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Leaf and canopy conductance in aspen and aspen-birch forests under free-air enrichment of carbon dioxide and ozone

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Summary Increasing concentrations of atmospheric carbon dioxide (CO_2) and tropospheric ozone (O_3) have the potential to affect tree physiology and structure, and hence forest feedbacks on climate. Here, we investigated how elevated concentrations of CO_2 (+45%) and O_3 (+35%), alone and in combination, affected conductance for mass transfer at the leaf and canopy levels in pure aspen (*Populus tremuloides* Michx.) and in mixed aspen and birch (*Betula papyrifera* Marsh.) forests in the free-air CO_2–O_3 enrichment experiment near Rhinelander, Wisconsin (Aspen FACE). The study was conducted during two growing seasons, when steady-state leaf area index (L) had been reached after > 6 years of exposure to CO_2- and O_3-enrichment treatments. Canopy conductance (g_c) was estimated from stand sap flux, while leaf-level conductance of sun leaves in the upper canopy was derived by three different and independent methods: sap flux and L in combination with vertical canopy modelling, leaf ^{13}C discrimination methodology in combination with photosynthesis modelling and leaf-level gas exchange. Regardless of the method used, the mean values of leaf-level conductance were higher in trees growing under elevated CO_2 and/or O_3 than in trees growing in control plots, causing a $CO_2 \times O_3$ interaction that was statistically significant ($P \leq 0.10$) for sap flux- and (for birch) ^{13}C-derived leaf conductance. Canopy conductance was significantly increased by elevated CO_2 but not significantly affected by elevated O_3. Investigation of a short-term gap in CO_2 enrichment demonstrated a +10% effect of transient exposure of elevated CO_2-grown trees to ambient CO_2 on g_c. All treatment effects were similar in pure aspen and mixed aspen-birch communities. These results demonstrate that short-term primary stomatal closure responses to elevated CO_2 and O_3 were completely offset by long-term cumulative effects of these trace gases on tree and stand structure in determining canopy- and leaf-level conductance in pure aspen and mixed aspen-birch forests. Our results, together with the findings from other long-term FACE experiments with trees, suggest that model assumptions of large reductions in stomatal conductance under rising atmospheric CO_2 are very uncertain for forests.

Keywords: ^{13}C discrimination, FACE, photosynthesis, sap flow, stomata, tree community.

Introduction

Stomata constitute a crucial interface between the terrestrial biosphere and the atmosphere, regulating their exchange of gas and energy. As such, stomatal responses to atmospheric change have important implications for ecosystem hydrology (Betts et al. 2007), plant air pollution sensitivity (Sitch et al. 2007), biogeographic distributions (Gutschick 2007) and land surface energy partitioning affecting local and regional climate (Bonan 2008). Of the radiatively forcing trace gases (greenhouse gases) altered by human activities (IPCC 2007), only atmospheric carbon dioxide (CO_2) and tropospheric ozone (O_3) have the potential to strongly affect plant canopy physiology and structure. Both elevated CO_2 and elevated O_3 increase the leaf intercellular CO_2 concentration (c_i) as a result of increased ambient CO_2 concentration and impaired photosynthesis (Reich and Amundson 1985), respectively (Figure 1). Stomata of most plant species investigated thus far partially close in response to short-term increases in c_i (Mott 1988, Morison 1998) and stomatal conductance (g_s) might therefore be

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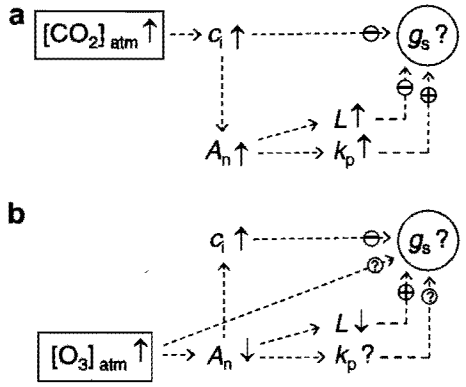

Figure 1. Hypothesized response pathway for effects of elevated (a) CO_2 and (b) O_3 on stomatal conductance (g_s). See text for hypotheses. c_i, intercellular CO_2 concentration; A_n, net photosynthesis; L, leaf area index; and k_p, whole-plant hydraulic conductance per unit ground area.

expected to decrease under growth in elevated CO_2 and O_3, as has been consistently observed in most experiments (e.g., Medlyn et al. 2001, Wittig et al. 2007).

In addition to the expected response of stomata to increased c_i under elevated CO_2 and O_3, stomata also respond to perturbations in the soil–plant–atmosphere hydraulic continuum. Stomatal conductance is constrained by the balance between water uptake and transfer capacity and total transpiring leaf area (e.g., Sperry 2000, Buckley 2005) and this may have a similarly strong, or stronger, effect on g_s as does increased c_i. Consequently, secondary effects of elevated CO_2 and O_3 on g_s come into play if these trace gases also affect the hydraulic and allocational properties of plants in the long term (Figure 1). Possible primary stomatal closure responses to elevated CO_2 may be counteracted or overridden by positive indirect effects on g_s, which are caused by enhanced root production and hydraulic capacity under elevated CO_2 (Figure 1a). The net effect of elevated CO_2 on leaf area-specific hydraulic conductance (k_l), and hence hydraulic constraints of g_s, is determined by the balance between possible increases in leaf area index (L) and whole-plant hydraulic conductance (k_p). Similarly, the c_i-mediated stomatal closure responses to elevated O_3 may be counteracted by, or act in concert with, long-term hydraulic and allocational adjustments under growth in elevated O_3, depending on the net effect of changes in L and k_p on k_l (Figure 1b). In addition, O_3 may also cause sluggishness in stomatal responses as guard cells and the surrounding epidermal cells become damaged (e.g., Mansfield 1998, Matyssek et al. 2006).

Coupled stomatal-photosynthesis models (Ball et al. 1987, Leuning 1995) are often incorporated into land surface schemes that are designed for General Circulation Models (e.g., Hadley Centre climate models, NCAR; Pitman 2003 and references therein). The stomatal-photosynthesis model formulation assumes that g_s and A_n are tightly linked by maintaining a constant ratio of intercellular to ambient CO_2 concentration (c_i/c_a), leading to substantial reductions in g_s under rising CO_2 (at given climatic conditions). While large decreases in g_s were typically observed in elevated CO_2 experiments with tree seedlings grown in controlled environments (e.g., Norby et al. 1999), the generality of this response has been challenged by recent findings from free-air CO_2 enrichment (FACE) experiments that were carried out in forests with steady-state L. There were no significant ($P \leq 0.10$) changes in g_s in a *Pinus taeda* L. dominated forest (Ellsworth 1999), a plantation with fast-growing *Populus* spp. (Bernacchi et al. 2003*a*; pre-coppice canopy closure), and in a multi-species mature deciduous forest (Keel et al. 2007), whereas a decreased g_s was reported for a *Liquidambar styraciflua* L. plantation (Gunderson et al. 2002). In these studies, L was unaffected by CO_2 treatment (Gielen et al. 2001, Schäfer et al. 2002, Körner et al. 2005), whereas below-ground activity (metabolism, fine root biomass, etc.) was substantially enhanced under elevated CO_2 (King et al. 2004, Körner 2006). Doubled O_3 concentration reduced g_s by 25% in both *Fagus sylvatica* L. and *Picea abies* (L.) Karst in a free-air O_3 enrichment experiment in a mature mixed forest in Germany (Nunn et al. 2006, Warren et al. 2006).

Given the uncertainties with regard to the mechanisms and magnitudes of effects of elevated CO_2 and elevated O_3 on g_s in closed forests, we estimated g_s or leaf conductance (g_l, conductance through stomata and leaf boundary layer) in closed pure aspen (*Populus tremuloides* Michx.) and mixed aspen and birch (*Betula papyrifera* Marsh.) communities in the free-air CO_2–O_3 enrichment experiment near Rhinelander, Wisconsin (Aspen FACE) in 2004 and 2005 using three different and independent methods: (i) sap flux and L in combination with vertical canopy modelling of radiation and g_l, (ii) leaf ^{13}C discrimination methodology in combination with photosynthesis modelling and (iii) leaf-level gas exchange. Whole-canopy sap flux-derived conductance (g_c) was also determined. There are advantages and disadvantages of all the three methods in estimating the leaf-level conductance in terms of temporal and spatial integration and dependence on modelling assumptions. The use of different methods serves to increase certainty if the results obtained agree, or to prevent precipitous conclusions drawn from one type of measurement if they do not. Although g_c is more relevant than leaf-level conductance (g_s or g_l) in studies of stand-level hydrology and energy partitioning, estimates of leaf-level conductance are critical for attributing effects on canopy transpiration to changes at the leaf (i.e., g_s or g_l) and canopy (i.e., L) levels, for separating stomatal and non-stomatal limitations of photosynthesis and for assessing plant sensitivity to O_3.

In the Aspen FACE experiment, stand development has occurred under the atmospheric gas treatments and the forests had reached steady-state L in 2004 (Uddling et al. 2008). Greater tree biomass, L and fine root biomass have been sustained by elevated CO_2 throughout the experiment,

whereas tree biomass and L but not fine root biomass were decreased by elevated O_3 (King et al. 2005). Stand-level tree sap flux of closed aspen and aspen-birch stands was increased by elevated CO_2 but not significantly affected by elevated O_3 after steady-state L had been reached (Uddling et al. 2008). The primary objective of this study was to determine how long-term exposure to elevated CO_2 and O_3, alone and in combination, affects conductance at the canopy and leaf levels in pure aspen and mixed aspen-birch forests. Furthermore, we separated the effect of long-term growth in elevated CO_2 from the short-term primary stomatal response to CO_2 by investigating sap flux responses during a transient exposure of high CO_2-grown trees to ambient CO_2 concentrations. Sap flux data were also used to examine the possible effects of elevated CO_2 and O_3 on the stomatal response to vapour pressure deficit of air (D).

Materials and methods

Site description

The Aspen FACE experiment near Rhinelander, Wisconsin (45.6° N and 89.5° W) has a randomized complete block design with orthogonal combinations of atmospheric CO_2 and O_3 treatments, and tree community composition as a split-plot factor. It consists of twelve 30-m-diameter circular plots with three control plots and three replicate plots each receiving elevated atmospheric CO_2 ($+CO_2$), elevated O_3 ($+O_3$) or both elevated CO_2 and O_3 ($+CO_2+O_3$) distributed over three blocks. The experiment was performed with 3–6-month-old plants at 1 m × 1 m spacing in July 1997 and fumigation treatments have been running since spring 1998. Each plot is divided into three sections with different tree community composition. This study was conducted in the eastern half with five clones of trembling aspen and in the south-western quadrant with an alternating mixture of trembling aspen and paper birch. All measurements were done within the core area of the plots, which is buffered from edge effects by five rows of trees on the outer edge of the treatment plots. After the growing season 2005, tree heights ranged between 5.7 and 7.6 m in the different treatment and community type combinations.

The Aspen FACE experiment is carried out on an old agricultural field and the soil is classified as an Alfic Haplorthod with a sandy loam soil texture. 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 CO_2 and O_3 aimed at maintaining target concentrations of 560 ppm CO_2 and 1.5 × ambient O_3 during hours when sun elevation was > 6°. Ozone enrichment was restricted to dry canopies on days when the maximum temperature was projected to be at least 15 °C. Daytime (i.e., sunrise to sunset) mean elevated concentrations during the 2004–2005 summers (mid-June through August) were 531 μmol CO_2 mol^{-1} and 1.35 × ambient O_3. Ambient mean daytime O_3 concentrations at the site were 32 and 38 nmol mol^{-1} during the 2004 and 2005 summers, respectively. Meteorological measurements that were used here include photosynthetically active photon flux density (PPFD) measured at the site in an open field, and air temperature and relative humidity, both measured above the canopy at 10 m above the ground in the centre of four plots. Temperature and relative humidity data were averaged across these four plots. Details about the site, micrometeorological monitoring and fumigation technology can be found in Dickson et al. (2000) and Hendrey et al. (1999).

Sap flux-derived conductance

Xylem sap flux density was measured using thermal dissipation sensors that were somewhat modified from Granier's original design (Granier 1985, 1987, Uddling et al. 2008). Our probes differed from the original design 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 that was surrounded by coiled heating wire inside a cylindrical aluminium tube. The thermocouple was 10 mm long and centred in the middle of the needle, while the coiled heating wire extended its full length (i.e., 20 mm). Stand-level tree water use in pure aspen and mixed aspen-birch stands during the growing seasons 2004–2005 was calculated as described by Uddling et al. (2008), except that in this study, sap flux estimates based on Granier's original calibration were scaled according to results from a gravimetric potometer experiment (see below).

Canopy conductance (g_c, ground area basis, including the pathway from the leaf intercellular space to the micrometeorological sensors above the canopy) was calculated from stand sap flux, atmospheric pressure and air D above the canopy (Monteith and Unsworth 1990), without accounting for the effects of canopy energy balance on leaf temperature and, hence, calculated conductance (Jarvis and McNaughton 1986). The Penman–Monteith equation could not be reliably used to calculate g_c as the experimental forest stands do not form a continuous canopy with their surroundings and equations for wind speed extinction over and within canopies therefore do not apply. Turbulent resistance to gas and sensible heat flux was probably small as the horizontal wind speed measured in the upper canopy in plots with fumigation did not differ from the horizontal wind speed measured above the canopy, 10 m above the ground (data not shown). Upper canopy wind speed did not significantly differ among treatments. The canopies studied were thus well coupled to the atmosphere and differences between air and canopy surface temperature were likely to be comparatively small.

The vertical profile of g_l was calculated from sap flux and L data together with modelling of canopy transmission of photosynthetically active radiation (PAR) and stomatal light response functions for sun and shade leaves, in an

approach similar to that used by Schäfer et al. (2002). Leaf area index was reported by Uddling et al. (2008), and canopy transmission of PAR was calculated using the canopy radiation model described in that study, assuming that 50% of the global radiation was PAR (Monteith and Unsworth 1990). A LI6400 (Li-Cor Inc., Lincoln, NE) was used to measure the g_s light responses of one sun leaf and one shade leaf each of one aspen and one birch tree in each of the control plots during 22–28 July 2005. Measurements were conducted between 7:30 and 11:30 h at a leaf temperature of 26 °C. The leaves were allowed to acclimate to each of five values of PPFD for ~ 20 min (the time required for > 90% change in g_s) and measured at PPFD steps of 0, 100, 300, 800 and 1800 μmol m^{-2} s^{-1}. As there were no differences in the shape of the relative (0–1) g_s light response between sun and shade leaves or between species, a generic relative stomatal light response function could be determined as

$$g_{s_rel} = 0.25 + (1 - 0.25)(1 - \exp(-0.0018 \times \mathrm{PPFD})). \quad (1)$$

The magnitude of g_s at light saturation was 50% higher for sun leaves than for shade leaves. The stomatal light response function and the difference in light-saturated g_s between sun and shade leaves were used, together with modelled PPFD at different canopy depths, for calculating the vertical profile of relative g_l. The sun leaf part of the canopy was defined as positions where a horizontal leaf received at least 35% of the integrated PPFD above the canopy during June and July (1.85 m^2 one-sided leaf area m^{-2} ground). The vertical profile of relative g_l was then integrated for the entire canopy and rescaled to match values of g_c derived from sap flux data. Sap flux-derived g_l data presented in this study are mean values for the sun leaf fraction of the canopy. Effects on g_l probably reflect well the effects on g_s in the upper canopy, where wind speed did not significantly differ among treatments.

Sap flux-derived g_c and g_l data from mid-June through August when $D \geq 0.6$ kPa and PPFD ≥ 500 μmol m^{-2} s^{-1} were fitted to D using the function proposed by Oren et al. (1999)

$$g_s = g_{ref} - m \ln(D), \quad (2)$$

where g_{ref} is the conductance at $D = 1$ kPa and m is a parameter describing the absolute sensivity of g_s to D. The relative sensitivity of g_s to D is described by m/g_{ref}. Data with $D < 0.6$ kPa were excluded to minimize uncertainties in estimates of sap flux-derived conductance (Ewers and Oren 2000).

Potometer experiment

Potometer experiments were conducted in 2006 to evaluate the appropriateness of Granier's (1985, 1987) original calibration for our modified sensors, tree size and species, as several previous sap flow studies using the Granier-type sap flow system (modified or unmodified) together with the original calibration, have reported on sap flux estimates that are low compared to other, independent estimates of water flux (Hogg et al. 1997, Lundblad et al. 2001, Wilson et al. 2001, Bovard et al. 2005). Sap flow sensors were installed on two aspen trees and three birch trees during conditions when canopies were wet. The 20-mm-long sensors were inserted at 0–20 mm sapwood depth where stem sapwood diameters were 46–54 mm. Aspen trees had sensors inserted from both north and south, whereas birch trees had only north-facing sensors. The aspen and birch trees were cut before dawn when leaves were still wet on 12 August and 14 September, respectively, and were immediately placed in buckets filled with tap water. During the time of cutting and the few seconds after the cut was completed until the cut end was placed in the bucket, the cut surface was continuously sprayed with water. Trees were secured in a vertical position to an articulating arm of a lift bucket and positioned so that the cut stems were immersed in water buckets that were placed on electronic balances. The buckets were covered to prevent evaporation. Balance readings were taken every 30th min until sunset during the first day and once again before dawn the following morning. Gravimetric tree water use was calculated adjusting for the volume of water displaced by the immersed part of the stem.

The potometer experiment revealed that actual water uptake was 2.5 (±0.3, SEM) times greater than the sap flux that was estimated with the modified Granier-type sensors and Granier's original calibration (Figure 2). The relationship between water uptake and estimated sap flux was strong ($r^2 = 0.88$ for a linear regression) and not

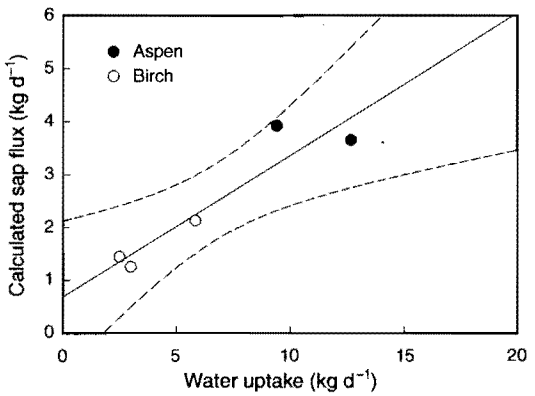

Figure 2. Tree water use estimated by sap flow sensors (modified Granier-type sensor and Granier's original calibration; Granier 1985, 1987) versus actual water uptake in gravimetric cut-tree experiments with aspen and birch trees on 12 August and 14 September, respectively, in Rhinelander 2006. Linear regression (solid line; $r^2 = 0.88$) is shown together with 95% confidence limits (dashed lines).

significantly different from a proportional relationship. Sap flux density of sensors inserted from north and south was similar, which was in agreement with an earlier investigation in 2003. Based on these results, sap flux data calculated using Granier's original calibration were multiplied by 2.5. Although our modified sensors (used with Granier's original calibration) substantially underestimated actual tree water use, the directions and the relative magnitudes of effects of trace gas treatments and species/community type reported in Uddling et al. (2008) should be correct because there was no significant deviation from proportionality in the relationship between water uptake and estimated sap flux (Figure 2). The ability of the sensors to adequately capture treatment-induced variation in sap flux was also supported by the good agreement between sap flux-derived g_l and leaf-level conductance determined by ^{13}C methodology and leaf gas exchange (Figure 3), as well as between treatment effects on sap flux and soil moisture (Uddling et al. 2008). Underestimations with our sap flow sensors (using Granier's original calibration) may have been caused by sensor modifications from the original design, small tree size and/or inappropriateness of the original calibration for our species (Lu et al. 2004).

^{13}C discrimination- and photosynthesis-derived g_l

The leaves for stable C isotope analysis were collected between 12:00 and 16:00 h on comparatively clear days in mid-August in 2004 and 2005 and in mid-September 2005. The selected leaves were fully developed and had insertion numbers of 6–9 (aspen) and 4–6 (birch) from the shoot tip on current year shoots in the upper canopy. Leaves with major signs of herbivory or pathogen attack were avoided. Particular care was taken to sample trees near the centre of the plots, to minimize the influence of temporal variations in CO_2 and O_3 concentrations. August samples consisted of three leaves from each of two trees per species and section (i.e., 3+3 leaves each for aspen in monoculture, aspen in species mixture and birch). August leaf samples from pure aspen stands consisted of leaves that were taken from randomly selected trees, with no respect to clonal identity. September samples, however, consisted of 3+3 leaves from each of the aspen clones 271, 42E (the two clones predominantly sampled in August) and 216 (the clone interplanted with birch in mixed stands) in pure aspen stands of control, $+CO_2$ and $+O_3$ plots (i.e., not $+CO_2+O_3$ plots). The three leaves from an individual tree were combined to one sample before chemical analysis. Leaf samples were frozen, dried, ground and analysed for stable C isotope ratio ($\delta^{13}C$, as defined by Farquhar et al. 1989) and N concentration using a Finnigan Delta plus isotope ratio mass spectrometer interfaced to a CE Elantech NC2500 elemental analyser with a Conflo II unit (Thermo Electron,. San Jose, CA) located at the University of Michigan. The mass:area ratio of the same leaves was also determined.

Data of leaf bulk tissue $\delta^{13}C$ and canopy air $\delta^{13}C$ were used to calculate the leaf ^{13}C discrimination (Δ) and the c_i/c_a ratio according to the equations

$$\Delta = \frac{(\delta^{13}C_{air} - \delta^{13}C_{leaf})}{(1 + \delta^{13}C_{leaf})}, \quad (3)$$

$$c_i/c_a = \frac{\Delta - a}{b - a}, \quad (4)$$

where a is the isotopic discrimination due to the slower diffusion of $^{13}CO_2$ to $^{12}CO_2$ in air (4.4‰) and b is the net isotopic discrimination by mesophyll CO_2 transfer and carboxylation activities (27‰) (Farquhar et al. 1982, 1989).

The $\delta^{13}C$ value of the CO_2 inside each of the 12 plots (air $\delta^{13}C$) was measured bi-weekly during the 2004–2005 summers. Sampling was conducted at a height of 2–2.5 m in the centre of each plot. In 2004, samples were collected with a 20-ml syringe near mid-day. In 2005, samples were collected by filling a 5 l Tedlar gas sampling bag over several hours during the middle of the day with mini-rotary pumps. In both cases, a syringe was used to fill a He-flushed 5-ml Exetainer vial with 5 ml of air from the bag or directly from the plot. The $\delta^{13}C$ of CO_2 was analysed on a GasBench II connected to a ThermoFinnigan Delta^{plus} Continuous Flow Isotope Ratio Mass Spectrometer (IRMS) located at the Michigan Technological University Forest Ecology Analytical Laboratory within a week of sampling. Samples were measured against a CO_2 reference gas calibrated with International Atomic Energy Agency (IAEA) reference materials. The standard deviation of repeated measurements of a laboratory CO_2 standard was 0.1‰ for $\delta^{13}C$. The IAEA reference materials had a reported precision of 0.20–0.25‰. Measured air $\delta^{13}C$ data were preferred over modelled air $\delta^{13}C$ data (calculated with a simple two-source single isotope-mixing model) as a positive relationship between measured air $\delta^{13}C$ and leaf tissue $\delta^{13}C$ in CO_2 enriched plots ($P = 0.012$ and $r^2 = 0.83$ for a temporally extended air $\delta^{13}C$ data set) indicated that much of the variation in leaf tissue $\delta^{13}C$ was caused by a rather large variation in air $\delta^{13}C$ among individual plots.

Mean air $\delta^{13}C$ during June–July in 2004–2005 was used in this study, as most of the bulk leaf tissue biomass was formed during this time period. June–July mean canopy CO_2 concentration during daylight hours and c_i data were combined with photosynthesis modelling to calculate instantaneous g_l by closing Fick's law of diffusion equation

$$g_l = \frac{A_n}{(c_a - c_i)}, \quad (5)$$

where A_n is the net photosynthesis. As leaf tissue $\delta^{13}C$ is an assimilation-weighed integrated measure, mean ^{13}C-derived g_l for June–July was calculated by weighing instantaneous g_l by photosynthetic rate. The biochemical

model of C_3 photosynthesis described by Farquhar et al. (1980) with recent modifications of photosynthetic temperature dependencies (Bernacchi et al. 2001, 2003*b*) was used to calculate A_n from c_i, PPFD, air temperature and photosynthetic parameters. Values of the maximum rate of carboxylation ($V_{c,max}$) were determined from the measurements of leaf gas exchange at near canopy air conditions that are described below, assuming mitochondrial respiration of 1.2 μmol m^{-2} s^{-1} at leaf temperature of 26 °C as observed for both aspen and birch leaves under darkness in control plots. Gas exchange data were used to fit $V_{c,max}$ only if g_s was above 0.05 mol m^{-2} s^{-1} and if the c_i value was at least 50 μmol mol^{-1} below the transition point between Rubisco limitation and ribulose-1,5-bisphosphate limitation, as determined from the values of $V_{c,max}$ and the maximum rate of electron transport (J_{max}) that was previously reported from the Aspen FACE experiment (Ellsworth et al. 2004). This caused the exclusion of 8 of 72 measured leaves. Transition points calculated for aspen and birch in the control and $+CO_2$ treatment were also applied for $+O_3$ and $+CO_2+O_3$ plots, respectively, because the elevated O_3 had no significant effects on light-saturated A_n in this study (see below). The values of J_{max} were calculated from $V_{c,max}$ using the relationships between J_{max} and $V_{c,max}$ in Ellsworth et al. (2004).

Leaf gas exchange

Gas exchange of aspen and birch leaves in the aspen-birch community type was measured between 10:00 and 14:00 h on three clear days (21 and 22 July, 12 August) in 2005; one block on each day. In each plot, three aspen leaves and three birch leaves, all attached to different trees, were measured. The selected leaves were similar to those measured for leaf tissue $\delta^{13}C$ (see above). The sequence in which different experimental plots were measured was arranged to minimize differences in hours of measurements among treatments. Leaf chamber conditions were set to PPFD = 1800 μmol m^{-2} s^{-1}, leaf temperature a few degrees above ambient air temperature and chamber CO_2 concentrations of 375 and 560 μmol mol^{-1} in ambient and elevated CO_2 plots, respectively. Each measurement took only a couple of minutes, which minimized stomatal acclimation to conditions inside the chamber.

Fumigation off/on experiments

A gap in CO_2 enrichment on day of year (DoY) 172 in 2004 was used to investigate the short-term response of trees growing under elevated CO_2 to transient exposure to ambient CO_2. During the time period 10:30–12:00 h, the CO_2 concentration was around 370 μmol mol^{-1} in all plots. Sap flux-derived g_c during 10:30–12:00 h on DoY 172 was compared with g_c during the same hours on seven reference days (DoY 167, 168, 169, 177, 178, 179 and 182) with good data capture. The comparison included only trees with data available for both DoY 172 and the seven respective reference days. All days were relatively clear and warm, but D was higher on the day with the gap in CO_2 enrichment. Mean PPFD, D, temperature and volumetric soil moisture (mean value at 0–15 cm soil depth in aspen and aspen-birch stands in control plots) during 10:30–12:00 h were 1304 μmol m^{-2} s^{-1}, 1.09 kPa, 19.3 °C and 0.22 m^3 m^{-3}, respectively, on DoY 172 and were 995 μmol m^{-2} s^{-1}, 0.67 kPa (range 0.36–1.03 kPa), 18.5 °C and 0.21 m^3 m^{-3} on the reference days.

Mathematical analyses

All data were statistically tested for effects of block, CO_2, O_3, community type (or species) and time (year) and their relevant interactions, using split-plot ANOVA appropriate for the Aspen FACE experiment (King et al. 2001). Tukey's HSD post hoc test was used for multiple comparisons of treatments. Effects were regarded as significant at $P \leq 0.10$. All tests were performed using SAS software Version 9.3.1 (SAS Institute, Cary, NC).

Fits between sap flux-derived conductance and D as well as modelling of photosynthesis, ^{13}C-derived g_l and vertical profiles of PFFD and sap flux-derived g_l were performed in MatLab, Version 7.5 (The MathWorks, Inc., Natick, MA).

Results

Sap flux-derived g_c at D = 1 kPa (g_{ref_c}) was significantly increased by elevated CO_2 (+17% averaged across years, O_3 regimes and community types, P = 0.088), and was significantly higher in pure aspen stands than in the aspen-birch mixture in 2004 but not in 2005 (Tables 1 and 2). Sap flux-derived leaf conductance of sun leaves at D = 1 kPa (g_{ref_l}) was significantly higher in the aspen than in aspen-birch community type, particularly in 2004. There was a significant $CO_2 \times O_3$ interaction (P = 0.079) on g_{ref_l} caused by lower values in control plots than in plots with elevated CO_2 and/or O_3. The difference between $+O_3$ and control plots was statistically significant in the mixed aspen-birch community type but not in aspen monoculture (Figure 3a). Canopy and leaf conductance were relatively more responsive to D (i.e., higher m/g_{ref}) in pure aspen than in mixed aspen-birch stands (+8%, P = 0.005, Tables 1 and 2). The responsiveness of g_c and g_l to D was significantly reduced by elevated O_3 in pure aspen stands (−7%, P = 0.096). However, aspen trees growing in species mixture did not exhibit reduced sensitivity of stomata to D under elevated O_3 (data not shown).

Leaf $\delta^{13}C$ data had a low coefficient of variation (CV; ~ 3%) in all treatments, whereas air $\delta^{13}C$, and hence also leaf Δ data, had CV twice as high as CV in elevated CO_2 (mean CV 11%) compared to ambient CO_2 plots (mean CV 5%; Table 3). The values of Δ or c_i/c_a were always

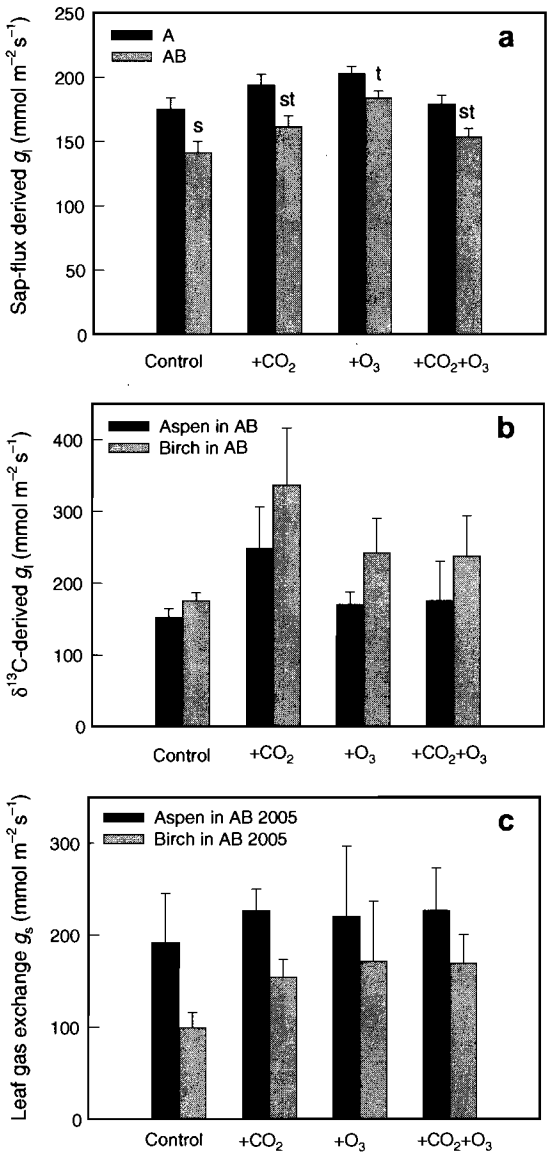

Figure 3. Leaf conductance (g_l) or stomatal conductance (g_s; both g_l and g_s on a one-sided leaf area basis) of upper canopy sun leaves in pure aspen (A) and mixed aspen-birch (AB) communities, derived using data of (a) sap flux and L (g_l at 1.0 kPa vapour pressure deficit), (b) $^{13}C/^{12}C$ and photosynthesis modelling and (c) leaf gas exchange. Data in (a) and (b) are mean values for 2004 and 2005. Significant ($P \leq 0.10$) differences among treatments determined by Tukey's HSD test are denoted by different letters.

lowest in the control and highest in the $+CO_2$ treatment, causing a $CO_2 \times O_3$ interaction, which was statistically significant in birch ($P = 0.069$) but not in aspen (Table 4; Figure 4a and b). Tukey's HSD test did not reveal any significant differences among individual treatments ($P \geq 0.13$). The values of Δ and c_i/c_a were significantly higher for birch than for aspen leaves. The significant species/community type × year interaction on Δ and c_i/c_a ($P = 0.018$) was caused by values for aspen in species mixture being similar to values for aspen in monoculture in 2004 but similar to values for birch in 2005 (Figure 4a and b). Values of c_i/c_a were highest in elevated CO_2 for all three aspen clones sampled in September 2005, but the effect was not statistically significant according to Tukey's HSD test ($P \geq 0.12$; Figure 4c). Treatment effects were weaker in clone 271, causing a significant clone × treatment interaction ($P = 0.071$). Results on g_l determined from $\delta^{13}C$ data and photosynthesis modelling were similar to results on Δ and c_i/c_a (Table 4; Figure 3b).

Leaf gas exchange measurements revealed no significant treatment effects on g_s (Figure 3c). In contrast with ^{13}C-derived g_l, gas exchange-derived g_s was significantly ($P = 0.002$) higher in aspen than in birch within the mixed community type. The mean CV of these data (37%) was higher than for sap flux-derived g_{ref_l} (13%) but similar to the CV of ^{13}C-derived g_l (35%) data. This variation was probably partly caused by diurnal changes in g_s between 10:00 and 14:00 h, but could not be attributed to variation in D. Elevated CO_2 significantly increased A_n, but neither CO_2 nor O_3 treatment significantly affected $V_{c,max}$ or area-based N concentration (Table 5). Another gas exchange data set, collected before noon after > 30 min acclimation to standard climatic conditions inside the leaf chamber, agreed well with the data presented here, but had even higher CV, probably because of the influence of individual plots being measured on different days with different soil moisture (data not shown).

Stand sap flux was on average 25% higher and g_c was 27% lower on the day with a gap in CO_2 enrichment on 20 June 2004 than on the seven reference days (Figure 5). The reduction in g_c was significantly ($P = 0.031$) smaller in elevated CO_2 (−23%) than in ambient CO_2 (−30%) plots, demonstrating a +10% effect of transient exposure of elevated CO_2-grown trees to ambient CO_2 on g_c. A similar short-term stomatal CO_2 response was observed at the leaf level in controlled gas exchange measurements (unpublished data). The effect was not likely to result from confounding influences of higher D during the fumigation gap (1.09 kPa during 10:30–12:00 h) compared to reference days (on average 0.67 kPa, range 0.36–1.03 kPa), as the positive effect of elevated CO_2 on relative g_c (i.e., g_c on DoY 172 relative to g_c on reference day) did not significantly vary with values of D on individual reference days. Furthermore, the results were similar for two alternative reference days with high D during mid-July (DoY 196 and 197; mean D of 1.16 kPa; data not shown).

Discussion

Effects of CO_2 and O_3 on conductance

The results obtained by different methods broadly agree, demonstrating that leaf-level conductance was not reduced by elevated CO_2 and/or O_3 in pure aspen and mixed

Table 1. Regression parameters for the dependence of canopy conductance (g_c, expressed on a ground area basis) and leaf conductance of sun leaves (g_l, expressed on a one-sided leaf area basis) on vapour pressure deficit of the air (D). Nonlinear regression techniques were used to fit data with $D \geq 0.6$ kPa and PPFD > 500 μmol m^{-2} to the equation (g_c or g_l) = $g_{ref} - m \ln(D)$, where g_{ref} is the conductance at $D = 1$ kPa (Oren et al. 1999).

Year	Community type[1]	Treatment	Canopy		Sun leaves[2]		Canopy and sun leaves
			g_{ref_c} (mmol m^{-2} ground s^{-1})	m (mmol m^{-2} s^{-1} ln(kPa)$^{-1}$)	g_{ref_l} (mmol m^{-2} s^{-1})	m (mmol m^{-2} s^{-1} ln(kPa)$^{-1}$)	m/g_{ref} (ln(kPa)$^{-1}$)
2004	A	Control	494 (44)	418 (53)	184 (17)	156 (21)	0.84 (0.04)
		$+CO_2$	654 (48)	530 (56)	216 (9)	175 (15)	0.81 (0.07)
		$+O_3$	482 (46)	353 (45)	210 (22)	154 (19)	0.73 (0.03)
		$+CO_2+O_3$	529 (72)	402 (68)	190 (18)	143 (20)	0.75 (0.03)
	AB	Control	432 (44)	302 (32)	144 (11)	101 (9)	0.70 (0.02)
		$+CO_2$	586 (22)	422 (19)	177 (3)	128 (2)	0.72 (0.02)
		$+O_3$	437 (42)	328 (37)	181 (8)	136 (5)	0.75 (0.02)
		$+CO_2+O_3$	486 (36)	342 (63)	159 (11)	115 (23)	0.70 (0.08)
2005	A	Control	468 (32)	341 (31)	166 (10)	122 (10)	0.73 (0.02)
		$+CO_2$	569 (69)	429 (48)	172 (24)	130 (17)	0.76 (0.03)
		$+O_3$	460 (58)	326 (49)	195 (17)	140 (15)	0.71 (0.02)
		$+CO_2+O_3$	499 (78)	354 (56)	168 (23)	120 (17)	0.71 (0.01)
	AB	Control	435 (35)	292 (22)	138 (11)	93 (7)	0.67 (0.01)
		$+CO_2$	493 (20)	328 (8)	146 (16)	97 (9)	0.67 (0.02)
		$+O_3$	469 (47)	334 (19)	186 (6)	134 (6)	0.72 (0.05)
		$+CO_2+O_3$	483 (22)	314 (7)	145 (4)	95 (4)	0.65 (0.02)

Values are treatment mean with standard error in parentheses.

[1]Pure aspen (A) and mixed aspen-birch (AB) community types.

[2]Values calculated using L and sap flux data together with stomatal light response curves and vertical canopy modelling (see text).

Table 2. Statistical significance (P values) of block, CO_2, O_3, community type and year and their relevant interactions on regression parameters for the dependence of conductance on vapour pressure deficit of the air. See Table 1 for explanations of the regression parameters as well as directions and magnitudes of effects.

Source	g_{ref_c}	g_{ref_l}	m/g_{ref}
Block	0.55	0.69	0.30
CO_2	**0.088**	0.74	0.68
O_3	0.39	0.32	0.38
$CO_2 \times O_3$	0.33	**0.079**[2]	0.57
Community	0.15	**0.008**	**0.005**
$CO_2 \times$ community	0.74	0.88	0.30
$O_3 \times$ community	0.51	0.52	**0.039**[3]
$CO_2 \times O_3 \times$ community	0.90	0.80	0.18
Year	0.12	0.20	0.15
$CO_2 \times$ year	0.16	0.14	0.99
$O_3 \times$ year	0.21	0.33	0.36
$CO_2 \times O_3 \times$ year	0.40	0.65	0.61
Community × year	**0.088**[1]	**0.034**[1]	0.24
$CO_2 \times$ community × year	0.25	0.52	0.28
$O_3 \times$ community × year	0.30	0.96	0.23
$CO_2 \times O_3 \times$ community × year	0.83	0.46	0.21

P values ≤ 0.10 are given in bold fonts.
[1]The community type difference was stronger in 2004 than in 2005.
[2]Lowest in control and highest in $+O_3$, results of Tukey's HSD in Figure 3a.
[3]A significant effect of elevated O_3 was found in pure aspen stands ($P = 0.096$, -7%) but not in mixed aspen-birch stands ($+2\%$).

aspen-birch canopies with steady-state L (Figure 3). On the contrary, the mean values of g_l and g_s were always higher in trees growing under elevated CO_2 and/or O_3 than in trees growing in control plots ($+19\%$ across community types/species, years, experimental treatments and methods to derive conductance), causing a $CO_2 \times O_3$ interaction that was statistically significant for sap flux-derived g_l and (for birch) ^{13}C-derived g_l (Tables 2 and 4). Results on Δ and c_i/c_a were similar to results on ^{13}C-derived g_l, as atmospheric gas treatments did not affect the parameters of the photosynthesis model (Table 5). Results on g_c were similar to results on stand sap flux, which have been discussed in a previous paper (Uddling et al. 2008).

Our results may be contrary to expectations, given the leaf-level stomatal closure responses to both trace gases (Figure 1) and the numerous reports of reduced g_s in trees growing under elevated CO_2 or O_3 in shorter-term experiments (e.g., Medlyn et al. 2001, Wittig et al. 2007). In addition, recent meta-analyses of FACE experiments reported on 16–19% significant reductions in g_s in trees growing under elevated CO_2 (Ainsworth and Long 2005, Ainsworth and Rogers 2007). It should be noted, however, that in these reviews, data from different years and species/clones were regarded as independent and no separation was made between overstorey/dominant and understorey/less abundant species. With this study demonstrating that the CO_2-induced reduction in g_s reported for aspen during an early stage of the experiment (Noormets et al. 2001) did not persist after canopy closure, four of five FACE experiments showed no significant ($P \leq 0.10$) reductions in g_s of abundant overstorey species in response to elevated CO_2 at steady-state L (Ellsworth 1999, Schäfer et al. 2002, Bernacchi et al. 2003*a* (pre-coppice canopy closure), Keel et al. 2007, but see Gunderson et al. 2002). In both the Aspen and Duke FACE experiments, the two largest and longest running FACE experiments, the effect of elevated CO_2 on leaf-level conductance was dependent on the stage of stand development. In the Duke experiment, leaf-level transpiration and conductance were unaffected by CO_2 treatment during the third year of the experiment, but were significantly increased by 10% in elevated CO_2 plots during the fifth year (Schäfer et al. 2002). In the Aspen FACE experiment, g_s of aspen was significantly reduced by elevated CO_2 during the second year (Noormets et al. 2001), when plants were still small and far from canopy closure, but not as the stands had reached steady-state L (Figure 3). Increased stand sap flux and g_c under elevated CO_2 in this experiment were attributed to an increased hydraulic efficiency caused by long-term cumulative effects on plant and stand structure (i.e., increased tree size, L and fine root biomass; Uddling et al. 2008). A similar interpretation can be made of the results on leaf-level conductance presented here, because the presence of a significant short-term response of stomata to CO_2 concentration (Figure 5) did not result in a lower g_s or g_l after long-term growth under elevated CO_2.

Table 3. Air and leaf ^{13}C isotope ratio ($\delta^{13}C$) and leaf ^{13}C discrimination (Δ) in control, elevated CO_2, elevated O_3 and elevated $CO_2 + O_3$ plots.

Source	Air $\delta^{13}C$	Leaf $\delta^{13}C$			Leaf Δ		
		Aspen in A	Birch in AB	Aspen in AB	Aspen in A	Birch in AB	Aspen in AB
Control	−8.4 (0.1)	−26.3 (0.3)	−27.1 (0.5)	−26.9 (0.3)	18.4 (0.3)	19.2 (0.4)	19.0 (0.3)
$+CO_2$	−19.9 (1.8)	−40.0 (0.6)	−41.4 (0.9)	−40.3 (0.6)	20.9 (1.4)	22.4 (1.0)	21.3 (1.3)
$+O_3$	−8.0 (0.4)	−27.7 (0.5)	−27.8 (0.5)	−27.3 (0.3)	20.2 (0.9)	20.4 (0.8)	19.8 (0.5)
$+CO_2+O_3$	−22.4 (1.3)	−40.4 (1.4)	−42.2 (0.8)	−41.5 (0.8)	18.7 (1.8)	20.6 (1.2)	19.9 (1.5)

Values are mean $\delta^{13}C$ and Δ (‰) across years (2004–2005; June–July data only for air $\delta^{13}C$) with standard error in parentheses. A, pure aspen community type and AB, mixed aspen-birch community type.

Table 4. Statistical significance (P values) of block, CO_2, O_3, species/community type and year and their relevant interactions on leaf ^{13}C discrimination (Δ), the ratio between intercellular and ambient CO_2 concentration (c_i/c_a) and ^{13}C-derived leaf conductance (g_l) of upper canopy sun leaves. See Figures 3b and 4a and b for directions and magnitudes of effects.

Source	All species/community combinations		Birch in mixed stands		Aspen in mixed stands		Aspen in monoculture
	Δ & c_i/c_a	g_l	Δ & c_i/c_a	g_l	Δ & c_i/c_a	g_l	Δ & c_i/c_a
Block	0.17	**0.063**	**0.075**	**0.042**	0.29	0.13	0.22
CO_2	0.25	0.11	**0.043**	**0.079**	0.28	0.19	0.66
O_3	0.79	0.55	0.68	0.68	0.79	0.46	0.89
$CO_2 \times O_3$	0.13	0.11	**0.069**[3]	**0.066**[3]	0.28	0.23	0.12
Species/community	**0.001**	**< 0.001**	na	na	na	na	na
CO_2 × species/community	**0.055**	0.26	na	na	na	na	na
O_3 × species/community	0.96	0.64	na	na	na	na	na
$CO_2 \times O_3$ × species/community	0.21	0.14	na	na	na	na	na
Year	0.24	0.99	0.14	0.30	0.86	0.46	**0.085**
CO_2 × year	0.23	0.23	0.97	0.86	0.12	0.11	0.72
O_3 × year	0.32	0.51	0.60	0.47	0.80	0.85	0.34
$CO_2 \times O_3$ × year	0.94	0.56	0.29	0.32	0.80	0.79	0.27
Species/community × year	**0.018**[1]	**0.081**[2]	na	na	na	na	na
CO_2 × species/community × year	0.32	0.24	na	na	na	na	na
O_3 × species/community × year	0.92	0.62	na	na	na	na	na
$CO_2 \times O_3$ × species/community × year	0.23	0.28	na	na	na	na	na

Values of Δ, c_i/c_a and g_l are derived from carbon isotope ratio and photosynthesis data/modelling.
na, not applicable.
P values ≤ 0.10 are given in bold fonts.
[1]Values for aspen in aspen-birch stands were similar to values for aspen in pure aspen stands in 2004 but similar to values for birch in 2005.
[2]The species difference birch > aspen in mixed stands was stronger in 2004 (+59%, $P < 0.001$) than in 2005 (+14%, $P = 0.30$).
[3]Lowest in control and highest in $+CO_2$, but Tukey's HSD post hoc test revealed no significant differences among individual treatments.

Also, the lack of a negative effect of elevated O_3 on g_s and g_l may be explained in a hydraulic context. In birch, the measurements of predawn and mid-day leaf water potential in 2005 revealed no significant effects of atmospheric gas treatments on the whole-plant water potential gradient (data not shown). This result, together with a similar sap flux in ambient and elevated O_3, suggests that whole-plant hydraulic conductance per unit ground (k_p) was unaffected by O_3, which would in turn imply increased k_l as L was significantly reduced by elevated O_3 (Uddling et al. 2008). In pure aspen stands, the stomatal closure response to increasing D was less sensitive (Tables 1 and 2) and mid-day leaf water potential was lower (J. King, personal communication) under elevated O_3, indicating O_3-induced impairment of stomatal control over transpiration in this community type. Water uptake capacity may have been largely unaffected by O_3 treatment, as fine root biomass was similar (aspen-birch stands) or even increased (pure aspen stands) in elevated O_3 compared to ambient O_3 (King et al. 2005). The c_i-mediated effect on g_s (Figure 1) was probably quite weak in this study, where the O_3 elevation was rather moderate (1.35 × ambient O_3) and light-saturated A_n of upper canopy leaves was not significantly affected by O_3 treatment (Table 5). Reductions in g_s usually occur at high elevations of O_3 but not under more moderately elevated O_3 exposure comparable to that in this study (Wittig et al. 2007). This may also explain the difference between results obtained here and those reported from the free-air O_3 fumigation experiment in a mature mixed forest in Germany, where both A_n and g_s of *F. sylvatica* and *P. abies* were decreased by ~ 25% under doubled O_3 concentrations (Nunn et al. 2006, Warren et al. 2006). It should also be noted that O_3 treatment was initiated on already developed canopies in the 3-year-long German study, allowing for limited structural adjustments under elevated O_3. The results on g_s and g_l presented here are not contradicted by recent Aspen FACE studies reporting on significantly reduced g_s under elevated O_3 (Calfapietra et al. 2007, data for aspen only; Riikonen et al. 2008, reduction in birch but not in aspen), as these reductions were observed during dry periods in 2005 when drought was particularly pronounced in elevated O_3 plots (Uddling et al. 2008).

The $CO_2 \times O_3$ interaction on g_l (Tables 2 and 4; Figure 3) was most likely a consequence of similar effects of elevated CO_2 on stand sap flux and in both O_3 regimes (Uddling et al. 2008) but a relatively larger CO_2-induced stimulation of L under elevated O_3 (54% across communities and years) than under ambient O_3 (26%). The interaction was not likely to be caused by interacting effects of CO_2 and O_3 on leaf physiology, as A_n, $V_{c,max}$ and stomatal sensitivity to D (i.e., m/g_{ref}) were not differently affected by O_3 treatment in the two CO_2 regimes (Tables 2 and 5).

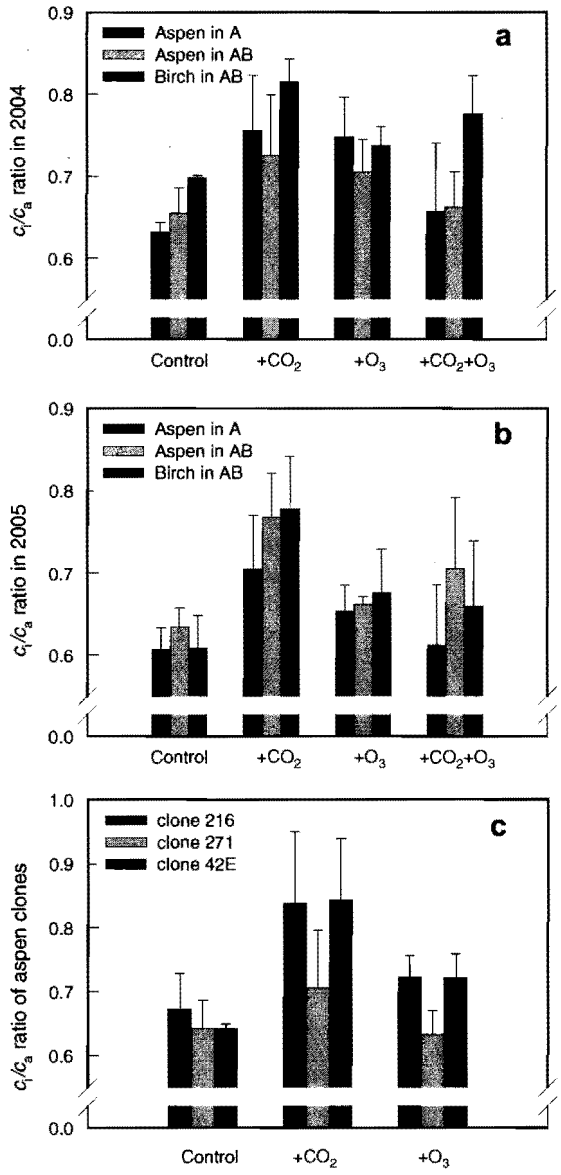

Figure 4. Intercellular to ambient CO_2 concentration (c_i/c_a) ratio of upper canopy sun leaves in pure aspen (A) and mixed aspen-birch (AB) communities in (a) 2004 and (b) 2005, as well as in (c) three different aspen clones in A stands in 2005. Statistical results for data in (a) and (b) are given in Table 4. In (c) there was a significant effect of clone ($P = 0.001$) as well as a significant clone × treatment interaction ($P = 0.071$). Tukey's HSD test detected no significant ($P \leq 0.10$) differences among individual treatments (a–c).

This study demonstrates that the effects of elevated atmospheric CO_2 and O_3 may be highly dependent on the stage of plant ontogeny and stand development. During the second year of the experiment, leaves in elevated CO_2 had a significantly lower g_s (Noormets et al. 2001; aspen data only), N concentration (Lindroth et al. 2001) and $V_{c,max}$ (Ellsworth et al. 2004), whereas O_3 treatment reduced leaf N concentration and, as leaves aged, light-saturated A_n and g_s (g_s reduced in sensitive aspen clone only). None of these effects were present after > 6 years of elevated CO_2 and/or O_3 exposure, when canopies, but not root systems, had reached steady-state L under the experimental atmospheric treatments (Table 5; Figure 3; Zak et al. 2007).

Observed changes in leaf tissue composition may only partly explain the ^{13}C discrimination patterns that are observed. With lignin being more depleted in ^{13}C and with carbohydrates being less depleted in ^{13}C compared to that in bulk leaf tissue (Badeck et al. 2005), positive effects of both O_3 and CO_2 treatments on non-structural carbohydrates (Lindroth et al. 2001, Liu et al. 2005, Holton et al. 2003) counteract observed patterns (Tables 3 and 4; Figure 4a and b). It is possible, however, that the $CO_2 \times O_3$ interaction on Δ and c_i/c_a (significant in birch only) was partly caused by a higher concentration of lignin in leaf litter in the $+CO_2+O_3$ treatment (Liu et al. 2005).

Effects of community type and species identity

Treatment effects on g_s and g_l were similar in both species/community types, but while g_s determined by leaf-level gas exchange measurements was lower in birch than in aspen within the mixed stands the opposite was true for ^{13}C-derived g_l (Figure 3). This discrepancy was probably caused by different degrees of hydraulic constraints on g_s in the two different data sets. While bulk leaf tissue $\delta^{13}C$ was determined primarily by structural C, assimilated during the comparatively wet and cool early part of the summer, leaf gas exchange was measured on days in July and August when both soil and air were relatively dry. Higher ^{13}C-derived g_l in birch may thus reflect a higher maximum g_s at low hydraulic limitation of stomatal opening in this species compared to that in aspen, while lower gas exchange g_s in birch measured on drier days may reflect the more efficient isohydric nature of stomatal control of leaf water potential (at a higher, i.e., less negative, value) in birch compared to aspen (A. Sôber, personal communication). Gas exchange data from August 2006, after 80-mm rainfall in a few days, support this hypothesis, as g_s of birch was then significantly ($P = 0.03$) higher in birch than in aspen in the mixed community type (unpublished data). Furthermore, stand sap flux was higher in aspen monoculture than in mixed aspen-birch stands on days with moderate to high values of D, but not on days with low D (Uddling et al. 2008). Such species × climate interaction probably also explains the significant species/community type × year interactions on Δ, c_i/c_a and ^{13}C-derived g_l (Table 4; Figure 4a and b). In the cool and humid summer of 2004, the values of Δ, c_i/c_a and g_l were higher in birch than in aspen, while in the warmer and drier summer of 2005, there was no significant difference between species within the mixed aspen-birch community type, most likely as a result of hydraulic constraints on g_s being stronger this year.

Table 5. Effects of species identity, CO_2 and O_3 on light-saturated net photosynthesis (A_n), maximum rate of carboxylation ($V_{c,max}$) and N concentration of upper canopy sun leaves of aspen and birch leaves in the mixed aspen-birch community type in 2005.

Species	Treatment	A_n[1] (μmol m^{-2} s^{-1})	$V_{c,max}$ (μmol m^{-2} s^{-1})	N concentration (g m^{-2})
Aspen	Control	12.0^a (2.2)	75.5^a (3.9)	1.66^a (0.12)
	$+CO_2$	21.2^b (1.6)	72.1^a (5.3)	1.59^a (0.06)
	$+O_3$	11.3^a (2.5)	70.6^a (4.3)	1.75^a (0.04)
	$+CO_2+O_3$	19.7^b (1.6)	65.7^a (1.7)	1.67^a (0.15)
Birch	Control	9.5^a (1.1)	83.7^a (4.6)	1.69^a (0.11)
	$+CO_2$	20.4^b (1.3)	82.4^a (1.2)	1.57^a (0.07)
	$+O_3$	12.1^a (2.3)	88.8^a (6.7)	1.59^a (0.06)
	$+CO_2+O_3$	19.9^b (3.0)	82.4^a (5.1)	1.90^a (0.25)

Values are treatment mean with standard error in parentheses, expressed on a one-sided leaf area basis. Within columns and species, mean values with the same superscript lowercase letter are not significantly different at $\alpha = 0.10$ according to Tukey's HSD test. Birch had a significantly higher $V_{c,max}$ than aspen ($P = 0.001$).
[1] A_n was measured at leaf chamber CO_2 concentrations of 365 and 560 μmol mol^{-1} in ambient and elevated CO_2 plots, respectively.

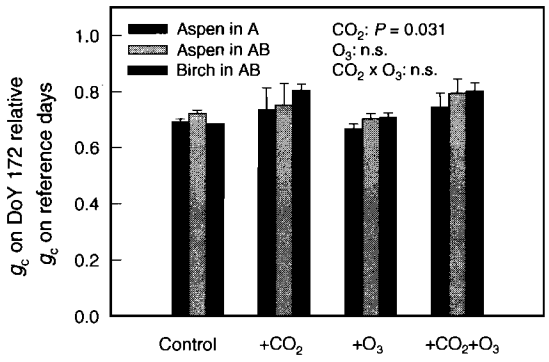

Figure 5. Canopy conductance (g_c) during 10:30–12:00 h on day of year (DoY) 172 relative to the average g_c during the same hours on seven reference days (DoY 167, 168, 169, 177, 178, 179 and 182). Significant ($P \leq 0.10$) treatment effects are denoted in the graph.

The less sensitive stomatal closure response to increasing D in aspen stands exposed to elevated O_3 (Tables 1 and 2) indicates an impaired stomatal control over transpiration and leaf water potential in these stands, which may have contributed to predisposing them to drought (Uddling et al. 2008). This $O_3 \times$ community type interaction was probably related to aspen clonal identity rather than to differences between species, as aspen trees growing in species mixture (clone 216) did not exhibit reduced sensitivity of stomata to D in elevated O_3 (data not shown). Less efficient stomatal control under elevated O_3 has been reported for several tree species before (e.g., Mansfield 1998, Matyssek et al. 2006).

Implications

We have demonstrated that short-term primary stomatal closure responses to elevated CO_2 and O_3 were completely offset by long-term cumulative effects of these trace gases on tree and stand structure in determining canopy- and leaf-level conductance in pure aspen and mixed aspen-birch forests. Despite a direct stomatal response to short-term altered CO_2 concentration (Figure 5), there was no significant reduction in g_s or g_l and a significant increase in g_c after long-term growth under elevated CO_2 (Tables 1, 2 and 4; Figure 3). Lack of reductions in leaf-level conductance reported here differs from the results obtained during an early phase of the experiment (Noormets et al. 2001), further emphasizing the dependence of g_s on tree and stand structure and the importance of structural adjustments to altered atmospheric trace gas composition in long-lived organisms like trees (Figure 1). Our results, together with findings from other long-term FACE experiments with trees, suggest that model assumptions of large reductions in g_s under rising atmospheric CO_2 are very uncertain for forests, raising questions with regard to model predictions of reduced land evapotranspiration (Betts et al. 2007) and reduced air pollution sensitivity (Sitch et al. 2007) of forests in the future with a higher atmospheric CO_2 concentration.

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References

Ainsworth, E.A. and S.P. Long. 2005. What have we learned from fifteen years of free air carbon dioxide enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytol. 165:351–372.

Ainsworth, E.A. and A. Rogers. 2007. The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. Plant Cell Environ. 30:258–270.

Badeck, F.-W., G. Tcherkez, S. Nogués, C. Piel and J. Ghashghaie. 2005. Post-photosynthetic fractionation of stable carbon isotopes between plant organs – a widespread phenomenon. Rapid Commun. Mass Spectrom. 19:1381–1391.

Ball, J.T., I.E. Woodrow and J.A. Berry. 1987. A model predicting stomatal conductance and its contribution to the control of photosynthesis under different environmental conditions. *In* Progresses in Photosynthesis Research. Vol. IV. Ed. I. Biggins. Martinus Nijhoff Publishers, Netherlands, pp 221–224.

Bernacchi, C.J., E.L. Singsaas, C. Pimentel, A.R. Portis and S.P. Long. 2001. Improved temperature response functions for models of Rubisco-limited photosynthesis. Plant Cell Environ. 24:253–260.

Bernacchi, C.J., C. Calfapietra, P.A. Davey, V.E. Wittig, G.E. Scarascia-Mugnozza, C.A. Raines and S.P. Long. 2003*a*. Photosynthesis and stomatal conductance responses of poplars to free air CO_2 enrichment (PopFACE) during the first growth cycle and immediately following coppice. New Phytol. 159:609–621.

Bernacchi, C.J., C. Pimentel and S.P. Long. 2003*b*. In vivo temperature response functions of parameters required to model RuBP-limited photosynthesis. Plant Cell Environ. 26:1419–1430.

Betts, R.A., O. Boucher, M. Collins et al. 2007. Projected increase in continental runoff due to plant responses to increasing carbon dioxide. Nature 448:1037–1042.

Bonan, G.B. 2008. Forests and climate change: forcings, feedbacks, and the climate benefits of forests. Science 320: 1444–1449.

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.

Buckley, T.N. 2005. The control of stomata by water balance. New Phytol. 168:275–291.

Calfapietra, C., A.E. Wiberly, T.G. Falbel, A.R. Linskey, G.S. Mugnozza, D.F. Karnosky, F. Loreto and T.D. Sharkey. 2007. Isoprene synthase expression and protein levels are reduced under elevated O_3 but not under elevated CO_2 (FACE) in field-grown trees. Plant Cell Environ. 30: 654–661.

Dickson, R.E., K.F. Lewin, J.G. Isebrands et al. 2000. Forest atmosphere carbon transfer and storage (FACTS-II) the aspen free-air CO_2 and O_3 enrichment (FACE) project: an overview. General Technical Report NC-214. USDA Forest Service, North Central Research Station, Rhinelander, WI, 68 p.

Ellsworth, D.S. 1999. CO_2 enrichment in a maturing pine forest: are CO_2 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 pCO_2 across four free-air CO_2 enrichment experiments in forest, grassland and desert. Global Change Biol. 10:2121–2138.

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.

Farquhar, G.D., S. von Caemmerer and J.A. Berry. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta 149:78–90.

Farquhar, G.D., M.H. O'Leary and J.A. Berry. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust. J. Plant Physiol. 9:121–137.

Farquhar, G.D., J.R. Ehleringer and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40:503–537.

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.

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 CO_2 enrichment. Plant Cell Environ. 25:379–393.

Gutschick, V.P. 2007. Plant acclimation to elevated CO_2 – from simple regularities to biogeographic chaos. Ecol. Model. 200:433–451.

Hendrey, G.R., D.S. Ellsworth, K.F. Lewin and J. Nagy. 1999. A free-air CO_2 enrichment system for exposing tall forest vegetation to elevated atmospheric CO_2. Global Change Biol. 5:293–309.

Hogg, E.H., T.A. Black, G. den Hartog et al. 1997. A comparison of sap flow and eddy fluxes of water vapor from a boreal deciduous forest. J. Geophys. Res. 102:28929–28937.

Holton, M.K., R.L. Lindroth and E.V. Nordheim. 2003. Foliar quality influences tree herbivore-parasitoid interactions: effects of elevated CO_2, O_3, and genotype. Oecologia 137: 233–244.

IPCC. 2007. The physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the IPCC. 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.

Keel, S.G., S. Pepin, S. Leuzinger and C. Körner. 2007. Stomatal conductance in mature deciduous forest trees exposed to elevated CO_2. Trees-Struct. Funct. 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 CO_2 and tropospheric O_3. Oecologia 128:237–250.

King, J.S., P.J. Hanson, E. Bernhardt, P. De Angelis, R.J. Norby and K.S. Pregitzer. 2004. A multi-year synthesis of soil respiration responses to elevated atmospheric CO_2 from four forest FACE experiments. Global Change Biol. 10:1027–1042.

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 O_3 compromises net primary production in young stands of trembling aspen, paper birch and sugar

maple in response to elevated atmospheric CO_2. New Phytol. 168:623–635.

Körner, C. 2006. Plant CO_2 responses: an issue of definition, time and resource supply. New Phytol. 172:393–411.

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 CO_2. Science 309:1360–1362.

Leuning, R. 1995. A critical appraisal of a combined stomatal-photosynthesis model for C_3 plants. Plant Cell Environ. 18:339–355.

Lindroth, R.L., B.J. Kopper, W.F.J. Parsons, J.G. Bockheim, D.F. Karnosky, G.R. Hendrey, K.S. Pregitzer, J.G. Isebrands and J. Sober. 2001. Consequences of elevated carbon dioxide and ozone for foliar chemical composition and dynamics in trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*). Environ. Pollut. 115:395–404.

Liu, L., J.S. King and C.P. Giardina. 2005. Effects of elevated atmospheric CO_2 and tropospheric O_3 on leaf litter production and chemistry in trembling aspen and paper birch ecosystems. Tree Physiol. 15:1511–1522.

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.

Lundblad, M., F. Lagergren and A. Lindroth. 2001. Evaluation of heat balance and heat dissipation methods for sapflow measurements in pine and spruce. Ann. For. Sci. 58:625–638.

Mansfield, T.A. 1998. Stomata and plant water relations: does air pollution create problems? Environ. Pollut. 101:1–11.

Matyssek, R., D. Le Thiec, M. Löw, P. Dizengremel, A.J. Nunn and K.-H. Häberle. 2006. Interactions between drought stress and O_3 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 CO_2 concentration: a synthesis. New Phytol. 149:247–264.

Monteith, J.L. and M.H. Unsworth. 1990. Principles of environmental physics. 2nd Edn. Edward Arnold Publishers, London, pp 154–156.

Morison, J.I.L. 1998. Stomatal response to increased CO_2 concentration. J. Exp. Bot. 49:443–452.

Mott, K.A. 1988. Do stomata respond to CO_2 concentrations other than intercellular? Plant Physiol. 86:200–203.

Noormets, A., A. Sôber, E.J. Pell, R.E. Dickson, K.G. Podila, J. Sôber, J.G. Isebrands and D.F. Karnosky. 2001. Stomatal and non-stomatal limitation to photosynthesis in two trembling aspen (*Populus tremuloides* Michx.) clones exposed to elevated CO_2 and/or O_3. 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 CO_2 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.

Oren, R., J.S. Sperry, G.G. Katul, D.E. Pataki, B.E. Ewers, N. Phillips and K.V.R. Schäfer. 1999. Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit. Plant Cell Environ. 22:1515–1526.

Pitman, A.J. 2003. The evolution of, and revolution in, land surface schemes designed for climate models. Int. J. Climatol. 23:479–510.

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 CO_2 and O_3. Tree Physiol. 28: 243–254.

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 CO_2 concentration. Global Change Biol. 8:895–911.

Sitch, S., P.M. Cox, W.J. Collins and C. Huntingford. 2007. Indirect radiative forcing of climate change through ozone effects on the land-carbon sink. Nature 448:791–795.

Sperry, J.S. 2000. Hydraulic constraints on plant gas exchange. Agric. For. Meteorol. 104:13–23.

Uddling, J., R.M. Teclaw, M.E. Kubiske, K.S. Pregitzer and D.S. Ellsworth. 2008. Sap flux in pure aspen and mixed aspen-birch forests exposed to elevated carbon dioxide and ozone. Tree Physiol. 28:1231–1243.

Warren, C.R., M. Löw, R. Matyssek and M. Tausz. 2006. Internal conductance to CO_2 transfer of adult *Fagus sylvatica*: variation between sun and shade leaves and due to free-air ozone fumigation. Environ. Exp. Bot. 59:130–138.

Wilson, K.B., P.J. Hanson, P.J. Mulholland, D.D. Baldocchi and S.D. Wullschleger. 2001. A comparison of methods for determining forest evapotranspiration and its components: sap-flow, soil water budget, eddy covariance and catchment water balance. Agric. For. Meteorol. 106:153–168.

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.

Zak, D.R., W.E. Holmes and K.S. Pregitzer. 2007. Atmospheric CO_2 and O_3 alter the flow of ^{15}N in developing forest ecosystems. Ecology 88:2630–2639.