.3 |
| | Ambient | eO ₃ | 403.8 ± 11.4 | 367.4 ± 13.9 | 36.5 ± 9.8 | 9 ± 2.3 |
| | eCO ₂ | eO ₃ | 376.3 ± 8.6 | 340.4 ± 17.7 | 35.8 ± 13.1 | 9.6 ± 3.5 |
| | eO ₃ | eO ₃ | 416.9 ± 25.6 | 376.2 ± 25.3 | 40.7 ± 4.4 | 9.8 ± 1.2 |
| | eCO ₂ + eO ₃ | eO ₃ | 392.7 ± 18 | 351.2 ± 18.6 | 41.5 ± 4.5 | 10.6 ± 1.2 |
| | Ambient | eCO ₂ + eO ₃ | 416.8 ± 11.3 | 384.3 ± 15.3 | 32.5 ± 9 | 7.8 ± 2.2 |
| | eCO ₂ | eCO ₂ + eO ₃ | 388.7 ± 16.8 | 360.5 ± 26.4 | 28.1 ± 9.9 | 7.5 ± 2.7 |
| | eO ₃ | eCO ₂ + eO ₃ | 401.7 ± 2.8 | 366 ± 18.2 | 35.7 ± 15.6 | 8.9 ± 3.9 |
| | eCO ₂ + eO ₃ | eCO ₂ + eO ₃ | 386.5 ± 9.7 | 359.3 ± 10.5 | 27.2 ± 9.4 | 7 ± 2.3 |
| Across Treatments Means | | | 398.2 ± 8.2 | 363.8 ± 7.9 | 34.4 ± 0.5 | 8.6 ± 0.1 |

Table 3-8 *P*-values for effects of elevated CO₂ and / or O₃ on percent wood density loss in birch and aspen clones 42, 271 (species). Elevated CO₂ (= eCO₂); elevated O₃ (=eO₃). Highlighted *P*-values are either significant (*P*-values ≤ 0.05) or marginally significant (*P*-values ≤ 0.10).

| Parameter | Percent wood density loss |
|---|---------------------------|
| eCO ₂ | 0.4192 |
| Species | 0.0334 |
| Source | 0.31115 |
| eCO ₂ × Species | 0.5257 |
| eCO ₂ × Source | 0.4345 |
| Species × Source | 0.9887 |
| eCO ₂ × Species × Source | 0.1019 |
| eO ₃ | 0.4280 |
| Species | 0.0929 |
| Source | 0.9391 |
| eO ₃ × Species | 0.9765 |
| eO ₃ × Source | 0.9147 |
| Species × Source | 0.6447 |
| eO ₃ × Species × Source | 0.5113 |
| eCO ₂ + eO ₃ | 0.9525 |
| Species | 0.0161 |
| Source | 0.9843 |
| eCO ₂ + eO ₃ × Species | 0.4933 |
| eCO ₂ + eO ₃ × Source | 0.9552 |
| Species × Source | 0.7490 |
| eCO ₂ + eO ₃ × Species × Source | 0.7702 |

CHAPTER 4: Effects of elevated CO₂ and O₃ on wood anatomical properties of trembling aspen, paper birch and sugar maple³

ABSTRACT

Physiological functions of woody plants and quality of wood are related to their anatomical properties. Anatomical properties of woody plants are influenced by the activity of the cambium and the growth characteristics of wood cells, which are in turn influenced by a range of environmental factors. Current background concentrations of CO₂ and O₃ are about 40% higher than during the pre-industrial era. The alterations in the background concentrations of atmospheric CO₂ and / or O₃ could impact wood anatomical structure and consequently impact wood quality. We evaluated the effects of 12 years of growth under elevated CO₂ and / or O₃ on the wood anatomical properties of birch (*Betula papyrifera* Marshall), sugar maple (*Acer saccharum* Marshall var. *saccharum*) and four aspen (*Populus tremuloides* Michx.) clones (8, 42, 216, and 271) at the Aspen Free Air CO₂ and O₃ enrichment (FACE) project near Rhinelander, WI, USA. Wood anatomical properties varied significantly with species, aspen genotype and stem radial position. Elevated CO₂ did not have statistically significant effects on wood anatomical properties, except that it marginally increased the number of vessels per square millimeter in aspen 271 and birch, compared to the control ($P = 0.0771$). Under elevated O₃, mean vessel lumen diameter decreased significantly in maple and marginally in birch compared to the control. Additionally, vessel lumen diameters were unaltered in all species and aspen genotypes at the inner and middle radial positions of growth rings, except for a significant decrease for maple in the middle radial position under elevated O₃, compared to ambient. However, vessel lumen diameter decreased significantly at the periphery of the growth ring in all species and clones, except for aspen 8, under elevated O₃. Vessel lumen diameter was also significantly narrower at the periphery than the middle and inner radial positions of growth rings, independent of treatments. As a result it is unclear if the reduction in vessel lumen diameter at

³ Manuscript, in progress

the peripheral radial position is solely due to elevated O₃ or not. The effects of elevated CO₂ and O₃, alone, on wood anatomical properties of aspen genotypes and birch were ameliorated when the gases were applied in combination. Based on the results, it is predicted that future higher levels of elevated CO₂ and / or O₃ concentrations could have moderate impacts on wood quality of northern hardwoods, but for utilization purposes these likely would not be considered significant.

INTRODUCTION

Wood quality and physiological function of woody plants are closely related to their anatomical properties (Dickison 2000). Anatomical properties of woody plants are influenced by the activity of the cambium and the growth characteristics of wood cells, which are in turn influenced by a range of environmental factors (Zobel and Buijtenen 1989; Dickison 2000; Barnett and Jeronimidis 2003). For example, soil nutrient enrichment stimulates woody plant growth and can result in less dense wood due to increased production of thin-walled cells with larger lumens (Zobel 1981; Zobel and Buijtenen 1989; Dickison 2000; Barnett and Jeronimidis 2003). The general body of evidence also indicates that elevated CO₂ has stimulating effects on photosynthesis, leading to increased biomass production (Ainsworth and Long 2005; Norby et al. 2005; Leakey et al. 2009; Dawes et al. 2011; Norby and Zak 2011). However, unlike elevated CO₂, elevated O₃ disrupts important physiological processes (Fuhrer and Booker 2003; Wittig et al. 2007; Wittig et al. 2009; Lindroth 2010; Street et al. 2011), injures woody plants (Karnosky et al. 1996; Karnosky et al. 2003; Karnosky et al. 2007) and induces an overall reduction in plant growth and productivity (Karnosky et al. 2007; Wittig et al. 2009). Hence changes in the environment due to the rising concentrations of atmospheric CO₂ and / or O₃ (IPCC 2007) could impact wood anatomical properties and consequently affect wood quality for its intended use (Ceulemans et al. 2002; Kostianinen et al. 2004; Kostianinen et al. 2006; Kostianinen et al. 2008; Kostianinen et al. 2009).

A number of investigations have been initiated to provide scientific understanding of the influence of rising concentrations of atmospheric CO₂ and / or O₃ on wood anatomical properties, helping assess future wood quality (Telewski et al. 1999; Kaakinen et al. 2004; Kostianen et al. 2004; Yazaki et al. 2005; Kostianen et al. 2006; Kostianen et al. 2008; Kostianen et al. 2009). Nevertheless, accumulating evidence has been divergent. For example, elevated CO₂ significantly decreased cell wall thickness and proportion of cell wall area and increased fiber lumen diameter and parenchyma proportions in three *Populus* species (Luo et al. 2005). Tracheid radial lumen diameter decreased in *Picea abies* L. grown under elevated CO₂ without nutrient enhancement (Kostianen et al. 2004). Kostianen et al. (2009) also observed a significant decrease in the cell wall thickness of earlywood and an increase in tracheid diameter of latewood in *Picea abies* grown under elevated CO₂. Similarly, tracheid walls and the proportion of resin canals decreased; and tracheid lumen increased in *Pinus sylvestris* L. grown under elevated CO₂ compared to the control (Ceulemans et al. 2002). Vessel frequency and diameter increased in *Quercus robur* L., and cell wall thickness increased in *Prunus avium* L. grown under elevated CO₂ (Atkinson and Taylor 1996). Under elevated CO₂, growth ring width increased in *Picea abies* L. (Kostianen et al. 2004); *Betula pendula* Roth (Kostianen et al. 2006); *Pinus sylvestris* L. (Ceulemans et al. 2002); *Pinus taeda* L. (Telewski et al. 1999); and *Populus tremuloides* Michx. (Kaakinen et al. 2004; Kostianen et al. 2008).

In contrast to the aforementioned reports, elevated CO₂ did not have a significant effect on vessel and cell wall area proportions, vessel lumen diameter, fiber lumen diameter and fiber wall thickness in *Populus tremuloides* (Kaakinen et al. 2004; Kostianen et al. 2008). Wood anatomical properties responsible for water transport in *Quercus mongolica* and *Alnus hirsute* were also not affected by elevated CO₂ (Watanabe et al. 2008). Similarly, fiber length, vessel length, vessel lumen diameter, vessel area and cell wall area proportions in two clones of *Betula pendula* Roth were not affected by elevated CO₂, but clone identity had significant effects (Kostianen et al. 2006). Cell wall thickness of *Picea abies* (L.) Karst. grown under elevated CO₂ was not significantly different from the ambient (Kostianen et al. 2004). Likewise, Telewski et al.

(1999) reported that the proportion of resin canals and cell wall:lumen area of *Pinus taeda* L. were not influenced by elevated CO₂. Elevated CO₂ also did affect the wood anatomy of *Larix kaempferi* and *Larix sibirica* seedlings (Yazaki et al. 2001; Yazaki et al. 2004).

A review of literature indicated that the response of wood anatomical properties of trees to elevated CO₂ could be species, clone, environment and age dependent (Yazaki et al. 2005). A more recent study by Watanabe et al. (2010) observed that the response of anatomical properties to elevated CO₂ was species dependent. They observed that elevated CO₂ did not affect vessel anatomy of *Quercus mongolica*, *Betula maximowicziana* and *Acer mono* but significantly modified the vessel properties and cambial activity of *Kalopanax septemlobus*. Luo et al. (2005) also observed elevated CO₂ × genotype interactions, but Kaakinen et al. (2004) observed no such interactions. However, variation in anatomical properties existed in different clones of *Populus tremuloides* (Kaakinen et al. 2004; Kostianen et al. 2008).

The impact of elevated O₃ concentrations on wood anatomical properties has also been variable. Kurczynska et al. (1998) studied the effects of elevated O₃ and soil nitrogen content on wood anatomical properties of *Picea abies* saplings in open top chambers. They observed that tracheid frequency decreased and latewood tracheid diameter increased in the wood of *Picea abies* produced under elevated O₃ on nitrogen enriched soils compared to the ambient environment. Independent of nitrogen level of the soil, sieve cell wall thickness increased under elevated O₃, but sieve cell frequency and latewood tracheid diameter also decreased under elevated O₃ in non N-enriched soil. Likewise, Kaakinen et al. (2004) observed a significant reduction in the vessel lumen diameter and an increase in cell wall thickness in *Populus tremuloides* after 3 years of exposure, but after 5 years of exposure for the same species, anatomical properties were unaffected except for a slight decrease in vessel lumen diameter (Kostianen et al. 2008). A significant reduction in vessel proportion and increased cell wall proportion in *Betula pendula* Roth (silver birch) due to elevated O₃ have been reported (Kostianen et al. 2006). Significant interactions of elevated O₃ with genotype on some anatomical properties have been observed (Kaakinen et al. 2004; Kostianen et al. 2006; Kostianen et al. 2008). Furthermore, reports of

combined impacts of elevated CO₂ and O₃ on wood structure are rare and have been shown to be insignificant (Kaakinen et al. 2004; Kostianen et al. 2006; Kostianen et al. 2008).

Although evidence on the effects of elevated CO₂ and / or O₃ on wood anatomical properties is growing, a large number of the studies were conducted on saplings and seedlings in growth chambers and greenhouses exposed to the gases for relatively short durations (Telewski et al. 1999; Yazaki et al. 2001; Ceulemans et al. 2002; Kostianen et al. 2004; Yazaki et al. 2004; Kostianen et al. 2006; Watanabe et al. 2008; Kostianen et al. 2009; Watanabe et al. 2010).

Additionally, those conducted in FACE experiments were conducted on immature trees (Kaakinen et al. 2004; Luo et al. 2005; Kostianen et al. 2008). Wood anatomical properties exhibit significant variation with respect to age, provenance, species and position along the radial and axial directions of the wood (Zobel 1981; Zobel and Buijtenen 1989; Dickison 2000; Barnett and Jeronimidis 2003; Yeh et al. 2006; Wheeler et al. 2007). Nevertheless, the investigations on the effects of elevated CO₂ and /or O₃ were typically done on the lower portions of woody stems (Kaakinen et al. 2004; Kostianen et al. 2004; Kostianen et al. 2006; Kostianen et al. 2008; Kostianen et al. 2009). The divergent reports suggest that further investigations with a different approach are necessary to improve understanding the effects of elevated CO₂ and / or O₃ on anatomy and quality of wood. We therefore investigated the effects of elevated CO₂ and / or O₃ on the wood anatomical properties of the upper portions of stems of birch, sugar maple and aspen clones 8, 42, 216 and 271 after twelve years of growth at the Aspen FACE site.

The Aspen FACE project provides an *in vivo* field approach for further investigations on the effects of elevated CO₂ and / or O₃ on wood anatomical properties. Aspen FACE is located at Harshaw, near Rhinelander, WI. It was a unique, long term experiment evaluating the impact of elevated CO₂ and O₃ and their interactions on northern forest ecosystems on a large scale. Measurements from the 12-year-long Aspen FACE experiment have shown a persistent 26% increase in net primary productivity (NPP) under elevated CO₂; the aspen clones community alone increased by 24-35% during the 10th to 12th years (Zak et al. 2011). However, growth of the individual tree species responded differently to elevated CO₂ fumigation (Isebrands et al.

2001; King et al. 2005; Kubiske et al. 2007). The differences in enhanced growth were attributed to the belowground competitive advantage of aspen clones 271 and 42 over other congeners for growth limiting nitrogen (Zak et al. 2007a; Zak et al. 2012). This observation parallels accruing evidence from Aspen FACE, that growth responses under elevated CO₂ differed among the aspen clones (8, 42, 216, 259 and 271) (Isebrands et al. 2001; Karnosky et al. 2003; King et al. 2005; Kubiske et al. 2007; Norby and Zak 2011; Zak et al. 2011; Zak et al. 2012). Aspen clones 271 and 42 grew faster than their congeners under elevated CO₂ (Isebrands et al. 2001; Kubiske et al. 2007), with the strongest response occurring for clone 271 (Kubiske et al. 2007). The growth of sugar maple under elevated CO₂, independent of O₃ concentration, was not different from the ambient (Kubiske et al. 2007), but the diameter growth of birch was significantly enhanced under elevated CO₂ (Kubiske et al. 2007).

Aspen 271 was reported to have the largest fiber lumen diameter among the clones under elevated CO₂ (Kaakinen et al. 2004). Data pooled for all the aspen clones showed that fiber lumen tended to increase under elevated CO₂ after 3 and 5 years of exposure at Aspen FACE (Kaakinen et al. 2004; Kostianen et al. 2008). Similarly, vessel diameter tended to increase under elevated CO₂ (Kostianen et al. 2008). Additionally after 5 years of exposure to elevated CO₂, aspen 271 was reported have reduced uronic acid (a constituent of hemicellulose) and a significant increase in starch content (Kostianen et al. 2008). Although diameter growth of birch and sugar maple under elevated O₃ was not significantly different from the ambient, elevated O₃ significantly reduced diameter growth of all the aspen clones at the end of 3rd and 7th years with the exception of the less ozone sensitive clone 8, for which diameter growth increased (Isebrands et al. 2001; Kubiske et al. 2007). Kaakinen et al. (2004) and Kostianen et al. (2008) also observed a reduction in radial growth and growth rings of the aspen clones at the end of the 3rd and 5th year of elevated O₃ fumigation. They reported a decrease in cell lumen area and an increase in cell wall area in aspen clones grown under elevated O₃ (Kaakinen et al. 2004; Kostianen et al. 2008).

Previous investigations at the Aspen FACE site, observed no effect of the combined elevated O₃ and elevated CO₂ treatments on growth parameters of birch, sugar maple and aspen clones (Isebrands et al. 2001; Kubiske et al. 2007; Zak et al. 2011; Zak et al. 2012) or on anatomical structure of the five aspen clones, 8 42, 259, 261 and 271 (Kaakinen et al. 2004; Kostianen et al. 2008). Based on the accruing evidences from Aspen FACE and other sources of information it was hypothesized that: 1) wood species / clonal effects would be observed in the wood anatomical properties of birch, sugar maple, aspen clones 8, 42, 216 and 271; 2) wood anatomical properties of birch, sugar maple and aspen clones would be altered under elevated CO₂ and elevated O₃ – specifically, the growth ring width, fiber length, fiber lumen diameter, vessel lumen diameter, vessel lumen area proportions and number of vessels per mm² (vessel frequency) would increase and cell wall area proportions would decrease under elevated CO₂, and the converse will hold for elevated O₃, except for clone 8, which is less sensitive to O₃, which would exhibit anatomical properties similar to the control; and 3) the combined treatment (elevated CO₂ + O₃) would have no effect on anatomical properties of birch, sugar maple and aspen clones 8, 42, 216 and 271.

MATERIALS AND METHODS

Site description

Samples for this research were obtained from the Aspen FACE study during the summer of 2009 (for a detailed description of site and experimental design of Aspen FACE see Dickson et al. (2000)). Aspen FACE research was conducted on a 32 ha USDA Forest Service Experimental Farm at Harshaw, near Rhinelander, in Wisconsin, USA (longitude 45.6° N, latitude 89.5° W). Potatoes and small grains were cultivated on the site for more than 50 years before the Forest Service acquired the land in 1972 to serve as a forest research station. Prior to the Aspen FACE research in 1997, the site was planted with poplar clones and larch. However, all the poplar clones and larch were cleared and stumps removed in 1996. The study site is nearly flat and the soil type is sandy loam. A thorough soil analyses was done for all treatment plots in 1997 and no

significant differences were observed, except for mean percent carbon and nitrogen, which were significantly greater in CO₂ rings than CO₂ + O₃ rings (Dickson et al. 2000).

The Aspen FACE experiment was a 2² factorial randomized complete block design with each treatment level replicated three times, once each in the northern, central and southern portions of the site. The main and crossed factors were carbon dioxide (CO₂), ozone (O₃) and CO₂+O₃ respectively. The treatment levels were ambient CO₂ and O₃ as the controls, elevated CO₂ (ambient + 200 ppm), and elevated O₃ (1.5 × ambient). The treatments were applied in twelve 30-m diameter rings, located at least 100 m apart. Each ring was partitioned into east and west sections. The eastern portion was planted with five *Populus tremuloides* Michx. (aspen) clones (8L, 42E, 216, 259 and 271) in random order, with a planting spacing of 1 m × 1 m. The western portion was further divided into north and south subplots. The northwest and southwest subplots were mixed plantations of aspen clone 216, with *Acer saccharum* Marshall var. *saccharum* (sugar maple) and *Betula papyrifera* Marshall (paper birch) respectively. All planting was completed in 1997, and exposure of treatment rings to elevated CO₂ and O₃ was done during the growing seasons of 1998 through 2009 between 0700 hrs and 1700 hrs each day when foliage was not wet.

Sampling and laboratory analysis

For this study, two trees each of 12-year-old sugar maple, paper birch and four aspen clones (8L, 42E, 216, and 271) were randomly sampled from each of the 12 rings. All the aspen clones were sampled from the eastern portion of the rings, while maple and paper birch were sampled from the northwestern and southwester sectors, respectively. Total height and diameter at breast height for all sampled trees were measured. A table saw was used to cut 4-cm thick discs at the midpoint of the 2004 height growth increment for anatomical analysis. From these, 2-cm thick disc subsamples were prepared using a band saw. These samples were softened and preserved in vials containing ethanol and glycerol (1:1) and transported to Michigan Technological University for anatomical analysis.

About five transverse sections of thickness 15-25 μm were cut from each of the 2-cm disc subsamples using a sliding microtome (Reichert-Jung, Heidelberg, Germany). Half to full cross sections were made depending on the diameter of the disc. The cut sections were first washed in distilled water and then stained in 1% safranin in 50% ethanol solution for about 10-20 minutes for contrast enhancement. After staining, they were rinsed in distilled water and dehydrated in increasing concentrations of ethanol: 30, 50, 70, 85, 90, 100 and 100 %, and later mounted in Canada balsam. All prepared slides were then dried at 60°C overnight. Splits of matchstick size were also taken from the 2-cm disk subsamples and kept in separate vials containing mixtures of 6% hydrogen peroxide and 97% acetic acid (1:1). To obtain a complete maceration, the specimens were incubated at 60°C for about 24 hours. Each macerated specimen was thoroughly rinsed in distilled water. Portions of the macerated specimen were teased with a pin and mounted temporarily in dilute glycerol.

Microphotographs were made from both permanent transverse sections and temporary macerated slides of the 2008 growth ring with a Leica digital camera attached to a Leica compound microscope. Anatomical properties analysis was done on the microphotographs using ImageJ software (National Institute of Health, Bethesda, MD, USA). Ten 3281 \times 2461 μm size images at 20 x magnifications at 2048 \times 1536 pixels resolution were captured randomly from each macerated specimen for fiber length measurements. About 100 - 120 straight fibers were measured per sample. Five 516 \times 387 μm size images at 100 x magnification at 1600 \times 1200 pixels resolution were also captured randomly from the mid portion of the growth ring for vessel frequency (number of vessels per mm^2) estimation. Vessel lumen diameter was determined for the growth ring at three different radial positions (inner, middle and periphery) on 3282 \times 2461 μm size images at 20 x magnification at 2048 \times 1536 pixels resolution. Additionally, three 328 \times 246 μm size images at 200 x magnification at 2048 \times 1536 pixels resolution were taken randomly from the mid portion of each growth ring for measurement of fiber lumen diameter and fiber lumen area, vessel lumen area, and ray parenchyma area proportions. Cell wall area proportion per unit area (CWA %) were then estimated from the equation $\text{CWA \%} = 100 - (\text{ray parenchyma area \%} +$

vessel lumen area % + fiber lumen area %) by Luo et al. (2005). Growth ring width measurements were done at three different positions on 16760 × 12570 μm size images at 15 × magnifications at 3264 × 2448 pixels resolution.

Analysis of data

Split-plot ANOVA (Montgomery 2009) was used to analyze all the wood anatomical parameters using the GLM procedure of SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA). The fixed main treatment factors of CO₂ (ambient vs. elevated CO₂); O₃ (ambient and elevated O₃), CO₂ + O₃ (ambient vs. elevated CO₂ + elevated O₃) were considered as the whole plot factor and species / aspen genotypes (birch, maple, aspen clones 8, 42, 216 and 271) as the sub-plot factor. There were three replicate rings / blocks along the north, central and southern regions of the experimental site. The analyses were carried out separately for each main treatment factor on all species / aspen clones. The error term for testing for each main treatment effect was block × main treatment with 2 degrees of freedom. The error term for testing for block, species / aspen genotypes and species / aspen genotypes × main treatment effects was the total error term of the split-plot ANOVA model (block × species / aspen genotype-nested-within-treatment) with 20 degrees of freedom (Appendix tables 4-2 to 4-6). When significant interactions were detected between species and any of the main treatment factors (elevated CO₂, elevated O₃ or elevated CO₂ + elevated O₃), a separate one-way ANOVA analysis was done for each species / aspen clone to identify the species or aspen clone which exhibited statistically significant main treatment factor effects (Appendix tables 4-8 to 4-13). Additionally, because vessel lumen diameter was sampled from three different radial positions of the growthring (inner, middle and periphery), a split-split-plot ANOVA was employed for analyzing the effects of main treatments, species and radial positions. In this case, the radial position of the vessel lumen data was considered as the sub-sub-plot. Hence the error term for testing for block and main treatment effects was block × treatment with 2 degrees of freedom. The error term for testing for species / aspen genotypes, and species / aspen genotypes × treatment effects was block × species / aspen genotype-nested-within-treatment with 20 degrees of freedom. The error term for testing the effects of radial

position on vessel lumen diameter and its interaction with species / aspen genotypes and /or treatment was the total error term of the split-split-plot ANOVA model with 48 degrees of freedom (Appendix tables 4-3, 4-5 and 4-7).

Descriptive statistics (means and standard errors) were computed for each parameter. In addition, data were examined for the normality and homogeneity of variance assumptions of analysis of variance before analysis was done. Data in percentages were arcsine transformed before the split-plot analysis was implemented. Tukey's post-hoc test was used to compare dependent variable means among all treatments. The effect of a factor was considered significant at $P = \leq 0.05$.

RESULTS

Species / genotypic effects

Anatomical properties varied significantly among species and / or genotypes (Table 4-1). Among the four aspen clones, 216 had the lowest mean growth ring width of 2.3 ± 0.3 mm (Table 4-1). Mean fiber length of the four aspen clones were not statistically different from each other. Mean fiber lumen diameter of aspen 271 was statistically different from aspen 8, 42 and 216. The mean vessel area proportion in birch and maple were similar but significantly less than the four aspen clones. Likewise mean vessel proportions in aspen 216 and 271 were similar, but significantly lower than in aspen 8 and 42, which also were similar (Table 4-1). Mean vessel diameter was widest in aspen 8 (55.8 ± 1.2 μm) and narrowest in aspen 216 (44.9 ± 1.1 μm), which was significantly different from aspens 42 and 271. Additionally, vessel lumen diameter varied along the radial position within a growth ring and was significantly narrower at the periphery than the middle and inner radial positions of the 2008 growth ring ($P = < 0.0001$, Fig. 4-2). Aspen 216 and 271 had the highest and lowest mean number of vessels per square millimeter, with 154 ± 7 and 129 ± 4 , respectively. Mean cell wall area proportion also varied significantly among species and aspen genotypes (Table 4-1).

Effects of elevated CO₂

Compared to ambient conditions, elevated CO₂ did not have statistically significant effects on mean growth ring width, mean fiber length, mean fiber lumen diameter, mean vessel area proportion, mean vessel lumen diameter, or mean cell wall area proportion in all species and aspen genotypes. Nonetheless, elevated CO₂ had a marginally significant effect on mean number of vessels per square millimeter ($p = 0.0771$; Table 4-2). The number of vessels per square millimeter tended to increase in aspen 8, 216, 271 and birch but not in aspen 42 and maple under elevated CO₂. There were no significant two-way-interactions between elevated CO₂ and species / aspen genotypes nor elevated CO₂ and radial position of vessel lumen diameter for all anatomical parameters (Table 4-2; Table 4-3). Additionally, no significant three-way-interaction was observed amongst elevated CO₂, species / aspen genotypes and radial position of vessel lumen diameter (Table 4-3). Elevated CO₂ did not significantly affect vessel lumen diameter sampled at the inner, middle or periphery radial positions of the growth ring, compared to the control (Table 4-3).

Effects of elevated O₃

The effects of elevated O₃ on wood anatomical properties were species or genotype and radial position dependent (Tables 4-4 and 4-5). Compared to ambient conditions, elevated O₃ did not have significant effects on mean growth ring width, mean fiber length, mean fiber lumen diameter and mean vessels per square millimeter (Table 4-4). However, significant or marginally significant two-way-interactions between elevated O₃ and species / aspen genotypes were observed in mean growth ring width ($P = 0.0499$), mean vessel area proportion ($P = 0.088$), mean vessel lumen diameter ($P = 0.0551$), and mean cell wall area proportion ($P = 0.0427$). The observed interactions were a result of mean growth ring width tending to increase under elevated O₃ in aspen 8 and 42 but decrease in aspen 216 and birch, with no effect on aspen 271 and maple. Mean vessel lumen area proportion tended to decrease in aspen 8, 42 and maple but increased in 216, 271 and birch under elevated O₃. Mean vessel lumen diameter tended to increase in aspen 8 but decrease slightly in aspen 42, 216, 271, birch and maple. Mean cell wall

area proportion tended to increase in aspen 8 and 42, decrease in aspen 216, 271 and birch, and be unaffected in maple under elevated O₃.

A one-way ANOVA performed separately for each species / aspen genotype (Appendix tables 4-8 to 4-12) showed that the effects of elevated O₃ on mean growth ring width was not significant for any species / aspen genotype ($P > 0.05$) but was marginally significant for mean vessel lumen area proportion in aspen 8 ($P = 0.0758$); for mean cell wall area proportion in aspen 271 ($P = 0.0535$); and for mean vessel lumen diameter in birch ($P = 0.0985$), and significant in maple for vessel lumen diameter ($P = 0.0241$), independent of radial position. In addition, the effects of elevated O₃ on vessel lumen diameter were radial position dependent, resulting in a significant interaction between radial position and elevated O₃ ($P = 0.0110$).

The interactive effect between radial position and elevated O₃ on vessel lumen diameter was because the impact of elevated O₃ on vessel lumen diameter was significant at the periphery ($P = 0.0026$) and non-significant at the inner ($P = 0.5485$) and middle ($P = 0.1304$) radial positions. A significant interaction between elevated O₃ and species / aspen genotypes was also detected for vessel lumen diameter ($P = 0.0232$) sampled from the middle radial position only. This was the result of vessel lumen diameter sampled from the middle radial position tending to increase in aspen 8 ($P = 0.4736$) and decrease for all other species and clones, significantly so for maple ($P = 0.0127$). There were no significant 3-way-interactions among elevated O₃, species / aspen genotypes and radial position for vessel lumen diameter (Table 4-5).

Effects of elevated CO₂ + O₃

Combined elevated CO₂ + elevated O₃ did not produce any statistically significant impact on growth ring width, fiber length, fiber lumen diameter, vessel area proportion, vessel lumen diameter, number of vessels per square millimeter, or cell wall area proportion in any species or aspen genotype, compared to ambient CO₂ + O₃ (Table 4-6). However, there was a marginally significant interaction between the combined elevated CO₂ + O₃ and species / aspen genotype for mean vessel lumen area proportion ($P = 0.0724$; Table 4-6). The interaction was the result of

mean vessel lumen area proportion tending to increase in aspen 216 and 271 and decrease in aspen 8, 42, birch and maple. The effect of combined elevated CO₂ + elevated O₃ on vessel lumen diameter did not differ significantly from ambient CO₂ + O₃ when sampling was done at inner, middle or periphery radial positions for all species / aspen genotypes (Table 4-7). Nevertheless, combined elevated CO₂ + elevated O₃ tended to increase vessel lumen diameter in aspen 8 and tended to decrease it in aspen 42, 216, 271, birch and maple, more at the middle and periphery than the inner radial positions. As a result there was a significant interaction between combined elevated CO₂ + elevated O₃ and radial position of vessel lumen diameter ($P = 0.0463$). Interaction between combined CO₂ + O₃ and species / aspen genotype were not significant ($P = 0.4622$), and those among combined CO₂ + O₃ and species / aspen genotype and radial position for vessel lumen diameter also were not significant.

DISCUSSION

In this study it was predicted that: (1) wood species /clonal effects would be observed in the wood anatomical properties of birch, sugar maple, aspen clones 8, 42, 216 and 271; (2) wood anatomical properties of birch, sugar maple and aspen clones would be altered under elevated CO₂ and elevated O₃, and under elevated O₃, aspen clone 8 which is less sensitive to O₃, would exhibit anatomical properties similar to the control; (3) the combined treatment (elevated CO₂ + elevated and O₃) would have no statistically significant effect on anatomical properties of birch, sugar maple and aspen clones 8, 42, 216 and 271. Results of this study supported hypotheses 1 and 3 and only occasionally portions of 2.

Species and genotypic effects

Species and aspen genotypes differed significantly in almost all anatomical properties investigated. Maple had significantly narrower growth ring width, vessel and fiber lumen diameters, shorter fiber length, lower vessel lumen area proportion, lower vessel frequency and greater cell wall area than aspen and birch. Birch had significantly longer fibers than aspen. Aspen had wider growth ring width, fiber lumen diameter, greater vessel area proportion and

vessel lumen diameter, higher vessels per square millimeter and lower cell wall area than birch and maple. These differences in the anatomical properties of aspen, birch and maple are consistent with existing reports (Panshin and Zeeuw 1980; Wheeler et al. 2007; Wheeler 2011). Differences in anatomical properties were also very conspicuous among aspen genotypes. The widest and narrowest growth ring widths were observed in aspen 42 and 216, respectively. But the differences in growth ring width of aspen 271, 42, and 8 were not statistically significant. Although slight differences were observed in the fiber length of the aspen genotypes, they were not significant. Aspen 271 had a significantly wider fiber lumen diameter than aspen genotypes 8, 42 and 216. Vessel lumen area proportion was significantly higher in aspen 8 and 42 than aspen 216 and 271. Aspen 216 had a narrower vessel lumen diameter and a greater number of vessels per square millimeter than the other aspen genotypes. Aspen 8 had a significantly lower fiber cell wall proportion than clones 42, 216 and 271. However, cell wall proportions in aspen 216 and 271 were significantly greater than in aspen 42. The observed anatomical properties differences in aspen genotypes are in agreement with earlier findings from Aspen FACE (Kaakinen et al. 2004; Kostianinen et al. 2008). For example, Kaakinen et al. (2004) observed the widest fiber lumen diameter in aspen 271 and narrowest fiber lumen and vessel lumen diameter, lowest vessel proportion and highest cell wall area proportion in clone 216. Differential growth and physiological patterns by species and aspen genotypes have also been observed at Aspen FACE (Isebrands et al. 2001; Karnosky et al. 2003; King et al. 2005; Kubiske et al. 2007; Norby and Zak 2011; Zak et al. 2011; Zak et al. 2012).

In addition to species and genotypic differences, vessel lumen diameter was narrower at the periphery than the inner and middle radial positions of the growth ring. Variations in the wood anatomical properties with respect to species / genotypes and positions along radial and axial directions have long been recognized (Panshin and Zeeuw 1980; Zobel and Buijtenen 1989; Zobel and Jett 1995; Dickison 2000; Barnett and Jeronimidis 2003; Wheeler et al. 2007)

Effects of elevated CO₂

The results of the current study showed no significant effects of elevated CO₂ on wood anatomical properties investigated with the exception of a nearly significant effect of elevated CO₂ on number of vessels per square millimeter in relation to the ambient treatment. In addition, vessel lumen diameter was not affected by elevated CO₂ at the inner, middle and periphery radial positions of the growth ring, compared to the control, implying that effects of elevated CO₂ on vessel lumen diameter are not radial position dependent. The current observations are consistent with earlier reports (Kaakinen et al. 2004; Kostianen et al. 2008), which indicated no significant effects of elevated CO₂ on the wood anatomical properties of the same species and aspen genotypes at ages 3 and 5 at the Aspen FACE research site on samples taken from breast height (1.37 m) on the main stem. Growing evidence also indicates no significant effects of elevated CO₂ on wood anatomical properties for other wood species. For example, fiber and vessel length, vessel lumen diameter, vessel area and cell wall proportions in two clones of *Betula pendula* were not affected by elevated CO₂ (Kostianen et al. 2006). Cell wall thickness of *Picea abies* (L.) Karst. grown under elevated CO₂ was not significantly different from the control (Kostianen et al. 2004). Likewise, Telewski et al. (1999) reported that cell wall:lumen area of *Pinus taeda* L. was not influenced by elevated CO₂. Elevated CO₂ also did not have effects on the wood anatomy of *Larix kaempferi* and *Larix sibirica* seedlings (Yazaki et al. 2001; Yazaki et al. 2004).

Although, elevated CO₂ did not have significant effects on wood anatomical properties in the current study, there was a nearly significant effect of elevated CO₂ for number of vessels per square millimeter. Specifically, number of vessels per square millimeter tended to increase in aspen 8, 216, 271, and birch but was unaltered in aspen 42 and maple under elevated CO₂, compared to the control. In line with the current study, the number of vessels per square millimeter also increased in *Quercus robur* L. grown under elevated CO₂ compared to the control (Atkinson and Taylor 1996). At Aspen FACE elevated CO₂ has been reported to increase diameter growth compared to the ambient treatment in aspen genotypes and birch (Isebrands et

al. 2001; Kaakinen et al. 2004; Kubiske et al. 2007; Kostianen et al. 2008). In addition net primary productivity increased by 26% persistently for about a decade under elevated CO₂. In this study, cell dimensions and cell wall area proportions were not significantly influenced by elevated CO₂, but marginal increases occurred for number of vessels per square millimeter in aspen genotypes and birch. It is therefore reasoned that the increase in growth parameters at Aspen FACE may have been due to increases in the number of cells of xylem tissues via rapid and prolonged cambial activity, rather than cell wall deposition and expansion (Yazaki et al. 2005). Secondly, xylem structure is determined by the activity of cambium and the developmental characteristics of wood cells, which are in turn influenced by environmental factors such as elevated CO₂. Elevated CO₂ can affect wood structure and dimensions via duration and rate of cell division by cambium, cell expansion or cell wall deposition (Yazaki et al. 2005). Longer duration of rapid cell division, coupled with absence of cell wall deposition and / or cell expansion under elevated CO₂ may increase radial growth but result in a decrease of wood density (Yazaki et al. 2005) due to increased void space (Barnett and Jeronimidis 2003).

Effects of elevated O₃

The effects of elevated O₃ on wood anatomical properties varied with species and radial position. Elevated O₃ in the presence of ambient CO₂ had no effect on mean fiber length, mean fiber lumen diameter and mean number of vessels per square millimeter for any species or aspen genotype, compared to the control. At Aspen FACE, elevated O₃ significantly increased cell wall proportion and decreased fiber lumen and vessel lumen diameter of aspen after 3 years of fumigation (Kaakinen et al. 2004), but the effects diminished after five years of fumigation (Kostianen et al. 2008). Similarly, elevated O₃ had no effect on vessel lumen diameter, vessel and fiber length and growth ring width but had significant and opposing effects on cell wall and vessel lumen area proportions in two silver birch clones (Kostianen et al. 2006).

Although not statistically significant, mean growth ring width tended to increase in aspen 8, decrease in aspen 216 and birch and be unaffected in aspen 42 and 271 and maple, resulting in a significant interaction between elevated O₃ and species and aspen genotype. Growth ring

width has a strong positive correlation with tree volume growth. Therefore the tendency for the mean growth ring width to increase in aspen 8; decrease in 216 and birch and be unaffected in aspen 42, 271 and maple under elevated O_3 implies growth parameters for aspen 8 were stimulated and those of birch and aspen 216 were dampened whereas maple, aspen 42 and 271 were unaltered under elevated O_3 .

In line with this reasoning, diameter growth was reported to decrease in aspen 42, 216, 271, and birch but increase in aspen 8 and be unaffected in maple under elevated O_3 , compared to the control in earlier studies at the Aspen FACE (Isebrands et al. 2001; Kubiske et al. 2007).

Furthermore, diameter from pith to bark and growth ring width tended to decrease in aspen after three and five years of elevated O_3 fumigation at Aspen FACE, respectively (Kaakinen et al. 2004; Kostianinen et al. 2008). The ozone-tolerance and greater competitive abilities of aspen 8 to mine growth limiting soil nitrogen relative to the other aspen clones under elevated O_3 (Zak et al. 2007b; Zak et al. 2012) might have contributed to its enhanced growth ring width and radial growth under elevated O_3 (Kubiske et al. 2007). In addition, the elimination of ozone-sensitive aspen clone 259 in the O_3 treatment rings during the Aspen FACE experiment might have provided aspen 8 and the other congener's greater growing space, resulting in enhanced radial growth and growth ring width under elevated O_3 .

Mean vessel lumen area proportion tended to decrease in aspen 8 and cell wall area proportion tended to increase, whereas mean vessel lumen, fiber lumen diameter and vessel frequency remained unaltered. These results suggest that radial growth increment tendency in aspen 8 under elevated O_3 might have been coupled with cell wall deposition. As a result, it is likely for aspen 8 to have moderate gain in wood density under future projected increases in background ozone concentration. In contrast, mean cell wall area proportion tended to decrease in aspen 271 and mean vessel lumen area proportion and vessel frequency tended to increase, whereas fiber lumen and vessel lumen diameter remained unaltered under elevated O_3 compared to control, which could contribute to slight reduction in wood density. This is in agreement with the slight reduction in wood density observed at the 0.5 m and 1.5 m height levels of aspen 271 stem

(Chapter 2, this dissertation). The anatomical study was performed on upper portions of tree stems. Wood anatomy and density vary along and across tree stems.

The effects of elevated O_3 on vessel lumen diameter were species or genotype and radial position specific. Compared to the control, mean vessel lumen diameter decreased significantly in maple and marginally in birch independent of radial position, under elevated O_3 . Size of vessels correlates positively with stem size (Schume et al. 2004). Reduction in growth of birch and maple has been observed under elevated O_3 (Karnosky et al. 2005; Kubiske et al. 2007). The reduction in vessel diameter of birch may have reduced void space and contributed to the observed increase in wood density under elevated ozone compared to ambient as observed in the earlier study (Chapter 2). With respect to radial position of vessels within the growth ring, vessel lumen diameter was not significantly modified at the inner and middle positions under elevated O_3 compared to the control, except it decreased significantly in maple at the middle position. Vessel lumen diameter decreased significantly at the peripheral radial position in all species, except for a slight increase in aspen 8 under elevated O_3 compared to the ambient. However, vessel lumen diameter generally decreased at the periphery independent of treatment. The reduction in vessel lumen diameter at the periphery position corresponds to the late growing period, when soil moisture may be limited, and therefore reduced vessel lumen diameter could be a physiological strategy to avoid cavitation. Wider vessel lumen diameters are more efficient in hydraulic conductivity but more vulnerable to cavitation, whereas narrower vessel lumen diameters are inefficient but less prone to cavitation (Hacke and Sperry 2001). It is therefore unclear if the reduction in vessel lumen diameter under elevated O_3 at the periphery radial position is due solely to elevated O_3 or not.

Effects of elevated $CO_2 + O_3$

No statistically significant effects of combined elevated $CO_2 + O_3$ were detected compared to the control for all anatomical properties in all species and aspen genotypes. However, there was a nearly significant decrease in the vessel lumen area proportion in maple under elevated $CO_2 + O_3$ compared to the control. In agreement with the current results, Kaakinen et al. (2004) and

Kostiainen et al. (2008) also reported no effect of elevated CO₂ + O₃ on the wood anatomical properties of aspen genotypes (8, 42, 216, 259 271) at the 3rd and 5th year of fumigation, respectively, at the Aspen FACE research site. Additionally, elevated CO₂ + O₃ had no significant impact on wood anatomical properties of two silver birch clones but caused a marginally significant increase in the vessel lumen area proportion in one silver birch clone, compared to the control (Kostiainen et al. 2006). These observations are parallel to accumulated evidence from the Aspen FACE research site, where no significant effect of elevated CO₂ + O₃ on growth parameters of the aspen genotypes, birch and maple were observed. It appears elevated CO₂ counteracts the effects of elevated O₃ on wood anatomical properties.

Conclusions

Species and genotype exhibited statistically significant effects on wood anatomical properties, independent of treatments. Compared to the control, elevated CO₂ did not have statistically significant effects on mean growth ring width, mean fiber length, mean fiber lumen diameter, mean vessel area proportion, mean vessel lumen diameter, or mean cell wall area proportion across all species and aspen genotypes. Nonetheless, elevated CO₂ marginally increased the number of vessels per square millimeter in aspen genotypes 8, 216, 271 and birch. Elevated O₃ influenced growth ring, cell wall and vessel lumen area proportions and vessel lumen diameter, especially at the periphery radial position of a growth ring, but effects were species and genotype specific. The effects of elevated CO₂ and O₃ alone on wood anatomical properties of aspen genotypes and birch were ameliorated when the gases were applied in combination. However, elevated CO₂ + O₃ marginally reduced vessel lumen area proportion in maple. Because wood density depends on wood anatomical properties, it is likely that future higher concentrations of CO₂ and / or O₃ could cause slight alterations in the strength of northern hardwoods, but for utilization purposes, the degree of changes would likely not be considered important based on the current anatomical data. Due to the variation in responses of aspen genotypes, birch and maple to elevated O₃, CO₂ alone, and in combination, it is recommended that future management of

natural stands of northern hardwoods should promote mixed species and genotypes in order to offset the potential minor effects of elevated CO₂ and / or elevated O₃ on wood quality.

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Figures

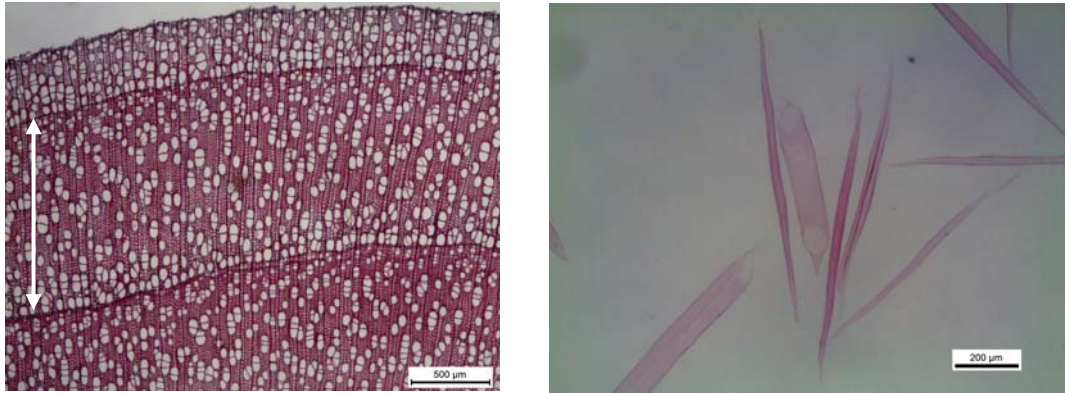


Fig 4-1 Micrographs of aspen illustrating the 2008 growth ring (Arrowed line, left) and a macerated wood sample for wood anatomical analyses (Right).

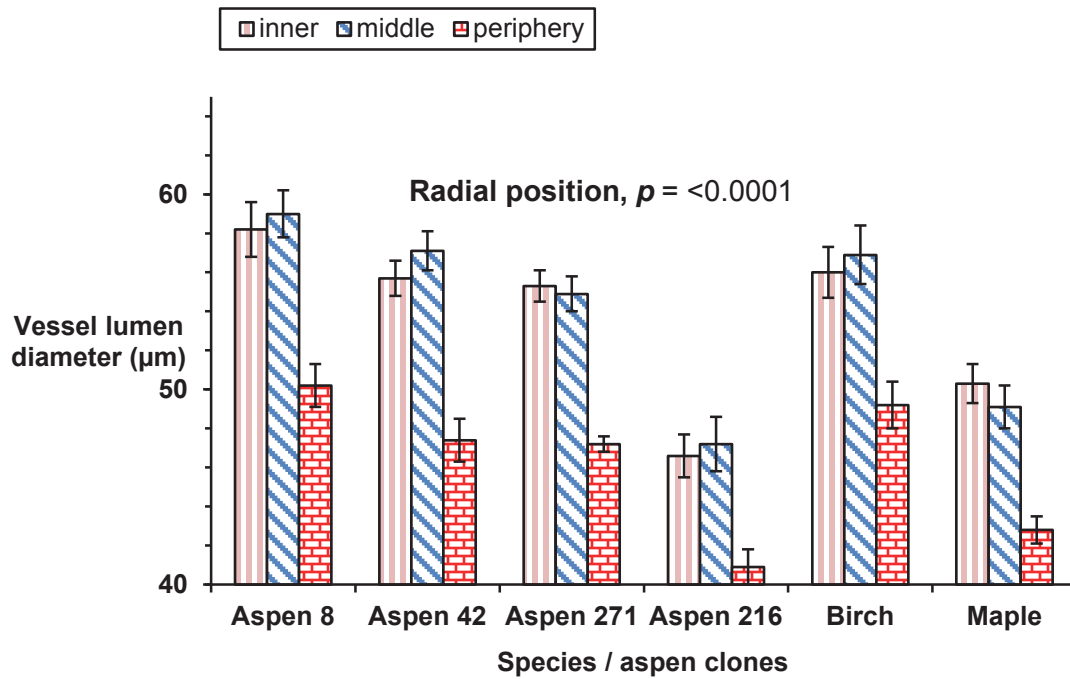


Figure 4-2 Vessel lumen diameter of aspen clones 8, 42, 216, 271, birch and maple in relation to radial position, independent of elevated CO₂ and / or O₃ treatments at Aspen FACE. Bars are means ± SE across all treatments and n =12.

Tables

Table 4-1 Effects of species / aspen clone on wood anatomical properties of aspen clones 8, 42, 216, 271, birch and maple independent of elevated CO₂ and / or elevated O₃ treatments at Aspen FACE. Values are means (± 1SE) across all treatments; means followed by the same letter in a column are not significantly different ($P > 0.05$). Highlighted P -values are significantly different ($p \leq 0.05$) and $n = 12$.

| Species / Clone | Ring width (mm) | Fiber length (mm) | Fiber lumen diameter (µm) | Vessel % | Vessel lumen diameter (µm) | Number of vessels / mm ² | Cell wall area % |
|-----------------|-------------------|-------------------|---------------------------|-------------------|----------------------------|-------------------------------------|-------------------|
| 8 | 3.3 ± 0.4ab | 0.8 ± 0.02a | 12.0 ± 0.2a | 33.6 ± 0.6a | 55.8 ± 1.2a | 142 ± 6ab | 34.8 ± 1.1a |
| 42 | 3.6 ± 0.3a | 0.7 ± 0.01a | 11.8 ± 0.2a | 31.0 ± 0.6a | 53.4 ± 0.9a | 134 ± 3b | 46.8 ± 0.8b |
| 216 | 2.3 ± 0.3b | 0.8 ± 0.02a | 12.1 ± 0.2a | 25.3 ± 0.8b | 44.9 ± 1.1b | 154 ± 7a | 50.6 ± 1.0bc |
| 271 | 3.2 ± 0.1ab | 0.8 ± 0.02a | 13.5 ± 0.3b | 26.9 ± 0.8b | 52.5 ± 0.7a | 129 ± 4b | 53.1 ± 1.4c |
| Birch | 2.4 ± 0.2b | 1.1 ± 0.03b | 12.3 ± 0.2a | 18.2 ± 0.9c | 54.0 ± 1.3a | 88 ± 4c | 52.9 ± 1.4c |
| Maple | 1.1 ± 0.1c | 0.7 ± 0.02a | 7.6 ± 0.1c | 16.1 ± 0.5c | 47.4 ± 0.9b | 87 ± 3c | 61.7 ± 0.9d |
| P -values | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

Table 4-2 Effects of CO₂ on wood anatomical properties of aspen clones 8, 42, 216, 271, birch and maple after 12 years of fumigation at the Aspen FACE research site, compared to the ambient. Values are means (\pm 1SE). Highlighted *p*-values are either significant ($P \leq 0.05$) or marginally significant ($P \leq 0.10$) and *n* = 3. Elevated CO₂ (= eCO₂).

| Species / Clone | Treatment | Ring width (mm) | Fiber length (mm) | Fiber lumen diameter (μ m) | Vessel % | Number of vessels / mm ² | Cell wall area % |
|------------------|------------------------|-------------------|-------------------|---------------------------------|-------------------|-------------------------------------|-------------------|
| 8 | Ambient | 2.4 \pm 0.2 | 0.8 \pm 0.05 | 12.0 \pm 0.5 | 35.6 \pm 1.0 | 145 \pm 13 | 30.6 \pm 0.8 |
| | eCO ₂ | 2.5 \pm 0.7 | 0.8 \pm 0.003 | 11.3 \pm 0.1 | 32.6 \pm 1.0 | 161 \pm 13 | 35.2 \pm 2.2 |
| 42 | Ambient | 3.9 \pm 0.1 | 0.7 \pm 0.01 | 11.6 \pm 0.4 | 32.4 \pm 0.6 | 141 \pm 6 | 46.4 \pm 3.6 |
| | eCO ₂ | 3.9 \pm 0.7 | 0.8 \pm 0.04 | 12.7 \pm 0.3 | 29.7 \pm 1.8 | 126 \pm 7 | 46.2 \pm 0.9 |
| 216 | Ambient | 2.7 \pm 0.4 | 0.8 \pm 0.05 | 12.1 \pm 0.5 | 24.6 \pm 0.8 | 144 \pm 2 | 50.8 \pm 1.9 |
| | eCO ₂ | 1.5 \pm 0.5 | 0.8 \pm 0.05 | 11.8 \pm 0.6 | 24.9 \pm 2.9 | 158 \pm 19 | 50.6 \pm 2.5 |
| 271 | Ambient | 3.3 \pm 0.2 | 0.8 \pm 0.02 | 12.8 \pm 0.8 | 24.0 \pm 0.8 | 121 \pm 1 | 57.6 \pm 3.2 |
| | eCO ₂ | 3.0 \pm 0.5 | 0.8 \pm 0.03 | 14.1 \pm 0.3 | 26.9 \pm 0.4 | 131 \pm 4 | 55.2 \pm 0.7 |
| Birch | Ambient | 2.9 \pm 0.3 | 1.2 \pm 0.03 | 12.9 \pm 0.2 | 17.6 \pm 1.7 | 80 \pm 5 | 54.1 \pm 3.3 |
| | eCO ₂ | 1.7 \pm 0.1 | 1.1 \pm 0.04 | 12.3 \pm 0.4 | 19.4 \pm 1.0 | 105 \pm 9 | 49.7 \pm 1.9 |
| Maple | Ambient | 1.2 \pm 0.1 | 0.7 \pm 0.08 | 7.7 \pm 0.1 | 17.1 \pm 0.6 | 89 \pm 2 | 63.1 \pm 0.9 |
| | eCO ₂ | 1.0 \pm 0.1 | 0.7 \pm 0.02 | 7.8 \pm 0.1 | 16.8 \pm 1.7 | 88 \pm 10 | 57.8 \pm 2.0 |
| <i>P</i> -values | Clone / spp | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| | eCO ₂ | 0.2037 | 0.6984 | 0.4079 | 0.9547 | 0.0771 | 0.2383 |
| | eCO ₂ x spp | 0.4032 | 0.2893 | 0.1452 | 0.2219 | 0.4278 | 0.3123 |

Table 4-3 Effects of CO₂ on vessel lumen diameter of aspen clones 8, 42, 216, 271, birch and maple, in relation to radial position within the growth ring, after 12 years of fumigation at the Aspen FACE research site compared to the ambient. Values are means (\pm 1SE). Highlighted *P*-values are significant ($P \leq 0.05$) and $n = 3$. Elevated CO₂ (= eCO₂).

| Species/clone | Treatment | Radial Position | | | |
|---|------------------|-----------------|----------------|----------------|-------------------|
| | | Inner | Middle | Periphery | Mean |
| 8 | Ambient | 56.7 \pm 2.8 | 57.8 \pm 2.3 | 50.3 \pm 2.4 | 55 \pm 2.5 |
| | eCO ₂ | 55.3 \pm 3.7 | 56.0 \pm 2.5 | 47.0 \pm 2.5 | 52.8 \pm 2.9 |
| 42 | Ambient | 54.9 \pm 2.3 | 58.6 \pm 1.6 | 50.2 \pm 3.5 | 54.6 \pm 2.2 |
| | eCO ₂ | 58.6 \pm 1.2 | 58.4 \pm 2.6 | 48.9 \pm 1.7 | 55.3 \pm 1.8 |
| 216 | Ambient | 47.0 \pm 2.3 | 48.5 \pm 2.5 | 42.4 \pm 1.8 | 46.0 \pm 2.1 |
| | eCO ₂ | 44.8 \pm 3.0 | 47.0 \pm 3.9 | 39.4 \pm 2.0 | 43.7 \pm 2.9 |
| 271 | Ambient | 54.8 \pm 1.5 | 55.0 \pm 0.6 | 47.1 \pm 0.2 | 52.3 \pm 0.6 |
| | eCO ₂ | 57.8 \pm 1.9 | 58.3 \pm 1.7 | 48.7 \pm 0.2 | 55.0 \pm 1.2 |
| Birch | Ambient | 57.8 \pm 2.4 | 60.0 \pm 1.8 | 52.0 \pm 1.8 | 56.6 \pm 2.0 |
| | eCO ₂ | 54.0 \pm 1.0 | 55.7 \pm 3.0 | 48.1 \pm 2.6 | 52.6 \pm 1.8 |
| Maple | Ambient | 53.1 \pm 2.0 | 53.1 \pm 0.3 | 45.3 \pm 0.5 | 50.5 \pm 0.7 |
| | eCO ₂ | 50.3 \pm 2.0 | 49.4 \pm 2.0 | 42.3 \pm 1.1 | 47.3 \pm 1.5 |
| <i>P</i>-values | | | | | |
| Species / clone | | 0.0004 | 0.0003 | 0.0003 | 0.0001 |
| eCO ₂ | | 0.8092 | 0.4274 | 0.1218 | 0.4263 |
| eCO ₂ \times species /clone | | 0.4137 | 0.5630 | 0.6857 | 0.5049 |
| Position | | | | | <0.0001 |
| eCO ₂ \times position | | | | | 0.2027 |
| Species / clone \times position | | | | | 0.5148 |
| eCO ₂ \times species / clone \times position | | | | | 0.8565 |

Table 4-4 Effects of elevated O₃ on wood anatomical properties of aspen clones 8, 42, 216, 271, birch and maple after 12 years of fumigation at the Aspen FACE research site, compared to the ambient. Values are means (\pm 1SE). Highlighted *P*-values are either significant ($P \leq 0.05$) or marginally significant ($P \leq 0.10$) and $n = 3$. Elevated O₃ (=eO₃).

| Species / Clones | Treatment | Ring width (mm) | Fiber length (mm) | Fiber lumen diameter (μ m) | Vessel % | Number of vessels / mm ² | Cell wall area % |
|------------------|-----------------------|-------------------|-------------------|---------------------------------|-------------------|-------------------------------------|-------------------|
| 8 | Ambient | 2.4 \pm 0.2 | 0.8 \pm 0.05 | 12.0 \pm 0.5 | 35.6 \pm 1.0 | 145 \pm 13 | 30.6 \pm 0.8 |
| | eO ₃ | 4.2 \pm 0.9 | 0.8 \pm 0.07 | 12.1 \pm 0.4 | 32.8 \pm 0.5 | 132 \pm 13 | 37.4 \pm 2.0 |
| 42 | Ambient | 3.9 \pm 0.1 | 0.7 \pm 0.01 | 11.6 \pm 0.4 | 32.4 \pm 0.6 | 141 \pm 6 | 46.4 \pm 3.6 |
| | eO ₃ | 4.3 \pm 0.4 | 0.7 \pm 0.02 | 11.8 \pm 0.2 | 30.1 \pm 1.0 | 128 \pm 2 | 48.3 \pm 0.3 |
| 216 | Ambient | 2.7 \pm 0.4 | 0.8 \pm 0.05 | 12.1 \pm 0.5 | 24.6 \pm 0.8 | 144 \pm 2 | 50.8 \pm 1.9 |
| | eO ₃ | 2.1 \pm 0.4 | 0.7 \pm 0.04 | 12.4 \pm 0.4 | 26.1 \pm 2.0 | 155 \pm 7 | 47.2 \pm 1.2 |
| 271 | Ambient | 3.3 \pm 0.2 | 0.8 \pm 0.02 | 12.8 \pm 0.8 | 24.0 \pm 0.8 | 121 \pm 1 | 57.6 \pm 3.2 |
| | eO ₃ | 3.3 \pm 0.3 | 0.7 \pm 0.02 | 13.2 \pm 0.3 | 28.4 \pm 1.4 | 131 \pm 2 | 49.0 \pm 1.9 |
| Birch | Ambient | 2.9 \pm 0.3 | 1.2 \pm 0.03 | 12.9 \pm 0.2 | 17.6 \pm 1.7 | 80 \pm 5 | 54.1 \pm 3.3 |
| | eO ₃ | 2.3 \pm 0.4 | 1.0 \pm 0.03 | 11.6 \pm 0.5 | 19.9 \pm 1.9 | 91 \pm 5 | 50.8 \pm 2.6 |
| Maple | Ambient | 1.2 \pm 0.1 | 0.7 \pm 0.08 | 7.7 \pm 0.1 | 17.1 \pm 0.6 | 89 \pm 2 | 63.1 \pm 0.9 |
| | eO ₃ | 1.1 \pm 0.1 | 0.7 \pm 0.03 | 7.5 \pm 0.3 | 16.0 \pm 0.7 | 92 \pm 5 | 62.9 \pm 0.3 |
| <i>P</i> -values | Clone / spp | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| | eO ₃ | 0.6773 | 0.1475 | 0.7363 | 0.5205 | 0.7185 | 0.4049 |
| | eO ₃ x spp | 0.0499 | 0.1807 | 0.3695 | 0.0803 | 0.2268 | 0.0427 |

Table 4-5 Effects of O₃ on vessel lumen diameter of aspen clones 8, 42, 216, 271, birch and maple, in relation to radial position within the growth ring, after 12 years of fumigation at the Aspen FACE research site, compared to the ambient. Values are means (\pm 1SE). Highlighted *P*-values are either significantly ($P \leq 0.05$) or marginally significantly different ($P \leq 0.10$) and *n* = 3. Elevated O₃ (=eO₃).

| Species / clone | Treatment | Position | | | Mean |
|--|-----------------|-------------------|-------------------|-------------------|-------------------|
| | | Inner | Middle | Periphery | |
| 8 | Ambient | 56.7 \pm 2.8 | 57.8 \pm 2.3 | 50.3 \pm 2.4 | 55 \pm 2.5 |
| | eO ₃ | 59.4 \pm 2.0 | 61.5 \pm 3.1 | 51.8 \pm 1.9 | 57.6 \pm 2.2 |
| 42 | Ambient | 54.9 \pm 2.3 | 58.6 \pm 1.6 | 50.2 \pm 3.5 | 54.6 \pm 2.2 |
| | eO ₃ | 56.8 \pm 0.8 | 57.8 \pm 0.4 | 46.1 \pm 0.2 | 53.6 \pm 0.3 |
| 216 | Ambient | 47.0 \pm 2.3 | 48.5 \pm 2.5 | 42.4 \pm 1.8 | 46.0 \pm 2.1 |
| | eO ₃ | 47.1 \pm 0.6 | 45.9 \pm 1.8 | 39.8 \pm 1.6 | 44.3 \pm 1.3 |
| 271 | Ambient | 54.8 \pm 1.5 | 55.0 \pm 0.6 | 47.1 \pm 0.2 | 52.3 \pm 0.6 |
| | eO ₃ | 54.8 \pm 1.3 | 53.3 \pm 1.0 | 46.6 \pm 0.9 | 51.6 \pm 1.1 |
| Birch | Ambient | 57.8 \pm 2.4 | 60.0 \pm 1.8 | 52.0 \pm 1.8 | 56.6 \pm 2.0 |
| | eO ₃ | 53.2 \pm 3.2 | 52.4 \pm 3.8 | 45.6 \pm 2.7 | 50.4 \pm 3.2 |
| Maple | Ambient | 53.1 \pm 2.0 | 53.1 \pm 0.3 | 45.3 \pm 0.5 | 50.5 \pm 0.7 |
| | eO ₃ | 46.8 \pm 1.3 | 44.8 \pm 1.5 | 40.2 \pm 1.5 | 43.9 \pm 1.4 |
| <i>P</i>-values | | | | | |
| Species / clone | | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| eO ₃ | | 0.5485 | 0.1304 | 0.0026 | 0.1301 |
| eO ₃ \times species /clone | | 0.1040 | 0.0232 | 0.1404 | 0.0551 |
| position | | | | | <0.0001 |
| eO ₃ \times position | | | | | 0.0110 |
| Species \times position | | | | | 0.0363 |
| eO ₃ \times spp/clone \times position | | | | | 0.1168 |

Table 4-6 Effects of elevated CO₂ + O₃ on wood anatomical properties of aspen clones 8, 42, 216, 271, birch and maple after 12 years of fumigation at the Aspen FACE research site, compared to the ambient. Values are means (\pm 1SE). Highlighted *P*-values are either significantly (*P* \leq 0.05) or marginally significantly different (*P* \leq 0.10) and *n* = 3. Elevated CO₂ (= eCO₂) and elevated O₃ (=eO₃).

| Species / Clone | Treatment | Ring width (mm) | Fiber length (mm) | Fiber lumen diameter (μ m) | Vessel % | Number of vessels / mm ² | Cell wall area % |
|------------------|---|-------------------|-------------------|---------------------------------|-------------------|-------------------------------------|-------------------|
| 8 | Ambient | 2.4 \pm 0.2 | 0.8 \pm 0.05 | 12.0 \pm 0.5 | 35.6 \pm 1.0 | 145 \pm 13 | 30.6 \pm 0.8 |
| | eCO ₂ + eO ₃ | 3.9 \pm 0.3 | 0.8 \pm 0.04 | 12.5 \pm 0.3 | 33.2 \pm 1.9 | 130 \pm 4 | 35.8 \pm 1.6 |
| 42 | Ambient | 3.9 \pm 0.1 | 0.7 \pm 0.01 | 11.6 \pm 0.4 | 32.4 \pm 0.6 | 141 \pm 6 | 46.4 \pm 3.6 |
| | eCO ₂ + eO ₃ | 2.3 \pm 0.6 | 0.7 \pm 0.03 | 11.2 \pm 0.3 | 31.9 \pm 1.2 | 141 \pm 7 | 46.2 \pm 0.7 |
| 216 | Ambient | 2.7 \pm 0.4 | 0.8 \pm 0.05 | 12.1 \pm 0.5 | 24.6 \pm 0.8 | 144 \pm 2 | 50.8 \pm 1.9 |
| | eCO ₂ + eO ₃ | 2.7 \pm 1.2 | 0.8 \pm 0.01 | 12.0 \pm 0.7 | 25.5 \pm 1.2 | 159 \pm 24 | 53.8 \pm 0.9 |
| 271 | Ambient | 3.3 \pm 0.2 | 0.8 \pm 0.02 | 12.8 \pm 0.8 | 24.0 \pm 0.8 | 121 \pm 1 | 57.6 \pm 3.2 |
| | eCO ₂ + eO ₃ | 3.3 \pm 0.2 | 0.8 \pm 0.02 | 13.7 \pm 0.5 | 28.1 \pm 2.1 | 133 \pm 16 | 50.7 \pm 2.1 |
| Birch | Ambient | 2.9 \pm 0.3 | 1.2 \pm 0.03 | 12.9 \pm 0.2 | 17.6 \pm 1.7 | 80 \pm 5 | 54.1 \pm 3.3 |
| | eCO ₂ + eO ₃ | 2.7 \pm 0.8 | 1.2 \pm 0.03 | 12.4 \pm 0.5 | 16.0 \pm 1.9 | 75 \pm 3 | 56.9 \pm 1.9 |
| Maple | Ambient | 1.2 \pm 0.1 | 0.7 \pm 0.08 | 7.7 \pm 0.1 | 17.1 \pm 0.6 | 89 \pm 2 | 63.1 \pm 0.9 |
| | eCO ₂ + eO ₃ | 1.2 \pm 0.1 | 0.7 \pm 0.03 | 7.5 \pm 0.3 | 14.5 \pm 0.3 | 79 \pm 4 | 63.0 \pm 1.6 |
| <i>P</i> -values | Clone / spp | <0.0037 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| | eCO ₂ + eO ₃ | 0.8186 | 0.4639 | 0.8915 | 0.6704 | 0.9109 | 0.2714 |
| | eCO ₂ + eO ₃ \times spp | 0.1262 | 0.5513 | 0.6619 | 0.0724 | 0.4863 | 0.1403 |

Table 4-7 Effects of CO₂ + O₃ on vessel lumen diameter of aspen clones 8, 42, 216, 271, birch and maple, in relation to radial position within the growth ring, after 12 years of fumigation at the Aspen FACE research site, compared to the ambient. Values are means (± 1SE). Highlighted *P*-values are either significantly (*P* ≤ 0.05) or marginally significantly different (*P* ≤ 0.10) and n = 3. Elevated CO₂ (= eCO₂) and elevated O₃ (=eO₃).

| Species / clone | Treatment | Radial position | | | Mean |
|---|------------------------------------|-----------------|-------------------|---------------|-------------------|
| | | Inner | Middle | Periphery | |
| 8 | Ambient | 56.7 ± 2.8 | 57.8 ± 2.3 | 50.3 ± 2.4 | 55 ± 2.5 |
| | eCO ₂ + eO ₃ | 61.3 ± 2.1 | 60.6 ± 0.1 | 51.8 ± 2.0 | 57.9 ± 1.4 |
| 42 | Ambient | 54.9 ± 2.3 | 58.6 ± 1.6 | 50.2 ± 3.5 | 54.6 ± 2.2 |
| | eCO ₂ + eO ₃ | 52.4 ± 1.3 | 53.6 ± 1.8 | 44.5 ± 0.9 | 50.2 ± 1.3 |
| 216 | Ambient | 47.0 ± 2.3 | 48.5 ± 2.5 | 42.4 ± 1.8 | 46.0 ± 2.1 |
| | eCO ₂ + eO ₃ | 47.6 ± 3.0 | 47.6 ± 3.9 | 41.9 ± 2.5 | 45.7 ± 3.1 |
| 271 | Ambient | 54.8 ± 1.5 | 55.0 ± 0.6 | 47.1 ± 0.2 | 52.3 ± 0.6 |
| | eCO ₂ + eO ₃ | 53.8 ± 1.5 | 53.1 ± 1.8 | 46.2 ± 1.2 | 51.0 ± 1.5 |
| Birch | Ambient | 57.8 ± 2.4 | 60.0 ± 1.8 | 52.0 ± 1.8 | 56.6 ± 2.0 |
| | eCO ₂ + eO ₃ | 58.8 ± 2.6 | 59.6 ± 2.4 | 50.9 ± 1.5 | 56.4 ± 2.1 |
| Maple | Ambient | 53.1 ± 2.0 | 53.1 ± 0.3 | 45.3 ± 0.5 | 50.5 ± 0.7 |
| | eCO ₂ + eO ₃ | 50.9 ± 1.0 | 48.9 ± 1.0 | 43.4 ± 1.0 | 47.7 ± 0.9 |
| <i>P</i>-values | | | | | |
| Species / clone | | 0.0003 | <0.0001 | 0.0001 | <0.0001 |
| eCO ₂ + eO ₃ | | 0.9747 | 0.2182 | 0.2989 | 0.4374 |
| eCO ₂ + eO ₃ × species /clone | | 0.5909 | 0.4102 | 0.4761 | 0.4622 |
| position | | | | | <0.0001 |
| eCO ₂ + eO ₃ × position | | | | | 0.0463 |
| Species × position | | | | | 0.1200 |
| eCO ₂ + eO ₃ × spp / clone | | | | | 0.8875 |
| ×position | | | | | |

CHAPTER 5: Dissertation Synthesis

Introduction

Atmospheric CO₂ and O₃ concentrations have increased by about 40% since the onset of the industrial revolution and are predicted to rise further due to anthropogenic activities such as fossil fuel combustion and changing land use systems (IPCC 2007). Available evidence indicates that elevated CO₂ concentrations stimulate woody plant growth, but elevated O₃ retards it (Karnosky et al. 1996; Karnosky et al. 2007; Kubiske et al. 2007; Norby and Zak 2011; Zak et al. 2011). Additionally the biochemistry of woody plants grown under elevated CO₂ or O₃ is altered (Kaakinen et al. 2004; Kostianen et al. 2004; Parsons et al. 2004; Liu et al. 2005; Kostianen et al. 2006; Kostianen et al. 2008; Parsons et al. 2008). Wood density, which is an important measure of wood quality, depends on wood anatomical properties, which in turn are influenced by cambial activity and cellular development (Dickison 2000). Cambial activity and cellular development of woody plants are also largely influenced by a range of environmental factors (Dickison 2000; Barnett and Jeronimidis 2003). Hence, changing concentrations of atmospheric CO₂ and O₃, could impact wood anatomical properties and quality. Furthermore, because many fungi depend on plant detritus for growth and development, modifications in the structure and chemical properties of woody plants under elevated CO₂ and / or O₃ could affect the wood-decaying fungal community and wood decomposition rates. Subsequently carbon and nutrient cycling and terrestrial forest ecosystem productivity could be altered. The general goal of this dissertation was therefore to investigate the long term effects of twelve years of ecosystem development under elevated CO₂ and / or O₃ on wood density, wood anatomical properties, wood-decaying fungal community composition and rates of wood decomposition for common northern hardwood tree species.

Summary of Results

Chapter 1 introduced the dissertation. A brief overview of the theoretical framework and justification for the dissertation were provided. The weaknesses in existing methodologies for

investigating the effects of elevated CO₂ and / or O₃ on wood properties and woody plant litter decomposition were highlighted and the general goal, objectives and hypotheses were outlined. The case was made that Aspen FACE provided a unique opportunity and more realistic approach for examining the long term effects of elevated CO₂ and / or O₃ on wood properties, wood-decaying fungal community dynamics, and wood decomposition than the short-term use of seedlings and saplings in greenhouses and growth chambers.

In Chapter 2, the long-term effects of elevated CO₂ and / or O₃ on wood density of birch and three clones of trembling aspen (42, 216 and 271) grown at Aspen FACE for twelve years were investigated. It was observed that wood density of aspen clone 271 decreased significantly under elevated CO₂ compared to the control. In contrast, wood density of aspen clone 42 and birch increased significantly under elevated O₃ compared to the control. The wood densities of aspen or birch under combined treatments of elevated CO₂ and O₃ were not significantly different from the ambient control. Species or clone effects on wood density were significant, with birch exhibiting a significantly higher wood density than aspen, independent of elevated CO₂ and / or O₃ treatments. Among the aspen clones, aspen 42 had significantly lower density than clones 216 and 271.

Chapter 3 examined the effects of birch and aspen (clones 42 and 271) produced or decomposing in elevated CO₂ and / or O₃ on wood-decaying fungal community composition and initial decomposition rates. Results indicated that production or decomposition of birch and aspen under elevated CO₂ and / or O₃ did not significantly alter wood-decaying fungal community composition or wood decomposition rates. However, wood species had a clear impact on wood-decaying fungal community composition and initial rates of wood decomposition. Wood-decaying fungal community composition within decaying birch and aspen logs were significantly different, and birch decomposed faster than aspen. A total of 14 wood-decaying basidiomycete fungal species, largely from the order Polyporales, Agaricales, and Russulales were isolated, independent of elevated CO₂ and / or O₃ treatments.

Wood anatomical traits are known to influence wood density, wood-decaying fungal community composition and decomposition rates (Eaton and Hale 1993; Dickison 2000; Barnett and Jeronimidis 2003; Cornwell et al. 2008; Cornwell et al. 2009; Weedon et al. 2009). Hence, in Chapter 4, the long term effects of growth under elevated CO₂ and / or O₃ on wood anatomical properties of four trembling aspen clones (8, 42, 216, and 271), birch and maple were evaluated. Compared to the ambient, anatomical properties of birch, maple and trembling aspen were not significantly affected by elevated CO₂, except for a marginal increase in number of vessels per square millimeter in one aspen genotype (271) and birch. However, under elevated O₃, vessel lumen diameter decreased significantly in maple and marginally in birch compared to the ambient. The combined treatment of elevated CO₂ and O₃ did not have a significant effect on any of the anatomical properties compared to the ambient. Species or genotype effects on all wood anatomical properties were very conspicuous. Vessel lumen diameter was observed to be significantly lower at the periphery than the inner and middle radial positions of the growth ring, independent of elevated CO₂ and / or O₃ treatments.

Conclusions / implications of study

Information on the effects of elevated O₃ alone and in combination with elevated CO₂ on wood density is rare. Nonetheless, mounting experimental evidence indicates that, elevated CO₂ alone could enhance, reduce or have no effects on wood density depending on species, genotype, age and soil nutrients status (Rogers et al. 1983; Conroy et al. 1990; Telewski et al. 1999; Maherali and DeLucia 2000; Oren et al. 2001; Beismann et al. 2002; Ceulemans et al. 2002; Atwell et al. 2003; Calfapietra et al. 2003; Kilpelainen et al. 2005; Qiao et al. 2008). Similarly, growing experimental evidence indicates that anatomical properties of woody plants grown under elevated CO₂ or O₃ alone could be modified or not depending on age, species or genotype (Telewski et al. 1999; Kaakinen et al. 2004; Kostianen et al. 2004; Luo et al. 2005; Yazaki et al. 2005; Kostianen et al. 2006; Kostianen et al. 2008; Kostianen et al. 2009; Watanabe et al. 2010). However, wood anatomical properties of woody plants grown under combined CO₂ and O₃ are not altered (Kostianen et al. 2004; Kostianen et al. 2006; Kostianen et al. 2008).

In line with existing accumulating evidence, wood density and anatomical properties of common northern hardwoods (birch, maple and trembling aspen) responses to long-term growth under elevated CO₂ and / or O₃ were observed to be species or genotype specific in this study. The reduction in wood density of aspen genotype 271 may be explained by the observed marginally significant increase in number of vessels per square millimeter under elevated CO₂ compared to the control. Additionally, the vessel and fiber lumen diameters of aspen 271 tended to increase under elevated CO₂ compared to the ambient, confirming earlier reports from Aspen FACE (Kaakinen et al. 2004; Kostianen et al. 2008). As a result void space in aspen 271 increased under elevated CO₂ compared to the ambient resulting in a significant reduction in wood density (Panshin and Zeeuw 1980; Dickison 2000; Barnett and Jeronimidis 2003). Aspen genotype 271 grew at a faster rate under elevated CO₂ compared to the ambient at Aspen FACE (Isebrands et al. 2001; Kubiske et al. 2007). Typically, fast growth of woody plants could cause modifications in anatomical properties and result in reduction in wood density.

In contrast to elevated CO₂ responses, wood density of aspen genotype 42 and birch increased significantly under elevated O₃ compared to the ambient in this study. Moderate modifications in the anatomical properties of birch and aspen 42 observed under elevated O₃, compared to the ambient, might have accounted for the increase in wood density. There was a marginally significant decrease in vessel lumen diameter in birch and cell wall area proportion tended to increase in aspen 42 under elevated O₃, compared to the ambient. This implies that void space in birch and cell wall area proportion in aspen genotype 42 decreased and increased, respectively, thereby causing significant increases in wood density under elevated O₃ compared to the ambient. Wood density is a measure of the ratio of cell wall material to void space (Barnett and Jeronimidis 2003). Additionally, lignin, starch and extractives which influence wood density (Grabner et al. 2005), increased in birch under elevated O₃ compared to the ambient at the same experiment site (Kaakinen et al. 2004; Kostianen et al. 2008). Hence chemical and cellular modifications which occurred in some aspen clones and birch under elevated CO₂ or O₃ may have resulted in the moderate modifications of wood density compared to ambient. This

suggests that future higher concentrations of CO₂ or O₃ alone could modify anatomical structure and wood density and consequently wood quality of common northern hardwoods, but effects will be species or genotype specific. Based on our results however, effects of elevated CO₂ or O₃ alone on wood quality may not be considered significant for utilization purposes, since the density changes were relatively small and mean density values observed in this study were well within the utilization range density of 400 to 750 kg / m³ (Panshin and Zeeuw 1980). The combined treatment of elevated CO₂ and O₃ did not show any significant effects on either wood density or anatomical properties. Implying that, the effects of elevated CO₂ or O₃ alone on wood density and anatomical properties are ameliorated when in combination.

Anatomical properties of birch and trembling aspen genotypes were moderately modified under elevated CO₂ or O₃ compared to the ambient, but growth or decomposition of birch and aspen wood in elevated CO₂ and / or O₃ did not have significant effects on wood-decaying fungal community composition or rates of wood decomposition. To our knowledge, no studies have investigated the effects of elevated CO₂ and / or O₃ on the wood-decaying fungal community. The effects of elevated CO₂ and / or O₃ on the fungal community and plant litter decomposition are assumed to be mediated via modifications in litter quality (Strain and Bazzaz 1983).. In this study, species / clone exhibited significant effects on wood-decaying fungal community composition and decomposition rates, while growth or decomposition of birch and aspen wood in elevated CO₂ and / or O₃ had only a minor effect. At Aspen FACE, modifications in forest community composition (relative proportions of birch, maple and aspen genotypes) have occurred due to differential growth response to elevated CO₂ and O₃ (Kubiske et al. 2007). Hence because wood-decaying fungal community and decomposition vary significantly with species or genotype, it is possible that future high levels of elevated CO₂ or O₃ will influence wood-decaying fungal community and decomposition via changes in substrate quality associated with alteration of the species / genotype composition of natural stands, as well as changes in the total amount of woody detritus produced due to effects of elevated CO₂ and O₃ on stand productivity.

Finally, because wood density, wood-decaying fungal community composition, and wood decomposition, and wood anatomical properties responses to elevated CO₂ and / or O₃ were species or genotype specific, it is important that the genetic resources of common hardwoods are conserved at all levels of stand management. A prudent conservation of the genetic resources of northern hardwoods has an enormous potential to offset the effects of projected future higher levels of CO₂ and / or O₃.

Limitations of study and future research

In the wood density studies, density estimations were done for both bark and secondary xylem (wood). The bark and secondary xylem (wood) tissues differ in structure and composition and may respond differently to elevated CO₂ and / or O₃. Nevertheless, the results are reliable for stem density long term response to elevated CO₂ and / or O₃ of common northern hardwoods. Hence it is recommended that future research consider separating the effects of elevated CO₂ and / or O₃ on bark and wood density.

Although, the effects of CO₂ and / or O₃ on wood-decaying fungal community composition and wood decomposition were done in the source environment, they were performed after all trees in the Aspen FACE rings were cut and new canopy development was minimal. It is therefore possible that fungal spores, which are airborne, could have circulated across all treatments. Additionally, wood detrital inputs during the 12 years of the Aspen FACE experiment prior to harvest were minor except small dead branches. The duration of the wood decomposition study was relatively short (one year) relative to the inherent slow decomposition rates of wood. Micro-fungi and bacteria do not cause considerable mass loss of wood detritus during decay, but their presence modifies the micro- environment and therefore could affect the activities and composition for wood-decaying basidiomycetes. In this study, micro-fungi and bacteria were not our focus. Also colonization and decay of wood occur in succession, and the isolated fungi showed that the colonization of the wood samples were from the primary and secondary stages. It is therefore not clear how the tertiary suite of wood-decaying fungi would respond to elevated CO₂ and / or O₃. Furthermore, litter quality is presumed to influence fungal community and

decomposition of plant litter, but the biochemistry of the logs samples were not examined. Although, the biochemistry of the same species of earlier reports was referenced, they were done on saplings and could have changed by the time of this study. To our knowledge, this is the first time the long term effects of CO₂ and / or O₃ on wood-decaying fungi and decomposition is being reported. In spite of the aforementioned limitations, careful interpretation of the results could serve as valuable basis for future research. It is recommended that future studies should have a longer decomposition period, consider micro-fungi response to elevated CO₂ and / or O₃, and possibly, the experiment could be performed under undisturbed canopy. Additionally, a thorough evaluation of the biochemical constituents of the samples under investigation should occur to provide information on the effects of elevated CO₂ and / or O₃ on woody litter quality.

Wood anatomical properties vary considerable between and within species, from pith to bark and along the longitudinal axis. Only two trees of each of the six species / clones were sampled from each of the 12 treatment rings. Additionally, anatomical measurements were done only on one growth ring in the upper portions of the trees. In view of the limited sampling, it is likely that the total variations within and between the six species and clones were not captured. However, our results are consistent with earlier experiments. It is recommended that a more intensive sampling, which considers all growth rings at different height levels of the trees, is done in order to capture all variations with respect to the effects of elevated CO₂ and / or O₃ on wood anatomical properties.

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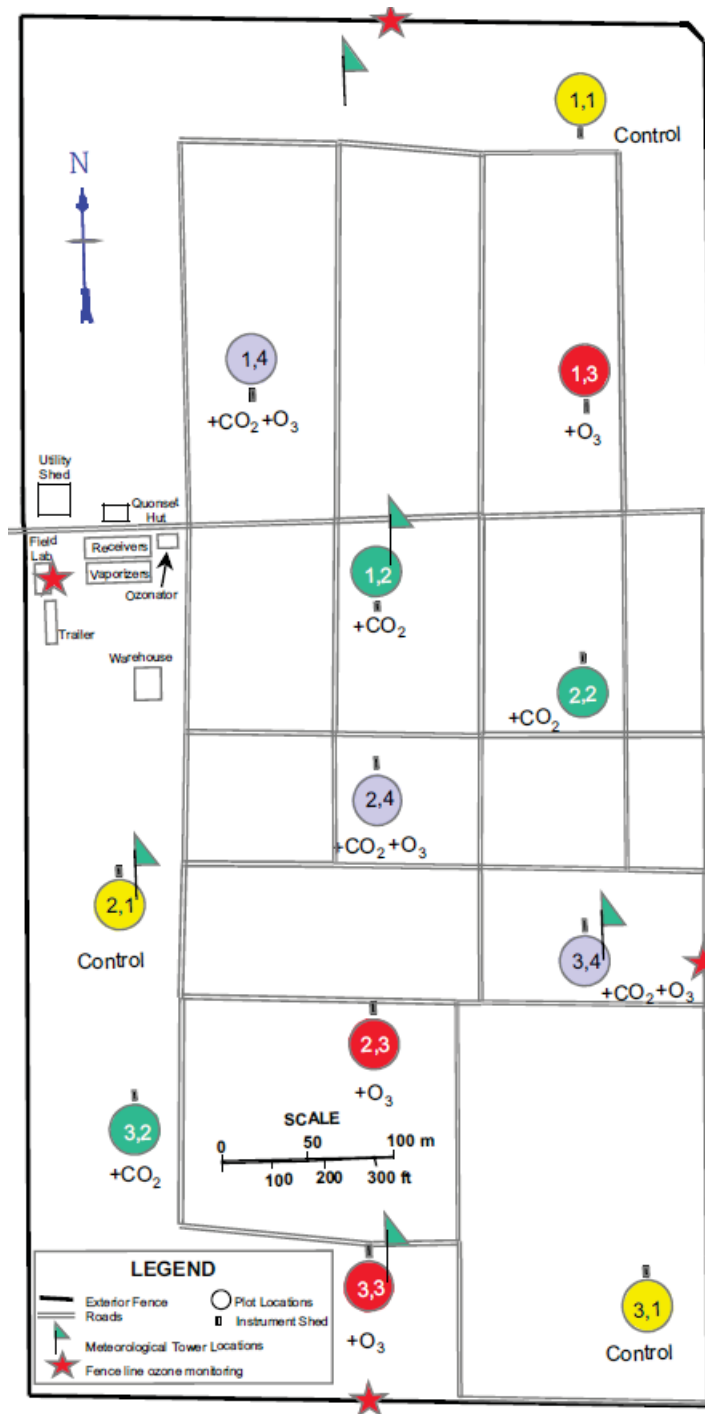
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APPENDIX



Appendix Figure 2-1 Positions of the treatment rings and facilities at the Aspen FACE project site, Harshaw, WI, USA. Total area of site is 32 ha (Dickson et al. 2000).

Appendix Table 2-1 Repeated measures analysis of variance for the effects of elevated CO₂ on wood density of birch, aspen clones 216, 271 and 42. The first table is the combined analysis for all species/clones and the next four tables are the separate analysis for birch, aspen clones 216, 271 and 42, respectively.

All species / clones

| Source | SS | df | MS | F ratio | P > F |
|---|-----------|-----|-----------|---------|---------|
| <i>Between subjects</i> | | | | | |
| Elevated CO ₂ | 108.304 | 1 | 108.30 | 0.09 | 0.7633 |
| Species | 762157.97 | 3 | 254052.66 | 215.62 | <0.0001 |
| Elevated CO ₂ × species | 10476.77 | 3 | 3492.26 | 2.96 | 0.0435 |
| Error | 47129.45 | 40 | 1178.24 | | |
| <i>Within subjects</i> | | | | | |
| Position | 22132.29 | 4 | 5533.07 | 11.77 | <0.0001 |
| Position × elevated CO ₂ | 4334.19 | 4 | 1083.55 | 2.30 | 0.0606 |
| Position × species | 8187.81 | 12 | 682.32 | 1.45 | 0.1480 |
| Position × elevated CO ₂ × species | 4476.11 | 12 | 373.01 | 0.79 | 0.6567 |
| Error | 75215.58 | 160 | 470.09 | | |

Birch

| Source | SS | df | MS | F ratio | P > F |
|-------------------------------------|---------|----|--------|---------|--------|
| <i>Between subjects</i> | | | | | |
| Elevated CO ₂ | 3611.7 | 1 | 3611.7 | 1.64 | 0.2293 |
| Error | 22033.1 | 10 | 2203.3 | | |
| <i>Within subjects</i> | | | | | |
| Position | 3663.1 | 4 | 920.8 | 3.93 | 0.0087 |
| Position × elevated CO ₂ | 197.2 | 4 | 49.3 | 0.21 | 0.9310 |
| Error | 9363.2 | 40 | 234.1 | | |

Aspen 216

| Source | SS | df | MS | F ratio | P > F |
|-------------------------------------|------------|----|-----------|---------|--------|
| <i>Between subjects</i> | | | | | |
| Elevated CO ₂ | 2387.7419 | 1 | 2387.7419 | 1.82 | 0.2068 |
| Error | 13099.9696 | 10 | 1309.9970 | | |
| <i>Within subjects</i> | | | | | |
| Position | 3430.75861 | 4 | 857.6897 | 1.19 | 0.3280 |
| Position × elevated CO ₂ | 3541.0958 | 4 | 1.23 | 1.23 | 0.3122 |
| Error | 28714.4885 | 40 | | | |

Aspen 271

| Source | SS | df | MS | F ratio | P > F |
|-------------------------------------|------------|----|-----------|---------|--------|
| <i>Between subjects</i> | | | | | |
| Elevated CO ₂ | 3738.8513 | 1 | 3738.8513 | 13.65 | 0.0041 |
| Error | 2738.2846 | 10 | 273.8285 | | |
| <i>Within subjects</i> | | | | | |
| Position | 17900 | 4 | 4475.0862 | 6.76 | 0.0003 |
| Position × elevated CO ₂ | 2452.1889 | 4 | 613.0472 | 0.93 | 0.4582 |
| Error | 26466.6755 | 40 | 661.6669 | | |

Aspen 42

| Source | SS | df | MS | F ratio | P > F |
|-------------------------------------|------------|----|-----------|---------|--------|
| <i>Between subjects</i> | | | | | |
| Elevated CO ₂ | 3611.7145 | 1 | 3611.7145 | 1.64 | 0.2293 |
| Error | 22033.0886 | 10 | 2203.3087 | | |
| <i>Within subjects</i> | | | | | |
| Position | 3683.1058 | 4 | 920.7765 | 3.93 | 0.0087 |
| Position × elevated CO ₂ | 197.2112 | 4 | 49.3028 | 0.21 | 0.9310 |
| Error | 9363.2338 | 40 | 234.0808 | | |

Appendix Table 2-2 Repeated measures analysis of variance for the effects of elevated O₃ on wood density of birch, aspen clones 216, 271 and 42. The first table is the combined analysis for all species/clones and the next four tables are the separate analysis for birch, aspen clones 216, 271 and 42, respectively.

All species / clones

| Source | SS | df | MS | F ratio | P > F |
|--|-----------|-----|-----------|---------|---------|
| <i>Between subjects</i> | | | | | |
| Elevated O ₃ | 8558.39 | 1 | 8558.39 | 8.52 | 0.0057 |
| Species | 780333.45 | 3 | 260111.15 | 259.04 | <0.0001 |
| Elevated O ₃ × species | 8238.28 | 3 | 2746.09 | 2.73 | 0.0562 |
| Error | 40165.27 | 40 | 1004.13 | | |
| <i>Within subjects</i> | | | | | |
| Position | 11571.70 | 4 | 2892.93 | 7.80 | <0.0001 |
| Position × elevated O ₃ | 2700.66 | 4 | 675.16 | 1.82 | 0.1272 |
| Position × species | 3011.08 | 12 | 250.92 | 0.68 | 0.7718 |
| Position × elevated O ₃ × species | 11041.68 | 12 | 920.14 | 2.48 | 0.0053 |
| Error | 59304.81 | 160 | 370.66 | | |

Birch

| Source | SS | df | MS | F ratio | P > F |
|------------------------------------|------------|----|------------|---------|--------|
| <i>Between subjects</i> | | | | | |
| Elevated O ₃ | 13041.3811 | 1 | 13041.3811 | 7.52 | 0.0208 |
| Error | 17348.9504 | 10 | 1734.8950 | | |
| <i>Within subjects</i> | | | | | |
| Position | 712.6555 | 4 | 178.1639 | 0.79 | 0.5381 |
| Position × elevated O ₃ | 1366.7048 | 4 | 341.6762 | 1.52 | 0.2158 |
| Error | 9012.5154 | 40 | 225.3129 | | |

Aspen 216

| Source | SS | df | MS | F ratio | P > F |
|------------------------------------|------------|----|-----------|---------|--------|
| <i>Between subjects</i> | | | | | |
| Elevated O ₃ | 17.5774 | 1 | 17.5774 | 0.01 | 0.906 |
| Error | 11971.9014 | 10 | 1197.1901 | | |
| <i>Within subjects</i> | | | | | |
| Position | 6345.5126 | 4 | 1586.3781 | 4.40 | 0.0048 |
| Position × elevated O ₃ | 912.6699 | 4 | 228.1675 | 0.63 | 0.6418 |
| Error | 14414.4350 | 40 | 360.3609 | | |

Aspen 271

| Source | SS | df | MS | F ratio | P > F |
|------------------------------------|------------|----|-----------|---------|--------|
| <i>Between subjects</i> | | | | | |
| Elevated O ₃ | 256.4739 | 1 | 256.4739 | 0.28 | 0.6067 |
| Error | 9081.9448 | 10 | 908.1945 | | |
| <i>Within subjects</i> | | | | | |
| Position | 1660.7411 | 4 | 415.1853 | 0.73 | 0.5750 |
| Position × elevated O ₃ | 7704.3881 | 4 | 1926.0970 | 3.40 | 0.0174 |
| Error | 22661.4965 | 40 | 566.5374 | | |

Aspen 42

| Source | SS | df | MS | F ratio | P > F |
|------------------------------------|------------|----|------------|---------|--------|
| <i>Between subjects</i> | | | | | |
| Elevated O ₃ | 13041.3811 | 1 | 13041.3811 | 7.52 | 0.0208 |
| Error | 17348.9504 | 10 | 1734.8950 | | |
| <i>Within subjects</i> | | | | | |
| Position | 712.6555 | 4 | 178.1639 | 0.79 | 0.5381 |
| Position × elevated O ₃ | 1366.7048 | 4 | 341.6762 | 1.52 | 0.2158 |
| Error | 9012.5154 | 40 | 225.31289 | | |

Appendix Table 2-3 Repeated measures analysis of variance for the effects of elevated CO₂ + O₃ on wood density of birch, aspen clones 216, 271 and 42. The first table is the combined analysis for all species/clones and the next four tables are the separate analysis for birch, aspen clones 216, 271 and 42, respectively.

All species / clones

| Source | SS | df | MS | F ratio | P > F |
|--|-----------|-----|-----------|---------|---------|
| <i>Between subjects</i> | | | | | |
| Elevated CO ₂ + O ₃ | 1716.06 | 1 | 1716.06 | 1.69 | 0.2009 |
| Species/Clones | 737649.83 | 3 | 245883.28 | 242.33 | <0.0001 |
| Elevated CO ₂ + O ₃ × species | 5157.25 | 3 | 1719.08 | 1.69 | 0.1836 |
| Error | 40586.25 | 40 | 1014.67 | | |
| <i>Within subjects</i> | | | | | |
| Position | 26511.75 | 4 | 6627.94 | 16.90 | <0.0001 |
| Position × elevated CO ₂ + O ₃ | 1655.61 | 4 | 413.90 | 1.06 | 0.3805 |
| Position × species/clones | 7671.98 | 12 | 639.33 | 1.63 | 0.0879 |
| Position × elevated CO ₂ + O ₃ × species | 5992.62 | 12 | 499.39 | 1.27 | 0.2390 |
| Error | 62739.45 | 160 | 392.12 | | |

Birch

| Source | SS | df | MS | F ratio | P > F |
|--|------------|----|-----------|---------|--------|
| <i>Between subjects</i> | | | | | |
| Elevated CO ₂ + O ₃ | 3136.5839 | 1 | 3136.5839 | 2.33 | 0.1575 |
| Error | 13436.6039 | 10 | 1343.6604 | | |
| <i>Within subjects</i> | | | | | |
| Position | 2383.1988 | 4 | 595.7997 | 2.58 | 0.0519 |
| Position × elevated CO ₂ + O ₃ | 1266.7706 | 4 | 316.6927 | 1.37 | 0.2613 |
| Error | | 40 | | | |

Aspen 216

| Source | SS | df | MS | F ratio | P > F |
|--|-------------|----|-----------|---------|--------|
| <i>Between subjects</i> | | | | | |
| Elevated CO ₂ + O ₃ | 1317.2695 | 1 | 1317.2695 | 1.1 | 0.3160 |
| Error | 11824.6303 | 10 | 1182.4630 | | |
| <i>Within subjects</i> | | | | | |
| Position | 7937.7747 | 4 | 1984.4437 | 5.56 | 0.0012 |
| Position × elevated CO ₂ + O ₃ | 15555.0811 | 4 | 388.7703 | 1.09 | 0.3751 |
| Error | 14283.05233 | 40 | 357.0763 | | |

Aspen 271

| Source | SS | df | MS | F ratio | P > F |
|--|------------|----|-----------|---------|--------|
| <i>Between subjects</i> | | | | | |
| Elevated CO ₂ + O ₃ | 1535.3192 | 1 | 1535.3192 | 1.15 | 0.3080 |
| Error | 13304.8416 | 10 | 1330.4842 | | |
| <i>Within subjects</i> | | | | | |
| Position | 15566.5145 | 4 | 3891.6286 | 6.22 | 0.0005 |
| Position × elevated CO ₂ + O ₃ | 3944.9211 | 4 | 986.2303 | 1.58 | 0.1991 |
| Error | 25012.8246 | 40 | 625.3206 | | |

Aspen 42

| Source | SS | df | MS | F ratio | P > F |
|--|------------|----|-----------|---------|--------|
| <i>Between subjects</i> | | | | | |
| Elevated CO ₂ + O ₃ | 3136.5839 | 1 | 3136.5839 | 2.33 | 0.1575 |
| Error | 13436.6039 | 10 | 1343.6604 | | |
| <i>Within subjects</i> | | | | | |
| Position | 2383.1988 | 4 | 595.7997 | 2.58 | 0.0519 |
| Position × elevated CO ₂ + O ₃ | 1266.7706 | 4 | 316.6927 | 1.37 | 0.2613 |
| Error | 9241.3493 | 40 | 231.0337 | | |

Appendix Table 3-1 PerMNOVA table for the effects of Species / Forest ecosystem community.
(Permutations = 1,000)

| Source | SS | df | MS | F ratio | P > F |
|----------------------------|---------|----|---------|---------|--------|
| Species / forest ecosystem | 1.24274 | 1 | 1.24274 | 7.7135 | 0.0009 |
| Error | 3.5445 | 22 | 0.16111 | | |
| Total | 4.7872 | 23 | | | |

Appendix Table 3-2 PerMNOVA table for the treatment effects of elevated CO₂ and / or O₃ on fungal community composition in aspen-birch forest ecosystem community only.
(Permutations = 1,000)

| Source | SS | df | MS | F ratio | P > F |
|-------------------|---------|----|---------|---------|--------|
| Treatment Effects | 0.70352 | 3 | 0.2345 | 1.4024 | 0.2567 |
| Error | 1.33775 | 8 | 0.16722 | | |
| Total | 2.04127 | 11 | | | |

Appendix Table 3-3 PerMNOVA table for the treatment effects of elevated CO₂ and / or O₃ on fungal community composition in the aspen clones forest ecosystem community only.
(Permutations = 1,000)

| Source | SS | df | MS | F ratio | P > F |
|-------------------|---------|----|----------|---------|--------|
| Treatment Effects | 0.51922 | 3 | 0.173072 | 1.8535 | 0.1029 |
| Error | 0.74702 | 8 | 0.093377 | | |
| Total | 1.26624 | 11 | | | |

Appendix Table 3-4 Split-split-plot ANOVA table for the effects of elevated CO₂ on percentage wood density loss in birch and aspen clones 42 and 271. Data were arcsine transformed.

| Source | SS | df | MS | F ratio | P > F |
|--|--------|----|--------|---------|--------|
| Elevated CO ₂ | 0.0037 | 1 | 0.0037 | 1.02 | 0.4192 |
| Block | 0.0066 | 2 | 0.0033 | 0.90 | 0.5257 |
| Error - main | 0.0073 | 2 | 0.0037 | | |
| Species | 0.0286 | 2 | 0.0143 | 5.36 | 0.0334 |
| Elevated CO ₂ × Species | 0.0037 | 2 | 0.0019 | 0.70 | 0.5257 |
| Error - subplot | 0.0214 | 8 | 0.0027 | | |
| Source of wood | 0.0146 | 3 | 0.0049 | 1.23 | 0.3115 |
| Elevated CO ₂ × Source | 0.0111 | 3 | 0.0037 | 0.93 | 0.4345 |
| Species × Source of wood | 0.0035 | 6 | 0.0006 | 0.15 | 0.9887 |
| Elevated CO ₂ × Species × Source of wood | 0.0459 | 6 | 0.0076 | 1.93 | 0.1019 |
| Error - sub - subplots | 0.1423 | 36 | 0.0039 | | |
| Total | 0.2888 | 71 | | | |

Appendix Table 3-5 Split-split-plot ANOVA table for the effects of elevated O₃ on percentage wood density loss in birch and aspen clones 42 and 271. Data were arcsine transformed.

| Source | SS | df | MS | F ratio | P > F |
|--|--------|----|--------|---------|--------|
| Elevated O ₃ | 0.0009 | 1 | 0.0009 | 0.97 | 0.4280 |
| Block | 0.0231 | 2 | 0.0116 | 12.68 | 0.0731 |
| Error - main | 0.0018 | 2 | 0.0009 | | |
| Species | 0.0175 | 2 | 0.0088 | 3.24 | 0.0929 |
| Elevated O ₃ × Species | 0.0001 | 2 | 0.0001 | 0.02 | 0.9765 |
| Error - subplot | 0.0216 | 8 | 0.0027 | | |
| Source | 0.0013 | 3 | 0.0004 | 0.13 | 0.9391 |
| Elevated O ₃ × Source | 0.0016 | 3 | 0.0005 | 0.17 | 0.9147 |
| Species × Source | 0.0136 | 6 | 0.0023 | 0.71 | 0.6447 |
| Elevated O ₃ × Species × Source | 0.0171 | 6 | 0.0028 | 0.89 | 0.5113 |
| Error - sub - subplots | 0.1149 | 36 | 0.0032 | | |
| Total | 0.2136 | 71 | | | |

Appendix Table 3-6 Split-split-plot ANOVA table for the effects of elevated CO₂ + O₃ on percentage wood density loss in birch and aspen clones 42 and 271. Data were arcsine transformed.

| Source | SS | df | MS | F ratio | P > F |
|--|---------|----|---------|---------|--------|
| Elevated CO ₂ + O ₃ | 0.00001 | 1 | 0.00001 | 0.00 | 0.9525 |
| Block | 0.0069 | 2 | 0.0034 | 1.08 | 0.4802 |
| Error - main | 0.0064 | 2 | 0.0032 | | |
| Species | 0.0444 | 2 | 0.0222 | 7.23 | 0.0161 |
| Elevated CO ₂ + O ₃ × Species | 0.0047 | 2 | 0.0024 | 0.77 | 0.4933 |
| Error - subplot | 0.0246 | 8 | 0.0031 | | |
| Source | 0.0006 | 3 | 0.0002 | 0.05 | 0.9843 |
| Elevated CO ₂ + O ₃ × Source | 0.0013 | 3 | 0.0004 | 0.11 | 0.9552 |
| Species × Source | 0.0136 | 6 | 0.0023 | 0.57 | 0.7490 |
| Elevated CO ₂ + O ₃ × Species × Source | 0.0129 | 6 | 0.0022 | 0.55 | 0.7702 |
| Error - sub - subplots | 0.1422 | 36 | 0.0039 | | |
| Total | 0.2575 | 71 | | | |

Appendix Table 4-1 One way- ANOVA table for the effects of species / clone on wood anatomical properties in aspen 8, 42, 216, 271, birch and maple. Data was pooled across all treatments, n = 12

Growth ring width

| Source | SS | df | MS | F ratio | P > F |
|-----------------|-------------|----|-------------|---------|---------|
| Species / clone | 50013018.62 | 5 | 10002603.72 | 11.85 | <0.0001 |
| Block | 1756517.68 | 2 | 878258.84 | 1.04 | 0.3592 |
| Error | 54023904.9 | 64 | 844123.5 | | |
| Total | 105793441.2 | 71 | | | |

Fiber length

| Source | SS | df | MS | <i>F</i> ratio | <i>P</i> > <i>F</i> |
|-----------------|-------------|----|------------|-------------------|---------------------|
| Species / clone | 1346239.509 | 5 | 269247.902 | 58.63 | <0.0001 |
| Block | 29083.588 | 2 | 14541.794 | 3.17 | 0.0488 |
| Error | 293927.967 | 64 | 4592.624 | | |
| Total | 1669251.064 | 71 | | | |

Fiber lumen diameter

| Source | SS | df | MS | <i>F</i> ratio | <i>P</i> > <i>F</i> |
|-----------------|-------------|----|------------|-------------------|---------------------|
| Species / clone | 240.7386831 | 5 | 48.1477366 | 90.97 | <0.0001 |
| Block | 1.6925578 | 2 | 0.8462789 | 1.60 | 0.2101 |
| Error | 33.8741078 | 64 | 0.5292829 | | |
| Total | 276.3053487 | 71 | | | |

Vessel lumen area proportion

| Source | SS | df | MS | <i>F</i> ratio | <i>P</i> > <i>F</i> |
|-----------------|------------|----|------------|-------------------|---------------------|
| Species / clone | 0.16127893 | 5 | 0.03225579 | 95.64 | <0.0001 |
| Block | 0.00181144 | 2 | 0.00090572 | 2.69 | 0.0759 |
| Error | 0.02158472 | 64 | 0.00033726 | | |
| Total | 0.18467508 | 71 | | | |

Number of vessels per square millimeter

| Source | SS | df | MS | <i>F</i> ratio | <i>P</i> > <i>F</i> |
|-----------------|-------------|----|------------|-------------------|---------------------|
| Species / clone | 48188.74940 | 5 | 9637.74988 | 36.45 | <0.0001 |
| Block | 1454.61382 | 2 | 727.30691 | 2.75 | 0.0714 |
| Error | 16921.12976 | 64 | 264.39265 | | |
| Total | 66564.49298 | 71 | | | |

Cell wall area proportion

| Source | SS | df | MS | <i>F</i> ratio | <i>P</i> > <i>F</i> |
|-----------------|------------|----|------------|-------------------|---------------------|
| Species / clone | 0.19949187 | 5 | 0.03989837 | 63.18 | <0.0001 |
| Block | 0.00060859 | 2 | 0.00030429 | 0.48 | 0.6199 |
| Error | 0.04041572 | 64 | 0.00063150 | | |
| Total | 0.24051619 | 71 | | | |

Vessel lumen diameter (pooled data for inner, middle and periphery)

| Source | SS | df | MS | <i>F</i> ratio | <i>P</i> > <i>F</i> |
|-----------------|-------------|----|------------|-------------------|---------------------|
| Species / clone | 1077.566169 | 5 | 215.513234 | 18.63 | <0.0001 |
| Block | 74.162469 | 2 | 37.081234 | 3.21 | 0.0471 |
| Error | 740.267434 | 64 | 11.566679 | | |
| Total | 1891.996073 | 71 | | | |

Appendix Table 4-2 Split-plot ANOVA table for the effects of elevated CO₂ and species on wood anatomical properties:

Growth ring width

| Source | SS | df | MS | <i>F</i> ratio | <i>P</i> > <i>F</i> |
|------------------------------------|-------------|----|-------------|-------------------|---------------------|
| Elevated CO ₂ | 1915001.998 | 1 | 1915001.998 | 3.46 | 0.2037 |
| <i>Error - main</i> | 1105357.03 | 2 | 552678.51 | | |
| Block | 297419.57 | 2 | 148709.78 | 0.30 | 0.7447 |
| Species | 27576032.57 | 5 | 5515206.51 | 11.10 | <0.0001 |
| Elevated CO ₂ × Species | 2673529.40 | 5 | 534705.88 | 1.08 | 0.4032 |
| <i>Error - subplot</i> | 9939489.69 | 20 | 496974.48 | | |
| Total | 43506830.25 | 35 | | | |

Fiber length

| Source | SS | df | MS | <i>F</i> ratio | <i>P</i> > <i>F</i> |
|------------------------------------|-------------|----|-------------|-------------------|---------------------|
| Elevated CO ₂ | 59.25649420 | 1 | 59.25649420 | 0.20 | 0.6984 |
| <i>Error - main</i> | 592.3726 | 2 | 296.1863 | | |
| Block | 14226.8380 | 2 | 7113.4190 | 1.63 | 0.2215 |
| Species | 681630.0946 | 5 | 136326.0189 | 31.18 | <0.0001 |
| Elevated CO ₂ × Species | 29234.2741 | 5 | 5846.8548 | 1.34 | 0.2893 |
| <i>Error - subplot</i> | 87456.5597 | 20 | 4372.8280 | | |
| Total | 813199.3955 | 35 | | | |

Fiber lumen diameter

| Source | SS | df | MS | F ratio | P > F |
|------------------------------------|-------------|----|------------|---------|---------|
| Elevated CO ₂ | 0.13622690 | 1 | 0.13622690 | 1.08 | 0.4079 |
| Error - main | 0.2523379 | 2 | 0.1261689 | | |
| Block | 0.0680002 | 2 | 0.0340001 | 0.06 | 0.9443 |
| Species | 117.6354272 | 5 | 23.5270854 | 39.76 | <0.0001 |
| Elevated CO ₂ × Species | 5.5281032 | 5 | 1.1056206 | 1.87 | 0.1452 |
| Error - subplot | 11.8341141 | 20 | 0.5917057 | | |
| Total | 135.4542094 | 35 | | | |

Vessel lumen area proportion

| Source | SS | df | MS | F ratio | P > F |
|------------------------------------|--------------|----|--------------|---------|---------|
| Elevated CO ₂ | 3.1660226E-6 | 1 | 3.1660226E-6 | 0.00 | 0.9547 |
| Error - main | 0.00154255 | 2 | 0.00077127 | | |
| Block | 0.00066202 | 2 | 0.00033101 | 1.17 | 0.3309 |
| Species | 0.07517757 | 5 | 0.01503551 | 53.11 | <0.0001 |
| Elevated CO ₂ × Species | 0.00218211 | 5 | 0.00043642 | 1.54 | 0.2219 |
| Error - subplot | 0.00566159 | 20 | 0.00028308 | | |
| Total | 0.08522900 | 35 | | | |

Number of vessels per square millimeter

| Source | SS | df | MS | F ratio | P > F |
|------------------------------------|-------------|----|-------------|---------|---------|
| Elevated CO ₂ | 596.7379252 | 1 | 596.7379252 | 11.49 | 0.0771 |
| Error - main | 103.85931 | 2 | 51.92965 | | |
| Block | 372.28963 | 2 | 186.14482 | 0.64 | 0.5359 |
| Species | 23550.66703 | 5 | 4710.13341 | 16.29 | <0.0001 |
| Elevated CO ₂ × Species | 1487.03955 | 5 | 297.40791 | 1.03 | 0.4278 |
| Error - subplot | 5784.54806 | 20 | 289.22740 | | |
| Total | 31895.14150 | 35 | | | |

Cell wall area proportion

| Source | SS | df | MS | F ratio | P > F |
|------------------------------------|------------|----|------------|---------|---------|
| Elevated CO ₂ | 0.00060184 | 1 | 0.00060184 | 2.76 | 0.2383 |
| Error - main | 0.00043554 | 2 | 0.00021777 | | |
| Block | 0.00137066 | 2 | 0.00068533 | 1.06 | 0.3645 |
| Species | 0.11589780 | 5 | 0.02317956 | 35.91 | <0.0001 |
| Elevated CO ₂ × Species | 0.00412289 | 5 | 0.00082458 | 1.28 | 0.3123 |
| Error - subplot | 0.01290922 | 20 | 0.00064546 | | |
| Total | 0.13533794 | 35 | | | |

Vessel lumen diameter (pooled data for inner, middle and periphery)

| Source | SS | df | MS | F ratio | P > F |
|------------------------------------|-------------|----|-------------|---------|---------|
| Elevated CO ₂ | 16.89415275 | 1 | 16.89415275 | 0.98 | 0.4263 |
| Error - main | 34.4354912 | 2 | 17.2177456 | | |
| Block | 33.1948411 | 2 | 16.5974206 | 1.52 | 0.2424 |
| Species | 493.1804286 | 5 | 98.6360857 | 9.05 | <0.0001 |
| Elevated CO ₂ × Species | 48.6282423 | 5 | 9.7256485 | 0.89 | 0.5049 |
| Error - subplot | 218.0120680 | 20 | 10.9006034 | | |
| Total | 844.3452241 | 35 | | | |

Vessel lumen diameter (Inner region only)

| Source | SS | df | MS | F ratio | P > F |
|------------------------------------|-------------|----|-------------|---------|--------|
| Elevated CO ₂ | 3.15029245 | 1 | 3.15029245 | 0.08 | 0.8092 |
| Error - main | 83.3582191 | 2 | 41.6791095 | | |
| Block | 6.8249658 | 2 | 3.4124829 | 0.24 | 0.7899 |
| Species | 550.3530758 | 5 | 110.0706152 | 7.70 | 0.0004 |
| Elevated CO ₂ × Species | 75.4546616 | 5 | 15.0909323 | 1.06 | 0.4137 |
| Error - subplot | 286.010163 | 20 | 14.300508 | | |
| Total | 1005.151378 | 35 | | | |

Vessel lumen diameter (middle region only)

| Source | SS | df | MS | F ratio | P > F |
|------------------------------------|-------------|----|-------------|---------|--------|
| Elevated CO ₂ | 16.45057629 | 1 | 16.45057629 | 0.98 | 0.4274 |
| Error - main | 33.7271525 | 2 | 16.8635763 | | |
| Block | 54.4681425 | 2 | 27.2340712 | 1.92 | 0.1727 |
| Species | 558.7392871 | 5 | 111.7478574 | 7.88 | 0.0003 |
| Elevated CO ₂ × Species | 56.7135057 | 5 | 11.3427011 | 0.80 | 0.5630 |
| Error - subplot | 283.721309 | 20 | 14.186065 | | |
| Total | 1003.819973 | 35 | | | |

Vessel lumen diameter (Periphery region only)

| Source | SS | df | MS | F ratio | P > F |
|------------------------------------|-------------|----|-------------|---------|--------|
| Elevated CO ₂ | 42.24885382 | 1 | 42.24885382 | 6.74 | 0.1218 |
| Error - main | 12.5325876 | 2 | 6.2662938 | | |
| Block | 53.5759688 | 2 | 26.7879844 | 2.61 | 0.0982 |
| Species | 401.9100276 | 5 | 80.3820055 | 7.84 | 0.0003 |
| Elevated CO ₂ × Species | 31.8247330 | 5 | 6.3649466 | 0.62 | 0.6857 |
| Error - subplot | 205.1214461 | 20 | 10.2560723 | | |
| Total | 747.2136170 | 35 | | | |

Appendix Table 4-3 Split-split-plot ANOVA table for the effects of elevated CO₂, species and position on vessel lumen diameter

| Source | SS | df | MS | F ratio | P > F |
|---|-------------|-----|-------------|---------|---------|
| Elevated CO ₂ | 50.68245825 | 1 | 50.68245825 | 0.98 | 0.4263 |
| Block | 99.58452340 | 2 | 49.79226170 | 0.96 | 0.5092 |
| Error - main | 103.306474 | 2 | 51.653237 | | |
| Species | 1479.541286 | 5 | 295.908257 | 9.05 | 0.0001 |
| Elevated CO ₂ × Species | 145.884727 | 5 | 29.176945 | 0.89 | 0.5049 |
| Error - subplot | 654.036204 | 20 | 32.701810 | | |
| Radial Position | 1362.947315 | 2 | 681.473657 | 201.40 | <0.0001 |
| Elevated CO ₂ × Position | 11.167264 | 2 | 5.583632 | 1.65 | 0.2027 |
| Species × Position | 31.461105 | 10 | 3.146110 | 0.93 | 0.5148 |
| Elevated CO ₂ × Species × Position | 18.108173 | 10 | 1.810817 | 0.54 | 0.8565 |
| Error - sub - subplots | 162.412753 | 48 | 3.383599 | | |
| Total | 4119.132282 | 107 | | | |

Appendix Table 4-4 Split-plot ANOVA table for the effects of elevated O₃ and species on wood anatomical properties:

Growth ring width

| Source | SS | df | MS | F ratio | P > F |
|-----------------------------------|-------------|----|-------------|---------|---------|
| Elevated O ₃ | 115411.7545 | 1 | 115411.7545 | 0.23 | 0.6773 |
| Error – main | 992719.66 | 2 | 496359.83 | | |
| Block | 1421607.05 | 2 | 710803.53 | 1.56 | 0.2339 |
| Species | 31057534.12 | 5 | 6211506.82 | 13.67 | <0.0001 |
| Elevated O ₃ × Species | 6164252.75 | 5 | 1232850.55 | 2.71 | 0.0499 |
| Error – subplot | 9090727.24 | 20 | 454536.36 | | |
| Total | 48842252.58 | 35 | | | |

Fiber length

| Source | SS | df | MS | F ratio | P > F |
|-----------------------------------|-------------|----|-------------|---------|--------|
| Elevated O ₃ | 24209.24322 | 1 | 24209.24322 | 5.32 | 0.1475 |
| Error – main | 9102.0491 | 2 | 4551.0245 | | |
| Block | 29594.3439 | 2 | 14797.1720 | 3.68 | 0.0435 |
| Species | 633124.5236 | 5 | 126624.9047 | 31.52 | <.0001 |
| Elevated O ₃ × Species | 34147.4396 | 5 | 6829.4879 | 1.70 | 0.1807 |
| Error – subplot | 80353.1410 | 20 | 4017.6570 | | |
| Total | 810530.7405 | 35 | | | |

Fiber lumen diameter

| Source | SS | df | MS | F ratio | P > F |
|-----------------------------------|-------------|----|------------|---------|---------|
| Elevated O ₃ | 0.05156205 | 1 | 0.05156205 | 0.15 | 0.7363 |
| Error – main | 0.6897966 | 2 | 0.3448983 | | |
| Block | 0.3474790 | 2 | 0.1737395 | 0.31 | 0.7342 |
| Species | 113.1835066 | 5 | 22.6367013 | 40.88 | <0.0001 |
| Elevated O ₃ × Species | 3.1712808 | 5 | 0.6342562 | 1.15 | 0.3695 |
| Error – subplot | 11.0746509 | 20 | 0.5537325 | | |
| Total | 128.5182760 | 35 | | | |

Vessel lumen area proportion

| Source | SS | df | MS | F ratio | P > F |
|-----------------------------------|------------|----|------------|---------|---------|
| Elevated O ₃ | 0.00007193 | 1 | 0.00007193 | 0.60 | 0.5205 |
| Error – main | 0.00024093 | 2 | 0.00012046 | | |
| Block | 0.00009430 | 2 | 0.00004715 | 0.17 | 0.8430 |
| Species | 0.07802007 | 5 | 0.01560401 | 57.02 | <0.0001 |
| Elevated O ₃ × Species | 0.00318975 | 5 | 0.00063795 | 2.33 | 0.0803 |
| Error – subplot | 0.00547357 | 20 | 0.00027368 | | |
| Total | 0.08709054 | 35 | | | |

Number of vessels per square millimeter

| Source | SS | df | MS | F ratio | P > F |
|-----------------------------------|-------------|----|-------------|---------|---------|
| Elevated O ₃ | 24.70870342 | 1 | 24.70870342 | 0.17 | 0.7185 |
| Error – main | 287.07978 | 2 | 143.53989 | | |
| Block | 228.15066 | 2 | 114.07533 | 0.86 | 0.4393 |
| Species | 20965.49771 | 5 | 4193.09954 | 31.52 | <0.0001 |
| Elevated O ₃ × Species | 1014.46453 | 5 | 202.89291 | 1.52 | 0.2268 |
| Error – subplot | 2660.94742 | 20 | 133.04737 | | |
| Total | 25180.84880 | 35 | | | |

Cell wall area proportion

| Source | SS | df | MS | F ratio | P > F |
|-----------------------------------|------------|----|------------|---------|---------|
| Elevated O ₃ | 0.00044672 | 1 | 0.00044672 | 1.10 | 0.4049 |
| Error – main | 0.00081465 | 2 | 0.00040733 | | |
| Block | 0.00006165 | 2 | 0.00003082 | 0.05 | 0.9524 |
| Species | 0.11296340 | 5 | 0.02259268 | 35.86 | <0.0001 |
| Elevated O ₃ × Species | 0.00894562 | 5 | 0.00178912 | 2.84 | 0.0427 |
| Error – subplot | 0.01260049 | 20 | 0.00063002 | | |
| Total | 0.13583254 | 35 | | | |

Vessel lumen diameter (pooled data for inner, middle and periphery)

| Source | SS | df | MS | F ratio | P > F |
|-----------------------------------|-------------|----|-------------|---------|---------|
| Elevated O ₃ | 45.97381877 | 1 | 45.97381877 | 6.22 | 0.1301 |
| Error – main | 14.7758245 | 2 | 7.3879122 | | |
| Block | 86.1608060 | 2 | 43.0804030 | 6.05 | 0.0088 |
| Species | 555.9213366 | 5 | 111.1842673 | 15.62 | <0.0001 |
| Elevated O ₃ × Species | 93.6798614 | 5 | 18.7359723 | 2.63 | 0.0551 |
| Error – subplot | 142.3813825 | 20 | 7.1190691 | | |
| Total | 938.8930298 | 35 | | | |

Vessel lumen diameter (Inner region only)

| Source | SS | df | MS | F ratio | P > F |
|-----------------------------------|-------------|----|-------------|---------|---------|
| Elevated O ₃ | 9.62621580 | 1 | 9.62621580 | 0.51 | 0.5485 |
| Error – main | 37.5986121 | 2 | 18.7993061 | | |
| Block | 71.7023048 | 2 | 35.8511524 | 3.92 | 0.0366 |
| Species | 516.7942258 | 5 | 103.3588452 | 11.31 | <0.0001 |
| Elevated O ₃ × Species | 97.2365044 | 5 | 19.4473009 | 2.13 | 0.1040 |
| Error – subplot | 182.8153828 | 20 | 9.1407691 | | |
| Total | 915.7732456 | 35 | | | |

Vessel lumen diameter (middle region only)

| Source | SS | df | MS | F ratio | P > F |
|-----------------------------------|-------------|----|-------------|---------|---------|
| Elevated O ₃ | 74.31620230 | 1 | 74.31620230 | 6.20 | 0.1304 |
| Error – main | 23.9540411 | 2 | 11.9770206 | | |
| Block | 88.5148392 | 2 | 44.2574196 | 4.91 | 0.0184 |
| Species | 762.0741029 | 5 | 152.4148206 | 16.90 | <0.0001 |
| Elevated O ₃ × Species | 151.2054427 | 5 | 30.2410885 | 3.35 | 0.0232 |
| Error – subplot | 180.330752 | 20 | 9.016538 | | |
| Total | 1280.395380 | 35 | | | |

Vessel lumen diameter (Periphery region only)

| Source | SS | df | MS | F ratio | P > F |
|-----------------------------------|-------------|----|-------------|---------|---------|
| Elevated O ₃ | 74.26819867 | 1 | 74.26819867 | 387.58 | 0.0026 |
| Error – main | 0.3832357 | 2 | 0.1916179 | | |
| Block | 102.0359390 | 2 | 51.0179695 | 7.25 | 0.0043 |
| Species | 433.2955141 | 5 | 86.6591028 | 12.31 | <0.0001 |
| Elevated O ₃ × Species | 66.6754686 | 5 | 13.3350937 | 1.89 | 0.1404 |
| Error – subplot | 140.8035449 | 20 | 7.0401772 | | |
| Total | 817.4619010 | 35 | | | |

Appendix Table 4-5 Split-split-plot ANOVA table for the effects of elevated O₃, species and radial position on vessel lumen diameter

| Source | SS | df | MS | F ratio | P > F |
|--|-------------|-----|-------------|---------|---------|
| Elevated O ₃ | 137.9214563 | 1 | 137.9214563 | 6.22 | 0.1301 |
| Block | 258.4824180 | 2 | 129.2412090 | 5.83 | 0.1464 |
| Error – main | 44.327473 | 2 | 22.163737 | | |
| Species | 1667.764010 | 5 | 333.552802 | 15.62 | <0.0001 |
| Elevated O ₃ × Species | 281.039584 | 5 | 56.207917 | 2.63 | 0.0551 |
| Error – subplot | 427.144147 | 20 | 21.357207 | | |
| Radial Position | 1299.019381 | 2 | 649.509691 | 317.53 | <0.0001 |
| Elevated O ₃ × Position | 20.289160 | 2 | 10.144580 | 4.96 | 0.0110 |
| Species × Position | 44.399833 | 10 | 4.439983 | 2.17 | 0.0363 |
| Elevated O ₃ × Species × Position | 34.077831 | 10 | 3.407783 | 1.67 | 0.1168 |
| Error - sub - subplots | 98.184612 | 48 | 2.045513 | | |
| Total | 4312.649908 | 107 | | | |

Appendix Table 4-6 Split-plot ANOVA table for the effects of elevated CO₂ + O₃ and species on wood anatomical properties:

Growth ring width

| Source | SS | df | MS | F ratio | P > F |
|---|-------------|----|-------------|---------|--------|
| Elevated CO ₂ + O ₃ | 22176.58727 | 1 | 22176.58727 | 0.07 | 0.8186 |
| Error – main | 651629.17 | 2 | 325814.59 | | |
| Block | 2010146.24 | 2 | 1005073.12 | 1.38 | 0.2735 |
| Species | 18353420.26 | 5 | 3670684.05 | 5.06 | 0.0037 |
| Elevated CO ₂ + O ₃ × Species | 7177465.14 | 5 | 1435493.03 | 1.98 | 0.1262 |
| Error – subplot | 14521761.85 | 20 | 726088.09 | | |
| Total | 42736599.26 | 35 | | | |

Fiber length

| Source | SS | df | MS | F ratio | P > F |
|---|-------------|----|-------------|---------|---------|
| Elevated CO ₂ + O ₃ | 1180.045594 | 1 | 1180.045594 | 0.81 | 0.4639 |
| Error – main | 2925.7160 | 2 | 1462.8580 | | |
| Block | 3607.0407 | 2 | 1803.5203 | 0.44 | 0.6494 |
| Species | 967457.9775 | 5 | 193491.5955 | 47.32 | <0.0001 |
| Elevated CO ₂ + O ₃ × Species | 16714.6614 | 5 | 3342.9323 | 0.82 | 0.5513 |
| Error – subplot | 81771.642 | 20 | 4088.582 | | |
| Total | 1073657.083 | 35 | | | |

Fiber lumen diameter

| Source | SS | df | MS | F ratio | P > F |
|---|-------------|----|------------|---------|---------|
| Elevated CO ₂ + O ₃ | 0.01311254 | 1 | 0.01311254 | 0.02 | 0.8915 |
| Error – main | 1.1011001 | 2 | 0.5505500 | | |
| Block | 0.7296098 | 2 | 0.3648049 | 0.58 | 0.5669 |
| Species | 121.7715224 | 5 | 24.3543045 | 38.99 | <0.0001 |
| Elevated CO ₂ + O ₃ × Species | 2.0430645 | 5 | 0.4086129 | 0.65 | 0.6619 |
| Error – subplot | 12.4927489 | 20 | 0.6246374 | | |
| Total | 138.1511582 | 35 | | | |

Vessel lumen area proportion

| Source | SS | df | MS | F ratio | P > F |
|---|------------|----|------------|---------|---------|
| Elevated CO ₂ + O ₃ | 0.00011704 | 1 | 0.00011704 | 0.24 | 0.6704 |
| Error – main | 0.00096060 | 2 | 0.00048030 | | |
| Block | 0.00167510 | 2 | 0.00083755 | 3.64 | 0.0447 |
| Species | 0.09891593 | 5 | 0.01978319 | 86.09 | <0.0001 |
| Elevated CO ₂ + O ₃ × Species | 0.00277291 | 5 | 0.00055458 | 2.41 | 0.0724 |
| Error – subplot | 0.00459616 | 20 | 0.00022981 | | |
| Total | 0.10903773 | 35 | | | |

Number of vessels per square millimeter

| Source | SS | df | MS | F ratio | P > F |
|---|-------------|----|------------|---------|---------|
| Elevated CO ₂ + O ₃ | 3.58103069 | 1 | 3.58103069 | 0.02 | 0.9109 |
| Error – main | 447.31273 | 2 | 223.65636 | | |
| Block | 1900.11650 | 2 | 950.05825 | 4.09 | 0.0324 |
| Species | 29194.19636 | 5 | 5838.83927 | 25.15 | <0.0001 |
| Elevated CO ₂ + O ₃ × Species | 1071.80029 | 5 | 214.36006 | 0.92 | 0.4863 |
| Error – subplot | 4642.73700 | 20 | 232.13685 | | |
| Total | 37259.74391 | 35 | | | |

Cell wall area proportion

| Source | SS | df | MS | F ratio | P > F |
|---|------------|----|------------|---------|---------|
| Elevated CO ₂ + O ₃ | 0.00017088 | 1 | 0.00017088 | 2.26 | 0.2714 |
| Error – main | 0.00015101 | 2 | 0.00007551 | | |
| Block | 0.00094484 | 2 | 0.00047242 | 0.78 | 0.4711 |
| Species | 0.12939285 | 5 | 0.02587857 | 42.83 | <0.0001 |
| Elevated CO ₂ + O ₃ × Species | 0.00572550 | 5 | 0.00114510 | 1.90 | 0.1403 |
| Error – subplot | 0.01208523 | 20 | 0.00060426 | | |
| Total | 0.14847031 | 35 | | | |

Vessel lumen diameter (pooled data for inner, middle and periphery)

| Source | SS | df | MS | F ratio | P > F |
|---|-------------|----|-------------|---------|---------|
| Elevated CO ₂ + O ₃ | 8.96092945 | 1 | 8.96092945 | 0.93 | 0.4374 |
| Error – main | 19.3469839 | 2 | 9.6734920 | | |
| Block | 32.4635461 | 2 | 16.2317731 | 1.65 | 0.2165 |
| Species | 520.7613237 | 5 | 104.1522647 | 10.61 | <0.0001 |
| Elevated CO ₂ + O ₃ × Species | 47.3827950 | 5 | 9.4765590 | 0.97 | 0.4622 |
| Error – subplot | 196.3166421 | 20 | 9.8158321 | | |
| Total | 825.2322203 | 35 | | | |

Vessel lumen diameter (Inner region only)

| Source | SS | df | MS | F ratio | P > F |
|---|-------------|----|-------------|---------|--------|
| Elevated CO ₂ + O ₃ | 0.01957684 | 1 | 0.01957684 | 0.00 | 0.9747 |
| Error – main | 30.6826275 | 2 | 15.3413138 | | |
| Block | 26.8404548 | 2 | 13.4202274 | 0.98 | 0.3922 |
| Species | 556.0613906 | 5 | 111.2122781 | 8.13 | 0.0003 |
| Elevated CO ₂ + O ₃ × Species | 51.7797785 | 5 | 10.3559557 | 0.76 | 0.5909 |
| Error – subplot | 273.5292773 | 20 | 13.6764639 | | |
| Total | 938.9131056 | 35 | | | |

Vessel lumen diameter (middle region only)

| Source | SS | df | MS | F ratio | P > F |
|---|-------------|----|-------------|---------|---------|
| Elevated CO ₂ + O ₃ | 22.70821440 | 1 | 22.70821440 | 3.15 | 0.2182 |
| Error – main | 14.4399064 | 2 | 7.2199532 | | |
| Block | 45.2665346 | 2 | 2.6332673 | 2.06 | 0.1541 |
| Species | 638.2534235 | 5 | 127.6506847 | 11.60 | <0.0001 |
| Elevated CO ₂ + O ₃ × Species | 58.4616720 | 5 | 11.6923344 | 1.06 | 0.4102 |
| Error – subplot | 220.1402112 | 20 | 11.0070106 | | |
| Total | 999.2699620 | 35 | | | |

Vessel lumen diameter (Periphery region only)

| Source | SS | df | MS | F ratio | P > F |
|---|-------------|----|-------------|---------|--------|
| Elevated CO ₂ + O ₃ | 18.96643498 | 1 | 18.96643498 | 1.93 | 0.2989 |
| Error – main | 19.6210651 | 2 | 9.8105326 | | |
| Block | 39.5350792 | 2 | 19.7675396 | 2.16 | 0.1411 |
| Species | 405.3383229 | 5 | 81.0676646 | 8.87 | 0.0001 |
| Elevated CO ₂ + O ₃ × Species | 43.0023154 | 5 | 8.6004631 | 0.94 | 0.4761 |
| Error – subplot | 182.7750984 | 20 | 9.1387549 | | |
| Total | 709.2383159 | 35 | | | |

Appendix Table 4-7 Split-split-plot ANOVA table for the effects of elevated CO₂ + O₃, species and radial position on vessel lumen diameter

| Source | SS | df | MS | F ratio | P > F |
|--|-------------|-----|-------------|---------|---------|
| Elevated CO ₂ + O ₃ | 26.88278835 | 1 | 26.88278835 | 0.93 | 0.4374 |
| Block | 97.39063832 | 2 | 48.69531916 | 1.68 | 0.3734 |
| Error – main | 58.040952 | 2 | 29.020476 | | |
| Species | 1562.283971 | 5 | 312.456794 | 10.61 | <0.0001 |
| Elevated CO ₂ + O ₃ × Species | 142.148385 | 5 | 28.429677 | 0.97 | 0.4622 |
| Error – subplot | 588.949926 | 20 | 29.447496 | | |
| Radial Position | 1259.411159 | 2 | 629.705579 | 278.71 | <0.0001 |
| Elevated CO ₂ + O ₃ × Position | 14.811438 | 2 | 7.405719 | 3.28 | 0.0463 |
| Species × Position | 37.369166 | 10 | 3.736917 | 1.65 | 0.1200 |
| Elevated CO ₂ + O ₃ × Species × Position | 11.095381 | 10 | 1.109538 | 0.49 | 0.8875 |
| Error - sub - subplots | 108.448738 | 48 | 2.259349 | | |
| Total | 3906.832542 | 107 | | | |

Appendix Table 4-8 One way- ANOVA table for the effects of elevated O₃ on growth ring width in aspen 8, 42, 216, 271, birch and maple.

Aspen 8

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|-------------|----|-------------|---------|--------|
| Elevated O ₃ | 4806166.707 | 1 | 4806166.707 | 3.83 | 0.1894 |
| Block | 3092280.317 | 2 | 1546140.158 | 1.23 | 0.4479 |
| Error | 2508765.20 | 2 | 1254382.60 | | |
| Total | 10407212.22 | 5 | | | |

Aspen 42

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|-------------|----|-------------|---------|--------|
| Elevated O ₃ | 177354.8500 | 1 | 177354.8500 | 0.99 | 0.4252 |
| Block | 675271.9554 | 2 | 337635.9777 | 1.88 | 0.3474 |
| Error | 359515.798 | 2 | 179757.899 | | |
| Total | 1212142.603 | 5 | | | |

Aspen 216

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|-------------|----|------------|---------|--------|
| Elevated O ₃ | 698767.616 | 1 | 698767.616 | 1.90 | 0.3022 |
| Block | 1449557.147 | 2 | 724778.573 | 1.97 | 0.3368 |
| Error | 736113.296 | 2 | 368056.648 | | |
| Total | 2884438.059 | 5 | | | |

Aspen 271

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|-------------|----|-------------|---------|--------|
| Elevated O ₃ | 4205.92441 | 1 | 4205.92441 | 0.02 | 0.9125 |
| Block | 99314.51808 | 2 | 49657.25904 | 0.18 | 0.8460 |
| Error | 545487.1846 | 2 | | | |
| Total | 649007.6271 | 5 | | | |

Birch

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|-------------|----|------------|---------|--------|
| Elevated O ₃ | 578843.685 | 1 | 578843.685 | 2.41 | 0.2607 |
| Block | 1412604.122 | 2 | 578843.685 | 2.41 | 0.2607 |
| Error | 480084.483 | 2 | 240042.241 | | |
| Total | 2471532.290 | 5 | | | |

Maple

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|-------------|----|-------------|---------|--------|
| Elevated O ₃ | 14325.71949 | 1 | 14325.71949 | 0.21 | 0.6903 |
| Block | 11065.75328 | 2 | 5532.87664 | 0.08 | 0.9242 |
| Error | 134994.1780 | 2 | 67497.0890 | | |
| Total | 160385.6508 | 5 | | | |

Appendix Table 4-9 One way - ANOVA table for the effects of elevated O₃ on vessel lumen area proportion in aspen 8, 42, 216, 271, birch and maple.

Aspen 8

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|------------|----|------------|---------|--------|
| Elevated O ₃ | 0.00052254 | 1 | 0.00052254 | 11.71 | 0.0758 |
| Block | 0.00023106 | 2 | 0.00011553 | 2.59 | 0.2787 |
| Error | 0.00008928 | 2 | 0.00004464 | | |
| Total | 0.00084288 | 5 | | | |

Aspen 42

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|------------|----|------------|---------|--------|
| Elevated O ₃ | 0.00039420 | 1 | 0.00039420 | 2.45 | 0.2583 |
| Block | 0.00004937 | 2 | 0.00002469 | 0.15 | 0.8672 |
| Error | 0.00032229 | 2 | 0.00016114 | | |
| Total | 0.00076586 | 5 | | | |

Aspen 216

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|------------|----|------------|---------|--------|
| Elevated O ₃ | 0.00018022 | 1 | 0.00018022 | 0.67 | 0.4981 |
| Block | 0.00087422 | 2 | 0.00043711 | 1.63 | 0.3797 |
| Error | 0.00053510 | 2 | 0.00026755 | | |
| Total | 0.00158954 | 5 | | | |

Aspen 271

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|------------|----|------------|---------|--------|
| Elevated O ₃ | 0.00150499 | 1 | 0.00150499 | 4.28 | 0.1743 |
| Block | 0.00014839 | 2 | 0.00007419 | 0.21 | 0.8256 |
| Error | 0.00070245 | 2 | 0.00035122 | | |
| Total | 0.00235582 | 5 | | | |

Birch

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|------------|----|------------|---------|--------|
| Elevated O ₃ | 0.00052445 | 1 | 0.00052445 | 0.51 | 0.5508 |
| Block | 0.00038366 | 2 | 0.00019183 | 0.18 | 0.8439 |
| Error | 0.00207490 | 2 | 0.00103745 | | |
| Total | 0.00298301 | 5 | | | |

Maple

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|------------|----|------------|---------|--------|
| Elevated O ₃ | 0.00039420 | 1 | 0.00039420 | 2.45 | 0.2583 |
| Block | 0.00004937 | 2 | 0.00002469 | 0.15 | 0.8672 |
| Error | 0.00032229 | 2 | 0.00016114 | | |
| Total | 0.00076586 | 5 | | | |

Appendix Table 4-10 One way- ANOVA table for the effects of elevated O₃ on cell wall area proportion in aspen 8, 42, 216, 271, birch and maple.

Aspen 8

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|------------|----|------------|---------|--------|
| Elevated O ₃ | 0.00314394 | 1 | 0.00314394 | 6.52 | 0.1252 |
| Block | 0.00023444 | 2 | 0.00011722 | 0.24 | 0.8045 |
| Error | 0.00096469 | 2 | 0.00048235 | | |
| Total | 0.00434308 | 5 | | | |

Aspen 42

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|------------|----|------------|---------|--------|
| Elevated O ₃ | 0.00021245 | 1 | 0.00021245 | 0.31 | 0.6339 |
| Block | 0.00175059 | 2 | 0.00087529 | 1.28 | 0.4394 |
| Error | 0.00137230 | 2 | 0.00068615 | | |
| Total | 0.00333533 | 5 | | | |

Aspen 216

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|------------|----|------------|---------|--------|
| Elevated O ₃ | 0.00074993 | 1 | 0.00074993 | 1.29 | 0.3743 |
| Block | 0.00008390 | 2 | 0.00004195 | 0.07 | 0.9328 |
| Error | 0.00116543 | 2 | 0.00058271 | | |
| Total | 0.00199926 | 5 | | | |

Aspen 271

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|------------|----|------------|---------|--------|
| Elevated O ₃ | 0.00150499 | 1 | 0.00150499 | 4.28 | 0.1743 |
| Block | 0.00014839 | 2 | 0.00007419 | 0.21 | 0.8256 |
| Error | 0.00070245 | 2 | 0.00035122 | | |
| Total | 0.00235582 | 5 | | | |

Birch

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|------------|----|------------|---------|--------|
| Elevated O ₃ | 0.00067364 | 1 | 0.00067364 | 1.44 | 0.3532 |
| Block | 0.00329247 | 2 | 0.00164624 | 3.52 | 0.2214 |
| Error | 0.00093643 | 2 | 0.00046821 | | |
| Total | 0.00490254 | 5 | | | |

Maple

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|------------|----|------------|---------|--------|
| Elevated O ₃ | 0.00000138 | 1 | 0.00000138 | 0.02 | 0.9064 |
| Block | 0.00009835 | 2 | 0.00004918 | 0.63 | 0.6134 |
| Error | 0.00015605 | 2 | 0.00007803 | | |
| Total | 0.00025578 | 5 | | | |

Appendix Table 4-11 One way- ANOVA table for the effects of elevated O₃ on vessel lumen diameter in aspen 8, 42, 216, 271, birch and maple.

Aspen 8

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|-------------|----|-------------|---------|--------|
| Elevated O ₃ | 10.40974732 | 1 | 10.40974732 | 0.83 | 0.4586 |
| Block | 40.16581672 | 2 | 40.16581672 | 1.60 | 0.3846 |
| Error | 25.10313271 | 2 | 12.55156636 | | |
| Total | 75.67869676 | 5 | | | |

Aspen 42

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|-------------|----|------------|---------|--------|
| Elevated O ₃ | 1.48361321 | 1 | 1.48361321 | 0.27 | 0.6559 |
| Block | 19.25832951 | 2 | 9.62916475 | 1.74 | 0.3645 |
| Error | 11.04350592 | 2 | 5.52175296 | | |
| Total | 31.78544864 | 5 | | | |

Aspen 216

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|-------------|----|-------------|---------|--------|
| Elevated O ₃ | 4.26885491 | 1 | 4.26885491 | 0.76 | 0.4757 |
| Block | 26.65026675 | 2 | 13.32513338 | 2.37 | 0.2971 |
| Error | 11.26210137 | 2 | 5.63105068 | | |
| Total | 42.18122303 | 5 | | | |

Aspen 271

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|------------|----|------------|---------|--------|
| Elevated O ₃ | 0.79773352 | 1 | 0.79773352 | 0.22 | 0.6883 |
| Block | 1.74016362 | 2 | 0.87008181 | 0.23 | 0.8099 |
| Error | 7.41168166 | 2 | 3.70584083 | | |
| Total | 9.94957880 | 5 | | | |

birch

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|-------------|----|-------------|---------|--------|
| Elevated O ₃ | 57.62094531 | 1 | 57.62094531 | 8.68 | 0.0985 |
| Block | 72.14669925 | 2 | 36.07334962 | 5.43 | 0.1555 |
| Error | 13.2834791 | 2 | 6.6417396 | | |
| Total | 143.0511237 | 5 | | | |

Maple

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|-------------|----|-------------|---------|--------|
| Elevated O ₃ | 65.07278594 | 1 | 65.07278594 | 40.08 | 0.0241 |
| Block | 12.00593746 | 2 | 6.00296873 | 3.70 | 0.2129 |
| Error | 3.24689884 | 2 | 1.62344942 | | |
| Total | 80.32562223 | 5 | | | |

Appendix Table 4-12 One way- ANOVA table for the effects of elevated O₃ on vessel lumen diameter in aspen 8, 42, 216, 271, birch and maple at the middle radial position.

Aspen 8 (middle radial position)

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|-------------|----|-------------|---------|--------|
| Elevated O ₃ | 20.57094562 | 1 | 20.57094562 | 0.77 | 0.4736 |
| Block | 35.36010675 | 2 | 17.68005338 | 0.66 | 0.6028 |
| Error | 53.6614623 | 2 | 26.8307311 | | |
| Total | 109.5925147 | 5 | | | |

Aspen 42 (middle radial position)

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|-------------|----|------------|---------|--------|
| Elevated O ₃ | 0.95126479 | 1 | 0.95126479 | 0.30 | 0.6396 |
| Block | 10.78058121 | 2 | 5.39029061 | 1.69 | 0.3715 |
| Error | 6.37275235 | 2 | 3.18637618 | | |
| Total | 18.10459836 | 5 | | | |

Aspen 216 (middle radial position)

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|-------------|----|-------------|---------|--------|
| Elevated O ₃ | 10.24272356 | 1 | 10.24272356 | 1.04 | 0.4148 |
| Block | 36.74230783 | 2 | 18.37115392 | 1.87 | 0.3486 |
| Error | 19.66631227 | 2 | 9.83315614 | | |
| Total | 66.65134366 | 5 | | | |

Aspen 271 (middle radial position)

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|-------------|----|------------|---------|--------|
| Elevated O ₃ | 4.10504784 | 1 | 4.10504784 | 2.31 | 0.2680 |
| Block | 4.43895093 | 2 | 2.21947547 | 1.25 | 0.4448 |
| Error | 3.55666065 | 2 | 1.77833033 | | |
| Total | 12.10065942 | 5 | | | |

Birch (middle radial position)

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|-------------|----|-------------|---------|--------|
| Elevated O ₃ | 85.08805136 | 1 | 85.08805136 | 8.25 | 0.1028 |
| Block | 87.81562961 | 2 | 43.90781480 | 4.26 | 0.1902 |
| Error | 20.6211291 | 2 | 10.3105645 | | |
| Total | 193.5248101 | 5 | | | |

Maple (middle radial position)

| Source | SS | df | MS | F ratio | P > F |
|-------------------------|-------------|----|-------------|---------|--------|
| Elevated O ₃ | 58.77682963 | 1 | 58.77682963 | 77.44 | 0.0127 |
| Block | 32.65859078 | 2 | 16.32929539 | 21.51 | 0.0444 |
| Error | 1.51805616 | 2 | 0.75902808 | | |
| Total | 92.95347657 | 5 | | | |

Appendix Table 4-13 One way- ANOVA table for the effects of elevated CO₂ + O₃ on vessel lumen area proportion in aspen 8, 42, 216, 271, birch and maple.

Aspen 8

| Source | SS | df | MS | F ratio | P > F |
|---|------------|----|------------|---------|--------|
| Elevated CO ₂ + O ₃ | 0.00041666 | 1 | 0.00041666 | 2.85 | 0.2335 |
| Block | 0.00096863 | 2 | 0.00048431 | 3.31 | 0.2320 |
| Error | 0.00029254 | 2 | 0.00014627 | | |
| Total | 0.00167783 | 5 | | | |

Aspen 42

| Source | SS | df | MS | F ratio | P > F |
|---|------------|----|------------|---------|--------|
| Elevated CO ₂ + O ₃ | 0.00002250 | 1 | 0.00002250 | 0.60 | 0.5187 |
| Block | 0.00044972 | 2 | 0.00022486 | 6.03 | 0.1423 |
| Error | 0.00007463 | 2 | 0.00003731 | | |
| Total | 0.00054684 | 5 | | | |

Aspen 216

| Source | SS | df | MS | F ratio | P > F |
|---|------------|----|------------|---------|--------|
| Elevated CO ₂ + O ₃ | 0.00007626 | 1 | 0.00007626 | 3.50 | 0.2021 |
| Block | 0.00066362 | 2 | 0.00033181 | 15.25 | 0.0615 |
| Error | 0.00004352 | 2 | 0.00002176 | | |
| Total | 0.00078339 | 5 | | | |

Aspen 271

| Source | SS | df | MS | F ratio | P > F |
|---|------------|----|------------|---------|--------|
| Elevated CO ₂ + O ₃ | 0.00127293 | 1 | 0.00127293 | 2.29 | 0.2695 |
| Block | 0.00052769 | 2 | 0.00026385 | 0.47 | 0.6783 |
| Error | 0.00111277 | 2 | 0.00055638 | | |
| Total | 0.00291339 | 5 | | | |

Birch

| Source | SS | df | MS | F ratio | P > F |
|---|------------|----|------------|---------|--------|
| Elevated CO ₂ + O ₃ | 0.00031122 | 1 | 0.00031122 | 0.74 | 0.4803 |
| Block | 0.00206152 | 2 | 0.00103076 | 2.45 | 0.2898 |
| Error | 0.00084125 | 2 | 0.00042062 | | |
| Total | 0.00321399 | 5 | | | |

Maple

| Source | SS | df | MS | F ratio | P > F |
|---|------------|----|------------|---------|--------|
| Elevated CO ₂ + O ₃ | 0.00079038 | 1 | 0.00079038 | 11.37 | 0.0778 |
| Block | 0.0005698 | 2 | 0.0002849 | 0.41 | 0.7092 |
| Error | 0.00013900 | 2 | 0.00006950 | | |
| Total | 0.00098636 | 5 | | | |
