Title: Interactive Influences of Ozone and Climate on Streamflow of Forested Watersheds

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Ozone Effects on Streamflow of Forest Watersheds

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ABSTRACT

The capacity of forests to mitigate global climate change can be negatively influenced by tropospheric ozone that impairs both photosynthesis and stomatal control of plant transpiration, thus affecting ecosystem productivity and watershed hydrology. We have evaluated individual and interactive effects of ozone and climate on late season streamflow for six forested watersheds (38-970,000 ha) located in the southeastern United States. Models were based on 18-26 year data records for each watershed and involved multivariate analysis of interannual variability of late season streamflow in response to physical and chemical climate during the growing season. In all cases, some combination of ozone variables significantly improved model performance over climate-only models. Effects of ozone and ozone×climate interactions were also consistently negative and were proportional to variations in actual ozone exposures, both spatially across the region and over time. Conservative estimates of the influence of ozone on the variability (R^2) of observed flow ranged from 7% in the area of lowest ozone exposure in West Virginia to 23% in the areas of highest exposure in Tennessee. Our results are supported by a controlled field study using free-air concentration enrichment (FACE) methodology which indicated progressive ozone-induced loss of stomatal control over tree transpiration during the summer in mixed aspen-birch stands. Despite the frequent assumption that ozone reduces tree water loss, our findings support increasing evidence that ozone at near ambient concentrations can reduce stomatal control of leaf transpiration, and increase water use. Increases in evapotranspiration and associated streamflow reductions in response to ambient ozone exposures are expected to episodically increase the frequency and severity of drought and affect flow-dependent aquatic biota in forested watersheds. Regional and global models of hydrologic cycles and related ecosystem functions should consider potential

interactions of ozone with climate under both current and future warmer and ozone enriched climatic conditions.

Introduction

Forests cover 30% of the world's land surfaces, generate 50% of global net primary productivity, and play a significant role in sequestering atmospheric CO_2 and regulating water supply (Bonan, 2008). Forests influence energy redistribution (Ryan et al., 2010) and evapotranspiration (Sun et al., 2011a; 2011b), thus play a key role in the global hydrologic cycle (Jung et al., 2010). Studies have projected that increasing CO_2 fertilization will increase water use efficiency and thus has the potential to increase ecosystem productivity and streamflow (Gedney et al., 2006). However, both carbon assimilation and forest water use efficiency can be negatively impacted by tropospheric ozone. Ozone is a very important global scale pollutant (The Royal Society, 2008) that acts both as greenhouse gas that contributes to global warming (Alley et al., 2007) and a phytotoxic pollutant that affects many interrelated forest physiological processes (Skarby et al., 1998; McLaughlin and Percy, 1999; Samuelson and Kelly, 2001). Tropospheric concentrations of ozone have doubled in the past century and are projected to follow increases in NO_X emissions in the 21st century (Vingarzan, 2004). The proportion of the world' forests that experience phytotoxic levels of ozone (> 60 nl l^{-1}h) is expected to increase from 24% in 1990 to 50% by 2100 (Fowler, 1999). Both empirical and modeling studies also show that tropospheric ozone can have negative impacts on carbon assimilation and growth and thus may limit the capacity of terrestrial vegetation to attenuate rising CO_2 levels (Ollinger et al., 2002; Hanson et al., 2005; Friedlingstein et al., 2006; McLaughlin et al., 2007a, 2007b; Noormets et al., 2010; Ren et al., 2011).

Although the capacity of ozone at ambient levels to reduce photosynthesis and growth of forest trees through internal biochemical changes is well documented (Skarby et al., 1998; McLaughlin and Percy, 1999), reported ozone effects on stomatal conductance (g_s) have been much more variable in both direction and magnitude of change (Mansfield, 1998). While reduced g_s has typically been observed in many controlled experiments with tree seedlings and

saplings (Wittig et al., 2007), the relatively high ozone concentrations used, effects of containment and exposure of plants in chambers, and the relatively short duration of most experiments and measures of response necessarily limit the relevance of such studies for large trees in a forest environment. In addition, ozone has been found to increase g_s and/or impair stomatal responsiveness and closure in response to a variety of environmental variables including drought (McAinsh et al., 2002; Pearson and Mansfield, 1993), vapour pressure deficit (Grulke et al., 2007; Maier-Maercker, 1999; Maier-Maercker and Koch, 1991; Uddling et al., 2009), light (Barnes and Brown, 1990; Grulke et al., 2002; 2004; 2007; Paoletti and Grulke, 2010; Reiling and Davison, 1995), and CO_2 concentration (Onandia et al. 2011). The consequences of such stomatal sluggishness can be increasing g_s and increased water use under conditions which normally induce stomatal closure (e.g., drought, high vapor pressure deficit, low light).

Recent studies with grassland species (Mills et al., 2009; Wilkinson and Davies, 2009; 2010;) have also shown ozone - induced increases in stomatal conductance even under reduced water supply (Wilkinson and Davies, 2009; Hayes et al., 2012). An important mechanism for these responses is ozone-induced reduction in stomatal sensitivity to abscissic acid (ABA), a plant hormone stimulating stomatal closure under drought conditions. Reduced stomatal sensitivity to ABA has occurred in association with increased ethylene production, which is stimulated by ozone exposure (Wilkinson and Davies, 2009; 2010)

Leaf gas exchange models currently employed in ecosystem models (Morales et al., 2005), as well as in Dynamic Global Vegetation Models (DGVM) (Prentice et al., 2007) and General Circulation Models (GCM) (Sellers et al., 1996; Pitman, 2003;) assume a tight link between g_s and photosynthesis that acts to maintain an approximately constant intercellular to ambient CO_2 concentration ratio (Ball et al., 2007; Lening, 1995). These combined stomatal-photosynthesis models predict photosynthesis-mediated reduction in g_s by ozone, but do not account for direct effects of ozone on stomatal responsiveness to other environmental variables. They may thus be in error estimating the effects of ozone on plant water use, especially under conditions with limited soil water availability (e.g., Hayes et al., 2012). Importantly, impairment of stomatal responsiveness to environmental variables may occur at moderately elevated ozone concentrations and in the absence of negative effects on photosynthesis (Onandia et al. 2011).

Chronic and episodic droughts that affect soil water availability mediate plant-soil and plant-plant interactions on a worldwide basis (Schulze et al., 1987), and potential changes in drought frequency and severity have been considered a key scenario in projecting the ecological consequences of future climate change (Wigley et al., 1984; Zhao and Running, 2010). Thus, possible increases in plant water use under current and/or future higher regional ozone concentrations are of particular concern for ecosystem hydrology and productivity under current warming trends.

Our previous studies detected ozone-induced amplification of drought effects on stem growth of mature loblolly pine trees (McLaughlin and Downing, 1995). In subsequent studies in a mixed deciduous forest in East Tennessee (McLaughlin et al., 2007a; 2007b) peak hourly ozone exposures per day averaged over days to weeks were found to play a significant role in reducing stem growth, stimulating sapflow (a measure of whole tree water use) increasing soil drying rate, and, over longer time frames, reducing streamflow of a nearby experimental watershed (McLaughlin et al., 2007b). Observed linkages between process level responses to ozone at the tree, stand, and watershed levels led to the present study to explore the magnitude and consistency of these relationships across forested watersheds at a regional scale. Our working hypothesis was that episodic increases in ambient ozone concentrations in the southeastern US study region would lead to increases in forest leaf canopy conductance, increases in ecosystem-level evapotranspiration, reduced soil moisture, and ultimately reduced late season (August-October) streamflow.

Materials and Methods

In the present study, we have analyzed hydrologic response to ozone and climate variables over time periods of 18-26 years for six watersheds located in the Appalachian states of Tennessee (Walker Branch and Little River), North Carolina (Cataloochee Creek), Virginia (James River and New River), and West Virginia (Fernow Experimental Watershed) (Figure 1). All watersheds were predominantly forested with mixed deciduous forests and catchment size ranging from 38 ha (Walker Branch Watershed in Oak Ridge, Tennessee) to 970,000 ha (New River watershed in Virginia and West Virginia. See Supplementary Information (SI 1.0) for more detailed watershed descriptions, sources of climate and ozone data and a summary of

environmental data for each watershed. We have used average monthly flow over the interval August through October, to represent the seasonal low flow conditions (Smakhtin, 2001) for the study region. The August through October streamflow was the focus of these analyses because it is a period of low rainfall when streamflow is most sensitive to tree transpiration, a major part of total watershed evapotranspiration. During this time, soil moisture and groundwater are primary sources of streamflow and both are responsive to cumulative tree water use over the entire growing season. Several other flow intervals, ranging from minimum weekly flow to growing season (April through October) flow were examined and found to be useful indicators, but are not reported here.

Model Development

We have developed multivariate linear regression models to systematically analyze annual variations in late season streamflow in response to typically 7-11 environmental variables. We used "best subset regression" techniques (Kleinbaum at al., 1998) to evaluate combinations of both ozone and climate variables and precedent time intervals as predictors of annual late season streamflow. Exploratory analyses indicated that neither non-linear models nor Principal Components Analysis improved the detection and partitioning of environmental effects in the linear regression models we present here. Best Regression analysis examines model structure and associated performance at successively more complex levels (n=1 to n=x) to identify the strongest combination of predictor variables for describing annual streamflow for each watershed over time. In our analyses we started with the single best (n=1) predictor variable and then defined the best possible combinations of variables (and models) as N was increased to a level at which model fit was no longer improved by further addition of candidates from the available predictor variable pool. The selection process minimizes covariance among selected variables by using "strongest predictive gain" as the criterion for including each new variable in the current mix of variables included with each successive increase in model complexity.

The candidate environmental variables considered in these analyses were developed to describe potentially relevant combinations of physical and chemical climate and seasonal phenology of forests across the region. Climatic variables included monthly values of temperature, precipitation, ozone exposure, and Palmer Drought Severity Index (PDSI) (Table 1).

Both the timing and duration of forest canopy development were also considered in selecting the most appropriate time intervals for influencing streamflow. The most frequent intervals examined included April through October (the interval of active canopy retention), July through September, and August through October. PDSI, a hybrid index based on antecedent precipitation, temperature, and potential evapotranspiration demand, was included as it is a well-established indicator of regional soil water stress in global change studies (Zhao and Running, 2010). We also examined vapor pressure deficit and solar radiation as predictors of watershed yield at the 39-ha Fernow watershed that had complete climatic data. Neither vapor pressure deficit nor solar radiation significantly improved model performance or estimates of ozone and ozone×climate interactions of models that included PDSI for that the site.

Ozone variables were derived from hourly data over the same monthly intervals as climate variables and were developed to capture three significant aspects of ozone exposure that provide indicators of potential phytotoxicity: These included summed absolute hourly values at or above 60 nl l^{-1} (SUM06); summed exceedances of a 60 nl l^{-1} threshold (AOT60); and finally averages of peak hourly concentration per day (MxH). The values were derived for a 24 hour day in all cases. Other ozone thresholds, including 40 nl l^{-1} were also examined and found to be useful indicators of ozone stress (see SI 3.0). In addition, four product terms were developed to evaluate the potential amplification of drought stress by ozone exposure as previously reported (McLaughlin et al, 2007a). Combinations of ozone and Palmer drought Severity Index included in all of these interaction variables were those that were frequently related to streamflow on an individual basis. These terms were products of MxHO$_3$ and PDSI over 3 time intervals (April- October; May–September; and July-September) and SUM06 (April-October).

All predictor variables were expressed as selected combinations of monthly averages for overlapping two to seven month intervals during April to October. The same time intervals were used for each of the three climate and three ozone variables evaluated. The predictor variables included in each model were initially evaluated by developing the best preliminary models based on the strongest climate-based predictors of flow, then the strongest ozone related predictors of flow. The capacity of added ozone terms to improve model predictive capacity of climate-only models was verified quantitatively and statistically at all stages of these analyses. The variables included in the final models were selected by best regression analyses

from a list comprised of equal numbers of the strongest predictors of flow from both categories of variables. The ozone×climate variables were included in each pre-selection subset.

Model validation and covariance analysis

We have evaluated statistical integrity, strength, and consistency of all regression models developed using several criteria. First, because many of the environmental variables we used are inter-correlated through their linkages to temperature driven climate, we performed several tests to quantify and limit the influence of covariance on both model form and fit to the data. Second, to test for influences of sample size on model parameterization, we evaluated the consistency of model composition and predictive capacity when they were developed from subsets of data within the same historical record. Third, we evaluated predictive capacity of models of varying complexity to accurately fit to streamflow data from other watersheds within the study region. Finally we used both growth patterns of mature trees and measurements of canopy water use of younger tree stands to test mechanistic aspects of the underlying hypothesis for these studies, that ambient ozone levels in the region were sufficiently high to increase forest water use, a prerequisite for reduced stream flow.

Covariance among predictor variables was addressed by statistically isolating the effects of ozone, climate, and climate×ozone interactive terms on model performance by Multiple-Partial Correlation Analysis (Kleinbaum et al., 1998) and by assessing temporal autocorrelation that could lead to spurious results because of non-causative association of parallel trends. Low levels of serial autocorrelation were verified for both flow and physical climate data by testing for the correlation between actual and 1-year lagged data within each series using Pearson Correlation Analysis.

To ensure that we were not simply over-fitting the models, where a large pool of potential input variables and a limited number of observations could result in spurious models, we evaluated the form and strength of models developed by splitting the data into halves and evaluating the consistency of models developed from each of the two independent halves. We found a high consistency (similar R^2 and similar specific predictor variables included) of models from the split data halves, both with each other and with a model developed from the whole

data set. We therefore focused our analysis on models based on the full dataset for each watershed.

The influence of covariance among predictor variables in developed streamflow models was addressed by isolating and individually quantifying the unique contributions of ozone, climate, and ozone×climate interactions to overall model performance for each watershed model developed using Multiple-Partial Correlation Analysis (Kleinbaum et al., 1998). This technique measures predictive power lost from the complete model by subtracting the effects of each variable class (ozone or climate) from overall model performance with that class included. The significance of contributions of each variable class was then tested with a partial F test for statistical significance to overall model R^2. To further isolate ozone and climate effects partial correlation coefficients were also determined for each variable included in each watershed model. This approach evaluated influences of each variable on streamflow with all other variables held constant. Summed effects of all variables within a class, which sometimes included positive and negative coefficients for the same type of variable over time, were also determined to measure the net effect of all variables of that class on streamflow.

Interregional comparisons of model performance

In developing models of each of the six watersheds evaluated in this manuscript, we have sought to maximize model performance by selecting the combinations of predictor variables that provided the best performance (highest R^2_{adj} and highest statistical significance) for each watershed. We have also evaluated how well these individual watershed models might fit the data from other watersheds within the approximate 125,000 km^2 area study region. To do this we used a less complex 7- variable "universal model", to assess model fit to data from other watersheds within the region. The universal model was based on definition of the strongest predictor variables derived from the Walker Branch Watershed. This model was then parameterized for each of the remaining watersheds. Parameterization involved developing a linear regression model using WBWS input variables with the actual environmental and flow data for each respective watershed.

A second method of evaluating the relative importance of climate and ozone variables as contributors to annual variations in streamflow patterns involved a differential analysis of the

input variables contributing most significantly to yearly differences in flow between two comparison watersheds. The two watersheds chosen were WBWS and JRWS, representing respectively relatively higher and lower ozone exposures within the watershed set we examined. Here the patterns of year to year differences in normalized annual flow between the two watersheds over time were analyzed by linear regression against annual differences in values of environmental input parameters between the two watersheds. Relative contributions of climate and ozone to observed flow differences were then quantified and tested statistically.

Cross-Scale Model Verification with Dendroecological Data and Free-Air Concentration Enrichment (FACE) Experiment

Tests of interrelationships among tree and watershed responses to climate and ozone involved two approaches: 1) dendroecological analyses involving parameterization and cross comparisons of a multispecies model of annual tree growth derived from mature trees in Southwest Virginia with the streamflow data and model for the 2100 sq. mile James River watershed from the same region, and 2) measurements of seasonal patterns of canopy level water use of mixed aspen-birch stands under controlled ozone levels using FACE methodology.

Dendroecological analyses of linkages between tree growth and stream flow

The only direct role that ozone can play in affecting watershed scale streamflow is through impacts on tree water use. As a further test and validation of those relationships we examined a 20 year growth record for five tree species from an area within the regional air shed of the James River Watershed. The growth data were derived from 175 increment cores (unpublished data). Sampled trees were from high elevation sites (typically >1000 meters) from the Blue Ridge Mountains of Virginia and 30-60 km northeast of Buchanan, VA, the gauging station for the James River Watershed. The average tree age at coring was 170 years. Five species were included: red oak (*Quercus rubrus*), chestnut oak (*Quercus prinus*), pignut hickory (*Carya glabra*), and yellow poplar (*Liriodendron tulipifera*) as well as shagbark hickory (*Carya ovata*), Four were of the same species or genus included in our previous mechanistic study of ozone effects on tree growth and water use in Tennessee (McLaughlin et al. 2007a). The test applied in this case was

development of a growth model of the five species mean growth chronology from candidate predictor variable subsets used in the development of regional streamflow models. Dendroecological analyses involved parameterization and cross comparisons of a multispecies model of annual tree growth derived from mature trees in Southwest Virginia with the streamflow data and model for the 2100 sq. mile James River watershed from the same region.

Ozone effects on sap flux in the Aspen FACE experiment

Responses of sap flow to ozone exposure have been derived from the Aspen FACE experiment near Rhinelander, Wisconsin (45.6°N, 89.5°W) (Uddling et al., 2008, 2009). The experiment consists of twelve 30-m-diameter circular plots with three control plots and three replicate plots each receiving elevated CO_2, elevated ozone, or both elevated CO_2 and elevated ozone. Ozone exposure levels in the elevated ozone treatment of the Aspen FACE experiment (AOT60 3.6 µl l^{-1} h over 90 days) were very similar to the 26-year mean ambient level in East Tennessee area (AOT60 3.85 µl l^{-1} h) over the same approximate time interval. Ozone and CO_2 treatments were distributed across three blocks. The experiment used 3-to-6-month-old seedlings at 1 m × 1 m spacing in July 1997 and fumigation treatments were initiated in the spring 1998. Each plot is divided into three sub-plots with different tree community composition. Here, we present data for mixed aspen-birch communities (*Betula papyrifera* Marsh. and *Populus tremuloides* Michx., clone 216) growing in control plots and elevated ozone plots in 2004, when steady-state leaf area had been reached.

Sap flux of mixed aspen-birch stands was measured in 66 trees (33 in control + 33 in elevated ozone) in 2004 and scaled to the stand level in this study as described by Uddling et al. (2008, 2009). Birch dominated over aspen with respect to both biomass and sap flux in ambient as well as elevated ozone stands (Kubiske et al., 2007; Uddling et al., 2008). Data were statistically tested for main effects of Ozone and Block and their interactions with Time (repeated measures) by analysis of variance using SAS Proc GLM, version 9.3.1 (SAS Institute, Cary, NC, USA).

Results

Streamflow Model Structure and Performance

Exploratory analyses were used to define the most useful predictor variables from combinations of the 3 climate and 3 ozone predictors of streamflow over various time intervals chosen to represent the period of active forest canopy development and function. Table 2 summarizes results of model testing to determine the influence of adding terms describing the three measures of seasonal ozone exposure to the three variable models developed around the three indicators of climate. These comparisons were made over four time intervals over the growing season. Late season flow (August to October, indicated $Flow_{810}$) was the dependent variable and data in Table 2 compare results of model analysis for one of the smallest watersheds in a high ozone area, Walker Branch Watershed (WBWS) in Tennessee, with analyses from the largest watershed , New River Watershed (NRWS), in a much lower ozone area in Southwest Virginia. Results indicated that models developed around the four time intervals were rather similar in strength, statistical significance, and provided generally similar attribution of the influences of ozone and climate across times within each watershed. The addition of ozone variables significantly improved performance of all models examined in Table 2. However, the ozone contribution for the simple six-variable models was stronger (19% in average) and highly significant statistically for the higher ozone area in Tennessee. By contrast overall models were stronger (higher R^2), but the estimated ozone signal was relatively weaker (4%) and less significant for the New River watershed.

Our primary analyses were of more complex models that included both interaction terms and multiple time intervals for predictor variables. Model R2, significance, and estimates of the relative influences of ozone, climate, and ozone×climate interaction terms were all improved using Best Regression Analysis to identify the strongest sets of predictor variables. Ozone variables were frequently identified along with climate variables as significant contributors to variance in late season flow as the total number of predictor variables was increased from n=3 up to n= 11, the maximum size included in these studies. Detection of effects on streamflow of climate, ozone, and ozone×climate interactions and overall model fit to

the streamflow data increased with increased numbers of predictor variables from n=3 up to n=11 (data not shown).

Comparative model structure and fit to the late season streamflow data for each of the six watersheds are shown in Table 3. These models typically contained at least 7 predictor variables, and ozone terms were consistently represented among the most significant contributors to overall model performance. These empirical models fit the streamflow data very closely (Table 3 and Figures 2a and 2b) and streamflow was strongly predicted by combined climate and ozone variables (R^2= 0.78-0.96). All models were highly significant statistically (p<0.005). As noted in exploratory studies, the addition of ozone variables significantly improved the performance of all climate models. This result was consistent for watersheds from both higher and lower ends of the ozone exposure spectrum and across a 10,000 fold watershed size spectrum. Improvement in predictive capacity of flow models for both the 97 ha Walker Branch Watershed (WBWS) (Figure 2a) in Tennessee and the 970,000 ha New River (NR) watershed in Virginia (Figure 2b) was substantial. The predictive R^2 for the best climate-only model improved from 0.51 to 0.78 for WBWS and from 0.78 to 0.96 for NRWS with the inclusion of ozone variables in the models.

Partitioning of model predictive capacity by Multiple Partial Correlation Analysis (Kleinbaum, 1998) allowed us to isolate the unique contributions of each predictive variable class (predictive R^2) and to test the statistical significance of these contributions to overall model performance. The partitioned effects of ozone, climate, and ozone×climate interaction variables and their statistical significance as contributors to overall model fit to streamflow data are shown in Table 4. Climate and ozone effects on model fit were shown to be individually significant (typically p << 0.02) in all models. ozone×climate interaction terms were also significant for all watersheds except Cataloochie Creek (p< 0.19). Ozone influences were highest (27%) in the areas of highest ozone exposure in Tennessee and lowest (7%) in West Virginia, the least industrialized area. Ozone effects identified by this process were, as expected, typically smaller than climate effects. An analysis of the variance indicated that the magnitude of ozone effects detected by the models were significantly (p <0.05) related to average annual ozone levels from air quality monitoring across the region.

The addition of ozone×climate variables to the models significantly improved overall model fit to the streamflow data. Combined climate influences (Climate + ozone×climate terms)

were improved more by the addition of interaction terms than were estimates of overall ozone (ozone + ozone×climate) influences (Table 4). In addition, the summed effects of ozone and ozone interaction terms, which sometimes combined positive and negative coefficients for different component time intervals (see Table 3), produced net negative effects on streamflow as evidenced by partial correlation coefficients of combined model terms summarized in Table 5. For these simple correlations Climate effects were held constant for each model system within which partial correlations were determined.

Interregional Comparisons of Model Performance

Tests of the predictive capability of a generalized 7-variable model developed for the WBWS (R^2 = 0.74) across the larger study region indicated that the WBWS model had strong predictive capabilities at other watersheds within the region (R^2 = 0.77 to 0.91 at other locations). This versatility was apparently limited to models that considered the influences of the relatively high ozone levels at the southern end of the study region as poor performance was achieved for models developed in the northern region and applied to the South. For example, the JRWS 6-variable model (R^2 = 0.89) that best predicted flow for this watershed did a relatively poor job of predicting WBWS flow (R^2 = 0.41). Differential analysis of the influence of inter watershed differences in annual climate and ozone parameters on annual differences in flow between WBWS and JRWS also supported the role of ozone in influencing differences in outflow patterns from these watersheds over time. Our analyses indicated that model input variables related to annual differences in ozone exposure and ozone×climate interactions explained 46% of observed differences in annual flow between the James River and Walker Branch Watersheds over time while interregional differences in climate terms explained only 17% of the variability in those annual flow differences.

Linkages between Tree Growth and Watershed Streamflow Patterns

To evaluate common responses to climate of mature trees and streamflow within the region, we tested a multi-species model based on annual radial tree growth as a predictor of annual streamflow patterns for the nearby James River Watershed (JRWS). The tree growth regression

model, which was based on 20 years of annual growth data for 175 individual trees representing five species provided a good fit to the annual patterns in radial increment growth (R^2=0.71 and Figure 3a) . The 11-variable tree growth model identified significant influences of PDSI (3 terms), precipitation (2 terms), O3×PDSI (3 terms), and Ozone alone (3 terms) on annual tree growth patterns. Partial F analysis of the tree growth model provided estimates of the influence of climate alone (50%), O_3 alone (15%), and ozone×drought (O_3×PDSI) (47%) on the variability (R^2) in annual tree growth patterns. Both climate/ozone parameters and time intervals identified as significant by the tree growth model also provided strong prediction of late season flows of the nearby 525,000 ha James River Watershed (R^2=0.78 and Figure 3b). Multiple Partial Correlation (MPC) analysis indicated that detection sensitivity of the streamflow model optimized to tree growth compared to the model optimized based on streamflow was reduced for estimated influences on streamflow of climate (12% *vs* 50%), but comparable for influences of both O_3 alone (8% *vs* 8 %) and climate×O_3 (7% *vs* 5%). Thus, both tree growth and streamflow appeared to be influenced significantly by the same set of environmental input variables and both tree and watershed systems responded significantly and negatively to the influence of ozone and ozone×climate variables.

Ozone effects on sap flux in the Aspen FACE experiment

The Aspen FACE experiment (Uddling et al., 2008) provided a more specific experimental test of ozone-induced changes in forest water use based on observations of stand tree water use in mixed aspen-birch communities of 5-8 m tall trees and steady state leaf area index in response to free-air delivery of ozone. There was no significant main effect of ozone on stand sap flux, with reduced leaf area index being compensated for by increased sap flux per unit leaf area (Uddling et al. 2008). However, there was a statistically significant ozone×time interaction (*p* = 0.016), with stand sap flux in elevated compared to ambient ozone increasing progressively during the summer (Figure 4). Upper soil moisture (at 0-15 cm) was decreased by ozone treatment during the first half of the summer (Uddling et al. 2008). This was most likely influenced by a combination of greater understory biomass, greater incident light reaching the understory under the ozone affected overstory (Bandeff et al. 2006), and possibly greater transpiration rate per unit leaf area of the understory, as was measured in the overstory trees, in the ozone treated plots. The lack of differences in overstory transpiration rate per unit of plot

ground area during the early season (Figure 4) suggests that ozone treatment effects on overstory trees did not contribute directly to this observed soil moisture reduction.

Similar Ozone ×Time interactions were found in aspen-birch stands exposed to elevated CO_2 (i.e. elevated CO_2+Ozone plots vs. elevated CO_2 plots) as well as for another year with good seasonal data capture (2006), but was not observed in the pure aspen community type. Further evidence of stomatal loss of sensitivity in the Aspen FACE experiment was provided by observations of ozone-induced reduction in stomatal responsiveness to short-term changes in CO_2 concentration in birch leaves (Onandia et al. 2011).

Discussion

The biological and conceptual foundation for these analyses was previously measured changes in tree growth, canopy conductance and water use in response to ozone (McLaughlin et al., 2007a; 2007b). Evidence that ambient ozone levels in East Tennessee were high enough to increase water use by individual trees, to reduce soil moisture in the rooting zone of those trees, and to reduce water yield of three local watersheds led to our testing the hypothesis that ozone would reduce streamflow over much larger basins across the Appalachian region.

The empirical models we have developed for six watersheds over a 5-State area indicated that climate and ozone acted both individually and interactively to reduce late season streamflow during the 18-26 years examined. These responses were consistent and statistically significant across watersheds representing a wide size range (38 ha to 970,000 ha) and over a large geographical range (430 km latitude by 340 km longitude). Our regression modeling results indicate that ozone effects were consistently important in improving modeling accuracy beyond levels achieved by models based on physical climate only.

Ozone and climate were found to affect streamflow interactively in these experiments; however we were able to provide conservative estimates of their individual effects by several analytical techniques. These included the use of Best Regression Analysis in variable selection during model development, Multiple Partial Correlation analysis in evaluating unique influences of each variable class (ozone, climate, and ozone climate interactions) as they contributed to overall model performance, and Partial Correlation Analysis to evaluate the significance of each

of the three variable classes with climate held constant. These tests and an additional differential analysis of the relative importance of climate and ozone input variables in explaining year to year variation in streamflow between paired watersheds, strongly support the statistical significance of ozone effects on streamflow across our region. Estimates of streamflow effects attributed to ozone in the models were also significantly related to observed differences in ozone exposure levels across the region. Additionally, the form of the ozone variables determined to be significant in the present watershed-scale studies, specifically the importance of peak hourly ozone concentrations per day, was often similar to that of variables identified in previous physiologically-based studies of responses of individual trees (McLaughlin et al., 2007a and 2007b).

Our dendroecological analyses of mature forest trees in the region showed that both tree growth and streamflow were negatively affected by the same set of climate and ozone predictor variables. Ozone can affect tree growth directly by inducing cumulative loss in production of photosynthate and indirectly by increasing water stress. Secondary effects on forest growth and forest hydrology may also occur through changes in root mass and capacity and associated soil organic matter (Loya et al., 2003) and soil moisture retention. Reduced allocation of photosynthate to roots and reduced root mass has frequently been observed in controlled experiments with ozone (Cooly and Manning, 1998). There is also very limited information on reduced root mass in the field, which includes studies at a high pollution site (both O3 and NO_X) in a ponderosa pine stand in California (Grulke et al., 1998) as well as in FACE studies in Wisconsin (Loya et al. 2003). Reduced root growth coupled with increased transpiration would be expected to amplify soil-plant moisture stress and growth reduction of forest trees by ozone, but more information is needed on how these stresses are developed, and compensated for by assimilate allocation processes in forest ecosystems. In our studies increases in water use, water stress, and decreases in soil moisture and streamflow represent a common linkage to ozone exposure that these dendroecological analysis infer, but cannot prove. However, these linkages were supported by measurements of concurrent influences of ozone on sap flow, moisture stress patterns and growth of tree stems, and soil moisture availability in our precedent studies at an intensive research site in Tennessee (McLaughlin et al., 2007a and 2007b).

Our analyses of seasonal changes in canopy transpiration in response to elevated ozone at the Aspen FACE site further support the role of ozone in potentially increasing forest water use. The progressive increase in relative water use of aspen-birch communities under continuous ozone exposure (Figure 4) provide stand level support for the hypothesis of dose-dependent, ozone-induced predisposition of forests to drought through reduced stomatal control of water loss (Mansfield, 1998; Maier-Maercker, 1999). The present study also adds to previous findings from many sources that moderate elevation of ozone concentrations may impair stomatal control of water loss by causing less sensitive (or 'sluggish') stomatal responses. While very high ozone levels and high moisture stress may certainly cause stomatal closure, we expect that under moderate ozone levels found field conditions, ozone affects g_s in two principally different ways: (i) through a stomatal closure response to increased intercellular CO_2 concentrations as photosynthesis is reduced, and (ii) by impairment of stomatal responsiveness to environmental variables (Uddling et al. 2009). While (i) may dominate in plants with ample water supply, it is possible that (ii) may be more important in water-limited environments where stomatal regulation of water losses during times of low soil water availability are critical.

Results from the Aspen FACE site are compatible with our previous findings with mature trees in several ways, including negative effects of ozone on tree growth, a progressive seasonal effect of ozone on canopy water use that was most apparent late in the growing season, and a reduction in soil moisture in the upper soil profile (McLaughlin et al. 2007a and 2007b; Uddling et al. 2008). Since annual scale ozone effects on streamflow can come only through its effects on the rate and/or duration of transpiration during the growing season, we infer that increased plant water use, reduced soil moisture, decreased tree growth, and decreased streamflow are linked components in ozone×climate interactions contributing to the apparent changes in forest water use efficiency observed in our study.

The results of this study support our hypothesis that ambient ozone levels can interact with climate stress to increase water use by the forests in the study region. Our findings on the late season streamflow and ozone relationships and a wide range of previous field studies with forest trees using diverse experimental systems (Maier-Maercker, 1999; Grulke et al., 2002, 2004, and 2007; McLaughlin et al. 2007a and 2007b;and Uddling et al., 2008; 2009) challenge that the limits of the generalization derived from controlled studies that ozone typically reduces g_s (Wittig et al., 2007). Our results suggest that longer term exposure of trees to moderate

ozone concentrations rather cause progressive loss of stomatal control over transpiration that may increase g_s and forest water use in ways not predicted by short term exposures of smaller well-watered plants under higher ozone levels.

Ozone-induced increases in stomatal conductance have other important implications in addition to increased water use, including notably, increased fluxes of ozone to metabolically active tissues within leaves. In a grassland species, Hayes et al., (2012) measured a 30-40% increase in O_3 flux due to ozone induced increases in g_s. Among the many previously cited studies in which g_s has been stimulated or stomatal closure reduced, are also reports of stomata's remaining partially open at night. Nighttime g_s values have ranged from 10-20% of daytime values for ponderosa pine (Grulke et al. 2004) to 50% of daytime gs values for birch cuttings (Mattyssek et al., 1995). Thus the uptake and effects of future ozone levels as ozone increases may be amplified for both forests (Grulke et al., 2002; 2007) and grassland (Hayes et al., 2012) systems because existing models do not reflect observed responses of stomatal conductance to ambient ozone exposure levels.

Our study indicated that the detected increase in water use by mature forests exposed to ambient ozone levels is a generalizable property of forest ecosystems in this study region and not a response of a few species under a narrow range of conditions. Both the direction and magnitude of streamflow responses in our region suggest that ambient levels of ozone will episodically increase the frequency and duration of low flow periods. These measured effects on water use by forests are opposite to those predicted based on assumed stomatal closure induced by O_3 and CO_2 in most current forest ecosystem models (Alley et al., 2007; Sitch et al., 2007; Felzer et al., 2004; Hanson et al., 2005; Ren et al., 2011; Gedney et al., 2006) . We believe that the distinction between responses of mature forests to moderate ozone concentrations in water-limited environments, as measured in the present study, and those (i.e., reduced g_s) sometimes observed with high ozone levels in well watered controlled experiments is important and should be considered in predicting current and future ozone effects on forest ecosystems.

Increasing evapotranspiration and reduced streamflow under regionally elevated ozone exposure have important implications for both the health and function of forest ecosystems. Results for our study region suggest that the frequency and severity of projected drought will likely be amplified by ozone-induced increases in water loss by forest transpiration. Existing ecosystem and global climate models that do not account for these climate-ozone interactions

may be biased towards underestimation of ozone uptake, drought stress, and growth limitations of terrestrial ecosystems under projected future atmospheric conditions. Further evaluation of the feedbacks between current and future tropospheric ozone levels, a warming climate, and forest evapotranspiration in other regions is warranted.

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(2a) with climate only $R^2 = 0.51$ and climate plus ozone $R^2 = 0.78$) and the 970,000 ha New River Watershed in Virginia (2b) with climate only $R^2 = 0.75$; climate plus ozone $R^2 = 0.92$).

Figure 3. Both annual variations in tree radial growth and in late season streamflow from the James River Watershed were linked to ozone and climate in similar ways. The same combinations of climate, ozone, and climate×ozone interactions selected to optimize prediction of annual tree growth of a 5-species mean growth model for 175 trees shown in (Figure 3a, triangles) with $R^2_{adj} = 0.71$, also had high predictive capacity ($R^2_{adj} = 0.78$) in explaining annual variations in late season streamflow of the nearby James River Watershed (Figure 3b, triangles). Symbols are solid circles for observed tree growth (Figure 3a) and streamflow rates (Figure 3b). Flow predictions based on a flow- based model are also shown in 3b (diamonds).

Figure 4. The effect of elevated O_3 ($+O_3$) on stand sap flux (i.e., per unit ground area) of mixed aspen-birch communities in the Aspen FACE experiment at Rhinelander, WI, during the summer of 2004. The O_3 ×Time interaction was statistically significant ($p = 0.016$) while the main effect of elevated O_3 was not ($p = 0.53$). Error bars represent standard error of the mean.

Table 1. Definition and Nomenclature of Terms in Watershed Models

Parameter		Units
Streamflow		
F810	Mean monthly stream flow from August to October	(mm month^{-1})
Ozone Exposure:		
O_3MxH	Monthly mean daily maximum hourly ozone concentration	(nl l^{-1})
O_3AOT60	Monthly sum of hourly O_3 exposures above 60 ppb	(ul l^{-1}*h)
O_3SumO60	Monthly sum of hourly O_3 exposures at or above 60 ppb	(ul l^{-1}*h)

Temperature

TMP	Monthly mean daily temperature	(^{0}C)

Precipitation:

PPT	Monthly mean daily precipitation	(mm d^{-1})

Drought:

PDSI	Monthly mean Palmer Drought Severity Index	None
WDF	Monthly mean water deficit	mm month^{-1}

Monthly mean is defined by the suffix with the initiating and concluding months in the series;

410=April-October; 59=May-September , etc.

Interaction Terms

O$_3$MxH*PDSI	Products of MaxHO$_3$ and PDSI for intervals 410, 59, and 79
SumO6*PDSI	Product of O$_3$SumO60 and PDSI for interval 410

Table 2. Summary of Multiple Partial Correlation Analysis of the importance of predictor time intervals in evaluating climate and ozone influences on late season streamflow for Walk Branch Watershed and New River Watershed. Models were developed around four Predictor Time Intervals to predict streamflow over the August-October time interval.

	Monthly Time Interval for Predictor Variables[1]			
	410	59	79	810
Model (v)^2		Model R^2		

C only (3)	R^2	0.4	0.51	0.4	0.43
O only (3)	R^2	0.37	0.4	0.38	0.21
C+ O (6)[3]	R^2	0.58	0.61	0.67	0.52
		Partial R^2 attributable to Ozone [4]			
Ozone					
Contribution	PR^2	0.21	0.22	0.27	0.31
	p <	0.04	0.00003	0.007	0.002

New River Watershed		Monthly Time Interval for Predictor Variables[1]			
		410	59	79	810
Model (v)[2]		Model R^2			
C only (3)	R^2	0.77	0.75	0.77	0.82
O only (3)	R^2	0.27	0.31	0.22	0.24
C+ O (6)[2]	R^2	0.82	0.79	0.83	0.85
		Partial R^2 attributable to Ozone [3]			
Ozone					
Contribution	PR^2	0.04	0.04	0.03	0.04
	p <	0.07	0.24	0.13	0.31

1. Predictor time intervals are April-October (410), May-September (59), July-September(79), and August - October (810).

2. v represents the number of predictor variables in each model.

3. All C+O (6) models were significant at a p <0.02 level.

4. Partial R^2 values were determined by Multiple Partial Correlation Analysis. A partial F test was used to evaluate the significance of the ozone contribution to each 6 variable (3C + 30) model.

Table 3. Model Form and Parameter Values for Six Appalachian Mountain Watersheds. All non-bolded model terms included are significant at the *p*< 0.05 level.

Watershed	Model	R^2_{adj}	*p*
Walker Branch Watershed (WBWS)	$Flow_{810}$ = 1.365 - 0.0192($O3MxH_{79}$) +0.0226 ($O3MxH_{810}$) - 0.01 15($O3MxHMaxMo$) −0.0126(**$O3MxH_{68}$**) + 0.054 ($O3SumO6_{68}$) +0.422 ($PDSI_{410}$) -0.408($PDSI_{59}$) − 0.0245(**$PDSI_{810}$**) − 0.208(PPT_{410}) + 0.217 (PPT_{59}) +0.0073($PRO3SUMO6*PDSI_{410}$)	0.78	<0.0001
Little River	$Flow_{810}$ = 2143 − 0.37($O3AOT60_{410}$) +55.19($O3MxH_{410}$) + 190($O3AOT60_{810}$) − 61.24($O3MxH_{810}$) + 49.36($O3SUMO60_{410}$)- 119.64($PDSI_{410}$) + 118.67($PDSI_{59}$) -29.05(**TMP_{59}**) + 5.60($O3MxH*PDSI_{410}$)−1.32($O3MxH*PDSI_{79}$) - 16.35($O3SUMO60*PDSI_{410}$)	0.97	<0.0001
Cataloochie Creek	$Flow_{810}$ = -208.2 -294.31($O3AOT60_{410}$)+ 96.70($O3AOT60_{59}$) + 108.14($O3AOT60_{79}$) +14.89($O3MxH_{510}$) -9.48($O3MxH_{79}$) - 10.29($O3SUMO60_{79}$) + 17.68(PPT_{79}) +0.15(**$O3MxH*PDSI_{59}$**) - 0.17(**$O3MxH*PDSI_{79}$**)	0.92	<0.0001
New River	Flow810 = 17921 + 8511($O3AOT60_{410}$) -6423($O3AOT60_{59}$) - 1351(**$O3AOT60_{810}$**) - 625. ($O3MxH_{410}$) +494($O3MxH_{59}$) − 610.3(TMP_{79}) +1429.9($PDSI_{410}$) +493.3(TMP_{59}) -108 ($O3MxH*PDSI_{79}$) 67.06($O3SUMO60*PDSI_{410}$)	0.92	<0.0001
James River (JRWS)	Flow79 = **-6020** +227.7($AOT60_{79}$) + 66.3 (MHO_{410}) − 199.6($O3SUMO60_{79}$)+ 159.3(TMP_{68}) -366.9(TMP_{810}) + 269.4(TMP_{910}) -616.3($PDSI_{79}$)+ 18.28 ($PRO3MxH*PDSI_{410}$) - 9.26($O3MxH*PDSI_{59}$) + 15.07(**$O3MxH*PDSI_{79}$**) -70(**$O3SUMO60*PDSI_{410}$**)	0.91	<0.0001
Fernow Experimental	Flow810 = **-0.659** +0.01($O3MxH_{810}$) +0.287($O3AOT60_{57}$) - 0.367($O3AOT60_{59}$) -0.0697(PPT_{59})	0.92	<0.003

Watershed	$-0.212(TMP_{410}) +0.204(TMP_{59}) - 0.0162 (WDF_{410})$
	$+0.0026(\mathbf{WDF_{59}}) +0.0005(O3MxH*WDF_{410}) -$
	$0.0019(O3SUMO60*WDF_{410})$

Note: unit of flow rate in this table is in cubic feet per second.

Table 4. Contributions of ozone(O), climate (C), and ozone-climate interactions to explain late season flow of six forested watersheds in the southeastern U.S.

Watershed[2]		Complete Model	Climate	C + C*O	Ozone	O +C*O	C*O
						Partial R^2 % for Each Model Components[1]	
Walker Branch	R^2 %	88	23	52	27	27	7
Oak Ridge, TN	v[3]	11	5	5+1	5	5+1	1
(98 ha); n = 26 yrs.	p<[4]	0.0001	0.0001		0.0001		0.04
Little River, GSMNP(W) , TN,	R^2 %	99	1	53	18	34	15
(28,000 ha)	v	11	3	3+3	5	5+3	3
n = 19 yrs.	p<	0.0001	0.001		0.02		0.02
Cataloochie Creek, GSMNP (E), NC	R^2 %	95	8	12	20	23	1
(12,500 ha)	v	9	1	1+2	6	6+2	2
n= 26 yrs	p<	0.0001	0.0005		0.0001		ns (0.19)
New River Glen Lynn, VA	R^2 %	96	17	61	12	15	7

(970,000 ha)	v	10	3	3+2	5	5+2	2
n =19 yr	$p<$		0.0001		0.0001		0.005
Parsons Branch	R^2 %	97	20	61	7	13	10
Fernow, WV	v	10	5	5+2	3	3+2	2
(38 ha); n =18 yrs.	$p<$	0.003	0.0005		0.02		0.005
James River	R^2 %	95	9	57	8	12	7
Buchanan, VA	v	11	4	4+4	3	3+4	4
(550,000 ha, n=26 yrs.	$p<$	0.0001	0.0001		0.0001		0.0001

NOTE:

1. The percentage of total variance explained by each variable class was estimated by determining R2 reduction realized by excluding those variables from the complete model.

2. Monitoring location and watershed area are indicated. Little River and Cataloochie Creek drain the western and eastern slopes of the Great Smoky Mountains National Park (GSMNP).

3. *v* is the number of predictor variables in each model.

4. p is the probability of a greater F for the effects of ozone variables in the full model.

Table 5.Partial correlation coefficients (R) for ozone (O) and ozone (O)*climate (C) interaction terms in watershed and tree growth models. Correlations were for measured late-season stream flow or annual tree growth determined with climate held constant.

Watershed	Variable	R
Walker Branch	C*O	-0.62
Oak Ridge, TN	O+O*C	-0.93
Little River	C*O	-0.62
GSMNP(W) Townsend, TN	O + O*C	-0.99
Cataloochie Creek	C*O	-0.74
GSMNP (E), NC	O + O*C	-0.68
New River	C*O	-0.84
Glen Lynn, VA	O + O*C	-0.7
Fernow	C*O	-0.77
Parsons, WV	O + O*C	-0.97
James River	C*O	-0.62
Buchanan, VA	O + O*C	-0.92
Annual Tree Growth[1]	C*O3	-0.72
Blue Ridge Parkway, VA	O + O*C	-0.93

Walker Branch , TN

Predicted *vs* Actual Tree Growth, Blue Ridge Mountains, VA

Predicted vs Actual Annual Streamflow Based on a Tree Growth Model James River , VA