Sap flux in pure aspen and mixed aspen–birch forests exposed to elevated concentrations of carbon dioxide and ozone

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Summary Elevated concentrations of atmospheric carbon dioxide ([CO₂]) and tropospheric ozone ([O₃]) have the potential to affect tree physiology and structure and hence forest water use, which has implications for climate feedbacks. We investigated how a 40% increase above ambient values in [CO₂] and [O₃], alone and in combination, affect tree water use of pure aspen and mixed aspen–birch forests in the free air CO₂–O₃ enrichment experiment near Rhinelander, Wisconsin (Aspen FACE). Measurements of sap flux and canopy leaf area index (L) were made during two growing seasons, when steady-state L had been reached after more than 6 years of exposure to elevated [CO₂] and [O₃]. Maximum stand-level sap flux was not significantly affected by elevated [O₃], but was increased by 18% by elevated [CO₂] averaged across years, communities and O₃ regimes. Treatment effects were similar in pure aspen and mixed aspen–birch communities. Increased tree water use in response to elevated [CO₂] was related to positive O₃ treatment effects on tree size and L (+40%). Tree water use was not reduced by elevated [O₃] despite strong negative O₃ treatment effects on tree size and L (–22%). Elevated [O₃] predisposed pure aspen stands to drought-induced sap flux reductions, whereas increased tree water use in response to elevated [CO₂] did not result in lower soil water content in the upper soil or decreasing sap flux relative to control values during dry periods. Maintenance of soil water content in the upper soil in the elevated [CO₂] treatment was at least partly a function of enhanced soil water-holding capacity, probably a result of increased organic matter content from increased litter inputs. Our findings that larger trees growing in elevated [CO₂] used more water and that tree size, but not maximal water use, was negatively affected by elevated [O₃] suggest that the long-term cumulative effects on stand structure may be more important than the expected primary stomatal closure responses to elevated [CO₂] and [O₃] in determining stand-level water use under possible future atmospheric conditions.

Keywords: climate, FACE, leaf area index, soil water, transpiration, tree community.

Introduction

There is currently great interest in understanding how vegetation properties affect local and regional climate, as well as how atmospheric forcing of ecosystem physiology and structure may feed back to affect climate–vegetation interactions with changing atmospheric trace gas composition (Cramer et al. 2001, Felzer et al. 2005). Of the radiative-forcing trace gases (greenhouse gases) altered by human activities (IPCC 2007), only carbon dioxide (CO₂) and tropospheric ozone (O₃) have the potential to strongly affect plant canopy physiology and structure, and hence feedbacks with climate. The increases in concentrations of atmospheric CO₂ ([CO₂]) and tropospheric O₃ ([O₃]) observed in recent decades and predicted for the coming decades (Dentener et al. 2006, IPCC 2007) may portend large alterations in plant canopy function, particularly for forests (Cramer et al. 2001, Felzer et al. 2005). Given that plant transpiration comprises a major regulator of mass and energy exchange between vegetation and the atmosphere (Baldocchi and Meyers 1998), knowledge of the long-term impacts of increasing atmospheric CO₂ and O₃ concentrations on stand-level tree water use is critical to inform models for predicting the feedbacks between forests and climate under possible future atmospheric conditions.

Higher atmospheric [CO₂] as well as O₃-induced photosynthetic impairment (Reich and Amundson 1985) act to increase the intercellular [CO₂] in leaves. Stomatal conductance (gₛ) might therefore be expected to decrease in response to both higher [CO₂] and [O₃], as has been consistently observed in most chamber studies (e.g., Norby et al. 1999, Wittig et al. 2007). Reduced gₛ and resulting water savings at the leaf-level,
in the absence of other major adjustments, may permit tree canopies to retain greater leaf area because steady-state canopy leaf area index (L) is dependent on resource availability (e.g., Woodward 1987). Photosynthetic enhancement in response to elevated \( \text{CO}_2 \) (Noormets et al. 2001, Ellsworth et al. 2004) should also act to increase L by improving leaf carbon balance, whereas the opposite would be expected in response to increased \( \text{O}_3 \) (Ollinger et al. 2002). Furthermore, leaves that are damaged by \( \text{O}_3 \) are prematurely lost in many tree species (e.g., Matyssek and Sandermann 2003). These responses suggest that, at equilibrium, stand-level tree water use should be reduced by increased \( \text{O}_3 \), whereas the effect of increased atmospheric \( \text{CO}_2 \) depends on the magnitudes of the opposing effects on \( g_s \) and L.

Although large decreases in \( g_s \) and increases in total leaf area have typically been observed in elevated \( \text{CO}_2 \) experiments with tree seedlings grown in controlled environments (e.g., Norby et al. 1999), the generality of these responses and their implications for stand-level tree water use have been challenged by recent findings from free-air \( \text{CO}_2 \) enrichment (FACE) experiments in forests. There were no significant \((P < 0.1)\) treatment effects on \( g_s \) or L in a Pinus taeda L. forest (Ellsworth 1999, Schäfer et al. 2002) or in a multi-species mature deciduous forest (Körner et al. 2005, Keel et al. 2007), resulting in mostly nonsignificant effects of the elevated \( \text{CO}_2 \) treatments on stand-level sap flux in these experiments (Schäfer et al. 2002, Cech et al. 2003). Similarly, there was likely no net effect of elevated \( \text{CO}_2 \) on stand-level tree water use in a plantation with fast-growing Populus spp., because neither \( L \) (Gielen et al. 2001) nor \( g_s \) (Bernacchi et al. 2003) was significantly affected by the elevated \( \text{CO}_2 \) treatment after canopy closure. In a Liquidambar styraciflua L. plantation, however, stand sap flux was reduced 13% by elevated \( \text{CO}_2 \) as a result of substantial reductions in \( g_s \) (Wullschleger and Norby 2001, Gunderson et al. 2002, Wullschleger et al. 2002). Little is known about the effects of \( \text{O}_3 \) on stand-level tree water use and steady-state L, because most experiments have been conducted on young plants growing in open-top chambers (Matyssek and Sandermann 2003).

Given the uncertainties concerning the mechanisms and magnitudes of effects of elevated \( \text{CO}_2 \) and elevated \( \text{O}_3 \) on stand-level tree-water use and the importance of both trace gases in industrialized regions, we undertook measurements of xylem sap flux and L within the Aspen FACE experiment (Rhinelander, Wisconsin, USA). We designed our experiment to assess how elevated \( \text{CO}_2 \) and \( \text{O}_3 \) alone and in combination, affect stand-level tree water use, and the extent to which these effects are related to changes in L. We employed sap flow methodology (Granier 1987) to quantify stand tree water use in aspen and aspen–birch communities in the Aspen FACE experiment during two contrasting growing seasons (i.e., relatively cool and moist versus relatively warm and dry) after more than 6 years of exposure to elevated \( \text{CO}_2 \) and \( \text{O}_3 \). Specifically, we sought answers to three questions. Is there an effect of elevated \( \text{CO}_2 \) on stand-level tree sap flux, and if so, how is it related to changes in leaf area index? Does elevated \( \text{O}_3 \) significantly reduce stand-level tree sap flux, and are there interactions between elevated \( \text{CO}_2 \) and \( \text{O}_3 \) on tree water use? Does species composition affect sap flux responses to elevated \( \text{CO}_2 \) and \( \text{O}_3 \)?

Materials and methods

Site and meteorological instrumentation

The Aspen FACE experiment near Rhinelander, Wisconsin (45°36'N, 89°30' W) has a randomized complete block design with orthogonal combinations of atmospheric \( \text{CO}_2 \) and \( \text{O}_3 \) treatments, and tree community composition as a split-plot factor. It consists of twelve 30-m-diameter rings with three control rings, and three replicate rings each receiving elevated atmospheric \( \text{CO}_2 \) (1,000 \( \text{ppm} \)), \( \text{O}_3 \) (25 \( \text{ppb} \)), or both elevated \( \text{CO}_2 \) and \( \text{O}_3 \) (1,000 \( \text{ppm} \) \( \text{CO}_2 \), 25 \( \text{ppb} \) \( \text{O}_3 \)), or both elevated \( \text{CO}_2 \) and \( \text{O}_3 \) (1,000 \( \text{ppm} \) \( \text{CO}_2 \), 25 \( \text{ppb} \) \( \text{O}_3 \)). The experiment was planted with 3- to 6-month-old plants at 1 x 1 m spacing in July 1997 and fumigation treatments have been running since 1998. Each ring is divided into three sections differing in community composition: the eastern half with five clones of trembling aspen (Populus tremuloides Michx.), the south-western quadrant with an alternating mixture of aspen and paper birch (Betula papyrifera Marsh.) and the north-western quadrant with an alternating mixture of aspen and sugar maple (Acer saccharum Marsh.). Our study was conducted in the core areas of the pure aspen and the mixed aspen–birch communities. The core area is buffered from edge effects by five rows of trees on the outer edge of the treatment rings, and measures 166 and 76 m² for the aspen and aspen–birch sections, respectively. At the end of the 2005 growing season, mean tree heights ranged between 5.7 and 7.6 m in the different treatments and community type combinations.

The Aspen FACE experiment is situated on an old agricultural field and the soil is classified as an Alfic Haplorthod with sandy loam soil texture (Dickson et al. 2000). Mean annual temperature at Rhinelander is 4.9 °C, mean July temperature is 19.7 °C and mean annual precipitation is 810 mm. Fumigation with \( \text{CO}_2 \) and \( \text{O}_3 \) aims at maintaining target concentrations of 560 ppm \( \text{CO}_2 \) and 1.5x ambient \( \text{O}_3 \) at the center of each treatment ring during daylight hours. Details about the site and fumigation technology can be found in Dickson et al. (2000) and Hendrey et al. (1999), respectively.

Meteorological measurements included photosynthetically active photon flux (\( Q_{\text{ph}} \)) measured at the site in an open field, air temperature (\( T \)) and relative humidity, both measured above the canopy at 10 m above ground in the centers of four rings. Temperature and relative humidity data were averaged across these four rings. Volumetric soil water content (\( \theta_s \)) was measured continuously in four rings (one of each treatment and one control) and bi-weekly in all 12 rings. Continuous \( \theta_s \) measurements were made at 5- to 35-cm depth with CS616 water content reflectometers (Campbell Scientific, Logan, U.K.), and bi-weekly measurements were made at 0–15-cm depth with a Tensile TDR (Soil Moisture Equipment, Goleta, CA). Both continuous and bi-weekly \( \theta_s \) data were collected in the centers of both the aspen and aspen–birch sections of a ring.
Sap flux measurements

Xylem sap flux density was measured with Granier-type sensors (Granier 1985, 1987). The Granier sap flow system consists of a heated (0.2 W) and an unheated reference probe, 20 mm in length and 2 mm in diameter. Thermocouples measure the difference in voltage (ΔV) between the probes as a measure of thermal dissipation. Our probes differed from the original design by Granier (1985) in that both the thermocouple and the heating wire were placed inside a cylindrical steel needle embedded in the tree, rather than placing the thermocouple in the midpoint of a thinner needle surrounded by coiled heating wire inside a cylindrical aluminum tube. This change was made to improve sensor durability and reusability. The thermocouple was 10-mm long and centered in the middle of the needle, and the coiled heating wire extended its full length.

Sensors were installed on April 26–30, 2004, before leaf development, and left in place during the winter between the 2004 and 2005 growing seasons. Granier-type sensors have been successfully used for more than one growing season by other investigators (Köstner et al. 1998; R. Oren, personal communication). The upper probe, containing the heated thermocouple, was placed 15 cm above the lower unheated probe on the north side of the stem. Azimuthal patterns in sap flux density were evaluated in 2003 and were found to be non-significant. To sample the full cross-sectional radius of the sapwood, the upper probes were inserted at tree heights where the stem diameter was about 44 mm and below the live crown. After the 2005 growing season, mean tree diameter at top-probe height (54 mm) still accounted for more than 95% of the cross-sectional sapwood area. Radial gradients in sap flux, which have typically been found to be significant only for depths greater than 40 mm and for larger trees in other diffuse-porous forest tree species (Edwards and Booker 1984; Phillips et al. 1996; but see Wullschleger and Norby 2001), should therefore be of minor importance in the calculation of stand-level sap flux in our study. The probes were covered by a highly reflective 30–40-cm-wide polyester film, mounted around the stem a few cm above the top probe. Visual inspections made at the beginning, middle, and end of each growing season revealed no problems with probe displacement caused by callus formation, radial growth, and low temperature effects.

Sap flow sensors were installed on 252 trees, partitioned as follows: 21 each in all 12 rings; nine and 12 in the aspen and aspen–birch sections of a ring, respectively; nine on birch and three on aspen trees in the aspen–birch section; 33, 34, 28 and 13 trees in the aspen sections from the clones 216, 271, 42E and 8L, respectively. Clone 259, which was greatly suppressed and contributed only 6% to total basal area in the pure aspen sections in 2004, was not sampled at all. In each ring, the sensors of one birch as well as one aspen tree within the pure aspen section were unheated to measure temperature differences between the upper and lower probes not caused by the heating. More birch trees than aspen trees were measured in the mixed stands because of the dominance of birch over aspen in these stands. Trees were selected to represent the major size classes within each individual ring. Measurements used in this study covered mid-June through September in 2004 and mid-June through August in 2005.

Sap flux calculations

Before converting the ΔV data to sap flux, the ΔV values measured in the unheated reference sensors were subtracted from the original sensor output data as recommended by Lu et al. (2004). On days without precipitation, the unheated sensors showed a distinct diurnal pattern with a brief dip in the morning and a broader peak during the afternoon and night. A test with all 252 sensors kept unheated for 26 h showed that unheated reference signals differed significantly (P < 0.05) among treatments in the early afternoon as well as during a 2-h period following sunrise, but that differences between species, community types or among rings within a treatment were not significant (data not shown). The subtraction of unheated reference sensor data was therefore done by treatment (average of six sensors) rather than by stand. These treatment differences in the unheated reference signals may have been caused by micrometeorological differences at sensor positions resulting from differences in below-canopy radiation and different sensor heights above the ground.

Adjusted ΔV data (i.e., after subtraction of unheated sensor values) were converted to sap flux density (J_o, g m^-2 s^-1) by Granier’s (1987) original calibration. Assuming that J_o before sunrise is always zero, a maximum ΔV baseline was defined for each individual sensor and 24-h cycle by linear regression between two stable predawn periods at the start and end of each cycle. Although nocturnal or predawn sap flux may occur under certain conditions, it is probably negligible for the young trees in our experiment, which were growing under conditions where relative humidity almost always reached saturation before dawn. To minimize uncertainties related to baseline positioning (Ewers and Oren 2000), we converted sap flux sensor data only if there were stable baselines before dawn.

Sap flux per unit basal area (J_o,B) was calculated by multiplying sap flux density at sensor height by the ratio of top-probe to basal (10 cm above soil surface) cross-sectional area (excluding bark). Size-weighted, stand-level mean J_o,B was calculated by summing the total sap flux for all measured trees and dividing it by their summed basal area (A_o; excluding bark). This was done for aspen and birch separately in the mixed stands. Because there was no relationship between tree size and J_o,B within treatments, stand-level sap flux per unit ground area (J_o,G, g m^-2 ground s^-1) was calculated by multiplying stand-level J_o,B with the stand-level A_o to ground area (A_o) ratio.

Stand-level A_o was calculated from measurements of basal stem diameter of all trees in the core areas after the growing seasons 2003–2005, correcting for bark thickness. Individual trees contributed to stand-level A_o estimates only if they were judged to be alive. Stem circumference and bark thickness of trees measured for sap flux were also measured at top-probe height in April 2004, as well as in November after the 2004
and 2005 growing seasons. Top-probe circumference of trees in the pure aspen stands was not measured in November 2004, and was estimated by assuming that the relative growth-specific growth development between sampling dates were similar in aspen and aspen–birch stands. Seasonal $A_b$ growth development was modeled from day number based on a sigmoid growth function derived from seasonal stem growth data for *Populus grandidentata* Michx. growing under similar climatic conditions at the University of Michigan Biological Station, located at a similar latitude (45° 30’ N; 84° 42’ W; C Vogel, personal communication), during 2001–2003. According to this model, almost 90% of the yearly $A_b$ increment occurs in June–July. Stem diameter data from July 5, 2005 revealed no significant treatment effect on the timing of seasonal $A_b$ growth in the Aspen FACE experiment, justifying the application of a common relative growth function for all treatments.

### Sap flux data analysis

Sap flux density scaled to the stand level was integrated over 24 h, beginning at 0500 h, to include nocturnal refilling. These data were then used to determine maximum ($J_{\text{max}}$) and seasonal mean ($J_{\text{mean}}$) sap flux, as well as sap flux responses to climate and interactions between treatments and other environmental variables. We determined $J_{\text{mean}}$ for the 5-day period with the highest sap flux. Daily $J_{\text{G}}$ data were related to daily mean $Q_p$ and vapor pressure deficit of the air ($D$) with the equation $J_{\text{G}} = a + bQ_p + cD$. From the fitted empirical coefficients $a$, $b$, and $c$, $J_{\text{G}}$ at $Q_p = 0.5$ mmol m$^{-2}$ s$^{-1}$ and $D = 0.6$ kPa was determined ($J_{\text{G}}$), representing typical clear-sky summer conditions at the site. For estimates of $J_{\text{mean}}$ during peak season (mid-June through August), data gaps were filled based on the stand-specific relationships between climate and $J_{\text{G}}$ (mean $r^2 = 0.74$; range 0.51–0.81). We calculated $J_{\text{mean}}$ only for 2004, because lower data capture in 2005, with data missing during different phases of drying cycles, precluded estimation of $J_{\text{mean}}$ for 2005.

For the determination of $J_{\text{mean}}$ and $J_{\text{G}}$, we used only data based on a minimum of five trees per stand (e.g., community type within each ring; a minimum of three birch and two aspen trees in mixed stands). For estimates of $J_{\text{mean}}$ and analyses of $\theta_\circ$–climate–treatment interactions, data based on a minimum of three trees per stand (two birch and one aspen trees in mixed stands) were used to minimize gaps in the dataset. However, when data were available for only three or four trees, stand-level $J_{\text{G}}$ was adjusted by dividing with the ratio between mean $J_{\text{G}}$ of these trees and mean $J_{\text{G}}$ of all trees in the stand during times when such data were available.

Mean data capture of all 24 stands during 2004 was 76 and 63% when three and five trees per stand were required, respectively. Corresponding values for 2005 were 62 and 49%. Data were completely lacking for aspen within the mixed aspen–birch stand of one $CO_2$ ring in both years. Stand-level $J_{\text{G}}$ of that stand was estimated assuming that $J_{\text{G}}$ for birch and aspen in that stand (i.e., the mixed section of that $CO_2$ ring) were similar, which was the case in the other rings (see below). Aspen accounted for only 20% of total $A_b$ in the stand.

### Leaf area index and canopy absorbance

Canopy transmittance of photosynthetically active radiation (PAR) was measured with a ceilometer AccuPAR Model LP-80 (Decagon Devices, Pullman, WA) on August 15, 2004 and July 19 and August 15, 2005 between 1000 and 1400 h under a clear sky. All 24 stands were measured on both days in 2005, but five aspen sections (one each in control, $CO_2$ and $O_3$, and two in $CO_2$+$O_3$) were not measured in 2004. Thirty-two below-canopy readings were taken in the core area of each stand, one measurement each facing south and west in sixteen 1-m$^2$ grids. Before and after the below-canopy measurements in each stand, five measurements were taken outside the ring under a clear sky, representing above-canopy $Q_p$. Mean below-canopy $Q_p$ was divided by mean above-canopy $Q_p$ to calculate the fraction of PAR transmitted by the canopy ($\tau_{\text{PAR}}$).

We estimated $L$ from the $\tau_{\text{PAR}}$ data by inverting the canopy light interception model given by Welles (1990), and including effects of leaf clumping:

$$
L = \frac{f_b}{(0.283 + 0.785a - 0.159a^2)(1 - 0.47f_b)} - 1
$$

where $f_b$ is the fraction of incident PAR which is beam, $\Omega$ is the canopy clumping factor, $K_s$ is the canopy extinction coefficient for black leaves in direct radiation and $a$ is leaf absorptivity in the PAR waveband. We assumed $a = 0.85$ (Jones 1992) and used a $\Omega$ of 0.84, which is a representative value for temperate broad-leaved forests (Baldocchi and Meyers 1998). Diffuse PAR was modeled from atmospheric transmittance and optical air mass number (Campbell and Norman 1998) to calculate $f_b$, which was always between 0.88 and 0.90. Assuming a spherical leaf angle distribution, which has been reported for aspen by Chen et al. (1997), $K_s$ was modeled as a function of solar zenith angle ($\Psi$) (Campbell and Norman 1998):

$$
K_s = \frac{1}{2\cos\Psi}
$$

Leaf area indices of the five aspen stands for which there were no ceilometer data in 2004 were estimated from their annual litter fall (C. Giardina, personal communication) and the ratio between litter fall and $L$ in the remaining 19 stands. This ratio ($66 \pm 2$ g m$^{-2}$) was not significantly affected by community type or treatment, justifying also the use of a common $\Omega$ value for all stands.

Canopy absorbance of short-wave radiation ($A_s$) was calculated as the ratio of absorbed to incoming short-wave radiation integrated from mid-June through August, using stand-specific $L$ estimates, $a = 0.5$, and leaf angle distribution and $\Omega$ as above. Global radiation was partitioned into diffuse and directional (direct + circumsolar diffuse) radiation according to Spitters et al. (1986). Canopy transmission of diffuse and directional radiation was modeled according to Goudriaan (1977) and Campbell and Norman (1998), respectively. The
radiation model performed well, accounting for 92% of the variation in PAR observed below the canopies in eight stands in 2005 (data not shown).

**Statistical analysis**

All data were statistically tested for effects of block, CO$_2$, O$_3$, community (or species) and time (year) and their relevant interactions, by split-plot analysis of variance (ANOVA) appropriate for the Aspen FACE experiment (King et al. 2001). When there were significant interactions including time, community type or species, data were analyzed for the two years/communities/species separately. Effects were regarded as significant at $P < 0.10$.

**Results**

Summer 2005 was warmer than summer 2004 (Table 1), and for the experimental period (1998–2005), the 2004 and 2005 summers were the coolest and warmest in Rhinelander, respectively. Both the air and soil were drier in 2005 than in 2004. Ambient O$_3$ was unusually low in 2004, but typical for this region in 2005 (U.S. Environmental Protection Agency; http://www.epa.gov/ttn/airs/airsaqsl/). Mean daytime O$_3$ concentrations were 1.31× and 1.40× ambient in the elevated [O$_3$] treatments during the 2004 and 2005 summers, respectively. Mean daytime elevated CO$_2$ concentrations were about 525 ppm in both years.

Both L (Figure 1a) and the A$_{L}$A$_{G}$ ratio (Figure 1b) were significantly ($P < 0.011$) increased by elevated [CO$_2$] and decreased by elevated [O$_3$]. Species composition also affected L and the A$_{L}$A$_{G}$ ratio ($P < 0.028$), with higher values in mixtures than in monocultures. Leaf area index did not differ significantly between years ($P > 0.10$). Treatment effects on L, averaged across years and communities, were +27% in CO$_2^{*}$, −33% in O$_3^{*}$ and +11% in CO$_2^{*}$+O$_3^{*}$. Corresponding treatment effects on the A$_{L}$A$_{G}$ ratio were +39%, −18 and +9%. There were no main effects of CO$_2$, O$_3$ or community type on leaf to sapwood area ratio (A$_{L}$:A$_{G}$; Figure 1c). However, because A$_{L}$:A$_{G}$ was higher for aspen–birch stands in the control rings, there was a significant CO$_2$ × O$_3$ × Community interaction ($P = 0.085$). Analyzing the aspen–birch community data separately, the CO$_2$ × O$_3$ interaction was significant at $P = 0.026$. Canopy absorbance of short-wave radiation was increased by elevated [CO$_2$] and decreased by elevated [O$_3$] (Figure 1d). There was also a significant CO$_2$ × O$_3$ interaction on A$_{G}$.

Values of J$_{max,0}$ were increased by elevated [CO$_2$] ($P = 0.061$), but were not significantly affected by elevated [O$_3$] or community type (Figure 2a, Table 2). It was significantly higher in 2004 compared with 2004 ($P = 0.047$), and there was a near-significant CO$_2$ × O$_3$ × Year interaction ($P = 0.101$) caused by a stronger stimulation by elevated [CO$_2$] in ambient [O$_3$] than in elevated [O$_3$] in 2004, but similar enhancement in both O$_3$ regimes in 2005. The mean CO$_2$ effect on J$_{max,0}$ across communities was +34% in ambient [O$_3$] and +11% in elevated [O$_3$] in 2004, and corresponding values for 2005 were +13 and +15%. Analyzing data for the two years separately, the CO$_2$ effect was strong in 2004 ($P = 0.030$) and not statistically significant in 2005 ($P = 0.15$). Values of J$_{max,0}$ were significantly reduced by elevated [CO$_2$] ($P = 0.053$) and increased by elevated [O$_3$] ($P = 0.027$) and higher in aspen stands compared with aspen–birch stands ($P = 0.009$; Figure 2b, Table 2). Values of J$_{max,0}$ expressed per unit total leaf area (J$_{max,1}$) were higher in aspen stands than in aspen–birch stands ($P < 0.001$), and substantially higher in the O$_3^{*}$ treatment compared with control values, as indicated by the significant CO$_2$ × O$_3$ interaction ($P = 0.018$; Figure 2c, Table 2). A CO$_2$ × Year interaction on J$_{max,0}$ was caused by a stronger negative effect of elevated [CO$_2$] in 2005 than in 2004. The value of J$_{max,0}$, normalized for canopy absorbance was not significantly affected by the atmospheric treatments, but was lower ($P = 0.024$) in aspen–birch stands than in aspen stands (Figure 2d, Table 2).

Table 1. Mean environmental conditions during June–August in 2004 and 2005 at the Aspen FACE site, WI, USA. Temperature (T), vapor pressure deficit of the air (D), and photosynthetic photon flux (Q$_{p}$) are calculated for 24 h, whereas O$_3$ and CO$_2$ concentrations are calculated for the daytime period only.

<table>
<thead>
<tr>
<th></th>
<th>2004</th>
<th>2005</th>
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<tbody>
<tr>
<td>T (°C)</td>
<td>16.3</td>
<td>19.9</td>
</tr>
<tr>
<td>D (kPa)</td>
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<td>0.51</td>
</tr>
<tr>
<td>Q$_{p}$ (mmol m$^{-2}$ s$^{-1}$)</td>
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<td>0.40$^1$</td>
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<tr>
<td>Precipitation (mm)</td>
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<td>183</td>
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<tr>
<td>Mean volumetric soil water ($\theta_h$)</td>
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<td>0.11$^2$</td>
</tr>
<tr>
<td>Minimum volumetric soil water ($\theta_x$)</td>
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<td>0.06$^3$</td>
</tr>
<tr>
<td>Ambient O$_3$ (ppbw)</td>
<td>32.8</td>
<td>37.0</td>
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<tr>
<td>Elevated O$_3$ (ppbw)</td>
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<tr>
<td>Ambient CO$_2$ (ppmv)</td>
<td>370</td>
<td>368</td>
</tr>
<tr>
<td>Elevated CO$_2$ (ppmv)</td>
<td>525</td>
<td>527</td>
</tr>
</tbody>
</table>

1 Data missing for day of year 221–227.
2 Value for 0–15-cm soil depth in pure aspen control stands.
3 Value for 0–15-cm soil depth in mixed aspen–birch control stands.

Elevated [CO$_2$] increased J$_{50,G}$ ($P = 0.077$) and there was a similar CO$_2$ × O$_3$ × Year interaction on J$_{50,G}$ ($P = 0.086$) as on J$_{max,0}$ (Figure 2e, Table 2). In contrast to J$_{max,0}$, however, J$_{50,G}$ did not differ significantly between years and was higher in aspen stands than in aspen–birch stands ($P = 0.090$). Fits between J$_{G}$ and Q$_{p}$ and D on which J$_{50,G}$ was based had a mean $r^2$ of 0.74 (range 0.51–0.81). Mean peak-season J$_{G}$ in 2004 was significantly increased by elevated [CO$_2$] ($P = 0.044$) and higher in aspen stands than in aspen–birch stands ($P = 0.060$; Figure 2f, Table 2). Treatment effects on J$_{G}$ were consistent throughout most of the 2004 growing season (data not shown).

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However, the $J_{s,c}$ relative control increased progressively (from $-1.3$ to $-1.55$) in CO$_2$ during September in both community types, and in aspen stands there was a tendency for O$_3$ to have a negative effect (from $-1.0$ to $-0.9$) in late September. Values of $J_{s,c}$ decreased in all treatments during September 2004 in response to decreasing $D$ and $L$ (data not shown).

The magnitude of the elevated [CO$_2$] and [O$_3$] effects on $J_{s,c}$ did not vary with $D$ (Table 3). Fumigation treat-
Table 2. Statistical significance (P values) of block, CO₂, O₃, community, year and their relevant interactions on maximum (J_{max}), mean (J_{mean}) and fitted (J_{fit}) daily sap flux density (kg m⁻² day⁻¹) from mid-June through August. Values of J_{max} are expressed per unit ground (J_{max,G}), leaf (J_{max,L}) or basal (J_{max,B}) area, as well as on a canopy absorptance basis (J_{max,C}) divided by the canopy absorptance (A_c) of short-wave radiation. Effects are considered significant at P < 0.10.

<table>
<thead>
<tr>
<th>Source</th>
<th>J_{fit,G}</th>
<th>J_{max,G}</th>
<th>J_{max,B}</th>
<th>J_{max,L}</th>
<th>J_{max,C}/A_c</th>
<th>J_{mean,G}</th>
</tr>
</thead>
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<tr>
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<td>ns</td>
</tr>
<tr>
<td>CO₂</td>
<td>0.077</td>
<td>0.061</td>
<td>0.053</td>
<td>0.012</td>
<td>ns</td>
<td>0.044</td>
</tr>
<tr>
<td>O₃</td>
<td>ns</td>
<td>ns</td>
<td>0.027</td>
<td>0.005</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>CO₂ × O₃</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.018</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Community</td>
<td>0.090</td>
<td>ns</td>
<td>0.009</td>
<td>&lt;0.001</td>
<td>0.024</td>
<td>0.060</td>
</tr>
<tr>
<td>CO₂ × Community</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>O₃ × Community</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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<tr>
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<tr>
<td>Year</td>
<td>ns</td>
<td>0.047</td>
<td>ns</td>
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<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>CO₂ × Year</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.056²</td>
<td>ns</td>
<td>ns</td>
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<tr>
<td>O₃ × Year</td>
<td>0.077</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>CO₂ × O₃ × Year</td>
<td>0.064¹</td>
<td>ns (0.010)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Community × Year</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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<td>ns</td>
</tr>
<tr>
<td>CO₂ × Community × Year</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>O₃ × Community × Year</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>CO₂ × O₃ × Community × Year</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

¹ Analyzing the two years separately, the positive effect of CO₂ was significant in 2004 (P = 0.043) but not in 2005 (P = 0.18).
² The CO₂ effect was stronger in 2005 than in 2004 (Figure 2c), but statistically significant for both years when years were analyzed separately.

Birch and aspen had similar J_{max,B} in the mixed stands, but because birch had higher A_B than aspen (+51%), J_{max,G} was significantly higher in birch (+58%, P = 0.063; Table 4). The J_{max,B} in birch was significantly (P = 0.023) lower than in aspen in monoculture.

Effects of elevated [CO₂], elevated [O₃] and community type on θ, of the upper soil layer varied with time (Figure 5). Before and early during the growing season, θ, was significantly increased by elevated [CO₂] (significant effects with 0.015 ≤ P ≤ 0.078), whereas elevated [O₃] often had a negative effect on θ, during the growing season (0.016 ≤ P ≤ 0.086). Soil water content was often significantly lower in aspen stands than in aspen–birch stands (0.0008 ≤ P ≤ 0.090) during the peak and (in 2004) late growing season. There were no significant interacting effects of CO₂ and O₃ on θ, and the few interacting effects of fumigation treatments and community type on θ, that were found were not consistent between years (data not shown).

Table 3. Daily sap flux per unit ground area (J_{G,G}, mean ± SE) of aspen (A) and aspen–birch (AB) communities from mid-June through August, stratified according to vapor pressure deficit of the air (D).

<table>
<thead>
<tr>
<th>D (kPa)</th>
<th>Number of days (A, AB)</th>
<th>J_{G,G} in control (kg m⁻² day⁻¹)</th>
<th>Percent change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>AB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CO₂⁺</td>
<td>O₃⁺</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>AB</td>
</tr>
<tr>
<td>&lt;0.3</td>
<td>35, 26</td>
<td>0.91 ± 0.06</td>
<td>0.91 ± 0.05</td>
</tr>
<tr>
<td>0.3 ≤ D ≤ 0.6</td>
<td>41, 39</td>
<td>1.39 ± 0.09</td>
<td>1.24 ± 0.11</td>
</tr>
<tr>
<td>D ≥ 0.6</td>
<td>18, 12</td>
<td>1.52 ± 0.09</td>
<td>1.42 ± 0.12</td>
</tr>
<tr>
<td>All data</td>
<td>95, 77</td>
<td>1.24 ± 0.08</td>
<td>1.17 ± 0.08</td>
</tr>
</tbody>
</table>

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Figure 3. Daily sap flux relative to the treatment control versus volumetric soil water content ($\theta_s$, 0–15-cm depth) of pure aspen stands in (a) 2004 and (b) 2005. Fully replicated bi-weekly $\theta_s$ data were temporally interpolated based on relative changes in continuous $\theta_s$ data available from one ring per treatment. Data were excluded for days with mean photosynthetic photon flux below 0.3 mmol m$^{-2}$ s$^{-1}$. Each value represents the binned average of 7–15 days, and error bars represent the standard error calculated for the mean of days to illustrate the significance of changes in treatment effects with $\theta_s$ rather than of differences among treatments.

Figure 4. Sap flux density ($J_a$) of aspen (A) and aspen–birch (AB) communities in relation to (a) photosynthetic photon flux ($Q_p$) and (a, b) vapor pressure deficit of the air ($D$). The $J_a$ data are (a) daily integrals and (b) half-hourly values, expressed per unit ground ($J_a, g$) and basal ($J_a, b$) area, respectively. Data with half-hourly $Q_p < 0.5$ mmol m$^{-2}$ s$^{-1}$ were excluded in (a). Values represent binned averages of data from all 12 rings.

Discussion

Effects of elevated [CO$_2$] and [O$_3$], alone and in combination, on physiological processes over long periods are likely to be manifested in differences in plant size and stand structure. This study broadly demonstrates the importance of such cumulative effects of elevated [CO$_2$] and [O$_3$] on stand structure and water-use processes of land surfaces. Our results suggest that the effects of elevated [CO$_2$] and [O$_3$] on tree and stand structure may be more important than the expected primary stomatal closure responses in determining tree water use of northern hardwood forests growing in a changed atmospheric trace gas composition.

Effects of CO$_2$ and O$_3$ on sap flux capacity

We found evidence that long-term exposure to elevated [CO$_2$] resulted in larger trees with greater stand leaf area that used significantly more water (mean +18%; $P = 0.044$ to 0.077) than trees in current ambient [CO$_2$] (Table 2, Figures 2a, 2c and 2f). Elevated [CO$_2$] increased $A_g$ by 36% and $L_e$ by 40%, averaged across years, O$_3$ regimes and communities (Figures 1a and 1b). A previous study reported a substantially enhanced fine root biomass in response to elevated [CO$_2$] (+62% at the end of 2003; King et al. 2005). These results suggest that trees in elevated [CO$_2$] used more water than trees in ambient [CO$_2$] because they were larger and had more transpiring leaf area and more extensive absorbing root systems. Although elevated [CO$_2$] has been observed to increase tree size and tree water use in shorter-term studies with potted temperate deciduous tree species (Beering et al. 1996, Heath and Kerstiens 1997, Kubiske and Pregitzer 1997, Engel et al. 2004), our results differ from other FACE studies where similar $L_e$ between CO$_2$ treatments either decreased (Wullschleger and Norby 2001) or mostly did not significantly affect (Schäfer et al. 2002, Cech et al. 2003) $J_a, g$ in elevated [CO$_2$]. The Aspen FACE experiment contrasts with these FACE experiments in that exposures began at the time of planting and continued through all subsequent phases of stand development (including reaching steady-state $L_e$; Figure 1a and unpublished data for 2006). In our study, sap flux responses are thus fully acclimated to the elevated [CO$_2$] and [O$_3$] treatments (though perhaps not in a steady state if root water uptake capacity and soil properties were still changing), whereas sap flux results reported from the other FACE experiments were obtained from trees during the first few years of CO$_2$ fumigation of canopies already developed (Wullschleger and Norby 2001, Schäfer et al. 2002, 2003, 2004).
Table 4. Statistical significance (P values) of block, CO₂, O₃, species, year and their relevant interactions on maximum daily sap flux density, \( J_{\text{smax}} \) (kg m⁻² day⁻¹) and basal to ground area (A₉G/A₉G) ratio, comparing birch (B) with aspen (A) growing in either monoculture or in aspen-birch mixture. Values of \( J_{\text{smax}} \) are expressed per unit ground \( J_{\text{smax_G}} \) or basal \( J_{\text{smax_B}} \) area. Positive and negative effects, as well as the year or species with the higher value, are indicated in brackets. Effects are considered significant at \( P < 0.10 \).

<table>
<thead>
<tr>
<th>Source</th>
<th>Birch versus aspen in monoculture ( J_{\text{smax_B}} )</th>
<th>Birch versus aspen in species mixture ( A_{\text{BG}}/A_{\text{AG}} ) ratio²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>CO₂</td>
<td>0.055 (−)</td>
<td>ns</td>
</tr>
<tr>
<td>O₃</td>
<td>0.062 (+)</td>
<td>0.012 (−)</td>
</tr>
<tr>
<td>CO₂ × O₃</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Species</td>
<td>0.023 (A +15%)</td>
<td>0.023 (±)</td>
</tr>
<tr>
<td>CO₂ × Species</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>O₃ × Species</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>CO₂ × O₃ × Species</td>
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<td>ns</td>
</tr>
<tr>
<td>Year</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>CO₂ × Year</td>
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<td>ns</td>
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<tr>
<td>O₃ × Year</td>
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</tr>
<tr>
<td>CO₂ × O₃ × Year</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Species × Year</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>CO₂ × Species × Year</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>O₃ × Species × Year</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>CO₂ × O₃ × Species × Year</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

¹ Positive CO₂ effect (\( P < 0.05 \)) in aspen but not in birch.
² Test made on data measured at the end of the 2004 growing season only, because \( A_{\text{BG}} \) is a cumulative measure.

Cech et al. (2003). During the third and last year of the Schäfer et al. (2002) study at the Duke FACE experiment, tree water use increased by 10% in the elevated [CO₂] treatment, an effect that was attributed to increased \( \theta \), resulting from increased litter build-up and decreased forest floor evapotranspiration. Litter build-up was also significantly increased by elevated [CO₂] in our study \( (P = 0.072; +19, −37 \text{ and } +3 \text{ in } \text{CO}_2^\text{a}, \text{O}_3^\text{a} \text{ and } \text{CO}_2^\text{a} + \text{O}_3^\text{a}, \text{respectively, in May 2003; C. Giardina, personal communication}). Furthermore, \( \theta \), of the upper soil before and early during the growing season was significantly increased in elevated [CO₂] (Figure 5), implying increased water-holding capacity, probably as a result of increased inputs of aboveground (King et al. 2005) and belowground (King et al. 2001) litter. Because trees growing in elevated [CO₂] used more water compared with their ambient-grown counterparts, the positive effect of elevated [CO₂] on \( \theta \), did not persist later during the growing season.

Similar \( J_{\text{s,1}} \), and stand sap flux on an absorbed radiation basis in trees in the control and \( \text{CO}_2^\text{a} \) treatment (Figure 2c and 2d) may be contrary to expectations, given the numerous reports of lower \( g_s \) in temperate deciduous tree species growing in elevated [CO₂] (e.g., Norby et al. 1999, Medlyn et al. 2001), including aspen during the second year of the Aspen FACE experiment (Noormets et al. 2001). However, effects of elevated [CO₂] on \( g_s \) of young trees must be interpreted carefully. Because leaf-level \( g_s \) is routinely constrained by hydraulic properties of the soil and plant (e.g., Sperry 2000), \( g_s \) is affected by the timing of increases in water uptake capacity and leaf area during plant and stand development. Because plant ontogeny and stand development are affected by elevated [CO₂] (Norby et al. 1999), early effects on \( g_s \) may be transient and not persist.
after steady state has been reached. At steady-state \( L \), long-term cumulative effects of elevated [CO\(_2\)] on tree size and soil properties probably resulted in an increased capacity for water uptake and transfer that overrode stomatal closure responses to elevated [CO\(_2\)]. The importance of structural adjustments in response to elevated [CO\(_2\)] was demonstrated in a chamber study with paper birch, where \( g_s \) increased in response to elevated [CO\(_2\)] as a result of increased hydraulic efficiency (Kuviske and Pregitzer 1997).

The reason for the greater CO\(_2\)-induced stimulation of \( J_{s,g} \) in CO\(_2\) in 2004 than in 2005 (Figure 2, Table 2) is unclear. Stratification of data according to \( D \) and \( \theta \), did not support the hypothesis that the difference was a result of the pronounced climatic differences between the two summers.

Counter to expectations, elevated atmospheric [O\(_3\)] did not reduce \( J_{s,g} \) despite substantial increases in \( L \) (22% across years, communities and CO\(_2\) regimes) and \( A_b \) (20%) (Table 2, Figures 1a, 1b and 2a). Values of \( J_{smax,1} \) were substantially increased in O\(_3\) only, indicating higher mean canopy \( g_s \) in this treatment compared to control stands, but similar \( g_s \) compared to control values in CO\(_2\) and CO\(_2\)+O\(_3\) (Figure 2c, Table 2). Most of the increase in \( J_{smax,1} \) in response to elevated [O\(_3\)] can be attributed to a higher proportion of sun leaves in O\(_3\) trees compared with control trees, and stand sap flux on an absorbed radiation basis was not significantly affected by atmospheric gas treatments (Figure 2d). Similar stand water use capacity in elevated [O\(_3\)] and ambient [O\(_3\)] despite reductions in tree size and \( L \) in response to elevated [O\(_3\)] may also be partly explained by stronger coupling between the atmosphere and leaves in canopies with lower \( L \) (Jarvis and McNaughton 1986), and by similar (aspen–birch mixture) or even increased (pure aspen) fine root biomass in elevated [O\(_3\)] compared with ambient [O\(_3\)] (King et al. 2005). The relative importance of possible effects of elevated [O\(_3\)] on \( g_s \), root uptake capacity and atmospheric coupling in explaining constancy in water use despite the reductions in tree size and \( L \) caused by elevated [O\(_3\)] could not be assessed with the data we obtained. The negative effect of elevated [O\(_3\)] on \( \theta \) of the upper soil layer (Figure 5) was probably caused by tree water use similar to ambient [O\(_3\)] treatments in combination with increased forest floor evaporanspiration in stands with lower \( L \) and canopy absorbance (Figures 1a and 1d) and higher ground vegetation cover (Bandelt et al. 2006).

Similar tree water use in ambient and elevated [O\(_3\)] treatments, despite lower \( L \) in elevated [O\(_3\)], differs from results reported from a free-air O\(_3\) fumigation experiment in a mature mixed forest in Germany, where \( g_s \), in Fagus sylvatica L. and Picea abies (L.) H. Karst. decreased 25% in response to a doubling in ambient [O\(_3\)] (Nunn et al. 2006, Warren et al. 2006). This difference may be explained by the strong enhancement of [O\(_3\)] in the German study (2× ambient [O\(_3\)]) compared with our study (1–1.4× ambient [O\(_3\)]), because stomatal closure usually occurs at high [O\(_3\)] but not at the more moderately elevated [O\(_3\)] used in our study (Wittig et al. 2007). It should also be noted that the O\(_3\) treatment was initiated on already developed canopies in the 3-year German study, allowing for little structural acclimation.

Our results are not in conflict with a recent report of significantly reduced \( g_s \) in birch (but not aspen) by both elevated [CO\(_2\)] and elevated [O\(_3\)] (Riikonen et al. 2008). The reduction in \( g_s \) in elevated [CO\(_2\)] found by Riikonen et al. (2008) was observed during late September–early October and may thus be related to delayed leaf shedding, whereas the reduction in \( g_s \) in elevated [O\(_3\)] was observed only during a dry period in late August–early September, when drought was particularly pronounced in stands in the elevated [O\(_3\)] treatment (Figure 2b). Estimates of \( g_s \) derived from our sap flux data (together with data on \( L \) and vertical canopy scaling) compare well with estimates of \( g_s \) based on \(^{13}\)C data and photosynthesis modeling (author’s unpublished data).

**Effects of climate and treatment by climate interactions**

Both aspen and birch showed asymptotic relationships between \( D \) and \( J_{s,g} \), with half-hourly sap flux density increasing little at \( D \) values above 1.0 kPa (Figure 4b). This so called isohydric stomatal behavior has been reported previously in many tree species, including both trembling aspen (Hogg et al. 2000) and paper birch (Bovard et al. 2005). Daily sap flux increased approximately linearly with daily mean \( Q \), as reported for other fast-growing deciduous tree species (e.g., Wullschleger and Norby 2001, Bovard et al. 2005).

Declining sap flux relative to control values (Figure 3b) and lower \( \theta \), of the upper soil (Figure 5) during dry periods in pure aspen stands exposed to elevated [O\(_3\)] may be explained in a hydrological context. The aspen stands in elevated [O\(_3\)] had stand-level tree water use similar to control stands in combination with presumably higher understory and forest floor evaporanspiration below tree canopies with greater radiation transmittance (Figure 1d). Pure aspen stands in the O\(_3\) treatment, where the drought–O\(_3\) interaction was particularly pronounced, had the lowest \( L \) (Figure 1a) and \( \theta \), (data not shown) of all treatment and community combinations, and higher ground vegete cover than aspen stands in the other treatments (Bandelt et al. 2006). This is the first stand-level study to corroborate the long-standing hypothesis that elevated [O\(_3\)] may predispose trees to drought stress (e.g., Matyssek et al. 2005).

The observed stimulation of tree water use by pure aspen stands in the CO\(_2\) treatment at low \( \theta \), in 2004 but not in 2005 (Figure 3) is puzzling. The absence of increased drought vulnerability despite increased tree water use in elevated [CO\(_2\)] may be explained by treatment effects on canopy transmittance, soil properties and possibly also vertical root distribution. Less radiation transmitted by the tree canopies (Figure 1d), lower ground vegetation cover, more litter build-up (C. Giardina, personal communication) and higher waterholding capacity of the upper soil (Figure 5) are all factors that act favorably on soil water status in elevated [CO\(_2\)].

**Community/species differences**

Our interpretation of differences between community types is complicated by the use of one aspen clone in the mixed aspen–birch stands and five aspen clones in the pure aspen
stands. Any effect of community type may therefore, in principle, be the result of either differences in species composition or in aspen clonal composition. Sap flux density expressed on a ground area, basal area, leaf area and absorbed radiation basis were all higher in the pure aspen stands than in the mixed aspen–birch communities (Figure 2, Table 2), and both, of the upper soil was consequently lower in aspen stands than in aspen–birch stands (Figure 5). Leaf area index, A\textsubscript{B} and canopy absorbance were higher in aspen–birch stands than in aspen stands (Figures 1a, 1b and 1d). Higher A\textsubscript{B} despite lower wood biomass (King et al. 2005) of aspen–birch stands compared with aspen stands is explained by different shapes of the stems of the two species.

Tree water use integrates effects on water uptake capacity, hydraulic architecture, leaf area and stomatal behavior, and in pure aspen and mixed aspen–birch communities, this response variable was similarly sensitive to elevated concentrations of CO\textsubscript{2} or O\textsubscript{3}, or both, under most conditions (Figure 2, Table 2). The lower O\textsubscript{3} sensitivity of total biomass observed for mixed aspen–birch stands compared with pure aspen stands (King et al. 2005) was therefore unaccompanied by a similar difference in O\textsubscript{3} sensitivity of stand-level tree water use.

Conclusion

Contrary to prevailing hypotheses, stand-level tree water use of the pure aspen and mixed aspen–birch forests was increased by elevated [CO\textsubscript{2}], but was not significantly affected by elevated [O\textsubscript{3}]. Stand-level leaf area responses to elevated [CO\textsubscript{2}] and [O\textsubscript{3}] were consistent with hypothesized carbon-balance effects of these gases, e.g., a positive CO\textsubscript{2} effect and a negative O\textsubscript{3} effect. However, there were unanticipated effects of these gases on stand water use per unit leaf area. Sap flux per unit leaf area was similar in stands fertilized with elevated [CO\textsubscript{2}] and in control stands, and was significantly higher in stands in the O\textsubscript{3}\textsuperscript{+} treatment. Trees in elevated [CO\textsubscript{2}] were larger with more transpiring leaf area and water-absorbing fine roots, and were growing in soil with CO\textsubscript{2}-induced increases in litter build-up and water-holding capacity of the upper soil, factors that act to increase tree water use. The relative importance of possible effects of elevated [O\textsubscript{3}] on root uptake capacity and atmospheric coupling in explaining the lack of significant effects on water use despite reductions in tree size and L in response to [O\textsubscript{3}] could not be assessed from the data we obtained. Our results suggest that long-term cumulative effects of elevated [CO\textsubscript{2}] and [O\textsubscript{3}] on tree and stand structure may be more important than the expected primary stomatal closure responses to elevated [CO\textsubscript{2}] and [O\textsubscript{3}] for understanding how these trace gases affect stand-level water use.

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