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Integrating O_3 influences on terrestrial processes: photosynthetic and stomatal response data available for regional and global modeling

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Abstract. Plants have a strong influence on climate by controlling the transfer of carbon dioxide and water between the biosphere and atmosphere during the processes of photosynthesis and transpiration. Chronic exposure to surface ozone (O_3) differentially affects photosynthesis and transpiration because it damages stomatal conductance, the common link that controls both processes, in addition to the leaf biochemistry that only affects photosynthesis. Because of the integral role of O_3 in altering plant interactions with the atmosphere, there is a strong motivation to incorporate the influence of O_3 into regional and global models. However, there are currently no analyses documenting both photosynthesis and stomatal conductance responses to O_3 exposure through time using a standardized O_3 parameter that can be easily incorporated into models. Therefore, models often rely on photosynthesis data derived from the responses of one or a few plant species that exhibit strong negative correlations with O_3 exposure to drive both rates of photosynthesis and transpiration, neglecting potential divergence between the two fluxes. Using data from the peer-reviewed literature, we have compiled photosynthetic and stomatal responses to chronic O_3 exposure for all plant types with data available in the peer-reviewed literature as a standardized function of cumulative uptake of O_3 (CUO), which integrates O_3 flux into leaves through time. These data suggest that stomatal conductance decreases $\sim$11 % after chronic O_3 exposure, while photosynthesis independently decreases $\sim$21 %. Despite the overall decrease in both variables, high variance masked any correlations between the decline in photosynthesis or stomatal conductance with increases in CUO. Though correlations with CUO are not easily generalized, existing correlations demonstrate that photosynthesis tends to be weakly but negatively correlated with CUO while stomatal conductance is more often positively correlated with CUO. Results suggest that large-scale models using data with strong negative correlations that only affect photosynthesis need to reconsider the generality of their response. Data from this analysis are now available to the scientific community and can be incorporated into global models to improve estimates of photosynthesis, global land-carbon sinks, hydrology, and indirect radiative forcing that are influenced by chronic O_3 exposure.

1 Introduction

Surface ozone (O_3) is a greenhouse gas that has increased by 30–60 % globally since industrialization (Karnosky, 2005), resulting in large direct economic costs because O_3 is a strong oxidant that causes foliar damage and reductions in crop yield (Skärby et al., 1987; Mortensen, 1992; Ashmore, 2005; Morgan et al., 2006; Feng et al., 2008). Fossil fuel combustion and industrial processes have increased atmospheric concentrations of nitrogen oxides (NO_x) and volatile organic compounds (Denman, 2007) that produce O_3 through photochemical reactions. Ozone concentrations are predicted to continue increasing in polluted regions by 1–10 ppb in coming decades due to climate change (Jacob and Winner, 2009). At the same time, higher water vapor concentrations in future climates are expected to decrease global background O_3 concentrations (Jacob and Winner, 2009),

with expected summertime decreases in the United States of 2–15 ppb (Wu et al., 2008).

In addition to directly increasing radiative forcing through its role as a greenhouse gas, O_3 alters regional and global climate through changing water and carbon exchange between plants and the atmosphere when transpiration and photosynthesis are affected (e.g., Sitch et al., 2007). Once O_3 has entered the leaf, it has the potential to damage several aspects of photosynthesis (Fig. 1a). Ozone (1) enters the leaf through the stomata; (2) alters mesophyllic processes and oxidizes cellular membranes (Fiscus et al., 2005; Francini et al., 2007; Noormets et al., 2001); and (3) decreases carbon fixation by reducing Rubisco enzyme content and activity (Ojanperä et al., 1998; Farage and Long, 1999; Fiscus et al., 2005) as well as chlorophyll content (Heagle et al., 1996; Bortier et al., 2000; Sharma, 2003; Fiscus et al., 2005; Herbinger et al., 2007). Ozone also changes stomatal conductance, which regulates both photosynthesis and transpiration (Fig. 1b), directly by changing guard cell turgor pressure and signaling pathways (e.g., abscisic acid, K^+; Freer-Smith and Dobson, 1989; Maier-Maercker and Kock, 1991; Hassan et al., 1994; Torsethaugen et al., 1999; Manes et al., 2001; Mills et al., 2009) and indirectly by increasing internal leaf CO_2 concentration, signaling stomatal cells to close (Calatayud et al., 2007; Herbinger et al., 2007). On average, all of these mechanisms reduce transpiration and photosynthesis, though the damage to internal leaf biochemistry often causes chronic O_3 exposure to decrease photosynthesis more than transpiration, leading to significant decoupling between the two over time (Mikkelsen, 1995; Tjoelker et al., 1995; Lippert et al., 1996; Maurer et al., 1997; Soldatini et al., 1998; Novak et al., 2005; Paoletti, 2005; Calatayud et al., 2007; Francini et al., 2007). It should also be noted that some studies find an increase in stomatal conductance with chronic O_3 exposure (e.g. Paoletti, 2005; McLaughlin et al., 2007).

Overall photosynthetic reductions after chronic O_3 exposure are common in many types of plants, though stomatal responses are more variable. Meta-analyses of trees (Wittig et al., 2007), wheat (Feng et al., 2008), soybeans (Morgan et al., 2003) and studies comparing plants from multiple functional groups (Reich and Amundson, 1985; Volin et al., 1998) all suggest approximately a 20 % average decrease in photosynthesis after chronic exposure (typically defined as exposures longer than seven days) to O_3. In contrast, average stomatal responses to O_3 exposure show decreases of 6–10 % in trees (Wittig et al., 2007) and ∼ 20 % in crops (Morgan et al., 2003; Feng et al., 2008), suggesting that stomatal conductance does not always respond similarly to photosynthesis and that responses are variable among plant types. Ozone exposure can also have inconsistent effects on leaf water loss. Several studies have reported that canopy evapotranspiration decreases with O_3 exposure (Bernacchi et al., 2011; VanLoocke et al., 2012), while others suggest that chronic O_3 exposure results in sluggish stomatal responses in many plants types (Mills et al., 2009; Paoletti and Grulke, 2010),

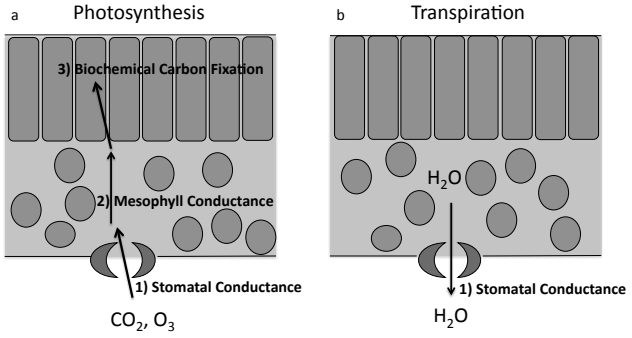

Fig. 1. An illustration of leaf cross sections. The process of photosynthesis **(a)** relies on three steps: (1) CO_2 enters the leaf through the stomata, which is regulated by the size of the opening between the stomatal cells, or the stomatal conductance; (2) CO_2 travels through the intercellular spaces to the chloroplasts (mesophyllic processes); and (3) inside the chloroplasts, CO_2 is changed into sugars through biochemical carbon fixation. At the leaf-level, the process of transpiration **(b)** is only controlled by stomatal conductance (1). Stomatal conductance is a common mechanism that controls both photosynthesis and transpiration, so these processes are often closely coupled and will continue to be coupled if O_3 primarily effects stomatal conductance. However, experimental evidence shows that O_3 damage primarily effects biochemical carbon fixation (3) and mesophyllic processes (2), leading to a decoupling of photosynthesis and transpiration.

and can increase leaf-level and ecosystem-scale transpiration rates (Paoletti, 2005; McLaughlin et al., 2007).

Despite differences in photosynthetic and stomatal responses to O_3, many modeling studies assume a fixed relationship between photosynthesis and stomatal conductance (Ollinger et al., 1997; Felzer et al., 2004; Sitch et al., 2007) and typically modify photosynthesis values using data from four or fewer species per plant functional type. Simulating O_3 changes to photosynthesis in this manner typically overestimates the decrease in stomatal conductance (Lombardozzi et al., 2012a). If stomatal conductance decreases less than photosynthesis during O_3 exposure as suggested by experiments, then hydrologic changes regulated by the biosphere, including precipitation, latent heat flux and surface runoff, are incorrectly predicted in most current model formulations. Predictions of O_3 damage and impacts on carbon and water cycling can be improved by separately incorporating photosynthetic and stomatal responses to chronic O_3 exposure (Lombardozzi et al., 2012b). However, few data are available documenting responses of stomatal conductance to O_3 exposure through time, and large-scale models use specific photosynthetic responses of plants sensitive to O_3 exposure rather than a photosynthetic response generalized over multiple plant species.

Plant responses to chronic O_3 exposure through time depend on the concentration of O_3, length of exposure time, and the vulnerability of the plant to O_3. Plant vulnerability

is a function of both stomatal conductance, which can limit the amount of O_3 entering the leaf, and antioxidant defenses within the leaf, which prevent oxidative damage by scavenging O_3 (Dizengremel et al., 2008). One metric of standardizing plant responses to chronic O_3 exposure is to calculate cumulative uptake of O_3 (CUO), which integrates O_3 flux into the leaf through time. Because CUO accounts for changes in stomatal conductance and therefore describes the amount of O_3 that enters the leaf, it estimates the ability of O_3 to oxidize biochemical components of the leaf that regulate photosynthesis and stomatal conductance. A flux threshold is sometimes used in CUO calculations to account for the ability of the plant to detoxify using antioxidants found within plant leaves. However, CUO does not directly account for internal defenses that can vary widely by plant and assumes that plants exposed to high concentrations for short periods of time will have similar CUO to plants exposed to lower concentrations for longer periods of time. Regardless, several studies have demonstrated strong negative correlations between CUO and photosynthesis (Reich, 1987; Wittig et al., 2007), biomass (Karlsson et al., 2004; Uddling et al., 2004) or relative crop yield (Pleijel et al., 2002; Pleijel et al., 2004), making CUO a better metric for predicting plant responses to O_3 than concentration or cumulative O_3 exposure (Reich, 1987; Wieser, 1997; Musselmann and Massmann, 1999; Matyssek et al., 2007). To our knowledge, there is no comprehensive analysis that documents responses of stomatal conductance to CUO in multiple plant types. Additionally, most available CUO data are based on responses of temperate deciduous trees and crops, though other plant functional types may respond differently.

The objective of this work was to determine generalized responses of photosynthesis and stomatal conductance to chronic O_3 exposure in multiple plant types, using CUO as an index for damage to plant physiological processes. This study improves upon work presented in previous syntheses, which document specific plant type responses to O_3 concentrations (Morgan et al., 2003; Wittig et al., 2007; Feng et al., 2008), because it determines the physiological responses to chronic O_3 exposure as a function of both O_3 concentration and time in multiple plant functional types. We collected all available data from the peer-reviewed literature to determine whether photosynthesis and stomatal conductance responded differently to chronic O_3 exposure, and whether responses varied by plant functional types. Modeling CUO using continuous environmental data is the ideal method to estimate O_3 uptake and damage (e.g. Emberson et al., 2000; Karlsson et al., 2004; Mills et al., 2011; Pleijel et al., 2004). However, simultaneous high-resolution environmental data and O_3 concentrations are rarely presented in conjunction with response variables within manuscripts. In our assessment of the literature, only eleven publications that documented photosynthetic and stomatal responses to O_3 also calculated high resolution CUO data, so an alternative method for calculating CUO was needed to determine generalized responses across many plant species and functional types. CUO was calculated using stomatal conductance, O_3 concentration, and exposure duration data available within the literature (see Appendix A). Through incorporating all available information in the literature, these data will provide a comprehensive data set for use in models that will greatly expand the number of species that are represented in modeled photosynthetic and stomatal responses to O_3 exposure.

2 Methods

2.1 Data collection

A database documenting the effects of O_3 on photosynthesis and stomatal conductance was compiled by surveying peer-reviewed literature using key word searches in the Web of Science (ISI). A total of 156 papers published between 1970 and June 2011 that chronically (> 7 days, consistent with methods used by Morgan et al., 2003, Wittig et al., 2007) exposed plants to O_3 using growth chambers, open-top chambers, branch chambers, or other fumigation methods contained data sufficient to calculate CUO (see Eqs. 1 and 2) and reported changes in photosynthesis and stomatal conductance. Individual measurements within papers were considered independent if they were not previously reported and were measured for different species or genotypes or on different days, similar to methods used by Wittig et al. (2007). Data from papers were eliminated if O_3 concentration or exposure duration used to calculate CUO were unclear; if both control and treatment responses were not reported; if photosynthesis was not reported in conjunction with stomatal conductance; if units of stomatal conductance or photosynthesis were not reported per leaf area; if the type of gas that stomatal conductance was measured for was unclear; if other environmental interactions were included so that the effect of just O_3 was unclear; or if studies were shorter than seven days. In total, data were collected from 108 papers representing 79 species providing sample sizes of 670 for photosynthesis and 772 for stomatal conductance for analyses. A list of publications used in this analysis is provided in Appendix A and data are available upon request.

Values of photosynthesis, stomatal conductance, V_{cmax}, which reflects the amount and activity of enzymes used in biochemical carbon fixation and is often damaged with O_3 exposure (Calatayud et al., 2010; Cardoso-Vilhena et al., 2004; Farage and Long, 1999; Feng et al., 2008; Fiscus et al., 2005; Noormets et al., 2001; Ojanperä et al., 1998; Pellegrini et al., 2010; Zheng et al., 2002), and CUO (if available) from control and elevated O_3 treatments were collected from tables, figures, and text within papers and compiled into a database. If data were presented graphically, data were extracted using PlotDigitizer (PlotDigitizer 2.5.1, Sourceforge, Japan). Additional information about factors that might influence the response of photosynthesis and

stomatal conductance to CUO was recorded for each data point. These factors included plant type, plant age, type of air to which control plants were exposed (e.g., ambient or charcoal-filtered), O_3 exposure system, O_3 concentration, and rooting environment. Confidence in CUO calculations and estimated vulnerability of the plant to O_3 were also documented (see Table 1).

Confidence levels for CUO calculations were assigned based on the quality of data presented in the publication. Data were assigned *high* confidence if CUO was presented in the publication or *medium* confidence if the publication contained multiple stomatal conductance measurements throughout the course of the experiment, allowing calculations to account for changes in stomatal conductance through time and resulting in more accurate calculations of CUO. Data were assigned *low* confidence if only an end-point stomatal conductance measurement was reported or if any assumptions were made about duration of O_3 exposure.

Vulnerability was estimated using the ratio of photosynthesis to stomatal conductance in control plants, assuming that higher stomatal conductance allowed higher flux of O_3 into the leaf (Reich and Amundson, 1985) and that leaf internal defense was a function of photosynthesis (Massman, 2004). Volin et al. (1998) found a strong positive correlation between reduction in biomass due to O_3 and the ratio of photosynthesis to stomatal conductance. Therefore, a plant with low photosynthesis relative to stomatal conductance (i.e., photosynthesis:conductance $< 0.02\,\mu mol\,CO_2\,mol^{-1}\,H_2O$) was considered vulnerable because it was susceptible to higher CUO relative to its ability to defend itself against O_3 damage internally. Ratios of photosynthesis to stomatal conductance (photosynthesis : conductance) were grouped into quartiles and data with high values assigned to *low* vulnerability (high photosynthesis per unit conductance, $> 0.06\,\mu mol\,CO_2\,mol^{-1}\,H_2O$) and low values assigned to *high* vulnerability (high conductance per unit photosynthesis, $< 0.02\,\mu mol\,CO_2\,mol^{-1}\,H_2O$).

2.2 CUO calculations

If available, CUO was collected from tables, figures, or text in publications. Otherwise, CUO was estimated using data contained within the papers. From each publication, stomatal conductance and cumulative exposure to O_3 (CEO_3) was recorded. Cumulative O_3 exposure was either presented in the paper (i.e., AOT00, SUM00, etc.) or calculated as

$$CEO_3\ (nmol\ mol^{-1} - h) = [O_3] \times H \times D, \quad (1)$$

where H was the number of daytime hours the plant was exposed to elevated O_3; D was the total number of days; and $[O_3]$ is the external O_3 concentration in ppb that plants were exposed to during daytime hours. AOT00 and SUM00 values are calculations of O_3 concentrations above 0 ppb over total exposure time and are therefore equivalent to calculations of CEO_3; AOT and SUM values with thresholds higher than 0 ppb (e.g., AOT40 or SUM06) were not used in this analysis. Cumulative uptake of O_3 was calculated using CEO_3 (similar to Reich, 1987; Nunn et al., 2006; Wittig et al., 2007; Lombardozzi et al., 2012a):

$$CUO\ (mmol\ m^{-2}) = CEO_3 \times g_s \times k_{O_3} \times 3600 \times 10^{-6} \quad (2)$$

where g_s is the mean leaf-level stomatal conductance in units of mol $H_2O\,m^{-2}\,s^{-1}$; $k_{O_3} = 1.67$ and is the ratio of leaf resistance for O_3 to leaf resistance for water vapor (Sitch et al., 2007; Wittig et al., 2007); 3600 is the number of seconds per hour; and 10^{-6} is the conversion from nmol to mmol. Because stomatal conductance likely changes over the duration of the experiments, CUO calculations were adjusted to account for these changes when papers reported multiple measurements of stomatal conductance. For example, when stomatal conductance values were presented at day 10 and day 20 of an experiment, uptake was calculated between days 0 and 10 using the mean stomatal conductance value from day 10, and uptake between days 10 and 20 was calculated using the stomatal conductance value from day 20. The uptake from days 0 through 10 was added to the uptake from days 10 through 20 to get a CUO value at day 20. This process was repeated at every time point for each paper reporting multiple stomatal conductance values, allowing for the most accurate CUO estimates possible with the available data by accounting for changes in stomatal conductance throughout each experiment. Calculations of CUO using this method were strongly correlated to CUO presented within manuscripts ($r^2 = 0.92$, Fig. B1).

2.3 Statistical analyses

A linear regression framework was used to analyze data for relationships between change in photosynthesis or stomatal conductance relative to plants exposed to little or no O_3 (% of control) with CUO (an indicator of O_3 plant physiological damage through time) using the generalized linear model (glm) function in R© version 2.11.1. Both response variables were normally distributed, though graphs depict log-transformed CUO for visual purposes. Linear and log-linear relationships between the variables and CUO were tested, though linear relationships were almost always stronger and used in all analyses. Linear models were constructed to test relationships using individual and combinations of factors (listed above) and their interactions, with models having p values ≤ 0.05 considered significant. The best combination of predictors that explained the relationships between photosynthesis or stomatal conductance and CUO was selected using r^2 values. In addition to linear analyses, overall means were compared using Student's t tests.

Table 1. Categories and levels describing the data collected from experiments studying O_3 effects on photosynthesis and stomatal conductance. Each row shows a category and the columns show various levels of that category. All tree categories are temperate unless otherwise noted. Numbers in parentheses are the number of studies and the number of data points within the associated categorical level: (# of studies, *n*).

Category	Categorical Level						
Plant Type	crop (34, 218)	evergreen shrub (1, 1)	grasses (C_3 & C_4) (2, 9)	herbaceous (3, 45)	deciduous tree (53, 396)	evergreen tree (17, 88)	tropical tree (3,15)
Plant Age (years)	< 1 (54, 388)	1 to 5 (46, 301)	> 5 (12,54)				
Control Air	ambient (29, 225)	charcoal filtered (78, 539)					
Exposure System	greenhouse (6, 50)	branch chamber (2, 30)	growth chamber (42, 287)	open-top chamber (50, 316)	free-air enrichment (8, 89)		
[O_3] (ppb)	25 to 50 (3, 8)	50 to 75 (27, 132)	75 to 100 (19, 126)	100 to 125 (23, 152)	125 to 150 (0, 0)	> 150 (11, 55)	
Rooting Environment	pot (84, 571)	ground (24, 181)					
Vulnerability	low (63, 290)	high (29, 178)					
Data Confidence	low (51, 227)	medium (46, 478)	high (11, 66)				

3 Results

3.1 All data

Across all data, average values for photosynthesis and stomatal conductance significantly decreased after chronic O_3 exposure ($p < 0.001$ for each, data not shown); photosynthesis decreased by 21 %, which was significantly greater than the 11 % decrease in stomatal conductance ($p < 0.001$). Additionally, there was a weak but significant positive relationship between photosynthesis and CUO (Fig. 2a; $r^2 = 0.009$, $p = 0.01$) that was strongly influenced by a single study measuring guava responses to O_3. There was no correlation between stomatal conductance and CUO (Fig. 2b; $p = 0.11$). Though photosynthesis is positively correlated with CUO, photosynthesis values were negative in response to chronic O_3 exposure until a CUO of $\sim 538\,\mathrm{mmol\,m^{-2}}$; at CUO values above $\sim 538\,\mathrm{mmol\,m^{-2}}$, which were only calculated in two studies, photosynthesis was stimulated by O_3 exposure. Removing the single study measuring guava responses to O_3 changed the results, leading to no significant correlation between photosynthesis and CUO ($p = 0.38$; data not shown).

Since V_{cmax} data were only available for a small subset of studies, data were not separated into sub-categories. Overall, V_{cmax} responded similarly to photosynthesis responses documented in the same study (Fig. 3; $p = 0.41$). Within these studies, neither V_{cmax} nor photosynthesis was significantly correlated with CUO (V_{cmax}: $p = 0.31$; photosynthesis: $p = 0.41$), though mean responses of both significantly decreased from control values (data not shown, $p < 0.001$).

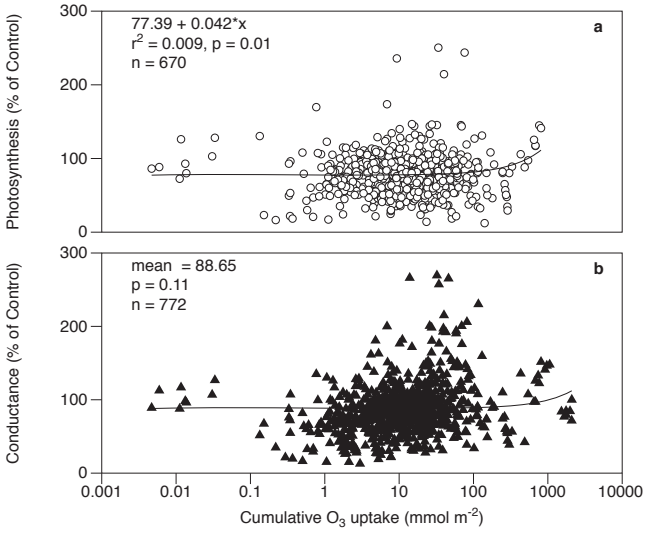

Fig. 2. The correlation of photosynthesis (open symbols; **a**) and conductance (closed symbols; **b**) to CUO across all plant types, ages, and vulnerabilities for all data. Response values are reported as % of control (treatment/control). CUO is shown on a log scale, but linear analyses (r^2 and line equations) were performed on non-log transformed data. r^2 values are only included for significant correlations, and *n* values are the number of data points included in the analyses.

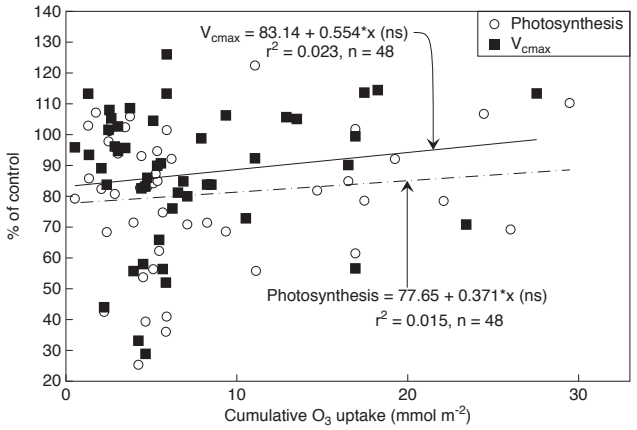

Fig. 3. The correlation of photosynthesis (open symbols) and V_{cmax} (closed symbols) to CUO from all studies that included information for both variables. Response values are reported as % of control (treatment/control).

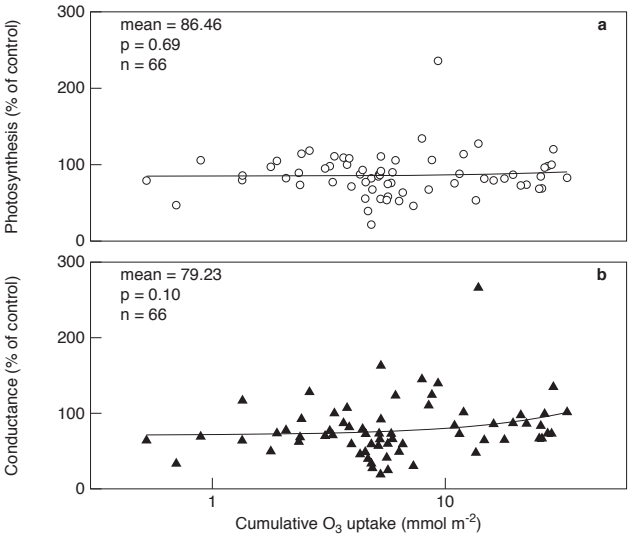

Fig. 4. The correlation between cumulative O_3 uptake (CUO) and photosynthesis (open symbols; **a**) or stomatal conductance (closed symbols; **b**) in all data that is compared to control plants exposed to charcoal-filtered air where CUO values were reported in the publication (e.g., high-confidence data).

3.2 Data subsets

While several sources of variation were considered in these analyses, two were considered important to remove from the analyses. One was the confidence in the CUO calculations. CUO data presented within a paper, or "high" confidence data, were only available for 11 studies, and there was no correlation between CUO and photosynthesis or stomatal conductance for high-confidence data (Fig. 4). Calculations of CUO in data categorized as "low" confidence relied on a single stomatal conductance value measured at the end of an experiment. Since stomatal conductance decreases in response to O_3, using the stomatal conductance value from the end of the experiment results in calculations of CUO that are likely smaller than the CUO the plants actually experienced. Therefore, analyses below remove all low-confidence data and instead subset the data to use only medium- and high-confidence data.

The second important source of variation considered in analyses was whether control plants were exposed to ambient air, which had background levels of O_3 specific to a particular location, or charcoal-filtered (CF) air, which removed nearly all O_3 from the environment. It is essential to separate analyses based on whether control plants were exposed to CF or ambient air because responses of photosynthesis and stomatal conductance in this analysis are made relative to a control treatment. Since ambient air varies by location, data presented within the rest of this manuscript focuses on responses relative to CF air so that comparisons are made to plants exposed to similar O_3 concentrations. Comparisons of elevated O_3 to ambient air are presented in Tables B1 and B2. It should be noted that all free-air enrichment exposure systems compare responses to ambient control air, so data collected using this type of exposure system are removed from further analyses.

When data were subset to remove low-confidence data and studies using ambient air, average photosynthesis decreased by $\sim 18\,\%$ and average stomatal conductance decreased by 16 % (Tables 2 and 3). There was a weak negative correlation between photosynthesis and CUO (Table 2; $r^2 = 0.02$, $p = 0.01$), but no correlation between stomatal conductance and CUO (Table 3; $p = 0.63$). Photosynthesis with large numbers of data points within categories of plant type (crop, $n = 134$), plant age (< 1, $n = 234$), O_3 exposure system (open-top chamber, $n = 146$), and rooting environment (pot, $n = 271$) were all weakly negatively correlated with CUO, with regression equations for each very similar to the overall regression (Table 2). There was a strong negative relationship between photosynthesis and CUO in plants grown in the ground ($r^2 = 0.17$, $p < 0.001$), though no correlations between CUO and photosynthesis within any other subcategory.

There were strong positive correlations between stomatal conductance and CUO in plants exposed to O_3 in growth chambers ($r^2 = 0.12$, $p < 0.001$) and plants with low vulnerability to O_3 ($r^2 = 0.23$, $p < 0.001$). There was also a strong positive correlation between stomatal conductance and CUO in temperate evergreen trees ($r^2 = 0.32$, $p < 0.001$), though this trend was largely driven by four data points above CUO values of $50\,\mathrm{mmol\,m^{-2}}$. There was a strong negative correlation between stomatal conductance and CUO in plants older than 5 yr ($r^2 = 0.34$, $p = 0.02$), though data were limited in this category ($n = 15$). Correlations between CUO and stomatal conductance were either weak or not significant in other subcategories or in the rooting environment category

Table 2. The number of data points (n), mean, regression, and statistics for photosynthesis when sorted by categories of plant type, plant age, O_3 exposure system, rooting environment, and vulnerability for all data that is compared to control plants exposed to charcoal-filtered air with medium or high confidence in cumulative O_3 uptake (CUO) calculations. All means are reported as the percent of control treatment. The p value for the overall data set (All data) designates whether the overall mean is significantly different from 100 (i.e., 0 % change). The p value for the mean within each category designates whether the mean of the category is statistically different from the mean of the overall data set (All data). All regressions are based on the correlation of photosynthesis to CUO for data within the category and are not included if the relationship is not significant (NS). The r^2 is calculated for each regression, and p values designate whether the relationship between photosynthesis and CUO for data within the category is statistically significant. P values are considered significant at $p = 0.05$, and significant values are indicated with *.

Charcoal-filtered air, medium or high confidence data: photosynthesis	n	Mean	p value	Regression	r^2	p value
All data	345	82.1	< 0.001*	$84.34 - 0.10$*x	0.02	0.01*
Plant type						
Crop	134	77.22	0.05*	$80.21 - 0.09$*x	0.08	< 0.001*
Evergreen shrub	0	NA	NA	NA	NA	NA
Grasses (C_3 and C_4)	8	80.18	0.87	NS	0.27	0.18
Herbaceous	41	83.27	0.8	NS	0.04	0.2
Temperate deciduous tree	113	87.52	0.22	NS	0.003	0.58
Temperate evergreen tree	47	83.9	0.66	NS	0.08	0.06
Tropical tree	2	44.13	0.19	NA	NA	NA
Plant age (years)						
< 1	234	79.71	0.29	$82.55 - 0.11$*x	0.06	< 0.001*
1–5	95	89.14	0.18	NS	0.002	0.64
> 5	7	81.41	0.93	NS	0.01	0.8
Exposure system						
Greenhouse	24	76.38	0.08	NS	0.08	0.18
Branch chamber	18	88.68	0.07	NS	0.12	0.16
Growth chamber	157	83.54	0.69	NS	0.00002	0.96
Open-top chamber	146	80.68	0.59	$84.48 - 0.11$*x	0.08	< 0.001*
Free-air enrichment	NA	NA	NA	NA	NA	NA
Rooting Environment						
Pot	271	81.64	0.87	$83.55 - 0.09$*x	0.01	0.05*
Ground	65	85.63	0.2	$91.74 - 0.19$*x	0.17	< 0.001*
Vulnerability						
Low	58	86.19	0.34	NS	0.01	0.42
High	135	81.52	0.88	NS	0.01	0.16

(Table 3). O_3 concentration also did not influence the correlations between CUO and photosynthesis or stomatal conductance (Fig. 5).

When plant functional types were considered, mean photosynthesis was lower in crops (Table 2; $p = 0.05$) but did not differ among other plant functional types. Crops are the only plant functional type where photosynthesis was significantly, though weakly, correlated with CUO (Table 2, Fig. 6c; $r^2 = 0.08$, $p < 0.001$). Mean stomatal conductance was significantly higher in temperate deciduous trees (Table 3; $p = 0.02$) and was significantly lower in crops (Table 3; $p = 0.007$), as compared to the overall mean. Stomatal conductance was positively correlated with CUO in temperate evergreen trees (Table 3, Fig. 6e; $r^2 = 0.32$, $p < 0.001$), but correlations were not significant for other plant types. Data pooled by O_3 concentrations demonstrated similar patterns (Fig. 7), with no strong correlations between O_3 concentration and photosynthesis or stomatal conductance in any plant functional type. Data presented in Figs. 6 and 7 focused only on plant functional types with abundant data and more than three publications reporting values (see Table 1), including temperate deciduous trees, crops and temperate evergreen trees (39, 35, and 13 % of the total data subset, respectively).

Table 3. The number of data points (n), mean, regression, and statistics for stomatal conductance when sorted by categories of plant type, plant age, O_3 exposure system, rooting environment, and vulnerability for all data that is compared to control plants exposed to charcoal-filtered air with medium or high confidence in cumulative O_3 uptake (CUO) calculations. All means are reported as the percent of control treatment. The p value for the overall data set (All data) designates whether the overall mean is significantly different from 100 (i.e., 0 % change). The p value for the mean within each category designates whether the mean of the category is statistically different from the mean of the overall data set (All data). All regressions are based on the correlation of stomatal conductance to CUO for data within the category and are not included if the relationship is not significant (NS). The r^2 is calculated for each regression, and p values designate whether the relationship between stomatal conductance and CUO for data within the category is statistically significant. P values are considered significant at $p = 0.05$, and significant values are indicated with *.

Charcoal-filtered air, medium or high confidence Data: Condutance	n	Mean	p value		Regression	r^2	p value
All data	393	84.44	< 0.001*	NS	0.0006	0.63	
Plant type							
Crop	136	75.11	0.007*	NS	0.005	0.43	
Evergreen shrub	0	NA	NA	NA	NA	NA	
Grasses (C_3 and C_4)	8	89.15	0.53	NS	0.2	0.27	
Herbaceous	41	88.19	0.37	NS	0.02	0.33	
Temperate deciduous tree	153	91.25	0.02*	NS	0.0001	0.9	
Temperate evergreen tree	53	86.45	0.54	$78.23 + 0.48^*x$	0.32	< 0.001*	
Tropical tree	2	48.3	0.17	NA	NA	NA	
Plant age (years)							
< 1	236	82.02	0.36	NS	0.00001	0.93	
1–5	133	89.9	0.08	$84.95 + 0.33^*x$	0.05	0.009*	
> 5	15	79.56	0.6	$108.37 - 3.14^*x$	0.34	0.02*	
Exposure system							
Greenhouse	30	89.1	0.31	NS	0.02	0.43	
Branch chamber	18	90.97	0.05*	NS	0.17	0.09	
Growth chamber	163	82.69	0.62	$74.25 + 0.57^*x$	0.12	< 0.001*	
Open-top chamber	182	84.59	0.95	$86.69 - 0.07^*x$	0.03	0.02*	
Free-air enrichment	NA	NA	NA	NA	NA	NA	
Rooting environment							
Pot	310	84.14	0.9	NS	0.0008	0.61	
Ground	74	86.8	0.39	NS	0.004	0.61	
Vulnerability							
Low	106	91.11	0.13	$78.24 + 1.13^*x$	0.23	< 0.001*	
High	135	79.14	0.04*	NS	0.004	0.49	

Very few studies (9 of 108) contained data for other plant functional types (Table 1).

Since correlations were either weak or not evident in plant functional types within the data set that removed both low-confidence data and studies using ambient air, smaller, more specific subsets were created. Due to the small number of data that fall within each category, the subsets focused on categories with the largest number of data: temperate deciduous trees and crops grown in pots that were exposed to 100–125 ppb O_3. Within this subset, photosynthesis and stomatal conductance in temperate deciduous trees that were 1 to 5 yr old were not correlated to CUO (photosynthesis: Fig. 8a, $r^2 = 0.02$, $p = 0.40$; stomatal conductance: Fig. 8c, $r^2 = 0.003$, $p = 0.71$). Similarly, photosynthesis in crops less than one year old (grown in pots, exposed to 100–125 ppb O_3) was not correlated with CUO (Fig. 8b, $r^2 = 0.004$, $p = 0.53$). However, stomatal conductance for the same subset of crops was positively correlated with CUO (Fig. 8d, $r^2 = 0.29$, $p = 0.004$).

Though our generalized responses largely do not demonstrate negative correlations between photosynthesis and CUO, several individual studies report negative correlations between photosynthesis and CUO. To determine how easily the negative relationship was lost, data from a single study

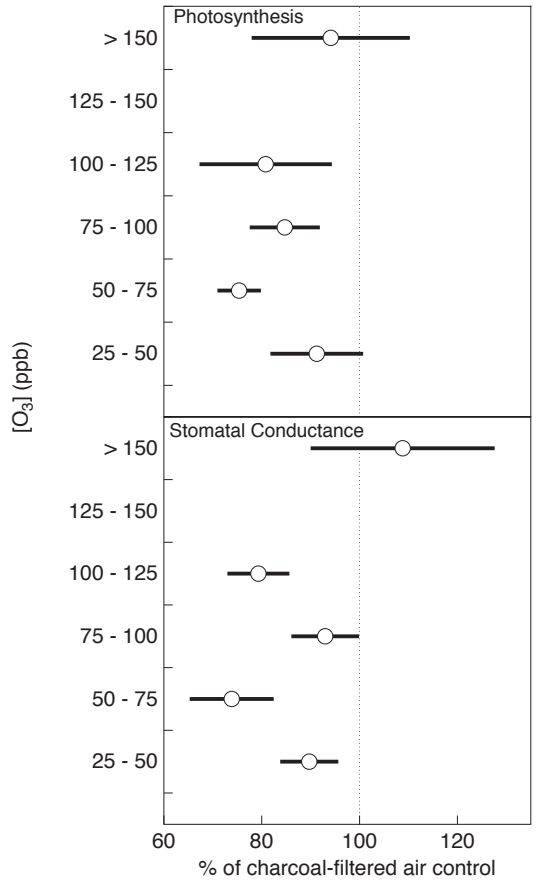

Fig. 5. Mean photosynthesis and stomatal conductance responses for plants exposed to chronic O_3 relative to control plants exposed to charcoal-filtered air, grouped by the O_3 concentration during exposure. Responses values are reported as % of control (treatment/control) and are plotted with 95 % confidence intervals.

was considered, and additional data added sequentially. In a single study measuring the response of wheat to chronic O_3 exposure that reported CUO values (plants > 1 yr old were grown in pots, compared to CF air, and exposed to 100 ppb O_3 in a growth chamber), photosynthesis and stomatal conductance were both negatively correlated with CUO (Fig. 9, open circles; photosynthesis: $r^2 = 0.59$, $p < 0.001$; stomatal conductance: $r^2 = 0.20$, $p = 0.05$). When other studies measuring responses of wheat to O_3 under similar conditions (plants > 1 yr old were grown in pots, compared to CF air, and exposed to 100 ppb O_3 in a growth chamber) are included, there is no correlation between photosynthesis and CUO (Fig. 9a; $p = 0.59$) and the correlation between stomatal conductance and CUO becomes positive (Fig. 9b; $r^2 = 0.20$, $p = 0.002$).

4 Discussion

Chronic O_3 exposure causes the plant physiological processes of photosynthesis and stomatal conductance to change, though how these processes change through time is not well known for many plant functional types. The results of this analysis suggest that chronic O_3 exposure depressed leaf-level photosynthesis and stomatal conductance in all plant types, ages, rooting environments and estimated sensitivities, though the variability within these data was too large to determine relationships with CUO. Overall, the 21 % average decrease in photosynthesis was larger than the 11 % average decrease in stomatal conductance, demonstrating that these variables respond differently to chronic O_3 exposure. A meta-analysis of tree responses to O_3 compared to charcoal-filtered air found a 19 % decrease in photosynthesis and a 10 % decrease in stomatal conductance (Wittig et al., 2007), similar to the means reported here. The difference between average photosynthesis (−18 %) and average stomatal conductance (−16 %) was less dramatic when low-confidence data and comparisons to ambient air were removed from the data set, but accounting for these factors did not strongly improve the correlations of either variable with CUO. This work additionally found that decreases in photosynthesis were typically larger than the decreases in stomatal conductance in most plant type, age, rooting environment, exposure system, O_3 concentration, and estimated vulnerability categories, though the magnitude of decrease in each variable differed based on the category.

To our knowledge, this is the first study that widely describes the relationship of stomatal conductance to chronic O_3 exposure through time, despite the known importance of stomatal conductance responses to O_3 on ecosystem-scale water dynamics like soil water content and stream flow (McLaughlin et al., 2007). This study found that stomatal conductance decreased less than photosynthesis after chronic O_3 exposure, but, unexpectedly, there was no strong correlation between stomatal conductance or photosynthesis and CUO in the overall data or in data subsets (Fig. 2, Tables 2 and 3). The only physiological responses that can be easily generalized from these data are that photosynthesis and stomatal conductance decrease after chronic O_3 exposure, suggesting that models incorporating negative correlations between photosynthesis and O_3 need to reconsider the generality of their responses. While calculations of CUO were not independent of stomatal conductance, there was also no consistent decrease in stomatal conductance based on O_3 concentration (Fig. 5), similar to results found by Wittig et al. (2007). Ozone concentration also did not affect the decreases in photosynthesis in a systematic manner (Fig. 5) in our study or in results found by Wittig et al. (2007).

Initial O_3 damage usually decreases enzyme activity and RuBP regeneration (Fiscus et al., 2005) and is apparent in V_{cmax} and J_{max} reductions after chronic O_3 exposure. Accounting for decreases in V_{cmax} and/or J_{max} is one

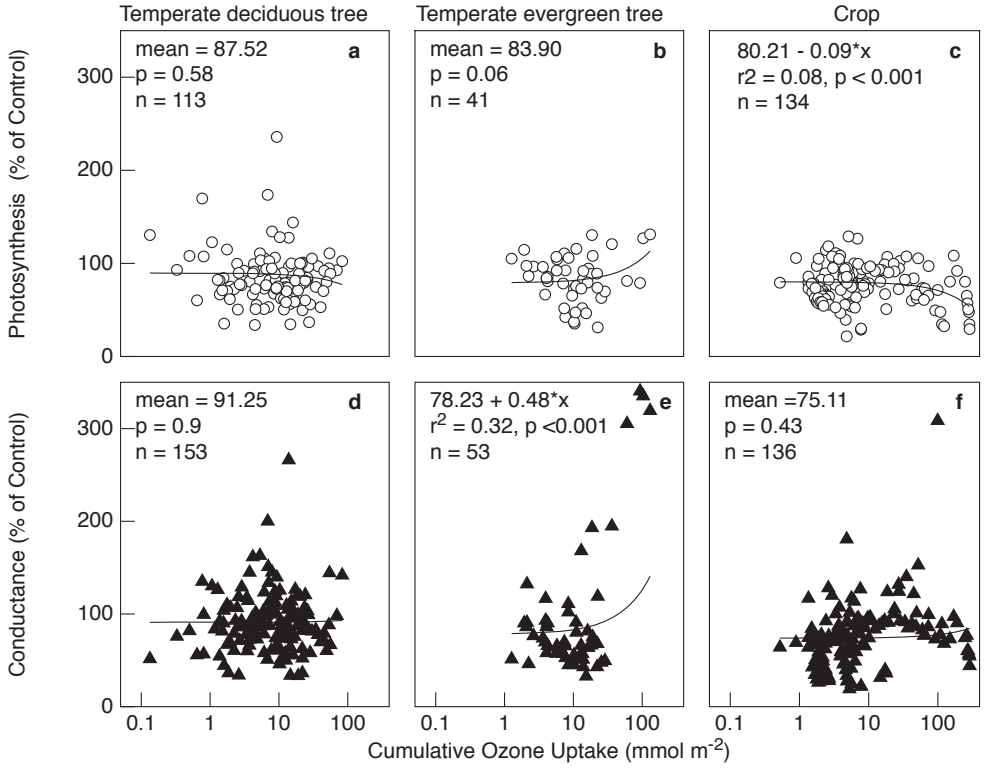

Fig. 6. The correlation of photosynthesis (open symbols; **a–c**) and conductance (closed symbols; **d–f**) to CUO for temperate deciduous trees **(a, d)**, temperate evergreen trees **(b, e)** and crops **(c, f)** for all data that is compared to control plants exposed to charcoal-filtered air with medium or high confidence in cumulative O_3 uptake (CUO) calculations. Response values are reported as % of control (treatment/control). CUO is shown on a log scale, but linear analyses (r^2 and line equations) were performed on non-log transformed data. r^2 values are only included for significant correlations, and n values are the number of data points included in the analyses.

mechanistic approach that physiological models can use to estimate O_3 damage to photosynthesis (Martin et al., 2000; Lombardozzi et al., 2012b), though little data are available to parameterize models in this way. Here, we chose to focus on decreases in V_{cmax} due to the higher availability of data. In the available data, average V_{cmax} followed similar trends as in photosynthesis and decreased in response to chronic O_3, similar to V_{cmax} measured in other studies (Cardosa-Vilhena et al., 2004; Calatyud et al., 2010; Kellomäki et al., 1997; Löw et al., 2007), though was not significantly correlated with CUO (Fig. 3). Cardosa-Vilhena et al. (2004) likewise found that decreases in V_{cmax} were not linearly correlated with CUO in wheat leaves exposed to O_3. Responses of V_{cmax} were not significantly different from photosynthetic responses, suggesting that either can be used to estimate photosynthetic decreases. However, photosynthesis responses can be used in models with more confidence given the lack of data estimating responses of V_{cmax} to chronic O_3 exposure.

Although we observed a similar overall decrease in photosynthesis, the lack of correlation between photosynthesis and CUO was different from the strong negative correlations found by Reich (1987) and Wittig et al. (2007) and was contrary to parameterizations used in many models incorporating the effects of O_3 on leaf-level physiology (e.g. Ollinger et al., 1997; Ollinger et al., 2002; Felzer et al., 2004; Felzer et al., 2005; Sitch et al., 2007; Ren et al., 2011). Demography of the data might play a role in the difference as the present study had a larger sample size ($n = 670$) and incorporated multiple types of plants, though temperate trees (evergreen and deciduous) comprised 58 % of these data and correlations were weak or non-existent when separated by plant type. While the methods used to calculate CUO were similar in all studies, the present study imposed strict standards on collected data to maintain confidence in CUO calculations and therefore rejected some data that were used by Wittig et al. (2007). It also seems likely that the differences between these studies is in part because the method used in the present study for calculating CUO accounted for changes in stomatal conductance through time whenever possible and resulted in a strong correlation with CUO presented in publications (Fig. B1, $r^2 = 0.92$), which was not factored into CUO calculations in other studies.

Most studies reporting responses of photosynthesis and stomatal conductance to chronic O_3 exposure do not provide CUO or fine-scale measurements of stomatal conductance to allow for accurate estimations of CUO, making it difficult to

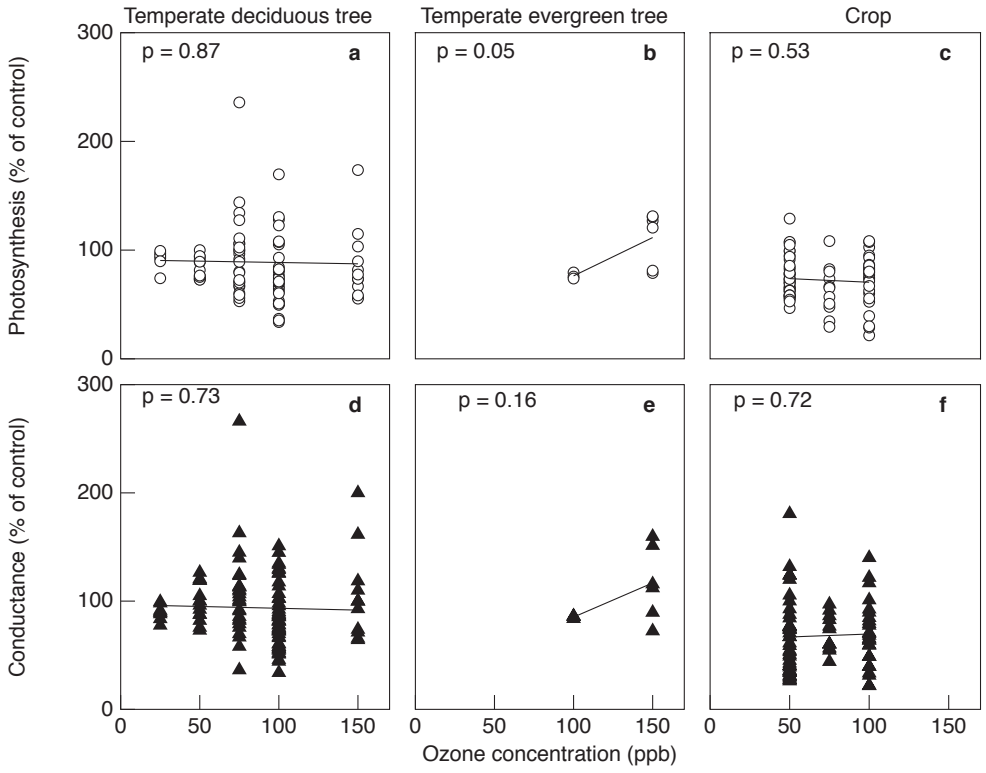

Fig. 7. The correlation of photosynthesis (open symbols; **a–c**) and conductance (closed symbols; **d–f**) to 25 ppb ozone concentration bins for temperate deciduous trees **(a, d)**, temperate evergreen trees **(b, e)** and crops **(c, f)** for all data that is compared to control plants exposed to charcoal-filtered air with medium or high confidence in cumulative O_3 uptake (CUO) calculations. Response values are reported as % of control (treatment/control). r^2 values are only included for significant correlations, and n values are the number of data points included in the analyses.

document responses of photosynthesis and stomatal conductance over a broad range of CUO. While statistical and modeling techniques have improved the accuracy of CUO by simulating hourly stomatal conductance values that can be incorporated into CUO calculations, (e.g. Grulke et al., 2002; Pleijel et al., 2002; Karlsson et al., 2004; Pleijel et al., 2004; Uddling et al., 2004), those techniques could not be employed in the present study due to the limitation of available environmental data within the studies. Though the accuracy of CUO calculations can be improved by accounting for hourly stomatal conductance and O_3 concentration, our methods of calculating CUO were strongly correlated with studies that presented CUO within their publication ($r^2 = 092$; Fig. B1), suggesting that any error in CUO calculations was systematic and did not influence correlations. Even combining the few studies that provided CUO values (high-confidence data) did not result in correlations between CUO and stomatal conductance or photosynthesis (Fig. 4). To improve the confidence of our findings, however, further analyses focus on a subset of data that contain only high- and medium-confidence data so that all CUO calculations account for the changes in stomatal conductance through time.

Plant functional type did not have a strong effect on the relationship of photosynthesis or stomatal conductance with either CUO (Fig. 6) or the O_3 concentration that plants were exposed to (Fig. 7). Wittig et al. (2007) found photosynthesis decreases that were similar in both angiosperms and gymnosperms in experiments that artificially increased O_3 concentration compared to charcoal-filtered control air, similar to the mean decreases found in this study ($p = 0.66$). Further, Reich and Amundson (1985) found that crop photosynthesis was more negatively affected than temperate evergreen trees after chronic O_3 exposure, also similar to our results ($p = 0.05$). Data used in this analysis additionally suggest that the decrease in mean crop stomatal conductance was 16 % larger than stomatal conductance in temperate deciduous trees. This result is consistent with responses of stomatal conductance determined in meta-analyses of multiple tree types and wheat; tree stomatal conductance decreased by 10 % (Wittig et al., 2007) and wheat stomatal conductance decreased by 22 % (Feng et al., 2008).

Manipulating the atmosphere around adult plants is difficult, making it hard to assess physiological responses of mature plants to chronic O_3 exposure. Given these challenges, literature data are most scarce for mature trees (in plants

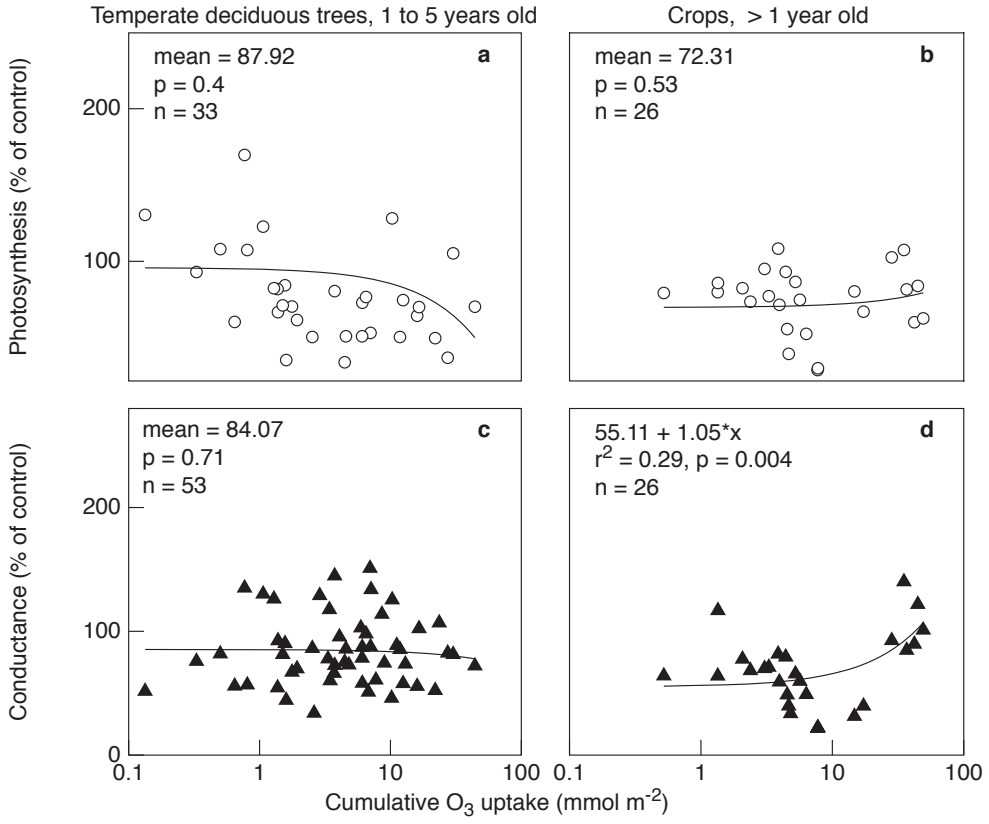

Fig. 8. The correlation of photosynthesis (open symbols; **a–b**) and conductance (closed symbols; **c–d**) to CUO for all temperate deciduous trees between one and five years old **(a, c)** and crops less than one year old **(b, d)** to chronic O_3 exposure for all plants grown in pots, exposed to 100–125 ppb O_3 in growth chambers, and are compared to control plants exposed to charcoal-filtered air with medium or high confidence in cumulative O_3 uptake (CUO) calculations. Response values are reported as % of control (treatment/control). CUO is shown on a log scale, but linear analyses (r^2 and line equations) were performed on non-log transformed data. r^2 values are only included for significant correlations, and n values are the number of data points included in the analyses.

older than 5 yr, stomatal conductance $n = 15$, Table 3; photosynthesis $n = 7$, Table 2), which make comparisons across plant ages challenging and modeling responses of different age classes uncertain. Within the published literature, a few studies found that O_3 decreased photosynthesis in mature trees more than juvenile trees (Edwards et al., 1994; Rebbeck et al., 1993), while Grulke and Miller (1994) conversely found that sensitivity to O_3 decreases as the age of trees increases. Results in the present study, which are generalized over several plant species, demonstrate no overall photosynthetic differences between mature and juvenile trees in response to O_3 (Table 2, $p = 0.93$), similar to measured responses in Ponderosa pine trees (Momen et al., 1997). Though photosynthetic correlations with CUO were not significant in mature plants (i.e., older than 5 yr; $p = 0.8$), similar to responses of mature giant sequoia (Grulke et al., 1996), stomatal conductance in mature plants decreased with CUO ($r^2 = 0.34$, $p = 0.02$). This strong negative relationship contrasts with the variable mean stomatal responses of mature trees documented by Karnosky et al. (2007) and suggests that CUO can help clarify stomatal responses in mature trees.

Though several individual studies find negative correlations between photosynthesis and CUO, driving the assumption in models that photosynthesis decreases linearly with CUO, the relationship is lost when generalizing across plant species (Fig. 8) and even within the same plant species (Fig. 9) when plant age, O_3 concentration, rooting environment and exposure system were similar. These results demonstrate that correlations with CUO can be difficult to document, even within a seemingly narrow range of variability. For example, photosynthesis in temperate deciduous trees from 1 to 5 yr old, and in crops that were less than one year old was not correlated with CUO (Fig. 8a and b; $p = 0.4$, 0.53). However, stomatal conductance was positively correlated with CUO in crops less than one year old (Fig. 8d; $r^2 = 0.29$, $p = 0.004$), suggesting that the mechanisms regulating stomatal closure in crops become damaged with chronic O_3 exposure, causing stomatal conductance to increase (Freer-Smith and Dobson, 1989; Hassan et al., 1994;

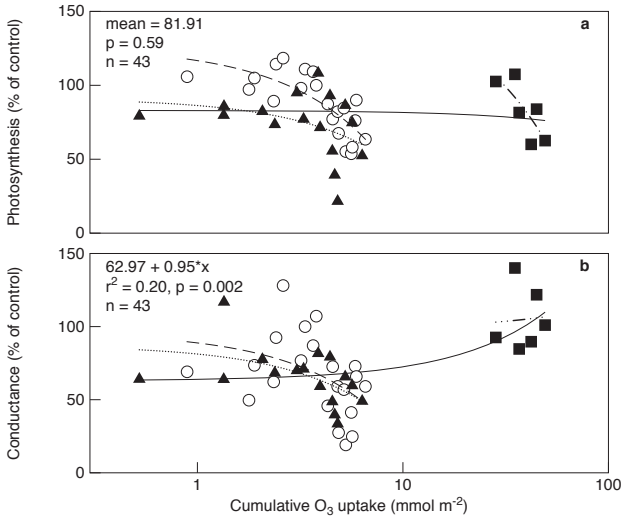

Fig. 9. The correlation of photosynthesis **(a)** and stomatal conductance **(b)** to CUO from all studies that measured responses of wheat plants grown in pots exposed to 100 ppb O_3 in growth chambers and compared to control plants exposed to charcoal-filtered air. CUO is shown on a log scale, but linear analyses (r^2 and line equations) were performed on non-log transformed data. Different symbols represent different studies. Initial data considered (open circles and dashed line) were from a single study and demonstrated negative correlations between CUO and both photosynthesis ($r^2 = 0.59$, $p < 0.001$) and conductance ($r^2 = 0.19$, $p = 0.05$). Adding data from other studies measuring the responses of wheat plants to O_3 in similar environments (closed symbols) resulted in no overall correlation with CUO, though overall correlations exist in some individual studies (photosynthesis: triangles and dotted line, $r^2 = 0.15$, $p = 0.15$; squares and dot-dashed line, $r^2 = 0.61$, $p = 0.06$; conductance: triangles and dotted line, $r^2 = 0.28$, $p = 0.03$; squares and dot-dashed line, $r^2 = 0.004$, $p = 0.91$). The text in the figure and the solid regression line summarizes the information for all data points included in the figure. r^2 values are only included for overall significant correlations, and n values are the number of data points included in the analyses.

Maiermaercker and Koch, 1991; Manes et al., 1998, 2001; McLaughlin et al., 2007; Mills et al., 2009). The trends are similar within data sets that are even more specific. Though all photosynthesis data used in Fig. 9a decreased in response to O_3, data from each study was not necessarily negatively correlated with CUO. Two of the studies were conducted by the same author and reported CUO values in the manuscript (circles and triangles in Fig. 9), though photosynthesis was significantly correlated with CUO in only one of these studies (circles, Fig. 9a). While generalized negative correlations between photosynthesis and CUO might have been evident if only studies with negative correlations between photosynthesis and CUO were used, handpicking data in this fashion can lead to biased responses that, when used in models, do not accurately represent generalized responses of plants to chronic O_3 exposure.

Correlations of CUO with photosynthesis or stomatal conductance were not evident within refined data sets that accounted for plant age, type, rooting environment, O_3 concentration, and exposure system (Figs. 8 and 9), although Karlsson et al. (2007) found negative correlations between biomass and CUO above a threshold of 1.6 mmol O_3 m^{-2} in three species of temperate deciduous trees. While direct comparisons for decreases in biomass and photosynthesis cannot be made, the strong negative correlations found by Karlsson et al. (2007) suggest that including an O_3 threshold, which was not possible in this study due to limitation of available data, might improve correlations with CUO. However, Wittig et al. (2007) found negative correlations between photosynthesis and CUO without accounting for an O_3 threshold. Additionally, changes in biomass reflect the whole-plant response to O_3 exposure, whereas measurements of photosynthesis, the focus of this study, only capture leaf-level responses. Ozone-induced plant-level phenological changes, such as the observed linear decreases in LAI with chronic O_3 exposure (Betzelberger et al., 2012), possibly contribute to the difference in plant-level compared to leaf-level response to O_3.

In addition to its role as a greenhouse gas, O_3 is likely to cause indirect changes in climate (Sitch et al., 2007) through altering carbon and water exchange between the biosphere and atmosphere, yet our ability to predict these responses on ecosystem or global scales is inadequate for several reasons. First, our understanding of plant responses is limited to temperate and crop ecosystems, which cover only 25 % of earth's land surface (Grace, 2004), leading to large uncertainty in global predictions. In the current literature, only three studies report data for tropical tree species or herbaceous plants, and two or fewer studies report data for grasses, and deciduous and evergreen shrubs (Table 1). The lack of available data for these ecosystems forces regional and global models (e.g. Felzer et al., 2004; Sitch et al., 2007) to base responses of tropical and grassland ecosystems on temperate tree and crop data (e.g., Reich, 1987; Karlsson et al., 2004; Pleijel et al., 2004). Further, most models incorporating plant responses to O_3 assume that photosynthesis declines linearly with CUO (e.g. Ollinger et al., 1997; Ollinger et al., 2002; Felzer et al., 2004; Felzer et al., 2005; Sitch et al., 2007; Ren et al., 2011), although the results of this study suggest this assumption is not accurate when generalized across several plant species. Finally, the common assumption that stomatal conductance declines linearly with photosynthesis should also be reconsidered since the responses of 750+ independent measurements collected in this study largely suggests that photosynthesis and stomatal conductance do not change at the same rate or with the same magnitude during chronic O_3 exposure. Simulating robust responses of both photosynthesis and stomatal conductance to chronic O_3 exposure using data collected by this study, rather than responses of a single or few studies, will improve the accuracy of carbon, water, and climate predictions. Nevertheless, global responses

to O_3, particularly for grassland and tropical forest ecosystems, will remain uncertain until more data are collected.

In conclusion, both photosynthesis and stomatal conductance decrease in response to chronic O_3 exposure, but there is little ability to predict a generalized response of photosynthesis or stomatal conductance through time. Relationships might be improved if future studies report physiological data in conjunction with CUO that is calculated based on fine-resolution O_3 concentrations and stomatal conductance measurements. Models can continue to use data that contain negative correlations between photosynthesis and CUO, though these responses are not necessarily representative of all plant responses, even within a single species. Data presented here suggest that studies using regional and global models, which generalize many plant physiological parameters, should also consider using generalized physiological responses to O_3 based on the modeled plant functional types. Ultimately, scientists can select data that best represents the needs of the model from data subsets included in this analysis, whether it is a generalized response, responses for particular plant types, or plants of a particular age, and allow the models to incorporate responses of photosynthesis and stomatal conductance independently (see methods in Lombardozzi et al., 2012b).

Additionally, data suggest that linear changes with CUO should be made when there are strong correlations, but do not support applying a linear relationship when there is no correlation between a variable and CUO. Including linear decreases in photosynthesis where data do not support this trend can possibly overestimate the decreases in the global land-carbon sink in the long term, and potentially underestimate the decreases in the short term. Instead, when correlations with CUO do not exist, models should reduce each variable by a specific proportion at every model time step based on the appropriate plant functional type data, regardless of CUO. Though these analyses were not able to incorporate any O_3 concentration or CUO threshold, the use of a threshold can be developed within a model to tune the predicted responses to match observations. Adjusting modeled values of photosynthesis and stomatal conductance independently using generalized data such as these allows for models to better capture the physiological responses of plants to chronic O_3 exposure and can potentially improve the accuracy of predicted responses in global carbon and hydrologic cycles.

Supplementary material related to this article is available online at http://www.biogeosciences.net/10/6815/2013/bg-10-6815-2013-supplement.zip.

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References

Ashmore, M.: Assessing the future global impacts of ozone on vegetation, Plant Cell Environ., 28, 949–964, 2005.

Bernacchi, C. J., Leakey, A. D. B., Kimball, B. A., and Ort, D. R.: Growth of soybean at future tropospheric ozone concentrations decreases canopy evapotranspiration and soil water depletion, Environ. Pollut, 159, 1464–1472, 2011.

Betzelberger, A. M., Yendrek, C. R., Sun, J., Leisner, C. P., Nelson, R. L., Ort, D. R., and Ainsworth, E. A.: Ozone Exposure Response for U.S. Soybean Cultivars: Linear Reductions in Photosynthetic Potential, Biomass, and Yield, Plant Physiol., 160, 1827–1839, 2012.

Bortier, K., Ceulemans, R., and De Temmerman, L.: Effects of ozone exposure on growth and photosynthesis of beach seedlings (*Fagus sylvatica*), New Phytol., 146, 271–280, 2000.

Calatayud, V., Cerveró, J., and Sanz, M. J.: Foliar, physiologial and growth responses of four maple species exposed to ozone, Water Air Soil Pollut., 185, 239–254, 2007.

Calatayud, V., Marco, F., Cerveró, J., Sánchez-Peña, G., and Sanz, M. J.: Contrasting ozone sensitivity in related evergreen and deciduous shrubs, Environ. Pollut., 158, 3580–3587, 2010.

Cardoso-Vilhena, J., Balaguer, L., Eamus, D., Ollerenshaw, J., and Barnes, J.: Mechanisms underlying the amelioration of O_3-induced damage by elevated atmospheric concentrations of CO_2, J. Experiment. Botany, 55, 771–781, 2004.

Denman, K., Brasseur, G., Chidthaisong, A., Ciais, P., Cox, P. M., Dickinson, R. E., Hauglustaine, D., Heinze, C., Holland, E., Jacob, D., Lohmann, U., Ramachandram, S., da Silva Dias, P. L., Wofsy, S. C., and Zhang, X.: Couplings between changes in the climate system and biogeochemistry, in: 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, New York, NY, 2007.

Dizengremel, P., Le Thiec, D., Bagard, M., and Jolivet, Y.: Ozone risk assessment for plants: Central role of metabolism-dependent changes in reducing power, Environ. Pollut., 156, 11–15, 2008.

Edwards, G. S., Wullschleger, S. D., and Kelly, J. M.: Growth and physiology of northern red oak: Preliminary comparisons of mature tree and seedling responses to ozone, Environ. Pollut., 83, 215–221, 1994.

Emberson, L. D., Ashmore, M. R., Cambridge, H. M., Simpson, D., and Tuovinen, J. P.: Modelling stomatal ozone flux across Europe, Environ. Pollut., 109, 403–413, 2000.

Farage, P. K. and Long, S. P.: The effects of O_3 fumigation during leaf development on photosynthesis of wheat and pea: An in vivo analysis, Photosyn. Res., 59, 1–7, 1999.

Felzer, B., Kicklighter, D., Melillo, J., Wang, C., Zhuang, Q., and Prinn, R.: Effects of ozone on net primary production and carbon sequestration in the conterminous United States using a biogeochemistry model, Tellus B, 56, 230–248, 2004.

Felzer, B., Reilly, J., Melillo, J., Kicklighter, D., Sarofim, M., Wang, C., Prinn, R., and Zhuang, Q.: Future effects of ozone on carbon sequestration and climate change policy using a global biogeochemical model, Climatic Change, 73, 345–373, 2005.

Felzer, B. S., Cronin, T. W., Melillo, J. M., Kicklighter, D. W., and Schlosser, C. A.: Importance of carbon-nitrogen interactions and ozone on ecosystem hydrology during the 21st century, J. Geophys. Res., 114, G01020, doi:10.1029/2008JG000826, 2009.

Feng, Z., Kobayashi, K., and Ainsworth, E. A.: Impact of elevated ozone concentration on growth, physiology, and yield of wheat (*Triticum aestivum* l.): A meta-analysis, Glob. Change Biol., 14, 2696–2708, 2008.

Fiscus, E., Booker, F., and Burkey, K.: Crop responses to ozone: Uptake, modes of action, carbon assimilation and partitioning, Plant Cell Environ., 28, 997–1011, 2005.

Francini, A., Nali, C., Picchi, V., and Lorenzini, G.: Metabolic changes in white clover clones exposed to ozone, Environ. Exp. Bot., 60, 11–19, 2007.

Freer-Smith, P. H. and Dobson, M. C.: Ozone flux to *Picea sitchensis* (bong) *carr* and *Picea abies* (l) *karst* during short episodes and the effects of these on transpiration and photosynthesis, Environ. Pollut., 59, 161–176, 1989.

Grace, J.: Understanding and managing the global carbon cycle, J. Ecol., 92, 189–202, 2004.

Grulke, N. E. and Miller, P. R.: Changes in gas exchange characteristics during the life span of giant sequoia: implications for response to current and future concentrations of atmospheric ozone, Tree Physiol., 14, 659–668, 1994.

Grulke, N. E., Miller, P. R., and Scioli, D.: Response of giant sequoia canopy foliage to elevated concentrations of atmospheric ozone, Tree Physiol., 16, 575–581, 1996.

Grulke, N. E., Preisler, H. K., Fan, C. C., and Retzlaff, W. A.: A statistical approach to estimate O_3 uptake of ponderosa pine in a Mediterranean climate, Environ. Pollut., 119, 163–175, 2002.

Hassan, I. A., Ashmore, M. R., and Bell, J. N. B.: Effects of O_3 on the stomatal behavior of Egyptian varieties of radish (*Raphanus sativus* l cv *baladey*) and turnip (*Brassica rapa* l cv *sultani*), New Phytol., 128, 243–249, 1994.

Heagle, A. S., Reinert, R. A., and Miller, J. E.: Response of white clover to ozone in different environments, J. Environ. Qual., 25, 273–278, 1996.

Herbinger, K., Then, C., Haberer, K., Alexou, M., Low, M., Remele, K., Rennenberg, H., Matyssek, R., Grill, D., Wieser, G., and Tausz, M.: Gas exchange and antioxidative compounds in young beech trees under free-air ozone exposure and comparisons to adult trees, Plant Biol., 9, 288–297, 2007.

Jacob, D. and Winner, D.: Effect of climate change on air quality, Atmos. Environ., 43, 51–63, 2009.

Karlsson, P., Uddling, J., Braun, S., Broadmeadow, M., Elvira, S., Gimeno, B. S., Le Thiec, D., Oksanen, E., Vandermeiren, K., Wilkinson, M., and Emberson, L.: New critical levels for ozone effects on young trees based on AOT40 and simulated cumulative leaf uptake of ozone, Atmos. Environ., 38, 2283–2294, 2004.

Karnosky, D. F., Skelly, J. M., Percy, K. E., and Chappelka, A. H.: Perspectives regarding 50 years of research on effects of tropospheric ozone air pollution on US forests, Environ. Pollut. 147, 489–506, 2007.

Karnosky, D., Pregitzer, K. S., Zak, D. R., Kubiske, M. E., Hendrey, G.R., Weinsten, D., Nosal, M., and Percy, K. E.: Scaling ozone responses of forest trees to the ecosystem in a changing climate, Plant Cell Environ., 28, 965–981, 2005.

Kellomäki, S. and Wang, K. Y.: Effects of elevated O_3 and CO_2 concentrations on photosynthesis and stomatal conductance in Scots pine, Plant Cell Environ., 20, 995–1006, 1997.

Lippert, M., Steiner, K., Payer, H. D., Simons, S., Langebartels, C., and Sandermann, H.: Assessing the impact of ozone on photosynthesis of European beech (*Fagus sylvatica* l) in environmental chambers, Trees, 10, 268–275, 1996.

Lombardozzi, D., Sparks, J. P., Bonan, G., and Levis, S.: Ozone exposure causes a decoupling of conductance and photosynthesis: Implications for the ball-berry stomatal conductance model, Oecologia, 169, 651–659, 2012a.

Lombardozzi, D., Levis, S., Bonan, G., and Sparks, J. P.: Predicting photosynthesis and transpiration responses to ozone: decoupling modeled photosynthesis and stomatal conductance, Biogeosciences, 9, 3113–3130, doi:10.5194/bg-9-3113-2012, 2012b.

Löw, M., Haeberle, K. H., Warren, C. R., and Matyssek, R.: O_3 flux-related responsiveness of photosynthesis, respiration, and stomatal conductance of adult *Fagus sylvatica* to experimentally enhanced free-air O_3 exposure, Plant Biol., 9, 197–206, 2007.

Maier-Maercker, U. and Koch, W.: Experiments on the control capacity of stomata of *Picea abies* (l) karst after fumigation with ozone and in environmentally damaged material, Plant Cell Environ., 14, 175–184, 1991.

Martin, M., Farage, P., Humphries, S., and Long, S.: Can the stomatal changes caused by acute ozone exposure be predicted by changes occurring in the mesophyll? A simplification for models of vegetation response to the global increase in tropospheric elevated ozone episodes, Aust. J. Plant Physiol., 27, 211–219, 2000.

Manes, F., Vitale, M., Donato, E., and Paoletti, E.: O_3 and $O_3 + CO_2$ effects on a Mediterranean evergreen broadleaf tree, holm oak (*Quercus ilex* L.), Chemosphere, 36, 801–806, 1998.

Manes, F., Donato, E., and Vitale, M.: Physiological response of *Pinus halepensis* needles under ozone and water stress conditions, Physiol. Plantarum, 113, 249–257, 2001.

Massman, W. J.: Toward an ozone standard to protect vegetation based on effective dose: A review of deposition resistances and a possible metric, Atmos. Environ., 38, 2323–2337, 2004.

Matyssek, R., Bahnweg, G., Ceulemans, R., Fabian, P., Grill, D., Hanke, D. E., Kraigher, H., Osswald, W., Rennenberg, H., Sandermann, H., Tausz, M., and Wieser, G.: Synopsis of the CASIROZ case study: Carbon sink strength of *Fagus sylvatica* l. in a changing environment – Experimental risk assessment of mitigation by chronic ozone impact, Plant Biol., 9, 163–180, 2007.

Maurer, S., Matyssek, R., Günthardt-Goerg, M. S., Landolt, W., and Einig, W.: Nutrition and the ozone sensitivity of birch (*Betula pendula*) .1. Responses at the leaf level, Trees, 12, 1–10, 1997.

McLaughlin, S. B., Wullschleger, S. D., Sun, G., and Nosal, M.: Interactive effects of ozone and climate on water use, soil moisture content and streamflow in a southern Appalachian forest in the USA, New Phytol., 174, 125–136, 2007.

Mikkelsen, T. N.: Physiological-responses of *Fagus sylvatica* l. exposed to low-levels of ozone in open-top chambers, Trees, 9, 355–361, 1995.

Mills, G., Hayes, F., and Wilkinson, S.: Chronic exposure to increasing background ozone impairs stomatal functioning in grassland species, Global Change Biol., 15, 1522–1533, 2009.

Mills, G., Hayes, F., Simpson, D., Emberson, L., Norris, D., Harmens, H., and Büker, P.: Evidence of widespread effects of ozone on crops and (semi-)natural vegetation in Europe (1990–2006) in relation to AOT40- and flux-based risk maps, Glob. Change Biol., 17, 592–613, 2011.

Momen, B., Anderson, P. D., and Helms, J. A.: Temperature dependency of acid-rain effect on photosynthesis of *Pinus ponderosa*, Forest Ecol. Manag., 113, 223–230, 1999.

Morgan, P., Ainsworth, E., and Long, S.: How does elevated ozone impact soybean? A meta-analysis of photosynthesis, growth and yield, Plant Cell Environ., 26, 1317–1328, 2003.

Morgan, P., Mies, T., Bollero, G., Nelson, R., and Long, S.: Season-long elevation of ozone concentration to projected 2050 levels under fully open-air conditions substantially decreases the growth and production of soybean, New Phytol., 170, 333–343, 2006.

Mortensen, L. M.: Effects of ozone concentration on growth of tomato at various light, air humidity and carbon dioxide levels, Sci. Hortic.-Amsterdam, 49, 17–24, 1992.

Musselman, R. C. and Massman, W. J.: Ozone flux to vegetation and its relationship to plant response and ambient air quality standards, Atmos. Environ., 33, 65–73, 1999.

Noormets, A., Sôber, A., Pell, E. J., Dickson, R. E., Podila, G. K., Sôber, J., Isebrands, J. G., and Karnosky, D. F.: Stomatal and non-stomatal limitation to photosynthesis in two trembling aspen (*Populus tremuloides* michx.) clones exposed to elevated CO_2 and/or O_3, Plant Cell Environ., 24, 327–336, 2001.

Novak, K., Schaub, M., Fuhrer, J., Skelly, J. M., Hug, C., Landolt, W., Bleuler, P., and Krauchi, N.: Seasonal trends in reduced leaf gas exchange and ozone-induced foliar injury in three ozone sensitive woody plant species, Environ. Pollut. 136, 33–45, 2005.

Nunn, A. J., Wieser, G., Reiter, I. M., Häberle, K.H ., Grote, R., Havranek, W. M., and Matyssek, R.: Testing the unifying theory of ozone sensitivity with mature trees of *Fagus sylvatica* and *Picea abies*, Tree Physiol., 26, 1391–1403, 2006.

Ojanperä, K., Patsikka, E., and Ylaranta, T.: Effects of low ozone exposure of spring wheat on net CO_2 uptake, rubisco, leaf senescence and grain filling, New Phytol., 138, 451–460, 1998.

Ollinger, S., Aber, J., and Reich, P.: Simulating ozone effects on forest productivity: Interactions among leaf-, canopy-, and stand-level processes, Ecol. Appl., 7, 1237–1251, 1997.

Ollinger, S., Aber, J., Reich, P., and Freuder, R.: Interactive effects of nitrogen deposition, tropospheric ozone, elevated CO_2 and land use history on the carbon dynamics of northern hardwood forests, Glob. Change Biol., 8, 545–562, 2002.

Paoletti, E.: Ozone slows stomatal response to light and leaf wounding in a Mediterranean evergreen broadleaf, *Arbutus unedo*, Environ. Pollut., 134, 439–445, 2005.

Paoletti, E. and Grulke, N. E.: Ozone exposure and stomatal sluggishness in different plant physiognomic classes, Environ. Pollut., 158, 2664–2671, 2010.

Pellegrini, E., Francini, A., Lorenzini, G., and Nali, C.: PSii photochemistry and carboxylation efficiency in *Liriodendron tulipifera* under ozone exposure, Environ. Exp. Bot., 70, 217–226, 2010.

Pleijel, H., Danielsson, H., Vandermeiren, K., Blum, C., Colls, J., and Ojanperä, K.: Stomatal conductance and ozone exposure in relation to potato tuber yield – results from the European CHIP programme, European J. Agron., 17, 303–317, 2002.

Pleijel, H., Danielsson, H., Ojanperä, K., de Temmerman, L., Högy, P., Badiani, M., and Karlsson, P. E.: Relationships between ozone exposure and yield loss in European wheat and potato – a comparison of concentration- and flux-based exposure indices, Atmos. Environ., 38, 2259–2269, 2004.

Rebbeck, J. and Jensen, K.: Ozone effects on grafted mature and juvenile red spruce: photosynthesis, stomatal conductance, and chlorophyll concentration, Canadian J. Forest Res., 23, 450–456, 1993.

Reich, P. B.: Quantifying plant response to ozone: A unifying theory. Tree Physiol., 3, 63–91, 1987.

Reich, P. B. and Amundson, R. G.: Ambient levels of ozone reduce net photosynthesis in tree and crop species, Science, 230, 566–570, 1985.

Ren, W., Tian, H., Tao, B., Chappelka, A., Sun, G., Lu, C., Liu, M., Chen, G., and Xu, X.: Impacts of tropospheric ozone and climate change on net primary productivity and net carbon exchange of China's forest ecosystems, Global Ecol. Biogeogr., 20, 391–406, 2011.

Sharma, P., Sôber, A., Sôber, J., Podila, G. K., Kubiske, M. E., Mattson, W. J., Isebrands, J. G., and Karnosky, D. F.: Moderation of CO_2-induced gas exchange responses by elevated tropospheric O_3 in trembling aspen and sugar maple, Ekologia (Bratislava), 22, 318–331, 2003.

Sitch, S., Cox, P. M., Collins, W. J., and Huntingford, C.: Indirect radiative forcing of climate change through ozone effects on the land-carbon sink, Nature, 448, 791–794, 2007.

Skärby, L., Troeng, E., and Bostrom, C. A.: Ozone uptake and effects on transpiration, net photosynthesis, and dark respiration in scots pine, Forest Sci., 33, 801–808, 1987.

Soldatini, G. F., Nali, C., Guidi, L., and Lorenzini, G.: Photosynthesis of *Hedera canariensis* var. *Azorica* variegated leaves as affected by ozone, Photosynthetica, 35, 247–253, 1998.

Tjoelker, M. G., Volin, J. C., Oleksyn, J., and Reich, P. B.: Interaction of ozone pollution and light effects on photosynthesis in a forest canopy experiment, Plant Cell Environ., 18, 895–905, 1995.

Torsethaugen, G., Pell, E., and Assmann, S.: Ozone inhibits guard cell K^+ channels implicated in stomatal opening, P. Natl. Acad. Sci., 96, 13577–13582, 1999.

Uddling, J., Günthardt-Goerg, M. S., Matyssek, R., Oksanen, E., Pleijel, H., Selldén, G., and Karlsson, P. E.: Biomass reduction of juvenile birch is more strongly related to stomatal uptake of ozone than to indices based on external exposure, Atmos. Environ., 38, 4709–4719, 2004.

VanLoocke, A., Betzelberger, A. M., Ainsworth, E. A., and Bernacchi, C. J.: Rising ozone concentrations decrease soybean evapotranspiration and water use efficiency whilst increasing canopy temperature, New Phytol., 195, 164–171, 2012.

Volin, J., Reich, P., and Givnish, T.: Elevated carbon dioxide ameliorates the effects of ozone on photosynthesis and growth: Species

respond similarly regardless of photosynthetic pathway or plant functional group, New Phytol., 138, 315–325, 1998.

Wieser, G.: Ozone impact on photosynthetic capacity of mature and young Norway spruce (*Picea abies* (l.) *karst*.): External versus internal exposure, Phyton-Ann. Rei Bot. A, 37, 297–302, 1997.

Wittig, V. E., Ainsworth, E. A., and Long, S. P.: 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 Environ., 30, 1150–1162, 2007.

Wu, S., Mickley, L. J., Leibensperger, E. M., Jacob, D. J., Rind, D., and Streets, D. G.: Effects of 2000–2050 global change on ozone air quality in the United States, J. Geophys. Res., 113, D006302, doi:10.1029/2007JD008917, 2008.

Zheng, Y., Shimizu, H., and Barnes, J. D.: Limitations to CO_2 assimilation in ozone-exposed leaves of plantago major, New Phytol., 155, 67–78, 2002.