WHEN PHYSIOLOGICAL MODELS FAIL: FIXING THE OZONE OXIDATION PROBLEM

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The plant physiological processes of photosynthesis and transpiration control the transfer of carbon dioxide and water, two powerful greenhouse gases between the biosphere and atmosphere and are therefore important in regulating climate. The rates of these processes can significantly change when plants are chronically exposed to surface ozone (O_3), a phytotoxic greenhouse gas that has globally increased in concentration over the past century. Since O_3 is integral in altering plant interactions with the atmosphere, there is a strong motivation to incorporate it into large-scale models. However, current methods incorporating physiological responses to O_3 assume that stomatal conductance changes linearly with photosynthesis despite empirical evidence suggesting otherwise. In chapter one, I developed a physiological modeling framework to modify Ball-Berry stomatal conductance predictions independently of photosynthesis and used experimental data to evaluate the results. The new model framework significantly improved the modeled ability to predict both photosynthesis and stomatal conductance responses to O_3. The second chapter tests the physiological model framework on a global scale using the Community Land Model. I found that the new framework, which directly modifies conductance,

reduces the effect of O_3 on both transpiration and GPP on a global scale compared to the standard modeling method of indirectly modifying conductance by changing photosynthesis. To create a comprehensive dataset available for large-scale modeling, the third chapter compiles photosynthetic and stomatal responses to chronic O_3 exposure through time using data from peer-reviewed literature. The results demonstrate that photosynthesis decreases more than stomatal conductance in many plant functional types, and O_3 affects plant types similarly through time. The fourth chapter combines the new modeling framework from the first two chapters with data from the third chapter to predict global changes in GPP and transpiration due to O_3 exposure. Through changing transpiration, O_3 increases runoff in many temperate and boreal regions and decreases latent heat flux. This body of work ultimately allows for more accurate predictions of plant and climate interactions.

BIOGRAPHICAL SKETCH

From early childhood, Danica Lombardozzi was curious about her environment and loved exploring the outdoors. Whether at her grandparents ranch in Melrose, MT or in her backyard in Easton, PA, she was often outside digging in the dirt or collecting flowers, leaves, and rocks. Following her passion for the environment, she majored in Environmental Sciences at Colorado College, where she worked with Dr. Sharon J. Hall studying biogeochemical changes caused by a nitrogen-fixing tree in Hawaii Volcanoes National Park and traveled to Kigoma, Tanzania to study how deforestation changed chemistry in tributaries to Lake Tanganyika with Dr. Catherine O'Reilly. After she graduated from college, Danica established a Scientist-in-Residence program at the Hayground School, an independent school for elementary and middle school students. Seeing the children's excitement for science, Danica realized her passion for using research as a teaching tool and decided to return to school to refine her research and teaching skills. Danica joined the lab of Dr. Jed Sparks at Cornell University to pursue a PhD and established collaborations with the National Center for Atmospheric Research (NCAR) where she worked with Dr. Sam Levis and Dr. Gordon Bonan to learn how to use global models. Upon finishing her degree at Cornell University, Danica will move to Boulder, Colorado to take a Post-Graduate Scientist position at NCAR.

ACKNOWLEDGMENTS

I am deeply indebted to all my family, friends and mentors who have supported and guided me through this scientific journey. I would first like to thank Dr. Jed Sparks, who helped me refine my scientific ideas and research approach, allowing me to gain insight into the scientific process within a few years that would have taken a lifetime on my own. Without fail, he pushed me to become a better thinker, speaker, and teacher by sharing his top-secret tips and enforcing rigorous practice. I can say without doubt that I am more confident in my scientific ability after working with Jed for the past six years.

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More than half of my dissertation would have been impossible without the extensive amounts of time Dr. Gordon Bonan and Dr. Sam Levis have spent teaching me to use their model and helping me think through new methods. Not many people are generous enough to devote several hours every week to meeting with a graduate student who is tangential to their work. They have helped me acquire a set of skills that will be invaluable for my scientific career. Gordon took on the tedious task of double-checking my code for mistakes, helped me interpret model results and develop tests to check that the model was responding as expected. Sam helped me wade through the model guts to show me how to make modifications, helped me think of appropriate methods of modifying the model, and spent numerous hours teaching me to troubleshoot code when I was stuck.

I would also like to thank my academic and scientific communities. In particular, thanks to all the past and present members of the Sparks lab for being a supportive scientific community and providing critical feedback on my manuscripts and presentations. Much of my experimental work was possible due to logistical and technical support provided by Kim Sparks and John Pollak. The community of folks at NCAR has been incredibly generous through immediately incorporating me into their energetic lunch table discussions, bike rides and other fun activities. They have also helped me figure out many model tricks that have improved my efficiency behind the computer. I look forward to being part of that community during upcoming years.

My experience as a graduate student has been greatly enhanced by the wonderful community of staff, faculty, and graduate students in the Department of Ecology and Evolutionary Biology and in the Biogeochemistry program. Special

thanks to the EEB staff members who have helped me make and maintain equipment for experiments, kept our halls, labs and offices clean, helped me reserve rooms for meetings, and kept me on track with grant proposals and finances. I feel very fortunate to be part of an amazing graduate student community who always pull together to work and socialize. I am constantly amazed at the creativity of my peers both in work and in fun. How many graduate student communities can organize a hugely successful grilled cheese competition?

I am lucky to have started graduate school with an amazing cohort of women in search of the illusive *Homo masculinis*. Despite having somewhat separate social lives, my cohort is one group of women that I can always turn to for support no matter how much time has passed. Good luck to all of us in our next endeavors!

Thanks to all my wonderful friends who have provided fun adventures and shoulders to cry on. Katie Wagner introduced me to Cornell University before graduate school was even a thought, and I would not be here without her. It is not often that a friend you meet on a summer program in Tanzania will remain one of your closest friends. Marie Nydam, Dena Vallano, Amy Parachnowitsch, April Melvin and Marc Lajeunesse are incredible friends and made the struggle through my early graduate years much more bearable. Susan Cook and Erica Larson have accompanied me on this journey from the beginning, and I could not have asked for better friends through the process. Thanks to many other good friends, including Billie Gould, Cayelan Carey, Quinn Thomas, and Jessica Bliss for making life more enjoyable. I owe an extra special thanks to my South Hill neighbors who have been my family during my years in Ithaca. Pat Brehl, Mat Langlois, Marjorie Weber, Chris Bliss,

Nancy Chen, Susan Cook, Erica Larson and Ben Dalziel: I would not have gotten through this without you! You have all listened to my complaints about failed experiments, helped me to remain engaged in work and life, and provided much needed comic relief. I will not ever live within a better community of people.

I would not have made it through this process without the support of my family: Jon DeCoste, Mizpah, my parents and my sister. Jon and Mizpah pull me back to reality when I am overwhelmed and have provided unconditional loving support. I look forward to many more adventures on our road ahead. My parents have watched me struggle throughout my life to find a path and have supported all my various endeavors with my dad's mantra, "be good and do your best." I will hear those words for the rest of my life. My sister and her three kids constantly remind me why I am doing my work, which is an attempt at understanding how humans are changing the world. I hope her children have a safe world to live and explore throughout their lifetime. I wish there were more powerful words to say thank you to all of you. I love you all.

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TABLE OF CONTENTS

CHAPTER 1

OZONE EXPOSURE CAUSES A DECOUPLING OF CONDUCTANCE AND PHOTOSYNTHESIS: IMPLICATIONS FOR THE BALL-BERRY STOMATAL CONDUCTANCE MODEL[1]

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ABSTRACT

Industrialization has significantly altered atmospheric chemistry by increasing concentrations of chemicals such as nitrogen oxides (NO_x) and volatile organic carbon that react in the presence of sunlight to produce tropospheric ozone (O_3). Ozone is a powerful oxidant that causes both visible and physiological damage to plants, impairing the ability of the plant to control processes like photosynthesis and transpiration. Damage to photosynthesis and stomatal conductance does not always occur at the same rate, which generates a problem when using the Ball-Berry model to predict stomatal conductance because the calculations directly rely on photosynthesis rates. The goals of this work were to develop a modeling framework to modify Ball-Berry stomatal conductance predictions independently of photosynthesis and to test the framework using experimental data. After exposure to elevated O_3 in open-top chambers, photosynthesis and stomatal conductance in tulip poplar changed at different rates through time. We were able to accurately model observed photosynthetic and stomatal conductance responses to chronic O_3 exposure in a Ball-Berry framework by adjusting stomatal conductance in addition to photosynthesis. This led to a significant improvement in the modeled ability to predict both photosynthesis and stomatal conductance responses to O_3.

KEY WORDS: *ozone, photosynthesis, conductance, stomata, Ball-Berry,* Liriodendron tulipifera

INTRODUCTION

Plants are an important control on climate through carbon and water exchange with the atmosphere during the processes of photosynthesis and transpiration (Bonan 2008). Photosynthesis is a multi-step process that depends on 1) carbon dioxide (CO_2) entering the leaf via the stomata (i.e., regulated by conductance) followed by 2) biochemical assimilation within the cells where carbon is fixed (carboxylation). Therefore, the rate of carbon assimilation is jointly controlled by both stomatal function and mesophyllic biochemical and transport processes (von Caemmerer and Farquhar 1981). In contrast, the ability of a plant to regulate leaf water loss in most situations is *singly* controlled by stomatal behavior (Cowan 1978). Oxidants like O_3 are capable of damaging the guard cells of stomata, the functioning of cellular membranes regulating diffusion and electron transport, and enzymes involved in the carboxylation process (Bortier et al. 2000; Kellomaki and Wang 1997; Reichenauer et al. 1997). Since leaf water loss depends primarily on a single plant-controlled parameter and photosynthesis depends on a series of factors, O_3 damage to any of these processes at unequal rates or magnitudes will cause photosynthesis and transpiration to become decoupled with potentially large impacts on global carbon and water cycling.

In low O_3 environments, photosynthesis and stomatal conductance are typically tightly coupled because the stomatal aperture equivalently controls the exchange of CO_2 and water at the leaf surface. A tight correlation usually exists between conductance and photosynthesis over a range of environmental conditions because stomata respond to changes in environmental cues like light and vapor pressure deficit.

In fact, the Ball-Berry stomatal conductance model (Ball et al. 1987; Collatz et al. 1991), commonly used to predict stomatal conductance in many physiological models, uses photosynthetic rates to predict conductance values. Therefore, any change in photosynthesis implies a direct, tightly coupled decrease in the calculation of stomatal conductance by the Ball-Berry model. If O_3 is causing photosynthesis and conductance to change at different rates or magnitudes, however, then stomatal conductance predicted by the Ball-Berry model is inaccurate.

Research over the past several decades indicates that chronic O_3 exposure significantly decreases photosynthetic rates in plants. Recent meta-analyses suggest ~20% decreases in photosynthesis in the crops wheat and soybean compared to carbon-filtered air (Feng et al. 2008; Morgan et al. 2004) and an 18% decrease in tree photosynthesis compared to ambient background O_3 (Wittig et al. 2007) due to chronic O_3 exposure. The reductions in photosynthesis typically are driven by both a reduction in leaf chlorophyll content (Heagle et al. 1996; Sharma 2003) and the quantity and activity of the primary carboxylation enzyme Rubisco (Fiscus et al. 2005; Ojanpera et al. 1998). Further, whole plant fumigation experiments suggest that decreases in photosynthesis can be independent of stomatal conductance (Francini et al. 2007; Noormets et al. 2001; Zhang et al. 2010), suggesting that decreases in carboxylation, rather than conductance, are responsible for decreases in light-saturated photosynthesis.

Instantaneous stomatal conductance also often decreases in response to O_3 exposure, though not always with the same magnitude as photosynthesis. In Wittig et al.'s (2007) meta-analysis of tree responses to O_3 compared to ambient background

air, O_3 caused an average stomatal conductance decrease of 6% compared to an 18% decrease in photosynthesis. Several studies suggest that decreases in conductance are a secondary response to chronic O_3 exposure and only occur following reduction in carboxylation efficiency (Farage et al. 1991; Manes et al. 2001; Noormets et al. 2001; Paoletti and Grulke 2005; Paoletti et al. 2007; Reich 1987).

Though decreasing conductance is a common response to O_3 exposure, many studies demonstrate that conductance and transpiration rates can also increase after chronic O_3 exposure (Freersmith and Dobson 1989; Maiermaercker and Koch 1991; Manes et al. 2001; Manes et al. 1998; McLaughlin et al. 2007; Mills et al. 2009) due to functional changes in stomata and stomatal signaling. For example, O_3 has been reported to cause stomata to respond sluggishly to stimuli, resulting in higher integrated daily transpiration rates (Paoletti 2005). Mills et al. (2009) suggested that some grass species increased stomatal conductance after chronic O_3 exposure due to a decreased sensitivity of stomatal cells to abscisic acid. Additionally, stomatal cells sometimes lose functionality after O_3 exposure through altering stomatal cell ion exchange (Manes et al. 2001; Torsethaugen et al. 1999) and causing the collapse of epidermal cells that surround the stomatal guard cells (Hassan et al. 1994) so that the stomata remain open in spite of external stimuli.

Because O_3 can directly impact the function of stomatal cells so that conductance no longer responds dynamically to changes in internal CO_2 concentrations (c_i) and the rate of carboxylation due to enzyme and membrane damage, the typical coupling of stomatal conductance and photosynthesis does not always hold under high O_3 conditions. Currently, the Ball-Berry stomatal conductance

model is only capable of predicting stomatal conductance decreases that are equivalent to photosynthetic decreases, even though data repeatedly demonstrate that conductance decreases less or even increases with O_3 exposure. Despite this knowledge, most studies that scale up plant physiological responses to O_3 assume that conductance decreases linearly with photosynthesis (e.g., Felzer et al. 2004; Martin et al. 2000; Ollinger et al. 1997b; Sitch et al. 2007). If conductance deviates from photosynthesis and stomatal responses are not explicitly considered in models, then changes in water cycling, arguably one of the most important controls on climate, will not be accurately predicted. To more clearly understand the impact that O_3 has on climate through changes in plant physiology, it is important to determine the mechanisms that govern photosynthetic and stomatal responses to O_3 independently as they change through time.

The objective of this work was to determine whether a leaf-level photosynthesis and stomatal conductance model could accurately predict *both* photosynthesis and stomatal conductance responses to chronic O_3 exposure observed in experiments. First, we conducted controlled open-top chamber experiments that exposed tulip poplar seedlings to elevated O_3 and measured physiological changes in photosynthesis and stomatal conductance as a function of chronic O_3 exposure. We then tested different methods of modifying a leaf photosynthesis/stomatal conductance model, which was based on combined Farquhar photosynthesis and Ball-Berry stomatal conductance models, to account for O_3 damage to both photosynthesis and stomatal conductance. Last, we compared observed declines in photosynthesis and stomatal conductance with those predicted by the various modifications to the leaf

photosynthesis/stomatal conductance model to determine the best method for modeling the effects of O_3 on both photosynthesis and stomatal conductance.

METHODS

Experimental Design.

Tulip poplar (*Liriodendron tulipifera*) seedlings were exposed to elevated O_3 in open top chambers at the Freese Road Experimental Fields in Ithaca, NY (42º27'50.75" N, 76º26'40.13" W) for 12 weeks beginning in June 2008. Sixteen chambers, one-cubic-meter in size, were constructed from ¾-inch PVC with a 45º frustum and covered in clear polyethylene. Air was circulated through each chamber using a duct fan to blow air through an aluminum duct manifold attached to 1-inch PVC housed inside the chamber. Temperature and humidity were continuously monitored and airflow adjusted so temperature did not exceed 5 ºC above ambient. Ozone, generated using an Ozonexia O_3 generator (Enaly, Inc), was introduced into eight of the chambers at concentrations 70 ppb above ambient for five hours per day, four days per week and defined as the elevated O_3 treatment. The remaining eight chambers had no additional O_3 and experienced ambient O_3 concentrations (mid-day peaks ~30 ppb; control). Ozone concentrations were chosen to simulate typically observed high (O_3 treatment) and background (Control treatment) concentrations and mimic the naturally episodic nature of elevated O_3 events on an O_3-sensitive broadleaf that grows throughout the eastern United States.

Tulip poplar was chosen as a study species because it is found in temperate forests throughout the eastern United States and is sensitive to O_3 (Skelly et al. 1999).

Seedlings (20-50 cm; 2-3 years old) were purchased from Lawyer Nursery, Inc. (Plains, MT) and potted in 5-gallon pots until bud break. After adjusting to external environmental conditions, four seedlings were planted directly into the ground inside each chamber. To minimize drought stress, seedlings were watered three times per day with 175 ml of water using a drip irrigation system (Raindrip, Inc.). A slow-release fertilizer (Osmocote®, Scotts Miracle-Gro Company) was applied at the beginning of the experiment according to manufacturers instructions. Plants were allowed to adjust to chamber conditions for 2 weeks prior to the start of measurements and fumigation.

Physiological Measurements.

We measured instantaneous transpiration and photosynthesis rates on all plants using a portable plant gas-exchange system (LiCor 6400, LiCor, Inc.) under approximately ambient CO_2 (400 μmol CO_2 mol^{-1} reference set point resulting in ambient CO_2 concentration of ~ 375 μmol CO_2 mol^{-1}) and light (1200 μmol m^{-2} s^{-1}) conditions. Other parameters, including conductance and internal leaf CO_2 concentrations (c_i), were simultaneously calculated by the portable plant gas-exchange system. Measurements were repeated on each plant (n = 16 for control; n = 24 for O_3 exposure) three times over the course of the 12-week experiment between 10:00 and 15:00. Dark respiration rates and minimum stomatal conductance were measured on the same days using the same portable gas-exchange system after dark between 22:00 and 24:00 without light and at CO_2 of 0 μmol CO_2 mol^{-1}. Leaf responses to O_3 exposure are cumulative with damage increasing with leaf age. Therefore, gas exchange was measured on leaves of each plant throughout their development. Leaves

measured at the start of the experiment were two weeks old and leaves at the end of the 12-week fumigation time were 14 weeks old.

To differentiate between diffusional and biochemical constraints on photosynthesis, photosynthetic responses to increasing CO_2 concentrations (A-c_i curves) were measured in five plants (n = 5) in each treatment at the same time intervals as above. Leaves were exposed to a range of CO_2 values from 50 to 2000 ppm at approximately ambient light (1200 μmol m^{-2} s^{-1}) and temperature (22 ºC) conditions. Estimates of light-saturated rates of electron transport (J_{max}) and substrate-saturated Rubisco carboxylation (V_{cmax}) were calculated from A-c_i curves using the A/C_i Fitting Utility from Sharkey et al. (2007).

Additionally, photosynthetic and stomatal responses to various light levels (light curves) were measured in the same five plants and at the same time intervals as described above. Leaves were exposed to a range of light values starting at 2000 μmol m^{-2} s^{-1} and decreasing to 0 μmol m^{-2} s^{-1} at approximately ambient CO_2 (400 ppm) and temperature (22 ºC) conditions.

Leaf-level Photosynthesis/Conductance Model Description.

The leaf photosynthesis/conductance model is a simple model constructed to predict photosynthesis and stomatal conductance over a range of environmental conditions. The model uses the biochemical equations of Farquhar et al (1980), modified by Harley and Sharkey (1991), and Harley et al. (1992), to predict leaf-level CO_2 assimilation. In this parameterization, the model represents photosynthetic uptake of CO_2 as limited by: i) Rubisco-limited photosynthesis, A_c, ii) RuBP-limited

photosynthesis, A_j, or iii) product-limited photosynthesis, A_p (see equations in Bonan et al. 2011). The net CO_2 assimilation rate, A_n, is:

$$A_n = min(A_c, A_j, A_p,) - R_d \quad \text{(equation 1)}$$

where R_d is dark respiration. The required internal leaf CO_2 concentration (c_i) is calculated from the diffusion equations:

$$A_n = (c_a - c_s)(g_b/1.4) = (c_s - c_i)(g_s/1.6) \quad \text{(equation 2)}$$

where c_a is the ambient CO_2 concentration, c_s is the CO_2 concentration at the leaf surface, g_b is the leaf boundary layer conductance, and g_s is the stomatal conductance. The photosynthesis model is coupled to the Ball-Berry stomatal conductance model (Ball et al. 1987; Collatz et al. 1991), in which stomatal conductance, g_s, is calculated based on the relationship:

$$g_s = b + mA_n h_s/c_s \quad \text{(equation 3)}$$

where b is the minimum stomatal conductance when $A_n \leq 0$, m is the Ball-Berry slope of the conductance-photosynthesis relationship, h_s is the fractional humidity at the leaf surface (dimensionless), and c_s is the CO_2 concentration ($[CO_2]$) at the leaf surface. Additional details are given in Bonan et al. (2011). The coupling of photosynthesis and stomatal conductance models results in a direct dependence of stomatal conductance on photosynthesis, while stomatal conductance plays a role in

predicting photosynthesis by controlling internal leaf CO_2 concentration (c_i), which is obtained from the diffusion equation and therefore depends on g_s. The model is forced with specified environmental variables to mimic field conditions, with c_a = 400 μmol CO_2 mol^{-1} air, light ranging from 0 – 2000 μmol photons $m^{-2}s^{-1}$, temperature = 22 ºC, h_s = 0.70, and g_b = 0.05 m s^{-1}.

Ozone Response Relationships

Photosynthesis. Photosynthesis, calculated using the Farquhar equations described above, was modified to account for decreases caused by O_3. The photosynthesis modification, denoted here as *Psn*, mimicked observed decreases in photosynthesis using an O_3 factor, F_{AO3}, calculated from a linear regression of treatment to control response ratios against cumulative O_3 uptake (CUO):

$$F_{AO3} = 1.0421\text{-}0.2399 \times CUO \qquad \text{(equation 4)}$$

where F_{AO3} is the response ratio of treatment to control photosynthesis, 1.0421 and 0.2399 are empirically derived intercept and slope coefficients, respectively, and CUO is cumulative O_3 uptake. The calculation for CUO assumes that the $[O_3]$ inside the leaf is zero and is calculated as:

$$CUO = \Sigma(k_{O3}/g_s)[O_3] \qquad \text{(equation 5)}$$

similar to Reich (1987), Nunn et al. (2006) and Wittig et al. (2007), where k_{O3} = 1.67 and is the ratio of leaf resistance for O_3 to leaf resistance to water, g_s is leaf-level stomatal resistance, and $[O_3]$ is the O_3 concentration. While boundary layer conductance is not directly included in this calculation, it is assumed to be inconsequential due to constant air circulation inside the open-top chambers (Farage et

al. 1991; Farage and Long 1999; Panek 2004). *CUO* is summed through time, with units of mmol m^{-2}. The *Psn* modification occurs *post hoc* in which net photosynthesis, after it is calculated using the leaf photosynthesis/stomatal conductance model, is multiplied by F_{AO3}. This method of modifying photosynthesis is similar to parameterizations in most other models that incorporate O_3 damage to physiology (e.g. Felzer et al. 2004; Ollinger et al. 1997a).

Stomatal Conductance. Two methods of simulating O_3-induced decreases in stomatal conductance were tested. The first method, *Psn*, modified stomatal conductance indirectly, using the photosynthesis modifications described in equation 3 and relying on decreases in photosynthesis to drive decreases in stomatal conductance, similar to methods used by Sitch et al. (2007), Ollinger et al (1997b), and Felzer et al. (2004). The second method of modifying stomatal conductance, denoted here as *Cnd*, used an O_3 factor, F_{GsO3}, calculated from a linear regression of treatment to control response ratios against cumulative O_3 uptake (CUO):

$$F_{GsO3} = 1.0884 - 0.1998 \times CUO \qquad \text{(equation 6)}$$

where F_{GsO3} is the response ratio of treatment to control stomatal conductance, and 1.0884 and 0.1998 are empirically derived intercept and slope coefficients, respectively. The *Cnd* modification multiplies stomatal conductance by F_{GsO3} after stomatal conductance is calculated and does not alter the photosynthesis calculations in the model.

Simulations

The leaf-level photosynthesis and stomatal conductance model simulated light curves that paralleled light curves measured in tulip poplar. Two simulations were run

with modifications to photosynthesis (F_{AO3}) and stomatal conductance (F_{AO3}, F_{GsO3}). The first simulation, *Psn*, modified photosynthesis and stomatal conductance (indirectly) using F_{AO3}. The second simulation, *Cnd*, modified photosynthesis using F_{AO3} and stomatal conductance using F_{GsO3}, which was applied after stomatal conductance was calculated using a photosynthesis value that was not yet modified for O_3. Each simulation ran at three cumulative O_3 uptake levels (CUO, see equation 4) of 0, 2.23 (+/- 0.12) and 4.24 (+/-0.23) mmol m^{-2}, which was equivalent to the mean +/- standard error CUO in pre-treatment, 6th, and 12th week of the open top chamber experiment under O_3 exposure of 70 ppb above ambient for five hours per day, four days per week. The simulated plants were individualized through changing dark respiration (R_d) rates to be based on measured rates for each experimental plant. Model parameters V_{cmax} and J_{max} were set to appropriate values based on measured rates for the experimental plants. The modifications were verified by comparing predictions of photosynthesis and stomatal conductance with measured plant responses from the open-top chamber experiment.

Statistical Analyses. Results of experimental work were analyzed for treatment differences using JMP software (SAS Institute, Inc.). Instantaneous photosynthesis, stomatal conductance, J_{max} and V_{cmax}, were analyzed using an analysis of variance (ANOVA) with time as a repeated measure.

Results of the simulations were analyzed to determine which modification allowed the model to best fit the observed data using R statistical software. Simulations were analyzed for root mean squared error (RMSE) to quantify variance and mean bias (MB) to determine the magnitude and direction of the model bias.

Linear mixed-effects regressions were used to determine the model that best fit the experimental data with individual plant as the random factor. The likelihoods of the models were compared, with the model having the likelihood closest to zero selected as the best model.

RESULTS

Instantaneous light-saturated photosynthesis decreased significantly in O_3-treated compared to control plants over the course of 12 weeks (Figure 1.1a; $p < 0.0001$). Photosynthetic rates were 57% lower in O_3 plants relative to control plants at a cumulative ozone uptake (CUO) of 2.23 mmol m^{-2}, and 74% lower at a CUO of 4.24 mmol m^{-2} (Figure 1.2). Further, instantaneous conductance to water vapor also decreased in O_3-treated compared to control plants over the course of the 12-week experiment (Figure 1.1b; $p < 0.0001$). At CUO of 2.23 mmol m^{-2}, conductance in treated plants decreased by 35% relative to control plants and was 49% lower than control plants at CUO of 4.24 mmol m^{-2} (Figure 1.2). Exposure to elevated O_3 did not significantly change dark respiration (data not shown; $p = 0.58$) or minimum stomatal conductance (data not shown; $p = 0.06$), though mean minimum conductance was higher in O_3-treated plants after 12 weeks (control = 0.049+/-0.0057; O_3 = 0.068+/-0.0077 mol H_2O m^{-2} s^{-1}).

Ozone significantly decreased both the maximum rate of electron transport, J_{max}, and substrate-saturated Rubisco carboxylation, V_{cmax}, in treated plants after 12 weeks of fumigation (Figures 1.3a-b; $p = 0.01$ and $p = 0.04$, respectively). J_{max} decreased by 35% compared to control plants after 6 weeks of O_3 exposure, and by

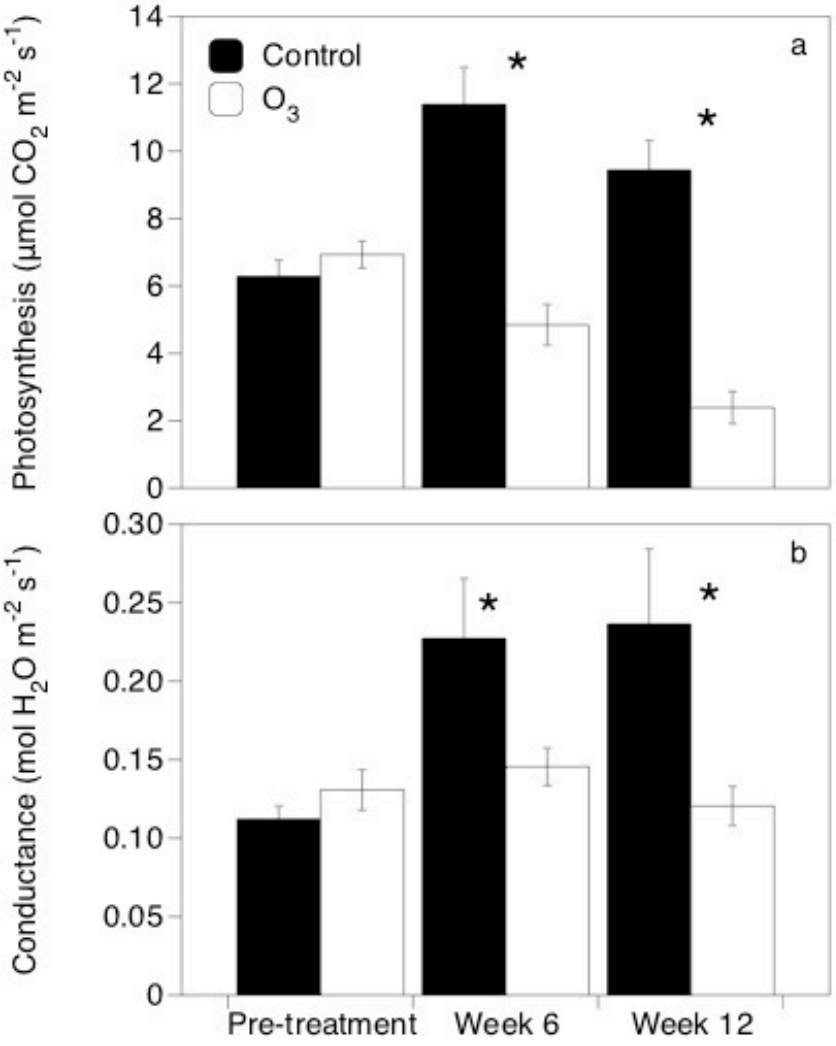

Figure 1.1: Instantaneous photosynthetic rates (a) and conductance (b) observed in tulip poplars exposed to ambient O_3 concentrations (black bars) and elevated O_3 concentrations (white bars) over the course of 12 weeks. Error bars represent +/- standard error and * indicates statistical significance at $p < 0.05$.

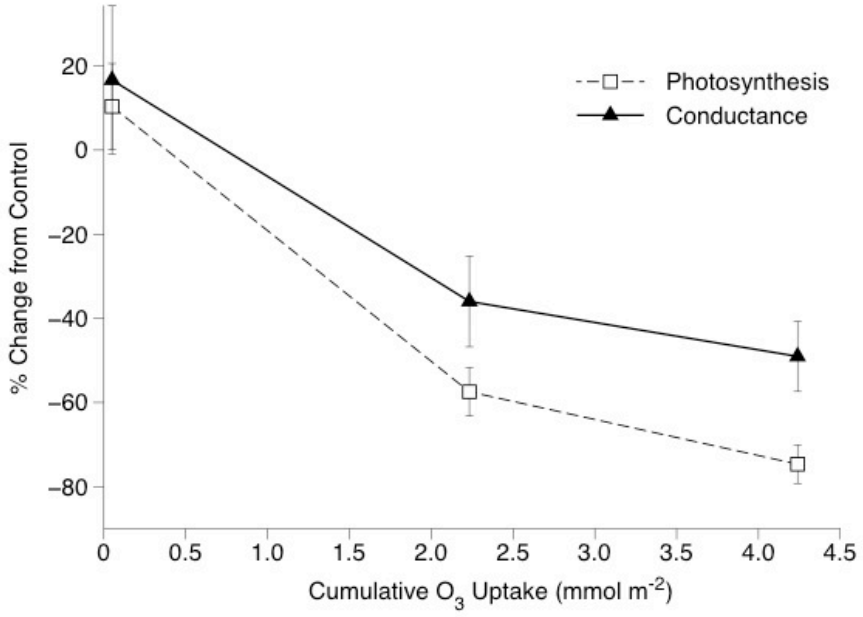

Figure 1.2: Percent changes in photosynthesis (open squares) and stomatal conductance (black triangles) over a range of cumulative ozone uptake (CUO). Differences in the rate of change over the same CUO demonstrate that photosynthesis and stomatal conductance do not change at the same rate. Error bars represent +/- bootstrap standard error.

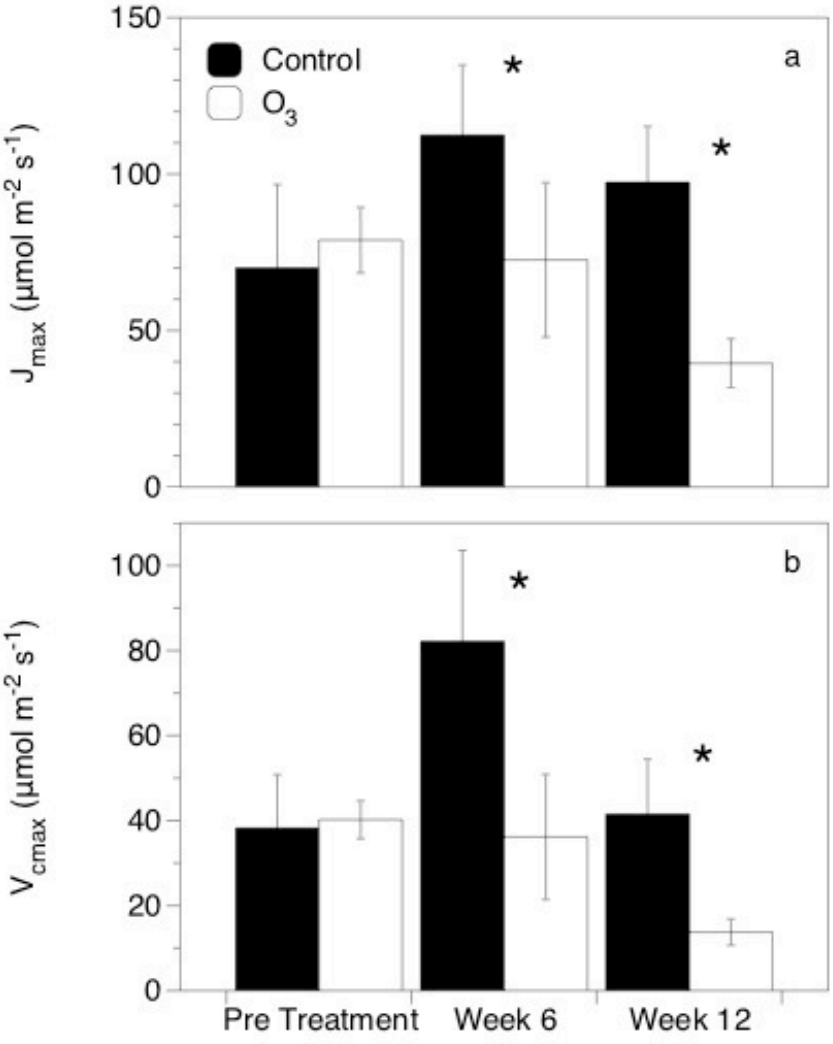

Figure 1.3: (a) J_{max} and (b) V_{cmax} estimated in tulip poplar exposed to ambient O_3 concentrations (black bars) and elevated O_3 concentrations (white bars) over the course of 12 weeks. Error bars represent +/- standard error and * indicates statistical significance at $p < 0.05$.

59% after 12 weeks of exposure. Ozone decreased V_{cmax} by 56% compared to control plants after 6 weeks of O_3 exposure, and by 66% after 12 weeks. After the first 6 weeks of O_3 exposure, the magnitude of decrease was larger in V_{cmax} than J_{max} and was approximately the same after 12 weeks of O_3 exposure.

Simulations of O_3-induced reductions in photosynthesis using *Psn* modifications in the leaf photosynthesis/stomatal conductance model accurately predicted photosynthetic responses at low and moderate levels of CUO over a range of light values, though under-predicted observations at high levels of CUO (Figure 1.4a-c; RMSE = 1.23; Bias = 0.07). Simulations using the *Cnd* modification better predict stomatal responses to saturating light at moderate and high levels of CUO than the *Psn* model (Figure 1.4d-f; *Psn* RMSE = 0.054, Bias = 0.029, log likelihood = 201.473; *Cnd* RMSE = 0.051, Bias = 0.020; log likelihood = 198.985). At low light levels (irradiance < 500), neither the *Psn* model nor the *Cnd* modification predicts stomatal conductance responses accurately.

DISCUSSION

Experimental results in this study indicate that photosynthesis and stomatal conductance change at different rates over the same CUO (Figure 1.2). As a result, the current formulation of the Ball-Berry model cannot accurately predict stomatal conductance because photosynthesis is used in the calculations (Figure 1.4d-f, *Psn* modification). Since conductance changes at a slower rate than photosynthesis, this ultimately leads to an underestimation of transpiration in most models incorporating the effects of O_3 on photosynthesis.

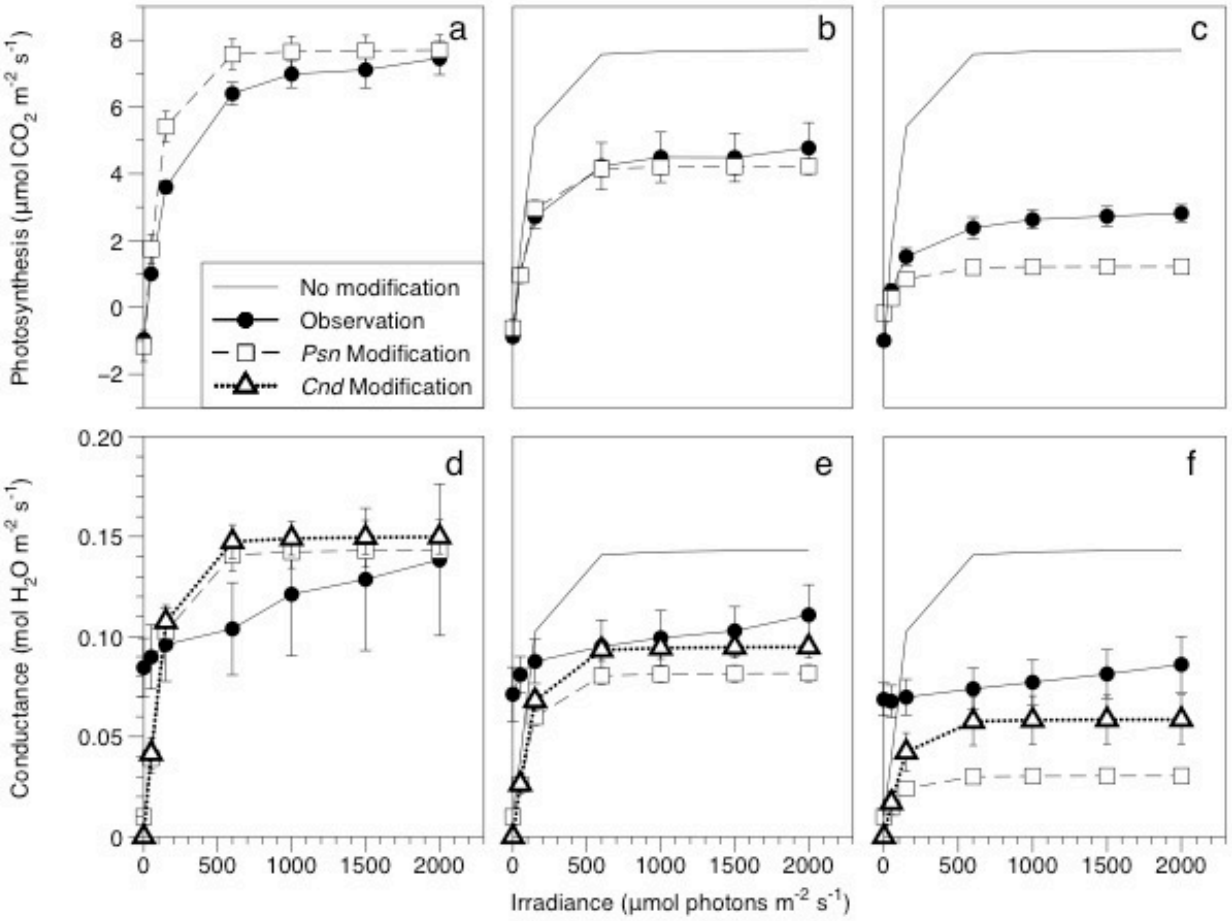

Figure 1.4: Observed and simulated responses of photosynthesis (a-c) and conductance (d-f) to a range of light values at cumulative ozone uptake (CUO) values of 0 (a, d), 2.23 (b, e) and 4.24 (c, f) mmol m^{-2}. Observations are represented by black circles; simulated responses from changes in photosynthesis (*Psn* modification) by open squares, and simulated responses from changes in conductance (*Cnd* modification) are represented by open triangles. Lines with no points represent the model before any modifications. Error bars represent +/- standard error.

What drives the differential responses of photosynthesis and stomatal conductance to O_3? Ozone might decrease photosynthesis through reductions in stomatal conductance, but in this study, decreases in photosynthesis are larger in magnitude than decreases in conductance suggesting that an additional mechanism is causing observed decreases in photosynthesis. Many studies (Francini et al. 2007, Noormets et al. 2001, Fiscus et al. 1997, Heagle et al. 1996, Sharma 2003, and Bortier et al. 2000) similarly found that O_3-induced differences in photosynthesis were the result of non-stomatal factors, potentially driven by either photosystem oxidation, limiting energy for RuBP regeneration, or decreased efficiency of Rubisco due to direct enzyme oxidation or reduced CO_2 transport to the enzymes. Decreases of J_{max} and V_{cmax} observed in this experiment suggest that O_3 reduces the biochemical capacity to fix CO_2. Thus, faster rates of change in photosynthesis compared to conductance are not surprising.

The *Psn* modification, which like many models (e.g. Martin et al. 2000; Sitch et al. 2007) uses photosynthesis to indirectly change conductance, results in a 45% decrease in photosynthesis and a 43% decrease stomatal conductance. Similarly, when simulating ponderosa pine responses to O_3 using the TREGRO model, Constable and Taylor (1997) found a 48% decrease in photosynthesis and a 45% decrease in stomatal conductance in var. *ponderosa* and a 10% photosynthetic decrease and 11% conductance decrease in var. *scopulorum*. In all of these simulations, photosynthetic decreases drove conductance decreases through the link in the Ball-Berry model so that conductance responses were similar in magnitude to photosynthetic decreases.

The results of these models, however, are not consistent with experimental data, such as this experiment on tulip poplar, studies by Francini et al. (2007) and Tjoelker et al (1995), or a meta-analysis by Wittig et al. (2007), which all found a decoupling of photosynthesis and stomatal conductance after chronic O_3 exposure. By allowing decreases in photosynthesis to drive decreases in stomatal conductance, modifying only photosynthetic responses to O_3 simulates less plant water loss than actually observed.

Despite the importance of plant water loss in regulating ecosystem water availability and atmospheric water vapor, few hydrologic or climate models have verified the accuracy of simulated stomatal responses to chronic O_3 exposure. The few studies that use leaf-level models to compare predicted with observed conductance find that altering photosynthesis in response to O_3 can result in inaccuracies. For example, de Beeck et al. (2007) found that stomatal responses to chronic O_3 exposure were only predicted accurately up to values of 40 mmol H_2O m^{-2} s^{-1}. Above that, it is likely that simulated changes in photosynthesis are driving larger decreases in simulated conductance than observed due to the dependence of conductance calculations on photosynthesis. Additionally, a study by Deckmyn et al. (2007) found that simulated O_3 uptake, which relies on conductance, underestimated measured O_3 uptake. While no conductance data is provided, it is likely that simulated conductance also underestimated measured conductance in this simulation. Similarly, the present study found that at saturating light levels, the *Psn* modification under-predicts conductance by 11% compared to observations at moderate CUO levels (Fig. 4e) and by 38% at high CUO levels (Fig. 4f). Given that experimental data regularly

demonstrate a decoupling of photosynthesis and conductance in response to O_3, it is necessary to verify and improve predicted stomatal responses to O_3.

To improve the accuracy of stomatal conductance predictions, we devised an additional variant of Farquhar/Ball-Berry model that predicts O_3-induced changes in stomatal conductance directly (i.e. no changes to photosynthesis). This simulation (*Cnd* modification) more accurately predicts the stomatal conductance decreases observed in the experiment at saturating light levels, though neither model accurately predicts stomatal conductance at low light values. Inaccuracy at low light levels is likely due to the fact that the minimum stomatal conductance value in the Ball-Berry equation (0.01 mol m^{-2} s^{-1}) is lower than the measured minimum stomatal conductance in tulip poplar.

Future efforts modeling the effects of O_3 on physiology can modify conductance independently of photosynthesis using the above method that first solves the Farquhar/Ball-Berry equations with O_3-modified photosynthesis and then, after calculating conductance using unmodified photosynthesis, alters conductance directly. This method is effective in modeling the effects of O_3 because it allows photosynthesis and stomatal conductance to both change, but change independently. It is important to note, however, the particular equations used in this parameterization are based on a single experiment using O_3 sensitive tree seedlings under open-top chamber conditions. Before scaling to regional and global levels, the complex interactions of O_3 with other environmental variables that affect ecosystems (e.g. drought, nutrient limitation, etc.) must also be considered. Additionally, as a steady-state model, the Farquhar/Ball-Berry model does not account for changes in dynamic stomatal

responses to environmental stimuli, such as stomatal sluggishness, a common result of chronic O_3 exposure (e.g. Paoletti and Grulke 2010), which are also likely to have impacts on plant water loss.

Reformulating the current method of incorporating O_3 damage into models to independently alter photosynthesis and stomatal conductance will significantly improve the accuracy of predicting plant water loss. On regional and global scales, plant water loss is important in regulating ecosystem water availability and atmospheric water vapor concentrations. Therefore, it is important to accurately predict plant water loss, leading to a better understanding of ecosystem water dynamics as well as regional and global climate. Implementing this new method of changing photosynthesis and stomatal conductance independently will improve predictions of water loss in the presence of O_3 and other oxidants and can be used whenever photosynthesis and stomatal conductance change independently. After exposure to elevated O_3, plant water loss is likely to be higher than currently predicted by models incorporating O_3 damage, resulting in more atmospheric water vapor and less saturated soils and groundwater.

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CHAPTER 2

PREDICTING PHOTOSYNTHESIS AND TRANSPIRATION RESPONSES TO OZONE: DECOUPLING MODELED PHOTOSYNTHESIS AND STOMATAL CONDUCTANCE

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Abstract

Plants exchange carbon dioxide and water, two key greenhouse gases, with the atmosphere through the processes of photosynthesis and transpiration, making them essential in climate regulation. Carbon dioxide and water exchange are typically coupled through the control of stomatal conductance, and the parameterization in many models often predict conductance based on photosynthesis values. Some environmental conditions, like exposure to high ozone (O_3) concentrations, alter photosynthesis independent of stomatal conductance, so models which couple these processes cannot accurately predict both. The goals of this study were to test direct and indirect photosynthesis and stomatal conductance modifications based on O_3 damage in a coupled Farquhar/Ball-Berry model. The same modifications were then tested in the Community Land Model (CLM) to determine the impacts on gross primary productivity (GPP) and transpiration. Modifying the V_{cmax} parameter and directly modifying stomatal conductance best predicts photosynthesis and stomatal conductance responses to chronic O_3 over a range of environmental conditions. On a global scale, directly modifying conductance reduces the effect of O_3 on both transpiration and GPP compared to indirectly modifying conductance, particularly in the tropics. The results of this study suggest that independently modifying stomatal conductance can improve the ability of models to predict hydrologic cycling, and therefore improve future climate predictions.

INTRODUCTION

Surface vegetation has a strong, direct effect on climate through regulating both carbon and hydrologic cycles on regional and global scales (Bonan 2008). Often, carbon and water exchange between plants and the atmosphere is closely coupled. On a leaf level, stomatal aperture controls the amount of carbon entering and water exiting the leaf, and responds to changes in many environmental parameters, such as light, temperature, and carbon dioxide concentrations (Jones 1998; Schroeder et al. 2001). Though regulation of stomatal conductance is the primary mechanism plants use to regulate water loss via transpiration, it is only one of the mechanisms controlling photosynthetic carbon gain; biochemical assimilation of carbon (carboxylation) also plays a large role (Cowan and Troughto 1971; Jones 1998; von Caemmerer and Farquhar 1981). Under circumstances where carboxylation is damaged or not limited by stomatal conductance, photosynthesis and conductance can become decoupled. For example, many C_3 plants do not completely close stomatal guard cells at night, resulting in water loss during a time when carboxylation does not occur due to a lack of light (Caird et al. 2007). Also, when plants are exposed to high light levels or high ozone (O_3) concentrations, membranes and photosystems become oxidized, decreasing carboxylation rates often without decreasing stomatal conductance at the same rate or magnitude (Calatayud et al. 2007; Demmig-Adams and Adams 2006; Francini et al. 2007; Maier-Maercker and Koch 1991; Matyssek et al. 1991; Paoletti 2005; Pearson and Mansfield 1993; Tjoelker et al. 1995). In all of these scenarios, photosynthesis and stomatal conductance can become decoupled, changing the relationship between carbon gain and water loss.

Models are a primary method of studying how vegetation interacts with climate on regional and global scales. Often, models scale leaf-level physiology to ecosystem and global levels by assuming that photosynthesis and transpiration are closely coupled and, in fact, calculate stomatal conductance based on photosynthetic values. For example, the physiological model of photosynthesis derived by Farquhar (Farquhar et al. 1980) and the Ball-Berry model of stomatal conductance (Ball 1987) are commonly used together in regional and global models and accurately predict observed photosynthesis and stomatal conductance under many conditions (Collatz et al. 1991; Harley et al. 1992; Misson et al. 2004; von Caemmerer and Farquhar 1981). In this formulation, the photosynthesis calculations are influenced by feedbacks from changes in stomatal conductance because conductance regulates internal carbon dioxide (CO_2) concentration (c_i), which drives the biochemical components of photosynthesis (see equation 1). The Ball-Berry conductance equation is calculated directly from photosynthetic rates (see equation 2), in addition to other factors like ambient CO_2 concentration ($[CO_2]$), a relative humidity gradient, and atmospheric partial pressure.

Despite the accuracy of the Farquhar/Ball-Berry physiological model in many situations, conditions that increase or decrease carboxylation without subsequent changes in stomatal conductance cannot be accurately predicted due to the direct dependence of stomatal conductance calculations on the photosynthetic rate. For example, the relationship between photosynthesis and transpiration changes after chronic O_3 exposure due to damage to functional aspects of both carboxylation and stomatal conductance, causing larger decreases in photosynthesis than transpiration

(Calatayud et al. 2007; Francini et al. 2007; Maier-Maercker and Koch 1991; Matyssek et al. 1991; Paoletti 2005; Pearson and Mansfield 1993; Tjoelker et al. 1995). Evidence suggests that models using Farquhar/Ball-Berry equations to predict O_3 damage to photosynthesis with proportional responses in conductance (Felzer et al. 2004; Ollinger et al. 1997; Sitch et al. 2007) likely predict overly large decreases in stomatal conductance (Lombardozzi et al. 2012), that, when scaled from leaf-level responses to regional and global responses through time, may result in large inaccuracies in predicted transpiration. Sitch et al. (2007) predicted that O_3 has a large, indirect impact on climate through suppressing photosynthesis, resulting in more CO_2 in the atmosphere. However, the method used assumes a proportional decrease in stomatal conductance. Considering differential responses of photosynthesis and stomatal conductance will allow transpiration to decrease less than predicted by such simulations, resulting in relative increases in atmospheric water vapor, an important greenhouse gas, in addition to increasing CO_2 concentrations, potentially exacerbating warming more than currently predicted.

Ozone damage to plants is a unique yet important scenario to incorporate into models because many regions already experience damaging concentrations (>40ppb) that change the ability of plants to exchange carbon and water with the atmosphere (Wittig et al. 2007), an important ecosystem service in regulating climate (Bonan 2008). Damage to photosynthesis, quantified in several meta-analyses and reviews (Feng et al. 2008; Morgan et al. 2003; Wittig et al. 2007), is caused by mechanisms that include reductions in leaf chlorophyll content that impact electron transport (Heagle et al. 1996; Sharma 2003), declines in carboxylation efficiency through

reductions in the quantity and activity of the primary carboxylation enzyme Rubisco (Fiscus et al. 2005), and/or direct damage to stomatal cells (Hassan et al. 1994; Manes et al. 2001; Torsethaugen et al. 1999). Though stomatal cells can impose a diffusional limitation to photosynthesis, several studies suggest that carboxylation and mesophyllic limitations are more important than stomatal limitation in trees exposed to O_3 (Francini et al. 2007; Matyssek et al. 1991; Noormets et al. 2001; Reichenauer et al. 1997). Typically, stomata close in response to O_3 as an indirect response to increasing internal CO_2 concentration (c_i) that results from decreases in carbon fixation (Paoletti 2005). However, the magnitude of stomatal decrease is seldom equal to the magnitude of total photosynthetic decrease under chronic O_3 exposure (Calatayud et al. 2007; Francini et al. 2007; Maier-Maercker and Koch 1991; Matyssek et al. 1991; Novak et al. 2005; Paoletti 2005; Pearson and Mansfield 1993; Tjoelker et al. 1995).

Acute instantaneous exposure at moderate or high concentrations of O_3 can cause instantaneous reductions in conductance similar in magnitude to photosynthesis (Farage et al. 1991). In contrast, chronic exposure often leads to sluggish stomatal responses to environmental stimuli due to loss of stomatal functioning and a decoupling of conductance from photosynthesis due to direct damage to biochemical carboxylation (Paoletti 2005; Tjoelker et al. 1995). In fact, several studies demonstrate that sluggish stomatal cells can also result in increases in conductance and/or integrated diurnal transpiration (Hassan et al. 1994; McLaughlin et al. 2007). These observations suggest that models can better represent the influence of chronic O_3 through modifying parameters that estimate responses of carboxylation rather than

total photosynthesis and directly modifying stomatal conductance because it often responds independent of photosynthesis.

To date, regional and global models that have incorporated O_3 damage to plants change only photosynthesis and assume a tight correlation between photosynthetic rate and stomatal conductance (Felzer et al. 2004; Felzer et al. 2005; Ollinger et al. 1997; Ollinger et al. 2002; Ren et al. 2011; Sitch et al. 2007), allowing photosynthesis to ultimately drive changes in transpiration. Since experimental data suggest O_3 damage to plants changes the relationship between photosynthesis and conductance, the standard Farquhar/Ball-Berry parameterization that couples these processes will not be capable of accurately predicting both processes. As a result, there are currently no accurate estimates of changes in some of the most important climate controls – transpiration, latent heat flux, hydrology and water cycling – due to O_3 damage to plants.

The objectives of this work were to: 1) determine the best photosynthesis and stomatal conductance parameterization to predict physiological responses to O_3 in a leaf-level Farquhar photosynthesis/Ball-Berry conductance model; and 2) to incorporate the most accurate O_3 parameterization into the Community Land Model version 4 (CLM4SP; described in Lawrence et al. 2011), the land component of the Community Earth System Model (CESM). To determine the best predictors of O_3 damage, we expanded upon work by Lombardozzi et al. (2012) to test an additional method of modifying photosynthesis and stomatal conductance in a Farquhar photosynthesis/Ball-Berry conductance model. Using CLM4SP, we then determined the differences in global gross primary productivity (GPP) and transpiration due to

direct changes to photosynthesis and stomatal conductance compared to indirect changes that alter transpiration by modifying photosynthesis. This study uniquely tests different methods of modifying photosynthesis and stomatal conductance and includes them into the framework of a global land model.

METHODS

FARQUHAR PHOTOSYNTHESIS/BALL-BERRY CONDUCTANCE MODEL DESCRIPTION

The Farquhar/Ball-Berry model predicts leaf-level photosynthesis and stomatal conductance over a range of environmental conditions. The specific implementation used here is the model used by Lombardozzi et al. (2012) and is a variant of the Ball-Berry stomatal conductance model (Ball 1987; Collatz et al. 1991), the Farquhar et al. (1980) C_3 photosynthesis model extended to include product-limited photosynthesis (Harley and Sharkey 1991; Harley et al. 1992) and C_4 photosynthesis (Collatz et al. 1992). In this parameterization, the model represents photosynthetic uptake of CO_2 as limited by: i) Rubisco-limited photosynthesis, A_c, ii) RuBP-limited photosynthesis, A_j, or iii) product-limited photosynthesis, A_p (see equations in Bonan et al. 2011). The net CO_2 assimilation rate, A_n, is:

$$A_n = min(A_c, A_j, A_p,) - R_d \quad \text{(equation 1)}$$

where R_d is dark respiration. The required internal leaf CO_2 concentration (c_i) is calculated from the diffusion equations:

$$A_n = (c_a - c_s)(g_b/1.4) = (c_s - c_i)(g_s/1.6) \quad \text{(equation 2)}$$

where c_a is the ambient [CO_2], c_s is the [CO_2] at the leaf surface, g_b is the leaf boundary layer conductance, and g_s is the stomatal conductance. The photosynthesis

model is coupled to the Ball-Berry stomatal conductance model (Ball 1987; Collatz et al. 1991), in which stomatal conductance, g_s, is calculated based on the relationship:

$$g_s = b + m\, A_n\, h_s/c_s \quad \text{(equation 2)}$$

where b is the minimum stomatal conductance when $A_n \leq 0$, m is the Ball-Berry slope of the conductance-photosynthesis relationship, and h_s is the fractional humidity at the leaf surface. Additional details are given in Bonan et al. (2011). The coupling of photosynthesis and stomatal conductance models results in a direct dependence of stomatal conductance on photosynthesis, while stomatal conductance plays a role in predicting photosynthesis by controlling c_i, which is obtained from the diffusion equation and therefore depends on g_s. The model is forced with specified environmental variables, with c_a ranging from 50 – 2000 μmol CO_2 mol^{-1} air, light ranging from 0 - 2000 μmol photons $m^{-2}s^{-1}$, temperature = 22 ºC, h_s = 0.70, and g_b = 0.05 m s^{-1}.

Ozone Response Relationships

Photosynthesis. Two methods of simulating O_3-induced decreases in photosynthesis were compared. The first method of modifying photosynthesis expanded on work by Lombardozzi et al. (2012) and is denoted here as the *Psn* modification. This modification mimicked observed decreases in photosynthesis using an O_3 factor developed by Lombardozzi et al. (2012), F_{AO3}, calculated from a linear regression of treatment to control response ratios against cumulative O_3 uptake (CUO):

$$F_{AO3} = 1.0421\text{-}0.2399 \times CUO \quad \text{(equation 3)}$$

where F_{AO3} is the response ratio of treatment to control photosynthesis, the constants are empirically derived intercept (1.0421, unitless) and slope (0.2399 m^2 $mmol^{-1}$) coefficients, and CUO is the cumulative O_3 uptake. The calculation for CUO assumes that the $[O_3]$ inside the leaf is zero and is calculated as:

$$CUO = \Sigma(k_{O3}/g_s)[O_3] \quad \text{(equation 4)}$$

similar to Reich (1987), Nunn et al. (2006) and Wittig et al. (2007), where $k_{O3} = 1.67$ and is the ratio of leaf resistance to O_3 to leaf resistance to water, g_s is leaf-level stomatal resistance, and $[O_3]$ is the O_3 concentration. *CUO* is summed through time, with units of mmol m^{-2}. The *Psn* modification occurs *post hoc* in which net photosynthesis, after it is calculated using the leaf photosynthesis/stomatal conductance model, is multiplied by F_{AO3}. This method of modifying photosynthesis is similar to parameterizations in most other models that incorporate O_3 damage to physiology (e.g. Felzer et al. 2004; Felzer et al. 2005; Ollinger et al. 1997; Ollinger et al. 2002; Ren et al. 2011; Sitch et al. 2007).

The second method of modifying photosynthesis, denoted here as *Rub,* impacts the biochemical aspects of photosynthesis directly through altering the V_{cmax} parameter in the Farquhar model. V_{cmax} integrates mesophyllic conductance with enzyme amount and activity and therefore estimates aspects of biochemical carbon fixation that are 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; Ojanpera et al. 1998; Pellegrini et al. 2010; Zheng et al. 2002). This modification mimicked observed decreases in V_{cmax} using an O_3 factor calculated from work by Lombardozzi et al. (2012), F_{RO3}:

$$F_{RO3} = 0.9888 - 0.1976 \text{ x } CUO \quad \text{(equation 5)}$$

where F_{RO3} is the response ratio of treatment to control V_{cmax}. The constants are empirically derived intercept (0.9888, unitless) and slope (0.1976 m^2 $mmol^{-1}$) coefficients. The *Rub* modification multiplies V_{cmax} by F_{RO3}, using the O_3-modified V_{cmax} in photosynthesis calculations. This method of modifying photosynthesis through changing V_{cmax} is similar to parameterizations used in Martin et al. (2000) to simulate photosynthetic responses to acute O_3 exposure and Constable and Taylor (1997) to simulate chronic exposure.

Stomatal Conductance. A new method of simulating O_3-induced decreases in stomatal conductance by altering the V_{cmax} parameter in photosynthesis calculations was compared to the *Cnd* modification developed by Lombardozzi et al. (2012). The *Cnd* modification altered stomatal conductance directly using an O_3-factor calculated by Lombardozzi et al. (2012), F_{GsO3}, calculated from a linear regression of treatment to control response ratios against cumulative O_3 uptake (CUO):

$$F_{GsO3} = 1.0884 - 0.1998 \times CUO \quad \text{(equation 5)}$$

where F_{GsO3} is the response ratio of treatment to control stomatal conductance, and the constants are empirically derived intercept (1.0884, unitless) and slope (0.1998 m^2 $mmol^{-1}$) coefficients, respectively. The *Cnd* modification multiplies stomatal conductance by F_{GsO3} after stomatal conductance is calculated and does not alter the photosynthesis calculations in the model.

The second method of altering stomatal conductance, denoted as *Rub*, modified conductance indirectly using the new modification to the photosynthesis model, which modified V_{cmax} by F_{RO3} (described above). This simulation relied on

photosynthetic decreases to indirectly drive stomatal conductance decreases, similar to methods used by Martin et al. (Martin et al. 2000) and Constable and Taylor (Constable and Taylor 1997).

Simulations

Each photosynthesis (*Psn*, *Rub*) and stomatal conductance (*Cnd*, *Rub*) modification simulated light curves at three O_3 uptake levels (0, 2 and 4.2 mmol m^{-2}) by calculating photosynthesis and stomatal conductance over a range of light values from 0 through 2000 µmol m^{-2} s^{-1} with [CO_2] at 380 ppm and a temperature of 25 ºC. Additionally, each photosynthesis modification simulated A-c_i curves at all three O_3 uptake levels by calculating photosynthesis over a range of [CO_2] from 50 to 2000 ppm with light equal to 2000 µmol m^{-2} s^{-1} and a temperature of 25 ºC. The simulated plants were individualized through changing dark respiration (R_d) rates and model parameters V_{cmax} and J_{max} were set to appropriate values based on measured rates for the experimental plants. The model was evaluated by comparing predictions of photosynthesis and stomatal conductance with plant responses measured in Lombardozzi et al. (2012).

Statistical Analysis

Results of the simulations were analyzed to determine which modification allowed the model to best fit the data using R© version 2.11.1. Simulations were analyzed for root mean squared error (RMSE) to quantify the variance and mean bias (MB) to determine the magnitude and direction of the model bias. Linear mixed-effects models with plant as the random factor were fit to the experimental data using

the nlme package in R (Pinheiro 2011). The model with the highest likelihood was selected as the best model.

TESTING THE COMMUNITY LAND MODEL

The CLM4SP represents biophysical land surface processes within the context of a global climate simulation model and is described in Lawrence et al. (2011). In this study, the model was run in offline mode forced with a historical atmospheric dataset that includes observed precipitation, temperature, downward solar radiation, surface windspeed, specific humidity, and air pressure from 1948 through 2004 (Qian et al. 2006). The CLM4SP uses coupled Farquhar photosynthesis and Ball-Berry stomatal conductance models (Bonan et al. 2011; Oleson 2010) to simulate plant physiology. A control simulation was run using the CLM4SP as described here without any effects of O_3.

Ozone Effects

To incorporate the effects of O_3 on photosynthesis and stomatal conductance into the CLM4SP, we used the *Rub* photosynthesis modification and the *Cnd* stomatal conductance modification to the Farquhar/Ball-Berry model. The *Rub* modification had the highest statistical likelihood of accurately predicting photosynthesis through modifying the V_{cmax} parameter in the model. Likewise, the *Cnd* modification had the highest statistical likelihood of predicting stomatal conductance through directly modifying the conductance variable. Additionally, the *Cnd* modification allowed the most flexibility in simulating other situations where stomatal conductance responds independently of photosynthesis. For example, stomata sometimes respond sluggishly after chronic O_3 exposure (e.g. Paoletti 2005), so the *Cnd* parameterization can be used

to simulate O_3-induced changes in diurnal transpiration. Nighttime transpiration and damage from photo-oxidation can also be simulated using the *Cnd* modification.

Ozone effects on both photosynthesis and stomatal conductance were included in the CLM4SP based on the O_3 response factors calculated for the Farquhar/Ball-Berry model. Cumulative O_3 uptake (CUO) was calculated by the CLM4SP in the canopy fluxes subroutine rather than being specified as in the leaf-level simulations. Therefore, four key differences existed in the calculations of CUO. First, CUO in the CLM was calculated using the sum of stomatal, boundary layer, and aerodynamical resistances. Second, a critical uptake threshold of 0.8 nmol O_3 m^{-2} s^{-1} was used as an instantaneous, flux-based threshold (similar to methods used in Sitch et al. 2007). Third, because O_3 damage is cumulative, we included a leaf-turnover O_3 decay rate so that accumulated O_3 damage did not accrue beyond the average leaf lifetime for evergreen plants. Last, CUO was only integrated over the time when leaf area index (LAI) was above a minimum value of 0.5. This threshold was chosen based on modeled LAI values because LAI values often asymptote in the model rather than reaching 0 (see Figure 2.1), causing O_3 accumulation to be too high. For a deciduous tree species, the 0.5 LAI threshold did not significantly change accumulated O_3 damage.

Simulations

Since photosynthesis and stomatal conductance were inherently linked in the CLM due to the traditional Ball-Berry formulation, calculations for both variables were made three times during each simulation to allow for separation between optimal rates and O_3-influenced rates. The first calculations provided optimal levels of

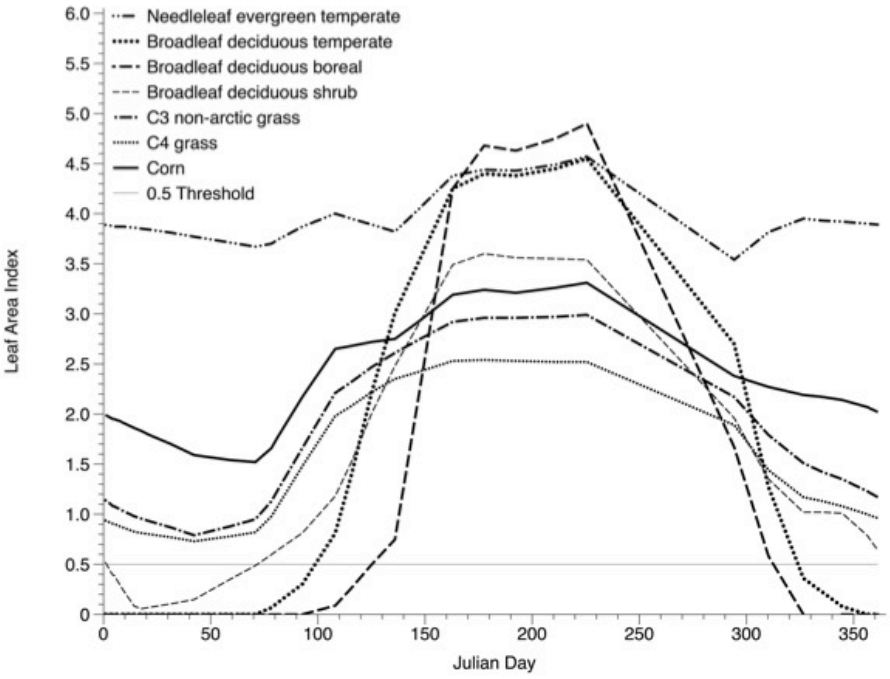

Figure 2.1: Annual leaf area index (LAI) cycle for several plant functional types (PFTs) that are simulated in the CLM. The 0.5-LAI threshold (grey horizontal line), above which O_3 accumulates, demonstrates that O_3 accumulates at most times when LAI is positive, but minimizes uptake when LAI does not mathematically reach 0 when it should equal 0, particularly relevant in the broadleaf deciduous temperate and boreal trees.

photosynthesis (psn_{opt}) and stomatal conductance (g_{sopt}) and were calculated in the absence of O_3. The second set of calculations directly modified photosynthesis (psn_{O3}) for O_3 and allowed stomatal conductance (g_{sfb}) to respond indirectly and through feedback loops. The third set of calculations modified stomatal conductance (g_{sO3}) for O_3 and allowed photosynthesis (psn_{fb}) to respond via feedback loops. We used this parameterization to run four different experimental simulations (see Table 2.1) that 1) directly modified photosynthesis and indirectly modified conductance (Pg); 2) directly modified both photosynthesis and conductance (PG); 3) modified only photosynthesis (P); and 4) modified only stomatal conductance (G). These simulations determined the magnitude of direct and indirect responses in addition to feedback loops between photosynthesis and stomatal conductance. This verified that the indirect responses could be eliminated. Simulations were named with letters "p" when photosynthesis was modified and "g" when stomatal conductance was modified, with a capital letter (e.g. P and G) signifying that the modification was direct and a lower case letter (e.g. p and g) signifying that the modification was indirect. The objective of each simulation was to determine the optimal or O_3-influenced photosynthesis and stomatal conductance values to be used in the downstream calculations like GPP and transpiration. For example, to determine whether O_3-modified photosynthesis could be calculated without influencing transpiration, the model used modified photosynthesis, psn_{O3}, and optimal conductance, g_{sopt}, for all downstream calculations.

To create a new modeling framework that would allow for decoupled behavior of photosynthesis and stomatal conductance, we took several steps to test the model behavior and make differences from the control simulation large. First, all simulations

Table 2.1: Description of the names, variables and simulations in the CLM.

Variables		
Name	**Variable**	**Description**
G	g_{so3}	O_3 affects conductance directly
g	g_{sfb}	O_3 indirectly affects conductance due to changes in photosynthesis
P	psn_{o3}	O_3 affects photosynthesis directly
p	psn_{fb}	O_3 indirectly affects photosynthesis due to changes in conductance
Experimental Modifications		
Name	**Variable**	**Description**
Pg	psn_{O3} g_{sfb}	O_3 affects photosynthesis directly while indirectly changing conductance. Standard parameterization used currently in models.
P	psn_{O3} g_{sopt}	O_3 affects photosynthesis but does not change conductance because the links between photosynthesis and conductance have been cut
G	psn_{opt} g_{sO3}	O_3 affects conductance but does not change photosynthesis because the links between conductance and photosynthesis have been cut
PG	psn_{O3} g_{sO3}	O_3 affects conductance without indirect changes to photosynthesis and O_3 affects photosynthesis without indirect changes to conductance

were run at a constant O_3 concentration of 100 ppb. This is an unrealistically high global concentration of O_3, but it helped to determine whether O_3 damage could be independently incorporated for photosynthesis and stomatal conductance and to identify hotspots where O_3 damage might have a large impact on GPP and transpiration. Second, for simplicity all O_3 modifications were based on data collected on tulip poplar seedling responses to O_3 (Lombardozzi et al. 2012). Plant species-specific responses representing multiple plant functional types should be used once these data become available. Each simulation was run for a total of 25 years, with the first 5 years being discarded in analyses to allow for stabilization of accumulated O_3 damage.

RESULTS & DISCUSSION

Farquhar Photosynthesis/Ball-Berry Conductance Model

Implementing stomatal conductance responses to O_3 independently of photosynthetic responses (the *Cnd* modification) improved the ability of the coupled Farquhar/Ball-Berry model to predict observed conductance values (Table 2.2; Figures 2.2a-c; neg. log likelihood = 199.0, bias = 0.020, RMSE = 0.051) compared to the simulation that indirectly changed predicted conductance (the *Rub* modification; neg. log likelihood = 200.7, bias = 0.027, RMSE = 0.051). This is different than the results of Martin et al. (2000), which found that altering V_{cmax} accurately predicted conductance. However, the simulations in Martin et al. (2000) were based on responses of wheat to acute, rather than chronic, O_3 exposure where conductance and photosynthesis decreased at similar rates in response to high [O_3] over a short period

Table 2.2: Statistical results calculated for the *Psn*, *Run*, and *Cnd* modifications to the Farquhar/Ball-Berry model. Intercept, slope, negative log likelihood, root mean square error (RMSE), and mean bias were calculated for both conductance and photosynthetic responses to varying levels of light (light curve) and photosynthetic responses to varying levels of CO_2 (A-c_i curve) at O_3 uptake values of 0, 2, and 4.2 mmol m^{-2}. Bolded values are the modifications within the response variable selected for use in the CLM simulations and are based on lowest negative log likelihood values.

Variable of Interest	Modification Name	Intercept	Slope	Negative Log Likelihood	RMSE	Mean Bias
A-c_i Curve						
Photosynthesis	*Psn*	2.21	1.07	-312.390	4.34	2.46
	Rub	**-0.04**	**1.32**	**-227.658**	**3.41**	**1.60**
Light Curve						
Photosynthesis	*Psn*	0.54	0.84	-164.606	1.23	0.07
	Rub	0.34	**0.89**	**-158.600**	**1.22**	**-0.008**
Conductance	*Psn*	0.068	0.39	201.473	0.054	0.030
	Rub	0.065	0.41	200.668	0.051	0.027
	Cnd	**0.067**	**0.34**	**198.985**	**0.051**	**0.020**

of time. Since chronic O_3 exposure often causes conductance and photosynthesis to decrease at different rates (Calatayud et al. 2007; Francini et al. 2007; Matyssek et al. 1991; Maurer et al. 1997; Mikkelsen 1995; Novak et al. 2005b; Paoletti and Grulke 2005; Soldatini et al. 1998; Tjoelker et al. 1995), as it did in the plants used to parameterize these simulations, the ability of a direct conductance modification (*Cnd*) to improve the model was expected. The parameters in this modification can be adjusted to capture different responses based on the type of plant being simulated, including plants that increase stomatal conductance and transpiration in response to O_3 exposure (Freersmith and Dobson 1989; Maier-Maercker and Koch 1991; Manes et al. 2001; Manes et al. 1998; McLaughlin et al. 2007; Mills et al. 2009).

The biochemical photosynthesis (*Rub*) modification to the coupled Ball-Berry/Farquhar model improved the ability of the model to predict photosynthetic responses to internal CO_2 (A-c_i; Figures 2.2g-i; *Rub* neg. log likelihood = -227.658; *Psn* neg. log likelihood = -312.390) and light curves (Figures 2.2d-f; Figures 2.2d-f; *Rub* neg. log likelihood = -158.6; *Psn* neg. log likelihood = -164.6) compared to a *post-hoc* photosynthesis (*Psn*) modification (Table 2.1) and was therefore selected for use in all CLM simulations. Both modifications predicted light curves (bias: *Psn* = 0.07, *Rub* = -0.008; RMSE: *Psn* = 1.23, *Rub* = 1.22) more accurately than A-c_i curves (bias: *Psn* = 2.46, *Rub* = 1.60; RMSE: *Psn* = 4.34; *Rub* = 3.41). Applying a *post hoc* decrease to the photosynthesis value (the *Psn* modification) is the method used in most models that incorporate the effects of O_3 on photosynthesis (Felzer et al. 2004; Ollinger et al. 1997; Sitch et al. 2007). While this method did predict decreases in

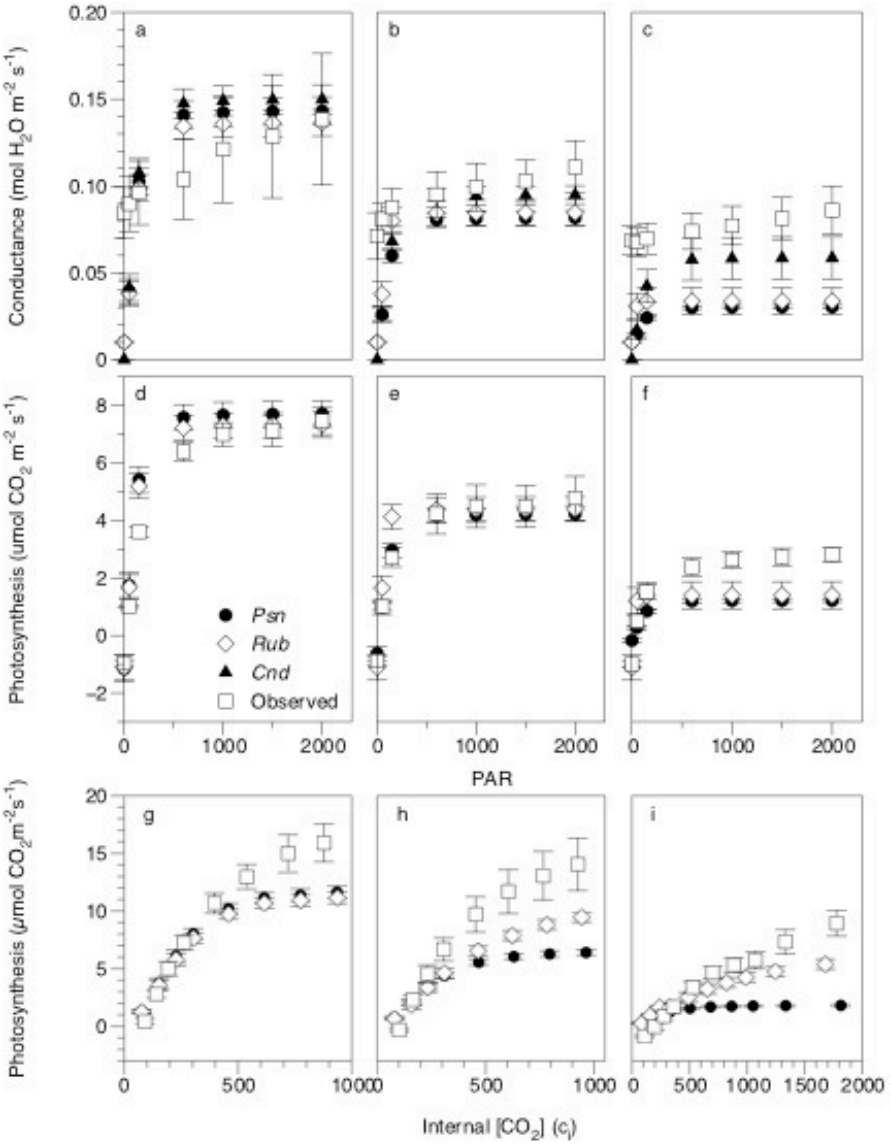

Figure 2.2: Mean light and A-c_i curves predicted by different Farquhar/Ball-Berry model parameterizations compared with observed plant responses at various cumulative O_3 uptake (CUO) values. Simulations were run before the effects of O_3 (CUO = 0; *a*, *d*, and *g*); at CUO of 2 mmol m^{-2} (*b*, *e*, and *h*); and at a CUO of 4.2 (*c*, *f*, and *i*). The *Cnd* model predicts observed stomatal conductance responses to different light values (*a*-*c*) more accurately than the *Psn* or *Rub* models. The *Rub* model predicts observed photosynthetic responses to light (*d*-*f*) more accurately than the *Psn* model. Neither model predicts observed photosynthetic responses to A-c_i (*g*-*i*) as accurately as light, though the *Rub* model predicts A-c_i curves more accurately than the *Psn* model. Error bars represent mean standard error. See Table 1 for a description of the simulations.

photosynthesis, the biochemical photosynthesis (*Rub*) modification is more representative of physiological responses to O_3 because it alters the biochemical aspects of photosynthesis directly, and therefore more accurately predicted photosynthesis over a range of environmental conditions.

CLM Simulations

Accounting for direct, independent O_3 damage to photosynthesis and stomatal conductance improves the accuracy of stomatal conductance predictions in the Farquhar photosynthesis/Ball-Berry stomatal conductance model, so we wanted to test this method in the CLM. The objective of the CLM simulations was to determine the magnitude of change in GPP and transpiration between direct modifications to stomatal conductance and indirect modifications driven by photosynthesis. The simulations were run at $[O_3]$ of 100ppb and parameterized on a single plant functional type. These simulations are not detailed depictions of global responses to O_3 because they do not include responses from a full array of plant functional type or continuous atmospheric $[O_3]$. However, they do demonstrate the differences in GPP and transpiration that may occur based on the parameterization used to estimate stomatal conductance.

We consider first the impact of modifications of the CLM on a 5x5 grid centered on Ithaca, NY, where photosynthetic and stomatal modifications were developed. Indirect changes to conductance (the Pg simulation) resulted in similar rates of change in photosynthesis (r^2 = -0.92; rate = -9.12) and transpiration (r^2 = -0.91; rate = -9.23; p = 0.781), comparable to results generated by simulations of ponderosa

pine using the TREGRO model (Constable and Taylor 1997), the only simulation to our knowledge that reports changes in transpiration caused by O_3. The parallel rates of decrease in photosynthesis and transpiration in these simulations, however, do not represent changes measured in many O_3-exposure experiments. Maier-Maercker (1997) and McLaughlin et al. (2007), for example, found that transpiration increased in trees exposed to elevated O_3, opposite of typical photosynthetic responses. Several studies similarly determined that photosynthesis decreases more than stomatal conductance, the primary plant control over transpiration, in response to O_3, uncoupling stomatal conductance from photosynthesis (Calatayud et al. 2007; Francini et al. 2007; Maurer et al. 1997; Mikkelsen 1995; Novak et al. 2005; Paoletti and Grulke 2005; Soldatini et al. 1998; Tjoelker et al. 1995). When O_3 directly altered photosynthesis and conductance (the PG simulation) in this study, transpiration decreased (r^2 = -0.90; rate = -8.68) at a slower rate than photosynthesis (r^2 = -0.89; rate = -10.44 p = 0.192; Figure 2.3a) for the same O_3 uptake, similar to trends in experimental observations. These results suggest that changing only photosynthesis likely over-predicts decreases in transpiration and is therefore not accurately capturing changes in atmospheric water vapor, a key greenhouse gas.

On regional and global scales, O_3 can have large impacts on hydrology and atmospheric water vapor by changing transpiration rates. In one of the few studies examining how hydrology responds to chronic O_3, Felzer et al. (2009) found that O_3, when coupled with nitrogen limitation, had a larger effect on runoff than did elevated CO_2, highlighting the importance of O_3 in the hydrologic cycle. The parameterization

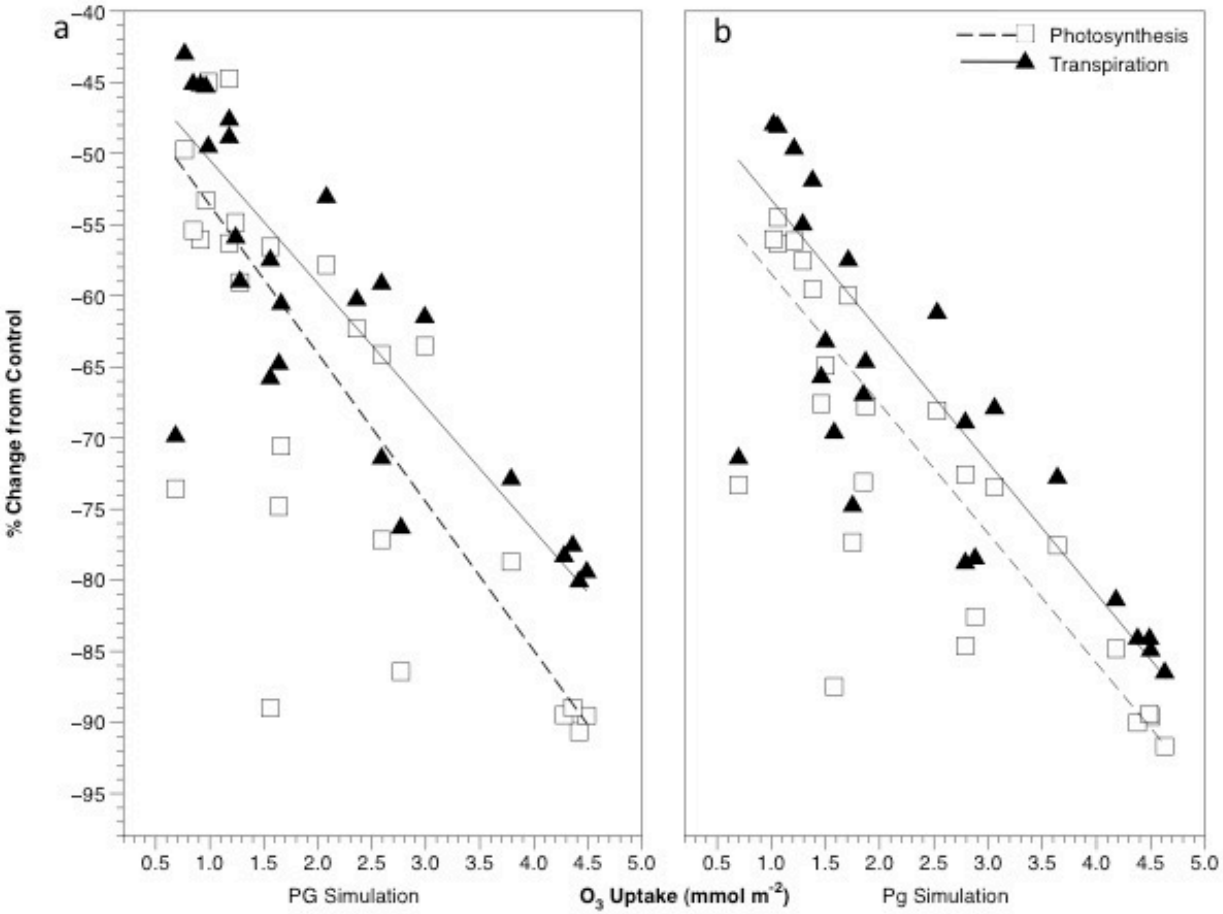

Figure 2.3: Percent change from control CLM simulations in photosynthesis and transpiration over a range of O_3 uptake in the PG (direct change to conductance; *a*) and Pg (indirect change to conductance; *b*) simulations. Results are from a 5x5 gridded region centered on Ithaca, NY (latitudes between 270-280 degrees; longitudes between 38-46 degrees) as modeled by the CLM. Points are gridcell averages from August of the 10th simulated year.

of TEM-Hydro used by Felzer et al. (2009) was similar to the Pg simulation in the CLM, where O_3 caused decreases in conductance indirectly through reduced photosynthesis. The accuracy of predicted hydrology can be improved by directly modifying stomatal conductance.

Global transpiration decreased in all simulations when O_3 modifications caused decreases in stomatal conductance (Figure 2.4). Compared to the control simulation (Figure 2.4a), transpiration rates decreased more than 50% in many areas when conductance changed indirectly (the Pg simulation), with the largest decreases in the tropics and other regions with high control transpiration rates (Figure 2.4b). In contrast, directly changing conductance (the PG simulation) produced smaller decreases in transpiration in tropical regions (Figure 2.4c), typically less than 50%, and similar decreases in mid- and high-latitudes (40-80%). Relative differences between the simulations that changed conductance indirectly (Pg) and directly (PG; Figure 2.4d) show that changing conductance directly (the PG simulation) results in higher transpiration rates than predicted by changing conductance indirectly (the Pg simulation) in almost all locations. Unsurprisingly, the differences driven by changing conductance directly (the PG simulation) compared to indirectly (the Pg simulation) were particularly evident in regions with high photosynthetic rates, such as tropical latitudes where indirect changes (the Pg simulation) drove decreases in transpiration that were 30% larger than direct changes (the PG simulation). Estimates of atmospheric water vapor, an important greenhouse gas, are therefore largely underestimated in these regions.

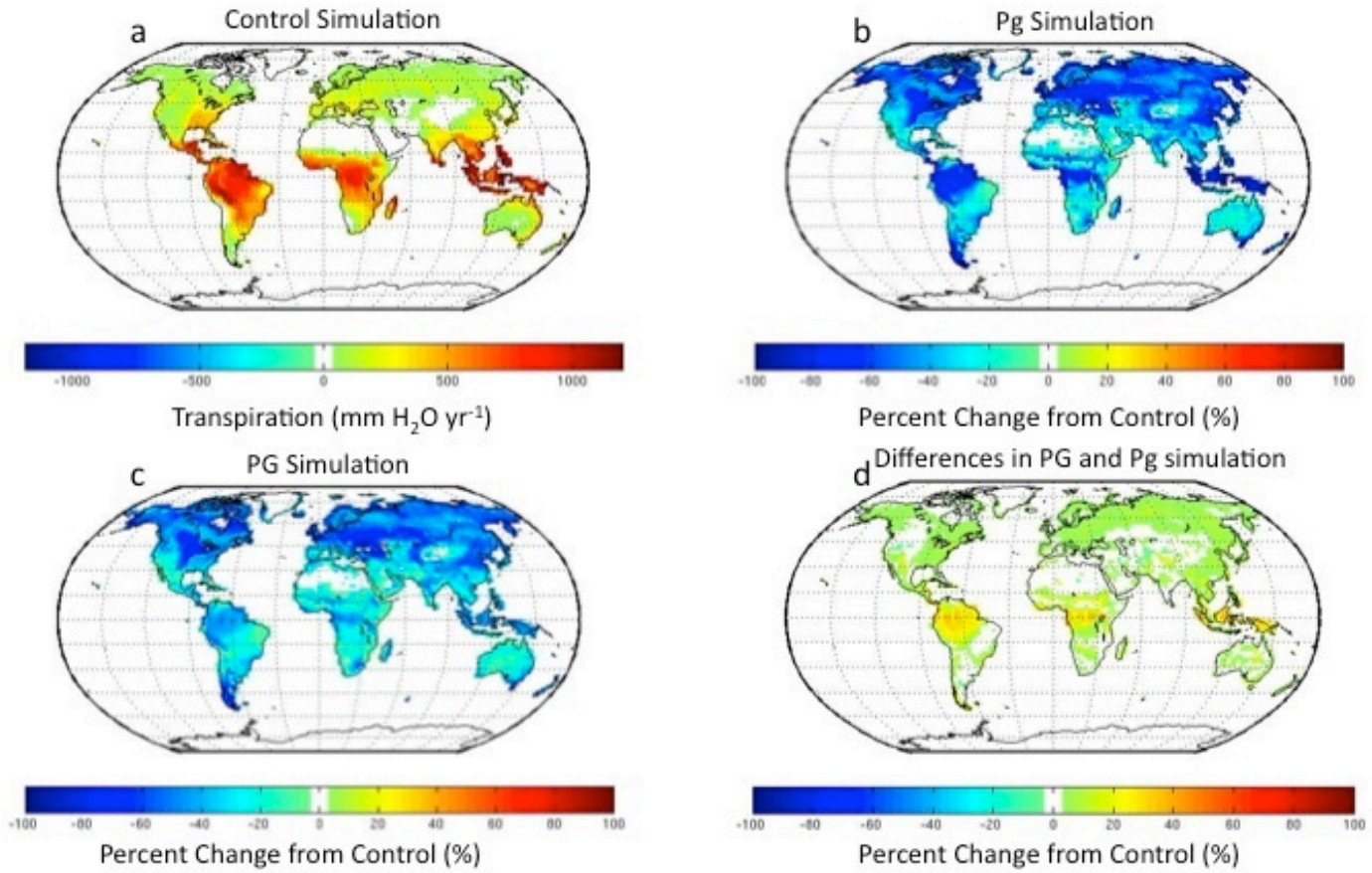

Figure 2.4: Mean annual transpiration predicted in 20-year CLM simulations run at 100 ppb O_3. The control simulation (*a*) shows the average amount of water lost via transpiration annually in the absence of O_3. The percent changes from control were mapped for the (*b*) Pg simulation, where a direct change in photosynthesis causes indirect changes in conductance and for the (*c*) PG simulation, where direct changes to photosynthesis and conductance occur independently. Panel *d* illustrates the differences in transpiration between the PG simulation compared to the Pg simulation, also mapped as a percent change from control.

Direct photosynthesis and indirect conductance modifications (the Pg simulation) decreased GPP in most locations, with decreases of more than 70% in the tropics and high latitudes (Figure 2.5b). Direct changes to photosynthesis and conductance (the PG simulation) also reduced GPP globally, though the decreases were less than 50% in most tropical regions (Figure 2.5c) and the largest decreases were in the high latitudes, which decrease GPP by 50% or more from control simulations. Though photosynthesis modifications were identical in the PG and Pg simulations, directly modifying conductance (the PG simulation) unexpectedly results in GPP nearly 50% higher in the tropics than predicted by indirect conductance modifications (the Pg simulation; Figure 2.5d), suggesting that differences in conductance changed the rate of O_3 uptake or altered the rate of carbon acquisition through changing c_i. In fact, directly modifying stomatal conductance (the PG simulation) in the CLM resulted in higher average rates of O_3 uptake (1-3 mmol m^{-2} in the mid- and high-latitudes and up to 5 mmol m^{-2} in the tropics; Figure 2.6a) than the simulation that indirectly modifies conductance (Pg; 1-3 mmol m^{-2} in the mid- and high-latitudes and up to 2.5-3 mmol m^{-2} in the tropics; Figure 2.6b).

We expected that the slower decrease in conductance due to a direct conductance modification (the PG simulation) compared to an indirect modification (the Pg simulation) would result in higher O_3 uptake rates, causing GPP to decrease more in the simulation that directly modified conductance (PG). Ollinger et al. (1997), using PnET to determine the impacts of O_3 on NPP in the northeastern United States, ran a sensitivity analysis in which O_3 caused conductance to increase, rather

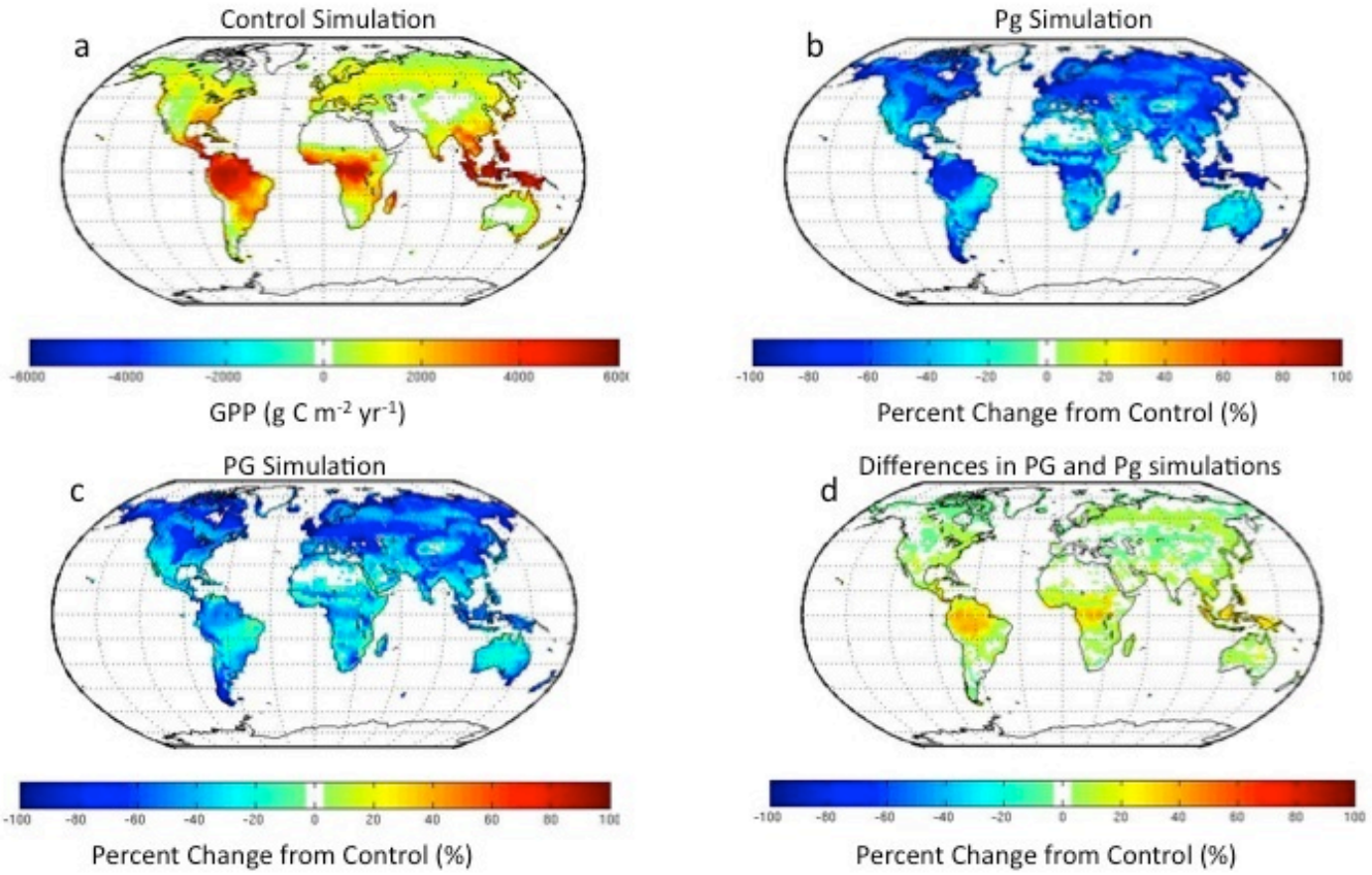

Figure 2.5: Mean annual gross primary productivity (GPP) predicted in 20-year CLM simulations run at 100 ppb O_3. The control simulation (*a*) shows the average amount of carbon gained via photosynthesis annually in the absence of O_3. The percent changes from control were mapped for the (*b*) Pg simulation, where a direct change in photosynthesis causes changes in conductance and for the (*c*) PG simulation, where direct changes to photosynthesis and conductance occur independently. Panel *d* illustrates the differences in GPP between the PG compared to the Pg simulation, also mapped as a percent change from control.

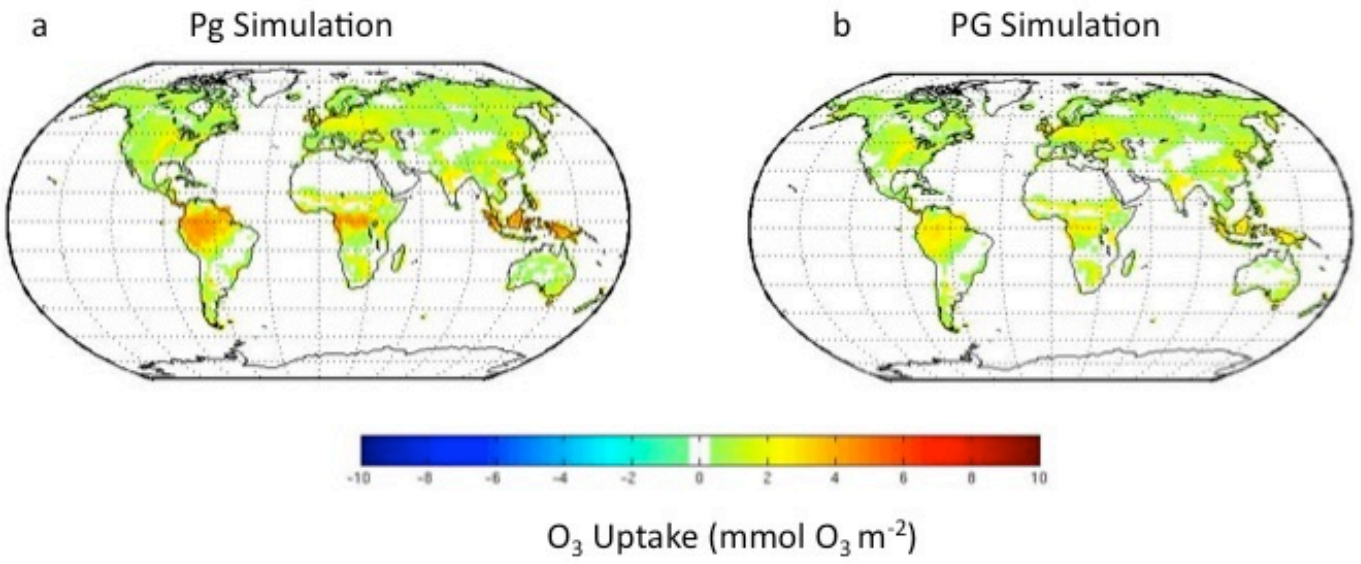

Figure 2.6: Mean annual O_3 uptake predicted in 20-year CLM simulations run at 100 ppb O_3 in the (*a*) Pg simulation, where a direct change in photosynthesis causes changes in conductance and for the (*b*) PG simulation, where direct changes to photosynthesis and conductance occur independently. Ozone uptake is calculated as a function of O_3 concentration and stomatal conductance integrated over time.

than decrease. In Ollinger et al.'s (1997) sensitivity analysis, increasing conductance resulted in higher O_3 uptake, doubling the decrease in NPP, similar to our expectations. However, in the present study, O_3 uptake was higher in simulations that indirectly modified conductance (the Pg simulation) compared to simulations that directly modified conductance (the PG simulation; Figure 2.6) and caused larger decreases in GPP, opposite of expected results.

Relative humidity (RH) is a factor that could be strongly related to O_3 uptake in the simulations because both factors directly or indirectly influence leaf conductance and therefore potentially influence each other. For example, differences in stomatal conductance and transpiration caused by O_3 uptake could potentially force changes in RH, initiating a positive feedback cycle among stomatal conductance, transpiration and RH that could lead to divergence in O_3 uptake between the PG and Pg simulations. In both simulations, relative humidity changed in a range of locations, but those changes were small, within 2% of RH in control simulations (Figures 2.7b-c). Further, there were only small differences between the two simulations in a few tropical locales, with RH increasing in the simulation that directly modified conductance (PG) compared to the simulation that indirectly modified conductance (Pg) by only 1% (Figure 2.7d). Given the small differences between the two simulations, changes in RH were likely not driving the larger differences in O_3 uptake observed in the tropics.

We conducted a sensitivity analysis to determine whether O_3 uptake was driving the differences in GPP. When the Pg and PG simulations were forced at a

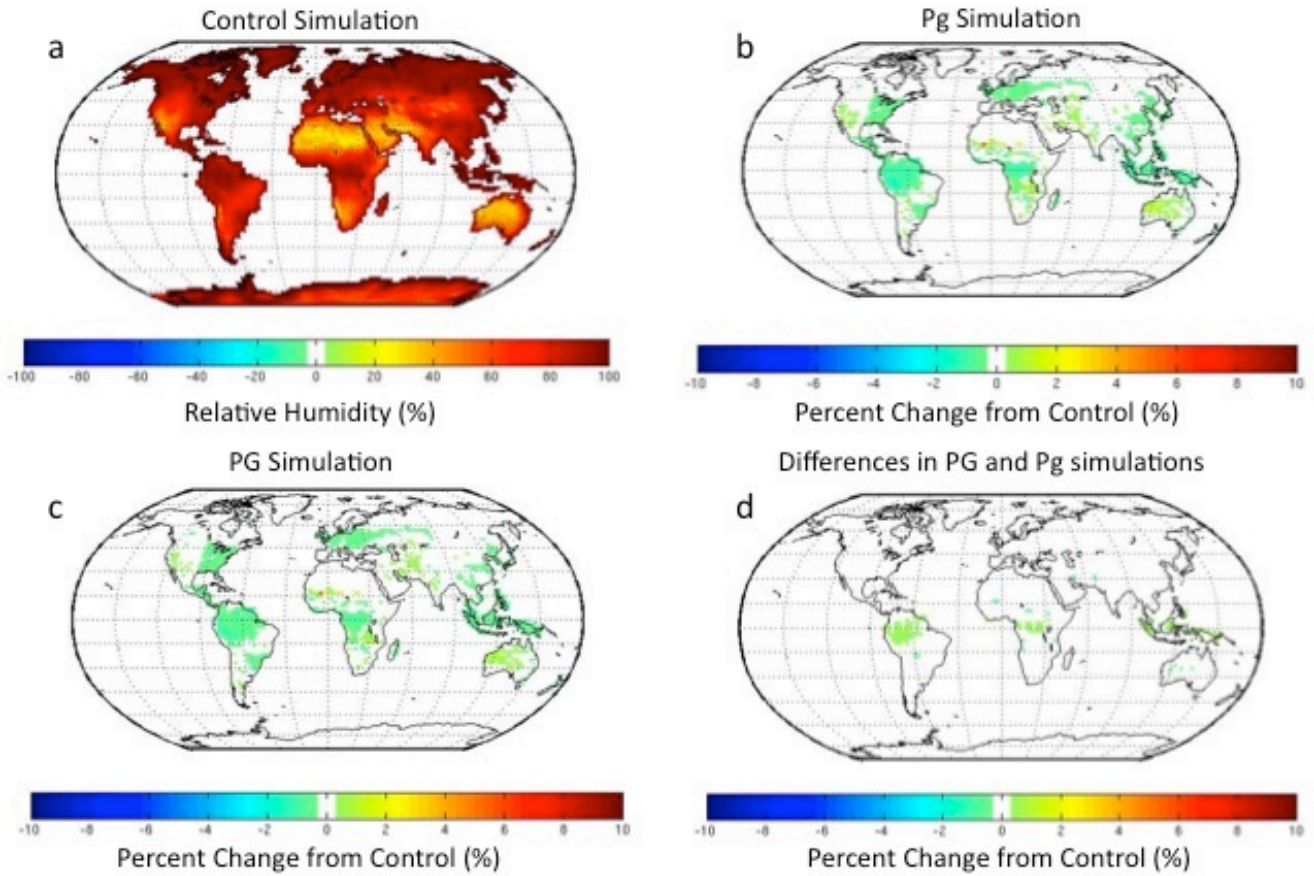

Figure 2.7: Mean annual relative humidity (RH) predicted in 20-year CLM simulations run at 100 ppb O_3. The control simulation (*a*) shows the average RH annually in the absence of O_3. The percent changes from control were mapped for the (*b*) Pg simulation, where a direct change in photosynthesis causes changes in conductance and for the (*c*) PG simulation, where direct changes to photosynthesis and conductance occur independently. Panel *d* illustrates the differences in RH between the PG compared to the Pg simulation, also mapped as a percent change from control. Note the difference in the scale of panels *b-d* compared to previous figures.

constant O_3 uptake of 3 mmol O_3 m^{-2}, one-year averages demonstrated no differences in O_3 uptake, and almost no differences in GPP (Figure 2.8a), though transpiration did change +/- 10% due to different methods of modifying stomatal conductance (Figure 2.8b). The similarity of GPP in both simulations at the same O_3 uptake suggest that the difference in O_3 uptake, which was higher in the simulation that indirectly modified conductance (Pg), was the primary factor driving larger decreases in GPP in the simulation that indirectly modified conductance (Pg). Higher O_3 uptake was expected in the simulation that directly modified conductance (PG) and it is not clear why O_3 uptake was lower in this simulation. Perhaps regions with initially high conductance rates in the simulation using a direct conductance modification (PG), such as tropical latitudes, allowed for high initial O_3 uptake rates. These initially high rates of O_3 uptake might act to reduce conductance quickly at the start of the simulation, resulting in lower conductance rates later in the simulation. Because conductance limits O_3 uptake, the lower conductance rates later in the simulation result in lower O_3 uptake. Ultimately, the differences in conductance caused changes in O_3 uptake, resulting in the differences in GPP between the PG and Pg simulations.

Photosynthesis and stomatal conductance modifications were run singly to determine the magnitude of feedbacks each modification caused (Figure 2.9). For example, stomatal conductance determines c_i, which is used in photosynthesis calculations. We therefore expected that changing only stomatal conductance (G simulation) would decrease photosynthesis due to feedbacks caused by a decrease in c_i values when conductance decreased. Implementing only stomatal conductance

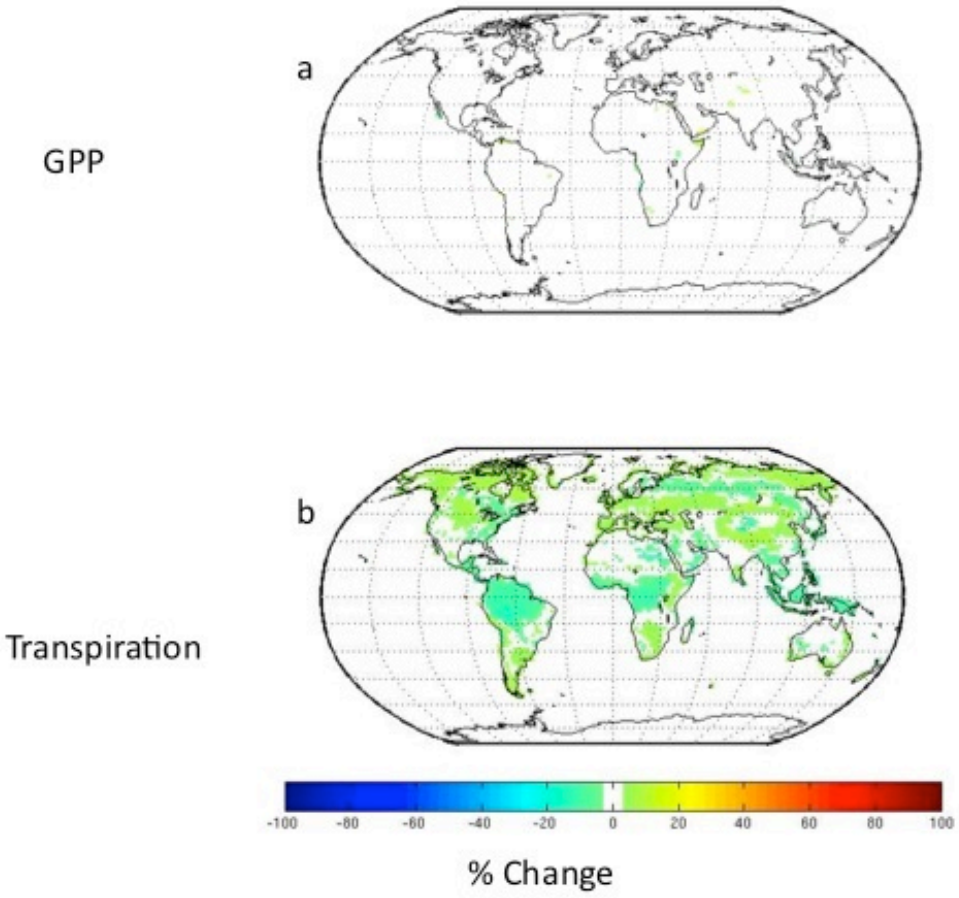

Figure 2.8: A sensitivity analysis comparing the PG and Pg simulations at the same O_3 uptake rate. The differences in (*a*) GPP and (*b*) transpiration between the PG compared to the Pg simulation, mapped as a percent change from control. Annual means were calculated from a single-year CLM simulation where O_3 uptake was set at a constant rate of 3 mmol m^{-2}.

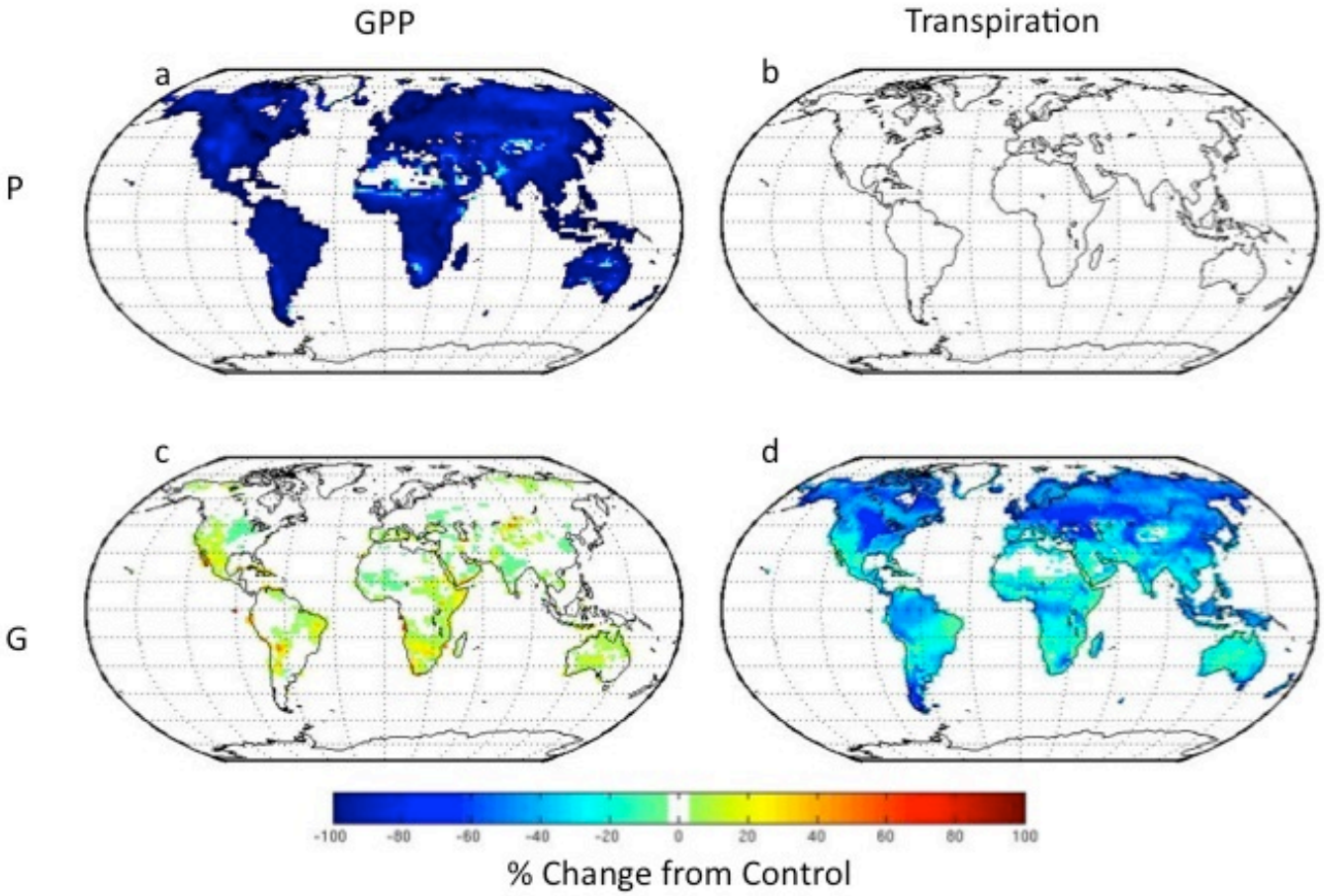

Figure 2.9: Mean annual GPP and transpiration predicted in 20-year CLM simulations run at 100 ppb O_3. Percent differences from control were mapped for the P simulation (*a*-*b*), where a direct change in photosynthesis caused decreases in GPP but did not cause indirect changes in conductance, and for the G simulation (*c*-*d*), where a direct change in conductance caused decreases in transpiration and only affects photosynthesis through feedback loops.

modifications (the G simulation) resulted in decreases in transpiration nearly identical to the simulation that directly modified photosynthesis and conductance (the PG simulation), with decreases up to approximately 50% in the tropics (Figure 2.9d). However, the same simulation caused GPP to increase relative to control simulations by 20% in many locations, though remained unchanged in the tropics and decreased in parts of North America, Africa, and Asia (Figure 2.9c). It appears that reductions in conductance stimulate photosynthesis compared to control simulations in several locations, though it is not clear why given the expected decrease c_i. The stimulation of photosynthesis in the simulation that changes only conductance (G) helps to explain why GPP increases in the simulation that modifies conductance directly (PG) compared to the simulation that modified conductance indirectly (Pg). When only photosynthesis was modified (P simulation), stomata did not close in response to increasing O_3 and resulted in extremely large O_3 uptake, which caused decreases in GPP of nearly 100% in most locations (Figure 2.9a). Transpiration did not change from control simulations (0% change; Figure 2.9b) in this simulation, suggesting that directly modifying photosynthesis did not create any feedbacks that altered conductance.

CONCLUSION

The results of this study demonstrate that independently modifying stomatal conductance in response to chronic O_3 exposure improved the ability of the coupled Farquhar/Ball-Berry model to predict conductance because conductance no longer decreased at the same rate as photosynthesis. Additionally, altering V_{cmax} improved

predictions of photosynthetic responses to O_3 exposure in the Farquhar/Ball-Berry model because it more mechanistically accounted for physiological changes after O_3 exposure. When scaled globally, independently modifying stomatal conductance (the PG simulation) allowed stomatal conductance to respond differently to O_3 exposure than photosynthesis, resulting in different rates of O_3 uptake that caused differences in GPP.

Many land surface components of climate models, such as the CLM, use the coupled Farquhar/Ball-Berry model to predict photosynthesis and stomatal conductance, which are then scaled to tree, canopy, ecosystem or global areas. However, changing photosynthesis in response to chronic O_3 exposure within the current Farquhar/Ball-Berry formulation does not accurately represent changes in stomatal conductance. Even small errors in stomatal conductance, when scaled to ecosystem or global areas, can propagate large errors in transpiration that then impact climate. Despite this fact, most studies modeling regional and global responses to O_3 (e.g. Felzer et al. 2004; Felzer et al. 2005; Ollinger et al. 1997; Ollinger et al. 2002; Ren et al. 2011; Sitch et al. 2007) focus primarily on changes in carbon and do not verify the accuracy of stomatal conductance predictions. We found that predictions of transpiration can be improved in regional and global models by directly modifying stomatal conductance.

Another key finding in all simulations is the potential importance of O_3 on tropical vegetation. Declines in GPP and transpiration were highest in tropical latitudes, similar to the findings in Sitch et al. (2007), and different methods of

incorporating reductions in stomatal conductance in the Pg and PG simulations resulted in the largest differences in the tropics. Despite these large predicted changes in the tropics, very little experimental data documenting the physiological responses of tropical plants to chronic O_3 exposure exists, making it difficult to determine the accuracy of responses. Since simulations predict this region to be largely impacted by chronic O_3, gas exchange data for tropical plants is a critical research need.

The ability of transpiration to respond differently than photosynthesis in the simulation that directly modifies conductance (PG) demonstrates that global models can allow for independent responses in photosynthesis and conductance. While this work focuses on O_3 oxidation damage, allowing photosynthesis and conductance to respond independently is critical for any situation that causes decoupling in these two parameters, such as oxidative damage caused by excessive light or nighttime transpiration. Though the parameterization of the CLM in these simulations is based on a single species at a single O_3 concentration, responses of multiple types of plants to realistic atmospheric O_3 can be incorporated into future simulations now that there is an established framework for varying photosynthesis and conductance separately. Ultimately, improving the ability of models to predict conductance allows for more accurate predictions of the water cycle, including atmospheric water vapor, a key gas in regulating climate.

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CHAPTER 3

INTEGRATING O_3 INFLUENCES ON TERRESTRIAL PROCESSES: PHOTOSYNTHETIC AND STOMATAL RESPONSE DATA AVAILABLE FOR LARGE-SCALE MODELING

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ABSTRACT

Plants have a strong influence on climate by controlling the transfer of carbon dioxide and water, two powerful greenhouse gases, between the biosphere and atmosphere through 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 biochemical aspects of 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 large-scale models. However, there are currently no existing 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. Using data from peer-reviewed literature, we have compiled photosynthetic and stomatal responses to chronic O_3 exposure as a standardized function of cumulative uptake of O_3 (*CUO*), which integrates O_3 flux into leaves through time. Crops, temperate deciduous and temperate evergreen trees currently make up 90% of available literature data, yet these plants only account for 13% of global net primary productivity (NPP). Tropical trees and grasslands account for 68% of global NPP, but only six studies included adequate information to calculate photosynthetic and stomatal responses to *CUO*. Results demonstrated that there was no overall correlation of photosynthesis with *CUO* and a positive correlation of conductance with *CUO*. Plant vulnerability was estimated using a ratio of photosynthesis to stomatal conductance in control treatments, assuming that higher conductance allowed higher O_3 flux rates and that internal defense was a function of

photosynthetic capacity to regenerate antioxidants. While plant types responded similarly to *CUO*, plant vulnerability plays a strong role in determining responses to O_3. Predicting global responses of the biosphere to chronic O_3 exposure will be improved if responses of photosynthesis and stomatal conductance to *CUO* are measured in tropical tree and grassland species.

INTRODUCTION

Surface ozone (O_3) concentrations have increased by 30 – 60% globally since industrialization (Karnosky 2005) resulting in large economic costs – billions of dollars in the US and Europe alone – because O_3 is a greenhouse gas and an oxidant causing visible foliar damage and reductions in crop yield (Skarby *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), which 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 the warmer temperatures resulting from climate change (Jacob and Winner 2009). At the same time, higher water vapor concentrations in the future climate are expected to decrease global background O_3 concentrations ($[O_3]$; 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). Ozone follows the same pathway through plant leaves as carbon dioxide (CO_2), damaging each step of photosynthesis (see Figure 3.1). After O_3 enters the leaf through the stomata (1) it: 2) decreases mesophyll conductance by oxidizing cellular membranes (Fiscus *et al*. 2005), and 3) decreases carbon fixation by reducing enzyme content and activity (Ojanpera *et al*. 1998; Farage and Long, 1999; Fiscus *et al*. 2005), and by decreasing chlorophyll

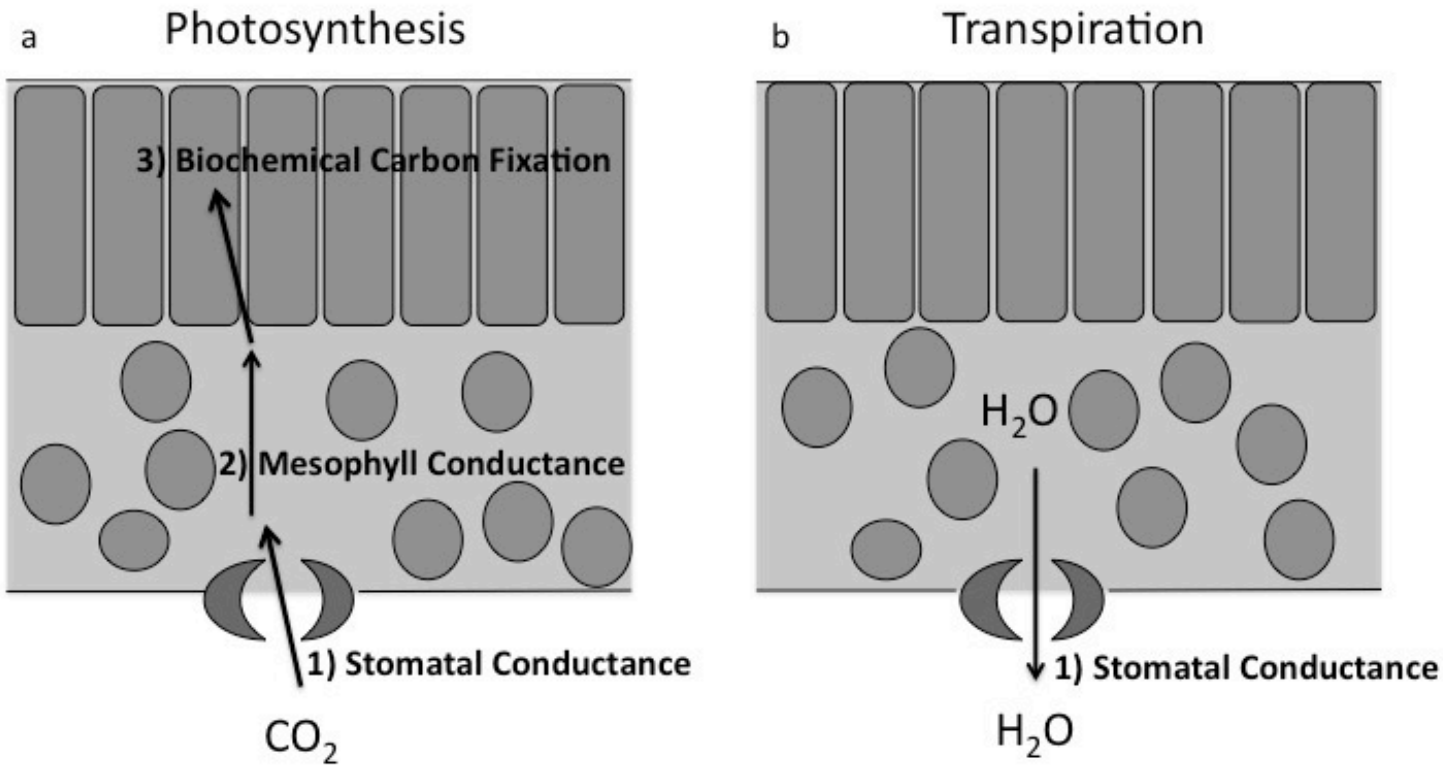

Figure 3.1: An illustration of leaf cross sections. The process of photosynthesis (a) relies on three steps: 1) CO_2 enters the leaf through the stomata, which is regulated by the size of the opening between the stomatal cells, or the stomatal conductance; 2) CO_2 travels through the intercellular spaces to the chloroplasts (mesophyll conductance); 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 mesophyll conductance (2), leading to a decoupling of photosynthesis and transpiration.

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, directly by changing guard cell turgor pressure and signaling pathways (e.g. absicic acid, K^+; Freersmith 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). While all these mechanisms tend to reduce transpiration and photosynthesis, the damage to internal leaf biochemistry causes O_3 to have an unequal effect on photosynthesis and transpiration (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).

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 exposure to chronic O_3. Average stomatal responses, on the other hand, 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 conductance does not always respond the same as photosynthesis and that responses are variable among plant types. Several studies demonstrate that chronic O_3 exposure results in sluggish stomatal responses in

many plants types (Mills *et al*. 2009; Paoletti and Grulke, 2010), and can increase leaf-level and ecosystem-scale transpiration rates (Paoletti 2005; McLaughlin *et al*. 2007).

Plant responses to chronic O_3 exposure 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 resistance and therefore describes $[O_3]$ inside the leaf, it estimates the ability of O_3 to oxidize physiological components of the leaf that regulate photosynthesis and stomatal conductance. However, *CUO* cannot account for internal defenses, such as antioxidants, and plants exposed to high concentrations for short periods of time will have similar *CUO* to plants exposed to lower concentrations for longer times. Regardless, several studies demonstrate strong negative correlations between photosynthesis (Reich 1987; Wittig *et al*. 2007), biomass (Karlsson *et al*. 2004; Uddling *et al*. 2004) and relative crop yield (Pleijel *et al*. 2002; Pleijel *et al*. 2004) and *CUO*, making *CUO* a better metric for predicting photosynthetic responses to O_3 than concentration or dose (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.

Despite differences in photosynthetic and stomatal responses, many modeling studies assume a fixed relationship between photosynthesis and stomatal conductance (Ollinger *et al*. 1997; Felzer *et al*. 2004; Sitch *et al*. 2007). Simulating O_3 changes to photosynthesis in this manner typically overestimates changes in conductance (Lombardozzi *et al*. 2012a). If conductance responds differently 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 under-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, very little data is available documenting responses of conductance through time.

The objective of this work was to determine responses of both photosynthesis and stomatal conductance to chronic O_3 exposure in multiple plant types, using *CUO* as an index for damage. We collected all available data from 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. These data will provide more robust estimates of plant-specific photosynthetic and stomatal responses to chronic O_3 exposure and can be incorporated into regional and global models to improve future predictions of changes in photosynthesis and transpiration.

METHODS

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 through June 2011 that chronically (>7 days) exposed plants to O_3 using growth chambers, open-top chambers, branch chambers, or another fumigation method contained data sufficient to calculate cumulative uptake of O_3 (*CUO*; see equation below) and reported changes in photosynthesis and 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 conductance; if units of conductance were not reported per leaf area; or if studies were shorter than seven days and plants were, therefore, not exposed to chronic O_3. In total, data were collected from 127 papers with 92 species providing a sample size of 1169 for analyses.

Values of photosynthesis, conductance, V_{cmax} 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

conductance to *CUO* was recorded for each data point. These factors included plant type, plant age, and type of air to which control plants were exposed (e.g. ambient or charcoal-filtered). Confidence in *CUO* calculations and estimated vulnerability were also documented (see Table 3.1).

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 conductance measurements throughout the course of the experiment, allowing calculations to account for changes in conductance through time and resulting in more accurate calculations of *CUO*. Data were assigned *low* confidence if only an end-point conductance measurement was reported or if any assumptions had to be made about duration of O_3 exposure.

Vulnerability was estimated using a ratio of photosynthesis to conductance in the control treatment, assuming that higher conductance allowed potentially 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 conductance. Therefore, a plant with high conductance relative to photosynthesis 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 conductance (photosynthesis/conductance) were grouped into quartiles and data with high values assigned to *low* vulnerability (high photosynthesis per unit conductance) and low values assigned to *high* vulnerability (high conductance

Table 3.1: Categories and levels describing the data collected from experiments studying O3 effects on photosynthesis (Amax) and stomatal conductance (gs). 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 (36, 241)	deciduous shrub (2, 14)	evergreen shrub (2, 9)	grasses (C_3 & C_4) (2, 9)	herbaceous (4, 50)	deciduous tree (59, 646)	evergreen tree (24, 183)	tropical tree (4, 17)
Plant Age (years)	< 1 (57, 443)	1 - 5 (60, 662)	> 5 (13, 55)					
Control Air	ambient (35, 349)	charcoal filtered (91, 812)						
Data Confidence	low (66, 461)	medium (49, 582)	high (12, 126)					
Sensitivity	low (72, 293)	medium-low (59, 292)	medium-high (51, 292)	high (33, 292)				

per unt photosynthesis). Intermediate quartiles were assigned a *medium-high* or *medium-low* vulnerability.

CUO calculations

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

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

where *H* was the number of hours per day the plant was exposed to elevated O_3, *D* was the total number of days and $[O_3]$ is the O_3 concentration in ppb. Cumulative uptake of O_3 was calculated using CEO_3:

$$CUO \text{ (mmol m}^{-2}) = CEO_3 \times g_s \times k_{O3} \times 3600 \times 10^{-6} \qquad \text{Equation 2}$$

where g_s is stomatal conductance in units of mol m^{-2} s^{-1}, $k_{O3} = 1.67$ and is the ratio of the diffusivity constants for water vapor and O_3, *3600* is the number of seconds per hour, and 10^{-6} is the conversion from nmol to mmol (similar to Reich, 1987; Nunn *et al*., 2006; Wittig *et al*., 2007; Lombardozzi *et al*., 2012a). Multiple measurements of conductance reported throughout the course of the experiment allowed for more accurate estimations of *CUO* by accounting for changes in conductance through time. *CUO* was calculated from these data for only the time period between the measurements using the appropriate conductance value. *CUO* for all time periods were then summed, accounting for changes in conductance throughout the experiment.

Statistical Analyses

A linear regression framework was used to analyze data for relationships between change in photosynthesis or conductance relative to plants exposed to little or no O_3 (% of control) with *CUO* using the 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 conductance and *CUO* was selected using r^2 values.

RESULTS

There was no correlation between photosynthesis and *CUO* (Figure 3.2a; $r^2 = 0.002$, $p = 0.14$), though there was a weak but significant positive relationship between conductance and *CUO* (Figure 3.2b; $r^2 = 0.012$, $p < 0.001$). Average values for both variables (data not shown) significantly decreased after chronic O_3 exposure ($p < 0.001$ for each); photosynthesis decreased by 20%, which was significantly greater than the 12% decrease in conductance ($p < 0.001$).

Confidence in *CUO* calculations did not change the mean value or alter the correlation of *CUO* with photosynthesis. There was no significant correlation between

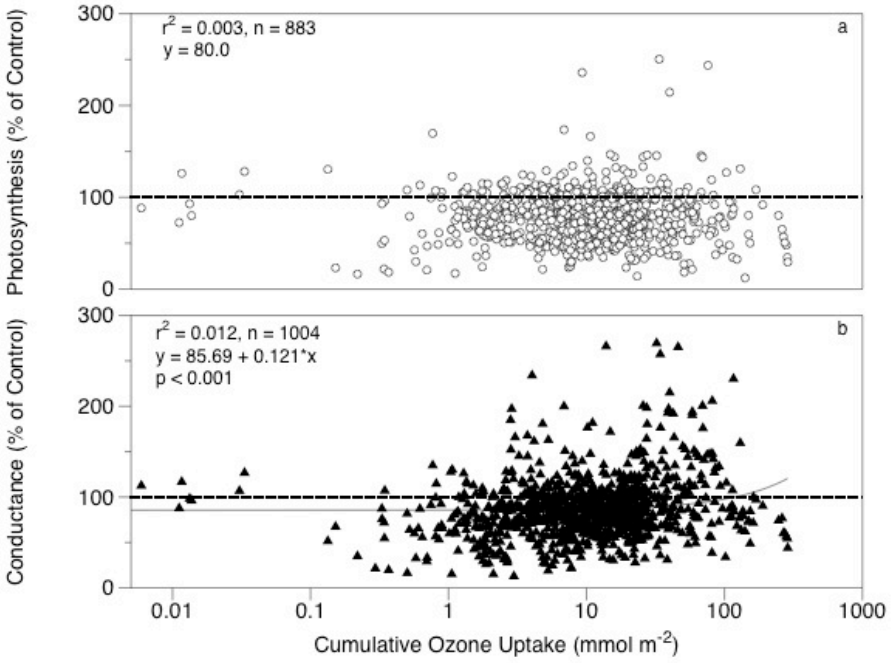

Figure 3.2: The correlation of photosynthesis (open symbols; a) and conductance (closed symbols; b) across all plant types, ages, sensitivities and confidence levels to *CUO*. Response values are reported as % of control (treatment/control). CUO is shown on a log scale, but linear analyses (r^2 and line equations) were performed on non-log transformed data. P-values are only included for significant correlations and the horizontal dotted line at 100% represents no change from control.

CUO and photosynthesis in *high* ($p = 0.71$), *medium* ($p=0.25$), or *low* ($p = 0.39$) confidence data. There was also no difference between the overall photosynthesis mean and the means of *high* ($p = 0.08$), *medium* ($p = 0.88$), and *low* ($p = 0.68$) confidence data (Figure 3.3a-c). There was no significant correlation between *CUO* and *high* confidence stomatal conductance data ($p = 0.11$), but there was a significant correlation in *medium* ($p = 0.02$) and *low* ($p = 0.02$) confidence data. Mean *high* confidence data was significantly lower than the overall mean stomatal conductance ($p = 0.05$), though there was no significant difference between the overall conductance mean and *medium* ($p = 0.38$) or *low* ($p = 0.69$) confidence data. The similarity between the whole dataset and data separated by *high*, *medium*, and *low* confidence suggests that uncertainty in *CUO* calculations did not influence the relationships between *CUO* and photosynthesis or conductance. Only 12 publications included *CUO* values (*high* confidence data) though *medium* and *low* confidence data were more widely available.

V_{cmax} estimates aspects of biochemical carbon fixation that are 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; Ojanpera et al. 1998; Pellegrini et al. 2010; Zheng et al. 2002). Though V_{cmax} data was only available for a small subset of manuscripts, it responded similarly to photosynthesis responses documented in those same manuscripts (Figure 3.4; $p = 0.41$). Neither V_{cmax} nor photosynthesis was significantly correlated with *CUO* (V_{cmax}: $p = 0.31$, $r^2 = 0.023$; photosynthesis: $p = 0.41$, $r^2 = 0.015$), though mean responses of both significantly decreased from control values ($p < 0.001$).

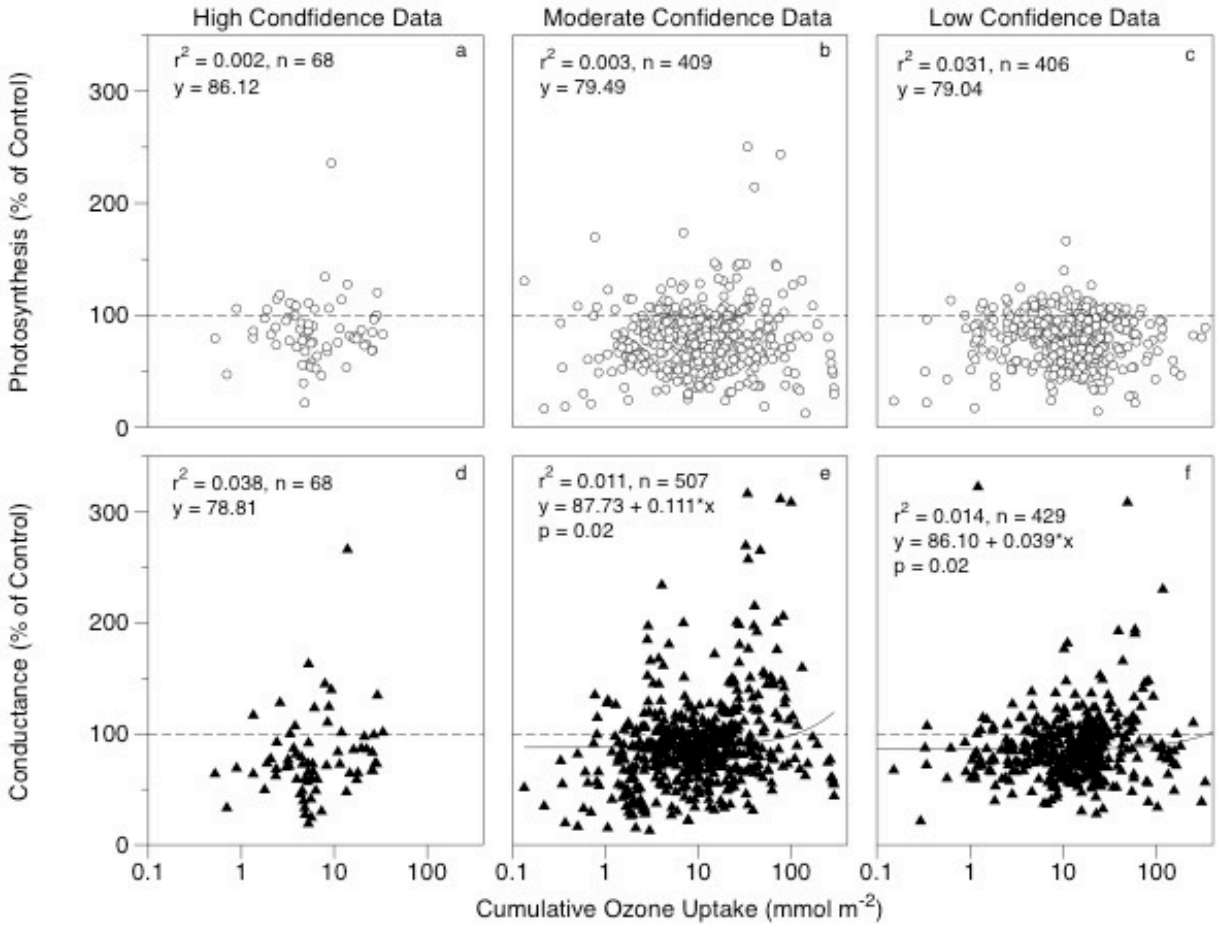

Figure 3.3: The correlation of photosynthesis (open symbols; a-c) and conductance (closed symbols; d-f) to *CUO* for *CUO* calculations with high (a, d), moderate (b, e) and low (c, f) confidence. Response values are reported as % of control (treatment/control). CUO is shown on a log scale, but linear analyses (r^2 and line equations) were performed on non-log transformed data. P-values are only included for significant correlations.

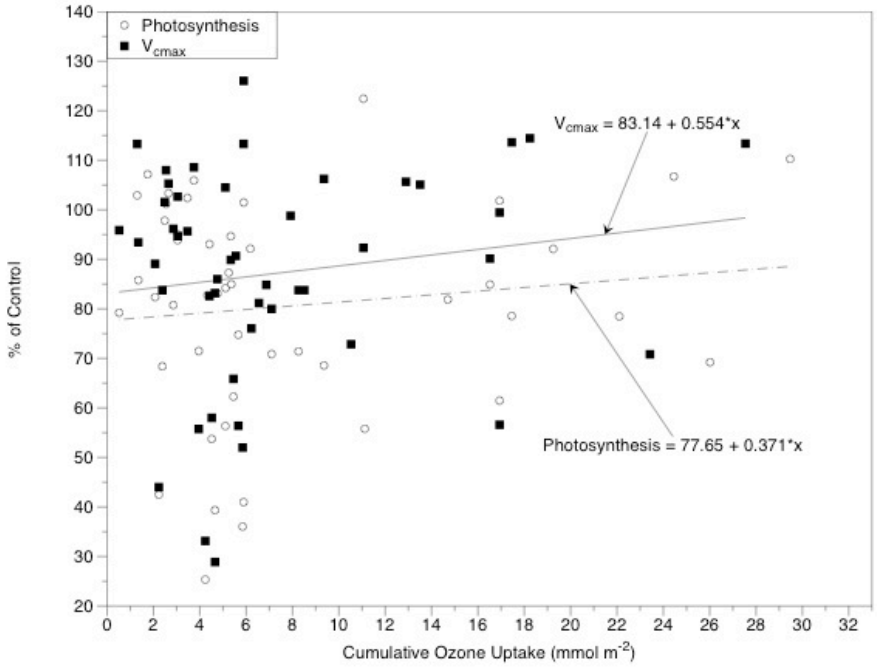

Figure 3.4: The correlation of photosynthesis (open symbols) and V_{cmax} (closed symbols) to *CUO* from all studies that included information for both variables. Response values are reported as % of control (treatment/control). P-values are only included for significant correlations.

Accounting for plant age did not improve overall correlations of either photosynthesis (Table 3.2) or conductance (Table 3.3) with *CUO*. Conductance significantly increased with *CUO* in plants one to five years old (Table 3.3; $p<0.001$, $r^2 = 0.074$) and photosynthesis significantly increased with *CUO* in plants less than one year old (Table 3.2; $p = 0.01$, $r^2 = 0.015$). Average photosynthesis was 7% higher and conductance was 9% higher in plants one to five years old compared to plants less than one year or older than five years (Table 3.2 and 3.3), though only 13 publications included data for plants older than five years.

Accounting for plant functional type (Figure 3.5) did not improve correlations of either variable with *CUO*; significant correlations were only found with temperate deciduous tree and crop conductance (Figure 3.5d: $p < 0.001$, $r^2 = 0.045$ and Figure 3.5f <0.001, $r^2 = 0.003$, respectively). Data presented here focused only on plant functional types with abundant data, including temperate deciduous trees, crops and temperate evergreen trees (55, 21, and 15% of the total data, respectively; Table 3.1). Very few studies (14 of 127) contained data for other plant functional types, with data for all other plant types reported in four or fewer publications. Average responses of photosynthesis and conductance varied slightly by plant type (data not shown). Photosynthesis was similar for crops (Figure 3.5c) and temperate deciduous trees (Figure 3.5a), though was significantly higher (9%) in temperate evergreen trees (Figure 3.5b), while conductance was similar in temperate deciduous (Figure 3.5d) and temperate evergreen trees (Figure 3.5e), but crop conductance (Figure 3.5f) was significantly lower (11%).

Table 3.2: The number of datapoints (n), mean, regression, and statistics for photosynthesis when sorted by categories of plant age, high vulnerability and low vulnerability. The p-value for the mean designates whether the mean of the category is statistically different from the mean of the overall dataset (All Data). All regressions are based on the correlation of photosynthesis to cumulative O_3 uptake (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 *.

		n	Mean	p-value	Regression	r^2	p-value
All Data		883	80.0		NS	0.003	
Plant Age (years)							
	< 1	418	76.86	0.11	75.47 + 0.385*x	0.015	0.01*
	1 – 5	421	83.46	0.07	NS	0.004	0.26
	> 5	47	75.71	0.19	NS	0.073	0.07
High Vulnerability							
All air types	Temperate Deciduous Tree	87	80.56	0.87	NS	0.021	0.18
	Temperate Evergreen Tree	16	81.42	0.81	111.10 – 3.390*x	0.385	0.01*
	Crop	66	79.77	0.99	82.74 – 0.067*x	0.068	0.03*
Charcoal-filtered air	Temperate Deciduous Tree	82	81.93	0.67	NS	0.027	0.14
	Temperate Evergreen Tree	12	78.49	0.89	112.36 – 3.743*x	0.453	0.02*
	Crop	66	79.77	0.99	82.74 – 0.067*x	0.068	0.03*
Low Vulnerability							
All air types	Temperate Deciduous Tree	120	89.13	0.02*	81.54 + 0.403*x	0.041	0.04*
	Temperate Evergreen Tree	75	89.96	0.001*	NS	0.022	0.20
	Crop	57	77.33	0.42	NS	0.001	0.82
Charcoal-filtered air	Temperate Deciduous Tree	49	88.94	0.14	NS	0.037	0.26
	Temperate Evergreen Tree	31	85.97	0.12	NS	0.083	0.12
	Crop	38	72.68	0.01*	NS	0.038	0.24

Table 3.3: The number of datapoints (n), mean, regression, and statistics for stomatal conductance when sorted by categories of plant age, high vulnerability and low vulnerability. The p-value for the mean designates whether the mean of the category is statistically different from the mean of the overall dataset (All Data). All regressions are based on the correlation of photosynthesis to cumulative O_3 uptake (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 *.

		n	Mean	p-value	Regression	r^2	p-value
All Data		1004	88.0		85.69 + 0.121*x	0.012	<0.001*
Plant Age (years)							
	< 1	443	86.32	0.36	NS	0.008	0.06
	1 – 5	497	91.18	0.16	82.08 + 0.507*x	0.074	<0.001*
	> 5	55	70.03	0.01*	NS	0.032	0.19
High Vulnerability							
All air types	Temperate Deciduous Tree	87	78.61	0.001*	NS	0.003	0.18
	Temperate Evergreen Tree	16	74.01	0.002*	84.41 – 1.154*x	0.246	0.05*
	Crop	66	69.22	<0.001	NS	0.000	0.96
Charcoal-filtered air	Temperate Deciduous Tree	82	81.08	0.01*	NS	0.022	0.18
	Temperate Evergreen Tree	12	73.68	0.004*	86.218 – 1.386*x	0.322	0.05*
	Crop	66	69.22	<0.001*	NS	0.000	0.96
Low Vulnerability							
All air types	Temperate Deciduous Tree	211	104.56	<0.001*	96.87 + 0.324*x	0.043	0.04*
	Temperate Evergreen Tree	89	96.07	0.03*	NS	0.041	0.06
	Crop	60	90.69	0.71	68.118 + 2.2893	0.578	<0.001*
Charcoal-filtered air	Temperate Deciduous Tree	92	95.73	0.04*	NS	0.003	0.63
	Temperate Evergreen Tree	37	89.14	0.83	79.35 + 0.592*x	0.324	<0.001*
	Crop	41	91.28	0.75	61.32 + 2.419*x	0.650	<0.001*

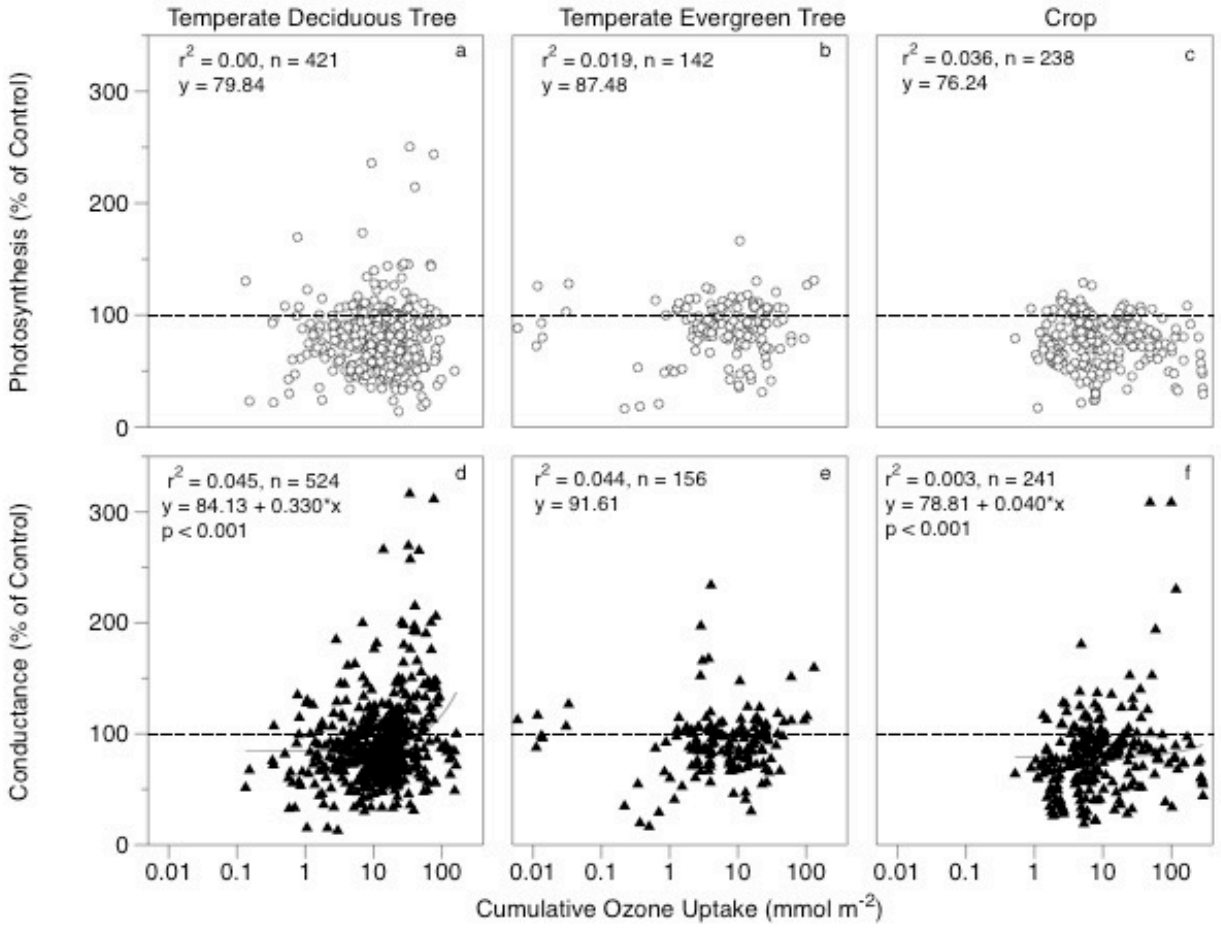

Figure 3.5: The correlation of photosynthesis (open symbols; a-c) and conductance (closed symbols; d-f) to *CUO* for temperate deciduous trees (a, d), temperate evergreen trees (b, e) and crops (c, f). Response values are reported as % of control (treatment/control). CUO is shown on a log scale, but linear analyses (r^2 and line equations) were performed on non-log transformed data. P-values are only included for significant correlations.

As discussed in the Methods section, plants with relatively high stomatal conductance and low photosynthesis rates are expected to be more vulnerable to O_3 exposure. Estimating plant vulnerability improved the overall relationships between photosynthesis and conductance with *CUO* (Figure 3.6), and both were positively correlated with *CUO* at high (photosynthesis: $p = 0.04$, $r^2 = 0.019$; conductance: $p = 0.04$, $r^2 = 0.020$) and low sensitivities (photosynthesis: $p = 0.04$, $r^2 = 0.017$; conductance: $p < 0.001$, $r^2 = 0.071$). When plant type was additionally included, temperate deciduous tree responses were correlated with *CUO* only for plants with low vulnerability (Tables 3.2 and 3.3; photosynthesis: $p = 0.04$, $r^2 = 0.041$; conductance: $p = 0.003$, $r^2 = 0.043$), whereas temperate evergreen tree responses were only correlated for plants with high sensitivities (Tables 3.2 and 3.3; photosynthesis: $p = 0.01$, $r^2 = 0.385$; conductance: $p = 0.05$, $r^2 = 0.246$). In crops, photosynthesis in plants with high vulnerability ($p = 0.03$, $r^2 = 0.068$) and conductance in plants with low vulnerability ($p < 0.001$, $r^2 = 0.578$) were correlated with *CUO* (Tables 3.2 and 3.3). Mean conductance values in all plant types with high vulnerability were significantly lower than overall mean conductance (Table 3.3), though average photosynthesis values for high vulnerability plants did not change from the overall mean (Table 3.2). Conductance and photosynthesis means for low vulnerability temperate deciduous and temperate evergreen trees were significantly higher than overall means (Tables 3.2 and 3.3).

In all analyses, control plants were exposed to ambient air or charcoal filtered (CF) air. Charcoal filtered air removed nearly all O_3 from the environment whereas exposure to ambient air meant control plants were exposed to the background level of

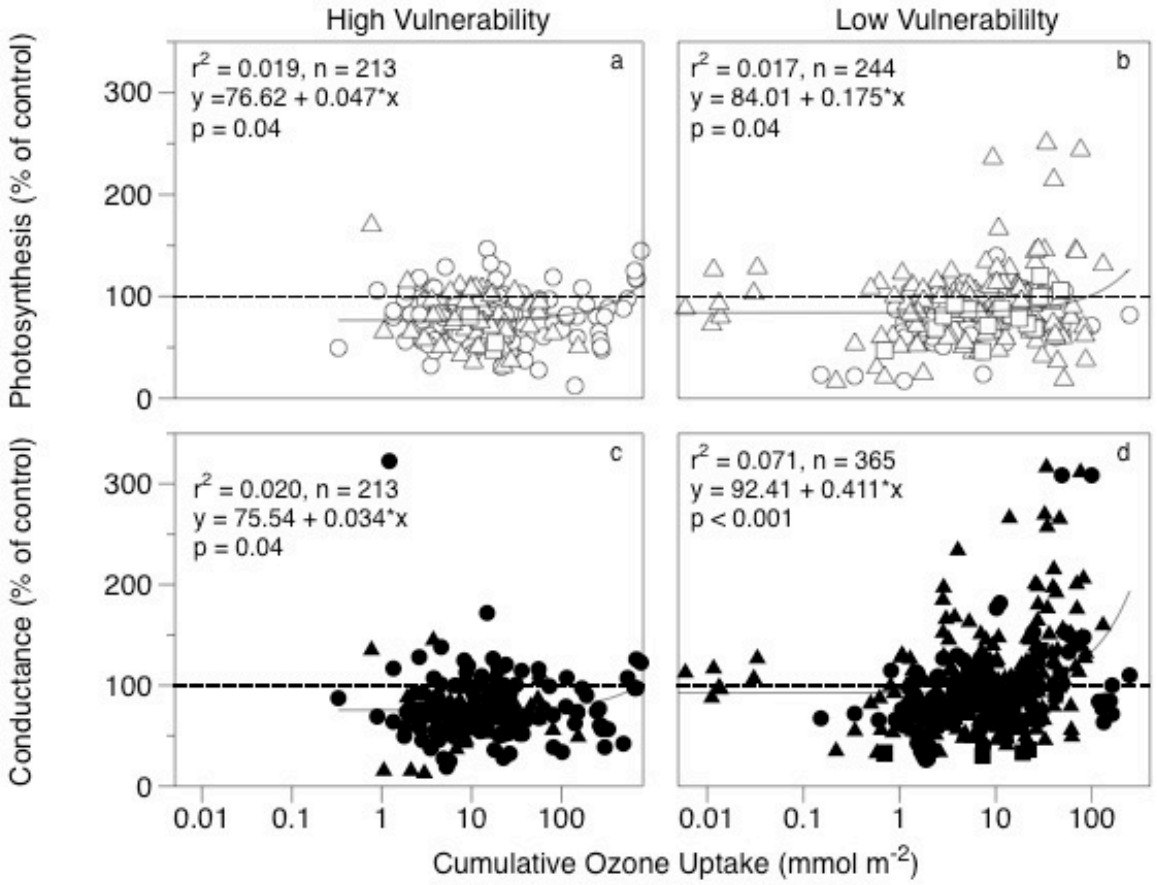

Figure 3.6: The correlation of photosynthesis (open symbols; a-b) and conductance (closed symbols; c-d) to *CUO* for all plants with high vulnerability (a, c) and low vulnerability (b, d) to chronic O_3 exposure. Circles represent plants less than one year old (<1), triangles represent plants between one and five years old (1-5) and squares represent plants older than five years (>5). 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. P-values are only included for significant correlations.

O_3 specific to a particular research location. Accounting for the type of air that control plants were exposed to did not improve correlations of photosynthesis or conductance with *CUO* in temperate deciduous trees (Tables 3.2 and 3.3; high vulnerability, photosynthesis: $p = 0.14$, $r^2 = 0027$; conductance: $p = 0.18$, $r^2 = 0.022$; low vulnerability, photosynthesis: $p = 0.26$, $r^2 = 0.037$; conductance: $p = 0.63$, $r^2 = 0.003$). However, analyses that included only CF air typically improved photosynthesis and conductance correlations in high and low sensitive temperate evergreen trees (Tables 3.2 and 3.3; high vulnerability photosynthesis: $p = 0.02$, $r^2 = 0.453$; conductance: $p = 0.05$, $r^2 = 0.322$; low vulnerability photosynthesis: $p = 0.12$, $r^2 = 0.083$; conductance: $p = <0.001$, $r^2 = 0.324$) and crops (Tables 3.2 and 3.3; high vulnerability, photosynthesis: $p = 0.03$, $r^2 = 0.068$; conductance: $p = 0.96$, $r^2 = 0.00$; low vulnerability, photosynthesis: $p = 0.24$, $r^2 = 0.038$; conductance: $p = <0.001$, $r^2 = 0.650$).

DISCUSSION

Chronic O_3 exposure causes the plant physiological processes of photosynthesis and conductance to change, though how these processes change through time is not well known for many plant functional types. Crops, temperate deciduous and temperate evergreen trees account for ~90% of the data available in the primary literature, yet these plant types cover only 25% of Earth's land surface (Grace 2004). Only four studies had data available for tropical tree species or herbaceous plants, and two or fewer studies contained data for grass species, deciduous and evergreen shrubs (Table 3.1). This important lack of information underscores the

challenge in understanding modeled O_3 responses and demonstrates the uncertainty of models to adequately represent global O_3 responses. Tropical trees account for approximately 12% of Earth's land surface and 35% of global net primary production (NPP), and grasslands cover 29% of Earth's land surface and contribute 33% to global NPP (Grace 2004), yet 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 these ecosystems on temperate tree and crop data (e.g., Reich 1987, Karlsson *et al*. 2004, Pleijel *et al*. 2004). More information is needed for several plant functional types and this should be a critical research priority if the scientific community is to confidently assess global impacts of O_3.

The results of this quantitative analysis suggest that chronic O_3 exposure depressed leaf-level photosynthesis and conductance in all plant types, ages, and estimated sensitivities. Overall, the 20% average decrease in photosynthesis was larger than the 12% average decrease in conductance, demonstrating that these variables responded differently to chronic O_3 exposure. A meta-analysis of tree responses to O_3 compared to charcoal-filtered air similarly found a 19% decrease in photosynthesis and a 10% decrease in conductance (Wittig *et al*. 2007). Our study found that decreases in photosynthesis were typically larger than the decreases in conductance in most plant type, age, and estimated vulnerability categories, though the magnitude of decrease in each variable differed based on the category.

Correlations of photosynthesis and conductance to *CUO* were not consistent and responses varied based on plant type, age, or estimated vulnerability. When all categories were combined, conductance was positively related to *CUO* (Figure 3.2),

suggesting that higher [O_3] or longer exposure times may result in increased conductance compared to plants exposed to low or no O_3. Unexpectedly, there was no correlation between photosynthesis and *CUO*, which 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) and results found by Wittig *et al*. (2007) and Reich (1987) for trees and crops. The absence of a correlation between photosynthesis and *CUO* found in this study suggests that increasing O_3 concentrations, which can increase *CUO*, will not cause larger decreases in plant carbon uptake than already observed.

Although we observed a similar overall decrease in photosynthesis, it is not clear why our study found no correlation between photosynthesis and *CUO* when the results of Reich (1987) and Wittig *et al*. (2007) demonstrate strong negative correlations. Demography of the data might play a role in the difference as the present study had a larger sample size (n = 883) and incorporated multiple types of plants, though temperate trees (evergreen and deciduous) comprised 70% of these data. While *CUO* calculations were nearly identical 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). Another potential factor driving differences was that our method of calculating *CUO* accounted for changes in conductance through time whenever possible, which was not factored into *CUO* calculations in other studies.

Most studies reporting responses of photosynthesis and conductance to chronic O_3 exposure do not provide *CUO* or fine-scale measurements of conductance to allow for accurate estimations of *CUO*, making it difficult to document responses of photosynthesis and conductance over a broad range of *CUO*. While statistical and modeling techniques have improved the accuracy of *CUO* by simulating hourly 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 data within most papers. Confidence in the *CUO* calculations did not influence the response of photosynthesis (Figure 3.3a-c), suggesting that including *medium* and *low* confidence data did not alter the correlations of photosynthesis to *CUO*. While *medium* and *low* confidence stomatal conductance data (Figure 3.3e-f) were not different from the overall mean, *high* confidence conductance data (Figure 3.3d) had a significantly lower mean and was not correlated with *CUO*. Variability is large, but similar in each confidence level, so the smaller sample sizes in *high* confidence data likely contribute to the lack of correlation between conductance and *CUO* in *high* confidence data.

To our knowledge, this is the first study to date that calculates the correlation between conductance and *CUO*, despite the known importance of conductance responses to O_3 on ecosystem-scale water dynamics like soil water content and stream flow (McLaughlin *et al*. 2007). In this study, conductance initially decreased and typically had no or a positive correlation with *CUO*, though was less affected by O_3 than photosynthesis. The increases in conductance with *CUO* might be due to O_3-

induced stimulation of conductance, though it is more likely indicative of sluggish conductance responses (as in Paoletti and Grulke, 2010), in which stomatal cells cannot respond to environmental cues and therefore remain open at higher *CUO*.

Initial O_3 damage usually decreases enzyme activity (Fiscus et al. 2005) and is apparent in V_{cmax} reductions after chronic O_3 exposure. Accounting for decreases in V_{cmax} is one mechanistic approach that physiological models can use to estimate O_3 damage to photosynthesis (Martin et al. 2000, Lombardozzi et al. 2012b). In the available data, average V_{cmax} decreased in response to chronic O_3, though was not significantly correlated with *CUO*. Responses of V_{cmax} were not significantly different from photosynthetic responses, suggesting that either can be used to estimate photosynthetic decreases. However, given the lack of data estimating responses of V_{cmax} to chronic O_3 exposure, responses of photosynthesis can be used with more confidence.

In this study, plant age did not have an overall effect on the relationship of photosynthesis or conductance with *CUO* (Tables 3.2 and 3.3). However, slightly higher mean photosynthesis and conductance in plants from one to five years old suggests the plants in this age bracket are not as strongly affected by O_3 as younger and older plants. Contrary to these results, Edwards *et al*. (1994) found that O_3 caused photosynthesis to decrease more in mature red oak trees compared to seedling conductance. The data in the present study cover a broader range of tree species than used by Edwards *et al*. (1994), but does not necessarily make direct comparisons between young and old trees of the same species. Manipulating the atmosphere around adult woody plants is difficult, making it hard to assess physiological

responses of mature trees to chronic O_3 exposure. Given these challenges, literature data are most scarce for mature trees, which make comparisons across plant ages challenging and modeling responses of different age classes more uncertain.

Plant functional type did not have a strong effect on the relationship of photosynthesis or conductance with *CUO* (Figure 3.5), although the magnitude of decrease in each variable did change by plant functional type. In general, photosynthesis decreased more than conductance for each plant functional type, with the largest difference in temperate deciduous trees. Chronic O_3 exposure caused smaller decreases in average photosynthesis in temperate evergreen trees compared to temperate deciduous trees or crops. Contrary to these results, Wittig *et al*. (2007) found photosynthesis decreases that were similar in both angiosperms and gymnosperms. However, Reich and Amundson (1985) found that crop photosynthesis was more negatively affected than a temperate evergreen tree after chronic O_3 exposure, similar to the results of our study. Data used in this analysis additionally suggests that the decrease in mean crop conductance was 11% larger than in either type of tree. This result is consistent with responses of conductance determined in meta-analyses of multiple tree types and wheat; tree conductance decreased by 10% (Wittig *et al*. 2007) and wheat conductance decreased by 22% (Feng *et al*. 2008).

Separating plants into groups based on high or low vulnerability to O_3 improved the correlations of photosynthesis and conductance with *CUO* (Figure 3.6). This result was not surprising given the importance of accounting for detoxification capacity in determining effective O_3 flux (Matyssek *et al*. 2007). We found that photosynthesis and conductance in plants with high and low vulnerability were

positively correlated with *CUO*, though plants with low vulnerability had considerably faster rates of increase per unit *CUO*. Across all data, plants with low vulnerability seemed to recover from or even be stimulated by O_3 and this trend was particularly obvious in temperate deciduous trees and crops. One possible explanation is that plants with low vulnerability respond to chronic O_3 exposure by up-regulating photosynthesis to increase detoxification capacity, which allows the plants to recover and might result in higher photosynthesis. High vulnerability plants only recovered photosynthetic and stomatal capacity at high CUO, a trend that was driven by plants less than 1-year old. While including detoxification capacity into O_3 flux calculations is unrealistic on large scales (Matyssek *et al*. 2007), estimating vulnerability based on the ratio of photosynthesis to conductance in plants not exposed to O_3 is easier to extrapolate to regional and global scales and improves correlations of both photosynthesis and conductance with *CUO*.

All responses were calculated by comparing O_3-treated plants to control plants that were exposed to either ambient air or CF air. Excluding responses that were calculated using control plants exposed to ambient air improved the correlations of temperate evergreen trees and crops with *CUO*, although it did not improve the correlation between photosynthesis or conductance and *CUO* in temperate deciduous trees (Tables 3.2 and 3.3). Control plants exposed to ambient air likely already experience a decrease in conductance and/or photosynthesis, damping the response of the variables as well as the calculated *CUO* if conductance had been reduced. Wittig *et al*. (2007) estimated that decreases from exposure to ambient O_3 were approximately 10% for photosynthesis and 13% for conductance, suggesting that

responses of plants compared to control ambient air in this study would likely be 10-13% larger if compared to control plants exposed to CF air.

Ozone damage to plants can have large impacts on ecosystems, yet our ability to predict these responses is inadequate. Our understanding of plant responses is limited to temperate and crop ecosystems, leading to large uncertainty in global predictions. Further, most models incorporating plant responses to O_3 assume 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 only accurate for high-vulnerability crops and evergreen trees rather than the mean response of most plant types. The common assumption that conductance declines linearly with photosynthesis also needs to be reconsidered since the responses of 1000+ independent measurements collected in this study largely suggests that photosynthesis and conductance do not change at the same rate during chronic O_3 exposure. Though O_3 is likely to cause changes in climate (Sitch *et al*. 2007), model predictions of the influence of O_3 on climate, carbon cycling and hydrology are inaccurate because they assume linear decreases in photosynthesis and a parallel decrease in conductance (e.g., Lombardozzi *et al*. 2012a, Lombardozzi *et al*., 2012b). Simulating robust responses of both photosynthesis and conductance to chronic O_3 exposure rather than responses of a single or few studies will improve the accuracy of predictions, though global responses, particularly for grasslands and tropical forests, will continue to be uncertain until more data are collected.

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CHAPTER 4

THE INFLUENCE OF CHRONIC OZONE EXPOSURE ON GLOBAL TRANSPIRATION AND GROSS PRIMARY PRODUCTIVITY: IMPLICATIONS FOR CLIMATE.

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ABSTRACT

Ozone (O_3) is a phytotoxic greenhouse gas that has globally increased from pre-industrial values of 10 ppb to a present day average of 40 ppb. In addition to directly increasing radiative forcing through its role as a greenhouse gas, O_3 can have indirect impacts on climate through alteration of the plant processes of photosynthesis and transpiration. While global estimates of gross primary productivity (GPP) have recently incorporated the effects of ozone, very few studies have explicitly determined the independent effects of O_3 on transpiration. In this study, we have included effects of present day ozone on photosynthesis and stomatal conductance obtained from a recent literature review to determine the global impact on GPP and transpiration. Using the Community Land Model, we found that chronic O_3 exposure reduces GPP and transpiration globally by 8% and less than 1%, respectively. Larger reductions in GPP compared to transpiration caused carbon and water cycles to become decoupled and changed patterns of surface runoff and energy fluxes.

INTRODUCTION

The world's vegetation regulates climate in part by controlling carbon and water fluxes between the biosphere and the atmosphere (Bonan et al. 2008), with terrestrial ecosystems sequestering an estimated 2.1 Pg of carbon annually (Luyssaert et al. 2007). Carbon and water fluxes through plants are linked by the common control of stomatal conductance, which regulates the rate of carbon dioxide (CO_2) entering and water exiting the leaf (Jones 1998). Land models that incorporate physiology couple carbon and water fluxes by calculating stomatal conductance, the primary plant control over water loss (transpiration), directly from rates of photosynthesis (carbon gain). While this method of predicting carbon and water fluxes is accurate under optimal environmental conditions (Niyogi and Raman 1997), stomatal conductance is not accurately predicted under the oxidative stress caused by chronic ozone (O_3) exposure (Lombardozzi et al. 2012a).

Ozone is a powerful oxidant that damages the physiology of most plant types (Lombardozzi et al. chapter 3), with the potential to alter global climate (Sitch et al. 2007) and hydrology (McLaughlin et al. 2007; Felzer et al. 2009). Most models directly couple photosynthetic and stomatal responses to chronic O_3 exposure. Experimental evidence suggests that this assumption is not correct (Mikkelsen 1995; Tjoelker et al. 1995; Maurer et al. 1997; Soldatini et al. 1998; Novak et al. 2005; Paoletti and Grulke 2005; Calatayud et al. 2007; Francini et al. 2007; Lombardozzi et al. 2012a; Lombardozzi et al. chapter 3) and can lead to incorrect predictions of transpiration (Lombardozzi et al., 2012b). Photosynthesis consistently decreases in response to chronic O_3 exposure because it oxidizes cellular membranes, enzymes, and

chlorophyll (Heagle et al. 1996; Ojanpera et al. 1998; Farage and Long, 1999; Bortier et al. 2000; Sharma 2003; Fiscus et al. 2005; Herbinger et al. 2007). Conductance, on average, also decreases in response to O_3, though with a smaller magnitude than photosynthesis (Lombardozzi et al., chapter 3). Ozone oxidizes components of the stomata, causing changes in guard cell turgor pressure and disrupting signaling pathways, both of which lead to increasing internal leaf CO_2 concentration (Freersmith and Dobson 1989; Maier-Maercker and Kock 1991; Hassan et al. 1994; Torsethaugen et al. 1999; Manes et al. 2001; Calatayud et al. 2007; Herbinger et al. 2007; Mills et al. 2009) and cause stomata to respond sluggishly to environmental cues, changing diurnal patterns of transpiration (Paoletti and Grulke, 2010). The difference in measured rates of change in photosynthetic and stomatal responses to O_3 suggests that models that assume equivalent rates of change over-predict stomatal closure and underestimate transpiration losses in responses to O_3 (Lombardozzi et al. 2012b).

Accurate predictions of stomatal responses, which regulate transpiration, are critical to understanding changes in terrestrial precipitation, runoff, and surface energy balances (Avissar et al. 1993; Gedney et al. 2006; Felzer et al. 2009; van der Ent et al. 2010) and are therefore important in predicting regional drought and flood potential and changes in surface temperatures. For example, simulations predicting the effects of elevated CO_2 suggest that increases in runoff occur due to a suppression of transpiration when stomata close (Gedney et al. 2006; Betts et al. 2007). Changes in conductance also alter the partitioning of sensible and latent heat flux and thereby change surface temperatures (Avissar et al. 1993). Despite the importance of O_3 in altering stomatal conductance, few studies document the resulting hydrologic and

surface energy effects in models and those that do have been limited by an inability to account for the decoupling of photosynthesis and transpiration (e.g. Felzer et al. 2009). While transpiration, runoff, and surface energy partitioning are expected to change in response to chronic O_3 exposure, the magnitude of these responses is still not documented.

The primary objective of this work was to predict global changes in transpiration, gross primary productivity (GPP), and runoff in response to chronic O_3 exposure through considering differential responses of GPP and transpiration. We used the parameterization created by Lombardozzi et al. (2012b) that independently alters photosynthesis and stomatal conductance in the Community Land Model version 4 (CLM4SP), and expanded it to include responses of multiple plant types to O_3 as discerned from a literature review (Lombardozzi et al., Chapter 3) and mean hourly ozone data from 2000-2005. Parameterizing the CLM4SP in this manner is the first time a global-scale model will use hourly O_3 values that can allow for variation in diurnal responses to chronic O_3 exposure resulting in more accurate estimations of O_3 uptake, and is novel in its method of modifying conductance independently of photosynthesis using a mechanistic approach that more realistically mimics physiological responses to chronic O_3 exposure.

METHODS

Model Description

This study simulated global biosphere responses to O_3 using the CLM4SP, described by Lawrence et al. (2011). The CLM4SP uses coupled Farquhar

photosynthesis and Ball-Berry stomatal conductance models (Oleson 2010; Bonan et al. 2011) to simulate plant physiology. The model was run in offline mode forced with a historical atmospheric dataset that includes observed precipitation, temperature, downward solar radiation, surface wind speed, specific humidity, and air pressure from 1948 through 2004 (Qian et al. 2006). Average hourly O_3 values used in the CLM4SP simulations were generated from the Community Atmosphere Model (CAM) simulations of O_3 for 2000 through 2005 (Brownstiener and Hess, 2011). Simulations were run for a total of 25 years, with the first 5 years being discarded in analyses to allow for stabilization of accumulated O_3 damage.

O_3 effects

The CLM4SP was modified to incorporate the effects of O_3 on photosynthesis and stomatal conductance using methods described by Lombardozzi et al. (2012b), in which V_{cmax} and stomatal conductance were directly modified based on responses to the cumulative uptake of O_3 (CUO) calculated in the model. V_{cmax} integrates mesophyllic conductance with enzyme amount and activity and therefore estimates aspects of biochemical carbon fixation that are 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; Ojanpera et al. 1998; Pellegrini et al. 2011; Zheng et al. 2002). The Lombardozzi et al. (2012b) method was improved upon by using plant functional type (PFT)-specific O_3 response equations presented in Lombardozzi et al. (chapter 3) based on a literature review rather than the single response equation used in Lombardozzi et al. (2012b). The critical O_3 threshold was eliminated because the

equations in Lombardozzi et al. (chapter 3) were generated without an O_3 threshold. This simulation used average photosynthetic and conductance responses of temperate deciduous trees, temperate evergreen trees, and crops to CUO from Figure 4.5 in Lombardozzi et al. (chapter 3). Ozone modifications to all broadleaf PFTs used the temperate deciduous tree response equation, needle-leaf PFTs used the temperate evergreen tree response equation, and grass and crop PFTs used the crop response equation. Response equations were adjusted to modify V_{cmax} using an equation that compared photosynthesis and V_{cmax} responses to CUO from all studies published between 1970 and 2011 (Lombardozzi et al., chapter 3):

$$V_{cmax}\text{-}O_3 = 1.49m \times CUO + 1.07b \qquad \text{Equation 1}$$

where V_{cmax}-O_3 was V_{cmax} adjusted for the effects of O_3, m was the slope and b was the intercept from the equations presented in Lombardozzi et al. (chapter 3), and the constants are the slope (1.49) and intercept (1.07) for the ratio of V_{cmax} to photosynthesis. The constants are unitless because they are derived from the ratio of V_{cmax} to photosynthesis.

In addition to mean responses to O_3, a high and a low sensitivity simulation were run. These simulations were parameterized with PFT-specific responses using plants that were either vulnerable or invulnerable to O_3 to determine the range of vegetation responses. The response equations used for each PFT were from Lombardozzi et al. (chapter 3), and are summarized in Table 4.1. Equations were adjusted for V_{cmax} as described above.

Table 4.1: The slopes and intercepts used to parameterize the responses of each plant functional type in CLM simulations based on values presented in Lombardozzi et al. (chapter 3). Photosynthesis slope and intercept values are modified for V_{cmax} by multiplying photosynthesis equations by ratio of V_{cmax} to photosynthesis from figure 3.4 in Lombardozzi et al. (chapter 3).

	Photosynthesis		**Conductance**	
Plant Group	Slope	Intercept	Slope	Intercept
Broadleaf	0	0.8549	0.0049	0.9008
Needleleaf	0	0.9367	0	0.9809
Crop	0	0.8163	0.0006	0.8439
High Vulnerability				
Broadleaf	0	0.8772	0	0.8681
Needleleaf	-1.037	1.4930	-0.0207	0.9231
Crop	-0.0010	0.8860	0	0.7411
Low Vulnerability				
Broadleaf	0	0.9522	0	1.0250
Needleleaf	0	0.9205	0.0088	0.8496
Crop	0	0.7782	0.0361	0.6565

RESULTS

Average [O_3] were highest in the eastern United States and western and central Asia, though concentrations were high in most mid-latitudes (Figure 4.1a). Average cumulative O_3 uptake was highest in mid-latitudes and was correlated with O_3 concentrations in these regions (Figure 4.1). Mean cumulative O_3 uptake ranged approximately from 0 to 600 mmol m^{-2} globally (Figure 4.1b). Regions with low cumulative O_3 uptake were correlated with either low O_3 concentrations, such as in tropical regions, or regions with low productivity, such as in northern Africa.

Gross primary productivity (GPP) was reduced globally by 8% in response to O_3 (Figure 4.2a-b) whereas global transpiration was reduced by less than 1% in response to O_3 (Figure 4.2c-d). The largest reductions in GPP (17%) and transpiration (12%) occurred in mid-latitudes where O_3 uptake, control GPP and transpiration were low. While GPP was reduced in most regions globally, reductions in transpiration were limited to mid- and high-latitudes and were unchanged in large areas of the tropics.

Chronic O_3 exposure caused larger changes in latent heat flux (Figure 4.3a-b) compared to sensible heat flux (Figure 4.3c-d) in mid- and high-latitudes, but both were largely unaffected in tropical and arid regions. In affected regions in the northern hemisphere, latent heat flux decreased approximately 2.5% while sensible heat flux increased approximately 1.5%, with 5% maximum changes in both.

Runoff increased globally by 1% in response to O_3 exposure (Figure 4.4a-b), though changes were minimal in most tropical and arid regions. Most of the increases were within 5% of the control simulation, though runoff was 10% higher in small

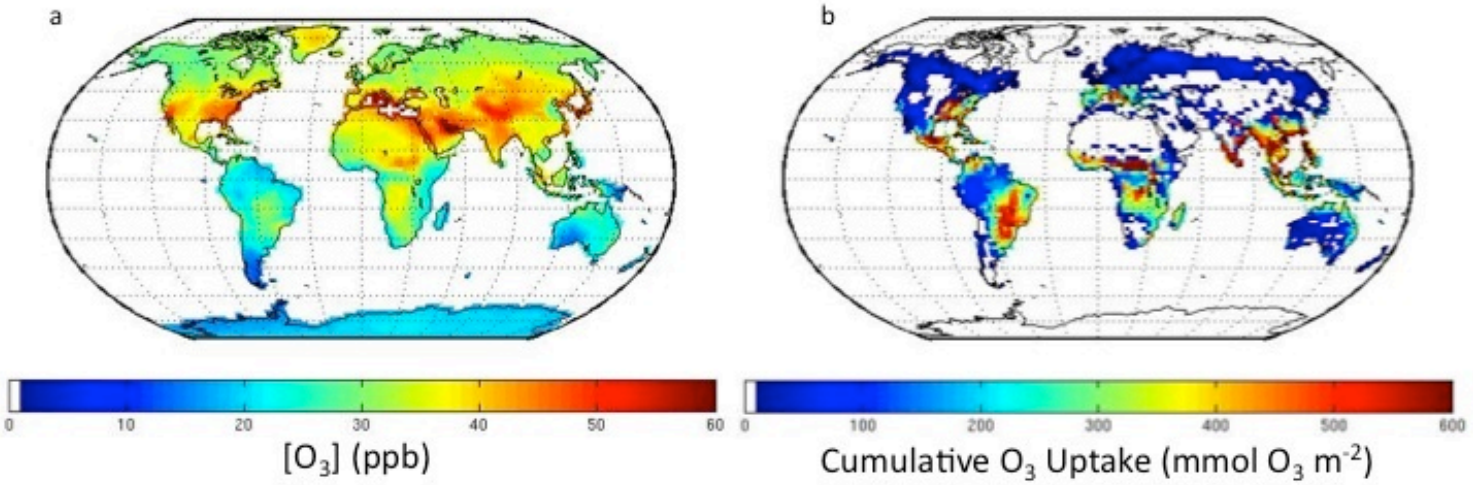

Figure 4.1: Annual mean hourly O_3 concentrations for 2000-2005 used in the CLM simulations (a) and cumulative O_3 uptake averaged over 20 years (b).

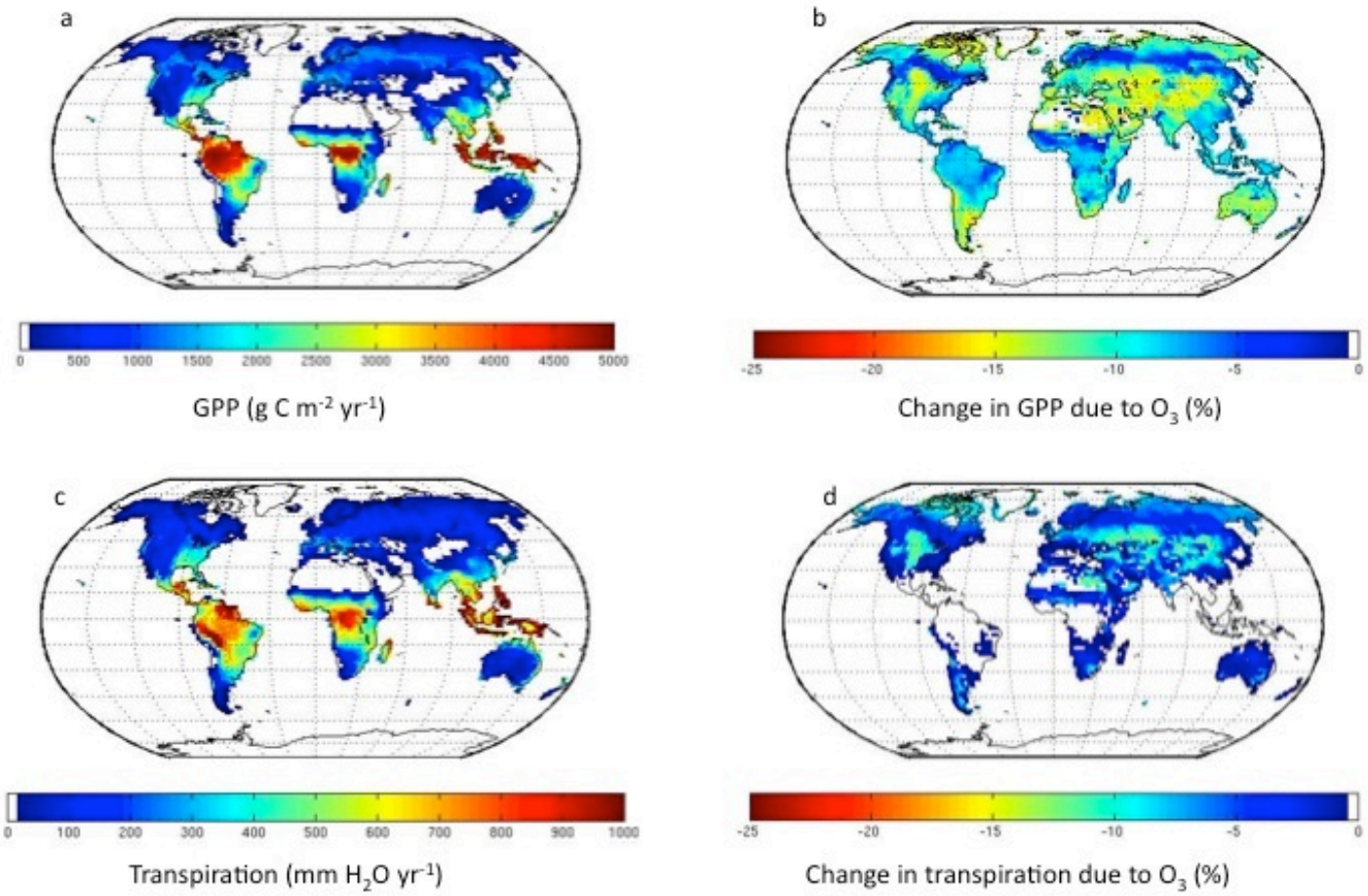

Figure 4.2: Simulated average gross primary productivity (a) and transpiration (c) after exposure to O_3 and percent change from control in GPP (b) and transpiration (d) due to O_3.

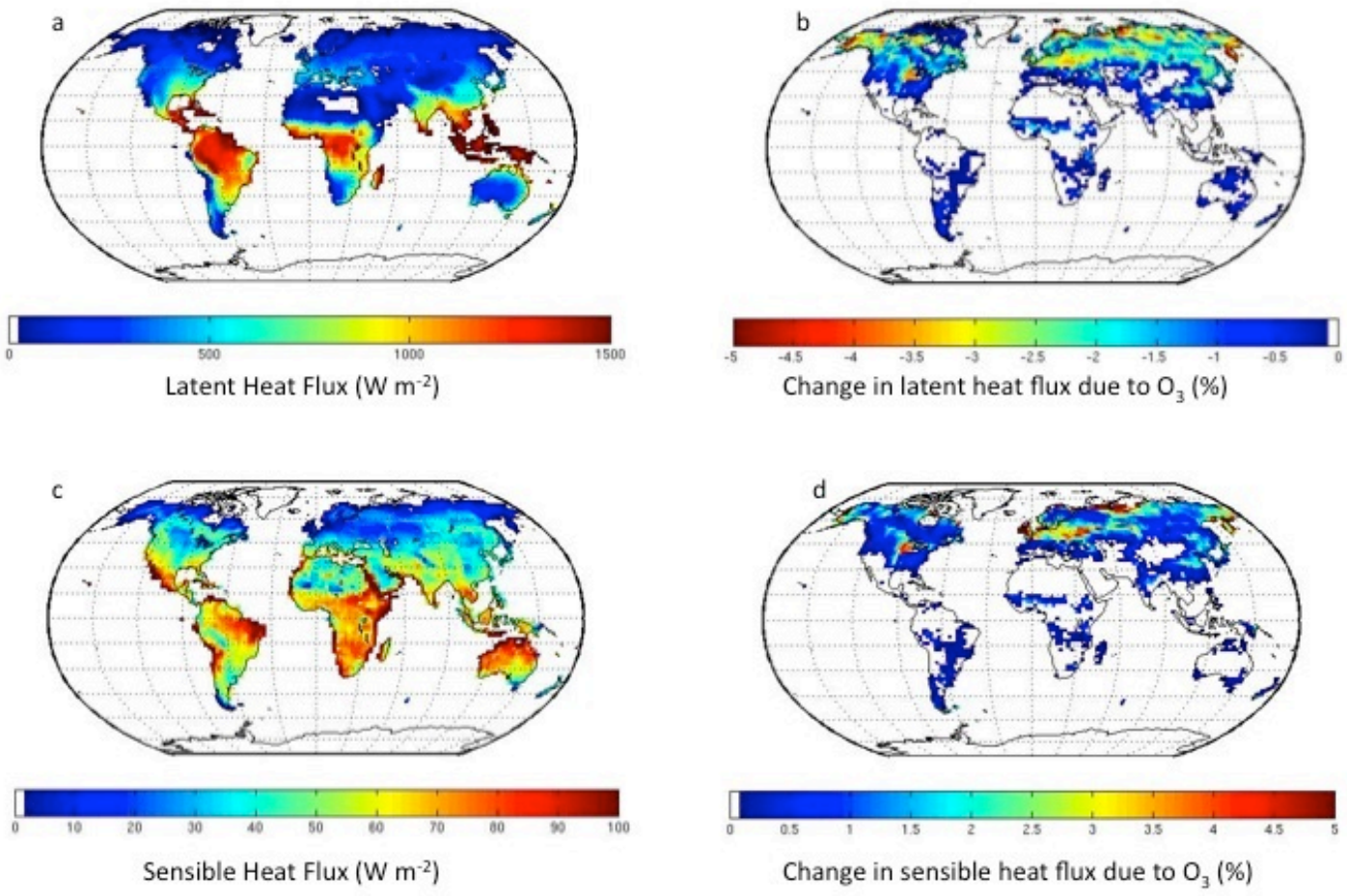

Figure 4.3: Average latent heat flux (a) and sensible heat flux (c) after exposure to O_3 and percent change from control in latent heat flux (b) and sensible heat flux (d) due to O_3.

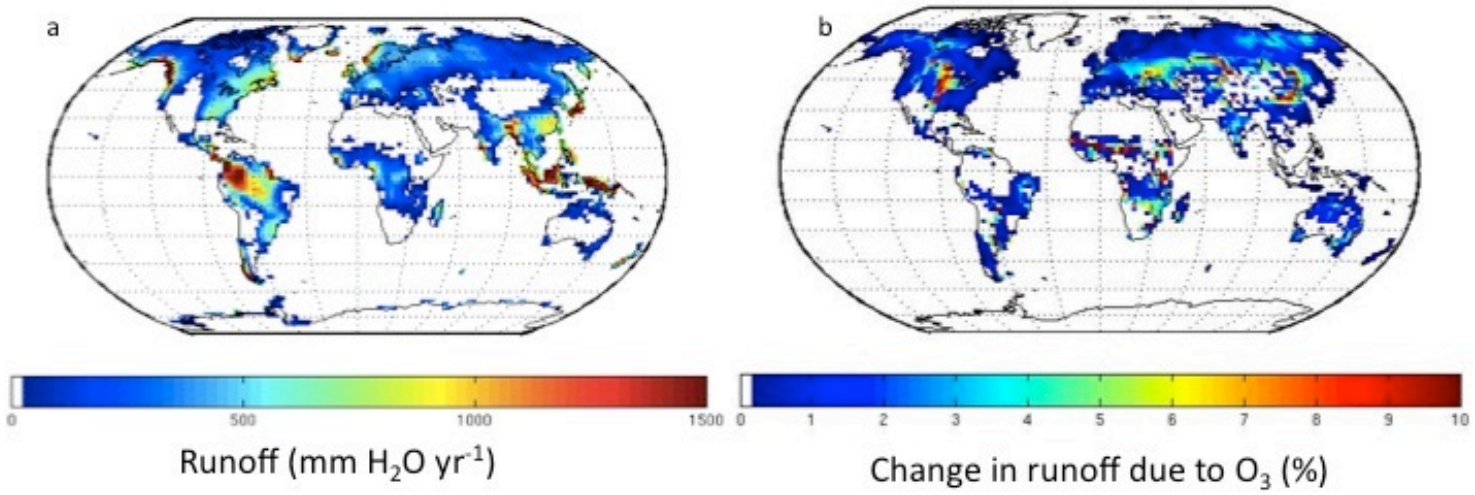

Figure 4.4: Average runoff after exposure to O_3 (a) and percent change from control in runoff due to O_3 (b).

regions in central North America, Africa, and Asia and up to 20% in a few isolated locations.

As expected, simulations running at low plant vulnerability to chronic O_3 resulted in smaller magnitude reductions of both GPP (6%; Figure 4.5b) and transpiration (>0.5%; Figure 4.5d) compared to mean responses to O_3, though were similar spatially. Simulations of high vulnerability plants caused larger decreases in GPP (Figure 4.5a) and transpiration (Figure 4.5c) and had different spatial patterns compared to mean O_3 responses. The high vulnerability simulation resulted in a 24% global reduction in GPP, with decreases near 100% in boreal forests and 50% in some Mediterranean and subtropical climates. Decreases in transpiration (5%) were globally widespread and spatially similar to GPP, with the largest decreases (50%) in boreal regions.

DISCUSSION

Carbon dioxide, water, and O_3 are all powerful greenhouse gases that directly influence climate. Models predict that O_3 reduces plant carbon uptake when photosynthesis is damaged, therefore also contributing indirectly to climate warming (Sitch et al. 2007). Changes in transpiration are equally important to regional climate dynamics through altering atmospheric water vapor concentrations and surface energy fluxes (Avissar et al. 1993), but few studies have quantified how O_3-induced changes in transpiration will impact climate. Felzer et al. (2009) found that elevated O_3, coupled with nitrogen-limitation, has a stronger effect on runoff than elevated CO_2, though the parameterization used photosynthetic modifications to drive changes in

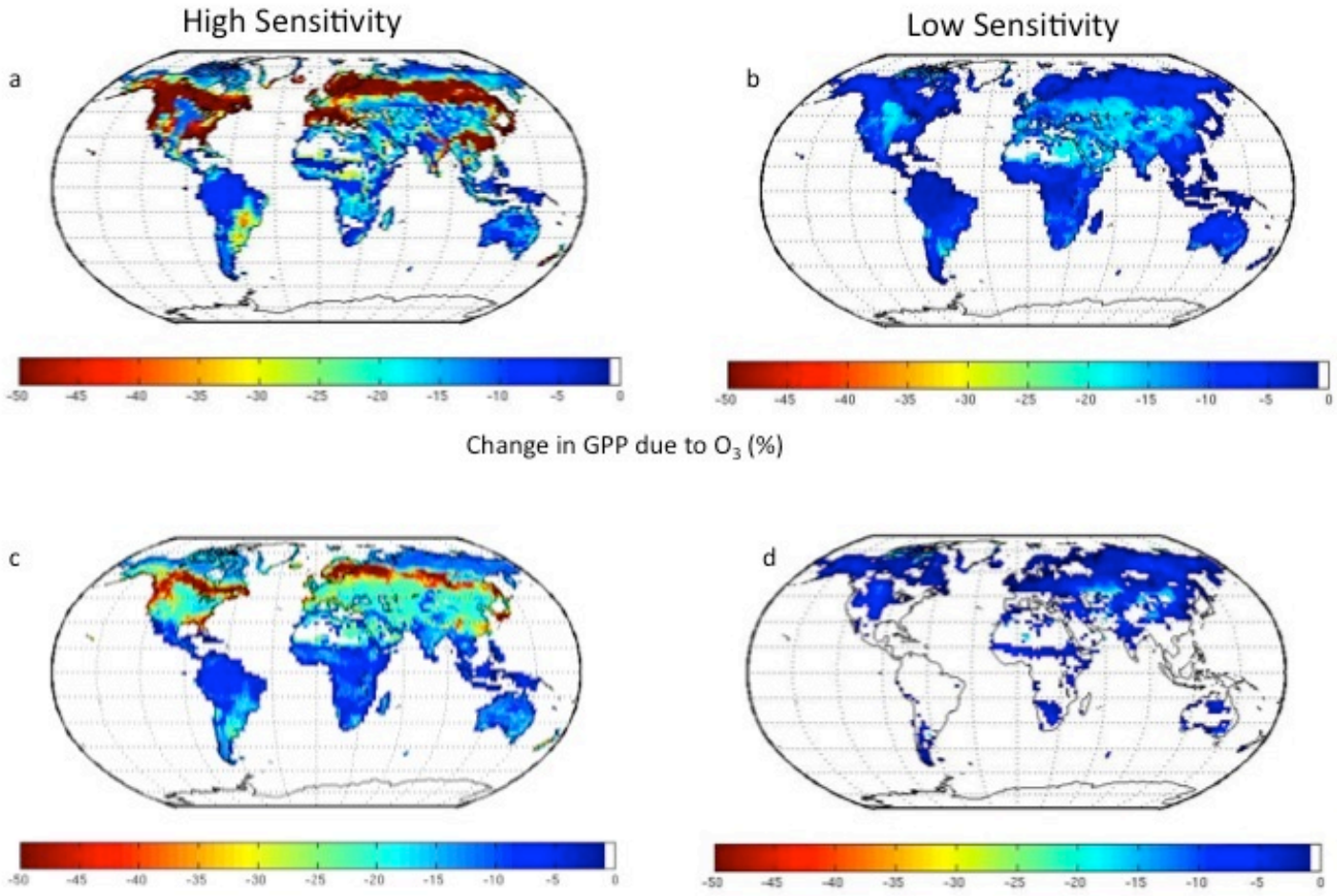

Figure 4.5: Simulated percent changes from control for high (a, c) and low (b, d) plant sensitivity O_3 responses in GPP (a, b) and transpiration (c, d).

conductance. While parameterizing O_3 responses in this manner can under-predict conductance (Lombardozzi et al. 2012a), the results observed in Felzer et al. (2009) demonstrate the large impact of O_3 on surface hydrology and suggest that O_3 might also indirectly affect climate through changing atmospheric water vapor concentrations and surface energy budgets. Our study shows that directly modifying transpiration to account for chronic O_3 exposure significantly alters surface energy budgets and hydrology (Figures 4.4, 4.5) in many regions.

Exposure to present day O_3 caused an average global decrease in transpiration less than 1%, though regional decreases ranged from 2-12% (Figure 4.2d). Transpiration only decreased in temperate regions and did not change in most of the tropics. To our knowledge, no regional- or global-scale models have documented the response of transpiration to O_3. The decreases found in this study are different from the increases in ecosystem-level transpiration rates measured in Appalachian forests under chronic O_3 exposure, which are likely due to increased nighttime transpiration or sluggishness of stomatal responses after chronic O_3 exposure (McLaughlin et al. 2007). Neither nighttime transpiration nor stomatal sluggishness was explicitly considered in the present simulations, perhaps driving the differences between the measured responses in the Appalachian forests and this simulation. The simulated decreases in global transpiration are also smaller than the 13-15% reductions in conductance observed by Lombardozzi et al. (chapter 3) and Wittig et al. (2007) from quantitative literature analyses. These differences are perhaps not surprising because plant responses measured in experiments (i.e. data compiled by Lombardozzi et al. chapter 3 and Wittig et al. 2007) are exposed to artificially high [O_3] and are often in

controlled environmental conditions. Additionally, transpiration data is not widely available in the literature, and comparison of transpiration to conductance is difficult because transpiration predictions are driven by vapor pressure deficit in addition to stomatal conductance.

Decreases in transpiration were not always largest in regions with high CUO. The results of Lombardozzi et al. (chapter 3), used to parameterize the simulations in this study, suggest that conductance decreases at low CUO and is positively correlated with CUO. Changes in conductance are 0 at a CUO of approximately 700 mmol m^{-2}, so regions with uptake in this high CUO range have little or no changes in conductance. Decreases in conductance at low CUO are indicative of the ability of plants to close stomata as a defense mechanism to minimize O_3 flux into the leaves, the main response implied in prior regional- or global-scale assessments of O_3 effects on transpiration. When stomatal cells become damaged by O_3 at high CUO values, the ability of stomata to respond to environmental cues, like humidity, light, or drought stress, becomes impaired and can increase both minimum and maximum conductance (Paoletti and Grulke 2010).

Reductions in simulated GPP were larger and more widespread than reductions in transpiration (Figure 4.2). Exposure to present day O_3 caused an 8% reduction in simulated GPP globally (Figure 4.2b), decreasing the terrestrial biosphere carbon sink and potentially exacerbating warming by increasing atmospheric CO_2 (Sitch et al. 2007). The 8% decrease found in this study using mean hourly O_3 values from 2000-05 is smaller than the 14-23% decrease in GPP between 1901 and 2100 found by Sitch et al. (2007) using monthly values of O_3 simulated from the STOCHEM model. The

larger decreases found by Sitch et al. (2007) are likely because the O_3 data used in their simulation assumes increases in [O_3] throughout the 21st century, which was not included in our study. Using hourly [O_3] rather than the monthly averages used by Sitch et al. (2007) reduces the uncertainty of GPP responses to O_3. The overall reduction in GPP found in this study is also smaller than the 11% reduction in tree photosynthesis found in a meta-analysis (Wittig et al. 2007) and less than 20% decrease after chronic O_3 exposure observed in several plant functional types (Lombardozzi et al., chapter 3), though these studies document responses of plants exposed to artificially high [O_3]. Global estimates of GPP from observations are quite variable, ranging from 119 Pg of carbon per year (Jung et al. 2011; Table 4.2) to 175 Pg of carbon per year (Welp et al. 2011). Estimates on the low end of this range (119 Pg C, Jung et al. 2011; 123 Pg C, Beer et al. 2010) are made from upscaling FLUXNET data that is collected using eddy covariance towers, while estimates on high end of this range (175 Pg C, Welp et al. 2011) use stable isotopes measured by Scripps Institution of Oceanography global flask network. While the unmodified CLM predictions of GPP are on the high end of this range (173 Pg; Table 4.2), the ~8% decrease in GPP caused by O_3 keeps GPP within the range estimated by observations.

Changes in transpiration can cause changes in atmospheric water vapor, cloud formation, and lapse rates that alter regional and global temperatures (Bala et al. 2007, Jung et al. 2010). By decreasing transpiration, O_3 causes latent heat flux to decrease more than sensible heat flux increases (Figure 4.3). Changes in these energy fluxes do not incorporate feedbacks from changing water vapor or atmospheric CO_2 because this

Table 4.2: Global gross primary productivity (GPP), evapotranspiration (ET) and runoff values for control, mean O_3 response, high and low O_3 sensitivity responses compared to observed values presented in [a] Bonan et al. (2011) and [b] Lawrence et al. (2011).

Simulation	GPP (Pg C yr^{-1})	ET (10^3 km^3 yr^{-1})	Runoff (10^3 km^3 yr^{-1})
Control	173	68.42	34.59
Mean O_3 response	159	68.12	34.87
High O_3 sensitivity	132	65.15	37.68
Low O_3 sensitivity	162	68.29	34.70
Observation	119[a] – 175[b]	65[a]	37.75[c]

study used prescribed atmospheric data. However, the changes that are observed suggest that O_3, through changing transpiration, alters surface energy fluxes and may contribute to changes in regional and global surface temperatures. Additionally, the possible increases in atmospheric CO_2 will likely affect changes in surface temperature and should be considered in future simulations.

In addition to altering surface energy budgets, changes in transpiration modify regional hydrology by changing precipitation patterns, soil moisture and runoff (Gedney et al. 2006, Qian et al. 2006, van der Ent et al. 2010). Chronic O_3 exposure elicited increases in runoff by decreasing transpiration (Figure 4.4). Increases in runoff were primarily restricted to temperate and boreal regions and were not observed in arid regions, where runoff is less impacted by rates of transpiration, and tropical regions, where there were no observed O_3-induced changes in transpiration. While global increases in runoff were less than 0.5%, regions affected by O_3 typically increased by 2-3% and a few areas increased by 10% or more. Felzer et al. (2009) similarly found that O_3 increased runoff in temperate forests in the United States and determined that O_3, in addition to nitrogen-limitation, doubled the increase in runoff compared to elevated CO_2. The changes in runoff in the present study were not as dramatic because stomatal conductance was directly modified, rather than decreased linearly with photosynthesis (the method used by Felzer et al. 2009). Overall global changes in runoff were not large. However, the observed regional changes in runoff are of a sufficient magnitude to influence both drought and flood potential in some regions.

Plant vulnerability to O_3 can play a large role in the magnitude of physiological responses (Bassin et al. 2007, Coleman et al. 1995, Lombardozzi et al. chapter 3), but the relative vulnerability of vegetation on regional or global scales is not well documented. Assessing ecosystem sensitivity to O_3 relies on species-level factors like detoxification and physical defense, community-level interactions with O_3 like competition and diversity, and ecosystem-level factors like nutrient availability (Bassin et al. 2007). Most of these factors are not studied thoroughly enough to extrapolate to regional and global vegetation sensitivity. Regardless of these limitations, we ran sensitivity analyses to simulate global high and low vulnerability responses to O_3 based on vulnerability calculations in Lombardozzi et al. (chapter 3) to determine the possible magnitude of change in GPP and transpiration if all vegetation was considered highly vulnerable or not vulnerable to O_3. Globally, GPP and transpiration in simulations run with low vulnerability responses to O_3 were similar to simulations using mean O_3 responses, though decreases were smaller in magnitude for both variables and less widespread in transpiration (Figures 4.5b, d). In contrast, large decreases in GPP and transpiration were observed in high vulnerability simulations and were most dramatic in boreal ecosystems despite the moderate O_3 concentrations these ecosystems experience (Figures 4.5a, c). Large decreases in GPP and transpiration in boreal ecosystems are likely due to the strong negative responses of high vulnerability evergreen trees to O_3 that were used to parameterize this simulation (Lombardozzi et al., chapter 3). While these simulations are informative, they are not realistic depictions of global responses to O_3 because of the limited information

available on the species-level, or even plant functional type, vulnerability to chronic O_3 exposure.

Through changing the physiological processes of photosynthesis and transpiration, O_3 indirectly affects climate by changing surface energy budgets and increasing atmospheric CO_2 concentrations (Sitch et al. 2007). The results of these simulations suggest that current day O_3 concentrations have acted to suppress GPP by ~8% and transpiration by less than 1%, with the strongest effects in temperate and boreal ecosystems. Additionally, by altering transpiration, O_3 has increased runoff and changed surface energy budgets. Simulations that modify transpiration indirectly through changing photosynthesis (e.g. Sitch et al. 2007) will therefore underestimate the indirect impact of O_3 on radiative forcing by overestimating the decreases in transpiration and latent heat flux. Future predictions of climate will benefit by incorporating the effects of chronic O_3 exposure on photosynthesis and transpiration independently to accurately capture changes in regional and global hydrology and surface energy budgets.

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