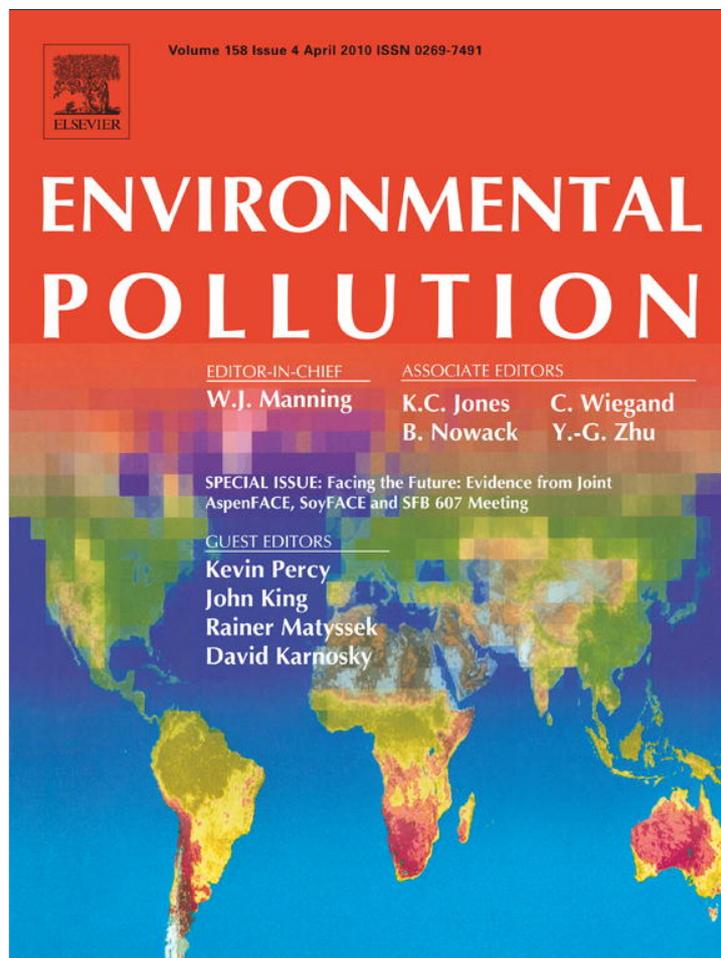


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## Effects of elevated atmospheric CO<sub>2</sub> and tropospheric O<sub>3</sub> on tree branch growth and implications for hydrologic budgeting

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Canopy architecture and stem flow are affected by elevated CO<sub>2</sub> and tropospheric O<sub>3</sub>.

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### ABSTRACT

The forest hydrologic budget may be impacted by increasing CO<sub>2</sub> and tropospheric O<sub>3</sub>. Efficient means to quantify such effects are beneficial. We hypothesized that changes in the balance of canopy interception, stem flow, and through-fall in the presence of elevated CO<sub>2</sub> and O<sub>3</sub> could be discerned using image analysis of leafless branches. We compared annual stem flow to the results of a computerized analysis of all branches from the 2002, 2004, and 2006 annual growth whorls of 97 ten-year-old trees from the Aspen Free-Air CO<sub>2</sub> and O<sub>3</sub> Enrichment (Aspen FACE) experiment in Rhinelander, WI. We found significant effects of elevated CO<sub>2</sub> and O<sub>3</sub> on some branch metrics, and that the branch metrics were useful for predicting stem flow from birch, but not aspen. The results of this study should contribute to development of techniques for efficient characterization of effects on the forest hydrologic budget of increasing CO<sub>2</sub> and tropospheric O<sub>3</sub>.

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### 1. 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 affected by forest evapotranspiration (Dingman, 1994). Atmospheric concentrations of gases, including CO<sub>2</sub> and O<sub>3</sub>, 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 CO<sub>2</sub> has been shown to increase plant growth, leaf area index and water use efficiency, while increases in tropospheric O<sub>3</sub> have been shown to decrease plant growth, leaf area display, and stomatal control (Karnosky, 2003; Karnosky et al., 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 CO<sub>2</sub> and O<sub>3</sub> on plant growth may offset one another

(Allen, 1990; Dickson et al., 1998; Volin et al., 1998; Loats and Rebbbeck, 1999; Karnosky et al., 1999), although such offsets have not been consistently 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). Measurement of interception is problematic, but is correlated to stem flow. 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; Matyssek and Sandermann, 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 angle can result from increasing CO<sub>2</sub> or O<sub>3</sub>. Significant changes in canopy architecture and species composition have been observed in some enhanced CO<sub>2</sub> studies due to alterations in the relative fitness of plant species (Reekie and Bazzaz, 1989; review by Pritchard et al., 1999). Others found lesser or insignificant canopy changes in the presence of elevated CO<sub>2</sub> (Kubiske et al., 1997; Norby et al., 2001; Gielen et al., 2002, 2003), but these studies were generally focused on leaf area, light

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interception, or stem growth and not branch architecture. None of these studies, however, investigated the influence of changes to canopy branching architecture caused by elevated CO<sub>2</sub> or O<sub>3</sub> on the hydrologic budget.

Studies relying on detailed characterization of canopy architecture are hampered by the difficulty in acquiring adequate datasets (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* [NIH]; *ImageJ* [NIH]).

During a tree harvest in the summer of 1997, we were able to generate a detailed photographic dataset for characterizing canopy architecture at the Aspen Free-Air CO<sub>2</sub> and O<sub>3</sub> Enrichment (Aspen FACE) experiment located near Rhinelander, WI. Aspen FACE was initiated in 1997 to study the effects of elevated atmospheric concentrations of CO<sub>2</sub>, O<sub>3</sub>, and their interaction on forest trees of north-temperate climates. The value of the existing information to hydrologic and forest growth modelers could be significantly increased by providing detailed information on the architecture of the tree canopies. Herein, we present the results of an analysis of changes in canopy architecture using branches collected from trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*) trees harvested during the summer of 2007, and relate those changes to the hydrologic budget through correlations of canopy changes to stem flow.

## 2. Methods

The Aspen FACE experiment consists of twelve 30-m diameter plots that have been grouped into three replicates of four treatments (randomized complete-block design). Each plot is subdivided into three subplots including a mixed trembling aspen and sugar maple community (AM), a mixed aspen and paper 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 O<sub>3</sub> sensitivity (8L = tolerant; 216 and 271 = somewhat tolerant; 42E = relatively sensitive; 259 = sensitive [Karnosky, 2003]) comparable to that which exists in natural populations (Percy et al., 2007) were randomized within the AA communities; clone 259 has since died out. Fumigation treatments are applied in the manner of the Brookhaven National Laboratory's design (Hendrey et al., 1999). Each ring is surrounded by PVC standpipes that are used to fumigate the ring, and wind/gas sensors within the ring are used to control the fumigation rate. The fumigation treatments include: (1) ambient conditions; (2) elevated CO<sub>2</sub> (~560 ppm); (3) elevated O<sub>3</sub> (~60 ppb, or 1.5 times ambient); and (4) both elevated CO<sub>2</sub> and O<sub>3</sub>. Fumigation has been 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](http://www.aspenface.mtu.edu).

### 2.1. Tree sample acquisition

Branch data included in the present study were collected during a harvest conducted at the Aspen FACE during August 2007. Stem flow data were collected throughout the 2007 and 2008 growing seasons, and diameter at breast height (d.b.h.) was also measured after leaf fall during the autumns of 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 rings where edge effects are mitigated and atmospheric mixing reduces variation in treatment concentrations of CO<sub>2</sub> and O<sub>3</sub>. The sample trees were measured for d.b.h., 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, and each branch emanating from a 2002, 2004, or 2006 annual stem increment ("whorl" hereafter) 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 4 m and small branches were photographed at a nominal distance of 1 m. Scales were included at multiple focal lengths in the periphery of each photograph to aid conversion of pixel counts to branch lengths.

### 2.2. 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<sup>®</sup> 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.

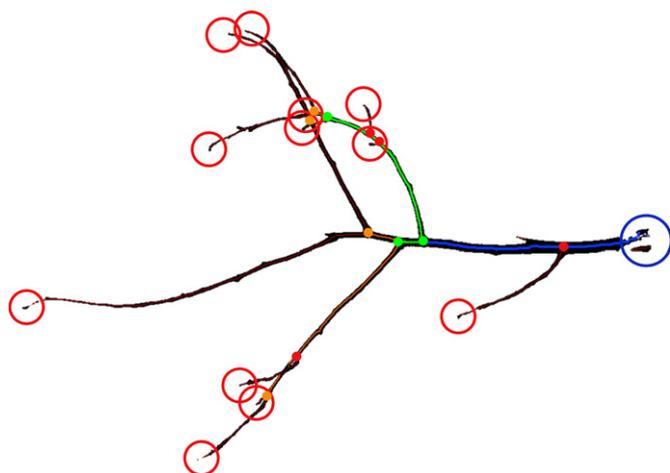
Initially, roi were reduced to a one-pixel-wide "skeleton" by iteratively eroding edge pixels until no areas of greater than one-pixel width along their minor axis remained. The skeleton was then superimposed on the parent, thresholded image. A minor radius was calculated for each pixel in the skeleton 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 (Fig. 1). 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.

### 2.3. Stem flow monitoring

Stem flows were monitored for at least one tree per genotype per subplot during the 2007 and 2008 growing seasons. Clear, 0.75-inch diameter nylon tubing was cut into 1-meter segments and split along 0.7 m of each segment using



**Fig. 1.** Example output image from the branch image analysis macro. The straight-line length of the branch is approximately 46 cm from the base to the farthest tip. Tips are indicated by red circles and the root base is indicated by a blue circle. Segment intersections (nodes) are indicated by solid dots. Actual segment lengths are corrected for tip location erosion during the image skeletonization process. Segments are differentiated by overlay color and overlay width is scaled to segment branch order. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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 sealed 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. As a result of overfills that occurred during several heavy storm events during the summer of 2007, stem flow was monitored during the 2008 growing season using larger catchment containers.

#### 2.4. 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, and the stem flow data were compared to stem d.b.h. and branch metrics using Pearson product moment and Spearman rank correlations. The ANOVA was based on individual branches to determine whether treatment effects on morphometrics such as branching length and volume were discernable. The purpose of the correlations was to discern the utility of using the branch metrics and d.b.h. to better discern treatment effects on stem flows, and thereby on ET and the hydrologic budget as a whole.

The ANOVA model for the data was based on that described by King et al. (2001). The CO<sub>2</sub> and O<sub>3</sub> 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 influenced 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).

All parameters included in the correlations were transformed as described above for the ANOVA prior to performing any other calculations. The significance of the Pearson moment-based and Spearman rank-based correlations were determined using Fischer Z-transformations. Correlations were considered significant at a *p*-value threshold of 0.05.

### 3. Results

#### 3.1. All-aspen community branch analysis

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 presented in Table 1.

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 (Table 1). Branch magnitude increased for most whorls under all treatments, but due to high variance, only significantly under elevated O<sub>3</sub> (35%, *p* = 0.043) (Table 2).

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 (Table 1). Branch order was significantly affected by genotype for 2004 whorls (*p* = 0.022) and CO<sub>2</sub> for 2006 whorls (*p* = 0.050) (Table 2). 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 CO<sub>2</sub> 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 (Table 1). The average length of first-order branch segments of the clones for 2004 whorls was differentially affected by elevated O<sub>3</sub> (O<sub>3</sub>\*clone *p* = 0.025) (Table 2). 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 (Table 1). The radii of the first-order segments of the 2004 whorls was affected by O<sub>3</sub> differentiable by clone (O<sub>3</sub>\*clone *p* = 0.047) and was also decreased overall by O<sub>3</sub> (O<sub>3</sub> *p* = 0.012) (Table 2). Clones 271 and 42E were reduced overall in the presence of the interaction by 46% and 66%, while the remaining clones were not significantly affected. Added O<sub>3</sub> 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 (Table 1). A significant CO<sub>2</sub> effect (*p* = 0.001) for overall branch length within the 2006 whorls was detected, but occurred in the presence of an interaction with genotype (*p* = 0.030) (Table 2). 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 CO<sub>2</sub> (216 = 14 cm; 42E = 8.5 cm; 271 = 2.6 cm; 8L = 1.2 cm). 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 CO<sub>2</sub>.

#### 3.2. Aspen-birch community branch analysis

Tree species significantly differentiated the treatment responses in the aspen-birch (AB) communities of 2004-whorl branch

**Table 1**  
Unadjusted (ordinary) means (standard errors) for branch metrics of 2002, 2004, and 2006 growth whorls of trembling aspen grown in an all-aspen community. The notation “–” indicates statistics not available.

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 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)
	+CO <sub>2</sub>	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)
		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)
	+O <sub>3</sub>	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)
	+CO <sub>2</sub> & O <sub>3</sub>	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)
		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)
	+CO <sub>2</sub>	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)
		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)
	+O <sub>3</sub>	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(–)
	+CO <sub>2</sub> & O <sub>3</sub>	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)
		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(–)

segments ( $p = 0.002$ ) and branching order ( $p = 0.014$ ), and was involved in significant interactions with elevated O<sub>3</sub> 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<sub>2</sub> ( $p = 0.036$ ) 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 3). The number of member segments per branch for the 2002 whorls was differentially affected between species (O<sub>3</sub>\*species  $p = 0.050$ ) (Table 4), with the LS-mean for aspen decreasing by 19% and that for birch increasing by 25% in the presence of elevated O<sub>3</sub>. The number of member segments per branch for the 2004 whorls was increased 39% by elevated CO<sub>2</sub> ( $p = 0.036$ ). An apparent effect of species for the 2004 whorls ( $p = 0.002$ ) was due to a near-significant interaction between CO<sub>2</sub> and species ( $p = 0.104$ ); after orthogonalization to other factors, 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; the respective

altitudes for birch were 11, 10, and 4 (Table 3). The branch altitude for the 2002 whorls of aspen and birch was differentially affected (O<sub>3</sub>\*species  $p = 0.026$ ) (Table 4), with elevated O<sub>3</sub> associated with an orthogonalized-effect 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; the respective orders for birch were 2.9, 3.2, and 1.6 (Table 3). The branch order of the 2004 whorls was slightly but significantly affected species ( $p = 0.014$ ) (Table 4), with birch having a higher average order than aspen (3.2 versus 2.8).

### 3.3. Branch cross-community responses

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

### 3.4. Branch metric correlations to d.b.h. and stem flow

Stem flow data for the 2008 growing season are summarized graphically in Fig. 2 and significant correlations ( $p < 0.05$ ) among

**Table 2**  
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 <sub>2</sub>	ns	ns	ns	ns	0.050	0.001
O <sub>3</sub>	0.043	ns	ns	0.012	ns	ns
Genotype	ns	0.022	ns	ns	ns	ns
CO <sub>2</sub> *O <sub>3</sub>	ns	ns	ns	ns	ns	ns
CO <sub>2</sub> *Genotype	ns	ns	ns	ns	ns	0.030
O <sub>3</sub> *Genotype	ns	ns	0.025	0.047	ns	ns
CO <sub>2</sub> *O <sub>3</sub> *Genotype	ns	ns	ns	ns	–	–

**Table 3**

Unadjusted (ordinary) 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. The notation “–” indicates statistics not available.

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 <sub>2</sub>	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 <sub>3</sub>	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 <sub>2</sub> & O <sub>3</sub>	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 <sub>2</sub>		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 <sub>3</sub>		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 <sub>2</sub> & O <sub>3</sub>		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(–)	

stem flow, d.b.h., and branch metrics are provided in Table 5. In the all-aspen community, significant Pearson (linear) and Spearman (rank) correlations to stem flow were indicated only for d.b.h. and branch volume, and the only significant correlation between d.b.h. and branch metrics was for the number of segments per branch (rank c.c. 0.33). The d.b.h. of the trees monitored for stem flow exhibited far better correlation to stem flow (c.c. 0.90–0.87) than branch volume (c.c. 0.36). For aspen in the aspen-birch community, only d.b.h. was significantly correlated to stem flow (c.c. 0.90–0.86) and there were no correlations between d.b.h. and branch metrics. For birch, however, the only parameters significantly correlated to stem flow were the branch metrics branch length (rank c.c. 0.61), branch magnitude (rank c.c. 0.63), and branch altitude (c.c. 0.87). In addition, there were several significant correlations between d.b.h. and branch metrics, including branch order (c.c. 0.69–0.64), branch magnitude (rank c.c. 0.61), and branch altitude (c.c. 0.73–0.64). These correlations occurred with d.b.h. of both trees not directly included in the branch analyses (those measured for stem flow) as well as those harvested for the branch analyses.

**Table 4**

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.

Effect	2002		2004	
	Branch segments	Branch altitude	Branch segments	Branch order
CO <sub>2</sub>	ns	ns	0.036	ns
O <sub>3</sub>	ns	ns	ns	ns
Species (genotype)	ns	ns	0.002	0.014
CO <sub>2</sub> *O <sub>3</sub>	ns	ns	ns	ns
CO <sub>2</sub> *Species	ns	ns	ns	ns
O <sub>3</sub> *Species	0.050	0.026	ns	ns

**4. Discussion**

The purpose of the present study was to determine if changes in branch architecture were caused by long-term exposure to elevated CO<sub>2</sub> or O<sub>3</sub>, and if that could impact the hydrologic budget through changes in apportionment of precipitation between interception, stem flow, and through-fall. Changes in the hydrologic budget due to O<sub>3</sub> have previously been reported by McLaughlin et al. (2007a,b), but their work was based on stem growth, sap flow, soil moisture, and stream base flow data rather than canopy architecture.

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).

The growth-enhancement effects of CO<sub>2</sub> 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’, *Populus alba* 2AS11, and *Populus xeuroamericana* I-214 conducted at the POPFACE CO<sub>2</sub> 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 CO<sub>2</sub> enrichment effects largely disappeared after canopy closure. Consistent with this interpretation, Norby et al. (2001) observed no effects of CO<sub>2</sub> 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 sweet gum experiment after the first, but not the second, year of treatment. Finally, in a multi-year study of the effects of CO<sub>2</sub> and O<sub>3</sub> on canopy, stem, and root

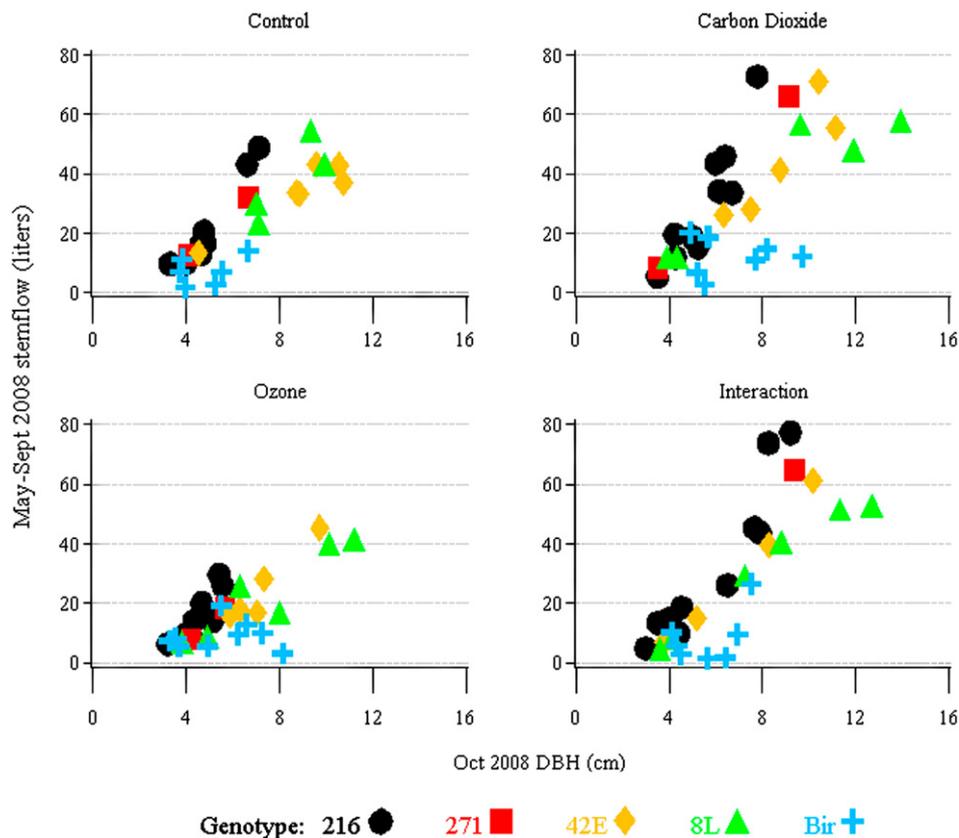


Fig. 2. Graphical summary of stem flow data for May through September 2008 at the Aspen FACE experiment located in Rhineland WI.

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 CO<sub>2</sub> (Körner, 2006), but this does not appear to be so for O<sub>3</sub> (Karnosky et al., 2007). The effects of O<sub>3</sub> found in the present study are generally consistent with previously reported reductions in overall growth under added O<sub>3</sub> at the site and the improved relative performance of the O<sub>3</sub>-resistant aspen clone 8L in the presence of added O<sub>3</sub> (Karnosky et al., 2005; Percy et al., 2007).

Although many previous studies at Aspen FACE have identified significant increases in growth metrics attributable to elevated CO<sub>2</sub> (Karnosky et al., 2005), in the present study the only significant increase associated with elevated CO<sub>2</sub> was for the number of segments per branch from 2004 whorls in the AB community. Elevated CO<sub>2</sub> 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 CO<sub>2</sub> treatment plots. Yet, elevated CO<sub>2</sub> 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. Perhaps the effects of CO<sub>2</sub> observed in the present study 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 CO<sub>2</sub> by Darbah et al. (2007).

In the present study, within the AA community elevated O<sub>3</sub> was associated with significant decreased lengths of first-order segments of branches from 2004 whorls of 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 O<sub>3</sub> are consistent with the generally observed reduction of growth. The increases other than that for branch magnitude were probably due to the increased relative performance of relatively O<sub>3</sub>-tolerant clones in the presence of competition with less O<sub>3</sub> tolerant genotypes, although Percy et al. (2007) mentions the possibility of hormetic O<sub>3</sub> effects.

Significant effects of O<sub>3</sub> 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 O<sub>3</sub>. 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 O<sub>3</sub>. 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 O<sub>3</sub> than aspen.

For aspen, in the all-aspen community the stem d.b.h. exhibited far higher correlation to stem flow (c.c. 0.90–0.87) than the only significantly correlated branch metric, branch volume

**Table 5**

Pearson product moment correlation coefficients (above diagonal) and Spearman rank correlation coefficients (below diagonal) between stem flow and: stem diameters of trees monitored for stem flow; stem diameters of trees harvested for the canopy branch characterization; and, canopy branch characterization parameters. All data were transformed for normality unless otherwise noted ("raw"). Effects are considered significant at  $p < 0.05$ . The abbreviation "ns" indicates non-significant correlations.

	Stem flow dia	Harvest dia	Stem flow	Segment count	Branch length	Branch volume	Branch order	Branch magnitude	Branch altitude	Branch ave angle
<b>AA Community Aspen</b>										
Stem flow stem dia		ns	0.87	ns	ns	ns	ns	ns	ns	ns
Harvest stem dia	ns		ns	ns	ns	ns	ns	ns	ns	ns
STEM FLOW	0.90	ns		ns	ns	0.36	ns	ns	ns	ns
Segment count	ns	0.33	ns		0.84	0.71	0.61	0.90	0.81	ns
Branch length	ns	ns	ns	0.87		0.88	0.59	0.84	0.82	ns
Branch volume	ns	ns	0.36	0.75	0.89		0.49	0.74	0.69	ns
Branch order	ns	ns	ns	0.58	0.54	0.47		0.67	0.58	ns
Branch magnitude	ns	ns	ns	0.89	0.83	0.74	0.59		0.89	ns
Branch altitude	ns	ns	ns	0.85	0.81	0.65	0.57	0.90		ns
Branch ave angle	ns	ns	ns	ns	ns	ns	ns	ns	ns	
<b>AB Community Aspen</b>										
Stem flow stem dia		ns	0.90	ns	ns	ns	ns	ns	ns	ns
Harvest stem dia	ns		ns	ns	ns	ns	ns	ns	ns	ns
STEM FLOW	0.86	ns		ns	ns	ns	ns	ns	ns	ns
Segment count	ns	ns	ns		0.96	0.88	0.70	0.88	0.75	ns
Branch length	ns	ns	ns	0.94		0.93	0.69	0.89	0.74	ns
Branch volume	ns	ns	ns	0.92	0.95		0.68	0.80	0.76	ns
Branch order	ns	ns	ns	0.69	ns	0.64		0.76	0.67	ns
Branch magnitude	ns	ns	ns	0.87	0.83	0.82	0.88		0.89	ns
Branch altitude	ns	ns	ns	0.83	0.70	0.73	0.71	0.83		ns
Branch ave angle	ns	ns	ns	ns	ns	ns	ns	ns	ns	
<b>AB Community Birch</b>										
Stem flow stem dia		ns	ns	ns	ns	ns	ns	ns	0.73	ns
Harvest stem dia	ns		ns	ns	ns	ns	0.64	ns	ns	ns
STEM FLOW	ns	ns		ns	ns	ns	ns	ns	0.87	ns
Segment count	ns	ns	ns		0.86	ns	0.87	0.81	ns	ns
Branch length	ns	ns	0.61	0.75		0.74	0.75	0.83	ns	ns
Branch volume	ns	ns	ns	ns	0.80		ns	ns	0.62	ns
Branch order	ns	0.69	ns	0.85	0.78	ns		0.78	ns	ns
Branch magnitude	0.61	ns	0.63	0.78	0.81	ns	0.70		0.66	ns
Branch altitude	0.64	ns	0.87	ns	0.70	0.66	ns	0.74		ns
Branch ave angle	ns	ns	ns	ns	ns	ns	ns	ns	ns	

(c.c. 0.36), and d.b.h. maintained this correlation within the aspen-birch community where no branch metrics were significantly correlated to stem flow. In contrast, d.b.h. was not significantly correlated to stem flow in the birch community, while significant correlations to stem flow were exhibited by branch length (rank c.c. 0.61), branch magnitude (rank c.c. 0.63) and branch altitude (c.c. 0.87). Thus, while branch metrics for the aspen included in this study appeared to be poor predictors of stem flow, they appeared to be quite valuable for predicting the stem flow of birch. Bark characteristics are known to influence stem flow (Xiao, 2000). 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.

## 5. Conclusions

In this study, several effects of elevated CO<sub>2</sub> and O<sub>3</sub> on branch architecture and stem flow were found using automated analysis of branch metrics, and branch metrics were shown to be useful predictors of stem flow for birch but not aspen. Significant treatment interactions and effects on branch architecture involving the main effect of CO<sub>2</sub> and O<sub>3</sub> with genotype, are a probable result of post-canopy closure light-competition in the control, +CO<sub>2</sub>, and +CO<sub>2</sub> + O<sub>3</sub> treatments reducing the growth-enhancement effects of CO<sub>2</sub>, and differing genotypic tolerance to O<sub>3</sub>. Canopy closure at the site has been described as having occurred circa 2003–2005 (Kostiainen et al., 2008). This is generally consistent with the

observations of site conditions by the authors, with the caveat that closure was visibly less advanced in the +O<sub>3</sub> plots in 2007, where significantly more light was able to penetrate to the understory (unpublished data).

For the present study, adequate canopy closure in the control, +CO<sub>2</sub>, and +CO<sub>2</sub> + O<sub>3</sub> may have been present in 2007 to reduce the growth-enhancement effects of CO<sub>2</sub> and decouple the canopy size from the stem volume of aspen (King et al., 2005) due to inter-tree competition for light, whereas the more open canopies in the +O<sub>3</sub> plots still allowed for the O<sub>3</sub> treatment effect to influence the trees with less interference from competition for light. Thus, responsiveness to CO<sub>2</sub> that may have been more apparent in prior years at the site could have been masked by canopy closure in 2007, while responsiveness to O<sub>3</sub> could have remained more apparent due to lesser inter-tree competition or an effectively less mature canopy. It is also possible that trees reached maturity faster in the presence of elevated CO<sub>2</sub> or the absence of elevated O<sub>3</sub>, resulting in reduction of carbon partitioning to branch growth.

The findings in the present study of significant treatment effects on branch architectural parameters, and correlations between canopy architecture parameters and stem flows, indicate that elevated CO<sub>2</sub> and O<sub>3</sub> have the potential to impact the forest hydrologic cycle. Given that many treatment effects on growth-related parameters at FACE sites seem to be small or insignificant after canopy closure (Norby et al., 2001; Gielen et al., 2002, 2003), and that stem diameters become uncoupled from canopy LAI after closure (King et al., 2005), branch architecture might be useful for characterizing changes in the forest hydrologic budget due to

increased concentrations of CO<sub>2</sub> and O<sub>3</sub> in the atmosphere. However, more efficient means of characterizing canopy architecture than the methods used in this study, such as remote-sensing-based technologies, would probably be needed for large-scale applications.

Based on the results of the present study, concentrations of CO<sub>2</sub> and tropospheric O<sub>3</sub> expected to occur within the century apparently have the potential to impact the forest hydrologic cycle. Branch architecture might be useful for characterizing such changes if practical methods of characterizing it become available.

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