

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

JOHAN UDDLING,^{1–3} RONALD M. TECLAW,⁴ MARK E. KUBISKE,⁴ KURT S. PREGITZER⁵
and DAVID S. ELLSWORTH⁶

¹ School of Natural Resources and Environment, University of Michigan, 440 Church Street, Ann Arbor, MI 48109, USA

² Present address: Department of Plant and Environmental Sciences, University of Gothenburg, P.O. Box 461, SE-405 30 Göteborg, Sweden

³ Corresponding author (johan.uddling@dps.gu.se)

⁴ USDA Forest Service, Northern Research Station, 5985 Hwy K, Rhinelander, WI 54501, USA

⁵ Ecosystem Science Center, School of Forest Resources and Environmental Science, Michigan Technological University, 1400 Townsend Ave., Houghton, MI 49931, USA

⁶ Centre for Plant and Food Science, University of Western Sydney, Locked Bag 1797, Penrith South DC NSW 1797, Australia

Received October 17, 2007; accepted February 28, 2008; published online June 2, 2008

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 CO₂ 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 (*g_s*) 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 *g_s* 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 O_3 are prematurely shed in many tree species (e.g., Matussek 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 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 (Wullschlegel and Norby 2001, Gunderson et al. 2002, Wullschlegel et al. 2002). Little is known about the effects of 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 (Matussek 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 (Rhineland, 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 $[\text{O}_3]$ and $[\text{CO}_2]$ 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 Rhineland, 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 $[\text{CO}_2]$ (CO_2^e), elevated $[\text{O}_3]$ (O_3^e), or both elevated $[\text{CO}_2]$ and $[\text{O}_3]$ ($\text{CO}_2^e + \text{O}_3^e$). The experiment was planted with 3- to 6-month-old plants at 1 × 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 Rhineland is 4.9 °C, mean July temperature is 19.7 °C and mean annual precipitation is 810 mm. Fumigation with CO_2 and O_3 aims at maintaining target concentrations of 560 ppm $[\text{CO}_2]$ and 1.5× 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_p) 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 (θ_v) was measured continuously in four rings (one of each treatment and one control) and bi-weekly in all 12 rings. Continuous θ_v measurements were made at 5- to 35-cm depth with CS616 water content reflectometers (Campbell Scientific, Loughborough, U.K.), and bi-weekly measurements were made at 0–15-cm depth with a Trase TDR (SoilMoisture Equipment, Goleta, CA). Both continuous and bi-weekly θ_v 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 Wullschlegel 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_S ; $\text{g m}^{-2} \text{s}^{-1}$) by Granier's (1987) original calibration. Assuming that J_S 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 ($J_{S,B}$) 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 $J_{S,B}$ was calculated by summing the total sap flux for all measured trees and dividing it by their summed basal area (A_B ; excluding bark). This was done for aspen and birch separately in the mixed stands. Because there was no relationship between tree size and $J_{S,B}$ within treatments, stand-level sap flux per unit ground area ($J_{S,G}$, $\text{g m}^{-2} \text{ground s}^{-1}$) was calculated by multiplying stand-level $J_{S,B}$ with the stand-level A_B to ground area (A_G) ratio.

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

For the determination of $J_{S_{max}}$ and $J_{S_{fit_G}}$, 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 $J_{S_{mean_G}}$ and analyses of θ /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_{S_B} was adjusted by dividing with the ratio between mean J_{S_B} of these trees and mean J_{S_B} 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^e ring in both years. Stand-level J_{S_B} of that stand was estimated assuming that J_{S_B} for birch and aspen in that stand (i.e., the mixed section of that CO_2^e 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 absorptance

Canopy transmission of photosynthetically active radiation (PAR) was measured with a ceptometer 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^e and O_3^e , and two in $\text{CO}_2^e + \text{O}_3^e$) 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-m² 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 (τ_{PAR}).

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

$$L = \frac{\left(f_b \left(1 - \frac{1}{2\Omega K_b} \right) - 1 \right) \ln \tau_{PAR}}{(0.283 + 0.785a - 0.159a^2)(1 - 0.47f_b)} \quad (1)$$

where f_b is the fraction of incident PAR which is beam, Ω is the canopy clumping factor, K_b 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 Ω of 0.84, which is a representative value for temperate broad-leaved forests (Baldochi 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_b was modeled as a function of solar zenith angle (Ψ) (Campbell and Norman 1998),

$$K_b = \frac{1}{2 \cos \Psi} \quad (2)$$

Leaf area indices of the five aspen stands for which there were no ceptometer 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 ($66 \pm 2 \text{ g m}^{-2}$) was not significantly affected by community type or treatment, justifying also the use of a common Ω value for all stands.

Canopy absorptance of short-wave radiation (A_c) 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 Ω 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, CO₂, O₃, 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 [O₃] was unusually low in 2004, but typical for this region in 2005 (U.S. Environmental Protection Agency; <http://www.epa.gov/ttn/airs/airsaqs/>). Mean daytime O₃ concentrations were 1.31× and 1.40× ambient in the elevated [O₃] treatments during the 2004 and 2005 summers, respectively. Mean daytime elevated CO₂ concentrations were about 525 ppm in both years.

Both L (Figure 1a) and the $A_B:A_G$ ratio (Figure 1b) were significantly ($P \leq 0.011$) increased by elevated [CO₂] and decreased by elevated [O₃]. Species composition also affected L and the $A_B:A_G$ ratio ($P \leq 0.028$), with higher values in mixtures than in monocultures. Leaf area index did not differ significantly between years ($P > 0.10$). Treatment effects on L , aver-

aged across years and communities, were +27% in CO₂^e, -33% in O₃^e and +11% in CO₂^e+O₃^e. Corresponding treatment effects on the $A_B:A_G$ ratio were +39, -18 and +9%. There were no main effects of CO₂, O₃ or community type on leaf to sapwood area ratio ($A_L:A_B$; Figure 1c). However, because $A_L:A_B$ was higher for aspen–birch stands in the control rings, there was a significant CO₂ × O₃ × Community interaction ($P = 0.085$). Analyzing the aspen–birch community data separately, the CO₂ × O₃ interaction was significant at $P = 0.026$. Canopy absorptance of short-wave radiation was increased by elevated [CO₂] and decreased by elevated [O₃] (Figure 1d). There was also a significant CO₂ × O₃ interaction on A_c .

Values of $J_{S_{max_G}}$ were increased by elevated [CO₂] ($P = 0.061$), but were not significantly affected by elevated [O₃] or community type (Figure 2a, Table 2). It was significantly higher in 2005 compared with 2004 ($P = 0.047$), and there was a near-significant CO₂ × O₃ × Year interaction ($P = 0.101$) caused by a stronger stimulation by elevated [CO₂] in ambient [O₃] than in elevated [O₃] in 2004, but similar enhancement in both O₃ regimes in 2005. The mean CO₂ effect on $J_{S_{max_G}}$ across communities was +34% in ambient [O₃] and +11% in elevated [O₃] in 2004, and corresponding values for 2005 were +13 and +15%. Analyzing data for the two years separately, the CO₂ effect was strong in 2004 ($P = 0.030$) and not statistically significant in 2005 ($P = 0.15$). Values of $J_{S_{max_B}}$ were significantly reduced by elevated [CO₂] ($P = 0.053$) and increased by elevated [O₃] ($P = 0.027$) and higher in aspen stands compared with aspen–birch stands ($P = 0.009$; Figure 2b, Table 2). Values of $J_{S_{max}}$ expressed per unit total leaf area ($J_{S_{max_L}}$) were higher in aspen stands than in aspen–birch stands ($P < 0.001$), and substantially higher in the O₃^e treatment compared with control values, as indicated by the significant CO₂ × O₃ interaction ($P = 0.018$; Figure 2c, Table 2). A CO₂ × Year interaction on $J_{S_{max_L}}$ was caused by a stronger negative effect of elevated [CO₂] in 2005 than in 2004. The value of $J_{S_{max_G}}$ normalized for canopy absorptance 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). Periods defining $J_{S_{max}}$ occurred on day of year (DOY) 196–200 in 2004 and DOY 190–194 in 2005. The 2004 $J_{S_{max}}$ period had daily mean $T = 18.8$ °C, $Q_p = 0.44$ mmol m⁻² s⁻¹, $D = 0.44$ kPa and $\theta_v = 0.12$ (0–15-cm depth in control, mean of aspen and aspen–birch stands), whereas the 2005 $J_{S_{max}}$ period was warmer ($T = 24.2$ °C) and somewhat clearer ($Q_p = 0.49$ mmol m⁻² s⁻¹) with drier air ($D = 0.81$ kPa) and soil ($\theta_v = 0.08$).

Elevated [CO₂] increased $J_{S_{fit_G}}$ ($P = 0.077$) and there was a similar CO₂ × O₃ × Year interaction on $J_{S_{fit_G}}$ ($P = 0.064$) as on $J_{S_{max_G}}$ (Figure 2e, Table 2). In contrast to $J_{S_{max_G}}$, however, $J_{S_{fit_G}}$ did not differ significantly between years and was higher in aspen stands than in aspen–birch stands ($P = 0.090$). Fits between J_{S_G} and Q_p and D on which $J_{S_{fit_G}}$ was based had a mean r^2 of 0.74 (range 0.51–0.81). Mean peak-season J_{S_G} in 2004 was significantly increased by elevated [CO₂] ($P = 0.044$) and higher in aspen stands than in aspen–birch stands ($P = 0.060$; Figure 2f, Table 2). Treatment effects on J_{S_G} were consistent throughout most of the 2004 growing season (data not shown).

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 O₃ and CO₂ concentrations are calculated for the daytime period only.

	2004	2005
T (°C)	16.3	19.9
D (kPa)	0.35	0.51
Q_p (mmol m ⁻² s ⁻¹)	0.37	0.40 ¹
Precipitation (mm)	147	183
Mean volumetric soil water (θ_v)	0.12 ²	0.11 ²
Minimum volumetric soil water (θ_v)	0.08 ²	0.06 ²
Ambient [O ₃] (ppbv)	32.8	37.0
Elevated [O ₃] (ppbv)	43.1	51.6
Ambient [CO ₂] (ppmv)	370	368
Elevated [CO ₂] (ppmv)	525	527

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.

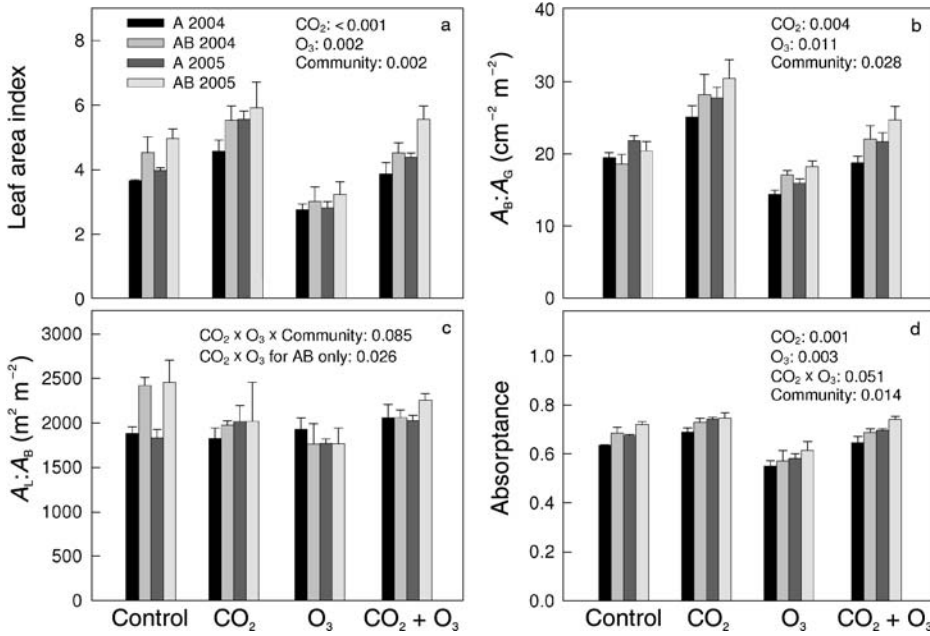


Figure 1. (a) Leaf area index, (b) basal to ground area ($A_B:A_G$) ratio, (c) leaf to basal area ($A_L:A_B$) ratio and (d) canopy absorbance of solar radiation of aspen (A) and aspen–birch (AB) communities in 2004 and 2005 in the Aspen FACE experiment. Absorbance is calculated for the period mid-June through August. The P values of significant effects ($P < 0.10$) are given in the panels. Basal area was measured at the end of each growing season and is statistically tested for 2004 data only because it is a cumulative measure.

However, the $J_{S,G}$ relative control increased progressively (from ~1.3 to ~1.55) in CO₂^e during September in both community types, and in aspen stands there was a tendency for O₃^e to have a negative effect (from ~1.0 to ~0.9) in late September.

Values of $J_{S,G}$ decreased in all treatments during September 2004 in response to decreasing D and L (data not shown).

The magnitude of the elevated [CO₂] and [O₃] effects on $J_{S,G}$ did not vary with D (Table 3). Fumigation treat-

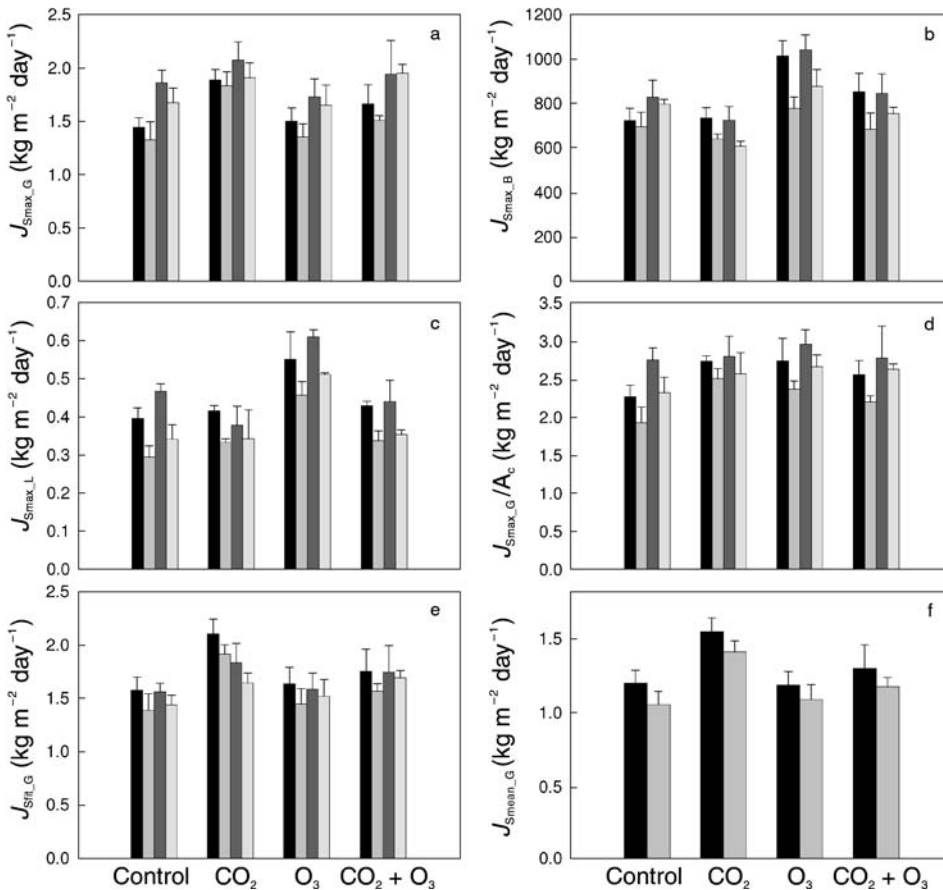


Figure 2. (a–d) Maximum (J_{Smax}), (e) fitted ($J_{Sfit,G}$) and (f) mean ($J_{Smean,G}$) sap flux density of aspen (A) and aspen–birch (AB) communities in 2004 and 2005. Values of J_{Smax} are expressed per unit (a) ground ($J_{Smax,G}$), (b) basal ($J_{Smax,B}$), and (c) leaf area ($J_{Smax,L}$), as well as (d) on a canopy absorbance (A_c) basis ($J_{Smax,G}$ divided by A_c of short-wave radiation). See Figure 1 for legend.

Table 2. Statistical significance (P values) of block, CO₂, O₃, community, year and their relevant interactions on maximum ($J_{S_{max}}$), mean ($J_{S_{mean}}$) and fitted ($J_{S_{fit_G}}$) daily sap flux density ($\text{kg m}^{-2} \text{day}^{-1}$) from mid-June through August. Values of $J_{S_{max}}$ are expressed per unit ground ($J_{S_{max_G}}$), leaf ($J_{S_{max_L}}$) or basal ($J_{S_{max_B}}$) area, as well as on a canopy absorptance basis ($J_{S_{max_G}}$ divided by the canopy absorptance (A_c) of short-wave radiation). Effects are considered significant at $P < 0.10$.

Source	$J_{S_{fit_G}}$	$J_{S_{max_G}}$	$J_{S_{max_B}}$	$J_{S_{max_L}}$	$J_{S_{max_G}}/A_c$	$J_{S_{mean_G}}$
Block	ns	ns	ns	ns	ns	ns
CO ₂	0.077	0.061	0.053	0.012	ns	0.044
O ₃	ns	ns	0.027	0.005	ns	ns
CO ₂ × O ₃	ns	ns	ns	0.018	ns	ns
Community	0.090	ns	0.009	< 0.001	0.024	0.060
CO ₂ × Community	ns	ns	ns	ns	ns	ns
O ₃ × Community	ns	ns	ns	ns	ns	ns
CO ₂ × O ₃ × Community	ns	ns	ns	ns	ns	ns
Year	ns	0.047	ns	ns	ns	–
CO ₂ × Year	ns	ns	ns	0.056 ²	ns	–
O ₃ × Year	0.077	ns	ns	ns	ns	–
CO ₂ × O ₃ × Year	0.064 ¹	ns (0.101)	ns	ns	ns	–
Community × Year	ns	ns	ns	ns	ns	–
CO ₂ × Community × Year	ns	ns	ns	ns	ns	–
O ₃ × Community × Year	ns	ns	ns	ns	ns	–
CO ₂ × O ₃ × Community × Year	ns	ns	ns	ns	ns	–

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.

ment– θ_v interactions on J_{S_G} were more pronounced in aspen stands than in aspen–birch stands (data not shown for aspen–birch). In pure aspen stands, stimulation of J_{S_G} by CO₂^e was strong during drier periods in 2004 (Figure 3a), whereas no such stimulation was observed during the drier year of 2005 (Figure 3b). In 2005, the J_{S_G} relative to the treatment control decreased with decreasing θ_v in aspen stands growing in elevated [O₃].

Relationships between daily mean Q_p and daily stand sap flux were approximately linear, whereas sap flux responses to D were asymptotic, increasing only slightly above a D of 0.4 kPa for 24-h data and above 1.0 kPa for 30-min data (Figure 4). Values of J_{S_G} were higher in aspen stands than in aspen–birch stands on days with relatively high Q_p and D , but similar in the two communities on days with low Q_p and D (Figure 4a, Table 3). Low soil water content in the upper soil layer had a small effect on daily sap flux in control rings in both years (data not shown).

Birch and aspen had similar J_{S_B} in the mixed stands, but because birch had higher A_B than aspen (+51%), J_{S_G} was significantly higher in birch (+58%, $P = 0.063$; Table 4). The J_{S_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_v of the upper soil layer varied with time (Figure 5). Before and early during the growing season, θ_v was significantly increased by elevated [CO₂] (significant effects with $0.015 \leq P \leq 0.078$), whereas elevated [O₃] often had a negative effect on θ_v during the growing season ($0.016 \leq P \leq 0.086$). Soil water content was often significantly lower in aspen stands than in aspen–birch stands ($0.0008 \leq P \leq 0.090$) during the peak and (in 2004) late growing season. There were no significant interacting effects of CO₂ and O₃ on θ_v , and the few interacting effects of fumigation treatments and community type on θ_v that were found were not consistent between years (data not shown).

Table 3. Daily sap flux per unit ground area (J_{S_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).

D (kPa)	Number of days (A, AB)	J_{S_G} in control ($\text{kg m}^{-2} \text{day}^{-1}$)		Percent change					
		A	AB	CO ₂ ^e		O ₃ ^e		CO ₂ ^e +O ₃ ^e	
				A	AB	A	AB	A	AB
$D < 0.3$	35, 26	0.91 ± 0.06	0.91 ± 0.05	+24	+21	0	+8	+13	+14
$0.3 \geq D < 0.6$	41, 39	1.39 ± 0.09	1.24 ± 0.11	+27	+28	+2	+5	+14	+14
$D \geq 0.6$	18, 12	1.52 ± 0.09	1.42 ± 0.12	+23	+26	+2	+3	+12	+18
All data	95, 77	1.24 ± 0.08	1.17 ± 0.08	+25.3	+25.3	+1.4	+5.6	+13.2	+14.9

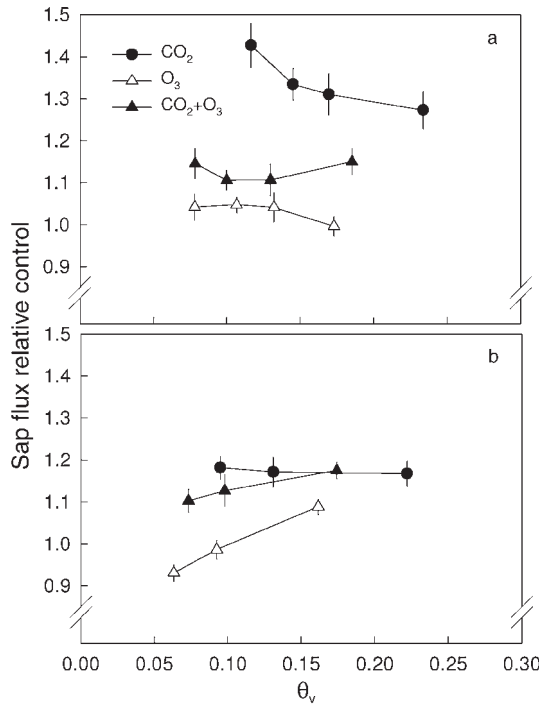


Figure 3. Daily sap flux relative to the treatment control versus volumetric soil water content (θ_v , 0–15-cm depth) of pure aspen stands in (a) 2004 and (b) 2005. Fully replicated bi-weekly θ_v data were temporally interpolated based on relative changes in continuous θ_v data available from one ring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

Discussion

Effects of elevated $[\text{CO}_2]$ and $[\text{O}_3]$, 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 $[\text{CO}_2]$ and $[\text{O}_3]$ on stand structure and water-use processes of land surfaces. Our results suggest that the effects of elevated $[\text{CO}_2]$ and $[\text{O}_3]$ 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 $[\text{CO}_2]$ 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 $[\text{CO}_2]$ (Table 2, Figures 2a, 2e and 2f). Elevated $[\text{CO}_2]$ increased A_B by 36% and L 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 $[\text{CO}_2]$ (+62%

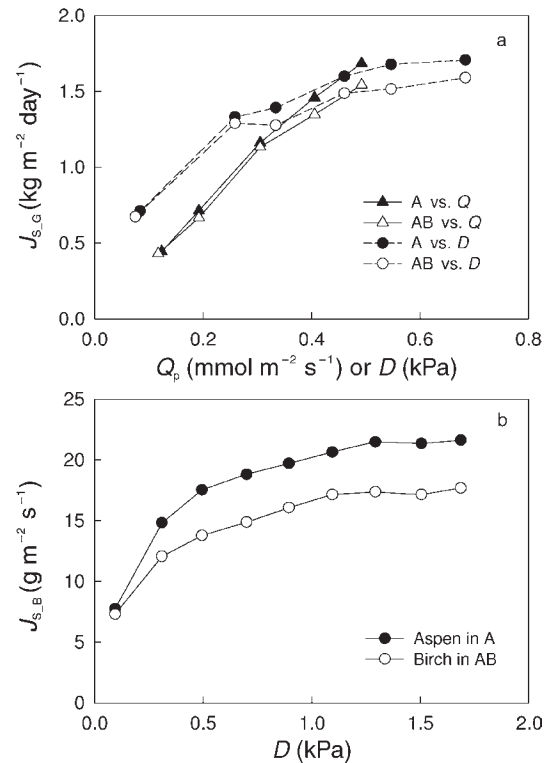


Figure 4. Sap flux density (J_S) of aspen (A) and aspen–birch (AB) communities in relation to (a) photosynthetic photon flux (Q_p) and (a, b) vapor pressure deficit of the air (D). The J_S data are (a) daily integrals and (b) half-hourly values, expressed per unit ground (J_{S-G}) and basal (J_{S-B})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

at the end of 2003; King et al. 2005). These results suggest that trees in elevated $[\text{CO}_2]$ used more water than trees in ambient $[\text{CO}_2]$ because they were larger and had more transpiring leaf area and more extensive absorbing root systems. Although elevated $[\text{CO}_2]$ has been observed to increase tree size and tree water use in shorter-term studies with potted temperate deciduous tree species (Beerling 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 between CO_2 treatments either decreased (Wullschlegel and Norby 2001) or mostly did not significantly affect (Schäfer et al. 2002, Cech et al. 2003) J_{S-G} in elevated $[\text{CO}_2]$. 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 ; Figure 1a and unpublished data for 2006). In our study, sap flux responses are thus fully acclimated to the elevated $[\text{CO}_2]$ and $[\text{O}_3]$ 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 (Wullschlegel and Norby 2001, Schäfer et al. 2002,

Table 4. Statistical significance (P values) of block, CO₂, O₃, species, year and their relevant interactions on maximum daily sap flux density (J_{Smax} ; kg m⁻² day⁻¹) and basal to ground area ($A_B:A_G$) ratio, comparing birch (B) with aspen (A) growing in either monoculture or in aspen–birch mixture. Values of J_{Smax} are expressed per unit ground (J_{Smax_G}) or basal (J_{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 $P < 0.10$.

Source	Birch versus aspen in monoculture		Birch versus aspen in species mixture		
	J_{Smax_B}		J_{Smax_B}	J_{Smax_G}	$A_B:A_G$ ratio ²
Block	ns		ns	ns	ns
CO ₂	0.055 (-)		0.012 (-)	ns	0.008 (+)
O ₃	0.062 (+)		0.023 (+)	ns	0.089 (-)
CO ₂ × O ₃	ns		ns	ns	ns
Species	0.023 (A +15%)		ns	0.063 (B +58%)	0.053 (B +51%)
CO ₂ × Species	ns		ns	0.092 ¹	ns
O ₃ × Species	ns		ns	ns	ns
CO ₂ × O ₃ × Species	ns		ns	ns	ns
Year	ns		ns	0.020 (2005)	–
CO ₂ × Year	ns		ns	ns	–
O ₃ × Year	ns		ns	ns	–
CO ₂ × O ₃ × Year	ns		ns	ns	–
Species × Year	ns		ns	ns	–
CO ₂ × Species × Year	ns		ns	ns	–
O ₃ × Species × Year	ns		ns	ns	–
CO ₂ × O ₃ × Species × Year	ns		ns	ns	–

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_B is a cumulative measure.

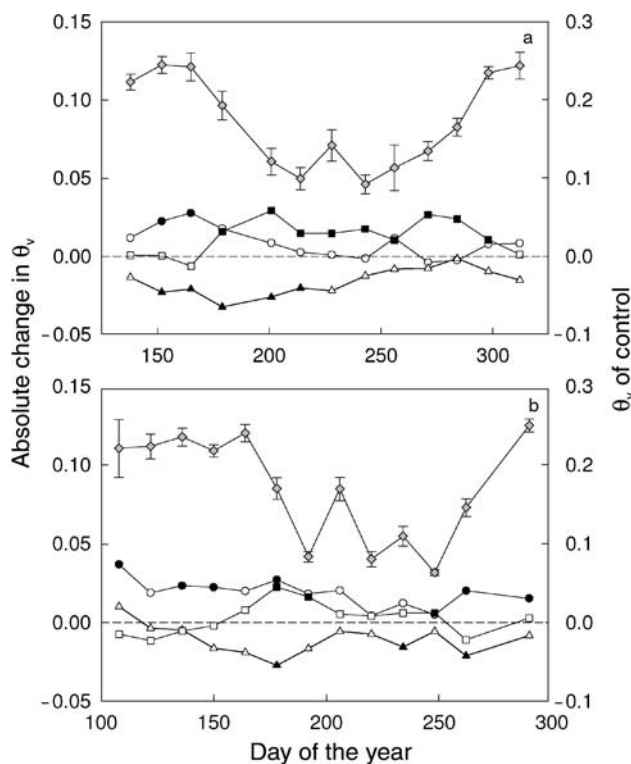


Figure 5. Volumetric soil water content (θ_v , 0–15-cm depth) of control stands (mean of aspen and aspen–birch stands \pm SE; \diamond) as well as absolute effects of CO₂ (\circ , \bullet) and O₃ (\triangle , \blacktriangle) treatments and community type (θ_v in aspen–birch mixture minus θ_v in aspen monoculture, \square , \blacksquare) on θ_v during (a) 2004 and (b) 2005. Significant effects ($P < 0.10$) of elevated [CO₂], elevated [O₃] and community type are indicated by filled symbols.

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 θ_v , 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 and +3 in CO₂^e, O₃^e and CO₂^e+O₃^e, respectively, in May 2003; C. Giardina, personal communication). Furthermore, θ_v 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 θ_v did not persist later during the growing season.

Similar J_{S_L} and stand sap flux on an absorbed radiation basis in trees in the control and CO₂^e 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 $[\text{CO}_2]$ on tree size and soil properties probably resulted in an increased capacity for water uptake and transfer that overrode stomatal closure responses to elevated $[\text{CO}_2]$. The importance of structural adjustments in response to elevated $[\text{CO}_2]$ was demonstrated in a chamber study with paper birch, where g_s increased in response to elevated $[\text{CO}_2]$ as a result of increased hydraulic efficiency (Kubiske 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 θ_v 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 $[\text{O}_3]$ did not reduce $J_{S,G}$ despite substantial decreases in L (-22% across years, communities and CO_2 regimes) and A_B (-20%) (Table 2, Figures 1a, 1b and 2a). Values of $J_{S_{\text{max},L}}$ 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 $\text{CO}_2^\circ + \text{O}_3^\circ$ (Figure 2c, Table 2). Most of the increase in $J_{S_{\text{max},L}}$ in response to elevated $[\text{O}_3]$ 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 $[\text{O}_3]$ and ambient $[\text{O}_3]$ despite reductions in tree size and L in response to elevated $[\text{O}_3]$ 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 $[\text{O}_3]$ compared with ambient $[\text{O}_3]$ (King et al. 2005). The relative importance of possible effects of elevated $[\text{O}_3]$ 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 $[\text{O}_3]$ could not be assessed with the data we obtained. The negative effect of elevated $[\text{O}_3]$ on θ_v of the upper-most soil layer (Figure 5) was probably caused by tree water use similar to ambient $[\text{O}_3]$ treatments in combination with increased forest floor evapotranspiration in stands with lower L and canopy absorptance (Figures 1a and 1d) and higher ground vegetation cover (Bandeff et al. 2006).

Similar tree water use in ambient and elevated $[\text{O}_3]$ treatments, despite lower L in elevated $[\text{O}_3]$, 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 $[\text{O}_3]$ (Nunn et al. 2006, Warren et al. 2006). This difference may be explained by the strong enhancement of $[\text{O}_3]$ in the German study ($2\times$ ambient $[\text{O}_3]$) compared with our study (1.3 – $1.4\times$ ambient $[\text{O}_3]$), because stomatal closure usually occurs at high $[\text{O}_3]$ but not at the more moderately elevated $[\text{O}_3]$ 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 $[\text{CO}_2]$ and elevated $[\text{O}_3]$ (Riikonen et al. 2008). The reduction in g_s in elevated $[\text{CO}_2]$ 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 $[\text{O}_3]$ was observed only during a dry period in late August–early September, when drought was particularly pronounced in stands in the elevated $[\text{O}_3]$ treatment (Figure 5b). 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 (authors' unpublished data).

Effects of climate and treatment by climate interactions

Both aspen and birch showed asymptotic relationships between D and J_s , 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 θ_v of the upper soil (Figure 5) during dry periods in pure aspen stands exposed to elevated $[\text{O}_3]$ may be explained in a hydrological context. The aspen stands in elevated $[\text{O}_3]$ had stand-level tree water use similar to control stands in combination with presumably higher understory and forest floor evapotranspiration 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 θ_v (data not shown) of all treatment and community combinations, and higher ground vegetation cover than aspen stands in the other treatments (Bandeff et al. 2006). This is the first stand-level study to corroborate the long-standing hypothesis that elevated $[\text{O}_3]$ may predispose trees to drought stress (e.g., Matussek et al. 2005).

The observed stimulation of tree water use by pure aspen stands in the CO_2° treatment at low θ_v in 2004 but not in 2005 (Figure 3) is puzzling. The absence of increased drought vulnerability despite increased tree water use in elevated $[\text{CO}_2]$ 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 water-holding capacity of the upper soil (Figure 5) are all factors that act favorably on soil water status in elevated $[\text{CO}_2]$.

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 θ_v of the upper soil was consequently lower in aspen stands than in aspen–birch stands (Figure 5). Leaf area index, A_B and canopy absorbance were higher in aspen–birch stands than in aspen stands (Figures 1a, 1b and 1d). Higher A_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 structure, and stomatal behavior, and in pure aspen and mixed aspen–birch communities, this response variable was similarly sensitive to elevated concentrations of CO₂ or O₃, or both, under most conditions (Figure 2, Table 2). The lower O₃ 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 O₃ 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 [CO₂], but was not significantly affected by elevated [O₃]. Stand-level leaf area responses to elevated [CO₂] and [O₃] were consistent with hypothesized carbon-balance effects of these gases, e.g., a positive CO₂ effect and a negative O₃ 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 fumigated with elevated [CO₂] and in control stands, and was significantly higher in stands in the O₃^c treatment. Trees in elevated [CO₂] were larger with more transpiring leaf area and water-absorbing fine roots, and were growing in soil with CO₂-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 [O₃] on g_s , 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 [O₃] could not be assessed from the data we obtained. Our results suggest that long-term cumulative effects of elevated [CO₂] and [O₃] on tree and stand structure may be more important than the expected primary stomatal closure responses to elevated [CO₂] and [O₃] for understanding how these trace gases affect stand-level water use.

Acknowledgments

The project was supported by the National Institute for Global Environmental Change, Midwestern Regional Office, via the U.S. Department of Energy. The first author was partly supported by Royal Swedish Academy of Agriculture (KSLA). In-kind support from the USDA Forest Service is gratefully acknowledged. The Aspen FACE Experi-

ment is funded principally by the Office of Science (BER), U.S. Department of Energy, with additional support from the USFS Global Change Program, Michigan Technological University, the Canadian Forest Service and the USFS Northern Research Station. We are grateful to Prof. Ram Oren for sharing sap flux calculation software with us, and to Kristine Crous, Matt Mioduszewski and Jeffrey Katz for assistance with measurements and sap flow sensor installation in the field.

References

- Baldocchi, D. and T. Meyers. 1998. On using eco-physiological, micrometeorological and biogeochemical theory to evaluate carbon dioxide, water vapor and trace gas fluxes over vegetation: a perspective. *Agric. For. Meteorol.* 90:1–25.
- Bandeef, J.M., K.S. Pregitzer, W.M. Loya, W.E. Holmes and D.R. Zak. 2006. Overstory community composition and elevated atmospheric CO₂ and O₃ modify understory biomass production and nitrogen acquisition. *Plant Soil* 282:251–259.
- Beerling, D.J., J. Heath, F.I. Woodward and T.A. Mansfield. 1996. Drought–CO₂ interactions in trees: observations and mechanisms. *New Phytol.* 134:235–242.
- Bernacchi, C.J., C. Calfapietra, P.A. Davey, V.E. Wittig, G.E. Scarascia-Mugnozza, C.A. Raines and S.P. Long. 2003. Photosynthesis and stomatal conductance responses of poplars to free air CO₂ enrichment (PopFACE) during the first growth cycle and immediately following coppice. *New Phytol.* 159:609–621.
- Bovard, B.D., P.S. Curtis, C.S. Vogel, H.-B. Su and H.P. Schmid. 2005. Environmental controls on sap flow in a northern hardwood forest. *Tree Physiol.* 25:31–38.
- Campbell, G.S. and J.M. Norman. 1998. An introduction to environmental biophysics. 2nd Edn. Springer-Verlag, New York, 281 p.
- Cech, P.G., S. Pepin and C. Körner. 2003. Elevated CO₂ reduces sap flux in mature deciduous forest trees. *Oecologia* 137:258–268.
- Chen, J.M., P.D. Blanken, T.A. Black, M. Guilbeault and S. Chen. 1997. Radiation regime and canopy architecture in a boreal aspen forest. *Agric. For. Meteorol.* 86:107–125.
- Cramer, W., A. Bondeau, F.I. Woodward et al. 2001. Global response of terrestrial ecosystem structure and function to CO₂ and climate change: from six dynamic global vegetation models. *Global Change Biol.* 7:357–373.
- Dentener, F., D. Stevenson, K. Ellingsen et al. 2006. The global atmospheric environment for the next generation. *Environ. Sci. Technol.* 40:3586–3594.
- Dickson, R.E., K.F. Lewin, J.G. Isebrands et al. 2000. Forest atmosphere carbon transfer and storage (FACTS-II) the aspen free-air CO₂ and O₃ enrichment (FACE) project: an overview. Technical Report NC-214. USDA For. Serv., North Central Res. Stn., Rhineland, WI, 68 p.
- Edwards, W.R.N. and R.E. Booker. 1984. Radial variation in the axial conductivity of *Populus* and its significance in heat pulse velocity measurement. *J. Exp. Bot.* 35:551–561.
- Ellsworth, D.S. 1999. CO₂ enrichment in a maturing pine forest: are CO₂ exchange and water status in the canopy affected? *Plant Cell Environ.* 22:461–472.
- Ellsworth, D.S., P.B. Reich, E.S. Naumburg, G.W. Koch, M.E. Kubiske and S.D. Smith. 2004. Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO₂ across four free-air CO₂ enrichment experiments in forest, grassland and desert. *Global Change Biol.* 10:2121–2138.
- Engel V.C., K.L. Griffin, R. Murthy, L. Patterson, C. Klimas and M. Potosnak. 2004. Growth CO₂ concentration modifies the transpiration response of *Populus deltoides* to drought and vapor pressure deficit. *Tree Physiol.* 24:1137–1145.

- Ewers, B.E. and R. Oren. 2000. Analyses of assumptions and errors in the calculation of stomatal conductance from sap flux measurements. *Tree Physiol.* 20:579–589.
- Felzer, B., J. Reilly, J. Melillo, D. Kicklighter, M. Sarofim, C. Wang, R. Prinn and Q. Zhuang. 2005. Future effects of ozone on carbon sequestration and climate change policy using a global biogeochemical model. *Climatic Change* 73:345–373.
- Gielen, B., C. Calfapietra, M. Sabatti and R. Ceulemans. 2001. Leaf area dynamics in a closed poplar plantation under free-air carbon dioxide enrichment. *Tree Physiol.* 21:1245–1255.
- Goudriaan, J. 1977. *Crop micrometeorology: a simulation study*. Centre for Agricultural Publishing Documentation (Pudoc), Wageningen, 249 p.
- Granier, A. 1985. Une nouvelle méthode pour la mesure du flux de sève brute dans le tronc des arbres. *Ann. Sci. For.* 42:193–200.
- Granier, A. 1987. Evaluation of transpiration in a Douglas-fir stand by means of sapflow measurements. *Tree Physiol.* 3:309–320.
- Gunderson, C.A., J.D. Sholtis, S.D. Wullschleger, D.T. Tissue, P.J. Hanson and R.J. Norby. 2002. Environmental and stomatal control of photosynthetic enhancement in the canopy of a sweetgum (*Liquidambar styraciflua* L.) plantation during three years of CO₂ enrichment. *Plant Cell Environ.* 25:379–393.
- Heath, J. and G. Kerstiens. 1997. Effects of elevated CO₂ on leaf gas exchange in beech and oak at two levels of nutrient supply: consequences for the sensitivity to drought in beech. *Plant Cell Environ.* 20:57–67.
- Hendrey, G.R., D.S. Ellsworth, K.F. Lewin and J. Nagy. 1999. A free-air CO₂ enrichment system for exposing tall forest vegetation to elevated atmospheric CO₂. *Global Change Biol.* 5:293–309.
- Hogg, E.H., B. Saugier, J.Y. Pontailier, T.A. Black, W. Chen, P.A. Hurdle and A. Wu. 2000. Responses of trembling and hazelnut to vapor pressure deficit in a boreal deciduous forest. *Tree Physiol.* 20:725–734.
- IPCC. 2007. The physical science basis. Contribution of working group I to the fourth assessment report of the IPCC. Available at: <http://www.ipcc.ch/>.
- Jarvis, P.G. and K.G. McNaughton. 1986. Stomatal control of transpiration: scaling up from leaf to region. *Adv. Ecol. Res.* 15:1–49.
- Jones, H.G. 1992. *Plants and microclimate: a quantitative approach to environmental plant physiology*. 2nd Edn. Cambridge University Press, Cambridge, 428 p.
- Keel, S.G., S. Pepin, S. Leuzinger and C. Körner. 2007. Stomatal conductance in mature deciduous forest trees exposed to elevated CO₂. *Trees* 21:151–159.
- King, J., K. Pregitzer, D. Zak, J. Sober, J. Isebrands, R. Dickson, G. Hendrey and D. Karnosky. 2001. Fine root biomass and fluxes of soil carbon in young stands of paper birch and trembling aspen as affected by elevated atmospheric CO₂ and tropospheric O₃. *Oecologia* 128:237–250.
- King, J.S., M.E. Kubiske, K.S. Pregitzer, G.R. Hendrey, E.P. McDonald, C.P. Giardina, V.S. Quinn and D.F. Karnosky. 2005. Tropospheric O₃ compromises net primary production in young stands of trembling aspen, paper birch and sugar maple in response to elevated atmospheric CO₂. *New Phytol.* 168:623–635.
- Kubiske, M.E. and K.S. Pregitzer. 1997. Ecophysiological responses to simulated canopy gaps of two tree species of contrasting shade tolerance in elevated CO₂. *Funct. Ecol.* 11:24–32.
- Körner, C., R. Asshoff, O. Bignucolo, S. Hättenschwiler, S.G. Keel, S. Peláez-Riedl, S. Pepin, R.T.W. Siegwolf and G. Zotz. 2005. Carbon flux and growth in mature deciduous forest trees exposed to elevated CO₂. *Science* 309:1360–1362.
- Köstner, B., A. Granier and J. Čermák. 1998. Sapflow measurements in forest stands: methods and uncertainties. *Ann. Sci. For.* 55: 13–27.
- Lu, P., L. Urban and P. Zhao. 2004. Granier's thermal dissipation probe (TDP) method for measuring sap flow in trees: theory and practice. *Acta Bot. Sin.* 46:631–646.
- Matyssek, R. and H. Sandermann. 2003. Impact of ozone on trees: an ecophysiological perspective. *In Progress in Botany* 64. Eds. K. Esser, U. Lüttge, W. Beyschlag and F. Hellwig. Springer-Verlag, Heidelberg, pp 349–404.
- Matyssek, R., D. Le Thiec, M. Löw, P. Dizengremel, A.J. Nunn and K.-H. Häberle. 2005. Interactions between drought stress and O₃ stress in forest trees. *Plant Biol.* 7:1–7.
- Medlyn, B.E., C.V.M. Barton, M.S.J. Broadmeadow et al. 2001. Stomatal conductance of forest species after long-term exposure to elevated CO₂ concentration: a synthesis. *New Phytol.* 149: 247–264.
- Noormets, A., A. Söber, E.J. Pell, R.E. Dickson, K.G. Podila, J. Söber, J.G. Isebrands and D.F. Karnosky. 2001. Stomatal and non-stomatal limitation to photosynthesis in two trembling aspen (*Populus tremul* Michx.) clones exposed to elevated CO₂ and/or O₃. *Plant Cell Environ.* 24:327–336.
- Norby, R.J., S.D. Wullschleger, C.A. Gunderson, D.W. Johnson and R. Ceulemans. 1999. Tree responses to rising CO₂ in field experiments: implications for the future forest. *Plant Cell Environ.* 22: 683–714.
- Nunn, A.J., G. Weiser, I.M. Reiter, K.-H. Häberle, R. Grote, W.M. Havranek and R. Matyssek. 2006. Testing the unifying theory of ozone sensitivity with mature trees of *Fagus sylvatica* and *Picea abies*. *Tree Physiol.* 26:1391–1403.
- Ollinger, S.V., J.D. Aber, P.B. Reich and R. Freuder. 2002. Interactive effects of nitrogen deposition, tropospheric ozone, elevated CO₂ and land use history on the carbon dynamics of northern hardwood forests. *Global Change Biol.* 8:545–562.
- Phillips, N., R. Oren and R. Zimmermann. 1996. Radial patterns of xylem sap flow in non-, diffuse- and ring-porous tree species. *Plant Cell Environ.* 19:983–990.
- Reich, P.B. and R.G. Amundson. 1985. Ambient levels of ozone reduce net photosynthesis in tree and crop species. *Science* 230: 566–570.
- Riikonen, J., K. Kets, J. Darbah, E. Oksanen, A. Sober, E. Vapaavuori, M.E. Kubiske, N. Nelson and D.F. Karnosky. 2008. Carbon gain and bud physiology in *Populus tremuloides* and *Betula papyrifera* grown under long-term exposure to elevated concentrations of CO₂ and O₃. *Tree Physiol.* 28:243–253.
- Schäfer, K.V.R., R. Oren, C.T. Lai and G.G. Katul. 2002. Hydrologic balance in an intact temperate forest ecosystem under ambient and elevated atmospheric CO₂ concentration. *Global Biol.* 8:895–911.
- Sperry, J.S. 2000. Hydraulic constraints on plant gas exchange. *Agric. For. Meteorol.* 104:13–23.
- Spitters, C.J.T., H.A.J.M. Toussaint and J. Goudriaan. 1986. Separating the diffuse and direct component of global radiation and its implications for modeling canopy photosynthesis. 1. Components of incoming radiation. *Agric. For. Meteorol.* 38:217–229.
- Warren, C.R., M. Löw, R. Matyssek and M. Tausz. 2006. Internal conductance to CO₂ transfer of adult *Fagus sylvatica*: variation between sun and shade leaves and due to free-air ozone fumigation. *J. Exp. Bot.* 59:130–138.
- Welles, J.M. 1990. Some indirect methods of estimating canopy structure. *In Instrumentation for Studying Vegetation Canopies for Remote Sensing in Optical and Thermal Infrared Regions*. Eds. N.S. Goel and J.M. Norman. *Remote Sens. Rev.* 5:31–43.

- Wittig, V.E., E.A. Ainsworth and S.P. Long. 2007. To what extent do current and projected increases in surface ozone affect photosynthesis and stomatal conductance of trees? A meta-analytic review of the last three decades of experiments. *Plant Cell Environ.* 30: 1150–1162.
- Woodward, F.I. 1987. *Climate and plant distribution*. Cambridge University Press, Cambridge, 174 p.
- Wullschleger, S.D. and R.J. Norby. 2001. Sap velocity and canopy transpiration for a 12-year-old sweetgum stand exposed to free-air CO₂ enrichment. *New Phytol.* 150:489–498.
- Wullschleger, S.D., C.A. Gunderson, P.J. Hanson, K.B. Wilson and R.J. Norby. 2002. Sensitivity of stomatal and canopy conductance to elevated CO₂ concentration—interacting variables and perspectives of scale. *New Phytol.* 153:485–496.