Environmental Pollution 159 (2011) 2350–2354

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Rapid Communication

Exposure to moderate concentrations of tropospheric ozone impairs tree stomatal response to carbon dioxide

Gabriela Onandia [a,b], Anna-Karin Olsson [a], Sabine Barth [a], John S. King [c], Johan Uddling [a,*]

[a] Department of Plant and Environmental Sciences, University of Gothenburg, P. O. Box 461, SE-405 30 Göteborg, Sweden
[b] Cavanilles Institute of Biodiversity and Evolutionary Biology, Department of Microbiology and Ecology, University of Valencia, E-46100 Burjassot, Valencia, Spain
[c] Department of Forestry and Environmental Resources, Campus Box 8002, North Carolina State University, Raleigh, NC 27695, USA

ARTICLE INFO

Article history:
Received 31 January 2011
Received in revised form
31 May 2011
Accepted 2 June 2011

Keywords:
Betula papyrifera
Carbon dioxide
Ozone
Sap flow
Stomata
Stomatal conductance

ABSTRACT

With rising concentrations of both atmospheric carbon dioxide (CO_2) and tropospheric ozone (O_3), it is important to better understand the interacting effects of these two trace gases on plant physiology affecting land-atmosphere gas exchange. We investigated the effect of growth under elevated CO_2 and O_3, singly and in combination, on the primary short-term stomatal response to CO_2 concentration in paper birch at the Aspen FACE experiment. Leaves from trees grown in elevated CO_2 and/or O_3 exhibited weaker short-term responses of stomatal conductance to both an increase and a decrease in CO_2 concentration from current ambient level. The impairement of the stomatal CO_2 response by O_3 most likely developed progressively over the growing season as assessed by sap flux measurements. Our results suggest that expectations of plant water-savings and reduced stomatal air pollution uptake under rising atmospheric CO_2 may not hold for northern hardwood forests under concurrently rising tropospheric O_3.

1. Introduction

Plant leaf stomata constitute a crucial interface between terrestrial vegetation and the atmosphere, regulating their exchange of matter and energy. Rising concentrations of both atmospheric carbon dioxide ($[CO_2]$) and tropospheric ozone ($[O_3]$; Meehl et al., 2007) are expected to reduce stomatal conductance (g_s), with future atmospheric change thus being projected to reduce plant water use, possibly resulting in increased continental runoff (Betts et al., 2007). Decreased g_s under elevated CO_2 (eCO_2) has also been predicted to have a protective effect by decreasing the flux of O_3 into the plant (Sitch et al., 2007). Reductions in g_s by eCO_2 and elevated O_3 (eO_3) observed in many experiments (Ainsworth and Rogers, 2007; Medlyn et al., 2001; Wittig et al., 2007) are generally attributed to the well-documented primary stomatal closure response to increased intercellular $[CO_2]$ (c_i; Morison, 1998; Mott, 1988). While eCO_2 directly increases c_i, eO_3 increases c_i through photosynthetic impairment (Wittig et al., 2007). Leaf gas exchange models represent the g_s response to c_i by assuming a tight link between g_s and photosynthesis that acts to maintain an approximately constant intercellular to ambient $[CO_2]$ ratio (Ball et al., 1987; Lening, 1995). These combined stomatal-photosynthesis models are now frequently employed in ecosystem models (Morales et al., 2005), as well as in Dynamic Global Vegetation Models (DGVM; Prentice et al., 2007) and General Circulation Models (GCM; Pitman, 2003; Sellers et al., 1996) used to predict future climate change.

Ozone may, however, affect stomatal regulation in ways that are independent of the c_i response. Elevated O_3 may directly affect guard cell functioning, leading to stomatal closure in the absence of effects on photosynthesis (Mansfield, 1998; McAinsh et al., 2002). In addition, exposure to eO_3 may disturb stomatal functioning as guard cells and surrounding epidermal cells become damaged (e.g., Mansfield, 1998), causing less sensitive, or 'sluggish' stomatal responses to drought (McAinsh et al., 2002; Pearson and Mansfield, 1993), vapour pressure deficit (VPD; Grulke et al., 2007; Maier-Maercker, 1999; Maier-Maercker and Koch, 1991; Uddling et al., 2009) and light (Barnes and Brown, 1990; Grulke et al., 2007; Reiling and Davison, 1995). In a recent study, Mills et al. (2009) demonstrated that stomatal responsiveness to severe water stress by leaf excision was impaired in two grassland species chronically exposed to eO_3. In the treatment with the highest $[O_3]$, stomata also lost their closure responsiveness to exogenous application of abscisic acid. As signalling in stomatal closure responses to abscisic acid and CO_2 overlap (Ainsworth and Rogers, 2007; Hetherington and Woodward, 2003), there is the possibility that eO_3 may also impair stomatal CO_2 responsiveness. To our knowledge, this hypothesis has not been tested.

* Corresponding author.
E-mail address: johan.uddling@dpes.gu.se (J. Uddling).

0269-7491/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envpol.2011.06.001

Another poorly understood aspect of stomatal regulation under atmospheric change is whether or not the primary stomatal response to CO_2 acclimates to prevailing growth [CO_2]. It has been suggested that the stomatal CO_2 response may acclimate to maintain its most sensitive range just below growth [CO_2] (Morison, 1998; i.e. within the normally encountered range of c_i). If so, this suggests that stomatal responses to CO_2 serve to reinforce stomatal opening and closing at dawn and dusk rather than to adjust the magnitude of g_s to varying atmospheric [CO_2]. Stomatal CO_2 responsiveness may also be partially lost in plants grown in eCO_2, as observed in a free-air CO_2 enrichment (FACE) experiment with scrub oak (*Quercus myrtifolia* Willd.; Lodge et al., 2001).

The Aspen FACE experiment (Dickson et al., 2000; Karnosky et al., 2003) with northern hardwood tree communities provides a unique opportunity to study the interacting effects of 40–50% elevation in [CO_2] and [O_3] on forest functioning under ecologically realistic conditions. By investigating the short-term response of g_s to altered [CO_2] (540 and 280 μmol mol^{-1} vs. 380 μmol mol^{-1}) in individual leaves of paper birch (*Betula papurifera* Marsh.) under controlled environmental conditions, the present study aims to answer the following two questions: (1) Is the primary stomatal response to [CO_2] impaired by chronic exposure to eO_3? (2) Is the short-term stomatal CO_2 responsiveness (in the [CO_2] range studied) reduced in leaves developed under eCO_2? To further assess if possible effect of eO_3 developed in a dose-dependent manner, continuous measurements of sap flux were used to investigate seasonal trends in stomatal control of stand tree water use.

2. Materials and methods

2.1. Site description

The study of stomatal CO_2 responsiveness was conducted at the factorial CO_2 and O_3 Aspen FACE experiment near Rhinelander, Wisconsin (45.6°N, 89.5°W) in August 2008. The experiment consists of twelve 30 m-diameter circular plots with three control plots and three replicate plots each receiving eCO_2 ($+CO_2$), eO_3 ($+O_3$), or both eCO_2 and eO_3 ($+CO_2+O_3$). The Aspen FACE experiment was situated on an old agricultural field and the soil is classified as an Alfic Haplorthod with sandy loam soil texture (Dickson et al., 2000). 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 seasons 1998–2008. Each plot is divided into three sub-plots with different tree community composition. The present study was conducted on paper birch (*Betula papyrifera* Marsh.) growing in the south-western sub-plot planted in an alternating pattern of birch and trembling aspen (*Populus tremuloides* Michx.; clone 216). Trees included in this study grew within the central core area of the plots, which is buffered from edge effects by five rows of trees on the outer edge of the treatment plots.

Mean annual temperature at Rhinelander is 4.9 °C, mean July temperature is 19.7 °C, and mean annual precipitation is 810 mm (Dickson et al., 2000). Fumigation with CO_2 and O_3 aimed at maintaining target concentrations of 560 μmol mol^{-1} CO_2 and 1.5 × ambient O_3 during hours when sun elevation was ≥6° from the horizon. Ozone enrichment was restricted to dry canopies on days when the maximum temperature was projected to be at least 15 °C. Ambient and elevated mean growing season daytime (7:00–19:00 h) O_3 concentrations in 2008 were 32.9 and 40.9 nmol mol^{-1}, respectively. Corresponding concentrations in 2006 were 37.1 and 44.9 nmol mol^{-1}.

2.2. Measurements and data analyses

The short-term stomatal response to CO_2 was studied in leaves from 12 excised shoots (one per plot) during 12 days during the period 8 August to 25 August, 2008. Shoots of about 50 cm, taken from current-year shoot growth in the upper fourth of the canopy (but avoiding the top shoot), were excised under water early in the morning when leaves were still wet from dew. They were immediately transported to the on-site laboratory, where one leaf with healthy appearance and insertion number 4–6 from the shoot tip was selected and enclosed in the leaf chamber of a gas exchange system with climate and CO_2 control (LI6400, LI-COR inc., Lincoln, NE, USA). The cut end of the stem was kept under water at all times. Measurements on excised shoots in the laboratory were preferred over field measurements on attached shoots, to avoid confounding influences of diurnal changes in, or treatment effects on, environmental conditions and whole-tree water relations on the leaf-level response studied. The influence from transpiration of the neighboring leaves on the shoot was probably minimal, as their g_s was low (data not shown), probably in response to low photosynthetic photon flux density (PPFD) inside the laboratory.

During the entire gas exchange measurements, leaf chamber conditions were set to saturating PPFD (1800 μmol m^{-2} s^{-1}), 25 °C leaf temperature, and 1 kPa leaf-to-air vapor pressure deficit. Vapor pressure deficit varied very little during the measurement of a leaf (on average by 0.03 kPa and never by > 0.10 kPa). Preliminary studies (data not shown) indicated that g_s remained stable for a few hours under these conditions. After an initial 20 min acclimation period to leaf chamber conditions ([CO_2] = 380 μmol mol^{-1}), a photosynthetic response measurement to varying c_i (a so-called Ac_i curve) was conducted. After the Ac_i curve, acclimated (i.e. steady-state) g_s was measured at leaf chamber [CO_2]s in the following order: 380, 540, 380, 280 and 380 μmol mol^{-1}. In this stomatal CO_2 response measurement, the leaf chamber [CO_2] was changed after g_s had reached a steady state with ≤1% change during the preceding 5 min. A minimum acclimation period of at least 30 min was given at each new [CO_2]. The average time between the first and third steady-state measurement at 380 μmol mol^{-1} was 3 h and the average time inside the leaf chamber was four and a half hours. The mean and minimum g_s at the first stomatal response measurement at 380 μmol mol^{-1} were 0.242 and 0.090 mol m^{-2} s^{-1}, respectively. The mean change in g_s between the first and second and between the second and third steady-state g_s measurements at 380 μmol mol^{-1} were −9% (standard deviation ± 15%) and +2% (standard deviation ± 19%), respectively. To account for these changes in g_s at similar conditions, a baseline of g_s at 380 μmol mol^{-1} was estimated by assuming a linear change in g_s over time between the steady-state g_s measurements at 380 μmol mol^{-1}. The relative g_s at increased or decreased CO_2 was calculated by dividing the steady-state g_s measurement at 540 or 280 μmol mol^{-1} with the estimated 380 μmol mol^{-1} baseline g_s at the time of the 540 or 280 μmol mol^{-1} steady-state g_s measurement (Fig. 1).

Sap flux data for mixed aspen-birch stands in 2006 presented in this study was measured and scaled to the stand level as described by Uddling et al. (2008, 2009). Birch dominated over aspen with respect to biomass and sap flux in control as well as treatment plots (Kubiske et al., 2007; Uddling et al., 2008; unpublished data for 2006).

After initial assurance of lack of significant heterogeneity of variances among treatments according to Cochran's test (Underwood, 1997), data were statistically tested for effects of CO_2 and O_3 and their interaction using two-way analysis of variance. Post-hoc comparison of each individual treatment with control was made using one-tailed (to test if treatments cause weaker response) Student's *t*-test assuming equal variances. Time was included as an additional factor (repeated measures) for sap flux data. Effects were regarded as significant at $P \leq 0.10$. All tests were performed using SAS Proc GLM, version 9.3.1 (SAS Institute, Cary, NC, USA).

3. Results

The response of g_s to a short-term increase in [CO_2] (540 vs. 380 μmol mol^{-1}) was significantly ($P = 0.033$–0.073) stronger in leaves from control plots (−13%) than in leaves from trees exposed to eCO_2 and/or O_3 (−1% to −3%; Fig. 2a), resulting in a significant interaction between CO_2 and O_3 treatment ($P = 0.090$). Excluding the one, two, three or four leaves with the largest changes in g_s between the first and second steady-state g_s measurements at

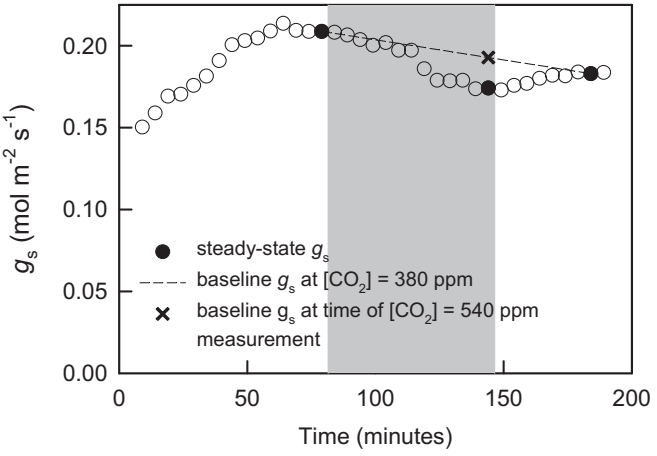

Fig. 1. Measurement of stomatal response to a short-term increase in [CO_2], with illustration of how this response was estimated accounting for a change in steady-state stomatal conductance (g_s) at [CO_2] = 380 ppm before and after the measurement of steady-state g_s at [CO_2] = 540 ppm. The response to increased [CO_2] was calculated from the steady-state measurement of g_s at 540 ppm compared to the estimated baseline g_s at 380 ppm at the time of the 540 ppm measurement. Data points are 5 min binned averages. The grey area indicates the period with [CO_2] = 540 ppm.

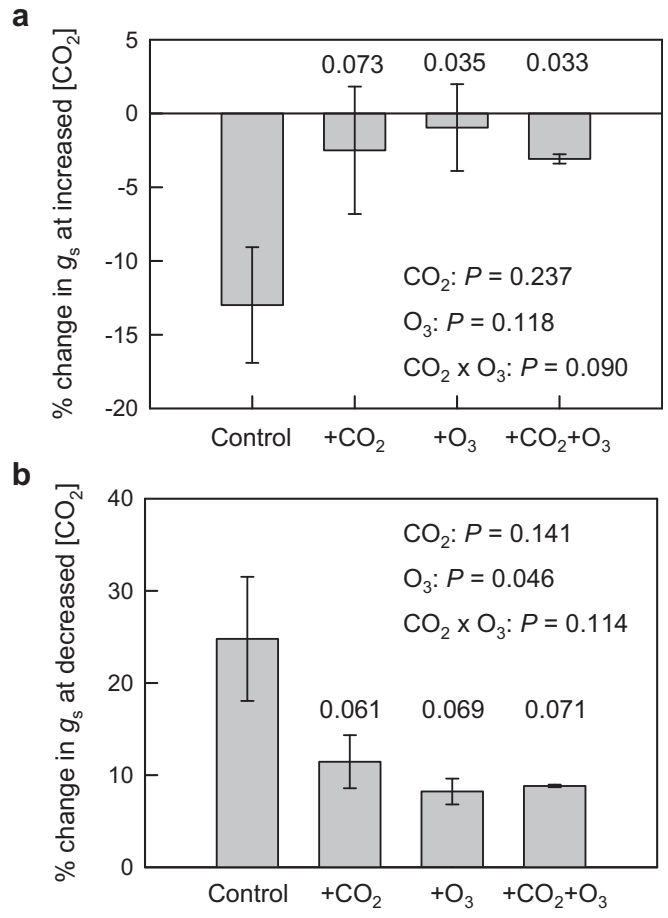

Fig. 2. Percent change (±standard error) in stomatal conductance (g_s) to a short-term (a) increase (540 μmol mol^{-1}) and (b) decrease (280 μmol mol^{-1}) in [CO_2] compared to g_s at 380 μmol mol^{-1} [CO_2] in birch grown in ambient air (Control), elevated CO_2 (+CO_2), elevated O_3 (+O_3) or both elevated CO_2 and O_3 (+CO_2+O_3) in the Aspen FACE experiment. Measurements were made on individual leaves under controlled environmental conditions (see text). P values shown are for main effects of CO_2 and O_3 treatment and their interaction (two-way ANOVA) as well as for comparisons of each individual treatment with control (Student's t-test).

[CO_2] of 380 μmol mol^{-1} resulted in either similar significant $CO_2 \times O_3$ interactions or simple main effects of eO_3. In all cases, leaves grown in eO_3 exhibited significantly smaller responses to increased [CO_2] compared to control leaves, while the response value of the +CO_2 treatment was strongly dependent on the inclusion/exclusion of one leaf with 6% stomatal opening response to increased [CO_2]. This implies that the estimated effect of eCO_2 on the stomatal closure response to increased CO_2 was sensitive to the potential problem of g_s changing over time (Fig. 1), but not the finding that this response was impaired by eO_3.

Similarly, the response of g_s to a short-term decrease in [CO_2] (280 vs. 380 μmol mol^{-1}) was significantly (P = 0.061–0.071) stronger in leaves from control plots (+25%) than in leaves from trees exposed to elevated CO_2 and/or O_3 (+8% to +11%; Fig. 2b). The main effect of elevated O_3 on the response was statistically significant (P = 0.046), while the $CO_2 \times O_3$ interaction was not (P = 0.114). For three of the twelve leaves, steady-state g_s data to estimate the stomatal response to a short-term decrease in CO_2 concentration were not available. Excluding the measurement with the largest change in g_s between the second and third steady-state g_s measurements at 380 μmol mol^{-1} did not qualitatively affect the results.

There was a progressive increase in elevated to ambient O_3 stand-level sap flux ratio from mid June until early September in 2006 (Fig. 3). The $O_3 \times$ Time interaction on stand sap flux was highly significant (P = 0.006). There was also a highly significant $CO_2 \times$ Time interaction on stand sap flux ($P < 0.001$), reflecting an increase in elevated to ambient CO_2 stand sap flux ratio during a very dry period in July (data not shown). The $O_3 \times$ Time interaction, however, mostly developed when soil water content was comparatively high during August. In August 2006, volumetric soil water content at 0–20 cm soil depth was ≥9% (data not shown), which was the threshold for negative effects of low soil water on stand sap flux in 2004–2005 (Uddling et al., 2010). There was no significant $CO_2 \times O_3 \times$ Time interaction on stand sap flux (P = 0.81). By restricting this analysis to the period of the growing season when canopy leaf area index was at its peak and before the onset of O_3-induced effects on leaf shedding (Karnosky et al., 2005), temporal trends of elevated O_3 on sap flux could be attributed to changes in g_s rather than changes in leaf area index.

There were no significant treatment effects on net photosynthesis at common c_i (266 or 378 μmol mol^{-1}) or on nitrogen content on a leaf area basis ($P \geq 0.40$; data not shown).

4. Discussion

Results presented here suggest that stomatal responsiveness to CO_2 is reduced by growth in eCO_2 and is impaired by chronic exposure to moderately elevated [O_3] (Fig. 2). Sap flux data indicate that the effect of O_3 is dose-dependent, with stomatal control over transpiration gradually being lost during the growing season (Fig. 3). While previous research has demonstrated that elevated O_3 may cause less sensitive ('sluggish') stomatal responses to drought (McAinsh et al., 2002; Pearson and Mansfield, 1993), VPD (Grulke et al., 2007; Maier-Maercker and Koch, 1991; Uddling et al., 2009), abscisic acid (Mills et al., 2009) and light (Barnes and Brown, 1990; Grulke et al., 2007; Reiling and Davison, 1995), the present study is the first to reveal that elevated O_3 may also impair stomatal responsiveness to CO_2. Although underlying mechanisms were not investigated in this admittedly small study, it is tempting to hypothesize that the observed effect of eO_3 on stomatal CO_2 responsiveness resulted from a general impairment of stomatal

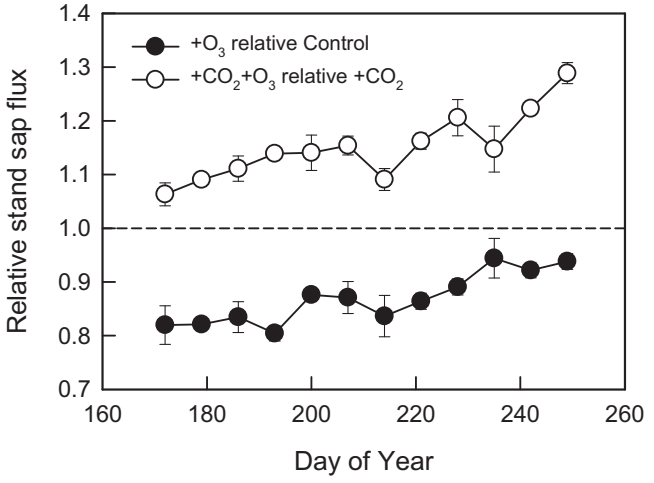

Fig. 3. The effect of elevated O_3 on stand sap flux of mixed aspen-birch communities in the Aspen FACE experiment. Elevated O_3 stands (+O_3) are compared to Control stands, while the combined elevated CO_2 and O_3 treatment (+CO_2+O_3) is compared to the elevated CO_2 (+CO_2) treatment. Standard error bars represent the standard error among days within binned 7-day periods, to illustrate the significance of temporal changes in O_3 effects rather than of treatment effects.

responsiveness by eO_3 (i.e., also responses to drought, VPD and light). Indeed, progressive loss of stomatal control over transpiration during the growing season was observed not only under eCO_2 ($+CO_2+O_3$ vs $+CO_2$), but to a similar extent under ambient CO_2 ($+O_3$ vs. Control; Fig. 3). Furthermore, the stomatal closure response to increasing VPD was less sensitive in pure aspen stands growing under eO_3 in the Aspen FACE experiment (Uddling et al., 2009).

Impairement of stomatal responsiveness to CO_2 in the absence of negative effects on photosynthesis suggests that stomatal functioning is very sensitive to chronic O_3 exposure and is likely already impaired over large areas of industrialized regions where ambient [O_3] equals or exceeds those in eO_3 treatment at in the Aspen FACE experiment (Dentener et al., 2006; Karnosky et al., 2003; Uddling et al., 2008). Loss of stomatal responsiveness may reduce growth and fitness, particularly under conditions of limited water availability. There are two current hypotheses regarding drought by O_3 interactions on plant growth. Either, drought may ameliorate negative effects of O_3 on growth as a consequence of reduced stomatal O_3 uptake under dry conditions, or elevated O_3 may predispose plants to more severe drought stress through loss of stomatal control over transpiration. The first type of interaction, observed for beech productivity in a free-air O_3 enrichment experiment in mature mixed beech-spruce forest (Matyssek et al., 2010), is potentially accounted for in O_3 risk assessments based on stomatal O_3 flux. Flux-based O_3 indices have been shown to perform better than O_3 indices based on external exposure in metaanalyses of experiments with both crops (Pleijel et al., 2007) and forest trees (Karlsson et al., 2007; Uddling et al., 2004). The second type of interaction was observed in the Aspen FACE experiment, where reduced stomatal sensitivity to VPD coincided with more severe drought in pure aspen stands exposed to eO_3 during the dry summer of 2005 (Uddling et al., 2008, 2009). Incorporation of this type of interaction in O_3 risk assessment requires not only additional parameters/functions to represent effects of elevated O_3 on stomatal responses to other environmental variables, but also improved ecosystem representation in O_3 flux modelling to account for coupled soil-plant-atmosphere interactions.

The finding of reduced stomatal CO_2 responsiveness in plants grown under eCO_2 is in agreement with results from a FACE experiment with scrub oak (Lodge et al., 2001). These results suggest that stomatal CO_2 responsiveness of plants grown in current ambient [CO_2] may have little relevance for the adjustment of g_s to rising atmospheric [CO_2]. In the Aspen FACE experiment, loss of stomatal CO_2 responsiveness in birch may be part of the explanation of the lack of significant effects of growth under eCO_2 on g_s in aspen-birch stands (Uddling et al., 2009). It may be difficult, however, to reconcile a hypothesis of loss of stomatal CO_2 responsiveness under growth in eCO_2 with the fact that g_s was reduced in most elevated CO_2 experiments conducted (Curtis and Wang, 1998), albeit mostly not in FACE experiments in closed forest stands (cf. Bernacchi et al., 2003 (pre-coppice canopy closure); Gunderson et al., 2002; Keel et al., 2007; Maier et al., 2008; Schäfer et al., 2002; Uddling et al., 2009). It remains a poorly explored possibility, however, that the effect of growth in eCO_2 on g_s is determined by tree plant hydraulics and water balance (e.g. Domec et al., 2009; Schäfer et al., 2002; Uddling et al., 2009) and/or coordination of adjustments of g_s and photosynthetic capacity rather than by the short-term response of stomatal guard cells to CO_2. Interestingly, negative effects of elevated CO_2 on leaf nitrogen (N) content, photosynthetic capacity and g_s observed in an earlier stage of the Aspen FACE experiment (Noormets et al., 2001; only aspen studied) did not persist after steady-state leaf area index had been reached (Uddling et al., 2009). Mechanisms evolved to maintain water and C:N balance under changing growth conditions but comparatively stable atmospheric [CO_2] are perhaps more likely than temporal extrapolation of current stomatal CO_2 responsiveness in determining g_s of plants under rising [CO_2], considering that the current [CO_2] already exceeds values affecting plant evolution during the last 14 million years (Tripati et al., 2009).

The observed loss of stomatal responsiveness to an increase in [CO_2] from 380 to 540 μmol mol^{-1} in leaves of trees grown in elevated CO_2 (Fig. 2a) is inconsistent with earlier findings of significantly enhanced elevated CO_2 to ambient CO_2 sap flux ratio in aspen and aspen-birch stands during a fumigation gap in June 2004 (Uddling et al., 2009). Possibly, the fumigation gap effect reported earlier was caused by a $CO_2 \times$ VPD interaction on transpiration, as VPD was higher on the day of the fumigation gap than on the reference days used to estimate the effect. It should also be noted that the 90% confidence interval of the stomatal CO_2 response to 540 vs. 380 μmol mol^{-1} observed here was comparatively large in the $+CO_2$ treatment (−10% to +5%) and does not exclude the +10% effect of transient exposure of eCO_2-grown trees to ambient CO_2 (i.e., 380 vs. 540 μmol mol^{-1}) on sap flux during the fumigation gap. As the effect of eO_3 on stomatal CO_2 responsiveness probably developed gradually over the growing season, the lack of CO_2 responsiveness in the $+CO_2+O_3$ treatment observed in August 2008 (Fig. 2a) does not contradict the earlier finding of a fumigation gap effect in June 2004.

In conclusion, our results demonstrate that the stomatal responsiveness to CO_2 may be significantly reduced in leaves from trees grown under eCO_2 and/or eO_3 concentrations. Further study of stomatal impairement/acclimation under altered trace gas composition is critically needed, as findings reported here potentially have strong implications for the understanding and prediction of plant functioning and vegetation–atmosphere interactions under atmospheric change.

Acknowledgements

This research was supported by Ångpanneföreningens Forskningsstiftelse, The Royal Society of Arts and Sciences in Göteborg (KVVS) and Helge Ax:son Johnssons Stitelse. Forest water balance and carbon cycling research at Aspen FACE was supported by USDA NRI (now AFRI-NIFA) grants 2001-35107-11262 and 2004-35102-16723 to John S King. The last author was supported by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Formas) and the strategic research area Biodiversity and Ecosystem services in a Changing Climate (BECC). Technical assistance in the field by Ronald M. Teclaw is gratefully acknowledged. The Aspen FACE Experiment is funded principally by the Office of Science (BER), US Department of Energy, with additional support from the USFS Global Change Program, Michigan Technological University, the Canadian Forest Service and the USFS Northern Research Station.

References

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

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

Barnes, J.D., Brown, K.A., 1990. The influence of ozone and acid mist on the amount and wettabihty of the surface waxes in Norway spruce [*Picea abies* (L.) Karst.]. New Phytologist 114, 531–535.

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

Betts, R.A., Boucher, O., Collins, M., Cox, P.M., Falloon, P.D., Gedney, N., Hemming, D.L., Huntingford, C., Jones, C.D., Sexton, D.M.H., Webb, M.J., 2007.

Projected increase in continental runoff due to plant responses to increasing carbon dioxide. Nature 448, 1037–1042.
Curtis, P.S., Wang, X., 1998. A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. Oecologia 113, 299–313.
Dentener, F., Stevenson, D., Ellingsen, K., Van Noije, T., Schultz, M.G., Amann, M., Atherton, C., Bell, N., Bergmann, D., Bey, I., Bouwman, L., Butler, T., Cofala, J., Collins, B., Drevet, J., Doherty, R., Eickhout, B., Eskes, H., Fiore, A.M., Gauss, M., Hauglustaine, D., Horowitz, L., Isaksen, I.S., Josse, B., Lawrence, M., Krol, M., Lamarque, J.F., Montanaro, V., Müller, J.F., Peuch, V.H., Pitari, G., Pyle, J., Rast, S., Rodriguez, I., Sanderson, M., Savage, N.H., Shindell, D., Strahan, S., Szopa, S., Sudo, K., Van Dingenen, R., Wild, O., Zeng, G., 2006. The global atmospheric environment for the next generation. Environmental Science and Technology 40, 3586–3594.
Dickson, R.E., Lewin, K.F., Isebrands, J.G., Coleman, M.D., Heilman, W.E., Reimenschneider, D.E., Sober, J., Host, G.E., Zak, D.R., Hendrey, G.R., Pregitzer, K.S., Karnosky, D.F., 2000. Forest Atmosphere Carbon Transfer and Storage (FACTS-II) - The Aspen Free-air CO_2 and O_3 Enrichment (FACE) Project: An Overview. Technical Report NC-214. USDA, Washington, DC.
Domec, J.C., Palmroth, S., Ward, E., Maier, C.A., Thérézien, M., Oren, R., 2009. Acclimation of leaf hydraulic conductance and stomatal conductance of *Pinus taeda* (loblolly pine) to long-term growth in elevated CO_2 (free-air CO_2 enrichment) and N-fertilizationpce_2014. Plant, Cell and Environment 32, 1500–1512.
Grulke, N.E., Neufeld, H.S., Davison, A.W., Roberts, M., Chappelka, A.H., 2007. Stomatal behaviour of ozone-sensitive and -insensitive coneflowers (*Rudbeckia laciniata* var. *digitata*) in Great Smoky Mountains National Park. New Phytologist 173, 100–109.
Gunderson, C.A., Sholtis, J.D., Wullschleger, S.D., Tissue, D.T., Hanson, P.J., Norby, R.J., 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 and Environment 25, 379–393.
Hetherington, A.M., Woodward, F.I., 2003. The role of stomata in sensing and driving environmental change. Nature 424, 901–908.
Karlsson, P.E., Braun, S., Broadmeadow, M., Elvira, S., Emberson, L., Gimeno, B.S., Le Thiec, D., Novak, K., Oksanen, E., Schaub, M., Uddling, J., Wilkinson, M., 2007. Risk assessments for forest trees: the performance of the ozone flux versus the AOT concepts. Environmental Pollution 146, 608–616.
Karnosky, D.F., Zak, D.R., Pregitzer, K.S., Awmack, C.S., Bockheim, J.G., Dickson, R.E., Hendrey, G.R., Host, G.E., King, J.S., Kopper, B.J., Kruger, E.L., Kubiske, M.E., Lindroth, R.L., Mattson, W.J., McDonald, E.P., Noormets, A., Oksanen, E., Parsons, W.F.J., Percy, K.E., Podila, G.K., Riemenschneider, R.E., Sharma, P., Thakur, R., Sôber, A., Sôber, J., Jones, W.S., Anttonen, S., Vapaavuori, E., Mankovska, B., Heilman, W., Isebrands, J.G., 2003. Tropospheric O_3 moderates responses of temperate hardwood forests to elevated CO_2: a synthesis of molecular to ecosystem results from the Aspen FACE project. Functional Ecology 17, 289–304.
Karnosky, D.F., Pregitzer, K.S., Zak, D.R., Kubiske, M.E., Hendrey, G.R., Weinstein, D., Nosal, M., Percy, K.E., 2005. Scaling ozone responses of forest trees to the ecosystem level in a changing climate. Plant, Cell and Environment 28, 965–981.
Keel, S.G., Pepin, S., Leuzinger, S., Körner, C., 2007. Stomatal conductance in mature deciduous forest trees exposed to elevated CO_2. Trees 21, 151–159.
Kubiske, M.E., Quinn, V.S., Marquardt, P.E., Karnosky, D.F., 2007. Effects of elevated atmospheric CO_2 and/or O_3 on intra-and interspecific competitive ability of Aspen. Plant Biology 9, 342–355.
Lening, R., 1995. A critical appraisal of a combined stomatal-photosynthesis model for C_3 plants. Plant, Cell and Environment 18, 339–355.
Lodge, R.J., Dijkstra, P., Drake, B.G., Morison, J.I.L., 2001. Stomatal acclimation to increased CO_2 concentration in a Florida scrub oak species *Quercus myrtifolia* Wild. Plant, Cell and Environment 24, 77–88.
Maier, C.A., Palmroth, S., Ward, E., 2008. Short-term effects of fertilization on photosynthesis and leaf morphology of field-grown loblolly pine following long-term exposure to elevated CO_2 concentration. Tree Physiology 28, 597–606.
Maier-Maercker, U., 1999. Predisposition of trees to drought stress by ozone. Tree Physiology 19, 71–78.
Maier-Maercker, U., Koch, W., 1991. Experiments on the control capacity of stomata of *Picea abies* (L.) Karst. After fumigation with ozone and in environmentally damaged material. Plant, Cell and Environment 14, 175–184.
Mansfield, T.A., 1998. Stomata and plant water relations: does air pollution create problems? Environmental Pollution 101, 1–11.
Matyssek, R., Wieser, G., Ceulemans, R., Rennenberg, H., Pretzsch, H., Haberer, K., Löw, M., Nunn, A.J., Werner, H., Wipfler, P., Oßwald, W., Nikolova, P., Hanke, D.E., Kraigher, H., Tausz, M., Bahnweg, G., Kitao, M., Dieler, J., Sandermann, H., Herbinger, K., Grebenc, T., Blumenröther, M., Deckmyn, G., Grams, T.E.E., Heerdt, C., Leuchner, M., Fabian, P., Häberle, K.H., 2010. Enhanced ozone strongly reduces carbon sink strength of adult beech (*Fagus sylvatica*) - Resume from the free-air fumigation study at Kranzberg Forest. Environmental Pollution 158, 2527–2532.
McAinsh, M.R., Evans, N.H., Montgomery, L.T., North, K.A., 2002. Calcium signalling in stomatal responses to pollutants. New Phytologist 153, 441–447.
Medlyn, B.E., Barton, C.V.M., Broadmedow, M.S.J., Ceulemans, R., De Angelis, P., Forstreuter, M., Freeman, M., Jackson, S.B., Kellomaki, S., Laitat, E., Rey, A., Roberntz, P., Sigurdsson, B.D., Strassemeyer, J., Wang, K., Curtis, P.S., Jarvis, P.G., 2001. Stomatal conductance of forest species after long-term exposure to elevated CO_2 concentration: a synthesis. New Phytologist 149, 247–264.
Meehl, G.A., Stocker, T.F., Collins, W.D., Friedlingstein, P., Gaye, A.T., Gregory, J.M., Kitoh, A., Knutti, R., Murphy, J.M., Noda, A., Raper, S.C.B., Watterson, I.G., Weaver, A.J., Zhao, Z.C., 2007. Global climate Projections. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.), Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, UK/USA, pp. 747–846.
Mills, G., Hayes, F., Wilkinson, S., Davies, W.J., 2009. Chronic exposure to increasing background ozone impairs stomatal functioning in grassland species. Global Change Biology 15, 1522–1533.
Morales, P., Sykes, M.T., Prenticew, I.C., Smith, P., Smith, B., Bugmann, H., Zierl, B., Friedlingstein, P., Viovy, N., Sabaté, S., Sánchez, A., Pla, E., Gracia, C.A., Sitch, S., Arneth, A., Ogee, J., 2005. Comparing and evaluating process-based ecosystem model predictions of carbon and water fluxes in major European forest biomes. Global Change Biology 11, 2211–2233.
Morison, J.I.L., 1998. Stomatal response to increased CO_2 concentration. Journal of Experimental Botany 49, 443–452.
Mott, K.A., 1988. Do stomata respond to CO_2 concentrations other than intercellular? Plant Physiology 86, 200–203.
Noormets, A., Sôber, A., Pell, E.J., Dickson, R.E., Podila, K.G., Sôber, J., Isebrands, J.G., Karnosky, D.F., 2001. Stomatal and non-stomatal limitation to photosynthesis in two trembling aspen (*Populus tremuoidesl* Michx.) clones exposed to elevated CO_2 and/or O_3. Plant, Cell and Environment 24, 327–336.
Pearson, M., Mansfield, T.A., 1993. Interacting effects of ozone and water stress on the stomatal resistance of beech (*Fagus sylvatica* L.). New Phytologist 123, 351–358.
Pitman, A.J., 2003. The evolution of, and revolution in, land surface schemes designed for climate models. International Journal of Climatology 23, 479–510.
Pleijel, P., Danielsson, H., Emberson, L., Ashmore, M.R., Mills, G., 2007. Ozone risk assessment for agricultural crops in Europe: further development of stomatal and flux-response relationships for European wheat and potato. Atmospheric Environment 41, 3022–3040.
Prentice, C., Bondeau, A., Cramer, W., Harrison, S.P., Hickler, T., Lucht, W., Sitch, S., Smith, B., Sykes, M.T., 2007. Dynamic global vegetation modelling: quantifying terrestrial ecosystem responses to large-scale environmental change. In: Canadell, J.G., Pataki, D., Pitelka, L.F. (Eds.), Terrestrial Ecosystems in a Changing World. Springer, Berlin, Germany, pp. 175–192.
Reiling, K., Davison, A.W., 1995. Effects of ozone on stomatal conductance and photosynthesis in populations of *Plantago major* L. New Phytologist 129, 587–594.
Schäfer, K.V.R., Oren, R., Lai, C.T., Katul, G.G., 2002. Hydrologic balance in an intact temperate forest ecosystem under ambient and elevated atmospheric CO_2 concentration. Global Change Biology 8, 895–911.
Sellers, P.J., Bounoua, L., Collatz, G.J., Randall, D.A., Dazlich, D.A., Los, S.O., Berry, J.A., Fung, I., Tucker, C.J., Field, C.B., Jensen, T.G., 1996. Comparison of radiative and physiological effects of doubled atmospheric CO_2 on climate. Science 271, 1402–1406.
Sitch, S., Cox, P.M., Collins, W.J., Huntingford, C., 2007. Indirect radiative forcing of climate change through ozone effects on the land-carbon sink. Nature 448, 791–795.
Tripati, A.K., Roberts, C.D., Eagle, R.A., 2009. Coupling of CO_2 and ice sheet stability over major climate transitions of the last 20 million years. Science 326, 1394–1397.
Uddling, J., Hogg, A.J., Teclaw, R.M., Caroll, M.A., Ellsworth, D.S., 2010. Stomatal uptake of O_3 in aspen and aspen-birch forests under free-air CO_2 and O_3 enrichment. Environmental Pollution 158, 2023–2031.
Uddling, J., Günthardt-Goerg, M.S., Matyssek, R., Oksanen, E., Pleijel, H., Selldén, G., Karlsson, P.E., 2004. Biomass reduction of juvenile birch is more strongly related to stomatal uptake of ozone than to indices based on external exposure. Atmospheric Environment 38, 4709–4719.
Uddling, J., Teclaw, R.M., Kubiske, M.E., Pregitzer, K.S., 2008. Sap flux in pure aspen and mixed aspen-birch forests exposed to elevated concentrations of carbon dioxide and ozone. Tree Physiology 28, 1231–1243.
Uddling, J., Teclaw, R.M., Pregitzer, K.S., Ellsworth, D.S., 2009. Leaf and canopy conductance in aspen and aspen-birch forests under free-air enrichment of carbon dioxide and ozone. Tree Physiology 29, 1367–1380.
Underwood, A.J., 1997. Experiments in Ecology. Cambridge University Press, UK. 504.
Wittig, V.E., Ainsworth, E.A., Long, S.P., 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 3 decades of experiments. Plant, Cell and Environment 30, 1150–1162.