

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

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ABSTRACT

The surface concentration of ozone ($[O_3]$) has risen from less than 10 ppb prior to the industrial revolution to a daytime mean concentration of approximately 40 ppb over much of the northern temperate zone. If current global emission trends continue, surface $[O_3]$ is projected to rise a further 50% over this century, with larger increases in many locations including Northern Hemisphere forests. This review uses statistical meta-analysis to determine mean effects, and their confidence limits, of both the current and projected elevations of $[O_3]$ on light-saturated photosynthetic CO_2 uptake (A_{sat}) and stomatal conductance (g_s) in trees. In total, 348 measurements of A_{sat} from 61 studies and 266 measures of g_s from 55 studies were reviewed. Results suggested that the elevation of $[O_3]$ that has occurred since the industrial revolution is depressing A_{sat} and g_s by 11% (CI 9–13%) and 13% (CI 11–15%), respectively, where CI is the 95% confidence interval. In contrast to angiosperms, gymnosperms were not significantly affected. Both drought and elevated $[CO_2]$ significantly decreased the effect of ambient $[O_3]$. Younger trees (<4 years) were affected less than older trees. Elevation of $[O_3]$ above current levels caused progressively larger losses of A_{sat} and g_s , including gymnosperms. Results are consistent with the expectation that damage to photosynthesis depends on the cumulative uptake of ozone (O_3) into the leaf. Thus, factors that lower g_s lessen damage. Where both g_s and $[O_3]$ were recorded, an overall decline in A_{sat} of 0.21% per $mmol\ m^{-2}$ of estimated cumulative O_3 uptake was calculated. These findings suggest that rising $[O_3]$, an often overlooked aspect of global atmospheric change, is progressively depressing the ability of temperate and boreal forests to assimilate carbon and transfer water vapour to the atmosphere, with significant potential effects on terrestrial carbon sinks and regional hydrologies.

Key-words: air pollution; atmospheric change; cumulative ozone uptake; forests; global change; stomata.

INTRODUCTION

Although altered precipitation, temperature and CO_2 concentrations ($[CO_2]$) have received the most attention with respect to the impacts of global change on terrestrial ecosystems (Geider, Delucia & Falkowski 2001; Prentice *et al.* 2001; Denman *et al.* 2007), tropospheric ozone (O_3) is also rising (Ehhalt *et al.* 2001; Forster *et al.* 2007; Meehl *et al.* 2007) and is now considered to be the most important air pollutant affecting vegetation in both rural and urban areas (Ashmore 2005; EPA 2006; Karnosky *et al.* 2007; Matyssek *et al.* 2007; Paoletti *et al.* 2007). O_3 is a secondary pollutant formed from the action of sunlight on nitrogen oxides (NO_x), produced mainly from automobiles and biomass burning, in the presence of volatile organic compounds (VOCs) of both natural and industrial origin (Fowler *et al.* 1999b; Denman *et al.* 2007; Forster *et al.* 2007). Limited measurements from the nineteenth century suggest that pre-industrial ground-level O_3 concentration ($[O_3]$) was less than 10 ppb (Volz & Kley 1988). Over the last century, surface ambient background $[O_3]$ over forested land in the Northern Hemisphere has increased to levels that are damaging to vegetation (Chappelka & Samuelson 1998; Skarby *et al.* 1998; Fowler *et al.* 1999a; Akimoto 2003; EPA 2006; Forster *et al.* 2007; Karnosky *et al.* 2007; Matyssek *et al.* 2007). Although $[O_3]$ varies across these regions depending on proximity to sources of pollutants and time of day and year, 40 ppb is representative of the mean day-time ambient background $[O_3]$ during spring and summer months (Fowler *et al.* 1999a; Appendix S1). Furthermore, projections based on the A2 storyline of the Special Report on Emissions Scenarios (SRES) and included in the Intergovernmental Panel on Climate Change (IPCC) Assessment Report Four (AR4) indicate that $[O_3]$ could rise 20–25% between 2015 and 2050, and further increase by 40–60% by 2100 if current emission trends continue (Meehl *et al.* 2007). The largest increases are projected for the Northern

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Hemisphere (Karnosky *et al.* 2005) because of both increasing precursor concentrations and climatic conditions more favourable to O₃ formation (Meehl *et al.* 2007).

O₃ has been suspected of causing visible foliar injury and reduced growth in vegetation since the 1950s (Middleton 1956; Darley & Middleton 1966). Mounting evidence compiled over the past several decades in the peer-reviewed literature and government criteria documents has confirmed that O₃ is the major pollutant responsible for visible foliar injury and reduced growth in trees (Reich 1987; Broadmeadow 1998; Chappelka & Samuelson 1998; Skarby *et al.* 1998; Fowler *et al.* 1999a; EPA 2006; Forster *et al.* 2007; Karnosky *et al.* 2007; Matyssek *et al.* 2007). For example, assuming that hourly O₃ above 60 ppb is most damaging to trees, Fowler *et al.* (1999a) projected that nearly a quarter of the earth's forests are currently at risk of damage and reduced productivity, and by 2100 this will expand to half of the world's forests. Although the molecular mechanisms leading to O₃ damage have not been fully elucidated (Kangasjarvi, Jaspers & Kollist 2005), physiological studies suggest that chronic elevation of [O₃] decreases productivity primarily by lowering photosynthesis (Heath 1994; Farage & Long 1995; Dizengremel 2001; Long & Naidu 2002). Photosynthesis or gross primary productivity (GPP) is the driving step of the global carbon cycle, with more than 50% of total terrestrial GPP and net primary productivity (NPP) (i.e. GPP–autotrophic respiration) accounted for by forests (Geider *et al.* 2001; Grace 2004). Rising [O₃] is therefore likely to decrease the capacity of the terrestrial biosphere to take up CO₂ and offset rising global [CO₂]. Recent reviews have documented responses of trees to O₃ on a case-by-case basis (EPA 2006; Karnosky *et al.* 2007; Matyssek *et al.* 2007), but without a comprehensive quantitative summary of known observations, it is difficult to quantify the current overall trend and future change in photosynthetic productivity.

O₃ is a strong oxidant, and significant damage to photosynthesis occurs when O₃ enters the leaf through the stomata. This leads to a progressive loss of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity (Farage *et al.* 1991; Farage & Long 1995; Pell, Schlaghauer & Arteca 1997; Dizengremel 2001), and is evident in a decline in light-saturated rate of leaf CO₂ uptake (A_{sat}) (Farage *et al.* 1991; Farage & Long 1995; Farage 1996). Reich (1987) proposed a 'unifying theory' of the impacts of O₃ on conifers, broadleaved trees and crops, and showed that the amount of damage to photosynthesis was linearly related to the rate of O₃ flux (FO_3) through the leaf stomata, rather than O₃ dose, i.e. the product of [O₃] and hours of exposure. This 'unifying theory' marked the shift towards estimating cumulative O₃ uptake (CU) rather than dose as a means to project O₃ impact (Ollinger, Aber & Reich 1997; Felzer *et al.* 2004). This approach was recently promoted for the level II assessment of O₃ impacts (Matyssek *et al.* 2007) and was shown to be superior to accumulated dose above a threshold of 40 ppb (Karlsson *et al.* 2007). The most recent reviews also suggest the approach would be further improved by including species-specific detoxification and

repair processes. However, species-specific information regarding these processes is limited at present, and thus unavailable for incorporation into a comprehensive quantitative analysis of O₃ impacts observed in the literature (Matyssek *et al.* 2007; Wieser & Matyssek 2007).

Although O₃ may directly decrease stomatal conductance (g_s) (Reich & Lassoie 1984), other analyses suggest that decreased g_s follows a decline in photosynthetic carboxylation capacity and that decreased g_s is likely a symptom rather than a cause of decline in A_{sat} (Farage *et al.* 1991; Farage & Long 1995; Martin *et al.* 2001). Any environmental stress that reduces g_s and therefore O₃ uptake, such as drought, elevated [CO₂] or nutrient deficiency, might be expected to lessen damage caused by O₃ (Long & Naidu 2002). If O₃ causes a large-scale decrease in g_s in forests, there are major implications for regional hydrology, surface temperatures and the global climate system (Sellers *et al.* 1996). Conversely, any environmental condition that causes a decoupling of A_{sat} to g_s , such as the heterogeneous light environment low in a forest canopy, which results in a higher conductance than is needed to support CO₂ uptake, is likely to exacerbate damage by O₃ uptake (Fredericksen *et al.* 1996). However, the response of trees is also likely to vary with age (Samuelson & Kelly 2001; Nunn *et al.* 2006; Karnosky *et al.* 2007) and capacity of the tree to detoxify O₃ (Matyssek *et al.* 2007). This complicates scaling to forests comprised of trees of various age classes (Samuelson & Kelly 2001; Karnosky *et al.* 2005), because scaling factors used in modelling analyses are based largely on juvenile trees in chambers (Reich 1987; Ollinger *et al.* 1997; Felzer *et al.* 2004). It has been suggested that conifers are less sensitive to O₃ compared to broadleaved trees, possibly because of their lower average conductance (Reich 1987; Chappelka & Samuelson 1998; Samuelson & Kelly 2001; Nunn *et al.* 2006). This is consistent with the view that O₃ damage is directly proportional to CU, which in any environment will be less for plants with lower g_s . Martin *et al.* (2001) showed that a decline in the rate of damage to A_{sat} with time in a leaf exposed to a constant elevation of [O₃] may be explained by a decline in g_s .

With the exception of free-air concentration enrichment (FACE) (Oksanen 2003; Karnosky *et al.* 2005; Low *et al.* 2006), all published experiments with trees grown at elevated [O₃] involve chambers of various designs. By nature of their design, chambers alter the soil–plant–atmosphere continuum and place restrictions on tree growth, which might modify the observed response to O₃ fumigation. Experiments also differ in many other respects including tree age, taxa, soils and fumigation techniques such as exposure duration and treatment [O₃] (Reich 1987; Chappelka & Samuelson 1998; Skarby *et al.* 1998; Matyssek & Innes 1999; Ashmore 2005; Karnosky *et al.* 2007). This variability complicates any assessment of the overall effect of rising [O₃] on tree A_{sat} and g_s .

From the wealth of individual studies, can we statistically determine the mean responses of A_{sat} and g_s to both current and projected elevations of [O₃]? A meta-analysis allows estimation of a mean relative response and its confidence

limits from disparate experiments investigating the effect of the same treatment, but administered in different ways and with different experimental designs. The technique has been widely used in ecology to analyse suites of observations from many varied independent sources (Hedges, Gurevitch & Curtis 1999; Rosenberg, Adams & Gurevitch 2000). Recent application of meta-analytic techniques to ecological and environmental studies has determined the magnitude and significance of the effect of elevated $[\text{CO}_2]$ on soybeans (Ainsworth *et al.* 2002) and trees (Curtis & Wang 1998), and the effect of elevated $[\text{O}_3]$ on soybean (Morgan, Ainsworth & Long 2003). The method is therefore suitable for a post-hoc analysis of the O_3 effects literature.

For this review, we compiled peer-reviewed studies published since the late 1960s, and from observations found in this literature we conducted a meta-analysis to determine the direction, magnitude and significance of O_3 impacts on A_{sat} and g_s of trees. This review includes observations from 73 primary research articles reporting over 1900 observations of A_{sat} and g_s of trees exposed to ambient and elevated $[\text{O}_3]$ relative to control conditions. This quantitative meta-analytic review of the literature addresses the following questions: (1) what is the impact of observed ambient background $[\text{O}_3]$ on A_{sat} and g_s relative to pre-industrial $[\text{O}_3]$; (2) how will future elevated $[\text{O}_3]$ affect A_{sat} and g_s relative to pre-industrial $[\text{O}_3]$; (3) what are the expected changes in A_{sat} and g_s with future $[\text{O}_3]$ relative to observed ambient background $[\text{O}_3]$; (4) in all cases, how do other factors modify this response; and (5) is there a linear decline in A_{sat} with CU when all studies of trees are pooled together? Quantitative answers to these questions should provide the best currently available estimates of how rising $[\text{O}_3]$ will affect A_{sat} and g_s , two key parameters in global and regional carbon cycle and hydrological models.

MATERIALS AND METHODS

Database

A database of the effects of O_3 on A_{sat} and g_s was compiled by surveying the peer-reviewed literature with the Web of Science (Thompson-ISI, Philadelphia, PA, USA) and Silver-Platter (Ovid Technologies, New York, NY, USA). Keyword searches were made for as far back as the search engines would allow, covering the period 1970 through October 2006. To avoid missing relevant references because of inadequate key-words or those published prior to 1970, reference lists of articles identified from the keyword searches were checked against the database to identify missing references. In total, 133 articles that reported $[\text{O}_3]$ effects on A_{sat} and 110 articles that reported g_s were found. Within these articles, individual measurements were considered independent if they were made on different species or distinct genotypes within a species, or if the measurements were made on different dates. Articles and measurements were excluded if: (1) the standard deviation (SD) could not be determined or there was no replication; (2) an exact measure of either A_{sat} or g_s , with their units, could not be

extracted; (3) the description of experimental design was insufficient to allow objective assignment to the categories of Table 1; (4) the data were previously or more completely reported in another article; and (5) the leaf exposure period was less than 7 d and therefore not representative of chronic exposure. After evaluating articles based on these exclusion criteria, 65 articles measuring A_{sat} and 51 articles measuring g_s were used for the meta-analysis, most exclusions having resulted from the first criterion (Appendix S1).

For each observation of A_{sat} or g_s , the value in the control and elevated $[\text{O}_3]$ treatment (X_C and X_E), the SDs (SD_C and SD_E) and replication (N_C and N_E) were entered into the database together with categorical information, including $[\text{O}_3]$ concentration and duration (Table 1). Values of A_{sat} and g_s were extracted from tables, text and/or figures of each primary article, and then compiled into spreadsheets specific to both A_{sat} and g_s . Values given only in the figures of publications were digitized using data extraction software (GRAFULA 3 v.2.10, Wesik SoftHaus, St. Petersburg, Russia). If measurements of A or g_s were made over the diurnal course, only values for light-saturating conditions were recorded in the database. Three databases were compiled: (1) trees grown in charcoal-filtered (CF) control were compared to trees grown in ambient background $[\text{O}_3]$; (2) trees grown in CF control were compared to trees grown in elevated $[\text{O}_3]$ treatments; and (3) trees grown in ambient background $[\text{O}_3]$ were compared to trees grown in elevated $[\text{O}_3]$ treatments.

Sources of variation

Eight categories were identified as important potential sources of variation that could alter the response of A_{sat} and/or g_s to $[\text{O}_3]$ (Table 1). Each observation of A_{sat} and g_s was objectively coded into the appropriate levels of each category shown in Table 1: (1) angiosperm versus gymnosperm; (2) tree age; (3) leaf age; (4) rooting environment; (5) fumigation method (e.g. FACE versus open-top chamber); (6) duration of leaf fumigation; (7) mean $[\text{O}_3]$; and (8) additional treatments (e.g. elevated $[\text{CO}_2]$ or drought) (Table 1). The mean $[\text{O}_3]$ in the control and in the treatment is defined as the average concentration for the leaf exposure period which varied from 4 to 24 h d⁻¹. Duration of leaf fumigation varied from 7 d to more than a year. In addition to these categories, the different tree genera in each database were examined.

Meta-analyses

To calculate the effect of O_3 on trees as a proportionate change in A_{sat} or g_s , the natural log of the response ratio, r , was used, where r is the ratio of the mean in the experimental treatment (X_E) divided by the mean in the control (X_C). Based on the assumption of random variation in effect sizes between studies, we used a weighted mixed-model analysis where each individual response was weighted by the reciprocal of the mixed-model variance (Gurevitch & Hedges 1999; Hedges *et al.* 1999). In the first analysis, X_C was the

Table 1. Categories and levels describing the experimental conditions in studies of ozone (O_3) effects on light-saturated photosynthesis (A_{sat}) and stomatal conductance (g_s) of trees

Category	Categorical level	
Tree classification	Angiosperm	Gymnosperm
Tree age (years)	<4	>4
Leaf age (years)	0 > 1	1–2
Rooting environment	Pot grown	Rooted in the ground
Method	Growth chamber	Greenhouse
Experiment duration (d)	7–29	30–59
Mean $[O_3]$ (ppb)	0–29	30–59
Additional treatment	No additional treatment	Elevated $[CO_2]$
		≥ 2
	Open-top chamber	Free-air enrichment
	60–89	120–149
	60–89	≥ 120
	Drought	Lower canopy
	Branch chamber	
	90–119	150–365
	90–119	≥ 365
	Low nutrient	≥ 730
		≥ 1095

measure in CF air, and X_E was ambient background $[O_3]$. In the second analysis, X_C was the measure in CF air, and X_E was the measure from elevated $[O_3]$ treatments. In the third analysis, X_C was the measure in ambient background $[O_3]$, and X_E was the measure in elevated $[O_3]$ treatments.

Effect sizes are reported as the unlogged r converted to the mean percentage change from the control $[(r - 1) \times 100]$ as in previous analyses (Curtis & Wang 1998; Ainsworth *et al.* 2002; Morgan *et al.* 2003; Ainsworth & Long 2005). Trees unaffected by $[O_3]$ have an $r = 1$, and therefore a 0% change from control. A negative percentage change indicates a decrease in A_{sat} or g_s in response to $[O_3]$, while positive values indicate an increase. If the 95% confidence interval (CI) did not overlap zero, response to O_3 treatment is considered significant (Curtis & Wang 1998). A meta-analytic software package was used to calculate all effect sizes and their 95% CIs (MetaWin 2.1.3.4, Sinauer Associates, Sunderland, MA, USA) (Rosenberg *et al.* 2000).

The eight categories described earlier were analysed to test differences in the response of different genera of trees grown under different experimental and environmental conditions. The analysis proceeded by partitioning the variance in two steps following the methods previously described by Curtis & Wang (1998). Firstly, between-group heterogeneity (Q_B) for each category was examined, then data were subdivided according to levels of those categorical variables with significant Q_B . If 95% CIs did not overlap, means were considered to be significantly different from one another (Curtis & Wang 1998; Gurevitch & Hedges 1999). Levels of each category were included in the analysis if there were at least 10 observations. If less than 10 observations were available, results were only discussed if they originated from at least three independent articles.

Cumulative O_3 uptake analysis

In addition to the mean $[O_3]$ (ppb) calculated over the leaf exposure period, the duration of leaf fumigation reported in days (d) and the number of hours (h) that fumigation was applied each day were recorded. Assuming that $[O_3]$ inside the leaf is zero (Laisk, Kull & Moldau 1989), then FO_3 into the leaf was calculated as:

$$FO_3 = [O_3] \cdot g_z \quad (1)$$

where g_z ($mmol\ m^{-2}\ s^{-1}$) is g_s to O_3 calculated from g_s ($mmol\ m^{-2}\ s^{-1}$) divided by 1.67, the ratio of the diffusion constants for water vapour and O_3 (Laisk *et al.* 1989). Finally, CU ($mmol\ m^{-2}$) was calculated by summing FO_3 over the time interval between consecutive observations of g_s :

$$CU = \sum_d \sum_h FO_3 \cdot 3600 \quad (2)$$

following Reich (1987) and Nunn *et al.* (2006). A_{sat} expressed as the percent reduction in leaves in elevated $[O_3]$ treatments relative to those in CF air was linearly regressed against CU. Studies were excluded from this

analysis based on three criteria: (1) rates of A_{sat} were not reported in conjunction with g_s ; (2) there was insufficient information to convert the reported units of g_s to mmol m^{-2} or to convert units of A_{sat} into $\mu\text{mol m}^{-2} \text{s}^{-1}$; and (3) cumulative uptake was less than 10 mmol m^{-2} ; these studies were excluded because either conductance was low and thus difficult to measure with any accuracy, or the exposure period was brief. Thirty-eight independent studies investigating 28 different species provided a sample size of 292 for the regression analysis.

RESULTS

CF air versus ambient background O_3

Across all studies, the ambient background $[\text{O}_3]$ caused an 11% decrease in A_{sat} ($n = 348$) and a 13% decrease in g_s ($n = 266$) compared to leaves in CF air. The average ambient $[\text{O}_3]$ in the experiments in which A_{sat} was measured was 47 ppb, with a range of 26–100 ppb, and was slightly higher than the average across the studies reporting effects on g_s , 42 ppb which ranged between 26 and 69 ppb (Fig. 1). This average however hides a marked difference between gymnosperms which showed no response in A_{sat} or g_s when exposed at ambient background $[\text{O}_3]$ ($Q_B = 26.97$, $P < 0.001$ for A_{sat} ; $Q_B = 29.86$, $P < 0.001$ for g_s) (Fig. 1) versus angiosperms which showed a highly significant decrease of 14 and 16%, respectively (Fig. 1). These were also significant differences between genera. A_{sat} and g_s were significantly reduced in *Fraxinus*, *Populus*, *Prunus* and *Viburnum* species grown in ambient background O_3 relative to CF controls, while there was no change in either parameter in *Picea*, *Pinus* or *Quercus* species (Table 2). The A_{sat} of *Fagus*



Figure 1. The percent change in light-saturated photosynthesis (A_{sat}) and stomatal conductance (g_s) for trees grown in ambient background ozone (O_3) relative to charcoal-filtered (CF) air including the difference between angiosperms and gymnosperms. Degrees of freedom (d.f.) and average ambient background $[\text{O}_3]$ are given on the y-axis.

species was not affected by background O_3 , while g_s was reduced by 16% on average (Table 2). One limitation in the comparison of genera is that $[\text{O}_3]$ differed between studies of different genera, and may confound the comparisons (Table 2).

Angiosperms growing under different growth environments or stress conditions showed different responses to ambient background $[\text{O}_3]$ (Table 3; Fig. 2). A_{sat} and g_s were not reduced significantly in drought-stressed trees exposed to ambient O_3 . No significant interaction of elevated $[\text{CO}_2]$ and $[\text{O}_3]$ or of nutrient deficiency with $[\text{O}_3]$ could be detected for trees grown in ambient $[\text{O}_3]$ relative to CF air. The reduction in both A_{sat} and g_s caused by current ambient elevation of $[\text{O}_3]$ was about 30% greater in lower canopy versus upper canopy leaves (Fig. 2). However, all of the measurements of lower canopy leaves came from a single study on three species (Novak *et al.* 2005), so results should be interpreted cautiously. When studies which imposed no additional treatments were separated out, then A_{sat} ($n = 194$) and g_s ($n = 136$) were reduced by 9 and 12%, respectively, by the current ambient $[\text{O}_3]$ (Fig. 2). Tree age significantly altered the mean response of A_{sat} and g_s to ambient background $[\text{O}_3]$ (Table 3; Appendix S2). In young trees age < 4 years, A_{sat} was reduced by 10% while the reduction in A_{sat} was more than double (22%) for trees > 4 years (Appendix S1). Ambient $[\text{O}_3]$ reduced g_s in trees < 4 years by 5%, while g_s of trees > 4 years was reduced by 23% compared to CF controls (Appendix S2).

Neither duration nor method of exposure significantly altered the mean response of A_{sat} or g_s of angiosperms to ambient background $[\text{O}_3]$ (Table 3). Ambient $[\text{O}_3]$ reduced g_s in trees grown in pots, while there was no effect on g_s in trees grown in the ground (Appendix S2).

CF air versus elevated O_3 treatments

Examination of all studies which compared trees grown in CF air with those grown in elevated $[\text{O}_3]$ treatments showed an average decrease in A_{sat} of 19% ($n = 460$) at a mean $[\text{O}_3]$ of 85 ppb and reduction in g_s of 10% ($n = 277$) (Fig. 3) at a mean $[\text{O}_3]$ of 91 ppb. The reduction in A_{sat} was progressively greater as the treatment $[\text{O}_3]$ increased, with a similar trend in g_s (Table 4; Fig. 3), except for treatments above 120 ppb where the small sample size and restriction to a few genera limit interpretation.

When comparing A_{sat} in trees grown in CF air versus elevated O_3 treatments, gymnosperms did not significantly differ from angiosperms; however, genera showed different responses for both parameters that were not explained by differences in the mean treatment $[\text{O}_3]$ (Table 5). Elevated O_3 treatments significantly reduced both A_{sat} and g_s for *Fagus*, *Pinus* and *Populus*, while only A_{sat} was reduced for *Prunus* species (Table 5). There was no response of A_{sat} or g_s to elevated O_3 treatments for *Picea* or *Liriodendron* species relative to CF controls (Table 5), and no response of g_s for *Abies* species. Further analysis revealed that the g_s studies with $[\text{O}_3]$ greater than 120 ppb were made with *Abies*,

Table 2. Percent change (% change), 95% confidence intervals (95% CI), degrees of freedom (d.f.) and mean ozone concentrations ($[O_3]$) for the estimate of the mean response of light-saturated photosynthesis (A_{sat}) and stomatal conductance (g_s) across all studies (cumulative effect size), and for angiosperms, gymnosperms and different genera grown in charcoal-filtered (CF) air versus ambient background ozone (O_3)

Genus	A_{sat}				g_s			
	% Change	95% CI	d.f.	$[O_3]$	% Change	95% CI	d.f.	$[O_3]$
Cumulative effect size	-11	-13 to -9	347	47	-13	-15 to -11	265	42
Gymnosperms	-2	-6 to 3	83	45	2	-4 to 9	57	44
<i>Picea</i>	4	-4 to 12	23	33	12	2-24	23	39
<i>Pinus</i>	-3	-7 to 2	58	49	-6	-14 to 3	33	48
Angiosperms	-14	-16 to -12	263	48	-16	-18 to -13	207	42
<i>Fagus</i>	-5	-11 to 2	14	33	-16	-27 to -3	8	36
<i>Fraxinus</i>	-17	-22 to -12	38	42	-9	-16 to -2	35	42
<i>Populus</i>	-24	-28 to -21	60	57	-27	-31 to -22	36	41
<i>Prunus</i>	-5	-9 to -1	59	54	-12	-19 to -4	19	44
<i>Quercus</i>	0	-8 to 9	22	42	-1	-5 to 6	36	43
<i>Viburnum</i>	-20	-23 to -15	36	41	-27	-32 to -22	36	41

Betula, *Picea* and *Pinus* species, which do not show a general decrease in g_s with elevated $[O_3]$.

Additional treatments affected the mean response of both A_{sat} and g_s (Table 4; Fig. 4). Both drought and elevated $[CO_2]$ ameliorated the effect of elevated $[O_3]$ on A_{sat} and g_s , while nutrient deficiency and lower canopy did not significantly affect the response (Fig. 4). Excluding studies with additional treatments, elevated $[O_3]$ reduced A_{sat} by 20% ($n = 374$) and g_s by 12% ($n = 230$). This was more than double the reduction of A_{sat} by ambient air relative to CF controls, while the reduction in g_s was of similar magnitude in both analyses in the absence of additional stress or changes to growth environment (Figs 2 & 4). Neither fumigation method nor leaf age significantly altered the mean response of either A_{sat} or g_s (Table 4). The mean response of A_{sat} to elevated $[O_3]$ was affected by tree age, while the mean response of g_s to elevated $[O_3]$ was affected by duration of exposure and rooting environment (Table 4; Appendix S3). The response of g_s to elevated $[O_3]$ treatments relative to CF air was greater for trees grown in the ground compared with pot-grown trees, but this might be explained

Table 3. Between-group heterogeneity (Q_B) for ozone (O_3) effect size, comparing angiosperms grown in charcoal-filtered (CF) air versus ambient background ozone concentration ($[O_3]$)

Category	A_{sat}		g_s	
	Q_B	P	Q_B	P
Genus	136.91	<0.001	112.93	<0.001
Tree age	16.75	<0.001	60.67	<0.001
Method	0.32	0.627	0.47	0.531
Rooting environment	0.22	0.901	19.01	0.001
Duration	11.43	0.167	6.47	0.385
Additional treatment	66.72	<0.001	41.04	<0.001

A_{sat} , light-saturated photosynthesis; g_s , stomatal conductance.

by the higher mean $[O_3]$ associated with experiments in which trees were grown in the ground, rather than in pots (Appendix S3).

Ambient background O_3 versus elevated O_3 treatments

In a third analysis, we compared trees grown at ambient background $[O_3]$, which averaged to 44 ppb for A_{sat} and 36 ppb for g_s , with trees grown under elevated O_3 treatments which averaged 81 ppb for A_{sat} and 71 ppb for g_s . Across all studies, elevated O_3 treatments decreased A_{sat} by 18% ($n = 349$) and decreased g_s by 6% ($n = 253$) (Fig. 5). There was a progressive decrease in A_{sat} and g_s with an increase in treatment $[O_3]$, but again no effect on g_s at

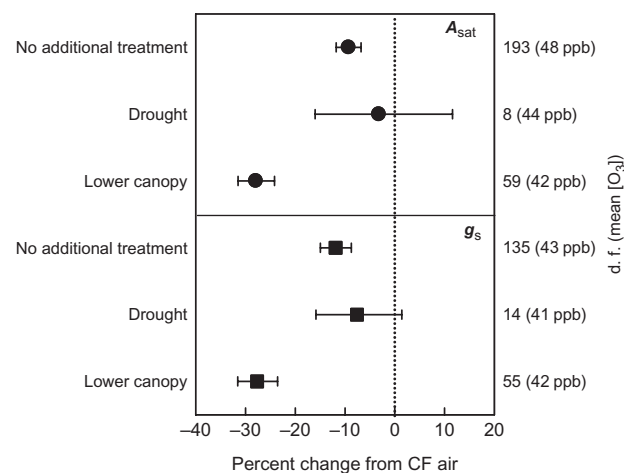


Figure 2. The effect of additional treatments on the response of light-saturated photosynthesis (A_{sat}) and stomatal conductance (g_s) of angiosperms to ambient background ozone (O_3) relative to charcoal-filtered (CF) controls. Degrees of freedom (d.f.) and average ambient background $[O_3]$ are given on the y-axis.

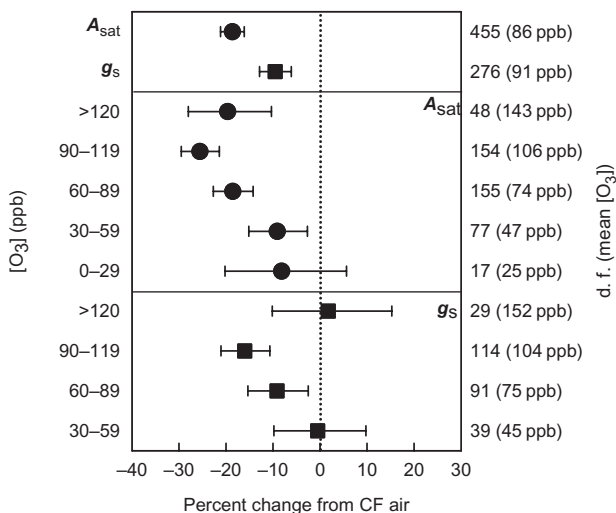


Figure 3. The percent change in light-saturated photosynthesis (A_{sat}) and stomatal conductance (g_s) for trees grown in elevated ozone (O_3) treatments relative to charcoal-filtered (CF) air, and the impact of different elevated ozone concentrations ($[O_3]$) on the response. Degrees of freedom (d.f.) and average treatment $[O_3]$ are given on the y-axis.

$[O_3] > 120$ ppb (Table 6; Fig. 5). In this comparison, neither gymnosperm versus angiosperm nor leaf age significantly altered the magnitude of the decrease because of elevation of $[O_3]$ (Table 6). Duration of exposure altered the mean response, but the trends were not easily explained (Appendix S4). The A_{sat} of trees <4 years old was reduced by 21%, which was significantly different from trees >4 years old for which A_{sat} was reduced by 10% (Appendix S4). A_{sat} was reduced more for trees grown in the ground compared to pot-grown trees, despite lower average $[O_3]$, while g_s was unaffected by elevated $[O_3]$ relative to ambient $[O_3]$ (Appendix S4). Neither branch chamber nor FACE significantly affected the response of g_s to elevated

Table 4. Between-group heterogeneity (Q_B) for ozone (O_3) effect size across descriptive categories, comparing trees grown in charcoal-filtered (CF) air versus elevated O_3 treatments

Category	A_{sat}		g_s	
	Q_B	P	Q_B	P
Angiosperms versus gymnosperms	0.88	0.387	2.52	0.157
Genus	26.77	0.041	38.53	0.006
Tree age	11.93	0.002	0.0001	0.992
Leaf age	2.30	0.378	6.71	0.073
Rooting environment	1.04	0.358	8.62	0.014
Method	1.66	0.695	2.01	0.457
Duration	18.75	0.056	21.35	0.027
$[O_3]$	24.17	0.001	13.21	0.018
Additional treatment	26.40	0.002	17.07	0.012

A_{sat} , light-saturated photosynthesis; g_s , stomatal conductance; $[O_3]$, O_3 concentration.

$[O_3]$ relative to ambient background controls; however, g_s was significantly reduced by elevated O_3 treatments in open-top chambers (Appendix S4). There was no significant effect of additional treatment (Table 6). *Acer*, *Betula*, *Fagus*, *Picea*, *Pinus*, *Populus* and *Prunus* species showed significant decreases in A_{sat} at elevated $[O_3]$ but not *Quercus*, while only *Pinus* and *Quercus* species showed a decrease in g_s (Table 7).

Cumulative O_3 uptake analysis of CF versus elevated O_3 treatments

When observations of A_{sat} for trees grown in elevated $[O_3]$ relative to CF air were combined, there was a significant negative correlation between percent change in A_{sat} from CF air and CU (Fig. 6) ($r^2 = 0.25$; $P < 0.01$). Linear regression analysis showed a significant and linear loss of

Table 5. Percent change (% change), 95% confidence intervals (95% CI), degrees of freedom (d.f.) and mean ozone concentrations ($[O_3]$) for the estimate of the mean response of light-saturated photosynthesis (A_{sat}) and stomatal conductance (g_s) across all studies (cumulative effect size), and for angiosperms, gymnosperms and different genera grown in charcoal-filtered (CF) air versus elevated ozone (O_3) treatments

Genus	A_{sat}			$[O_3]$	g_s			$[O_3]$
	% Change	95% CI	d.f.		% Change	95% CI	d.f.	
Cumulative effect size	-19	-21 to -16	455	86	-10	-13 to -6	276	91
Gymnosperms	-17	-21 to -12	160	92	-6	-12 to 0	109	96
<i>Abies</i>					2	-19 to 28	9	110
<i>Picea</i>	-8	-17 to 2	54	76	5	-4 to 16	45	90
<i>Pinus</i>	-21	-27 to -15	93	97	-17	-24 to -9	53	100
Angiosperms	-20	-23 to -16	294	82	-12	-16 to -7	166	88
<i>Betula</i>	-15	-23 to -8	48	86	1	-8 to 10	38	98
<i>Fagus</i>	-21	-32 to -8	17	49	-23	-35 to -8	11	57
<i>Liriodendron</i>	-10	-29 to 15	12	58	-10	-35 to 24	10	60
<i>Populus</i>	-21	-26 to -16	120	79	-19	-28 to -9	40	104
<i>Prunus</i>	-28	-34 to -21	52	106	-100			
<i>Quercus</i>	-3	-17 to 14	22	77	-15	-24 to -6	36	80

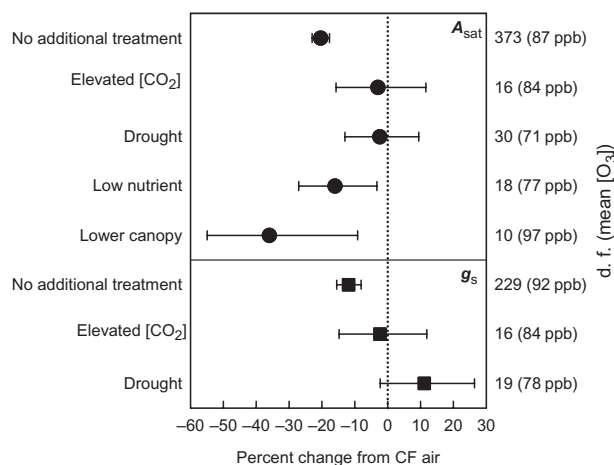


Figure 4. The effect of additional treatments on the response of light-saturated photosynthesis (A_{sat}) and stomatal conductance (g_s) to elevated ozone (O_3) treatments relative to charcoal-filtered (CF) controls. Degrees of freedom (d.f.) and average treatment [O_3] are given on the y-axis.

photosynthetic capacity, as measured by A_{sat} , of 22% for every 100 mmol m⁻² of CU per unit of leaf area (Fig. 6).

DISCUSSION

Are current background levels of O_3 affecting photosynthesis and g_s in trees?

This meta-analytic review of the peer-reviewed literature suggests that the elevation of surface level [O_3] that has occurred since the industrial revolution is depressing leaf

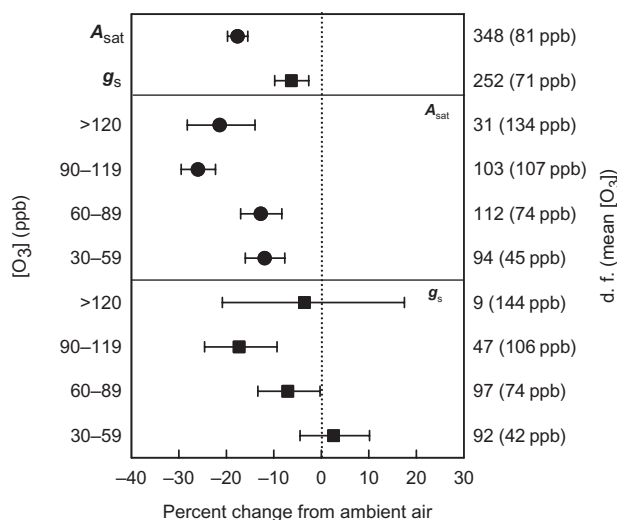


Figure 5. The percent change in light-saturated photosynthesis (A_{sat}) and stomatal conductance (g_s) for trees grown in elevated ozone (O_3) treatments relative to ambient background O_3 , and the impact of different elevated O_3 treatments on the response of both parameters. Degrees of freedom (d.f.) and average treatment [O_3] are given on the y-axis.

photosynthetic CO_2 uptake in trees by 11%, with a 95% CI of 9–13% (Table 2). This is based on 348 measurements, almost exclusively from studies representing trees from the temperate and boreal forest biomes of the Northern Hemisphere, the region of the globe that has seen the largest increase in surface level [O_3] (Ehhalt *et al.* 2001). Temperate and boreal forests account for approximately 17% of terrestrial NPP and 29% of the estimated total carbon sink of terrestrial ecosystems (0.82 Gt C year⁻¹) (Grace (2004). Assuming that photosynthesis or GPP is proportional to NPP, our findings suggest that these regions could be an even larger current carbon sink in the absence of O_3 effects.

This review also suggests that the rise in [O_3] since the industrial revolution has also caused a 13% decrease in average g_s with a 95% CI of 11–15% (Table 2; Fig. 1). Because the response of A_{sat} to intercellular CO_2 concentration (c_i) is non-linear, this would be insufficient to explain the 9–13% decrease in A_{sat} . However, such a large decrease in g_s across forest landscapes could have impacts on regional and continental hydrology. Sellers *et al.* (1996) showed via coupled plant physiology and global circulation models that a decrease in g_s of similar magnitude because of rising [CO_2] affected regional climate, by both decreasing transfer of water to the atmosphere so lowering precipitation and increasing surface temperature. In soy bean fields growing under FACE, a 10% decrease in average g_s across three complete growing seasons resulted in an 8.6% decrease in system evapo-transpiration and 0.4 °C increase in day-time surface temperature (Bernacchi *et al.* 2007). As the third strongest greenhouse gas, O_3 directly contributes to global warming (Denman *et al.* 2007); this analysis also suggests that in its role as the most important secondary pollutant impacting trees, current [O_3] has potential to indirectly alter regional climate change through reduced g_s leading to increased surface temperature and decreased atmospheric humidity.

O_3 effects are not uniform across taxa: gymnosperms are apparently unaffected by the current increase in ambient

Table 6. Between-group heterogeneity (Q_B) for ozone (O_3) effect size across descriptive categories, comparing trees grown in ambient background ozone concentration ([O_3]) versus elevated O_3 treatments

Category	A_{sat}		g_s	
	Q_B	P	Q_B	P
Angiosperms versus gymnosperms	1.98	0.256	2.30	0.178
Genus	24.67	0.083	19.52	0.103
Tree age	4.38	0.076	3.3	0.148
Leaf age	3.39	0.336	4.55	0.183
Rooting environment	5.70	0.050	0.19	0.697
Method	3.31	0.703	15.67	0.015
Duration	20.27	0.044	20.57	0.015
[O_3]	34.31	0.001	14.18	0.030
Additional treatment	5.92	0.563	5.23	0.535

A_{sat} , light-saturated photosynthesis; g_s , stomatal conductance.

Table 7. Percent changes (% change), 95% confidence intervals (95% CI), degrees of freedom (d.f.) and mean ozone concentrations ([O₃]) for the estimate of the mean response of light-saturated photosynthesis (A_{sat}) and stomatal conductance (g_s) across all studies (cumulative effect size), and for angiosperms, gymnosperms and different genera grown in ambient background ozone (O₃) versus elevated O₃ treatments

Genus	A_{sat}			[O ₃]	g_s			[O ₃]
	% Change	95% CI	d.f.		% Change	95% CI	d.f.	
Cumulative effect size	-18	-20 to -15	348	81	-6	-10 to -3	252	71
Gymnosperms	-16	-19 to -12	134	87	-10	-15 to -4	101	82
<i>Picea</i>	-16	-22 to -10	55	73	-7	-15 to 1	60	77
<i>Pinus</i>	-16	-21 to -10	76	94	-13	-22 to -3	40	90
Angiosperms	-19	-22 to -16	213	77	-4	-9 to 1	150	63
<i>Acer</i>	-20	-31 to -8	14	67	4	-12 to 22	12	68
<i>Betula</i>	-14	-22 to -6	43	45	8	-2 to 18	52	40
<i>Fagus</i>	-11	-20 to -2	19	53	-12	-26 to 5	11	57
<i>Liriodendron</i>					-6	-41 to 50	7	68
<i>Populus</i>	-26	-32 to -20	40	89				
<i>Prunus</i>	-24	-30 to -19	50	108				
<i>Quercus</i>	-7	-17 to 5	22	77	-11	-19 to -2	38	80

background [O₃], while A_{sat} and g_s are generally decreased in angiosperms. Gymnosperms consistently have lower average g_s compared to angiosperms and therefore lower uptake of [O₃]. Samuelson & Kelly (2001) reported g_s for a range of gymnosperm and angiosperm temperate trees grown at different life stages and grown under the same conditions. From this broad sample, we calculated a mean g_s

of 100 mmol m⁻² s⁻¹ for gymnosperms and 185 mmol m⁻² s⁻¹ for angiosperms. At these conductances, O₃ uptake in the gymnosperms would be 0.54 or roughly half that of the angiosperms.

In the absence of additional treatments such as drought or nutrient deficiency, A_{sat} was 10% lower for angiosperms in ambient background O₃ compared to CF air (Fig. 2). Relative to pre-industrial conditions, elevated [CO₂] projected for the middle of this century ameliorates the impact of ambient background [O₃] on angiosperms (Fig. 2). This implies that the impact of the current ambient [O₃] will lessen with time. This is consistent with the fact that g_s decreases with rising [CO₂] (Long *et al.* 2004) and will therefore decrease CU. However, current projections suggest that [O₃] will rise at a similar rate to [CO₂], and more in some regions of the temperate Northern Hemisphere (Ehhalt *et al.* 2001; Denman *et al.* 2007). So, any decrease in g_s caused by rising [CO₂] is likely to be offset by a rise in [O₃]. Further, Karnosky *et al.* (2003) reported that despite decreased g_s in some trees, damage remains the same for a given [O₃] treatment, implying that decreased O₃ uptake may be offset by decreased capacity for detoxification in trees grown in elevated [CO₂].

The current meta-analysis shows that the detrimental effect of ambient [O₃] is less in droughted trees, again a situation where g_s and therefore O₃ uptake will be decreased (Fig. 2). However, individual studies have suggested that high [O₃] exposure may result in increased transpiration and exacerbate drought effects (Maier-Maercker & Koch 1991; McLaughlin *et al.* 2007), although these studies examined natural variation in [O₃] where effects may be confounded with other environmental variables. Grulke *et al.* (2004) observed night-time opening of stomata in ponderosa pine exposed to elevated [O₃]. However, g_s was 5–10 times lower than during the day and also less in older trees and at the end of the growing season. Given the diurnal variation in water vapour pressure deficit, this

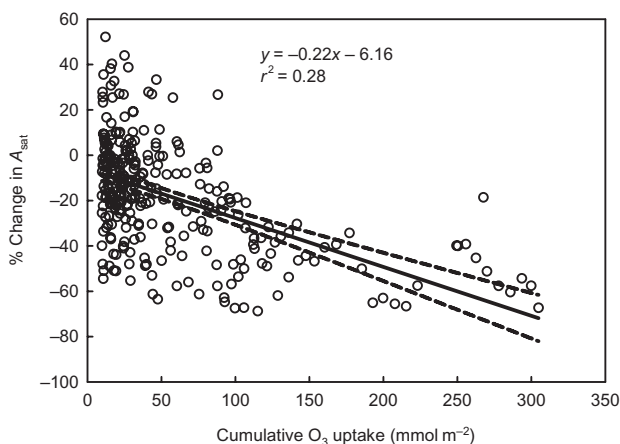


Figure 6. The relationship between cumulative ozone (O₃) uptake (mmol m⁻²) and the percent change in light-saturated photosynthesis (A_{sat}) for trees grown in elevated O₃ treatments relative to charcoal-filtered (CF) air. The solid line shows the linear regression, and dashed lines show 95% confidence intervals (CIs). Twenty-eight species were included in this analysis: *Acer saccharum*, *Betula pendula*, *Ceratonia siliqua*, *Fagus sylvatica*, *Fraxinus pennsylvanica*, *Liriodendron tulipifera*, *Malus pumila*, *Olea europaea*, *Picea abies*, *Pinus echinata*, *Pinus halepensis*, *Pinus ponderosa*, *Pinus taeda*, *Populus maximowiczii* × *trichocarpa*, *Populus tremuloides*, *Populus tristis* × *balsamifera*, *Prunus armeniaca*, *Prunus avium*, *Prunus domestica*, *Prunus dulcis*, *Prunus persica*, *Prunus salicina*, *Prunus serotina*, *Pyrus pyrafolia*, *Quercus ilex*, *Quercus ilex ilex*, *Quercus ilex rotundifolia* and *Quercus rubra*.

nocturnal opening would likely have a minimal effect on water use. Grulke *et al.* (2002) noted a sluggish response of the stomata of ponderosa pine exposed to elevated $[O_3]$. However, predicted g_s for these plants based on light and water potential was not statistically different from control. Despite these effects of $[O_3]$, our analysis shows that on average g_s is significantly reduced, suggesting that loss of control may be isolated to certain species and does not appear to reflect the average trend.

Less easily predicted was the finding that lower canopy leaves are more affected than upper canopy leaves. However, this result came from a single study that investigated three species (Novak *et al.* 2005), so the generality of the result is unknown. It is possible that low or dynamic light conditions cause a decoupling of A_{sat} and g_s in shaded leaves of seedlings and saplings, and thus create greater potential for O_3 uptake per unit of net photosynthesis (Fredericksen *et al.* 1996). It is also possible that the shaded leaves are older, and therefore might have a higher CU.

How will future levels of tropospheric O_3 impact photosynthesis and g_s in trees?

The average current day-time background $[O_3]$ in the studies reviewed in this analysis was 40 ppb. Given current emission trends, tropospheric $[O_3]$ is projected to rise globally by 20–25% between 2015 and 2050, and 40–60% by 2100 (Meehl *et al.* 2007). Based on the mean ambient $[O_3]$ calculated from data in this analysis, the IPCC projections imply an increase in $[O_3]$ from 40 to 48–50 ppb by 2050 and to 56–64 ppb by 2100 for the sites in which the data for this analysis were obtained. Based on the current meta-analysis, this could drive a further 8–16% decrease in A_{sat} caused by rising $[O_3]$ (Appendix S4). However, O_3 is not the only element of global change, and increasing $[CO_2]$, temperature and drought stress will interact with O_3 stress.

Surprisingly, angiosperms and gymnosperms showed a similar reduction in A_{sat} under elevated $[O_3]$. One interpretation of this finding is that while current elevations have been insufficient to affect gymnosperms, the increase projected for later in this century will be sufficient not only to further reduce A_{sat} in angiosperms, but also reduce A_{sat} of gymnosperms. This suggests greater sensitivity of Northern Hemisphere forest carbon sinks in the future compared with present conditions (Karnosky *et al.* 2007; McLaughlin *et al.* 2007).

Because of the difficulty of studying large and mature trees, young trees and often seedlings and saplings have formed the larger part of most studies of O_3 impacts. Older trees were under-represented in the data so preventing a comprehensive analysis of age effects. There were sufficient data to separate effects on trees under and over 4 years. Trees over 4 years showed a greater reduction in A_{sat} and g_s because of current background than did trees under 4 years, suggesting greater sensitivity with age (Appendix S3). This is of concern because it suggests by focusing on younger trees, the effect of ambient $[O_3]$ on photosynthesis and productivity may be underestimated. The finding is consistent with the

observation that during the first 2 years of growth under free-air $[O_3]$ enrichment, there were no observed effects on productivity at elevated $[O_3]$ for some *Populus tremuloides* clones, but highly significant effects were apparent in later years (Karnosky *et al.* 2005). However, this meta-analysis suggests that when $[O_3]$ was elevated to 83–84 ppb, damage to A_{sat} appeared greater in the younger trees. This lack of consensus highlights the need for experiments which examine impact of future $[O_3]$ on trees from planting through to maturation, as is being conducted in the FACE experiment in Rhinelander, WI, USA (Karnosky *et al.* 2007).

In the absence of additional environmental modifications, such as elevated $[CO_2]$, A_{sat} was reduced by 20% in elevated $[O_3]$ of 87 ppb compared to CF air. This reduction was ameliorated when elevated $[O_3]$ was combined with elevated $[CO_2]$. Similarly, the detrimental effect of elevated $[O_3]$ was ameliorated by drought, likely because g_s and therefore $[O_3]$ uptake were lower in drought treatments (Fig. 4). Results from aspen FACE suggest that elevated $[O_3]$ is sufficient to offset the enhancement in NPP by $[CO_2]$ elevated to 550 ppm, and that in some species elevated $[O_3]$ will reduce NPP despite a higher $[CO_2]$ (King *et al.* 2005). In all cases in this analysis, the A_{sat} of *Populus* species was significantly and negatively impacted by $[O_3]$, often appearing most sensitive angiosperm. This is significant because *Populus* trees are major components of forests across the northern temperate and boreal zone and also include important candidate bioenergy crops (Isebrands *et al.* 2001; Karnosky *et al.* 2007).

Is there a linear decline in A_{sat} with increasing CU?

The analysis has been limited to the effects based on day-time $[O_3]$. However, CU has been shown to be a more effective predictor of O_3 damage (Reich 1987; Nunn *et al.* 2006; Karlsson *et al.* 2007; Karnosky *et al.* 2007; Matyssek *et al.* 2007). This measure is theoretically more satisfactory, but more difficult to estimate because it requires a simultaneous record of both g_s and A_{sat} under various $[O_3]$. Reich (1987) in a review of the limited observations of O_3 impacts on tree productivity that had been made at this date showed linear relationships between reduction of A_{sat} and CU. Here, there were sufficient data to undertake this analysis with 28 tree species (Fig. 6). With the variability of species and genotypes within species, coupled with a wide range of locations and experimental conditions, far less of the variability is accounted for by CU, but a significant negative correlation was found (Fig. 6). Some of this variability may also be accounted for by differing capacities for detoxification (Matyssek *et al.* 2007; Wieser & Matyssek 2007). This suggests that the concept of CU as a predictor of O_3 damage (Karnosky *et al.* 2007; Matyssek *et al.* 2007) is robust and supports the 'unifying theory' proposed by Reich (1987). Our estimated slope predicts a 0.21% decrease in A_{sat} for every $mmol\ m^{-2}$ of uptake. This is substantially less than the 0.64% per $mmol\ m^{-2}$ averaged across the values given by Reich (1987) for pines and hardwoods, but this may result

from the larger database now available and still represents a significant loss of photosynthetic capacity. Our estimate matches annual biomass reduction for less sensitive species predicted by Karlsson *et al.* (2007) of 0.28% per mmol m^{-2} . Because we use all known data in the peer-reviewed literature to date, 0.21% per mmol m^{-2} represents the current literature average for loss of photosynthetic capacity with CU. If we assume an average of 14 h daylight over a 150 d growing season, at a mean $[\text{O}_3]$ of 40 ppb and a mean g_s of $185 \text{ mmol m}^{-2} \text{ s}^{-1}$, CU would be 48 mmol m^{-2} which would cause an 11% decline in A_{sat} based on the relationship of Fig. 6. If $[\text{O}_3]$ rises to 60 ppb by the end of the century, then uptake would rise to 72 mmol m^{-2} and the decline in A_{sat} would reach 17%, with even larger losses for forests in more polluted areas.

While the effects shown here are not new, we have quantified the current state of knowledge providing the averages and their error limits for two important forest parameters for both terrestrial carbon cycle and regional hydrology models. The significant decreases in both A_{sat} and g_s , and the projected further declines, indicate that rising $[\text{O}_3]$ is negatively affecting both carbon sequestration and water vapour transfer to the atmosphere. Rising surface $[\text{O}_3]$ and its interaction with forests are an often overlooked aspect of global change. The statistically significant evidence for deleterious impacts on photosynthetic potential averaged across over 350 measurements demonstrates the need to fully incorporate this into future projections of how atmospheric change and forest biomes will interact in effecting future climatic change.

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REFERENCES

- Ainsworth E.A. & Long S.P. (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy. *New Phytologist* **165**, 351–371.
- Ainsworth E.A., Davey P.A., Bernacchi C.J., *et al.* (2002) A meta-analysis of elevated $[\text{CO}_2]$ effects on soybean (*Glycine max*) physiology, growth and yield. *Global Change Biology* **8**, 695–709.
- Akimoto H. (2003) Global air quality and pollution. *Science* **302**, 1716–1719.
- Ashmore M.R. (2005) Assessing the future global impacts of ozone on vegetation. *Plant, Cell & Environment* **28**, 949–964.
- Bernacchi C.J., Kimball B.A., Quarles D.R., Long S.P. & Ort D.R. (2007) Decreases in stomatal conductance of soybean under open-air elevation of $[\text{CO}_2]$ are closely coupled with decreases in ecosystem evapotranspiration. *Plant Physiology* **143**, 134–144.
- Broadmeadow M. (1998) Ozone and forest trees. *New Phytologist* **139**, 123–125.
- Chappelka A.H. & Samuelson L.J. (1998) Ambient ozone effects on forest trees of the eastern United States: a review. *New Phytologist* **139**, 91–108.
- Curtis P.S. & Wang X.Z. (1998) A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. *Oecologia* **113**, 299–313.
- Darley E.F. & Middleton J.T. (1966) Problems of air pollution in plant pathology. *Annual Review of Phytopathology* **4**, 103–118.
- Denman K.L., Brasseur A., Chidthaisong A., *et al.* (eds) (2007) *Couplings Between Changes in the Climate System and Biogeochemistry*. Cambridge University Press, Cambridge, UK/New York, NY, USA.
- Dizengremel P. (2001) Effects of ozone on the carbon metabolism of forest trees. *Plant Physiology and Biochemistry* **39**, 729–742.
- Ehhalt D., Prather M., Dentener F., *et al.* (eds) (2001) *Atmospheric Chemistry and Greenhouse Gases*. Cambridge University Press, Cambridge, UK/New York, NY, USA.
- EPA US. (2006) *Air Quality Criteria for Ozone and Related Photochemical Oxidants (Final)*. US Environmental Protection Agency, Washington, DC, USA.
- Farage P.K. (1996) The effect of ozone fumigation over one season on photosynthetic processes of *Quercus robur* seedlings. *New Phytologist* **134**, 279–285.
- Farage P.K. & Long S.P. (1995) An *in vivo* analysis of photosynthesis during short-term O_3 exposure in three contrasting species. *Photosynthesis Research* **43**, 11–18.
- Farage P.K., Long S.P., Lechner E.G. & Baker N.R. (1991) The sequence of change within the photosynthetic apparatus of wheat following short-term exposure to ozone. *Plant Physiology* **95**, 529–535.
- Felzer B., Kicklighter D., Melillo J., Wang C., Zhuang Q. & Prinn R. (2004) Effects of ozone on net primary production and carbon sequestration in the conterminous United States using a biogeochemistry model. *Tellus Series B – Chemical and Physical Meteorology* **56**, 230–248.
- Forster P., Ramaswamy V., Artaxo P., *et al.* (eds) (2007) *Changes in Atmospheric Constituents and in Radiative Forcing*. Cambridge University Press, Cambridge, UK/New York, NY, USA.
- Fowler D., Cape J.N., Coyle M., Flechard C., Kuylenstierna J., Hicks K., Derwent D., Johnson C. & Stevenson D. (1999a) The global exposure of forests to air pollutants. *Water, Air, & Soil Pollution* **116**, 5–32.
- Fowler D., Cape J.N., Coyle M., Smith R.I., Hjellbrekke A.G., Simpson D., Derwent R.G. & Johnson C.E. (1999b) Modelling photochemical oxidant formation, transport, deposition and exposure of terrestrial ecosystems. *Environmental Pollution* **100**, 43–55.
- Fredericksen T.S., Kolb T.E., Skelly J.M., Steiner K.C., Joyce B.J. & Savage J.E. (1996) Light environment alters ozone uptake per net photosynthetic rate in black cherry trees. *Tree Physiology* **16**, 485–490.
- Geider R.J., Delucia E.H., Falkowski P.G., *et al.* (2001) Primary productivity of planet Earth: biological determinants and physical constraints in terrestrial and aquatic habitats. *Global Change Biology* **7**, 849–882.
- Grace J. (2004) Understanding and managing the global carbon cycle. *Journal of Ecology* **92**, 189–202.
- Gulke N.E., Preisler H.K., Fan C.C. & Retzlaff W.A. (2002) A statistical approach to estimate O_3 uptake of ponderosa pine in a mediterranean climate. *Environmental Pollution* **119**, 163–175.
- Gulke N.E., Alonso R., Nguyen T., Cascio C. & Dobrowolski W. (2004) Stomata open at night in pole-sized and mature ponderosa pine: implications for O_3 exposure metrics. *Tree Physiology* **24**, 1001–1010.
- Gurevitch J. & Hedges L.V. (1999) Statistical issues in ecological meta-analyses. *Ecology* **80**, 1142–1149.

- Heath R.L. (1994) Possible mechanisms for the inhibition of photosynthesis by ozone. *Photosynthesis Research* **39**, 439–451.
- Hedges L.V., Gurevitch J. & Curtis P.S. (1999) The meta-analysis of response ratios in experimental ecology. *Ecology* **80**, 1150–1156.
- Isebrands J.G., McDonald E.P., Kruger E., Hendrey G., Percy K., Pregitzer K., Sober J. & Karnosky D.F. (2001) Growth responses of *Populus tremuloides* clones to interacting elevated carbon dioxide and tropospheric ozone. *Environmental Pollution* **115**, 359–371.
- Kangasjarvi J., Jaspers P. & Kollist H. (2005) Signalling and cell death in ozone-exposed plants. *Plant, Cell & Environment* **28**, 1021–1036.
- Karlsson P., Braun S., Broadmeadow M., et al. (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. et al. (2003) Tropospheric O₃ moderates responses of temperate hardwood forests to elevated CO₂: 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 & Environment* **28**, 965–981.
- Karnosky D., Skelly J., Percy K. & Chappelka A. (2007) Prospectives regarding 50 years of research on effects of tropospheric ozone air pollution on US forests. *Environmental Pollution* **147**, 489–506.
- King J.S., Kubiske M.E., Pregitzer K.S., Hendrey G.R., McDonald E.P., Giardina C.P., Quinn V.S. & Karnosky D.F. (2005) Tropospheric O₃ compromises net primary production in young stands of trembling aspen, paper birch and sugar maple in response to elevated atmospheric CO₂. *New Phytologist* **168**, 623–635.
- Laisk A., Kull O. & Moldau H. (1989) Ozone concentration in leaf intercellular air spaces is close to zero. *Plant Physiology* **90**, 1163–1167.
- Long S.P. & Naidu S. (2002) Effects of oxidants at the biochemical, cell and physiological levels, with particular reference to ozone. In *Air Pollution and Plants* (ed. M. Treshow), pp. 69–88. John Wiley, London, England.
- Long S.P., Ainsworth E.A., Rogers A. & Ort D.R. (2004) Rising atmospheric carbon dioxide: plants FACE the future. *Annual Review of Plant Biology* **55**, 591–628.
- Low M., Herbinger K., Nunn A.J., et al. (2006) Extraordinary drought of 2003 overrules ozone impact on adult beech trees (*Fagus sylvatica*). *Trees – Structure and Function* **20**, 539–548.
- 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 & Environment* **14**, 175–184.
- Martin M.J., Host G.E., Lenz K.E. & Isebrands J.G. (2001) Simulating the growth response of aspen to elevated ozone: a mechanistic approach to scaling a leaf-level model of ozone effects on photosynthesis to a complex canopy architecture. *Environmental Pollution* **115**, 425–436.
- Matyssek R. & Innes J.L. (1999) Ozone: a risk factor for trees and forest in Europe? *Water, Air, & Soil Pollution* **116**, 199–226.
- Matyssek R., Bytnerowicz A., Karlsson P.E., Paoletti E., Sanz M., Schaub M. & Wieser G. (2007) Promoting the O₃ flux concept for European forest trees. *Environmental Pollution* **146**, 587–607.
- McLaughlin S.B., Nosal M., Wullschlegel S.D. & Sun G. (2007) Interactive effects of ozone and climate on tree growth and water use in a southern Appalachian forest in the USA. *New Phytologist* **174**, 109–124.
- Meehl G.A., Stocker T.F., Collins W.D., et al. (eds) (2007) *Global Climate Projections*. Cambridge University Press, Cambridge, UK/New York, NY, USA.
- Middleton J. (1956) Response of plants to air pollution. *Journal of the Air Pollution Control Association* **6**, 7–9.
- Morgan P.B., Ainsworth E.A. & Long S.P. (2003) How does elevated ozone impact soybean? A meta-analysis of photosynthesis, growth and yield. *Plant, Cell & Environment* **26**, 1317–1328.
- Novak K., Schaub M., Fuhrer J., Skelly J.M., Hug C., Landolt W., Bleuler P. & Krauchi N. (2005) Seasonal trends in reduced leaf gas exchange and ozone-induced foliar injury in three ozone sensitive woody plant species. *Environmental Pollution* **136**, 33–45.
- Nunn A.J., Wieser G., Reiter I.M., Haberle K.H., Grote R., Havranek W.M. & Matyssek R. (2006) Testing the unifying theory of ozone sensitivity with mature trees of *Fagus sylvatica* and *Picea abies*. *Tree Physiology* **26**, 1391–1403.
- Oksanen E. (2003) Physiological responses of birch (*Betula pendula*) to ozone: a comparison between open-soil-grown trees exposed for six growing seasons and potted seedlings exposed for one season. *Tree Physiology* **23**, 603–614.
- Ollinger S.V., Aber J.D. & Reich P.B. (1997) Simulating ozone effects on forest productivity: interactions among leaf-, canopy-, and stand-level processes. *Ecological Applications* **7**, 1237–1251.
- Paoletti E., Bytnerowicz A., Andersen C., et al. (2007) Impacts of air pollution and climate change on forest ecosystems – emerging research needs. *The Scientific World Journal* **7**, 1–8.
- Pell E.J., Schlagnhauer C.D. & Arteca R.N. (1997) Ozone-induced oxidative stress: mechanisms of action and reaction. *Physiologia Plantarum* **100**, 264–273.
- Prentice I.C., Farquhar G.D., Fasham M.J.R., Goulden M.L., Heimann M., Jaramillo V.J., Kheshgi H.S., Le Quere C., Scholes R.J. & Wallace D.W.R. (2001) The carbon cycle and atmospheric carbon dioxide. In *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change* (eds J.T. Houghton, Y. Ding, D.J. Griggs, M. Noguer, P.J. van der Linden, X. Dai, K. Maskell & C.A. Johnson), pp. 183–237. Cambridge University Press, Cambridge, UK/New York, NY, USA.
- Reich P.B. (1987) Quantifying plant response to ozone: a unifying theory. *Tree Physiology* **3**, 63–91.
- Reich P.B. & Lassoie J.P. (1984) Effects of low-level O₃ exposure on leaf diffusive conductance and water-use efficiency in hybrid poplar. *Plant, Cell & Environment* **7**, 661–668.
- Rosenberg M.S., Adams D.C. & Gurevitch J. (2000) *MetaWin: Statistical Software For Meta-Analysis. Version 2.0*. Sinauer Associates, Sunderland, MA, USA.
- Samuelson L. & Kelly J.M. (2001) Scaling ozone effects from seedlings to forest trees. *New Phytologist* **149**, 21–41.
- Sellers P.J., Bounoua L., Collatz G.J., et al. (1996) Comparison of radiative and physiological effects of doubled atmospheric CO₂ on climate. *Science* **271**, 1402–1406.
- Skarby L., Ro-Poulsen H., Wellburn F.A.M. & Sheppard L.J. (1998) Impacts of ozone on forests: a European perspective. *New Phytologist* **139**, 109–122.
- Volz A. & Kley D. (1988) Evaluation of the montsouris series of ozone measurements made in the 19th-century. *Nature* **332**, 240–242.
- Wieser G. & Matyssek R. (2007) Linking ozone uptake and defense towards a mechanistic risk assessment for forest trees. *New Phytologist* **174**, 7–9.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Appendix S1. References included in the database for meta-analysis and cumulative ozone uptake analysis.

Appendix S2. Effect Sizes (E) 95% confidence intervals (CI), degrees of freedom (d.f.) and mean ozone concentrations ($[O_3]$) for categories with significant between-group heterogeneity for trees grown in charcoal-filtered air relative to ambient background ozone concentrations.

Appendix S3. Effect Sizes (E) 95% confidence intervals (CI), degrees of freedom (d.f.) and mean ozone concentrations ($[O_3]$) for categories with significant between-group heterogeneity for trees grown in charcoal-filtered air relative to elevated ozone treatments.

Appendix S4. Effect Sizes (E) 95% confidence intervals (CI), degrees of freedom (d.f.) and mean ozone concentrations ($[O_3]$) for categories with significant between-group heterogeneity for trees grown in ambient background ozone relative to elevated ozone treatments.

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