Stomatal uptake of O_3 in aspen and aspen-birch forests under free-air CO_2 and O_3 enrichment

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Stomatal ozone flux in aspen and aspen-birch forests was not reduced by elevated CO_2 concentration.

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ABSTRACT

Rising atmospheric carbon dioxide (CO_2) may alleviate the toxicological impacts of concurrently rising tropospheric ozone (O_3) during the present century if higher CO_2 is accompanied by lower stomatal conductance (g_s), as assumed by many models. We investigated how elevated concentrations of CO_2 and O_3, alone and in combination, affected the accumulated stomatal flux of O_3 (AFst) by canopies and sun leaves in closed aspen and aspen-birch forests in the free-air CO_2–O_3 enrichment experiment near Rhinelander, Wisconsin. Stomatal conductance for O_3 was derived from sap flux data and AFst was estimated either neglecting or accounting for the potential influence of non-stomatal leaf surface O_3 deposition. Leaf-level AFst (AFst$_l$) was not reduced by elevated CO_2. Instead, there was a significant $CO_2 \times O_3$ interaction on AFst$_l$, as a consequence of lower values of g_s in control plots and the combination treatment than in the two single-gas treatments. In addition, aspen leaves had higher AFst$_l$ than birch leaves, and estimates of AFst$_l$ were not very sensitive to non-stomatal leaf surface O_3 deposition. Our results suggest that model projections of large CO_2-induced reductions in g_s alleviating the adverse effect of rising tropospheric O_3 may not be reasonable for northern hardwood forests.

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1. Introduction

The impact of tropospheric ozone (O_3) on trees is a major concern for large areas of global forest (e.g., Felzer et al., 2004; Fowler et al., 1999; Karnosky et al., 2003). Effects of O_3 on the metabolism of plants are typically dose-dependent, related to the uptake through the stomata (Reich, 1987). Efforts have therefore been made within the Convention on Long-Range Transboundary Air Pollution (CLRTAP), promoted by the United Nations Economic Commission for Europe (UNECE), to relate effects of O_3 to the accumulated flux of O_3 through stomata (AFst) rather than to concentration-based O_3 indices (Karlsson et al., 2004, 2007; Uddling et al., 2004a; UNECE, 2004). Projections of future O_3 impacts on vegetation under global environmental change must therefore account for changes not only in O_3 concentrations, but also in all environmental variables affecting stomatal conductance (g_s). Most notably, the potential negative impact of a predicted increase in tropospheric O_3 during coming decades (Dentener et al., 2006) is expected to be ameliorated by decreasing g_s under rising atmospheric carbon dioxide (CO_2; e.g., Harmens et al., 2007; Sitch et al., 2007). However, although reductions in g_s were observed in most elevated CO_2 enclosure experiments with trees (e.g., Medlyn et al., 2001), the generality of this effect after long-term exposure under ecologically realistic conditions has been challenged by results from free-air CO_2 enrichment (FACE) experiments. Stomatal conductance of abundant overstorey species was significantly ($P \leq 0.10$) reduced by elevated CO_2 in only one out of five FACE experiments on forest stands after canopy closure (i.e., in: Gunderson et al., 2002; not in: Bernacchi et al., 2003; Ellsworth, 1999; Keel et al., 2007; Uddling et al., 2009). It should be noted that effects of elevated CO_2 on g_s is highly dependent on the stage of plant and stand development (Schäfer et al., 2002; Uddling et al., 2009), and that recent meta-analyses of FACE experiments, reporting on 16–19% significant reductions in g_s of trees growing

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Nomenclature

AFnst	accumulated non-stomatal flux
AFst	accumulated stomatal flux
AFstY	accumulated stomatal flux over a cut-off threshold of Y nmol m^{-2} s^{-1}
AOT40	accumulated exposure over a cut-off threshold of 40 nmol mol^{-1} during daylight hours
CLRTAP	Convention on Long-Range Transboundary Air Pollution
D	vapor pressure deficit of the air
EMEP	European Monitoring and Evaluation Programme
FACE	free-air carbon dioxide enrichment
Fst	stomatal flux of O_3
g_b	leaf-boundary layer conductance
g_{bs}	conductance through stomata and leaf-boundary layer

g_c	canopy conductance
g_s	stomatal conductance
L	leaf area index
Q_p	photosynthetic photon flux density
T	air temperature
u	horizontal wind speed
UNECE	United Nations Economic Commission for Europe
θ_v	volumetric soil moisture

Subscripts

AFstY and Fst	"max" and "min" refer to stomatal flux calculated neglecting and accounting for potential non-stomatal O_3 flux respectively
c	flux at the canopy
l	flux at the leaf levels

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under elevated CO_2 (Ainsworth and Long, 2005; Ainsworth and Rogers, 2007), treated data from different years and species/clones within an experiment as independent observations and made no separation between overstorey/dominant and understorey/less abundant species. Elevated O_3 typically reduces g_s of juvenile trees in shorter-term experiments (Wittig et al., 2007), but both the direction and magnitude of the response vary widely among experiments and little data exist to assess the likely effect of O_3 on g_s under field conditions after steady-state leaf area index (L) has been reached.

The fraction of non-stomatal to total O_3 flux in forests is typically large (30–70%; Hogg et al., 2007 and references therein), but its partitioning to gas-phase reactions and non-stomatal deposition to leaf, bark and soil surfaces remains unclear (Altimir et al., 2006; Cieslik, 2004; Hogg et al., 2007; Kurpius and Goldstein, 2003). In most elevated O_3 experiments, stomatal O_3 flux is calculated without accounting for possible competing non-stomatal O_3 sinks at the leaf surface (e.g., Karlsson et al., 2004; Pleijel et al., 2007). This approach has been criticized for overestimating AFst (Grünhage et al., 2004), but the magnitude of this overestimation has rarely been assessed. A study accounting for non-stomatal leaf surface conductance for O_3 (g_{ns}) concluded that estimates of AFst of upper canopy sun leaves were largely insensitive to g_{ns} in nine European O_3 experiments with birch, as a result of turbulent conditions within the experimental tree crowns/canopies (Uddling et al., 2004a). However, the parameterization of g_{ns} in that study was taken from the European Monitoring and Evaluation Programme (EMEP) photo-oxidant model that is used to provide estimates of O_3 concentrations across Europe (Emberson et al., 2000). The EMEP model, as well as most canopy-scale O_3 deposition models (e.g., Massman, 2004; Zeller and Nikolov, 2000; Zhang et al., 2002b) assume that the non-stomatal sink is a passive receptor of O_3, with constant and low affinity for O_3. On the contrary, several studies have shown that g_{ns} is both high and dependent on environmental conditions such as radiation, temperature, air humidity and surface wetness (e.g., Altimir et al., 2006; Fowler et al., 2001; Hogg et al., 2007; Kurpius and Goldstein, 2003; Pleijel et al., 1995; Zhang et al., 2002a).

Given the uncertainties in the interacting effects of elevated O_3 and CO_2 on stomatal O_3 uptake of forest trees and the projected increases of both trace gases (Dentener et al., 2006; IPCC, 2007), we quantified stomatal O_3 flux in pure aspen and mixed aspen-birch canopies exposed to elevated concentrations of CO_2 and O_3, alone and in combination, in the free-air CO_2–O_3 enrichment experiment near Rhinelander, Wisconsin (Aspen FACE). The Aspen FACE experiment offers a unique opportunity to study the interactive

effects of elevated CO_2 and O_3 on forest ecosystem functioning under ecologically realistic conditions. Stand-level net primary production and tree uptake of nitrogen (N) and water were significantly increased by elevated CO_2, while productivity and N uptake but not stand sap flux were decreased under elevated O_3 (King et al., 2005; Uddling et al., 2008; Zak et al., 2007). In this study, we employed a sap flux-based approach for quantifying AFst (e.g., Matyssek et al., 2008) at the canopy (AFst$_c$) and leaf (AFst$_l$) levels, with or without flux cut-off thresholds (AFstY, Y = flux threshold), during two contrasting growing seasons (e.g., relatively cool and moist versus relatively warm and dry) after more than six years of exposure to CO_2 and O_3 enrichment treatments. To also asses the potential influence of competing non-stomatal O_3 sinks at the leaf surface on estimates of stomatal O_3 fluxes, we quantified AFYst$_l$ on the assumptions that g_{ns} was either negligible ($\rightarrow$ AFst$_{max}$) or high and dynamic ($\rightarrow$ AFst$_{min}$). The parameterization of g_{ns} and its environmental dependencies was derived from a study in a northern mixed hardwood forest dominated by *Populus grandidentata* located at a similar latitude in Northern Michigan (Hogg, 2007; Hogg et al., 2007). With the over-all aim to improve estimations of present and future stomatal uptake of O_3, we set out to answer the following two research questions:

(1) Is AFstY$_l$ reduced by elevated CO_2?
(2) How sensitive are estimates of AFstY$_l$ potentially to competing non-stomatal O_3 sinks at the leaf surface; i.e., how large is the difference between AFstY$_{max,l}$ and AFstY$_{min,l}$?

We also discuss the effects of atmospheric gas treatments and community type on AFstY in relation to effects reported on stand productivity in the Aspen FACE experiment.

Materials and methods

2.1. Site description and environmental measurements

The Aspen FACE experiment near Rhinelander, Wisconsin (45.6°N, 89.5°W) has a randomized complete block design with orthogonal combinations of atmospheric CO_2 and O_3 treatments, and tree community type 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), and both elevated CO_2 and O_3 (+CO_2 + O_3). The experiment was planted with 3-to-6-month-old plants at 1 m × 1 m spacing in July 1997 and fumigation treatments have been running during the growing season since 1998. Each plot is divided into three sections with different tree community composition. The present study was conducted in the eastern half with five clones of trembling aspen (*Populus tremuloides* Michx.) and the south-western quadrant with an alternating mixture of aspen (clone 216) and paper birch (*Betula papyrifera* Marsh.). All measurements were conducted 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 situated on an old agricultural field and the soil is classified as an Alfic Haplorthod with 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 aims at maintaining target concentrations of 560 μmol CO_2 mol^{-1} and 1.5 × ambient O_3 when the sun altitude is greater than +6° (denoted "daytime" period in the following). Details about the site and fumigation technology can be found in Dickson et al. (2000) and Hendrey et al. (1999).

Meteorological measurements include photosynthetically active photon flux density on a horizontal surface (Q_p) measured at the site in an open field, horizontal wind speed (u) measured in the upper canopy of all plots with CO_2 and/or O_3 fumigation (nine plots), approximately 1 m below the top of the dominating trees, and air temperature (T) and relative humidity measured above the canopy at 10 m above ground in the centre of four plots (one of each treatment and one control). Temperature and relative humidity data were averaged across these four plots. Plot-specific upper canopy u data were used in plots with atmospheric gas treatments, while mean values of these nine plots were used in control plots since upper canopy u did not significantly differ among treatments. Volumetric soil moisture (θ_v) was measured continuously in four plots and bi-weekly in all twelve plots. Continuous θ_v measurements were made at 5–35 cm depth with CS616 Water Content Reflectometers (Campbell Scientific, Loughborough, UK), and bi-weekly measurements were made at 0–15 cm depth with a Trase TDR (SoilMoisture Equipment Corp., Goleta, CA, USA). Both continuous and bi-weekly θ_v data were collected in the centres of both the aspen and aspen-birch sections of a plot. Soil moisture data used in this study are fully replicated bi-weekly θ_v data, temporally interpolated using relative changes in continuous θ_v data available from one plot per treatment. All meteorological data used here were averaged for half-hour periods.

2.2. Conductance

Results on conductance and AFstY at the canopy and leaf levels presented in this study are for the peak season from mid-June through August (day number 168–243) in 2004 and 2005, the period with fairly good sap flux data capture (>60% of the time) and maximum and stable L. Sap flux was measured with Granier-type sensors (Granier, 1985, 1987) inserted on the main trunk of 252 aspen and birch trees during the 2004 and 2005 growing seasons, as previously described in Uddling et al. (2008). A subsequent gravimetric potometer experiment revealed a substantial but proportional underestimation of tree water use by Granier's original equation, and sap flux rates used here were adjusted accordingly (Uddling et al., 2009). Canopy conductance (g_c) and conductance through stomata and leaf-boundary layer of upper canopy sun leaves (g_{bs}) were calculated from stand-level sap flux and L, measurements of atmospheric pressure and vapor pressure deficit of the air (D), and (for g_{bs}) vertical canopy modeling of radiation and leaf-level conductance, as described by Uddling et al. (2009). Canopy conductance is expressed per unit ground area while g_{bs} is expressed on a one-sided leaf area basis. The sun leaf fraction of the canopy was defined as the top 1.85 m^2 one-sided leaf area m^{-2} ground where Q_p integrated over June–July was at least 35% of its above-canopy value. Stomatal conductance of sun leaves was calculated from g_{bs} and leaf-boundary layer conductance (g_b),

$$g_s = \frac{g_{bs}g_b}{g_b - g_{bs}}, \tag{1}$$

with g_b estimated from characteristic leaf dimensions (0.044 and 0.060 m for aspen and birch leaves, respectively; 0.052 m for aspen-birch mixture) and upper canopy u according to Campbell and Norman (1998).

Canopy leaf temperature could not be reliably estimated using the energy balance approach, as the experimental forest stands do not form a continuous canopy with their surroundings and the equations for wind speed extinction over and within canopies (Campbell and Norman, 1998) therefore do not apply. Turbulent resistance to gas flux was probably small for these well-coupled canopies, where upper canopy u did not differ from u measured above the canopy, 10 m above ground and about 2–4 m above the canopy (data not shown). Possible errors in conductance estimation resulting from the assumption of negligible turbulent resistance were unlikely to affect experimental treatment effects reported here, as u in the upper canopy did not differ significantly among treatments.

Canopy conductance for water vapor was converted into g_c for O_3 by multiplying with 0.613. This value, representing the ratio of the molecular diffusivities of O_3 to water vapor (Campbell and Norman, 1998), was used on the assumption that resistance to turbulent transport was negligible (see above). The small difference in conductance ratio for O_3 and water vapor in molecular diffusion (through stomatal pores) and forced convection (through leaf-boundary layer; 0.721) was neglected in the conversion of g_c but taken into account in conversions of g_s and g_b of upper canopy leaves, since g_b could be reliably estimated for upper canopy sun leaves (u data available), but not at the canopy level (wind speed laws do not apply).

Sap-flux-derived conductance was calculated when sap flux data were available from a minimum of three trees in a stand (stand = a community type within a plot). Such data were available during 76% and 62% of the peak seasons in 2004 and 2005,

respectively. During periods when sap flux data were available from less than three trees per stand, or when data were absent for one species in the mixed aspen-birch stands, or when low D (<0.6 kPa) or Q_p (<50 μmol m^{-2} s^{-1}) caused large uncertainties in the estimation of conductance, g (i.e., g_c and g_{bs}) was modeled as

$$g = \frac{g(D)}{l_\theta} \times f(Q_p) \times f(\theta_v) \tag{2}$$

$$f(Q_p) = 1 - \exp(-0.006 \times Q_p) \tag{3}$$

$$f(\theta_v) = \min(1, \ 0.086 + 10.2 \times \theta_v) \tag{4}$$

where $g(D)$ is the stand-specific relationship between D and sap-flux-derived g_c or g_{bs} during peak season fitted to the function proposed by Oren et al. (1999; parameters in Uddling et al. (2009)), $f(Q_p)$ and $f(\theta_v)$ are relative response functions between zero and one for Q_p (in μ mol m^{-2} s^{-1}) and θ_v, respectively, and l_θ is the mean $f(\theta_v)$ for data used to parameterize $g(D)$. Values of $g(D)$ and l_θ are stand- and year-specific. Values of l_θ averaged 0.98 in 2004 and 0.94 in 2005 and ranged from 0.84 to 1.00. The $g(D)$ function was fitted to data with $0.6 \le D \le 3.1$ kPa. Below this range, $g(D)$ was assumed to plateau at its value at 0.6 kPa rather than to continue to increase with decreasing D. Potentially large errors in sap flux-derived conductance estimation at low D arise from uncertainties in positioning of a diurnal baseline sensor signal at zero sap flux, as well as from possible micrometeorological measurement errors (Ewers and Oren, 2000). Uncertainties at low Q_p are related to possible time lags between stem sap flux and transpiration in the morning and evening (e.g., Schulze et al., 1985). In our data, correlation between sap flux and D in the morning was best if no time lag was applied, while sap flux with 1 h time lag correlated best with falling Q_p in the evening, indicating a diurnally dynamic time lag. The $f(\theta_v)$ predicts a linear decrease in g below θ_v of 0.09, with 40% reduction in g at the lowest value of θ_v, 0.05, observed in pure aspen stands in +O_3 plots in 2005. The function was based on the relationship between daily sap flux and θ_v on 15 days with similar mean D (0.48 kPa $\le D \le$ 0.55 kPa) and θ_v ranging from 0.06 to 0.27 during the warm and dry summer 2005. This relationship was independent of community type or atmospheric gas treatments. A previous statement that "soil moisture in the upper soil layer had a small impact on daily sap flux in control rings" (Uddling et al., 2008) was incorrect and based on the failure to separate influences of D and θ_v (days with very high D and very low θ_v co-occurred). The $f(Q_p)$ is the generic stomatal light-response function for deciduous tree species employed in the EMEP O_3 flux model for Europe (UNECE, 2004), which is based on field studies on Betula pendula (Uddling et al., 2004b, 2005). This function predicts complete stomatal closure in darkness, an assumption supported by observations of very low (0.008 ± 0.004 mol m^{-2} s^{-1}) g_s of aspen and birch leaves in control plots at midnight in mid-August 2005.

The parameterization of g_{ns} for O_3 and its environmental dependencies was based on data from an O_3 flux study in a northern mixed hardwood forest dominated by P. grandidentata located at a similar latitude in Northern Michigan (Hogg et al., 2007), complemented by data for three additional years (Hogg, 2007). Relative g_{ns} response functions, between zero and one, for $T(f_{ns}(T))$ and Q_p ($f_{ns}(Q_p)$) were determined for data in the T and Q_p ranges 10–35 °C and 0–1700 mol m^{-2} s^{-1}, respectively:

$$f_{ns}(T) = \max\left(0, -1.14 + 0.19 \times T - 0.0043 \times T^2\right) \tag{5}$$

$$f_{ns}(Q_p) = \min\left(1, 0.081 + 2.6 \times 10^{-4}Q_p + 1.8 \times 10^{-7}Q_p^2\right) \tag{6}$$

Non-stomatal conductance for O_3 increased monotonically with increasing Q_p (in μ mol m^{-2} s^{-1}), while it was highest in an intermediate temperature range (20–25 °C) and substantially lower below 15 °C and above 30 °C. Parameterizations were based on July–August data only, in order to minimize confounding influences of seasonality on L and plant physiology. Analyzing subsets of data, Hogg et al. (2007) demonstrated independent and strong responses of g_{ns} to both T and Q_p, and we therefore employed a multiplicative model to predict g_{ns} from T and Q_p:

$$g_{ns} = g_{ns,max} \times f_{ns}(T) \times f_{ns}(Q_p), \tag{7}$$

where $g_{ns,max}$ is the mean value of g_{ns} observed under conditions when it was presumably weakly limited by both T (17.5–22.5 °C) and Q_p (>1400 μmol m^{-2} s^{-1}), adjusted for the small limitations predicted by $f_{ns}(T)$ and $f_{ns}(Q_p)$. The value of $g_{ns,max}$ was 0.55 mol O_3 m^{-2} s^{-1} at the canopy level and 0.23 mol O_3 m^{-2} s^{-1} (one-sided leaf area basis) for sun leaves, assuming that the vertical gradient of g_{ns} was similar to that modeled for g_s in a canopy with L of 4, like the P. grandidentata dominated canopy studied by Hogg et al. (2007). Similar vertical profiles of g_{ns} and g_s seem likely given the strong dependence of both variables on radiation (Hogg et al., 2007; Uddling et al., 2008). These $g_{ns,max}$ values should be regarded as maximum estimates, as they are based on the assumption that the ecosystem-level O_3 fluxes reported by Hogg (2007) occur entirely as deposition to leaves.

2.3. Stomatal O_3 flux

Stomatal flux of O_3 at the leaf level (Fst$_l$, expressed on a one-sided leaf area basis) was calculated using the resistance analogue principle derived from Ohm's law (e.g.,

Campbell and Norman, 1998), considering the leaf-boundary layer as a resistance to O_3 flux connected in series with the parallel resistances of stomata and non-stomatal leaf surfaces:

$$Fst = \frac{[O_3]_{can}-[O_3]_{int}}{r_b + r_s + \frac{r_b\, r_s}{r_{ns}}} \tag{8}$$

where $[O_3]_{can}$ and $[O_3]_{int}$ are the O_3 concentrations within the canopy and in the intercellular air space of the leaves, respectively, and r_b, r_s and r_{ns} are the resistances for O_3 flux through the leaf-boundary layer, stomata, and for non-stomatal surface deposition, respectively. Assuming zero g_{ns}, equation (8) can be written:

$$Fst = \frac{[O_3]_{can}-[O_3]_{int}}{r_b + r_s} = \frac{[O_3]_{can}-[O_3]_{int}}{r_{bs}} \tag{9}$$

$[O_3]_{int}$ was assumed to be zero; an assumption with empirical support (Laisk et al., 1989). Stomatal fluxes calculated with equation (8) and the g_{ns} parameterization described above represent low estimates of Fst_l, relevant if all non-stomatal flux occurs at the leaf surface ($Fst_{min,l}$), whereas stomatal fluxes calculated with equation (9) represent high estimates if non-stomatal leaf surface fluxes are negligible ($Fst_{max,l}$).

The accumulated leaf-level stomatal O_3 flux over a cut-off threshold of Y nmol m^{-2} s^{-1} ($AFstY_l$) was calculated by integrating positive values of ($Fst_l - Y$) from mid-June through August (day of year 168–243):

$$AFstY_l = \sum (Fst_l - Y) \quad \text{for } Fst_l > Y \tag{10}$$

Values of Y applied were 0, 1.6 or 3.0 nmol m^{-2} s^{-1}. The threshold value 1.6 nmol m^{-2} s^{-1} was used for forest trees in the O_3 flux–response relationships established for forest trees within the UNECE CLRTAP (UNECE, 2004), and the value 3.0 nmol m^{-2} s^{-1} has been suggested as a more conservative value, reducing the need of response extrapolations in O_3 experiments with plants lacking charcoal-filtered control treatments (Uddling, 2004). The currently used O_3 flux index within the UNECE CLRTAP is $AFst_l1.6$ for upper canopy sun leaves.

Stomatal flux of O_3 at the canopy level (Fst_c, expressed on a ground area basis) was calculated without cut-off threshold and assuming negligible non-stomatal leaf surface flux (i.e., $AFst0_{max,c}$).

2.4. Mathematical analysis

All calculations of conductances and fluxes were performed in MatLab, version 7.5 (The MathWorks, Inc., Natick, MA, USA). 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 (Steel and Torrie, 1980; King et al., 2001). Tukey's HSD post hoc test was used for multiple comparisons of treatments. Effects were regarded as significant at $P \leq 0.10$. Statistical tests were performed using SAS software version 9.3.1 (SAS Institute, Cary, NC, USA).

3. Results

Environmental conditions were markedly different during the two summers (Table 1 and Fig. 1). The summer of 2005 was warmer

Table 1
Mean environmental conditions from mid-June through August (day number 168–243) in 2004 and 2005 at the Aspen FACE site, WI USA.

Environmental variable	2004		2005	
	Ambient air	Elevated treatment	Ambient air	Elevated treatment
$[CO_2]$ (µmol mol^{-1})	363	545 (6)	362	547 (6)
$[O_3]$ (nmol mol^{-1})	32.4	44.4 (0.4)	39.3	53.6 (1.1)
AOT40[a] (µmol mol^{-1} h)	1.3	11.3 (0.6)	4.6	19.9 (0.7)
T (°C)	16.3		19.9	
D (kPa)	0.35		0.51	
Q_p (mmol m^{-2} s^{-1})	0.37		0.40	
Mean vol. soil moisture[b] (θ_v)	0.14		0.11	
Precipitation[c] (mm)	258		198	

Temperature (T), vapor pressure deficit of the air (D), and photosynthetic photon flux density (Q_p) are calculated for 24 h, while O_3 and CO_2 concentrations are calculated for the period with sun altitude >6°. Values in parentheses represent standard deviation ($n = 6$).

[a] Accumulated exposure Over a cut-off Threshold of 40 nmol mol^{-1} during daylight hours (i.e. $Q_p > 106$ µmol m^{-2} s^{-1} to comply with UNECE (2004) definitions).

[b] Mean value for 0–15 cm soil depth in pure aspen and mixed aspen-birch stands in control plots.

[c] Total precipitation during May–August.

than the summer of 2004, and for the duration of the experiment (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, but mean Q_p and upper canopy u were fairly similar. Upper canopy u did not significantly differ among atmospheric treatments (not shown). Ambient levels of O_3 were unusually low in 2004, and typical for this region in 2005 (U.S. Environmental Protection Agency; http://www.epa.gov/ttn/airs/airsaqs/). Mean daytime (sun elevation >6°) ambient O_3 concentrations were 32 nmol mol^{-1} in 2004 and 39 nmol mol^{-1} in 2005 during the periods studied (day number 168–243 in 2004 and 2005; Table 1). Elevated O_3 concentrations were 1.37 × ambient in both years. Mean daytime ambient and elevated CO_2 concentrations were 362 and 546 µmol mol^{-1}, respectively. The fumigation system delivered CO_2 and O_3 enrichment during 97.7% and 56.6% (51.7% in 2004 and 61.5% in 2005) of the total daytime, respectively. The lower number for O_3 is due to O_3 enrichment being restricted to dry canopies on days when the maximum temperature was projected to be at least 15 °C. Values of AOT40 in the ambient air were below or at the current critical level for O_3 of 5 µmol mol^{-1} h for sensitive tree species in Europe (UNECE, 2004), while values in elevated O_3 at least doubled that value. It should be noted that the critical level AOT40 value was based on the exposure during a six months long growing season, while AOT40 values presented here refer to the peak season only (mid-June through August).

The model used to calculate conductance during periods when sap flux data were missing was evaluated by comparing modeled g_c
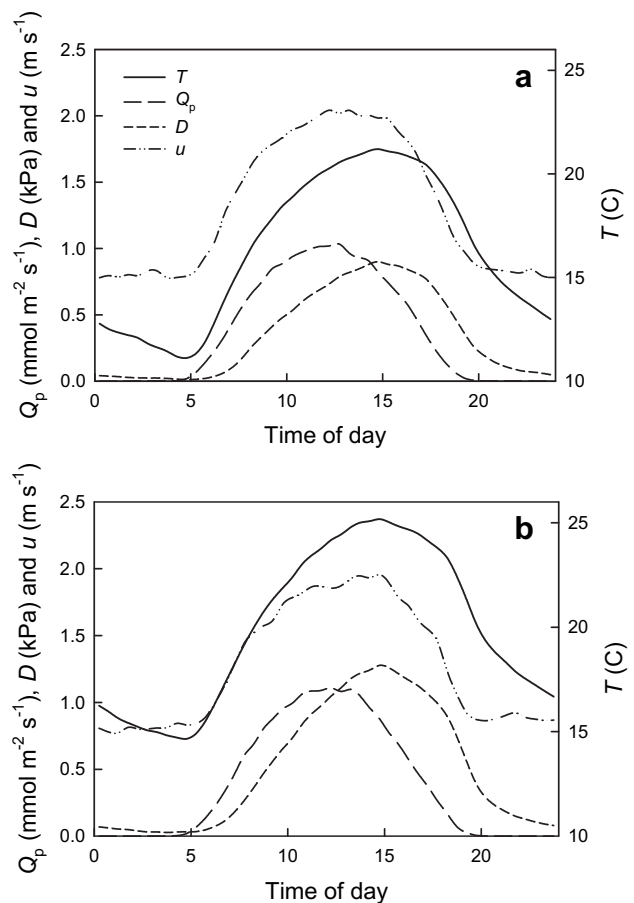

Fig. 1. Mean diurnal temperature (T), photosynthetic photon flux density (Q_p), vapor pressure deficit of air (D), and horizontal wind speed in the upper canopy (u) from mid-June through August (day number 168–243) in (a) 2004 and (b) 2005 at the Aspen FACE site, WI, USA.

with sap-flux-derived g_c during periods when sap flux data were available. The conductance model explained on average 64% of the variation in sap-flux-derived g_c (r^2 of the 1:1 relationship); a value only slightly lower than the mean r^2 value of 66% for linear regressions of modeled g_c plotted against sap-flux-derived g_c. On average, the model overestimated g_c by 1.6%. Based on this small overestimation of g_c and the small difference between the r^2 values of 1:1 relationships and linear regressions, model performance was judged as good enough to justify its use for gap filling.

Canopy conductance at noon was marginally significantly increased by elevated CO_2 by 16%, averaged across O_3 regimes, years and community types ($P = 0.10$; Table 2 and Fig. 2). Aspen stands had 11% higher g_c than aspen-birch stands in 2004, while the difference was only 6% in 2005, causing a significant community type × year interaction ($P = 0.054$). There were significant $CO_2 \times O_3$ interactions on both g_{bs} and g_s at noon ($P = 0.073$ and 0.078, respectively), with values for +CO_2 and +O_3 plots being higher than those for control and +CO_2 + O_3 plots. The only significant difference in conductance among individual treatments, according to Tukey's HSD post hoc test, was that g_{bs} and g_s of mixed aspen-birch communities were higher in +O_3 than in control plots (Fig. 2b and c). Community type × year interactions on g_{bs} and g_s were similar as that on g_c. Values of noon g_s were on average 23% higher than values of noon g_{bs} and mean noon g_b was 0.65 mol O_3 m^{-2} s^{-1}.

Canopy stomatal O_3 flux (AFst0$_{max,c}$) was significantly increased by 37% by elevated O_3 ($P = 0.011$), averaged across CO_2 regimes, years and community types (Tables 2 and 3). AFst0$_{max,c}$ was not significantly increased by CO_2 treatment ($P = 0.16$) in spite of noon g_c being marginally so ($P = 0.10$), as a consequence of O_3 concentrations being slightly (+2.3%) but significantly ($P = 0.040$) higher in +O_3 compared to +CO_2 + O_3 plots in 2004 (not shown). At the leaf level, AFstY$_l$ was increased by elevated O_3 and there was a significant $CO_2 \times O_3$ interaction ($P = 0.060–0.71$), with positive or small effects of elevated CO_2 under ambient O_3 but negative effects of elevated CO_2 under elevated O_3. AFstY$_l$ was always significantly higher in +O_3 plots compared to control plots according to Tukey's HSD test. AFstY$_l$ of +CO_2 + O_3 plots were higher compared to control plots in 2005 but not significantly so in 2004. As for conductance, there were significant community type × year interactions on AFstY$_l$. Effects on AFstY$_l$ were similar regardless of flux cut-off thresholds or assumptions regarding non-stomatal flux.

Effects of atmospheric treatments on the fraction of stomatal to total leaf-level O_3 flux reflected those on g_s. The $CO_2 \times O_3$ interaction on non-stomatal O_3 flux (AFnst0$_l$) mirrored (i.e., were opposite to) those on AFstY$_l$ as a consequence of the interplay between g_s, g_b and g_{ns} (Eq. (8)); i.e., a positive effect on g_s will lead to increased stomatal O_3 flux and hence decreased O_3 flux to the competing non-stomatal leaf surface sink).

During 8:00–16:00 h, g_{bs} decreased while O_3 concentration increased, resulting in a small net variation in Fst0$_l$ during this time period in 2004 and a fairly small decrease in Fst0$_l$ in 2005 (Fig. 3). The higher O_3 concentrations in 2005 compared to 2004 were cancelled by an opposite difference in g_{bs}, for no net difference in Fst0$_l$ between the two years (Table 2, Fig. 3). Values of AFst0$_{min,l}$ were on average 10% lower than values of AFst0$_{max,l}$ (Table 3, Fig. 3c and d).

4. Discussion

Altered atmospheric concentrations of CO_2 and O_3, alone and in combination, did not reduce g_s in aspen and aspen-birch canopies with steady-state L after more than six years of exposure (Fig. 2). As a consequence, elevated CO_2 did not appear to "protect" plants by reducing stomatal O_3 flux, and elevated O_3 did not induce partial stomatal closure limiting further O_3 uptake (Tables 2 and 3). These findings are contrary to expectations based on leaf-level primary stomatal closure responses to both elevated CO_2 and O_3 (e.g., Morison, 1998; Mott, 1988; Paoletti and Grulke, 2005), observations in most earlier experiments (e.g., Norby et al., 1999; Medlyn et al., 2001; Wittig et al., 2007), and assumptions made by models projecting O_3 fluxes and effects on plants under rising CO_2 (e.g., Sitch et al., 2007). Previous studies at the Aspen FACE experiment have demonstrated that long-term cumulative effects of elevated CO_2 and O_3 on tree and stand structure were more important than primary stomatal closure responses in determining stand sap flux and canopy- and leaf-level conductance in pure aspen and mixed aspen-birch forests (Uddling et al., 2008, 2009). Increased canopy transpiration and g_c under elevated CO_2 were related to positive treatment effects of CO_2 on tree size, L and root biomass, while stand tree water use and g_c were not significantly reduced by O_3 treatment in spite of negative effects of O_3 on tree size and L. The absence of O_3 effects on tree water use and g_c were probably at least partly due to unaffected (aspen-birch) or even increased (aspen)

Table 2

Statistical significance (P values) of block, CO_2, O_3, community type and year and their relevant interactions on noon conductance and accumulated flux of O_3 at the canopy and leaf levels during mid-June through August in 2004 and 2005 in the Aspen FACE experiment. P values ≤ 0.10 are shown in bold.

Source	g_c	g_{bs}	g_s	AFst0$_{max,c}$	AFst0$_{max,l}$	AFst0$_{min,l}$	AFnst0$_l$	% stomatal flux[a]	AFst1.6$_{max,l}$	AFst3.0$_{max,l}$
Block	0.48	0.64	0.66	0.51	0.56	0.58	0.52	0.49	0.57	0.60
CO_2	**0.10**	0.65	0.65	0.16	0.37	0.35	0.53	0.53	0.36	0.32
O_3	0.39	0.31	0.33	**0.011**	**<0.001**	**<0.001**	**<0.001**	0.32	**<0.001**	**<0.001**
$CO_2 \times O_3$	0.31	**0.073**	**0.078**	0.54	**0.063**	**0.060**	**0.098**	**0.049**	**0.065**	**0.071**
Com	0.15	**0.013**	**0.014**	0.12	**0.008**	**0.006**	0.56	**0.008**	**0.008**	**0.009**
$CO_2 \times$ Com	0.68	0.84	0.88	0.72	0.82	0.78	0.69	0.85	0.82	0.78
$O_3 \times$ Com	0.51	0.55	0.59	0.66	0.94	0.99	0.57	0.38	0.97	0.85
$CO_2 \times O_3 \times$ Com	0.99	0.73	0.83	0.99	0.72	0.68	0.57	0.47	0.73	0.78
Year	**0.015**	**0.050**	**0.052**	0.97	0.47	0.41	**0.002**	**0.056**	0.47	0.47
$CO_2 \times$ Year	0.16	0.26	0.37	0.46	0.30	0.28	0.28	0.16	0.30	0.32
$O_3 \times$ Year	0.17	0.44	0.55	0.23	0.44	0.48	**<0.001**	0.31	0.46	0.71
$CO_2 \times O_3 \times$ Year	0.28	0.40	0.26	0.38	0.60	0.69	0.94	0.53	0.61	0.69
Com $\times$ Year	**0.054**	**0.012**	**0.008**	**0.056**	**0.045**	**0.056**	**0.067**	0.16	**0.045**	**0.036**
$CO_2 \times$ Com $\times$ Year	0.33	0.44	0.51	0.35	0.49	0.44	0.43	0.60	0.49	0.46
$O_3 \times$ Com $\times$ Year	0.38	0.79	0.73	0.19	0.79	0.94	0.89	0.96	0.79	0.86
$CO_2 \times O_3 \times$ Com $\times$ Year	0.80	0.54	0.43	0.93	0.31	0.28	0.26	0.56	0.29	0.23

Directions and magnitudes of effects as well as significant differences among individual treatments according to Tukey's HSD post hoc test are shown in Fig. 2 and Table 3. Abbreviations: g_c, canopy conductance; g_{bs}, conductance through stomata and leaf-boundary layer; g_s, stomatal conductance; AFstY, accumulated stomatal flux over a cut-off threshold of Y nmol m^{-2} s^{-1} during day of year 168–243; AFnstY, ditto non-stomatal flux; subscripts "max" and "min" refer to stomatal fluxes calculated neglecting and accounting for potential non-stomatal O_3 flux, respectively; subscripts "c" and "l" refer to fluxes at the canopy and leaf levels, respectively.

[a] Fraction of stomatal to total accumulated O_3 flux of upper canopy sun leaves (i.e., AFst0$_{min,l}$/(AFst0$_{min,l}$ + AFnst0$_l$), assuming that the leaf surface is the only non-stomatal sink for O_3 (see *Stomatal O_3 flux* subsection).
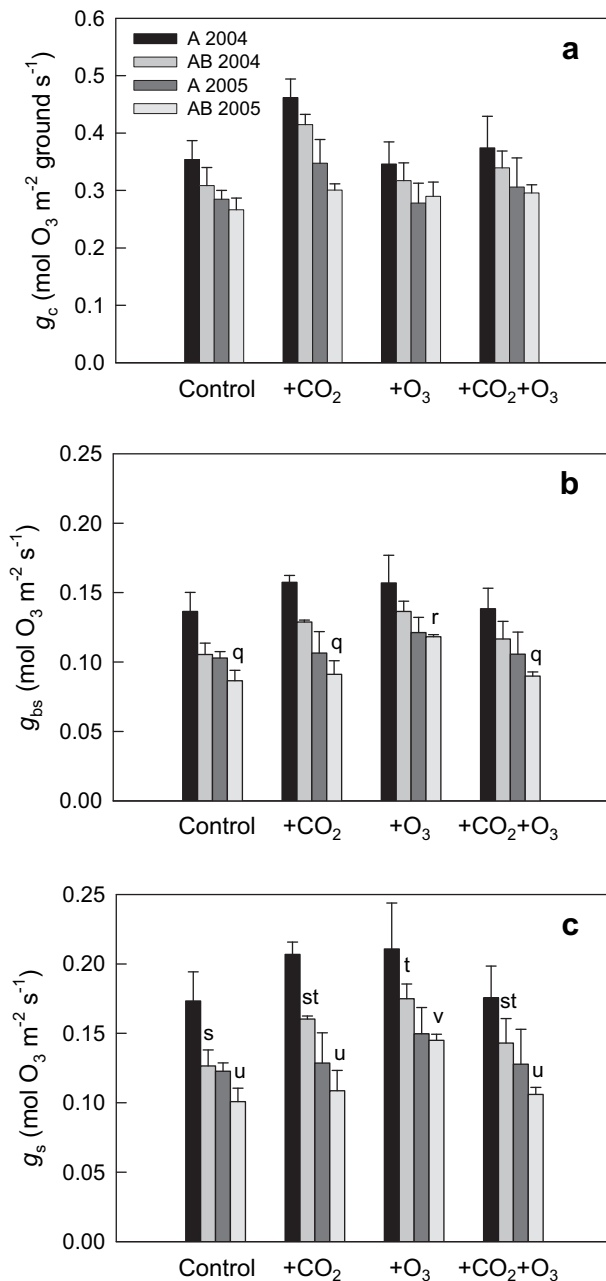
Fig. 2. Noon median values (+SEM, $n = 3$) of (a) canopy conductance (g_c), (b) conductance through boundary layer and stomata (g_{bs}), and (c) stomatal conductance (g_s) for O_3 of pure aspen (A) and mixed aspen-birch (AB) stands from mid-June through August (day number 168–243) in 2004 and 2005 in the Aspen FACE experiment. Values of g_{bs} and g_s are for upper canopy sun leaves. There were no significant ($P \leq 0.10$) differences among treatments within a year and community type, according to Tukey's HSD test.

fine root biomass under elevated O_3 (King et al., 2005; Uddling et al., 2008, 2009). Interestingly, a CO_2-induced significant reduction in g_s observed in aspen during an early stage of the Aspen FACE experiment (Noormets et al., 2001) thus did not persist after canopy closure. At steady-state L, four out 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 (Ellsworth, 1999; Bernacchi et al., 2003; Keel et al., 2007, but see Gunderson et al., 2002), suggesting that the common model assumption of substantial reductions in g_s under rising atmospheric CO_2 (e.g., Pitman, 2003 and references therein) is very uncertain for forests.

These findings raise questions regarding model predictions of reduced O_3 sensitivity of forests in a future with high atmospheric CO_2 concentration (Sitch et al., 2007).

There was a significant $CO_2 \times O_3$ interaction on $AFstY_l$ ($P = 0.060$–0.071) with positive and negative effects of elevated CO_2 on stomatal O_3 flux in the ambient and elevated O_3 regimes, respectively (Tables 2 and 3). This interaction on $AFstY_l$ was the result of a significant $CO_2 \times O_3$ interaction on g_s and g_{bs} ($P = 0.073$–0.078), with higher values in $+CO_2$ and $+O_3$ than in control and $+CO_2 + O_3$ plots (Fig. 2). The interaction on $AFstY_l$ suggests that adverse effects of elevated O_3 on plant productivity and resource acquisition should be stronger under ambient CO_2 than under elevated CO_2. Effects of elevated CO_2 and O_3 on stand-level biomass production (King et al., 2005; data up to 2003), nitrogen uptake (Zak et al., 2007; data up to June 2004) and tree water use (Uddling et al., 2008; 2004–2005 data) reported from the Aspen FACE experiment thus far have been additive, without significant $CO_2 \times O_3$ interactions. A significant $CO_2 \times O_3 \times year \times community$ type interaction was, however, found on yearly stand basal sap wood area increments in 2004 and 2005 ($P = 0.042$), with significant $CO_2 \times O_3$ interactions in pure aspen stands in 2004 ($P = 0.005$) and mixed aspen-birch stands in 2005 ($P = 0.023$; unpublished data). Elevated O_3 decreased yearly basal area increment under ambient CO_2 but not under elevated CO_2, consistent with the $CO_2 \times O_3$ interaction found on $AFstY_l$. However, conclusions on the relationship between stomatal O_3 fluxes and growth should not be drawn until estimates of year-specific stand net primary production are available, a topic that will be addressed in forthcoming studies. The higher stomatal O_3 flux in the pure aspen community type is consistent with its higher O_3 sensitivity of biomass production compared to the aspen-birch community type (King et al., 2005).

This study clearly illustrates the climate-induced implications of using a flux-based O_3 index rather than a concentration-based index. Although O_3 concentrations were markedly higher in 2005 than in 2004, stomatal O_3 fluxes were not, as a result of the lower g_c and g_s during the considerable warmer and drier 2005 compared to the cool and moist summer of 2004 (Tables 2 and 3, Fig. 3). Similarly, but on a different temporal scale, the increasing O_3 concentration during the day was counteracted by falling g_{bs}, for a small net effect on stomatal O_3 flux during 8:00–16:00 h (Fig. 3).

The potential influence of non-stomatal leaf surface O_3 sinks on stomatal fluxes (Eq. (8)) was neglected in the calculation of $AFst1.6_l$ in the open-top chamber experiments on which current critical levels and flux–response relationships for O_3 within the UNECE CLRTAP are based (Karlsson et al., 2004; Pleijel et al., 2007). As a result, stomatal O_3 fluxes in these experiments may have been overestimated (Grünhage et al., 2004). Data presented here show that even if non-stomatal leaf surface O_3 flux is large (32–50%; Table 3), its reducing effect on stomatal fluxes of sun leaves may be relatively small under free-air conditions (-10% for $AFst0_l$ and -16% for $AFst1.6_l$; Fig. 1). The effect is presumably smaller for well-ventilated chamber experiments (Uddling et al., 2004a). Values of $AFstY_{min,l}$ represent minimum estimates of $AFstY_l$, based on the assumption that all non-stomatal O_3 sinks are located at the leaf surfaces and contribute to lowering the O_3 concentration at the leaf surface (i.e., inside the leaf-boundary layer) where stomata and the non-stomatal leaf surface are competing sinks for O_3. Leaf-level studies have indicated that non-stomatal O_3 deposition is very small compared to stomatal flux (Grulke et al., 2007; Pleijel et al., 2004), and work in a pine forest suggest that the non-stomatal O_3 deposition is dominated by gas-phase reactions rather than leaf surface deposition (Goldstein et al., 2004; Kurpius and Goldstein, 2003). If substantial non-stomatal O_3 consumption occurs at non-leaf surfaces or as gas-phase chemical reactions outside the leaf-boundary layer (in the Aspen FACE experiment and/or in the study

Table 3
Mean ($\pm$SEM in parentheses, $n = 3$) accumulated stomatal fluxes of O_3 (AFst$Y$, mmol m^{-2}) for the period mid-June through August in 2004 and 2005 at the canopy and leaf levels of pure aspen and mixed aspen-birch communities in the Aspen FACE experiment. Fluxes are calculated with or without flux cut-off thresholds, neglecting (AFstY_{max}) or accounting for potential non-stomatal leaf surface O_3 deposition (AFstY_{min}).

Variable	Pure aspen					Mixed aspen-birch				
	Control	+CO_2	+O_3	+CO_2 + O_3	Tukey's HSD[a]	Control	+CO_2	+O_3	+CO_2 + O_3	Tukey's HSD[a]
2004										
AFst0$_{max,c}$	37.1 (3.4)	48.8 (3.6)	52.3 (5.0)	55.8 (7.4)	a-a-a-a	31.8 (3.2)	43.0 (1.6)	47.3 (4.5)	50.3 (3.8)	a-ab-b-b
AFst0$_{max,l}$	14.3 (1.4)	16.8 (0.6)	23.9 (2.6)	20.8 (1.9)	a-a-b-ab	10.9 (0.9)	13.4 (0.2)	20.5 (0.8)	17.4 (1.7)	a-a-b-ab
AFst0$_{min,l}$	13.1 (1.3)	15.4 (0.6)	21.7 (2.3)	19.0 (1.8)	a-a-b-ab	9.9 (0.8)	12.2 (0.2)	18.4 (0.8)	15.7 (1.6)	a-a-b-ab
% stomatal flux	64 (3)	68 (1)	67 (3)	64 (3)	a-a-a-a	56 (2)	62 (0)	64 (1)	59 (3)	a-a-a-a
AFst1.6$_{max,l}$	8.5 (1.3)	10.8 (0.6)	17.8 (2.5)	14.8 (1.8)	a-a-b-ab	5.2 (0.8)	7.5 (0.2)	14.5 (0.8)	11.5 (1.6)	a-a-b-ab
AFst1.6$_{min,l}$	7.2 (1.3)	9.4 (0.6)	15.7 (2.3)	13.0 (1.7)	a-a-b-ab	4.2 (0.7)	6.3 (0.2)	12.5 (0.8)	9.9 (1.5)	a-a-b-ab
AFst3.0$_{max,l}$	4.4 (1.2)	6.4 (0.6)	13.3 (2.4)	10.5 (1.7)	a-a-b-ab	1.8 (0.5)	3.5 (0.2)	10.1 (0.8)	7.4 (1.5)	a-a-b-ab
AFst3.0$_{min,l}$	3.4 (1.0)	5.1 (0.5)	11.2 (2.2)	8.7 (1.6)	a-a-b-ab	1.1 (0.3)	2.5 (0.1)	8.2 (0.7)	5.8 (1.4)	a-a-b-ab
2005										
AFst0$_{max,c}$	36.7 (2.0)	43.4 (5.1)	51.3 (6.3)	56.7 (9.2)	a-a-a-a	33.6 (2.5)	37.5 (1.6)	52.2 (5.0)	54.5 (3.1)	a-a-b-b
AFst0$_{max,l}$	13.4 (0.6)	13.5 (2.0)	22.6 (2.0)	19.8 (3.0)	a-a-b-b	11.0 (0.9)	11.5 (1.3)	21.5 (0.8)	16.8 (0.5)	a-a-b-c
AFst0$_{min,l}$	12.2 (0.6)	12.2 (1.8)	20.5 (1.8)	17.8 (2.7)	a-ab-c-bc	9.8 (0.9)	10.2 (1.2)	19.4 (0.7)	14.7 (0.6)	a-a-b-c
% stomatal flux	56 (1)	55 (4)	59 (2)	55 (4)	a-a-a-a	50 (2)	51 (3)	59 (1)	51 (1)	a-a-a-a
AFst1.6$_{max,l}$	7.6 (0.6)	7.7 (1.9)	16.6 (1.9)	13.8 (2.9)	a-a-b-b	5.2 (0.9)	5.7 (1.2)	15.5 (0.7)	10.9 (0.5)	a-a-b-c
AFst1.6$_{min,l}$	6.4 (0.5)	6.4 (1.7)	14.5 (1.8)	11.9 (2.6)	a-ab-c-bc	4.2 (0.8)	4.5 (1.1)	13.4 (0.7)	8.8 (0.6)	a-a-b-c
AFst3.0$_{max,l}$	3.8 (0.5)	3.9 (1.4)	12.0 (1.8)	9.4 (2.8)	a-a-b-b	2.0 (0.6)	2.3 (0.9)	11.0 (0.7)	6.6 (0.5)	a-a-b-c
AFst3.0$_{min,l}$	2.9 (0.4)	2.9 (1.2)	10.0 (1.6)	7.6 (2.4)	a-a-b-b	1.3 (0.4)	1.6 (0.7)	9.0 (0.6)	4.8 (0.5)	a-a-b-c

See Table 2 for explanations of variables.
[a] Significant ($P \leq 0.10$) differences among treatments according to Tukey's HSD post hoc test are indicated by different letters, in the order Control, +CO_2, +O_3 and +CO_2 + O_3.

of Hogg et al., 2007), actual values of AFstY_l calculated from g_{bs} and within-canopy O_3 concentrations are probably closer to AFst$Y_{max,l}$ than to AFst$Y_{min,l}$. The overestimation of stomatal O_3 flux when neglecting g_{ns} may therefore be small for upper canopy leaves as well as in the well-ventilated chamber experiments used to derive current critical levels and flux–response relationships for O_3 within the UNECE CLRTAP.

5. Conclusions

Returning to our specific research questions, we conclude that:

(1) AFstY_l was not reduced by elevated CO_2, but there was a significant $CO_2 \times O_3$ interaction on AFstY_l caused by a significant $CO_2 \times O_3$ interaction on g_s, which in turn was the
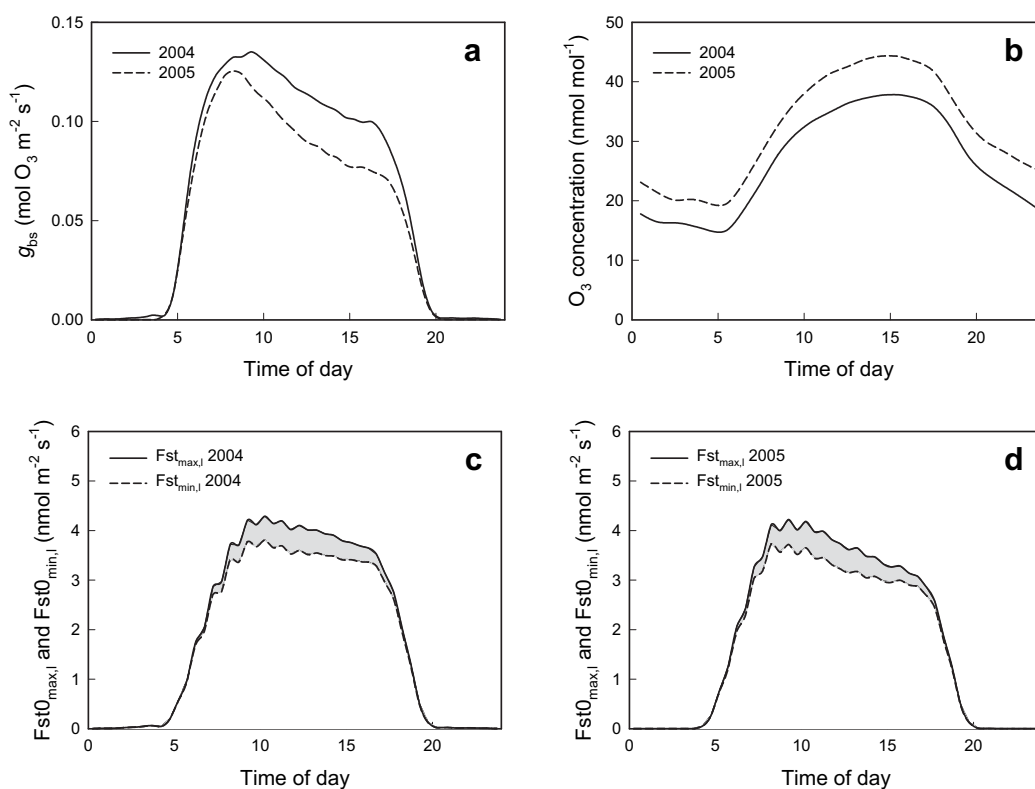

Fig. 3. Mean diurnal (a) conductance through boundary layer and stomata (g_{bs}), (b) O_3 concentration, and leaf-level stomatal O_3 flux without flux cut-off threshold (Fst0$_l$) in control plots from mid-June through August in (c) 2004 and (d) 2005 in the Aspen FACE experiment. Subscripts "max" and "min" refer to stomatal fluxes calculated neglecting and accounting for potential non-stomatal O_3 flux, respectively.

result of higher values of g_s in +CO_2 and +O_3 than in control and +CO_2 + O_3 plots.

(2) Estimates of stomatal O_3 flux were not very sensitive to potential non-stomatal leaf surface fluxes of O_3, as the application of a model with high and dynamic g_{ns} resulted in a large fraction of non-stomatal O_3 flux (32–50%) but a comparatively small (10%) reduction in AFst0$_l$.

Further studies are required to investigate if atmospheric treatment effects on AFst0$_l$ are consistent with effects on year-specific stand productivity in the Aspen FACE experiment. The higher stomatal O_3 flux in pure aspen stands was consistent with higher O_3 sensitivity of this community type compared to aspen-birch mixture.

Acknowledgements

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