

ABSTRACT

RHEA, LEE KIRK. Implications of Elevated Atmospheric Carbon Dioxide and Tropospheric Ozone for Water Use in Stands of Trembling Aspen and Paper Birch. (Under the direction of John S. King.)

Projected increases in the atmospheric concentration of CO₂ and tropospheric O₃ over the next 50 years are of concern due in part to their potential to affect forest water budgets. I conducted a series of studies at the Aspen Free Air CO₂ and O₃ Enrichment experiment near Rhinelander, WI to determine the effect of projected concentrations of these gases for the year 2050 on the water budget in stands of trembling aspen and paper birch.

In order to determine the effects of elevated CO₂ (eCO₂) and O₃ (eO₃) on rainfall partitioning between interception, through fall, and stem flow I performed a computerized analysis of photographed canopy branches and compared the results to hydrologic measurements. Elevated O₃ significantly decreased total aspen and birch branch length, resulting in net decreases for 2002 whorls of -18 % and 2006 whorls of -16 %. Some of these changes had measurable effects on rainfall partitioning.

The biomass of fine roots has been observed to change in response to eCO₂ and eO₃ at shallow depths, but little work has been done to assess deeper roots. I

characterized fine root responses to eCO₂ and eO₃ to a depth of one meter. Fumigation with O₃ increased small root biomass in shallow soil 30 % in all-aspen plots and decreased root biomass in shallow soil 46 % in aspen-birch plots. Increases in root length up to 131 % and specific root length up to 77 % occurred under eO₃ in middle and deep soil layers, indicating more extensive soil exploration at depth. Small root biomass in shallow soils increased 20 % to 24 % under eCO₂, indicative of more intensive soil exploration near the surface.

Previous studies of sapwood from Aspen-FACE indicated that anatomical structures related to hydraulic conductance (K) differed between aspen clones and that they responded to the treatments differently. I constructed embolism curves for stem wood samples collected below the base of the live crowns. There were no significant treatment effects on K at full water saturation, although there was trend for a decrease in K under eO₃. Clones 42E and 216 responded to all treatment combinations with increased cavitation resistance, requiring from 16 % to 85 % increased pressure to reach 50 % embolization (P₅₀). Clones 8L and 271 exhibited minor responses in embolizing pressure to reach P₅₀ under eCO₂ and eCO₂×eO₃ (-3.3 % to +3.2 %) but reductions under eO₃ (-43 % to -48 % for clone 8L and -8.9 % to -14 % for clone 271).

Both eCO₂ and eO₃ have been shown to decrease stomatal conductance, but the potential water savings can be offset by increased canopy growth under eO₂ and increased understory growth under eO₃. Previous workers have attempted to determine the influence on stand water use of eCO₂ and eO₃ and found increased growth under eCO₂ and no significant change under eO₃ at Aspen FACE, but at other sites eCO₂ had relatively minor effects on total water use and eO₃ resulted in a net decrease in water use. I conducted a combined sap flux and stratified soil water content study and saw only weak effects of eCO₂ on sap flux but increases in shallow soil water content and deep soil drainage, and decreased sap flux under eO₃ in conjunction with decreased soil water content in deep soils and a larger increase in deep soil drainage.

Implications of Elevated Atmospheric Carbon Dioxide and Tropospheric Ozone
for Water Use in Stands of Trembling Aspen and Paper Birch

by
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DEDICATION

To my wife and parents.

BIOGRAPHY

The author was born in Alameda, California in 1968 to the daughter of a farmer from Iowa and the son of a rancher from Montana. He was raised from the age of four in Easton, Pennsylvania until graduating from the Easton Area High School in 1986. He then attended Penn State, graduated in 1990 with a B.S. in earth science with a minor in marine science. He entered an M.S. program in oceanography at the University of New Hampshire in the fall of 1990 but changed his major to hydrology soon after arrival. After three years he took a full-time job at an environmental consulting company, finally finishing a thesis on Monte-Carlo simulation of petroleum migration, and graduating, in 1994. He continued to work for the same company until moving to North Carolina with his wife and son in 1998, and there worked as a consulting hydrogeologist until matriculating at NC State in 2005.

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CHAPTER 1

Introduction

Availability of water for human use and natural ecosystems during the coming decades is a significant concern, and increasing concentrations of carbon dioxide (CO₂) and tropospheric ozone (O₃) may significantly affect the hydrologic budget (IPCC 2001, 2007; UNWWAP 2003, 2006). Shifts in the frequency and intensity of precipitation are expected as a consequence of projected global warming attributed to anthropogenic changes in atmospheric chemistry (IPCC 2001, 2007). IPCC (2007) concluded that anthropogenic sources of greenhouse gases are almost certainly the cause of a significant portion of global warming, and that global warming will have a significant effect on many ecosystems. They identified CO₂ as the dominant anthropogenic greenhouse gas, e.g. constituting 77 % of total anthropogenic greenhouse gas emissions in 2004. During the last 160,000 years atmospheric CO₂ usually ranged between 180 to 250 ppm (Finlayson-Pitts and Pitts, 1997, 2000; Fowler et al., 1999; IPCC, 2001; IPCC, 2007) and global temperatures were highly correlated to the concentration of CO₂ during that period (Barnola et al., 1995; Raynaud et al., 1993). Since the 19th century Industrial Revolution the CO₂ concentration has steadily risen at an increasing rate to about 380 ppm (Friedli et al., 1986; Keeling et al., 1995), and is projected to reach or exceed 700 ppm within the next 100 to 150 years (IPCC, 2000).

The responses of ecosystems to changes in atmospheric chemistry are not limited to responses to altered temperatures and precipitation patterns caused by greenhouse effects. In the absence of other limiting resources, elevated CO₂ has also been shown to directly increase plant growth, leaf area index and water use efficiency (Ceulemans and Mousseau, 1994; Strain and Cure, 1994; Wullschleger et al., 1997; Curtis and Wang, 1998; Oren et al., 2001; Delucia et al., 1999; King et al.; 2001, 2005) and may lead to changes in phenology such as bud burst or bud set (Murray et al., 1994; Olszyk et al., 1998; Jach and Ceulemans, 1999; Sigurdsson, 2001; Kilpeläinen et al., 2006; Slaney et al., 2007). Increases in growth are not limited to aboveground organs, occurring below ground as well (Rogers and Runion, 1994; Pregitzer et al., 1995; King et al., 1996, 2001, 2005). These effects are generally larger in species that use the C₃ photosynthetic pathway (see Dickson et al., 2000), because these species are more CO₂- limited than C₄ and CAM species, and in species with higher photosynthetic and respiratory rates, such as early successional species (Ackerly and Bazzazz, 1995; Poorter 1998) and species with indeterminate growth. A meta-analysis by Ainsworth and Rogers (2007) found that on average for free-air CO₂ enrichment (FACE) experiments, elevated CO₂ reduced stomatal conductance by 22 % but only a statistically insignificant 5 % reduction in stomatal density occurred, and some acclimatization over time did occur. They found that light-saturated

photosynthesis of C3 plants increased 31 % on average but was dependent on functional type, available nitrogen supply and sink capacity, and environment. Photosynthetic limitation by Rubisco (carboxylation) shifted toward limitation by Ribulose biphosphate (RubP) regeneration (electron transport) with increased atmospheric CO₂, but unlike shrubs and crops, trees and C3 grasses did not become RubP limited under elevated CO₂. Many of the effects of elevated CO₂ are believed to be a consequence of increased concentrations of CO₂ in sub-stomatal cavities (Strain and Cure, 1994). Increased atmospheric concentration of CO₂ is thought to allow C3 plants to reduce stomatal aperture during photosynthesis, which may have the effect of also reducing transpiration and therefore potentially increasing water use efficiency, lowering water use, and increasing drought tolerance (Sionit and Kramer, 1986; Morison 1993). Some small-scale experiments have found decreased stand water use (Scarascia-Mugnozza and De Angelis, 1998; Jackson et al., 1994; Owensby et al., 1996) but increased leaf area commonly observed under elevated CO₂ (e.g. Curtis et al., 1995; Ceulemans et al., 1999) may compensate for or dominate increased water use efficiency (Jones et al., 1985; Nijs et al., 1989). Increased xylem hydraulic efficiency associated with faster growth might also offset predicted water savings (Ceulemans and Mousseau, 1994).

In contrast to the effects of elevated CO₂, the relatively minor greenhouse gas ozone (O₃) is nonetheless a dominant air pollutant (Ashmore, 2005; EPA, 2006; Karnosky et al., 2007; Matyssek et al., 2007; Paoletti et al., 2007) and phytotoxin (Pye, 1988; Bortier et al., 2000) formed by photochemical reactions of nitrogen oxides (Fowler et al., 1999b; Denman et al., 2007; Forster et al., 2007). It has risen from background concentrations of 10-20 ppb to ambient concentrations in many areas in excess of 40 ppb today (IPCC, 2001, 2007), and has been shown at present and projected future concentrations (Percy, 2003) to decrease plant growth, leaf area display, leaf longevity, stomatal control, photosynthesis, phloem loading, fine root growth, disease and predation, plant water balance, and plant species diversity (Barbo et al., 1998; Pell et al., 1999; Oksanen and Rousi, 2001; Fluckiger et al., 2002; Karlsson et al., 2003b; Kopper and Lindroth, 2003; Grantz, 2003, Karnosky et al., 2003; Panek, 2004; Karnosky, 2005, 2006; Nunn et al., 2005; McLaughlin et al., 2007a, b). Based on a meta-analysis including 263 studies, Wittig et al. (2009) found that current ambient O₃ concentrations averaging about 40 ppb reduced total biomass of trees by 7 %; reduced the root-to-shoot ratio, indicating higher belowground sensitivity; and decreased transpiration rates. The phytotoxic effects of ozone are a result of its high oxidative power. After entering through stomates it dissolves into the aqueous phase of the apoplast and reacts with fatty acids of the plasmalemma to form

aldehydes and reactive oxygen species such as hydrogen peroxide, superoxide, and oxidative radicals (Hippelli and Elstner, 1996) that can destroy cell membranes (Winston, 1990) and lead to a reduction in photosynthesis and growth (Pye, 1988; Saxe, 1991; Bortier et al., 2000). Reduced photosynthesis typically is accompanied by a reduction in stomatal aperture, which can result in a partial decoupling between atmospheric ozone concentrations and observed effects on plant growth. For this same reason several extraneous factors can reduce uptake of O₃, including low respiratory rates, high atmospheric vapor pressure deficit, low soil water content, or elevated atmospheric CO₂. Thus, young leaves and early successional species with relatively high rates of respiration and photosynthesis tend to be more susceptible to damage by O₃. Over time, however, cumulative oxidative damage to the cell wall lignin of guard cells (Maier-Maercker, 1998) may eventually result in sluggish stomatal responses, resulting in loss of stomatal control and decrease in water use efficiency (Reich and Lassoie, 1984; Matyssek et al., 1991; Maurer and Matyssek, 1997). Effects of O₃ on photosynthesis may not be observed until the rate of O₃ uptake exceeds the plant's production rate of antioxidant defense compounds, although visible foliar injury may already be apparent (Ashmore, 2005). Although increased insect predation has been observed under O₃, it is also known to stimulate the phenylpropanoid pathway, resulting in increased concentrations of phenolics (e.g.

Wustman et al., 2001). Increased apportionment to secondary metabolites and repair has been identified as a possible cause of decreased root growth under elevated O₃ (Dickson, 1986, 1991; Coleman et al., 1996; Wulff et al., 1996; Anderson et al., 1997), and may be exacerbated by increased leaf aging and earlier senescence (Karnosky et al., 1996) which may disproportionately affect the mature leaves at the base of the canopy of *Populus* species, which export carbon to support root and stem growth and respiration. Thus, reduced growth may lead to decreased water use, but this effect may be offset by lower water use efficiency or increased understory growth due to increased light availability.

Combined elevated CO₂ and O₃ have sometimes been shown to have offsetting effects on plant growth (Allen, 1990; McKee et al., 1995; Mortensen, 1995; Volin and Reich, 1996; Dickson et al., 1998; Volin et al., 1998; Loats and Rebbeck, 1999; Karnosky et al., 1999), although such offsets have not been universally observed (Balaguer et al., 1995; Barnes et al., 1995; Kull et al., 1996). It is thought that findings of inconsistent interactions between these gases are related to inter- and intra-species differences in stomatal responses (Reich, 1987); stomatal closure in response to high atmospheric vapor pressure deficit and low soil water content (Grunehage and Jager, 2003; Vollenweider et al., 2003b; le Thiec and Manninen, 2003), differing sensitivity to O₃ by leaf ontogenetic stage

and respiration rate (Fredrickson et al., 1996; Wieser et al., 2002), sun exposure (Tjoelker et al., 1995; Samuelson and Kelly, 2001; Kolb and Matyssek, 2001; Wei et al., 2004), partitioning of photosynthate at the time of exposure (Fuhrer and Booker, 2003), and variation in antioxidants (Busotti and Gerosa, 2001; Nali et al., 2004).

Effects of global change on climate and ecosystems are expected to increase toward the poles, and to be greater in the northern hemisphere due to its greater land area. Increased temperature, more frequent droughts in the west, and more variable and possibly increased precipitation in the east are projected for the U.S. mainland. The direct effects of these climatic changes on the hydrologic cycle and water supply will be modified by the response of vegetation, which will be responding not only to the changes in climate but also to altered atmospheric chemistry. This thesis investigates the effects on the hydrologic budget of responses of some important early-successional boreal tree species, trembling aspen (*Populus tremuloides* Michx.) and paper birch (*Betula papyrifera* Marsh.), to increases in CO₂ and O₃ predicted to occur within the next 50 to 100 years. Trembling aspen is an economically important species that has the widest distribution of any tree species in North America (Perala, 1990) and paper birch is

a strong competitor of aspen where these species co-occur (Barnes and Wagner, 1981).

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CHAPTER 2

**Effects of elevated atmospheric CO₂ and tropospheric O₃ on tree branch
growth and implications for hydrologic budgeting**

Abstract

Atmospheric concentrations of CO₂ and tropospheric O₃ have been rapidly increasing since the Industrial Revolution. Because CO₂ and tropospheric O₃ greatly affect growth and display of leaves, the amount of light and precipitation intercepted by the canopy are likely to change with impacts on evapotranspiration (ET). We hypothesized that increased CO₂ would increase branching and ozone would decrease branching, and that these changes would have commensurate effects on interception, throughfall, and stem flow. Numerous remote sensing methods have been used to characterize canopy architecture with some success, but resolution down to the scale of individual branches, currently incorporated into the best computer models of interception, has been elusive. An established method for analysis of structures exhibiting dendritic topologies such as tree canopies, roots, blood vessel systems, and neurons is computerized analysis of digital images. In order to determine the effects of elevated CO₂ and O₃ on canopy architecture we performed a computerized analysis of all branches from the 2002, 2004, and 2006 annual growth whorls of 97 trees from the Aspen Free Air CO₂ and O₃ Enrichment experiment (Rhinelander, WI). Specifically, we present the results of an analysis of changes in tree morphological and topological metrics including branching length, branch volume, number of tips per branch (magnitude), maximum branch hydraulic path-length / maximum number of

nodes traversed by evaporating water (branch altitude), and secondary branching angles from trembling aspen and paper birch trees grown for 10 years in a combined CO₂ - O₃ - community - genotype factorial experiment. Logically, greater total branching lengths were associated with older whorls for each species. Tropospheric O₃ significantly decreased total aspen and birch branch length, resulting in net decreases in the presence of other influencing factors for 2002 whorls of -18% and 2006 whorls of -16%. Increases in total branching length for whorls seemed apparent in the raw data with added CO₂, but the effects were insignificant due to the amount of unexplained variation and differing responses amongst aspen clones and between communities. Differential CO₂ effects were significant for the 2004 second-order segment lengths and 2006 total branch lengths of some aspen clones, and a decrease in order of 2006 branches across clones (-13%). Branch metrics were generally inferior to standard stem metrics for predicting apportionment of precipitation between interception, throughfall, and stemflow. Throughfall was decreased in the presence of CO₂ by an average of 5.4 % across communities and usually insignificantly affected by CO₂ × O₃, but was differentially affected across communities by O₃ and the direction of the effects was inconsistent. Stem flow increased under CO₂ an average of 11 % in the AA community and 16 % in the AB community, was usually insignificantly affected by combined CO₂ and O₃, and decreased under O₃ an average of 18 % in

the AA community and 16 % in the AB community. Interception was also differentiated by community, and increased under CO₂ 4.9 % in the AA community and 4.5 % in the AB community, usually insignificantly effected under combined CO₂ and O₃, and increased by O₃ 2.5 % in the AA community but decreased 18 % in the AB community. In this study predicted future atmospheric concentrations of CO₂ and tropospheric O₃ were shown to significantly affect canopy growth and partitioning of precipitation within the hydrologic budget.

Introduction

Availability of water for human use and natural ecosystems during the coming decades is a significant concern due to the rapidly rising mean global temperature and expected shifts in the frequency and intensity of precipitation (IPCC 2001; UNWWAP 2003, 2006). Groundwater and surface water resources are significantly reduced by forest evapotranspiration (Dingman, 1994). Atmospheric concentrations of gases, including CO₂ and O₃, have been increasing since the Industrial Revolution (Finlayson-Pitts and Pitts, 1997, 2000; Fowler et al., 1999; IPCC, 2001) and evapotranspiration (ET) could be significantly impacted by changes in forest tree physiology and growth. Elevated atmospheric CO₂ has been shown to increase plant growth, leaf area index and water use efficiency, while increases in tropospheric O₃ have been shown to decrease plant growth, leaf area display, and stomatal control (Karnosky et al., 2003; Karnosky, 2005; McLaughlin et al., 2007a; Wittig et al., 2007) as well as to directly affect plant water balance (Panek, 2004; McLaughlin et al., 2007a, b). The antagonistic effects of elevated CO₂ and O₃ on plant growth may offset one another (Allen, 1990; Dickson et al., 1998; Volin et al., 1998; Loats and Rebbeck, 1999; Karnosky et al., 1999), although such offsets have not been universally observed (Barnes et al., 1995; Kull et al., 1996).

Evapotranspiration is affected by tree crown interception of precipitation (Horton, 1919; Pypker et al., 2005; Roth et al., 2007), and tree crown architecture is controlled by natural environmental variation as well as genetic potential (Tremmel and Bazzaz, 1995). Apportionment of precipitation to interception, throughfall, and stem flow is problematic and associated with large relative errors (Kimmins, 1973) but is important because it influences the amount and areal distribution of water contacting the soil surface, and thereby recharge of subsurface water supplies (Clements, 1971). Canopy architectural parameters such as stem surface index, branch roughness, and branch orientation have been shown to be important predictors of canopy interception, in addition to more commonly used parameters such as leaf area index (Xiao, 2000). Previous work with European aspen and birch (Matyssek et al., 2002, 2003) and trembling aspen (Coleman et al., 1996; Karnosky et al., 1996; Dickson et al., 2001) has shown that morphological or topological changes in tree branching, such as terminal branch angles, can result from increasing CO₂ or O₃. In addition, significant changes in canopy architecture and species composition have been observed in some enhanced CO₂ studies due to alterations in the relative fitness of plant species (Reekie and Bazzaz, 1989; review by Pritchard et al., 1999). Others have found

lesser or insignificant canopy changes in the presence of elevated CO₂ (Kubiske, 1997; Norby et al., 2001; Gielen et al., 2002, 2003), but these studies were generally focused on leaf area, light interception, or stem growth and not branch architecture. None of these studies investigated the influence of changes to canopy branching architecture caused by elevated CO₂ or O₃ on the hydrologic budget.

Studies relying on detailed characterization of canopy architecture are hampered by the difficulty in acquiring adequate data (Singh and Woolhiser, 2002).

Numerous remote sensing methods such as interferometric synthetic aperture radar (InSAR), multispectral imagery, synthetic aperture radar (SAR) and light interception detection and ranging (LIDAR) have been used to characterize gross canopy architecture (Mougin et al., 1999; Slatton et al., 2001; Parker et al., 2004; Houldcroft et al., 2005; Butson and King, 2006; Roth et al., 2007), but resolution down to the scale of individual branches has been elusive. An established method for detailed analysis of structures exhibiting dendritic topologies such as tree canopies, roots, blood vessel systems, and neurons is computerized analysis of photographs, and several readily available freeware and proprietary platforms are

available for this purpose (e.g. *NIHImage* [<http://rsbweb.nih.gov/ij/>]; ImageJ [NIH]).

During a tree harvest in the summer of 2007, we were able to generate a detailed photographic dataset for characterizing canopy architecture at the Aspen Free-Air CO₂ and O₃ Enrichment (Aspen FACE) experiment located near Rhineland, WI. Aspen FACE was initiated in 1997 to study the effects of elevated atmospheric concentrations of CO₂, O₃, and their interaction on forest trees of north-temperate climates. Herein, we present the results of an analysis of changes in canopy architecture using branches collected from trembling aspen and paper birch trees harvested at Aspen FACE during the summer of 2007, and relate those changes to the hydrologic budget through regression analysis. Our hypotheses were that the apportionment of rainfall between interception, throughfall, and stem flow would be altered by elevated CO₂ and O₃ and that the alterations could be explained by changes in branch metrics. Specifically, we hypothesized that elevated CO₂ would result in additional branch growth and elevated O₃ would result in decreased branch growth, while combined elevated CO₂ and O₃ would have offsetting effects on branch growth. In addition, increases in branch growth would result in a greater proportion of interception and stem flow relative to

control, while decreases in branch growth would result in decreased interception and stem flow relative to control.

Methods

The Aspen FACE experiment consisted of twelve 30-m diameter plots grouped into three replicates of four treatments (randomized complete-block design). Each plot was subdivided into three subplots that included a mixed aspen and maple community (AM), a mixed aspen and birch community (AB), and an all-aspen community (AA). Each community was planted during 1997 with seedlings of birch or maple, or cuttings of aspen clones, on one-meter centers. Aspen clone 216 was alternated with birch and maple in the AB and AM communities, respectively. A selection of aspen clones (8L, 42E, 216, 259, and 271) representing a wide range of O₃ sensitivity (8L= tolerant; 216 and 271 = somewhat tolerant; 42E = relatively sensitive; 259 = sensitive [Karnosky et al., 2003]) comparable to that which exists in natural populations (Percy et al., 2007) were randomized within the AA communities; clone 259 died out during the experiment. Fumigation treatments were applied in the manner of the Brookhaven National Laboratory's design (Hendrey et al., 1999). Each plot was surrounded by PVC standpipes that were used to fumigate the ring, and wind/gas

sensors within the ring were used to control the fumigation rate. The fumigation treatments included: (1) ambient conditions; (2) elevated CO₂ (~560 ppm); (3) elevated O₃ (~60 ppb, or 1.5 times ambient); and (4) both elevated CO₂ and O₃. Fumigation was performed during the daylight hours of each growing season since inception of the project. Additional description of the project is available elsewhere (Dickson et al., 2000) and documentation of the fumigation system performance is available at www.aspenface.mtu.edu.

Precipitation, Stemflow, Throughfall, and Interception

Climatic conditions were generally similar during the duration of the present study (**Figure 2.1**). Precipitation (P) was automatically monitored at a central micro-meteorological station and at one treatment ring within each study replication throughout the duration of the study. Throughfall and stem flow were monitored during the 2006-2008 growing seasons. Three monitoring stations for throughfall were established in each of the all-aspen and aspen-birch communities of the 12 treatment rings in the study. Throughfall was collected using purpose-fabricated plastic collection troughs of known gathering-area (0.13 m²) that were mounted just above the top of the understory and plumbed into covered 5-gallon

collection buckets. The contents of these buckets was measured and emptied after each precipitation event that occurred during the study.

Stem flow monitoring during the study was problematic. Stem flows were monitored for at least one tree per genotype per subplot during the 2007 and 2008 growing seasons. Six monitoring stations for stem flow were established in each of the all-aspen and aspen-birch communities of the 12 treatment rings in the study. Stem flow was monitored using clear, 0.75-inch diameter nylon tubing cut into 1-meter segments and split along 0.7-meter of each segment using a box cutter. The split end was tacked to a tree stem at breast height using a steel brad, wrapped down the stem with the interior facing upward, tacked to the stem with another brad at the end of the split section, and sealed to the tree along its entire split length with clear, aquarium-safe silicone caulk. The un-split end of the tubing was fed through a tight fitting cap and inserted into a plastic jug, with the cap seated to minimize evaporative loss. The volume of water in the container was recorded after each precipitation event, and the water was then emptied onto the ground at the base of the tree. Field technicians were unable during the first year of the study to capture all of the stem flow in the containers, leading to significant right-censoring of those data. Larger containers were again overfilled

during several heavy storm events during the summer of 2007, but overfills did not occur during 2008.

Tree Branch Sample Acquisition

Branch data included in the present study were collected during a harvest conducted at Aspen FACE during August 2007. Diameter at breast height (d.b.h) was measured after leaf fall during the autumns of 2006, 2007 and 2008. The AA and AB communities of each treatment plot were sampled. The sampling plan included one each of aspen clones 8L, 42E, 216, and 271 from each AA community; and, one aspen clone 216 and one birch from each AB community. Sample trees were randomly selected from locations within designated core regions of the plots where edge effects are mitigated and atmospheric mixing reduces variation in treatment concentrations of CO₂ and O₃. The sample trees were measured for diameter at breast height (d.b.h.) and height, cut approximately 10 cm from the ground surface, immediately enclosed in plastic, and transported to the USDA Northern Research Station located in Rhinelander, WI. Any trees not immediately processed were held in cold storage at the facility. During processing, all branches for each growth whorl were counted and cut from the tree. The leaves from each branch were then removed manually. To obtain a

representative sample of the live crown, which usually started at or adjacent to the 2001 stem increment (“whorl” hereafter); each branch emanating from a 2002, 2004, or 2006 whorl was color-photographed with a digital camera against a white background. Both transverse and longitudinal views were photographed. Large branches were photographed at a nominal distance of four meters and small branches were photographed at a nominal distance of one meter. Scales were included at multiple focal lengths in the periphery of each photograph to aid dimensional analysis.

Branch Sample Imaging

Computerized analysis of branch morphology and topology was performed using the photographs of all branches from the 2002, 2004, and 2006 growth whorls of each harvested tree. A Java plug-in to the NIH ImageJ freeware (<http://rsb.info.nih.gov/ij/>) was written to perform the branch analyses, and SAS® software was used to perform ANOVA of the image data. A set of 717 plan-view tiff images were manually loaded into ImageJ, thresholded, despeckled, and converted to jpeg format for storage. The cleaned images were batch processed using a purpose-written macro. The macro loaded and processed each image sequentially. Upon loading, each image was automatically subdivided into

regions of interest (roi) corresponding to contiguous regions of non-background color (individual branches). Each roi was processed sequentially.

Initially, roi were reduced to a one-pixel-wide “skeleton” by iteratively eroding edge pixels until no areas of greater than one pixel width along their minor axis remained. The skeleton was then superimposed on the parent, thresholded image. A minor radius was calculated for each pixel in the skeleton by expanding a circle about the skeletal pixel until at least one background pixel was encountered; a major radius was similarly calculated by continuing the search until background color was encountered on two opposite points on the circle. A cross-sectional area was then calculated using the average radius and the formula for a circle. The skeletal pixels were then organized by comparisons to their neighbors. Pixels with two neighbors were designated as segment members. Pixels with one neighbor were designated as tips, or terminal bounds. Pixels with three or more neighbors were designated as potential branch points/crossover points, or internal bounds. Initial segments were then designated as all adjoining segment pixels isolated between two bounds. Some initial segments were then joined or designated as crossovers across common bounds, based on comparisons of the angles formed by the proximal segment ends, the similarity of their local radii,

and the distance separating them relative to the radii of the parent segments. To mitigate against small-scale extension effects caused by the erosion process (Kimura et al., 1999), segment lengths were calculated by summing the number of steps of length radius to traverse the segment, plus a correction at tips for the erosion process. Segment lengths were also extended at tip-ends to offset reductions during the skeletonization process. The connectivity of the final segments was then stored to form a complete branch. Segment volumes were estimated by summing the stored cross-sectional areas of each skeletal pixel within each segment.

The base of the branch was selected from among the tips using a scoring process. Each tip of the branch was initially scored based on the proximal branch radius. The score was then modified using the positional relationship of the tip to all of the forks in the branch. The score modification was based on the angle between a vector defined by the tip and the vertex of a given fork in the branch, and a vector defined by the bisector of the fork. After the base of the root was selected, branch orders for the segments were calculated by setting the tip orders to one and working upward toward the base, increasing the order by one at each junction of

two segments with the same order and maintaining the higher order at junctures of differing orders. Segments were then split across any changes in branch order.

Output included annotated images and a csv-format file. The annotated images included overlay of color coded skeletal segments, with the colored width determined by the segment order; tips identified by red circles; branch points identified by solid dots; and the branch base circled in blue (**Figure 2.2**). The csv file output included one run for only segment lengths and radii, and a second run for root topology. Summary parameter output from the root topology run included the number of segments per branch; total branch length; total branch volume; total number of branch angles with average and standard deviation; the order at the base of the branch (“branch order”); the number of tips excluding the base (“branch magnitude”); and the longest minimum path from a tip to the base (“branch altitude”). Detailed topological output also included breakouts of intersection angles by topological location (i.e. between tips or interior bounds); and breakouts by branch order of segment length, and segment radii counts, averages, and standard deviations.

Statistical Analysis

Statistical analyses were performed using the Statistical Analysis System version 9.1 (SAS Institute, Cary, North Carolina, USA). The branch data were analyzed using ANOVA to avoid loss of degrees of freedom associated with MANOVA. The ANOVA was based on individual branches to determine whether treatment effects on morphometrics such as branching length and volume were discernable. Monthly subplot-mean interception, throughfall, and stem flow data were also subjected to ANOVA to screen for treatment effects. Interception, throughfall, and stem flow data were also compared to stem d.b.h., area at breast height (a.b.h.) estimated from d.b.h., height, and branch metrics using stepwise regression via SAS v. 9.2 proc Reg.

The ANOVA model for the data was based on that described by King et al. (2001). The CO₂ and O₃ treatments were treated as whole-plot effects; community was treated as a subplot effect, genotype was nested in community, whorl was treated as a stripped (non-randomized) effect, and branches were repeated on parent tree. An initial analysis indicated that community and its interactions were insignificant effects for the branch-level data. However, each community was analyzed separately with all genotypes (species/clones) included

in the analysis because insufficient power was available for single-species analyses of birch. Due to the multi-factorial nature of the study and the potential for interactions between effects, percentage and absolute differences between effects and among factor levels are expressed herein in terms of adjusted means, or least-squares (LS) means, when influences by effects extraneous to the current discussion are significant.

A significance level of 0.05 was used. Replicate (block) was initially treated as a fixed effect in the ANOVA due to a known soil fertility gradient across the site, but was changed to random effect to increase statistical power after initial analyses indicated the fixed fertility effect was not significant.

Highly skewed parameters characteristic of survival data, such as the distribution of branch lengths, were transformed to fit a normal distribution prior to conducting ANOVA. The branch length and radius data for the whorl-level analysis were log-transformed. The data for the branch-level analysis were subjected to an optimizing (iterated) Box-Cox transformation, followed by a monotonic, inverse-error-function correction to mitigate negative kurtosis caused by excessive tail compression by the Box-Cox transformation (Klein and Fischer,

2006). All transformed data beyond four standard deviations about the mean were excluded from the analyses (<0.1% of the total datasets).

Stepwise regressions was used to discern the utility of using the branch metrics versus stem metrics to better predict treatment effects on stem flow, through-fall, and interception, and thereby on ET and the hydrologic budget as a whole. Both linear and transformed data were tried in the regressions but improvements with transformed data were minimal. An entry significance level of 0.4 and a retention significance level of 0.15 were used in the stepwise regression. All regression analyses were performed with SAS v.9.2 proc Reg.

Results

Precipitation, Stemflow, Throughfall, and Interception

Precipitation recorded at the site during the growing season was generally consistent during the study years, averaging about 325 mm for the growing season, and was close to the climate normal for the vicinity (<http://www.ncdc.noaa.gov/oa/climate/online/ccd/nrmcp.txt>). During May-September 2006-2008 control treatment mean throughfall averaged $50.8 \text{ mm}\cdot\text{mo}^{-1}$ in the all-aspen community and $50.5 \text{ mm}\cdot\text{mo}^{-1}$ in the aspen-birch community

(**Table 2.1**); mean stemflow was $6.4 \text{ mm}\cdot\text{mo}^{-1}$ in the all-aspen community and $4.6 \text{ mm}\cdot\text{mo}^{-1}$ in the aspen-birch community (**Table 2.2**); and, by differencing, mean interception was $20.1 \text{ mm}\cdot\text{mo}^{-1}$ in the all-aspen community and $22.4 \text{ mm}\cdot\text{mo}^{-1}$ in the aspen-birch community (**Table 2.3**). Stemflow, however, was greatly underestimated during 2006 due to equipment problems (**Figure 2.3**). Negative interception, often associated with windy conditions, was sometimes calculated during 2007 (**Figure 2.4**). No problems were noted for throughfall, stem flow, and interception in the 2008 data (**Figure 2.5**).

Consistent with our hypotheses, throughfall was decreased in the presence of CO_2 by an average of 5.4 % across communities and usually insignificantly affected by $\text{CO}_2 \times \text{O}_3$. Counter to our hypotheses, however, throughfall was differentially affected across communities by O_3 and the direction of the effects was inconsistent through time. Stem flow was statistically differentiated by community in 2007 and 2008. Consistent with our hypotheses, stemflow increased under CO_2 an average of 11 % in the AA community and 16 % in the AB community, was usually insignificantly affected by combined CO_2 and O_3 , and decreased under O_3 an average of 18 % in the AA community and 16 % in the AB community. In a complementary fashion, interception was also differentiated by community, and increased under CO_2 by 4.9 % in the AA community and 4.5

% in the AB community, usually insignificantly affected under combined CO₂ and O₃, and increased by O₃ 2.5 % in the AA community but decreased 18 % in the AB community.

Branch Metrics

All-aspen community

Several significant main effects were detected, but those for 2004 first-order segment radii and 2006 total branch lengths occurred in the presence of significant interactions. Those effects are, therefore, discussed below in terms of the orthogonalized LS-means and their standard errors rather than the ordinary (unadjusted) means and standard errors.

Branch magnitude (the total number of tips) of aspen 2002, 2004, and 2006 whorls grown under ambient conditions in the AA communities averaged 1375, 519, and 51, respectively (**Table 2.4**). Branch magnitude increased for most whorls under all treatments, but due to high variance, only significantly under elevated O₃ (35 %, $P=0.043$) (**Table 2.5**).

Branch order of aspen 2002, 2004, and 2006 whorls grown under ambient conditions in the AA communities averaged 2.9, 2.6, and 2.0, respectively (**Table 2.4**). Branch order was significantly affected by genotype for 2004 whorls ($P=0.022$) and CO₂ for 2006 whorls ($P=0.050$) (**Table 2.5**). The only significant factor-level differences were between the 2004 whorls of clones 271 and 8L (2.7 versus 2.3, respectively), and a decrease of 10% associated with elevated CO₂ for the 2006 whorls.

Average lengths for first-order branch segments of aspen 2002, 2004, and 2006 whorls grown under ambient conditions in the AA communities averaged 4.5 cm, 5.3 cm, and 13 cm, respectively (**Table 2.4**). The average length of first-order branch segments of the clones for 2004 whorls was differentially affected by elevated O₃ (O₃ × clone $P=0.025$) (**Table 2.5**). First-order segment lengths decreased for clones 216 and 42E, (LS-mean differences of 28 % and 33 %), while clones 271 and 8L LS-means increased 33 % and 21.5 %, respectively.

Average radii for first-order branch segments of aspen 2002, 2004, and 2006 whorls grown under ambient conditions in the AA communities averaged 0.17 cm, 0.16 cm, and 0.96 cm, respectively (**Table 2.4**). The radii of the first-order

segments of the 2004 whorls was affected by O₃ differentiable by clone (O₃ × clone $P=0.047$) (**Table 2.5**). Clones 271 and 42E were reduced by 46% and 66%, respectively, while the remaining clones were not significantly affected. Added O₃ was associated with a significant LS-mean decrease of 27 % in first order segment radius for the 2004 whorls.

Total lengths for individual branches of aspen 2002, 2004, and 2006 whorls grown under ambient conditions in the AA communities averaged 170 cm, 116 cm, and 34 cm, respectively (**Table 2.4**). A significant CO₂ interaction with genotype ($P=0.030$) was present for overall branch length (**Table 2.5**). The interaction was attributable to rank changes between clones under control (8L = 111 cm; 271 = 85 cm; 216 = 24 cm; 42E = 7.1 cm) versus those under elevated CO₂ (216 = 14 cm; 42E = 8.5 cm; 271 = 2.6 cm; 8L = 1.2cm). All treatments were associated with overall decreases from the control value of 42 cm across whorls (weighted average), but the only decrease identified as significant was an LS-mean change to 4.2 cm (90 % decrease) associated with elevated CO₂.

Aspen-birch community

Tree species significantly differentiated the treatment responses in the aspen-birch (AB) communities of 2004-whorl branch segments ($P = 0.002$) and branching order ($P = 0.014$), and was involved in significant interactions with elevated O_3 for 2002 whorl branch segment counts ($P = 0.050$) and branch altitudes ($P = 0.026$). The only other significant effect for the AB community was that of elevated CO_2 ($P = 0.036$) for branch segment counts of the 2004 whorls.

Segments per branch for the 2002, 2004, and 2006 aspen whorls grown in the AB communities under ambient conditions averaged 32, 23, and 1.8; the respective counts for birch were 26, 24, and 1.6 (**Table 2.6**). The number of member segments per branch for the 2002 whorls was differentially affected between species ($O_3 \times \text{species } P = 0.050$) (**Table 2.7**), with the LS-mean for aspen decreasing by 19 % and that for birch increasing by 25 % in the presence of elevated O_3 . The number of member segments per branch for the 2004 whorls was increased 39 % by elevated CO_2 ($P = 0.036$). An apparent effect of species for the 2004 whorls ($P = 0.002$) was due to a near-significant interaction between CO_2 and species ($P = 0.104$); LS-means for aspen and birch were 7.7 and 12.5, respectively.

Branch altitudes (the maximum number of segment/node traversals from a branch tip to the branch base) for the 2002, 2004, and 2006 aspen whorls grown in the AB communities under ambient conditions averaged 14, 11, and 3.8, respectively; the respective altitudes for birch were 11, 10, and 4 (**Table 2.6**). The branch altitude for the 2002 whorls of aspen and birch was differentially affected ($O_3 \times$ species $P=0.026$) (**Table 2.7**), with elevated O_3 associated with an LS-mean decrease for aspen of 29 % and increase of 9.2 % for birch.

Branch orders for the 2002, 2004, and 2006 aspen whorls grown in the AB communities under ambient conditions averaged 3.1, 2.8, and 1.8, respectively; the respective orders for birch were 2.9, 3.2, and 1.6 (**Table 2.6**). The branch order of the 2004 whorls was slightly but significantly affected by species ($P=0.014$) (**Table 2.7**), with birch having a higher average order than aspen (3.2 versus 2.8, respectively).

Cross-Community Responses

Aspen clone 216 is the only genotype present in both communities. No statistically significant community effects were identified for this clone despite notable differences in means due to high variance.

Branch and Stem Metric Correlations to Hydrologic Parameters

Despite using monthly means for interception, throughfall, and stemflow rather than individual storm totals, stem and branch metrics were still able to explain from 51 % to 96 % of the variation in the throughfall, stem flow, and interception compartments of the hydrologic budget, and all regression models were highly significant ($P < .0001$) (**Table 2.8**). Predictive capability was similar across communities, but counter to our expectations stem metrics were better predictors than branch metrics. Branch metrics were usually insignificant predictors of throughfall and interception but were more often successful predictors of stem flow. Branch order and second order branch length were useful in one instance each to predict throughfall, and branch order and average forking angle were useful in one instance each to predict interception. Branch altitude, average forking angle, second order branch length, branch magnitude, branch order, and

second order branch radius were significant predictors for stem flow in at least one instance each.

Discussion

The purpose of the present study was to determine if changes in branch architecture were caused by long-term exposure to elevated CO₂ or O₃, and if that could impact the hydrologic budget through changes in apportionment of precipitation between interception, stem flow, and through-fall. Changes in forest hydrologic budget due to O₃ have previously been reported for the U.S. Southeast by McLaughlin et al. (2007a, b), but their work was based on stem growth, sap flow, soil water content, and stream base flow data rather than differences in canopy architecture between and within species. Significant differences in the performance of cultivated varieties of plants under varying site conditions, including rank-order changes within and between species, are very well known (Zobel and Talbert, 2003). The existence of rank-order changes among the genotypes in the present study is not surprising given that they were selected in part based on varying tolerance to ozone (Dickson et al., 2000).

Relative to the Control, we found few indications of increased branch growth in the present study after a decade of exposure to elevated CO₂ and the occurrence of canopy closure. Although many previous studies at Aspen FACE have identified significant increases in growth metrics attributable to elevated CO₂ (Karnosky et al., 2005), in the present study the only significant increase associated with elevated CO₂ was for the number of segments per branch from 2004 whorls in the AB community. Elevated CO₂ was in fact associated with a decrease in the branch order of 2006 whorls in the AA community, indicative of less secondary branching. This result, however, may be attributable to an increased elongation response due to greater light-competition as a result of enhanced canopy development in the elevated CO₂ treatment plots. Yet, elevated CO₂ also had a clone-specific negative effect in the AA community, decreasing total branching length for 2006 whorls of clones 8L and 271, without an apparent commensurate increase for clones 216 and 42E as might be expected if this effect was due to inter-clone competition. McDonald et al. (2002) found that clonal responses to treatments at Aspen-FACE were influenced by competitive status, a factor not included in the present study. Also, the effects of CO₂ observed in the present study may reflect some influence other than resource competition associated with canopy closure, such as an alteration of carbon partitioning. Increased maturation

rate and changes in reproductive structures and root to shoot ratio for birch at Aspen FACE were attributed to elevated CO₂ by Darbah et al. (2007).

The growth-enhancement effects of CO₂ have been observed to decrease or disappear after canopy closure in previous studies at other FACE sites. In a study of *Populus nigra* 'Jean Pourtet', *P. alba* 2AS11, and *P. x euroamericana* I-214 conducted at the Pop-FACE CO₂ enrichment experiment in central Italy, Gielen et al. (2002) found species- and season-specific increases in branch dimensions, sylleptic branching, and live canopy depth during the first few years of the study when the canopy had not yet closed. However, Gielen et al. (2003) found insignificant effects on LAI and light-related parameters after canopy closure and concluded that CO₂ enrichment effects largely disappeared after canopy closure. Consistent with this interpretation, Norby et al. (2001) observed no effects of CO₂ enhancement on LAI, basal area, stem height, or wood density over the initial two years of treatment in a closed stand of sweet gum (*Liquidambar styraciflua*) at the Oak Ridge, TN FACE experiment. However, a significant increase of above-ground dry matter production was observed in the sweetgum experiment after the first, but not the second, year of treatment. Finally, in a multi-year study of the effects of CO₂ and O₃ on canopy, stem, and root biomass conducted at the Aspen

FACE site (King et al., 2005), it was found that the relative treatment effects decreased through time. The decreases were consistent with increasing competition as the canopy approached closure.

There are many investigations in the literature indicating that plants may acclimate over time to added CO₂ (Körner, 2006), but this does not appear to be so for O₃ (Karnosky et al., 2007). The effects of O₃ on branch growth found in the present study are generally consistent with previously reported reductions in overall growth under added O₃ at the site, and the improved relative performance of the O₃-resistant aspen clone 8L in the presence of added O₃ (Karnosky, 2005; Percy et al., 2007).

In the present study, within the AA community elevated O₃ was associated with significant decreased lengths of first-order segments of branches from 2004 whorls for clones 216 and 42E (although increases occurred for clones 271 and 8L, but were statistically insignificant); decreased radii of the first-order segments of the 2004 branches of clones 271 and 42E; and increased magnitudes of the 2002 branches. Similar increases in branch magnitude were noted for other treatments but were not statistically significant, possibly due to high variance.

The decreases attributed to elevated O₃ are consistent with the generally observed reduction of growth (King et al. 2005). The increases other than that for branch magnitude were probably due to the increased relative performance of relatively O₃-tolerant clones in the presence of competition with less O₃ tolerant genotypes, although Percy et al. (2007) mentions the possibility of hormetic (low-dose stimulatory) O₃ effects.

Significant effects of O₃ in the AB community were differentiated by species. The number of member segments and the branch altitudes for the 2002 branches decreased for aspen but increased for birch in the presence of elevated O₃. The reduced branch segments and altitudes of the aspen are indications of reduced branch complexity and, therefore, overall branch development, that is consistent with the detrimental effects of O₃. The increases in segment members and branch altitude for the birch indicate that increased secondary branching and likely overall growth could be occurring, which is consistent with birch exhibiting greater tolerance to O₃ than aspen.

The apportionment of precipitation found in this study between interception, throughfall, and stem flow was within the typical range reported for north-

temperate forests, given the exception of low stem flow recoveries in 2006. Throughfall and stemflow typically account for 70 % to 90 % of incident precipitation in temperate forests with stemflow usually accounting for less than 5 % to 10 % of total precipitation (Levia and Frost, 2003) (see **Figure 2.3** through **Figure 2.5**). Clements (1971) studied 55-year old bigtooth aspen (*Populus grandidentata* Michx.) and red maple (*Acer rubrum* L.) growing in Ontario, Canada with a canopy leaf area index of 1.6 (somewhat lower than at Aspen-FACE). He reported monthly stemflow for these species ranged from 0.4 % to 2.2 % of total rainfall and monthly interception ranged from 7.7 % to 25.2 % of rainfall. Some other reports of interception by aspen species include 16 % for aspen in Colorado USA at age 32 (Dunford and Niederof, 1944), 25 % to 30 % for aspen 25 to 30 years of age and 19 % to 30 % for aspen 37 to 43 years of age in Russia (Molchanov, 1960).

There are other factors such as leaf area index, branch orientation, and bark roughness that have been shown in other studies to significantly influence stem flow (Levia and Frost, 2003) but these were not incorporated into the present study due to logistical constraints during the tree harvest. Leaf area index is higher in the CO₂ plots and lower in the O₃ plots at Aspen-FACE and the

correlation to the apportionment of precipitation in the present study is evident. Herwitz (1987) found using a rainfall simulator that stemflow could account for as much as 80 % of simulated rainfall when branches were oriented at greater than 60 ° above the horizontal. Universally for the stems included in the present study, the branch bark was relatively smooth for both the aspen and birch, but the birch stems were exfoliating and rough while the aspen stems remained quite smooth.

Conclusions

In this study, effects of elevated CO₂ and O₃ on branch architecture and hydrologic parameters were found using automated analysis of branch metrics, and branch metrics were shown to be useful predictors of the partitioning of precipitation. The effects of CO₂ were relatively weak on branch architecture relative to effects on hydrologic parameters. The converse was generally true for the effects of elevated O₃, given an exception for stem flow for an effect on elevated O₃ was usually present. Significant treatment interactions and effects on branch architecture involving the main effect of CO₂ and O₃ with genotype, are a probable result of post-canopy-closure light competition in the control, CO₂, and CO₂ × O₃ treatments reducing the growth-enhancement effects of CO₂, and differing genotypic tolerance to O₃. Based on the results of the present study, it is

likely that concentrations of CO₂ and tropospheric O₃ expected to occur by mid-century have the potential to significantly impact the forest hydrologic cycle.

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Table 2.1. Treatment effects, means (standard errors) for control, and percentage deviation for throughfall (mm) at Aspen-FACE during the study period.

Throughfall	May-06	Jun-06	Jul-06	Aug-06	Sep-06	Oct-06	May-07	Jun-07	Jul-07	Aug-07	Sep-07	Oct-07	May-08	Jun-08	Jul-08	Aug-08	Sep-08
Rep (block)	ns	ns	ns	ns	ns	ns	na	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Community	ns	ns	ns	ns	ns	ns	na	ns	0.1443	ns	ns	ns	ns	ns	ns	ns	ns
CO ₂	ns	0.0010	0.0310	ns	0.0483	0.0006	na	0.0492	0.0561	0.0191	ns	0.0373	0.0622	0.0485	0.0229	0.0322	0.1288
CO ₂ × Community	ns	ns	ns	ns	ns	ns	na	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
O ₃	ns	ns	ns	ns	ns	0.0036	na	ns	0.0502	ns	ns	0.0171	ns	0.1949	0.1063	0.1091	ns
O ₃ × Community	ns	0.0619	ns	ns	ns	0.0097	na	ns	ns	ns	ns	0.0256	ns	ns	0.1572	ns	ns
CO ₂ × O ₃	ns	ns	ns	ns	ns	0.1092	na	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CO ₂ × O ₃ × Community	ns	ns	ns	ns	ns	ns	na	ns	ns	0.0666	ns	ns	ns	0.1227	ns	ns	0.1173
All-aspen community																	
Control	76.6 (3.33)	36.0 (0.08)	41.0 (1.72)	108. (2.71)	20.9 (1.30)	40.9 (0.79)	na	70.4 (1.35)	87.3 (4.46)	62.9 (1.72)	14.8 (na)	100. (7.61)	19.8 (0.96)	30.6 (2.07)	53.4 (0.27)	29.0 (0.33)	21.5 (12.1)
eCO ₂	-7.6 (-10.%)	-4 (-11.%)	-6 (-14.%)	-10. (-9.9%)	-2.9 (-13.%)	-3.2 (-7.8%)	na	-4.2 (-5.9%)	-9.2 (-10.%)	-6 (-9.5%)	0.6 (4.05%)	-9.8 (-9.8%)	-2 (-10.%)	-3.6 (-11.%)	-5.3 (-9.9%)	-2.6 (-8.9%)	-2.1 (-9.8%)
eO ₃	5.2 (6.78%)	0.70 (1.94%)	-1.4 (-3.4%)	5 (4.62%)	-0.5 (-2.3%)	2.7 (6.60%)	na	4.39 (6.24%)	6.8 (7.78%)	1.9 (3.02%)	na	8 (8%)	-0.1 (-0.5%)	2.8 (9.15%)	4.3 (8.05%)	1.6 (5.51%)	0.91 (4.67%)
eCO ₂ × eO ₃	-2.8 (-3.6%)	-0.2 (-0.8%)	-1.4 (-3.4%)	-3 (-2.7%)	-3.5 (-16.%)	0.5 (1.22%)	na	-3.6 (-5.1%)	1.10 (1.26%)	-3.6 (-5.7%)	na	-0.7 (-0.7%)	-1 (-5.0%)	-1.9 (-6.2%)	-0.5 (-0.9%)	-0.6 (-2.4%)	-0.1 (-0.4%)
Aspen-birch Community																	
Control	78.0 (1.2)	36.4 (1.82)	42.3 (2.8)	106. (5.10)	21. (1.58)	42. (0.64)	na	67. (2.48)	84.3 (5.58)	60.8 (1.44)	18.5 (na)	98.4 (4.70)	19.5 (1.06)	29.1 (3.83)	55.2 (1.48)	28. (1.33)	20.9 (0.57)
eCO ₂	-9.2 (-11.%)	-2.8 (-7.6%)	-4.2 (-9.9%)	-8.4 (-7.9%)	-3 (-14.%)	-4.3 (-10.%)	na	-4 (-5.9%)	-8.3 (-9.8%)	-3.7 (-6.0%)	-5.2 (-28.%)	-10. (-10.%)	-0.6 (-3.5%)	0.69 (2.40%)	-3.9 (-7.0%)	0.10 (0.35%)	0.30 (1.43%)
eO ₃	4.2 (5.38%)	-0.2 (-0.8%)	0 (0%)	7 (6.60%)	-0.3 (-1.4%)	0.70 (1.66%)	na	8.59 (12.8%)	10.7 (12.6%)	8.10 (13.3%)	na	15.6 (15.8%)	2 (10.2%)	4.5 (15.4%)	3.09 (5.61%)	2.8 (10%)	1.6 (7.65%)
eCO ₂ × eO ₃	-6.7 (-8.5%)	-3.7 (-10.%)	-4.3 (-10.%)	-4 (-3.7%)	-3 (-14.%)	-1.9 (-4.5%)	na	-3.7 (-5.5%)	-0.5 (-0.7%)	-5.3 (-8.7%)	na	5.59 (5.69%)	-0.5 (-2.5%)	-0.7 (-2.4%)	-4.2 (-7.6%)	-0.1 (-0.7%)	-0.3 (-1.9%)

Table 2.2. Treatment effects, means (standard errors) for control, and percentage deviation for stemflow (mm) at Aspen-FACE during the study period.

Stemflow	May-06	Jun-06	Jul-06	Aug-06	Sep-06	Oct-06	May-07	Jun-07	Jul-07	Aug-07	Sep-07	Oct-08	May-08	Jun-08	Jul-08	Aug-08	Sep-08
Rep (block)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Community	ns	ns	0.1481	ns	0.1627	ns	ns	0.0004	0.0003	0.0035	ns	0.0002	0.0004	0.0009	0.0025	0.0186	0.0130
CO ₂	ns	ns	ns	ns	ns	ns	ns	0.0174	0.0178	0.0908	ns	0.0015	0.0034	0.0005	0.0251	0.0984	0.1427
CO ₂ × Community	ns	ns	ns	ns	0.0192	ns	ns	0.1204	ns	ns	ns	ns	ns	ns	ns	ns	ns
O ₃	ns	0.1066	ns	ns	0.0785	0.1854	ns	0.0041	0.0044	0.0295	ns	0.0147	ns	ns	0.0941	ns	0.1606
O ₃ × Community	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.0448	ns	ns	0.0511	0.0877	ns	ns	ns
CO ₂ × O ₃	ns	ns	ns	ns	ns	0.1950	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CO ₂ × O ₃ × Community	ns	ns	ns	ns	ns	0.1070	ns	ns	0.1030	ns	ns	ns	ns	ns	ns	ns	ns
All-aspen community																	
Control	0.92 (0.03)	1.97 (0.12)	1.49 (0.03)	3.20 (0.13)	0.66 (0.04)	1.00 (0.02)	na	11.9 (0.59)	16.7 (0.55)	9.98 (0.76)	2.72 (na)	23.2 (1.35)	4.52 (0.4)	3.92 (0.49)	10.2 (0.84)	5.83 (0.84)	4.93 (0.80)
eCO ₂	0.01 (2.17%)	-0.0 (-3.5%)	0.01 (0.67%)	0.15 (4.68%)	-0.1 (-21.%)	-0.0 (-3%)	na	1.5 (12.6%)	0.5 (2.99%)	0.42 (4.20%)	1.23 (45.2%)	1.7 (7.32%)	1.52 (33.6%)	2.16 (55.1%)	2 (19.6%)	0.74 (12.6%)	0.23 (4.66%)
eO ₃	0.02 (3.26%)	-0.2 (-12.%)	-0.0 (-2.0%)	-0.1 (-5.9%)	-0.1 (-18.%)	-0.0 (-8%)	na	-2.1 (-18.%)	-4.7 (-28.%)	-1.7 (-17.%)	-0.5 (-19.%)	-5.9 (-25.%)	-1.3 (-29.%)	-0.9 (-23.%)	-2.8 (-28.%)	-1.7 (-30.%)	-1.7 (-36.%)
eCO ₂ × eO ₃	0 (0%)	-0.3 (-18.%)	-0.0 (-5.3%)	-0.1 (-3.1%)	-0.1 (-24.%)	-0.0 (-6.9%)	na	-1.6 (-13.%)	-1.1 (-6.5%)	0.01 (0.20%)	0.13 (4.77%)	0.40 (1.72%)	0.61 (13.4%)	1 (25.5%)	-0.0 (-0.9%)	-0.2 (-3.6%)	-0.6 (-14.%)
Aspen-birch Community																	
Control	1.00 (0.02)	1.96 (0.08)	1.31 (0.16)	3.17 (0.27)	0.47 (0.09)	1.00 (0.01)	na	8.16 (0.82)	11.7 (1.28)	8.54 (0.99)	2.33 (na)	15.1 (0.96)	2.36 (0.39)	2.43 (0.63)	6.95 (0.40)	3.59 (0.2)	2.80 (0.08)
eCO ₂	-0.0 (-2%)	-0.1 (-7.6%)	-0.0 (-3.0%)	0.06 (1.89%)	0.14 (29.7%)	-0.0 (-6.0%)	na	3.34 (40.9%)	4.4 (37.6%)	1.86 (21.7%)	0.02 (1.28%)	5.2 (34.4%)	0.65 (27.5%)	0.67 (27.5%)	0.43 (6.18%)	0.67 (18.6%)	0.62 (22.1%)
eO ₃	0.02 (2%)	-0.1 (-7.1%)	0.13 (9.92%)	-0.4 (-15.%)	-0.0 (-10.%)	-0.1 (-12%)	na	-1.9 (-23.%)	-3.9 (-33.%)	-2.9 (-34.%)	-0.4 (-18.%)	-3.5 (-23.%)	-0.3 (-13.%)	-0.2 (-11.%)	-1.9 (-28.%)	-0.8 (-22.%)	-0.7 (-25%)
eCO ₂ × eO ₃	-0.1 (-14%)	-0.0 (-5.1%)	-0.0 (-2.2%)	-0.4 (-13.%)	0.03 (6.38%)	-0.0 (-4%)	na	0.10 (1.34%)	-1 (-8.5%)	-1.3 (-15.%)	0.53 (22.7%)	2.5 (16.5%)	1.61 (68.2%)	1.6 (65.8%)	1.29 (18.5%)	0.91 (25.3%)	0.78 (27.8%)

Table 2.3. Treatment effects, means (standard errors) for control, and percentage deviation for interception (mm) at Aspen-FACE during the study period.

Interception	May-06	Jun-06	Jul-06	Aug-06	Sep-06	Oct-06	May-07	Jun-07	Jul-07	Aug-07	Sep-07	Oct-07	May-08	Jun-08	Jul-08	Aug-08	Sep-08
Rep (block)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Community	ns	ns	ns	ns	ns	ns	ns	0.0289	0.0038	0.1176	ns	0.0220	0.0190	0.1303	0.1904	0.0847	0.0217
CO ₂	ns	0.0008	0.0325	ns	0.0492	0.0043	ns	0.1304	0.1849	0.0676	ns	ns	ns	ns	0.0825	ns	ns
CO ₂ × Community	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.1861
O ₃	ns	ns	ns	ns	ns	0.0196	ns	ns	ns	ns	ns	0.0838	ns	ns	ns	ns	ns
O ₃ × Community	ns	0.0819	ns	ns	ns	0.0260	ns	ns	ns	ns	ns	0.0362	0.0354	ns	ns	ns	ns
CO ₂ × O ₃	ns	ns	ns	ns	ns	0.1575	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CO ₂ × O ₃ × Community	ns	ns	ns	ns	ns	ns	ns	ns	0.1000	0.0386	ns	ns	ns	ns	ns	ns	0.0802
All-aspen community																	
Control	39.9 (3.3)	-11 (0.13)	59.3 (1.74)	-27. (2.68)	51.0 (1.25)	34.3 (0.77)	na	-3.5 (1.87)	19.5 (3.97)	8.53 (2.4)	90.1 (na)	-37. (8.95)	44.7 (1.34)	12.1 (1.76)	6.00 (0.73)	6.59 (1.18)	28.8 (1.06)
eCO ₂	7.6 (19.0%)	4 (-36%)	5.10 (8.60%)	11 (-40%)	3.9 (7.64%)	3.2 (9.32%)	na	2.8 (-80%)	8.8 (45.1%)	5.57 (65.2%)	-1.8 (-1.9%)	9 (-24%)	0.5 (1.11%)	1.5 (12.3%)	3.35 (55.8%)	1.86 (28.2%)	2.4 (8.33%)
eO ₃	-5.2 (-13.%)	0 (0%)	1.40 (2.36%)	-5 (18.5%)	1.5 (2.94%)	-2.6 (-7.5%)	na	-1. (66.6%)	-2 (-10.%)	-0.1 (-1.2%)	na	-2 (5.40%)	1.5 (3.35%)	-1.8 (-14.%)	-1.4 (-23.%)	0.18 (2.73%)	1.3 (4.51%)
eCO ₂ × eO ₃	2.8 (7.01%)	1 (-9.%)	1.40 (2.36%)	2 (-7.4%)	4.5 (8.82%)	-0.3 (-1.1%)	na	5.09 (-145%)	-0.5 (-2.5%)	3.47 (40.6%)	na	2 (-5.4%)	0.39 (0.89%)	1 (8.26%)	0.61 (10.1%)	0.81 (12.2%)	1.2 (4.16%)
Aspen-birch Community																	
Control	38.0 (1.19)	-11. (1.89)	58.1 (3.01)	-26 (5.00)	51.4 (1.67)	33.2 (0.63)	na	3.10 (3.25)	27.6 (6.77)	12.1 (2.43)	86.8 (na)	-26. (5.09)	47.2 (1.4)	15.2 (4.44)	7.54 (1.83)	9.31 (1.28)	32.0 (0.5)
eCO ₂	9.6 (25.2%)	2.1 (-19.%)	4.3 (7.40%)	9 (-34.%)	3.4 (6.61%)	4.4 (13.2%)	na	0.9 (29.0%)	3.4 (12.3%)	1.8 (14.8%)	5.2 (5.99%)	5 (-19.%)	0 (0%)	-1.4 (-9.2%)	3.36 (44.5%)	-0.2 (-2.5%)	-1 (-3.1%)
eO ₃	-4 (-10.%)	0 (0%)	-0.1 (-0.1%)	-6 (23.0%)	0.89 (1.75%)	-0.4 (-1.2%)	na	-6 (-193%)	-7.6 (-27.%)	-5.2 (-43.%)	na	-12 (46.1%)	-1.7 (-3.6%)	-5.2 (-34.%)	-1.1 (-15.%)	-1.5 (-16.%)	-0.8 (-2.8%)
eCO ₂ × eO ₃	7.2 (18.9%)	3 (-27.%)	4.4 (7.57%)	5 (-19.%)	3.3 (6.42%)	2 (6.02%)	na	4.09 (131.%)	1.5 (5.43%)	6.5 (53.7%)	na	-9 (34.6%)	-1.1 (-2.3%)	-1 (-6.5%)	2.02 (26.7%)	-0.2 (-2.2%)	-0.3 (-1.2%)

Table 2.4. Unadjusted means (standard errors) for branch metrics of 2002, 2004, and 2006 growth whorls of trembling aspen grown in an all-aspen community.

Community /Genotype	Treatment	Whorl	Branch Length (cm)	Branch Volume (cm ³)	Branch Segments	Branch Order	Branch Magnitude	Branch Altitude	Segment Length (cm)		Segment Radius (cm)	
									Order 1	Order 2	Order 1	Order 2
Subplot AA Aspen	Control	2002	170(23)	28(4.9)	27(3.3)	2.9(0.11)	1375(288)	13(0.94)	4.5(0.84)	11(1.6)	0.17(8.1)	0.23(1.8)
		2004	116(20)	22(6.8)	18(2.5)	2.6(0.13)	519(103)	11(0.88)	5.3(0.69)	8.0(2.0)	0.16(1.4)	0.22(2.1)
		2006	34(13)	1.8(0.86)	5.2(1.3)	2.0(0.15)	51(15)	4.8(0.70)	13(7.6)	4.8(1.9)	0.096(0.99)	0.12(1.8)
	+ CO ₂	2002	212(50)	35(9.5)	36(8.5)	3.0(0.20)	3032(990)	13(1.9)	3.7(0.49)	10(1.7)	0.20(1.9)	0.22(0.022)
		2004	131(22)	27(6.2)	24(3.4)	2.7(0.11)	1175(248)	12(1.1)	3.3(0.47)	9.8(1.7)	0.17(1.3)	0.24(3.5)
		2006	4.6(1.6)	0.63(0.48)	3.9(0.95)	1.8(0.16)	37(12)	4.4(0.71)	2.0(1.0)	6.3(5.3)	0.14(4.1)	0.082(0.021)
	+ O ₃	2002	141(27)	20(4.7)	27(4.6)	2.9(0.15)	1857(488)	13(1.3)	3.5(0.47)	7.6(1.5)	0.14(1.5)	0.19(3.4)
		2004	91(15)	18(4.6)	17(2.3)	2.6(0.078)	812(206)	10(0.84)	2.9(0.35)	8.0(1.2)	0.14(0.010)	0.19(1.5)
		2006	13(3.8)	0.40(0.16)	2.1(0.61)	1.9(0.13)	37(15)	4.0(0.82)	7.8(3.1)	4.9(2.6)	0.082(1.3)	0.095(2.3)
	+ CO ₂ & O ₃	2002	206(39)	47(12)	31(5.6)	2.9(0.13)	2000(587)	13(1.2)	5.4(0.77)	11(2.3)	0.20(1.8)	0.21(2.5)
		2004	95(16)	14(5.2)	18(2.2)	2.6(8.9)	723(142)	11(0.92)	3.8(0.85)	11(2.7)	0.13(7.6)	0.17(1.4)
		2006	9.2(6.3)	0.31(0.23)	2.8(0.31)	2.0(0.00)	19(7.5)	3.3(0.49)	3.6(2.4)	0.74(0.52)	0.10(1.7)	0.16(0.084)
Subplot AA Aspen 216	Control	2002	163(45)	17(5.0)	28(7.3)	3.0(0.24)	1387(568)	14(2.1)	4.2(1.0)	7.4(1.5)	0.16(1.1)	0.18(1.7)
		2004	185(71)	18(12)	22(7.8)	2.8(0.65)	751(471)	13(2.6)	8.5(1.9)	10(3.3)	0.11(1.0)	0.20(2.7)
		2006	24(9.3)	0.89(0.47)	5.4(2.3)	2.0(0.22)	51(28)	4.6(1.1)	3.7(1.6)	10(2.0)	0.080(1.4)	0.099(9.1)
	+ CO ₂	2002	387(144)	62(24)	53(20)	3.5(0.43)	4505(1975)	18(5.2)	4.5(0.45)	13(4.1)	0.19(2.8)	0.17(4.0)
		2004	70(44)	8.9(6.8)	11(4.1)	2.1(0.14)	288(140)	9.0(1.9)	5.1(1.4)	5.1(2.9)	0.13(0.021)	0.18(5.5)
		2006	14(-)	0.44(-)	2.0(-)	2.0(-)	15(-)	3.0(-)	2.1(-)	12(-)	0.084(-)	0.10(-)
	+ O ₃	2002	198(61)	22(13)	34(12)	3.7(0.47)	2179(1489)	16(2.5)	2.7(1.3)	11(3.2)	0.13(2.5)	0.15(0.035)
		2004	28(9.9)	4.2(2.8)	9.4(2.7)	2.3(0.19)	213(83)	7.3(1.8)	1.3(0.29)	5.9(2.0)	0.16(3.5)	0.21(4.5)
		2006	12(7.1)	0.32(0.20)	3.0(1.1)	2.0(0.00)	69(19)	5.8(1.0)	2.8(1.6)	4.7(4.5)	0.069(2.3)	0.11(0.032)
	+ CO ₂ & O ₃	2002	173(70)	23(8.5)	31(14)	2.8(0.32)	2067(1343)	13(2.7)	5.6(1.4)	7.2(1.6)	0.18(3.8)	0.16(1.4)
		2004	44(15)	6.1(2.8)	10(2.5)	2.3(0.14)	246(85)	6.9(1.3)	2.5(0.60)	5.0(1.1)	0.12(1.8)	0.16(3.1)
		2006	21(16)	0.71(0.58)	3.0(0.58)	2.0(0.00)	26(20)	4.0(1.2)	7.3(5.3)	0.22(-)	0.12(1.3)	0.24(-)

Table 2.5. Statistical significance (*P* values) for total branching length in meters of 2002, 2004, and 2006 growth whorls of trembling aspen clones 216, 271, 42E, and 8L grown in an all-aspen community. Effects are considered significant at *P* < 0.05. The abbreviation “ns” indicates non-significant effects and the notation “-“ indicates effects not evaluated.

Source	2002		2004		2006	
	Branch Magnitude	Branch Order	Order 1 Seg. Avg. Length (cm)	Order 1 Seg. Radius (cm)	Branch Order	Total Length (cm)
CO ₂	ns	ns	ns	ns	0.050	0.001
O ₃	0.043	ns	ns	0.012	ns	ns
Genotype	ns	0.022	ns	ns	ns	ns
CO ₂ *O ₃	ns	ns	ns	ns	ns	ns
CO ₂ *Genotype	ns	ns	ns	ns	ns	0.030
O ₃ *Genotype	ns	ns	0.025	0.047	ns	ns
CO ₂ *O ₃ *Genotype	ns	ns	ns	ns	-	-

Table 2.6. Unadjusted means (standard errors) for branching metrics of 2002, 2004, and 2006 growth whorls of trembling aspen and paper birch grown in an aspen-birch community. Insufficient sampling is denoted by “-”.

Community /Genotype	Treatment	Whorl	Branch Length (cm)	Branch Volume (cc)	Branch Segments	Branch Order	Branch Magnitude	Branch Altitude	Segment Length (cm)		Segment Radius (cm)	
									Order 1	Order 2	Order 1	Order 2
Subplot AB Aspen 216	Control	2002	260(87)	67(39)	32(8.9)	3.1(0.21)	1571(553)	14(2.5)	3.8(0.71)	20(8.1)	0.17(2.3)	0.21(3.4)
		2004	152(47)	28(11)	23(6.9)	2.8(0.24)	747(313)	11(1.9)	5.8(1.3)	7.4(1.6)	0.14(2.0)	0.19(2.6)
		2006	11(6.0)	0.63(0.42)	1.8(0.58)	1.8(0.20)	36(20)	3.8(1.2)	2.9(1.8)	17(16)	0.13(3.7)	0.18(0.038)
	+ CO ₂	2002	287(80)	35(9.2)	45(14)	2.8(0.17)	3287(1963)	17(1.6)	4.4(0.72)	16(4.8)	0.16(3.4)	0.22(3.6)
		2004	207(77)	28(11)	31(9.7)	3.3(0.56)	1807(504)	13(2.7)	4.1(0.71)	14(4.1)	0.17(1.4)	0.25(2.2)
		2006	-	-	-	-	-	-	-	-	-	-
	+ O ₃	2002	95(42)	5.5(3.3)	25(8.5)	3.0(0.24)	1402(777)	11(2.4)	1.9(0.41)	12(5.8)	0.070(8.8)	0.086(1.7)
		2004	69(27)	3.1(1.8)	16(5.4)	2.7(0.24)	678(275)	10(2.6)	2.2(0.71)	4.3(1.6)	0.07(0.84)	0.099(2.8)
		2006	40(9.1)	1.4(0.72)	12(6.0)	2.0(0.00)	51(4.5)	11(4.0)	6.6(0.11)	2.4(0.18)	0.11(0.029)	0.14(8.0)
	+ CO ₂ & O ₃	2002	240(100)	35(13)	34(12)	3.2(0.31)	1963(893)	14(1.8)	4.7(0.74)	30(14)	0.20(4.0)	0.24(3.5)
		2004	91(50)	9.2(4.3)	15(9.2)	3.0(0.58)	662(554)	10(4.4)	2.7(2.0)	11(2.1)	0.16(1.9)	0.17(3.1)
		2006	16(0.90)	0.30(6.3)	1.5(0.50)	2.0(0.00)	6.0(0.00)	2.0(0.00)	8.4(-)	15(-)	0.12(-)	0.070(-)
Subplot AB Birch	Control	2002	210(57)	20(9.0)	26(8.0)	2.9(0.21)	1683(640)	11(2.0)	4.6(0.69)	18(3.4)	0.14(1.8)	0.15(1.9)
		2004	205(50)	16(7.2)	24(6.5)	3.2(0.21)	978(330)	10(1.4)	7.2(0.79)	16(4.1)	0.12(1.4)	0.14(1.7)
		2006	31(15)	0.74(0.43)	1.6(0.40)	1.6(0.24)	18(8.8)	4.0(0.84)	15(6.0)	21(10)	0.069(2.6)	0.11(0.010)
	+ CO ₂	2002	96(30)	4.9(2.0)	13(3.0)	2.4(0.20)	559(144)	9.1(1.8)	4.7(1.2)	13(4.2)	0.096(0.90)	0.11(2.1)
		2004	119(49)	6.0(3.4)	17(5.6)	2.8(0.26)	851(349)	8.3(1.5)	6.8(3.3)	12(3.7)	0.088(1.1)	0.12(0.030)
		2006	64(39)	3.3(2.3)	5.8(3.2)	2.2(0.20)	201(143)	6.6(2.2)	9.2(4.8)	12(7.9)	0.095(1.5)	0.095(1.4)
	+ O ₃	2002	287(68)	39(12)	28(6.5)	3.1(0.27)	1337(436)	13(1.5)	7.3(1.3)	20(4.1)	0.20(3.2)	0.21(0.022)
		2004	167(41)	12(4.6)	24(7.0)	2.9(0.22)	975(310)	11(1.2)	8.3(1.7)	9.2(3.1)	0.12(1.2)	0.13(1.9)
		2006	56(-)	1.1(-)	2.0(-)	2.0(-)	21(-)	5.0(-)	41(-)	15(-)	0.076(-)	0.089(-)
	+ CO ₂ & O ₃	2002	132(56)	12(7.1)	18(3.2)	3.1(0.14)	468(141)	11(0.91)	5.6(1.0)	16(5.6)	0.13(1.5)	0.17(0.033)
		2004	196(45)	18(4.6)	17(3.5)	2.9(0.23)	699(272)	10(1.2)	8.3(1.4)	31(7.7)	0.14(0.020)	0.18(3.9)
		2006	60(14)	14(11)	4.8(0.85)	2.3(0.25)	51(18)	4.8(1.0)	19(8.8)	74(-)	0.18(0.045)	0.16(-)

Table 2.7. Statistical significance (*P*-values) for 2002, 2004, and 2006 growth whorls of trembling aspen clone 216, and paper birch grown in a mixed aspen-birch community. Effects are considered significant at $P < 0.05$. The abbreviation “ns” indicates non-significant effects and the notation “-“ indicates effects not evaluated.

Effect	2002		2004	
	Branch Segments	Branch Altitude	Branch Segments	Branch Order
CO ₂	ns	ns	0.036	ns
O ₃	ns	ns	ns	ns
Species (genotype)	ns	ns	0.002	0.014
CO ₂ *O ₃	ns	ns	ns	ns
CO ₂ *Species	ns	ns	ns	ns
O ₃ *Species	0.050	0.026	ns	ns

Table 2.8. Results of stepwise regression for predicting interception, stemflow, and throughfall at Aspen-FACE using stem and branch metrics collected during 2007. Significance (*P*) values are provided beneath each model coefficient.

Commu	Year	Model	Precip	Stem metrics			Branch metrics											
		Rsq	Height	DBH	ABH	Altitude	Angles	Length			Magnitude	Order	Radius			Volume		
								Total	Order 1	Order 2	Order 3			Order 1	Order 2	Order 3		
Interception																		
AA	2006	0.50553	-0.595 (<.0001)	- -	-3.12 (0.0017)	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
	2007	0.52417	-0.555 (<.0001)	-1493 (0.0011)	- -	3.15 (0.0708)	- -	- -	- -	- -	- -	- -	- -	5.72 (0.1393)	- -	- -	- -	- -
	2008	0.71696	-0.554 (<.0001)	- -	-1.38 (0.0623)	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
AB	2006	0.51619	-0.605 (<.0001)	-974 (0.0023)	- -	3.87 (0.0915)	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
	2007	0.65999	-0.559 (<.0001)	- -	-9.59 (0.0276)	5.90 (0.0001)	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
	2008	0.74039	-0.538 (<.0001)	- -	- -	- -	- -	-0.0924 (0.0798)	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -

Table 2.8 (Continued)

Commu	Year	Model	Precip	Stem metrics			Branch metrics											
		Rsq	Height	DBH	ABH	Altitude	Angles	Length				Magnitude	Order	Radius			Volume	
Stemflow																		
AA	2006	0.7467	0.00515 (0.0646)	-	0.347 (<.0001)	-0.0649 (0.0722)	-	-	-	-	-	-	-	-	-	-	-	-
	2007	0.93733	-0.0799 (<.0001)	-124 (0.1049)	1.42 (0.0501)	-	-	-	-	-	-	-	-	-	-	-	-4.95 (0.1495)	-
	2008	0.89042	-0.0883 (<.0001)	-196 (0.0006)	1.67 (0.0392)	-	-	-0.0158 (0.1810)	-	-	-	-	-	-	-	-	-	-
AB	2006	0.76496	0.00596 (0.0490)	-	0.375 (<.0001)	-0.123 (0.0333)	-	-	-	-	-	-	-	-	-	-	-	-
	2007	0.93428	-0.0585 (<.0001)	-793 (<.0001)	5.23 (0.2889)	0.850 (0.0527)	-	-	-	-	-0.151 (0.0968)	-	0.000915 (0.0231)	-	-	-	-	-
	2008	0.86714	-0.0591 (<.0001)	-559 (0.0082)	2.81 (<.0001)	0.542 (0.0499)	-0.168 (0.0291)	0.0224 (0.0412)	-	-	-	-	-	1.53 (0.0835)	-	-	-	-

Table 2.8 (Continued)

Commu	Year	Model	Precip	Stem metrics			Branch metrics											
		Rsqr	Height	DBH	ABH	Altitude	Angles	Length			Magnitude	Order	Radius			Volume		
								Total	Order 1	Order 2			Order 3	Order 1	Order 2		Order 3	
Throughfall																		
AA	2006	0.78656	-0.408	-	2.88	-	-	-	-	-	-	-	-	-	-	-	-	-
			(<.0001)	-	(0.0029)	-	-	-	-	-	-	-	-	-	-	-	-	-
	2007	0.94304	-0.358	1376	-	-2.86	-	-	-	-	-	-	-	-5.32	-	-	-	-
			(<.0001)	(0.0005)	-	(0.0631)	-	-	-	-	-	-	(0.1158)	-	-	-	-	
AB	2006	0.79814	-0.400	912	-	-3.69	-	-	-	-	-	-	-	-	-	-	-	-
			(<.0001)	(0.0041)	-	(0.0990)	-	-	-	-	-	-	-	-	-	-	-	-
	2007	0.9561	-0.366	1559	-	-8.01	-	-	-	0.366	-	-	-	-	-	-	-	-
			(<.0001)	(0.0008)	-	(0.0190)	-	-	-	(0.1215)	-	-	-	-	-	-	-	
2008	0.86692	-0.314	483	-	-1.80	-	-	-	-	-	-	-	-	-	-	-	-	
			(<.0001)	(0.0606)	-	(0.0860)	-	-	-	-	-	-	-	-	-	-	-	

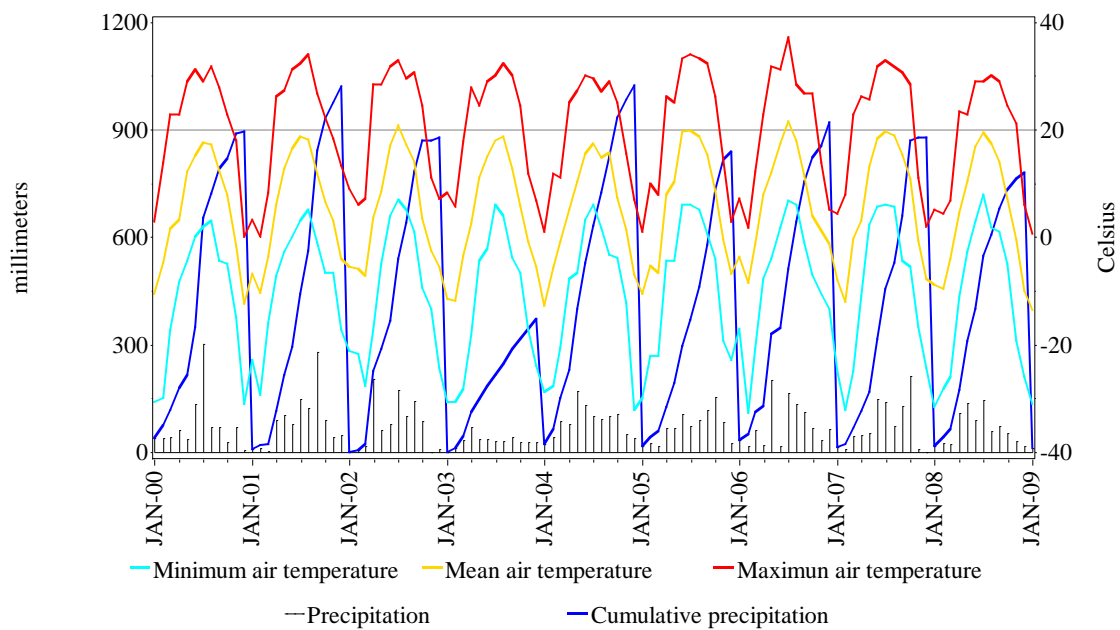


Figure 2.1. Air temperature (Celsius) and cumulative precipitation (mm) at Aspen FACE.

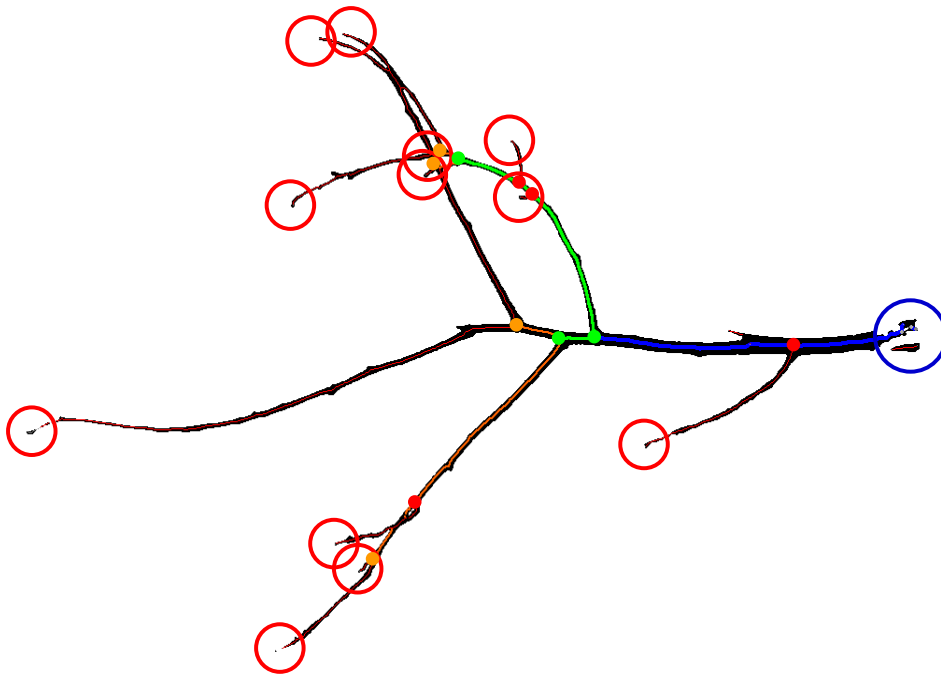


Figure 2.2. Example of an output image from the branch image analysis macro. The aspen branch is approximately 46 cm long along the main longitudinal axis. Some portions of the branch base were obscured by a rope used to suspend the branch, and appear white in this figure. Tips are indicated by red circles and the base is indicated by a blue circle. Segment orders are differentiated by overlay-line color and width. Internal nodes are indicated by solid dots. Segment length measurements were corrected for tip location erosion during the image skeletonization process.

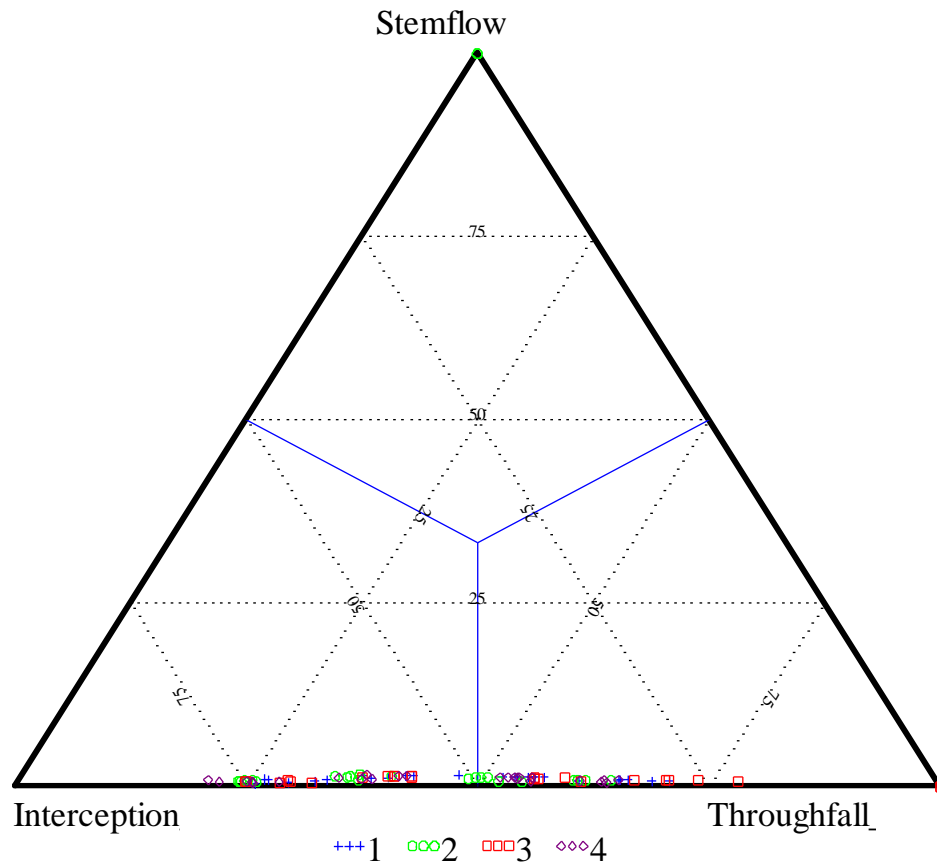


Figure 2.3. Comparison of measured to predicted monthly interception, throughfall and stemflow during May-September 2006 at the Aspen FACE experiment located in Rhinelander WI. Obtaining stem flow measurements in 2006 were problematic due to equipment malfunctions.

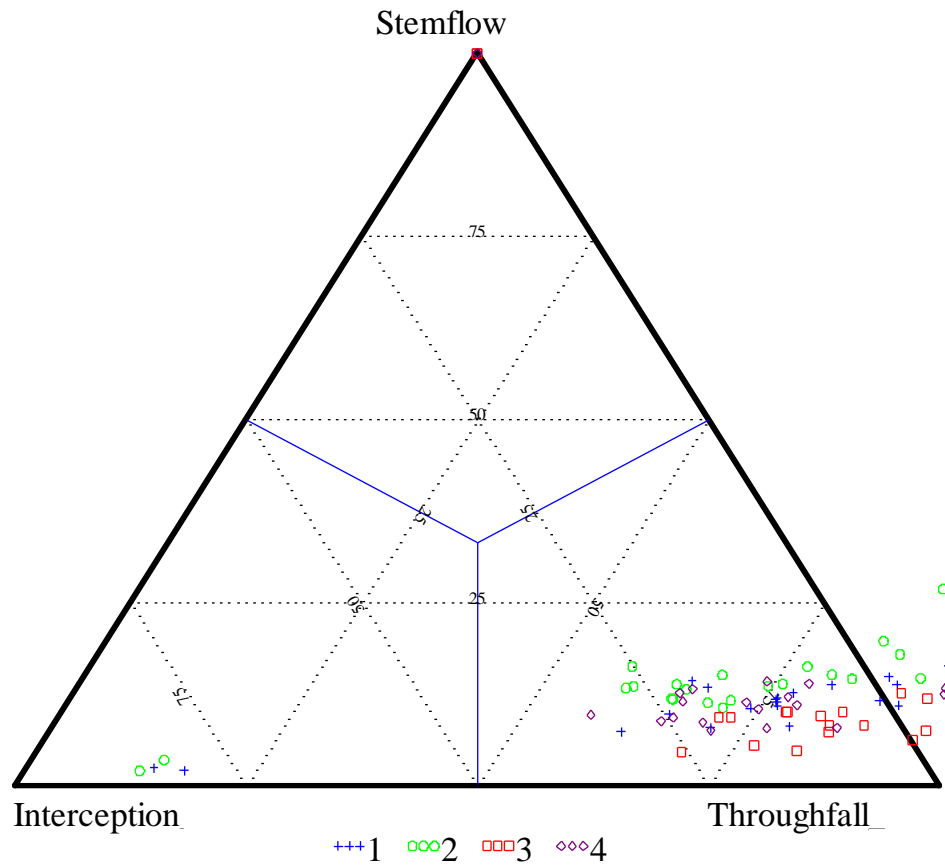


Figure 2.4. Comparison of measured to predicted monthly interception, throughfall and stemflow during May-September 2007 at the Aspen FACE experiment located in Rhinelander WI. Under windy conditions throughfall and stem flow can exceed vertical precipitation, resulting in a calculation of negative interception (as indicated by the points outside the ternary diagram).

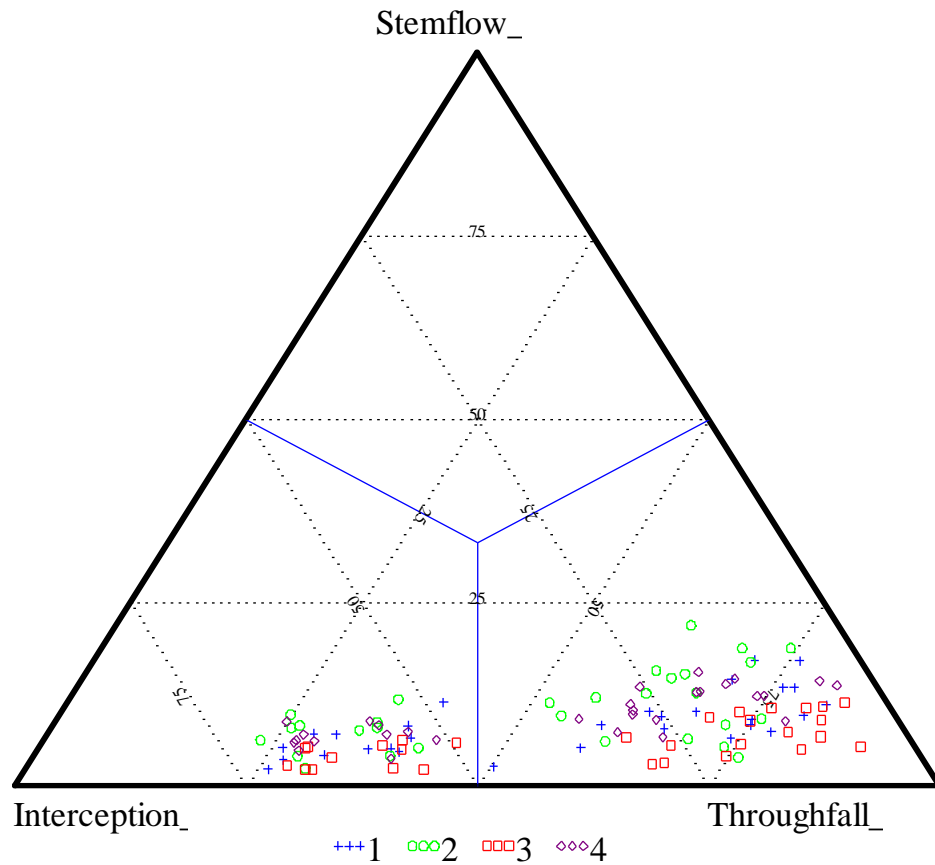


Figure 2.5. Comparison of measured to predicted monthly interception, throughfall and stemflow during May-September 2008 at the Aspen FACE experiment located in Rhinelander WI.

CHAPTER 3

**Dependency of fine-root biomass and morphology responses to elevated CO₂
and O₃ in young stands of trembling aspen and paper birch**

Abstract

Projected changes in the atmospheric concentration of CO₂ and tropospheric O₃ over the next 50 years are of significant concern due in part to their potential to affect the global water cycle and the ability of forests to sequester carbon. Responses of tree roots to elevated CO₂, elevated O₃, and to a lesser extent, combined CO₂ and O₃ have been characterized based primarily on biomass studies of relatively shallow roots. Yet deeper roots often play a disproportionately large role in water acquisition relative to their biomass, and might also increase in biomass relative to shallower roots, either as an avoidance mechanism from drought stress or in response to increased competition for resources in shallower soils. Such shifts in patterns of soil exploration by tree root systems (changes in spatial distribution), would be better characterized by use of root morphological characteristics than biomass alone.

In the current study, I characterized small root biomass and morphometric responses to elevated CO₂ and O₃ at the Aspen-FACE Experiment in Rhinelander, Wisconsin, in 10-cm increments down to a depth of one meter. Changed root responses to treatments relative to previous studies of shallow roots are consistent with stand development and the progression from a decoupled system

characterized by an abundance of non-carbon resources towards a coupled system nearing full canopy development.

Root responses to fumigation were differentiated by depth, with the greatest magnitudes of biomass and RAI responses occurring in the shallowest of the three soil layers studied (e.g. for biomass in the aspen-birch community, $71 \text{ g}\cdot\text{m}^2$ to $159 \text{ g}\cdot\text{m}^2$ in the shallow layer versus $6 \text{ g}\cdot\text{m}^2$ to $10 \text{ g}\cdot\text{m}^2$ in the deep layer) and the greatest relative morphometric responses occurring at depth (e.g. for SRL in the aspen-birch community, $26.7 \text{ m}\cdot\text{g}^{-1}$ to $35.7 \text{ m}\cdot\text{g}^{-1}$ in the shallow layer, versus $31.5 \text{ m}\cdot\text{g}^{-1}$ to $55.8 \text{ m}\cdot\text{g}^{-1}$ in the deep layer). Fumigation with O_3 increased small root biomass in shallow soil 30 % in all-aspen plots and decreased root biomass in shallow soil 46 % in aspen-birch plots. However, elevated ozone increased root length up to 131 % and specific root length up to 77 % in mid and deep soil layers, indicating more extensive soil exploration at depth, while elevated CO_2 increased small root biomass in shallow soils between 20 % to 24 %, indicative of more intensive soil exploration near the surface. Interactions between eCO_2 and eO_3 affecting root morphology were not detected. The combined biomass and morphometric data suggest that nutrient and water resource demands are

increased proportionally under eCO₂, and that water requirements relative to nutrient requirements are greater in the presence of eO₃.

Introduction

Understanding the effects of elevated atmospheric CO₂ (eCO₂) and tropospheric O₃ (eO₃) on forest net primary production is important for predicting climate feedbacks via carbon sequestration, but it is also critical for estimating future water availability as the cycling of carbon and water are intimately linked.

Elevated CO₂ has been shown to increase aboveground and belowground growth and water use efficiency (WUE), while eO₃ generally has the opposite effect (Curtis and Wang 1998; Anderson, 2003). The effects of combined eCO₂ and eO₃ have been less consistent, but also less well studied (Rogers et al., 1994; King et al., 2001, 2005; Kasurinen et al., 2004; Norby et al., 2004). To circumvent problems identified with container-based studies such as lessening of growth response through time due to root restriction (Ceulemans and Mousseau, 1994), studies of the effects of eCO₂ on tree root biomass have been undertaken at several free-air CO₂-enrichment (FACE) experiments, including Duke-FACE located in Durham, NC, USA (Oren et al., 1998; Pritchard et al., 2008), ORNL-FACE of Oak Ridge, TN, USA (Norby et al., 2004; Iversen et al., 2008), Pop-FACE in central Italy (Lukac et al., 2003), and at Aspen-FACE, located in Rhinelander, WI, USA (King et al., 2001; Karnosky et al., 2003; King et al., 2005; Pregitzer et al., 2008). Duke-FACE and ORNL-FACE were initiated in

closed canopy stands of loblolly pine and sweetgum, respectively, and Pop-FACE, similar to Aspen-FACE, was initiated as planted poplar cuttings on former agricultural land. The Pop-FACE experiment was maintained under short-rotation coppicing.

Enhanced root biomass was observed under elevated CO₂ at each of the forest FACE sites and was found to be greater at depth based on relatively coarse vertical stratifications of sampling, generally limited to about 0.5 meter. The increase in small root production at ORNL-FACE dominated the growth stimulation of CO₂ in that experiment, but was a minor response at Duke-FACE which is underlain by a shallow clay pan (Oren et al., 1998; Schlesinger and Lichter, 2001; Lichter et al., 2005; Stoy et al., 2008; <http://websoilsurvey.nrcs.usda.gov>). The studies conducted at Duke, ORNL, and for one of three poplar species at POP-FACE, found preferential root proliferation occurring deeper in the soil in response to eCO₂. The site-specificity of root biomass response to eCO₂ and changing responses through time at Duke- and ORNL-FACE have been explained using optimization models to nitrogen limitation (Oren et al., 2001; Luo et al., 2004; Dewar et al., 2009; Franklin et al., 2009) or multiple-resource optimization constrained by nitrogen and soil-water

availability (McMurtrie et al., 2008). Kubiske et al. (2007) attributed inter-annual variation in growth at Aspen-FACE to summer PAR and previous autumn temperatures.

Responses of tree roots to eCO₂, eO₃, and to a lesser extent, combined CO₂ and O₃ have been relatively modest at the forest FACE experiments, and based primarily on biomass studies of relatively shallow roots. Yet deeper roots often play a disproportionately large role in water acquisition relative to their biomass (Stone and Kalisz, 1991), and might also increase in biomass relative to shallower roots, either as an avoidance mechanism to drought stress, or in response to increasing resource competition in shallower soils. Deep-root avoidance responses to competition or environmental stress can be expected to result in changes in root spatial distributions in the soil, which might be better characterized by use of root architecture (morphometrics) than biomass alone (Forde and Lorenzo, 1991; López-Bucio et al., 2003). Eissenstat et al. (2000) reviewed the utility of root morphometric parameters in assessing root longevity, a parameter of paramount concern to studies of belowground carbon sequestration under changing climatic conditions. Longevity of leaves has been correlated to morphometric parameters (Reich et al. 1992; 1997) analogous to root diameter,

tissue density, and specific root length, and root longevity has been positively correlated with root branching order (Eissenstat, 2000). Root morphometric responses may help explain differing belowground responses at the various FACE facilities (e.g. McMurtrie et al., 2008; Franklin et al., 2009) based on growth-limiting factor(s) (Liebig, 1855; Bloom et al., 1985) (Eissenstat et al., 2000). For example, in trees nitrogen concentration has been correlated to root diameter (Pregitzer et al., 2002) and specific root length (SRL) has been correlated to nitrogen uptake (Reich et al., 1998), while in grasses tissue density (TD), but not SRL nor diameter, have been correlated with the root longevity (Ryser 1996, 1998). It also seems plausible that nitrogen limitation (see Franklin et al., 2009) might be reflected in increased SRL (Ryser, 2006) in shallow soils, while water stress (see McMurtrie et al., 2008) might be reflected in increased SRL at depth.

Körner (2006) suggested that results of eCO₂ experiments be interpreted as a function of soil water content in three categories: (1) decoupled systems characterized by a pre-existing abundance of non-carbon resources; (2) aerially expanding systems; and (3) coupled systems close to a steady-state nutrient cycle and full canopy development. Aspen-FACE may well provide an example of a single experiment that has developed through these stages. Previous root biomass

studies conducted at Aspen-FACE provide a unique opportunity to investigate such responses because they document the effects of eO₃ in combination with eCO₂ on shallow roots, and because they span the period from stand establishment to canopy closure. During the first five years of the experiment, that generally corresponded with the open-canopy stage, small root biomass was greatest under eCO₂, lowest under eO₃, and similar to control under combined eCO₂ and eO₃ (King et al. 2001; 2005; Kostianen et al., 2008). Allometric relationships between aboveground and belowground plant biomass pools were not affected by the treatments, consistent with other eCO₂ studies (Gebauer et al. 1996; King et al. 1996; Norby et al. 1999), but decoupling of the aboveground and belowground allometric relationships was predicted for the latter years of the experiment after canopy closure occurred (King et al. 2005). Kostianen et al., (2008) reported that closed-canopy conditions were developing at the Aspen-FACE site circa 2002, and Pregitzer et al. (2008) found aboveground to belowground allometric ratios had shifted by that time. By 2005, LAI was temporally stable, and similar across communities and treatments with the exception of eO₃, where it was lower. LAI across communities during 2004 and 2005 ranged from 3.7 to 5.1 for control, 4.7 to 6.2 for eCO₂, 2.8 to 3.3 for eO₃, and 3.9 to 5.7 for eCO₂ × eO₃ (Uddling et al., 2008, their figure 1). Thus, the root “canopy” probably was well developed in 2005, and aboveground responses to

the treatments at that time were also reflected in the root zone (Pregitzer et al. 2008).

Characterization of subsurface responses to $e\text{CO}_2$ and $e\text{O}_3$ based on biomass studies of shallow roots assumes implicitly that any changes in deeper roots are either proportional to those of shallow roots in kind and magnitude, or are negligible. But this is not required under the growth maximization paradigm of carbon allocation by trees to acquisition of limiting resource(s). Within this conceptual framework and our preexisting knowledge of the site, we believed that after over-story canopy closure occurred at Aspen FACE differences in the shallower portion of the root canopy would become less pronounced while any within the deeper portion of the root canopy might remain. We also expected that lower LAI observed under $e\text{O}_3$ could result in a lower root biomass under $e\text{O}_3$, but were unsure to what extent this effect might be counterbalanced by root growth increases due to reduced WUE under $e\text{O}_3$. Therefore, in the present study we characterized roots over a greater depth than typical using both biomass and morphometric parameters. Our hypotheses were that relative differences in shallow root biomass across treatments would be smaller than prior to canopy closure, and that enhanced root growth at depth should have occurred and be

detectable by morphometric analysis. We expected that root biomass at depth would be more influenced under the fuller eCO₂ canopies by competition in shallow soil, whereas deeper roots under the more open eO₃ canopies would exhibit morphometrics indicative of stress avoidance or a disproportionate need for water due to decreased WUE.

Methods

The Aspen-FACE experiment is located in northern Wisconsin on former agricultural land. Dickson et al. (2000) provide a generalized soil profile description for the Aspen-FACE site. A 15-cm thick sandy loam topsoil grades into a clay loam approximately 30-cm thick (at 15-45 cm depth), in turn grading into a sandy loam, stratified sand, and gravel substratum at about 100-cm deep. Sporadic clay lenses occur at depths of 30- to 60-cm, and the water table is at least 10-m deep. Fumigation treatments were applied using the Brookhaven National Laboratory FACE design (Hendrey et al., 1999). For this experiment, the fumigation treatments included ambient conditions, elevated CO₂ (eCO₂; ~560 ppm), elevated O₃ (eO₃; ~60 ppb, or 1.5 times ambient), and both eCO₂ and eO₃. Fumigation was performed during the daylight hours of each growing season since inception of the project (1997). The experimental units consist of twelve

30-m diameter plots that have been blocked into three replicates of the four treatments (randomized complete-block design). Each plot is subdivided into three subplots comprised of a mixed trembling aspen (*Populus tremuloides* Michx.) and sugar maple (*Acer saccharum* Marsh.) community (AM), a mixed aspen and paper birch (*Betula papyrifera* Marsh.) community (AB), and an all-aspen community (AA). Each community was planted during 1997 with seedlings or cuttings on one-meter centers. Aspen clone 216 was alternated with seedling birch and seedling maple in the AB and AM communities, respectively. A selection of aspen clones (8L, 42E, 216, 259, and 271) representing a range of O₃ sensitivity (8L= tolerant; 216 and 271 = somewhat tolerant; 42E = relatively sensitive; 259 = sensitive [Dickson et al., 2000]) comparable to that which exists across groups of natural clonal stands (Percy et al., 2007) was randomized within the AA communities. Clone 259 has since died out. Additional description of the project is available in Dickson et al. (2000) and documentation of the fumigation system performance and other information is available at www.aspenface.mtu.edu.

Root Biomass Sampling

Seven soil samples per treatment plot were collected during July and August, 2005, including three from the AA community, three from the AB community, and one from the AM community. Samples were collected as 10-cm diameter cores extending from the ground surface to one-meter depth. The cores were obtained using a 10 cm diameter steel corer advanced to 100 cm depth by rapid percussion with a jack hammer. The corer consisted of a steel cylinder equipped with a threaded cutting shoe, transparent plastic liner, and drive head. The corer was cleaned and fitted with a new liner between samplings. The liner containing the soil sample was removed from the corer, labeled, sealed with vinyl caps at each end, and frozen at -20 °C. At the end of the sampling period, the soil samples were shipped frozen to our laboratory at North Carolina State University.

The frozen soil cores were cut into 10-cm long sections, one at a time, for processing. The 10 cm sections were individually thawed and processed for root biomass. Roots were manually sieved from the soil, washed with cool tap water, placed in labeled plastic bags, and re-frozen in water in preparation for analysis. Roots were thawed in a refrigerator prior to analysis, allowing any excess water to drain. Thawed root samples were spread on trays and manually cleaned of any

remaining soil or detritus. Roots were separated into the following classes: small tree roots (S) of 0-2 mm diameter, coarse tree roots (C) of >2 mm diameter, dead tree roots (D), and weed roots (W). The roots were then patted dry with paper towels, weighed, and imaged. Imaging was performed by spreading roots on the plate of a flatbed scanner to minimize overlaps and intersections, and raw images were stored as black and white .tiff files to manage disk storage volume. The images were then processed using a Java plug-in macro written for the U.S. National Institutes of Health (NIH) Image-J image analysis software, version 1.38 (Rhea et al., 2010). Image data generated included root segment counts, lengths, and diameters. Diameters were calculated by the macro along a pixel-by-pixel walk down the longitudinal axis of roots, and surface areas and volumes were calculated continuously along the axis assuming a circular root cross-section. After imaging, the roots were dried at 50 °C to constant mass. The dry roots were then re-weighed and root dry masses, fresh (imaged) lengths, diameters, surface areas, and volumes were converted to rooting densities by dividing them by their respective total soil sample volumes and normalizing the results to a one-square-meter ground area, yielding dry-mass rooting density (mass), fresh-length rooting density (length), fresh-diameter rooting density (diameter), fresh rooting surface area index (RAI), and fresh-volume rooting density (volume). Specific root length (SRL) was calculated by dividing the fresh root lengths by respective dry

masses. Tissue density (TD) was calculated by dividing the fresh root mass by volume.

Data Analysis

Root dry mass was related to fresh mass by linear regression with an r-squared value of 0.97 and a p-value of <0.0001 by the equation $\text{dry mass} = 0.4674 \times \text{fresh mass}$. The data were normalized by Box-Cox transformation using SAS v9.1, Proc TransReg (SAS Institute, Cary, North Carolina, USA). Outliers were removed based on inspection of histograms, scatterplots, and residual plots, with less than 5 % of the data ultimately excluded. Summary statistics (means and standard errors) were calculated in transform-space and back-transformed to express treatment means and standard errors.

Individual ANOVA for each parameter investigated were performed to assess the significance of experimental effects. An ANOVA for all individual levels of root classifications (S, C, D, W) and depths (0-10 cm, 10-20 cm, ... , 90-100 cm) was unsuccessful due to the paucity of D and W types at depth, and high variability in the C type roots. Consequently, further analyses were restricted to small live tree

roots. The distribution of small root diameters, however, mitigated this effect across treatments (**Figure 3.1**) and depths (**Figure 3.2**). The final ANOVA model was based on a hierarchal randomized complete-block design (King et al. 2001). The ANOVA were run incorporating individual depths into three soil layers defined as shallow (0-30 cm), middle (30-70 cm) and deep (70-100 cm) based on inspection of the data and knowledge of soil stratification at the site. Block (replication) was included as a fixed effect in the ANOVA due to a known soil fertility gradient across the site (Dickson et al., 2000). Elevated CO₂ and eO₃ were treated as main-plot effects, community was treated as a sub-plot effect, and the autocorrelation by depth within sampling location was accounted for in the analyses by using random coefficients modeling (e.g. individual growth modeling). Similar to the methodology of Norby et al. (2005) for work conducted at ORNL-FACE, a primary significance level of 0.05 was used but effects satisfying a marginal significance level of 0.10 are also included in the discussion.

The ANOVA were performed using the Statistical Analysis System version 9.1.3 (SAS Institute, Cary, NC). Factor-level separations within effects and interactions were performed at a 0.95 confidence level using Tukey's LSD and the PDMIX800 macro (Saxton, 1998). SAS Proc Mixed was used to perform

ANOVA because of its capability to analyze datasets with correlated factors incorporating trends via a random coefficients model. The random coefficients model was used to model a strong trend in transformed rooting parameters with depth that included smooth trends within soil layers separated by discontinuous breaks corresponding to changes in soil layers, and because it was better able to achieve numerical convergence than use of the SAS “Repeated” statement in the presence of data censoring at depth. The typical trend-profile with depth was not well suited to modeling by a low-order, curvilinear polynomial. Therefore, depth was nested within the three soil layers corresponding to changes in soil texture and marked changes in the slope of dependent variables with depth generally occurring at roughly 0.33 m and 0.67 m depth.

Results

Biomass, Volume, RAI, and Length

Small root biomass, volume, RAI, and length responded similarly to the treatments, and the data from weighing and image analysis were very highly correlated (**Figure 3**). All increased in response to eCO₂, and to eO₃ in the AA community, but decreased in response to eO₃ in the AB community (**Figure 4**).

There was also a decreasing trend in biomass with depth in both communities (**Figure 5**). Within the AA community the respective Control root masses (standard errors) within the shallow, middle, and deep layers were 113 (28.3) $\text{g}\cdot\text{m}^{-2}$, 16 (8.5) $\text{g}\cdot\text{m}^{-2}$, and 6 (4.8) $\text{g}\cdot\text{m}^{-2}$, and within the AB community were 133 (31.5) $\text{g}\cdot\text{m}^{-2}$, 25 (11.2) $\text{g}\cdot\text{m}^{-2}$, and 39 (22.3) $\text{g}\cdot\text{m}^{-2}$ (**Table 1**). The respective root to soil volume ratios were $0.0017 \text{ m}^3\cdot\text{m}^{-3}$, $0.00034 \text{ m}^3\cdot\text{m}^{-3}$, and $0.00013 \text{ m}^3\cdot\text{m}^{-3}$ for the AA community and $0.0024 \text{ m}^3\cdot\text{m}^{-3}$, $0.00033 \text{ m}^3\cdot\text{m}^{-3}$, and $0.00048 \text{ m}^3\cdot\text{m}^{-3}$ for the AB community. The respective RAI were $7.0 \text{ m}^2\cdot\text{m}^{-2}$, $1.5 \text{ m}^2\cdot\text{m}^{-2}$, and $0.8 \text{ m}^2\cdot\text{m}^{-2}$ for the AA community and $9.4 \text{ m}^2\cdot\text{m}^{-2}$, $1.5 \text{ m}^2\cdot\text{m}^{-2}$, and $1.9 \text{ m}^2\cdot\text{m}^{-2}$ for the AB community. The respective lengths were $2733 \text{ m}\cdot\text{m}^{-2}$, $630 \text{ m}\cdot\text{m}^{-2}$, and $429 \text{ m}\cdot\text{m}^{-2}$ for the AA community and $3537 \text{ m}\cdot\text{m}^{-2}$, $617 \text{ m}\cdot\text{m}^{-2}$, and $726 \text{ m}\cdot\text{m}^{-2}$ for the AB community.

ANOVA for root mass (**Table 2**) indicated significant interactions for $\text{CO}_2\times\text{Layer}$, $\text{O}_3\times\text{Layer}$, and $\text{O}_3\times\text{Depth (Layer)}$ that superseded main effects for soil layer and soil depth within layer. A marginally significant interaction for $\text{O}_3\times\text{Community}$ was also indicated. The ANOVA for volume yielded similar results with the exceptions that the $\text{CO}_2\times\text{Layer}$ and $\text{O}_3\times\text{Layer}$ interactions were only marginally significant, and there was a marginally significant interaction for

CO₂×O₃×Community×Depth (Layer). The ANOVA results for RAI and length were very similar to those for volume, with the exception that the Community×Depth (Layer) interaction was significant. Few individual factor-levels were statistically differentiable (e.g. **Figure 3**). Elevated CO₂ had a stimulatory effect across communities in the shallow layer. Elevated O₃ had a stimulatory effect in the shallow and middle layers in the AA community and a deleterious effect in the shallow layer of the AB community. The effects of eO₃ were differentiable with depth within the shallow layer but not the middle or deep layers.

Diameter

The distribution of rooting diameters was equivalent across treatments (**Figure 1**) and depths (**Figure 2**), but mean root diameter followed a slightly decreasing trend with increasing depth (**Figure 5**). Average small root diameter within the AA community under Control within the shallow, middle, and deep layers was 0.39 (0.0134) mm, 0.37 (0.0150), and 0.28 (0.0197) mm, respectively, and within the AB community for the same respective layers was 0.41 (0.0131) mm, 0.35 (0.0154) mm, and 0.34 (0.0221) mm (**Table 1**). The only significant interaction

indicated was Community×Depth(Layer) (**Table 2**). There were marginally significant interactions for CO₂×Layer and CO₂×O₃×Community×Layer.

Specific Root Length

Within the AA community under the Control, the shallow, middle, and deep layer SRL were 23.8 (7.12) m·g⁻¹, 42.1 (12.6) m·g⁻¹, and 54.4 (17.6) m·g⁻¹, and within the AB community were 26.7 (7.8) m·g⁻¹, 36.3 (11.0) m·g⁻¹, and 31.5 (14.4) m·g⁻¹, respectively (**Table 1**). There was a significant interaction of Community×Depth (Layer) that superseded a main effect for Depth (Layer), and main effects for Layer and O₃ (**Table 2**). Elevated O₃ was associated with increased diameter relative to control at depth in the AA community (**Figure 4**).

Tissue Density

Tissue density in the shallow, middle, and deep layers of the AA community was 0.072 (0.021) g·cm⁻³, 0.051 (0.015) g·cm⁻³, and 0.067 (0.024) g·cm⁻³, respectively, and in the same respective layers in the AB community was 0.062 (0.017) g·cm⁻³, 0.077 (0.025) g·cm⁻³, and 0.089 (0.045) g·cm⁻³ (**Table 1**). There was a significant interaction of O₃×Community×Depth (Layer) (**Table 2**).

Elevated O₃ was associated with decreased TD relative to control at depth in the AA community and across all depths in the AB community (**Figure 4**).

Discussion

Comparison to Previous Aspen FACE Root Studies

Based on cores to 25 cm depth and allometric ratios, estimates of fine (<1 mm diameter) root biomass for the site from 1997 to 2003 ranged from of 26 to 80 g·m⁻² (King et al., 2005). More recently, Pregitzer et al. (2008) estimated small root biomass (< 2 mm diameter) at Aspen FACE for 2005 to range from 150 to 250 g m⁻² (e.g. see their Figure 3) but used hydropneumatic elutriation, which the authors reported increased root recovery by an average 39 %. In the 0-30 cm depth interval, which is most comparable to other studies, our non-elutriated recovery of small roots (0-2 mm diameter) in the present study ranged from 71 to 182 g·m⁻², on the low side of many destructively sampled estimates (Jackson et al., 1997; Bauhus and Messier, 1998; Steinaker and Wilson, 2005; Iversen et al., 2008; Jackson et al., 2009), but comparable to Pregitzer et al. (2008) if adjusted for elutriation. Fine root biomass and production are notoriously difficult to estimate and differences between studies can be attributed to soil sampling and

sample processing methodology, time of year, soil type and water content, inter-annual variation in climate, or numerous other factors (Kurz and Kimmins 1987; Smucker 1990; Taylor et al. 1991; Publicover and Vogt 1993; Vogt et al. 1998). In the current study, I had to drive a very large corer (10 cm diameter x 1 m depth) into very dry soil with a jack hammer, which may have affected root recovery relative to other studies done at Aspen FACE. However, our sampling and sample processing methodology were internally consistent, so interpretation of relative treatment responses within and between years is valid.

Relative Treatment Responses and Allometric Shifts

Relative responses to the treatments have been consistent between Aspen FACE root biomass studies, but allometric ratios believed to be have been stable in the past appear to have changed later in stand development. In general, King et al. (2005), Pregitzer et al. (2008), and the current study all report greater small root biomass associated with eCO₂; increased small root biomass in the AA community and decreases in the AB community associated with eO₃; and increased small root biomass under eCO₂×eO₃ in both communities.

Increased small root growth under eO₃ has only recently begun to appear in the literature (King et al. 2005; Pregitzer et al. 2008). King et al. (2001) found that under eCO₂, the very fine (<0.5 mm diameter) live root biomass increased 113 % and 83 % in the all-aspen and aspen-birch communities, respectively; and that that live fine (<1 mm diameter) root biomass increased 72 % across communities; there were no significant effects associated with either eO₃ or combined eCO₂ and eO₃ fumigation. King et al. (2005) using allometric relationships found that from 1997 through 2003 eCO₂ was associated with average respective increases of fine root biomass in the AA and AB plots of +45 % and +64 %, respectively; eO₃ was associated with respective changes of +44 % and -15 %; and eCO₂×eO₃ was associated with changes of +107 % and +46 %, respectively. Pregitzer et al. (2008) found increases in small root biomass associated with eCO₂, eO₃, and eCO₂×eO₃ of 48 %, 37 %, and 113 % for 2002 and 27 %, 36 %, and 60 % for 2005, respectively. In contrast to the previous studies, however, Pregitzer et al. (2008) found that increases in fine root biomass within the AA community associated with eO₃, and within the AA and AB communities associated with eCO₂×eO₃, were not accompanied by commensurate increases in aboveground biomass as prior changes in root biomass had been (Kubiske et al., 2006, 2007). Consistent with the onset of canopy closure beginning at the site circa 2002 (Kostiainen et al., 2008), increasing competitive interactions and compensatory

growth among tree genotypes were advanced as explanations for these differences from previous results.

Transitory Responses of Biomass to eCO₂ and eO₃

The strong effects of eCO₂ reported in early investigations at Aspen-FACE and the decrease of many of them through time (e.g. Karnosky et al., 2003; King et al., 2001, 2005; Pregitzer et al., 2008) are consistent with the onset of competition at canopy closure. Accelerated stand development under eCO₂ has apparently been at the cost of a shift in genotypic frequencies (see Kubiske et al., 2006, 2007), and higher metabolic and resource cycling rates (see Karnosky et al., 2003; and, Pregitzer et al., 2008), but was not accompanied by shifts in aboveground to belowground allometry prior to leaf canopy or “root canopy” closure (King et al., 2005). At full site occupancy, resource competition mediated by space constraints can be expected to become the dominant constraint to growth (Körner, 2006).

The ongoing effects of eO₃ at the site could be due to an increased maintenance cost associated with transpiration, which would necessitate an increased relative

expenditure of carbon on resource acquisition rather than growth (Chapin et al., 2002), and a shift toward tolerant genotypes (Karnosky et al., 2003; Kubiske et al., 2006, 2007). The partitioning of carbon under the influence of eO₃ might shift in favor of nutrient-gathering structures (roots) even after spatially-mediated nutrient constraints become significant. The relative increases in total root biomass under eO₃ reported by Pregitzer et al. (2008) and in the present study within the AA community, are consistent with a genotypic adjustment to eO₃ followed by increased soil exploration by the adjusted community.

The effect of eCO₂×eO₃ at the site on aboveground biomass has been fairly similar to control throughout the experiment, as it was on root biomass in the early years of the experiment (King et al., 2005). However, small root biomass under eCO₂×eO₃ increased relative to control after canopy closure. Such increases appear disproportionate to stem biomass in the eCO₂×eO₃ and AA eO₃ plots in the present study, and were also disproportionate in the eCO₂×eO₃ reported by Pregitzer et al. (2008).

Vertical Differentiation of Root Biomass

Vertically-integrated studies of root biomass do not characterize depth-differentiated root responses to atmospheric pollution. Such responses potentially afford means to adapt to atmospheric pollution by accessing additional resources or avoiding drought or other environmental stresses. In the current study, soil layer and depth-within-layer were significant as main effects and the effects of $e\text{CO}_2$ and $e\text{O}_3$ were significantly differentiated by layer (depth). Not surprisingly, the magnitudes of the responses were greatest in the shallow soil layer, where the overwhelming majority (average percentage) of root biomass occurred. The responses in the middle and deep layers were generally not large enough to offset the shallow soil responses. Responses expressed as percentage change were often quite large in the middle and deep soil layers (**Table 1**), but the variances were also large and the relative amount of root biomass was very small. The profile of root density with depth found in the present study is generally consistent with the commonly observed asymptotic root depth distribution (Jackson et al., 1997), and overall the findings are consistent with our hypothesis of the depth-dependence of root responses to air pollution.

The small root proliferation in the shallow soil layer under eCO₂ found in the present study is consistent with some FACE studies of herbaceous material (e.g. Prior et al., 1994) and some species of poplar (Lukac, 2003; Afas et al., 2008). Our results were not entirely consistent, however, with the pattern of increased root proliferation at depth reported in studies conducted in warmer climates at Duke- and ORNL-FACE (Norby et al, 2004; Iversen et al., 2008; Pritchard et al., 2008), for white poplar at Pop-FACE (Lukac et al., 2003), and Holm oak in Spain (López et al., 2001). López et al. (2001) suggested that the decrease in rooting within the shallowest soils was a consequence of summer heat and drought. Indeed, the differences between these FACE studies in root proliferation with depth might be partly attributable to differences in heating and desiccation of shallow soils, or differing edaphic characteristics (Oren et al., 1998; Schlesinger and Lichter, 2001; Lichter et al., 2005; Stoy et al., 2008; <http://websoilsurvey.nrcs.usda.gov>; USDA Soil Conservation Service, 1967; Norby and Iversen, 2006; Calfapietra et al, 2003; Hoosbeek et al., 2006). Summers are relatively hot at Duke-FACE, ORNL-FACE, and Pop-FACE in comparison to Aspen-FACE, although Pop-FACE is irrigated, and more significant droughts and high ambient ozone levels have occurred in the vicinity of the Duke and ORNL than Wisconsin experiments (US Drought Monitor cooperative data at http://www.drought.unl.edu/dm/dmtabs_archive.htm;

Southeast Regional Climate Data Center data at

<http://www.sercc.com/climateinfo/historical/historical.html>;

<http://epa.gov/airtrends/ozone.html#oznat>); University of Wisconsin Cooperative

Extension data at

<http://www.soils.wisc.edu/asigServlets/asos/SelectHourlyAsos.jsp>).

Root Morphology

Eissenstat et al. (2000) postulated that increased carbon gain under elevated CO₂ might increase root length density and greater branching in shallow soils. In the present study we detected morphometric responses to the eCO₂ and eO₃ treatments, which could have significant implications for tree access to soil resources, especially water. Our results supported our hypotheses regarding the utility of root morphometrics to differentiate treatment responses, as it was only root morphometrics such as SRL, not biomass, that best differentiated treatment effects at depth.

Length

In the present study root length was similar to the ranges reported by Jackson et al. (1997) who estimated the small root length to be $2.6 \text{ km}\cdot\text{m}^{-2}$ in boreal forests and $5.4 \text{ km}\cdot\text{m}^{-2}$ in temperate deciduous forests based on a database of 253 field studies. These values are considerably higher, however, than is commonly reported based on minirhizotron image analysis data (Bauhus and Messier, 1998; Norby, 2004; Steinaker and Wilson, 2005; Pritchard et al., 2008). In the present study the relative responses of root length to treatments were very similar to those of biomass, and our interpretations for these two parameters in the present study are generally equivalent. It is noteworthy, however, that although the root lengths were greatest in the shallow soils ($2733 \text{ m}\cdot\text{m}^{-2}$ in the AA community and $3537 \text{ m}\cdot\text{m}^{-2}$ in the AB community), considerable root length was present even in the deepest soils investigated ($617 \text{ m}\cdot\text{m}^{-2}$ in the AA community and $726 \text{ m}\cdot\text{m}^{-2}$ in the AB community). Clearly, this amount of deeper rooting indicates a significant potential for resource acquisition below depths typically characterized in most root studies, and has special significance in the context of forest water cycling.

Diameter

Small root diameters found in the present study were comparable to those reported elsewhere for a range of species (Eissenstat et al., 2000; Comas et al., 2002; Bauhus and Messier, 1998; Pregitzer et al., 2000). Diameter slightly decreased with increasing depth in the present study (**Figure 5**), contrary to that of Wells et al., (2002). Consistent with our results, however, Pritchard et al. (2008) and Iversen et al. (2008) found that eCO₂ had no effect on small root diameter for loblolly pine or sweetgum. We did however find marginally significant interactions with depth for eCO₂ (possibly increased in the AA community in shallow and deep, but not middle layer soils) and eCO₂×eO₃ (apparently increased in deep soils). Increased root diameters in shallow and deep soil under eCO₂ relative to Control may indicate balanced increases in demands for nutrients and water, while increased root diameters only at depth under eCO₂×eO₃ may indicate a shift in relative resource acquisition towards greater water uptake relative to nutrients.

RAI

The response of RAI to the was very similar to that of biomass and volume. RAI has been shown to be correlated with LAI (e.g. Afas et al., 2008), facilitating study of allocation using the ratio of the two, and the range of RAI to LAI ratios were within the range of about three to five, consistent with ratios for various species (Jackson et al., 1997; Afas et al., 2008; López et al., 2001; Bolte and Villanueva, 2006; Meinen et al., 2009).

Taylor et al. (2008) reported control LAI on Julian day 240 of 2003 and 2004 at Aspen FACE as 2.7 and 2.3, respectively, and under eCO₂ on the same respective dates as 2.9 and 2.6. McGrath et al. (2010) reported LAI at Aspen FACE for control, eCO₂, eO₃, and eCO₂×eO₃ as 3.7, 4.1, 3.2, and 4.0, respectively.

Although direct comparisons between LAI and RAI are not available for Aspen FACE, ratios of RAI from the present study to these reported LAI values for eCO₂, eO₃, and eCO₂×eO₃ differed from Control by about +2.4 %, +20 %, and +96 % in the AA community and +6.3 %, -46 %, and +27 % in the AB community, respectively. These ratios in conjunction with biomass results indicate that both aboveground and belowground growth was stimulated equally by eCO₂ across communities, the effects of eO₃ were community-dependent, and

allometric ratios of surface area were not conserved in the presence of eO_3 or $eCO_2 \times eO_3$. Shifts in allometry can occur when the relative difficulty of acquiring limiting resources is altered (McCarthy and Enquist, 2007). It is commonly reported that eCO_2 is associated with higher WUE, increased aboveground growth, and faster onset of competition, while eO_3 has essentially opposite effects (see Karnosky et al., 2003). In this context, the shifts in RAI and LAI may indicate that the degree of growth enhancement caused by eCO_2 at the relatively resource-rich Aspen-FACE Experiment has not been adequate to cause a shift in resource limitations to growth, whereas aboveground-belowground allometric shifts have occurred under eO_3 . If true, this implies that in environments similar to northeast Wisconsin, eCO_2 will not be as likely as eO_3 to alter ecosystems through shifts in resource limitations or biomass partitioning.

SRL

Specific root length is a highly sensitive (nonlinear) parameter, but is important because it is used to estimate root biomass from minirhizotron images and to characterize soil exploration strategies of roots. SRL has also been positively correlated to disturbed soils (Eissenstat, 1991) and root hydraulic conductivity (Eissenstat, 1997; Huang and Eissenstat, 2000). SRL has been found to range

widely over species, from $4.4 \text{ m}\cdot\text{g}^{-1}$ in grasses to $280 \text{ m}\cdot\text{g}^{-1}$ in some deciduous tree species (Ryser, 1996; Pregitzer et al., 1997, 1998; Wells, 1999; Comas et al., 2002; Comas and Eissenstat, 2004; Norby, 2004; Bolte and Villanueva, 2006) and Iversen et al. (2008) reported the reciprocal of SRL, root mass per unit length ($\text{mg}\cdot\text{cm}^{-1}$), at ORNL-FACE was well characterized by three times the diameter squared. In the current study, SRL increased with soil depth, from an average 28.2 to $53.6 \text{ m}\cdot\text{g}^{-1}$ in the shallow and deep soil layers, respectively, averaged across treatments and communities. In general, the increases in SRL in the presence of eO_3 in the deep soil layer were about $25 \text{ m}\cdot\text{g}^{-1}$ compared to the Control (**Figure 5**). Given that there appeared to be no correlated changes in small root biomass or volume at this depth, this may indicate a shift toward a more extensive soil exploration strategy, consistent with an increased demand for water.

Volume and TD

Root volume in the present study served the dual purposes of providing a secondary means of quantifying small root biomass and, in conjunction with root mass, to estimate root tissue density. TD of a sample of tree species has been reported to range between $0.06 \text{ g}\cdot\text{cm}^{-3}$ to $0.20 \text{ g}\cdot\text{cm}^{-3}$ (Eissenstat, 2000; Comas et

al., 2002). Our reported values ranging from $0.034 \text{ g}\cdot\text{cm}^{-3}$ to $0.089 \text{ g}\cdot\text{cm}^{-3}$ TD are somewhat low compared to these previous studies, possibly attributable to taxonomic differences or finer discrimination of very small root volumes by our image analysis based system of estimating root morphometry, but this does not invalidate relative comparisons between treatments. The author is unaware of other FACE studies that have investigated effects on subsurface TD, but such effects could be important to carbon cycling and sequestration.

Conclusions

Taken together, the work presented here and in previous studies at Aspen FACE illustrate temporally changing effects of $e\text{CO}_2$ and $e\text{O}_3$ on belowground dynamics during early-stand development. The beneficial effects on growth of $e\text{CO}_2$ in the absence of $e\text{O}_3$ appear to decrease with canopy closure and presumably the onset of light limitation. Aboveground-to-belowground partitioning was stable at Aspen-FACE until canopy closure. Responses to $e\text{O}_3$ appear to be species-specific and dependent upon interspecific competition, and changes in root biomass or morphometry at depth may be a mechanism used by trees to compensate for these deleterious effects. The morphometric data from the present study indicate that nutrient and water resource demands are increased

proportionally under eCO₂ alone, but the water requirements relative to nutrient requirements are greater in the presence of eO₃ and are not offset by the effects of eCO₂.

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Table 3.1. Least-squares means, standard errors, and percent differences (%) from Control treatment for small (0-2 mm dia.) live tree roots, by soil layer, community (AA = All-aspen, AB = Aspen-birch), and treatment (1 = Control, 2 = eCO₂, 3=eO₃, 4=eCO₂+eO₃). Volume and RAI were computed assuming a circular root cross-section. Derived parameters were calculated for individual samples rather than group means (e.g. SRL ~ length/mass, TD ~ 1E-6·mass·volume⁻¹).

Comm / Trt	Mass g·m ⁻²	Volume m ³ ·m ⁻³	RAI m ² ·m ⁻²	Length m·m ⁻²	Diameter millimeters	SRL m·g ⁻¹	TD g·cm ⁻³
Shallow soil layer (0-30 cm)							
AA	1 113 (28.3)	0.00169 (0.00047)	7.0 (1.94)	2733 (783)	0.39 (0.0134)	23.8 (7.12)	0.072 (0.021)
	2 140 (32.6) 24%	0.00207 (0.00052)	23% 8.1 (2.09)	16% 2962 (809)	8% 0.41 (0.0130)	4% 24.4 (7.23) 3%	0.065 (0.019) -9%
	3 146 (29.5) 30%	0.00220 (0.00049)	30% 9.2 (2.05)	31% 3603 (834)	32% 0.38 (0.0121)	-4% 30.7 (7.76) 29%	0.068 (0.017) -6%
	4 182 (36.4) 62%	0.00281 (0.00060)	66% 11.8 (2.53)	69% 4699 (1033)	72% 0.38 (0.0129)	-4% 28.4 (7.71) 20%	0.064 (0.017) -11%
AB	1 133 (31.5)	0.00236 (0.00057)	9.4 (2.29)	3537 (905)	0.41 (0.0131)	26.7 (7.80)	0.062 (0.017)
	2 159 (28.4) 20%	0.00270 (0.00053)	14% 11.3 (2.22)	20% 4398 (904)	24% 0.39 (0.0108)	-4% 27.3 (6.38) 2%	0.070 (0.016) 13%
	3 71 (18.2) -46%	0.00134 (0.00036)	-43% 5.6 (1.52)	-40% 2217 (619)	-37% 0.39 (0.0118)	-3% 35.7 (8.88) 34%	0.051 (0.012) -18%
	4 145 (27.8) 9%	0.00250 (0.00051)	6% 10.3 (2.13)	10% 4009 (865)	13% 0.38 (0.0115)	-7% 28.3 (6.87) 6%	0.061 (0.014) -1%
Middle soil layer (30-70 cm)							
AA	1 16 (8.5)	0.00034 (0.00018)	1.5 (0.80)	630 (333)	0.37 (0.0150)	42.1 (12.63)	0.051 (0.015)
	2 9 (5.1) -48%	0.00018 (0.00011)	-48% 0.9 (0.53)	-42% 404 (235)	-36% 0.34 (0.0146)	-8% 43.3 (11.87) 3%	0.057 (0.016) 10%
	3 39 (12.9) 140%	0.00072 (0.00025)	109% 3.3 (1.13)	117% 1459 (493)	131% 0.33 (0.0139)	-11% 42.8 (10.99) 2%	0.055 (0.014) 8%
	4 20 (8.7) 19%	0.00049 (0.00021)	43% 2.4 (0.98)	59% 1104 (435)	75% 0.33 (0.0151)	-13% 74.6 (19.04) 77%	0.046 (0.012) -11%
AB	1 25 (11.2)	0.00033 (0.00017)	1.5 (0.76)	617 (321)	0.35 (0.0154)	36.3 (11.03)	0.077 (0.025)
	2 11 (4.5) -56%	0.00024 (0.00011)	-26% 1.1 (0.49)	-28% 430 (206)	-30% 0.35 (0.0111)	-2% 44.8 (9.47) 23%	0.052 (0.011) -32%
	3 18 (7.7) -28%	0.00038 (0.00017)	14% 1.6 (0.71)	7% 605 (285)	-2% 0.37 (0.0129)	5% 48.3 (12.19) 33%	0.042 (0.010) -46%
	4 14 (6.2) -43%	0.00030 (0.00014)	-11% 1.4 (0.63)	-6% 602 (274)	-2% 0.34 (0.0129)	-4% 45.0 (10.85) 24%	0.053 (0.013) -31%
Deep soil layer (70-100 cm)							
AA	1 6 (4.8)	0.00013 (0.00012)	0.8 (0.62)	429 (302)	0.28 (0.0197)	54.4 (17.57)	0.067 (0.024)
	2 19 (10.2) 233%	0.00039 (0.00021)	191% 1.9 (0.97)	129% 844 (424)	97% 0.34 (0.0174)	21% 52.2 (16.63) -4%	0.051 (0.017) -24%
	3 9 (5.3) 63%	0.00024 (0.00014)	83% 1.2 (0.64)	45% 536 (285)	25% 0.30 (0.0158)	8% 71.1 (18.35) 31%	0.040 (0.010) -41%
	4 6 (3.9) 2%	0.00016 (0.00011)	24% 0.8 (0.51)	3% 378 (225)	-12% 0.34 (0.0148)	20% 80.6 (20.36) 48%	0.034 (0.009) -49%
AB	1 39 (22.3)	0.00048 (0.00032)	1.9 (1.30)	726 (517)	0.34 (0.0221)	31.5 (14.42)	0.089 (0.045)
	2 15 (6.2) -60%	0.00027 (0.00013)	-43% 1.3 (0.58)	-33% 555 (252)	-24% 0.33 (0.0126)	-5% 36.0 (8.50) 14%	0.081 (0.020) -8%
	3 19 (9.4) -51%	0.00044 (0.00021)	-8% 2.1 (0.98)	13% 929 (422)	28% 0.35 (0.0156)	1% 55.8 (16.07) 77%	0.047 (0.014) -47%
	4 10 (4.6) -75%	0.00015 (0.00008)	-69% 0.6 (0.38)	-66% 256 (155)	-65% 0.37 (0.0117)	6% 47.6 (10.78) 51%	0.062 (0.014) -29%

Table 3.2. ANOVA significance test results for small roots (0-2 mm in diameter) collected over 0.1 meter depth increments to one meter depth at the Aspen-FACE experiment, Rhinelander, WI, in 2005. Variates analyzed include dry root mass-density (Mass), fresh root volume-density (Volume), fresh root surface area index (RAI), fresh root length-density (Length), fresh root diameter (Diameter), specific root length (SRL) and tissue density (TD). Interactions are denoted by “×” and nesting variables are denoted by brackets “()”. Analyses were performed separately for data summed over depth (for increased testing power for main and lower-order interactions) and data within individual soil layers. Trends with depth were modeled by augmenting the ANOVA model with intercepts for each layer and slopes within each layer based on trends with depth. Both analyses were performed using Proc Mixed of SAS v. 9.1. P-values ≤ 0.05 are shown in bold, $p \leq 0.10$ are shown in italic, and $p > 0.20$ are indicated by “ns”.

Effect	Mass	Volume	RAI	Length	Diameter	SRL	TD
Block	0.1465	0.1907	ns	ns	ns	ns	ns
Soil Layer	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002	0.1247
Depth (Layer)	<.0001	<.0001	<.0001	<.0001	0.0121	0.0001	0.0022
Community	ns	ns	ns	ns	0.0203	<i>0.0974</i>	ns
Community × Layer	ns	ns	ns	ns	ns	ns	ns
Community × Depth (Layer)	ns	0.1031	0.0474	0.0190	0.0483	0.0087	<i>0.0577</i>
CO ₂	ns	ns	ns	ns	ns	ns	ns
CO ₂ × Community	ns	ns	ns	ns	ns	ns	ns
CO ₂ × Layer	0.0352	<i>0.0817</i>	<i>0.0952</i>	0.1072	<i>0.0567</i>	ns	ns
CO ₂ × Depth (Layer)	ns	ns	ns	0.1682	ns	ns	ns
CO ₂ × Community × Layer	ns	ns	ns	ns	ns	ns	ns
CO ₂ × Community × Depth (Layer)	ns	ns	0.1793	0.1587	ns	ns	ns
O ₃	<i>0.0853</i>	ns	ns	ns	0.1808	0.0167	0.0254
O ₃ × Community	ns	ns	ns	ns	ns	ns	ns
O ₃ × Layer	0.0469	<i>0.0750</i>	<i>0.0679</i>	<i>0.0628</i>	ns	0.1987	0.1049
O ₃ × Depth (Layer)	0.0307	0.0140	0.0064	0.0046	ns	ns	0.1603
O ₃ × Community × Layer	ns	ns	ns	ns	ns	ns	ns
O ₃ × Community × Depth (Layer)	ns	ns	ns	ns	ns	0.1527	0.0393
CO ₂ × O ₃	ns	ns	ns	ns	ns	ns	ns
CO ₂ × O ₃ × Community	0.1171	ns	ns	ns	ns	ns	ns
CO ₂ × O ₃ × Layer	ns	ns	ns	ns	ns	ns	ns
CO ₂ × O ₃ × Depth (Layer)	ns	ns	ns	ns	ns	0.1042	0.1133
CO ₂ × O ₃ × Community × Layer	ns	ns	ns	ns	<i>0.0718</i>	ns	ns
CO ₂ × O ₃ × Community × Depth (Layer)	0.1311	<i>0.0805</i>	<i>0.0843</i>	0.1084	ns	ns	ns

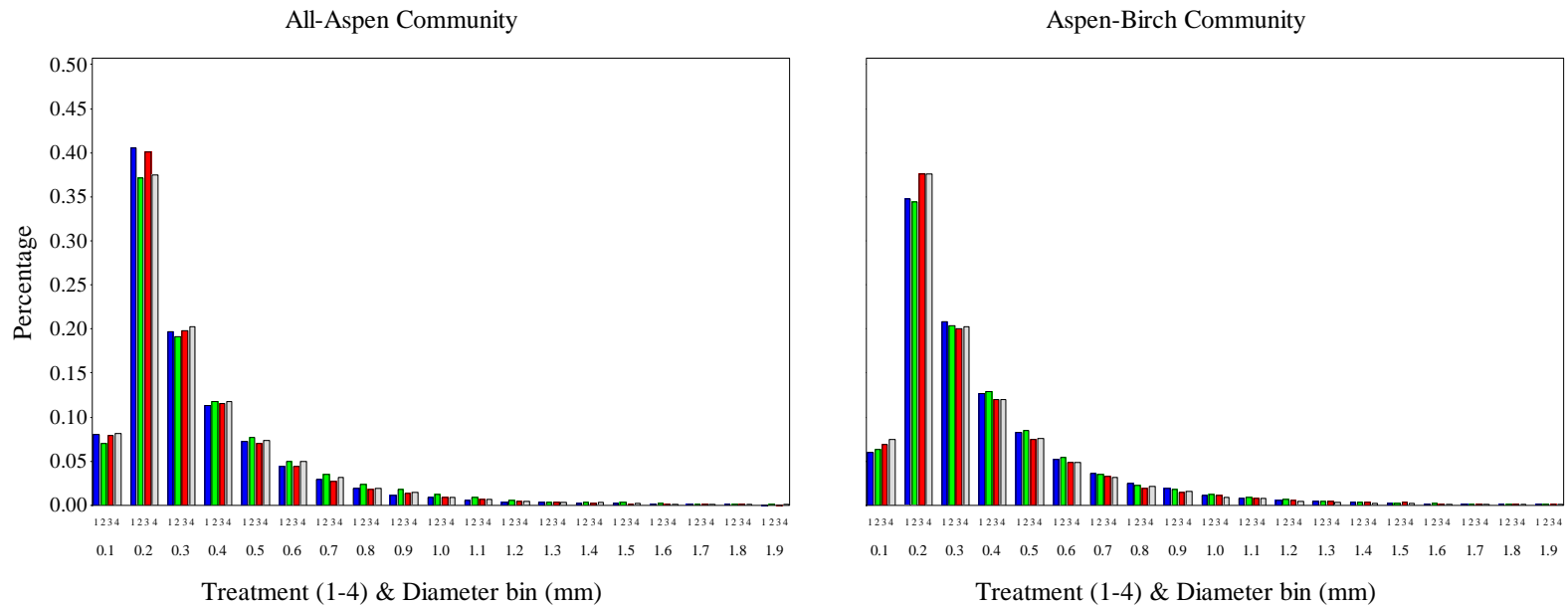


Figure 3.1. Histograms of tree root diameter frequencies by community and treatment, averaged across replications and depths. Samples were collected at the Aspen FACE experiment during July and August 2005 to a maximum depth of one meter. Diameter bins span 0.1 mm and are identified using the maximum diameter contained. Treatments Control (1), eCO₂ (2), eO₃ (3), and eCO₂+eO₃ (4) are shown from left-to-right within each diameter bin. The shapes of the histograms were not significantly differentiated by depth, although means decreased with increasing depth.

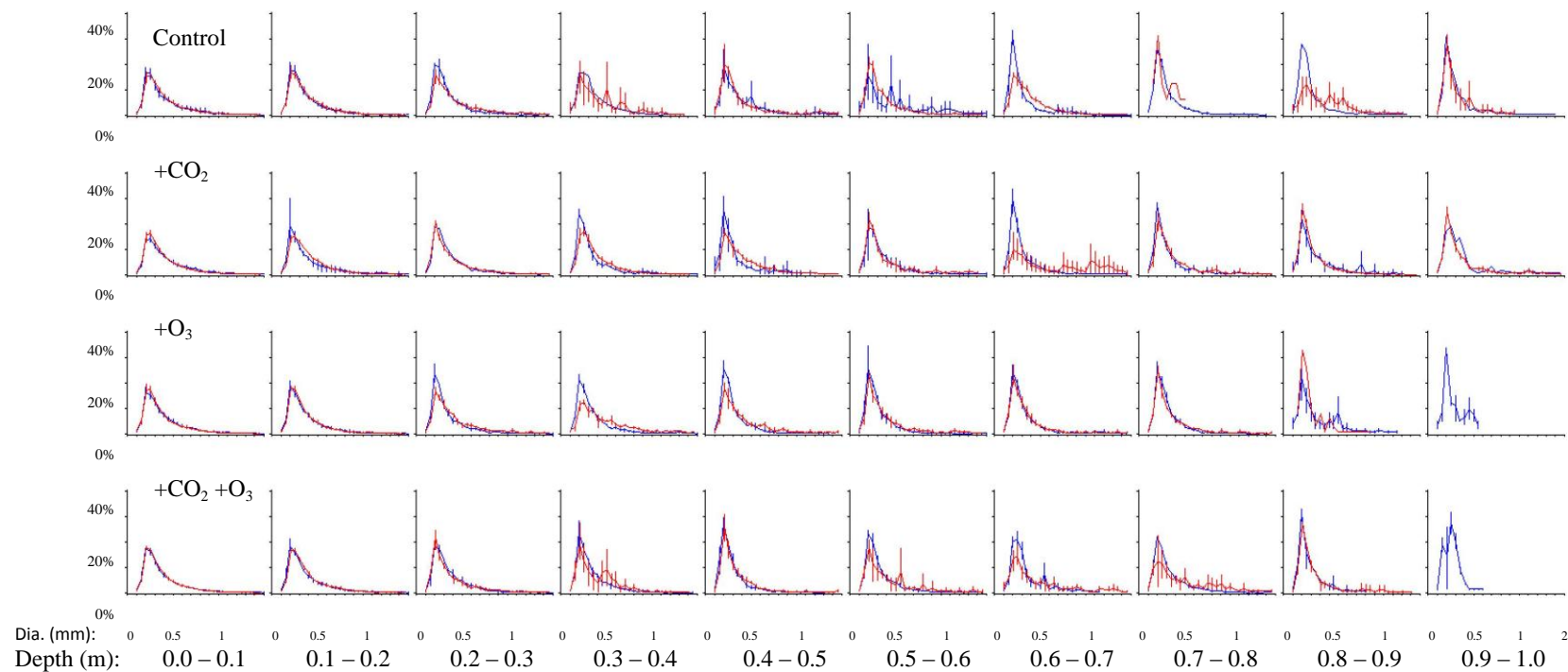


Figure 3.2. Hi-lo plots of tree root diameter frequency distributions by block, community, treatment, and depth. Community AA (all-aspens) is depicted in red and community AB (aspens and birch) is depicted in blue. The highest and lowest values for the three replicates correspond to the tops and bottoms of the vertical bars and the intermediate values are joined by lines. The treatments Control, eCO₂, eO₃, and eCO₂+eO₃ are arranged vertically, and 0.1 meter increments in depth are arranged horizontally. Samples were collected at the Aspen FACE experiment during July and August 2005 to a maximum depth of one meter.

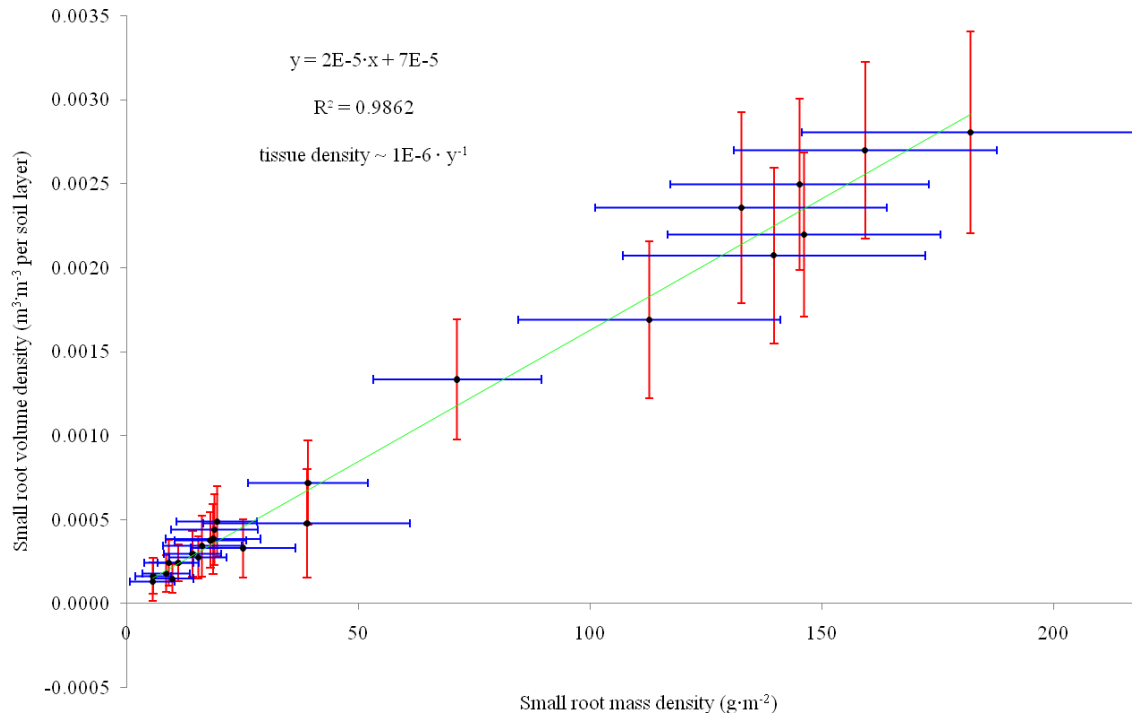


Figure 3.3. Comparison of means and standard errors ($\alpha=0.05$) for small tree root (<2 mm diameter) mass and volume densities factored by communities (AA, AB), treatments (Control, eCO_2 , eO_3 , eCO_2+eO_3), and soil layers (0-30 cm, 30-70 cm, and 70-100 cm). Samples were collected at the Aspen FACE experiment during July and August 2005 to a depth of one meter. The mass estimates are from root weighing and the volume estimates are from image analyses. The mass and volumes were linearly related and very highly correlated ($R^2 = 0.9862$). Because of the linear relationship independent estimates of tissue density could be used to easily correct for any systematic bias in the volume estimates from the image analysis program, such as could be caused by poor image quality (e.g. shadows).

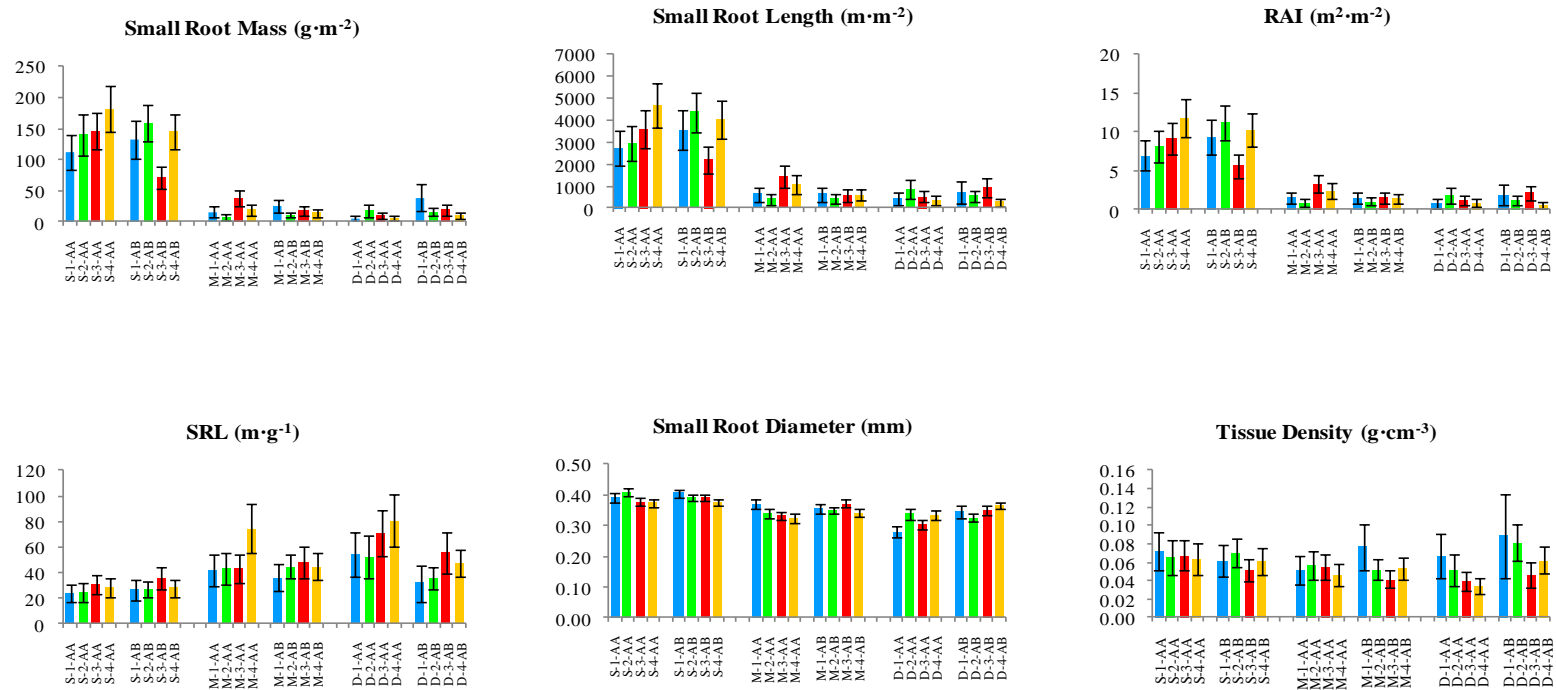


Figure 3.4. Means and standard errors ($\alpha=0.05$) for small tree root (<2 mm diameter) parameters by soil layer (S = 0 to 30 cm, M = 30 to 70 cm, D = 70 to 100 cm), experimental treatment (1 = Control, 2 = eCO_2 , 3 = eO_3 , 4 = eCO_2+eO_3) and community (AA = all aspen, AB = aspen and birch). Samples were collected at the Aspen FACE experiment during July and August 2005 to a maximum depth of one meter.

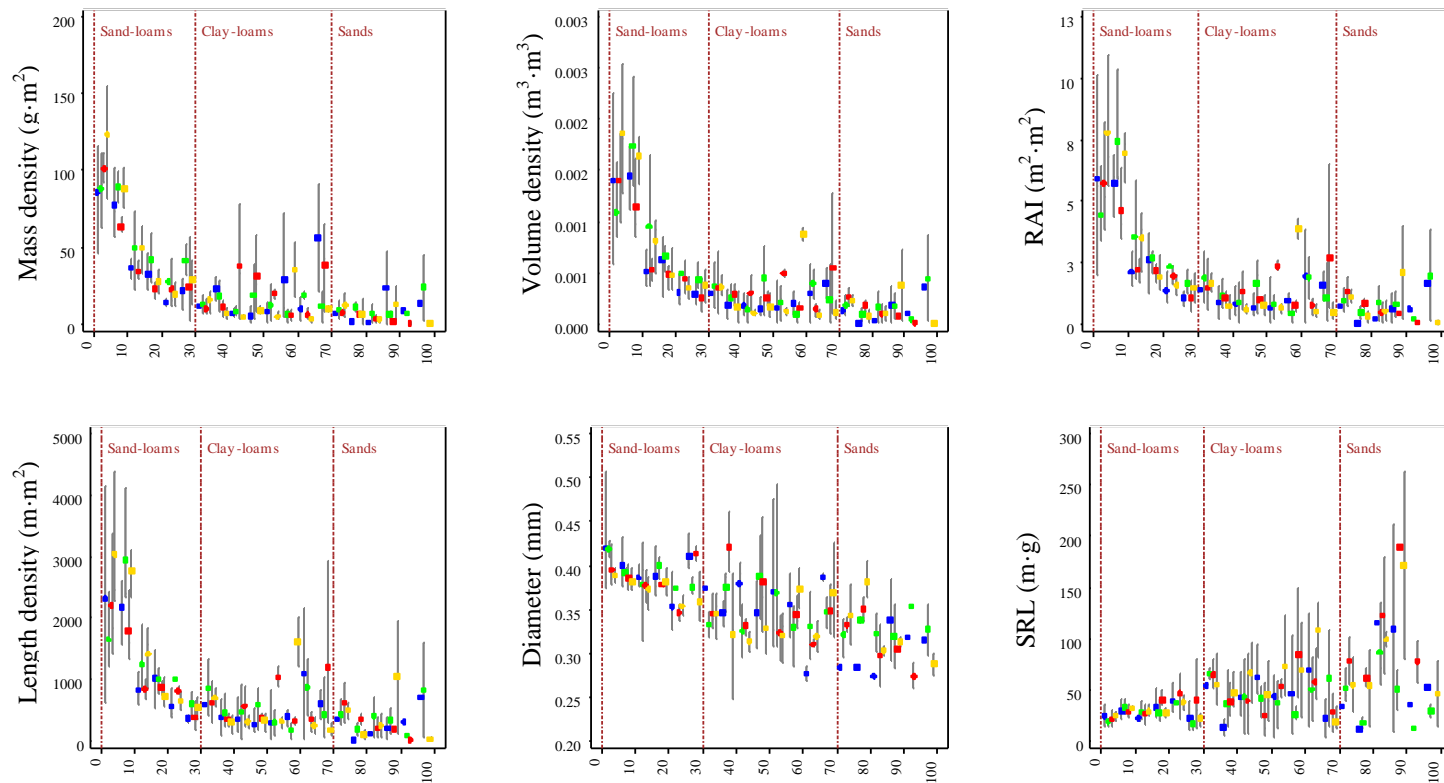


Figure 3.5. Raw univariate means by block for selected small root parameters, hierarchically grouped from left-to-right by increasing depth, community, and treatment. Intermediate values are indicated by colored symbols and the range of the high-low values are indicated by gray lines. The approximate boundaries between major soil layers are indicated by vertical brown lines and the dominant soil texture of each layer is indicated at the top of each graph panel. Depth increases from 0-1 meter from left to right in 10-cm increments. Within each depth interval data are grouped from left to right by all-aspen (AA) versus aspen and birch (AB) communities, and sub-grouped from left to right by treatments Control, eCO₂, eO₃, and eCO₂+eO₃. Means for the AA treatments are indicated by colored dots and means for the AB treatments are indicated by colored squares. Blue dots indicate Control, green dots indicate eCO₂, red dots indicate eO₃, and gold dots indicate eCO₂+eO₃.

CHAPTER 4

**Effects of elevated atmospheric CO₂ and tropospheric O₃ on the anatomy
and conductivity to water of sapwood in trembling aspen (*Populus
tremuloides* Michx.)**

Abstract

Availability of water for human use and natural ecosystems during the coming decades is a significant concern due to rapidly rising mean global temperature and expected shifts in the frequency and intensity of precipitation and drought.

Atmospheric concentrations of CO₂ and O₃ have been significantly increasing since the Industrial Revolution and resultant changes in forest tree physiology and growth could affect forest water balance and evapotranspiration at regional scales, thereby affecting net water yield to replenish aquifers and reservoirs, and maintain stream base flows. One consequence of projected climatic change identified as a significant concern is large-scale tree mortality due to temperature-sensitive carbon starvation or cavitation under extreme water stress. Changes in hydraulic conductivity of a sample of boreal tree species of different ontogenetic stages and soil water status have been shown to limit transpiration. Sapwood anatomical changes in response to air pollution have been documented at the Aspen-FACE experiment. We hypothesized that wood anatomical changes could result in alterations to maximum sapwood conductance, and possibly resistance to embolism under water stress. Maximum sap wood conductance averaged 25 (0.19) cm·min⁻¹ and sapwood xylem vessel mean density was 82.7 (4.27) vessels mm⁻², and neither of these parameters was significantly affected by elevated CO₂,

or O₃. Xylem vessel element mean (se) area was 2173 (30) μm² and was differentially affected by elevated CO₂ between genotypes. Median resistance to embolism (Km) increased under CO₂ up to 85 % and decreased as much as -48 % under O₃, but these effects were highly variable between genotypes. The changes in Km appeared to be at least partly mediated by the area of individual sapwood vessels but not vessel density, indicating that some other factor(s) may be important as well. Results also suggest trees grown under elevated O₃ may be somewhat limited in maximum sustainable transpiration rates, and that trees grown under elevated CO₂ may benefit from enhanced resistance to embolism at times of stress.

Introduction

Availability of water for human use and natural ecosystems during the coming decades is a significant concern due to the rapidly rising mean global temperature and expected shifts in the frequency and intensity of precipitation (UNWWAP 2006; IPCC 2007; NRC 2011). Increased variability in the frequency and intensity of precipitation and drought events has been identified as a significant concern in addition to shifts in mean conditions (Sheffield and Wood, 2008). Atmospheric concentrations of CO₂ and O₃, have been significantly increasing since the Industrial Revolution (IPCC, 2001) and resultant changes in forest physiology and growth (Karnosky, 2003; Wittig et al., 2007) could affect forest water cycling directly (Panek, 2004; McLaughlin et al., 2007), and therefore net water drainage available to replenish aquifers and reservoirs, and maintain stream base flows. Large-scale shifts in the geographic ranges of vegetation communities has been predicted in response to climate change, including extensive tree mortality due to either temperature-mediated carbon starvation or to catastrophic hydraulic failure under extreme water stress (xylem cavitation) (Adams et al., 2009). Carbon starvation is postulated as a result of unsustainably elevated respiration rates under elevated temperatures, and could be exacerbated by closing of leaf stomata in response to concurrent water stress. Physiological

changes in trees accompanying climate change, such as altered xylem hydraulic conductance and resistance to embolism, could then be expected to significantly influence the extent of forest mortality.

Changes in hydraulic conductivity of a sample of boreal species corresponding to differences in ontogenetic stage and soil water status have been documented (Schume et al, 2004; Wang, 2005) and shown to limit transpiration (Sperry et al., 1991). Various xylem anatomical parameters have been considered as potential predictors of xylem conductance and resistance to embolism (Logullo et al., 1995; Zwieiniecki et al., 2001; Sperry et al., 2005; Christman et al., 2009). Xylem susceptibility to embolism is believed to be dependent on factors related to conductance such as lumen diameter, with increased lumen diameters associated with higher conductance but also increased susceptibility to embolism (Sperry and Sullivan, 1992). Susceptibility to embolism may also change with pit-membrane degradation through time (Sperry et al., 1991) and between genotypes within species (Sperry and Saliendra, 1994). Xylem susceptibility to embolism can also be expected to be correlated with growth rate or dominance status, as wood properties such as vessel size have been correlated with stem growth and climatic conditions (Joyce and Steiner, 1995; Schume et al., 2004). The pit area

hypothesis (Wheeler et al., 2005) suggests that characteristics of the pits in a vessel element should control embolism susceptibility rather than lumen diameter, as described by the Hagen-Poiseuille equation. Cai and Tyree (2010) state, however, that experimental data do not provide strong support for this hypothesis. They investigated the relationship between the hydraulic conductivity of xylem vessels and vessel diameter for trembling aspen, and found that the pressure or tension (MPa) causing median conductivity loss due to embolism (P_{50}) was successfully predicted as a function of lumen diameter (μm) alone ($R^2=0.995$):

$$P_{50} = 6.166 \cdot (\text{lumen diameter})^{-0.3134}$$

Few direct studies of the effects of changing atmospheric chemistry on xylem conductance have been performed and study results have been variable, but in general ring-porous species such as oaks are more affected than diffuse-porous species such as aspen and birch, and responses of gymnosperms are mixed. Conroy et al. (1988) found that shoot conductance of Radiata pine (*Pinus radiata* D.) was not changed under eCO₂. Atkinson and Taylor (1996) found that English oak (*Quercus robur* L.) responded to eCO₂ by increasing vessel number and size, while little effect occurred for hybrid cherry (*Prunus avium* L. \times *Prunus pseudocerasus* Lind.). Ceulemans et al. (2002) found a fairly large (25 %)

increase in the number of tracheids and a similar (24 %) increase in the mean tracheid diameter of Scotch pine (*Pinus Sylvestris* L.) under eCO₂. Gartner et al. (2003) found that seedlings of the evergreen holly oak (*Quercus ilex* L.) had similar vessel frequency and wood density, but larger average vessel lumen area, under eCO₂. Luo et al. (2005) found wider vessels in black poplar (*Populus nigra* L.) and hybrid poplar (*Populus × euroamericana* Rehd.), but not white poplar (*Populus alba* L.). Kostianen et al., (2006) found little effect of eCO₂ on European birch (*Betula pendula* Roth.). Watanabe et al. (2008) found little effect of eCO₂ on wood structures related to hydraulic conductance in the ring-porous Mongolian oak (*Quercus mongolica* Fisch.) and the diffuse-porous Manchurian alder (*Alnus hirsute* Spach). Finally, Domec et al. (2010) found growth under elevated CO₂ to result in slightly increased maximum hydraulic conductivity and decreased median embolizing pressure for sweetgum (*Liquidambar styraciflua* L.) and flowering dogwood (*Cornus florida* L.), decreased conduit diameter for winged elm (*Ulmus alata*), and increased conduit diameter for flowering dogwood.

The long term (1997-2009) Aspen-FACE experiment near Rhinelander Wisconsin provided a means to test the effects of both elevated CO₂ and O₃ on xylem

anatomy and hydraulic conductance. Previous studies of sapwood xylem at Aspen-FACE indicated that anatomical structures potentially related to hydraulic conductance, such as vessel lumen diameter, differed between aspen clones and that they responded to the treatments differentially by species, genotype, and canopy stage. Isebrands et al. (2001) found that the xylem anatomy of aspen clone 216 was notably different from the other clones, having the smallest vessel (and fiber) lumen diameters, and the smallest vessel percentage. Kaakinen et al (2004) investigated treatment effects on stem xylem of trembling aspen, paper birch, and sugar maple fumigated for three growing seasons at Aspen-FACE (1998-2000). They found that eCO₂ had no statistically significant effect on wood anatomy, although there was a trend of increased vessel lumen diameter, and that eO₃ significantly decreased vessel lumen diameter in aspen, but simultaneous exposure to eCO₂ counteracted this effect. Kostianen et al. (2008) found in a follow-up study that while some xylem anatomical parameters appeared to have changed after canopy closure in 2002, they found no significant effects on wood anatomy under eCO₂, but decreased vessel lumen diameter under eO₃.

In the current study, I investigated the effects of elevated CO₂ (eCO₂) and ozone (eO₃) on aspen stem xylem anatomy and hydraulic conductivity to support a comprehensive site water balance analysis for the Aspen-FACE experiment. Aspen-FACE has experienced both relatively wet and dry years and soil water content has reached levels in summer at which water stress could occur (Uddling et al, 2008). Under these conditions, stem conductance is expected to be decreased by embolism due to increased capillary tension (Taiz and Zeiger, 2002). Based on our understanding of wood anatomical responses to eCO₂ and eO₃, we hypothesized that: (1) increased hydraulic conductivity of sapwood (K) and its susceptibility to embolism would result from eCO₂ alone; (2) that decreases in K and susceptibility to embolism would result from exposure to eO₃ alone; and, (3) that exposure to eCO₂ and eO₃ together would result in wood structure/function not significantly different from control conditions.

Methods

Aspen FACE was initiated in 1997 to study the effects of eCO₂, eO₃, and their interaction on forest trees of north-temperate climates. The Aspen FACE

experiment consists of twelve 30-m diameter plots that have been grouped into three replicates of four treatments. Each plot is subdivided into three subplots including communities of mixed aspen and maple (AM), mixed aspen and birch (AB), and all-aspen (AA). Treatments are applied in the manner of the Brookhaven National Laboratory's design (Hendrey et al., 1999), including: (1) ambient conditions; (2) elevated CO₂ (~560 ppm); (3) elevated O₃ (~60 ppb, or 1.5 times ambient); (4) and both elevated CO₂ and O₃. Additional documentation is available in Dickson et al. (2000) and at www.aspenface.mtu.edu.

Sample Acquisition

Samples analyzed for the present study were collected during a harvest conducted at Aspen FACE during August 2007. Diameter at breast height (d.b.h) was measured after leaf fall during the autumns of 2006, 2007 and 2008. The AA and AB communities of each treatment plot were sampled. The sampling plan included one each of aspen clones 8L, 42E, 216, and 271 from each AA community; and, one aspen clone 216 and one birch from each AB community. Sample trees were randomly selected from locations within designated core regions of the plots where edge effects are mitigated and atmospheric mixing reduces variation in treatment concentrations of CO₂ and O₃. The sample trees

were measured for diameter at breast height (d.b.h.) and height, cut approximately 10 cm from the ground surface, immediately enclosed in plastic, and transported to the USDA Northern Research Station located in Rhinelander, WI. Any trees not immediately processed were held in cold storage at the facility. Trees were processed by teams divided into task subgroups. Initially total stem length and annual growth increments on the stem were identified. Branches were then counted, cut, and grouped by annual whorl. Each branch was then manually stripped of leaves, the total leaf area was electronically calculated, and leaf samples for chemical analyses were prepared and packaged. Maximum branch order was calculated for each branch, where order started at each branch tip (leaf) and increased by one each time two branch segments of equal order converged at a fork. Branches from the 2002, 2004, and 2006 whorls were then photographed, and all branches were cut into segments binned by branch order. The weights of the binned segments were recorded and samples for chemical analysis were prepared and packaged. Simultaneously, stem samples from approximately two feet below the base of the live portion of the crown of each tree were cut, sealed with beeswax, packaged in sealable plastic bags, and frozen. Stem samples were sealed at the ends with wax, frozen, and transported to our laboratory at North Carolina State University in Raleigh, NC.

Hydraulic Conductivity

Maximum (saturated) hydraulic conductance and embolism curves were computed for the aspen stem samples from the all-aspen community using methods described by Domec and Gartner (2001). The samples were thawed in sealed plastic bags and a chisel was used to cut approximately 1-cm square, 12-14 cm long, longitudinal sections from the outermost rings of the thawed sapwood. Generally 2-4 rings were included in the samples. The samples were then de-embolized in filtered deionized water under a vacuum. The de-embolized samples were fitted with perforated heat-shrink plastic sleeves and placed in a pressure chamber (Model 1000, PMS Instrument Co., Albany, OR, USA). One end of each sample was connected to a tube filled with ultra-filtered pH2 (2 micromolar HCl) deionized water containing an algaecide (Dynamax Inc., Houston, TX), maintained under a constant hydraulic head of 50-cm. The other end of the sample was attached to a pipette, used to monitor the amount of water passing through the sample. Hydraulic conductivity at full de-embolization (K_{sat}) was measured and then the samples were gradually re-embolized using increasing pressures of 5, 10, 20, 30, and 40 bar applied to the chamber.

Hydraulic conductivity was calculated from the sample dimensions and rate of volumetric water flux through the samples.

The K_{sat} data were Box-Cox transformed to attain normality and subjected to an analysis-of-variance using proc Mixed of SAS v. 9.2. Effects included in the model were replication (3), treatment (control, eCO₂, eO₃, and eCO₂ with eO₃) as a whole-plot factor, aspen genotype (8L, 42E, 216, 271) as a sub-plot factor, and stem d.b.h. as a covariate to account for tree size/dominance status.

Embolism curves are normally defined as the percentage loss of K with increasing tension (pressurization). These curves are typically analyzed by fitting a sigmoid equation (Pammenter and Willigen, 1998):

$$\% K_{\text{loss}} = 100 \cdot \{1 + \exp[\alpha \cdot (\Psi - \beta)]\}^{-1}$$

In this equation β identifies the pressure at which 50% loss of K occurs, α provides the slope at β , and Ψ is pressure (tension). Normally the middle of the sigmoid curve, β , corresponding to both the mean and the median (K_m), is used as a relative measure of cavitation susceptibility and the slope at this point on the

curve, α , is used as a measure of the dispersion of conductivity (Kd). Domec and Gartner (2001) took the derivative of the curve at β and took the zero-intercept as the air-entry point Ψ_e :

$$\Psi_e = 2/\alpha + \beta$$

Inspection of the sigmoid function, however, reveals that it is a form of the cumulative density function (CDF) of the two-parameter logistic distribution, a function of the mean (μ) and standard deviation (σ):

$$CDF_{\text{logistic}} = 1 \cdot \{1 + \exp[-(\Psi - \mu)/\sigma]\}^{-1}$$

Therefore, the descriptive statistics for these curves such as the mean and standard deviation are directly recoverable from the fitting parameters, where the mean, μ , is given by β and the standard deviation σ is given by $-\alpha^{-1}$. Thus, the air-entry point has been fixed by Domec and Gartner (2001) as two standard deviations from the mean.

Understanding the embolism sigmoid curve in this way elucidates a potential shortcoming. The logistic distribution is symmetrical, and therefore somewhat

limited in that it cannot accommodate skewed or asymmetrically distributed data without a transformation, such as might be encountered if some sampled xylem was formed under unusual conditions. Also, it has heavier tails than the normal distribution and so could lead to biased predictions if used to model normal data. A less commonly used alternative to fit embolism curves, however, is a form of the CDF of the Weibull distribution (e.g. Sperry et al., 1998) that is capable of incorporating skew and from which standard descriptive statistics such as the mean, median, variance, skew, and kurtosis are recoverable from the fitting parameters.

There was some skew in the embolism data from the current study. This may have contributed to issues with nonlinear-regression convergence for our curve fits using SAS v.9.2 proc Model. We therefore modeled un-transformed K values here, using a form of the Weibull CDF. Transformation of our curves to standard embolism curves is, however, trivial. We fitted the converse of the CDF (1-CDF) for a 2-parameter Weibull distribution to the untransformed hydraulic conductivities, equivalent to a rotation of the curve about the median loss of conductivity. Statistics for each K-loss curve including the mean, median, standard deviation, and skewness were then recovered from the fitting parameters.

The means, medians and standard deviations were then subjected to ANOVA using the model described above with d.b.h. as a covariate to discern whether there were significant treatment effects on Km pressure and whether there were significant treatment effects on the range over which embolism occurred.

Xylem Anatomy

To support interpretation of the hydraulic conductivity investigation and to compare results from early and later in the Aspen FACE experiment, stem xylem was also analyzed for vessel lumen area, a surrogate for diameter. Samples were prepared by manual microtoming the most recent two years of xylem and staining. Color images of the stained samples were then generated under microscope fitted with a digital camera. Images were obtained at 100× and 200× magnification. The images were loaded into the National Institute of Health (NIH) public domain software ImageJ for analysis. Scale conversion was selected based on magnification and the images were thresholded and adjusted as necessary to sharpen boundaries of anatomical elements. Lumen density (per 2.2192 mm² sapwood) and individual lumen areas were obtained by thresholding and the "analyze-particle" command with a lower area bound of 500 μm². The resulting data were imported into SAS v.9.2 for statistical analyses. Within SAS

the data were Box-Cox transformed for normality and means were calculated for individual xylem rings within each tree. The samples were then analyzed using SAS proc Mixed with block as a fixed replicate factor, to account for a known fertility gradient across the site, CO₂ and O₃ as cross main treatment effects, aspen genotype as a nested subplot factor, and individual stems identified in a repeated statement with an unstructured covariance matrix. Ring was initially included as a stripped subplot factor but removed due to lack of significance.

Results

Hydraulic Conductivity

The Weibull function described the embolism curves well, as indicated by the lack of any curvilinearity in the relationship between the measured and fitted values (**Figure 4.1, Figure 4.2**). The mean (se) saturated K was 25 (0.19) cm·min⁻¹ or 1.5 (0.17) kg·m⁻¹·MPa⁻¹·s⁻¹. As is typically observed, the rate of reduction in K with pressure was greatest near K_m, typically between embolizing pressures of 2 and 3 MPa (20 and 30 bar) (**Table 4.1**).

I detected no significant treatment effects for K_{sat} (all $p > 0.1000$), although there was a trend for a decrease in K_{sat} under eO₃ ($p = 0.1344$). The ANOVA results were equivalent when the covariate d.b.h. was excluded. No significant effects on the standard deviations of the embolism curves, a proxy for K_d.

Significant or marginally significant main effects and interactions were found for K_m using the mean and the median, but were superseded by the interaction eCO₂ × eO₃ × Genotype (p -mean = 0.0643, p -median = 0.0970) (**Table 4.2**). The interaction was caused by opposite responses to the treatments of clones 8L and 271 compared to 42E and 216, and by non-additivity of the eCO₂ and eO₃. Clones 42E and 216 both responded to all treatment combinations with increased resistance to embolism, requiring from 16% to 85% increased pressure to reach K_m, while clones 8L and 271 exhibited minor responses in embolizing pressure to reach K_m under eCO₂ and eCO₂ × eO₃ (-3.3% to +3.2%) but reductions under eO₃ (-43% to -48% for clone 8L and -8.9% to -14% for clone 271). The only significant factor-level separations were between clone 271 under Control versus clone 8L under eO₃, which had the highest and lowest pressures at K_m, respectively.

Xylem Anatomy

Sapwood xylem vessel mean (se) density was 82.7 (4.27) vessels mm⁻² and there were no significant effects or interactions for xylem vessel density. These parameters were generally negatively correlated (**Figure 4.3**). Estimating total conductivity as the product of lumen density and the mean lumen conductance calculated using Poiseuille–Hagen law, total conductivity was not conserved because the density of lumen was not proportional to the fourth power of the radii of the lumen. Xylem vessel element mean (se) area was 2173 (30) μm², corresponding to an approximate diameter of 52.6 μm, statistically indistinguishable from that reported by Kostianen et al. (2008) of 51.5 (9.3) μm. Two treatment effects were significant for xylem vessel element area, including CO₂ × Genotype (*P*=0.0229), and Genotype (*P*=0.0455) (**Table 4.3**). Across genotypes, eCO₂, eO₃, and eCO₂ × eO₃ were associated with respective decreases of 21%, 28%, and 13% in vessel element area, which illustrates the non-additivity of the eCO₂ and eO₃ effects. Clone 216 had the smallest average lumen area, 1374 (59.2) μm², and clone 271 had the largest average lumen area, 2959 (96.6) μm² (**Figure 4.4**). Clones 8L, 42E, and 271 exhibited decreases in lumen areas under eCO₂ of -46 %, -21 %, and -32 %, respectively, whereas clone 216 showed no change (-1 %).

Discussion

The mean (se) K_{sat} of 25 (0.19) cm/min or 1.5 (0.17) kg/m/MPa/s, is within the range of specific conductivity values reported for aspen and birch branch samples reported by Wang (2005) and stems of juvenile aspen whips reported by Gálvez and Tyree (2009), lending confidence to my sampling and analysis procedures .

My hypothesis regarding eCO_2 treatment effects on K_{sat} were not supported, as I detected no significant treatment effects for this parameter. These results are consistent with Kostianen et al. (2008), who found no significant effects on the anatomy of xylem under eCO_2 at Aspen FACE. However, we did see a decrease in lumen diameter for clones 8L, 42E, and 271 with no change in vessel density. Most stems grew larger in diameter under eCO_2 , however, so a larger total number of vessels should have been present.

There was a weak indication of decreased K under eO_3 that is consistent with the findings for aspen under eO_3 of decreased lumen diameter by Kostianen et al. (2008). In the present study I saw no significant effects of eO_3 , although clones 42E and 271 both exhibited average decreases in lumen diameter of about 46 %

under this treatment, respectively. The lack of significant treatment effects on K under combined $eCO_2 \times eO_3$ was consistent with Kostianen et al. (2008), and our hypotheses of offsetting effects under this treatment regime. We did not see a significant $eCO_2 \times eO_3$ effect on lumen area, but there was considerable variability in the data that could have obscured any such effect (**Table 4.3**).

The $eCO_2 \times eO_3 \times$ Genotype interaction was due to clones 42E and 216 exhibiting considerably higher (+36 % to +85 %) median resistance to embolism under all treatment combinations (greater embolizing pressure to attain K_m), while clones 8L and 271 generally exhibited losses (+0.7 % to -48 %) of resistance to embolism under eO_3 , but minor responses to other treatments (**Table 4.2**). The response to eCO_2 differed from that of Kostianen et al. (2008), who reported no significant effects of eCO_2 on xylem anatomy, and does not support our hypothesis of greater susceptibility to embolism due to faster growth under eCO_2 . This result appears consistent with our finding of decreased vessel lumen diameter under eCO_2 for all clones except 216.

The observed decrease in pressure to reach K_m under eO_3 for clones 8L and 271 also failed to support my hypothesis, but is consistent with both the results of

Kostiainen et al (2008), who found increased vessel lumen diameter for aspen under eO₃, and with the paradoxical increased growth exhibited by clone 8L under eO₃ (Kubiske et al., 2007). We found no significant changes in vessel lumen diameter associated with eO₃, but nonetheless there were sizable decreases for clones 42E and 271 of approximately 46 %.

The responses of Km to combined eCO₂ × eO₃ treatment were somewhat similar for clones 8L, 216, and 271, which were generally more similar to control under these conditions than under either treatment alone. Clone 42E, however, showed the greatest resistance to embolism under combined eCO₂ × eO₃ treatment.

Changes in vessel lumen diameter for clones 8L, 42E, and 216 were generally much less than observed for either treatment alone in the present study, although the opposite was true for clone 271.

Other studies have generally found that eCO₂ has greater effect on the hydraulic conductivity of xylem in ring porous species than diffuse porous species such as aspen. Although fewer studies have been done on the effects of eO₃ on xylem conductance are compared to eCO₂, there are indications from the literature that ozone could affect xylem anatomical features relevant to hydraulic conductance,

principally the diameter (area) of vessel lumen (Kaakinen, 2004; Kostianen, 2008). Our results indicate that generalization of the effects of atmospheric gases on aspen, and reasonably, other species as well, probably requires study of numerous genotypes before conclusions are drawn. We also found that lumen diameter (area) alone may not be an adequate predictor of xylem conductance in aspen, and lumen density did not appear significantly vary with treatment or genotype. Therefore, our results suggest that there may be other parameters that affect xylem K.

Conclusions

Responses to treatments appeared to be genotype-specific, and relationships between factors such as stem diameter growth and vessel or fiber lumen diameter are apparently inconsistent at Aspen FACE across aspen clones, or ontogenic stages, or both. Nonetheless, there were indications that physiological adjustments to changes in atmospheric chemistry could in some instances ameliorate and in other cases exacerbate projected wide-scale tree mortality or decreases in productivity due to xylem embolism under projected increases in drought stress. In the present study, significant changes in maximum xylem hydraulic conductance were not found, but there were indications that some

genotypes might have higher resistance to xylem embolism due to eCO₂, while others may become more prone to cavitation due to eO₃. Therefore, genotypic shifts in populations would be likely under increased drought stress under projected changes in atmospheric chemistry for the coming century.

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Table 4.1. AVOVA significance test results (p-values) for plot mean and plot median effects of eCO₂, eO₃, and aspen genotype (8L, 42E, 216, 271) on pressure at which mean or median (50%) K-loss occurred. Data are for samples of the outer 2 to 4 growth rings of stem samples collected beneath the base of the live crown of trees at aspen FACE harvested during the summer of 2007.

Effect	p-mean	p-median
Block	ns	ns
CO₂	0.1213	0.0718
O ₃	ns	ns
CO ₂ × O ₃	ns	ns
Genotype	0.0150	0.0987
CO ₂ × Genotype	ns	ns
O₃ × Genotype	0.0757	ns
CO₂ × O₃ × Genotype	0.0643	0.0970

Table 4.2. AVOVA of means (standard errors) [deviations] and percent change of treatment effects for eCO₂, eO₃, and aspen genotype (8L, 42E, 216, 271) on pressure at which 50%-loss (median) or average loss (mean) of K occurred. Data are for samples of the outer 2 to 4 growth rings of stem samples collected beneath the base of the live crown of trees at Aspen FACE harvested during the summer of 2007.

Treatment	Aspen genotype			
	8L	42E	216	271
Bars at mean K-loss				
Control	24.4 (1.90)	16.5 (2.77)	20.4 (1.90)	28.4 (1.50)
eCO ₂	23.6 (1.89) [-0.80] -3.3%	24.5 (1.50) [8.00] 49%	27.2 (1.89) [6.80] 33%	not available
eO ₃	13.8 (2.88) [-10.8] -43%	23.9 (2.78) [7.40] 45%	27.5 (2.78) [7.10] 35%	24.4 (2.88) [-4.00] -14%
eCO ₂ × eO ₃	23.3 (1.94) [-1.10] -4.5%	28.3 (2.77) [11.8] 72%	23.6 (2.88) [3.20] 16%	29.3 (2.77) [0.90] 3.2%
Bars at median K-loss				
Control	24.0 (3.13)	17.3 (4.63)	14.8 (3.13)	28.2 (2.44)
eCO ₂	20.1 (3.11) [-3.90] -16%	24.8 (2.44) [7.50] 43%	27.4 (3.11) [12.6] 85%	not available
eO ₃	12.5 (4.63) [-11.5] -48%	23.5 (4.47) [6.20] 36%	27.2 (4.47) [12.4] 84%	25.7 (4.63) [-2.50] -8.9%
eCO ₂ × eO ₃	22.8 (3.13) [-1.20] -5%	28.3 (4.58) [11.0] 64%	24.7 (4.63) [9.90] 67%	28.4 (4.58) [0.20] 0.7%

Table 4.3 AVOVA significance test results (*P*-values), Control treatments means (standard errors), and deviations from Control for plot mean and plot median effects of eCO₂, eO₃, and aspen genotype (8L, 42E, 216, 271) on sapwood xylem lumen area (μm²).

Effect	<i>P</i> -value	
Rep	ns	
CO ₂	ns	
O ₃	ns	
CO ₂ × O ₃	0.1011	
Genotype	0.0455	
CO ₂ × Genotype	0.0229	
O ₃ × Genotype	0.1726	
CO ₂ × O ₃ × Genotype	ns	

Treatment	All		8L		42E		216		271	
Control	2173	(30.0)	2496	(48.8)	1981	(154)	1374	(59.2)	2959	(96.6)
eCO ₂	-464	-21%	-1158	-46%	-411	-21%	-10	-0.75%	-950	-32%
eO ₃	-611	-28%	362	+15%	-914	-46%	861	+63%	-1397	-47%
eCO ₂ × eO ₃	-286	-13%	185	+7.4%	-3	-0.14%	-72	-5.2%	-1923	-65%

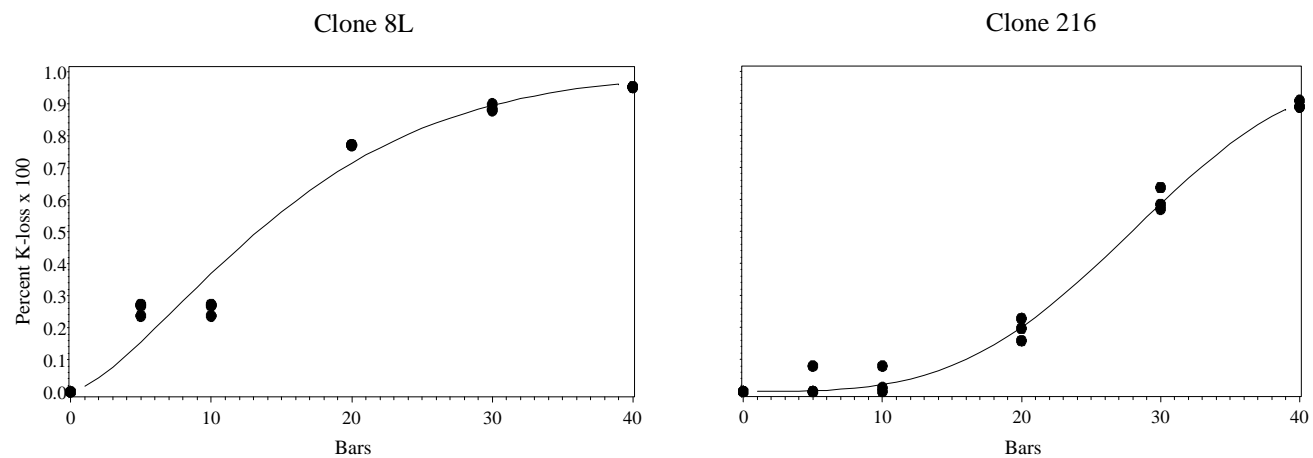


Figure 4.1. Fitted versus measured values of stem xylem hydraulic conductivity (centimeters per minute). Up to three samples were used to populate each curve with data. Fits were made using the converse of the cumulative density function for the 2-parameter Weibull distribution via SAS version 9.2 proc Model. Raw hydraulic conductivity and the Weibull cdf were fitted rather than percent-loss of conductivity and the standard curvilinear function. Use of a cdf-related function to fit the data allowed for direct recovery of the statistical parameters of the data distributions, and the ability of the Weibull distribution to accommodate distributional skewness resulted in much better fitting of the data. Example embolism curves (Percent loss of conductivity versus bars of embolizing pressure applied) are shown for aspen clone 8L under eO_3 and clone 216 under combined $eCO_2 \times eO_3$. Dots indicate measured values and curves indicate fitted values. Here, clone 8L embolizes more readily than clone 216.

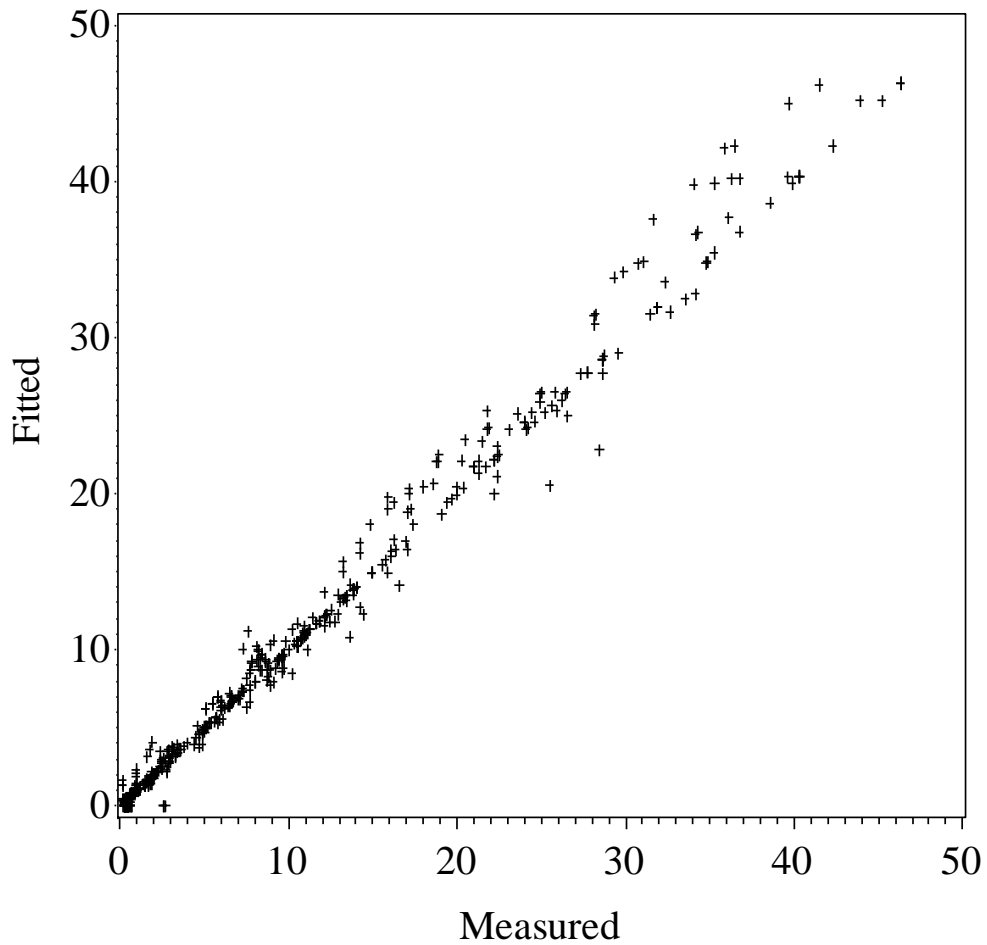


Figure 4.2. Fitted versus measured values of stem xylem hydraulic conductivity (centimeters per minute). Fits were made using the converse of the cumulative density function for the 2-parameter Weibull distribution via SAS version 9.2 proc Model. Raw hydraulic conductivity and the Weibull cdf were fitted rather than percent-loss of conductivity and the standard curvilinear function. Use of a cdf-related function to fit the data allowed for direct recovery of the statistical parameters of the data distributions, and the ability of the Weibull distribution to accommodate distributional skewness resulted in much better fitting of the data.

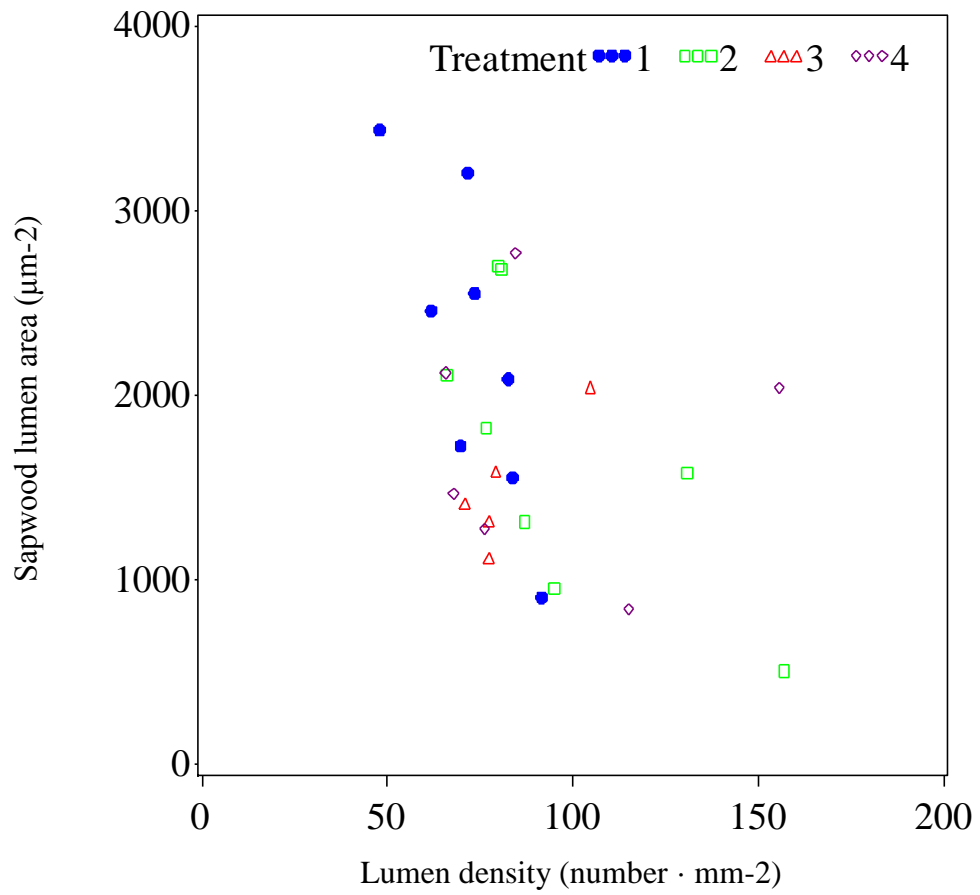
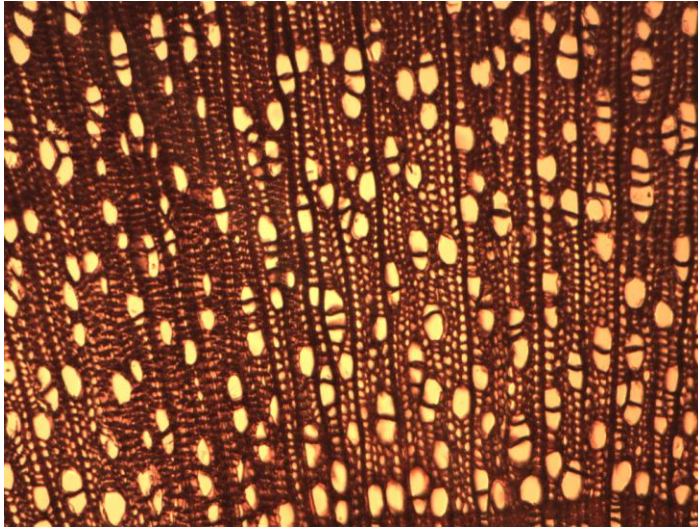


Figure 4.3. Relationship between sapwood xylem lumen area and lumen density. Data shown are individual stem means.

Clone 216



Clone 271

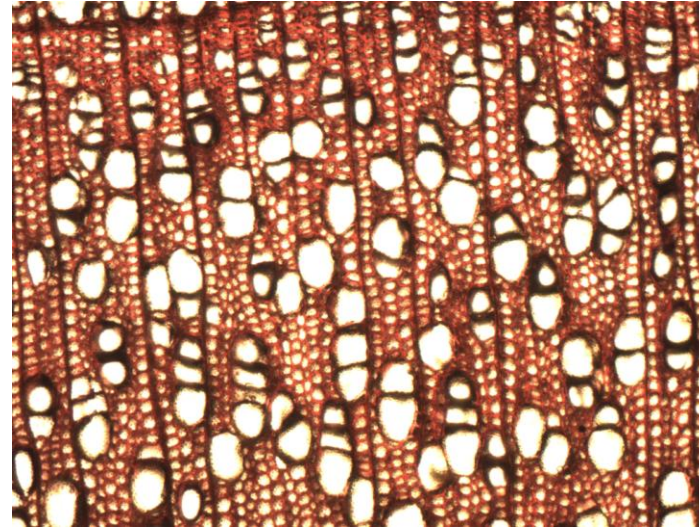


Figure 4.4. Stained sections (originally 100 \times , rescaled to 17% here) of aspen sapwood xylem from Aspen-FACE. ANOVA indicated that there was a significant genotype effect on lumen area. Left: clone 216 (average lumen area 1,374 μm^2). Right: clone 271 (average lumen area 2,959 μm^2). Both clones shown were grown under Control treatment.

CHAPTER 5

**Effects of elevated CO₂ and tropospheric O₃ on transpiration and soil water
content within stands of trembling aspen and paper birch.**

Abstract

Availability of water for human use and natural ecosystems during the coming decades is a significant concern due to the rapidly rising mean global temperature and expected shifts in the frequency and intensity of precipitation. Atmospheric concentrations of CO₂ and O₃ have been rapidly increasing since the Industrial Revolution, and resultant changes in forest tree physiology and growth could affect plant water balance and evapotranspiration, and therefore net water drainage to replenish aquifers and maintain stream base flows. We conducted a study at the Aspen free-air CO₂ and O₃ enrichment experiment (Aspen-FACE) to understand the effects of changes in atmospheric concentrations of these gases on the hydrologic budget. We collected sap flux data during the 2006 and 2007 growing years, when detailed micrometeorological and soil water content data were also available. We also evaluated understory ET and modeled soil water drainage below the root zone. We expected that sap flux should have been similar in the control, eCO₂, and eCO₂ × eO₃ plots with closed, ostensibly light-limited canopies similar to other studies undertaken in closed canopy situations. We also expected that sap flux should be discernibly different in the eO₃ plots where the canopies are less developed than the other treatments, resulting in lower canopy ET but potentially higher understory ET. We hypothesized that the greatest

differences in transpiration should be in the eO₃ canopy and understory, rather than in the eCO₂ treatments where light limitation due to canopy closure had set in by the time of this study. Furthermore, we hypothesized that potential for increased transpiration in the eO₃ canopy (due to loss of stomatal control) would be offset by increased competition for water from a larger understory biomass, also suffering from loss of stomatal control, as well as increased evaporation from the soil due to increased solar heating. We found that canopy sap flow, understory ET, soil water content, and deep soil drainage were affected by eCO₂ and eO₃ similarly in all-aspen and aspen-birch communities when summed over a growing season, and that the effects of eO₃ were usually greater than eCO₂. Averaged over communities and two growing seasons, eCO₂, eO₃, and eCO₂ with eO₃ had modest effects on sap flow (+1.3%, -11 %, and -12 %, respectively) and soil water content (-7.2 %, -3.3 % , and -5.7 %, respectively); but considerable effects on deep soil drainage (+52 %, +77 %, and +109 %, respectively [concentrated in 2007]), although the volumes of soil drainage were all small and relatively similar in comparison to volumes of annual precipitation and sap flow. Understory ET mitigated the effects of eCO₂ and eO₃ on the canopy, which was consistent with our understanding of canopy leaf area dynamics. There was more light reaching the understory under eO₃ (Kostiainen, 2008) providing for greater soil evaporation and herbaceous transpiration. Understory ET under eO₃ was

probably not only increased by the availability of additional light, but by higher understory g_s there as well caused by loss of stomatal control. Results of this study indicate that changes in atmospheric chemistry forecast for the next century could alter the forest hydrologic budget and deep soil drainage to groundwater at middle latitudes. Changes to soil water content and drainage due to alterations in canopy water use under eCO_2 and eO_3 were mitigated by compensatory responses of the understory. These effects would probably vary during stand development and succession, however, because the understory should be influenced by competitive feedbacks due to increased rates of canopy development under eCO_2 and decreased rates of development under eO_3 . Nonetheless, after nine to ten years of stand development soil water drainage below the root zone was significantly increased by the treatments.

Introduction

Groundwater and surface water resources are significantly influenced by forest evapotranspiration (ET), which in turn could be affected by changes in forest tree physiology and growth in response to increasing concentrations of atmospheric CO₂ and tropospheric O₃ (Dingman, 1994; Finlayson-Pitts and Pitts, 1997, 2000; Fowler et al., 1999; IPCC, 2001). Elevated atmospheric CO₂ has been shown to increase plant growth, leaf area index and water use efficiency, while increases in tropospheric O₃ have been shown to decrease plant growth, leaf area display, and stomatal control (Karnosky et al., 2003; Karnosky, 2005; McLaughlin et al., 2007a; Wittig et al., 2007) as well as to directly affect plant water balance (Panek, 2004; McLaughlin et al., 2007a, b). Studies of the effects on tree water use of eCO₂ alone or in conjunction with eO₃ have been undertaken at several free-air CO₂- (O₃-) enrichment (FACE) experiments, including: Duke-FACE located in Durham, NC, USA; a forest stand in northwest Switzerland near the town of Basel; ORNL-FACE of Oak Ridge, TN, USA; Pop-FACE in central Italy; and, Aspen-FACE near Rhinelander, WI, USA (Schafer et al., 2002; Cech et al., 2003; Gielen et al., 2001; Bernacchi et al., 2003; Wullschleger and Norby, 2001; Gunderson et al., 2002; Wullschleger et al., 2002; Uddling et al., 2008; Rhea et al. 2010). Duke-FACE, the Swiss experiment, and ORNL-FACE were initiated in

closed canopy stands of loblolly pine, mixed deciduous forest, and sweetgum, respectively. Pop-FACE, similar to Aspen-FACE, was initiated as planted poplar cuttings on former agricultural land.

Sap flux (J_s) and stomatal conductance (G_s) have commonly been used to characterize forest ET due to their relative reliability, ease of measurement, and low cost in comparison to other methods of characterizing ET, such as satellite imagery, soil water content probes, or atmospheric eddy-flux covariance towers, or because other methods are inappropriate for site conditions (as is the case for eddy-flux covariance at Aspen-FACE, for example). Sap flux in the Duke-FACE and Swiss experiments was relatively unaffected by eCO_2 (Schafer et al., 2002; Cech et al., 2003) and data on leaf area and leaf stomatal conductance (Gielen et al., 2001; Bernacchi et al., 2003) are consistent with a lack of significant effect on sap flux at Pop-FACE as well (Uddling et al., 2008). At ORNL-FACE, stand-level sap flux was reduced 13% and correlated to reductions in leaf stomatal conductance (Wullschleger and Norby, 2001; Gunderson et al., 2002; Wullschleger et al., 2002). After canopy closure occurred at Aspen-FACE (Kostiainen, 2008), Uddling et al. (2008) studied the effects of eCO_2 , eO_3 , and their combined effects on sap flux and surface soil volumetric water content (θ_v).

They found effects due to community and eO_3 on maximum sap flux rates, and in contrast to other studies, an 18% increase in sap flux under eCO_2 averaged across years, but with considerable inter-annual variation, possibly due to contrasting wet and dry years included in their study. The effects of eCO_2 on water use were correlated to increased tree size and leaf area (+40%) associated with eCO_2 , but there was decreased tree size and leaf area (-22%) associated with eO_3 that did not correlate with a change in sap flux (Uddling et al. 2008). They also found that soil water content was relatively unchanged under eCO_2 but lower under eO_3 , and attributed these results to increased litter buildup under eCO_2 and better atmospheric coupling or enhanced understory ET under eO_3 .

Additional effects of eCO_2 and eO_3 on stand-level water use might be discernable at Aspen FACE than have been found to date. In their analysis, Uddling et al. (2008) had reasonable justification for the assumption of no genotypic effects across aspen clones and that nocturnal sap flux was negligible. They also conservatively included data only when a stable, zero pre-dawn sap flux was believed to exist. Also, because data capture in the all-aspen and mixed aspen-birch stands in 2004 was 76% and 63%, respectively, and in 2005 it was 62% and 49%, considerable gap filling was done. Gap filling was performed using a linear

function based on vapor pressure deficit (VPD) and photosynthetically active radiation (PAR). Current evidence suggests that nocturnal sap flux is often significant (Oishi et al., 2008), and that transpiration of trembling aspen growing in the southern boreal forest is related non-linearly to VPD and PAR, as well as air temperature and soil water content (Hogg et al., 1997; Hogg and Hurdle, 1997; Ewers et al., 2002). Genotypic differences in the hydraulic conductivity of the stem xylem of the aspen clones at Aspen FACE is also likely (Kostiainen et al., 2008) and genotypic shifts in growth under the various treatments at the site have been documented (McDonald et al. 2002; Kubiske et al., 2007).

My expectations for post-canopy closure sap flux at Aspen-FACE differ somewhat from the findings of Uddling et al. (2008). Based on the allometric study by King et al. (2005), with the potential exception of the eO₃ treatment plots where lower LAI persists (Uddling, 2008), we expected that sap flux should have been similar in the control, eCO₂, and eCO₂ × eO₃ plots with closed, ostensibly light-limited canopies similar to other studies undertaken in closed canopy stands (Schäfer et al., 2002, Cech et al., 2003). We also expected that sap flux should be discernibly different in the eO₃ plots where the canopies were less developed than the other treatments, resulting in lower canopy ET as seen by others (Nunn et al.,

2006 and Warren et al., 2006) and facilitating greater understory ET (Bandeff et al., 2006). As indicated by Uddling et al. (2008), increased understory ET might compete for water with increased canopy transpiration due to better atmospheric coupling. To understand patterns of water use for Aspen FACE at a later stage of canopy development, we collected sap flux data during the 2006 and 2007 growing years, when detailed micrometeorological data were also available to aid interpretation. We hypothesized that the greatest differences in transpiration should be in the eO₃ canopy and understory, rather than in the eCO₂ treatments where light limitation should have set in with canopy closure. Furthermore, we hypothesized that any increased potential for transpiration in the eO₃ canopy due to loss of stomatal control would be offset by increased competition for water from a larger understory biomass (Bandeff et al., 2003), also suffering from loss of stomatal control, as well as increased evaporation from the soil due to increased solar heating. In light of these expectations and in light of the lower canopy LAI in the eO₃ plots (Uddling, 2008), we also expected that the differences in tree sap flux between the eO₃ and other treatments would be greater than the differences in soil water content, due to differential water use by the understories.

Methods

Aspen FACE was initiated in 1997 to study the effects of eCO₂, eO₃, and their interaction on north-temperate forest ecosystems. Dickson et al. (2000) provide a generalized soil profile description for the Aspen-FACE site that indicates the presence of sandy soils, which typically have low volumetric moisture content θ_v (**Figure 5.2**). A 15-cm thick sandy loam topsoil grades into a clay loam approximately 30-cm thick (at 15-45-cm depth), in turn grading into a sandy loam, stratified sand, and gravel substratum at about 100-cm deep. Sporadic clay lenses occur at depths of 30- to 60-cm, and the water table is at least 10-m deep. On these soils, twelve 30-m diameter treatment plots were grouped into three replicates (designated 1 through 3) of four treatments (1 = Control, 2 = eCO₂, 3 = eO₃, 4 = eCO₂ with eO₃) and identified by replicate and plot number (e.g. ring 1-2 is replicate 1, treatment 2). Each plot was subdivided into three subplots consisting of communities of mixed trembling aspen (*Populus tremuloides* Michx.) and sugar maple (*Acer saccharum* Marshall) (AM), mixed aspen and paper birch (*Betula papyrifera* Marshall) (AB), and all-aspen (AA), planted in alternating pattern on one-meter centers. The pure aspen community was planted with 5 clones of varying sensitivity to eCO₂ and eO₃, planted as randomized pairs. Treatments were applied during daylight hours during the growing season in the

manner of the Brookhaven National Laboratory's design (Hendrey et al., 1999), including: (1) ambient conditions; (2) eCO₂ (~560 ppm); (3) eO₃ (~60 ppb, or 1.5 times ambient); (4) and both eCO₂ and eO₃. Additional documentation is available at www.aspenface.mtu.edu. Measurements for the current study were collected during the growing seasons (roughly May-October), of 2006, 2007, and except for sap flux, 2008. Meteorological conditions (**Figure 5.3**) and projected evapotranspiration (**Figure 5.4**) were relatively consistent during these periods. Data from the AM subplots was not included in the present study because the site was not adequately instrumented there to obtain experimental-level data of sufficient quality.

Micrometeorological Measurements

A 20-m tall central micro-meteorological station was established at the site at the beginning of the Aspen-FACE experiment that collected data continuously for the duration of the study. Data collected at the tower included photosynthetically active radiation (PAR), net radiation (NR), relative humidity (RH), leaf wetness (L_w), precipitation (P), pan evaporation (E), air temperature (T_a), soil temperature (T_s), soil water content (M_s), wind speed (W_s), wind direction (W_d), and barometric pressure (B). Atmospheric profiles for W_s, W_d, T_a, and RH were

monitored at elevations of 2, 5, 10, 15, and 20 meters, a subsurface profile for M_s was monitored at depths of 0, 5, 10, 20, 50, and 100 cm, and a subsurface profile for T_s was monitored at depths of 0-30, 30-60, and 100-130 cm. Additional above-canopy atmospheric monitoring was performed above the center of rings 1-2, 2-1, 3-3, and 3-4 for T_a , P, RH, PAR, NR, W_s , and W_d . Within each subplot of each ring, PAR, RH, and T_a were monitored at 2 m elevation; T_s is monitored at depths of 0, 5, 10, 20, 50, and 100 cm, and M_s was monitored at depths of 0-20 cm, 40-60 cm, and 80-100 cm. There were three locations in subplots AA and AM where M_s profiles were monitored. Campbell CS500 probes (Campbell Scientific, Inc., Logan, UT) were used to monitor T_a and RH; T_s was measured with copper/constantan thermocouples referenced to a Campbell T107 temperature probe (Fenwall Electronics, Milford, MA); W_s and W_d were monitored with the Young 03001-5 wind sentry (R.M. Young Co., Traverse City, MI); PAR was monitored with LI-1905B quantum sensors (LI-COR, Inc., Lincoln, NE); NR was monitored with Q7.1 net radiometers (Radiation and Energy Balance Co., Bellevue, WA); P was monitored with TE525 electronic rain gauges (Texas Electronics, Inc., Dallas, TX); and T_s with Cambell CS615 reflectometer probes (tower) or Decagon Echo-2 soil water content probes (Decagon Devices Inc, Pullman, WA) (rings). All data were recorded on 30-

minute intervals with the exception of soil water content, which was recorded on 2-hour intervals. Additional details are described by Dickson et al. (2000).

The Decagon soil probes were installed during the summer of 2005, vertically in soil core holes (10 cm diameter \times 100 cm depth) that were back-filled with horizon/depth-specific native soil collected at the site, and re-packed to approximate native soil bulk density. We used 20 cm long probes installed over the depth intervals 0-20 cm, 40-60 cm, and 80-100 cm. Within each all-aspen and aspen-birch community of each treatment plot, three probes were placed in each of the three soil layers. Within the aspen-maple communities, one probe was placed in each soil layer. Soil probes were allowed to equilibrate with the soil environment during repeated wetting/drying cycles from August 2005 until May 2006, before beginning to collect data for the current study.

To develop a site-specific calibration for the soil probes, a soil 1-m deep pit was excavated near the center of the experimental site. A coarse sand and gravel layer was encountered at 100-cm depth. A grid of 13 moisture probes was laterally inserted in the south wall of the test pit using the manufacturer's pilot tool. Four probes were placed horizontally in a sandy loam at 15-cm depth. Four more

probes were placed in silty soils at a depth of 40-cm. A series of four probes was installed between 60- to 70-cm depths in clayey silty sand. One probe was installed at 110-cm depth in sand, overlaying a sand and gravel hardpan. The probes were then connected to a data logger and the pit was back-filled with the excavated material by depth-horizon specific soil layers. The entire area was then flooded for seven hours with a water tank to saturate the soil, and covered to decrease ET. The soil water content decay curves during the drying cycle were used to evaluate the variation in probe response within similar soils and to estimate soil field capacity (θ_{fc}). After 24 hours the curves were relatively flat. Volumetric moisture content (θ_v) for the deepest probe, installed in the lower sand, had exponentially decayed to a surprisingly low θ_{fc} of 6%. Probes from the overlying silty, clayey, loams had very high θ_{fc} , $27\pm 3\%$. Probes in the surficial topsoil also had a fairly high θ_{fc} of $23\pm 1\%$. Interestingly, the probes from the near-surface sandy loams showed a strong logarithmic decay after saturation, reaching a 24-hour θ_{fc} of $12\pm 2\%$.

Soil probe measurements of θ_v at ambient soil water content against laboratory gravimetric measurements were also obtained as the difference between fresh and desiccated soils (θ_g) (**Figure 5.5**). Soils from a depth of about 20-cm had

laboratory θ_g of $11\pm 1\%$ while probe θ_v readings ranged from 6% to 9%. Soils from a depth of 40-cm had ambient θ_g of $11\pm 1\%$ but probe θ_v measurements ranged from -4% to 2%. For soils from 60 cm depth laboratory measurements of θ_v were $11.5\pm 1.5\%$ while probe responses varied from 9% to 22%. In both sets of readings a sharp drop in soil water content occurred between 60-cm and 80-cm depth, and the only probe reading below 80 cm depth was -6% θ_v at 110 cm depth. Available laboratory θ_g included readings at 85-cm ($\theta_g 3\pm 0.5\%$), 105-cm depth ($\theta_g 3.5\pm 2.5\%$), and 130 cm depth ($\theta_g 7\pm 0.75\%$).

Soil Lysimeters

Seven soil lysimeters (McJannet et al. 2000) were located in each of the twelve treatment rings, including three in the all-aspen community, three in the aspen-birch community, and one in the aspen-maple community. The cylindrical soil lysimeters were ten centimeters in diameter and ten centimeters deep. The walls were fabricated from clear acrylic plastic and the bases were closed with fine-mesh ($16 \text{ squares cm}^{-1}$) stainless steel screen to minimize impedance to water drainage. Native topsoil was used to fill the lysimeters. The surfaces of the soil in the lysimeters were open to the ground and were frequently invaded by understory weed and grass roots, and any top growth was carefully removed at the

time of weighing. Surface soil evaporation was monitored by determining changes in lysimeter mass through time. Readings were generally taken following storm events when other hydrologic measurements were also being collected.

Canopy Sap Flow

Sap flux instrumentation at Aspen-FACE is described in detail by Uddling et al. (2008). Granier-type (Granier, 1987) sensors were custom-fabricated for the site and installed in 252 trees during late April 2004 below the base of the live crown, prior to leaf-out. Nine sensors were placed in the all-aspen communities of each treatment ring and 12 sensors were placed in the mixed aspen-birch communities of each ring. Sensors in the all-aspen communities were relatively evenly distributed among the viable clones 8L, 42E, 216, and 271 (clone 259 was severely suppressed by this time of the experiment), and three of the twelve sensors in each mixed aspen-birch community were placed in aspen (clone 216). Stems were selected to represent the range of size classes in each ring. One sensor in each subplot was left unheated to use for correction as necessitated by extraneous thermal effects such as insolation and differences in early morning air and sap temperatures (Perämäki et al., 2001; Lu et al., 2004). The sensors were

left in place continuously until the end of the experiment in 2009, and annual inspections, and data QA/QC, revealed no problematic wound responses.

Azimuthal patterns of sap flux were evaluated by others in 2003 and found to be insignificant.

Calibration of the heated to the unheated sensors was attempted by turning off all heating for several days and recording the relative responses to scale the responses between sensors (Uddling, 2008). Relationships between the sensors appeared to vary in quality and form, however, so a different approach was used. The responses of individual heated sensors were each modeled in SAS v.9.2 proc Model. Every heated sensor was individually modeled on a monthly basis to accommodate any drift in sensor responses over time. The model included terms for an intercept (the base voltage), the unheated component, and components for response to VPD, potential PAR, and soil water content. Actual PAR and net radiation were originally included in the model but discarded due to lack of predictive power; the enhanced response of trees to diffuse PAR, which was a greater fraction of total PAR during cloudy conditions, appeared to offset the commensurate decrease in direct PAR. Lagging of variables was also tried, but found to be unnecessary to obtain adequate fits to evaluate the unheated signal

component. Potential PAR was modeled using a parameterization of the non-rectangular hyperbola (Ridout, 1994) because other functions, such as the rectangular hyperbola, were too gradual in the transition to saturation. VPD was also modeled using this function because visual inspection of the data indicated that decreases in sap flux with very high VPD were not present in the data set, and therefore did not need to be accounted for in the model. Volumetric soil water content (θ_v) was modeled using the CDF of the 2-parameter Weibull distribution so that the tendency for sap flux to only be affected by relatively low θ_v could be emulated by the model (**Figure 5.2**). The critical parameter, the unheated signal to be removed, was modeled as a linear combination of all unheated signals within a given treatment. The model output was used to correct the individual heated sap flux signals by subtracting the unheated component predicted by the model, which seldom exceeded an amplitude increase for the composite unheated signal of about 25 % (e.g. **Figure 5.6**). The problem of accurate baseline determination (Oishi et al., 2008) was addressed by using the model results. The sensor time series corrected with unheated signal component were scanned for days with reasonably low (0-0.5 kPa) pre-dawn VPD. The model was then used to predict the baseline at that time by substituting zero for VPD. The baseline was then extended between these points using linear interpolation, providing values of maximum signal response (ΔmV_{\max}) to be used in the sap flux calculations.

Linear sap flux rates (meters per second) were calculated using the equation described in Granier (1987), where millivoltage between the top and bottom Granier sensor probes was substituted for temperature changes (James et al., 2002):

$$\text{Sap flux density (m} \cdot \text{s}^{-1}\text{)} = 0.000119 \cdot [(\Delta mV_{\text{max}} - \Delta mV) / \Delta mV]^{1.231}$$

Sap flux was converted to tree-specific volumetric flow rates (cubic meters per second), also known as sap flux density (J_s), by multiplying the linear sap flux rate by the stem cross-sectional area at instrument height (the entire stem of the relatively young, diffuse-porous aspen and birch was assumed to be active sapwood). Conservation of sap volume and constant xylem hydraulic conductance was assumed along the axis of the stem below the crown, such that the linear sap flux rate would scale with the taper of stem cross-sectional area to maintain a constant volumetric flow along the stem axis. Based on this assumption, J_s in the non-instrumented trees were calculated using SAS v.9.2 proc Mixed by regressing the volumetric sap flows from instrumented trees against cross-sectional stem area at breast height (ABH) for all stems in the experiment. The regression equations included partitioning by main effects and interactions of

replication, treatment, community, and genotype. Data were then aggregated to the stand level by these effects on a daily basis, and re-aggregated on a monthly basis while excluding missing values.

Soil Water Drainage

Soil water drainage was incorporated into the modeling of the heated and unheated signal responses from the canopy sap flux sensors. The site-specific soil matric potential – water content curve (Dickson et al., 2000) was used to develop a soil diffusivity – water content curve for the model. The curve in the model was used to relate soil water content to ease of water extraction by evaporation and transpiration, and to the soil water gravitational drainage rate. The model was allowed to adjust the diffusivity – water content curve independently for shallow (0-30 cm), intermediate (30-70 cm), and deep (70-100 cm) soil horizons at each sensor location. Curve adjustment was made to optimize the volumetric balance of water between ET and θ_v , where ET was modeled as a function of canopy Js. ET was estimated by partitioning canopy Js between soil layers based on soil water content and diffusivity, and a model-optimized constant to represent evaporation from the soil surface and transpiration by the understory.

Gravitational soil drainage from each soil layer was then calculated using the adjusted curves and soil water content data.

Hydraulic lift by roots was not explicitly included in the model. Soil water content was only allowed to increase in the shallow soil as a lagged function of precipitation, with a percentage up to a maximum amount, each optimized by the model, removed to represent interception. Soil water content in the intermediate and deep layers was only increased by the calculated drainage from the immediately overlying layer.

Understory Evapotranspiration and Stomatal Conductance

Aspen understory ET and g_s measurements were performed during late July and early August of 2008. Measurements were made between two hours before and after solar noon, during fair weather. A minimum of two days was allowed to elapse prior to conducting measurements after any precipitation event. Replicates and treatments within replicates were sampled in random order. If all treatment rings were not sampled on the same day within a replicate, then all of the data for the replicate were discarded. Each replicate was sampled twice.

Stomatal conductance was characterized for the dominant understory species, goldenrod and dandelion (Awmack et al., 2007). Composite samples for each species were collected from the northern and southern ends of each AA community understory. Each composite sample was comprised of the 20% trimmed-mean of a minimum of 20 individual leaf measurements. Understory ET was characterized using a 0.5-meter wide, 2-meters long, and 1-meter high static chamber. The chamber was constructed using 0.75-in. dia. PVC pipe and a plastic film) resistant to condensation formation and transparent to PAR. A 0.3-meter wide plastic skirt was attached around the perimeter of its base and weighted to seal the chamber. Two fans and an air pump were placed inside the chamber. The air pump was connected to a LiCor 840 CO₂/H₂O detector (manufacturer location). Fluxes to the atmosphere were calculated as the slope of a plot of concentration versus time, and averaged by individual community (Figure 5.7).

Data Analysis

Data were reduced to subplot means and analyzed by ANOVA using Proc Mixed of SAS v. 9.2. Data were transformed as necessary prior to statistical analysis

using a Box-Cox transformation. The ANOVA model was based on a statistical model for the site developed by King et al. (2001). Replication was treated as a fixed effect because of a known fertility gradient across the site. The treatments CO₂ and O₃ were treated as main-plot factors, and community and genotype nested within community were treated as subplot factors. When data were modeled across time or elevation or depth a random coefficients model was used instead of a repeated measures analysis.

Results

We found that canopy sap flow, understory ET, soil water content, and deep soil drainage were affected by eCO₂ and eO₃ similarly in all-aspen and aspen-birch communities when summed over a growing season, and that the effects of eO₃ were usually greater than eCO₂. Averaged over communities and two growing seasons, eCO₂, eO₃, and eCO₂ with eO₃ had modest effects on sap flux (+1.3%, -11 %, and -12 %, respectively); had small effects on soil water content (-7.2 %, -3.3 % , and -5.7 %, respectively); but considerable effects on deep soil drainage (+52 %, +77 %, and +109 %, respectively [concentrated in 2007]). Considerable variation was present between responses during individual months, however.

Soil Water Content

The only ANOVA effect that was significant for surficial soil water content, other than Block in June 2006 ($P=0.0062$) and Community in September 2006 ($P=0.0649$), was the interaction $O_3 \times$ Community in June 2006 ($P=0.0444$), June-August 2007 ($P=0.0410$ to 0.0684), and June-September 2008 ($P=0.0201$ to 0.0739) (**Table 5.1**). Surficial soil water under eO_3 increased 111 % in the AA community and 78.2 % in the AB community during June 2006. During June through August 2007 surficial soil water increased under eO_3 78.0 % to 123 % in the AA community but only 7.04 % to 29.4 % during the same period in the AB community. During May through October 2008 surficial soil water increased under eO_3 40 % to 64 % in the AA community and 6.83 % to 21.3 % in the AB community.

Subsurface soil water content was differentiated between the lower soil layer and the upper layers (**Table 5.2, Figure 5.8**). Soil water content in the shallow sandy loam layer (0-30 cm) was often the highest and showed a high degree of responsiveness to precipitation. The silty to clayey loam middle layer (30-70 cm) also showed responsiveness to precipitation events but was somewhat subdued in comparison to the shallow layer. The sandy deep layer (70-100 cm) had a much

lower water content throughout the growing season and was unresponsive to precipitation events. Summed over the top meter of soil, soil water content was slightly higher under eCO₂ and frequently lower under eO₃, while being similar to Control under combined eCO₂ and eO₃.

During 2006 soil water exhibited no responses to treatments that were discernable by ANOVA, although there was a persistent effect of soil layer ($p < 0.0001$ to $p = 0.0030$) caused by a large decrease in θ_v in the deep layer relative to the shallow and intermediate layers (**Table 5.2**). More treatment effects were evident in 2007, when in addition to continued soil layer effects of the same nature as previously described, a persistent community effect was detected and interactions between CO₂, O₃, Community, and Layer occurred. A fourth order interaction between CO₂, O₃, Community, and Layer occurred during May 2007 ($p = 0.0945$) that superceded an interaction between O₃ and Layer ($p = 0.0836$) and a main effect for Layer ($p < 0.0001$). There was a CO₂ by layer interaction during June 2007 due to a large drop in the soil water content of the middle layer. There was also a persistent Community effect during the growing season ($p = 0.0002$ to $p = 0.0139$) for which the all aspen communities maintained the lowest soil water contents and the aspen-maple communities maintained the highest soil water contents. During the 2008 growing season, a slightly drier year, more significant

or nearly significant treatment effects were discerned. The soil layer and community effects continued in a similar fashion, but there was a significant interaction between CO₂ and soil layer (p=0.0705) caused by a sharp decrease in soil water content within the middle soil layer from the underlying layer. Interactions of O₃ with layer occurred during May and September when the deep soil water content was quite low relative to the overlying layers. An ozone by community by layer interaction also occurred in June.

Canopy Sap Flow

Canopy sap flow (Js) magnitudes were low in 2006 (**Figure 5.9**), consistent with the previous study by Uddling et al. (2008). Based on a later gravimetric study, Uddling et al. (2009) estimated the sap flows reported in Uddling et al. (2008) to be about 2.5 times too low. Sensors were serviced or replaced between the 2006 and 2007 growing seasons, and in spite of similar meteorological conditions sap flow was higher in 2007 (**Figure 5.9**). Sap flow in the AA community was relatively stable during the 2006 growing season until tailing off in August and September, and sapflow during 2007 in the AA community and both 2006 and 2007 in the AB community gradually increased to a peak in midsummer and gradually decreased thereafter. These trends appeared to be present for individual

genotypes (**Figure 5.10**) as well as stand-aggregated responses (**Figure 5.9**).

Defining the active growing season here as spanning the last ten days of May through the first three weeks of September, sap flow in 2006 under the treatments was highest under Control (AA = 200 mm, AB = 181 mm), with general decreases under eCO₂ (AA = 154 mm, AB = 181 mm) eO₃ (AA = 153 mm, AB = 142 mm), and combined eCO₂ and eO₃ (AA=164 mm, AB = 142 mm). Sap flow in 2007 was fairly similar for Control (AA = 384 mm, AB = 298 mm), eCO₂ (AA = 376 mm, AB = 403 mm), and eO₃ (AA = 354 mm, AB = 333 mm) treatments but was lower under combined eCO₂ and eO₃ (AA=327 mm, AB = 325 mm). Aspen clone 216 typically contributed the least to stand sap flow across all treatments, with the remaining aspen clones and birch usually contributed roughly equally to stand sap flow. Under treatment with eCO₂, however, clones 271 and 42E accounted for an increased proportion of stand sap flow at the expense of clone 8L. In the AB community, the proportion of sap flow attributed to aspen clone 216 and birch was relatively similar and stable across treatments.

One or more significant effects or interactions on Js were present for Community, Genotype, eCO₂, and eO₃ (**Table 5.3**). There were significant Community by Genotype interactions in May 2006 ($P=0.0749$), August 2006 ($P=0.0382$), and July 2007 ($P=0.0523$) due to lower sap flows for aspen clone 216 in the AA (1.17

mm·d⁻¹, 1.15 mm·d⁻¹, and 2.73 mm·d⁻¹, respectively) relative to the AB community (1.67 mm·d⁻¹, 1.32 mm·d⁻¹, and 3.75 mm·d⁻¹, respectively). There were also significant main effects for Genotype in June, July, and September 2006 ($P=0.0006$, $<.0001$, $<.0001$, respectively); and May, June, August, and September 2007 ($P=0.0004$, $<.0001$, 0.0229 , 0.0004 respectively). Sap flows were generally similar during these periods between aspen clone 216 and birch in the AB community. With the exception of September 2007, however, sap flows within the AA community were ordered by genotype with clone 216 having the lowest flows, followed by clone 271, then clone 8L, and clone 42E having the highest flows (**Table 5.4**). During September 2007 clone 8L had the lowest flow (0.59 mm·d⁻¹), clone 42E had an intermediate flow (0.63 mm·d⁻¹), and clones 271 and 216 had the highest flows (0.76 mm·d⁻¹). Main effects for Community were indicated in September 2006 ($P=0.0229$), and June and August 2007 ($P=0.0758$ and 0.0284 , respectively, with flows in the AA community exceeding those in the AB community (**Table 5.4**).

Significant effects involving eCO₂ included a main effect for eCO₂ during September 2007 ($P=0.0383$); interactions between eCO₂ and Genotype during May 2006, July 2006, June 2007, and August 2007 ($P=0.0549$, $.0628$, 0.0009 , and 0.0388 respectively); and an interaction between CO₂, Community, and Genotype

in June 2007 ($P=0.0094$). Sap flow was 72 % greater under eCO_2 in September 2007. During May 2006, July 2006, June 2007, and August 2007 sap flow under eCO_2 in the AA community decreased for clone 216 between -7.8 % to -22.7 %, increased for clone 271 between 8.79 % to 56.5 %, decreased for clone 8L between -44.1 % and -63.1 %, and for clone 42E decreased -28.1 % in September 2006 and increased 42.5 % and 27.1 % in July 2006 and June 2007, respectively. During May 2006, July 2006, June 2007, and August 2007 sap flow under eCO_2 in the AA community of clone 216 changed -30.5 %, -4 %, +157 %, and -17.1 %, respectively and of birch changed +21.6 %, +17.7 %, +145 %, and -14 %.

There were no significant main effects for eO_3 , but there were significant interactions of eO_3 with Community and eO_3 with Genotype. Significant interactions between eO_3 and Community occurred during May 2006 ($P=0.0853$), July 2006 ($P=0.0316$), and September 2007 ($P=0.0013$). During May and July 2006 decreases in sap flow associated with eO_3 in the AB community were roughly twice those in the AA community, and sap flows under eO_3 actually increased in September 2007 in the AA community but not the AB community (**Table 5.4**). Significant interactions between eO_3 and Genotype occurred during May 2006 ($P=0.0776$) and June, July, and August 2007 ($P=0.0067$, 0.0240, and 0.0011, respectively). Sapflow was decreased for all genotypes during these

periods except for increases in the AA community for clone 216 during May and July 2006 of 17.9 % and 34 %, respectively; increases of 11.5 % and 13.8 % for clone 8L in June and July 2007, respectively; and an (insignificant) increase of 1.64 % for clone 271 in July 2007.

The only significant interaction involving eCO₂ and eO₃ was CO₂×O₃×Genotype during August and September 2007 ($P=0.0578$ and 0.0704 , respectively). Under combined treatment with eCO₂ and eO₃, during August 2007 sap flow in the AA community increased for clone 216 37.5 % and 21.9 % for clone 271 but decreased -41.9 % for clone 42E and 30.1 % for clone 8L. During September 2007, sap flow in the AB community decreased -9.97 % for clone 216 but increased 1.31 % for birch.

Canopy sap flux was related to total soil water loss needed to balance soil water content, given infiltration from precipitation and drainage from overlying soil layers. Approximately 90 % of the coefficient magnitudes summed over the shallow, middle, and deep soil layers were in the shallow layer, indicating that the vast majority of soil water extraction for ET occurred from that layer (data not shown). Canopy sap flow accounted for the largest proportion of ET under combined eCO₂ and eO₃ treatment, followed closely by eCO₂ treatment (**Figure**

5.11). Canopy sap flow accounted for lower proportions of total ET under Control and eO₃, with the lowest proportions occurring under eO₃, generally during the late spring or early summer.

Understory Measurements

There were no statistically significant interactions. The only statistically significant main effect identified for understory gs was eCO₂ (p=0.072) (**Table 5.4**), which was associated with a decrease of 23% in comparison to control. Elevated O₃ was the only significant main effect for understory ET (p=0.031), associated with a 52% decrease relative to control.

Soil Water Drainage

There was some variability in deep soil drainage between communities on a monthly basis (**Figure 5.12**) but cumulative drainage over each growing season was quite similar across communities (**Figure 5.13**). Defining the active growing season here as that which spanned the last ten days of May through the first three weeks of September, during 2006 season total deep soil drainage averaged about 20 % of precipitation and similar under Control (AA = 137 mm, AB = 125 mm)

and eCO₂ (AA = 122 mm, AB = 120 mm), while drainage was generally higher under eO₃ (AA = 174 mm, AB = 182 mm) and combined eCO₂ and eO₃ (AA = 161 mm, AB = 166 mm). During the 2007 season deep soil drainage averaged about 30 % of precipitation and progressively elevated above Control (AA = 66.7 mm, AB = 83.7 mm) under eCO₂ (AA = 159 mm, AB = 156 mm), eO₃ (AA = 157 mm, AB = 167 mm), and combined eCO₂ with eO₃ (AA = 219 mm, AB = 216 mm). Significant effects and interactions for soil water drainage were indicated for eCO₂, eO₃, and Community but these were almost entirely limited to the 2007 growing season (**Table 5.5**). The only significant effect for the 2006 growing season was during August for the interaction between eCO₂ and Community ($P=0.0944$). During this period drainage under Control was low and quite similar across communities, averaging 15.1 mm·month⁻¹ in the AA community and 15.9 mm·month⁻¹ in the AB community, while there was virtually no change under eCO₂ in the AB community but a 10.5 % increase in the AA community. There were significant three-way interactions between eCO₂, eO₃ and Community in May 2007 ($P=0.0916$) and June 2007 ($P=0.0948$). During May 2007 drainage increased under combined eCO₂ and eO₃ 18.9 % in the AA community but 41.9 % in the AB community, while in June 2007 it increased 337 % in the AA community and 108 % in the AB community. These changes did not appear to be additive relative to the concurrent changes under eCO₂ and eO₃ alone (**Table 5.5**).

During July 2007 eCO₂ was associated with a 61.9 % increase in the AA community and a 57 % increase in the AB community, and eO₃ was associated with a 58.1 % increase in the AA community and a 45.4 % increase in the AB community. Least-squares means were not available for Control in September 2007 but the means for the treatment responses appeared to follow trends similar to those observed in the preceding month.

Discussion

Uddling et al. (2008) found decreased surficial soil water content due to eO₃, which could be consistent with increased understory ET under eO₃ found in the present study (>2 mm·d⁻¹). Our surficial soil water content data were collected after storm events, and we found no significant effects on surficial θ_v , other than increases under eO₃ that were larger in the AA relative to the AB community, and an overall trend of increased surficial soil water relative to control under all treatments. Decreased understory ET under eCO₂ could result from decreased understory LAI and solar heating. Increased surficial soil water content following storms under eO₃ could result from decreased canopy interception as a result of lower canopy LAI, and decreased time-averaged surficial soil water content under

eO₃ could result from greater understory ET due to increased understory growth and atmospheric coupling (Uddling et al., 2008).

Subsurface soil water generally decreased throughout the growing season except following precipitation events, was lower in the deep layer relative to the shallow and intermediate layers and became more temporally stable with increasing depth. It was usually higher under eCO₂, lower under eO₃, and similar to Control or slightly lower under combined eCO₂ and eO₃. Also, with the exception of 2008, subsurface soil water content was higher in the AA than the AB community. Also, significant effects of eCO₂ or eO₃ only occurred in the spring or fall and were differentiated by layer, with eCO₂ usually having the greatest impacts in the shallow and deep layers and eO₃ having the greatest impacts in the middle and deep layers. During the 2006 and 2008 growing seasons eO₃ was associated with significantly decreased moisture at the greatest depth relative to control, suggesting that tree or understory roots were adapting to the results of eO₃ stress by increased reliance on water from depth. Conversely, during the fall of 2006, summer of 2007, and spring and summer of 2008, eCO₂ was associated with relatively large increases in soil water content in the deepest soils. Based on the dependence of soil water drainage on soil water content, direct interpretation of

these data indicates a potential net increase in deep recharge under eCO₂, a net decrease under eO₃, and little change under combined eCO₂ and eO₃. More accurate predictions should be available, however from incorporating available ET data and fine tuning of soil water retention curves.

Uddling et al., (2008) found that sap flux varied significantly between the 2005 and 2006 growing seasons, but averaged over years found higher sap flux under eCO₂, higher leaf conductance (gs) under all treatment combinations relative to control, and an increase in canopy conductance (Gs) associated with eCO₂, some genotypic effects on gs but not sap flux, and no community effects. Our findings for canopy water use at the Aspen-FACE site differ somewhat from the findings of Uddling et al., (2008) in that we found effects involving eO₃ as well as eCO₂, and that effects were usually modulated by genotype or genotype and community. We found decreased water use with all treatments in 2006, although the decreases under eCO₂ were often insignificant, and similar responses during the summer of 2007. During the spring and fall of 2007, however, we found increased water use under eCO₂ and eO₃, with the greatest use under eCO₂, and also increased water use under combined eCO₂ and eO₃ in the fall of 2007. Thus, our canopy sap flux results were generally consistent with our hypothesis that sap flux should have

been more similar in the Control, eCO₂, and eCO₂ × eO₃ plots with closed, ostensibly light-limited canopies, as observed in other studies undertaken in closed canopy forests (Schäfer et al., 2002, Cech et al., 2003), than in the relatively open eO₃ canopies (Uddling et al., 2008) with greater understory growth (Bandeff et al., 2006), as was the case in some recent studies of European beech and Norway spruce (Nunn et al., 2006 and Warren et al., 2006).

The understory decrease in g_s associated with eCO₂ and the increase in ET associated with eO₃ found in the present study are consistent with documented effects of these gases (Karnosky, 2003), and generally support our hypotheses that the understory would exhibit higher stomatal conductance (g_s) and ET under eO₃, due to increased light dominating the typical effects of mid-range eO₃ on g_s , and lower g_s and ET under eCO₂ due to decreased light acting in concert with the typical effects on g_s of eCO₂. The increase in ET relative to control under combined eCO₂ × eO₃, however, and its similarity to eO₃, was unexpected. Elevated CO₂ has been shown to typically offset the effects of eO₃ in the general literature as well as studies specific to this site (King et al., 2005). Elevated g_s under eCO₂ is atypical (Wang and Curtis, 1998), but could be consistent with increased growth. Elevated g_s under eO₃ occurs in some species suffering loss of stomatal regulation after significant O₃ exposure (Maier-Maercker 1998). My

results for the understory are consistent with our understanding of the canopy. There is more light reaching the understory under eO₃ (Kostiainen, 2008) providing for greater soil evaporation and herbaceous transpiration. Understory ET under eO₃ is probably not only increased by the availability of additional light, but by higher understory g_s there as well.

Few large decreases in deep soil drainage relative to Control were associated with the treatments, with the exception of September 2007, while increases were common. The largest increases were frequently associated with combined eCO₂ and eO₃ treatment, although the largest increases in August 2006 were associated with eO₃.

We hypothesized lesser eCO₂ effects than eO₃ effects due to the onset of light limitation in the non-eO₃ plots, and greater eO₃ effects for sap flux than for soil water content. Decreased water use could be attributed to decreased stomatal conductance under eCO₂ or eO₃, decreased understory LAI and soil heating under eCO₂, and decreased canopy LAI under eO₃, while increased water use could be attributed to greater canopy LAI under eCO₂, increased understory LAI and atmospheric coupling under eO₃, or increased soil heating under eO₃ (Uddling, 2008).

Our sap flow results indicate that the eCO₂ plots appeared to have not become fully light-limited, but to have effects of lesser magnitude than the eO₃ plots. Sap flow in 2006 under the treatments was slightly lower than Control, with the largest decreases under eO₃. Sap flow in 2007 was similar for Control, eCO₂, and eO₃ treatments but was lower under combined eCO₂ and eO₃. The computed sap flows were more variable than the depth-integrated soil water contents and did not fully correlate to changes in deep drainage. However, the modeled apportionment of canopy sap flow indicated that an increased proportion of total ET was due to the understory under Control and eO₃, in comparison to eCO₂ and eCO₂ with eO₃. These results are consistent with decreased understory growth and understory ET under eCO₂, and increased understory growth and understory ET under eO₃. The general trend of decreased canopy sap flow accompanied by increased deep soil drainage under the treatments is intuitively obvious, and in the instance of eCO₂ where an appreciable reduction in sap flow did not occur, the increase in drainage could be attributed to lower understory ET due to increased canopy LAI (shading), whereas increased drainage under eO₃ indicates that increased understory ET under the lower canopy LAI was not sufficient to offset the reduction in canopy ET. The decreases in soil water content accompanying these changes, although small, might be considered counterintuitive because drainage

rates should increase at higher soil water contents. However, deep roots supporting decreased ET might not lift as much water into the active root zone at night for transpiration during the day.

Conclusions

The results of this study indicate that changes in atmospheric chemistry forecast for the next century could alter the forest hydrologic budget and deep soil drainage to groundwater at middle latitudes. Changes to soil water content and drainage due to alterations in canopy water use under $e\text{CO}_2$ and $e\text{O}_3$ were mitigated by compensatory responses of the understory. Studies of the water balance under altered atmospheric chemistry should account for treatment effects on the understory in addition to the canopy.

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Table 5.1. Results of ANOVA for soil water (mm) contained in 10-cm deep lysimeters at Aspen-FACE during 2006-2008 growing seasons. ANOVA was performed using SAS v.9.2 proc Mixed. Control *P*-values and means (se) are provided all-aspen and aspen-birch communities, and differences (percentage) from Control for other treatments are also shown. *P*-values >0.2 are denoted “ns” and unavailable data are denoted “na”.

Effect	May-06	Jun-06	Jul-06	Aug-06	Sep-06	Oct-06	May-07	Jun-07	Jul-07	Aug-07	Sep-07	Oct-07	May-08	Jun-08	Jul-08	Aug-08	Sep-08
Block	na	0.0062	0.1904	na	na	na	na	na	na	na	na	na	na	na	na	na	na
Community	na	na	na	na	0.0649	na	na	na	na	na	na	na	na	na	na	na	na
CO ₂	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
CO ₂ × Community	na	na	na	na	na	na	na	na	0.1937	na	na	na	na	na	na	na	na
O ₃	na	na	0.1576	na	0.1045	na	na	na	na	na	na	na	na	na	na	na	na
O₃ × Community	na	0.0444	0.1480	0.1237	na	na	na	0.0546	0.0684	0.0410	na	na	na	0.0631	0.0201	0.0739	0.0660
CO ₂ × O ₃	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	0.1398
CO ₂ × O ₃ × Community	na	na	na	na	na	na	na	0.1522	na	na	na	na	na	na	na	na	na

Table 5.1. (Continued.)

Effect	May-06	Jun-06	Jul-06	Aug-06	Sep-06	Oct-06	May-07	Jun-07	Jul-07	Aug-07	Sep-07	Oct-07	May-08	Jun-08	Jul-08	Aug-08	Sep-08
All-aspens community																	
Control	na	18.2 (4.96)	11.4 (7.76)	25.0 (6.72)	22.0 (5.60)	na	na	16.4 (6.18)	14.9 (6.55)	10.0 (5.58)	20.3 (na)	na	16.5 (5.36)	21.6 (5.30)	22.7 (5.12)	19.0 (4.41)	18.1 (4.57)
eCO ₂	na	26.3 (5.89) 44.5%	18.8 (5.08) 64.9%	28.0 (5.7) 12.0%	20.8 (5.9) -5.45%	na	na	22.3 (0.9) 35.9%	20.0 (1.93) 34.2%	15.1 (0.7) 51.0%	20.9 (na) 2.95%	na	21.3 (1.3) 29.0%	24.8 (1.48) 14.8%	25. (1.26) 10.1%	21.9 (2.36) 15.2%	23.2 (1.98) 28.1%
eO ₃	na	38.5 (4.43) 111.1%	24.2 (4.62) 112.1%	40.6 (2.87) 62.4%	33.6 (5.13) 52.7%	na	na	29.2 (4.86) 78.0%	28.1 (5.88) 88.5%	22.3 (5.88) 123.3%	na	na	23.1 (4.26) 40.0%	33.0 (4.97) 52.7%	33.9 (4.40) 49.3%	27.7 (3.72) 45.7%	29.7 (3.13) 64.0%
eCO ₂ × eO ₃	na	34.3 (1.13) 88.4%	20. (3.56) 75.4%	37.0 (2.67) 48.0%	31.5 (2.18) 43.1%	na	na	21.3 (2.01) 29.8%	19.3 (3.50) 29.5%	14.7 (1.20) 47.0%	na	na	19.0 (1.00) 15.1%	24.0 (1.92) 11.1%	25.5 (1.41) 12.3%	20.5 (2.06) 7.89%	21.8 (2.31) 20.4%
Aspen-birch Community																	
Control	na	17.9 (4.18)	13.1 (2.74)	39.6 (5.28)	21.3 (2.37)	na	na	18.9 (1.55)	15.3 (3.63)	14.2 (2.37)	23. (na)	na	17.8 (3.38)	23.4 (2.77)	24.1 (3.06)	21.6 (2.7)	20.1 (4.65)
eCO ₂	na	24.9 (8.77) 39.1%	17.8 (5.36) 35.8%	32.9 (6.26) -16.9%	20.8 (7.15) -2.34%	na	na	22.2 (1.06) 17.4%	23. (2.60) 50.3%	14.3 (0.77) 0.70%	12.2 (na) -46.9%	na	21.3 (1.13) 19.6%	25.6 (1.91) 9.40%	26.4 (2.12) 9.54%	25.4 (3.98) 17.5%	25.9 (4.2) 28.8%
eO ₃	na	31.9 (13.) 78.2%	25.9 (4.09) 97.7%	38.5 (12.1) -2.77%	34.9 (8.57) 63.8%	na	na	21.2 (2.49) 12.1%	19.8 (1.90) 29.4%	15.2 (2.84) 7.04%	na	na	21.6 (4.24) 21.3%	25. (2.17) 6.83%	26.6 (2.59) 10.3%	24.3 (2.8) 12.5%	22.6 (1.56) 12.4%
eCO ₂ × eO ₃	na	31. (2.31) 73.1%	24.2 (0.28) 84.7%	31.2 (3.60) -21.2%	32.2 (2.35) 51.1%	na	na	22.5 (1.45) 19.0%	20.6 (0.97) 34.6%	17.1 (2.16) 20.4%	na	na	20.3 (2.2) 14.0%	24.1 (1.2) 2.99%	25.3 (1.43) 4.97%	21.0 (1.22) -2.77%	21.1 (2.04) 4.97%

Table 5.2. ANOVA *P*-values and supporting statistics for subsurface volumetric soil water content at Aspen-FACE during May-September 2006-2008. *P*-values greater than 0.2 are designated “ns”.

Effect	May-06	Jun-06	Jul-06	Aug-06	Sep-06
Rep (block)	ns	ns	ns	ns	ns
Community	ns	ns	0.0765	0.0300	0.0203
Layer	<.0001	<.0001	0.0030	<.0001	0.0003
Community × Layer	ns	ns	ns	ns	0.0643
CO ₂	ns	0.1765	ns	ns	ns
CO ₂ × Community	ns	ns	ns	ns	0.1358
CO₂ × Layer	ns	0.0705	0.1992	ns	ns
CO ₂ × Community × Layer	ns	ns	ns	ns	ns
O ₃	ns	0.1340	ns	ns	ns
O ₃ × Community	ns	ns	ns	ns	ns
O₃ × Layer	0.0368	ns	0.1756	ns	0.0728
O₃ × Community × Layer	0.1799	0.0508	ns	ns	ns
CO ₂ × O ₃	ns	ns	ns	ns	ns
CO ₂ × O ₃ × Community	ns	ns	ns	ns	ns
CO ₂ × O ₃ × Layer	0.1146	0.1880	ns	ns	ns
CO ₂ × O ₃ × Community × Layer	ns	ns	ns	ns	ns
Selected means (se)					
Control	15.57 (1.173)	11.27 (1.225)	5.935 (1.276)	8.477 (1.308)	6.488 (1.370)
eO ₃	15.20 (1.178)	9.924 (1.225)	5.339 (1.273)	8.150 (1.306)	6.326 (1.365)
eCO ₂	15.42 (1.178)	11.57 (1.228)	5.829 (1.27)	7.883 (1.310)	5.874 (1.368)
eCO ₂ × eO ₃	15.38 (1.197)	11.17 (1.233)	5.618 (1.28)	8.115 (1.312)	5.669 (1.370)
Community all-aspen	15.82 (1.15)	10.85 (1.153)	5.382 (1.18)	7.607 (1.185)	5.603 (1.217)
Community aspen-birch	15.34 (1.148)	10.92 (1.150)	5.590 (1.179)	8.264 (1.180)	6.018 (1.220)
Community aspen-maple	15.02 (1.166)	11.15 (1.155)	6.071 (1.18)	8.000 (1.187)	6.654 (1.226)
Soil layer - deep	10.16 (1.149)	6.372 (1.173)	3.280 (1.238)	2.763 (1.209)	2.407 (1.262)
Soil layer - middle	17.49 (1.168)	13.36 (1.181)	6.564 (1.241)	10.62 (1.213)	7.022 (1.261)
Soil layer - shallow	19.08 (1.153)	13.82 (1.173)	7.529 (1.235)	12.40 (1.205)	9.716 (1.257)
eCO ₂ × Layer (% differences from Control)					
Deep	-3.5%	6.1%	-4.2%	-7.3%	20%
Middle	-2.8%	-3.0%	-7.5%	-9.0%	-16%
Shallow	5.9%	19%	15%	2.4%	-7.9%
eO ₃ × Layer (% differences from Control)					
Deep	-14%	-19%	-16%	-13%	-34%
Middle	-0.6%	-2.3%	-15%	-4.6%	-2.9%
Shallow	7.1%	0.7%	7.7%	8.4%	13%

Table 5.2 (continued)

Effect	May-07	Jun-07	Jul-07	Aug-07	Sep-07
Rep (block)	ns	ns	ns	ns	ns
Community	ns	0.0139	0.0002	0.0012	0.0121
Layer	<.0001	<.0001	<.0001	0.0007	0.0004
Community × Layer	ns	ns	ns	ns	ns
CO ₂	ns	ns	ns	ns	ns
CO ₂ × Community	ns	ns	ns	ns	ns
CO₂ × Layer	0.1299	0.0700	0.1759	ns	ns
CO ₂ × Community × Layer	ns	ns	ns	ns	ns
O ₃	ns	ns	ns	ns	ns
O ₃ × Community	ns	ns	ns	ns	ns
O₃ × Layer	0.0836	ns	ns	ns	ns
O ₃ × Community × Layer	ns	ns	ns	ns	ns
CO ₂ × O ₃	ns	ns	ns	ns	ns
CO ₂ × O ₃ × Community	ns	ns	ns	ns	ns
CO ₂ × O ₃ × Layer	0.1330	ns	ns	ns	ns
CO₂ × O₃ × Community × Layer	0.0945	ns	ns	ns	ns
Selected means (se)					
Control	14.62 (1.212)	11.84 (1.339)	8.854 (1.483)	5.217 (1.301)	na
eO ₃	14.27 (1.159)	11.02 (1.253)	8.200 (1.361)	5.248 (1.225)	7.233 (1.233)
eCO ₂	15.02 (1.159)	12.12 (1.260)	9.305 (1.365)	5.349 (1.232)	6.433 (1.237)
eCO ₂ × eO ₃	14.67 (1.167)	11.65 (1.263)	8.670 (1.368)	4.972 (1.236)	6.487 (1.241)
Community all-aspen	14.68 (1.143)	10.85 (1.000)	7.420 (1.244)	4.477 (1.170)	6.003 (1.193)
Community aspen-birch	14.00 (1.143)	11.67 (1.182)	8.865 (1.244)	5.174 (1.167)	6.963 (1.200)
Community aspen-maple	14.77 (1.162)	12.46 (1.196)	10.04 (1.260)	5.974 (1.182)	na
Soil layer - deep	9.979 (1.156)	6.496 (1.190)	3.931 (1.256)	2.308 (1.221)	na
Soil layer - middle	16.19 (1.147)	13.54 (1.189)	10.55 (1.253)	6.640 (1.215)	8.787 (1.220)
Soil layer - shallow	18.24 (1.145)	15.71 (1.000)	12.75 (1.250)	7.203 (1.213)	10.51 (1.225)
eCO ₂ × Layer (% differences from Control)					
Deep	5.0%	1.2%	29%	16%	na
Middle	-4.2%	-4.3%	-8.2%	-14%	-19%
Shallow	8.0%	14%	8.2%	3.1%	-1.0%
eO ₃ × Layer (% differences from Control)					
Deep	-6.5%	-6.6%	-10%	-6.7%	na
Middle	-7.7%	-11%	-14%	-13%	-0.9%
Shallow	5.6%	0.6%	1.6%	8.2%	7.9%

Table 5.2 (continued)

Effect	May-08	Jun-08	Jul-08	Aug-08	Sep-08
Rep (block)	ns	ns	ns	ns	0.0461
Community	ns	ns	0.1318	ns	0.0721
Layer	<.0001	<.0001	0.0008	0.0030	<.0001
Community × Layer	ns	ns	ns	ns	ns
CO ₂	ns	ns	ns	ns	ns
CO ₂ × Community	ns	ns	ns	ns	ns
CO ₂ × Layer	ns	ns	ns	ns	ns
CO ₂ × Community × Layer	ns	ns	ns	ns	ns
O ₃	ns	ns	ns	ns	0.1698
O ₃ × Community	ns	ns	ns	ns	ns
O ₃ × Layer	ns	ns	ns	ns	ns
O ₃ × Community × Layer	ns	ns	ns	ns	ns
CO ₂ × O ₃	ns	ns	ns	ns	ns
CO ₂ × O ₃ × Community	ns	ns	ns	ns	ns
CO ₂ × O ₃ × Layer	ns	ns	ns	ns	ns
CO ₂ × O ₃ × Community × Layer	ns	ns	ns	ns	ns
Selected means (se)					
Control	14.60 (1.342)	11.10 (1.380)	6.115 (1.435)	3.802 (1.349)	4.048 (1.278)
eO ₃	13.50 (1.321)	11.05 (1.389)	5.703 (1.440)	3.689 (1.350)	3.693 (1.270)
eCO ₂	na	12.41 (1.382)	7.280 (1.430)	4.739 (1.342)	4.439 (1.253)
eCO ₂ × eO ₃	na	10.62 (1.380)	6.076 (1.440)	3.628 (1.346)	na
Community all-aspen	14.35 (1.222)	11.27 (1.228)	5.829 (1.240)	3.927 (1.205)	4.300 (1.177)
Community aspen-birch	14.38 (1.219)	11.48 (1.228)	6.451 (1.248)	3.972 (1.20)	4.033 (1.163)
Community aspen-maple	na	11.12 (1.24)	6.586 (1.260)	3.970 (1.220)	na
Soil layer - deep	na	6.012 (1.256)	3.150 (1.273)	1.900 (1.256)	na
Soil layer - middle	na	13.62 (1.257)	7.320 (1.274)	4.975 (1.252)	4.477 (1.221)
Soil layer - shallow	na	15.08 (1.246)	8.938 (1.267)	5.362 (1.246)	6.310 (1.216)
eCO ₂ × Layer (% differences from Control)					
Deep	na	14%	24%	30%	na
Middle	na	-2.2%	6.3%	-0.80%	-7.3%
Shallow	na	3.4%	14%	15%	5.9%
eO ₃ × Layer (% differences from Control)					
Deep	na	-24%	-28%	-27%	na
Middle	na	-5.0%	-11%	-11%	-6.1%
Shallow	na	-0.7%	-3.8%	-10%	-9.4%

Table 5.3. Results of ANOVA for sap flow (Js) at Aspen-FACE during May-September 2006 and 2007. ANOVA was performed using SAS v.9.2 proc Mixed.

Effect	2006					2007				
	May	June	July	August	September	May	June	July	August	September
Block	ns	0.1648	ns	ns	ns	ns	0.1509	ns	0.1456	ns
Community	0.0723	0.1411	ns	0.0018	0.0229	ns	0.0758	<.0001	0.0284	ns
Genotype	<.0001	0.0006	<.0001	<.0001	<.0001	0.0004	<.0001	<.0001	<.0001	0.0004
Community × Genotype	0.0749	ns	ns	0.0382	0.1353	ns	ns	0.0523	ns	0.1391
CO₂	ns	0.1432	ns	ns	ns	ns	ns	0.1226	ns	0.0383
CO ₂ × Community	ns	ns	0.1524	ns	ns	0.1698	ns	ns	ns	ns
CO₂ × Genotype	0.0549	ns	0.0628	ns	ns	ns	0.0009	ns	0.0388	0.1064
CO₂ × Community × Genotype	ns	0.1327	ns	ns	ns	ns	0.0094	ns	ns	ns
O ₃	ns	ns	0.1417	ns	0.1390	ns	ns	ns	ns	ns
O₃ × Community	0.0853	ns	0.0316	ns	ns	ns	0.1422	0.1258	ns	0.0013
O₃ × Genotype	0.0776	ns	ns	0.1410	ns	ns	0.0067	0.0240	0.0011	ns
O₃ × Community × Genotype	ns	ns	ns	ns	ns	ns	0.0416	ns	ns	ns
CO ₂ × O ₃	ns	ns	ns	ns	ns	ns	0.1802	ns	ns	ns
CO ₂ × O ₃ × Community	ns	ns	ns	0.1976	ns	ns	0.1295	0.1580	ns	ns
CO₂ × O₃ × Genotype	0.1831	ns	ns	ns	ns	ns	0.1983	ns	0.0578	0.0704
CO₂ × O₃ × Community × Genotype	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 5.4. Means, standard errors (se), and percent changes from Control for canopy sap flow (Js) at Aspen-FACE during the 2006 and 2007 growing seasons. Units are mm·day⁻¹.

Community and treatment	Genotype	2006					2007				
		May	June	July	August	September	May	June	July	August	September
All-aspen community mean, se, %change											
Control	216	1.17(1.34)	1.30(1.43)	1.00(1.35)	1.15(1.32)	0.96(1.30)	2.29(1.69)	2.72(1.74)	2.73(1.46)	2.05(1.40)	0.76(1.59)
	271	1.94(1.34)	1.65(1.43)	1.82(1.35)	1.50(1.32)	1.11(1.30)	2.74(1.69)	3.36(1.74)	3.65(1.46)	2.87(1.40)	0.76(1.59)
	42E	3.06(1.34)	2.39(1.43)	2.14(1.35)	1.95(1.32)	1.33(1.30)	3.27(1.69)	4.76(1.74)	5.83(1.46)	5.34(1.40)	0.63(1.43)
	8L	2.92(1.34)	2.31(1.43)	1.97(1.35)	1.38(1.32)	1.13(1.30)	2.60(1.69)	4.15(1.74)	3.96(1.46)	4.34(1.40)	0.59(1.43)
eCO ₂	216	1.05(1.34) -10.2	0.88(1.34) -32.3	0.90(1.35) -10.0	0.60(1.32) -47.8	0.52(1.26) -45.8	1.17(1.75) -48.9	2.10(1.42) -22.7	1.82(1.43) -33.3	1.89(1.36) -7.80	1.50(1.41) 97.3
	271	2.19(1.34) 12.8	1.59(1.34) -3.63	1.98(1.35) 8.79	1.21(1.32) -19.3	0.98(1.26) -11.7	3.16(1.75) 15.3	5.26(1.42) 56.5	4.24(1.43) 16.1	3.80(1.36) 32.4	3.09(1.41) 306.
	42E	2.20(1.34) -28.1	1.61(1.34) -32.6	3.05(1.35) 42.5	1.65(1.32) -15.3	1.05(1.26) -21.0	2.48(1.75) -24.1	6.05(1.42) 27.1	5.20(1.43) -10.8	5.27(1.36) -1.31	3.99(1.41) 533.
	8L	1.40(1.34) -52.0	0.90(1.34) -61.0	1.10(1.35) -44.1	0.81(1.32) -41.3	0.58(1.26) -48.6	0.58(1.75) -77.6	2.10(1.42) -49.3	1.63(1.43) -58.8	1.60(1.36) -63.1	1.13(1.41) 91.5
eO ₃	216	1.38(1.34) 17.9	1.22(1.60) -6.15	1.34(1.35) 34.0	0.97(1.32) -15.6	0.43(1.26) -55.2	1.79(1.30) -21.8	2.41(1.5) -11.3	2.71(1.35) -0.73	1.70(1.31) -17.0	1.56(1.34) 105.
	271	1.88(1.34) -3.09	1.41(1.60) -14.5	1.48(1.35) -18.6	1.08(1.32) -28.0	0.56(1.26) -49.5	2.40(1.30) -12.4	2.23(1.5) -33.6	3.71(1.35) 1.64	2.30(1.31) -19.8	1.80(1.34) 136.
	42E	2.21(1.34) -27.7	1.79(1.60) -25.1	1.68(1.35) -21.4	1.24(1.32) -36.4	0.50(1.26) -62.4	3.33(1.30) 1.83	3.90(1.5) -18.0	4.45(1.35) -23.6	2.89(1.31) -45.8	2.20(1.34) 249.
	8L	2.34(1.34) -19.8	1.52(1.60) -34.1	1.53(1.35) -22.3	1.11(1.32) -19.5	0.74(1.26) -34.5	3.72(1.30) 43.0	4.63(1.5) 11.5	4.51(1.35) 13.8	2.79(1.31) -35.7	1.99(1.34) 237.
eCO ₂ ×eO ₃	216	1.27(1.34) 8.54	1.05(1.34) -19.2	1.41(1.35) 41.0	0.94(1.32) -18.2	0.71(1.33) -26.0	1.56(1.30) -31.8	1.99(1.42) -26.8	2.14(1.35) -21.6	2.82(1.36) 37.5	2.51(1.34) 230.
	271	1.68(1.34) -13.4	1.55(1.34) -6.06	1.97(1.35) 8.24	1.38(1.32) -8.00	0.99(1.33) -10.8	2.26(1.30) -17.5	2.79(1.42) -16.9	3.08(1.35) -15.6	3.50(1.36) 21.9	2.74(1.34) 260.
	42E	1.48(1.34) -51.6	1.37(1.34) -42.6	1.81(1.35) -15.4	1.14(1.32) -41.5	1.03(1.33) -22.5	2.19(1.30) -33.0	2.71(1.42) -43.0	2.86(1.35) -50.9	3.10(1.36) -41.9	2.65(1.34) 320.
	8L	1.46(1.34) -50.0	1.42(1.34) -38.5	1.69(1.35) -14.2	1.68(1.32) 21.7	0.98(1.33) -13.2	2.52(1.30) -3.07	2.70(1.42) -34.9	2.88(1.35) -27.2	3.03(1.36) -30.1	2.57(1.34) 335.
Aspen-birch community mean, se, %change											
Control	216	1.67(1.34)	1.39(1.34)	1.75(1.35)	1.32(1.32)	1.14(1.30)	1.84(1.48)	1.77(1.55)	3.75(1.46)	2.57(1.40)	2.33(1.43)
	Birch	1.57(1.34)	1.91(1.34)	1.75(1.35)	1.37(1.32)	1.11(1.30)	1.79(1.48)	1.72(1.55)	3.70(1.46)	2.00(1.40)	2.28(1.43)
eCO ₂	216	1.16(1.39) -30.5	1.18(1.34) -15.1	1.68(1.35) -4.00	1.39(1.32) 5.30	1.04(1.30) -8.77	3.06(1.48) 66.3	4.56(1.42) 157.	4.10(1.35) 9.33	2.13(1.31) -17.1	2.86(1.34) 22.7
	Birch	1.91(1.39) 21.6	1.23(1.34) -35.6	2.06(1.35) 17.7	1.68(1.32) 22.6	1.66(1.30) 49.5	3.94(1.48) 120.	4.23(1.42) 145.	4.04(1.35) 9.18	1.72(1.31) -14.0	2.57(1.34) 12.7
eO ₃	216	0.95(1.34) -43.1	1.26(1.44) -9.35	0.63(1.35) -64.0	1.39(1.32) 5.30	0.67(1.33) -41.2	2.20(1.45) 19.5	3.05(1.50) 72.3	4.01(1.35) 6.93	2.20(1.31) -14.3	0.87(1.34) -62.6
	Birch	1.20(1.34) -23.5	1.60(1.44) -16.2	1.49(1.35) -14.8	1.47(1.32) 7.29	0.67(1.33) -39.6	2.51(1.45) 40.2	3.10(1.50) 80.2	4.10(1.35) 10.8	2.30(1.31) 15.0	1.92(1.34) -15.7
eCO ₂ ×eO ₃	216	1.02(1.34) -38.9	1.29(1.34) -7.19	1.60(1.35) -8.57	1.01(1.39) -23.4	0.86(1.50) -24.5	2.26(1.30) 22.8	2.83(1.42) 59.8	2.33(1.35) -37.8	3.06(1.31) 19.0	2.10(1.34) -9.87
	Birch	1.21(1.34) -22.9	1.06(1.34) -44.5	1.54(1.35) -12.0	1.03(1.39) -24.8	0.94(1.50) -15.3	2.54(1.30) 41.8	2.96(1.42) 72.0	2.80(1.35) -24.3	2.93(1.31) 46.5	2.31(1.34) 1.31

Table 5.5. Results of ANOVA for computed deep soil drainage (mm) at Aspen-FACE during May-September 2006 and 2007. Drainage was computed using a volumetric balance between measured soil water content, precipitation, and sapflux coupled with gravimetric drainage rates computed using site-specific soil matric potential curves. ANOVA was performed using SAS v.9.2 proc Mixed. Control *P*-values and means (se) are provided all-aspen and aspen-birch communities, and differences (percentage) from Control for other treatments are shown. *P*-values >0.2 are denoted “ns” and unavailable data are denoted “na”.

	2006					2007				
	May	June	July	August	September	May	June	July	August	September
ANOVA effect										
Block	ns	ns	ns	ns	ns	ns	ns	0.0213	ns	0.0003
Community	ns	ns	ns	ns	ns	0.1382	0.0227	ns	ns	ns
CO₂	ns	ns	ns	ns	ns	0.1050	0.1019	0.0174	ns	0.0748
CO₂×Community	ns	ns	ns	0.0944	ns	ns	0.0237	ns	ns	ns
O₃	ns	ns	ns	0.1641	ns	ns	ns	0.0196	ns	0.0020
O₃×Community	ns	ns	ns	ns	ns	0.0607	0.0671	0.0726	ns	ns
CO ₂ ×O ₃	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CO₂×O₃×Community	ns	ns	ns	ns	ns	0.0916	0.0948	ns	ns	ns
All-aspen community mean (se) %-change										
Control	46.8(8.95)	32.5(5.53)	36.1(4.79)	15.1(4.68)	54.7(7.25)	46.9(3.57)	12.8(4.14)	23.4(1.13)	14.9(7.71)	na
eCO ₂	61.0(8.67) 30.3%	27.4(4.37) -15.%	36.8(4.79) 1.93%	16.7(4.61) 10.5%	31.0(7.25) -43.%	69.7(2.86) 48.6%	44.4(4.14) 246.%	37.9(1.00) 61.9%	35.1(5.09) 135.%	26.6(1.62) na
eO ₃	50.6(6.93) 8.11%	54.2(4.20) 66.7%	37.0(3.68) 2.49%	40.1(4.61) 165.%	38.0(3.53) -30.%	37.3(2.86) -20.%	30.4(4.14) 137.%	37.0(1.00) 58.1%	42.0(5.09) 181.%	50.8(1.62) na
eCO ₂ ×eO ₃	50.9(8.95) 8.76%	38.8(4.20) 19.3%	56.1(3.68) 55.4%	31.5(3.53) 108.%	26.4(3.41) -51.%	55.8(2.86) 18.9%	56.0(3.00) 337.%	55.2(1.09) 135.%	59.4(3.57) 298.%	42.7(1.62) na
Aspen-birch community mean (se) %-change										
Control	41.2(8.95)	30.2(5.98)	26.2(4.79)	15.9(4.61)	55.8(7.25)	49.8(3.57)	26.7(4.14)	24.2(1.13)	16.2(7.71)	na
eCO ₂	55.3(10.8) 34.2%	27.4(4.20) -9.2%	37.2(4.79) 41.9%	16.0(4.61) 0.62%	31.0(7.25) -44.%	63.4(2.86) 27.3%	45.0(4.14) 68.5%	38.0(1.00) 57.0%	35.5(5.09) 119.%	24.2(1.62) na
eO ₃	55.0(6.18) 33.4%	50.4(4.20) 66.8%	37.5(3.68) 43.1%	45.9(4.61) 188.%	43.7(3.41) -21.%	41.1(2.86) -17.%	33.8(4.14) 26.5%	35.2(1.00) 45.4%	46.5(5.09) 187.%	55.3(1.62) na
eCO ₂ ×eO ₃	86.0(8.95) 108.%	32.5(4.20) 7.61%	55.2(3.68) 110.%	28.5(3.53) 79.2%	30.8(3.53) -44.%	70.7(2.86) 41.9%	55.8(3.00) 108.%	53.9(1.09) 122.%	51.3(3.57) 216.%	46.0(1.62) na

Table 5.6. Means, standard errors (se), and *P*-values from the understory mid-day stomatal conductance (g_s) and evapotranspiration (ET) ANOVAs. Stomatal conductance is expressed in units of $\text{milliMol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and ET is expressed in units of $\text{mm} \cdot \text{d}^{-1}$. *P*-values greater than 0.2 are designated “ns” and standard errors are expressed at a 90% confidence level. Understory species surveyed for g_s included clover, goldenrod, and dandelion. In herbaceous species $e\text{CO}_2$ is often associated with reduced g_s and ET, and at adequate concentration, $e\text{O}_3$ is associated with increased g_s and ET.

mean (se)	mid-day g_s	mid-day ET
Control	114.1 (10.83)	1.41 (0.269)
$e\text{CO}_2$	87.3 (13.50)	1.02 (0.182)
$e\text{O}_3$	139.2 (14.40)	2.14 (0.597)
$e\text{CO}_2 \times e\text{O}_3$	107.2 (15.57)	2.88 (0.956)
p-values		
Rep (block)	ns	0.118
CO_2	0.072	ns
O_3	0.159	0.031
$\text{CO}_2 \times \text{O}_3$	ns	0.148
Species	ns	
$\text{CO}_2 \times \text{Species}$	ns	
$\text{O}_3 \times \text{Species}$	ns	
$\text{CO}_2 \times \text{O}_3 \times \text{Species}$	ns	

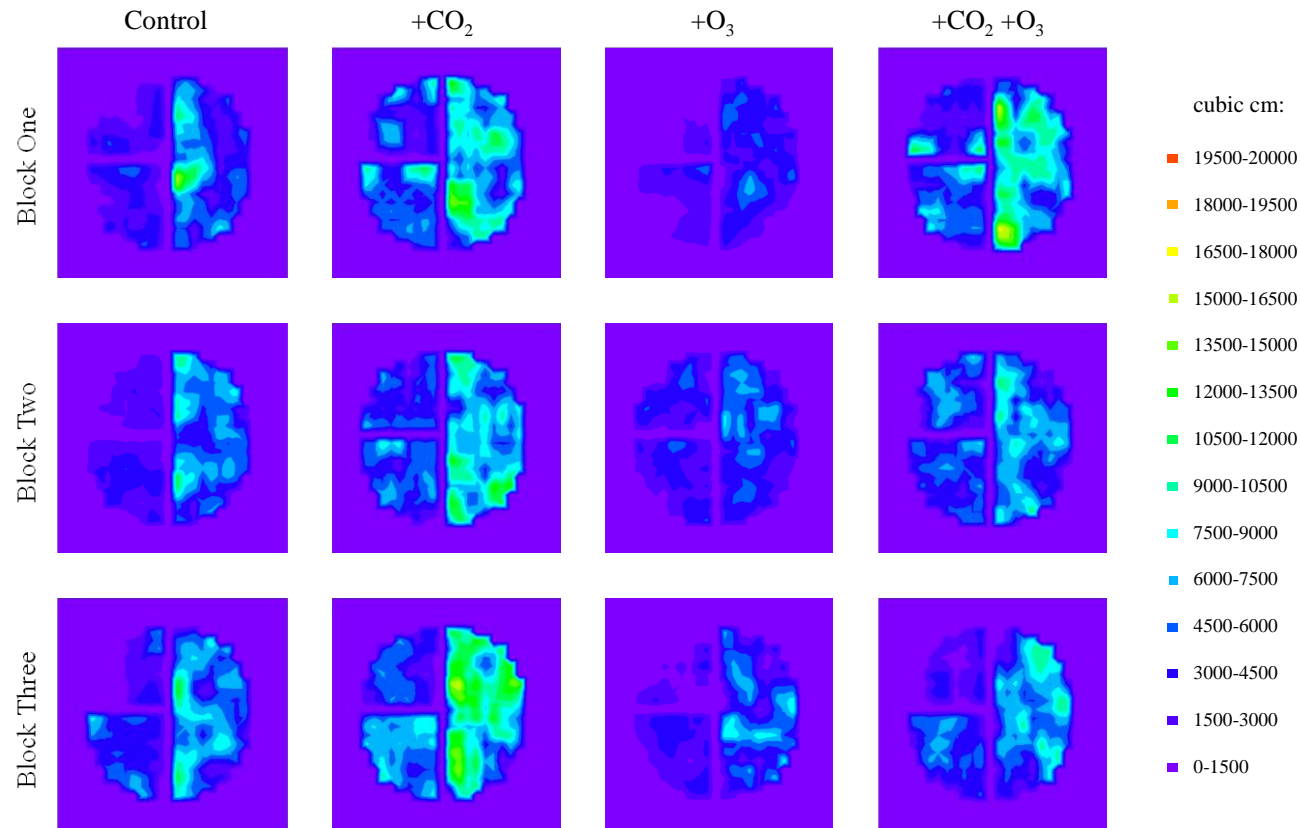


Figure 5.1. Stem volumes within the core areas of treatment rings at Aspen-FACE during October 2006. Volumes were estimated from height and DBH as per Kubiske et al. (2007). The upper-left quadrants are planted with alternated sugar maple and aspen clone 216. The lower-left quadrants are planted with alternated paper birch and aspen clone 216. The right quadrants are planted with aspen clones 8L, 42E, 216, 259, and 271, but the 259 clone had been severely repressed or eliminated at some time prior to the end of the 2006 growing season. All plots were planted in 1997 on one-foot centers with rooted cuttings (aspen) or seedlings (birch and maple) from local seed sources.

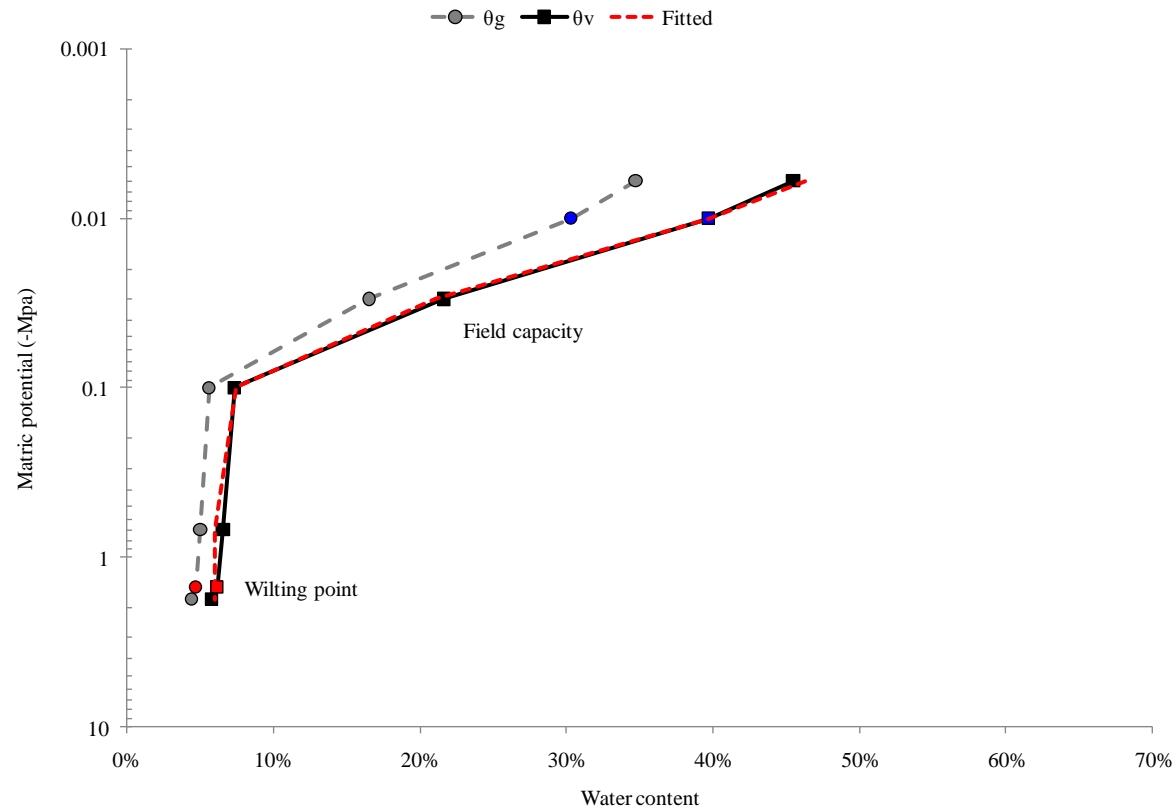


Figure 5.2. Soil matric properties at Aspen-FACE near Rhinelander, WI USA (after Dickson et al., 2000). The fitted function is based on the Weibull cdf: $\theta_v = \exp\{-[(\Psi+0.01)/0.018]^{0.8}\} + 0.06$, or $\Psi = -0.01 + 0.018 \cdot \{[-\log(\theta_v - 0.06)]^{1/0.8}\}$, where θ_v is soil volumetric water content Ψ is the matric potential. If linear flux rate $q = L \cdot d\theta_v/dt \cdot n_e^{-1} = K_\Psi \cdot \Psi \cdot L^{-1}$, where L is vertical distance, t is time, and n_e is effective porosity, then $K_\Psi = d\theta_v \cdot (dt \cdot \Psi \cdot n_e)^{-1}$.

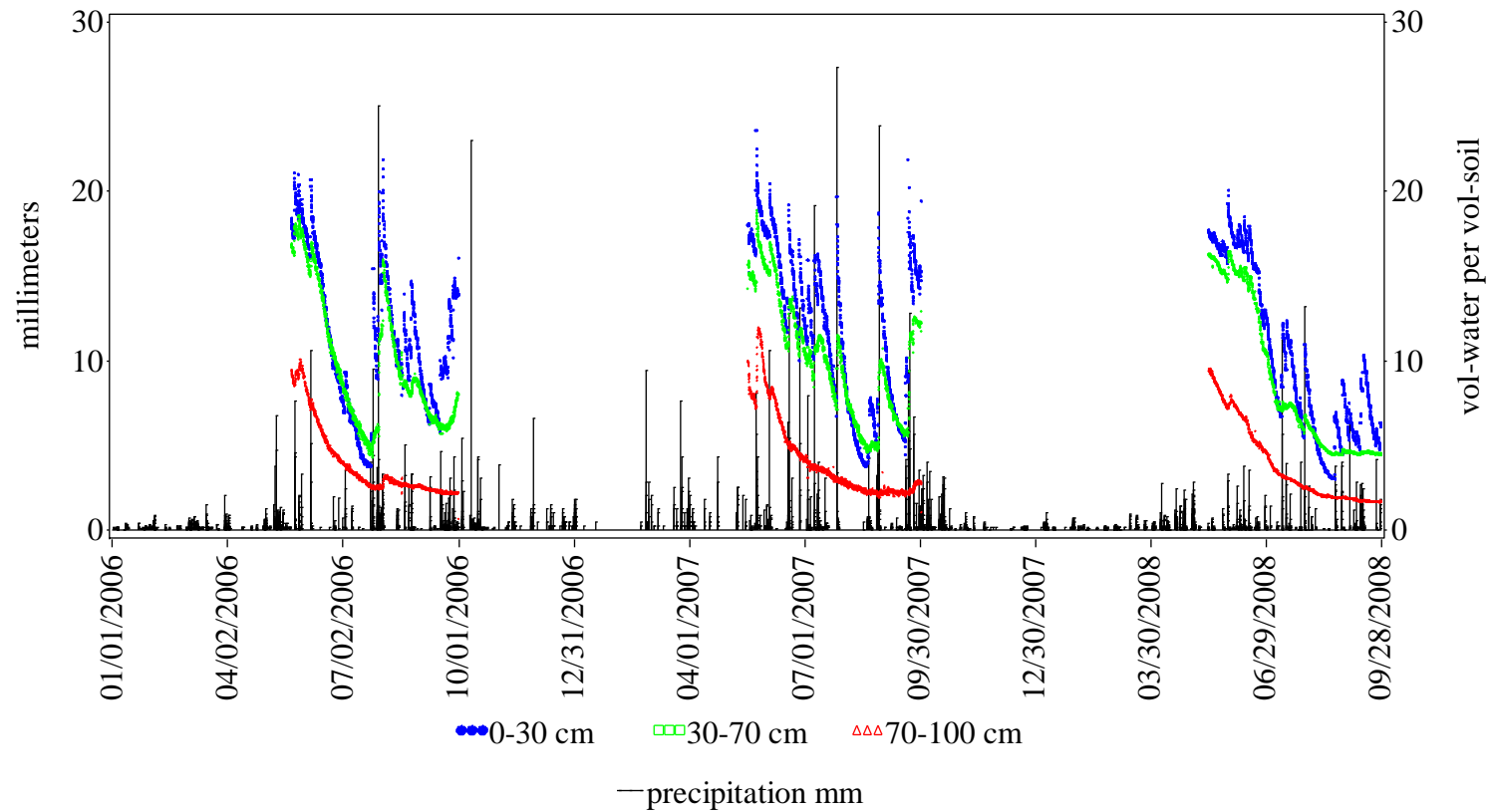


Figure 5.3. Precipitation, median volumetric soil water content, and standard errors at Aspen-FACE for 2006-2008. The soil water content data are probably biased low by several percent based on sensor calibration studies conducted at the site and in the laboratory. Nonetheless, they provide a means to compare inter- and intra-annual temporal trends.

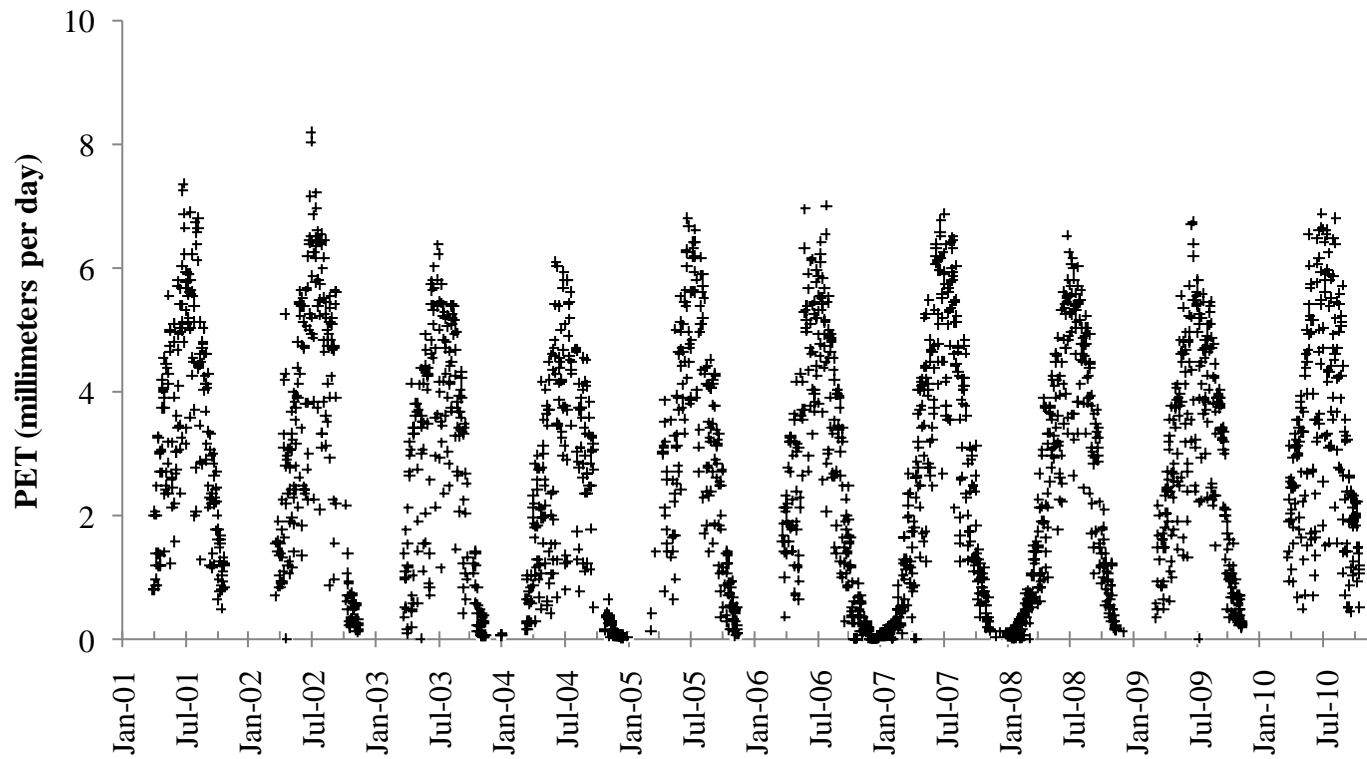


Figure 5.4. Reference evapotranspiration (ET_0) at 45.5N 89.6W, estimated by the University of Wisconsin Extension Agricultural Weather evapotranspiration model. (http://www.soils.wisc.edu/uwex_agwx/sun_water/et_wimn)

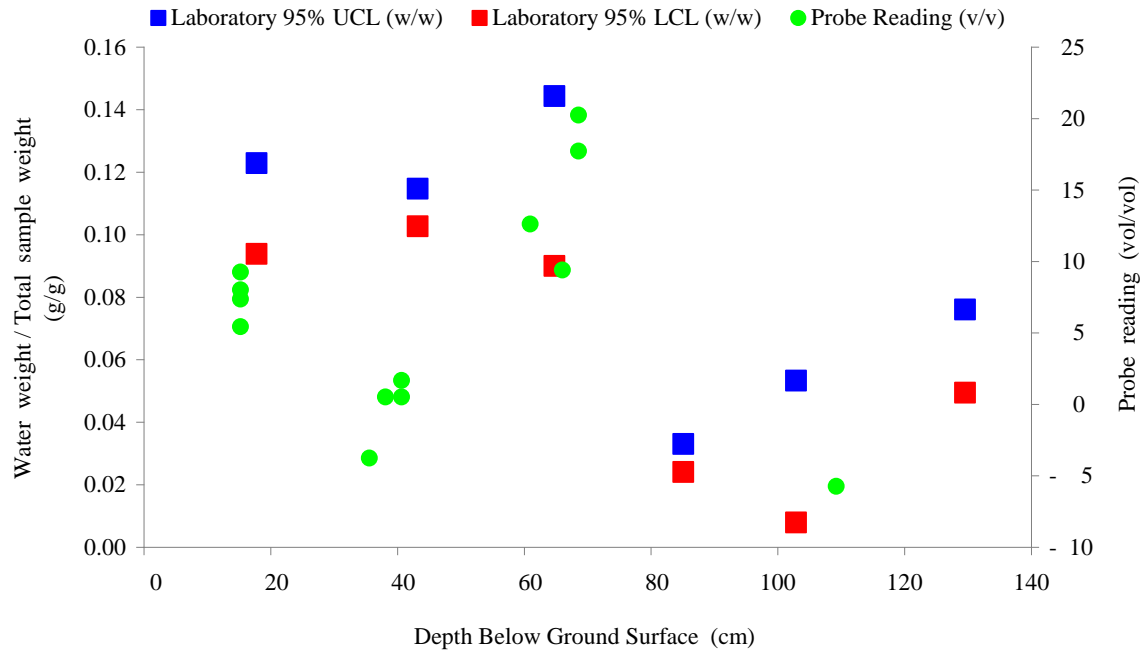


Figure 5.5. Soil water content probe calibration. Soil samples were collected from a test pit at the centroid of the Aspen-FACE experimental area at noon on June 17, 2008 after soil water content probe readings (green circles) were recorded. Four samples were collected from depths of about 18 cm, 40 cm, 65 cm, 85 cm, 105 cm, and 130 cm. The samples were individually packaged, labeled, and sealed prior to being shipped refrigerated during to our laboratory at North Carolina State University. Upon arrival the samples were transferred to glassware, wet-weighed, and placed in a desiccation oven set to 50 degrees Celsius for two weeks. The samples were than dry-weighed. For each group of samples, the upper confidence intervals about the mean is shown in blue and the lower confidence interval about the mean is shown in red. An obvious break in soil water content occurs between 60 cm and 80 cm depth, where a transition to sands was observed in the test pit.

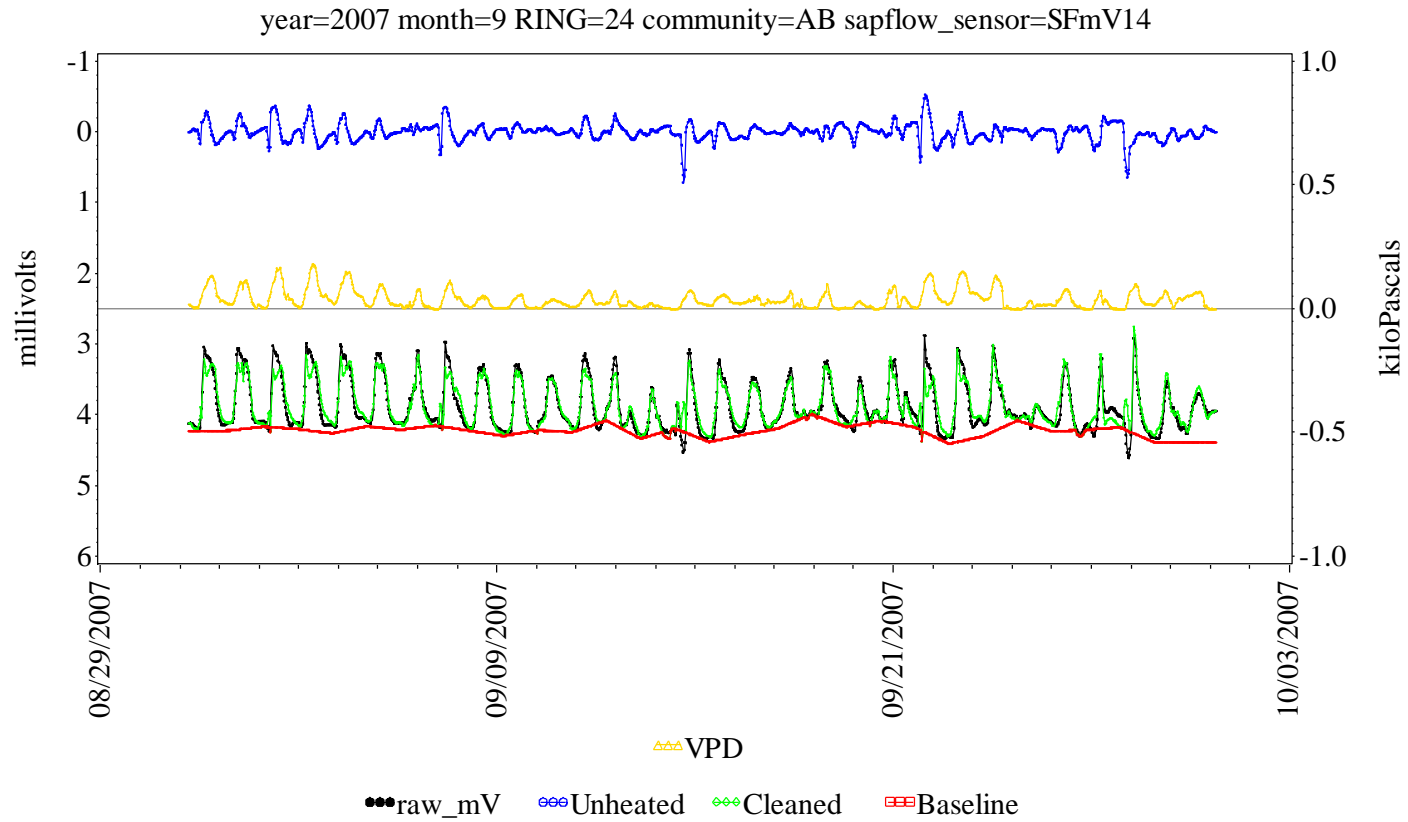


Figure 5.6. Sap flux signal correction. A model adequate to estimate the unheated component of sap flux sensor signals was developed for the present study. The model included an intercept to represent the sensor base voltage, curvilinear relationships relating the sensor response to VPD, potential PAR, and volumetric soil water content, and a linear combination of all unheated sensor signals within the same treatment as the heated sensor response being modeled. Each heated sensor was modeled separately over a period of one month to accommodate any drift in sensor responses over time.

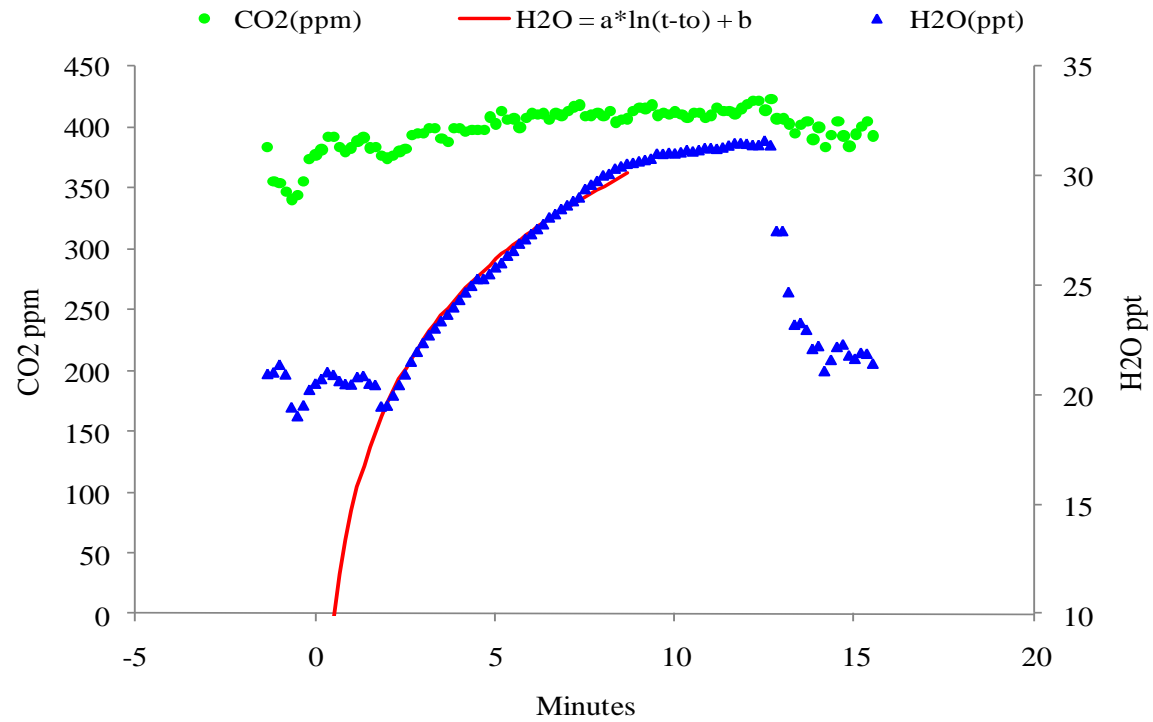


Figure 5.7. Example closed chamber data for understory ET at Aspen FACE. Data shown were collected August 3, 2008 in the all-aspen subplot of treatment ring 1.3 (replicate 1 elevated O₃ treatment). The carbon dioxide efflux (parts per million) represents the net rate of soil respiration versus understory photosynthesis. The derivative of the curve fitted to the water vapor efflux (parts per thousand) was used to recover the undisturbed water vapor efflux rate at time of initial chamber placement ($t_0 = 2$ minutes).

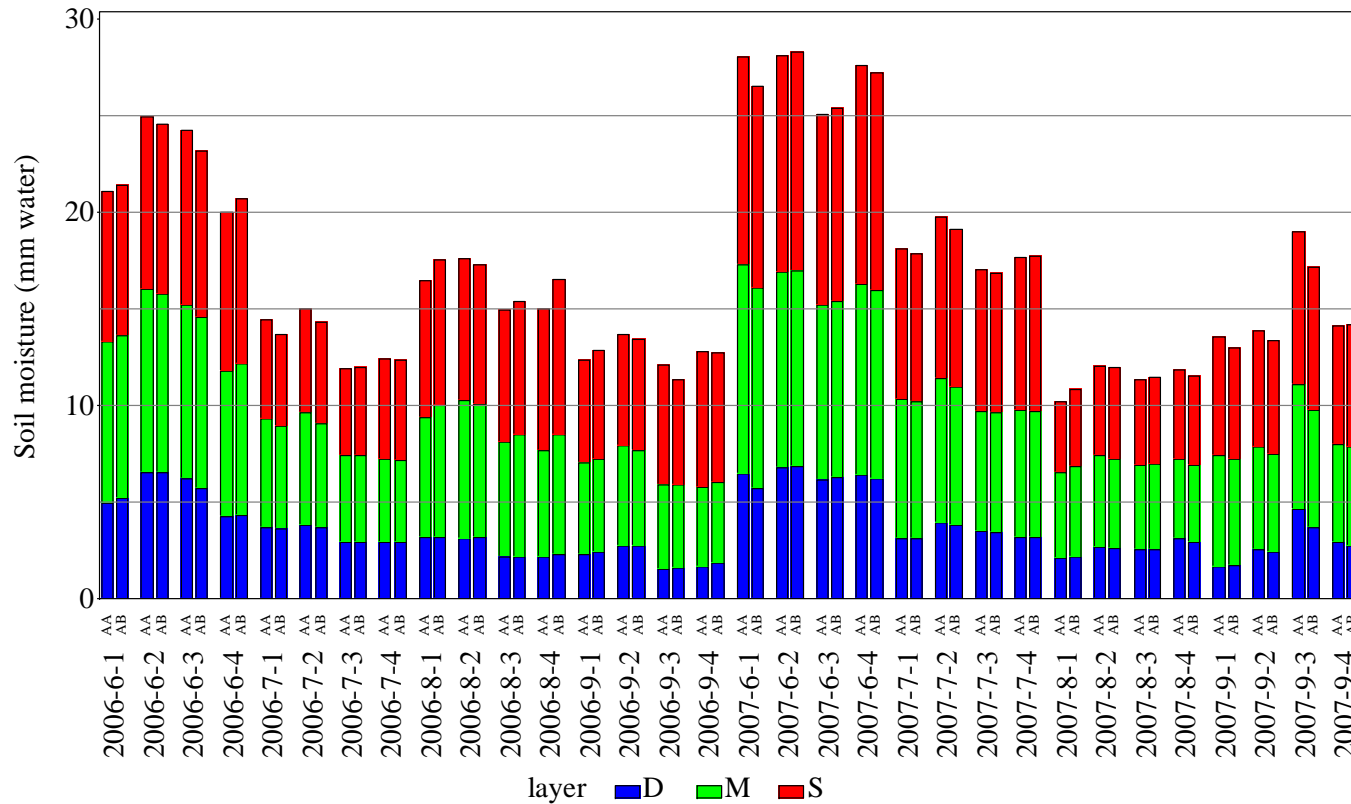


Figure 5.8. Soil water content for a 1-meter soil profile for shallow (S) (0-30 cm), middle (M) (30-70 cm) and deep (D) (70-100 cm) soil layers expressed as millimeters of water. Results for treatments (1) Control, (2) eCO₂, (3) eO₃, and (4) eCO₂×eO₃ for May through September 2006 and 2007 are grouped by all-aspen (AA) and aspen-birch (AB) communities.

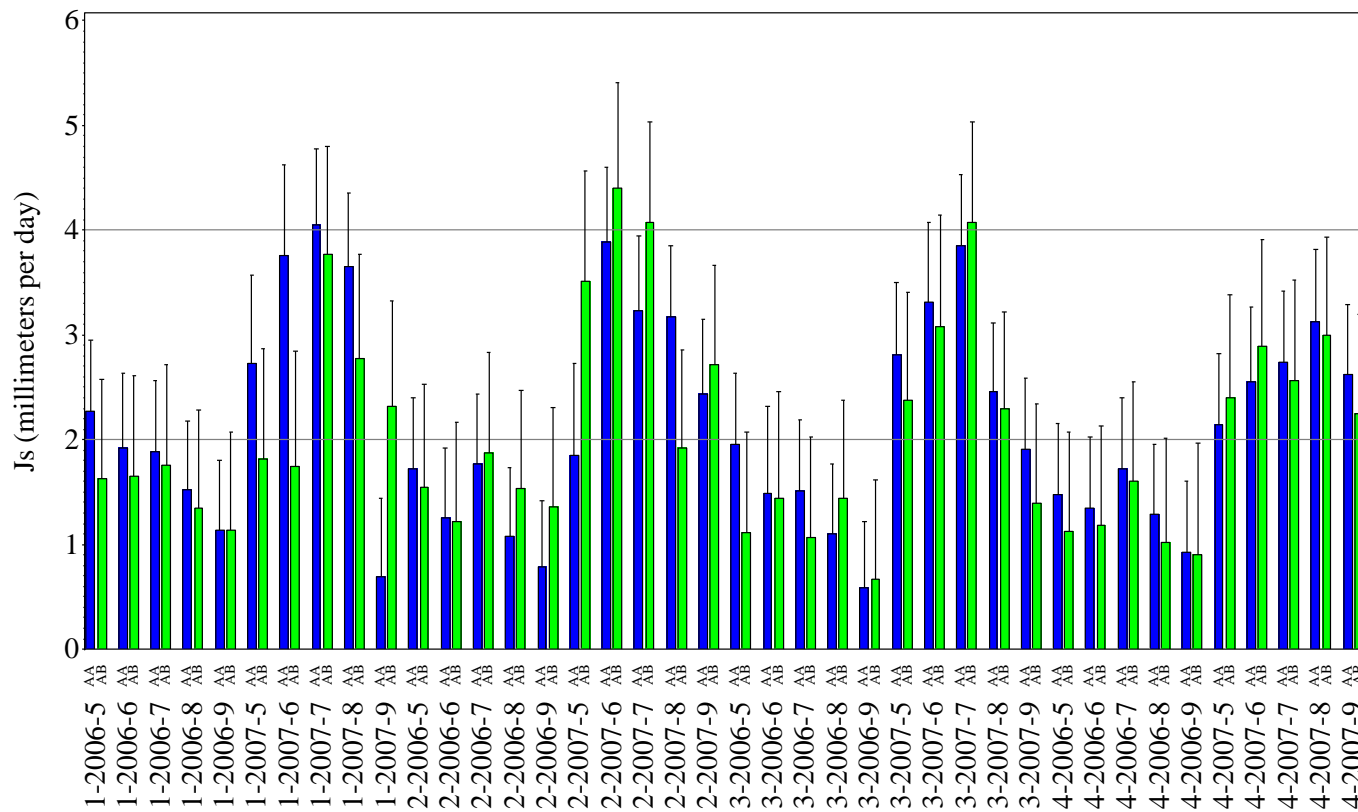


Figure 5.9. Aspen-FACE mean volumetric sap fluxes and univariate (single-factor) standard errors for instrumented trees scaled to the stand level. Results for treatments (1) Control, (2) eCO₂, (3) eO₃, and (4) eCO₂×eO₃ for May through September 2006 and 2007 are grouped by all-aspen (AA) and aspen-birch (AB) communities. Sap flows were computed using the standard Granier equation.

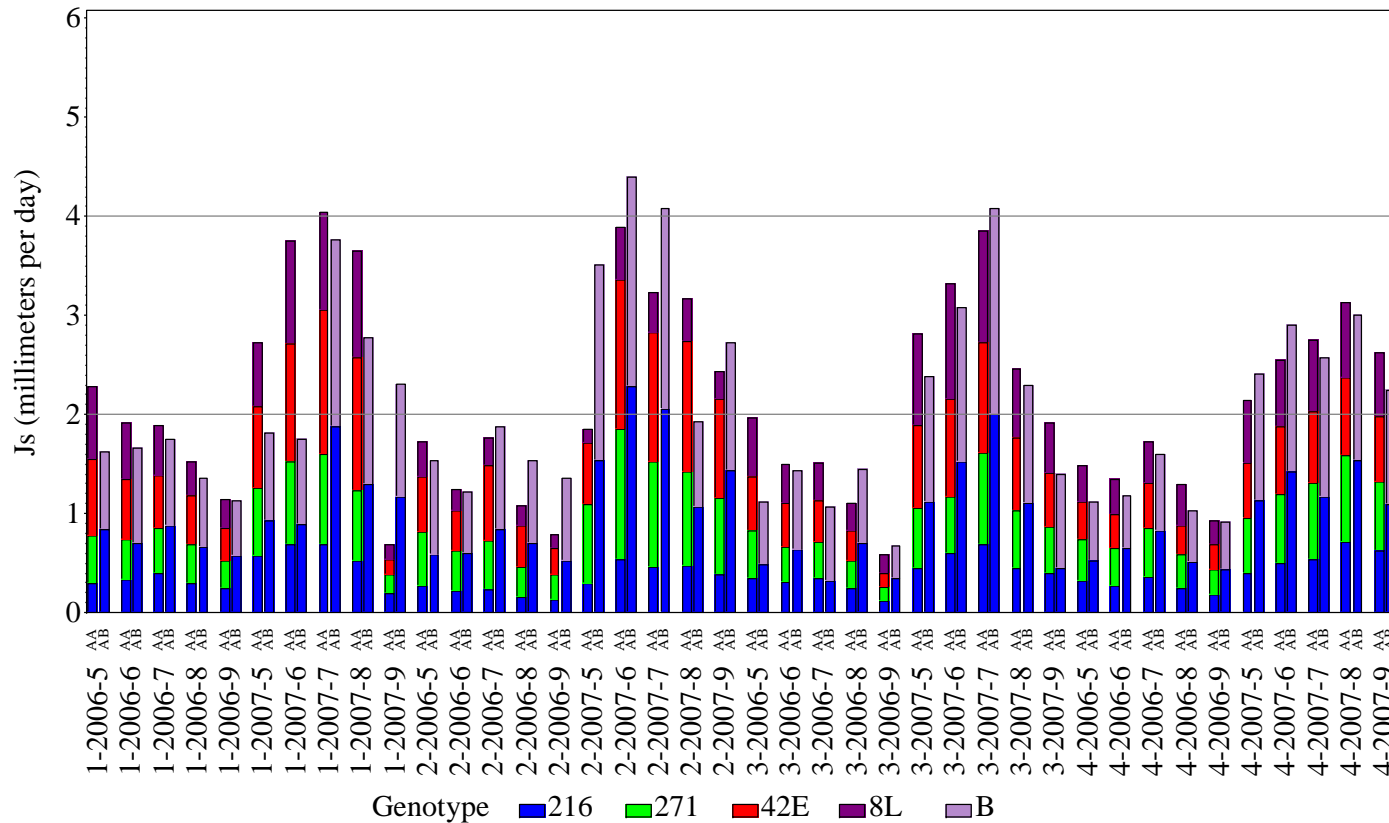


Figure 5.10. Aspen FACE mean daily volumetric sapfluxes for May-September 2006 and 2007, scaled up to all stems in treatment ring core areas. Results for treatments (1) Control, (2) eCO₂, (3) eO₃, and (4) eCO₂×eO₃ for May through September 2006 and 2007 are grouped by all-aspens (AA) and aspen-birch (AB) communities. Individual genotypic responses have been scaled down to proportions of total sap flux by community within treatment (subplot).

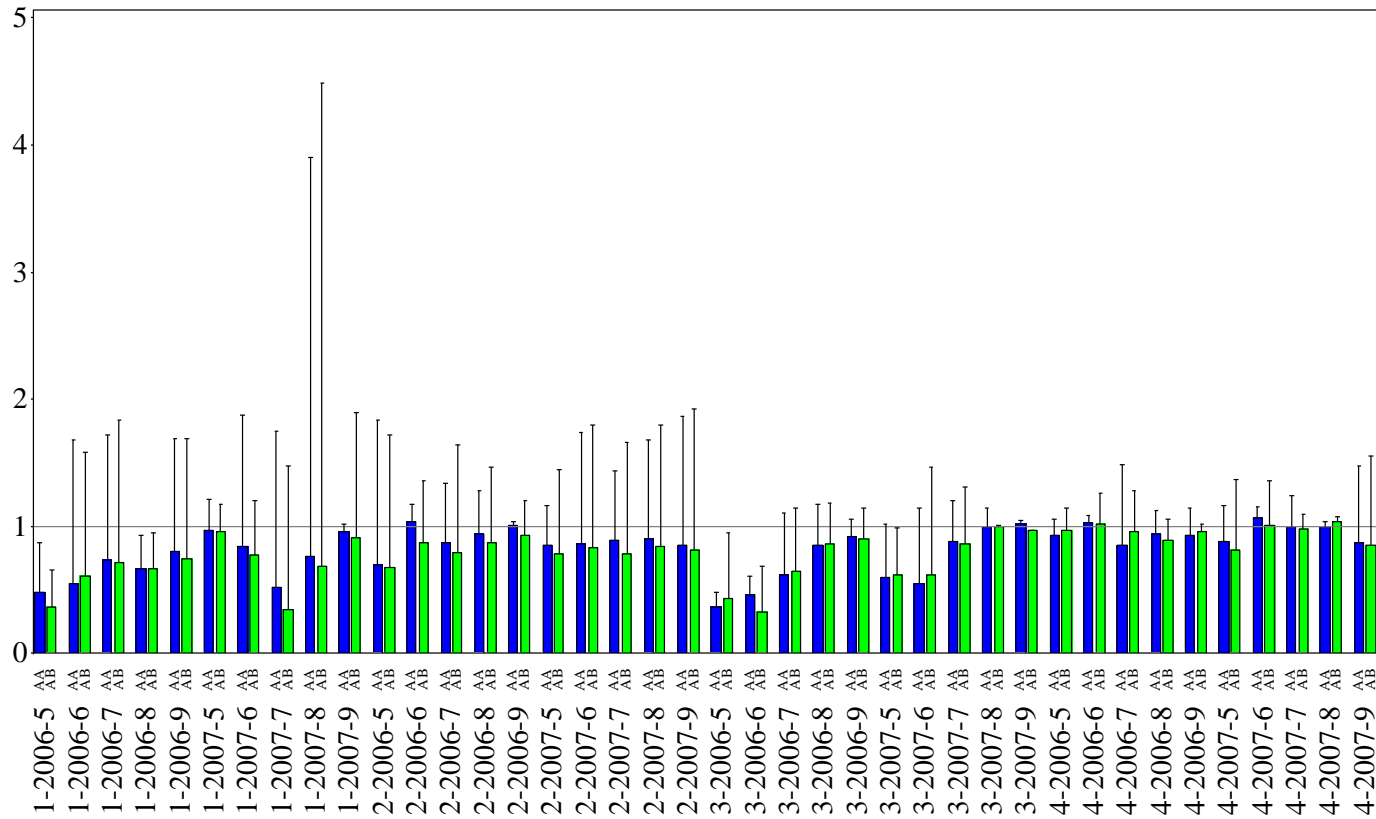


Figure 5.11. Coefficients for shallow (0-30 cm), middle (30-70 cm), and deep (70-100 cm) soil layers relating canopy sap flow to total estimated ET. Data are grouped by treatment (1 = Control, 2 = eCO₂, 3 = eO₃, 4=eCO₂ eO₃), year (2006 or 2007), month (5 through 9), and community (AA = all-aspen, AB = aspen-birch). Coefficient sums less than one indicate canopy sap flux accounts for less than total ET.

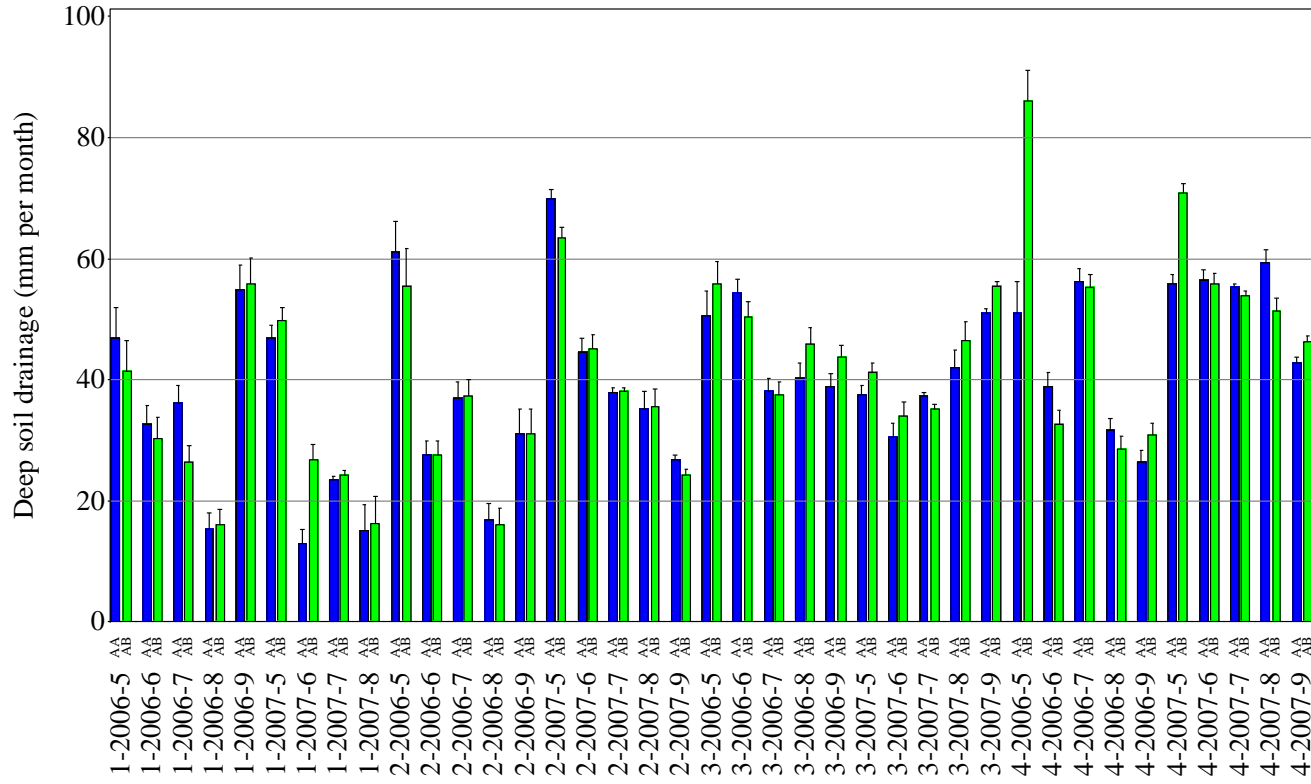


Figure 5.12. Soil drainage from deep soil (70-100 cm depth) expressed as millimeters of water. Data are grouped by treatment (1 = Control, 2 = eCO₂, 3 = eO₃, 4=eCO₂ eO₃), year (2006 or 2007), month (5 through 9), and community (AA = all-aspen, AB = aspen-birch).

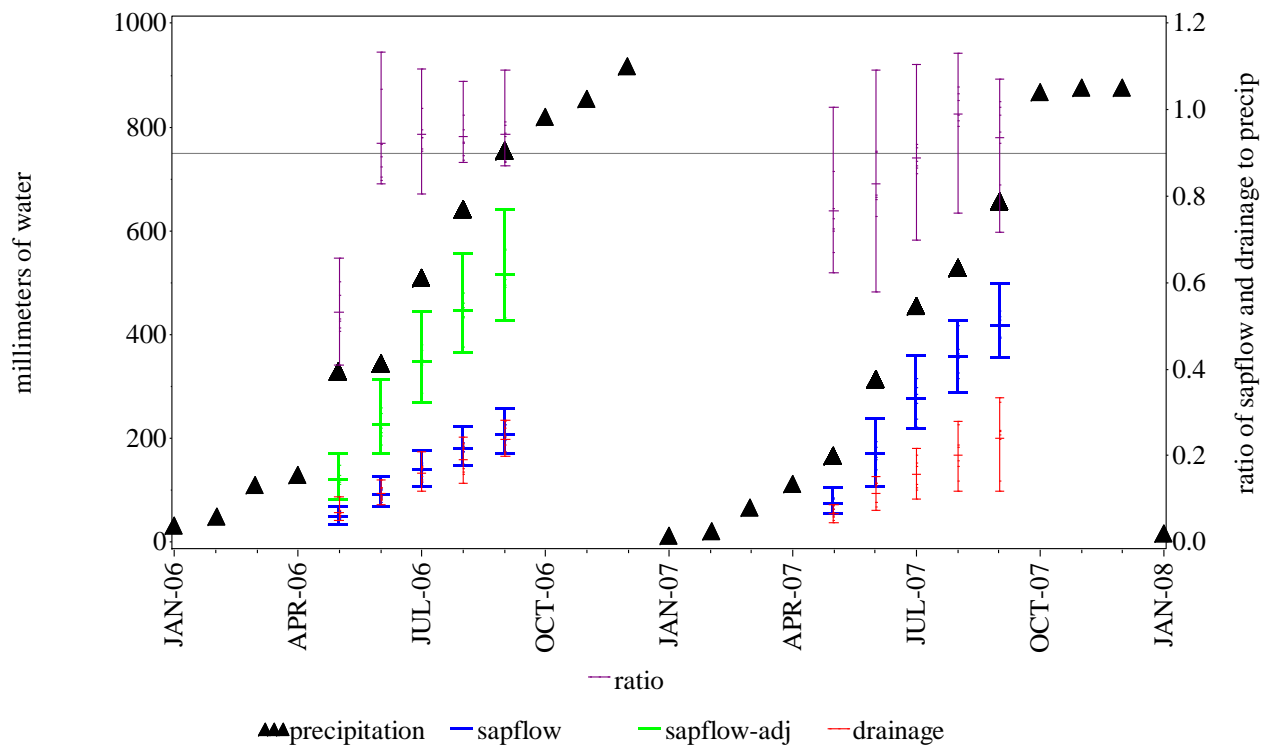


Figure 5.13. Annual cumulative precipitation, canopy transpiration (sapflow) via the method of Granier (1987), and modeled drainage at Aspen-FACE. Sapflow estimates for 2006 by Uddling et al., 2008 were similar to the values for 2006 in the present study, but based on a gravimetric flux calibration at the site Uddling et al., 2009 suggested a factor of 2.5 (sapflow-adj). Vertical bars indicate the mean and range of values that occurred for the Control, eCO₂, eO₃, and eCO₂ with eO₃ treatments within the all-aspen (AA) and aspen-birch (AB) communities. Sensors were serviced between the 2006 and 2007 growing seasons. Canopy sap flux and deep soil drainage account for about 90% of precipitation (ratio).

CHAPTER 6

Summary

The purpose of this thesis was to contribute to the understanding of the effects of projected concentrations of atmospheric CO₂ and tropospheric O₃ on the water budgets and water use of important north-temperate forest ecosystems. Seemingly minor effects in the environment are often rendered significant when scaled to large areas, so some of the statistically significant effects that may seem small here could be of practical concern. In the first study, I found that tropospheric O₃ significantly decreased total aspen and birch branch length, resulting in net decreases for 2002 whorls of -18 % and 2006 whorls of -16 %. Some of these changes had measurable effects on stem flow, and the finding that stem flow could be characterized using branch metrics independent of orientation to the stem could be of practical use to watershed managers. These metrics can be characterized using overhead or fisheye photography and image analysis software, and could therefore reduce the need for comparatively difficult or time consuming measurements such as branch orientation or DBH to characterize aboveground components of the forest hydrologic budget, such as stem flow. Under dry conditions stem flow is much more likely to escape the root zone than distributed through fall, so an accurate estimation of it may become more important as human populations experience water stress.

It is common for studies involving root biomass to focus on the most efficiently collected material nearest the ground surface. However, the second study shows that characterizing subsurface water use by stands based solely on aboveground measures and conditions in very shallow soils can miss important processes such as vertical stratification of water withdrawal, either to escape the effects of contaminants in the overlying atmosphere or as an ecological competition-escape strategy. Fumigation with O₃ increased small root biomass in shallow soil 30 % in all-aspen plots and decreased root biomass in shallow soil 46 % in aspen-birch plots, but given the study design I was unable to determine whether this was due to an adjustment across species or within aspen genotypes. Also, elevated ozone increased root length up to 131 % and specific root length up to 77 % in mid and deep soil layers, indicating more extensive soil exploration at depth, while elevated CO₂ increased small root biomass in shallow soils between 20 % to 24 %, indicative of more intensive soil exploration near the surface. Interactions between eCO₂ and eO₃ affecting root morphology were not detected. The deeper soil explorations may have revealed shifts from intensive soil explorations for nutrients within the rich surface soils, supporting increased aboveground growth, to a more extensive search strategy at depth by ozone-stressed trees seeking water or less concentrated nutrients where understory competition and possible ozone infusion would be decreased.

Much work has been done on water use rates of trees based on generalized methods and equations such as sap flux monitoring. Sap flux work is well known to be quite sensitive to the method used to scale up from the monitored trees to the stand. There has been work in recent years to refine the use of sap flux methodology, such as developing better methods for accounting for night-time fluxes and for correcting for differences in sap flux rate with sapwood depth. Although dogma tells us that the important resistances to sap water flow along the SPAC are at the roots and in or proximal to the leaves, it is also well known that xylem cavitation during water stress can limit ET. In my third study on sapwood xylem hydraulic conductivity and susceptibility to embolization under drought stress, I found that there were no significant treatment effects on hydraulic conductance at full water saturation although there was a trend for a decrease in conductance under eO_3 . Genotypic differences were evident with clones 42E and 216 both responding to all treatment combinations with increased cavitation resistance, requiring from 16 % to 85 % increased pressure to reach 50 % embolization. Clones 8L and 271 exhibited minor responses in embolizing pressure to reach K_m under eCO_2 and $eCO_2 \times eO_3$ (-3.3 % to +3.2 %), but reductions under eO_3 (-43 % to -48 % for clone 8L and -8.9 % to -14 % for clone 271). The (generally) faster growing trees under eCO_2 actually had higher

resistance to embolism while the (generally) slower growing trees under eO₃ had lower resistance to embolism. We found significant embolism in the range of 25 bars, similar to mid-day leaf tensions we measured at the study site during the relatively “typical” summer of 2007.

I believe that the water balance (deep drainage) study at Aspen-FACE provides a valuable benchmark in the struggle to answer the questions of (1) whether trees exposed to eCO₂ will either use more water to enhanced growth rates or less water due to better water use efficiency; and (2) whether stands exposed to eO₃ will use less water due to slower canopy growth and reduced stomatal conductance, or increased water use due to reduced water use efficiency and proliferation of the understory under the less light-limiting canopies. Previous workers have attempted to determine the influence on total stand water use of eCO₂ and eO₃, and found that in closed stands eCO₂ had relatively minor effects on total water use and that eO₃ results in a net decrease in water use. Yet in a study at Aspen-FACE, others found increased growth under eCO₂ and no significant change under eO₃. In my study, however, there were only weak effects of eCO₂ on sap flux but an increase in shallow soil water content, and decreased sap flux under eO₃ in conjunction with decreased soil water content in the deeper, infrequently studied soils. Somewhat surprisingly, there was also increased drainage from

deep soil under all treatment combinations, with the greatest increase occurring under eO₃.

I believe that eCO₂ may result in greater water use under open-canopy conditions, but the higher rate of water use diminishes as the stand develops and becomes light rather than CO₂ limited. It also appears that stands under the conditions we investigated probably use less water under eO₃, in spite of increased ET from the greater understory biomass afforded by additional light penetration of the canopy.

It has been posited in the literature that eO₃ could predispose plants to drought stress due to commonly observed responses that were also in the studies presented here, such as the decreased tree root growth, increased susceptibility to sapwood xylem embolism, increased understory competition, and eventual loss of stomatal control. Conversely, eCO₂ could increase tolerance to drought stress due to commonly observed responses also seen herein, such as decreased stomatal conductance and increased root growth. There is also some evidence from the studies herein, however, that inferences on forest tree response within the ecosystem based on segregated interpretation of response variables, or only on those commonly studied variables that show the greatest responses, might lead to incorrect conclusions. While the aforementioned responses were observed in the

present study, there were also compensatory responses such as increased root growth in deep soils under eO₃. Also, there have been shifts in competitive status under both eCO₂ and eO₃ documented at the site. Others have documented that aspen clone 8L grew unexceptionally under all treatment combinations except for eO₃, where ostensibly due to a competitive advantage it grew faster than it did in the Control plots. Although clone 8L was at an advantage under eO₃, it developed with greater susceptibility to xylem cavitation there and it remains untested whether this genotypic shift will result in a stand less able to withstand periodic climatic extremes such as drought or disease outbreaks. Also, while clone 42E was shown by others to be dominant in both the Control and eCO₂ plots, its increased resistance to cavitation resistance under eCO₂ could increase its tolerance to periodic stressors such as drought, potentially narrowing the genetic base for the species. Finally, the finding of significant genetic interactions amongst aspen clones for sap flows in the last study presented herein requires that caution be used in generalizing the results of this work. Shifting genotypic compositions through time might be sufficient to significantly alter stand-level characteristics such as drought tolerance, understory growth, and water use.