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Effects of elevated atmospheric CO2 and O3 on wood density, antomical proerties and decomposition of Northern Hardwoods

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EFFECTS OF ELEVATED ATMOSPHERIC CO_2 AND O_3 ON WOOD DENSITY, ANTOMICAL PROPERTIES AND DECOMPOSITION OF NORTHERN HARDWOODS

By

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In loving memory

of

Professor David F. Karnosky

Table of Contents

PREFACE

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DISSERTATION ABSTRACT

Anthropogenic activities continue to drive atmospheric CO_2 and O_3 concentrations to levels higher than during the pre-industrial era. Accumulating evidence indicates that both elevated CO_2 and elevated O_3 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 CO_2 and / or O_3 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 CO_2 and / or O_3 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 CO_2 and / or O_3 on wood anatomical properties, wood density, wood-decaying fungi and wood decomposition of northern hardwood tree species at the Aspen Free-Air CO_2 and O_3 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 CO_2 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 O_3 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 CO_2 and O_3, alone, significantly modified wood density but effects were species and / or genotype specific. However, the effects of elevated CO_2 and O_3, 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 CO_2 and / or O_3.

Polyporales, Agaricales, and Russulales were the dominant orders of fungi isolated. Based on the current results, future higher levels of CO_2 and O_3 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 CO_2 and O_3.

CHAPTER 1: Introduction

The atmospheric concentration of CO_2 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 O_3 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 CO_2 and O_3 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; Kostiainen 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 CO_2 and O_3 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 CO_2 and / or O_3 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 CO_2 and / or O_3 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; Kostiainen et al. 2008; Kostiainen et al. 2009). Although wood density and

anatomical properties vary with position along the stem (Panshin and Zeeuw 1980; Zobel and Buijtenen 1989; Dickison 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; Kostiainen 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 CO_2 and / or O_3 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 CO_2 and / or O_3 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 CO_2 and / or O_3) (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 CO_2 and /or O_3 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 CO_2 and O_3, 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 CO_2 and elevated O_3, 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 CO_2 and O_3 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 CO_2 and O_3 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 CO_2 and reduced cellobiohydrolase activity in soil under elevated O_3 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 CO_2 and / or O_3 on the wood properties of common northern hardwood tree species (four *Populus tremuloides* Michx. clones, including three relatively O_3 tolerant (8, 216, 271) and one relatively O_3 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 CO_2 (ambient + 200 ppm), elevated O_3 (1.5 × ambient), and elevated CO_2 with elevated O_3. Additionally, the effects of twelve years of forest and soil development under elevated CO_2 and /or O_3 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 CO_2 and / or O_3 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 CO_2 and O_3 on wood density are being reported for these species.

In Chapter 3, the effects of 12 years of forest and soil development under elevated CO_2 and / or O_3 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 CO_2 and / or O_3 for 12 years would cause significant alterations in the wood-decaying basidiomycete fungal community; (3) elevated CO_2 and / or O_3 fumigation environment would have no direct impact on wood decomposition rates; and (4) rates of decomposition of aspen and birch wood produced under elevated CO_2 and O_3 would decrease significantly.

Chapter 4 examined the long term effects of growth under elevated CO_2 and /or O_3 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 CO_2 and O_3; and (3) the combined treatment (elevated CO_2 + elevated O_3) 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 CO_2 and O_3 concentrations.

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CHAPTER 2: Effects of elevated atmospheric CO_2 and / or O_3 on wood density of paper birch and trembling aspen[1]

ABSTRACT

Current background concentrations of CO_2 and O_3 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 CO_2 and / or O_3 on wood density of northern hardwoods are not well understood. We evaluated the effects of elevated CO_2 and O_3 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 CO_2 and O_3 Enrichment (Aspen FACE) project near Rhinelander, WI, USA. Elevated CO_2 significantly decreased wood density of aspen clone 271, compared with trees grown under ambient conditions. In contrast, elevated O_3 increased wood density of aspen clone 42 and birch compared to the ambient. The combined effects of elevated CO_2 and elevated O_3 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 O_3 in the presence of elevated CO_2. 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 CO_2 and /or O_3 will be species and / or genotype dependent.

INTRODUCTION

Atmospheric CO_2 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

[1] Manuscript, in progress

predicted to increase further at the rate of 14-19 ppm every 10 years (IPCC 2007). The continuous increase in the background concentration of CO_2 is attributed to anthropogenic activities such as fossil fuel combustion (IPCC 2007). Atmospheric CO_2 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 CO_2 increases photosynthesis in C3 plants by enhancing carboxylation by rubisco and reducing photorespiration. Generally, elevated CO_2 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 O_3 concentration may offset the stimulating growth effects of CO_2 (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 O_3. This process is the major driving force for tropospheric O_3 increase (Fowler et al. 1999). The concentration of tropospheric O_3 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 O_3 concentrations higher than 60 nL L^{-1} by 2100 (Fowler et al. 1999; IPCC 2007). Unlike CO_2, 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 O_3 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 O_3 and CO_2, 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 CO_2 and O_3 could impact wood quality via their effects on growth patterns. Consequently, evidence of the effects of rising concentrations of CO_2 and / or O_3 on wood quality are accumulating (Conroy et al. 1990; Telewski et al. 1999; Beismann et al. 2002; Kaakinen et al. 2004; Kostiainen et al. 2004; Kostiainen et al. 2006; Kostiainen et al. 2008; Kostiainen 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; Kostiainen 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; Kostiainen 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 CO_2 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 CO_2 exposure in growth chambers. Beismann et al. (2002) and Kostiainen et al. (2009) reported no effects of elevated CO_2 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 CO_2 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 CO_2. In contrast, *Abies faxoniana* seedlings exposed to elevated CO_2 in closed top chambers exhibited significantly lower wood density when compared to ambient conditions (Qiao et al. 2008). In a FACE experiment, elevated CO_2 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 CO_2 or O_3 on hardwoods. Elevated CO_2 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 CO_2 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 O_3 and CO_2 co-occur naturally, there are virtually no studies on the effects of elevated O_3, alone, or in combination with CO_2, 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 O_3 and CO_2 alone or in combination (Kaakinen et al. 2004; Kostiainen et al. 2004; Kostiainen et al. 2006; Kostiainen et al. 2008; Kostiainen et al. 2009; Street et al. 2011).

The objective of this study was to determine the effects of elevated CO_2 and / or O_3 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 O_3, alone and in combination with elevated CO_2, 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 CO_2 and O_3 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^{o} N, latitude 89.5^{o} 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 CO_2 rings than CO_2 + O_3 rings (Dickson et al. 2000).

The Aspen FACE experiment was a 2^2 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 CO_2, O_3 and CO_2 + O_3, respectively. The four treatments were ambient CO_2 and O_3 as the control, elevated CO_2 (ambient + 200 ppm), elevated O_3 (1.5 × ambient), and elevated CO_2 with elevated O_3. 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 CO_2 and O_3 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^{o}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 (CO_2, O_3, and $CO_2 + O_3$) 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 / m^3, respectively, while overall mean wood density for aspen clones 216 and 271 were 409.2 ± 3.5 and 398.4 ± 3.3 kg / m^3, 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 / m^3 in birch; 398.3 ± 3.9 to 427.2 ± 6.2 kg / m^3 in clone 216; 384.3 ± 5.7 to 421.1 ± 4.4 kg / m^3 in clone 271 and 350.9 ± 3.9 to 381.9 ± 4.7 kg / m^3 in clone 42, independent of treatments.

Effects of elevated CO_2 and / or O_3 on wood density

Compared to the control, elevated CO_2 with ambient O_3 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 O_3 (with ambient CO_2) 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 CO_2 and elevated O_3 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 CO_2 with ambient O_3 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 CO_2 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 CO_2 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 O_3 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 CO_2 and O_3 treatments (Figs. 2-4 to 2-7).

DISCUSSION

This study demonstrates that elevated CO_2 and / or O_3, 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 CO_2 and O_3 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

CO_2, with overall mean density ranging between 348 and 409 kg / m^3. 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 CO_2 and / or O_3 on wood density in this study.

Effects of elevated CO_2 and / or O_3 on wood density

The wood density of birch, aspen 42 and aspen 216, under elevated CO_2 was not different from the control. Calfapietra et al. (2003) also reported no effects of elevated CO_2 on wood density for three *Populus* species. Several studies also have shown no significant effects of elevated CO_2 for many woody plants species. For example elevated CO_2 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 CO_2 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 CO_2. A persistent 26 % increase in ecosystem net primary productivity (NPP) occurred under elevated CO_2 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 CO_2 increased NPP of the aspen clones community by 24-35% during the 10^{th}-12^{th} 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 CO_2 (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 CO_2 (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 CO_2 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 CO_2 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 CO_2 (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 CO_2 alone either increased vessel diameters or reduced cell wall thickness in three *Populus* species, but nitrogen fertilization alone and in combination with elevated CO_2 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 CO_2 and enhanced nutrient availability. It is therefore likely that elevated CO_2 '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 CO_2 could be changes in its wood chemistry (Kaakinen et al. 2004; Kostiainen et al. 2008). Under elevated CO_2 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 CO_2 aspen 271 was reported have reduced uronic acid (a constituent of hemicellulose) and significant increases in starch content (Kostiainen 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 O_3 fumigation at the Aspen FACE experiment was genotype and species specific. Wood density of aspen clones 216 and 271 was unaffected by elevated O_3 treatment but significantly increased in birch and aspen clone 42 under elevated O_3 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 O_3 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 Kostiainen 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 O_3 fumigation. They also reported a decrease and increase in the cell lumen and cell wall areas, respectively in aspen clones (Kaakinen et al. 2004; Kostiainen et al. 2008). Implying that elevated O_3 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; Kostiainen et al. 2008) and significant increase in wood density of aspen 42. Vessel lumen diameter of birch grown under elevated O_3 from Aspen FACE decreased marginally compared to the control (Chapter 4, this dissertation). Therefore changes in cellular structure in birch in response to elevated O_3 (Street et al. 2011) might have contributed to the observed increase in birch wood density. Kostiainen 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 O_3 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 O_3 at the same experimental site (Kaakinen et al. 2004; Kostiainen 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 O_3 and elevated CO_2 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; Kostiainen 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 CO_2 in combination with elevated O_3 on wood density compared to the control for birch and the three aspen clones under investigation. Implying that, the effects of either elevated CO_2 or elevated O_3 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 O_3 and was largely enhanced in the elevated O_3 in combination with elevated CO_2 with a more pronounced impact in aspen 42 than birch. This observed trend suggest that lower concentrations (ambient) of O_3 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 CO_2 and elevated O_3 on biochemistry of the wood of aspen and birch. They reported that the combined effects of elevated CO_2 and elevated O_3 significantly increased aspen wood nitrogen by 15%, but in elevated CO_2 alone had no effects on aspen wood nitrogen. Likewise, Kostiainen et al. (2006) indicated that elevated O_3 alone reduced vessel proportion, but in combination with elevated CO_2 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; Kostiainen 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

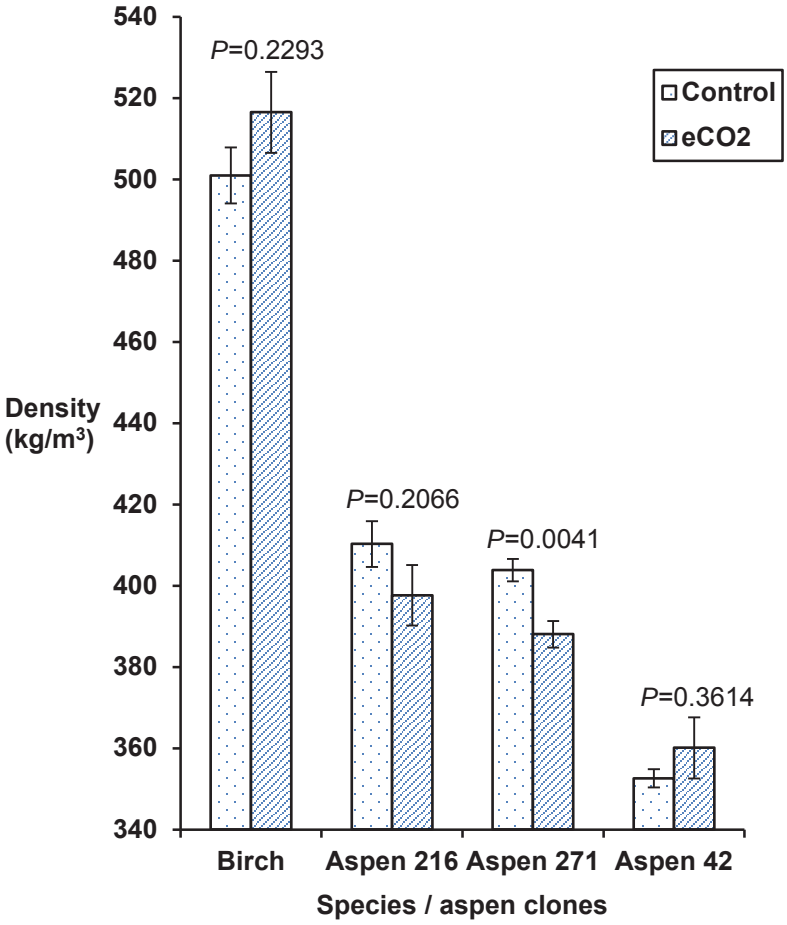

Figure 2-1 Wood density of *B. papyrifera* (birch) and *P. tremuloides* clones (aspen 216, 271 and 42) grown under either ambient CO_2 (control) or elevated CO_2 (eCO2) for 12 years. Values shown are means ± SE, (n=6).

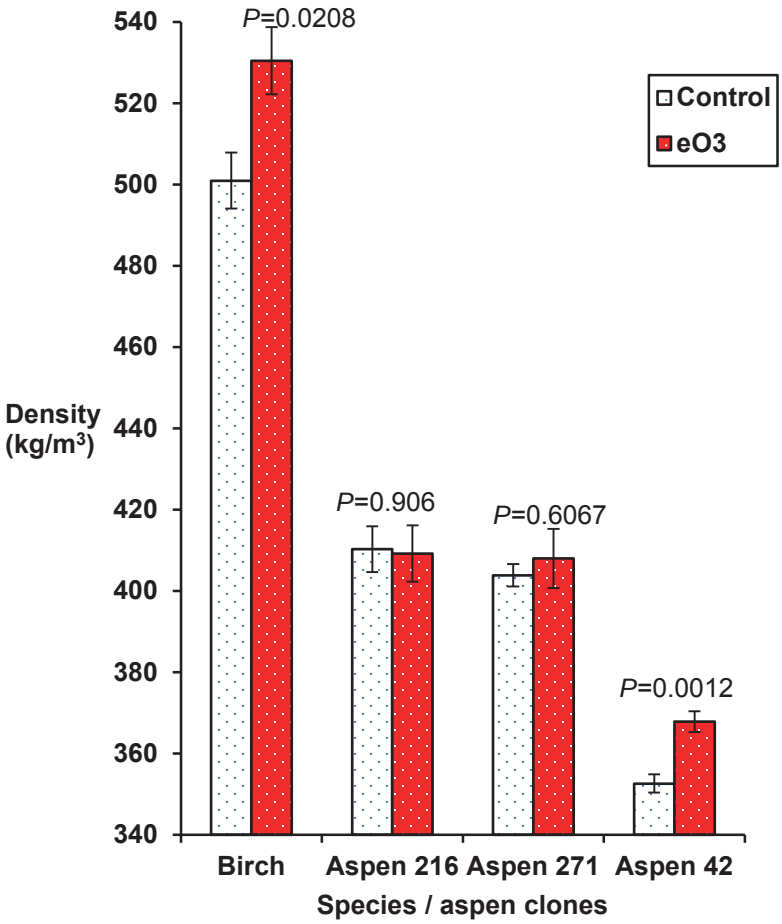

Figure 2-2 Wood density of *B. papyrifera* (birch) and *P. tremuloides* clones (aspen 216, 271 and 42) grown under either ambient O_3 (control) or elevated O_3 (eO3) for 12 years. Values shown are means ± SE, (n=6).

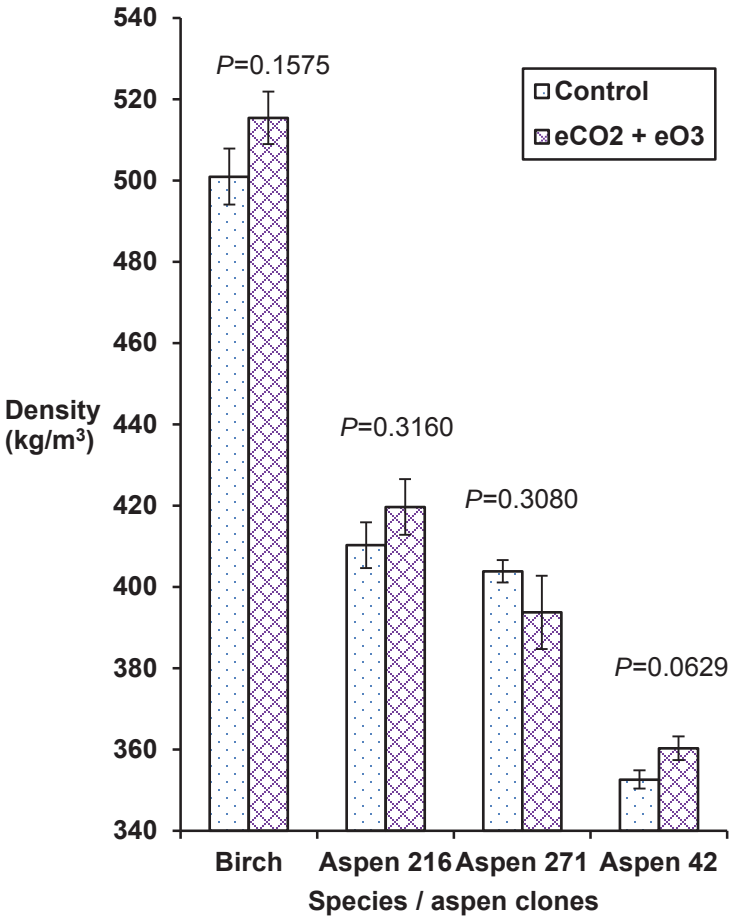

Figure 2-3 Wood density of *B. papyrifera* (birch) and *P. tremuloides* clones (aspen 216, 271 and 42) grown under either ambient CO_2 + O_3 (control) or elevated CO_2 + O_3 (eCO2 + eO3) for 12 years. Values shown are means ± SE, (n=6).

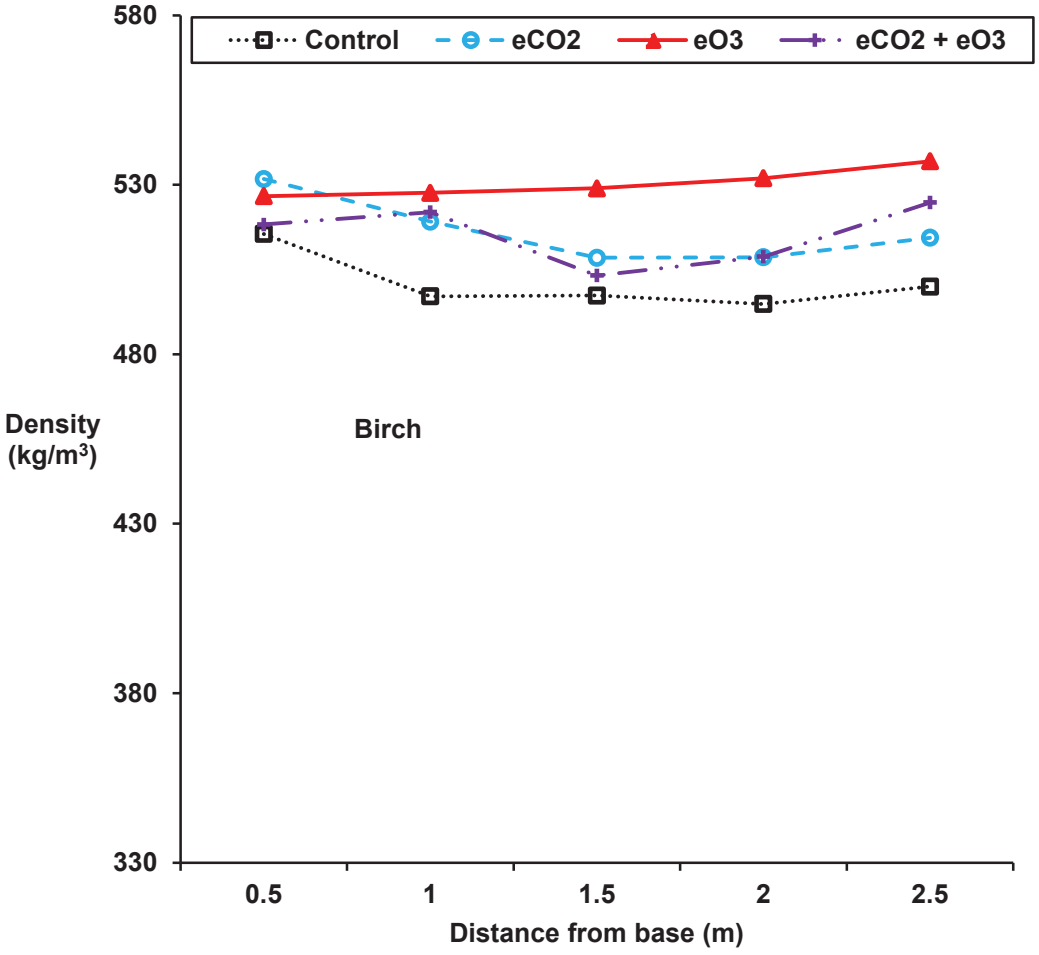

Figure 2-4 Wood density of *B. papyrifera* (birch) with respect to position along the stem, grown under either ambient CO_2 + O_3 (control) or elevated CO_2 (eCO2), elevated O_3 (eO3) and elevated CO_2 + O_3 (eCO2 + eO3) for 12 years. Values shown are means (n=6).

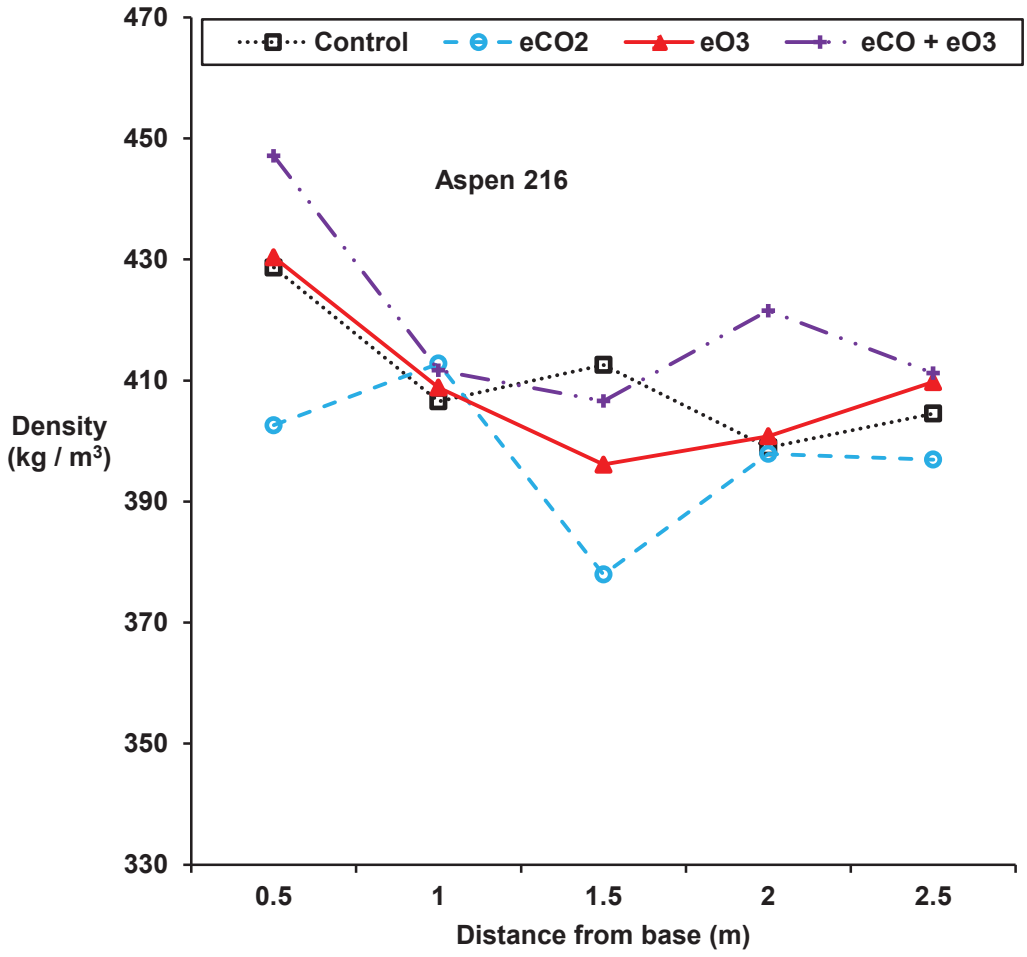

Figure 2-5 Wood density of *P. tremuloides* clone (aspen 216) with respect to position along the stem, grown under either ambient CO_2 + O_3 (control) or elevated CO_2 (eCO2), elevated O_3 (eO3) and elevated CO_2 + O_3 (eCO2 + eO3) for 12 years. Values shown are means (n=6).

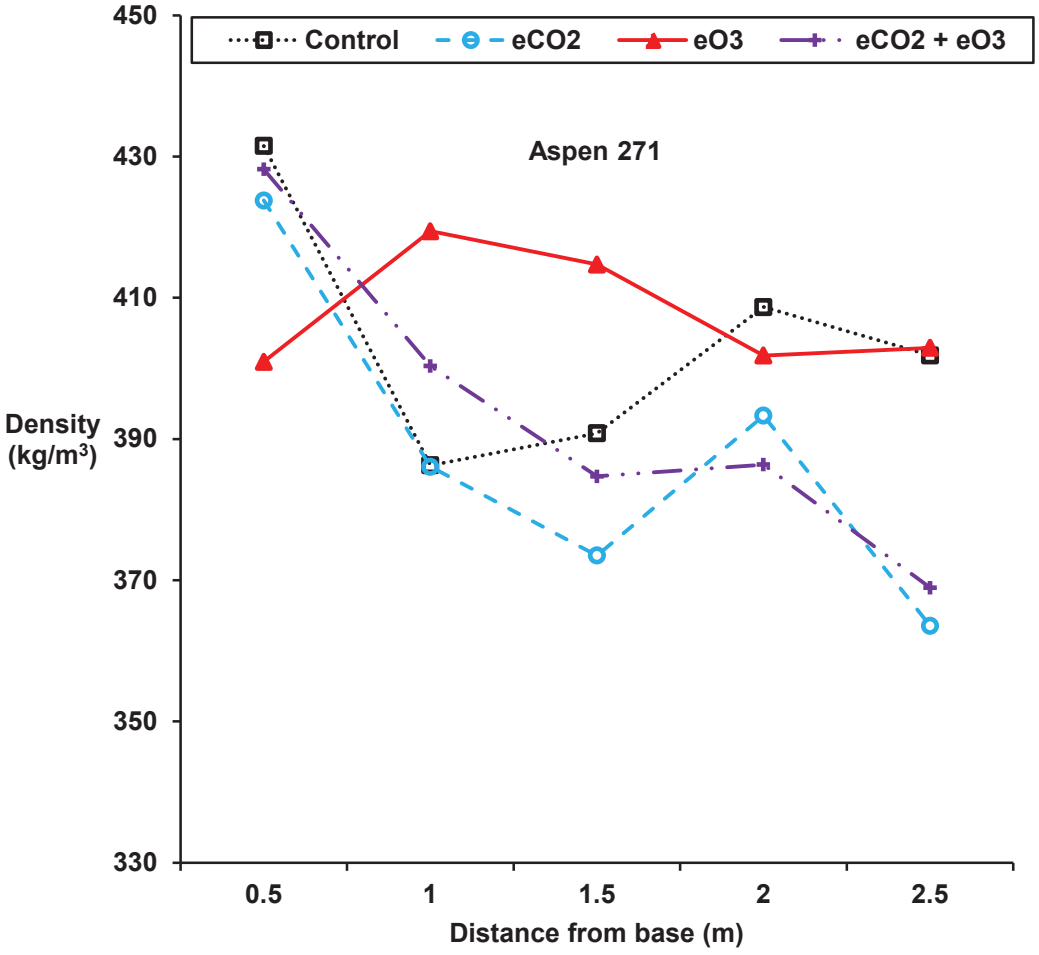

Figure 2-6 Wood density of *P. tremuloides* clone (aspen 271) with respect to position along the stem, grown under either ambient CO_2 + O_3 (control) or elevated CO_2 (eCO2), elevated O_3 (eO3) and elevated CO_2 + O_3 (eCO2 + eO3) for 12 years. Values shown are means (n=6).

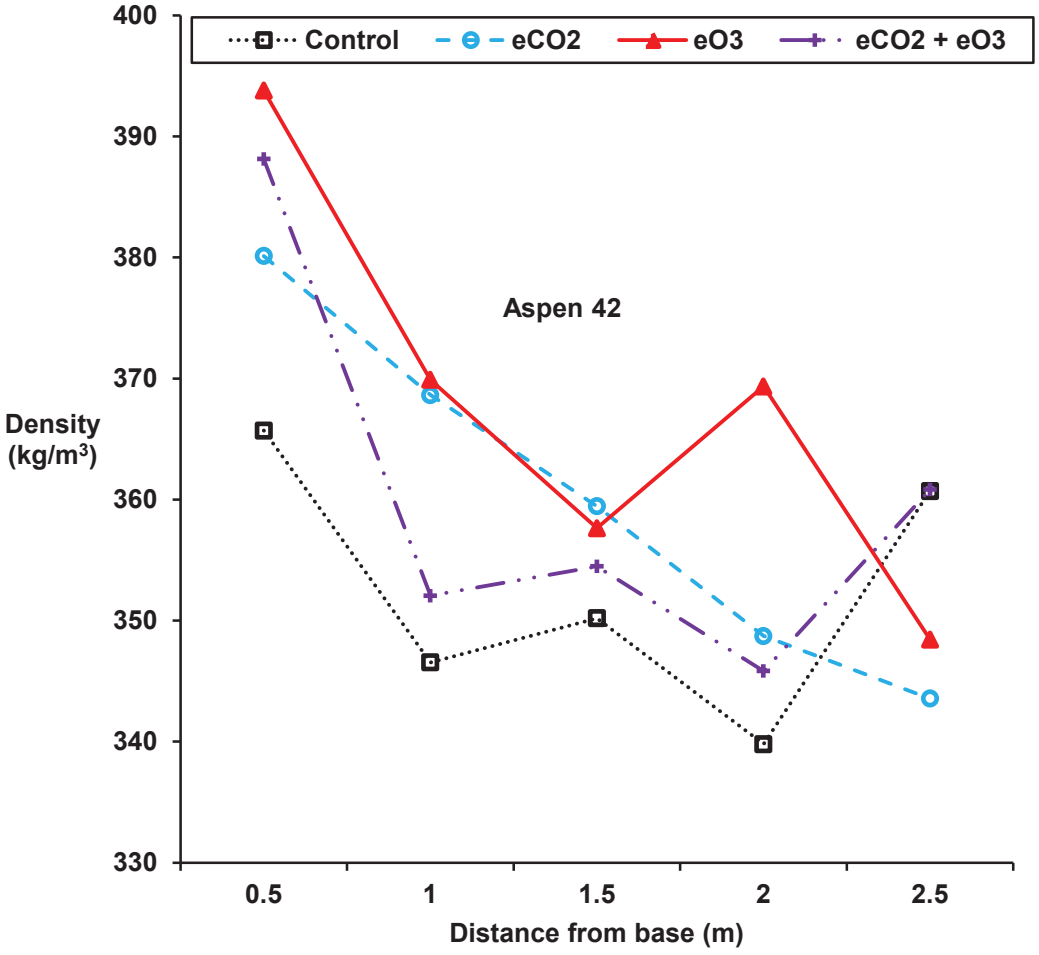

Figure 2-7 Wood density of *P. tremuloides* clone (aspen 42) with respect to position along the stem, grown under either ambient CO_2 + O_3 (control), or elevated CO_2 (eCO2), elevated O_3 (eO3) and elevated CO_2 + O_3 (eCO2 + eO3) for 12 years. Values shown are means (n=6).

CHAPTER 3: Effects of elevated CO_2 and O_3 on wood decomposition and wood-decaying fungal community composition[2]

ABSTRACT

Anthropogenic activities continue to drive atmospheric CO_2 and O_3 concentrations to levels higher than in the pre-industrial era. Accumulating evidence indicates that both elevated CO_2 and elevated O_3 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 CO_2 and / or O_3 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 CO_2 and / or O_3 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 CO_2 and / or O_3 on the wood-decaying basidiomycete fungal community and initial rates of wood decomposition at the Aspen Free-Air CO_2 and O_3 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 CO_2 and / or O_3 for 12 years, were reciprocally transplanted and fumigated with elevated CO_2 and / or O_3 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 CO_2 and / or O_3 fumigation tended to reduce the number of fungal species, the fungal communities under elevated CO_2 and / or O_3 were not statistically different from the fungal communities under the ambient conditions.

[2] Manuscript, in progress

Independent of origin of wood production and elevated CO_2 and / or O_3 fumigation, birch showed higher initial decomposition rate than the aspen clones. However, elevated CO_2 and / or O_3 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 CO_2 and / or O_3.

INTRODUCTION

Atmospheric concentration of CO_2 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 O_3 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 CO_2 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 CO_2, elevated O_3 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 CO_2 and / or O_3 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; Kostiainen et al. 2004; Kostiainen et al. 2006; Kostiainen et al. 2008). For example, Liu et al. (2005) observed a significant increase in the C:N ratio and tannin concentration under elevated CO_2 and O_3, 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 O_3 (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 CO_2 and / or O_3 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 CO_2 and / or O_3 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 CO_2. Sporocarp biomass of ectomycorrhizal fungi increased under elevated CO_2 and decreased under elevated O_3 compared to the control (Andrew and Lilleskov 2009). Ectomycorrizhal fungal community composition was significantly altered under elevated CO_2 and elevated O_3 but the effects diminished with time (Andrew and Lilleskov 2009).

Strnadova et al. (2004) observed no effect of elevated CO_2 on the saprotrophic fungal community. Likewise, Chung et al. (2006) did not detect significant effects of elevated CO_2 on fungal community composition, but found it was significantly modified under elevated O_3 at the Aspen FACE experiment. They also found no effects of either elevated CO_2 and / or eO_3 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 CO_2 and elevated O_3. The metabolism of plant and fungal cell walls was augmented significantly under elevated CO_2 (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 CO_2 (Edwards and Zak 2011) but was significantly reduced by elevated O_3 (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 CO_2 or O_3 (Edwards and Zak 2011).

The outcomes of investigations aimed at evaluating the effects of the rising CO_2 and / or O_3 on litter decomposition have also had divergent results (Norby et al. 2001a; Lindroth 2010). For example, Strain and Bazzaz (1983) suggested that elevated CO_2 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 CO_2. Contrarily, elevated CO_2 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 CO_2 showed no significant effects on litter decomposition (Norby et al. 2001a).

The effects of elevated ozone on decomposition also show contrasting results. Elevated O_3 increased decomposition rates in litter of *Betula papyrifera* (Parsons et al. 2008). However, elevated O_3 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 O_3 (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 CO_2 and / O_3 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 CO_2 and O_3 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 CO_2 and O_3 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 CO_2 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 O_3 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 CO_2 and elevated O_3 have been observed at Aspen FACE. Elevated CO_2 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 O_3 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 CO_2 and / or O_3 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 CO_2 and O_3, respectively, and increased in birch wood under either elevated CO_2 or O_3 (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 be observed under elevated CO_2 and O_3 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 CO_2 and reduced cellobiohydrolase activity in soil under elevated O_3 has been reported (Larson et al. 2002; Chung et al. 2006; Edwards and Zak 2011). Elevated CO_2 and / or O_3 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 CO_2 decreased significantly, while that of birch and aspen leaf produced under elevated O_3 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 CO_2 and / or O_3 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 CO_2 and /or O_3 for 12 years would cause

significant alterations in the wood-decaying basidiomycete fungal communities; (3) elevated CO_2 and / or O_3 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 CO_2 or O_3 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 CO_2 and O_3 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^{o} N, latitude 89.5^{o} 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 CO_2 rings than CO_2 + O_3 rings (Dickson et al. 2000).

The Aspen FACE experiment was a 2^2 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 CO_2, O_3 and CO_2 + O_3, respectively. The treatment levels were ambient CO_2 and O_3 as the control and elevated CO_2 (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 CO_2 and O_3, 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, CO_2 and O_3 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^{o}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 CO_2, O_3 and $CO_2 + O_3$ 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 mm^3 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 CO_2, elevated O_3 and elevated CO_2 + elevated O_3) 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 CO_2 and / or O_3 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 CO_2 (ambient vs. elevated CO_2); O_3 (ambient and elevated O_3), and CO_2 + O_3 (ambient vs. elevated CO_2 + elevated O_3) 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 CO_2 and / or O_3) 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 CO_2 and or / O_3. 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 CO_2 and elevated O_3, and 12 species present in their combination, independent of species of log (Table 3-3). The aspen logs under ambient conditions, elevated CO_2, elevated O_3 and elevated

CO_2 + O_3 had 11, 9, 8 and 8 fungal species, respectively. In addition the birch logs under ambient conditions, elevated CO_2, elevated O_3 and elevated CO_2 + elevated O_3 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 CO_2 and / or O_3 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 CO_2 and / or O_3 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 / m^3 with percent loss of 13.2 ± 1.1 in birch; (Table 3-5); from 357.4 ± 2.2 to 317.8 ± 4.9 Kg / m^3 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 / m^3 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 CO_2 and / or O_3 fumigation and growth source of log (Table 3-8, Appendix tables 3-4 to 3-6). Independent of wood source, elevated CO_2 and /or O_3 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 CO_2 and / or O_3 fumigations, birch or aspen logs originally produced under either ambient conditions or elevated CO_2 and / or O_3 (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 CO_2 treatment (*P* = 0.1001). This was the result of percent density loss in birch and aspen 271 wood produced under elevated CO_2 tending to decrease and that of aspen 42 wood produced under elevated CO_2 tending to increase elevated CO_2 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 CO_2 and / or O_3 for 12 years would cause significant alterations in wood-decaying basidiomycete fungal community (3) elevated CO_2 and / or O_3 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 CO_2 and O_3 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 CO_2 and / or O_3 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 CO_2 and / or O_3 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 CO_2 on saprotrophic micro-fungal community composition at the Swiss FACE experiment.

At Aspen FACE a number of studies on the effects of elevated CO_2 and /or O_3 on forest floor and soil microbial communities and function have been carried out. In one assessment, elevated CO_2 had no effect on soil fungal communities, but elevated O_3 did (Chung et al. 2006). Earlier in the Aspen FACE experiment the overall soil microbial community under elevated CO_2 was found to be different from ambient, but elevated O_3 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 CO_2 and / or eO_3 treatments (Edwards and Zak 2011). For example, elevated CO_2 and O_3 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 CO_2 altered the Agaricomycetes fungal community in the forest floor compared to ambient CO_2. 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 CO_2 and / or O_3 (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*-acetylgluccosaminidase (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*-acetylgluccosaminidase activities under elevated CO_2, with no effects under elevated O_3 after three years of fumigation. At the end of five years of fumigation, elevated CO_2 was observed still to increase cellobiohydrolase and *N*-acetylgluccosaminidase activities, while elevated O_3 reduced cellobiohydrolase and had no effect on *N*-acetylgluccosaminidase activities (Chung et al. 2006). After ten years of fumigation, the effects of elevated CO_2 and O_3 on cellobiohydrolase and *N*-acetylgluccosaminidase activities appeared to have dampened and were no longer statistically significant compared to the control (Edwards and Zak 2011).

Cellobiohydrolase and *N*-acetylgluccosaminidase 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 O_3. It is therefore reasoned that the diminishing effect of elevated CO_2 and O_3 on cellobiohydrolase and *N*-acetylgluccosaminidase activities after ten years of fumigation (Edwards and Zak 2011) is in agreement with the current studies observation which was conducted after the 12^{th} year of Aspen FACE. To our knowledge, this is the first time effects of elevated CO_2 and / or O_3 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 CO_2 and / or O_3 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 CO_2 and / or O_3 fumigation did not have any significant impact on percent density loss. This implies that elevated CO_2 and / or O_3

fumigation environment did not have direct effects on initial decomposition rates of birch and aspen wood. Elevated CO_2 and / or O_3 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 CO_2 and / or O_3 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 CO_2, 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 O_3 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 CO_2 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 CO_2 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 CO_2 and / or O_3 were minor with regard to those needed to alter decomposition. Percent density loss in aspen 271 produced under elevated CO_2

tended to decrease when placed in the elevated CO_2 treatment compared to the control. Surprisingly, percent density loss of aspen 271 produced under ambient or elevated CO_2 tended to increase under ambient, suggesting that the observed reduction in percent density loss in aspen 271 produced under elevated CO_2 and placed in elevated CO_2 treatment may not be directly explained by either source of wood (substrate quality) or elevated CO_2 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 of decreased significantly under elevated CO_2 and increased under elevated O_3 and CO_2+O_3 (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 CO_2 and lignin and extractives increased and decreased, respectively, under elevated O_3 in aspen. In birch, wood extractives increased under elevated CO_2 and elevated O_3 and C:N tended to decrease in all treatments (Kaakinen et al. 2004; Kostiainen 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 elevate CO_2 native placement in this study. The low C:N in birch may explain high percent wood density loss under elevated O_3 and elevated CO_2 + elevated O_3

and high extractives may have contributed to the lower percent wood density loss under elevated CO_2.

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 CO_2 and / or O_3 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 CO_2 and elevated O_3 (Kubiske et al. 2007). Hence because wood decomposition varies with species or genotype, it is possible that future high levels of elevated CO_2 or elevated O_3 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 CO_2 and / or O_3 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 CO_2 and / or O_3 differentially, it is reasoned that, future higher levels of atmospheric CO_2 and O_3 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 CO_2 and / O_3 fumigations.

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Figures

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

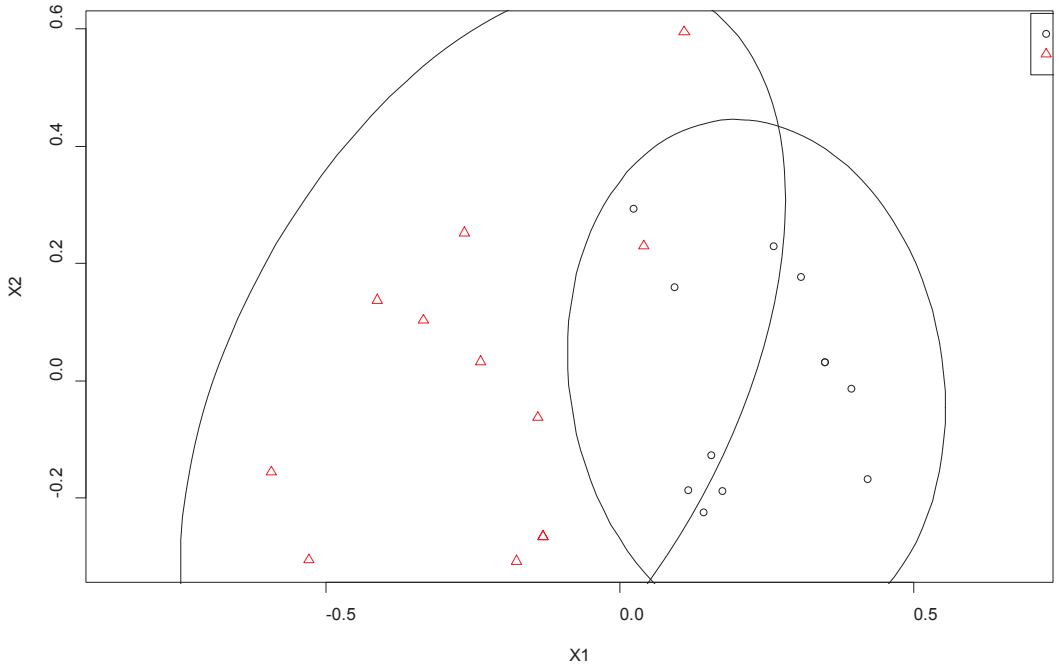

Figure 3-2 NMDS plot of wood-decaying basidiomycete fungal community composition, at Aspen FACE. Communities are displayed with respect to aspen logs and birch logs across all treatment rings and blocks. An ellipse is a 95% confidence level for each fungal community. The open circles and the red triangle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

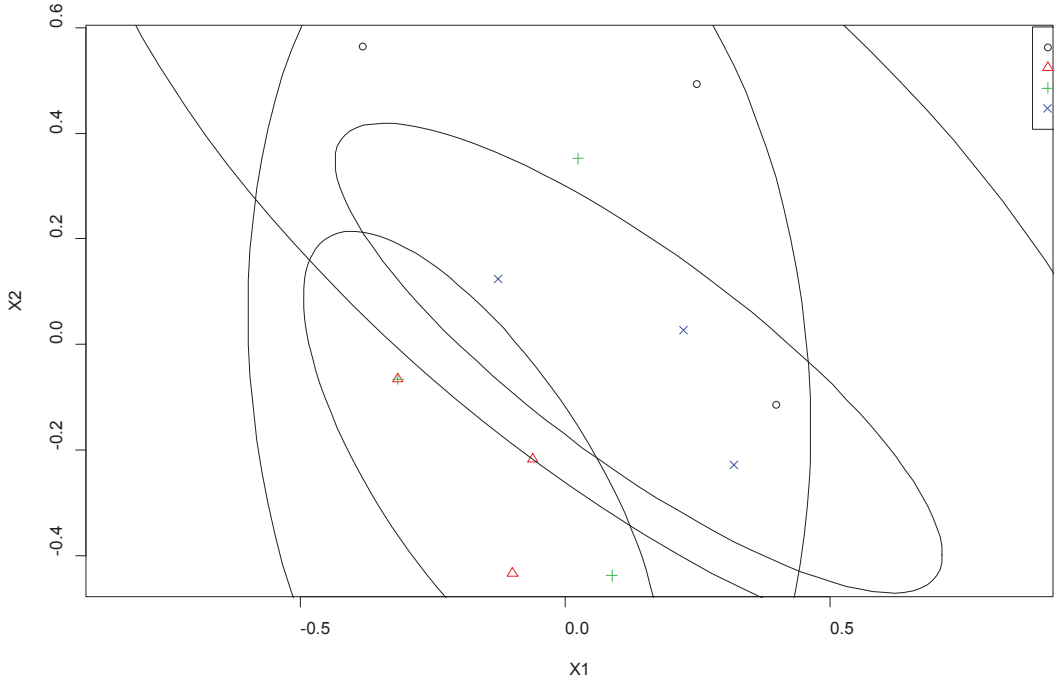

Figure 3-3 NMDS plot of wood-decaying basidiomycete fungal community composition for birch logs, at Aspen FACE. Communities are displayed with respect to ambient (open circle), elevated CO_2 (red triangle), elevated O_3 (green +) and elevated CO_2 + elevated O_3 (blue ×) across all treatment rings and blocks. An ellipse is a 95% confidence level for each fungal community.

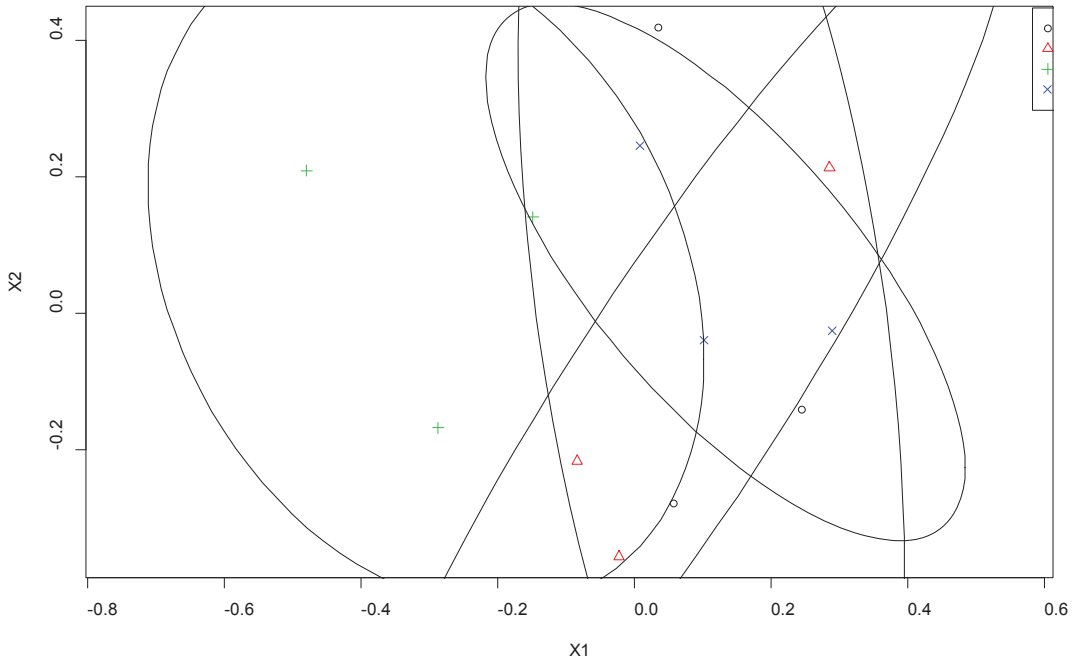

Figure 3-4 NMDS plot of wood-decaying basidiomycete fungal community composition for aspen logs, at Aspen FACE. Communities are displayed with respect to ambient (open circle), elevated CO_2 (red triangle), elevated O_3 (green +) and elevated CO_2 + elevated O_3 (blue ×) across all treatment rings and 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 CO_2 and /or O_3. 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.

	Treatment Assigned for Log Decomposition			
Log Growth Source	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.

	Fungus Species	Genus	Family	Order
	Bjerkandera adusta	Bjerkandera	Meruliaceae	Polyporales
	Cerrena unicolor	Cerrena	Polyporaceae	Polyporales
	Chondrostereum purpureum	Chondrostereum	Cyphellaceae	Agaricales
	Cylindrobasidium laeve	Cylindrobasidium	Physalacriaceae	Agaricales
	Irpex lacteus	Irpex	Meruliaceae	Polyporales
	Peniophora aurantiaca	Peniophora	Peniophoraceae	Russulales
	Peniophora sp	Peniophora	Peniophoraceae	Russulales
	Schizophyllum commune	Schizophyllum	Schizophyllaceae	Agaricales
	Sistotrema brinkmannii	Sistotrema	Hydnaceae	Cantharellales
	Stereum rugosum	Stereum	Stereaceae	Russulales
	Stereum sp.	Stereum	Stereaceae	Russulales
	Trametes gibbosa	Trametes	Polyporaceae	Polyporales
	Trametes versicolor	Trametes	Polyporaceae	Polyporales
	Unidentified sp	unknown	unknown	unknown
Total	14	8	7	3

Table 3-3 Fungal species present (1) or absent (0) with respect to elevated CO_2 and / or O_3 treatments and log species.

	Aspen logs				Birch logs			
	Treatments				Treatments			
Fungal species	Ambient	CO_2	O_3	CO_2 + O_3	Ambient	CO_2	O_3	CO_2 + O_3
Bjerkandera adusta	1	1	1	1	1	1	0	0
Cerrena unicolor	0	0	0	0	1	1	1	0
Chondrostereum purpureum	1	1	1	1	1	0	0	0
Cylindrobasidium laeve	1	1	1	1	1	1	1	1
Irpex lacteus	1	1	0	1	1	0	0	1
Peniophora aurantiaca	1	1	1	0	1	1	1	1
Peniophora sp	1	0	1	0	1	0	1	1
Schizophyllum commune	1	0	0	0	0	0	0	0
Sistotrema brinkmannii	0	0	0	0	1	0	0	0
Stereum rugosum	1	1	1	1	1	0	0	0
Stereum sp.	1	1	1	1	0	0	0	1
Trametes gibbosa	1	1	0	1	1	1	1	0
Trametes versicolor	1	1	1	1	1	0	1	0
Unidentified sp	0	0	0	0	1	0	0	1
Fungal species per treatment	11	9	8	8	12	5	6	6
Fungal species per community	Aspen 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 CO_2 and / or O_3 on wood-decaying basidiomycete fungal community composition. Highlighted *P*-value is significant (*P*-values ≤ 0.05).

Parameter		Fungal community
Wood species		**0.0009**
***Aspen logs*:-**		
	Elevated CO_2 and / or O_3	0.1029
***Birch logs*:-**		
	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 CO_2 (= eCO_2); elevated O_3 (=eO_3). 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	13.2 ± 1.1

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 CO_2 (= eCO_2); elevated O_3 (=eO_3). Values are mean (±1SE), n=3.

			Density (Kg / m^3)			
Species	Source	Treatment	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 CO_2 (= eCO_2); elevated O_3 (=eO_3). Values are mean (±1SE), n=3.

			Density (Kg / m3)			
SPP	Source	Treatment	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	eC_{O2}	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 CO_2 and / or O_3 on percent wood density loss in birch and aspen clones 42, 271 (species). Elevated CO_2 (= eCO_2); elevated O_3 (=eO_3). 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 CO_2 and O_3 on wood anatomical properties of trembling aspen, paper birch and sugar maple[3]

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 CO_2 and O_3 are about 40% higher than during the pre-industrial era. The alterations in the background concentrations of atmospheric CO_2 and / or O_3 could impact wood anatomical structure and consequently impact wood quality. We evaluated the effects of 12 years of growth under elevated CO_2 and / or O_3 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 CO_2 and O_3 enrichment (FACE) project near Rhinelander, WI, USA. Wood anatomical properties varied significantly with species, aspen genotype and stem radial position. Elevated CO_2 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 O_3, 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 O_3, 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 O_3. 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 O_3 or not. The effects of elevated CO_2 and O_3, 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 CO_2 and / or O_3 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 CO_2 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 CO_2, elevated O_3 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 CO_2 and / or O_3 (IPCC 2007) could impact wood anatomical properties and consequently affect wood quality for its intended use (Ceulemans et al. 2002; Kostiainen et al. 2004; Kostiainen et al. 2006; Kostiainen et al. 2008; Kostiainen et al. 2009).

A number of investigations have been initiated to provide scientific understanding of the influence of rising concentrations of atmospheric CO_2 and / or O_3 on wood anatomical properties, helping assess future wood quality (Telewski et al. 1999; Kaakinen et al. 2004; Kostiainen et al. 2004; Yazaki et al. 2005; Kostiainen et al. 2006; Kostiainen et al. 2008; Kostiainen et al. 2009). Nevertheless, accumulating evidence has been divergent. For example, elevated CO_2 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 CO_2 without nutrient enhancement (Kostiainen et al. 2004). Kostiainen 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 CO_2. Similarly, tracheid walls and the proportion of resin canals decreased; and tracheid lumen increased in *Pinus sylvestris* L. grown under elevated CO_2 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 CO_2 (Atkinson and Taylor 1996). Under elevated CO_2, growth ring width increased in *Picea abies* L. (Kostiainen et al. 2004); *Betula pendula* Roth (Kostiainen 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; Kostiainen et al. 2008).

In contrast to the aforementioned reports, elevated CO_2 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; Kostiainen et al. 2008). Wood anatomical properties responsible for water transport in *Quercus mongolic*a and *Alnus hirsute* were also not affected by elevated CO_2 (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 CO_2, but clone identity had significant effects (Kostiainen et al. 2006). Cell wall thickness of *Picea abies* (L.) Karst. grown under elevated CO_2 was not significantly different from the ambient (Kostiainen 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 CO_2. Elevated CO_2 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 CO_2 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 CO_2 was species dependent. They observed that elevated CO_2 did not affect vessel anatomy of *Quercus mongolica, Betula maximowicziana* and *Acer mono* but significantly modified the vessel properties and cambial activity of *Kalopanax septemtobus.* Luo et al. (2005) also observed elevated CO_2 × 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; Kostiainen et al. 2008).

The impact of elevated O_3 concentrations on wood anatomical properties has also been variable. Kurczynska et al. (1998) studied the effects of elevated O_3 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 O_3 on nitrogen enriched soils compared to the ambient environment. Independent of nitrogen level of the soil, sieve cell wall thickness increased under elevated O_3, but sieve cell frequency and latewood tracheid diameter also decreased under elevated O_3 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 (Kostiainen et al. 2008). A significant reduction in vessel proportion and increased cell wall proportion in *Betula pendula* Roth (silver birch) due to elevated O_3 have been reported (Kostiainen et al. 2006). Significant interactions of elevated O_3 with genotype on some anatomical properties have been observed (Kaakinen et al. 2004; Kostiainen et al. 2006; Kostiainen et al. 2008). Furthermore, reports of

combined impacts of elevated CO_2 and O_3 on wood structure are rare and have been shown to be insignificant (Kaakinen et al. 2004; Kostiainen et al. 2006; Kostiainen et al. 2008).

Although evidence on the effects of elevated CO_2 and / or O_3 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; Kostiainen et al. 2004; Yazaki et al. 2004; Kostiainen et al. 2006; Watanabe et al. 2008; Kostiainen 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; Kostiainen 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 CO_2 and /or O_3 were typically done on the lower portions of woody stems (Kaakinen et al. 2004; Kostiainen et al. 2004; Kostiainen et al. 2006; Kostiainen et al. 2008; Kostiainen et al. 2009). The divergent reports suggest that further investigations with a different approach are necessary to improve understanding the effects of elevated CO_2 and / or O_3 on anatomy and quality of wood. We therefore investigated the effects of elevated CO_2 and / or O_3 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 CO_2 and / or O_3 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 CO_2 and O_3 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 CO_2; the aspen clones community alone increased by 24-35% during the 10^{th} to 12^{th} years (Zak et al. 2011). However, growth of the individual tree species responded differently to elevated CO_2 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 CO_2 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 CO_2 (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 CO_2, independent of O_3 concentration, was not different from the ambient (Kubiske et al. 2007), but the diameter growth of birch was significantly enhanced under elevated CO_2 (Kubiske et al. 2007).

Aspen 271 was reported to have the largest fiber lumen diameter among the clones under elevated CO_2 (Kaakinen et al. 2004). Data pooled for all the aspen clones showed that fiber lumen tended to increase under elevated CO_2 after 3 and 5 years of exposure at Aspen FACE (Kaakinen et al. 2004; Kostiainen et al. 2008). Similarly, vessel diameter tended to increase under elevated CO_2 (Kostiainen et al. 2008). Additionally after 5 years of exposure to elevated CO_2, aspen 271 was reported have reduced uronic acid (a constituent of hemicellulose) and a significant increase in starch content (Kostiainen et al. 2008). Although diameter growth of birch and sugar maple under elevated O_3 was not significantly different from the ambient, elevated O_3 significantly reduced diameter growth of all the aspen clones at the end of 3^{rd} and 7^{th} 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 Kostiainen et al. (2008) also observed a reduction in radial growth and growth rings of the aspen clones at the end of the 3^{rd} and 5^{th} year of elevated O_3 fumigation. They reported a decrease in cell lumen area and an increase in cell wall area in aspen clones grown under elevated O_3 (Kaakinen et al. 2004; Kostiainen et al. 2008).

Previous investigations at the Aspen FACE site, observed no effect of the combined elevated O_3 and elevated CO_2 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; Kostiainen 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 CO_2 and elevated O_3 – specifically, the growth ring width, fiber length, fiber lumen diameter, vessel lumen diameter, vessel lumen area proportions and number of vessels per mm^2 (vessel frequency) would increase and cell wall area proportions would decrease under elevated CO_2, and, the converse will hold for elevated O_3, except for clone 8, which is less sensitive to O_3, which would exhibit anatomical properties similar to the control; and 3) the combined treatment (elevated CO_2 + O_3) 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^{o} N, latitude 89.5^{o} 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 CO_2 rings than CO_2 + O_3 rings (Dickson et al. 2000).

The Aspen FACE experiment was a 2^2 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 (CO_2), ozone (O_3) and CO_2+O_3 respectively. The treatment levels were ambient CO_2 and O_3 as the controls, elevated CO_2 (ambient + 200 ppm), and elevated O_3 (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 CO_2 and O_3 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 × 2461 µm size images at 20 x magnifications at 2048 × 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 × 387 µm size images at 100 x magnification at 1600 × 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 × 2461 µm size images at 20 x magnification at 2048 × 1536 pixels resolution. Additionally, three 328 × 246 µm size images at 200 x magnification at 2048 × 1536 pixels resolution were taken randomly from the mid portion of each growthring 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 CWA % = 100 - (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 CO_2 (ambient vs. elevated CO_2); O_3 (ambient and elevated O_3), $CO_2 + O_3$ (ambient vs. elevated CO_2 + elevated O_3) 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 CO_2, elevated O_3 or elevated CO_2 + elevated O_3), 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 CO_2

Compared to ambient conditions, elevated CO_2 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 CO_2 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 CO_2. There were no significant two-way-interactions between elevated CO_2 and species / aspen genotypes nor elevated CO_2 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 CO_2, species / aspen genotypes and radial position of vessel lumen diameter (Table 4-3). Elevated CO_2 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 O_3

The effects of elevated O_3 on wood anatomical properties were species or genotype and radial position dependent (Tables 4-4 and 4-5). Compared to ambient conditions, elevated O_3 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 O_3 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 O_3 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 O_3. 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 O_3.

A one-way ANOVA performed separately for each species / aspen genotype (Appendix tables 4-8 to 4-12) showed that the effects of elevated O_3 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 O_3 on vessel lumen diameter were radial position dependent, resulting in a significant interaction between radial position and elevated O_3 ($P = 0.0110$).

The interactive effect between radial position and elevated O_3 on vessel lumen diameter was because the impact of elevated O_3 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 O_3 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 O_3, species / aspen genotypes and radial position for vessel lumen diameter (Table 4-5).

Effects of elevated CO_2 + O_3

Combined elevated CO_2 + elevated O_3 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 CO_2 + O_3 (Table 4-6). However, there was a marginally significant interaction between the combined elevated CO_2 + O_3 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 CO_2 + elevated O_3 on vessel lumen diameter did not differ significantly from ambient CO_2 + O_3 when sampling was done at inner, middle or periphery radial positions for all species / aspen genotypes (Table 4-7). Nevertheless, combined elevated CO_2 + elevated O_3 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 CO_2 + elevated O_3 and radial position of vessel lumen diameter (P = 0.0463). Interaction between combined CO_2 + O_3 and species / aspen genotype were not significant (P = 0.4622), and those among combined CO_2 + O_3 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 CO_2 and elevated O_3, and under elevated O_3, aspen clone 8 which is less sensitive to O_3, would exhibit anatomical properties similar to the control; (3) the combined treatment (elevated CO_2 + elevated and O_3) 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; Kostiainen 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 CO_2

The results of the current study showed no significant effects of elevated CO_2 on wood anatomical properties investigated with the exception of a nearly significant effect of elevated CO_2 on number of vessels per square millimeter in relation to the ambient treatment. In addition, vessel lumen diameter was not affected by elevated CO_2 at the inner, middle and periphery radial positions of the growth ring, compared to the control, implying that effects of elevated CO_2 on vessel lumen diameter are not radial position dependent. The current observations are consistent with earlier reports (Kaakinen et al. 2004; Kostiainen et al. 2008), which indicated no significant effects of elevated CO_2 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 CO_2 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 CO_2 (Kostiainen et al. 2006). Cell wall thickness of *Picea abies* (L.) Karst. grown under elevated CO_2 was not significantly different from the control (Kostiainen et al. 2004). Likewise, Telewski et al. (1999) reported that cell wall:lumen area of *Pinus taeda* L. was not influenced by elevated CO_2. Elevated CO_2 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 CO_2 did not have significant effects on wood anatomical properties in the current study, there was a nearly significant effect of elevated CO_2 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 CO_2, 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 CO_2 compared to the control (Atkinson and Taylor 1996). At Aspen FACE elevated CO_2 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; Kostiainen et al. 2008). In addition net primary productivity increased by 26% persistently for about a decade under elevated CO_2. In this study, cell dimensions and cell wall area proportions were not significantly influenced by elevated CO_2, 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 CO_2. Elevated CO_2 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 CO_2 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 O_3

The effects of elevated O_3 on wood anatomical properties varied with species and radial position. Elevated O_3 in the presence of ambient CO_2 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 O_3 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 (Kostiainen et al. 2008). Similarly, elevated O_3 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 (Kostiainen 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 O_3 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; Kostiainen 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 increased 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 CO_2 + O_3 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 CO_2 + O_3 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 CO_2 + O_3 on growth parameters of the aspen genotypes, birch and maple were observed. It appears elevated CO_2 counteracts the effects of elevated O_3 on wood anatomical properties.

Conclusions

Species and genotype exhibited statistically significant effects on wood anatomical properties, independent of treatments. Compared to the control, elevated CO_2 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 CO_2 marginally increased the number of vessels per square millimeter in aspen genotypes 8, 216, 271 and birch. Elevated O_3 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 CO_2 and O_3 alone on wood anatomical properties of aspen genotypes and birch were ameliorated when the gases were applied in combination. However, elevated CO_2 + O_3 marginally reduced vessel lumen area proportion in maple. Because wood density depends on wood anatomical properties, it is likely that future higher concentrations of CO_2 and / or O_3 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 O_3, CO_2 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 CO_2 and / or elevated O_3 on wood quality.

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Figures

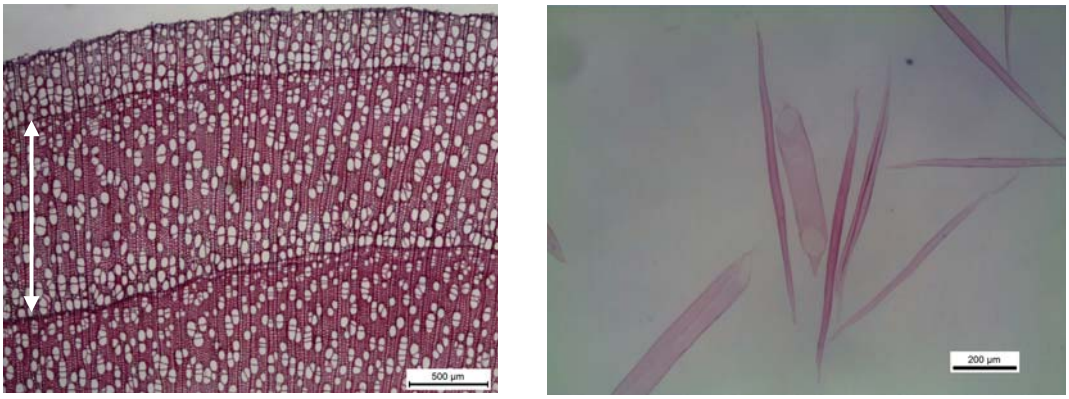

Fig 4-1 Micrographs of aspen illustrating the 2008 growth ring (Arrowed line, left) and a macerated wood sample for wood anatomical analyses (Right).

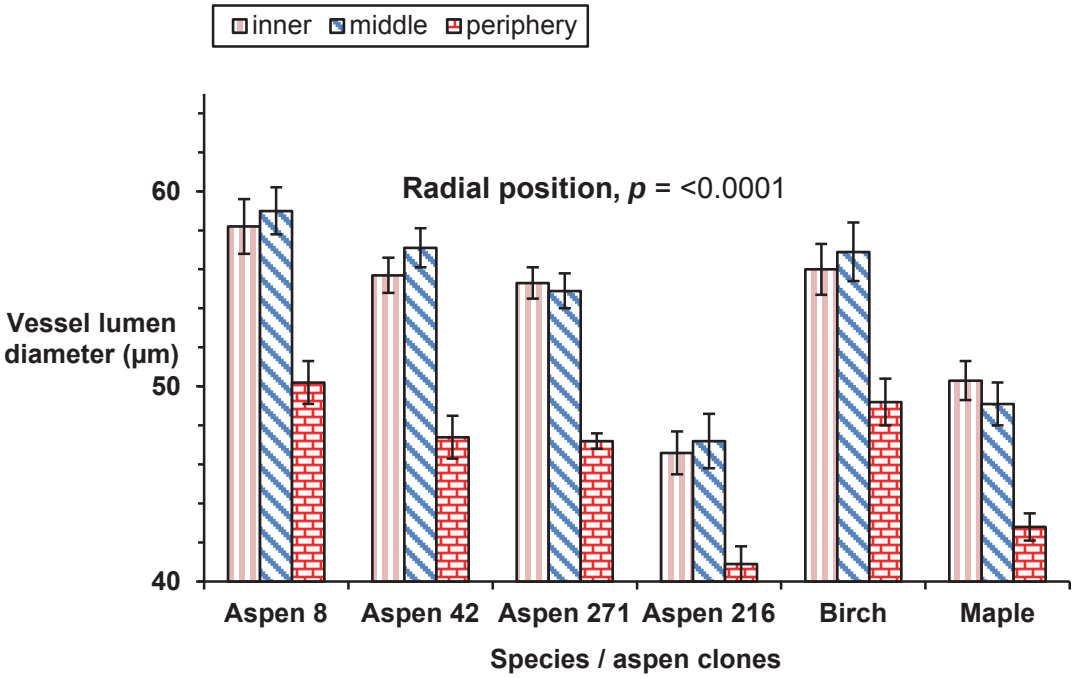

Figure 4-2 Vessel lumen diameter of aspen clones 8, 42, 216, 271, birch and maple in relation to radial position, independent of elevated CO_2 and / or O_3 treatments at Aspen FACE. Bars are means ± SE across all treatments and n =12.

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 CO_2 and / or elevated O_3 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 CO_2 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 (± 1SE). Highlighted *p*-values are either significant ($P \leq 0.05$) or marginally significant ($P \leq 0.10$) and n = 3. Elevated CO_2 (= eCO_2).

Species / Clone	Treatment	Ring width (mm)	Fiber length (mm)	Fiber lumen diameter (µm)	Vessel %	Number of vessels / mm^2	Cell wall area %
8	Ambient	2.4 ± 0.2	0.8 ± 0.05	12.0 ± 0.5	35.6 ± 1.0	145 ± 13	30.6 ± 0.8
	eCO_2	2.5 ± 0.7	0.8 ± 0.003	11.3 ± 0.1	32.6 ± 1.0	161 ± 13	35.2 ± 2.2
42	Ambient	3.9 ± 0.1	0.7 ± 0.01	11.6 ± 0.4	32.4 ± 0.6	141 ± 6	46.4 ± 3.6
	eCO_2	3.9 ± 0.7	0.8 ± 0.04	12.7 ± 0.3	29.7 ± 1.8	126 ± 7	46.2 ± 0.9
216	Ambient	2.7 ± 0.4	0.8 ± 0.05	12.1 ± 0.5	24.6 ± 0.8	144 ± 2	50.8 ± 1.9
	eCO_2	1.5 ± 0.5	0.8 ± 0.05	11.8 ± 0.6	24.9 ± 2.9	158 ± 19	50.6 ± 2.5
271	Ambient	3.3 ± 0.2	0.8 ±0.02	12.8 ± 0.8	24.0 ± 0.8	121 ± 1	57.6 ± 3.2
	eCO_2	3.0 ± 0.5	0.8 ± 0.03	14.1 ± 0.3	26.9 ± 0.4	131 ± 4	55. 2 ± 0.7
Birch	Ambient	2.9 ± 0.3	1.2 ± 0.03	12.9 ± 0.2	17.6 ± 1.7	80 ± 5	54.1 ± 3.3
	eCO_2	1.7 ± 0.1	1.1 ± 0.04	12.3 ± 0.4	19.4 ± 1.0	105 ± 9	49.7 ± 1.9
Maple	Ambient	1.2 ± 0.1	0.7 ± 0.08	7.7 ± 0.1	17.1 ± 0.6	89 ± 2	63.1 ± 0.9
	eCO_2	1.0 ± 0.1	0.7 ± 0.02	7.8 ± 0.1	16.8 ± 1.7	88 ± 10	57.8 ± 2.0
P-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 × spp	0.4032	0.2893	0.1452	0.2219	0.4278	0.3123

Table 4-3 Effects of CO_2 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 significant ($P \leq 0.05$) and n = 3. Elevated CO_2 (= eCO_2).

		Radial Position			
Species/clone	Treatment	Inner	Middle	Periphery	Mean
8	Ambient	56.7 ± 2.8	57.8 ± 2.3	50.3 ± 2.4	55 ± 2.5
	eCO_2	55.3 ± 3.7	56.0 ± 2.5	47.0 ± 2.5	52.8 ± 2.9
42	Ambient	54.9 ± 2.3	58.6 ± 1.6	50.2 ± 3.5	54.6 ± 2.2
	eCO_2	58.6 ± 1.2	58.4 ± 2.6	48.9 ± 1.7	55.3 ± 1.8
216	Ambient	47.0 ± 2.3	48.5 ± 2.5	42.4 ± 1.8	46.0 ± 2.1
	eCO_2	44.8 ± 3.0	47.0 ± 3.9	39.4 ± 2.0	43.7 ± 2.9
271	Ambient	54.8 ± 1.5	55.0 ± 0.6	47.1 ± 0.2	52.3 ± 0.6
	eCO_2	57.8 ± 1.9	58.3 ± 1.7	48.7 ± 0.2	55.0 ± 1.2
Birch	Ambient	57.8 ± 2.4	60.0 ± 1.8	52.0 ± 1.8	56.6 ± 2.0
	eCO_2	54.0 ± 1.0	55.7 ± 3.0	48.1 ± 2.6	52.6 ± 1.8
Maple	Ambient	53.1 ± 2.0	53.1 ± 0.3	45.3 ± 0.5	50.5 ± 0.7
	eCO_2	50.3 ± 2.0	49.4 ± 2.0	42.3 ± 1.1	47.3 ±1.5
***P*-values**					
Species / clone		**0.0004**	**0.0003**	**0.0003**	**0.0001**
eCO_2		0.8092	0.4274	0.1218	0.4263
eCO_2 × species /clone		0.4137	0.5630	0.6857	0.5049
Position					**<0.0001**
eCO_2 × position					0.2027
Species / clone × position					0.5148
eCO_2 × species / clone ×position					0.8565

Table 4-4 Effects of elevated O_3 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 (± 1SE). Highlighted *P*-values are either significant ($P \leq 0.05$) or marginally significant ($P \leq 0.10$) and n = 3. Elevated O_3 (=eO_3).

Species / Clones	Treatment	Ring width (mm)	Fiber length (mm)	Fiber lumen diameter (μm)	Vessel %	Number of vessels / mm^2	Cell wall area %
8	Ambient	2.4 ± 0.2	0.8 ± 0.05	12.0 ± 0.5	35.6 ± 1.0	145 ± 13	30.6 ± 0.8
	eO_3	4.2 ± 0.9	0.8 ± 0.07	12.1 ± 0.4	32.8 ± 0.5	132 ± 13	37.4 ± 2.0
42	Ambient	3.9 ± 0.1	0.7 ± 0.01	11.6 ± 0.4	32.4 ± 0.6	141 ± 6	46.4 ± 3.6
	eO_3	4.3 ± 0.4	0.7 ± 0.02	11.8 ± 0.2	30.1 ± 1.0	128 ± 2	48.3 ± 0.3
216	Ambient	2.7 ± 0.4	0.8 ± 0.05	12.1 ± 0.5	24.6 ± 0.8	144 ± 2	50.8 ± 1.9
	eO_3	2.1 ± 0.4	0.7 ± 0.04	12.4 ± 0.4	26.1 ± 2.0	155 ± 7	47.2 ± 1.2
271	Ambient	3.3 ± 0.2	0.8 ±0.02	12.8 ± 0.8	24.0 ± 0.8	121 ± 1	57.6 ± 3.2
	eO_3	3.3 ± 0.3	0.7 ± 0.02	13.2 ± 0.3	28.4 ± 1.4	131 ± 2	49.0 ± 1.9
Birch	Ambient	2.9 ± 0.3	1.2 ± 0.03	12.9 ± 0.2	17.6 ± 1.7	80 ± 5	54.1 ± 3.3
	eO_3	2.3 ± 0.4	1.0 ± 0.03	11.6 ± 0.5	19.9 ± 1.9	91 ± 5	50.8 ± 2.6
Maple	Ambient	1.2 ± 0.1	0.7 ± 0.08	7.7 ± 0.1	17.1 ± 0.6	89 ± 2	63.1 ± 0.9
	eO_3	1.1 ± 0.1	0.7 ± 0.03	7.5 ± 0.3	16.0 ± 0.7	92 ± 5	62.9 ± 0.3
P-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 × spp	**0.0499**	0.1807	0.3695	**0.0803**	0.2268	**0.0427**

Table 4-5 Effects of O_3 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 ($P \leq 0.05$) or marginally significantly different ($P \leq 0.10$) and n = 3. Elevated O_3 (=eO_3).

		Position			
Species / clone	Treatment	Inner	Middle	Periphery	Mean
8	Ambient	56.7 ± 2.8	57.8 ± 2.3	50.3 ± 2.4	55 ± 2.5
	eO_3	59.4 ± 2.0	61.5 ± 3.1	51.8 ± 1.9	57.6 ± 2.2
42	Ambient	54.9 ± 2.3	58.6 ± 1.6	50.2 ± 3.5	54.6 ± 2.2
	eO_3	56.8 ± 0.8	57.8 ± 0.4	46.1 ± 0.2	53.6 ± 0.3
216	Ambient	47.0 ± 2.3	48.5 ± 2.5	42.4 ± 1.8	46.0 ± 2.1
	eO_3	47.1 ± 0.6	45.9 ± 1.8	39.8 ± 1.6	44.3 ± 1.3
271	Ambient	54.8 ± 1.5	55.0 ± 0.6	47.1 ± 0.2	52.3 ± 0.6
	eO_3	54.8 ± 1.3	53.3 ± 1.0	46.6 ± 0.9	51.6 ± 1.1
Birch	Ambient	57.8 ± 2.4	60.0 ± 1.8	52.0 ± 1.8	56.6 ± 2.0
	eO_3	53.2 ± 3.2	52.4 ± 3.8	45.6 ± 2.7	50.4 ± 3.2
Maple	Ambient	53.1 ± 2.0	53.1 ± 0.3	45.3 ± 0.5	50.5 ± 0.7
	eO_3	46.8 ± 1.3	44.8 ± 1.5	40.2 ± 1.5	43.9 ± 1.4
***P*-values**					
Species / clone		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
eO_3		0.5485	0.1304	**0.0026**	0.1301
eO_3 × species /clone		**0.1040**	**0.0232**	0.1404	**0.0551**
position					**<0.0001**
eO_3 × position					**0.0110**
Species × position					**0.0363**
eO_3 × spp/clone ×position					0.1168

Table 4-6 Effects of elevated CO_2 + O_3 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 (± 1SE). Highlighted *P*-values are either significantly ($P \leq 0.05$) or marginally significantly different ($P \leq 0.10$) and n = 3. Elevated CO_2 (= eCO_2) and elevated O_3 (=eO_3).

Species / Clone	Treatment	Ring width (mm)	Fiber length (mm)	Fiber lumen diameter (μm)	Vessel %	Number of vessels / mm^2	Cell wall area %
8	Ambient	2.4 ± 0.2	0.8 ± 0.05	12.0 ± 0.5	35.6 ± 1.0	145 ± 13	30.6 ± 0.8
	eCO_2 + eO_3	3.9 ± 0.3	0.8 ± 0.04	12.5 ± 0.3	33.2 ± 1.9	130 ± 4	35.8 ±1.6
42	Ambient	3.9 ± 0.1	0.7 ± 0.01	11.6 ± 0.4	32.4 ± 0.6	141 ± 6	46.4 ± 3.6
	eCO_2 + eO_3	2.3 ± 0.6	0.7 ± 0.03	11.2 ± 0.3	31.9 ± 1.2	141 ± 7	46.2 ± 0.7
216	Ambient	2.7 ± 0.4	0.8 ± 0.05	12.1 ± 0.5	24.6 ± 0.8	144 ± 2	50.8 ± 1.9
	eCO_2 + eO_3	2.7 ± 1.2	0.8 ± 0.01	12.0 ± 0.7	25.5 ± 1.2	159 ± 24	53.8 ± 0.9
271	Ambient	3.3 ± 0.2	0.8 ±0.02	12.8 ± 0.8	24.0 ± 0.8	121 ± 1	57.6 ± 3.2
	eCO_2 + eO_3	3.3 ± 0.2	0.8 ± 0.02	13.7 ± 0.5	28.1 ± 2.1	133 ± 16	50.7 ± 2.1
Birch	Ambient	2.9 ± 0.3	1.2 ± 0.03	12.9 ± 0.2	17.6 ± 1.7	80 ± 5	54.1 ± 3.3
	eCO_2 + eO_3	2.7 ± 0.8	1.2 ± 0.03	12.4 ± 0.5	16.0 ± 1.9	75 ± 3	56.9 ± 1.9
Maple	Ambient	1.2 ± 0.1	0.7 ± 0.08	7.7 ± 0.1	17.1 ± 0.6	89 ± 2	63.1 ± 0.9
	eCO_2 + eO_3	1.2 ± 0.1	0.7 ± 0.03	7.5 ± 0.3	14.5 ± 0.3	79 ± 4	63.0 ± 1.6
P-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 × spp	0.1262	0.5513	0.6619	**0.0724**	0.4863	0.1403

Table 4-7 Effects of CO_2 + O_3 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 ($P \leq 0.05$) or marginally significantly different ($P \leq 0.10$) and n = 3. Elevated CO_2 (= eCO_2) and elevated O_3 (=eO_3).

		Radial position			
Species / clone	Treatment	Inner	Middle	Periphery	Mean
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
***P*-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 ×position					0.8875

CHAPTER 5: Dissertation Synthesis

Introduction

Atmospheric CO_2 and O_3 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 CO_2 concentrations stimulate woody plant growth, but elevated O_3 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 CO_2 or O_3 is altered (Kaakinen et al. 2004; Kostiainen et al. 2004; Parsons et al. 2004; Liu et al. 2005; Kostiainen et al. 2006; Kostiainen 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 CO_2 and O_3, 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 CO_2 and / or O_3 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 CO_2 and / or O_3 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 CO_2 and / or O_3 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 CO_2 and / or O_3 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 CO_2 and / or O_3 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 CO_2 compared to the control. In contrast, wood density of aspen clone 42 and birch increased significantly under elevated O_3 compared to the control. The wood densities of aspen or birch under combined treatments of elevated CO_2 and O_3 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 CO_2 and / or O_3 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 CO_2 and / or O_3 on wood-decaying fungal community composition and initial decomposition rates. Results indicated that production or decomposition of birch and aspen under elevated CO_2 and / or O_3 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 CO_2 and / or O_3 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 CO_2 and / or O_3 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 CO_2, except for a marginal increase in number of vessels per square millimeter in one aspen genotype (271) and birch. However, under elevated O_3, vessel lumen diameter decreased significantly in maple and marginally in birch compared to the ambient. The combined treatment of elevated CO_2 and O_3 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 CO_2 and / or O_3 treatments.

Conclusions / implications of study

Information on the effects of elevated O_3 alone and in combination with elevated CO_2 on wood density is rare. Nonetheless, mounting experimental evidence indicates that, elevated CO_2 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 CO_2 or O_3 alone could be modified or not depending on age, species or genotype (Telewski et al. 1999; Kaakinen et al. 2004; Kostiainen et al. 2004; Luo et al. 2005; Yazaki et al. 2005; Kostiainen et al. 2006; Kostiainen et al. 2008; Kostiainen et al. 2009; Watanabe et al. 2010). However, wood anatomical properties of woody plants grown under combined CO_2 and O_3 are not altered (Kostiainen et al. 2004; Kostiainen et al. 2006; Kostiainen 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 CO_2 and / or O_3 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 CO_2 compared to the control. Additionally, the vessel and fiber lumen diameters of aspen 271 tended to increase under elevated CO_2 compared to the ambient, confirming earlier reports from Aspen FACE (Kaakinen et al. 2004; Kostiainen et al. 2008). As a result void space in aspen 271 increased under elevated CO_2 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 CO_2 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 CO_2 responses, wood density of aspen genotype 42 and birch increased significantly under elevated O_3 compared to the ambient in this study. Moderate modifications in the anatomical properties of birch and aspen 42 observed under elevated O_3, 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 O_3, 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 O_3 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 O_3 compared to the ambient at the same experiment site (Kaakinen et al. 2004; Kostiainen et al. 2008). Hence chemical and cellular modifications which occurred in some aspen clones and birch under elevated CO_2 or O_3 may have resulted in the moderate modifications of wood density compared to ambient. This

suggests that future higher concentrations of CO_2 or O_3 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 CO_2 or O_3 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 / m^3 (Panshin and Zeeuw 1980). The combined treatment of elevated CO_2 and O_3 did not show any significant effects on either wood density or anatomical properties. Implying that, the effects of elevated CO_2 or O_3 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 CO_2 or O_3 compared to the ambient, but growth or decomposition of birch and aspen wood in elevated CO_2 and / or O_3 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 CO_2 and / or O_3 on the wood-decaying fungal community. The effects of elevated CO_2 and / or O_3 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 CO_2 and / or O_3 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 CO_2 and O_3 (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 CO_2 or O_3 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 CO_2 and O_3 on stand productivity.

Finally, because wood density, wood-decaying fungal community composition, and wood decomposition, and wood anatomical properties responses to elevated CO_2 and or / O_3 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 CO_2 and / or O_3.

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 CO_2 and / or O_3. Nevertheless, the results are reliable for stem density long term response to elevated CO_2 and / or O_3 of common northern hardwoods. Hence it is recommended that future research consider separating the effects of elevated CO_2 and / or O_3 on bark and wood density.

Although, the effects of CO_2 and / or O_3 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 basidiomyecetes. 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 CO_2 and / or O_3. 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 CO_2 and / or O_3 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 CO_2 and / or O_3, 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 CO_2 and / or O_3 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 CO_2 and / or O_3 on wood anatomical properties.

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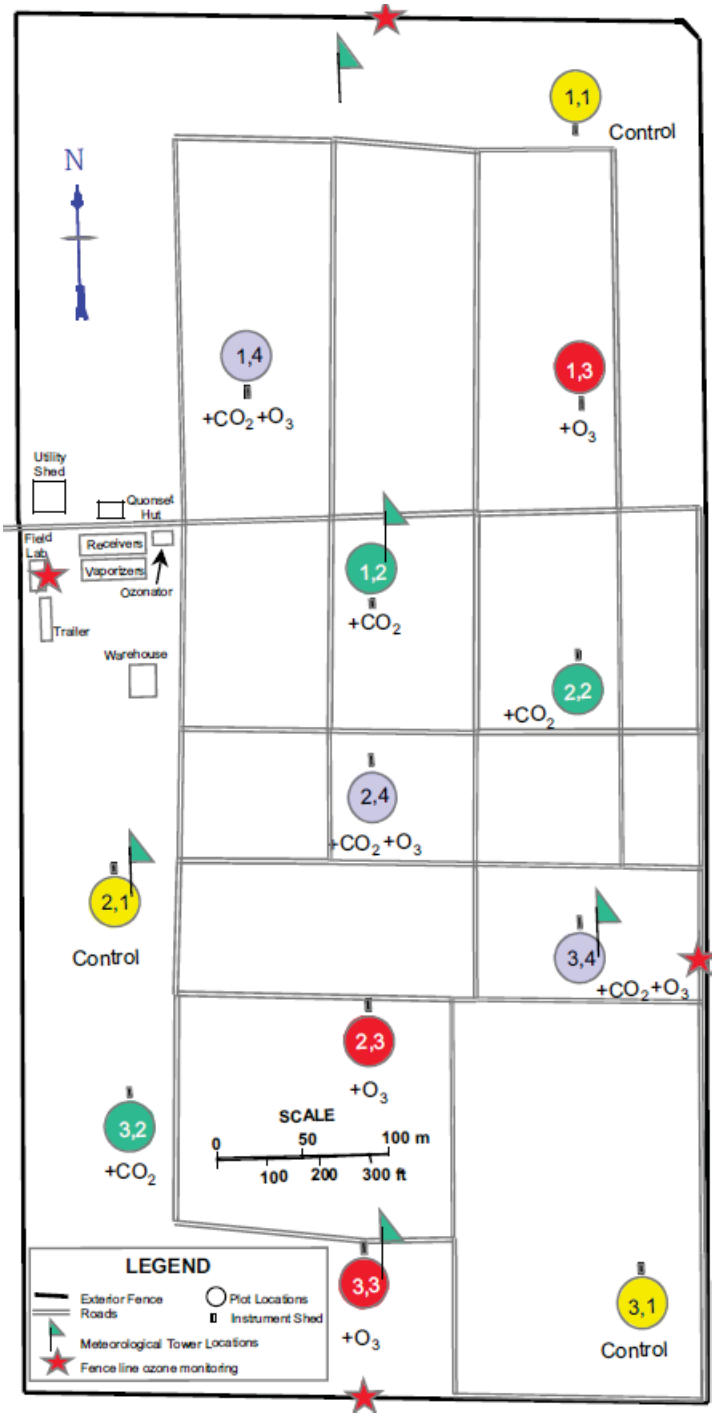
APPENDIX



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 CO_2 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*
Between subjects					
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		
Within subjects					
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*
Between subjects					
Elevated CO_2	3611.7	1	3611.7	1.64	0.2293
Error	22033.1	10	2203.3		
Within subjects					
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*
Between subjects					
Elevated CO_2	2387.7419	1	2387.7419	1.82	0.2068
Error	13099.9696	10	1309.9970		
Within subjects					
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*
Between subjects					
Elevated CO_2	3738.8513	1	3738.8513	13.65	0.0041
Error	2738.2846	10	273.8285		
Within subjects					
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*
Between subjects					
Elevated CO_2	3611.7145	1	3611.7145	1.64	0.2293
Error	22033.0886	10	2203.3087		
Within subjects					
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 O_3 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*
Between subjects					
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		
Within subjects					
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*
Between subjects					
Elevated O_3	13041.3811	1	13041.3811	7.52	0.0208
Error	17348.9504	10	1734.8950		
Within subjects					
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*
Between subjects					
Elevated O_3	17.5774	1	17.5774	0.01	0.906
Error	11971.9014	10	1197.1901		
Within subjects					
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*
Between subjects					
Elevated O_3	256.4739	1	256.4739	0.28	0.6067
Error	9081.9448	10	908.1945		
Within subjects					
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*
Between subjects					
Elevated O_3	13041.3811	1	13041.3811	7.52	0.0208
Error	17348.9504	10	1734.8950		
Within subjects					
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 CO_2 + O_3 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*
Between subjects					
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		
Within subjects					
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*
Between subjects					
Elevated CO_2 + O_3	3136.5839	1	3136.5839	2.33	0.1575
Error	13436.6039	10	1343.6604		
Within subjects					
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*
Between subjects					
Elevated CO_2 + O_3	1317.2695	1	1317.2695	1.1	0.3160
Error	11824.6303	10	1182.4630		
Within subjects					
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*
Between subjects					
Elevated CO_2 + O_3	1535.3192	1	1535.3192	1.15	0.3080
Error	13304.8416	10	1330.4842		
Within subjects					
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*
Between subjects					
Elevated CO_2 + O_3	3136.5839	1	3136.5839	2.33	0.1575
Error	13436.6039	10	1343.6604		
Within subjects					
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 CO_2 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 CO_2 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 CO_2 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 O_3 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 CO_2 + O_3 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	*F* ratio	*P* > *F*
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	*F* ratio	*P* > *F*
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	*F* ratio	*P* > *F*
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	*F* ratio	*P* > *F*
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	*F* ratio	*P* > *F*
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	*F* ratio	*P* > *F*
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 CO_2 and species on wood anatomical properties:

Growth ring width

Source	SS	df	MS	*F* ratio	*P* > *F*
Elevated CO_2	1915001.998	1	1915001.998	3.46	0.2037
Error - main	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
Error - subplot	9939489.69	20	496974.48		
Total	43506830.25	35			

Fiber length

Source	SS	df	MS	*F* ratio	*P* > *F*
Elevated CO_2	59.25649420	1	59.25649420	0.20	0.6984
Error - main	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
Error - subplot	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 CO_2, 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 O_3 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 O_3, 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 CO_2 + O_3 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 CO_2 + O_3, 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 O_3 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 O_3 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 O_3 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 O_3 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 O_3 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 CO_2 + O_3 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	00005698	2	0.00002849	0.41	0.7092
Error	0.00013900	2	0.00006950		
Total	0.00098636	5			